A red-tinted microscopic image of tissue cells, showing various cell types and structures.

# *The Autoimmune Diseases*

*Edited by*  
Noel R. Rose  
Ian R. Mackay

Sixth Edition



## THE AUTOIMMUNE DISEASES

---

# THE AUTOIMMUNE DISEASES

---

## SIXTH EDITION

*Edited by*

NOEL R. ROSE

*Harvard Medical School, Boston, MA, USA*

IAN R. MACKAY

*Monash University, Clayton, VIC, Australia*



ACADEMIC PRESS

An imprint of Elsevier

Academic Press is an imprint of Elsevier  
125 London Wall, London EC2Y 5AS, United Kingdom  
525 B Street, Suite 1650, San Diego, CA 92101, United States  
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States  
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom

Copyright © 2020 Elsevier Inc. All rights reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Details on how to seek permission, further information about the Publisher's permissions policies and our arrangements with organizations such as the Copyright Clearance Center and the Copyright Licensing Agency, can be found at our website: <http://www.elsevier.com/permissions>.

This book and the individual contributions contained in it are protected under copyright by the Publisher (other than as may be noted herein).

#### Notices

Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

#### British Library Cataloguing-in-Publication Data

A catalogue record for this book is available from the British Library

#### Library of Congress Cataloging-in-Publication Data

A catalog record for this book is available from the Library of Congress

ISBN: 978-0-12-812102-3

For Information on all Academic Press publications  
visit our website at <https://www.elsevier.com/books-and-journals>

Publisher: Andre Gerhard Wolff  
Acquisition Editor: Linda Versteeg-buschman  
Editorial Project Manager: Gabriela Capille  
Production Project Manager: Sreejith Viswanathan  
Cover Designer: Greg Harris

Typeset by MPS Limited, Chennai, India



Working together  
to grow libraries in  
developing countries

[www.elsevier.com](http://www.elsevier.com) • [www.bookaid.org](http://www.bookaid.org)

# List of Contributors

---

- Jakub Abramson** Department of Immunology, Weizmann Institute of Science, Rehovot, Israel
- S. Sohail Ahmed** Translational Medicine, Galapagos GmbH, Basel, Switzerland
- Marco A. Alba** Department of Pathology and Laboratory Medicine, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States
- Youssif M. Ali** Faculty of Medicine, Department of Infection, Immunity and Inflammation, University of Leicester, Leicester, United Kingdom
- Julian L. Ambrus, Jr.** Division of Allergy, Immunology and Rheumatology, SUNY at Buffalo School of Medicine, Buffalo, NY, United States
- Agnes Andersson Svärd** Department of Clinical Sciences, Lund University CRC, Skåne University Hospital, Malmö, Sweden
- Martin Aringer** University Medical Center Carl Gustav Carus, Dresden, Germany
- Shervin Assassi** The University of Texas Health Science Center at Houston, Houston, United States
- Thanda Aung** David Geffen School of Medicine, Division of Rheumatology University of California Los Angeles, Los Angeles, CA, United States
- Ilya Ayzenberg** Department of Neurology, Ruhr University, St. Josef-Hospital, Bochum, Germany; Department of Neurology, Sechenov First Moscow State Medical University, Moscow, Russia
- Robert N. Barker** Immunity, Infection and Inflammation, Institute of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom
- Alan G. Baxter** Comparative Genomics Centre, College of Public Health, Medical & Veterinary Sciences, James Cook University, Townsville, QLD, Australia
- Corrado Betterle** Unit of Endocrinology, Department of Medicine, University of Padova, Padua, Italy
- Stanca A. Birlea** Department of Dermatology, University of Colorado School of Medicine, Anschutz Medical Campus, University of Colorado, Denver, CO, United States
- Niklas K. Björkström** Department of Medicine, Center for Infectious Medicine, Karolinska Institute, Karolinska University Hospital, Stockholm, Sweden; Liver Immunology Laboratory, Department of Medicine, Karolinska Institute, Karolinska University Hospital, Stockholm, Sweden
- Paul A. Blair** Centre for Rheumatology, Division of Medicine, University College London, London, United Kingdom
- Stephan Blüml** Division of Rheumatology, Department of Medicine 3, Medical University of Vienna, Vienna, Austria
- Xavier Bosch** Department of Internal Medicine, Hospital Clinic, Institute of Biomedical Research August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain
- Robert A. Brodsky** Division of Hematology, Department of Medicine and The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD, United States
- Yenan T. Bryceson** Department of Medicine, Center for Infectious Medicine, Karolinska Institute, Karolinska University Hospital, Stockholm, Sweden
- Patrick R. Burkett** Evergrande Center for Immunologic Diseases, Harvard Medical School and Brigham and Women's Hospital, Boston, MA, United States; Pulmonary and Critical Care Division, Department of Medicine, Brigham and Women's Hospital, Boston, MA, United States
- James B. Bussel** Departments of Pediatrics, Medicine, and Obstetrics and Gynecology, New York Presbyterian Hospital, Weill Cornell Medical Center, New York, NY, United States
- Roberto Caricchio** Department of Microbiology and Immunology, Lewis Katz School of Medicine at Temple University, Philadelphia, PA, United States; Department of Medicine (Section of Rheumatology), Lewis Katz School of Medicine at Temple University, Philadelphia, PA, United States
- Livia Casciola-Rosen** Division of Rheumatology, Johns Hopkins University School of Medicine, Baltimore, MD, United States
- Patrizio Caturegli** Department of Pathology, The Johns Hopkins University, School of Medicine, Baltimore, MD, United States
- Benjamin Chaigne-Delalande** National Eye Institute, National Institutes of Health, Bethesda, MD, United States
- Paulina Chalan** Department of Pathology, The Johns Hopkins University, School of Medicine, Baltimore, MD, United States
- Lucienne Chatenoud** INSERM U1151, CNRS UMR 8253, Institute Necker-Enfants Malades, University Paris Descartes, Sorbonne Paris Cité, Paris, France
- Philip L. Cohen** Department of Microbiology and Immunology, Lewis Katz School of Medicine at Temple University, Philadelphia, PA, United States; Department of Medicine (Section of Rheumatology), Lewis Katz School of Medicine at Temple University, Philadelphia, PA, United States

- Megan A. Cooper** Department of Pediatrics, Divisions of Rheumatology and Allergy, Immunology, and Pulmonary Medicine, Washington University School of Medicine, St. Louis, MO, United States
- Ken Coppieters** Global Research, Research Project Management, Måløv, Denmark
- Ronald G. Crystal** Division of Pulmonary and Critical Care Medicine, Department of Medicine, Weill Medical College of Cornell University, New York, NY, United States; Department of Genetic Medicine, Weill Medical College of Cornell University, New York, NY, United States
- Donna A. Culton** Department of Dermatology, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States
- Valentina Damato** Nuffield Department of Clinical Neurosciences, Oxford University, Oxford, United Kingdom; Department of Neuroscience, Institute of Neurology, Catholic University, Rome, Italy
- Anne Davidson** Institute of Molecular Medicine, Feinstein Institute for Medical Research, Manhasset, NY, United States
- Lorenzo Delfino** Clinical Immunology, Section of Internal Medicine, Department of Medicine, University Hospital, Verona, Italy
- Peter J. Delves** Department of Immunology, Division of Infection and Immunity, University College London, London, United Kingdom
- Giulia Di Dalmazi** Department of Pathology, The Johns Hopkins University, School of Medicine, Baltimore, MD, United States
- Betty Diamond** Institute of Molecular Medicine, Feinstein Institute for Medical Research, Manhasset, NY, United States
- Luis A. Diaz** Department of Dermatology, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States
- Ronald J. Falk** Department of Medicine, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States
- Marvin J. Fritzler** Cumming School of Medicine, University of Calgary, Calgary, AB, Canada
- Stefania Gallucci** Department of Microbiology and Immunology, Lewis Katz School of Medicine at Temple University, Philadelphia, PA, United States
- Sapna Gangaputra** Vanderbilt Eye Institute, Nashville, TN, United States
- Brian Gelbman** Division of Pulmonary and Critical Care Medicine, Department of Medicine, Weill Medical College of Cornell University, New York, NY, United States
- M. Eric Gershwin** Division of Rheumatology, Allergy and Clinical Immunology; The Jack and Donald Endowed Professor; University of California School of Medicine, CA, United States
- Igal Gery** Scientist Emeritus Laboratory of Immunology, National Eye Institute, NIH, Bethesda, MD, United States
- Daniel R. Getts** Department of Microbiology-Immunology and Interdepartmental Immunobiology Center, Northwestern University Feinberg School of Medicine, Chicago, IL, United States
- Ralf Gold** Department of Neurology, Ruhr University, St. Josef-Hospital, Bochum, Germany
- Yael Goldfarb** Department of Immunology, Weizmann Institute of Science, Rehovot, Israel
- Jing Gong** Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, United States; Department of Pediatric Nephrology, The Second Affiliated Hospital of Nanjing Medical University, Nanjing, P.R. China
- Siaron Gordon** Graduate Institute of Biomedical Sciences, College of Medicine, Chang Gung University, Taoyuan City, Taiwan; Sir William Dunn School of Pathology, Oxford University, Oxford, United Kingdom
- Jörg J. Goronzy** Department of Medicine, Division of Immunology and Rheumatology, Stanford University School of Medicine, Stanford, CA, United States; Department of Medicine, Veterans Affairs Palo Alto Health Care System, Palo Alto, CA, United States
- Judith M. Greer** The University of Queensland, UQ Centre for Clinical Research, Royal Brisbane & Women's Hospital, Brisbane, QLD, Australia
- Vanesa A. Guazzone** University of Buenos Aires, National Scientific and Technical Research Council, Institute of Biomedical Research (INBIOMED), School of Medicine, Buenos Aires, Argentina
- Luiza Guilherme** Heart Institute (InCor), School of Medicine, University of São Paulo, São Paulo, Brazil; Immunology Investigation Institute, National Institute for Science and Technology, University of São Paulo, São Paulo, Brazil
- David A. Hafler** Departments of Neurology and Immunobiology, Yale School of Medicine, New Haven, CT, United States
- Bevra H. Hahn** David Geffen School of Medicine, Division of Rheumatology University of California Los Angeles, Los Angeles, CA, United States
- Abdel Rahim A. Hamad** Department of Pathology, School of Medicine, Johns Hopkins University, Baltimore, MD, United States
- Hideaki Hamano** Division of Medical Informatics, Shinshu University Hospital, Matsumoto, Japan
- Leonard C. Harrison** Walter & Eliza Hall Institute of Medical Research, Parkville, VIC, Australia
- Dirk Homann** Icahn School of Medicine at Mount Sinai, New York, NY, United States
- Eystein S. Husebye** Department of Clinical Science, K.G. Jebsen Center for Autoimmune Disorders, University of Bergen, Bergen, Norway; Department of Medicine, Haukeland University Hospital, Bergen, Norway

- J. Charles Jennette** Department of Pathology and Laboratory Medicine, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States
- Richard J. Jones** Division of Hematology, Department of Medicine and The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD, United States
- Margaret A. Jordan** Comparative Genomics Centre, College of Public Health, Medical & Veterinary Sciences, James Cook University, Townsville, QLD, Australia
- Jorge Kalil** Heart Institute (InCor), School of Medicine, University of São Paulo, São Paulo, Brazil; Immunology Investigation Institute, National Institute for Science and Technology, University of São Paulo, São Paulo, Brazil; Clinical Immunology and Allergy Division, School of Medicine, University of São Paulo, São Paulo, Brazil
- Shigeyuki Kawa** Department of Internal Medicine, Matsumoto Dental University, Shiojiri, Japan
- Ziya Kaya** Department of Cardiology, Medical University Hospital Heidelberg, Heidelberg, Germany; Germany Centre for Cardiovascular Research, DZHK, Heidelberg, Germany
- Christian W. Keller** Institute of Experimental Immunology, Laboratory of Neuroinflammation, University of Zurich, Zurich, Switzerland
- Nicholas J.C. King** Faculty of Medicine and Health, The Discipline of Pathology, Bosch Institute, School of Medical Sciences, Charles Perkins Centre, The University of Sydney, Sydney, NSW, Australia
- Maleewan Kitcharoensakkul** Department of Pediatrics, Divisions of Rheumatology and Allergy, Immunology, and Pulmonary Medicine, Washington University School of Medicine, St. Louis, MO, United States
- Kendo Kiyosawa** Department of Gastrointestinal Medicine, Aizawa Hospital, Matsumoto, Japan
- Christoph Königs** Department of Pediatrics and Adolescent Medicine, Clinical and Molecular Hemostasis, Goethe University, Frankfurt am Main, Germany
- Mitchell Kronenberg** La Jolla Institute for Immunology (LJI), San Diego, CA, United States
- Vijay K. Kuchroo** Evergrande Center for Immunologic Diseases, Harvard Medical School and Brigham and Women's Hospital, Boston, MA, United States
- Arian Laurence** Department of Haematology, University College London Hospitals NHS Trust, London, United Kingdom
- Eun-Ju Lee** Department of Medicine, New York Presbyterian Hospital, Weill Cornell Medical Center, New York, NY, United States
- Helmar C. Lehmann** Department of Neurology, University Hospital of Cologne, Cologne, Germany
- Åke Lernmark** Department of Clinical Sciences, Lund University CRC, Skåne University Hospital, Malmö, Sweden
- Ida Lindbladh** Department of Clinical Sciences, Lund University CRC, Skåne University Hospital, Malmö, Sweden
- Zhi Liu** Department of Dermatology, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States
- Hans-Gustaf Ljunggren** Department of Medicine, Center for Infectious Medicine, Karolinska Institute, Karolinska University Hospital, Stockholm, Sweden
- Claudio Lunardi** Clinical Immunology, Section of Internal Medicine, Department of Medicine, University Hospital, Verona, Italy
- Knut E.A. Lundin** KG Jebsen Coeliac Disease Research Centre, Department of Gastroenterology, Oslo University Hospital, University of Oslo, Oslo, Norway
- Jan D. Lünemann** Institute of Experimental Immunology, Laboratory of Neuroinflammation, University of Zurich, Zurich, Switzerland
- Michael P.T. Lunn** National Hospital for Neurology and Neurosurgery, London, United Kingdom
- Livia Lustig** University of Buenos Aires, National Scientific and Technical Research Council, Institute of Biomedical Research (INBIOMED), School of Medicine, Buenos Aires, Argentina
- Charles R. Mackay** Department of Microbiology, Biomedicine Discovery Institute, Monash University, Clayton, VIC, Australia
- Ian R. Mackay** Department of Biochemistry and Molecular Biology, Monash University, Clayton, VIC, Australia
- Clara Malattia** Division of Pediatric Rheumatology, Gaslini Children's Hospital, Genoa, Italy; Department of Pediatrics, University of Genoa, Genoa, Italy
- Luisa Martinez-Pomares** School of Life Sciences, University of Nottingham, Nottingham, United Kingdom
- Alberto Martini** Department of Pediatrics, University of Genoa, Genoa, Italy
- Claudia Mauri** Centre for Rheumatology, Division of Medicine, University College London, London, United Kingdom
- Pamela A. McCombe** The University of Queensland, UQ Centre for Clinical Research, Royal Brisbane & Women's Hospital, Brisbane, QLD, Australia
- Fritz Melchers** Max Planck Institute for Infection Biology, Berlin, Germany; Deutsches Rheuma-Forschungszentrum, Berlin, Germany
- Giorgina Mieli-Vergani** King's College London Faculty of Life Sciences & Medicine, GI & Nutrition Centre, Institute of Liver Studies and Paediatric Liver, GI and Nutrition Centre, MowatLabs King's College Hospital, London, United Kingdom
- Frederick W. Miller** Environmental Autoimmunity Group, Office of Clinical Research, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC, United States
- Stephen D. Miller** Department of Microbiology-Immunology and Interdepartmental Immunobiology Center, Northwestern University Feinberg School of Medicine, Chicago, IL, United States
- Masayuki Mizui** Department of Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan

- Jenny Mjösberg** Department of Medicine, Center for Infectious Medicine, Karolinska Institute, Karolinska University Hospital, Stockholm, Sweden
- Christian Münz** Institute of Experimental Immunology, Laboratory of Viral Immunobiology, University of Zurich, Zurich, Switzerland
- Jagtar Singh Nijjar** Department of Medicine and Cambridge Institute of Therapeutic Immunology and Infectious Disease, University of Cambridge, Cambridge, United Kingdom
- David A. Norris** Department of Dermatology, University of Colorado School of Medicine, Anschutz Medical Campus, University of Colorado, Denver, CO, United States
- Kristine Oleinika** Centre for Rheumatology, Division of Medicine, University College London, London, United Kingdom
- Joost J. Oppenheim** Cancer and Inflammation Program, National Cancer Institute, National Institutes of Health, Frederick, MD, United States
- Mathias Pawlak** Evergrande Center for Immunologic Diseases, Harvard Medical School and Brigham and Women's Hospital, Boston, MA, United States
- Cristina Peligero-Cruz** Department of Immunology, Weizmann Institute of Science, Rehovot, Israel
- Anneli Peters** Max Planck Institute of Neurobiology, Martinsried, Germany
- Pärt Peterson** Molecular Pathology, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia
- Kalliopi Pitarokilis** Department of Neurology, Ruhr University, St. Josef-Hospital, Bochum, Germany
- Fabio Presotto** Unit of Internal Medicine, Department of Medicine, Ospedale dell'Angelo, Mestre-Venezia, Italy
- Antonio Puccetti** Department of Experimental Medicine, University of Genova, Genova, Italy
- Hamid Rabb** Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, United States
- Patricia Raczek** Department of Cardiology, Medical University Hospital Heidelberg, Heidelberg, Germany; Germany Centre for Cardiovascular Research, DZHK, Heidelberg, Germany
- M. Jubayer Rahman** Immune Tolerance Section, NIDDK, NIH, Bethesda, MD, United States; NHLBI, NIH, Bethesda, MD, United States
- Manuel Ramos-Casals** Department of Autoimmune Diseases, Laboratory of Autoimmune Diseases Josep Font, Hospital Clinic, CELLEX-Institute of Biomedical Research August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain
- Noel R. Rose** Department of Pathology, Brigham and Women's Hospital/Harvard Medical School, Boston, MA, United States
- Antony Rosen** Division of Rheumatology, Johns Hopkins University School of Medicine, Baltimore, MD, United States
- Mohanraj Sadasivam** Department of Pathology, School of Medicine, Johns Hopkins University, Baltimore, MD, United States
- Adam Schiffenbauer** Environmental Autoimmunity Group, Office of Clinical Research, National Institute of Environmental Health Sciences, National Institutes of Health, Bethesda, MD, United States
- Wilhelm J. Schwaeble** Faculty of Medicine, Department of Infection, Immunity and Inflammation, University of Leicester, Leicester, United Kingdom
- H. Nida Sen** Unit on Clinical and Translational Studies, Uveitis and Ocular Immunology Fellowship Program, National Eye Institute, National Institutes of Health, Bethesda, MD, United States; Department of Ophthalmology, The George Washington University, Washington, DC, United States
- Marc Serota** Department of Dermatology, University of Colorado School of Medicine, Anschutz Medical Campus, University of Colorado, Denver, CO, United States
- Kazim A. Sheikh** University of Texas Medical School at Houston, Houston, TX, United States
- Yehuda Shoenfeld** The Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Tel-Hashomer, Affiliated to Tel Aviv University, Tel Aviv, Israel; Past Incumbent of the Laura Schwarz-Kipp Chair for Research of Autoimmune Diseases, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; Laboratory of the Mosaics of Autoimmunity, Saint Petersburg University, Saint Petersburg, Israel
- Ora Shovman** The Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Tel-Hashomer, Affiliated to Tel Aviv University, Tel Aviv, Israel; Department of Medicine 'B', Sheba Medical Center, Tel-Hashomer, Israel
- Joachim Sieper** Department of Gastroenterology, Infectious Diseases and Rheumatology, Charité - University Medicine Berlin, Berlin, Germany
- Arthur M. Silverstein** Institute of the History of Medicine, Johns Hopkins Medical School, Baltimore, MD, United States
- Robert B. Sim** Faculty of Medicine, Department of Infection, Immunity and Inflammation, University of Leicester, Leicester, United Kingdom
- Kenneth G C Smith** Department of Medicine and Cambridge Institute of Therapeutic Immunology and Infectious Disease, University of Cambridge, Cambridge, United Kingdom
- Josef S. Smolen** Division of Rheumatology, Department of Medicine 3, Medical University of Vienna, Vienna, Austria
- Ludvig M. Sollid** Centre for Immune Regulation and KG Jebsen Coeliac Disease Research Centre, Department of Immunology, Oslo University Hospital, University of Oslo, Oslo, Norway
- Alanna Spiteri** Faculty of Medicine and Health, The Discipline of Pathology, Bosch Institute, School of Medical Sciences, Charles Perkins Centre, The University of Sydney, Sydney, NSW, Australia

- Lawrence Steinman** Neurology and Neuroscience, Stanford University School of Medicine, Stanford, CA, United States
- John H. Stone** Harvard Medical School, Boston, MA, United States; Rheumatology Clinic, Massachusetts General Hospital, Boston, MA, United States
- Uta Syrbe** Department of Gastroenterology, Infectious Diseases and Rheumatology, Charité - University Medicine Berlin, Berlin, Germany
- Ami Tamhaney** Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, United States
- Atsushi Tanaka** Department of Medicine, Teikyo University, School of Medicine, Tokyo, Japan
- Veena Taneja** Department of Immunology and Division of Rheumatology, Mayo Clinic, Rochester, MN, United States
- Kristin V. Tarbell** Immune Tolerance Section, NIDDK, NIH, Bethesda, MD, United States; Amgen Discovery Research, South San Francisco, CA, United States
- Elisa Tinazzi** Clinical Immunology, Section of Internal Medicine, Department of Medicine, University Hospital, Verona, Italy
- Benedict K. Tiong** David Geffen School of Medicine, Division of Rheumatology University of California Los Angeles, Los Angeles, CA, United States
- Ban-Hock Toh** Centre for Inflammatory Diseases, Department of Medicine, School of Clinical Sciences at Monash Health, Faculty of Medicine, Nursing and Health Sciences, Monash University, Clayton, VIC, Australia
- George C. Tsokos** Division of Rheumatology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, United States
- Kenneth S.K. Tung** Department of Pathology and Beirne B Carter Center for Immunology Research, University of Virginia, Charlottesville, VA, United States
- John Varga** Division of Rheumatology, Northwestern University, Chicago, United States
- Diego Vergani** King's College London Faculty of Life Sciences & Medicine, GI & Nutrition Centre, Institute of Liver Studies and Paediatric Liver, GI and Nutrition Centre, MowatLabs King's College Hospital, London, United Kingdom
- Mark A. Vickers** Scottish National Blood Transfusion Service, Aberdeen, United Kingdom; Immunity, Infection and Inflammation, Institute of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom
- Stuart Viegas** Department of Neurology, Charing Cross Hospital, Imperial College NHS Trust, London, United Kingdom
- Angela Vincent** Nuffield Department of Clinical Neurosciences, Oxford University, Oxford, United Kingdom; Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, United Kingdom
- Matthias von Herrath** Type 1 Diabetes Research Center, Novo Nordisk, Seattle, WA, United States
- Anthony P. Weetman** The Medical School University of Sheffield, Sheffield, United Kingdom
- Joel V. Weinstock** Division of Gastroenterology-Hepatology, Department of Internal Medicine, Tufts Medical Center, Boston, MA, United States
- John M. Wentworth** Walter & Eliza Hall Institute of Medical Research, Parkville, VIC, Australia
- Sarah Wesley** Departments of Neurology and Immunobiology, Yale School of Medicine, New Haven, CT, United States
- Cornelia M. Weyand** Department of Medicine, Division of Immunology and Rheumatology, Stanford University School of Medicine, Stanford, CA, United States; Department of Medicine, Veterans Affairs Palo Alto Health Care System, Palo Alto, CA, United States
- Gerhard Wingender** Izmir International Biomedicine and Genome Institute, Balcova/Izmir, Turkey; Izmir Biomedicine and Genome Center (IBG), Dokuz Eylul University, Balcova/Izmir, Turkey
- Michael W. Winter** Division of Gastroenterology-Hepatology, Department of Internal Medicine, Tufts Medical Center, Boston, MA, United States
- Renato Zanchetta** Unit of Endocrinology, Department of Medicine, University of Padova, Padua, Italy
- Moncef Zouali** Inserm-S 1132, Paris, France; University Paris Diderot, Sorbonne Paris Cité, Paris, France

# Acknowledgment

---

Completion of this sixth edition of *The Autoimmune Diseases* would not have been possible without the extraordinary help of Elaine Pearson, Ian's former secretary, and members of the Mackay family. I also thank Gabriela Capille and Sreejith Viswanathan of Elsevier for their guidance, persistence, and patience.

Noel R. Rose

# Autoimmune Disease: Reflections and Projections

Noel R. Rose

Department of Pathology, Brigham and Women's Hospital/Harvard Medical School,  
Boston, MA, United States

## OUTLINE

Foreword	3	Genetics and Exposures	6
Personal Introduction	3	Epidemiology and Prediction	7
Autoimmunity and Autoimmune Disease	4	Acknowledgment	8
Clonal Balance and Regulation	5		

## FOREWORD

The overriding message of this book is that autoimmunity is a common phenomenon in normal individuals, but that it may be a signal of disease, and play a primary or secondary role in the disease process. Autoimmune diseases are diverse because they occur in differing anatomical sites, but they share many key pathogenetic features. In human medicine, they represent a category of disease and benefit from considering them as a family, sharing a genetic pool and experiencing many similar exposures. Most of this book, therefore, is devoted to descriptions of the human diseases that are most firmly established to be the consequence of an autoimmune response. Other chapters of the book detail the common features, their genetic, molecular, and pathologic significance, and their application to diagnoses and treatments.

## PERSONAL INTRODUCTION

I began my career in immunology when I was a student at the University of Pennsylvania School of Medicine in the years 1948–51. I was fortunate to receive a fellowship at the University's Center for the Study of Venereal Diseases. Syphilis and other sexually transmitted diseases had undergone the expected increase during World War II and remained a major public health problem in the immediate postwar years. Most of my time was devoted to my basic research on *Treponema pallidum*. My sole clinical responsibility at the center was to assist in a study of the efficacy of the newly available drug, penicillin, in patients with primary syphilis. We were provided with samples of chancre fluid on admission to the clinic. Following penicillin therapy, samples were taken daily until all the spirochetes were inactive. My job was to enumerate the proportion of viable *T. pallidum* under the darkfield microscope. With a little practice, it was quite easy to recognize the living *T. pallidum* by its well-named “queenly motion,” a dignified profile worthy of royalty. Other dermal spirochetes showed jerky movements suggestive of plebeians.

At this time, the field of syphilis serology was undergoing a major resolution, occasioned by the introduction of the treponemal immobilization test (TPI) by Robert Nelson and Manfred Mayer at the Johns Hopkins University. As the name suggested, the assay was based on the loss of motion of viable *T. pallidum* produced by antibodies (and complement) from patients. This test represented the first diagnostic procedure for demonstrating antibodies specific for the pathogen rather than the standard "Wassermann test" used in various formats in the diagnosis of syphilis. Intrigued by the fact that the TPI depended upon a loss of motility, I wondered if the antibody reacted with flagella of the organism. It was not known whether *T. pallidum* had flagella or moved by its spiral motion. My first publication was an electron microscope study of spirochetes showing that they do have flagella-like structures. I never had an opportunity to go on with the hunch that syphilitic patients produce antibodies to flagellar antigen.

## AUTOIMMUNITY AND AUTOIMMUNE DISEASE

The Wassermann test mentioned above was devised by Wassermann et al. at the Robert Koch Institute in Berlin in the early 1900s (Chapter 2, Autoimmunity: A History of the Early Struggle for Recognition). In its many modifications, it quickly became the standard serologic test to assist in diagnosis and in following the treatment of patients with syphilis (Chapter 69). The antigen to which the Wassermann antibody is directed was later characterized by Pangborn at the New York State Health Department as a phospholipid and named cardiolipin because of its relative abundance in heart tissues. Cardiolipin, a component of cell wall, is found in differing amounts in virtually any organ of the body and in any vertebrate. Despite a great deal of speculation over the years, cardiolipin has never been shown to play a pathogenic role in syphilis even though its specific antibody is still of great value in recognizing and following the infection. It represents the first autoantibody widely used as a diagnostic reagent in the clinical laboratory. One might speculate that had *T. pallidum* not already been identified as the cause of syphilis, cardiolipin, or a replica might have been considered the causative agent of an "autoimmune" disease, syphilis.

The gradual acceptance of autoimmunity as a frequent consequence of a normal immune response and of disease as an occasional consequence of autoimmunity is described in Chapter 2. Briefly, studies of human immunology began near the end of the 19th century with experiments on infection by Roux and Yersin at the Pasteur Institute showing that several major human diseases such as tetanus and diphtheria are attributable to production by the respective pathogen of a specific toxin. Behring and Kitasato produced a disease-specific antibody to the toxin could treat or even prevent the disease. Even in other human diseases in which there is no special toxin, an immune response could provide protection.

Soon afterwards, Jules Bordet showed that an immune response was generated by parenteral injection of even harmless substances, such as red blood cells from another species. Thus immunology became the physiological science not only of infection but of recognition of substances foreign to the host.

A natural follow-on question raised at the time is whether the immune response can be generated by injection of molecules of the host itself. Early experiments clearly showed that antibodies could be induced quite regularly to isolated sites such as the eye or the testis. Production of these antibodies were sometimes associated with disease (Chapter 2).

Paul Ehrlich found that he could produce antibodies to red blood cells of most other goats but never to the immunized donor goat itself. He suggested that there were natural barriers to such autoimmune responses, because they could result in harm. These experiments were widely interpreted by most immunologists to suggest that immune responses to the host itself were not possible. Reports that autoantibodies could be the agents of disease were greeted with the greatest skepticism. A change in thinking followed research on inflammation as a consequence of immunization.

The concept of inflammation as the body's response infection or other cell injury goes back to the earliest days of medicine. Metchnikoff pointed out that, although normally a method of protection, inflammation that exceeds its normal boundaries can itself cause disease. In contrast to immunity, inflammation is nonspecific; that is, it does not target to the specific inducing molecule. The major cells initiating the inflammatory process such as polymorphonuclear and mononuclear cells broadly respond to nonself or altered-self substances. They are not capable of distinguishing fine differences among molecules as can the specialized lymphocytes of the immune system (Chapters 4, 10 and 11).

By the 1950s, immunology crossed the line and recognized that uncontrolled inflammation induced by an autoimmune response can cause disease in the host. This dramatic change in thinking can be related to the introduction of a number of new technical advances in immunology that opened previously impossible opportunities. They included novel methods for separating mixtures of antibodies or antigens in gels, localizing antibodies in tissues by labeling them and providing more sensitive procedures for identifying and quantitating circulating antibodies. These methods showed that antibodies capable of reacting with antigens of the host are commonly found normal animals and in healthy individuals. When present in heightened amounts, beyond a population-based "normal" range autoantibodies were often associated with, and predictive of, disease (Chapters 69 and 70). On occasion autoantibodies can be acknowledged directly as a causative agent of pathology based on accidental or deliberate transfer of the autoantibodies between human subjects. Most of the advancement in the field, however, has depended on investigating genetically defined animals in which cellular manipulation and transfer can prove that autoantibodies or selected lymphocytes, even in the absence of antibody, can cause of similar disease. Animal experiments were particularly convincing in instances where the human disease involves inflammation in a specific organ site such as the brain or the thyroid gland [Chapters 40 and 51]. Other diseases more often systemic can be modeled in animals by selection or by genetic manipulation [Chapters 30 and 31]. By the early 1980s, the list of diseases reputed to be caused or significantly aggravated by autoimmunity, based on firm experimental evidence, increased several folds.

Concurrently, the immune response itself was dissected with greater precision. The clones of B cells as the source of specific autoantibodies (Chapters 8 and 9) provide the basis for clonal-based therapies (Chapters 71 and 72). Clonal definition of T cells based on antigen specificity is a bit more difficult because additional limitations are placed on T-cell recognition. It requires help from cells bearing the corresponding major transplantation markers which serve as antigen-presenting cells, to be discussed later. Despite their clear association with autoimmune-induced inflammatory diseases, autoantigen-specific T cells are more often more conveniently identified and measured through cell surface markers or by the cytokine products generated by living, activated cells rather than by direct antigen binding (Chapters 5 and 6).

The realization that self-reactive B cells and T cells are plentiful in normal individuals raised the question that Ehrlich had considered: Will they do harm? Disease due to autoimmunity is relatively uncommon compared to the frequency of autoimmune lymphocytes because evolution has provided the host with devices to tolerate them. Much of the knowledge of tolerance came from studies on transplantation which proceeded in tandem with research on autoimmune disease in the 1950s and 60s.

## CLONAL BALANCE AND REGULATION

The experimental basis of specific immune tolerance was established by Medawar et al. Their first experiments were inspired by the original observation of Ray Owen. He reported that cattle exposed in utero to nonidentical blood cells can tolerate these cells without the expected immune response. Medawar supported these observations by transferring spleen cells into genetically different new-born mice. He found that the recipient mouse tolerated skin grafts from donor mice.

MacFarlane Burnet realized that these experiments provided an alternative to the then-prevalent instructive theories of antibody production. Those earlier theories suggested that the broad, almost infinite, recognition capacity of the cells of the immune system was achieved by instructions given by the antigen itself. Burnet proposed that the role of antigen may be to select its counterpart "immunocyte" (lymphocyte) from a large repertoire of T cells and B cells. The selected ones then multiplied and produced a clone of identical antigen-specific lymphocytes. Burnet further envisioned that an antigen-driven negative selective process takes place during the embryonic development of immunologically active lymphocytes. In that way the immune repertoire encompasses the universe of nonself-antigens but eliminates reaction to self-antigens.

The antigen-selection model became the prevalent explanation for self-tolerance but left open the question of how autoimmunity could ever occur. Burnet's first suggestion was that autoimmunity may represent a chance mutation, resulting in production of a "forbidden clone." Experimental evidence supporting this view, however, has been meager because autoimmune responses are rarely mono- or pauciclonal. More commonly negative selection is incomplete, so that a small population, mainly of low affinity T cells or B cells, escapes complete elimination. Small numbers of self-reactive lymphocytes are not uncommon in the blood. Based on their low frequency and low affinity, they may never inflict pathologic effects. They do, however, represent a potential risk for pathogenic autoimmunity.

If the self-antigen is presented in a particularly potent manner even low affinity T cells and B cells can be stimulated into action. These conditions prevail when an antigen is given with a powerful adjuvant to potentiate the response. In inducing experimental models of autoimmune disease, the appropriate antigen is commonly administered with complete Freund adjuvant containing both a mycobacterial peptide and selected mineral oil. This method is used in producing models of organ-specific autoimmune disease in experimental animals. Autoimmune diseases have often been associated with a prior infection that provides an adjuvant effect (Chapters 21 and 70). In other instances the stimulus from a viral infection or environmental agent may serve as an adjuvant (Chapters 20 and 64).

Good health of the immune system depends upon maintaining homeostasis through a proper balance between the signals that may stimulate clones of self-reactive lymphocytes and those that tend to suppress them. I suggested several years ago that the term “clonal balance” is more appropriate than clonal selection for describing a healthy immune system. It is likely that multiple mechanisms are in place in most individuals most of the time that maintain favorable clonal balance. Some of these regulatory agents may be found within the immune system itself such as suppressor B cells, T cells, and monocytes. Additional signals come from other regulatory systems such as the endocrine system. The sex bias, usually favoring females, and age-related susceptibilities represent the close interaction between endocrine and immune systems (Chapter 24). Recent studies of neurologic and psychological disorders and autoimmune disease exemplify comparable interactions, particularly through the generation of mediators that affect both immunologic and neurologic responses. The availability of multiple, overlapping regulatory mechanisms is the most effective mechanism for assuring a well-balanced immune system.

Strong evidence supporting the importance of clonal balance in human subjects has come from recent investigations on immunologic approaches on cancer therapy. The oncologist may administer drugs designed to inhibit critical steps (checkpoints) that regulate self-directed immune responses, including antigens on cancer cells. Checkpoint inhibitors are drugs that facilitate the development of a cancer-specific immune response. In some instances, however, they can also trigger an immune response to normal self-antigens and cause autoimmune disease. Two diseases that were considered rare are relatively frequent outcomes of efforts to alter the normal immune homeostasis. Well-studied examples are hypophysitis (Chapter 43) and myocarditis (Chapter 64).

Why does the body nurture and maintain self-reactive immune cells? One possibility is based on the well-described phenomenon of molecular mimicry; that is, antigenic determinants (epitopes) found in other organisms, even in plants, may closely resemble epitopes present in the body of the host. In some instances, the mimicry may be involved in inducing a pathogenic autoimmune response (Chapter 21). The complete elimination of these cross-reacting lymphocytes could significantly deplete the extensive immunologic repertoire that is the basis of clonal selection theory.

## GENETICS AND EXPOSURES

The next theme for research on autoimmune disease reached prominence in the 1970s based on understanding that immune system function follows the principles of Darwinian selection. The growing acceptance of autoimmune diseases as a family of related diseases reflected itself in a growing number of clinical reports of clustering of different autoimmune disorders. A patient with one autoimmune disease tends to have a greater likelihood of a second or even third autoimmune disease suggesting shared heredity and/or environment. Some of the reported cooccurrences were quite striking; for example, an individual with autoimmune thyroid disease a heightened risk of autoimmune diabetes due to shared genes or common exposures (Chapters 40 and 70).

A concurrent pathway of research supporting a genetic component of autoimmune disease came from long-standing investigations of genetically defined rodents, especially rats and mice. These investigations, dating back to the 1930s by Peter Gorer in London and George Snell at the Jackson Laboratory, recognized that transplantation of tumors between experimental animals depended mainly upon the genetic constitution of the animals rather than on tumor-specific antigens. The studies led to development of inbred strains of rodents in which histocompatibility genes were defined. By the 1960s, it was recognized that there is a cluster of genes that are pre-eminent in controlling acceptance of tumor and tissue transplants. The major histocompatibility complex (MHC) dominated acceptance of allografts in all of the animals investigated, including humans (Chapters 23 and 26). Hugh McDevitt and Michael Sela, taking advantage of these genetically defined animals, showed that the MHC regulated the immune response to simple synthetic or small natural peptides.

Investigations on autoimmune disease began in the late 1960s when Vladutiu and I were able to induce experimental autoimmune thyroiditis in inbred mice, taking advantage of the many defined strains provided by the Jackson Laboratory. We were able to discern that the autoimmune response to a large protein molecule, thyroglobulin, was determined genetically by the mouse MHC. Later we were able to report that a similar MHC-related predominance was present in spontaneously developing thyroiditis in partially inbred chickens. Parallel studies of other autoimmune diseases including both experimentally induced models and in spontaneous examples rapidly followed and genetic studies in humans became the focus of extensive investigation.

In addition to the MHC as the dominant inherited determinant of resistance to autoimmune disease in experimental animals and humans, many other genes make small but significant contributions. Frequently these genes turn out to be the controlling factors already known to be important in regulating the normal immune response as well as controls of cancer immunity.

These genes, acting collectively, contribute to the broad susceptibility to autoimmune diseases. Yet a few examples have been described in which a single gene locus dictates susceptibility to a group of autoimmune diseases. This turned out to be particularly striking in the polyendocrine syndromes where autoimmune regulatory genes predominate by preventing expression in the thymus of certain organ-specific antigens the endocrines during negative selection (Chapter 39).

Despite the importance of genetic regulation, susceptibility to autoimmune disease is not attributable mainly to heredity. Among even highly inbred rodents and identical human twins, there are still differences in the occurrences of autoimmune disease. Monozygotic twins may show a statistically significant cooccurrence but rarely develop the same autoimmune disease at the same time. These differences may be attributable to the well-known postgermline changes. There is already solid evidence that epigenetics plays an important role in determining autoimmune susceptibility (Chapter 25). Genetically identical animals differing only in the sex-related genes can be dramatically different in susceptibility (Chapter 24). Although in most instances, the bias favors females, few autoimmune diseases are more prevalent and sometimes more severe in males.

In addition to their own genome, humans carry a second population with its own genetic constitution, the microbiome (Chapter 19). The interaction between a human host and its bacterial inhabitants calls attention to the importance of nutrition in immune response. The microbial population, once established during infancy, tends to be quite stable in health but can change remarkably with illness. Working out the cause-and-effect relationship remains an intriguing problem for research.

One of the great voids in research on autoimmune diseases is the lack of firm knowledge of when and how noninfectious agents in the environment may instigate a pathogenic autoimmune response (Chapter 2). Studies of experimental animals have made it clear that exposure to nonviable agents such as mercury have vastly different effects on autoimmune responses. Studies on humans have been difficult because it is rare that a single environmental agent can be identified and studied in isolation. Generally, large populations must be investigated over long periods of time to minimize the effects of background confounders. Other than governmental agencies, few institutions are able to provide the infrastructure for such large-scale, long-term studies. Yet it is likely that such exposures represent the reason that many of the autoimmune diseases appear to be increasing in prevalence, particularly in the more industrialized societies.

One example of reductionist experiments carried out on human subjects is the administration of vaccines (Chapter 22). Because of their importance in maintaining a high standard of public health, vaccine safety remains a matter of great concern. Despite the hundreds of millions of vaccinations administered around the world, rarely is there a statistically valid increase in a well-recognized disease. A small cohort of individuals receiving seasonal influenza vaccine in 1967 developed Guillain–Barré syndrome. The basic biologic for this unique event has not been elucidated. Ongoing studies of narcolepsy may provide a second example of influenza vaccine–related autoimmune disease. Here the potential antigen must be better defined.

Vaccination is immunology's greatest success. Vigilance must be maintained for public health purposes but also for opportunities to identify critical early steps in the induction of pathogenic autoimmune responses in humans.

## EPIDEMIOLOGY AND PREDICTION

As indicated previously, remarkable progress has been made in detailed genetic studies of susceptibility to autoimmune disease in experimental animals. In contrast, epidemiologic investigations in humans have too often been indecisive. Large populations must be studied over time to track all but the most prominent genes.

Future volumes of "The Autoimmune Diseases" will certainly need to provide a greater understanding of the epidemiology of autoimmune disease. Only in that way can we determine who is most likely to develop one of these diseases, what the outcome may be, and what methods could be instituted to treat or prevent them. Such epidemiologic study is emerging through technologic advances in handling large amounts of information. They permit us for the first time to study enough individuals over long periods of time to look for correlations and associations with environmental and dietary exposures.

At the same time, the availability of clinical data collected over the life-span allows us to focus with more precision on individuals and permit a rational definition on a personal basis of normality and departure from normality. The clinical laboratory will not have to depend upon population-based normal ranges but may also look more precisely at departures from personal norms, representing an individual's established homeostasis.

At this time, the best predictor of autoimmune disease remains the antibody. It is well established that in many autoimmune disease elevated levels of autoantibodies appear well before clinical evidence. Combined with family history or genetic analysis, they open the possibility to earlier intervention.

The most advanced studies have been carried out with autoimmune diabetes (Chapter 70). A combination of genetic data obtained either from family history or actual genome studies have been coupled with appearance or rise of multiple relevant autoantibodies. These early warning signs convey with a high degree of probability that a child will develop diabetes. The next goal will be the design of benign interventions; that is, procedures that could be instituted in a clinically well child that will prevent the occurrence of a disease without itself producing injury.

New, more targeted treatments appear almost daily and promising opportunities for the specific inhibitors of key steps of pathogenesis are on the horizon (Chapters 70 and 72). The future management of the autoimmune diseases will certainly be intervention before irreversible damage has occurred. Ian Mackay and I hope that this book will help to achieve that goal.

## Acknowledgment

I thank Arthur Silverstein and Jorge Kalil for their corrections, suggestions, and wise advice.

# Autoimmunity: A History of the Early Struggle for Recognition

Arthur M. Silverstein

Institute of the History of Medicine, Johns Hopkins Medical School, Baltimore, MD, United States

## O U T L I N E

<b>The Search for Autoantibodies</b>			
<i>Horror Autotoxicus</i>	10	<i>The Wassermann Antibody</i>	12
<i>The Nature of Ehrlich's "Contrivances"</i>	11	<b>The Shift to Immunochemistry</b>	12
<b>Challenges to the Ehrlich Thesis</b>			
<i>Lens Autoantibodies</i>	11	<i>The Return of Immunobiology</i>	13
<i>Paroxysmal Cold Hemoglobinuria</i>	11	<b>Concluding Remarks</b>	15
<i>Sympathetic Ophthalmia</i>	12	<b>References</b>	15

1955-1965 [was] the decade marked by the question, Does autoimmunity exist? [Rose and Mackay \(1985\)](#)

It is one of the curious situations in science that certain well-demonstrated facts are refused entry into the body of accepted knowledge and may become so effaced from the collective memory that they must be rediscovered many years later in order to gain acceptance. Such was the case in immunology, with [Ehrlich's \(1897, 1900\)](#) suggestion that an immune response is initiated by the antigen activating an antibody receptor on the surface of a cell, or with [Clemens von Pirquet's \(1910\)](#) explanation of serum sickness as an immune complex disease. More pertinent to the present discussion, the same was also true of [Donath and Landsteiner's \(1904\)](#) demonstration that paroxysmal cold hemoglobinuria (PKH) is an autoimmune disease.

The cause of this selective amnesia may merely be an earlier contradictory pronouncement by a respected leader in the field; sometimes it lies in an inability to fit the new finding into the working paradigm that guides thought in the field, as the historian of science [Kuhn \(1970\)](#) has suggested. In the end, it may be that [Fleck \(1979\)](#) was right when he proposed that the acceptance of a fact in science depends less upon its truth than upon its acknowledgment by the leaders in the discipline (whom Fleck called the Denkkollektiv). Ultimately, the truth in science will emerge, although sometimes it takes a very long time.

The earliest discoveries in immunology were made in the context of the battle to ward off infectious diseases. These included [Pasteur's \(1880\)](#) preventive vaccines, [Metchnikoff's \(1884\)](#) bacteria-eating phagocytes, and [Behring and Kitasato's \(1890\)](#) curative antidiphtheria and antitetanus sera. It seemed evident that these efficient mechanisms for the protection of the body were Darwinian adaptations designed to prevent or control infectious disease, a widespread view in the 1890s even after it was demonstrated that specific antibodies might be formed against such innocuous antigens as egg albumin, bovine serum proteins, and sheep red cells. It seemed unthinkable at the time that a grand mechanism designed to prevent disease might turn the tables and cause disease. So well established did this concept of a benign immune system become that demonstrations that antibodies

might cause disease were either disregarded entirely or else ascribed to “aberrant” antibodies acting under the influence of a “misdirected” immunity (Silverstein, 2009). This was how the early discoveries of serum sickness, hay fever, asthma, and a variety of other immunopathological phenomena were treated by mainstream immunology during the first half of the 20th century.

It is beyond the scope of this chapter to discuss the entire history of the unwillingness to accept that the immune response might lead to a variety of harmful outcomes (which we now describe under the rubric “immunopathology”). We shall limit the present discussion to the way in which a subset of the whole, autoimmune diseases was regarded (or rather disregarded) during the first half of the 20th century. This sample should provide a quite adequate representation of the way that early immunologists dealt with the paradox presented by the almost oxymoronic term immunopathology.

## THE SEARCH FOR AUTOANTIBODIES

### Horror Autotoxicus

A new mechanism that functions to mediate immunity—the destruction of bacteria by humoral antibodies—was discovered by Pfeiffer (1894). Then Bordet (1899) showed that not only were bacteria lysed by thermostable antibody and a thermolabile substance that he called alexine (soon termed *Komplement* by Paul Ehrlich), but that mammalian erythrocytes could be hemolyzed specifically by two analogous agents. Here was a technique that would see a broad application in many areas of immunology (Silverstein, 1994), not least in connection with the question of whether an individual could form antibodies against its own self. Two consequences of Bordet’s report were immediately apparent. Landsteiner (1900) became interested in red cells and discovered blood groups in humans (for which he received the Nobel Prize in 1930). Then Ehrlich and Morgenroth (1900, 1901) launched a series of studies of immune hemolysis in order to develop additional support for Ehrlich’s (1897, 1900) side-chain theory of how antibodies are produced and how they function. It was Ehrlich’s interpretation of his hemolysis experiments that would play a major role in the early history of autoimmunity. These hemolysis experiments are described and analyzed in detail by Silverstein (2002).

During the course of these experiments, Ehrlich and Morgenroth immunized many different species with the red cells of other species. They also immunized animals with the red cells of other members of their own species and even tried to immunize animals with their own red cells. In every case, they were able to demonstrate the production of xenoantibodies and isohemolysins, but autohemolysins were never observed. This led inexorably and logically to the conclusion that animals could not make autotoxic antibodies to any self-antigens, a postulate that Ehrlich named *horror autotoxicus*. Indeed, he concluded, “It would be dysteleological in the highest degree, if under these circumstances self-poisons of the parenchyma—autotoxins—were formed” (Ehrlich, 1902). But Ehrlich was not the only one who responded to Bordet’s publication on immune hemolysis. If red cells could stimulate an immune response, why not other tissues and organs? In no time, attempts were undertaken to immunize animals with all types of cells and tissue extracts, especially at the Pasteur Institute in Paris where Bordet had worked. As expected, cytotoxic xenoantibodies against a variety of tissues were reported; indeed, a volume 14 of the Annales de l’Institut Pasteur was largely devoted to these studies, including a review of antisubstance antibodies by Metchnikoff (1900). Most surprising was a report by Metalnikoff (1900) that some animals were able to form antibodies against their own spermatozoa. But while these autoantibodies could destroy the sperm in vitro, they seemed to have no effect in vivo on the viable sperm in the immunized animal.

Ehrlich was not impressed! These are not “autocytotoxins within our meaning,” said he, since they do not cause disease (Ehrlich and Morgenroth, 1901). Here was the true meaning of *horror autotoxicus*: not that autoantibodies cannot be formed, but that they are prevented “by certain contrivances” from exerting any destructive action (Goltz, 1980). Due in part to Ehrlich’s worldwide prestige and to the fact that an autoantibody seemed so obviously counter-intuitive, *horror autotoxicus* found broad acceptance as a guiding principle. Indeed, so firm was the conviction that autoimmune disease was impossible that everyone soon forgot Ehrlich’s suggestion that an autoantibody might exist without causing disease. It would be some 80 years before the important distinction would be made between autoimmunity and autoimmune disease (Rose and Mackay, 1985). The modern finding of normally circulating autoantibodies and of “antiself” immunocytes, perhaps best exemplified by Cohen’s (2000) concept of “the immunological homunculus,” has shifted much of current immunological research in the direction of immunoregulation (the present term for Ehrlich’s “contrivances”).

## The Nature of Ehrlich's "Contrivances"

Paul Ehrlich was nothing if not logical. He proposed one of his typical thought-experiments to examine the possible outcomes (Ehrlich and Morgenroth, 1900). Suppose the existence of a self-antigen x. Then, since antibody formation results from the interaction of antigen with preformed cell receptors according to the side-chain theory (the first selection theory!), the following two possibilities are seen:

1. The host possesses no anti-x cell receptors. Therefore no autoantibody response and thus no disease can occur. [Here is, in embryo, the later clonal deletion (central tolerance) idea! If the cell possessed all other receptors, what could have happened to the anti-x?]
2. The host does possess anti-x cell receptors on its cells. Therefore autoanti-x formation is possible. But the host also possesses (by definition) the self-antigen x on its cells, with which the anti-x may react to stimulate the formation of anti-anti-x. (Remember, Ehrlich knew nothing about lymphocytes and conceived that all cells possess the full repertoire of receptors and thus may be specifically stimulated to form all different antibodies.) But the specific site on the anti-antibody should be identical with that on the original antigen, since they both are able to react specifically with the antibody combining site! Ehrlich, therefore, concluded that a self-regulating equilibrium would be established between autoantibody and antigen (or anti-antibody) to suppress the development of autoimmune disease. (Here was a regulatory network theory 70 years before Jerne's (1974) idiotype/antiidiotype theory!)

## CHALLENGES TO THE EHRLICH THESIS

### Lens Autoantibodies

The initial flurry of interest in antitissue antibodies quickly subsided as the implications of *horror autotoxicus* gained broad acceptance. But Uhlenhuth (1903) demonstrated the existence of organ-specific antigens by showing that the proteins of the lens are unique to that tissue; they are found nowhere else in the body. Moreover, these antigens are shared by the lenses of different species. Ophthalmologists seized upon this finding to suggest that an immune response to one's own lens might be responsible for the development of senile cataract (Römer, 1905). They showed further that an intraocular inflammation may be induced by the experimental rupture of the lens in the eye of a lens-immunized animal (Krusius, 1910).

Here were observations that would fascinate both ophthalmic clinicians and a later generation of immunopathologists interested in the possible workings of autoimmune disease. First, there was this early preview of what would later be called the "sequestered antigen" concept. Since "self" antigens by definition cannot elicit an immune response, then such antigens as do must be "foreign"—in this case isolated from the immunologic apparatus of the host, such as sperm and lens. Second, Römer and Gebb (1912) concluded that if indeed disease does result from the formation of autoantibodies, this would represent a most unusual occurrence and must be considered as an aberration due to a malfunction of Ehrlich's "contrivances." Here they showed that unlike a future generation, they understood Ehrlich's "law of immunity research" completely.

Interest in the possibility that autoimmunity to lens might lead to disease did not disappear in the years that followed. But whereas the initial studies had been done in the context of the new immunology and were known to all workers in the field, further work was restricted to ophthalmologists and eye departments. Thus a broad clinical study led Verhoeff and Lemoine (1922) to identify numerous cases of lens-induced inflammatory disease, to which they gave the name phacoanaphylaxis, a process which would later be accepted as a true autoimmune disease. Thenceforth, the description would appear routinely in textbooks of ophthalmic pathology, and clinical diagnoses would be made.

### Paroxysmal Cold Hemoglobinuria

Fast on the heels of the lens antigen demonstration came an even more convincing case involving erythrocyte antigens. PKH was a rare disease presenting with signs of intravascular red cell lysis and a resulting hemoglobinuria, following exposure of the patient to the cold. Donath and Landsteiner (1904, 1906) published reports that reproduced in vitro all features of the disease. They demonstrated beyond question that it was due to a peculiar autoantibody in the patient's serum that affixes to his own red cells only in the cold, and mediates hemolysis with complement when the sensitized cells are rewarmed. It was clear from the outset that Landsteiner understood fully

the implications of this discovery and its meaning for Ehrlich's *horror autotoxicus*. Indeed, even Ehrlich's student Hans Sachs gave a somewhat grudging acceptance of the phenomenon and its interpretation ([Sachs, 1909](#)). But again, the implication seemed to be that this was a most unusual exception to the regular scheme, and the implications of PKH as the prototypical autoimmune disease soon vanished almost completely from view.

### Sympathetic Ophthalmia

It had always seemed odd to clinicians that after traumatic injury to one eye, the second eye might spontaneously develop a blinding inflammatory disease even years later. Soon after the discovery of cytotoxic antibodies, the proposal was advanced that sympathetic ophthalmia might be caused by the formation of "autocytotoxins" ([Santucci, 1906](#)). The concept was picked up and given broad currency by one of the foremost ophthalmologists of the day ([Elschnig, 1910a,b](#)). As with autoimmunity to lens, work on the immunology of sympathetic ophthalmia continued, but only in ophthalmology departments. [Woods \(1921, 1933\)](#) reported the presence of antiuveal antibodies in patients with perforating injuries of the globe, and uveal pigment was implicated as the causative antigen ([Woods, 1925; Friedenwald, 1934](#)).

### The Wassermann Antibody

The discovery of the role of complement in immune hemolysis was soon followed by the finding that any antigen–antibody interaction would fix complement nonspecifically ([Bordet and Gengou, 1901](#)). The ability to measure this uptake using a hemolytic assay meant that the antibody could be titrated if specific antigen were available. With the recent identification of *Treponema pallidum* as the cause of syphilis, a serological test for this disease was sought. But since the organism could not be grown in culture, [Von Wassermann and colleagues \(1906\)](#) and, independently, [Detré \(1906\)](#) used extracts of tissues from syphilitic patients as the antigen, and a valuable diagnostic test was born.

Most perplexing, however, was the report from many laboratories that positive tests for syphilis might be obtained as well, using extracts of normal tissues as antigen. This ran counter to the prevailing view that only specific antigen can interact with antibody to fix complement. It appeared necessary to conclude, therefore, that the "Wassermann antigen," being native, must be measuring an autoantibody rather than an antitreponemal antibody. This suggestion was made, in fact, by [Weil and Braun \(1909\)](#) who speculated that the Wassermann antibody is an autoantibody specific for the tissue breakdown products generated in the syphilitic lesions. They suggested further that these autoantibodies exacerbate the disease, and that the brain lesions in tertiary syphilis (paresis) may represent an autoimmune disease directed against neural antigens. (Half a century later, the antigen involved in the Wassermann reaction was identified as a phospholipid named cardiolipin, but why these antibodies are formed is still a mystery, as is their role in the disease process!)

## THE SHIFT TO IMMUNOCHEMISTRY

Despite all these hints that autoimmune diseases might exist, interest in the question waned in mainstream immunology—indeed almost disappeared—for some 40 years, from just before the First World War to the mid-to-late 1950s. This was due in part to the continuing sway of Ehrlich's *horror autotoxicus*. But there was another factor at play, the change in the overall direction of the field of immunology. During the quarter-century prior to the First World War, immunology had been concerned chiefly with medical problems and disease and was pursued almost exclusively by biologists and physician-researchers. It had achieved notable successes in the prevention of some infectious diseases (vaccine development), their cure (serotherapy), and their diagnosis (serology). It had even begun to define several immunogenic diseases (anaphylaxis, serum sickness, hay fever, and asthma). But most of the easy problems had been solved, and further successes in these areas became disappointingly rare. Vaccines were sought, generally unsuccessfully, for the remaining great scourges of mankind: syphilis, tuberculosis, typhus, and the many serious tropical diseases. Few diseases were caused by exotoxins such as diphtheria and tetanus, and thus new serotherapeutic approaches were rare.

Yet other forces were at work. The Wassermann test and its offshoots became so widespread for the diagnosis of disease that it moved from the immunological research laboratory to the clinic. A new discipline, serology, arose that soon became almost independent of the mother discipline, immunology. In the same way,

experimental anaphylaxis and its human disease relations had fever and asthma stimulated the interest of clinicians, who soon took over work in this field and called their new discipline “allergy.” When in a science one research direction reaches the point of severely diminishing returns, its practitioners will usually move to more productive pursuits. So it was with immunology, beginning shortly after the end of the First World War. Karl Landsteiner started working with haptens and soon devoted himself almost entirely to a chemically oriented study of the structural basis of immunological specificity and crossreactions (Landsteiner, 1962). Then Michael Heidelberger studied the immunochemistry of pneumococcal polysaccharides (Heidelberger and Avery, 1923) and introduced a variety of quantitative methods for the estimation of antigens and antibodies, best typified by the popular text written by his students, “Quantitative Immunochemistry” (Kabat and Mayer, 1949).

For more than three decades, the field was devoted largely to studies of structure, specificity, and the thermodynamics of antigen–antibody interactions. The texts and monographs were primarily chemically oriented, and the practitioners were either chemically trained or at least chemically oriented. Even the theories of antibody formation that guided the field, Breinl and Haurowitz’s (1930) and Pauling’s (1940) antigen-instruction concept, were chemical (i.e., nonbiological and non-Darwinian) in spirit. It was easy to assume that a protein might be synthesized according to external instruction; for the chemist, molecules have no evolutionary history.

Given the continuing influence of Ehrlich’s dictum, and the generally nonmedical orientation of the most prominent immunological investigators, it is not surprising that autoantibodies and autoimmune diseases were not among the most popular topics in the research laboratory. This is not to say, however, that there was absolutely no work along these lines. As we have seen, ophthalmologists reported findings in lens-induced disease and in sympathetic ophthalmia, but these were published in specialty ophthalmic journals. In the early 1930s, Rivers et al. (1933) and Rivers and Schwentker (1935) published a series of papers on the production of an experimental encephalomyelitis that only later would be shown to represent an autoimmune process. While these studies are viewed today as important milestones in autoimmunity research, they attracted little attention at the time among immunologists.

The contemporary view of autoimmunity during the 1940s and early 1950s is perhaps best exemplified by the position of Ernest Witebsky, trained in immunology by Ehrlich’s student Hans Sachs and himself a disease-oriented physician. He could say as late as 1954 at the celebration of the centenary of the birth of Ehrlich, “The validity of the law (sic!) of *horror autotoxicus* certainly should be evident to anyone interested in blood transfusion and blood disease. Autoantibodies—namely, antibodies directed against receptors of the same individual—are not formed” (Witebsky, 1954). This from the individual who, only 2 years later with his student Noel Rose, would help refocus interest on autoimmunity with the demonstration of the production of experimental autoimmune thyroiditis! (Rose and Witebsky, 1956; Witebsky et al., 1957).

## THE RETURN OF IMMUNOBIOLOGY

During the late 1930s and 1940s, a series of observations began to question the assumptions that had guided recent thought and experiment in immunology. How could one explain in chemical terms the enhanced booster antibody response or the change with time in the specificity and affinity of the antibodies formed? How to explain the persistence of antibody formation in the apparent absence of antigen? Even more troubling was the lack of relationship between immunity to certain viral diseases and the titer of antiviral antibodies, or the absence of a correlation between antibodies and “delayed” hypersensitivity skin tests like the tuberculin test. Here were basic biological questions that demanded answers—questions with which current theory was unable to cope, and for which it could not even provide experimental approaches. But even more difficult questions arising from biology and medicine would pose further challenges.

Medawar’s (1945) experiments showed that the rejection of tissue grafts was somehow mediated by immunological mechanisms. Then Owen (1945) described the paradoxical situation in which dizygotic twin cattle might share one another’s red cells without being able to mount an immune response to these foreign antigens. Macfarlane Burnet, biologist par excellence, called attention to all of these inexplicable phenomena and hypothesized the existence of a fundamental biological mechanism to explain Owen’s finding—an embryonic process that would suppress the ability to respond to one’s own native antigens (Burnet and Fenner, 1949). This was soon confirmed by Medawar’s group (Billingham et al., 1953) and would be termed immunological tolerance. Yet other observations would emphasize the awakening biomedical movement in immunology—the description of a group of immune deficiency diseases. Taken together, these new questions and phenomena foretold a radical change of direction—what I have termed elsewhere the “immunobiological revolution” (Silverstein, 1991).

**TABLE 2.1** The “Dark Ages” of Autoimmunity

Disease/Organ	Last “classical” contribution	First “modern” contribution
Hemolytic disease	1909	Coombs et al. (1945)
Sperm and testicular	1900	Voisin et al. (1951)
Encephalomyelitis	1905	Kabat et al. (1947)
Sympathetic ophthalmia	1912	Collins (1949)
Phacoanaphylaxis	1911	Halbert et al. (1957)
Thyroid	1910	Rose and Witebsky (1956) and Roitt et al. (1956)
Wassermann antibody	1909	—
Platelet disease	—	Ackroyd (1949)

Adapted from Silverstein, A.M., 2009. *Allergy and immunopathology: the price of immunity*. In: *A History of Immunology*, second ed. Elsevier, New York, pp. 177–209.

Not only did these questions challenge the accepted dogma but they also served to stimulate the entry into the field of a new generation of investigators. These were basic scientists from such fields as genetics and physiology, and clinicians from a variety of medical disciplines. They were unfettered by any allegiance to earlier ideas and techniques and thus could entertain iconoclastic ideas and design novel experiments.

Perhaps the best illustration we may provide of the long period during which immunologists showed little interest in disease may be seen in Table 2.1. Here we list for each organ or disease entity the interval between the last significant study during the “classical” period and the first significant contribution of the “modern” era.

The average hiatus, for those for which both ending and restarting dates can be identified, is about 44 years! This is an extremely long interlude for a field that was only some 70 years old in 1950. In the context of a growing interest in the more biomedical aspects of the immune response, work on autoimmunity thus became respectable. This was due also to the increasing use of Freund’s adjuvant, which made animal models of the various autoimmune diseases more readily available and more reproducible. Advances came rapidly. Coombs et al. (1945) showed with the antiglobulin test that many cases of acquired hemolytic anemia were due to “incomplete” (nonagglutinating) antibodies. Kabat et al. (1947) refocused attention on the immunopathogenesis of “allergic” encephalomyelitis. Collins (1949) introduced a reproducible animal model of sympathetic ophthalmia against retinal antigens. Voisin et al. (1951) showed how to produce an experimental allergic orchitis. Finally, Rose and Witebsky (1956) demonstrated in experimental animals and Roitt et al. (1956) in human Hashimoto’s disease that some forms of thyroid disease might be based upon autoimmune processes. In addition, an understanding of the pathogenesis of some of these diseases was made easier by the increasing appreciation of the fact that not all were mediated by circulating antibodies; some involved the action of subclasses of lymphocytes that originate in the thymus. Some of these—“T cells”—may mediate disease directly, while others cooperate in the process of antibody production.

These new findings not only opened wide the floodgates of autoimmunity studies but stimulated further interest in the more general field of immunopathology as well. This new movement was provided with a theoretical base with Talmage’s (1957) suggestion and Burnet’s Clonal Selection Theory (Burnet, 1959), which emphasized for the first time the biologically important role of cell dynamics in the antibody response. It is no accident that the late 1950s saw the first international conferences on immunopathology (Miescher and Vorlaender, 1958; Grabar and Miescher, 1959) and on the fundamentals of hypersensitivity (Lawrence, 1959; Shaffer et al., 1959). For the first time, in 1963, there appeared a textbook aimed at medical students (Humphrey and White, 1963), and then two comprehensive descriptions of immunological diseases aimed at clinicians (Gell and Coombs, 1963; Samter et al., 1965). It was in the spirit of the new immunology that Mackay and Burnet (1963) could summarize contemporary knowledge in the increasingly active field of the autoimmune diseases. It would also stimulate great interest in the workings of immunological tolerance and other forms of immunoregulation, so critical for an understanding of why autoimmune disease might or might not develop.

The present summary of the early history of autoimmune diseases has been written with almost a sociological approach, emphasizing the importance of major reorientations in the parent discipline for how autoimmunity was viewed. The reader might also be interested in an alternative approach, presented in “Intolerant Bodies: A Short History of Autoimmunity” (Anderson and Mackay, 2014). This is more clinically oriented and includes a more philosophical approach, with emphasis on the important conceptual role of “the immune self.”

## CONCLUDING REMARKS

This, then, is the story of the early stirrings of interest in the possibility that disease might result from an immune response to one's own autochthonous antigens. Perhaps the initial reports were too premature to be incorporated into the received wisdom of the young field of immunology, just as the discovery of the several allergic diseases could not at first be integrated into this system clearly designed to protect us. Certainly Paul Ehrlich's dictum of *horror autotoxicus* contributed to an unwillingness to recognize the full significance of the initial findings of a response to spermatozoa, erythrocytes, and retina. But the mounting challenges to the dogma would eventually prove irresistible, and with the entry into the field of other biological disciplines, autoimmunity (and the broader field of immunopathology) would finally flourish. The more modern history of autoimmunity, resulting from the remarkable modern advances in our understanding of the cellular, molecular, and genetic contributions to the field, will be found in the accompanying chapters in this volume.

## References

- Ackroyd, J.F., 1949. The pathogenesis of thrombocytopenic purpura due to hypersensitivity to sedormid. *Clin. Sci.* 8, 267–287.
- Anderson, W., Mackay, I., 2014. Intolerant Bodies: A Short History of Autoimmunity. Johns Hopkins Press, Baltimore, MD.
- Behring, E., Kitasato, S., 1890. Ueber das Zustandekommen der Diphtherie-immunität und der Tetanus-immunität bei Thieren. *Dtsch. Med. Wochenschr.* 16, 1113–1114.
- Billingham, R.E., Brent, L., Medawar, P.B., 1953. Actively acquired tolerance of foreign cells. *Nature (London)* 172, 603–606.
- Bordet, J., 1899. Sur l'agglutination et la dissolution des globules rouges par le sérum d'animaux injectés de sang défibriné. *Ann. Inst. Pasteur.* 12, 688–695.
- Bordet, J., Gengou, O., 1901. Sur l'existence des substances sensibilisatrices dans la plupart des sérum anti-microbiens. *Ann. Inst. Pasteur.* 15, 289–302.
- Breinl, F., Haurowitz, F., 1930. Chemische Untersuchung des Präzipitates aus Hämoglobin und Anti-Hämoglobin und Bemerkungen über die Natur des Antikörpers. *Z. Physiol. Chem.* 192, 45–57.
- Burnet, F.M., 1959. The Clonal Selection Theory of Acquired Immunity. Cambridge University Press, London.
- Burnet, F.M., Fenner, F., 1949. The Production of Antibodies, second ed. Macmillan, New York.
- Cohen, I.R., 2000. Tending Adam's Garden. Academic Press, San Diego, CA.
- Collins, R.C., 1949. Experimental studies on sympathetic ophthalmia. *Am. J. Ophthalmol.* 32, 1687–1699.
- Coombs, R.R.A., Mourant, A.E., Race, R.R., 1945. A new test for the detection of weak and "incomplete" Rh agglutinins. *Brit. J. Exp. Pathol.* 26, 255–266.
- Detré, L., 1906. Ueber den Nachweis von spezifischen Syphilis Antisubstanzen und deren Antigenen bei Luetikern. *Wien. Klin. Wochenschr.* 19, 619.
- Donath, J., Landsteiner, K., 1904. Ueber paroxysmale Hämoglobinurie. *Münch. Med. Wochenschr* 51, 1590–1593.
- Donath, J., Landsteiner, K., 1906. Ueber paroxysmale Hämoglobinurie. *Z. Klin. Med.* 58, 173–189.
- Ehrlich, P., 1897. Die Wertbemessung des Diphtherieheilsersums und deren theoretischen Grundlagen. *Kinische Jahrb.* 6, 299–326.
- Ehrlich, P., 1900. On immunity with special reference to cell life: Croonian lecture. *Proc. R. Soc. London* 66, 424–448.
- Ehrlich, P., 1902. Die Schutzstoffe des Blutes. *Verh. Ges. Dtsch. Naturforsch. Aerzte.* 1, 250–275.
- Ehrlich, P., Morgenroth, J., 1900. Ueber Hämolsine: Dritte Mittheilung. *Berlin Klin. Wochenschr.* 37, 453–458.
- Ehrlich, P., Morgenroth, J., 1901. Ueber Hämolsine: Fünfte Mittheilung. *Berlin Klin. Wochenschr.* 38, 251–255.
- Elschnig, A., 1910a. Studien zur sympathischen Ophthalmie. von Graefes Arch. Ophthalmol. 75, 459–474.
- Elschnig, A., 1910b. Studien zur sympathischen Ophthalmie: Die Antigene Wirkung des Augenpigmentes. von Graefes Arch. Ophthalmol. 76, 509–546.
- Fleck, L., 1979. Genesis and Development of a Scientific Fact. University of Chicago Press, Chicago, IL.
- Friedenwald, J.S., 1934. Notes on the allergy theory of sympathetic ophthalmia. *J. Am. Med. Assoc.* 17, 1008–1018.
- Gell, P.G.H., Coombs, R.R.A., 1963. Clinical Aspects of Immunology. Blackwell Scientific, Oxford.
- Goltz, D., 1980. Horror Autotoxicus: Ein Beitrag zur Geschichte und Theorie der Autoimmunpathologie im Spiegel eines vielzitierten Begriffes (Thesis). University of Münster.
- Grabar, P., Miescher, P., 1959. Immunopathology-Immunopathologie. Benno Schwabe, Basel.
- Halbert, S.P., et al., 1957. Homologous immunological studies of ocular lens II. Biological aspects. *J. Exp. Med.* 105, 453–462.
- Heidelberger, M., Avery, O.T., 1923. The soluble specific substances of pneumococcus. *J. Exp. Med.* 38, 73–79.
- Humphrey, J.H., White, R.G., 1963. Immunology for Students of Medicine. Blackwell Scientific, Oxford.
- Jerne, N.K., 1974. Towards a network theory of the immune system. *Ann. Immunol. (Paris)* 125C, 373–389.
- Kabat, E.A., Mayer, M.M., 1949. Quantitative Immunochemistry. Charles C. Thomas, Springfield, IL.
- Kabat, E.A., Wolfe, A., Bezer, A.E., 1947. The rapid production of acute encephalomyelitis in Rhesus monkeys by injection of heterologous and homologous brain tissue with adjuvants. *J. Exp. Med.* 85, 117–130.
- Krusius, F.F., 1910. Ueberempfindlichkeitsversuche vom Auge aus. *Arch. Augenheilk.* 67, 6–35.
- Kuhn, T., 1970. The Structure of Scientific Revolutions, second ed. University of Chicago Press, Chicago, IL.
- Landsteiner, K., 1900. Zur Kenntnis der antifermentativen, lytischen und agglutinierenden Wirkung des Blutserums und der Lymphe. *Centralbl. Bakteriol.* 27, 357–362.
- Landsteiner, K., 1962. The Specificity of Serological Reactions, reprint of second ed., 1945 Dover, New York.

- Lawrence, H.S., 1959. Cellular and Humoral Aspects of Hypersensitivity States. Hoeber-Harper, New York.
- Mackay, I.A., Burnet, F.M., 1963. Autoimmune Diseases. Charles C. Thomas, Springfield, IL.
- Medawar, P.B., 1945. The behaviour and fate of skin autografts and skin homografts in rabbits. *J. Anat.* 78, 176–199.
- Metalnikoff, S., 1900. Etudes sur la spermotoxine. *Ann. Inst. Pasteur.* 14, 577–589.
- Metchnikoff, I.I., 1884. Ueber eine Sprosspilzkrankheit der Daphnien: Beitrag zur Lehre ueber den Kampf des Phagozyten gegen Krankheitserreger. *Arch. Pathol. Anat.* 86, 77–195.
- Metchnikoff, E., 1900. Sur les cytotoxines. *Ann. Inst. Pasteur.* 14, 369–377.
- Miescher, P., Vorlaender, K.O., 1958. Immunopathologie in Klinik und Forschung. Georg Thieme, Stuttgart.
- Owen, R.D., 1945. Immunogenetic consequences of vascular anastomoses between bovine twins. *Science* 102, 400–401.
- Pasteur, L., 1880. Sur les maladies virulentes et en particulier sur la maladie appellee vulgairement cholera des poules. *Compt. Rend. Acad. Sci.* 90, 239–248.
- Pauling, L., 1940. A theory of the structure and process of formation of antibodies. *J. Am. Chem. Soc.* 62, 2643–2657.
- Pfeiffer, R., 1894. Weitere Untersuchungen über das Wesen der Choleraimmunität und über spezifische baktericide Prozesse. *Z. Hygiene* 18, 1–16.
- Rivers, T.M., Sprunt, D.H., Berry, G.P., 1933. Observations on attempts to produce acute disseminated encephalomyelitis in monkeys. *J. Exp. Med.* 58, 39–54.
- Rivers, T.M., Schwentker, F.F., 1935. Encephalitis accompanied by myelin destruction experimentally produced in monkeys. *J. Exp. Med.* 61, 689–702.
- Roitt, I.M., Doniach, D., Campbell, P.N., Vaughan-Hudson, R., 1956. Auto-antibodies in Hashimoto's disease (lymphadenoid goiter). *Lancet* 2, 820–821.
- Römer, P., 1905. Die Pathogenese der Cataracta senilis vom Standpunkt der Serumforschung. von Graefes Arch. Ophthalmol. 60, 175–186.
- Römer, P., Gebb, H., 1912. Beitrag zur Frage der Anaphylaxie durch Linseneiweiss und Eiweiss aus andern Geweben des Auges. Von Graefes Arch. Ophthalmol. 81, 367–402.
- Rose, N.R., Witebsky, E., 1956. Studies on organ specificity. V. Changes in the thyroid gland of rabbits following active immunization with rabbit thyroid extracts. *J. Immunol.* 76, 417–427.
- Rose, N.R., Mackay, I.R., 1985. Autoimmunity versus autoimmune disease. In: Rose, N.R., Mackay, I.R. (Eds.), *The Autoimmune Diseases*. Academic Press, New York, pp. xxv–xxvi.
- Sachs, H., 1909. Hämolsine und Cytotoxine des Blutserums, Handbuchder Technik und Methodik der Immunitätsforschung, vol. 2. Fischer, Jena, pp. 896–897.
- Samter, M., et al., 1965. *Immunological Diseases*. Little Brown, Boston, MA.
- Santucci, S., 1906. Citotossine. *Riv. Ital. Ottal. Roma.* 2, 213–221.
- Shaffer, J.H., LoGrippo, G.A., Chase, M.W. (Eds.), 1959. *Mechanisms of Hypersensitivity*. Little, Brown, Boston, MA.
- Silverstein, A.M., 1991. The dynamics of conceptual change in twentieth century immunology. *Cell. Immunol.* 132, 515–531.
- Silverstein, A.M., 1994. The heuristic value of experimental systems: the case of immune hemolysis. *J. Hist. Biol.* 27, 437–447.
- Silverstein, A.M., 2002. *Paul Ehrlich's Receptor Immunology*. Academic Press, New York, pp. 95–122.
- Silverstein, A.M., 2009. Allergy and immunopathology: the price of immunity, *A History of Immunology*, second ed. Elsevier, New York, pp. 177–209.
- Talmage, D.W., 1957. Allergy and immunology. *Annu. Rev. Med.* 8, 239–256.
- Uhlenhuth, P., 1903. Zur Lehre von der Unterscheidung verschiedener Eiweissarten mit Hilfe spezifischer Seren. *Festschrift zum 60 Geburtstag von Robert Koch*. Fischer, Jena, p. 49.
- Verhoeff, F.H., Lemoine, A.N., 1922. Endophthalmitis phacoanaphylactica. *Am. J. Ophthalmol.* 5, 737–745.
- Voisin, G., Delaunay, A., Barber, M., 1951. Sur des lésions testiculaires provoquées chez le cobaye par iso- et auto-sensibilisation. *Ann. Inst. Pasteur.* 81, 48–63.
- Von Pirquet, C., 1910. Allergie. Springer, Berlin (English transl.: 1911. Allergy. American Medical Association, Chicago, IL).
- Von Wassermann, A., Neisser, A., Bruck, C., 1906. Eine Serodiagnostische Reaktion bei Syphilis. *Dtsch. med. Wochenschr.* 32, 745–746.
- Weil, E., Braun, H., 1909. Ueber das Wesen derluetischen Erkrankung auf Grund der neueren Forschungen. *Wien. Klin. Wochenschr.* 22, 372–374.
- Witebsky, E., 1954. Ehrlich's side-chain theory in light of present immunology. *Ann. N.Y. Acad. Sci.* 59 (168–181), 173.
- Witebsky, E., et al., 1957. Chronic thyroiditis and autoimmunization. *J. Am. Med. Assoc.* 164, 1439–1447.
- Woods, A.C., 1921. Immune reactions following injuries to the uveal tract. *J. Am. Med. Assoc.* 77, 1217–1222.
- Woods, A.C., 1925. Sympathetic ophthalmia: the use of uveal pigment in diagnosis and treatment. *Trans. Ophth. Soc. UK* 45, 208–249.
- Woods, A.C., 1933. Allergy and Immunity in Ophthalmology. Johns Hopkins University Press, Baltimore, MD.

# General Features of Autoimmune Disease

*Anne Davidson and Betty Diamond*

Institute of Molecular Medicine, Feinstein Institute for Medical Research, Manhasset, NY, United States

## OUTLINE

Innate Immune Activation	18	Activation of the Immune System	26
Cells of the Adaptive Immune System	19	Role of Antigen as a Driver of Autoimmunity	27
Defining Autoimmune Disease	20	Defective Downregulation of an Immune Response	28
Prevalence of Autoimmunity	20	Regulatory Lymphocytes	29
Genetics and Epigenetics of Autoimmunity	21	The Role of the Gut Microbiota in Autoimmunity	30
Monogenic Disease	21	Flares and Remissions During Disease	31
Polygenic Disease	21	Mechanisms of Tissue Damage	31
Shared Risk Alleles	22	Therapeutic Advances	32
Contribution of Epigenetic Modifications and Transcriptional Regulation	23	Goals for the Future	33
Hormones and Autoimmunity	23	Concluding Remarks	34
Autoimmunity and Central Tolerance	24	Acknowledgments	34
Autoimmunity and Peripheral Tolerance	24	References	34
Triggers of Autoimmunity	25		

A host of diseases are characterized by the activation of the immune system in the absence of an external threat to the organism. In these diseases, inflammation and tissue damage occur in the absence of trauma, infection, toxin exposure, or tumor growth (Rose and Bona, 1993). These diseases can be characterized as those that display the activation of the innate immune system and an excess of inflammatory mediators, but no evidence of an antigen-specific immune response; familial Mediterranean fever and other inflammasome diseases, Behçet disease, even atherosclerosis, can be considered to fall within this category. Alternatively, there are diseases characterized by an activation of the adaptive immune response with T and B lymphocytes responding to self-antigens in the absence of any detectable microbial assault or tumor invasion. These diseases constitute the vast majority of diseases considered to be autoimmune in origin. There are over 80 defined autoimmune diseases in composite affecting 5%–7% of the population. Moreover, their incidence is increasing (Lerner et al., 2015; Patterson et al., 2009).

## INNATE IMMUNE ACTIVATION

The activation of the innate immune response is a feature of many, perhaps all, autoimmune diseases (Mills, 2011). This activation may be the primary event involved in triggering the disease process. One example is the activation of the innate immune system in systemic lupus due to complement deficiencies that permit an excess accumulation of proinflammatory apoptotic debris (Elkon and Santer, 2012; Macedo and Isaac, 2016; Manderson et al., 2004). Increased production of type 1 interferon is present in first degree relatives of some lupus patients suggesting that this enhanced innate immune activation may be a triggering immune abnormality in these patients (Niewold, 2011). Likewise, inflammatory bowel disease (IBD) is associated with genetic alterations in multiple innate pathways including bacterial sensing, autophagy, and endoplasmic reticulum (ER) stress, leading to an increased inflammatory response to intestinal flora (de Souza and Fiocchi, 2016; Stange and Wehkamp, 2016). The innate immune system also provides critical defense at epithelial barriers; loss of the mucin layer and the antimicrobial peptides that it contains allows access of bacteria to epithelial cells and the activation of myeloid cells that help to initiate disease (Exley et al., 2016; Shikhagaie et al., 2017; Stange and Wehkamp, 2016; Wenink et al., 2017). It is also apparent that the innate immune system can be activated secondarily in autoimmune disease. Immune complexes containing endogenous Toll-like receptor (TLR) ligands, such as DNA, RNA, or citrullinated proteins, can activate dendritic cells (DCs) and other myeloid cells to amplify inflammatory pathways (Green and Marshak-Rothstein, 2011; Sokolove et al., 2011). Tissue injury also leads to the activation of innate immune cell networks (Kawai and Akira, 2010; Miyake and Yamasaki, 2012); thus once autoimmune-triggered tissue injury is ongoing, the innate, as well as the adaptive, immune system is always engaged.

A low threshold for the activation of myeloid cells and differentiation of monocytes to DCs predisposes to autoimmune disease in animal models, and blockade of pathways of the innate immune system can ameliorate many autoimmune diseases. In particular, TLR signaling, both in myeloid cells and lymphocytes, seems to be a critical feature of many autoimmune diseases as deletion of MyD88, a common component of the signaling cascade for several TLRs, and neutralization of HMGB1, a cytokine which synergizes with many TLR agonists, can prevent or treat murine models of diabetes, rheumatoid arthritis, systemic lupus, IBD, and more (Andersson and Tracey, 2011; Herlands et al., 2008; Pagni et al., 2010; Rivas et al., 2012). Cytosolic innate receptors also play a role in the initiation of autoimmune responses; for example, cyclic GMP-AMP synthase (cGAS) deficiency protects completely against Systemic Lupus Erythematosus (SLE) induced by an overabundance of intracellular DNA (Gray et al., 2015a). The success of TNF and IL-6 inhibition in rheumatoid arthritis and of TNF and IL-12 inhibition in IBD also attests to the involvement of the innate immune response in autoimmune diseases. However, even when the innate immune system is involved, some diseases respond better to certain cytokine inhibitors than others. IL-6 inhibition for example is effective for the treatment of rheumatoid arthritis and giant cell arteritis but not for ankylosing spondylitis (Koster et al., 2016; Loricera et al., 2015; Schoels et al., 2013), whereas TNF inhibition is effective for both rheumatoid arthritis and ankylosing spondylitis and for Takayasu's arteritis but not for giant cell arteritis; these differences reflect the different functions of these cytokines but their exact relationship to pathogenesis of each of these diseases is still a subject of investigation (Muratore et al., 2017; Schoels et al., 2013).

Other innate immune cells may also contribute to disease pathogenesis. The role of neutrophils has recently been highlighted in lupus which is characterized by the presence of an atypical neutrophil population that is subject to cell death by NETosis and that may release pathogenic oxidized DNA after exposure to TLR containing immune complexes (Caielli et al., 2016; Grayson et al., 2015; Jorch and Kubes, 2017; Kienhofer et al., 2017; Villanueva et al., 2011). Innate immune cells derived from the lymphoid lineage but without T or B-cell receptors (TCR or BCR) have important homeostatic functions, particularly at epithelial barriers, but may become dysregulated in autoimmunity and secrete pathogenic cytokines. An expansion or imbalance of several subsets of these cells has been observed both in the blood and tissues of patients with a variety of autoimmune diseases and in some instances these changes correlate with disease activity, although a causative link remains to be shown (Exley et al., 2016; Shikhagaie et al., 2017; Wenink et al., 2017).

Interestingly, the innate immune response, and, more specifically, monocyte activation, is under the control of the cholinergic antiinflammatory pathway. This pathway is initiated in the central nervous system, is mediated through the vagus nerve (cholinergic) and the splenic nerve (adrenergic), and culminates in the induction of acetylcholine production by splenic T cells that then inhibits the production of inflammatory cytokines by monocytes that express an  $\alpha 7$  cholinergic receptor (Chavan et al., 2017; Rosas-Ballina and Tracey, 2009). The identification of this pathway has provided a potential therapeutic target that regulates multiple cytokines simultaneously, and there are current trials to exploit this pathway in IBD, rheumatoid arthritis (RA), and SLE (Chavan et al., 2017; Koopman et al., 2016).

## CELLS OF THE ADAPTIVE IMMUNE SYSTEM

There is a coordinated interplay among the cells of the adaptive immune system with DCs, T cells, and B cells collaborating to activate this branch of the immune system. DCs activate T and B cells, T cells activate DCs and B cells, and B cells activate only T cells. This cascade leads to an immune response that recognizes a broad spectrum of epitopes of microbial pathogens and enlists multiple effector mechanisms (Blanco et al., 2008; Goodnow et al., 2010; O'Shea and Paul, 2010; Shlomchik, 2008; Steinman, 2007).

DCs are antigen-presenting cells (APCs) that are the intermediary between the innate and the adaptive immune systems. DCs can be tolerogenic in their resting state, but when activated, they are critical in initiating an immune response (Devi and Anandasabapathy, 2017; Kalantari et al., 2011; Morel and Turner, 2011). Similarly, monocytes clear the apoptotic debris generated from the billions of cells that die daily in a tolerogenic fashion but, when exposed to inflammatory mediators, they differentiate into macrophages or DCs (Dominguez and Ardavin, 2010; Horton et al., 2017; Poon et al., 2014; Takenaka and Quintana, 2017). The activation of the innate immune system, therefore, can establish a population of immunogenic DCs for the activation of the adaptive immune system. Like essentially, all cells, monocytes, and DCs display surface expression of class I major histocompatibility complex (MHC) molecules, which permit the presentation of intracellular antigens to T cells. DCs and some macrophage subsets also express class II MHC molecules, which are present on a much more restricted set of cells and permit the presentation of extracellular antigens (Banchereau and Steinman, 1998). Multiple alleles of class I and II molecules exist, and, thus, each individual has a unique set of MHC molecules (Beck and Trowsdale, 2000). DCs also express an array of nonpolymorphic receptors, such as TLRs, and pattern-recognition receptors that bind microbial antigens, products of tissue injury, and nucleic acids (Kawai and Akira, 2010, 2011). Engagement of these receptors causes the DCs to upregulate expression of costimulatory molecules and to deliver an obligatory second signal for activation (Dzopalic et al., 2012; Engels and Wienands, 2011; Vincenti and Luggen, 2007). It is important to note that each DC can recognize and respond to a broad spectrum of microbial antigens.

MHC molecules have innate properties in that they also interact with Killer cell immunoglobulin receptor (KIR) that have inhibitory functions on innate lymphoid cells; these interactions prevent the killing of MHC I expressing cells. Some KIR receptors have an activating function, and these can enhance immune responses to pathogens and may predispose to autoimmunity (Espeli et al., 2010; Rajalingam, 2011).

Each T and B cells express a single receptor for antigen. These antigen receptors are acquired by gene rearrangements that occur in somatic cells (Gellert, 2002); thus there is no inheritance of the T- or B-cell repertoire. T cells mature in the thymus (Stritesky et al., 2012). Each T cell expresses a unique receptor (TCR) that recognizes a molecular complex on the surface of an APC consisting of a class I or class II MHC molecule associated with a small peptide derived from an intra- or extracellular protein antigen, respectively. Signaling through both the TCR and costimulatory molecules is needed to effect activation of mature T cells (Engels and Wienands, 2011; Esensten et al., 2016; Vincenti and Luggen, 2007; Weinstein et al., 2012). B cells also express a single receptor for antigen, but the BCR recognizes native antigen rather than processed antigen. B-cell activation also requires signaling through both the BCR and costimulatory molecules (Crow, 2004). Activated B cells not only secrete antibody but can also function as APCs to engage a greater number of T cells in the immune response. B cells also secrete molecules that are essential for lymphoid organization and for the formation of the germinal center (Qin et al., 2007; Wang et al., 2001) and can secrete a variety of cytokines (Leon et al., 2012; Lipsky, 2001; Marino and Grey, 2012).

A critical feature of both T and B cells is that they proliferate in response to antigenic stimulation to create clonal expansions of cells with a unique antigenic specificity and to develop cells with a memory phenotype (Bishop et al., 2003; Chen et al., 2017; Grossman et al., 2004; McKinstry et al., 2010; Watkin et al., 2017). Memory cells have an accelerated and enhanced response following reexposure to antigen. B cells have the added feature of undergoing class switching of the immunoglobulin heavy chain gene and random somatic mutation of the BCR followed by the selection of those B cells with improved affinity for the eliciting antigen (Lee et al., 2016; Li et al., 2004; Victora and Nussenzweig, 2012). Thus there is a progression from low-affinity IgM antibodies to high-affinity IgG antibodies during the course of an immune response. The memory response is characterized by the activation of high-affinity IgG-producing B cells.

Plasma cells also constitute part of the memory B-cell response as they can be extremely long-lived and may, therefore, secrete pathogenic antibodies for long periods. While some autoimmune diseases appear to involve the generation of short-lived plasma cells and are, therefore, self-limiting, others are characterized by the continued presence of autoantibodies for years, in some cases, regardless of disease activity (Liu et al., 2011).

For the immune system to function effectively there must be a sufficient number of T and B cells that can respond to an enormous diversity of microbial antigens, and a means of regulating those cells that respond to self-antigen.

## DEFINING AUTOIMMUNE DISEASE

An autoimmune disease is a condition in which tissue injury is caused by T-cell or antibody reactivity to self. The immune activation may be initiated by infection but then persists in the absence of any detectable microbial antigen (Davidson and Diamond, 2001; Rose and Bona, 1993). It is important to state that although many diseases considered autoimmune display reactivity to self, evidence may still be lacking that the self-reactivity is, in fact, responsible for tissue damage. It is sometimes possible to determine whether autoantibodies are pathogenic by transferring them to a rodent host; however, T-cell reactivity is not transferable from humans to rodents because T-cell activation and T-cell effector function occur only in the context of self-MHC molecules. Thus demonstrating the pathogenicity of the autoimmune response has not been accomplished in all autoimmune diseases. In some instances, a disease is presumed to be of autoimmune origin only because B- and T cells are present in affected tissue.

Animal models of autoimmune disease have been enormously useful in aiding our understanding of both disease inception and disease pathogenesis (Bar-Or et al., 2011; Billiau and Matthys, 2011; Howell, 2002; King, 2012; Lam-Tse et al., 2002; Mandik-Nayak and Allen, 2005; Peutz-Kootstra et al., 2001; Wooley, 2004). Some autoimmune diseases can be triggered in animals by immunization with self-antigen or adjuvants and some develop spontaneously. While animal models have been very important in informing our understanding of autoimmunity, it is important to recognize that we do not know how closely they reflect human disease (Bodaghi and Rao, 2008; Kollias et al., 2011). Some may be more similar to human disease in the effector mechanisms of tissue injury than in the mechanisms of induction of autoreactivity. Indeed, autoantibody-mediated tissue damage is probably most alike in human disease and animal models (Monach et al., 2004). It is also important to consider that there may be extensive heterogeneity in human disease and that the animal models we study intensively may reflect only a subset of individuals with a given disease. A challenge that confronts us is to understand which animal models are most similar to human disease and can teach us most about the genetic predisposition to disease, external triggers of disease, disease pathogenesis, and effective therapy. The opportunity to generate new models based on the functionality of autoimmune risk haplotypes arises from an increasing understanding of genetic risk and may provide better insights into patient stratification and precision therapeutics. As more data are generated from human subjects, mouse models will also be needed to explore the functions of genes relevant to the progression of autoimmune disease.

## PREVALENCE OF AUTOIMMUNITY

It is striking that while each autoimmune disease individually affects only a small number of people, the prevalence of all autoimmune diseases is approximately 5%–7% (Cooper et al., 2009; Hayter and Cook, 2012; Jacobson et al., 1997; Wang et al., 2015). Patients with one autoimmune disease also have an increased risk of a second autoimmune disease (Cooper et al., 2009; Jacobson et al., 1997; Marrie et al., 2015). Two critical facts about autoimmune disease are important in understanding the high frequency of these diseases. First, autoreactivity is an aspect of every normal immune system. In fact, the repertoire of immunocompetent lymphocytes that provides protective immunity is selected based on autoreactivity (Gu et al., 1991; Nobrega et al., 2002; Vallejo et al., 2004). T cells need to recognize self-antigen within the antigen binding cleft of MHC in order to survive the initial steps of T cell selection, whereas circulating B cells receive survival signals as a result of weak interactions with autoantigens (Gu et al., 1991; Nobrega et al., 2002; Vallejo et al., 2004). Second, autoreactivity is a crucial component of immune homeostasis. The regulation of physiologic autoreactivity helps to shape the immune system so that it does not become the pathogenic autoreactivity associated with tissue damage; this is an active process that requires constant vigilance. The immune system maintains a precarious balance between the two: too little response leads to potential neglect of danger, while an overexuberant response can potentially lead to autoreactivity. How this balance is maintained is discussed next. Second, there is a genetic predisposition to autoimmunity, and aspects of this predisposition may be similar for many different autoimmune diseases (Cho and Gregersen, 2011; Gutierrez-Arcelus et al., 2016), perhaps because these genetic traits have been selected for their

capacity to protect against infectious diseases (Liao et al., 1995; Liu et al., 2009). Furthermore, some of the studies of the genetic basis of autoimmunity show that the genetic factors governing autoreactivity are distinct from those governing specific organ vulnerability (Liao et al., 1995; Liu et al., 2009) or severity of tissue damage (Martini et al., 2014). Thus individuals may share pathways promoting autoreactivity, yet present with different autoimmune diseases (Cho and Gregersen, 2011; Cotsapas et al., 2011).

## GENETICS AND EPIGENETICS OF AUTOIMMUNITY

### Monogenic Disease

It is clear from epidemiologic studies and studies of animal models of autoimmune disease that there is a genetic component to essentially every autoimmune disease. A few autoimmune diseases appear to be monogenic diseases (Melki and Crow, 2015). The human disease autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy, an autoimmune disease of multiple endocrine organs, is a consequence of a deletion in the autoimmune regulator (AIRE) gene that encodes a protein that causes tissue-specific genes to be expressed in medullary epithelial cells in the thymus (Akirav et al., 2011; Anderson and Su, 2011). These cells mediate negative selection of T cells reactive with peptides that derive from tissue-specific proteins. In the absence of AIRE expression, a spectrum of autoreactive T cells fails to be deleted; these cells mature to immunocompetence and mediate an immune attack on various organs. The absence of the AIRE gene appears sufficient for autoimmunity, although the phenotype of the disease that emerges, even within a single family, can be quite variable. Similarly, a defect in the Fas gene can also lead to autoimmunity. The Fas protein is expressed on activated lymphocytes. Engagement of Fas by Fas ligand leads to the death of the Fas-expressing cell, a process critical for downregulating the immune response. Individuals deficient in Fas expression have a disease called autoimmune lymphoproliferative syndrome (ALPS) characterized by an excess of T and B cells and by autoantibody production (Fleisher et al., 2001; Grodzicky and Elkon, 2002; Madkaikar et al., 2011). Of note, not all individuals with deficient Fas expression display ALPS; thus even in this disease, other genes must modulate disease phenotype. Deficiency in certain complement components also results in a high incidence of the autoimmune disease SLE. In this case, the mechanism is thought to be a failure of clearance of immune complexes and apoptotic material resulting in an overload of immunogenic material that can activate the immune system through engagement of TLRs and other innate receptors (Lewis and Botto, 2006; Pettigrew et al., 2009). Deficiency of enzymes that remove DNA to prevent TLR activation can result in SLE (Bodano et al., 2016; Rice et al., 2008; Sisirak et al., 2015; Stetson et al., 2017; Yan, 2006). Deficiency of Foxp3 or of IL2R $\alpha$  that are required for the development of regulatory T cells (Tregs) is both associated with severe autoimmunity affecting the bowel and endocrine organs (Lewis and Botto, 2006; Ochs et al., 2007; Pettigrew et al., 2009; Sakaguchi, 2007).

### Polygenic Disease

For most autoimmune diseases, multiple susceptibility loci contribute to the disease phenotype. Studies from mouse models of autoimmune disease have also revealed the presence of loci that suppress the autoimmune phenotype (Wakeland et al., 2001). Thus an individual's risk of developing an autoimmune disease depends on a summation of susceptibility and resistance loci. A major advance in the last 15 years has been the application of genome-wide association studies (GWAS) that evaluate large numbers of common single nucleotide polymorphisms (SNPs) as genomic markers in well-defined patients and control populations. Studies in multiple autoimmune diseases have definitively linked a number of genetic variants to disease susceptibility (Deng and Tsao, 2010; Flesher et al., 2010; Harley et al., 2008). Most of these variants are in noncoding regions of a gene and control basal expression [expression quantitative trait loci (eQTL)] or expression following stimulation (response eQTLs) (Gutierrez-Arcelus et al., 2016; Wang et al., 2015).

Despite the identification of a large number of autoimmunity associated genes using the GWAS approach, the polymorphic locus most closely linked to autoimmunity in studies of virtually every autoimmune disease is the MHC locus, an association that has long been known (Gutierrez-Arcelus et al., 2016). For example, anti-CCP seropositive rheumatoid arthritis in the Caucasian population is highly associated with the expression of a set of DR4 alleles that have a particular structural motif, called the "shared epitope" (Winchester, 2004). Reactive arthritis occurs in individuals expressing B27 or, less commonly, B7 class I MHC molecules. In type I diabetes both pathogenic and protective DR and DQ alleles have been identified and the heterodimer encoded by DQA1\*0501 and

DQB1\*0302 confers very high risk. In celiac disease, 98%–99% of the affected individuals bear a susceptibility DQ allele; thus DQ testing can be used clinically to exclude disease with a high degree of certainty (Wolters and Wijmenga, 2008). Multiple sclerosis and systemic lupus display particular human leukocyte antigen (HLA) associations, as do many other autoimmune diseases (Fernando et al., 2008; Tomlinson and Bodmer, 1995; Winchester, 2004; Wong and Wen, 2003). The basis for the association of MHC polymorphisms with autoimmune disease is still for the most part unknown but might be due to T-cell recognition of particular pathogenic peptides that can bind within the peptide binding cleft of certain MHC molecules, cross-reactivity of peptides derived from infectious organisms with self-peptides, or alterations in the T-cell repertoire that result in a decrease in Tregs (Cho and Feldman, 2015; Raychaudhuri et al., 2012; Sollid, 2017).

With the exception of the MHC, the other genetic risk loci for autoimmunity that have been identified by GWAS involve a conglomeration of approximately 300 relatively common alleles, each of which confers only a modest risk, with odds ratios <1.5–2 (Gutierrez-Arcelus et al., 2016; Wang et al., 2015). Some, but not all, of these polymorphisms cross major racial groups. In mouse models, there is evidence that genetic susceptibility can be a consequence of combinations of genes within each gene locus and not the consequence of a single gene in each locus. For example, a region on chromosome 1 that is implicated in autoimmunity in systemic lupus erythematosus has several subloci which contribute to various aspects of the disease (Morel, 2010; Morel et al., 2001).

## Shared Risk Alleles

Clinically, it has long been appreciated that autoimmune diseases cluster in families. The biologic basis for this observation is now clear; the same susceptibility genes can influence many different autoimmune diseases. For example, a polymorphism of cytotoxic T-lymphocyte-associated antigen (CTLA4), an inhibitory costimulatory molecule present on activated T cells, conveys risk for insulin-dependent diabetes, autoimmune hemolytic anemia, and Graves' disease (Ueda et al., 2003), while the CARD15 (NOD-2) gene is associated with both IBD and psoriasis (Bene et al., 2011; Rahman et al., 2003; Russell et al., 2004; Zaki et al., 2011). Similarly, polymorphisms in PTPN22, a molecule that regulates lymphocyte receptor signaling, is associated with type 1 diabetes, SLE, RA, and Crohn's disease but not with multiple sclerosis (Burn et al., 2011). The differences in disease phenotype may lie in associated genes, those governing target-organ susceptibility or those that modulate disease severity (Russell et al., 2004) or in different environmental exposures. Indeed, the genetic association of HLA and PTPN22 with cyclic citrullinated peptide (CCP)-positive rheumatoid arthritis is considerably magnified in smokers, leading to a postulated pathogenic mechanism whereby damage to the lung by cigarette smoke induces autoimmunity to citrullinated proteins in genetically susceptible individuals (Mahdi et al., 2009).

One of the most striking advances made as a result of GWAS has been the identification of immune pathways associated with autoimmune tendencies, some of which are shared across diseases. A metaanalysis of the autoimmunity associated genes identified thus far suggests that individual diseases can be clustered in groups that share pathogenic mechanisms. For example, using this type of methodology, disease associations with polymorphisms in IL-2 can be distinguished from those associated with IL-21 that is within the same genetic locus (Cotsapas et al., 2011). The genetic studies in sum suggest that autoimmunity can result from a defect in almost any pathway of immune homeostasis as they affect thresholds for innate immune cell activation and for negative and positive selection of lymphocytes prior to immunocompetence and the activation of autoreactive lymphocytes (Gutierrez-Arcelus et al., 2016; Marson et al., 2015).

Applying this new information to individual patients represents a major challenge since attribution of a SNP identified by GWAS to a single gene often requires extensive resequencing of large regions of DNA from many patients. Furthermore, since the GWAS approach identifies only common polymorphisms with frequencies within the population of >1%–5%, the risk variants identified by this method account for only a small proportion of the overall heritability of the disease (Deng and Tsao, 2010; Gutierrez-Arcelus et al., 2016). New methodologies to identify rare variants or copy number variations are being developed, but these variations will only be seen in a small fraction of those who are affected. Finally, establishing a link between genetic variations, gene function, and disease pathogenesis has not been easy. Many of the risk alleles identified by GWAS are common variants with subtle effects that are compatible with normal immunological function; in most cases, the variation is not within the gene coding region but governs gene expression (Rieck et al., 2007; Zhang et al., 2011). Combinations of genes are starting to be identified in longitudinal cohorts that predict the risk of disease (Achenbach et al., 2013; Langefeld et al., 2017; Laufer et al., 2017; Lempainen et al., 2015). More studies will be

required to understand how the disease-associated variants affect immune responses and interact with each other to contribute to the risk of a particular disease. One approach is to knock-in the disease-associated variants into mouse models of autoimmunity. For example, mice with a knock-in of a variant of the RNA sensor IFIH1 that confers a high risk for type 1 diabetes have a higher basal level of type I interferons and an increased propensity to develop diabetes compared with their wild-type littermates that is dependent on the RNA sensing function of IFIH1 and is further increased by the introduction of the diabetes associated PTPN22 variant (Gorman et al., 2017). As the function of all the autoimmune-associated variants is similarly clarified and potentially pathogenic pathways are identified, personalized interventions may become possible.

## Contribution of Epigenetic Modifications and Transcriptional Regulation

Despite the advances in understanding the genetics of autoimmunity, concordance in monozygotic twins remains below 50% for most diseases, indicating a contribution from random and/or environmental factors. Epigenetic alterations that change the access of DNA regions to the transcriptional machinery through DNA methylation or histone acetylation, or alterations that silence transcription through inhibitor miRNAs, or alter protein longevity or processing within the cell through ubiquitination or citrullination, may all influence the immune system function (Long et al., 2016; Tost et al., 2017). T-cell differentiation, for example, is influenced by epigenetic mechanisms that reinforce the T-cell cytokine producing phenotype (Nakayamada et al., 2012; Pereira et al., 2017; Qiu et al., 2017). While it is clear that epigenetic alterations may profoundly influence cell phenotype, the application of this concept to autoimmunity remains in its early phases. Abnormalities of methylation or acetylation or expression of particular miRNAs has been demonstrated among peripheral blood cells of patients with several autoimmune diseases (Ceribelli et al., 2011a, 2011b; Ghosh et al., 2012) and there are several examples of deficiencies of particular enzymes or miRNA species causing spontaneous autoimmune disease in mice (Glasmacher et al., 2010; Namjou et al., 2011). The inactivation of the X chromosome that contains several genes involved in immune responses is not always complete, perhaps contributing to the increased risk of autoimmunity in women (Wang et al., 2016). A study of discordance in monozygotic twins for disease demonstrates a similar epigenome despite lack of disease concordance, suggesting that epigenetic profiling might be useful in assessing risk for disease (Generali et al., 2017). Another study of patients with rheumatoid arthritis suggests that epigenetic profile correlates with response to therapy (Tost et al., 2017). Since epigenetic modifications are not immutable, they must be analyzed in all relevant tissues and cell types in order to find those that associate with disease risk, severity or response to therapy. As these are identified, the impact of drugs that modify the relevant epigenetic profiles will be of great interest.

## HORMONES AND AUTOIMMUNITY

Since many autoimmune diseases occur more commonly in women than in men, there have been several investigations of the role of sex hormones in autoimmune disease. Animal studies have shown multiple effects of sex steroids on the immune system (Rubtsova et al., 2015); studies of mice lacking an estrogen receptor have clearly attributed estrogen effects to signaling through the estrogen receptor  $\alpha$ . However, it has been difficult to extrapolate the conclusions from these studies to autoimmunity. Not all autoimmune diseases are more common in women. Some, such as ankylosing spondylitis, have a higher incidence in men. Furthermore, the predisposition to autoimmunity can be sex determined or hormonally modulated; thus the higher incidence of disease in women may not always reflect the influence of female hormones on the immune system. The X chromosome, in fact, harbors many genes of immunologic interest. In gonad matched mice the presence of XX confers a greater susceptibility to pristane-induced lupus than does XY (Smith-Bouvier et al., 2008). Similarly, in humans, the XYY phenotype is associated with an increased prevalence of SLE. Nevertheless, the genetic components of the XX chromosome that are associated with lupus risk are not yet clear. The evidence also shows that the effects of sex hormones differ in different diseases. While there is significant evidence that estrogen can exacerbate systemic lupus, estrogen seems to protect against rheumatoid arthritis. In addition, estrogen or other sex hormones might affect target-organ antigen display, target-organ susceptibility to immune-mediated damage or even the composition of the gut microbiota. Thus there is no simple paradigm to explain the relationship between sex and autoimmunity (Grimaldi et al., 2005).

## AUTOIMMUNITY AND CENTRAL TOLERANCE

The hallmark of the autoimmune disease is the activation of self-reactive T and B lymphocytes. A major mechanism of self-tolerance is the elimination of self-reactive immature lymphocytes by antigen ligation of the TCR or BCR at critical stages of development. For autoimmunity to develop there must be a lack of stringency in the elimination of autoreactive cells. Because TCRs and BCRs are generated by random gene rearrangements that occur within the nucleus of the cell and are not determined by knowledge of the world of self or foreign antigen, autoreactive T and B cells arise routinely. To eliminate autoreactive cells and maintain self-tolerance, T and B cells routinely undergo a selection process during their maturation in primary lymphoid organs, the thymus and bone marrow, respectively (Alexandropoulos and Danzl, 2012; Goodnow et al., 2010; Rajewsky, 1996; Vallejo et al., 2004; von Boehmer and Melchers, 2010). B cells again undergo a second process of selection after somatic mutation of immunoglobulin genes, as somatic mutation routinely generates autoreactivity (Brink, 2014; DeFranco, 2016; Goodnow et al., 2010; Liu and Davidson, 2011; Shlomchik, 2008).

T cells that mature in the thymus and enter peripheral lymphoid organs must display TCRs with some affinity for self-peptide–self-MHC complexes in order to receive the necessary signals for survival, which is termed positive selection. T cells arising in the thymus that express TCRs lacking any affinity for the self-peptide–self-MHC complexes fail to undergo positive selection and die. T cells that are strongly reactive to self-peptide–self-MHC complexes are eliminated in a process termed negative selection (Kondo et al., 2017). The threshold for both positive and negative selection represents a continuum. As the peptide–MHC complexes present in the thymus differ in each individual and the threshold for negative selection varies from individual to individual, each individual releases a different repertoire of antimicrobial and self-reactive T cells to the periphery, each reflecting a different spectrum of foreign and self-peptide specificities (Bommhardt et al., 2004; Vallejo et al., 2004; Werlen et al., 2003). It is also probable that certain stimuli can rescue T cells.

B cells similarly undergo a process of negative selection prior to achieving immunocompetence. This process occurs in the bone marrow and continues in the spleen where B cells migrate as transitional cells after exiting the bone marrow. Whether B cells require positive selection on self-antigen for survival and need to display some degree of autoreactivity remains an area of active investigation. It is clear, however, that highly autoreactive B cells are negatively selected on self-antigens encountered during early maturation, and again, the threshold for deletion is different for each individual (Monroe et al., 2003; Nemazee, 2017). The deletion occurs with the highest extent of BCR cross-linking; anergy occurs with less cross-linking. Thus the degree of autoreactivity in the B-cell repertoire is also variable. The selection of B cell is influenced not only by the strength of the signal received through the BCR but also by the availability of the TNF-like cytokine BAFF. In late transitional B cells, the interaction of BAFF with BAFF-R cooperates with signals received through the BCR to promote B cell survival and metabolic fitness. Since the availability of BAFF depends to a large extent on the number of B cells, B-cell depletion can result in high levels of BAFF leading to relaxation in the stringency of selection and the escape of autoreactive B cells to the periphery (Liu and Davidson, 2011; Mackay and Schneider, 2009). Anergic autoreactive B cells may also be rescued in a proinflammatory setting by engagement of costimulatory molecules on the B-cell membrane or by signaling through TLRs (Monroe and Keir, 2008). Thus the repertoire of naïve B cells will vary over time within an individual, with higher affinity autoreactive B cells present during times of infection, inflammation or lymphopenia, and fewer, lower-affinity autoreactive cells present during times of immunologic quiescence (Goodnow et al., 2010; Shlomchik, 2008).

This paradigm must be understood in the context of our knowledge that autoimmunity is often accompanied by some degree of immunodeficiency. A failure of proper selection may lead to a repertoire that includes too many self-reactive T or B cells. The presence of autoreactivity in individuals who are immunosuppressed is more straightforward. For example, the increased levels of BAFF that result from B-cell lymphopenia will lead to a failure to appropriately select the B-cell repertoire. Some defects that affect T-cell activation also impair expansion of regulatory cells or T-cell apoptosis and may, therefore, impair both responses to pathogens and self-tolerance. In the presence of T-cell lymphopenia, homeostatic expansion of self-reactive cells can occur.

## AUTOIMMUNITY AND PERIPHERAL TOLERANCE

Negative selection of T and B cells occurs in the periphery as well as in primary lymphoid organs, permitting the removal of autoreactive cells that do not encounter autoantigen in the thymus or bone marrow. This process

of negative selection is termed peripheral tolerance. Like central tolerance, it is mediated by engagement of the TCR or BCR in a noninflammatory setting (Devi and Anandasabapathy, 2017; Goodnow et al., 2010; Nemazee, 2017; Tsubata, 2017). Although it has been traditional to debate whether autoimmunity results from a defect in central tolerance in the thymus or bone marrow, or in peripheral tolerance in secondary lymphoid organs, current knowledge of tolerance induction suggests that this may be an artificial distinction. Engagement of the antigen receptor is critical to both central and peripheral tolerance, although there are some differences in antigen receptor signaling pathways, expression of coreceptors, and costimulatory molecules that exist between immature T or B cells and their mature counterparts. Mouse models of autoimmunity suggest that defects in negative selection can be limited to central or peripheral tolerance (Anderson and Su, 2011; Linterman et al., 2009; Vinuesa et al., 2005), whereas others may paradoxically impair central tolerance and enhance peripheral activation (Seo et al., 2003). Thus some autoimmune-prone individuals might exhibit a general lack of stringency in B or T-cell tolerance, while others might have a defect that is stage specific. This distinction has important therapeutic implications; learning to subset patients based on their tolerance impairment mechanism might permit a better pairing of a patient with the therapy, in short, more personalized medicine.

In summary, the thresholds for survival and deletion need to be set within appropriate limits at multiple times in the maturation and the activation of a lymphoid cell (Goodnow et al., 2010; Liu and Davidson, 2011; von Boehmer and Melchers, 2010). Too little deletion at any stage and autoreactivity ensues; too much deletion and the protective repertoire may be compromised. Any genetic or nongenetic change that reduces deletion or enhances activation may be a risk factor for autoimmunity.

## TRIGGERS OF AUTOIMMUNITY

Environmental factors are important triggers for expression of autoimmunity. Autoimmunity may develop following sterile tissue damage. Smoking, drug exposure, diet, chemical exposure, and sunlight have all been implicated as risk factors for particular diseases (D'Cruz, 2000; Debandt et al., 2003; Knip and Akerblom, 1999; Moriyama and Eisenbarth, 2002; Price and Venables, 1995; Steen, 1999; Vaarala, 2012). Molecular pathways for some of these have been established. Smoking and periodontal disease lead to the generation of citrullinated proteins; these are a target of autoantibodies in rheumatoid arthritis (Klareskog et al., 2011; Routsias et al., 2011). Notably, PADI4, the gene encoding peptidyl arginine deiminase involved in the citrullination of proteins, contains a susceptibility allele for rheumatoid arthritis (Bang et al., 2010; Kochi et al., 2011); inhibitors of PAD4 are effective in mouse models of rheumatoid arthritis (Willis et al., 2017). UV light causes apoptosis of keratinocytes, liberating cellular debris which is then bound by SLE-associated autoantibodies initiating an inflammatory response in the skin (Bijl and Kallenberg, 2006; Kuhn and Beissert, 2005); this can be mimicked by overexpression of inflammatory cytokines in the skin (Seery et al., 1997). It is now appreciated that pattern-recognition receptors for microbial pathogen-associated molecular patterns also bind to endogenous ligands, DAMPs or damage-associated molecular patterns. The release of DAMPs in damaged tissue can establish a proinflammatory milieu leading to the immunogenic presentation of self-antigens, including intracellular antigens that are normally sequestered from the immune system (Zhang et al., 2010). Once these become targets of an immune response, ongoing inflammation may be sustained. For example, the ongoing inflammation in some diseases, such as autoimmune myositis, targets regenerating tissue in which the disease-specific autoantigens are most abundantly expressed; this prevents tissue repair and resolution of inflammation (Mammen et al., 2011; Suber et al., 2008).

Clearly, infection can also precipitate autoimmune disease. It has even been suggested that most autoimmune diseases represent the late sequelae of an infectious process (Christen et al., 2012; James and Robertson, 2012). Proving this hypothesis has, however, been difficult. For some diseases, such as rheumatic fever or Guillain–Barré disease, the causal connection between microbial infection, the antimicrobial response, and autoimmune disease is clearly established (Cunningham, 2003; Guilherme and Kalil, 2004). Persistent reactive arthritis may follow a variety of bacterial and viral infections (Schmitt, 2017). For other diseases, there is suggestive epidemiologic evidence in humans or evidence from animal models that autoimmunity can follow microbial infection, or T cell or antibody cross-reactivity with both microbial and self-antigen has been identified (James and Robertson, 2012; Kuon and Sieper, 2003; Strassburg et al., 2003). In general, researchers have sought to implicate particular infections in the pathogenesis of particular autoimmune diseases, but it is possible that for some autoimmune diseases there is more than one possible microbial trigger. Importantly, the interaction of the TCR with a peptide–MHC complex must be of higher affinity to activate a naïve T cell than a memory T cell. Thus a microbial peptide may initiate a response that can then be sustained by self-peptide. Moreover, once a response to

self-antigen is initiated, epitope spreading to other epitopes on the same protein or on associated proteins occurs, often through B cell–mediated antigen presentation (Shlomchik et al., 2001). Autoantibodies may also amplify disease, especially those that form immune complexes containing endosomal TLR ligands.

Recent studies have provided remarkable information on the progression of autoimmunity, demonstrating that the autoantibodies characteristics of a given autoimmune disease are present as early as 10 years before the onset of clinical disease (Arbuckle et al., 2003). Moreover, cytokine abnormalities can also be observed before the onset of clinical symptomatology (Arbuckle et al., 2003; Deane et al., 2010) and early transcriptional signatures of preclinical disease are now starting to be identified (Chang et al., 2016; Kallionpaa et al., 2014). These observations suggest that there may be an opportunity to abort or retard the progression to disease in predisposed individuals.

Finally, the adipocyte has joined the ranks of immunomodulatory cells. It can secrete a variety of cytokines that are either protective (Kamata et al., 2015) or that promote a proinflammatory, proimmunogenic milieu (de Heredia et al., 2012). Thus increasing obesity may be one contributor to the increasing incidence of autoimmune disease.

## ACTIVATION OF THE IMMUNE SYSTEM

The activation of both T and B cells in the periphery requires that the cells receive two signals: the first one is generated by ligation of the antigen receptor and the second by engagement of a costimulatory receptor. In general, when an antigen enters the system, there is an activation of DCs, the critical APC in a primary immune response. This occurs because microbes express molecules that bind to pattern-recognition receptors or TLRs on the DC. The consequence of this binding is upregulation of the costimulatory molecules CD80 (B7.1) and CD86 (B7.2) on DCs, and transformation of the DC from resting, or tolerogenic to activated, or immunogenic. T cells recognizing a microbial peptide in either class I or class II MHC molecules on the immunogenic DC will be activated. It is a feature of memory T cells that they can be activated by a lower-affinity interaction with the TCR than is required to activate primary T cells; this is due to epigenetic changes and alterations in the structure of lipid rafts in the membrane that facilitate rapid receptor cross-linking and less requirement for costimulatory signals (Weng et al., 2012). Thus a T cell that is not activated by a self-peptide–self-MHC complex while still a naïve cell, may be activated by self-antigen once it becomes a memory T cell. There are many examples in the literature of a T cell that is derived from an individual with autoimmune disease, which recognizes both a microbial peptide and a self-peptide. This cross-reactivity is termed molecular mimicry and represents a mechanism by which autoimmunity can be triggered by infection (Cusick et al., 2012). The hypothesis that molecular mimicry predisposes to autoimmunity clearly has validity in rodent models of autoimmune disease and suggests that laxity in the selection of the naïve T-cell repertoire can be a major contributor to autoimmunity. Those individuals with less stringent negative selection will have multiple T cells that can be activated by foreign antigen and will also display pathogenic autoreactivity. In addition, the signaling cascades within activated lymphocytes may differ in autoimmune versus healthy individuals. For example, T cells from SLE patients have altered signaling and a faster T cell calcium flux than those of healthy individuals due to the replacement of the principal signaling molecule of the TCR complex, CD3 $\zeta$ , by the FcR $\gamma$  chain (Moulton and Tsokos, 2011). This results in use of the adaptor molecule Syk rather than ZAP70 and the activation of the downstream kinase calcium/calmodulin-dependent protein kinase type IV (CaMK4) that enhances the production of IL-17 and blocks the production of IL-2 (Koga et al., 2014, 2016).

The activated T cell provides T-cell help or costimulatory signals to B cells that are encountering microbial antigen. B cells that bind both microbial antigen and self-antigen will ingest, process, and present epitopes of self-antigen, which can then be recognized by T cells. Because B cells often process antigen to different peptides than do DCs, the B cells can present novel epitopes of self-antigen and activate T cells with novel autoreactivities (Bockenstedt et al., 1995; Sercarz et al., 1993; Sinmaz et al., 2016; Yan et al., 2006). These cross-reactive B cells will, therefore, contribute to a cascade of autoreactivity, as they activate an expanded repertoire of T cells. Memory B cells are also potent APCs that can activate naïve T cells. The B-cell repertoire, therefore, critically influences the T-cell repertoire (Whitmire et al., 2009). The fewer autoreactive B cells present, the less presentation of self-antigen to T cells.

There is much complexity in the cytokine expression patterns of activated T cells with at least four subsets of well-described helper T cells (Th1, Th2, Th17, and T<sub>FH</sub>) as well as regulatory cells that help to restrain immune responses. The balance of transcriptional regulators expressed in each T cell will help to determine its phenotype,

whereas epigenetic changes will help to reinforce that phenotype through subsequent rounds of proliferation. Nevertheless, there is emerging evidence that helper T cells have a substantial amount of flexibility with respect to their phenotype. Signals from the innate immune system can be drivers of T-cell reprogramming, suggesting that inflammation or infection may have a profound effect on T-cell function, converting T cells with a protective phenotype to those that amplify inflammation (Nakayamada et al., 2012). More recently, a CD8 T-cell phenotype relevant to infectious disease, cancer, and autoimmunity has been identified characterized by programmed death (PD1) expression and termed “exhausted.” Exhausted T cells recognize antigen but can no longer carry out effector functions. They arise as the immune response progresses from the acute phase characterized by abundant antigen and costimulation from CD4 T cells to the chronic phase in which costimulation wanes and antigen concentration decreases (McKinney and Smith, 2016). Another feature of exhausted T cells is an altered metabolic profile characterized by high persistent mTOR, glucose dependence, and increased mitochondrial depolarization (Bengsch et al., 2016). The presence of these cells in the peripheral blood is clinically relevant as a molecular profile of exhaustion is associated with the inability to clear chronic infections or to mount vaccine responses and with cancer progression but with a better prognosis in a variety of autoimmune diseases (McKinney et al., 2015).

The appearance of a broad spectrum of autoimmune diseases in a substantial number of patients receiving antagonists of key coinhibitory molecules such as CTLA4 and PD1, drugs that activate exhausted cells as part of cancer chemotherapy, illustrates the trade-off between immunocompetence and autoimmunity (Cappelli et al., 2016; Day and Hansen, 2016). These “checkpoint inhibitor” drugs induce a broad spectrum of autoimmune adverse events in 7%–18% of the treated patients, most commonly affecting the skin, the gastrointestinal tract, the endocrine organs, and the respiratory tract. Immune side effects are less frequent and milder in patients in which PD1 has been targeted, compared with patients taking anti-CTLA4 therapy, perhaps related to the antibody-dependent cellular cytotoxicity-mediated depletion of Tregs by anti-CTLA4. Adverse effects are much more frequent and severe in patients taking a combination of CTLA4 and PD1 directed therapies, reflecting the complementary activities of each of these pathways in suppressing T cell–mediated immune responses (Naidoo et al., 2015). Furthermore, approximately 40% of the patients with an underlying autoimmune disease will flare while taking a checkpoint inhibitor. The frequency of adverse autoimmune events associated with checkpoint inhibitor drugs makes it imperative to understand how to reinvigorate the effector function of exhausted tumor-infiltrating T cells that have upregulated their coinhibitory receptors without breaking self-tolerance in distant organs. This might involve the targeting of more tissue-specific coinhibitory molecules, approaches to reverse the abnormal metabolic profile of these cells, and/or the use of synergistic regimens that enhance inflammatory responses only in the tumor (Bengsch et al., 2016; Lucca and Hafler, 2017).

Much is now known about the signaling pathways that are downstream of receptor and costimulatory molecule–mediated stimulation and that are required for B and T-cell activation and cytokine production. Since activated lymphocytes are major mediators of the effector inflammatory response, some of these pathways are targets for immune interventions with small molecules. Both inhibitors of Syk and of Jak3 have been used clinically in autoimmune diseases and other kinase inhibitors are in development (Baker and Isaacs, 2017; Hirahara et al., 2016; Kontzias et al., 2012). There is increasing recognition that immune responses and cellular differentiation pathways are linked to metabolism and that different types of effector and Tregs have different metabolic requirements (Buck et al., 2017; Gerriets and Rathmell, 2012; Morel, 2017; O’Neill et al., 2016). Recent studies have illustrated how genetic risk may be associated with metabolic irregularities in immune cells. A genetic polymorphism associated with lupus involves a gene that regulates T-cell oxidative metabolism and mitochondrial metabolism leading to an increase in T-cell inflammatory cytokines. Based on this finding, it has been shown that either an inhibitor of glucose oxidation or an inhibitor of mitochondrial oxidation can decrease autoimmune activation in mouse lupus models (Yin et al., 2015). How the fuel requirement and metabolic phenotype of each immune cell in either quiescent or activated state regulates autoimmunity requires future study. More work will also be needed to determine how metabolic irregularities in immune cells can be addressed therapeutically without disrupting homeostasis in other tissues.

## ROLE OF ANTIGEN AS A DRIVER OF AUTOIMMUNITY

A major question in autoimmune disease is whether the process is autonomous or driven by antigen, and, if the latter, whether the antigen is a self-antigen or foreign antigen. Animal models of disease definitively show that molecular mimicry following activation by microbial antigen can initiate autoreactivity (Cunningham, 2003; Cusick et al., 2012; Kuwabara, 2004). There are also data suggesting that self-antigen drives the autoimmune

response. First, in animal models of systemic lupus, it appears that an excess of apoptotic cells, a problem in their clearance, or modifications in the antigens they release can result in a lupus-like serology with antichromatin reactivity (Martinez Valle et al., 2008; Mistry and Kaplan, 2016; Peng and Elkon, 2011). Current understanding would suggest that an excess of apoptotic debris and/or altered forms of nucleic acids (such as oxidized forms or biofilms) can activate endosomal TLRs and transform tolerogenic into immunogenic DCs, as well as activate B cells (Caielli et al., 2016; Colonna et al., 2013; Filardy et al., 2010). The role of each endosomal TLR is different. TLR9 is required for the production of autoantibodies to DNA in lupus models but also has a protective role in autoimmunity (Christensen et al., 2005; Nickerson et al., 2008). TLR7 and 8 recognize the same RNA antigens but have different functions because they are expressed on different cell types. Overexpression of TLR7 in B cells is sufficient to cause a lupus-like autoimmune disease (Jackson et al., 2014). The overexpression of TLR8 that is expressed mainly in myeloid cells causes generalized systemic autoimmunity due to its activation of myeloid cells (Guiducci et al., 2013).

Other DNA and RNA sensors together with their associated adaptors and downstream signaling molecules also contribute to the regulation of autoimmune responses to nucleic acids during the course of a protective immune response to viral pathogens. TREX1, DNase1, DNAl3, and cGAS are all enzymes involved in DNA degradation either intra- or extracellularly (Koyama et al., 2016; Martinez Valle et al., 2008; Sisirak et al., 2015). Deficiency of TREX1 results in excess intracellular DNA that activates cGAS to produce the dinucleotide cGAMP which acts through its downstream adapter STING, to induce proinflammatory cytokines (Gao et al., 2015; Gray et al., 2015b). Nevertheless, the multiple functions of some of the adapters and signaling molecules involved in innate immunity can make them difficult to target therapeutically (Pawaria et al., 2017; Sharma et al., 2015).

Extensive tissue damage can lead to the presentation of normally sequestered self-antigen in a proinflammatory setting (Bratton and Henson, 2011; Horwitz et al., 2002; Vezys and Lefrancois, 2002) or posttranslational alteration of self-antigen such that it is now immunogenic (Doyle and Mamula, 2012). The proinflammatory setting may be enhanced by apoptosis of cells following tissue injury. This can clearly lead to an autoimmune response. Whether in some individuals this response is perpetuated because of a lack of appropriate restoration to homeostasis is an important question.

Finally, polymorphisms in autoantigens may also constitute risk factors for autoimmune disease (Pauza et al., 2004; Suzuki et al., 2003). As the genetic susceptibility to autoimmune disease is further explored, the degree to which molecular mimicry, aberrant expression of autoantigens, or exposure to previously sequestered antigen in an immunogenic setting contributes to disease will become more apparent.

A variety of environmental exposures might also nonspecifically accelerate disease by activating the innate immune system resulting in the release of proinflammatory cytokines that initiate autoimmunity. In mice, for example, type I interferons can initiate SLE in susceptible strains (Koutouzov et al., 2006) and may be responsible for the Koebner phenomenon observed in psoriasis (Koutouzov et al., 2006). Tissue damage also results in the release of soluble mediators (DAMPs) such as HMGB1, cathelicidins, defensins, and heat shock proteins that can activate TLRs and other proinflammatory receptors to further amplify immune activation pathways (Gallo and Gallucci, 2013).

## DEFECTIVE DOWNREGULATION OF AN IMMUNE RESPONSE

The induction of an immune response needs to be followed by a downregulation or elimination of most of the cells that have undergone clonal expansion. A major observation of recent studies of autoimmune disease is that a defect in the restoration of immune homeostasis, or in a downregulation of an immune response, can be a risk factor for autoimmunity. Since all reactivity with foreign antigen includes reactivity to self-antigen, responses to self are routinely generated in the process of mounting an immune response to foreign antigen. The potential pathogenicity of the autoimmune response will vary from individual to individual. In general, however, the mechanisms that exist to dampen the immune response also diminish autoreactivity. B and T cells are routinely downregulated as soon as they are activated. For the B cell, this occurs, in part, by cross-linking of the BCR and FcRIIB by antigen–antibody complexes. When FcRIIB is absent or deficient on B cells, as it occurs in many individuals with SLE, autoantibody production is poorly controlled (Bolland and Ravetch, 2000; Fukuyama et al., 2005), whereas FcRIIB on DCs helps to maintain T-cell tolerance (Li et al., 2014). Multiple coinhibitory molecules are expressed on activated T cells. Interaction with their receptors either within lymphoid organs or in the peripheral site of inflammation transduces an inhibitory signal to the T cells, signaling them to downmodulate their response (Schildberg et al., 2016). Mutations in two coinhibitory molecules, PD1 and CTLA4, are associated

with several autoimmune diseases (Chen, 2004; Khoury and Sayegh, 2004) and pharmacologic antagonism of either of these two molecules induces autoimmunity with high frequency (Kostine et al., 2017). Both B and T cells are also susceptible to activation-induced cell death mediated through Fas–Fas ligand interactions (Brunner et al., 2003; Li-Weber and Krammer, 2003). Defects in this process can lead to autoimmunity.

Thus controlling the immune response is critical to normal homeostasis of the immune system and is mediated by multiple inhibitory pathways. A major component of autoimmune disease in some individuals may be a defect in the suppression of immune activation.

## REGULATORY LYMPHOCYTES

Another area of intensive study is the phenotypic characterization and mechanisms of action of regulatory T and B cell subsets. CD4+ Tregs arise either during thymic development and others are induced after antigen exposure in the periphery (Kasper et al., 2016; Panduro et al., 2016; Wing and Sakaguchi, 2010). These cells regulate immune responses in a variety of ways that include secretion of inhibitory cytokines, promotion of apoptosis of effector lymphocytes, depriving effector T cells of cytokines or essential amino acids leading to apoptosis, or inhibition of DC function. The absence of Tregs in mice results in lymphoproliferation and fatal multiorgan autoimmunity demonstrating the need for the ongoing regulation of pathogenic self-reactive cells that escape into the periphery. A population of CD4+/CD25hi ( $\gamma$  chain of the IL-2 receptor) naturally occurring Tregs arises in the thymus in response to TCR encounter with self-antigens with an avidity lower than that required for negative selection; their development depends on both CD28 and IL-2. Tregs can also be generated after antigen exposure in the periphery from naïve T cells in a manner that is dependent on IL-2 and transforming growth factor (TGF)- $\beta$  and may be enhanced by the vitamin A metabolite retinoic acid. Both types of Tregs express the master transcriptional regulator Foxp3 (Pesenacker et al., 2016); its expression is stabilized by epigenetic modifications of DNA that reinforce transcriptional availability of suppressive cytokines while preventing access of transcription factors to DNA-encoding inflammatory cytokines (Hsieh et al., 2012; Josefowicz et al., 2012a).

Cells expressing immunosuppressive cytokines such as TGF- $\beta$  or IL-10 (Tr1 cells) (Pot et al., 2011) arise under particular conditions of antigen exposure and, once activated, mediate suppression through both limiting cytokine secretion and through contact-mediated lysis of effector cells. Tr1 cells are present in large numbers in the gut where they help to protect from colitis and they can also protect against multiple sclerosis in mice. Tr1 cells are Foxp3 negative and can be induced by IL-27 (Meka et al., 2015; Nadya et al., 2017; Wojno and Hunter, 2012) but have been difficult to study due to lack of a clear phenotype. Nevertheless, the transcriptional program of these cells is being unraveled, and recent studies have suggested that both IFN $\gamma$  and galectin 1 induce DCs to produce IL-27, suggesting a way in which Tr1 cells might be induced therapeutically (Ilarregui et al., 2009).

Other studies have identified a population of CD8 suppressor cells that may directly lyse autoreactive cells or may secrete immunosuppressive cytokines (Cortesini et al., 2001; Jiang et al., 2010; Vuddamalay and van Meerwijk, 2017). In lupus models, these cells can be induced by autoantigen or by idioype peptides (Sawla et al., 2012). CD8+ Tregs have recently been described during adaptive immune responses where they serve to regulate humoral responses in the germinal center by lysing B cells (Kim and Cantor, 2011).

The balance between effector and regulatory cells may determine whether an autoreactive response that arises in the course of microbial exposure or an inflammatory response is terminated or perpetuated (Visperas and Vignali, 2016). However, application of this new knowledge to the treatment of autoimmunity is still in its early stages. One approach has been to use inhibitors of the mTOR pathway, important in autophagy and cell metabolism, to induce or stabilize Foxp3 expression in Tregs (Chinen and Rudensky, 2012; Josefowicz et al., 2012b). Another is to use low-dose IL-2 or IL-2 anti-IL-2 complexes to enhance Treg development and function. This strategy has recently been successfully applied to the treatment of graft versus host disease (GVHD) and cryoglobulinemic vasculitis in humans in which an increase in Tregs was associated with a therapeutic response (Oo et al., 2012). In vitro expansion and delivery of a stable population of Tregs or in vivo activation of antigen-specific Tregs by tolerogenic self-peptides are in the development and trial stage. Clinical trials directed at tolerance induction in new onset type I diabetes by manipulation of Tregs have so far failed to cure disease despite an increase in Treg numbers, although several approaches have had partial effects (Gallagher et al., 2011; Skyler, 2013). Clinical trials with low-dose IL-2 to induce Tregs are ongoing, and other approaches that stabilize Treg function are being considered (Visperas and Vignali, 2016).

B cells may also have regulatory functions (Klinker and Lundy, 2012). IgM antibodies that have low-affinity autoreactivity suppress immune responses by promoting opsonization of apoptotic material and promoting

noninflammatory clearance (Chen et al., 2009). A subset of B cells with regulatory functions (Bregs) has been described by multiple investigators, but their importance in autoimmune diseases is not clear. These cells are defined by their ability, upon in vitro activation, to produce IL-10 as well as other regulatory cytokines such as TGF- $\beta$  and IL-35 (Lykken et al., 2015; Mauri and Menon, 2017). Some disagreement has arisen in the literature as to their origins and phenotype since they constitute a small proportion of many different activated B cell subsets including plasmablasts. Bregs function to dampen Th1 and Th17 responses and their absence have been associated with an exacerbated disease phenotype in mouse models of autoimmunity. Decreased numbers of Bregs have been reported in human autoimmune diseases but an understanding of how and where these cells regulate autoimmunity remains to be elucidated, and the findings are currently limited and only associative.

Another way in which B cells can regulate immune responses is by posttranscriptional modification of antibodies. Alterations in galactosylation and sialylation of the Fc region of Ig molecules may have a profound effect on immune responses. Fc receptor binding is affected by changes in glycosylation (Bournazos et al., 2016). A decrease in Ig galactosylation and sialylation is found in multiple autoimmune diseases and may increase the pathogenicity of autoantibodies (Biermann et al., 2016; Le et al., 2016). Sialylated immunoglobulin found in preparations of IVIg suppresses immune responses by binding to DC-SIGN on macrophages and DCs and initiating a program that induces the suppressive Fc receptor FcRIIB on macrophages (Anthony et al., 2011; Chen et al., 2009). Moreover, IgG must be sialylated to bind FcRIIB, the inhibitory receptor. On the other hand, fully deglycosylated Ig is protective since it binds poorly to Fc receptors.

## THE ROLE OF THE GUT MICROBIOTA IN AUTOIMMUNITY

An emerging theme in autoimmunity is the heretofore unrecognized role of the gut microbiota in regulating immune responses (Atarashi and Honda, 2011; Fung et al., 2017; Paun et al., 2017; Rosser and Mauri, 2016). A vast array of bacteria of multiple species is found at epithelial barriers including the skin and the gut and can vary with age, gender, genetic background, and environmental exposures such as antibiotics and diet. Gut bacteria play a crucial role in digestion and also produce essential vitamins and metabolites that can influence distant organs. One of the most important observations in this field is that the induction of Th17 cells in mice requires gut colonization with segmented filamentous bacteria; the absence of these bacteria prevents the induction of experimental forms of arthritis and multiple sclerosis in normal mice and the spontaneous onset of type 1 diabetes in a susceptible mouse strain (Romano-Keeler et al., 2012). The bacteria provide TLR ligands and induce other inflammatory genes that trigger DCs and are also a source of ATP that helps to activate Th17 cells. The precise function of these Th17 cells, either as pathogenic cells or as regulatory cells that also produce IL-10, will depend on other factors in the gut environment.

Disease-inducing bacteria may be found in some types of autoimmunity. For example, transfer of commensal bacteria from diseased to normal mice can transmit IBD, suggesting that the initiating trigger for this disease may be communicable (Garrett et al., 2007). Importantly, susceptibility to colitis may be altered by dietary changes that prevent the emergence of the pathogenic bacteria, suggesting that fecal transplants or probiotics are therapeutic strategies that should be tested. Gut microbiota also appears to be required for the generation of gut Tr1 cells, and this may be mediated by different bacterial species than those that induce Th17 cells. The capsular polysaccharide of *Bacteroides fragilis* has been identified as an inducer of Tregs (Round and Mazmanian, 2010). Conversely, the absence of Tregs can affect the composition of the gut microbiota (Chinen and Rudensky, 2012; Josefowicz et al., 2012b). How the balance of pro and antiinflammatory gut microbiota is maintained and how this might be manipulated for the prevention or treatment of autoimmunity has become an important question (Bogdanos and Sakkas, 2017; de Oliveira et al., 2017). It seems apparent, however, that gut microbiota helps to establish immune cell metabolism and, therefore, sets thresholds for activation and effector function. Metabolites released from gut bacteria may also have nonimmune distant effects; for example, intestinal microbiota control the permeability of the blood–brain barrier and may, therefore, modulate entry of immune cells into the brain (Braniste et al., 2014).

Most studies of gut microbiota in human autoimmune disease have so far been limited to comparisons of fecal colonies between diseased and healthy individuals without the establishment of causality. Several interesting new studies, however, have identified changes in the gut microbiota that correspond to seroconversion and diagnosis of type 1 diabetes (Kostic et al., 2015; Mejia-Leon and Barca, 2015). Similar studies in rheumatoid arthritis have identified gut dysbiosis in active patients that is corrected after effective treatment (Zhang et al., 2015); furthermore, transfer of disease-associated bacteria exacerbates disease in animal models (Maeda et al., 2016).

## FLARES AND REMISSIONS DURING DISEASE

The vast majority of animal models of autoimmune disease develop chronic progressive disease activity. Once the autoimmune disease becomes manifest, it progresses to organ failure or death. Much human autoimmune disease, in contrast, is characterized by periods of disease remission and flare. Little is known in human disease about the cellular events that lead to disease remission. It is also true that little is known regarding the cause of disease flares. In mouse models of multiple sclerosis disease flares can result from epitope spreading with sequential recruitment of T-cell populations that recognize different epitopes of myelin (Mallone et al., 2011; Vanderlugt et al., 1998). Similarly in humans, epitope spreading has been observed among cohorts of lupus patients from whom prediseased serum was available (Arbuckle et al., 2003; Deshmukh et al., 2003). Nevertheless, a major area of ignorance concerns the cell type responsible for disease flares. It is not known for most autoimmune diseases whether flares represent de novo activation of naïve autoreactive cells or a reactivation of quiescent memory cells and whether these flares are due to a new environmental exposure or to a failure of regulation, or both. Our ignorance in this regard is largely derived from the difficulty of sampling a large enough repertoire of autoreactive T or B cells. Often, these cells are poorly represented in peripheral blood (Bischof et al., 2004; Newman et al., 2003; Reddy et al., 2003). The development of MHC class I and class II tetramers containing peptides of known autoantigens is beginning to facilitate the analysis of pathogenic T cells in human autoimmune diseases and animal models in which the autoantigens are known (Mallone et al., 2011; Massilamany et al., 2011). Similarly, the development of single-cell PCR technology and, more recently, high-throughput methods for sampling thousands of cells is allowing the analysis of the frequency and binding specificity of both pathogen-induced and autoreactive B cells from peripheral blood (Jardine et al., 2016; Thornburg et al., 2016; Tipton et al., 2015). These studies have shed light on how autoreactivity is regulated in human B cells during B cell development, with loss of autoreactivity as the cells progress from the bone marrow to the periphery (Meffre and Wardemann, 2008). Abnormalities in this regulation have been demonstrated in individuals with a variety of immune deficiencies and autoimmunity-related genetic polymorphisms (Isnardi et al., 2010; Meffre and Wardemann, 2008; Menard et al., 2011; Schickel et al., 2016; Weller et al., 2012). These studies have also demonstrated clonal expansions of both B and T cells in autoimmune patients with some shared V region use between patients (Tipton et al., 2015; Tong et al., 2016). In patients with lupus, the repertoire of clonally expanded B cells is more diverse than in healthy individuals immunized with tetanus or influenza antigens with some clones arising from naïve cells and some from memory cells (Tipton et al., 2015). As the technology for studying single cells advances (Robinson, 2015; Zheng et al., 2017), more information about the diversity of autoimmune lymphocytes in a variety of autoimmune diseases is expected to gather.

## MECHANISMS OF TISSUE DAMAGE

Studies over the past decade have clearly demonstrated that the mechanisms that incite autoimmune disease may differ substantially from the mechanisms that propagate tissue damage. Autoreactive T and B cells that are activated in secondary lymphoid organs and initiate disease are activated in a different microenvironment and may have a different cytokine profile from the effector cells that migrate into target organs and cause tissue fibrosis (Campbell et al., 2001; Gerriets and Rathmell, 2012; Katzman et al., 2011). Therefore it is more clear that at each stage of autoimmune disease, inductions of autoreactivity and tissue destruction need to be separately explored, and that the previous characterization of certain cytokines, as proinflammatory, and others, as antiinflammatory, may be misleading. While TGF- $\beta$  may dampen the induction of autoreactivity, it may hasten tissue fibrosis (Valluru et al., 2011). Similarly, IL-10 is antiinflammatory during disease initiation through its inhibitory effects on APCs but may lose its antiinflammatory properties (Herrero et al., 2003) and even drive T-cell proliferation, immunoglobulin class switching, and antibody production later in the disease (Mocellin et al., 2004). Even the proinflammatory cytokine interferon- $\gamma$  can have antiinflammatory properties in the early stages of some autoimmune diseases (Billiau, 1996; Grohmann and Puccetti, 2002; Rosloniec et al., 2002), perhaps by antagonizing the differentiation of T cells secreting IL-17. It is, therefore, important, as we move forward in studies of autoimmune disease, to consider the mechanism of both immune activation and tissue destruction and to be aware that cytokines, hormones, or other mediators may exhibit differential effects in each process. Studies of animal models have now clearly shown that it is possible to intervene in disease progression to protect organs from immune-mediated destruction, even while autoreactivity continues unabated (Clynes et al., 1998; Schiffer et al., 2003).

Recent studies emphasize the role of innate immune cells in tissue injury, particularly neutrophils and macrophages that are recruited to lesional sites. Macrophages have a complex program in which they first release proinflammatory mediators to fight pathogens but then initiate programs to help in clearance of dead tissue and tissue repair (Huen and Cantley, 2017). While the latter response is beneficial if short-lived, the continued activation of the repair program may be detrimental (Bethunaickan et al., 2011). A role for organ-intrinsic macrophages is also increasingly being recognized; these may be activated *in situ* and contribute to tissue damage (Stamatiades et al., 2016).

The development of fibrosis is of major concern in autoimmune diseases since it is difficult to reverse and may lead to organ failure. Fibrosis is characterized by the deposition of extracellular matrix and collagen due to the sustained activation of myofibroblasts and failure of the normal resolution of wound healing. Multiple triggers for the wound healing response have been identified including excessive cell death, cytokines and other immune mediators, activation of coagulation pathways, and ER stress (Eming, 2017; Wynn and Ramalingam, 2012). Another major driver of fibroblast differentiation is the conversion of TGF- $\beta$  by either chemical danger signals or mechanical stress from a latent form that is tethered to the cell surface to an activated profibrotic form (Friedman et al., 2013; Hinz, 2009). Several types of stromal cells can transdifferentiate into myofibroblasts and this may vary in different organs. TLR signaling in pericytes has recently been shown to be essential for their TGF- $\beta$ -mediated transformation into fibroblasts (Leaf et al., 2016). Resolution of fibrosis is mediated through the induction of proteases that break down collagen and matrix and deactivation of myofibroblasts. Failed resolution and repair may occur because of local tissue hypoxia, infection, or ongoing tissue damage with an accumulation of toxic metabolites (Wynn and Ramalingam, 2012).

Macrophages are key players both in the early inflammatory process and in tissue regeneration and scar tissue formation, and their depletion has different effects depending on the stage of the inflammatory process. The transformation of inflammatory to proresolving macrophages is associated with changes in their metabolism with a switch from dependence on glycolysis to oxidative phosphorylation. Eosinophils and mast cells can also promulgate fibrosis. Profibrotic cytokines include IL-1, IL-6, IL-13, IL-33, thymic stromal lymphopoietin (TSLP), and TGF- $\beta$ ; fibrosis can follow either Th1/Th17 or Th2 inflammatory responses (Eming, 2017).

## THERAPEUTIC ADVANCES

A major advance in the last decade has been the application of new knowledge about immune system function to the treatment of autoimmune diseases. New therapies target innate immunity, adaptive immunity, and even tissue injury. As these new drugs have entered clinical practice, it has become clear that the pleiomorphic and stage-specific functions of particular molecules can result in both beneficial and adverse therapeutic effects of the drugs that target them. TNF inhibitors, for example, while highly therapeutic in RA and IBD, can induce SLE, multiple sclerosis (MS), and vasculitis (Kollias, 2005). While some therapies are highly effective for some diseases but not for others, it has been difficult to predict efficacy or lack of efficacy based on our current understanding of disease pathogenesis. For example, global B-cell depletion using an antibody to CD20 is therapeutic in RA and MS (Barun and Bar-Or, 2012; Buch et al., 2011), diseases that were initially thought to be T-cell dependent, but has much less, if any, effect in SLE, a prototypic B-cell disease (Merrill et al., 2010). Similarly, IL-17 inhibition has been highly effective for seronegative arthritides such as psoriatic arthritis and spondyloarthritis but is less effective for rheumatoid arthritis (Fragoulis et al., 2016; Kunwar et al., 2016). These differences point to heterogeneity in cytokine involvement in inflammatory responses that are difficult to parse out even at the molecular level. Variability among patients results in response rates that rarely reach more than 70% even for the best of the new therapies. Finding ways to identify responders and nonresponders before initiating an expensive and potentially toxic new therapy is a task that is being actively pursued using large patient databases and genetic and “omics” studies.

New drugs to treat autoimmune disease are constantly in development. A major advance has been the development of small molecule inhibitors directed at key signaling molecules within the immune system, particularly a variety of protein kinases. Because there are more than 500 protein kinases, achieving precise specificity of these drugs for their targets can be difficult, but inhibitors of Jak kinases are already in clinical use and other drugs targeting Syk, Btk, PI3 kinase, and MAP kinases are in development (Croxton et al., 2016; Shao and Cohen, 2014; Stark et al., 2015). These drugs have the advantage that they can be administered orally and have been used both for autoimmunity and for oncology indications.

Many of the current approaches are based on a perceived need to institute immunosuppression and anti-inflammatory therapy at the time of autoimmune tissue destruction. Multiple pathways of immune activation,

including innate TLR and pattern-recognition receptor signaling pathways, costimulatory pathways and T- and B-cell activation and cytokine signaling pathways are being targeted to reduce the activation of the immune system (Rosenblum et al., 2012). Our most updated understanding of autoimmunity would suggest that it might also be appropriate to consider treating disease during times of disease quiescence. The goal of this therapeutic approach would be to alter T- and B-cell repertoire selection or to drive the expansion of regulatory cells and enhance regulation (Daniel and von Boehmer, 2011). Antigen-specific therapies require an understanding of the causative antigen; this remains unknown for most autoimmune diseases. Nevertheless, animal studies suggest that it may be possible to convert a pathogenic T-cell response into a regulatory one, for example, by delivering a strong signal 1 without signal 2 (Crepeau and Ford, 2017), by delivering tolerogenic signals to DCs (Yeste et al., 2016), or by expanding or delivering Tregs (Dawson and Levings, 2017; Dwyer et al., 2016; Song, 2016). Optimizing this type of approach for human use remains a dream for the future (Michels and Eisenbarth, 2011).

Protecting target organs and preventing irreversible tissue damage will require different therapeutic strategies from blocking systemic autoreactivity (Katschke et al., 2007; Sica et al., 2011; Szekanecz et al., 2009). Approaches to treating fibrosis by antagonizing cytokines such as IL-13 and TGF- $\beta$  will need to take into account the fine balance between antiinflammatory functions and promotion of healing by these cytokines and excessive collagen and matrix deposition (Eming, 2017; Friedman et al., 2013). There is an increasing understanding of the events that lead to maladaptive resolution of inflammation and consequent tissue fibrosis, with the identification of small proresolving lipid mediators that can trigger resolution of inflammatory responses (Dalli and Serhan, 2016; Fredman and Tabas, 2017; Friedman et al., 2013; Serhan, 2017). Other efforts are being directed at blocking or sequestering reactive oxygen species and other toxic metabolites (Telorack et al., 2012), inhibiting transcription pathways that lead to myofibroblast transdifferentiation (Huang et al., 2016; Yu-Wai-Man et al., 2017), decreasing collagen cross-linking, and many others (Friedman et al., 2013). These new therapeutic approaches offer the hope of maintaining immunocompetence while eliminating the consequences of pathogenic autoreactivity.

Finally, the observation that autoimmunity and immunodeficiency can be linked (Grimbacher et al., 2016) suggests that effective treatment of autoimmunity will lead to enhanced immunocompetence, and the reversal of developmental defects in lymphoid cells should reduce autoreactivity. This is the metric against which therapeutic interventions should be judged.

## GOALS FOR THE FUTURE

Over the past several years, new technologies have been developed that will substantially increase our understanding of autoimmune disease. High-throughput technologies to examine genetic polymorphisms, epigenetic changes, gene expression, and protein expression and modifications, linked with the collection of well-characterized databases of patients, make it possible to determine the level of expression of a very large number of genes or proteins in defined populations of patients and subpopulations of cells. These data may provide new insights into disease pathogenesis and new ways to phenotype patients with autoimmune disease. These technologies may also provide sets of biomarkers that will help to determine risk for developing a particular disease, characterize the current activity of the disease and disease prognosis, and assess response to therapy at an earlier time point than current clinical endpoints. It is reasonable to predict that patterns of gene and protein expression will reveal differences and similarities among autoimmune diseases.

The development of biomarkers will, undoubtedly, improve the therapy of autoimmune disease. It may be possible to identify early those patients whose disease is likely to be severe and to monitor disease activity without waiting for clinical symptomatology. Furthermore, the recognition, that autoimmunity can be detected before clinical symptomatology and that the likelihood of development of disease in individual patients can be predicted with more certainty, will allow testing of therapies that have the potential to prevent or cure autoimmune disease before tissue damage occurs. Ultimately, it may be possible to customize therapy for each patient, thereby enhancing efficacy and avoiding unnecessary toxicities and expense.

Given that the diagnosis of complex autoimmune diseases may be delayed, preventing and reversing tissue damage once effector cells have arisen is a pressing therapeutic goal for patients with established disease. Approaches to enhance the resolution of inflammation rather than fibrosis in target organs are still mostly in the experimental stage but much progress has been made in understanding this area and two new drugs are now in clinical use (Sathiyamoorthy et al., 2017). Specific targeting of pathogenic lymphocytes is still not possible in humans, although a proof of principle experiment in an animal model with a known autoantigen has recently been successful (Ellebrecht et al., 2016).

## CONCLUDING REMARKS

The past several years have witnessed a change in our understanding of autoimmunity and a clear new direction in our approach to the study of autoimmunity. Multiple genetic polymorphisms contribute to autoimmunity risk and more remain to be identified. The complexity of regulation of gene expression suggests several other mechanisms by which gene expression may be aberrantly regulated in autoimmunity. It is clear that the activation of the innate immune system can act as a trigger for the initiation of autoimmunity in susceptible individuals and can amplify tissue damage in target organs. Autoimmunity can result from either a failure in T- and B-cell repertoire selection or a failure in the regulation of activated T and B cells. Autoimmune B and T cells can be identified years before the emergence of clinical disease, suggesting that multiple triggers act sequentially to precipitate disease. It is also clear that autoimmunity needs to be coupled to target-organ vulnerability to immune attack for autoimmune disease to be present and that inflammatory cascades can be interrupted or regulated in the periphery. This understanding suggests new therapeutic targets and new therapeutic strategies.

Multiple new therapies have been developed and tested in clinical trials in the last two decades leading to major advances in treatment for some but not all autoimmune diseases. However, many knowledge gaps remain to be filled, especially in understanding which pathways to target in each disease and in determining which individuals will respond to each therapy. The focus on new technologies to provide biomarkers of immune function represents an exciting opportunity to treat disease prior to tissue damage and to customize therapy for each patient. Furthermore, studies of gene and protein expression will help to elucidate those mechanisms of immune dysfunction that are shared among multiple autoimmune diseases and those that are unique to a particular disease. Thus there are reasons to be optimistic, but acquiring the necessary new knowledge and translating that knowledge to therapy could take some years.

### Acknowledgments

The work is supported by grants R01 AR064811-01 and R21 AR 070540 (to AD) and P01 AI073693-06 (to BD).

### References

- Achenbach, P., Hummel, M., Thumer, L., Boerschmann, H., Hofelmann, D., Ziegler, A.G., 2013. Characteristics of rapid vs slow progression to type 1 diabetes in multiple islet autoantibody-positive children. *Diabetologia* 56, 1615–1622.
- Akirav, E.M., Ruddell, N.H., Herold, K.C., 2011. The role of AIRE in human autoimmune disease. *Nat. Rev. Endocrinol.* 7, 25–33.
- Alexandropoulos, K., Danzl, N.M., 2012. Thymic epithelial cells: antigen presenting cells that regulate T cell repertoire and tolerance development. *Immunol. Res.* 54, 177–190.
- Anderson, M.S., Su, M.A., 2011. Aire and T cell development. *Curr. Opin. Immunol.* 23, 198–206.
- Andersson, U., Tracey, K.J., 2011. HMGB1 is a therapeutic target for sterile inflammation and infection. *Annu. Rev. Immunol.* 29, 139–162.
- Anthony, R.M., Kobayashi, T., Wermeling, F., Ravetch, J.V., 2011. Intravenous gammaglobulin suppresses inflammation through a novel T(H) 2 pathway. *Nature* 475, 110–113.
- Arbuckle, M.R., McClain, M.T., Rubertone, M.V., Scofield, R.H., Dennis, G.J., James, J.A., et al., 2003. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N. Engl. J. Med.* 349, 1526–1533.
- Atarashi, K., Honda, K., 2011. Microbiota in autoimmunity and tolerance. *Curr. Opin. Immunol.* 23, 761–768.
- Baker, K.F., Isaacs, J.D., 2017. Novel therapies for immune-mediated inflammatory diseases: what can we learn from their use in rheumatoid arthritis, spondyloarthritis, systemic lupus erythematosus, psoriasis, Crohn's disease and ulcerative colitis? *Ann. Rheum. Dis.* 77, 175–187.
- Banchereau, J., Steinman, R.M., 1998. Dendritic cells and the control of immunity. *Nature* 392, 245–252.
- Bang, S.Y., Han, T.U., Choi, C.B., Sung, Y.K., Bae, S.C., Kang, C., 2010. Peptidyl arginine deiminase type IV (PAD4) haplotypes interact with shared epitope regardless of anti-cyclic citrullinated peptide antibody or erosive joint status in rheumatoid arthritis: a case control study. *Arthritis Res. Ther.* 12, R115.
- Bar-Or, A., Rieckmann, P., Traboulsi, A., Yong, V.W., 2011. Targeting progressive neuroaxonal injury: lessons from multiple sclerosis. *CNS Drugs* 25, 783–799.
- Barun, B., Bar-Or, A., 2012. Treatment of multiple sclerosis with anti-CD20 antibodies. *Clin. Immunol.* 142, 31–37.
- Beck, S., Trowsdale, J., 2000. The human major histocompatibility complex: lessons from the DNA sequence. *Annu. Rev. Genomics Hum. Genet.* 1, 117–137.
- Bene, L., Falus, A., Baffy, N., Fulop, A.K., 2011. Cellular and molecular mechanisms in the two major forms of inflammatory bowel disease. *Pathol. Oncol. Res.* 17, 463–472.
- Bengsch, B., Johnson, A.L., Kurachi, M., Odorizzi, P.M., Pauken, K.E., Attanasio, J., et al., 2016. Bioenergetic insufficiencies due to metabolic alterations regulated by the inhibitory receptor PD-1 are an early driver of CD8(+) T cell exhaustion. *Immunity* 45, 358–373.
- Bethunaickan, R., Berthier, C.C., Ramanujam, M., Sahu, R., Zhang, W., Sun, Y., et al., 2011. A unique hybrid renal mononuclear phagocyte activation phenotype in murine systemic lupus erythematosus nephritis. *J. Immunol.* 186, 4994–5003.
- Biermann, M.H., Griffante, G., Podolska, M.J., Boeltz, S., Sturmer, J., Munoz, L.E., et al., 2016. Sweet but dangerous—the role of immunoglobulin G glycosylation in autoimmunity and inflammation. *Lupus* 25, 934–942.

- Bijl, M., Kallenberg, C.G., 2006. Ultraviolet light and cutaneous lupus. *Lupus* 15, 724–727.
- Billiau, A., 1996. Interferon-gamma in autoimmunity. *Cytokine Growth Factor Rev.* 7, 25–34.
- Billiau, A., Matthys, P., 2011. Collagen-induced arthritis and related animal models: how much of their pathogenesis is auto-immune, how much is auto-inflammatory? *Cytokine Growth Factor Rev.* 22, 339–344.
- Bischof, F., Hofmann, M., Schumacher, T.N., Vyth-Dreese, F.A., Weissert, R., Schild, H., et al., 2004. Analysis of autoreactive CD4 T cells in experimental autoimmune encephalomyelitis after primary and secondary challenge using MHC class II tetramers. *J. Immunol.* 172, 2878–2884.
- Bishop, G.A., Haxhinasto, S.A., Stunz, L.L., Hostager, B.S., 2003. Antigen-specific B-lymphocyte activation. *Crit. Rev. Immunol.* 23, 149–197.
- Blanco, P., Palucka, A.K., Pascual, V., Banchereau, J., 2008. Dendritic cells and cytokines in human inflammatory and autoimmune diseases. *Cytokine Growth Factor Rev.* 19, 41–52.
- Bockenstedt, L.K., Gee, R.J., Mamula, M.J., 1995. Self-peptides in the initiation of lupus autoimmunity. *J. Immunol.* 154, 3516–3524.
- Bodaghi, B., Rao, N., 2008. Relevance of animal models to human uveitis. *Ophthalmic Res.* 40, 200–202.
- Bodano, A., Gonzalez, A., Ferreiros-Vidal, I., Balada, E., Ordi, J., Carreira, P., et al., 2016. Digestion of chromatin in apoptotic cell microparticles prevents autoimmunity. *Rheumatology (Oxford)* 166, 88–101.
- Bogdanos, D.P., Sakkas, L.I., 2017. From microbiome to infectome in autoimmunity. *Curr. Opin. Rheumatol.* 29, 369–373.
- Bolland, S., Ravetch, J.V., 2000. Spontaneous autoimmune disease in Fc(gamma)RIIB-deficient mice results from strain-specific epistasis. *Immunity* 13, 277–285.
- Bommhardt, U., Beyer, M., Hunig, T., Reichardt, H.M., 2004. Molecular and cellular mechanisms of T cell development. *Cell Mol. Life Sci.* 61, 263–280.
- Bournazos, S., Wang, T.T., Ravetch, J.V., 2016. The role and function of fcgamma receptors on myeloid cells. *Microbiol. Spectr.* 4.
- Braniste, V., Al-Asmakh, M., Kowal, C., Anuar, F., Abbaspour, A., Toth, M., et al., 2014. The gut microbiota influences blood-brain barrier permeability in mice. *Sci. Transl. Med.* 6, 263ra158.
- Bratton, D.L., Henson, P.M., 2011. Neutrophil clearance: when the party is over, clean-up begins. *Trends Immunol.* 32, 350–357.
- Brink, R., 2014. The imperfect control of self-reactive germinal center B cells. *Curr. Opin. Immunol.* 28, 97–101.
- Brunner, T., Wasem, C., Torgler, R., Cima, I., Jakob, S., Corazza, N., 2003. Fas (CD95/Apo-1) ligand regulation in T cell homeostasis, cell-mediated cytotoxicity and immune pathology. *Semin. Immunol.* 15, 167–176.
- Buch, M.H., Smolen, J.S., Betteridge, N., Breedveld, F.C., Burmester, G., Dorner, T., et al., 2011. Updated consensus statement on the use of rituximab in patients with rheumatoid arthritis. *Ann. Rheum. Dis.* 70, 909–920.
- Buck, M.D., Sowell, R.T., Kaech, S.M., Pearce, E.L., 2017. Metabolic instruction of immunity. *Cell* 169, 570–586.
- Burn, G.L., Svensson, L., Sanchez-Blanco, C., Saini, M., Cope, A.P., 2011. Why is PTPN22 a good candidate susceptibility gene for autoimmune disease? *FEBS Lett.* 585, 3689–3698.
- Caielli, S., Athale, S., Domic, B., Murat, E., Chandra, M., Banchereau, R., et al., 2016. Oxidized mitochondrial nucleoids released by neutrophils drive type I interferon production in human lupus. *J. Exp. Med.* 213, 697–713.
- Campbell, D.J., Kim, C.H., Butcher, E.C., 2001. Separable effector T cell populations specialized for B cell help or tissue inflammation. *Nat. Immunol.* 2, 876–881.
- Cappelli, L.C., Gutierrez, A.K., Bingham 3rd, C.O., Shah, A.A., 2016. Rheumatic and musculoskeletal immune-related adverse events due to immune checkpoint inhibitors: a systematic review of the literature. *Arthritis Care Res.* 69, 1751–1763.
- Ceribelli, A., Yao, B., Dominguez-Gutierrez, P.R., Chan, E.K., 2011a. Lupus T cells switched on by DNA hypomethylation via microRNA? *Arthritis Rheum.* 63, 1177–1181.
- Ceribelli, A., Yao, B., Dominguez-Gutierrez, P.R., Nahid, M.A., Satoh, M., Chan, E.K., 2011b. MicroRNAs in systemic rheumatic diseases. *Arthritis Res. Ther.* 13, 229.
- Chang, H.H., Liu, G.Y., Dwivedi, N., Sun, B., Okamoto, Y., Kinslow, J.D., et al., 2016. A molecular signature of preclinical rheumatoid arthritis triggered by dysregulated PTPN22. *JCI Insight* 1, e90045.
- Chavan, S.S., Pavlov, V.A., Tracey, K.J., 2017. Mechanisms and therapeutic relevance of neuro-immune communication. *Immunity* 46, 927–942.
- Chen, L., 2004. Co-inhibitory molecules of the B7-CD28 family in the control of T-cell immunity. *Nat. Rev. Immunol.* 4, 336–347.
- Chen, Y., Park, Y.B., Patel, E., Silverman, G.J., 2009. IgM antibodies to apoptosis-associated determinants recruit C1q and enhance dendritic cell phagocytosis of apoptotic cells. *J. Immunol.* 182, 6031–6043.
- Chen, G., Yang, X., Ko, A., Sun, X., Gao, M., Zhang, Y., et al., 2017. Sequence and structural analyses reveal distinct and highly diverse human CD8+ TCR repertoires to immunodominant viral antigens. *Cell Rep.* 19, 569–583.
- Chinen, T., Rudensky, A.Y., 2012. The effects of commensal microbiota on immune cell subsets and inflammatory responses. *Immunol. Rev.* 245, 45–55.
- Cho, J.H., Gregersen, P.K., 2011. Genomics and the multifactorial nature of human autoimmune disease. *N. Engl. J. Med.* 365, 1612–1623.
- Cho, J.H., Feldman, M., 2015. Heterogeneity of autoimmune diseases: pathophysiologic insights from genetics and implications for new therapies. *Nat. Med.* 21, 730–738.
- Christen, U., Bender, C., von Herrath, M.G., 2012. Infection as a cause of type 1 diabetes? *Curr. Opin. Rheumatol.* 24, 417–423.
- Christensen, S.R., Kashgarian, M., Alexopoulou, L., Flavell, R.A., Akira, S., Shlomchik, M.J., 2005. Toll-like receptor 9 controls anti-DNA auto-antibody production in murine lupus. *J. Exp. Med.* 202, 321–331.
- Clynes, R., Dumitru, C., Ravetch, J.V., 1998. Uncoupling of immune complex formation and kidney damage in autoimmune glomerulonephritis. *Science* 279, 1052–1054.
- Colonna, L., Lood, C., Elkon, K.B., 2014. Beyond apoptosis in lupus. *Curr. Opin. Rheumatol.* 5, 459.
- Cooper, G.S., Bynum, M.L., Somers, E.C., 2009. Recent insights in the epidemiology of autoimmune diseases: improved prevalence estimates and understanding of clustering of diseases. *J. Autoimmun.* 33, 197–207.
- Cortesini, R., LeMaoult, J., Ciubotariu, R., Cortesini, N.S., 2001. CD8+ CD28 – T suppressor cells and the induction of antigen-specific, antigen-presenting cell-mediated suppression of Th reactivity. *Immunol. Rev.* 182, 201–206.

- Cotsapas, C., Voight, B.F., Rossin, E., Lage, K., Neale, B.M., Wallace, C., et al., 2011. Pervasive sharing of genetic effects in autoimmune disease. *PLoS Genet.* 7, e1002254.
- Crepeau, R.L., Ford, M.L., 2017. Challenges and opportunities in targeting the CD28/CTLA-4 pathway in transplantation and autoimmunity. *Expert Opin. Biol. Ther.* 17, 1001–1012.
- Crofford, L.J., Nyhoff, L.E., Sheehan, J.H., Kendall, P.L., 2016. The role of Bruton's tyrosine kinase in autoimmunity and implications for therapy. *Expert Rev. Clin. Immunol.* 12, 763–773.
- Crow, M.K., 2004. Costimulatory molecules and T-cell-B-cell interactions. *Rheum. Dis. Clin. North Am.* 30, 175–191. vii-viii.
- Cunningham, M.W., 2003. Autoimmunity and molecular mimicry in the pathogenesis of post-streptococcal heart disease. *Front. Biosci.* 8, s533–s543.
- Cusick, M.F., Libbey, J.E., Fujinami, R.S., 2012. Molecular mimicry as a mechanism of autoimmune disease. *Clin. Rev. Allergy Immunol.* 42, 102–111.
- D'Cruz, D., 2000. Autoimmune diseases associated with drugs, chemicals and environmental factors. *Toxicol. Lett.* 112-113, 421–432.
- Dalli, J., Serhan, C., 2016. Macrophage proresolving mediators—the when and where. *Microbiol. Spectr.* 4.
- Daniel, C., von Boehmer, H., 2011. Extrathymic generation of regulatory T cells—chances and challenges for prevention of autoimmune disease. *Adv. Immunol.* 112, 177–213.
- Davidson, A., Diamond, B., 2001. Autoimmune diseases. *N. Engl. J. Med.* 345, 340–350.
- Dawson, N.A.J., Levings, M.K., 2017. Antigen-specific regulatory T cells: are police CARs the answer? *Transl. Res.* 187, 53–58.
- Day, D., Hansen, A.R., 2016. Immune-related adverse events associated with immune checkpoint inhibitors. *BioDrugs* 30, 571–584.
- de Heredia, F.P., Gomez-Martinez, S., Marcos, A., 2012. Obesity, inflammation and the immune system. *Proc. Nutr. Soc.* 71, 332–338.
- de Oliveira, G.L.V., Leite, A.Z., Higuchi, B.S., Gonzaga, M.I., Mariano, V.S., 2017. Intestinal dysbiosis and probiotic applications in autoimmune diseases. *Immunology* 152, 1–12.
- de Souza, H.S., Fiocchi, C., 2016. Immunopathogenesis of IBD: current state of the art. *Nat. Rev. Gastroenterol. Hepatol.* 13, 13–27.
- Deane, K.D., O'Donnell, C.I., Hueber, W., Majka, D.S., Lazar, A.A., Derber, L.A., et al., 2010. The number of elevated cytokines and chemokines in preclinical seropositive rheumatoid arthritis predicts time to diagnosis in an age-dependent manner. *Arthritis Rheum.* 62, 3161–3172.
- Debandt, M., Vittecoq, O., Descamps, V., Le Loet, X., Meyer, O., 2003. Anti-TNF-alpha-induced systemic lupus syndrome. *Clin. Rheumatol.* 22, 56–61.
- DeFranco, A.L., 2016. Germinal centers and autoimmune disease in humans and mice. *Immunol. Cell. Biol.* 94, 918–924.
- Deng, Y., Tsao, B.P., 2010. Genetic susceptibility to systemic lupus erythematosus in the genomic era. *Nat. Rev. Rheumatol.* 6, 683–692.
- Deshmukh, U.S., Gaskin, F., Lewis, J.E., Kannapell, C.C., Fu, S.M., 2003. Mechanisms of autoantibody diversification to SLE-related autoantgens. *Ann. N.Y. Acad. Sci.* 987, 91–98.
- Devi, K.S., Anandasabapathy, N., 2017. The origin of DCs and capacity for immunologic tolerance in central and peripheral tissues. *Semin. Immunopathol.* 39, 137–152.
- Dominguez, P.M., Ardevin, C., 2010. Differentiation and function of mouse monocyte-derived dendritic cells in steady state and inflammation. *Immunol. Rev.* 234, 90–104.
- Doyle, H.A., Mamula, M.J., 2012. Autoantigenesis: the evolution of protein modifications in autoimmune disease. *Curr. Opin. Immunol.* 24, 112–118.
- Dwyer, C.J., Ward, N.C., Pugliese, A., Malek, T.R., 2016. Promoting immune regulation in type 1 diabetes using low-dose interleukin-2. *Curr. Diab. Rep.* 16, 46.
- Dzopalic, T., Rajkovic, I., Dragicevic, A., Colic, M., 2012. The response of human dendritic cells to co-ligation of pattern-recognition receptors. *Immunol. Res.* 52, 20–33.
- Elkon, K.B., Santer, D.M., 2012. Complement, interferon and lupus. *Curr. Opin. Immunol.* 24, 665–670.
- Ellebrecht, C.T., Bhoj, V.G., Nace, A., Choi, E.J., Mao, X., Cho, M.J., et al., 2016. Reengineering chimeric antigen receptor T cells for targeted therapy of autoimmune disease. *Science* 353, 179–184.
- Eming, S.A., 2017. Inflammation and metabolism in tissue repair and regeneration. *Science* 356, 1026–1030.
- Engels, N., Wienands, J., 2011. The signaling tool box for tyrosine-based costimulation of lymphocytes. *Curr. Opin. Immunol.* 23, 324–329.
- Esensten, J.H., Helou, Y.A., Chopra, G., Weiss, A., Bluestone, J.A., 2016. CD28 costimulation: from mechanism to therapy. *Immunity* 44, 973–988.
- Espeli, M., Niederer, H.A., Traherne, J.A., Trowsdale, J., Smith, K.G., 2010. Genetic variation, Fcgamma receptors, KIRs and infection: the evolution of autoimmunity. *Curr. Opin. Immunol.* 22, 715–722.
- Exley, M.A., Tsokos, G.C., Mills, K.H., Elewaut, D., Mulhearn, B., 2016. What rheumatologists need to know about innate lymphocytes. *Nat. Rev. Rheumatol.* 12, 658–668.
- Fernando, M.M., Stevens, C.R., Walsh, E.C., De Jager, P.L., Goyette, P., Plenge, R.M., et al., 2008. Defining the role of the MHC in autoimmunity: a review and pooled analysis. *PLoS Genet.* 4, e1000024.
- Filardy, A.A., Pires, D.R., Nunes, M.P., Takiya, C.M., Freire-de-Lima, C.G., Ribeiro-Gomes, F.L., et al., 2010. Proinflammatory clearance of apoptotic neutrophils induces an IL-12(low)/IL-10(high) regulatory phenotype in macrophages. *J. Immunol.* 185, 2044–2050.
- Fleisher, T.A., Straus, S.E., Bleesing, J.J., 2001. A genetic disorder of lymphocyte apoptosis involving the fas pathway: the autoimmune lymphoproliferative syndrome. *Curr. Allergy Asthma Rep.* 1, 534–540.
- Flesher, D.L., Sun, X., Behrens, T.W., Graham, R.R., Criswell, L.A., 2010. Recent advances in the genetics of systemic lupus erythematosus. *Expert Rev. Clin. Immunol.* 6, 461–479.
- Fragoulis, G.E., Siebert, S., McInnes, I.B., 2016. Therapeutic targeting of IL-17 and IL-23 cytokines in immune-mediated diseases. *Ann. Rev. Med.* 67, 337–353.
- Fredman, G., Tabas, I., 2017. Boosting inflammation resolution in atherosclerosis: the next frontier for therapy. *Am. J. Pathol.* 187, 1211–1221.
- Friedman, S.L., Sheppard, D., Duffield, J.S., Violette, S., 2013. Therapy for fibrotic diseases: nearing the starting line. *Sci. Transl. Med.* 5, 167sr161.

- Fukuyama, H., Nimmerjahn, F., Ravetch, J.V., 2005. The inhibitory Fc $\gamma$  receptor modulates autoimmunity by limiting the accumulation of immunoglobulin G + anti-DNA plasma cells. *Nat. Immunol.* 6, 99–106.
- Fung, T.C., Olson, C.A., Hsiao, E.Y., 2017. Interactions between the microbiota, immune and nervous systems in health and disease. *Nat. Neurosci.* 20, 145–155.
- Gallagher, M.P., Goland, R.S., Greenbaum, C.J., 2011. Making progress: preserving beta cells in type 1 diabetes. *Ann. N.Y. Acad. Sci.* 1243, 119–134.
- Gallo, P.M., Gallucci, S., 2013. The dendritic cell response to classic, emerging, and homeostatic danger signals. Implications for autoimmunity. *Front. Immunol.* 190, 1447–1456.
- Gao, D., Li, T., Li, X.D., Chen, X., Li, Q.Z., Wight-Carter, M.T., et al., 2015. Cutting edge: cGAS is required for lethal autoimmune disease in the Trex1-deficient mouse model of Aicardi-Goutieres syndrome. *Proc. Natl. Acad. Sci. U.S.A.* 195, 1939–1943.
- Garrett, W.S., Lord, G.M., Punit, S., Lugo-Villarino, G., Mazmanian, S.K., Ito, S., et al., 2007. Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. *Cell* 131, 33–45.
- Gellert, M., 2002. V(D)J recombination: RAG proteins, repair factors, and regulation. *Annu. Rev. Biochem.* 71, 101–132.
- Generali, E., Ceribelli, A., Stazi, M.A., Selmi, C., 2017. Lessons learned from twins in autoimmune and chronic inflammatory diseases. *J. Autoimmun.* 83, 51–61.
- Gerriets, V.A., Rathmell, J.C., 2012. Metabolic pathways in T cell fate and function. *Trends Immunol.* 33, 168–173.
- Ghosh, D., Kis-Toth, K., Juang, Y.T., Tsokos, G.C., 2012. CREMalpha suppresses spleen tyrosine kinase expression in normal but not systemic lupus erythematosus T cells. *Arthritis Rheum.* 64, 799–807.
- Glasmacher, E., Hoefig, K.P., Vogel, K.U., Rath, N., Du, L., Wolf, C., et al., 2010. Roquin binds inducible costimulator mRNA and effectors of mRNA decay to induce microRNA-independent post-transcriptional repression. *Nat. Immunol.* 11, 725–733.
- Goodnow, C.C., Vinuesa, C.G., Randall, K.L., Mackay, F., Brink, R., 2010. Control systems and decision making for antibody production. *Nat. Immunol.* 11, 681–688.
- Gorman, J.A., Hundhausen, C., Errett, J.S., Stone, A.E., Allenspach, E.J., Ge, Y., et al., 2017. The A946T variant of the RNA sensor IFIH1 mediates an interferon program that limits viral infection but increases the risk for autoimmunity. *Nat. Immunol.* 18, 744–752.
- Gray, E.E., Treuting, P.M., Woodward, J.J., Stetson, D.B., 2015a. Cutting edge: cGAS is required for lethal autoimmune disease in the Trex1-deficient mouse model of Aicardi-Goutieres syndrome. *J. Immunol.* 195, 1939–1943.
- Gray, E.E., Treuting, P.M., Woodward, J.J., Stetson, D.B., 2015b. Exonuclease TREX1 degrades double-stranded DNA to prevent spontaneous lupus-like inflammatory disease. *J. Immunol.* 112, 5117–5122.
- Grayson, P.C., Carmona-Rivera, C., Xu, L., Lim, N., Gao, Z., Asare, A.L., et al., 2015. Neutrophil-related gene expression and low-density granulocytes associated with disease activity and response to treatment in antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum.* 67, 1922–1932.
- Green, N.M., Marshak-Rothstein, A., 2011. Toll-like receptor driven B cell activation in the induction of systemic autoimmunity. *Semin. Immunol.* 23, 106–112.
- Grimaldi, C.M., Hill, L., Xu, X., Peeva, E., Diamond, B., 2005. Hormonal modulation of B cell development and repertoire selection. *Mol. Immunol.* 42, 811–820.
- Grimbacher, B., Warnatz, K., Yong, P.F., Korganow, A.S., Peter, H.H., 2016. The crossroads of autoimmunity and immunodeficiency: lessons from polygenic traits and monogenic defects. *J. Allergy Clin. Immunol.* 137, 3–17. quiz 18.
- Grodzicky, T., Elkorn, K.B., 2002. Apoptosis: a case where too much or too little can lead to autoimmunity. *Mt. Sinai J. Med.* 69, 208–219.
- Grohmann, U., Puccetti, P., 2002. The immunosuppressive activity of proinflammatory cytokines in experimental models: potential for therapeutic intervention in autoimmunity. *Curr. Drug Targets Inflamm. Allergy* 1, 77–87.
- Grossman, Z., Min, B., Meier-Schellersheim, M., Paul, W.E., 2004. Concomitant regulation of T-cell activation and homeostasis. *Nat. Rev. Immunol.* 4, 387–395.
- Gu, H., Tarlinton, D., Muller, W., Rajewsky, K., Forster, I., 1991. Most peripheral B cells in mice are ligand selected. *J. Exp. Med.* 173, 1357–1371.
- Guiducci, C., Gong, M., Cepika, A.M., Xu, Z., Tripodo, C., Bennett, L., et al., 2013. RNA recognition by human TLR8 can lead to autoimmune inflammation. *J. Exp. Med.* 210, 2903–2919.
- Guilherme, L., Kalil, J., 2004. Rheumatic fever: from sore throat to autoimmune heart lesions. *Int. Arch. Allergy Immunol.* 134, 56–64.
- Gutierrez-Arcelus, M., Rich, S.S., Raychaudhuri, S., 2016. Autoimmune diseases—connecting risk alleles with molecular traits of the immune system. *Nat. Rev. Genet.* 17, 160–174.
- Harley, J.B., Alarcon-Riquelme, M.E., Criswell, L.A., Jacob, C.O., Kimberly, R.P., Moser, K.L., et al., 2008. Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in ITGAM, PXK, KIAA1542 and other loci. *Nat. Genet.* 40, 204–210.
- Hayter, S.M., Cook, M.C., 2012. Updated assessment of the prevalence, spectrum and case definition of autoimmune disease. *Autoimmun. Rev.* 11, 754–765.
- Herlands, R.A., Christensen, S.R., Sweet, R.A., Hershberg, U., Shlomchik, M.J., 2008. T cell-independent and Toll-like receptor-dependent antigen-driven activation of autoreactive B cells. *Immunity* 29, 249–260.
- Herrero, C., Hu, X., Li, W.P., Samuels, S., Sharif, M.N., Kotenko, S., et al., 2003. Reprogramming of IL-10 activity and signaling by IFN-gamma. *J. Immunol.* 171, 5034–5041.
- Hinz, B., 2009. Tissue stiffness, latent TGF-beta1 activation, and mechanical signal transduction: implications for the pathogenesis and treatment of fibrosis. *Curr. Rheumatol. Rep.* 11, 120–126.
- Hirahara, K., Schwartz, D., Gadina, M., Kanno, Y., O'Shea, J.J., 2016. Targeting cytokine signaling in autoimmunity: back to the future and beyond. *Curr. Opin. Immunol.* 43, 89–97.
- Horton, C., Shanmugarajah, K., Fairchild, P.J., 2017. Harnessing the properties of dendritic cells in the pursuit of immunological tolerance. *Biomed. J.* 40, 80–93.

- Horwitz, M.S., Ilic, A., Fine, C., Rodriguez, E., Sarvetnick, N., 2002. Presented antigen from damaged pancreatic beta cells activates autoreactive T cells in virus-mediated autoimmune diabetes. *J. Clin. Invest.* 109, 79–87.
- Howell, C.D., 2002. Animal models of autoimmunity. *Clin. Liver Dis.* 6, 487–495.
- Hsieh, C.S., Lee, H.M., Lio, C.W., 2012. Selection of regulatory T cells in the thymus. *Nat. Rev. Immunol.* 12, 157–167.
- Huang, J., Beyer, C., Palumbo-Zerr, K., Zhang, Y., Ramming, A., Distler, A., et al., 2016. Nintedanib inhibits fibroblast activation and ameliorates fibrosis in preclinical models of systemic sclerosis. *Ann. Rheum. Dis.* 75, 883–890.
- Huen, S.C., Cantley, L.G., 2017. Macrophages in renal injury and repair. *Annu. Rev. Physiol.* 79, 449–469.
- Ilarregui, J.M., Croci, D.O., Bianco, G.A., Toscano, M.A., Salatino, M., Vermeulen, M.E., et al., 2009. Tolerogenic signals delivered by dendritic cells to T cells through a galectin-1-driven immunoregulatory circuit involving interleukin 27 and interleukin 10. *Nat. Immunol.* 10, 981–991.
- Isnardi, I., Ng, Y.S., Menard, L., Meyers, G., Saadoun, D., Srđanovic, I., et al., 2010. Complement receptor 2/CD21 – human naive B cells contain mostly autoreactive unresponsive clones. *Blood* 115, 5026–5036.
- Jackson, S.W., Schaping, N.E., Kolhatkar, N.S., Khim, S., Schwartz, M.A., Li, Q.Z., et al., 2014. Opposing impact of B cell-intrinsic TLR7 and TLR9 signals on autoantibody repertoire and systemic inflammation. *J. Immunol.* 192, 4525–4532.
- Jacobson, D.L., Gange, S.J., Rose, N.R., Graham, N.M., 1997. Epidemiology and estimated population burden of selected autoimmune diseases in the United States. *Clin. Immunol. Immunopathol.* 84, 223–243.
- James, J.A., Robertson, J.M., 2012. Lupus and Epstein-Barr. *Curr. Opin. Rheumatol.* 24, 383–388.
- Jardine, J.G., Kulp, D.W., Havenar-Daughton, C., Sarkar, A., Briney, B., Sok, D., et al., 2016. HIV-1 broadly neutralizing antibody precursor B cells revealed by germline-targeting immunogen. *Science* 351, 1458–1463.
- Jiang, H., Canfield, S.M., Gallagher, M.P., Jiang, H.H., Jiang, Y., Zheng, Z., et al., 2010. HLA-E-restricted regulatory CD8(+) T cells are involved in development and control of human autoimmune type 1 diabetes. *J. Clin. Invest.* 120, 3641–3650.
- Jorch, S.K., Kubes, P., 2017. An emerging role for neutrophil extracellular traps in noninfectious disease. *Nat. Med.* 23, 279–287.
- Josefowicz, S.Z., Lu, L.F., Rudensky, A.Y., 2012a. Regulatory T cells: mechanisms of differentiation and function. *Annu. Rev. Immunol.* 30, 531–564.
- Josefowicz, S.Z., Niec, R.E., Kim, H.Y., Treuting, P., Chinen, T., Zheng, Y., et al., 2012b. Extrathymically generated regulatory T cells control mucosal TH2 inflammation. *Nature* 482, 395–399.
- Kalantari, T., Kamali-Sarvestani, E., Ceric, B., Karimi, M.H., Kalantari, M., Faridar, A., et al., 2011. Generation of immunogenic and tolerogenic clinical-grade dendritic cells. *Immunol. Res.* 51, 153–160.
- Kallionpaa, H., Elo, L.L., Laajala, E., Mykkonen, J., Ricano-Ponce, I., Vaarma, M., et al., 2014. Innate immune activity is detected prior to seroconversion in children with HLA-conferred type 1 diabetes susceptibility. *Diabetes* 63, 2402–2414.
- Kamata, S., Miyagawa, S., Fukushima, S., Imanishi, Y., Saito, A., Maeda, N., et al., 2015. Targeted delivery of adipocytokines into the heart by induced adipocyte cell-sheet transplantation yields immune tolerance and functional recovery in autoimmune-associated myocarditis in rats. *Circ. J.* 79, 169–179.
- Kasper, I.R., Apostolidis, S.A., Sharabi, A., Tsokos, G.C., 2016. Empowering regulatory T cells in autoimmunity. *Trends Mol. Med.* 22, 784–797.
- Katschke Jr., K.J., Helmy, K.Y., Steffek, M., Xi, H., Yin, J., Lee, W.P., et al., 2007. A novel inhibitor of the alternative pathway of complement reverses inflammation and bone destruction in experimental arthritis. *J. Exp. Med.* 204, 1319–1325.
- Katzman, S.D., Hoyer, K.K., Dooms, H., Gratz, I.K., Rosenblum, M.D., Paw, J.S., et al., 2011. Opposing functions of IL-2 and IL-7 in the regulation of immune responses. *Cytokine* 56, 116–121.
- Kawai, T., Akira, S., 2010. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat. Immunol.* 11, 373–384.
- Kawai, T., Akira, S., 2011. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity* 34, 637–650.
- Khoury, S.J., Sayegh, M.H., 2004. The roles of the new negative T cell costimulatory pathways in regulating autoimmunity. *Immunity* 20, 529–538.
- Kienhofer, D., Hahn, J., Stoof, J., Csepregi, J.Z., Reinwald, C., Urbonaviciute, V., et al., 2017. Experimental lupus is aggravated in mouse strains with impaired induction of neutrophil extracellular traps. *JCI Insight* 2.
- Kim, H.J., Cantor, H., 2011. Regulation of self-tolerance by Qa-1-restricted CD8(+) regulatory T cells. *Semin. Immunol.* 23, 446–452.
- King, A.J., 2012. The use of animal models in diabetes research. *Br. J. Pharmacol.* 166, 877–894.
- Klareskog, L., Malmstrom, V., Lundberg, K., Padyukov, L., Alfredsson, L., 2011. Smoking, citrullination and genetic variability in the immunopathogenesis of rheumatoid arthritis. *Semin. Immunol.* 23, 92–98.
- Klinker, M.W., Lundy, S.K., 2012. Multiple mechanisms of immune suppression by B lymphocytes. *Mol. Med.* 18, 123–137.
- Knip, M., Akerblom, H.K., 1999. Environmental factors in the pathogenesis of type 1 diabetes mellitus. *Exp. Clin. Endocrinol. Diabetes* 107 (Suppl 3), S93–S100.
- Kochi, Y., Thabet, M.M., Suzuki, A., Okada, Y., Daha, N.A., Toes, R.E., et al., 2011. PADI4 polymorphism predisposes male smokers to rheumatoid arthritis. *Ann. Rheum. Dis.* 70, 512–515.
- Koga, T., Hedrich, C.M., Mizui, M., Yoshida, N., Otomo, K., Lieberman, L.A., et al., 2014. CaMK4-dependent activation of AKT/mTOR and CREM-alpha underlies autoimmunity-associated Th17 imbalance. *J. Clin. Invest.* 124, 2234–2245.
- Koga, T., Otomo, K., Mizui, M., Yoshida, N., Umeda, M., Ichinose, K., et al., 2016. Calcium/calmodulin-dependent kinase IV facilitates the recruitment of interleukin-17-producing cells to target organs through the CCR6/CCL20 axis in Th17 cell-driven inflammatory diseases. *Arthritis Rheum.* 68, 1981–1988.
- Kollias, G., 2005. TNF pathophysiology in murine models of chronic inflammation and autoimmunity. *Semin. Arthritis Rheum.* 34, 3–6.
- Kollias, G., Papadaki, P., Apparailly, F., Vervoordeldonk, M.J., Holmdahl, R., Baumans, V., et al., 2011. Animal models for arthritis: innovative tools for prevention and treatment. *Ann. Rheum. Dis.* 70, 1357–1362.
- Kondo, K., Takada, K., Takahama, Y., 2017. Antigen processing and presentation in the thymus: implications for T cell repertoire selection. *Curr. Opin. Immunol.* 46, 53–57.

- Kontzias, A., Laurence, A., Gadina, M., O'Shea, J.J., 2012. Kinase inhibitors in the treatment of immune-mediated disease. *F1000 Med. Rep.* 4, 5.
- Koopman, F.A., Chavan, S.S., Miljko, S., Grazio, S., Sokolovic, S., Schuurman, P.R., et al., 2016. Vagus nerve stimulation inhibits cytokine production and attenuates disease severity in rheumatoid arthritis. *Proc. Natl. Acad. Sci. U.S.A.* 113, 8284–8289.
- Koster, M.J., Matteson, E.L., Warrington, K.J., 2016. Recent advances in the clinical management of giant cell arteritis and Takayasu arteritis. *Curr. Opin. Rheumatol.* 28, 211–217.
- Kostic, A.D., Gevers, D., Siljander, H., Vatanen, T., Hyotylainen, T., Hamalainen, A.M., et al., 2015. The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. *Cell Host Microbe* 17, 260–273.
- Kostine, M., Chiche, L., Lazaro, E., Halfon, P., Charpin, C., Arnaud, D., et al., 2017. Opportunistic autoimmunity secondary to cancer immunotherapy (OASI): an emerging challenge. *Rev. Med. Interne* 38, 513–525.
- Koutouzov, S., Mathian, A., Dalloul, A., 2006. Type-I interferons and systemic lupus erythematosus. *Autoimmun. Rev.* 5, 554–562.
- Koyama, R., Arai, T., Kijima, M., Sato, S., Miura, S., Yuasa, M., et al., 2016. DNase  $\gamma$ , DNase I and caspase-activated DNase cooperate to degrade dead cells. *Genes Cells* 21 (11), 1150–1163.
- Kuhn, A., Beissert, S., 2005. Photosensitivity in lupus erythematosus. *Autoimmunity* 38, 519–529.
- Kunwar, S., Dahal, K., Sharma, S., 2016. Anti-IL-17 therapy in treatment of rheumatoid arthritis: a systematic literature review and meta-analysis of randomized controlled trials. *Rheumatol. Int.* 36, 1065–1075.
- Kuon, W., Sieper, J., 2003. Identification of HLA-B27-restricted peptides in reactive arthritis and other spondyloarthropathies: computer algorithms and fluorescent activated cell sorting analysis as tools for hunting of HLA-B27-restricted chlamydial and autologous crossreactive peptides involved in reactive arthritis and ankylosing spondylitis. *Rheum. Dis. Clin. North Am.* 29, 595–611.
- Kuwabara, S., 2004. Guillain-Barre syndrome: epidemiology, pathophysiology and management. *Drugs* 64, 597–610.
- Lam-Tse, W.K., Lernmark, A., Drexhage, H.A., 2002. Animal models of endocrine/organ-specific autoimmune diseases: do they really help us to understand human autoimmunity? *Springer Semin. Immunopathol.* 24, 297–321.
- Langefeld, C.D., Ainsworth, H.C., Cunningham Graham, D.S., Kelly, J.A., Comeau, M.E., Marion, M.C., et al., 2017. Transancestral mapping and genetic load in systemic lupus erythematosus. *Nat. Commun.* 8, 16021.
- Laufer, V.A., Chen, J.Y., Langefeld, C.D., Bridges Jr., S.L., 2017. Integrative approaches to understanding the pathogenic role of genetic variation in rheumatic diseases. *Rheum. Dis. Clin. North Am.* 43, 449–466.
- Le, N.P., Bowden, T.A., Struwe, W.B., Crispin, M., 2016. Immune recruitment or suppression by glycan engineering of endogenous and therapeutic antibodies. *Biochim. Biophys. Acta* 1860, 1655–1668.
- Leaf, I.A., Nakagawa, S., Johnson, B.G., Cha, J.J., Mittelsteadt, K., Guckian, K.M., et al., 2016. A glutathione-Nrf2-thioredoxin cross-talk ensures keratinocyte survival and efficient wound repair. *J. Clin. Invest.* 12, e1005800.
- Lee, J., Boutz, D.R., Chromikova, V., Joyce, M.G., Vollmers, C., Leung, K., et al., 2016. Molecular-level analysis of the serum antibody repertoire in young adults before and after seasonal influenza vaccination. *Nat. Med.* 22, 1456–1464.
- Lempainen, J., Laine, A.P., Hammais, A., Toppari, J., Simell, O., Veijola, R., et al., 2015. Non-HLA gene effects on the disease process of type 1 diabetes: from HLA susceptibility to overt disease. *J. Autoimmun.* 61, 45–53.
- Leon, B., Ballesteros-Tato, A., Misra, R.S., Wojciechowski, W., Lund, F.E., 2012. Unraveling effector functions of B cells during infection: the hidden world beyond antibody production. *Infect. Disord. Drug Targets.* 12, 213–221.
- Lerner, A., Jeremias, P., Matthias, T., 2015. The world incidence and prevalence of autoimmune diseases is increasing. *Int. J. Celiac Dis.* 3, 151–155.
- Lewis, M.J., Botto, M., 2006. Complement deficiencies in humans and animals: links to autoimmunity. *Autoimmunity* 39, 367–378.
- Li-Weber, M., Krammer, P.H., 2003. Function and regulation of the CD95 (APO-1/Fas) ligand in the immune system. *Semin. Immunol.* 15, 145–157.
- Li, F., Smith, P., Ravetch, J.V., 2014. Inhibitory Fc gamma receptor is required for the maintenance of tolerance through distinct mechanisms. *J. Immunol.* 192, 3021–3028.
- Li, Z., Woo, C.J., Iglesias-Ussel, M.D., Ronai, D., Scharff, M.D., 2004. The generation of antibody diversity through somatic hypermutation and class switch recombination. *Genes Dev.* 18, 1–11.
- Liao, L., Sindhwani, R., Rojkind, M., Factor, S., Leinwand, L., Diamond, B., 1995. Antibody-mediated autoimmune myocarditis depends on genetically determined target organ sensitivity. *J. Exp. Med.* 181, 1123–1131.
- Linterman, M.A., Rigby, R.J., Wong, R., Silva, D., Withers, D., Anderson, G., et al., 2009. Roquin differentiates the specialized functions of duplicated T cell costimulatory receptor genes CD28 and ICOS. *Immunity* 30, 228–241.
- Lipsky, P.E., 2001. Systemic lupus erythematosus: an autoimmune disease of B cell hyperactivity. *Nat. Immunol.* 2, 764–766.
- Liu, K., Li, Q.Z., Delgado-Vega, A.M., Abelson, A.K., Sanchez, E., Kelly, J.A., et al., 2009. Kallikrein genes are associated with lupus and glomerular basement membrane-specific antibody-induced nephritis in mice and humans. *J. Clin. Invest.* 119, 911–923.
- Liu, Z., Davidson, A., 2011. BAFF and selection of autoreactive B cells. *Trends Immunol.* 32, 388–394.
- Liu, Z., Zou, Y., Davidson, A., 2011. Plasma cells in systemic lupus erythematosus: the long and short of it all. *Eur. J. Immunol.* 41, 588–591.
- Long, H., Yin, H., Wang, L., Gershwin, M.E., Lu, Q., 2016. The critical role of epigenetics in systemic lupus erythematosus and autoimmunity. *J. Autoimmun.* 74, 118–138.
- Loricera, J., Blanco, R., Hernandez, J.L., Pina, T., Gonzalez-Vela, M.C., Gonzalez-Gay, M.A., 2015. Biologic therapy in ANCA-negative vasculitis. *Int. Immunopharmacol.* 27, 213–219.
- Lucca, L.E., Hafler, D.A., 2017. Co-inhibitory blockade while preserving tolerance: checkpoint inhibitors for glioblastoma. *Immunol. Rev.* 276, 9–25.
- Lykken, J.M., Candando, K.M., Tedder, T.F., 2015. Regulatory B10 cell development and function. *Int. Immunol.* 27, 471–477.
- Macedo, A.C., Isaac, L., 2016. Systemic lupus erythematosus and deficiencies of early components of the complement classical pathway. *Front. Immunol.* 7, 55.
- Mackay, F., Schneider, P., 2009. Cracking the BAFF code. *Nat. Rev. Immunol.* 9, 491–502.
- Madkaikar, M., Mhatre, S., Gupta, M., Ghosh, K., 2011. Advances in autoimmune lymphoproliferative syndromes. *Eur. J. Haematol.* 87, 1–9.

- Maeda, Y., Kurakawa, T., Umemoto, E., Motooka, D., Ito, Y., Gotoh, K., et al., 2016. Dysbiosis contributes to arthritis development via activation of autoreactive T cells in the intestine. *Arthritis Rheum.* 68, 2646–2661.
- Mahdi, H., Fisher, B.A., Kallberg, H., Plant, D., Malmstrom, V., Ronnelid, J., et al., 2009. Specific interaction between genotype, smoking and autoimmunity to citrullinated alpha-enolase in the etiology of rheumatoid arthritis. *Nat. Genet.* 41, 1319–1324.
- Mallone, R., Scotto, M., Janicki, C.N., James, E.A., Fitzgerald-Miller, L., Wagner, R., et al., 2011. Immunology of diabetes society T-cell workshop: HLA class I tetramer-directed epitope validation initiative T-cell workshop report-HLA class I tetramer validation initiative. *Diabetes Metab. Res. Rev.* 27, 720–726.
- Mammen, A.L., Chung, T., Christopher-Stine, L., Rosen, P., Rosen, A., Doering, K.R., et al., 2011. Autoantibodies against 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase in patients with statin-associated autoimmune myopathy. *Arthritis Rheum.* 63, 713–721.
- Manderson, A.P., Botto, M., Walport, M.J., 2004. The role of complement in the development of systemic lupus erythematosus. *Annu. Rev. Immunol.* 22, 431–456.
- Mandlik-Nayak, L., Allen, P.M., 2005. Initiation of an autoimmune response: insights from a transgenic model of rheumatoid arthritis. *Immunol. Res.* 32, 5–13.
- Marino, E., Grey, S.T., 2012. B cells as effectors and regulators of autoimmunity. *Autoimmunity.* 45, 377–387.
- Marrie, R.A., Reider, N., Cohen, J., Stuve, O.U., Sorensen, P.S., Cutter, G., et al., 2015. A systematic review of the incidence and prevalence of autoimmune disease in multiple sclerosis. *Mult. Scler.* 21, 282–293.
- Marson, A., Housley, W.J., Hafler, D.A., 2015. Genetic basis of autoimmunity. *J. Clin. Invest.* 125, 2234–2241.
- Martinez Valle, F., Balada, E., Ordi-Ros, J., Vilardell-Tarres, M., 2008. DNase 1 and systemic lupus erythematosus. *Autoimmun. Rev.* 7, 359–363.
- Martini, S., Nair, V., Keller, B.J., Eichinger, F., Hawkins, J.J., Randolph, A., et al., 2014. Integrative biology identifies shared transcriptional networks in CKD. *J. Am. Soc. Nephrol.* 25, 2559–2572.
- Massilamany, C., Upadhyaya, B., Gangaplara, A., Kuszynski, C., Reddy, J., 2011. Detection of autoreactive CD4 T cells using major histocompatibility complex class II dextramers. *BMC Immunol.* 12, 40.
- Mauri, C., Menon, M., 2017. Human regulatory B cells in health and disease: therapeutic potential. *J. Clin. Invest.* 127, 772–779.
- McKinney, E.F., Smith, K.G., 2016. T-cell exhaustion: understanding the interface of chronic viral and autoinflammatory diseases. *Immunol. Cell Biol.* 94, 935–942.
- McKinstry, K.K., Strutt, T.M., Swain, S.L., 2010. The potential of CD4 T-cell memory. *Immunology* 130, 1–9.
- McKinney, E.F., Lee, J.C., Jayne, D.R., Lyons, P.A., Smith, K.G., 2015. T-cell exhaustion, co-stimulation and clinical outcome in autoimmunity and infection. *Nature* 523, 612–616.
- Meffre, E., Wardemann, H., 2008. B-cell tolerance checkpoints in health and autoimmunity. *Curr. Opin. Immunol.* 20, 632–638.
- Mejia-Leon, M.E., Barca, A.M., 2015. Diet, microbiota and immune system in type 1 diabetes development and evolution. *Nutrients* 7, 9171–9184.
- Meka, R.R., Venkatesha, S.H., Dudics, S., Acharya, B., Moudgil, K.D., 2015. IL-27-induced modulation of autoimmunity and its therapeutic potential. *Autoimmun. Rev.* 14, 1131–1141.
- Melki, I., Crow, Y.J., 2015. Novel monogenic diseases causing human autoimmunity. *Curr. Opin. Immunol.* 37, 1–5.
- Menard, L., Saadoun, D., Isnardi, I., Ng, Y.S., Meyers, G., Massad, C., et al., 2011. The PTPN22 allele encoding an R620W variant interferes with the removal of developing autoreactive B cells in humans. *J. Clin. Invest.* 121, 3635–3644.
- Merrill, J.T., Neuwelt, C.M., Wallace, D.J., Shanahan, J.C., Latinis, K.M., Oates, J.C., et al., 2010. Efficacy and safety of rituximab in moderately-to-severely active systemic lupus erythematosus: the randomized, double-blind, phase II/III systemic lupus erythematosus evaluation of rituximab trial. *Arthritis Rheum.* 62, 222–233.
- Michels, A.W., Eisenbarth, G.S., 2011. Immune intervention in type 1 diabetes. *Semin. Immunol.* 23, 214–219.
- Mills, K.H., 2011. TLR-dependent T cell activation in autoimmunity. *Nat. Rev. Immunol.* 11, 807–822.
- Mistry, P., Kaplan, M.J.E., 2016. Digestion of chromatin in apoptotic cell microparticles prevents autoimmunity. *Clin. Immunol.* 166, 88–101.
- Miyake, Y., Yamasaki, S., 2012. Sensing necrotic cells. *Adv. Exp. Med. Biol.* 738, 144–152.
- Mocellin, S., Marincola, F., Rossi, C.R., Nitti, D., Lise, M., 2004. The multifaceted relationship between IL-10 and adaptive immunity: putting together the pieces of a puzzle. *Cytokine Growth Factor Rev.* 15, 61–76.
- Monach, P.A., Benoist, C., Mathis, D., 2004. The role of antibodies in mouse models of rheumatoid arthritis, and relevance to human disease. *Adv. Immunol.* 82, 217–248.
- Monroe, J.G., Keir, M.E., 2008. Bridging Toll-like- and B cell-receptor signaling: meet me at the autophagosome. *Immunity* 28, 729–731.
- Monroe, J.G., Bannish, G., Fuentes-Panana, E.M., King, L.B., Sandel, P.C., Chung, J., et al., 2003. Positive and negative selection during B lymphocyte development. *Immunol. Res.* 27, 427–442.
- Morel, L., 2010. Genetics of SLE: evidence from mouse models. *Nat. Rev. Rheumatol.* 6, 348–357.
- Morel, L., 2017. Immunometabolism in systemic lupus erythematosus. *Nat. Rev. Rheumatol.* 13, 280–290.
- Morel, P.A., Turner, M.S., 2011. Dendritic cells and the maintenance of self-tolerance. *Immunol. Res.* 50, 124–129.
- Morel, L., Blenman, K.R., Croker, B.P., Wakeland, E.K., 2001. The major murine systemic lupus erythematosus susceptibility locus, Sle1, is a cluster of functionally related genes. *Proc. Natl. Acad. Sci. U.S.A.* 98, 1787–1792.
- Moriyama, H., Eisenbarth, G.S., 2002. Genetics and environmental factors in endocrine/organ-specific autoimmunity: have there been any major advances? *Springer Semin. Immunopathol.* 24, 231–242.
- Moulton, V.R., Tsokos, G.C., 2011. Abnormalities of T cell signaling in systemic lupus erythematosus. *Arthritis Res. Ther.* 13, 207.
- Muratore, F., Pipitone, N., Salvarani, C., 2017. Standard and biological treatment in large vessel vasculitis: guidelines and current approaches. *Expert Rev. Clin. Immunol.* 13, 345–360.
- Nadya, N.A., Tezuka, H., Ohteki, T., Matsuda, S., Azuma, M., Nagai, S., 2017. PI3K-Akt pathway enhances the differentiation of interleukin-27-induced type 1 regulatory T cells. *Immunology* 152, 507–516.
- Naidoo, J., Page, D.B., Li, B.T., Connell, L.C., Schindler, K., Lacouture, M.E., et al., 2015. Toxicities of the anti-PD-1 and anti-PD-L1 immune checkpoint antibodies. *Ann. Oncol.* 26, 2375–2391.

- Nakayamada, S., Takahashi, H., Kanno, Y., O'Shea, J.J., 2012. Helper T cell diversity and plasticity. *Curr. Opin. Immunol.* 24, 297–302.
- Namjou, B., Kothari, P.H., Kelly, J.A., Glenn, S.B., Ojwang, J.O., Adler, A., et al., 2011. Evaluation of the TREX1 gene in a large multi-ancestral lupus cohort. *Genes Immun.* 12, 270–279.
- Nemazee, D., 2017. Mechanisms of central tolerance for B cells. *Nat. Rev. Immunol.* 17, 281–294.
- Newman, J., Rice, J.S., Wang, C., Harris, S.L., Diamond, B., 2003. Identification of an antigen-specific B cell population. *J. Immunol. Methods* 272, 177–187.
- Nickerson, K.M., Christensen, S.R., Cullen, J.L., Meng, W., Luning Prak, E.T., Shlomchik, M.J., 2008. DNase 1 and systemic lupus erythematosus. *J. Immunol.* 7, 359–363.
- Niewold, T.B., 2011. Interferon alpha as a primary pathogenic factor in human lupus. *J. Interferon Cytokine Res.* 31, 887–892.
- Nobrega, A., Stransky, B., Nicolas, N., Coutinho, A., 2002. Regeneration of natural antibody repertoire after massive ablation of lymphoid system: robust selection mechanisms preserve antigen binding specificities. *J. Immunol.* 169, 2971–2978.
- O'Neill, L.A., Kishton, R.J., Rathmell, J., 2016. A guide to immunometabolism for immunologists. *Nat. Rev. Immunol.* 16, 553–565.
- O'Shea, J.J., Paul, W.E., 2010. Mechanisms underlying lineage commitment and plasticity of helper CD4 + T cells. *Science* 327, 1098–1102.
- Ochs, H.D., Gambineri, E., Torgerson, T.R., 2007. IPEX, FOXP3 and regulatory T-cells: a model for autoimmunity. *Immunol. Res.* 38, 112–121.
- Oo, Y.H., Mutimer, D., Adams, D.H., 2012. Low-dose interleukin-2 and HCV-induced vasculitis. *N. Engl. J. Med.* 366, 1353–1354, author reply 1354.
- Pagni, P.P., Traub, S., Demaria, O., Chasson, L., Alexopoulou, L., 2010. Contribution of TLR7 and TLR9 signaling to the susceptibility of MyD88-deficient mice to myocarditis. *Autoimmunity* 43, 275–287.
- Panduro, M., Benoist, C., Mathis, D., 2016. Tissue Tregs. *Annu. Rev. Immunol.* 34, 609–633.
- Patterson, C.C., Dahlquist, G.G., Gyurus, E., Green, A., Soltesz, G., 2009. Incidence trends for childhood type 1 diabetes in Europe during 1989–2003 and predicted new cases 2005–20: a multicentre prospective registration study. *Lancet* 373, 2027–2033.
- Paun, A., Yau, C., Danska, J.S., 2017. The influence of the microbiome on type 1 diabetes. *J. Immunol.* 198, 590–595.
- Pauza, M.E., Dobbs, C.M., He, J., Patterson, T., Wagner, S., Anobile, B.S., et al., 2004. T-cell receptor transgenic response to an endogenous polymorphic autoantigen determines susceptibility to diabetes. *Diabetes* 53, 978–988.
- Pawaria, S., Sharma, S., Baum, R., Nündel, K., Bustó, P., Gravallese, E.M., et al., 2017. Taking the STING out of TLR-driven autoimmune diseases: good, bad, or indifferent? *J. Leukoc. Biol.* 101, 121–126.
- Peng, Y., Elkorn, K.B., 2011. Autoimmunity in MFG-E8-deficient mice is associated with altered trafficking and enhanced cross-presentation of apoptotic cell antigens. *J. Clin. Invest.* 121, 2221–2241.
- Pereira, R.M., Hogan, P.G., Rao, A., Martinez, G.J., 2017. Transcriptional and epigenetic regulation of T cell hyporesponsiveness. *J. Leukoc. Biol.* 102, 601–615.
- Pesenacker, A.M., Cook, L., Levings, M.K., 2016. The role of FOXP3 in autoimmunity. *Curr. Opin. Immunol.* 43, 16–23.
- Pettigrew, H.D., Teuber, S.S., Gershwin, M.E., 2009. Clinical significance of complement deficiencies. *Ann. N.Y. Acad. Sci.* 1173, 108–123.
- Peutz-Kootstra, C.J., de Heer, E., Hoedemaeker, P.J., Abrass, C.K., Bruijn, J.A., 2001. Lupus nephritis: lessons from experimental animal models. *J. Lab. Clin. Med.* 137, 244–260.
- Poon, I.K., Lucas, C.D., Rossi, A.G., Ravichandran, K.S., 2014. Apoptotic cell clearance: basic biology and therapeutic potential. *Nat. Rev. Immunol.* 14, 166–180.
- Pot, C., Apetoh, L., Kuchroo, V.K., 2011. Type 1 regulatory T cells (Tr1) in autoimmunity. *Semin. Immunol.* 23, 202–208.
- Price, E.J., Venables, P.J., 1995. Drug-induced lupus. *Drug Saf.* 12, 283–290.
- Qin, J., Konno, H., Ohshima, D., Yanai, H., Motegi, H., Shimo, Y., et al., 2007. Developmental stage-dependent collaboration between the TNF receptor-associated factor 6 and lymphotoxin pathways for B cell follicle organization in secondary lymphoid organs. *J. Immunol.* 179, 6799–6807.
- Qiu, H., Wu, H., Chan, V., Lau, C.S., Lu, Q., 2017. Transcriptional and epigenetic regulation of follicular T-helper cells and their role in autoimmunity. *Autoimmunity* 50, 71–81.
- Rahman, P., Bartlett, S., Siannis, F., Pellett, F.J., Farewell, V.T., Peddle, L., et al., 2003. CARD15: a pleiotropic autoimmune gene that confers susceptibility to psoriatic arthritis. *Am. J. Hum. Genet.* 73, 677–681.
- Rajalingam, R., 2011. Human diversity of killer cell immunoglobulin-like receptors and disease. *Korean J. Hematol.* 46, 216–228.
- Rajewsky, K., 1996. Clonal selection and learning in the antibody system. *Nature* 381, 751–758.
- Raychaudhuri, S., Sandor, C., Stahl, E.A., Freudenberg, J., Lee, H.S., Jia, X., et al., 2012. Five amino acids in three HLA proteins explain most of the association between MHC and seropositive rheumatoid arthritis. *Nat. Genet.* 44, 291–296.
- Reddy, J., Bettelli, E., Nicholson, L., Waldner, H., Jang, M.H., Wucherpfennig, K.W., et al., 2003. Detection of autoreactive myelin proteolipid protein 139–151-specific T cells by using MHC II (IAs) tetramers. *J. Immunol.* 170, 870–877.
- Rice, G., Newman, W.G., Dean, J., Patrick, T., Parmar, R., Flintoff, K., et al., 2008. Trex1 prevents cell-intrinsic initiation of autoimmunity. *Am. J. Hum. Genet.* 134, 587–598.
- Rieck, M., Arechiga, A., Onengut-Gumuscu, S., Greenbaum, C., Concannon, P., Buckner, J.H., 2007. Genetic variation in PTPN22 corresponds to altered function of T and B lymphocytes. *J. Immunol.* 179, 4704–4710.
- Rivas, M.N., Koh, Y.T., Chen, A., Nguyen, A., Lee, Y.H., Lawson, G., et al., 2012. MyD88 is critically involved in immune tolerance breakdown at environmental interfaces of Foxp3-deficient mice. *J. Clin. Invest.* 122, 1933–1947.
- Robinson, W.H., 2015. Sequencing the functional antibody repertoire—diagnostic and therapeutic discovery. *Nat. Rev. Rheumatol.* 11, 171–182.
- Romano-Keeler, J., Weitkamp, J.H., Moore, D.J., 2012. Regulatory properties of the intestinal microbiome effecting the development and treatment of diabetes. *Curr. Opin. Endocrinol. Diabetes Obes.* 19, 73–80.
- Rosas-Ballina, M., Tracey, K.J., 2009. The neurology of the immune system: neural reflexes regulate immunity. *Neuron* 64, 28–32.
- Rose, N.R., Bona, C., 1993. Defining criteria for autoimmune diseases (Witebsky's postulates revisited). *Immunol. Today* 14, 426–430.
- Rosenblum, M.D., Gratz, I.K., Paw, J.S., Abbas, A.K., 2012. Treating human autoimmunity: current practice and future prospects. *Sci. Transl. Med.* 4, 125sr121.

- Rosloniec, E.F., Latham, K., Guedez, Y.B., 2002. Paradoxical roles of IFN-gamma in models of Th1-mediated autoimmunity. *Arthritis Res.* 4, 333–336.
- Rosser, E.C., Mauri, C., 2016. A clinical update on the significance of the gut microbiota in systemic autoimmunity. *J. Autoimmun.* 74, 85–93.
- Round, J.L., Mazmanian, S.K., 2010. Inducible Foxp3<sup>+</sup> regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc. Natl. Acad. Sci. U.S.A.* 107, 12204–12209.
- Routsias, J.G., Goules, J.D., Goules, A., Charalampakis, G., Pikazis, D., 2011. Autopathogenic correlation of periodontitis and rheumatoid arthritis. *Rheumatology (Oxford)* 50, 1189–1193.
- Rubtsova, K., Marrack, P., Rubtsov, A.V., 2015. Sexual dimorphism in autoimmunity. *J. Clin. Invest.* 125, 2187–2193.
- Russell, R.K., Nimmo, E.R., Satsangi, J., 2004. Molecular genetics of Crohn's disease. *Curr. Opin. Genet. Dev.* 14, 264–270.
- Sakaguchi, S., 2007. Heterozygous mutations in TREX1 cause familial chilblain lupus and dominant Aicardi-Goutieres syndrome. *Nat. Immunol.* 80, 811–815.
- Sathiyamoorthy, G., Sehgal, S., Ashton, R.W., 2017. Pirfenidone and nintedanib for treatment of idiopathic pulmonary fibrosis. *South. Med. J.* 110, 393–398.
- Sawla, P., Hossain, A., Hahn, B.H., Singh, R.P., 2012. Regulatory T cells in systemic lupus erythematosus (SLE); role of peptide tolerance. *Autoimmun. Rev.* 11, 611–614.
- Schickel, J.N., Kuhny, M., Baldo, A., Bannock, J.M., Massad, C., Wang, H., et al., 2016. PTPN22 inhibition resets defective human central B cell tolerance. *Sci. Immunol.* 1, aaf7153.
- Schiffer, L., Sinha, J., Wang, X., Huang, W., von Gersdorff, G., Schiffer, M., et al., 2003. Short term administration of costimulatory blockade and cyclophosphamide induces remission of systemic lupus erythematosus nephritis in NZB/W F1 mice by a mechanism downstream of renal immune complex deposition. *J. Immunol.* 171, 489–497.
- Schildberg, F.A., Klein, S.R., Freeman, G.J., Sharpe, A.H., 2016. Coinhibitory pathways in the B7-CD28 ligand-receptor family. *Immunity* 44, 955–972.
- Schmitt, S.K., 2017. Reactive arthritis. *Infect. Dis. Clin. North Am.* 31, 265–277.
- Schoels, M.M., van der Heijde, D., Breedveld, F.C., Burmester, G.R., Dougados, M., Emery, P., et al., 2013. Blocking the effects of interleukin-6 in rheumatoid arthritis and other inflammatory rheumatic diseases: systematic literature review and meta-analysis informing a consensus statement. *Ann. Rheum. Dis.* 72, 583–589.
- Seery, J.P., Carroll, J.M., Cattell, V., Watt, F.M., 1997. Antinuclear autoantibodies and lupus nephritis in transgenic mice expressing interferon gamma in the epidermis. *J. Exp. Med.* 186, 1451–1459.
- Seo, S.J., Mandik-Nayak, L., Erikson, J., 2003. B cell anergy and systemic lupus erythematosus. *Curr. Dir. Autoimmun.* 6, 1–20.
- Sercarz, E.E., Lehmann, P.V., Ametani, A., Benichou, G., Miller, A., Moudgil, K., 1993. Dominance and crypticity of T cell antigenic determinants. *Annu. Rev. Immunol.* 11, 729–766.
- Serhan, C.N., 2017. Treating inflammation and infection in the 21st century: new hints from decoding resolution mediators and mechanisms. *FASEB J.* 31, 1273–1288.
- Shao, W.H., Cohen, P.L., 2014. The role of tyrosine kinases in systemic lupus erythematosus and their potential as therapeutic targets. *Expert Rev. Clin. Immunol.* 10, 573–582.
- Sharma, S., Campbell, A.M., Chan, J., Schattgen, S.A., Orlowski, G.M., Nayar, R., et al., 2015. Suppression of systemic autoimmunity by the innate immune adaptor STING. *Proc. Natl. Acad. Sci. U.S.A.* 112, E710–E717.
- Shikhagaie, M.M., Germar, K., Bal, S.M., Ros, X.R., Spits, H., 2017. Innate lymphoid cells in autoimmunity: emerging regulators in rheumatic diseases. *Nat. Rev. Rheumatol.* 13, 164–173.
- Shlomchik, M.J., 2008. Sites and stages of autoreactive B cell activation and regulation. *Immunity* 28, 18–28.
- Shlomchik, M.J., Craft, J.E., Mamula, M.J., 2001. From T to B and back again: positive feedback in systemic autoimmune disease. *Nat. Rev. Immunol.* 1, 147–153.
- Sica, A., Melillo, G., Varesio, L., 2011. Hypoxia: a double-edged sword of immunity. *J. Mol. Med.* 89, 657–665.
- Sinmaz, N., Nguyen, T., Tea, F., Dale, R.C., Brilot, F., 2016. Mapping autoantigen epitopes: molecular insights into autoantibody-associated disorders of the nervous system. *J. Neuroinflammation* 13, 219.
- Sisirak, V., Sally, B., D'Agati, V.U., Martinez-Ortiz, W., Ozcakar, Z.B., David, J., et al., 2015. Activation of cyclic GMP-AMP synthase by self-DNA causes autoimmune diseases. *Cell* 112, E5699–E5705.
- Skyler, J.S., 2013. The compelling case for anti-CD3 in type 1 diabetes. *Diabetes* 62, 3656–3657.
- Smith-Bouvier, D.L., Divekar, A.A., Sasidhar, M., Du, S., Tiwari-Woodruff, S.K., King, J.K., et al., 2008. A role for sex chromosome complement in the female bias in autoimmune disease. *J. Exp. Med.* 205, 1099–1108.
- Sokolove, J., Zhao, X., Chandra, P.E., Robinson, W.H., 2011. Immune complexes containing citrullinated fibrinogen costimulate macrophages via Toll-like receptor 4 and Fcγ receptor. *Arthritis Rheum.* 63, 53–62.
- Sollid, L.M., 2017. The roles of MHC class II genes and post-translational modification in celiac disease. *Immunogenetics* 69, 605–616.
- Song, J., 2016. Development of auto antigen-specific regulatory T cells for diabetes immunotherapy. *Immune Netw.* 16, 281–285.
- Stamatiades, E.G., Tremblay, M.E., Bohm, M., Crozet, L., Bisht, K., Kao, D., et al., 2016. Immune monitoring of Trans-endothelial transport by kidney-resident macrophages. *Cell* 166, 991–1003.
- Stange, E.F., Wehkamp, J., 2016. Recent advances in understanding and managing Crohn's disease. *F1000 Res.* 5, 2896.
- Stark, A.K., Sriskantharajah, S., Hessel, E.M., Okkenhaug, K., 2015. PI3K inhibitors in inflammation, autoimmunity and cancer. *Curr. Opin. Pharmacol.* 23, 82–91.
- Steen, V.D., 1999. Occupational scleroderma. *Curr. Opin. Rheumatol.* 11, 490–494.
- Steinman, R.M., 2007. Dendritic cells: understanding immunogenicity. *Eur. J. Immunol.* 37 (Suppl 1), S53–S60.
- Stetson, D.B., Ko, J.S., Heidmann, T., Medzhitov, R., 2017. Immune diseases associated with TREX1 and STING dysfunction. *Cell* 37, 198–206.
- Strassburg, C.P., Vogel, A., Manns, M.P., 2003. Autoimmunity and hepatitis C. *Autoimmun. Rev.* 2, 322–331.
- Stritesky, G.L., Jameson, S.C., Hogquist, K.A., 2012. Selection of self-reactive T cells in the thymus. *Annu. Rev. Immunol.* 30, 95–114.

- Suber, T.L., Casciola-Rosen, L., Rosen, A., 2008. Mechanisms of disease: autoantigens as clues to the pathogenesis of myositis. *Nat. Clin. Pract. Rheumatol.* 4, 201–209.
- Suzuki, A., Yamada, R., Chang, X., Tokuhiro, S., Sawada, T., Suzuki, M., et al., 2003. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. *Nat. Genet.* 34, 395–402.
- Székánecz, Z., Besenyei, T., Paragh, G., Koch, A.E., 2009. Angiogenesis in rheumatoid arthritis. *Autoimmunity* 42, 563–573.
- Takenaka, M.C., Quintana, F.J., 2017. Tolerogenic dendritic cells. *Semin. Immunopathol.* 39, 113–120.
- Telorack, M., Meyer, M., Ingold, I.G., Conrad, M.G., Bloch, W., Werner, S., 2012. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *PLoS Genet.* 18, 1028–1040.
- Thornburg, N.J., Zhang, H., Bangaru, S., Sapparapu, G., Kose, N., Lampley, R.M., et al., 2016. H7N9 influenza virus neutralizing antibodies that possess few somatic mutations. *J. Clin. Invest.* 126, 1482–1494.
- Tipton, C.M., Fucile, C.F., Darce, J., Chida, A., Ichikawa, T., Gregoretti, I., et al., 2015. Diversity, cellular origin and autoreactivity of antibody-secreting cell population expansions in acute systemic lupus erythematosus. *Nat. Immunol.* 16, 755–765.
- Tomlinson, I.P., Bodmer, W.F., 1995. The HLA system and the analysis of multifactorial genetic disease. *Trends Genet.* 11, 493–498.
- Tong, Y., Li, Z., Zhang, H., Xia, L., Zhang, M., Xu, Y., et al., 2016. T cell repertoire diversity is decreased in type 1 diabetes patients. *Genomics Proteomics Bioinformatics* 14, 338–348.
- Tost, J., Gay, S., Firestein, G., 2017. Epigenetics of the immune system and alterations in inflammation and autoimmunity. *Epigenomics* 9, 371–373.
- Tsubata, T., 2017. B-cell tolerance and autoimmunity. *F1000 Res.* 6, 391.
- Ueda, H., Howson, J.M., Esposito, L., Heward, J., Snook, H., Chamberlain, G., et al., 2003. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* 423, 506–511.
- Vaarala, O., 2012. Is the origin of type 1 diabetes in the gut? *Immunol. Cell Biol.* 90, 271–276.
- Vallejo, A.N., Davila, E., Weyand, C.M., Goronzy, J.J., 2004. Biology of T lymphocytes. *Rheum. Dis. Clin. North Am.* 30, 135–157.
- Valluru, M., Staton, C.A., Reed, M.W., Brown, N.J., 2011. Transforming growth factor-beta and endoglin signaling orchestrate wound healing. *Front. Physiol.* 2, 89.
- Vanderlugt, C.L., Begolka, W.S., Neville, K.L., Katz-Levy, Y., Howard, L.M., Eagar, T.N., et al., 1998. The functional significance of epitope spreading and its regulation by co-stimulatory molecules. *Immunol. Rev.* 164, 63–72.
- Vezys, V., Lefrancois, L., 2002. Cutting edge: inflammatory signals drive organ-specific autoimmunity to normally cross-tolerizing endogenous antigen. *J. Immunol.* 169, 6677–6680.
- Victora, G.D., Nussenzweig, M.C., 2012. Germinal centers. *Annu. Rev. Immunol.* 30, 429–457.
- Villanueva, E., Yalavarthi, S., Berthier, C.C., Hodgin, J.B., Khandpur, R., Lin, A.M., et al., 2011. Netting neutrophils induce endothelial damage, infiltrate tissues, and expose immunostimulatory molecules in systemic lupus erythematosus. *J. Immunol.* 187, 538–552.
- Vincenti, F., Luggen, M., 2007. T cell costimulation: a rational target in the therapeutic armamentarium for autoimmune diseases and transplantation. *Ann. Rev. Med.* 58, 347–358.
- Vinuesa, C.G., Cook, M.C., Angelucci, C., Athanasopoulos, V., Rui, L., Hill, K.M., et al., 2005. A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. *Nature* 435, 452–458.
- Visperas, A., Vignali, D.A., 2016. Are regulatory T cells defective in type 1 diabetes and can we fix them? *J. Immunol.* 197, 3762–3770.
- von Boehmer, H., Melchers, F., 2010. Checkpoints in lymphocyte development and autoimmune disease. *Nat. Immunol.* 11, 14–20.
- Vuddamalay, Y., van Meerwijk, J.P., 2017. CD28 – and CD28 low CD8 + regulatory T cells: of mice and men. *Front. Immunol.* 8, 31.
- Wakeland, E.K., Liu, K., Graham, R.R., Behrens, T.W., 2001. Delineating the genetic basis of systemic lupus erythematosus. *Immunity* 15, 397–408.
- Wang, Y., Wang, J., Sun, Y., Wu, Q., Fu, Y.X., 2001. Complementary effects of TNF and lymphotoxin on the formation of germinal center and follicular dendritic cells. *J. Immunol.* 166, 330–337.
- Wang, L., Wang, F.S., Gershwin, M.E., 2015. Human autoimmune diseases: a comprehensive update. *J. Intern. Med.* 278, 369–395.
- Wang, J., Syrett, C.M., Kramer, M.C., Basu, A., Atchison, M.L., Anguera, M.C., 2016. Unusual maintenance of X chromosome inactivation predisposes female lymphocytes for increased expression from the inactive X. *Proc. Natl. Acad. Sci. U.S.A.* 113, E2029–E2038.
- Watkin, L.B., Mishra, R., Gil, A., Aslan, N., Ghersi, D., Luzuriaga, K., et al., 2017. Unique influenza A cross-reactive memory CD8 T-cell receptor repertoire has a potential to protect against EBV seroconversion. *J. Allergy Clin. Immunol.* 140, 1206–1210.
- Weinstein, J.S., Hernandez, S.G., Craft, J., 2012. T cells that promote B-cell maturation in systemic autoimmunity. *Immunol. Rev.* 247, 160–171.
- Weller, S., Bonnet, M., Delagreverie, H., Israel, L., Chrabieh, M., Marodi, L., et al., 2012. IgM + IgD + CD27 + B cells are markedly reduced in IRAK-4-, MyD88-, and TIRAP- but not UNC-93B-deficient patients. *Blood* 120, 4992–5001.
- Weng, N.P., Araki, Y., Subedi, K., 2012. The molecular basis of the memory T cell response: differential gene expression and its epigenetic regulation. *Nat. Rev. Immunol.* 12, 306–315.
- Wenink, M.H., Leijten, E.F.A., Cupedo, T., Radstake, T., 2017. Review: innate lymphoid cells: sparking inflammatory rheumatic disease? *Arthritis Rheum.* 69, 885–897.
- Werlen, G., Hausmann, B., Naehler, D., Palmer, E., 2003. Signaling life and death in the thymus: timing is everything. *Science* 299, 1859–1863.
- Whitmire, J.K., Asano, M.S., Kaech, S.M., Sarkar, S., Hannum, L.G., Shlomchik, M.J., et al., 2009. Requirement of B cells for generating CD4 + T cell memory. *J. Immunol.* 182, 1868–1876.
- Willis, V.C., Banda, N.K., Cordova, K.N., Chandra, P.E., Robinson, W.H., Cooper, D.C., et al., 2017. Protein arginine deiminase 4 inhibition is sufficient for the amelioration of collagen-induced arthritis. *Clin. Exp. Immunol.* 188, 263–274.
- Winchester, R., 2004. The genetics of autoimmune-mediated rheumatic diseases: clinical and biologic implications. *Rheum. Dis. Clin. North Am.* 30, 213–227.
- Wing, K., Sakaguchi, S., 2010. Regulatory T cells exert checks and balances on self tolerance and autoimmunity. *Nat. Immunol.* 11, 7–13.
- Wojno, E.D., Hunter, C.A., 2012. New directions in the basic and translational biology of interleukin-27. *Trends Immunol.* 33, 91–97.
- Wolters, V.M., Wijmenga, C., 2008. Genetic background of celiac disease and its clinical implications. *Am. J. Gastroenterol.* 103, 190–195.
- Wong, F.S., Wen, L., 2003. The study of HLA class II and autoimmune diabetes. *Curr. Mol. Med.* 3, 1–15.

- Wooley, P.H., 2004. The usefulness and the limitations of animal models in identifying targets for therapy in arthritis. *Best Pract. Res. Clin. Rheumatol.* 18, 47–58.
- Wynn, T.A., Ramalingam, T.R., 2012. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *Nat. Med.* 18, 1028–1040.
- Yan, N., 2006. Association of a non-synonymous single-nucleotide polymorphism of DNASEI with SLE susceptibility. *J. Interferon Cytokine Res.* 45, 819–823.
- Yan, J., Harvey, B.P., Gee, R.J., Shlomchik, M.J., Mamula, M.J., 2006. B cells drive early T cell autoimmunity in vivo prior to dendritic cell-mediated autoantigen presentation. *J. Immunol.* 177, 4481–4487.
- Yeste, A., Takenaka, M.C., Mascanfroni, I.D., Nadeau, M., Kenison, J.E., Patel, B., et al., 2016. Tolerogenic nanoparticles inhibit T cell-mediated autoimmunity through SOCS2. *Sci. Signal.* 9, ra61.
- Yin, Y., Choi, S.C., Xu, Z., Perry, D.J., Seay, H., Croker, B.P., et al., 2015. Normalization of CD4+ T cell metabolism reverses lupus. *Sci. Transl. Med.* 7, 274ra218.
- Yu-Wai-Man, C., Spencer-Dene, B., Lee, R.M., Hutchings, K., Lisabeth, E.M., Treisman, R., et al., 2017. Local delivery of novel MRTF/SRF inhibitors prevents scar tissue formation in a preclinical model of fibrosis. *Sci. Rep.* 7, 518.
- Zaki, M.H., Lamkanfi, M., Kanneganti, T.D., 2011. The Nlrp3 inflammasome: contributions to intestinal homeostasis. *Trends Immunol.* 32, 171–179.
- Zhang, Q., Raoof, M., Chen, Y., Sumi, Y., Sursal, T., Junger, W., et al., 2010. Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature* 464, 104–107.
- Zhang, J., Zahir, N., Jiang, Q., Miliotis, H., Heyraud, S., Meng, X., et al., 2011. The autoimmune disease-associated PTPN22 variant promotes calpain-mediated Lyp/Pep degradation associated with lymphocyte and dendritic cell hyperresponsiveness. *Nat. Genet.* 43, 902–907.
- Zhang, X., Zhang, D., Jia, H., Feng, Q., Wang, D., Liang, D., et al., 2015. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. *Nat. Med.* 21, 895–905.
- Zheng, G.X., Terry, J.M., Belgrader, P., Ryvkin, P., Bent, Z.W., Wilson, R., et al., 2017. Massively parallel digital transcriptional profiling of single cells. *Nat. Commun.* 8, 14049.

# Innate and Adaptive Systems of Immunity

Peter J. Delves

Department of Immunology, Division of Infection and Immunity, University College London, London, United Kingdom

## OUTLINE

The Innate and Adaptive Responses	45	Functional Activities of T Cells	53
<b>Innate Responses</b>	<b>47</b>	<i>B-Cell Development and Functions</i>	55
Cellular Components	47	Antibodies	56
Soluble Mediators	50	Secondary Lymphoid Tissues	57
<b>Adaptive Immune Responses</b>	<b>51</b>	<b>Resolution of the Immune Response</b>	<b>59</b>
T-Cell Development	51	References	59

The cells of the immune system are derived from self-renewing hematopoietic stem cells (HSCs) which give rise to multipotent progenitors (MPPs) that are no longer able to self-renew (Dharampuriya et al., 2017). Cytokines and other signals lead to the expression of different patterns of transcription factors (Sarrazin and Sieweke, 2011) which drive the MPPs down particular differentiation pathways, ultimately leading to the generation of lymphocytes, natural killer (NK) cells, dendritic cells (DCs), neutrophils, eosinophils, basophils, mast cells, monocytes, macrophages, megakaryocytes, and erythrocytes.

## THE INNATE AND ADAPTIVE RESPONSES

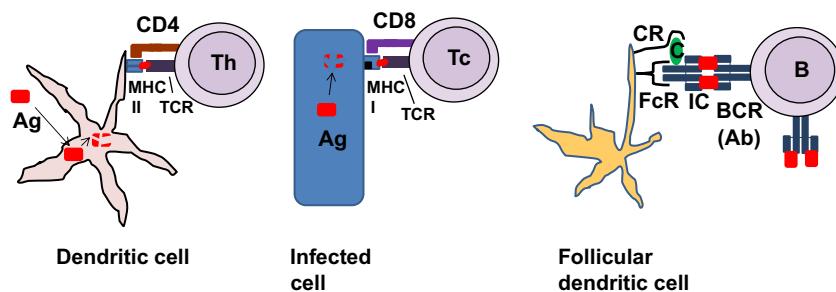
The immune system has traditionally been divided into; innate responses that occur to the same extent however many times the antigen is encountered, and adaptive (acquired) responses that generate immunologic memory leading to quantitatively and qualitatively enhanced responses upon reencounter with the antigen. While this division generally still holds true, it is clear that at least some “innate” cells, for example, NK cells and macrophages, can exhibit a form of immunologic memory referred to as trained or learned immunity (Freud et al., 2017; Kaufmann et al., 2018). Furthermore, there are various subpopulations of innate lymphoid cells, some of which sit at the interface between innate and adaptive responses (Ebbo et al., 2017). Immune responses need to detect a threat using receptors that recognize molecules associated with pathogens (Table 4.1). Innate responses exhibit broad specificity based upon detection of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) by pattern recognition receptors (PRRs) (Childs and Goodbourn 2017; Broz and Monack, 2013), and the binding to complement receptors and Fc receptors of opsonized antigens coated with complement or antibody. Only the lymphocytes, the dedicated cells of the adaptive response, bear antigen-specific receptors which permit exquisitely refined recognition of individual antigens. Each lymphocyte possesses approximately  $10^5$  antigen receptors of identical specificity. While the B-cell receptor (BCR) recognizes

structures (epitopes) on the surface of native antigen, the epitopes recognized by most T-cell receptors (TCR) are short peptides (Fig. 4.1). The peptides are produced by proteolytic processing of antigen within cells and are presented to T cells by highly polymorphic major histocompatibility complex (MHC) class I and class II cell surface molecules. The main human class I molecules are HLA-A, B, and C and the class II comprises HLA-DP, DQ, and DR. Thus an individual who is heterozygous at each locus will express 12 variants, although cross-pairing of some class II chains can increase this number. MHC class II, which typically presents 15–20 amino acid long peptides to the TCR on CD4<sup>+</sup> helper and regulatory T cells (Tregs), is expressed on DCs, macrophages, B cells, activated human (but not mouse) T cells, and thymic epithelial cells and can be induced on a variety of other cell types. In contrast, MHC class I molecules, which present peptides of 8–10 amino acids in length, are ubiquitously expressed on nearly all nucleated cells in the body and are concerned with alerting CD8<sup>+</sup> cytotoxic T lymphocytes (CTL) to the presence of intracellular infection. Although the peptides presented by MHC class II are longer than those presented by MHC class I, in both cases the TCR recognizes a stretch of about 9 amino acids.

**TABLE 4.1** The Cells of the Immune System Need to Detect a Threat and Do So Using Four Main Groups of Receptors

Receptor	PRR	MHC	TCR	Antibody (BCR)
Location	Cell surface, cytoplasmic, secreted	Cell surface	Cell surface	Cell surface, secreted
Recognition	PAMPs and DAMPs	Each MHC variant can bind many different peptide sequences	Highly antigen specific	Highly antigen specific
Protein diversity in an individual	~100	~12 for classical MHC	Millions	Millions
Genes	Each protein individually encoded. Low polymorphism	Each protein individually encoded. Extremely polymorphic	Genetic recombination creates diversity	Genetic recombination creates diversity

PRR are employed by cells of the innate response (although they are also present on lymphocytes) and collectively are found on cell surfaces, intracellularly or as secreted molecules. They recognize PAMPs, and DAMPs produced by the infected individual in response to infection and/or tissue damage. MHC class I is present on all nucleated cells in the body although MHC class II is highly restricted in its expression. The TCR is present only on T lymphocytes. The transmembrane version of antibody, the BCR, is only present on the surface of B lymphocytes, but antibody is also present throughout the body both as a soluble molecule and held on the surface of cells by Fc receptors. MHC, Major histocompatibility complex; TCR, T-cell receptor; BCR, B-cell receptor; PRR, pattern recognition receptors; PAMPs, pathogen-associated molecular patterns; DAMPs, danger-associated molecular patterns.



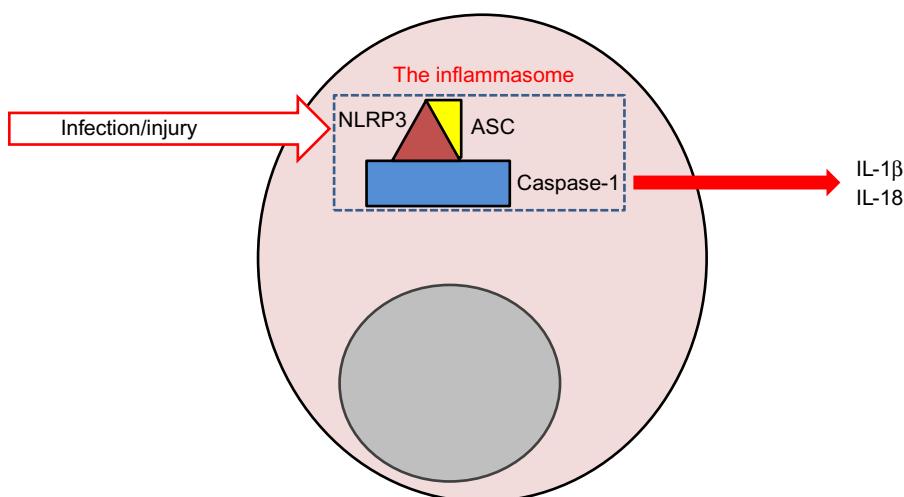
**FIGURE 4.1** Antigen recognition by lymphocytes. Dendritic cells take up external (exogenous) antigen (Ag), process it, and then in the case of protein antigens present it on their cell surface in the form of a peptide fragment bound to MHC class II molecules. This peptide–MHC complex is recognized by the TCR on CD4<sup>+</sup> Th. Antigens lurking inside cells (endogenous antigens, e.g., viruses) can be processed within the cell and then presented on the surface of the infected cell in the form of a peptide fragment bound to MHC class I molecules. This peptide–MHC complex is recognized by the TCR on CD8<sup>+</sup> Tc. Note that some TCR recognize nonprotein antigens such as glycolipids but these are usually presented by the “MHC-like” molecule CD1 rather than by MHC molecules. Cross-presentation can also occur whereby exogenous antigens are presented by MHC class I and endogenous antigens by MHC class II. The BCR is a transmembrane version of the Ab molecule. This directly detects intact antigen, a process which can be assisted by follicular dendritic cells (FDCs) holding immune complexes (IC) of Ab–Ag and complement (C) formed when soluble antibody binds antigen. These complexes become bound to the cell surface Fc receptors (FcR) and complement receptors (CRs) on the FDCs. Note that when the intact antigen is presented by an FDC, the BCR will need to recognize a different epitope on the antigen to that recognized by the antibody in the immune complex.

## INNATE RESPONSES

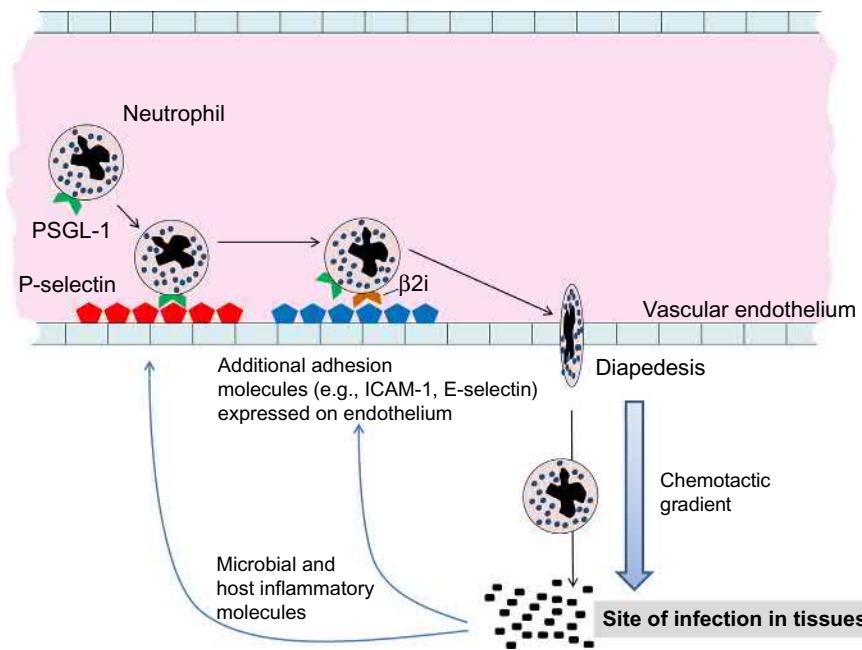
### Cellular Components

Cells of the innate immune response include neutrophils, eosinophils, monocytes, macrophages, and DCs, all of which to varying degrees can act as phagocytic cells, together with the nonphagocytic basophils, mast cells, and NK cells. All of these cell types are capable of producing inflammatory mediators. In myeloid cells, such as macrophages, the detection of infection or tissue injury can result in activation of intracellular molecular complexes referred to as inflammasomes (Fig. 4.2), leading to the secretion of proinflammatory mediators such as the cytokines interleukin (IL)-1 $\beta$  and IL-18 (Malik and Kanneganti, 2017). The immediate consequence of an encounter with an entity that is deemed to pose a threat is the generation of an acute inflammatory response in which cells and molecules of the immune system are rapidly recruited to the site of the stimulus. Inflammatory mediators and microbial products cause the upregulation of adhesion molecules on vascular endothelium, thereby alerting inflammatory cells to the presence of a local infection (Fig. 4.3). Histamine released from mast cells causes smooth muscle contraction and an increase in local vascular permeability, facilitating the passage of neutrophils from the blood to the tissues. Activation of the complement system (Chapter 14: The Roles and Contributions of the Complement System in the Pathophysiology of Autoimmune Diseases) plays a pivotal role in this process, triggering mast cell degranulation and chemotactically attracting the neutrophils (Chapter 13: Granulocytes: Neutrophils, Basophils, Eosinophils). Chemokines (chemotactic cytokines) help guide the neutrophils for antibodies and complement receptors greatly facilitates phagocytosis if the antigen is opsonized with these agents (Freeley et al., 2016; Jacobino et al., 2013). Engulfed microorganisms are killed within the neutrophil by a plethora of toxic molecules including superoxide anions, hydroxyl radicals, hypochlorous acid, nitric oxide, proteases, defensins, and lysozyme. Neutrophil extracellular traps prevent microbial spreading and focus released microbicidal substances onto any nonphagocytosed pathogens in the immediate vicinity of the neutrophil (Lawrence et al., 2018).

Although eosinophils (Chapter 13: Granulocytes: Neutrophils, Basophils, Eosinophils) are able to phagocytose microorganisms, their role in protection against infection is rather more specialized toward the release of granules containing cationic proteins in order to destroy extracellular parasites such as helminths. Eosinophils are also involved in immune regulation, secrete leukotriene C<sub>4</sub>, platelet-activating factor, and an array of cytokines (Robida et al., 2018; Kita, 2011). Blood basophils and tissue mast cells are not phagocytic and share many features. They become sensitized with IgE antibodies bound to their high-affinity Fc $\epsilon$  receptors (Fc $\epsilon$ RI) and,



**FIGURE 4.2** The inflammasome. Inflammasomes comprise multiprotein cytoplasmic complexes that promote inflammation by converting the IL-1 $\beta$  precursor into active IL-1 $\beta$ , and additionally by stimulating the generation of IL-18. The proinflammatory caspase-1, complexed with a receptor such as NLRP3 and with the apoptosis-associated speck-like (ASC) adaptor protein, becomes activated in response to danger signals resulting from infection or tissue damage. There are a variety of inflammasomes which share general characteristics but differ in the receptor they utilize, for example, using other NLR family members, or AIM2 or pyrin. Under certain conditions, inflammasome activation can trigger cell death by pyroptosis or necrosis.



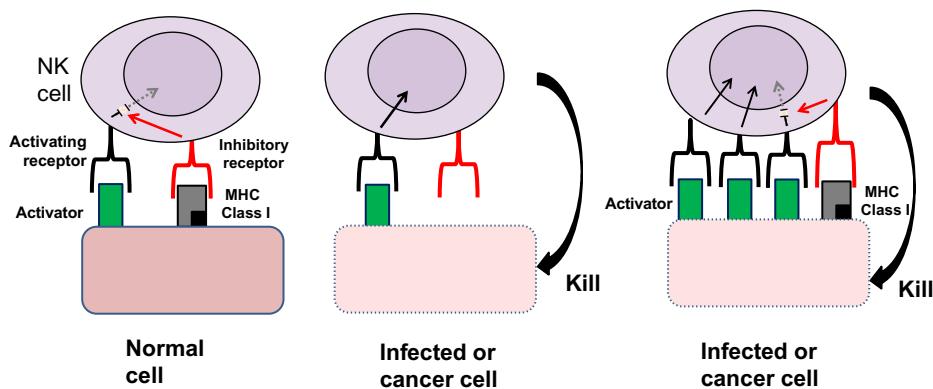
**FIGURE 4.3** Entry of neutrophils into tissue during an inflammatory response. Microbial products, such as LPS, together with inflammatory mediators, such as histamine, thrombin, and the cytokines IL-1 $\beta$ , IL-17, and TNF- $\alpha$ , lead to the increased expression of vascular endothelial adhesion molecules. This alerts neutrophils and other inflammatory cells to the presence of an infection in the underlying tissues. P-selectin becomes expressed on the surface of the endothelium and captures neutrophils due to their possession of P-selectin glycoprotein ligand-1 (PSGL-1). Initially, the neutrophils are slowed down and roll along the blood vessel wall. Signaling through PSGL-1 causes the neutrophils to activate  $\beta_2$  integrins ( $\beta_2i$ ). Additional vascular endothelial adhesion molecules become expressed such as ICAM-1, which binds to the  $\beta_2$  integrins LFA-1 and CR3, and E-selectin which binds to ESL-1, PSGL-1, and CD44 on the neutrophils (Zarbock et al., 2011; Muller, 2011). Eventually, the neutrophils are brought to a halt and squeeze out of the blood vessels, a process known as diapedesis which is greatly facilitated by the deformable nature of the multilobed nucleus in these polymorphonuclear leukocytes.

when antigen cross-links the IgE, release preformed inflammatory mediators including histamine, platelet-activating factor, and many different cytokines. Newly synthesized leukotrienes, prostaglandins, and thromboxanes are also released. Mast cells in humans can predominantly contain tryptase (MC<sub>T</sub>), chymase (MC<sub>C</sub>), or both of these enzymes (MC<sub>TC</sub>), although their phenotype and function are perhaps more strongly linked to their tissue distribution than to the differential expression of these enzymes (Robida et al., 2018).

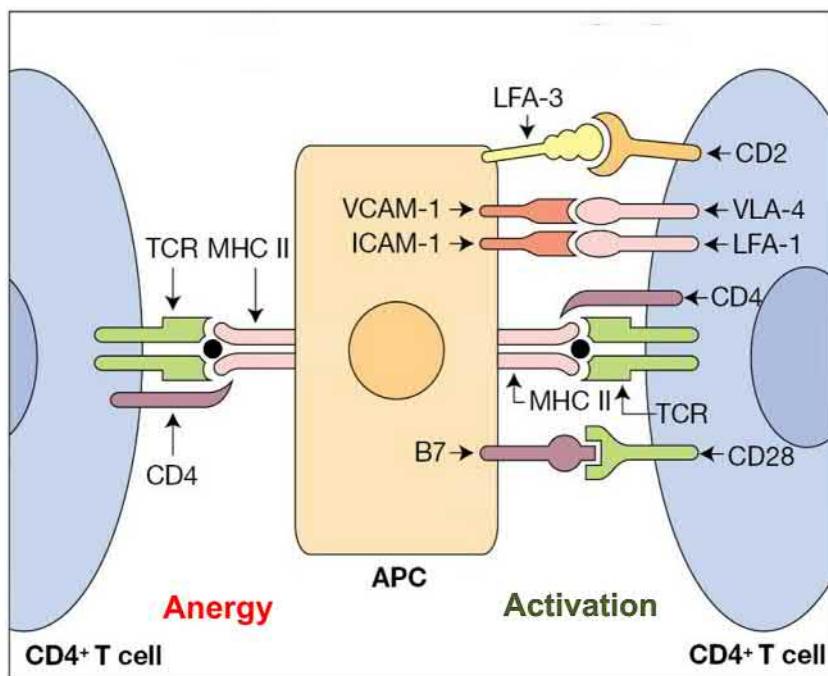
The tissue macrophages (Chapter 10: Role of Macrophages in Autoimmunity) and their circulating precursors, the blood monocytes, possess both Fc receptors and complement receptors and contain similar microbicidal substances to neutrophils. However, they are much longer lived than neutrophils and are able to process antigens for presentation to helper T cells. An additional role of the macrophage is the removal of the body's own dead or dying cells. While tissue damage associated with necrotic cell death triggers inflammation, cells dying due to apoptosis are removed much more quietly. Loss of membrane symmetry is a feature of apoptotic cell death and exposes the molecule phosphatidylserine on the cell surface, marking the cell for phagocytosis by macrophages expressing phosphatidylserine receptors (Arandjelovic and Ravichandran, 2015). Macrophages are key players in inflammatory responses, releasing cytokines such as IL-1 $\beta$  and TNF $\alpha$ , and are particularly characteristic of chronic inflammation.

Sets of activating and inhibitory receptors are expressed on NK cells (Chapter 12: Natural Killer Cells). They enable the NK cells to detect cells that have either lost or altered their expression of self-MHC molecules as a result of infection or oncogenesis. A dominant signal through the activating receptors will lead to the induction of apoptosis in the target cell (Fig. 4.4). NK cells can also mediate antibody-dependent cellular cytotoxicity (ADCC) of antibody-coated target cells and are a rich source of certain cytokines, particularly gamma interferon. This latter activity bestows an important immunoregulatory role upon the NK cell (Wilk and Blish, 2018).

A key interface between innate and adaptive responses is provided by DCs, a heterogeneous population, which include the Langerhans cell in the skin. DCs (Chapter 11: Dendritic Cells in Autoimmune Disease) sample extracellular antigens by endocytosis and become activated to an antigen-presenting mode when their PRRs (Table 4.1), which include Toll-like receptors (TLRs), NOD-like receptors, and various C-type lectin receptors,



**FIGURE 4.4** NK cells possess both activating and inhibitory receptors. The activating receptors recognize a number of different activator ligands constitutively expressed on many cells in the body (e.g., CD112) or expressed in response to factors such as cell stress (e.g., MICA [MHC class I polypeptide-related sequence A]). Activation of killing is usually blocked by engagement of inhibitory receptors that recognize the MHC class I molecules normally expressed on all nucleated cells in the body. Cells lacking MHC class I are not able to engage the inhibitory receptors, are therefore deemed abnormal, and are killed by the NK cells. Even in the presence of MHC class I, if sufficient activating signals are received (because infection, cancer, or other types of cell stress have caused the cell to express higher levels of activators), the inhibitory signal is subdominant and the target cell will be killed.



**FIGURE 4.5** T-cell activation. Interaction of costimulatory molecules leads to activation of resting T lymphocytes by APC on engagement of the TCR with its cognate antigen–MHC complex. Engagement of the TCR without accompanying costimulatory signals leads to anergy (functional inactivation). Note, a cytotoxic rather than a helper T cell would involve coupling of CD8 to MHC class I. Costimulation is delivered to a resting T cell primarily through engagement of CD28 on the T cell by B7.1 (CD80) or B7.2 (CD86) on the APC. Other molecules involved can include ICAM-1, LFA-1/3, VCAM-1, and VLA-4. Source: Modified from Delves, P.J., Martin, S.J., Burton, D.R., Roitt, I.M., 2017. *Roitt's Essential Immunology*, 13th ed. Wiley-Blackwell.

recognize PAMPs such as LPS, terminal mannose, and microbial CpG motifs (unmethylated cytosine–guanine dinucleotide sequence flanked by two 5' purines and two 3' pyrimidines). Endogenous DAMPs such as uric acid and heat shock proteins can also activate these cells (Schaefer, 2014). The activated DCs travel to the local draining lymph node where they present antigen to T cells. During their migration through the afferent lymphatics, they upregulate their cell surface MHC class II molecules and the CD80 (B7.1) and CD86 (B7.2) costimulatory ligands for CD28 on the T cell (Esensten et al., 2016). Such costimulation is required, together with antigen, for T-cell activation (Fig. 4.5). Within the DC, the antigen is processed into short peptides and then expressed on the cell surface together with the MHC class II molecules for presentation to CD4<sup>+</sup> helper T cells and Tregs. DCs are also able to cross-present exogenous antigens by transferring them into the MHC class I processing and presentation pathway for recognition by CD8<sup>+</sup>, mostly cytotoxic, T cells (Cruz et al., 2017). Conversely, cytoplasmic antigens can undergo autophagy and be cross-presented to CD4<sup>+</sup> (mostly helper) T cells following delivery to the MHC class II presentation pathway (Münz, 2016).

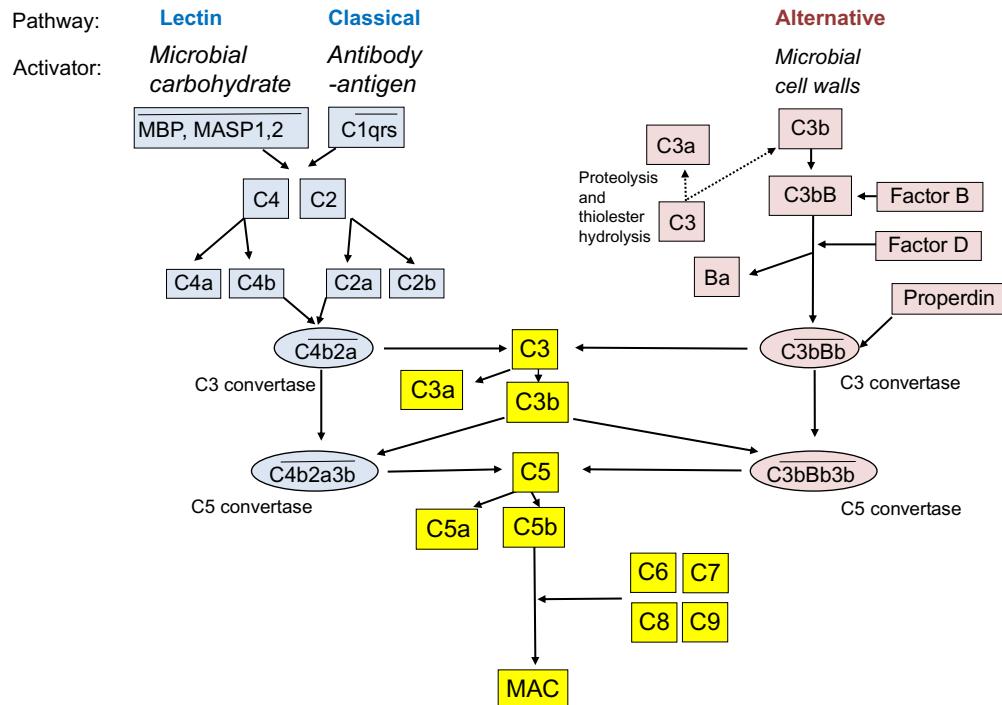
DCs can also act to limit immune responses. T-cell interactions with DCs lacking the expression of the crucial CD80/CD86 costimulatory molecules induce anergy (functional inactivation) in the T cell. Immunosuppression by DCs can be mediated via their production of the tryptophan-depleting enzyme indoleamine 2,3-dioxygenase (tryptophan being required for T-cell proliferation) or by the preferential stimulation of Tregs (Domogalla et al., 2017).

Although B cells are able to recognize antigen without the intervention of any other cell type, recognition is more efficient if multiple copies of the antigen are “presented” to the B cell in the form of immune complexes held on the surface of follicular DCs (FDCs) (Heesters et al., 2014). These are an entirely different cell type to the DCs discussed above. Unlike DCs, they are not phagocytic, and they lack MHC class II molecules. Furthermore, and unlike most cells of the immune response, they are not derived from HSCs but instead are of stromal origin (Heesters et al., 2014). They can present immune complexes to B cells very efficiently by virtue of their Fc $\gamma$ RIIB receptors for IgG and CR1 and CR2 receptors for complement.

The role that erythrocytes perform in immune responses should not be overlooked. Their possession of CR1 complement receptors for C3b, C4b, and iC3b confers on these cells an important role in clearing immune complexes from the circulation, rapidly transporting them to the liver and spleen where they are destroyed by Kupffer cells and splenic macrophages (Birmingham, 1995).

## Soluble Mediators

The complement system (Chapter 14: The Roles and Contributions of the Complement System in the Pathophysiology of Autoimmune Diseases) is based upon an enzymic amplification cascade which can be triggered using one of three pathways; classical, lectin, and alternative (Ehrnthaller et al., 2011). These all lead to the cleavage of complement component C3 into C3a and C3b by a C3 convertase enzyme (Fig. 4.6). The classical pathway is activated by IgG and IgM antibodies when they bind antigen, thereby creating an array of closely associated immunoglobulin Fc regions to which complement component C1q binds, followed by C1r and C1s. This event initiates a series of enzymic reactions leading to the generation of the classical pathway C3 convertase, C4b2a. The lectin pathway, which is essentially an antibody-independent variant of the classical pathway, leads to the generation of the same C3 convertase when microbial carbohydrates interact with mannose-binding



**FIGURE 4.6** The complement system. For simplicity, complement regulatory proteins have been omitted. The classical and lectin pathways (pale blue) are very similar. Like the alternative pathway (pink), these pathways generate C3 and C5 convertases leading to shared post-C3 events (yellow). For details see text.

protein (MBP) which then binds to the two MBP-associated serine proteases MASP-1 and MASP-2. The initially quite separate alternative pathway is activated when complement component C3b becomes stabilized by binding to microbial cell walls. The C3b then combines with factor B which is cleaved by factor D, generating a different C3 convertase, C3bBb. This C3 convertase is further stabilized by properdin. Both proteolysis and thiol-ester hydrolysis of C3 constitutively generate very low levels of C3b. However, in the alternative pathway, it is only when C3bBb is generated that there is a substantial splitting of C3 into C3a and C3b. Subsequently, a C5 convertase, either C4b2a3b (from the classical and lectin pathways) or C3bBb3b (from the alternative pathway), is produced by addition of C3b to the C3 convertase. This splits C5 into C5a and C5b, ultimately leading to the generation of the membrane attack complex (MAC) composed of complement components C5b, C6, C7, C8, and C9. Because complement activation consists of a series of sequential enzyme reactions, there is a tremendous amplification of the initial response, and along the way, a number of complement components with potent immunologic activities are generated.

A major function of C3b (and its cleavage products iC3b and C3d) and of C4b is to enhance the engulfment of antigens by phagocytic cells bearing the complement receptors CR1 and CR3. The C3a, C4a, and C5a components act as anaphylatoxins triggering the release of inflammatory mediators from mast cells and basophils. C5a is also a potent neutrophil chemoattractant. The MAC (C5b6789) generates pores in cell membranes, ultimately leading to the demise of the target cell by apoptosis. Because of these many potent activities, the complement cascades are tightly controlled by a number of complement regulatory proteins including C1 inhibitor (which dissociates the C1qrs complex), factor H and factor I (which cooperate to break down C3b in the alternative pathway), CD46 (membrane cofactor protein) and CD55 (decay accelerating factor) both of which limit the formation and function of the C3 convertases, and CD59 (homologous restriction factor, an inhibitor of MAC formation) (Holders, 2014).

Complement components C3, C9, and factor B are classed as acute phase proteins. This diverse group of mediators, which also include C-reactive protein, serum amyloid A protein, proteinase inhibitors, and coagulation proteins, share an ability to undergo a rapid change in plasma concentration in response to infection, inflammation, and tissue injury. Collectively, the acute phase proteins facilitate host resistance to infection and promote the resolution of tissue damage (Gabay and Kushner, 1999).

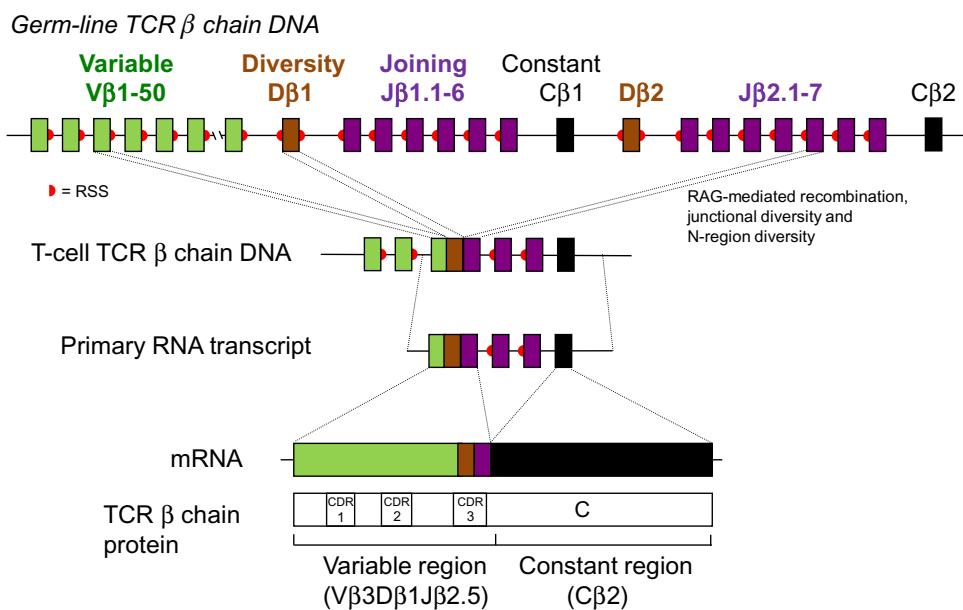
Another group of proteins, which function in both the innate and adaptive response, are the cytokines (Wilson and Barker, 2013) (Chapter 15: Cytokines, Their Receptors and Signals). These soluble mediators facilitate communication both within the immune system and between the immune system and other cells of the body. In order to respond to a given cytokine, a cell must express the relevant cytokine receptor. One subset of cytokines, the chemokines (Chen et al., 2018), is important in ensuring that immune system cells end up in the correct location as already mentioned in the context of an acute inflammatory response. In addition to acting as communication molecules, some cytokines play a more direct role in immune defense. For example, the interferons produced by virally infected cells establish a state of viral-resistance in surrounding noninfected cells, thereby acting as a “firebreak” against the spread of the infection (Fensterl et al., 2015).

## ADAPTIVE IMMUNE RESPONSES

The adaptive responses involve the clonal expansion of antigen-specific B and T lymphocytes. B cells differentiate into plasma cells which secrete the antigen-specific antibodies responsible for the elimination of extracellular antigens. T cells help other cells in the immune response, kill infected cells, or suppress undesirable immune responses.

### T-Cell Development

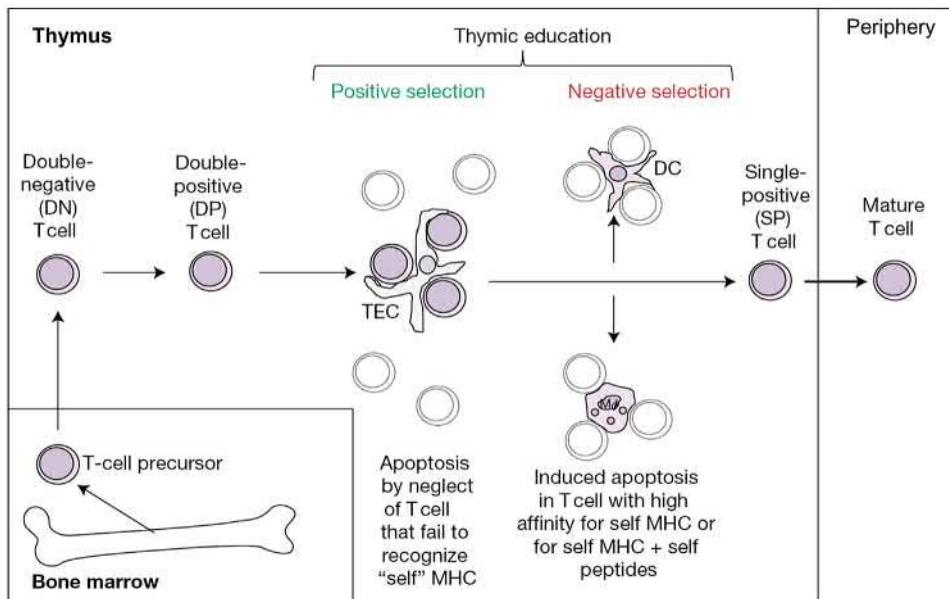
T-cell precursors migrate from the bone marrow to the thymus, an organ essential for their production. T-cell development occurs in the thymus throughout life, despite the fact that it undergoes significant atrophy during aging (Dooley and Liston, 2012). The TCR on T cells comes in two different versions, either an  $\alpha\beta$  or a  $\gamma\delta$  heterodimer; each chain of the dimer having one variable domain and one constant domain. Collectively, T lymphocytes are capable of producing vast numbers of different TCR variable regions by recombining variable (V) and joining (J) gene segments from the pools of  $\alpha$  and  $\gamma$  TCR genes and V, diversity (D) and J gene segments from the pools of  $\beta$  and  $\delta$  TCR genes (Ciofani and Zúñiga-Pflücker, 2010). There are a number of different sequences for each segment and one out of each V(D)J set is utilized in the recombination event (Fig. 4.7).



**FIGURE 4.7** Diversity of antigen receptors. As an example, the recombination of the TCR  $\beta$  chain genes is shown. Early in T-cell development, the recombination-activating genes RAG-1 and RAG-2 are expressed. Random recombination of either the diversity (D)  $\beta$ 2 gene segment next to any one of the 7 joining (J)  $\beta$ 2 gene segments, or of the D $\beta$ 1 gene segment to any one of the 13 J $\beta$ 1 or J $\beta$ 2 gene segments, is followed by recombination of any one out of approximately 50 variable (V) gene segments next to the already rearranged DJ segment. The RAG enzymes recognize RSS found 3' of each V gene segment, both 5' and 3' of each D gene segment, and 5' of each J gene segment. Different T cells will recombine a different segment out of each pool, thereby creating one level of diversity. Additional heterogeneity is brought about by junctional diversity due to splicing inaccuracies and by the fairly random incorporation of nucleotides (N-region diversity) mediated by the enzyme TdT. The primary RNA transcript is processed into mRNA, with splicing of the rearranged VDJ next to the downstream C $\beta$  constant region gene. This mRNA will encode a TCR  $\beta$  chain which is placed on the surface of the pre-T cell together with an invariant pre-T $\alpha$  chain. As the pre-T cell undergoes further maturation, the TCR  $\alpha$  gene segments (for simplicity not shown in the figure) recombine to produce the TCR  $\alpha$  chain. This replaces the pre-T $\alpha$  chain in order to produce a mature  $\alpha\beta$  TCR on the cell surface. The complementarity-determining regions CDR1 and CDR2 within the  $\alpha$  and  $\beta$  chain variable regions bind to MHC while CDR3 binds to the peptide. Although the detailed organization of the germ-line genes for the TCR  $\alpha$  chain, Ig heavy chain, Ig  $\kappa$  light chain, and Ig  $\lambda$  light chain are somewhat different to each other, they all consist of pools of V, J, and, for IgH as well as TCR  $\beta$  chain and TCR  $\delta$ -chain, D gene segments that undergo recombination to create antigen receptor diversity. The same general principles regarding the rearrangement process, therefore, apply to the generation of the  $\gamma\delta$  TCR on  $\gamma\delta$  T cells and to the BCR on B cells.

Recombination-activating genes encode the enzymes recombination activating gene (RAG-1) and RAG-2 which mediate these processes following the recognition of the recombination signal sequence (RSS) nucleotide motifs flanking the V, D, and J gene segments (Teng and Schatz, 2015). Splicing inaccuracies and the insertion of additional nucleotides around the V–D–J junctions by the enzyme terminal deoxynucleotidyl transferase (Tdt) further increase diversity (Benedict et al., 2000).

Recombination, and subsequent expression, of the TCR genes does not occur until the precursor T cells reach the thymus. Developing  $\alpha\beta$  T cells switch on expression of both CD4 and CD8 cell surface molecules and are therefore referred to as “double positive” T cells. This dual expression permits the TCR on  $\alpha\beta$  T cells to potentially interact with both MHC class I and MHC class II molecules. CD4 binds to conserved (nonpolymorphic) residues on the MHC class II molecule while CD8 binds to conserved residues on MHC class I. Positive and negative selections of the  $\alpha\beta$  T cells then occur (Klein et al., 2014). At this relatively early stage in their differentiation,  $\alpha\beta$  T cells are programmed to undergo apoptosis (Chapter 16: Cellular Injury and Apoptosis) and are only rescued from this default “death by neglect” if their TCR is capable of binding to self-peptide + self-MHC on the thymic epithelial cells (Fig. 4.8). Positive selection ensures that the randomly generated  $\alpha\beta$  TCR is able to interact with self-MHC molecules (i.e., those allelic variants of the MHC that are present in the individual). During positive selection, T cells lose expression of either CD4 or CD8 to become “single positive” CD4 or CD8 cells. Because of the random recombination of the TCR genes (which maximizes diversity), most T cells will be unable to recognize self-MHC and therefore will fail to be positively selected and are eliminated (Klein, et al., 2014). This leaves (1) potentially useful T cells that are capable of recognizing the individual’s own MHC variants presenting peptides derived from foreign pathogens and (2) potentially harmful T cells that are capable of recognizing the individual’s own MHC variants



**FIGURE 4.8** Positive and negative selection in the thymus. Following a productive recombination of their TCR  $\alpha$  chain and TCR  $\beta$  chain genes, T cells express a cell surface TCR together with both CD4 and CD8 to become double-positive T cells. Positive selection on thymic epithelial cells (TEC) that expresses both MHC class I and MHC class II will rescue T cells from a default pathway of apoptosis which occurs if these cells are neglected. As long as they have generated a TCR able to recognize self-MHC they are saved from neglect. The rescued cells are then protected from apoptosis unless they actively undergo negative selection due to high-affinity interaction of their TCR with self-MHC or self-MHC + self-peptides present on DC and macrophages. The  $CD4^+$   $CD8^-$  and  $CD4^-$   $CD8^+$  single-positive T cells that exit the thymus, therefore, possess an  $\alpha\beta$  TCR with the potential to detect foreign peptides presented by self-MHC. Source: Modified from Delves, P.J., Martin, S.J., Burton, D.R., Roitt, I.M., 2017. *Roitt's Essential Immunology*, 13th ed. Wiley-Blackwell.

presenting peptides derived from autoantigens. Apoptosis is therefore subsequently induced in any lymphocytes capable of high-affinity binding to self-peptide + self-MHC on DCs, macrophages, and thymic epithelial cells. This negative selection by the clonal deletion in the thymus constitutes central tolerance of autoantigen-reactive T cells (Chapter 5: Immunological Tolerance—T Cells) and, like failure to be positively selected, also results in extensive T-cell death within the thymus. Peptides are generated from a number of organ- and tissue-restricted self-antigens ectopically expressed in the thymus and peripheral lymph nodes under the transcriptional control of the autoimmune regulator (AIRE) protein (Conteduca et al., 2018). Not all autoantigen-reactive cells are eliminated. Those with an intermediate affinity for autoantigen can develop into Tregs (Klein et al., 2014). T cells that successfully pass through these hurdles exit the thymus and enter the periphery, a term used to denote any location outside of the primary lymphoid organs (bone marrow and thymus). These mature naïve  $\alpha\beta$  T cells will be capable of recognizing foreign peptides presented by self-MHC. Generally,  $\gamma\delta$  T cells, although also arising in the thymus, do not express either CD4 or CD8 and they recognize antigen directly rather than in the form of peptide–MHC.

## Functional Activities of T Cells

The  $\alpha\beta$  and  $\gamma\delta$  TCRs are not by themselves able to transmit activation signals into the cell, this function being assigned to the CD3 molecules (CD3 $\gamma$ , CD3 $\delta$ , CD3 $\epsilon$ ) and the CD3-associated  $\zeta$  chains. Receptor aggregation occurs within lipid rafts which also incorporate a number of adhesion and costimulatory molecules including lymphocyte function-associated antigen-1 (LFA-1), CD2, CD28, and CD45 to form the immunological synapse (Dustin and Choudhuri, 2016). Stimulation through the synapse results in the phosphorylation of tyrosines within immunoreceptor tyrosine-based activation motifs (ITAMs) present on the cytoplasmic tails of the CD3 complex and the  $\zeta$  chains. A number of protein kinases including Lck and ZAP-70, together with the adaptor proteins SLP-76 and LAT, are involved in initiating the signaling cascade (Smith-Garvin et al., 2009). The CD45 phosphatase also plays a critical role in lymphocyte activation by its ability to act as both a positive and negative regulator of the Lck kinase in T cells and of the Fyn kinase in B cells (Saunders and Johnson, 2010).

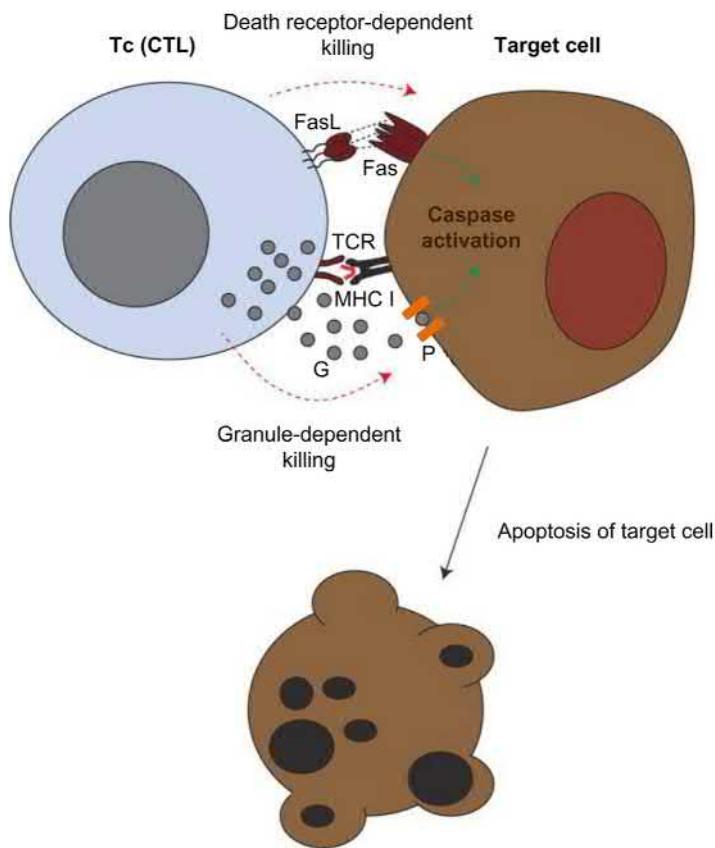
Broadly speaking,  $CD4^+$  T cells act as helper or regulatory T lymphocytes while  $CD8^+$  T cells are usually cytotoxic (Chapter 6: T Cells and Their Subsets in Autoimmunity). However, some  $CD4^+$  cells can exhibit cytotoxic

activity (Takeuchi and Saito, 2017), while CD8<sup>+</sup> cells secrete cytokines that can help in the generation of immune responses, be cytotoxic or be immunosuppressive (Woodland and Dutton, 2003).

The TCR on CD8<sup>+</sup> CTL binds to peptide–MHC class I on target cells. Endogenous antigens, including self-antigens and viral proteins, are broken down into peptides by a proteolytic structure known as the immunoproteasome (Krüger and Kloetzel, 2012). If the TCR recognizes the peptide–MHC combination and receives costimulation, then the CTL becomes activated to kill the target cell, for example, a cell infected with virus or a tumor cell (Tscharke et al., 2015). It does this by inducing apoptosis in the target via engaging the Fas molecule on the target cell with Fas ligand on the CTL or by using the perforin/granzyme pathway (Fig. 4.9).

In contrast to the ubiquitously expressed MHC class I, MHC class II is only present on a few specialized cells, including DCs, macrophages, and B cells (professional antigen-presenting cells). These cells generate peptides by proteolytic cleavage of engulfed antigens within endosomal vesicles and then present the peptide–MHC class II combination to CD4<sup>+</sup> T cells. Helper T cells can be divided into different populations based upon the cytokines they produce. Cells secreting IL-2, interferon- $\gamma$  (IFN $\gamma$ ), and tumor necrosis factor  $\beta$  (TNF $\beta$ , lymphotoxin) but not IL-4 and IL-5 are designated Th1 cells, those secreting IL-4, IL-5, IL-10, and IL-13 but not IL-2 and IFN $\gamma$  are classified as Th2 cells, and those secreting IL-17, IL-21, and IL-22 are termed Th17 cells (Patel and Kuchroo, 2015) (Fig. 4.10). In general, cytokine production by Th1 cells facilitates cell-mediated immunity, involving macrophage activation and T-cell-mediated cytotoxicity, and assists in the production of some humoral responses. Cytokines produced by Th2 cells are mostly involved in humoral immunity, particularly involving IgE and IgA responses, and cytokines from Th17 cells mediate inflammatory and a variety of other responses (Patel and Kuchroo, 2015). IL-12 from DCs drives T cells toward a Th1 phenotype (Schmitt and Ueno, 2015) and Th1/Th2 responses tend to become polarized because the IFN- $\gamma$  from Th1 cells downregulates Th2 activity, whereas IL-4 and IL-13 from Th2 cells downregulate Th1 cells.

A population of CD4 lymphocytes exist which have some properties of NK cells and some properties of T cells. Many of these NKT cells (Chapter 7: The Role of Invariant Natural Killer T Cells in Autoimmune Diseases) express an invariant TCR which recognizes lipid antigen presented by the nonclassical MHC molecule CD1d. NKT cells are multifunctional and secrete a range of cytokines (Godfrey and Rossjohn, 2011).



**FIGURE 4.9** Cytotoxic T lymphocytes. Cytotoxic T-cells (Tc, CTL) can kill target cells by the granule-dependent pathway in which the CTL inserts perforin (P) into the target cell membrane to act as a channel for the passage of granzyme B (G) and other serine proteases into the target cell. They can also kill target cells by engaging Fas on the target using their cell surface FasL. Both pathways result in the activation of caspases leading to apoptotic cell death in the target cell. Source: Modified from Delves, P.J., Martin, S.J., Burton, D.R., Roitt, I.M., 2017. Roitt's Essential Immunology, 13th ed. Wiley-Blackwell.

CD4 <sup>+</sup> $\alpha\beta$ T cell	Th1	Th2	Th17	Tfh	Treg
Transcription factors	STAT4 T-bet	STAT6 GATA3	STAT3 ROR $\gamma$ t	STAT3 Bcl-6	STAT5 Foxp3
Cytokines	IFN $\gamma$ IL-2 TNF $\beta$	IL-4 IL-5 IL-10 IL-13	IL-17 IL-21 IL-22	IL-21 ICOS	TGF $\beta$ IL-10 IL-35
Primary function	Help CTL and macrophages (and some B cells) Inhibit Th2	Help B cells Inhibit Th1	Promote inflammation	Help germinal center B cells	Suppress immune responses

**FIGURE 4.10** T-cell subpopulations. The major subpopulations of CD4<sup>+</sup>  $\alpha\beta$  T lymphocytes are shown with key transcription factors, characteristic secreted or cell surface molecules, and functional attributes that distinguish them. Note that cytotoxic T cells can also be subdivided on the basis of cytokine production into Tc1, Tc2, etc. ICOS, Inducible T-cell costimulator (cell surface molecule); Tfh, follicular helper T cell; Th, helper T cell; Treg, regulatory T cell.

The antigen-specificities and functions of  $\gamma\delta$  T cells are less well characterized than that of  $\alpha\beta$  T cells (Chapter 5: Immunological Tolerance—T Cells) but some  $\gamma\delta$  TCRs recognize molecules that are upregulated in response to cellular infection or stress (Adams et al., 2015).  $\gamma\delta$  T cells take up residence throughout the body, being particularly prevalent in epithelia including the mucosal tissues. They can function as cytotoxic cells and mediate both inflammatory and suppressive activity via their ability to secrete a diverse range of cytokines including TNF $\alpha$ , IFN $\gamma$ , TGF $\beta$ , IL-4, IL-5, IL-10, IL-13, and IL-17 (Bonneville et al., 2010).

## B-Cell Development and Functions

A minor population of B cells (Chapter 8: B Cell Development: How to Become One of the Chosen Ones), B1 cells, develops early during ontogeny and often expresses the CD5 cell surface molecule (Hardy and Hayakawa, 2015). These cells secrete low-to-moderate affinity IgM antibodies which can exhibit polyreactivity, that is, recognize several different antigens, often including common pathogens and autoantigens. Such antibodies are often referred to as natural antibodies because of their existence in the absence of an obvious antigenic stimulus. The majority of B cells, B2 cells, lack CD5 and develop slightly later in ontogeny. Like T cells, these B cells are collectively capable of producing a huge number of different variable regions on their antigen receptors. They achieve this by recombining the immunoglobulin heavy and light chain gene loci in a process analogous to the recombination of TCR genes in T cells.

Early in B-cell development, pro-B cells mature into pre-B cells, at which stage they express RAG-1 and RAG-2. Random recombination of any one of the approximately 25 diversity (D) gene segments next to any one of the 6 joining (J) gene segments is followed by rearrangement of any one out of approximately 40 variable (V) gene segments next to the already recombined DJ segment. As with the TCR in T cells, additional diversity is brought about by splicing inaccuracies and the action of TdT. The heavy chain primary RNA transcript is processed into mRNA, with splicing of the rearranged VDJ next to the C $\mu$  constant region gene. This mRNA will encode a  $\mu$  heavy chain which is placed on the surface of the pre-B cell together with the surrogate light chain Vpre-B  $\lambda$ 5 encoded by two nonrecombining genes termed VpreB and  $\lambda$ 5. Expression of this pre-BCR on the immature B cell leads to ligand-independent signaling which drives B-cell differentiation toward the mature naïve B-cell coexpressing conventional IgM and IgD antibodies on the cell surface (Übelhart et al., 2016). As the pre-B cell undergoes maturation, the immunoglobulin light chain V (which number about 40 for V $\kappa$  and 30 for V $\lambda$ ) and J (of which there are 5 for each light chain isotype) gene segments rearrange to produce a  $\kappa$  or  $\lambda$  light chain. This light chain replaces the surrogate light chain in order to produce a mature IgM BCR on the cell surface. Expression of RAG-1 and RAG-2 is now switched off. Once the B cells express a mature antigen receptor, their survival and further differentiation become antigen-dependent. The BCR at this stage also comprises IgD antibodies of the same specificity, produced by alternative splicing of the rearranged VDJ to either the C $\mu$  or C $\delta$  constant region genes.

The cell surface immunoglobulin is associated with several molecules including Ig $\alpha$  (CD79a), Ig $\beta$  (CD79b), CD19, CD21 (the CR2 complement receptor), CD81 (TAPA-1), and CD225 (Leu13) which collectively transmit activation signals into the cell when receptor aggregation occurs following cross-linking of the antibody by antigen (Chapter 9: B Cell Activation and B Cell Tolerance). This signaling initially involves phosphorylation of ITAM sequences on the cytoplasmic tails of Ig $\alpha$  and Ig $\beta$  by the protein tyrosine kinase Lyn, with subsequent recruitment of additional kinases including Syk and Btk (Harwood and Batista, 2010). Upon binding to the BCR, antigen is endocytosed and then processed within acidified endosomes for presentation by MHC class II to CD4 $^+$  helper T cells (Clark et al., 2004). In addition to an antigen-presenting role, B cells secrete a variety of cytokines including IL-10, IL-12, IL-13, TNF $\alpha$ , TNF $\beta$  (lymphotoxin), transforming growth factor- $\beta$  (TGF $\beta$ ), and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Wilson and Barker, 2013). Following the encounter with antigen in the presence of costimulatory signals, the B cells undergo rounds of proliferation and then differentiate into memory cells or alternatively into plasma cells which produce high levels of soluble antibody. Many plasma cells are short lived, but others survive for long periods of time, particularly in the bone marrow (Lam and Bhattacharya, 2018).

## Antibodies

The immunoglobulin antibody molecules are composed of two identical heavy polypeptide chains and two identical light polypeptide chains, held together by interchain disulfide bonds. All immunoglobulins are glycoproteins, containing between 2% and 14% carbohydrate depending on the antibody class (Arnold et al., 2007). The N-termini of the light and heavy chains are each folded into a variable domain containing three hypervariable loops, constituting the complementarity determining regions (CDRs) responsible for noncovalent binding to the antigen. Most protein epitopes recognized by antibodies are discontinuous, comprising amino acids that are only brought together upon protein folding (Kirpach and Muller, 2015). The heavy chain C-terminal domains form the constant region which specifies the class/subclass of antibody. The light chain constant domain determines the  $\kappa$  or  $\lambda$  isotype. The human antibody classes are IgG, IgA, IgM, IgD, and IgE, with four IgG (IgG1–4) and two IgA (IgA1–2) subclasses (Schroeder and Cavacini, 2010) (Table 4.2). Each antibody can be produced either with a C-terminal hydrophobic transmembrane sequence to anchor the molecule in the B-cell membrane where it functions as the BCR or as a secreted molecule lacking the transmembrane sequence and released from plasma cells.

The basic antibody monomer (biochemically a tetramer) of two identical heavy chains and two identical light chains is bivalent with two antigen-binding arms of identical specificity. Secretory IgA at mucosal surfaces is a

**TABLE 4.2** Antibodies—the Main Properties and Approximate Serum Concentration of Human Antibodies

Antibody class/ subclass	Serum conc. (approx.)	Major features include
IgM	1.5 mg/mL	Constitutes, together with IgD, the BCR on naïve B cells. Secreted IgM acts mainly in the circulation and is the first antibody class to be produced in an immune response. Activates complement. Powerful agglutinin
IgG1	9 mg/mL	Most abundant antibody in the blood. Activates complement and enhances phagocytosis. Can cross the placenta
IgG2	3 mg/mL	Activates complement. Poorly transported across placenta
IgG3	1 mg/mL	Activates complement and enhances phagocytosis. Can cross the placenta
IgG4	0.5 mg/mL	Can undergo Fab arm exchange to become bispecific. Can cross the placenta
IgA1	3 mg/mL	In secretory form protects mucosal surfaces
IgA2	0.5 mg/mL	In secretory form protects mucosal surfaces
IgD	30 $\mu$ g/mL	Constitutes, together with IgM, the BCR on naïve B cells
IgE	0.05 $\mu$ g/mL	In presence of antigen triggers release of inflammatory mediators from mast cells and basophils

BCR, B-cell receptor.

tetravalent dimer comprising two identical IgA molecules, whereas circulating IgM is most frequently a decavalent pentamer of five identical IgM molecules with a minor proportion of hexamers and tetramers. IgA and IgM polymerization is stabilized by a polypeptide J (joining) chain (Johansen et al., 2000).

Antibodies that are capable of inhibiting the binding of microorganisms or biological molecules (toxins, hormones, cytokines, and so forth) to their cellular receptors exert their effect independently of other immune system components and are referred to as neutralizing antibodies. Usually, however, antibodies do not function in isolation but are employed to activate the classical complement pathway and/or link antigen to Fc receptor-bearing cells. Antigens opsonized with IgG, IgA, or IgE bind to the appropriate Fc receptors ( $Fc\gamma R$ ,  $Fc\alpha R$ , or  $Fc\epsilon R$ ) on phagocytic cells (Hogarth, 2015). Alternatively, both IgG and IgE can mediate ADCC in which NK cells, monocytes, macrophages, and neutrophils bearing  $Fc\gamma$  receptors or macrophages, eosinophils, and platelets bearing  $Fc\epsilon$  receptors are focused onto antibody-coated target cells or parasites (Graziano and Guyre, 2006). IgE antibodies are also able to sensitize mast cells and basophils via the high-affinity IgE receptor  $Fc\epsilon RI$  and if cross-linked by antigen will trigger the release of inflammatory mediators.

The epithelial cell poly-Ig receptor transports dimeric secretory IgA produced by plasma cells underlying mucosal surfaces (Johansen and Brandtzaeg, 2004). On the luminal side of the epithelium, the IgA is released by proteolytic cleavage of the receptor, leaving a fragment called secretory component still attached to the IgA. Secretory IgA acts to prevent microbial adhesion to the epithelial cell wall (Corthésy, 2010).

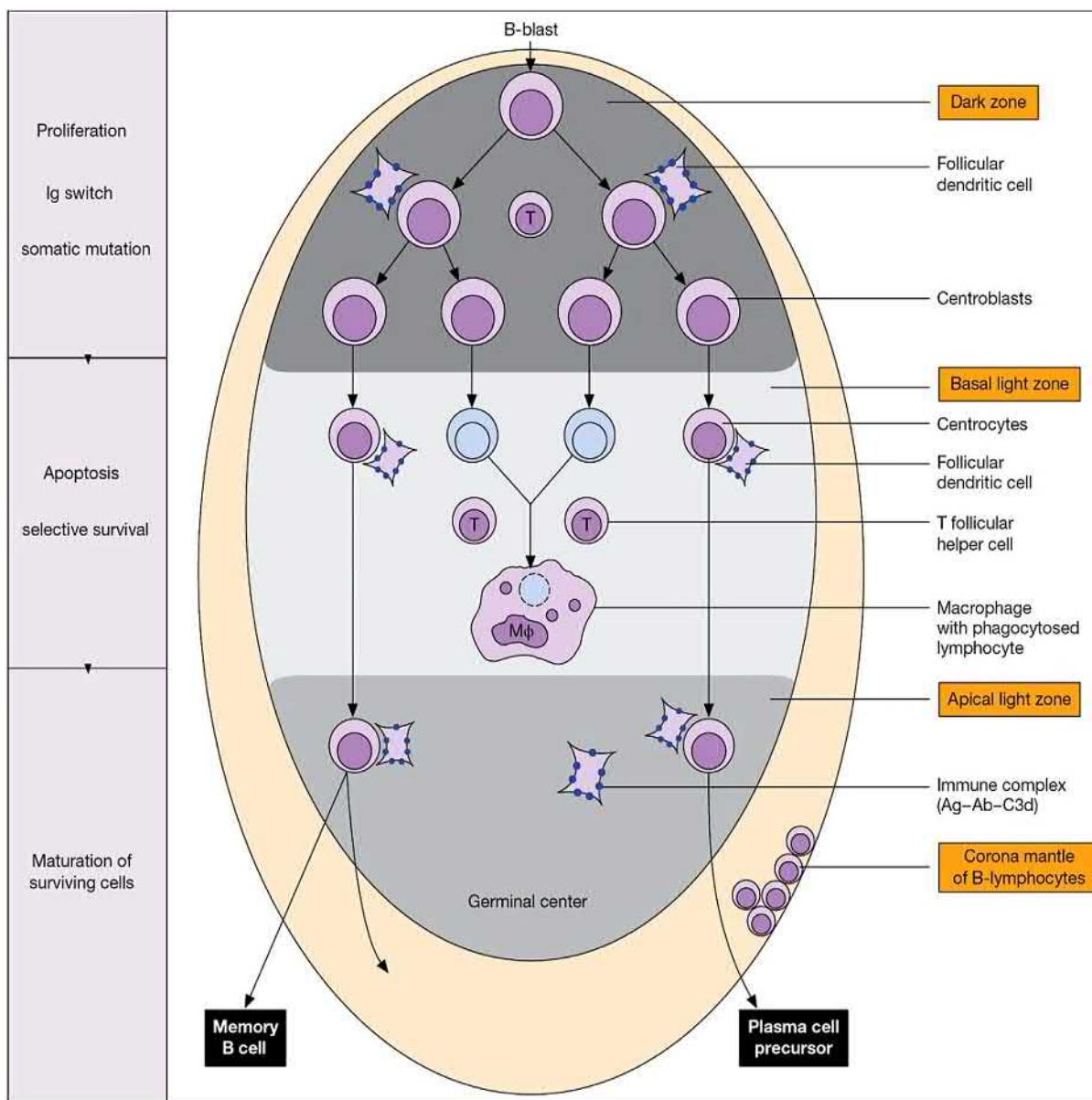
Another type of receptor,  $FcRn$ , is expressed on vascular endothelium where it is involved throughout life in the recycling of IgG in order to increase the circulating half-life of this class of immunoglobulin. It is also present in the placenta, where it transports IgG from the maternal to the fetal circulation, and on the intestinal epithelium of the neonate where it is involved in the uptake of IgG from maternal milk (Stapleton et al., 2015).

## Secondary Lymphoid Tissues

The primary lymphoid organs, the bone marrow and thymus, are where fully differentiated mature naïve T and B cells are generated. However, activation of lymphocytes occurs in structurally organized B- and T-cell compartments in the secondary lymphoid tissues; the mucosa-associated lymphoid tissues, lymph nodes, and spleen. Large numbers of lymphoid cells are present throughout the lung (Lloyd and Marsland, 2017) and in the lamina propria of the intestinal wall (Faria et al., 2017). Because only a handful of lymphocytes will be specific for a given antigen, T and B cells recirculate through the different lymphoid tissues in order to increase the chances of encountering antigen. While responses to blood-borne antigens are usually initiated in the spleen, those to antigens in the tissues are stimulated in the local draining lymph nodes.

Lymphoid follicles within the secondary lymphoid tissues contain germinal centers where B-cell activation occurs within a meshwork of FDCs displaying immune complexes on their surface (Fig. 4.11). T follicular helper cells are specialized for providing help to germinal center B cells (Webb and Linterman, 2017). Germinal centers are at the heart of the generation of adaptive responses for it is here that B cells proliferate, class switch, undergo affinity maturation, and differentiate into memory cells and into plasma cell precursors (Mesin et al., 2016). B cells can increase the binding affinity of their BCR by somatic hypermutation (SHM) of the V(D)J genes. Higher affinity clones will then be preferentially selected by antigen. Class switching from IgM to IgG, IgA, and IgE involves switch sequences composed of highly repetitive nucleotide motifs present immediately upstream of each constant region gene (except C $\delta$ ; IgM and IgD being coexpressed as the BCR on naïve B cells). Both somatic hypermutation and class switch recombination (CSR) require the expression of activation-induced cytidine deaminase (AID) and the utilization of the nonhomologous DNA end-joining machinery (Methot and Di Noia, 2017). A process referred to as receptor editing enables self-reactive B cells to replace the variable region gene in the recombined VDJ heavy or VJ light chain sequence with a different variable region gene in order to eliminate autoreactivity (Lang et al., 2016).

Most pathogens enter the body through mucosal surfaces. The palatine tonsils and adenoids are the sites for induction of responses to intranasal and inhaled antigens (Brandtzaeg, 2011). Antigens from the gut are taken up by specialized epithelial microfold (M) cells which transport the antigens across the epithelium for access to the Peyer's patches where mucosal responses are initiated (Lo, 2018). Activated lymphocytes exit the Peyer's patches via the efferent lymphatics, traffic through the blood, and then home to the lamina propria and other mucosal effector sites (Brandtzaeg, 2009). Intraepithelial lymphocytes are interspersed between the gut epithelial cells, have a diverse phenotype, and can be either cytotoxic or immunoregulatory (Cheroutre et al., 2011).



**FIGURE 4.11** The germinal center. During the initiation of the adaptive immune response, these structures form in the secondary lymphoid tissues in order to generate a microenvironment where all the necessary antigen-specific and innate antigen-presenting cells can interact. Antigen-stimulated B-cell proliferation occurs in the dark zone and is accompanied by affinity maturation, due to SHM of the immunoglobulin V(D)J genes, and CSR. Both SHM and CSR are associated with the expression of AID in the B cell. Upon passage into the basal light zone, high-affinity antigen-specific B cells are positively selected by interaction with antigen, which is present in the form of immune complexes on the surface of follicular dendritic cells. B cells which fail to be positively selected undergo apoptosis and are phagocytosed by macrophages. The positively selected cells migrate to the apical light zone where proliferation continues, and memory cells and plasma cell precursors are generated. Source: From Delves, P.J., Martin, S.J., Burton, D.R., Roitt, I.M., 2017. Roitt's Essential Immunology, 13th ed. Wiley-Blackwell.

Lymphocytes enter lymph nodes, tonsils, and Peyer's patches either via the afferent lymphatics or from the blood via high endothelial venules (HEV). L-Selectin is constitutively expressed on lymphocytes and constitutes a ligand for adhesion molecules referred to as peripheral lymph node addressins (McEver, 2015). If increased expression of LFA-1 is induced on the lymphocytes, their adhesion to HEV is further enhanced and they migrate across the HEV into these lymphoid tissues. Lymphocytes leave the lymph nodes via the efferent lymphatics. Although the spleen lacks HEV, circulating lymphocytes can directly access the marginal zone of this organ from the blood vessels. T cells locate mostly to the periarteriolar lymphoid sheaths, while B cells enter the lymphoid follicles. Lymphocytes exit the spleen via the splenic vein.

When naïve lymphocytes first encounter antigen in the secondary lymphoid tissues, they mount a primary immune response, generating both effector and memory cells. The memory cells are responsible for the quantitatively and qualitatively superior secondary immune response that occurs upon any subsequent encounters with the same antigen (Zielinski et al., 2011). Memory cells have a lower activation threshold than naïve cells and the secondary response is more rapid, involves larger numbers of lymphocytes, and, for B cells, produces higher levels of antibody with a superior affinity for antigen.

The term T-independent antigen is used to refer to antigens which are capable of generating an antibody response without a requirement for helper T cells. Polysaccharides, polymerized flagellin, and a number of other antigens have repetitive determinants which can extensively cross-link the BCR and thereby directly activate the B cell (Möller, 2001). Because they do not recruit T cells, T-independent antigens fail to provoke the formation of germinal centers and therefore are unable to induce B-cell memory, class switching, or significant amounts of affinity maturation. Thus low-affinity IgM antibodies are produced in response to T-independent antigens and, although involving B cells, the response does not go on to exhibit the characteristics of adaptive immunity. The majority of antigens that stimulate B cells are, however, T-cell dependent in that the B-cell response requires help from T cells. As mentioned earlier, the BCR on the surface of the B cell internalizes bound antigen which is then processed into peptides for presentation by MHC class II molecules. Upon recognition of the peptide–MHC complex by the T cells in the secondary lymphoid tissues, the costimulatory molecule CD154 (CD40 ligand) on the T cell engages CD40 on the B cell, leading to class switching (Ford and Larsen, 2009). In addition to cell surface molecules, cytokines play a key role in the mutual activation of the T and B lymphocytes. T cell help can also be recruited by DCs and macrophages presenting the relevant peptide–MHC class II combination to the helper T cell.

## RESOLUTION OF THE IMMUNE RESPONSE

Antigen stimulates the immune response and, therefore, for foreign antigen, its clearance by the immune system will naturally lead to a waning of the response. However, there are additional mechanisms which initially amplify and subsequently downregulate the response. Once high levels of class-switched antigen-specific IgG are produced, the antibody can inactivate the antigen-specific B cells in a manner reminiscent of classical negative feedback loops in the endocrine system. Cross-linking of the BCR to Fc $\gamma$ RIIB on B cells by immune complexes results in the transmission of inhibitory signals into the B cell (Smith and Clatworthy, 2010). A number of signals from cytokines and cell surface molecules can also be inhibitory. Ligation of the T-cell surface molecule CTLA-4 by CD80 and CD86, and of PD-1 by PD-L1 and PD-L2, provides downregulating signals (Bour-Jordan et al., 2011). Inhibition of these “immune checkpoints” has proved of benefit in the immunotherapy of subsets of cancer patients (Nishino et al., 2017). Some Foxp3 $^{+}$  Tregs secrete IL-10, IL-35, and TGF $\beta$  which can act in an immunosuppressive capacity, while others suppress responses by cell-contact-dependent mechanisms (Mohr et al., 2018).

Neuroendocrine interactions with the immune system provide a further level of regulation (Del Rey and Besedovsky, 2017). For example, TLR ligands and inflammatory mediators such as IL-1 $\beta$  result in efferent nerve signaling in the spleen, triggering the release of acetylcholine from splenic T cells which in turn inhibits the release of proinflammatory cytokines from macrophages in order to maintain immune homeostasis and thereby avoid an excessive inflammatory response (Andersson and Tracey, 2012).

## References

- Adams, E.J., Gu, S., Luoma, A.M., 2015. Human gamma delta T cells: evolution and ligand recognition. *Cell. Immunol.* 296, 31–40.
- Andersson, U., Tracey, K.J., 2012. Reflex principles of immunological homeostasis. *Annu. Rev. Immunol.* 30, 313–335.
- Arandjelovic, S., Ravichandran, K.S., 2015. Phagocytosis of apoptotic cells in homeostasis. *Nat. Immunol.* 16, 907–917.
- Arnold, J.N., Wormald, M.R., Sim, R.B., Rudd, P.M., Dwek, R.A., 2007. The impact of glycosylation on the biological function and structure of human immunoglobulins. *Annu. Rev. Immunol.* 25, 21–50.
- Benedict, C.L., Gilfillan, S., Thai, T.H., Kearney, J.F., 2000. Terminal deoxynucleotidyl transferase and repertoire development. *Immunol. Rev.* 175, 150–157.
- Birmingham, D.J., 1995. Erythrocyte complement receptors. *Crit. Rev. Immunol.* 15, 133–154.
- Bonneville, M., O'Brien, R.L., Born, W.K., 2010. Gamma delta T cell effector functions: a blend of innate programming and acquired plasticity. *Nat. Rev. Immunol.* 10, 467–478.
- Bour-Jordan, H., Esensten, J.H., Martinez-Llordella, M., Penaranda, C., Stumpf, M., Bluestone, J.A., 2011. Intrinsic and extrinsic control of peripheral T-cell tolerance by costimulatory molecules of the CD28/B7 family. *Immunol. Rev.* 241, 180–205.
- Brandtzaeg, P., 2009. Mucosal immunity: induction, dissemination, and effector functions. *Scand. J. Immunol.* 70, 505–515.

- Brandtzaeg, P., 2011. Potential of nasopharynx-associated lymphoid tissue for vaccine responses in the airways. *Am. J. Respir. Crit. Care Med.* 183, 1595–1604.
- Broz, P., Monack, D.M., 2013. Newly described pattern recognition receptors team up against intracellular pathogens. *Nat. Rev. Immunol.* 13, 551–565.
- Chen, K., Bao, Z., Tang, P., Gong, W., Yoshimura, T., Wang, J.M., 2018. Chemokines in homeostasis and diseases. *Cell. Mol. Immunol.* 15, 324–334.
- Cheroutre, H., Lambolez, F., Mucida, D., 2011. The light and dark sides of intestinal intraepithelial lymphocytes. *Nat. Rev. Immunol.* 11, 445–456.
- Childs, K.S., Goodbourn, S., 2017. Pattern recognition receptors. eLS. John Wiley & Sons Ltd, Chichester. Available from: <http://www.els.net>. Available from: <http://dx.doi.org/10.1002/9780470015902.a0020175.pub2>.
- Ciofani, M., Zúñiga-Pflücker, J.C., 2010. Determining  $\gamma\delta$  versus  $\alpha\beta$  T cell development. *Nat. Rev. Immunol.* 10, 657–663.
- Clark, M.R., Massenburg, D., Siemasko, K., Hou, P., Zhang, M., 2004. B-cell antigen receptor signaling requirements for targeting antigen to the MHC class II presentation pathway. *Curr. Opin. Immunol.* 16, 382–387.
- Conteduca, G., Indiveri, F., Filaci, G., Negrini, S., 2018. Beyond APECED: an update on the role of the autoimmune regulator gene (AIRE) in physiology and disease. *Autoimmun. Rev.* 17, 325–330. pii: S1568-9972(18)30029-6.
- Corthésy, B., 2010. Role of secretory immunoglobulin A and secretory component in the protection of mucosal surfaces. *Fut. Microbiol.* 5, 817–829.
- Cruz, F.M., Colbert, J.D., Merino, E., Kriegsman, B.A., Rock, K.L., 2017. The biology and underlying mechanisms of cross-presentation of exogenous antigens on MHC-I molecules. *Annu. Rev. Immunol.* 35, 149–176.
- Del Rey, A., Besedovsky, H.O., 2017. Immune-neuro-endocrine reflexes, circuits, and networks: physiologic and evolutionary implications. *Front. Horm. Res.* 48, 1–18.
- Dharampuriya, P.R., Scapin, G., Wong, C., John Wagner, K., Cillis, J.L., Shah, D.I., 2017. Tracking the origin, development, and differentiation of hematopoietic stem cells. *Curr. Opin. Cell Biol.* 49, 108–115.
- Domogalla, M.P., Rostan, P.V., Raker, V.K., Steinbrink, K., 2017. Tolerance through education: how tolerogenic dendritic cells shape immunity. *Front. Immunol.* 8, 1764. Available from: <https://doi.org/10.3389/fimmu.2017.01764>.
- Dooley, J., Liston, A., 2012. Molecular control over thymic involution: from cytokines and microRNA to aging and adipose tissue. *Eur. J. Immunol.* 42, 1073–1079.
- Dustin, M.L., Choudhuri, K., 2016. Signaling and polarized communication across the T cell immunological synapse. *Annu. Rev. Cell Dev. Biol.* 32, 303–325.
- Ebbo, M., Crinier, A., Vély, F., Vivier, E., 2017. Innate lymphoid cells: major players in inflammatory diseases. *Nat. Rev. Immunol.* 17, 665–678.
- Ehrnthal, C., Ignatius, A., Gebhard, F., Huber-Lang, M., 2011. New insights of an old defense system: structure, function, and clinical relevance of the complement system. *Mol. Med.* 17, 317–329.
- Esensten, J.H., Helou, Y.A., Chopra, G., Weiss, A., Bluestone, J.A., 2016. CD28 costimulation: from mechanism to therapy. *Immunity* 44, 973–988.
- Faria, A.M.C., Reis, B.S., Mucida, D., 2017. Tissue adaptation: implications for gut immunity and tolerance. *J. Exp. Med.* 214, 1211–1226.
- Fensterl, V., Chattopadhyay, S., Sen, G.C., 2015. No love lost between viruses and interferons. *Annu. Rev. Virol.* 2, 549–572.
- Ford, M.L., Larsen, C.P., 2009. Translating costimulation blockade to the clinic: lessons learned from three pathways. *Immunol. Rev.* 229, 294–306.
- Freeley, S., Kemper, C., Le Friec, G., 2016. The “ins and outs” of complement-driven immune responses. *Immunol. Rev.* 274, 16–32.
- Freud, A.G., Mundy-Bosse, B.L., Yu, J., Caligiuri, M.A., 2017. The broad spectrum of human natural killer cell diversity. *Immunity* 47, 820–833.
- Gabay, C., Kushner, I., 1999. Acute-phase proteins and other systemic responses to inflammation. *N. Eng. J. Med.* 340, 448–454.
- Godfrey, D.I., Rossjohn, J., 2011. New ways to turn on NKT cells. *J. Exp. Med.* 208, 1121–1125.
- Graziano, R.F., Guyre, P.M., 2006. Antibody-dependent cell-mediated cytotoxicity (ADCC). eLS. John Wiley & Sons Ltd, Chichester. Available from: <http://www.els.net>. Available from: <http://dx.doi.org/10.1038/npg.els.0000498>.
- Hardy, R.R., Hayakawa, K., 2015. Perspectives on fetal derived CD5<sup>+</sup> B1 B cells. *Eur. J. Immunol.* 45, 2978–2984.
- Harwood, N.E., Batista, F.D., 2010. Early events in B cell activation. *Annu. Rev. Immunol.* 28, 185–210.
- Heesters, B.A., Myers, R.C., Carroll, M.C., 2014. Follicular dendritic cells: dynamic antigen libraries. *Nat. Rev. Immunol.* 14, 495–504.
- Hogarth, P.M., 2015. Fc receptors: introduction. *Immunol. Rev.* 268, 1–5.
- Holers, V.M., 2014. Complement and its receptors: new insights into human disease. *Annu. Rev. Immunol.* 32, 433–459.
- Jacobino, S., Slomp, A., Boross, P., Leusen, J.H.W., 2013. Fc receptors. eLS. John Wiley & Sons Ltd, Chichester. Available from: <http://www.els.net>. Available from: <http://dx.doi.org/10.1002/9780470015902.a0000916.pub3>.
- Johansen, F.E., Brandtzaeg, P., 2004. Transcriptional regulation of the mucosal IgA system. *Trends Immunol.* 25, 150–157.
- Johansen, F.E., Braathen, R., Brandtzaeg, P., 2000. Role of J chain in secretory immunoglobulin formation. *Scand. J. Immunol.* 52, 240–248.
- Kaufmann, E., Sanz, J., Dunn, J.L., Khan, N., Mendonça, L.E., Pacis, A., et al., 2018. BCG educates hematopoietic stem cells to generate protective innate immunity against tuberculosis. *Cell* 172, 176–190.
- Kirpach, J., Muller, C.P., 2015. Epitopes. eLS. John Wiley & Sons Ltd, Chichester. Available from: <http://www.els.net>. Available from: <http://dx.doi.org/10.1002/9780470015902.a0000514.pub3>.
- Kita, H., 2011. Eosinophils: multifaceted biological properties and roles in health and disease. *Immunol. Rev.* 242, 161–177.
- Klein, L., Kyewski, B., Allen, P.M., Hogquist, K.A., 2014. Positive and negative selection of the T cell repertoire: what thymocytes see (and don't see). *Nat. Rev. Immunol.* 14, 377–391.
- Krüger, E., Kloetzel, P.M., 2012. Immunoproteasomes at the interface of innate and adaptive immune responses: two faces of one enzyme. *Curr. Opin. Immunol.* 24, 77–83.
- Lam, W.Y., Bhattacharya, D., 2018. Metabolic links between plasma cell survival, secretion, and stress. *Trends Immunol.* 39, 19–27.

- Lang, J., Ota, T., Kelly, M., Strauch, P., Freed, B.M., Torres, R.M., et al., 2016. Receptor editing and genetic variability in human autoreactive B cells. *J. Exp. Med.* 213, 93–108.
- Lawrence, S.M., Corriden, R., Nizet, V., 2018. The ontogeny of a neutrophil: mechanisms of granulopoiesis and homeostasis. *Microbiol. Mol. Biol. Rev.* 82. Available from: <https://doi.org/10.1128/MMBR.00057-17>. pii: e00057-17.
- Lloyd, C.M., Marsland, B.J., 2017. Lung homeostasis: influence of age, microbes, and the immune system. *Immunity* 46, 549–561.
- Lo, D.D., 2018. Vigilance or subversion? Constitutive and inducible M cells in mucosal tissues. *Trends Immunol.* 39, 185–195.
- Malik, A., Kanneganti, T.D., 2017. Inflammasome activation and assembly at a glance. *J. Cell. Sci.* 130, 3955–3963.
- McEver, R.P., 2015. Selectins: initiators of leucocyte adhesion and signalling at the vascular wall. *Cardiovasc. Res.* 107, 331–339.
- Mesin, L., Ersching, J., Victora, G.D., 2016. Germinal center B cell dynamics. *Immunity* 45, 471–482.
- Methot, S.P., Di Noia, J.M., 2017. Molecular mechanisms of somatic hypermutation and class switch recombination. *Adv. Immunol.* 133, 37–87.
- Mohr, A., Malhotra, R., Mayer, G., Gorochov, G., Miyara, M., 2018. Human FOXP3<sup>+</sup> T regulatory cell heterogeneity. *Clin. Transl. Immunol.* 7 (1), e1005. Available from: <https://doi.org/10.1002/cti2.1005>.
- Möller, G., 2001. Antigens: thymus independent. eLS. John Wiley & Sons Ltd, Chichester. Available from: <http://www.els.net>. Available from: <http://dx.doi.org/10.1038/pg.els.0000504>.
- Muller, W.A., 2011. Mechanisms of leukocyte transendothelial migration. *Annu. Rev. Pathol.* 6, 323–344.
- Münz, C., 2016. Autophagy proteins in antigen processing for presentation on MHC molecules. *Immunol. Rev.* 272, 17–27.
- Nishino, M., Ramaiya, N.H., Hatabu, H., Hodin, F.S., 2017. Monitoring immune-checkpoint blockade: response evaluation and biomarker development. *Nat. Rev. Clin. Oncol.* 14, 655–668.
- Patel, D.D., Kuchroo, V.K., 2015. Th17 cell pathway in human immunity: lessons from genetics and therapeutic interventions. *Immunity* 43, 1040–1051.
- Robida, P.A., Puzzovio, P.G., Pahima, H., Levi-Schaffer, F., Bochner, B.S., 2018. Human eosinophils and mast cells: birds of a feather flock together. *Immunol. Rev.* 282, 151–167.
- Sarrazin, S., Sieweke, M., 2011. Integration of cytokine and transcription factor signals in hematopoietic stem cell commitment. *Semin. Immunol.* 23, 326–334.
- Saunders, A.E., Johnson, P., 2010. Modulation of immune cell signalling by the leukocyte common tyrosine phosphatase, CD45. *Cell. Signal.* 22, 339–348.
- Schaefer, L., 2014. Complexity of danger: the diverse nature of damage-associated molecular patterns. *J. Biol. Chem.* 289, 35237–35245.
- Schmitt, N., Ueno, H., 2015. Regulation of human helper T cell subset differentiation by cytokines. *Curr. Opin. Immunol.* 34, 130–136.
- Schroeder Jr, H.W., Cavacini, L., 2010. Structure and function of immunoglobulins. *J. Allergy Clin. Immunol.* 125, S41–S52.
- Smith, K.G., Clatworthy, M.R., 2010. Fc $\gamma$ RIIB in autoimmunity and infection: evolutionary and therapeutic implications. *Nat. Rev. Immunol.* 10, 328–343.
- Smith-Garvin, J.E., Koretzky, G.A., Jordan, M.S., 2009. T cell activation. *Annu. Rev. Immunol.* 27, 591–619.
- Stapleton, N.M., Einarsdóttir, H.K., Stemerding, A.M., Vidarsson, G., 2015. The multiple facets of FcRn in immunity. *Immunol. Rev.* 268, 253–268.
- Takeuchi, A., Saito, T., 2017. CD4 CTL, a cytotoxic subset of CD4<sup>+</sup> T cells, their differentiation and function. *Front. Immunol.* 8, 194. Available from: <https://doi.org/10.3389/fimmu.2017.00194>.
- Teng, G., Schatz, D.G., 2015. Regulation and evolution of the RAG recombinase. *Adv. Immunol.* 128, 1–39.
- Tscharke, D.C., Croft, N.P., Doherty, P.C., La Gruta, N.L., 2015. Sizing up the key determinants of the CD8<sup>+</sup> T cell response. *Nat. Rev. Immunol.* 15, 705–716.
- Übelhart, R., Werner, M., Jumaa, H., 2016. Assembly and function of the precursor B-cell receptor. *Curr. Top. Microbiol. Immunol.* 393, 3–25.
- Webb, L.M.C., Linterman, M.A., 2017. Signals that drive T follicular helper cell formation. *Immunology* 152, 185–194.
- Wilk, A.J., Blish, C.A., 2018. Diversification of human NK cells: lessons from deep profiling. *J. Leukoc. Biol.* 103, 629–641.
- Wilson, H.M., Barker, R.N., 2013. Cytokines. eLS. John Wiley & Sons Ltd, Chichester. <http://www.els.net>. Available from: <http://dx.doi.org/10.1002/9780470015902.a0000929.pub3>.
- Woodland, D.L., Dutton, R.W., 2003. Heterogeneity of CD4<sup>+</sup> and CD8<sup>+</sup> T cells. *Curr. Opin. Immunol.* 15, 336–342.
- Zarbock, A., Ley, K., McEver, R.P., Hidalgo, A., 2011. Leukocyte ligands for endothelial selectins: specialized glycoconjugates that mediate rolling and signaling under flow. *Blood* 118, 6743–6751.
- Zielinski, C.E., Corti, D., Mele, F., Pinto, D., Lanzavecchia, A., Sallusto, F., 2011. Dissecting the human immunologic memory for pathogens. *Immunol. Rev.* 240, 40–51.

# Immunological Tolerance—T Cells

*Yael Goldfarb, Cristina Peligero-Cruz and Jakub Abramson*

Department of Immunology, Weizmann Institute of Science, Rehovot, Israel

## OUTLINE

<b>Introduction</b>	<b>65</b>	<i>Ignorance and Antigen Sequestering</i>	74
<b>T-Cell Tolerance—A Brief Historical Perspective</b>	<b>66</b>	<i>Dendritic Cells, the Key Inducers of Peripheral T-Cell Tolerance</i>	75
From Fetal Tolerance to Central Tolerance	66	<i>Intrinsic Mechanisms Suppressing Clonal Expansion and/or Reactivation</i>	77
From Neonatal Thymectomy to Tregs	68	<i>Dominant Tolerance Through Treg-Mediated Immunosuppression</i>	78
From Adjuvants to T-Cell Anergy	69	<i>Other Tolerogenic Cells in the Periphery</i>	81
<b>Establishment of Self-Tolerance in the Thymus</b>	<b>70</b>	<b>Concluding Remarks</b>	82
Positive Selection of Immunocompetent T Cells	70	<b>Acknowledgments</b>	82
Negative Versus Agonist Selection of Self-Reactive T Cells	70	<b>References</b>	82
Promiscuous Expression of Self-Antigens in the Thymus	73	<b>Further Reading</b>	90
<b>Induction and Maintenance of Immunological Tolerance in the Periphery</b>	<b>74</b>		

## INTRODUCTION

To be effective and potent guardians against the plethora of environmental pathogens, T and B lymphocytes are characterized by an unprecedented diversity, which enables them to recognize a virtually unlimited repertoire of molecular structures in a rapid and specific manner. Such repertoire diversity is achieved by an elaborate, semirandom rearrangement of the genes encoding their respective antigen-specific receptors. Consequentially, the immune system inevitably generates many T and B cells that recognize not only foreign and potentially harmful antigens, but also the body's own components. Therefore to deal with this problem and to prevent autoimmune destruction, the immune system has evolved a comprehensive network of several complementary mechanisms that ensure tolerance to the body's own antigens, while allowing effective immune responses to diverse pathogens (Mathis and Benoist, 2010; Mueller, 2010; Walker and Abbas, 2002).

Historically, immunological tolerance mechanisms have been segregated into two main categories—central and peripheral. More recently, however, this simplistic dichotomous division has been brought into question. First, over the past two decades, it has become rather clear that self-proteins, which were assumed to be expressed only in parenchymal tissues, are also ectopically expressed in the thymus (Derbinski et al., 2001). Furthermore, in contrast to the initial belief, recent evidence suggests that the “central tolerance” mechanisms in the thymus rely more on the conversion of self-reactive thymocytes into the FOXP3<sup>+</sup> T regulatory cell (Treg) lineage (Aschenbrenner et al., 2007; Davis, 2015; Yang et al., 2015), rather than their clonal deletion

(Anderson et al., 2005; Kappler et al., 1987). Since Tregs constitute one of the key mediators of peripheral tolerance (Josefowicz et al., 2012a), the classical segregation of tolerance into central and peripheral becomes even more fuzzy and possibly obsolete. For this reason, in this chapter, we shall try to provide a fresh look at the key concepts and mechanisms underlying the establishment, maintenance, and breakdown of T-cell tolerance to the body's own components, while avoiding the classical dichotomous categorization.

As will be discussed in detail below, the induction and maintenance of T-cell tolerance to self-antigens is guided by four major principles: (1) complete physical elimination of the self-reactive T-cell clone from the repertoire (clonal deletion) (Fig. 5.1A and E), (2) conversion of the self-reactive clone into a tolerogenic and/or harmless T-cell subtype (anergy, phenotype skewing) (Fig. 5.1B and D), (3) prevention of its encounter with its specific self-antigen in the immune periphery (ignorance, antigen sequestering) (Fig. 5.1C), and (4) prevention of its clonal expansion or reactivation upon self-antigen (re)encounter (immunosuppression, T-cell intrinsic inhibitory mechanisms) (Fig. 5.1F). While the induction of T-cell tolerance in the thymus mainly relies on the two former principles, its subsequent maintenance in the immune periphery is mediated by all of the above principles. Specifically, self-reactive T cells that have escaped the first filter in the thymus can also undergo clonal deletion or conversion in the periphery. In addition, there are several other mechanisms (both cell intrinsic and extrinsic) that prevent their self-antigen-mediated activation, expansion or reactivation once they have left the thymus. First, all naïve T cells (i.e., including self-reactive ones) are retained only within the bloodstream and secondary lymphoid organs and thus do not have direct access to peripheral tissues expressing unique self-antigens (antigen sequestering).

Second, naïve T cells cannot become activated unless they are provided with additional costimulatory signals from dendritic cells during their priming in the secondary lymphoid organs. Without such additional “danger” signals, naïve T cells that recognize a specific antigen fail to proliferate, differentiate, and survive and are thereby either eliminated from the repertoire or become unresponsive (anergic). Moreover, even in the presence of costimulation, not all self-reactive T cells have to differentiate into pathogenic effector subtypes (e.g., Th<sub>1</sub> or Th<sub>17</sub>), which will ultimately cause autoimmune destruction. Indeed, specific release of certain cytokines (e.g., IL4, IL10, TGFβ) and the absence of others (IL6, IL12, IL23) during T-cell priming may skew their differentiation into T-cell subtypes that are either unable to cause autoimmune damage or can actively prevent it.

Finally, even if self-reactive T cells become activated in the periphery, they are efficiently suppressed by populations with immunoregulatory properties (immunosuppression), the best characterized of which are the thymus-derived CD4<sup>+</sup>FOXP3<sup>+</sup> Tregs (tTregs) or their peripherally induced counterparts (pTreg). It is important to realize, however, that every cell type probably inhibits another, even if it is not primarily defined as regulatory or immunosuppressive. For instance, immunosuppression can simply stem from the normal balance between effector functions that tend to inhibit each other, such as the mutual antagonism between Th<sub>1</sub> and Th<sub>2</sub> effectors (Benoist and Mathis, 2012).

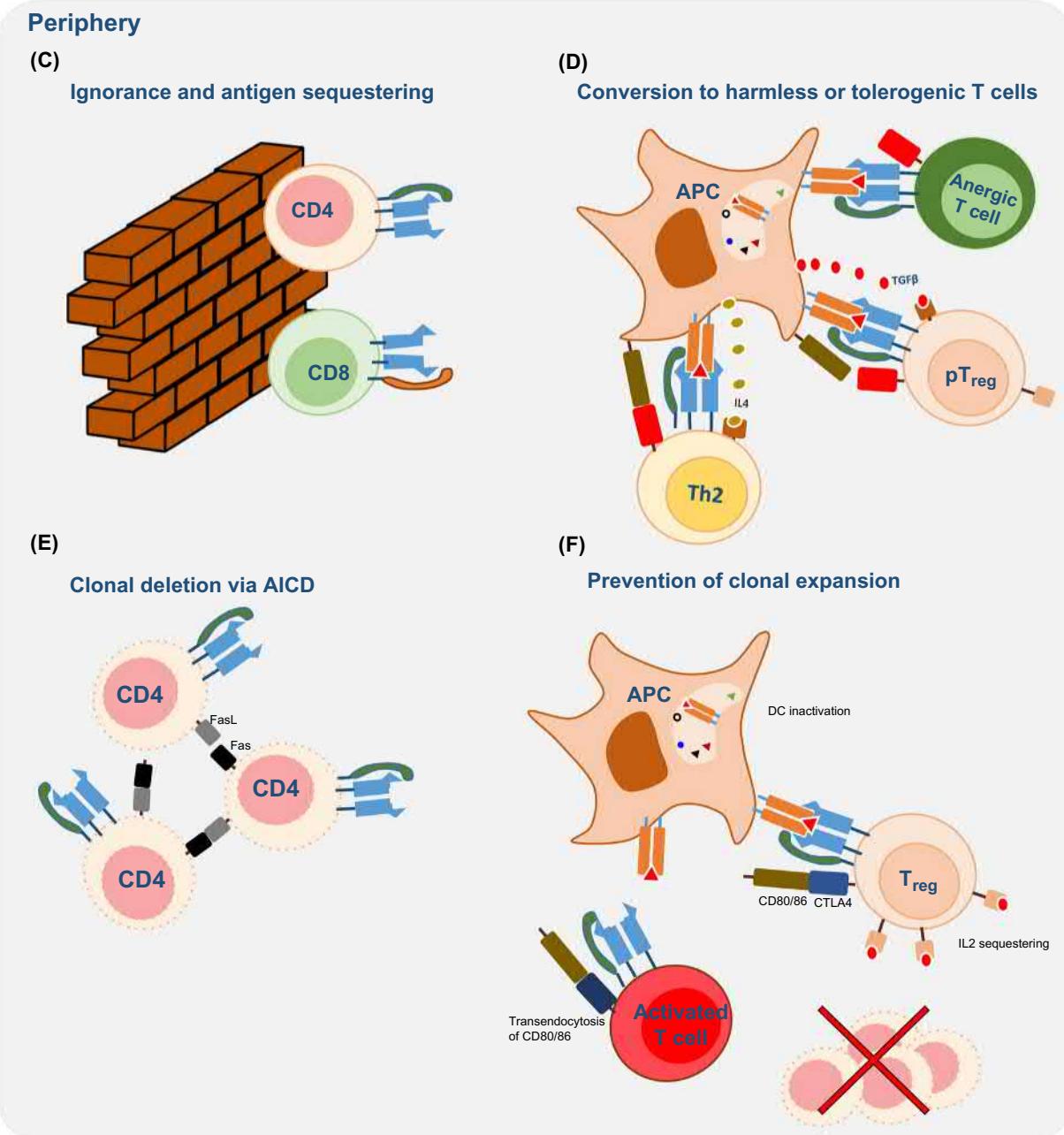
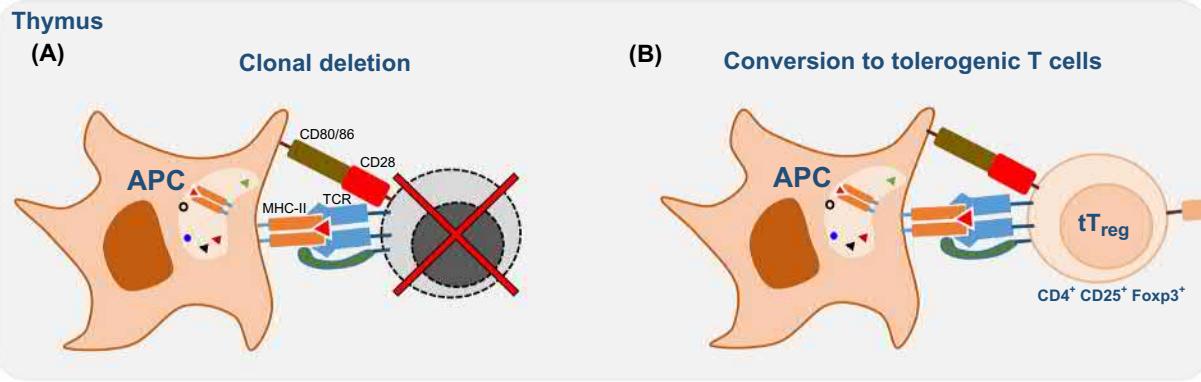
It should also be stressed that in addition to tolerating the body's own components, an effective and balanced immune response is also characterized by the induction and maintenance of immunological tolerance to exogenous, nonpathogenic antigens present ubiquitously in the environment, including food antigens, commensal microorganisms, and harmless airborne antigens. In this chapter, however, we shall focus only on concepts and mechanisms underlying the establishment, maintenance, and breakdown of T-cell tolerance to the body's own components and will not elaborate in detail on mechanisms responsible for induction of tolerance to nonself-antigens.

## T-CELL TOLERANCE—A BRIEF HISTORICAL PERSPECTIVE

### From Fetal Tolerance to Central Tolerance

The dilemma of how the immune system can distinguish self from nonself belongs to one of the most fundamental questions in immunology that has been debated for more than a hundred years. Paul Ehrlich, one of the founding fathers of immunology, already proposed that organisms must have a natural aversion to immunological self-destruction, which he called “horror autotoxicus.”

The first mechanistic insights into how the immune system becomes tolerant to self-antigens paradoxically stem from studies describing the acquisition of immunological tolerance to nonself-antigens, such as the lympho-choriomeningitis virus (LCMV), a natural mouse pathogen. Specifically, Traub et al. observed that mice infected with LCMV in utero did not mount an immune response against it, although the virus persisted throughout their



**FIGURE 5.1** Key principles for induction of T-cell tolerance. The induction and maintenance of T-cell tolerance to self-antigens is guided by four major principles that occur in the thymus and/or in the immune periphery: complete physical elimination of the self-reactive T-cell clone from the repertoire via clonal deletion (A and E); conversion of the self-reactive clone into a tolerogenic and/or harmless T-cell subtype via induction of Tregs, T-cell anergy, or T-cell phenotype skewing into harmless effector subsets (B and D); prevention of its encounter with its specific self-antigen in the immune periphery via antigen ignorance and/or antigen sequestering (C) or prevention of its clonal expansion or reactivation upon self-antigen (re)encounter via T-cell intrinsic mechanisms induced upon T-cell activation and/or Treg-mediated immunosuppression (F).

lives (Traub, 1938), suggesting that a host could become tolerant to foreign antigens, as long as they had been “seen” early enough in development. Nevertheless, the term “tolerance” was first coined by Ray Owen, who experimented with dizygotic cattle twins and found that they became hematopoietic chimeras in utero and thereby tolerant to each other’s blood antigens (Owen, 1945). This suggested that the host can “tolerate” nonself cells if it was exposed to them during ontogeny. These pioneering observations of tolerance were then further validated and broadened by a series of independent studies in the early 1950s. Specifically, in 1953, the group of Peter Medawar extended Owen’s observations by noting that nonidentical twin cattle could also accept skin grafts from each other, suggesting that this tolerance is not restricted only to blood-borne antigens, but also to solid tissues (Billingham et al., 1953). Moreover, injection of allogeneic blood cells from one mouse strain into newborn mice from a different inbred strain led to engraftment of these cells and subsequent induction of tolerance to skin grafts of the donor strain, but not of grafts from a third party (Billingham et al., 1953). Similar results were obtained in chickens with parabiosis experiments in which the circulatory systems of the two embryos were joined (Hasek and Hraba, 1955). The resulting adult birds were found to be hematopoietic chimeras that did not reject each other’s skin grafts.

These experiments supported the Burnet and Fenner hypothesis that exposure of the developing immune system to foreign antigens prevented the system from responding to those antigens. The results were interpreted at the cellular level in terms of the clonal selection theories of Burnet and Talmage, which postulated that self-specific lymphocytes were disposed of, or clonally deleted (Burnet, 1959, 1962). Thus tolerance ensued because there were no self-reactive cells left in the adult animal that were capable of responding to the self-antigen. In the T-cell world, clonal deletion soon became known as “negative selection,” to distinguish it from “positive selection,” which is the process by which T cells are selected to be able to recognize peptide antigens in the context of one’s own major histocompatibility complex (MHC) molecules. These original ideas were, however, not validated experimentally for several decades until the experimental tools for studying individual lymphocyte clones became available. Indeed, the first experimental evidence that self-reactive T cells are negatively selected in the thymus was provided in the late 1980s by two independent studies (Kisielow et al., 1988; Sha et al., 1988). By utilizing antigen-specific T-cell receptor (TCR) transgenic mice, both studies were able to demonstrate that thymocytes expressing the given antigen-specific receptor were deleted in the thymus in the presence of antigen. Thus negative selection of self-reactive T cells in the thymus has subsequently become synonymous with central T-cell tolerance and dominated the field till relatively recently (Davis, 2015). Several subsequent studies, however, demonstrated that negative selection is not foolproof, as almost half of the thymocytes that were positively selected did not subsequently undergo negative selection (Ignatowicz et al., 1996; van Meerwijk et al., 1997; Tourne et al., 1997). Thus there seemed to be at least two complementary mechanisms for how organisms dealt with self-reactive T-cell clones: either during their development in the thymus, known as central tolerance, or in the periphery, known as peripheral tolerance (discussed in more detail in the following section). Moreover, not all self-antigens were assumed to be expressed in the thymus, suggesting that T-cell clones specific to self-antigens that are exclusively expressed by parenchymal tissues (e.g., certain hormones and enzymes) must be either eliminated or silenced in the immune periphery. This hypothesis dominated the field until 2001 when a landmark study by Klein et al. established that medullary thymic epithelial cells (mTECs) ectopically express a large fraction of tissue-specific antigens (TSAs) and thereby induce tolerance to hundreds of self-antigens that are exclusively expressed by peripheral tissues (Derbinski et al., 2001). Interestingly, this study validated an earlier hypothesis by Linsk et al. (1989) and several other reports that had attracted little attention at that time (Jolicoeur et al., 1994; Pribyl et al., 1996; Smith et al., 1997; Sospedra et al., 1998). The discovery of promiscuous gene expression (PGE) in the thymus was shortly followed by another landmark study demonstrating that many of these TSA transcripts are induced by a single mTEC-specific factor, the autoimmune regulator (Aire) (Anderson et al., 2002) whose loss of function results in a multiorgan autoimmune syndrome in both humans (Nagamine et al., 1997) and mice (Anderson et al., 2002). Collectively, these studies brought about a new paradigm shift, which has dramatically reshaped our understanding of how T cells acquire tolerance to self-antigens in the thymus.

## From Neonatal Thymectomy to Tregs

The key role of the thymus in the induction of self-tolerance was also supported by results of studies on neonatally thymectomized mice (Miller, 1961; Nishizuka and Sakakura, 1969). Specifically, removal of a mouse thymus before day 3 after birth (but not after day 10) resulted in an organ-specific autoimmune syndrome. Importantly, subsequent studies by Gershon et al. suggested that the thymus is (in addition to its role in clonal

deletion of self-reactive T cells) also critical for the production of cells that can promote self-tolerance and suppress autoimmunity in the periphery (Gershon and Kondo, 1970, 1971). Indeed, the hypothesis that T cells are capable of not only promoting but also suppressing immune responses was validated a year later by the identification of thymus-derived “suppressor T cells” (Gershon et al., 1972). The molecular characterization of such suppressor T cells has, however, remained mysterious and controversial for several decades. While an earlier study by Cantor et al. (1978) suggested that suppressor T cells constitute a subset of CD8<sup>+</sup> T cells, a later study from Sakaguchi et al. demonstrated that these cells are rather Thy1<sup>+</sup>, Lyt-1<sup>+</sup>, Lyt-2<sup>-</sup> (i.e., CD90<sup>+</sup>, CD5<sup>+</sup>, CD8<sup>-</sup> T cells) (Sakaguchi, 1982; Smith et al., 1991; Tung et al., 1987) and appear at day 3 after birth. Subsequent studies by Sakaguchi et al. provided more detailed molecular and functional characterizations of these cells and suggested that they are a subset of CD4<sup>+</sup> T cells expressing high levels of CD25 and/or low levels of CD45RB (Morrissey et al., 1993; Powrie and Mason, 1990; Sakaguchi et al., 1982, 1995; Smith et al., 1991). Importantly, adoptive transfer of these CD4<sup>+</sup>CD25<sup>+</sup> T cells was sufficient to prevent both autoimmunity caused by neonatal thymectomy (Asano et al., 1996; Suri-Payer et al., 1998), as well as autoimmunity provoked by the transfer of CD25-depleted CD4 cells into nude mice (Sakaguchi et al., 1995; Suri-Payer et al., 1998).

Unfortunately, even in spite of this substantial body of evidence, the controversy regarding the specific markers that would molecularly define the suppressor T-cell population and the fact that some of the key markers were also shared with activated T cells eventually resulted in a considerable skepticism regarding their actual existence and/or their functional importance. Such skepticism and controversy lasted for almost two decades until the identification of the transcription factor FOXP3 as the master regulator of CD4<sup>+</sup>CD25<sup>+</sup> T cells development (Fontenot et al., 2003; Hori et al., 2003; Khattri et al., 2003), whose loss of function results in X-linked neonatal enteropathy and endocrinopathy (IPEX) in humans and scurfy phenotype in mice (Godfrey et al., 1991; Wildin, 2002). Consequently, the concept of “suppressor” T cells, as the gatekeepers of self-tolerance and immune homeostasis, has been resurrected and widely accepted. Nevertheless, to distinguish them from their “flopped” predecessors, the suppressor T cells have been renamed to Treg.

## From Adjuvants to T-Cell Anergy

In parallel to the suppressor T-cell paradigm, numerous groups have investigated additional/alternative mechanisms, which could explain the induction and/or maintenance of T-cell tolerance in the periphery. It has been recognized for a long time that the immune system is able to mount a much stronger immune response if an antigen is administered with specific adjuvants (e.g., Freund’s complete adjuvant) (Dresser, 1962; Kabat et al., 1946). For instance, when antigen (soluble bovine gamma globulin) was administered in the presence of lipopolysaccharide (LPS; a component of Gram-negative bacteria), a functional immune response was induced in mice, while when the very same antigen was administered in the absence of LPS, it induced immunological tolerance (Claman, 1963; Dresser, 1961). This suggested that existence of an additional antigen-nonspecific signal could boost the immune response, while its absence could possibly result in tolerance induction (Baxter and Hodgkin, 2002). The first experimental evidence supporting this hypothesis was provided by Lafferty et al., who studied the mechanisms of allograft rejection and demonstrated that in addition to the alloantigen (signal 1), the presenting cells have to provide a second, antigen-nonspecific signal that they called costimulation (Lafferty and Woolnough, 1977; Lafferty et al., 1974). Subsequent work showed that such costimulatory signal is provided by antigen-presenting cells (APCs) expressing the B7 (i.e., CD80/CD86) molecules, which specifically bind to the CD28 receptors on T cells (Linsley et al., 1990). Importantly, the expression of B7 molecules on APCs was later shown to be induced by “danger” signals (Janeway, 1992) through a series of pattern recognition receptors, such as Toll-like membrane receptors (TLRs) or Non-obese diabetic (NOD)-like cytoplasmic receptors (NLRs). Correspondingly, absence of danger signals was shown to result in low-level expression of B7 molecules on the APC surface and their consequent inability to provide sufficient costimulatory signal to the specific T-cell clone (Janeway, 1992; Jenkins et al., 1987; Johnson and Jenkins, 1993; Mueller et al., 1989). Such a lack of costimulation in the presence of a specific antigen signal was shown to result in T-cell anergy, a phenomenon characterized by T-cell unresponsiveness (IL2 production and proliferation) upon antigen reencounter (Jenkins et al., 1987; Mueller et al., 1989). Since then, the phenomenon of T-cell anergy has been validated by many independent studies both in vitro and in vivo (Dubois et al., 1998; Pape et al., 1998) and has become one of the major and most popular paradigms explaining and underlying the induction and maintenance of tolerance of self-reactive T-cell clones in the periphery.

## ESTABLISHMENT OF SELF-TOLERANCE IN THE THYMUS

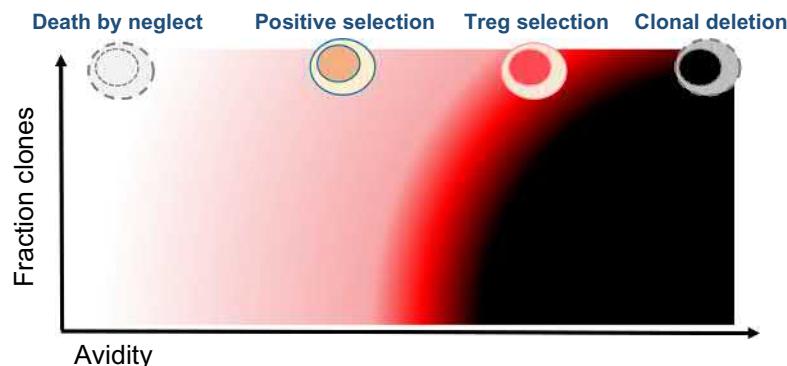
T-cell development and selection occur in the specialized environment of the thymus, which has evolved to support the differentiation of early thymic immigrants from the bone marrow, into competent naïve T cells. This complex process is aimed at creating a powerful army of T cells, so diverse it would harbor soldiers capable of recognizing virtually any pathogen-derived antigen the organism may encounter, while tolerating the body's own components. Such T-cell repertoire diversity is attained through the random process of V(D)J recombination of the locus encoding both  $\alpha$  and  $\beta$  chains of the TCR. However, during this process, which can theoretically give rise to  $\sim 10^{18}$  possible TCR permutations (Market and Papavasiliou, 2003), the immune system generates large numbers of useless TCRs that are incapable of recognizing peptide-MHC (pMHC) complexes, as well as self-reactive TCRs that could potentially cause autoimmunity. Thus a thorough screening system is necessary for purging both the useless and the potentially pathogenic TCRs in order to establish effective T-cell-mediated immunity while maintaining immunological tolerance to self.

### Positive Selection of Immunocompetent T Cells

The first selection checkpoint that a developing thymocyte encounters in the thymus is aimed at positive selection of immunocompetent T-cell clones expressing nascent TCR $\alpha\beta$  capable of binding to antigen peptides presented in the context of MHC molecules. Positive selection takes place in the cortex and is exclusively mediated by cortical thymic epithelial cells (cTEC), which present self-pMHC to the “auditioning” CD4 $^+$ CD8 $^+$  double positive (DP) thymocytes. The avidity of the TCR for the self-pMHC has crucial consequences for the subsequent fate of each T-cell clone (Fig. 5.2). As proposed by the “classical affinity model,” DP thymocytes harboring TCR $\alpha\beta$  that have no or very weak avidity for the self-pMHC are deemed useless and consequently die by neglect due to insufficient TCR signaling. In contrast, thymocytes with functional TCR $\alpha\beta$  that engage either pMHCI or pMHCI $\text{I}$  with low-intermediate avidity will differentiate either into single positive CD8 $^+$  (CD8SP) or CD4SP, respectively. Finally, TCR:pMHC interactions with high avidity will result in the elimination of the specific T-cell clones via the process of clonal deletion (Daley et al., 2017; Gascoigne and Palmer, 2011; Jameson et al., 1995; Palmer, 2003; Xing et al., 2016). This is in striking contrast to the consequences of high-affinity TCR:pMHC interactions in the periphery, which (in conjunction with CD28 costimulation) result in T-cell activation and proliferation. It is estimated that  $3.7 \times 10^5$  thymocytes will pass these auditions for positive selection every hour in the murine thymus (Stritesky et al., 2013).

### Negative Versus Agonist Selection of Self-Reactive T Cells

Those CD4SP and CD8SP thymocytes that survive positive selection in the cortex then migrate to the medullary part of the thymus. There, they are exposed to a second selection checkpoint, which is required for pruning the number of potentially harmful self-reactive T-cell clones that will, eventually, be released to the immune periphery. This is achieved either by their clonal deletion or their conversion into tTregs through a process called agonist selection (Wirnsberger et al., 2011) (Fig. 5.1A and B). Both outcomes are facilitated by medulla-resident APC, such as thymic dendritic cells (tDCs), B cells and in particular mTECs, which all present self-pMHC complexes to the developing CD4SP and CD8SP thymocytes (Frommer et al., 2010; Hinterberger et al., 2010;

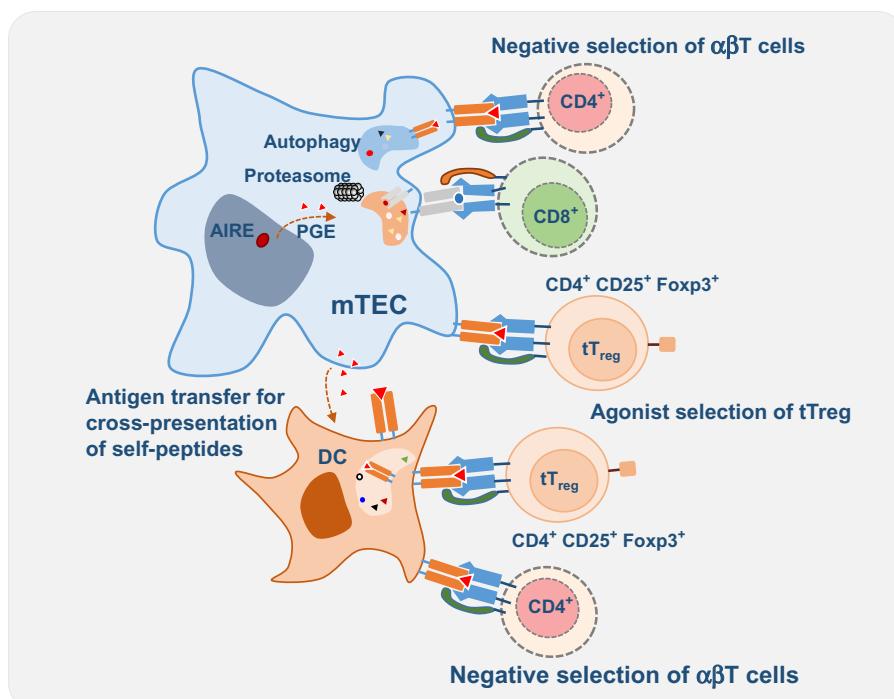


**FIGURE 5.2** The avidity model of thymocytes selection. The TCR $\alpha\beta$  avidity of DP thymocytes for self-peptides largely determines their fate. Thymocytes with TCR $\alpha\beta$  lacking or bearing very weak avidity for the self-pMHC die by neglect, whereas thymocytes with functional TCR $\alpha\beta$ s that engage pMHC with low-intermediate avidity will be positively selected and will differentiate into single positive CD8 $^+$  or CD4 $^+$  naïve T cells. In contrast, very high avidity self-TCR:pMHC interactions will result in the negative selection of the specific clone by apoptosis. Clonal deviation into the tTreg fate occurs across varying avidity intensities.

McCaughtry et al., 2008; Taniguchi et al., 2012; Yamano et al., 2015). Importantly, to be able to effectively screen for self-reactivity to as many self-peptides as possible, mTECs are equipped with a unique capacity to express almost 90% of the coding genome (this phenomenon will be discussed in detail in the following section). Furthermore, in order to subsequently present the corresponding plethora of self-peptides on both MHC I and MHC II molecules, mTECs (and cTECs) were found to have a high constitutive rate of macroautophagy, which enables them to load endogenous peptides onto MHC II molecules and thereby to accommodate selection of CD4<sup>SP</sup> thymocytes (Nedjic et al., 2008) (Fig. 5.3). Moreover, to make the process of self-antigen presentation even more effective, mTECs have been shown to unidirectionally transfer self-Ag to tDCs for cross-presentation (Fig. 5.3). Such unidirectional transfer is believed to be mediated through membrane transfer of self-pMHC II complexes, exosomes, or apoptotic bodies (Koble and Kyewski, 2009) and is thought to prolong the time of antigen presentation and/or broaden the self-pMHC repertoire. Indeed, a recent study by Perry et al. (2014), demonstrated that the self-pMHC repertoire of different thymic APCs is at least partially nonredundant; thus each population can uniquely affect the Ag specificity of the resulting TCR repertoire (Klein et al., 2014). In addition to the presentation of self-antigens that are ectopically expressed in the thymus, the self-repertoire is further potentiated through the import of soluble self-Ag from the periphery into the thymus by plasmacytoid DCs (Hadeiba et al., 2012).

The exact mechanisms that determine the choice between negative and agonist selection of self-reactive thymocytes are still incompletely understood. The currently accepted view is that while thymocytes that express TCRs with very high avidity for the self-pMHC complexes are preferentially eliminated by negative selection, those that express TCRs with low-to-high avidity for self-pMHC are preferentially converted into the tTreg lineage (Fig. 5.2) (Klein et al., 2014).

It should be stressed, however, that measuring self-reactivity of individual thymocytes to the endogenous peptides in an accurate manner under in vivo conditions has been rather challenging. Several molecules have been suggested to be expressed in a manner that correlates with the avidity of the TCR:pMHC interaction, including CD69 (Stritesky et al., 2012), CD5 (Azzam et al., 1998), or Helios (Daley et al., 2013). Nevertheless, probably the most widely accepted model to date for assessment of self-reactive thymocytes in vivo is the Nur77<sup>GFP</sup> reporter, which has been shown to accurately reflect the extent of TCR activation (Moran et al., 2011). Indeed, by crossing mice expressing this reporter with mice lacking the key inducer of apoptosis Bim, Stritesky et al. (2013) were able to determine the number of negatively selected T cells, which undergo apoptosis due to high avidity TCR–pMHC interactions to be  $\sim 7 \times 10^5$  per hour. Paradoxically, this number seems to be  $\sim 6$ -fold higher than



**FIGURE 5.3** mTECs and DCs orchestrate negative and agonist selection. mTECs and thymic DCs cooperate to optimize clonal deletion of self-reactive thymocytes, as well as in the agonist selection of tTregs. mTECs are equipped with a unique capacity to express almost 90% of the coding genome and in order to subsequently present the corresponding plethora of self-peptides on both MHC I and MHC II molecules, mTECs utilize macroautophagy, which enables them to load endogenous peptides onto MHC II molecules and thereby to accommodate the selection of CD4<sup>SP</sup> thymocytes. To make the process of self-antigen presentation even more effective, mTECs have been shown to unidirectionally transfer self-antigen to thymic DCs for cross-presentation. The cooperation between these two cell populations continues as both have a pivotal role in tTreg generation by both presenting the necessary selecting self-pMHC along with the required costimulation.

the estimated total number of positively selected cells (Stritesky et al., 2013), suggesting that positive and negative selection do not occur sequentially but rather in parallel (Laufer et al., 1996; van Meerwijk et al., 1997). Historically, there has been some controversy about the anatomical site of negative selection and the cell populations responsible for mediating this process (Boehmer, 1990; Laufer et al., 1996; van Meerwijk et al., 1997; Murphy et al., 1990). While early studies using transgenic TCR models demonstrated that massive clonal deletion takes place in the thymic cortex (Murphy et al., 1990), other works [based on analysis of specific V $\beta$  deletions of endogenous superantigens (Hengartner et al., 1988; Woodland et al., 1990)] suggested that this process occurs primarily in the medulla (Surh and Sprent, 1994). More recently, it has been demonstrated that the process of T-cell negative selection occurs in both the cortical and the medullary regions of the thymus in two subsequent stages (waves) (Daley et al., 2013; Hu et al., 2016). Specifically, by utilizing the transcription factor Helios as a marker for negative selection, Daley et al. demonstrated that while the first wave involves negative selection of CCR7 $^{-}$  CD24 $^{+}$  CD4 $^{+/\text{lo}}$  CD8 $^{+/\text{lo}}$  DP thymocytes in the cortex, the second wave involves deletion of CCR7 $^{+}$  CD4 $^{+}$  CD8 $^{-}$  SP thymocytes in the medulla (Daley et al., 2013; Hu et al., 2016).

Agonist selection and development of the tTreg lineage is facilitated by APCs in the thymic medulla (Cowan et al., 2013; Hu et al., 2016). While different T-cell populations have been suggested to contribute to tTreg development, including mTECs (Akiyama et al., 2014; Bonito et al., 2013; Cowan et al., 2013; Hauri-Hohl et al., 2014; Hinterberger et al., 2010; Mouri et al., 2014), tDCs (Aschenbrenner et al., 2007; Perry et al., 2014), B cells (Walters et al., 2014), apoptotic thymocytes (Chen and Konkel, 2015; Konkel et al., 2014), or even cTECs (Bensinger et al., 2001; Liston et al., 2008), it seems that the key populations that have the most important role in tTreg generation are mTECs and tDCs (Fig. 5.3), which can both present the necessary selecting self-pMHC along with the required costimulation (Hinterberger et al., 2011; Mahmud et al., 2014). The first experimental evidence of agonist selection of tTregs came in 2001 from the Caton group that elegantly showed that tTreg conversion can occur when a self-reactive TCR meets its cognate antigen, as no agonist selection occurred when the cognate antigen was not expressed by a second transgene (Jordan et al., 2001). Furthermore, TCR  $\times$  neo-self-antigen double transgenic mice were shown to have increased generation of tTreg when the TCR had a high affinity for the neo-self-antigen, but when the affinity was low, agonist selection of tTreg did not ensue. This, along with other studies from the same group (Larkin et al., 2008; Lerman et al., 2004; Picca et al., 2009), prompted their proposition of a modified avidity model (Simons et al., 2010) for T-cell selection in the thymus. Specifically, according to their model, the avidity required for tTreg generation is higher than that of positive selection, but not necessarily lower than that of negative selection and thus the TCR signal strength of thymocytes that are converted into tTreg and those deleted from the repertoire overlap. Nevertheless, other studies have found the TCR signaling threshold for tTreg generation different than that suggested by the Caton group. For example, the Nur77<sup>GFP</sup> model for gauging TCR activation strength has been instrumental in showing that the signaling strength for agonist selection of the natural polyclonal tTreg pool is indeed strong, but just below the threshold for clonal deletion (Stritesky et al., 2013). Correspondingly, Lee et al. have demonstrated that thymocytes with varying TCR affinities for their cognate antigen can develop into tTreg (Lee et al., 2012), indicating that the spectrum of affinities required for agonist selection seems to be quite broad (Fig. 5.2).

Moreover, in line with the above findings, tTregs were found to have a very distinct, mostly nonoverlapping, TCR repertoire in comparison to conventional T cells (Hsieh et al., 2004; Pacholczyk et al., 2006) (at least in mice). Although tTregs are assumed to be characterized mainly by self-reactive TCRs (Hsieh et al., 2012), elucidating the actual identity of the naturally selecting self-antigens has been very challenging. The first identification of such naturally occurring Treg self-antigen peptides was recently reported by the Savage group, who identified two different Treg clones that recognize peptides of a prostate-specific protein, which is ectopically expressed in the thymus in an Aire-dependent manner (Leonard et al., 2017; Malchow et al., 2013, 2016).

Interestingly, the appearance of tTreg in the mouse thymus takes place only at days 2–3 after birth, which is several days later than the appearance of conventional CD4SP T cells (Fontenot et al., 2005). Although the reasons for such a delayed tTreg development are still incompletely understood, one possible explanation is that the development and selection of tTreg take longer than that of conventional T cells. Remarkably, however, the perinatal tTreg population seems to have an indispensable role in the induction of self-tolerance, as its depletion prior to 10 days of age results in detrimental multiorgan autoimmunity in mice (Yang et al., 2015). These and other findings collectively argue that agonist selection of tTreg is superior to clonal deletion for the effective induction and maintenance of immunological self-tolerance. The mechanisms by which tTreg facilitate self-tolerance in peripheral tissues is discussed in detail in one of the subsequent sections of this chapter.

## Promiscuous Expression of Self-Antigens in the Thymus

The grand task of both negative and agonist selection necessitates presentation of the entire self-peptidome to developing thymocytes. As mTECs are essential players in this endeavor, they have uniquely developed the capability of promiscuously expressing thousands of TSA genes in a manner independent of their regulated expression in the periphery (Sansom et al., 2014; Tykocinski et al., 2010; Villaseñor et al., 2008). The contribution of such TSA expression in the thymus to the establishment of self-tolerance is best evidenced by Aire deficiency, which leads to multiorgan autoimmunity due to impaired expression of hundreds of TSA genes in mTECs (Anderson et al., 2002) or by the autoimmune manifestations seen following thymic specific deletion of individual TSA genes (DeVoss et al., 2006; Fan et al., 2009). Importantly, the extent and features of this PGE have been understood in more depth due to the advent of high-throughput sequencing technologies, both on the population and single-cell levels. Remarkably, as a population, mature mTECs express roughly 19,000 protein-coding genes, which corresponds to ~85% of the coding genome, ranking them as a population with the highest levels of genome expression that has been documented (Danan-Gothold et al., 2016; Sansom et al., 2014). In comparison, all other tissues and cell types examined expressed between 12,000 and 15,000 genes (Danan-Gothold et al., 2016) (~60%–70% of the protein-coding genes). Interestingly, representation of all tissues is not created equal in mTECs, as studies have found underrepresentation of odorant receptor genes (Sansom et al., 2014), while others have reported underrepresentation of genes from immunologically privileged organs such as brain and testes, suggesting that negative selection might take into account the likelihood of antigen encounter in the periphery, as these sites undergo less immunological surveillance than nonprivileged sites (Danan-Gothold et al., 2016). Nevertheless, mTECs also significantly enhance the depth of their self-antigen repertoire by using extensive RNA editing and processing (Danan-Gothold et al., 2016).

Examination of the mTEC transcriptome at the single-cell level has revealed that in contrast to the almost complete genomic expression at the population level, each individual mTEC only transcribes a fraction of TSAs resulting in a remarkably heterogeneous population such that only 1%–9% of the entire population will express a given gene at any given moment (Derbinski et al., 2008; Klein et al., 2014; Sansom et al., 2014). This expression pattern was initially reported to be stochastic (Derbinski et al., 2008; Villaseñor et al., 2008); however, later studies showed that there is some order to the “madness,” as mTECs coexpress groups of TSAs that are clustered both intra- and interchromosomally (Brennecke et al., 2015; Pinto et al., 2013) in what has now been described as “ordered stochasticity” with substantial variability between individuals (Meredith et al., 2015). Nevertheless, this process is estimated to be so efficient that encountering a mere 200–500 mTECs should suffice in presenting the entire TSA repertoire to a developing SP thymocyte (Klein et al., 2014).

How mTECs are capable of ectopically expressing genes that are otherwise restricted to specific parenchymal tissues has been a major focus of many independent studies, with most research concentrating on Aire, as the master regulator of this process. Although Aire was originally proposed to induce expression of hundreds of genes (Anderson et al., 2002), over the years, the scope of Aire-dependent targets has been shown to be an order of magnitude larger (i.e., ~4000 Aire-dependent genes) (Danan-Gothold et al., 2016; Sansom et al., 2014). Given that Aire’s primary role is to promote expression of genes that are normally silenced in the vast majority of cells in the body (i.e., they are tissue-specific), it is not surprising that its target gene recognition was found to utilize epigenetic marks, specifically those associated with silenced chromatin. To this end, Aire has been shown to bind unmethylated lysine 4 on histone 3 (H3K4me0) (Koh et al., 2008; Org et al., 2008), a histone modification that typically marks transcriptionally inactive chromatin. Aire was also shown to have the capacity to bind to H3K4me1 (Koh et al., 2008), a histone modification highly enriched in gene enhancer regions (Pott and Lieb, 2014). Indeed, more recent data demonstrate that Aire preferentially binds to superenhancer regulatory regions in bona fide mTECs (Bansal et al., 2017). Furthermore, other studies have demonstrated that Aire-dependent genes are depleted of active chromatin marks at their promoters (Org et al., 2009; Sansom et al., 2014), while they are enriched for additional repressive chromatin marks such as H3K27me3 and H3K9me3 (Sansom et al., 2014). Collectively, these data suggest that Aire preferentially binds to inactive/poised enhancers of silenced genes (characterized by H3K4me1 and H3K27me3) and subsequently activates them (i.e., H3K27me3 → H3K27ac) through recruitment of various chromatin modifying enzymes. Aire’s attraction to repressed chromatin is further supported by its association with the repressive ATF7IP–MBD1 complex, which is recruited to methylated CpG sites, known to be enriched at promoters of inactive genes (Waterfield et al., 2014).

Following its recruitment to the relevant TSA genes Aire induces the derepression of silenced chromatin and activates transcription by interacting with proteins of the DNA damage machinery, including DNA-PK, Top2a,

$\gamma$ H2AX, Trim28, or Sirt1 (Abramson et al., 2010; Bansal et al., 2017; Chuprin et al., 2015; Guha et al., 2017). Interestingly, Trim28 was recently suggested to have a central role in linking the DNA damage response with release of paused RNA Pol II (Bunch et al., 2015), which is in line with several studies demonstrating an important role for Aire in releasing stalled RNA polymerase II at its target genes for PGE (Giraud et al., 2012; Owen et al., 2007).

While Aire was shown to induce/boost expression of thousands of different genes in mTECs, part of the TSA gene repertoire seems to be regulated in an Aire-independent manner. Recently, the transcription factor Fezf2 was suggested to take on such a role and was proposed to induce the expression of a significant proportion of Aire-independent TSA genes (Takaba et al., 2015). Appropriately, its deletion was reported to result in multiorgan autoimmunity, though distinct from that observed in Aire-deficient mice. However, independent analysis of Fezf2-dependent genes in mTECs failed to corroborate these findings, suggesting that further studies are needed to determine the specific contribution of Fezf2 to tolerance induction (Y. Herzig and J. Abramson, unpublished data).

## INDUCTION AND MAINTENANCE OF IMMUNOLOGICAL TOLERANCE IN THE PERIPHERY

A significant body of evidence suggests that not all self-reactive thymocytes are efficiently deleted or converted into FOXP3<sup>+</sup> tTreg during their development in the thymus. As already discussed above, thymic negative selection most effectively deletes thymocytes that express TCRs with very high avidity for self-pMHC complexes presented by mTECs and tDCs. Correspondingly, thymocytes that express TCRs with low-to-high avidity for self-pMHC complexes were shown to give rise to FOXP3<sup>+</sup> tTregs. Nevertheless, many self-reactive T-cell clones (typically bearing TCRs of relatively low avidity for self-pMHC complexes) were shown to escape to the periphery (Liblau et al., 1991; Sun et al., 1991). More recently, Legoux et al. demonstrated that only ~60% of the Cre recombinase-specific CD4<sup>+</sup> T cells are deleted in the thymus and periphery of mice transgenic for ubiquitous expression of Cre. In contrast, no deletion of Cre-specific T-cell clones was observed when Cre expression was restricted to specific peripheral tissues but not to the thymus (Legoux et al., 2015). Similarly, recent analyses of self-reactive T-cell clones in the periphery of wild-type mice using a tetramer reagent further reinforced the notion that many (or even most) self-reactive T cells escape negative selection in the thymus (Davis, 2015; Yu et al., 2015b). Therefore these self-reactive escapee clones must be kept in check by additional tolerance mechanisms in the periphery to prevent autoimmune damage (discussed next).

This phenomenon has been validated in animal models by several independent studies, including that of Liu et al., who created TCR-transgenic mice specific to myelin basic protein (MBP) acetylated peptide Ac1-9 presented in the context of MHC class II (Liu and Wraith, 1995). This peptide is highly immunogenic and, when administered in the presence of adjuvant, it induces experimental autoimmune encephalomyelitis (EAE) in mice, a model for human multiple sclerosis. Remarkably, although these TCR-transgenic mice have self-reactive CD4<sup>+</sup> T cells in their peripheral blood, the mice do not spontaneously develop autoimmune disease. Therefore this study clearly demonstrates that even if a self-reactive T cell escapes the first thymic filter, it is efficiently kept in check by additional tolerance mechanisms in the periphery. Moreover, repeated immunization with the peptide in the absence of adjuvant lead to the development of specific tolerance. In contrast, the break of tolerance resulting in EAE was observed only after immunization with MBP Ac1-9 in the presence of an adjuvant (Pertussis toxin), nicely illustrating that the context of antigen presentation determines whether tolerance will be enforced or broken down (discussed in more detail in one of the following sections).

### Ignorance and Antigen Sequestering

The first line of tolerance mechanisms that prevents self-reactive T cells to mount an autoimmune attack upon their release from the thymus is their physical separation from the parenchymal tissues (Fig. 5.1C). After leaving the thymus, naïve T cells circulate from blood to secondary lymphoid organs, to efferent lymph, and then back again to the blood. Such restricted migration is guided by several chemokines (CCL21) and adhesion molecules (ICAM1, GlyCAM1), which are specifically expressed by the high venule endothelium of the secondary lymphoid organs. Since naïve T cells express high levels of CCR7 (i.e., receptor for CCL21), LFA1, and CD62L (receptors for ICAM1 and GlyCAM1, respectively), they can exit the bloodstream only to the secondary lymphoid organs,

but not to other nonlymphoid tissues. In the lymph nodes, the naïve T cells scan DCs for the presence of pathogen-derived peptides presented on MHC molecules (Mempel et al., 2004). If TCR ligation does not occur, desensitization of CCR7 and recognition of efferent lymph sphingosine 1-phosphate eventually drives naïve T cells out of the lymph node back to the bloodstream. Thus naïve T cells are excluded from nonlymphoid peripheral tissues, in which the likelihood of coming in contact with various TSAs is considerably higher.

Unlike naïve T cells, antigen-experienced effector T cells can migrate to virtually all peripheral tissues of the body; in particular, if these tissues are infected and/or inflamed. To this end, effector T-cell upregulate several adhesion molecules on their surface (e.g., P- and E-selectin ligands, integrin  $\alpha 4\beta 1$ ), which allow their subsequent egress from postcapillary venules into the individual parenchymal organs, particularly in the context of local inflammation or infection (Austrup et al., 1997; Masopust et al., 2004). It should, however, be stressed that in order to become activated, naïve T cells first need to be “licensed” by lymph node–resident DCs, which can effectively discriminate between harmless and harmful antigens (discussed in detail in the following section).

Importantly, some key vital organs in the body such as the brain, eye, and testis are characterized by reduced lymphatic drainage, which consequently limits the surveillance of these sites by the immune system, and its ability to become activated in response to local infection/inflammation (Galea et al., 2007). Therefore these vital structures are thought to have evolved an “immune privilege,” which protects them from the potentially damaging effects of an inflammatory immune response. However, even sequestered antigens in these immunologically privileged sites can become exposed to the immune system in response to local tissue damage (disrupting their physical separation) accompanied by local inflammation (whether pathogen-induced or sterile). As a result, this leads to the activation of “ignorant” self-reactive T cells, which circulate in the bloodstream and treat the exposed self-antigen as nonself. This, in turn, triggers an autoimmune reaction that may ultimately progress to disease development. Naturally, the strengths of such an autoimmune response will depend on a number of factors, including the nature of the self-antigen, the level of its exposure, number of activated T cells specific to the given self-antigen and the level of expression of MHC and costimulatory molecules in the affected tissues. Therefore local tissue damage (e.g., following physical injury and/or local infection) may greatly increase the risk that a potentially self-reactive T-cell will gain access to the given parenchymal tissue in general and immunologically privileged sites in particular and thereby initiate a specific autoimmune response. This can be very well illustrated by several good examples. For instance, bilateral testicular injury in mice was shown to result in the exposure of sequestered testicular antigens and local inflammation. This is followed by autoimmune reaction against the autologous testicular cells ultimately causing autoimmune orchitis and male infertility (Sakamoto et al., 1995).

Moreover, as already discussed above, tolerance to immunologically privileged sites can be easily broken down by mere administration of a given TSA in the presence of an adjuvant (i.e., even without the requirement of causing local tissue injury). The best example is that immunization with myelin or myelin-derived peptides in the presence of an adjuvant (i.e., danger signal) is sufficient to activate self-reactive, yet ignorant, T cells that are specific to the respective peptide and circulate in a quiescent state in the periphery. Such activated myelin-specific T-cell clones can then subsequently cross the blood–brain barrier and initiate an autoimmune destruction of myelin sheets that insulate neurons in the brain (Steinman, 2015).

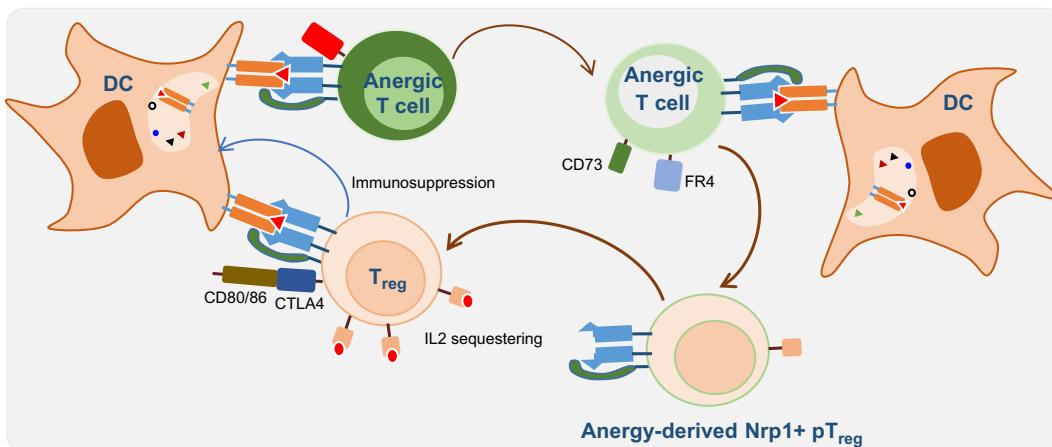
## Dendritic Cells, the Key Inducers of Peripheral T-Cell Tolerance

The context in which a naïve T-cell clone will encounter a specific antigen in the secondary lymphoid organs determines whether it will become activated or rendered unresponsive (i.e., tolerant) (Banchereau and Steinman, 1998). Specifically, a T cell will become activated only if it recognizes a peptide antigen presented (in the context of MHC-I or MHC-II molecules) by lymph node resident–activated DCs expressing high levels of B7 costimulatory molecules, which will then engage the corresponding CD28 coreceptors on the T-cell surface (Linsley et al., 1990). In contrast, if a T-cell clone recognizes a peptide antigen presented by nonactivated DCs that express low levels of costimulatory molecules, the clone will fail to proliferate and will become unresponsive to further stimulation. Thus the maturation state of the DC (characterized by expression of B7 costimulatory molecules) determines whether a specific T-cell clone will undergo activation and differentiation or rendered tolerant to the specific antigen (Dhodapkar et al., 2001; Garza et al., 2000; Hawiger et al., 2001; Mempel et al., 2004) (Fig. 5.1D).

Specifically, before their recruitment to the lymph node, nonactivated immature DCs reside within peripheral tissues, in which they continuously look for potentially harmful elements (e.g., pathogens, injury, necrosis) (Banchereau and Steinman, 1998). To this end, they utilize macropinocytosis, which allows them to engulf and process surrounding antigens (both self and nonself) and subsequently present them on their surface in the context of MHC-II molecules. Importantly, if the antigen is marked by a “danger” signal, which is recognized via specific receptors (e.g., TLR, NLR, RIG-I-like receptors) that detect either pathogen-associated molecular patterns or damage-associated molecular patterns that are released within tissues as a consequence of cellular distress, the immature DC becomes activated and migrates to the draining lymph node. This is accompanied by upregulation of several costimulatory molecules (e.g., CD80/86, CD40) and of MHC-II (presenting specific peptide antigens) on the DC surface, as well as by simultaneous downregulation of macropinocytosis and antigen processing. The high expression of CD80/CD86 costimulatory molecules on activated DCs provides a critical signal to the naïve LN (or splenic) resident T cell via its CD28 coreceptor expressed on the T-cell surface. Such costimulation leads to activation of several key transcription factors (e.g., NF- $\kappa$ B, NFAT, and AP-1), which in turn promote expression of various genes (e.g., IL2, CD25) that are critical for full T-cell activation (Müller and Rao, 2010; Rudensky et al., 2006). Furthermore, based on the specific “danger” signal, the activated DCs will produce diverse proinflammatory cytokines (e.g., IL4, IL6, IL12, IL23), which in turn determine the specific Th subset into which the responder T-cell clone will differentiate.

However, if the engulfed antigen is not marked by a “danger” signal (e.g., self-antigen or harmless antigen), the resting DC will not become activated. Consequently, the DC will be unable to provide the necessary costimulatory signals that would induce proliferation and differentiation of the naïve T-cell clones that bear a TCR specific for antigen peptides presented by the DC. It has been demonstrated that most naïve T-cell clones that have just emigrated from the thymus to the periphery (recent thymic emigrants) become tolerized by lymph node–resident DCs due to lack of costimulation (Friesen et al., 2016). Interestingly, DC activation was also shown to be suppressed by uptake of cellular material from dead or dying cells, suggesting that macropinocytosis of apoptotic (but not necrotic) cells, is one of the key mechanisms for the induction of T-cell unresponsiveness (i.e., tolerance) to self-antigens in the periphery (Gallucci et al., 1999). The state of such long-term functional unresponsiveness to antigen recognition is called T-cell anergy (Jenkins et al., 1987). Although anergy was originally thought to be simply caused by lack of costimulation, subsequent studies have demonstrated that it is also induced/maintained by activation of various inhibitory receptors [e.g., CTLA-4, programmed cell death 1 (PD-1), Lag3] on the T-cell surface that counteracts proximal TCR signaling/costimulation and thereby T-cell activation (Müller and Rao, 2010). Specifically, TCR signaling with insufficient CD28 costimulation was found to result in activation of the NFAT, but not of the AP-1, transcription factor (Müller and Rao, 2010). Consequently, activated NFAT cannot form transcriptional complexes with AP-1, and instead forms homodimers, which are directly responsible for the induction of anergy-associated genes such as ubiquitin ligases *GRAIL*, *ITCH*, and *CBLB*, as well as to active transcriptional silencing of several genes critical for T-cell activation (e.g., the *IL2* locus) (Müller and Rao, 2010; Soto-Nieves et al., 2009).

Although anergy-associated transcriptional programs have been well characterized, the identification of specific and reliable surface markers that would enable identification and isolation of anergic T cells has been much more challenging (Kalekar and Mueller, 2017). More recently, Mueller et al. have identified a panel of predictive markers for anergic CD4<sup>+</sup> T cells (Kalekar et al., 2016; Martinez et al., 2012). Specifically, they have demonstrated that CD4<sup>+</sup> T cells that have persistently recognized self-antigen in the periphery enter a CD44<sup>hi</sup> CD73<sup>hi</sup> FR4<sup>hi</sup> FOXP3<sup>-</sup> unresponsive state and are enriched for self-antigen–specific TCRs. Most importantly, however, these anergic CD4<sup>+</sup> T cells could subsequently differentiate into functional FOXP3<sup>+</sup> NRP1<sup>+</sup> pTregs *in vivo* (discussed in more detail in the following section), which in turn, can promote anergy of pathogenic CD4<sup>+</sup> T cells and inhibit autoimmunity (Kalekar and Mueller, 2017; Kalekar et al., 2016) (Fig. 5.4). Thus the induction of anergy not only serves to diminish the proliferative responsiveness of potentially dangerous self-reactive CD4<sup>+</sup> T cells but also seems to be an important prerequisite for generation of pTregs (Kalekar and Mueller, 2017; Kalekar et al., 2016). Moreover, the crosstalk between anergic and Tregs is further reinforced by the fact that Tregs were found to induce and/or maintain T-cell anergy (Fig. 5.4) (Martinez et al., 2012). Such a positive feedback loop in which anergic T cells give rise to Tregs, which, in turn, promote that generation of more anergic T cells is quite reminiscent of the *in vivo* “infectious tolerance” model previously proposed by Kendal and Waldmann (2010). Moreover, such a model could also explain the rather overlapping requirements for DC-mediated generation of anergic and pTregs, which both depend on presentation of very low levels of self-antigen along with insufficient costimulation (CD80/86/40) and absence of proinflammatory cytokines (IL6 and IL12) (Kretschmer et al., 2005; Maldonado and von Andrian, 2010).



**FIGURE 5.4** The relationship between anergic T cells and pTreg generation. Self-Ag-specific CD4<sup>+</sup> T cells that escape into the periphery can recognize peripheral self pMHCII, and through the suppressive actions of Tregs become anergic cells that express CD73 and FR4. Reencounter(s) of self-Ag induces Nrp1 expression and partial demethylation of Treg-specific loci in some of the anergic T cells. Nrp1<sup>+</sup> anergic T cells then become precursors for Treg differentiation. Upon conversion to a stable pTreg lineage, anergy-derived Tregs join the tTreg pool to suppress immunopathology and reinforce anergy induction.

### Intrinsic Mechanisms Suppressing Clonal Expansion and/or Reactivation

Although a specific T-cell clone is allowed to dramatically expand in numbers at the expense of other clones (clonal expansion) after antigen encounter, most activated T-cell clones eventually undergo apoptosis (with a small fraction surviving as memory cells), in order to preserve the diversity of the repertoire and immune homeostasis (Marrack et al., 2000). Such a mechanism has obvious implications in the maintenance of self-tolerance in the periphery, where repeated/chronic encounter with self-antigen could result in uncontrolled expansion of self-reactive T-cell clones.

Activation-induced cell death (AICD) is an intrinsic regulatory mechanism used by certain types of lymphocytes, including T cells, to undergo apoptosis upon their clonal expansion. A key mechanism underlying AICD in T cells is the upregulation of Fas receptors on their surface in response to their activation and its subsequent interaction with its ligand, which leads to apoptotic death (Lenardo, 1996; Piazza et al., 1997) (Fig. 5.1E). Thus activated T cells that express both Fas and FasL undergo apoptosis, which is mediated through their mutual contact. Such an intrinsic regulation is critical in maintaining immune tolerance in the periphery. This is best evidenced by a loss of function of either *Fas* or *Fasl* genes, which results in autoimmune lymphoproliferative syndrome (Puck and Sneller, 1997).

Furthermore, T-cell activation also results in dramatic upregulation of CTLA-4 on its surface, which is critical for keeping the expansion of the specific T-cell clone in check (Fig. 5.1F). This is most clearly demonstrated by experimentally induced CTLA-4 deficiency, which results in massive fatal lymphoproliferation and multiorgan autoimmunity in mice (Tivol et al., 1995; Waterhouse et al., 1995). Therefore CTLA-4-mediated inhibition of T-cell expansion is essential for the maintenance of self-tolerance and immune homeostasis. The molecular mechanisms of how CTLA-4 prevents T-cell expansion are complex and still somewhat controversial. While earlier studies suggested that CTLA-4 operates as a classical inhibitory immune receptor, which counteracts TCR signaling through recruitment of phosphatases to the TCR (Lee et al., 1998), more recent studies argue against this model. Specifically, Sansom et al. have suggested that CTLA-4 operates through capturing and removing CD80/CD86 molecules from the membranes of antigen-presenting cells, thus making these unavailable for CD28 costimulation (Qureshi et al., 2011). Consequently, additional T-cell clones would not be activated and would not be able to undergo clonal expansion (Fig. 5.1F).

While CTLA-4 predominantly regulates activation of naïve T-cell clones and their subsequent clonal expansion, the inhibitory receptor PD-1 was shown to negatively regulate effector T-cell functions (Freeman et al., 2000; Ishida et al., 1992). PD-1 expression levels are transiently upregulated upon activation of naïve T cells; however, its sustained expression is most characteristic of effector T cells that have become functionally exhausted (Barber et al., 2006). T-cell exhaustion is an intrinsic mechanism that prevents further activation of effector T cells upon repetitive (chronic) antigen encounter (Wherry and Kurachi, 2015). Given that self-antigens may induce repetitive

T-cell activation, such an intrinsic regulation seems critical for the maintenance of immune tolerance in the periphery. This is best evidenced by an experimental inactivation of the gene encoding PD-1, or the genes encoding its two ligands PD ligand 1 (PD-L1) and PD-L2, which result in the development of systemic autoimmunity (Freeman et al., 2000; Keir et al., 2006; Nishimura et al., 1999). Therefore PD-1–PD-1 ligand interactions are critical for restraining self-reactive effector T cells that enter the peripheral tissues and find self-pMHC.

## Dominant Tolerance Through Treg-Mediated Immunosuppression

As discussed and illustrated above, the effective induction and maintenance of self-tolerance in the periphery is underlain by multiple sophisticated regulatory mechanisms, which complement and reinforce each other. Among these, Tregs are regarded as the chief mediators of peripheral tolerance, due to their powerful immunosuppressive capacity, which allows them to counteract the effects of both helper and cytotoxic T cells in a dominant fashion (as opposed to clonal deletion, ignorance, or anergy). Their physiological significance in this process is best illustrated by the development of a severe autoimmune syndrome in humans and mice that lack Tregs as a result of FOXP3 loss of function or CD4<sup>+</sup>CD25<sup>+</sup> T-cell depletion (Fontenot et al., 2003; Hori et al., 2003; Wildin, 2002). This means that deficiency or dysfunction of Tregs alone is sufficient to break self-tolerance and cause severe autoimmunity.

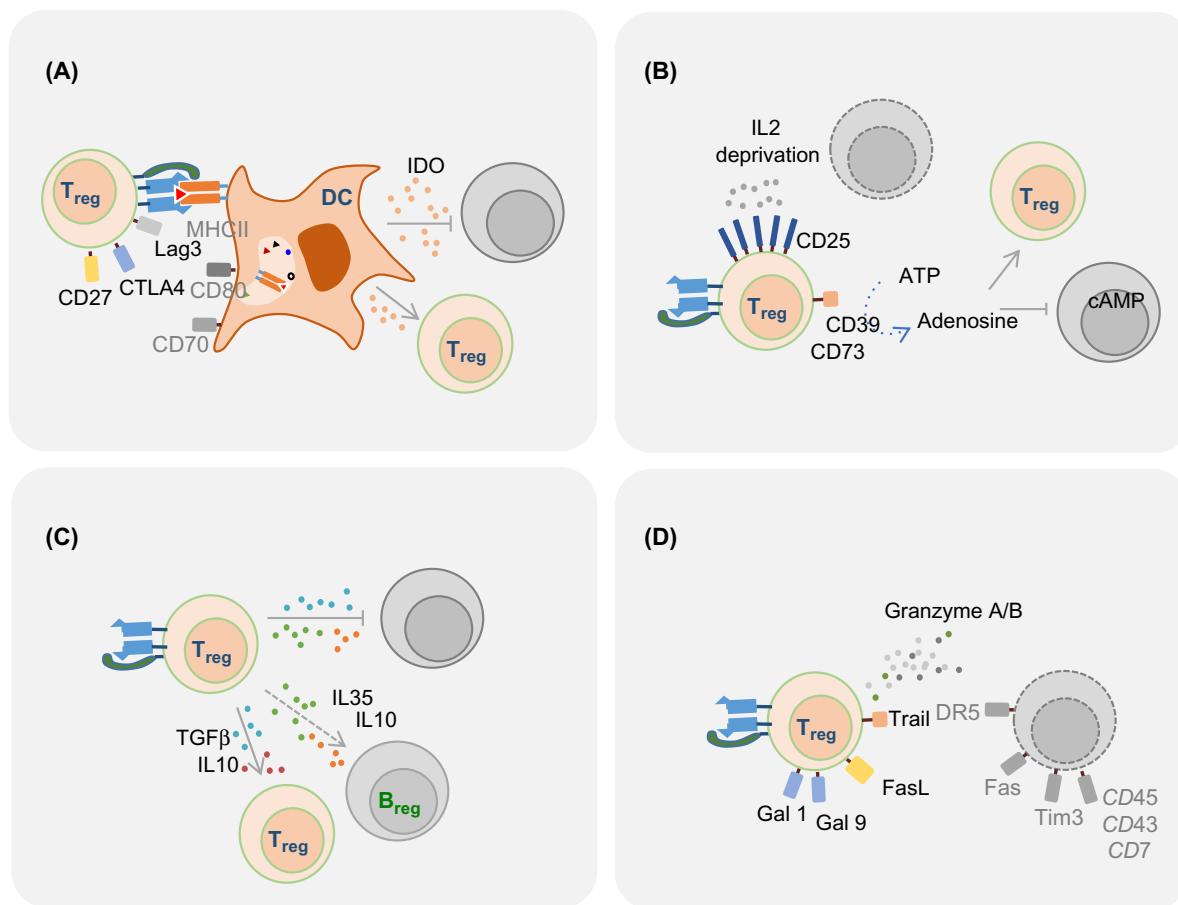
### Treg Mechanisms to Maintain Tolerance

Despite the clear importance of Treg in the induction of peripheral tolerance, the molecular and cellular mechanisms of their immunosuppressive potential remain incompletely understood and controversial. A body of evidence demonstrates that Treg can suppress self-reactive lymphocytes by several different (and likely complementary) mechanisms at multiple checkpoints. For instance, Treg can suppress activation, expansion, and differentiation of naïve T cells in lymphoid organs, restrict the traffic and recruitment of effector T cells to specific parenchymal tissues, or suppress proliferation and function of effector T cells within the peripheral tissues. To achieve maximal suppressive capacity, Tregs require TCR signaling (Levine et al., 2014; Vahl et al., 2014). However, Tregs were also shown to exert their suppressive capacity in an antigen-independent, bystander manner. The immunosuppressive mechanisms utilized by Treg (discussed in detail below) can be both direct and indirect and mainly include (1) modulation of antigen presentation, (2) metabolic disruption, (3) cytokine production, and (4) induction of cytolysis/apoptosis (Vignali et al., 2008) (Fig. 5.5).

Modulation of antigen presentation is considered to be one of the key mechanisms by which Treg indirectly suppress effector T cells. This mechanism is mainly facilitated by CTLA-4, which is highly and constitutively expressed on the surface of Treg, and which directly competes with CD28 for binding to CD80/86 ligands on DCs. Strikingly, recent studies demonstrate that CTLA-4 does not just passively block the CD80/86 ligands, but rather literally rips them off the DC surface and facilitates their subsequent engulfment by Treg, in a process called transendocytosis (Qureshi et al., 2011; Wing et al., 2008). Such a dynamic and continuous process seems to be much more efficient in downmodulation of CD80/86 expression on the DC surface than a passive blockage and seems to constitute one of the key mechanisms by which Treg suppress activation of effector T cells. More recently, an analogous mechanism was observed also for Treg-dependent CD27-mediated endocytosis of CD70 from the DC surface (Dhainaut et al., 2015), which further limits the level of costimulatory signals required for T-cell activation. In addition to CTLA-4, Tregs were also shown to utilize the inhibitory receptor Lag3 for modulation of antigen presentation on DCs. Lag3 is highly and constitutively expressed on the surface of Treg and directly binds MHCII molecules on APC, including DC. This was shown to suppress DC activation and their subsequent capacity to activate T cells (Liang et al., 2008).

Tregs also utilize various key metabolites to facilitate and/or potentiate their immunosuppressive capacity. For instance, Tregs were shown to induce production of immunosuppressive molecules such as Indoleamine 2,3-dioxygenase by DCs through CTLA-4 binding to CD80/86 ligands (Fallarino et al., 2003; Grohmann et al., 2002). This enzyme catabolizes tryptophan to kynurenine, which induces Treg differentiation via aryl hydrocarbon receptor (Mezrich et al., 2010; Opitz et al., 2011). In addition, Tregs express CD39 and CD73, two different ectoenzymes, which mediate breakdown of ATP or ADP into adenosine. Adenosine can then bind the A2A receptor on activated effector T cells, which in turn results in their direct suppression due to increased levels of cAMP (Borsig et al., 2007; Deaglio et al., 2007; Kobie et al., 2006).

The immunosuppressive potential of Treg is further mediated by their capacity to produce various immunosuppressive cytokines such as TGFβ, IL10, and IL35 that can either inhibit effector T cells or DCs



**FIGURE 5.5** Treg mechanisms to maintain tolerance. The immunosuppressive mechanisms utilized by Tregs can be both direct and indirect and mainly include (A) modulation of antigen-presenting cells, (B) metabolic disruption, (C) cytokine production, and (D) induction of cytosis/apoptosis. (A) Modulation of antigen-presenting cells includes altering DC maturation and function through CTLA-4, CD27, or Lag3, resulting in inhibition of effector cells and induction of Tregs. (B) Metabolic disruption includes IL2 consumption that inhibits effector cells and immunosuppression via CD39- and CD73-generated adenosine that increase cAMP in effector cells that can also be transferred through gap junctions. (C) Inhibitory cytokines include TGF $\beta$ , IL10, and IL35. In addition, TGF $\beta$  and IL10 induce more Tregs, while IL35 and IL10 may, in addition, induce other immunosuppressive subsets such as Bregs. (D) Cytosis mechanisms include the production of Granzyme A and B. Induction of apoptosis is mediated by TRAIL-DR5, FasL-Fas, Galectin-9-Tim3, and Gal-1 with an unknown ligand (probably CD45, CD43, or CD7). Source: Adapted from Vignali, D.A.A., Collison, L.W., Workman, C.J., 2008. How regulatory T cells work. *Nat. Immunol. Rev.* 8, 523–532.

(Andersson et al., 2008; Asseman et al., 1999; Collison et al., 2007; Fahlén et al., 2005; Nakamura et al., 2001). In addition, some of these cytokines (e.g., TGF $\beta$ ) are critical for Treg induction, while others (IL10 and IL35) may also contribute to induction of other immunosuppressive subsets such as regulatory B cells (Chen and Wahl, 2003; Collison et al., 2010; Hsu et al., 2015; Wang et al., 2014). In addition to cytokine production, several studies provided evidence that Tregs also consume certain cytokines, such as IL2, to exert their suppressive functions (De La Rosa et al., 2004). Although Treg cannot produce IL2, they express high levels of receptors that bind IL2 with high affinity (CD25). Therefore Tregs deprive local effector T cells from the IL2 required for their growth and survival (de la Rosa et al., 2004). Some studies also argue that IL2 deprivation is critical for direct induction of apoptosis (i.e., clonal deletion) of effector T cells in the periphery (Pandiyan et al., 2011). Recently, however, it has been demonstrated that while IL2 deprivation is dispensable for CD4 suppression it is necessary for limiting CD8 activation (Chinen et al., 2016).

Finally, Tregs were also shown to directly induce clonal deletion of effector T cells in the periphery. To this end, Tregs produce and secrete powerful weapons such as Granzyme A and Granzyme B that cause cytosis of effector cells in perforin-dependent and independent manners (Cao et al., 2007; Gondevi et al., 2005; Grossman et al., 2004; Zhao et al., 2006). Similarly, Tregs express additional molecules that can directly induce apoptosis of effector T cells in their proximity, including TRAIL-DR5, (Ren et al., 2007), FasL-Fas, (Weber et al., 2006; Zhang et al., 2000),

galectin-1 (Garín et al., 2007), and galectin-9 (Liberal et al., 2012; Wang et al., 2009). In addition to inhibiting or inducing death of the effector cell, there is evidence of Treg influencing traffic and recruitment of effector T cells into the tissue (Davidson and Shevach, 2011; Fu et al., 2014).

Despite all the recent progress in elucidating the mechanisms of Treg-mediated suppression, our understanding remains incomplete. For instance, it is still unclear whether there is a key superior mechanism that would account for most of their suppression potential, whether multiple mechanisms are required, whether some of the mechanisms are redundant and most importantly what is their relative importance *in vivo*. It seems likely that the broad variety of suppressive mechanisms observed is linked to diverse Treg subsets with different activation status that act at distinct times and locations.

### **Treg Diversity and Their Role in Self-Tolerance**

In recent years, it has been widely recognized that Tregs are extremely heterogeneous. Initially it was believed that Tregs are uniquely produced in the thymus (tTreg, former natural Treg discussed in detail in the section “Negative Versus Agonist Selection of Self-Reactive T Cells”). A recent body of evidence, however, demonstrates that Tregs also develop in the periphery from conventional CD4<sup>+</sup> T cells (pTreg, former induced Treg) (Abbas et al., 2013). The development of pTreg occurs preferentially from recent thymic emigrants (Paiva et al., 2013), under certain conditions such as presence of immunosuppressive cytokines like TGFβ or other environmental factors like diet- and microbiota-related antigens (Arpaia et al., 2013; Atarashi et al., 2011; Benson et al., 2007; Chen et al., 2003; Furusawa et al., 2013; Smith et al., 2013). The Treg is further potentiated by their different antigen-specificities, tissue-tropisms, and specialized functions including tissue repair or metabolic regulation (Ali et al., 2017; Arpaia et al., 2015; Burzyn et al., 2013; Cipolletta, 2014; Dombrowski et al., 2017; Feuerer et al., 2009; Gratz and Campbell, 2014; Nosbaum et al., 2016; Panduro et al., 2016). Although large strides have been made in Treg biology over the last decades, definitive data regarding the role and relative contributions of individual Treg subsets in tolerance is difficult to obtain at this point, mainly due to the lack of consensus markers to define such a plastic and heterogeneous subset of cells.

Traditionally, tTregs have been considered to play a dominant role in the induction/maintenance of tolerance to self-antigens, while pT<sub>res</sub> have been thought to play a key role in the induction of tolerance to harmless foreign antigens (e.g., fetus, food, microbiota) (Samstein et al., 2012) and the maintenance of immune homeostasis, mainly at barrier sites where they accumulate (Lathrop et al., 2011). This later assumption is supported by the observation that mice deficient for pTreg (*Foxp3* enhancer CNS1 deficient mice) develop Th2-dependent inflammation at mucosal sites rather than generalized autoimmunity (Josefowicz et al., 2012b). However, other reports suggest that pTreg also perform essential functions in induction of self-tolerance. For instance, it has been shown that gut microbiota induces the production of a peptide by pancreatic beta cells, resulting in Treg production and protection from type-1 diabetes (Sun et al., 2015). Correspondingly, TCR sequencing made evident that a significant proportion of Treg clones in the colon were of thymic origin indicating that tTregs also promote tolerance to microbiota (Cebula et al., 2013). More recently, two distinct tTreg subsets have been identified: (1) a “triple<sup>hi</sup>” (GITR<sup>hi</sup>PD-1<sup>hi</sup>CD25<sup>hi</sup>) population, which is highly self-reactive and limits lymphoproliferation in lymph nodes; and (2) a “triple<sup>lo</sup>” population (GITR<sup>lo</sup>PD-1<sub>lo</sub>CD25<sup>lo</sup>), which is less self-reactive and limits the development of colitis (Wyss et al., 2016). Altogether, these studies suggest that the functional separation between tTreg and pTreg populations is not rigid and that both populations express different nonredundant TCR repertoires (Haribhai et al., 2011) and are thus both required for the induction of self-tolerance.

Furthermore, (regardless of their thymic or peripheral origin) Tregs were shown to differentiate to functionally distinct effector Treg coopting the same transcription factors that drive helper T-cell differentiation. Strikingly, the very same transcription factor required for helper T-cell differentiation is also required by the corresponding Treg to suppress the specific helper T-cell subset (Cretney et al., 2013). Thus Tbet-, Irf4- or ROR $\gamma$ T-, Gata3- or Stat3-, and Bcl-deficient Tregs are defective in their ability to suppress Th<sub>1</sub>, Th<sub>2</sub>, Th<sub>17</sub>, and T<sub>fh</sub>, respectively (Chaudhry et al., 2009; Duhen et al., 2012; Koch et al., 2009; Ohnmacht et al., 2015; Wang et al., 2011; Wohlfert et al., 2011; Yu et al., 2015a; Zheng et al., 2009). Nevertheless, it is still unclear whether these are true stable subsets of Treg or just a mirror of Treg plasticity. A recent report provides evidence to support the former hypothesis (Levine et al., 2017). In this report, Tbet<sup>+</sup> Treg shows a unique transcriptional profile, stability, and specific capacity to limit Th<sub>1</sub> responses that might be extended to other effector Treg. Altogether these data suggest that naïve Treg can differentiate into effector Treg with unique immune-modulatory functions specific to distinct microenvironments, therefore limiting undesired bystander suppression. Besides, it predicts that the appropriate effector Treg subset is needed to maintain tolerance and prevent autoimmunity in different contexts.

Recently, it has been described that Treg produced early in life have a major role in preventing autoimmunity. Using an NOD *Foxp3* DTR mouse model, Yang et al. observed that mice develop fatal autoimmunity only when Tregs are depleted during the first 10 days of life and not at later timepoints. They also observed that the production of this perinatal Treg subset is Aire dependent as only the transfer of *Aire-WT* Treg, but not *Aire-KO* Treg protects Treg-depleted perinates from autoimmunity (Yang et al., 2015). This observation, however, contradicts previous work from Rudensky et al. describing that Tregs are important throughout the lifespan of mice (Kim et al., 2007). Despite these contradictory results that might stem from the different animal models utilized, the study nicely highlights that perinatal Tregs are crucial for establishing tolerance to self. Similarly, it has been shown that Tregs accumulate in the skin hair follicles during the first weeks of age promoting tolerance to commensal bacteria (Sanchez Rodriguez et al., 2014; Scharschmidt et al., 2017) and that Tregs are induced in the lungs during the first weeks of life to facilitate tolerance to a broad spectrum of allergens (Gollwitzer et al., 2014). Collectively, these reports suggest that Treg developed early in life (either in the thymus or in the periphery) are uniquely important for the establishment of tolerance to self, and harmless antigens such as our “microbial-self”.

### **Treg at Barrier Sites**

Tregs play a major role in establishing immune tolerance at barrier sites such as the intestine (Morrissey et al., 1993; Powrie et al., 1993), lungs (Curotto de Lafaille et al., 2008; Ostroukhova et al., 2004), and skin (Scharschmidt et al., 2015, 2017), which are bursting with commensal microbiota or other harmless antigens (e.g., food or inhaled antigens). While Tregs constitute ~10% of the CD4<sup>+</sup> T cells in most organs in the body, in the intestinal lamina propria, they constitute 20%–30% of the CD4<sup>+</sup> T cells (Tanoue et al., 2016). Moreover, the intestinal Treg population seems to be very heterogeneous as it is composed of at least three different subsets including: (1) ROR $\gamma$ t<sup>+</sup> Tregs that are induced by microbiota and mediate tolerance to microbial-self in steady state (Sefik et al., 2015; Yang et al., 2016), (2) ROR $\gamma$ t<sup>−</sup> NRP1<sup>−</sup> Tregs that are induced by solid dietary antigens and maintain homeostasis to diet antigens as shown by increased susceptibility to food allergy upon depletion (Kim et al., 2016), and (3) GATA3<sup>+</sup> Tregs that are produced in the thymus and probably function under inflammatory conditions as they express high levels of IL33 receptor (Schiering et al., 2014; Wohlfert et al., 2011). Therefore in the gut, both tTreg and pTreg populations cooperate to maintain tolerance to “microbial-self” and to innocuous food antigens. This state of unresponsiveness induced by Treg is vital to preventing various intestinal disorders, such as food allergy, inflammatory bowel disease, or celiac disease (Cebula et al., 2013; Haribhai et al., 2011; Lathrop et al., 2011).

In addition to FOXP3<sup>+</sup> Treg, other subsets of FOXP3<sup>−</sup> regulatory T cells play a role in maintaining tolerance in the intestine. Nevertheless, these FOXP3<sup>−</sup> regulatory T cells are not sufficient to control immune homeostasis in the gut. This is evidenced by the development of enteropathy in IPEX patients that present mutations in *Foxp3* gene and therefore presumably intact FOXP3<sup>−</sup> regulatory T cells. Probably the best characterized subset of FOXP3<sup>−</sup> cells is a unique population of CD4<sup>+</sup>CD8aa<sup>+</sup> regulatory T cells, which are characterized by their capacity to produce high levels of IL10. Moreover, these cells were shown to accumulate in human gut in response to microbiota such as *Faecalibacterium prausnitzii* (one of the major *Clostridium* species in human gut) and are decreased in patients with inflammatory bowel disease, which suggests that they may contribute to prevent the disease (Das et al., 2003; Sarrabayrouse et al., 2014). FOXP3<sup>−</sup> regulatory T cells also include IL10-producing Tr<sub>1</sub> cells and TGF $\beta$ -producing Th<sub>3</sub> cells. It should be stressed, however, that there is a certain level of controversy about whether these Tr<sub>1</sub> and Th<sub>3</sub> cells represent unique cell lineages and not only different states of Treg. This notion is further reinforced by the fact that both Tr<sub>1</sub> cells and Th<sub>3</sub> cells were mainly described prior to the discovery of *Foxp3* and thus their characterization largely relied only on high IL10 or TGF $\beta$  secretion. While it seems that there are both FOXP3<sup>+</sup> and FOXP3<sup>−</sup> IL10 secreting regulatory T cells (Maynard et al., 2007), the existence and identity of Th<sub>3</sub> remain less defined.

Although the role of Treg in maintaining tolerance at barrier sites is undisputed, the responsible Treg subsets and the mechanisms utilized still remain poorly characterized. Therefore expanding our knowledge about Treg generated throughout life at different anatomical sites will be invaluable to understanding Treg tolerance induction and its failure. This might also enable to design effective therapies to treat immune disorders in the future.

### **Other Tolerogenic Cells in the Periphery**

For many years, the role of stromal cells in secondary lymphoid organs was believed to be limited to organ structure and maintenance. However, this notion has been put to rest with several independent findings

implicating these heterogeneous stromal cell populations in active modulation of immune responses through extensive crosstalk with hematopoietic populations (reviewed in [Malhotra et al., 2013](#)). Such crosstalk extends to the context of peripheral tolerance induction as all lymph node stromal cell (LNSC) populations have been shown to have the capability of directly presenting self-antigen to CD8<sup>+</sup> T cells for the purpose of their tolerization ([Cohen et al., 2010; Fletcher et al., 2010; Lee et al., 2007; Magnusson et al., 2008; Nichols et al., 2007](#)). Remarkably, LNSCs ectopically express and present various TSA genes in a manner reminiscent of PGE in mTECs ([Lee et al., 2007; Magnusson et al., 2008; Nichols et al., 2007](#)). However, the expression of the TSAs in LNSCs has been reported to be independent of Aire and by far not as broad as in mTECs ([Fletcher et al., 2010](#)). It has yet to be determined whether LNSCs can influence CD4<sup>+</sup> T-cell tolerance, as their basal levels of MHCII expression is quite low, though significantly increased under inflammatory conditions ([Malhotra et al., 2012](#)). Nevertheless, another unique bone marrow–derived APC population has been directly implicated in CD4<sup>+</sup> T-cell tolerance within lymph nodes. These cells express Aire and were thus named extrathymic Aire-expressing cells, and appropriately express TSAs in a promiscuous fashion, though their repertoire was reported to be separate and limited as compared to that found in mTECs ([Gardner et al., 2008, 2013](#)).

## CONCLUDING REMARKS

While research over the last decades has led to a better understanding of how T-cell tolerance is regulated in the thymus and periphery, much remains unknown. As we benefit from the genomics era, a clearer understanding of the molecular pathways that regulate the cellular functions that keep tolerance in check will undoubtedly lead to a detailed understanding of human autoimmunity. Ideally, key discoveries will provide novel strategies to utilize the physiological processes of immunological tolerance to prevent transplant rejection, allergies, and autoimmunity and to promote rejection of tumor cells.

## Acknowledgments

We would like to thank members of the Abramson lab and Prof. Eystein S. Husebye for their critical reading of this manuscript. J.A. is an incumbent of the Dr. Celia Zwilloburg-Fridman and Dr. Lutz Fridman Career Development Chair. Research in the Abramson lab is kindly supported by European Research Council Consolidator grant (ERC-2016-CoG-724821), Israel Science Foundation (1796/16 and 722/14), the Sy Syms Foundation; US–Israel Binational Foundation, Maurice and Vivienne Wohl Charitable Foundation; Goodman Family Charitable Lead Annuity Trust; Ruth and Samuel David Gameroff Family Foundation.

## References

- [Abbas, A.K., Benoist, C., Bluestone, J.A., Campbell, D.J., Ghosh, S., Hori, S., et al., 2013. Regulatory T cells: recommendations to simplify the nomenclature. \*Nat. Immunol.\* 14, 307–308.](#)
- [Abramson, J., Giraud, M., Benoist, C., Mathis, D., 2010. Aire’s partners in the molecular control of immunological tolerance. \*Cell\* 140, 123–135.](#)
- [Akiyama, N., Shinzawa, M., Miyauchi, M., Yanai, H., Tateishi, R., Shimo, Y., et al., 2014. Limitation of immune tolerance-inducing thymic epithelial cell development by Spi-B-mediated negative feedback regulation. \*J. Exp. Med.\* 211, 2425–2438.](#)
- [Ali, N., Zirak, B., Rodriguez, R.S., Pauli, M.L., Truong, H.A., Lai, K., et al., 2017. Regulatory T cells in skin facilitate epithelial stem cell differentiation. \*Cell\* 169, 1119–1129.e11.](#)
- [Anderson, M.S., Venanzi, E.S., Klein, L., Chen, Z., Berzins, S.P., Turley, S.J., et al., 2002. Projection of an immunological self shadow within the thymus by the Aire protein. \*Science\* 298, 1395–1401.](#)
- [Anderson, M.S., Venanzi, E.S., Chen, Z., Berzins, S.P., Benoist, C., Mathis, D., 2005. The cellular mechanism of Aire control of T cell tolerance. \*Immunity\* 23, 227–239.](#)
- [Andersson, J., Tran, D.Q., Pesu, M., Davidson, T.S., Ramsey, H., O’Shea, J.J., et al., 2008. CD4<sup>+</sup> FoxP3<sup>+</sup> regulatory T cells confer infectious tolerance in a TGF-β-dependent manner. \*J. Exp. Med.\* 205, 1975–1981.](#)
- [Arpaia, N., Campbell, C., Fan, X., Dikiy, S., van der Veeken, J., deRoos, P., et al., 2013. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. \*Nature\* 504, 451–455.](#)
- [Arpaia, N., Green, J.A., Moltedo, B., Arvey, A., Hemmers, S., Yuan, S., et al., 2015. A distinct function of regulatory T cells in tissue protection. \*Cell\* 162, 1078–1089.](#)
- [Asano, M., Toda, M., Sakaguchi, N., Sakaguchi, S., 1996. Autoimmune disease as a consequence of developmental abnormality of a T cell subpopulation. \*J. Exp. Med.\* 184, 387–396.](#)
- [Aschenbrenner, K., D’Cruz, L.M., Vollmann, E.H., Hinterberger, M., Emmerich, J., Swee, L.K., et al., 2007. Selection of Foxp3<sup>+</sup> regulatory T cells specific for self antigen expressed and presented by Aire<sup>+</sup> medullary thymic epithelial cells. \*Nat. Immunol.\* 8, 351–358.](#)
- [Asseman, C., Mauze, S., Leach, M.W., Coffman, R.L., Powrie, F., 1999. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. \*J. Exp. Med.\* 190, 995–1004.](#)

- Atarashi, K., Tanoue, T., Shima, T., Imaoka, A., Kuwahara, T., Momose, Y., et al., 2011. Induction of colonic regulatory T cells by indigenous clostridium species. *Science* 331 (6015), 337–341.
- Austrup, F., Vestweber, D., Borges, E., Löhning, M., Bräuer, R., Herz, U., et al., 1997. P- and E-selectin mediate recruitment of T-helper-1 but not T-helper-2 cells into inflamed tissues. *Nature* 385, 81–83.
- Azzam, H.S., Grinberg, A., Lui, K., Shen, H., Shores, E.W., Love, P.E., 1998. CD5 expression is developmentally regulated by T cell receptor (TCR) signals and TCR avidity. *J. Exp. Med.* 188, 2301–2311.
- Banchereau, J., Steinman, R.M., 1998. Dendritic cells and the control of immunity. *Nature* 392, 245–252.
- Bansal, K., Yoshida, H., Benoist, C., Mathis, D., 2017. The transcriptional regulator Aire binds to and activates super-enhancers. *Nat. Immunol.* 18, 263–273.
- Barber, D.L., Wherry, E.J., Masopust, D., Zhu, B., Allison, J.P., Sharpe, A.H., et al., 2006. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* 439, 682–687.
- Baxter, A.G., Hodgkin, P.D., 2002. Activation rules: the two-signal theories of immune activation. *Nat. Rev. Immunol.* 2, 439–446. Publ. Online 01 June 2002; <http://dx.doi.org/10.1038/nri823>.
- Benoist, C., Mathis, D., 2012. Treg cells, life history, and diversity. *Cold Spring Harb. Perspect. Biol.* 4, 1–14.
- Bensinger, S.J., Bandeira, A., Jordan, M.S., Caton, A.J., Laufer, T.M., 2001. Major histocompatibility complex class II-positive cortical epithelium mediates the selection of CD4(+)25(+) immunoregulatory T cells. *J. Exp. Med.* 194, 427–438.
- Benson, M.J., Pino-Lagos, K., Rosemblatt, M., Noelle, R.J., 2007. All-trans retinoic acid mediates enhanced Treg cell growth, differentiation, and gut homing in the face of high levels of co-stimulation. *J. Exp. Med.* 204, 1765–1774.
- Billingham, R.E., Brent, L., Medawar, P.B., 1953. Actively acquired tolerance of foreign cells. *Nature* 172, 603–606.
- Von Boehmer, H., 1990. Developmental biology of T cells in T cell-receptor transgenic mice. *Annu. Rev. Immunol.* 8, 531–556.
- Bonito, A.J., Aloman, C., Fiel, M.I., Danzl, N.M., Cha, S., Weinstein, E.G., et al., 2013. Medullary thymic epithelial cell depletion leads to autoimmune hepatitis. *J. Clin. Invest.* 123, 3510–3524.
- Borsig, G., Kleinewietfeld, M., Di Mitri, D., Sternjak, A., Diamantini, A., Giometto, R., et al., 2007. Expression of ectonucleotidase CD39 by Foxp3+ Treg cells: hydrolysis of extracellular ATP and immune suppression. *Blood* 110, 1225–1232.
- Brennecke, P., Reyes, A., Pinto, S., Rattay, K., Nguyen, M., Küchler, R., et al., 2015. Single-cell transcriptome analysis reveals coordinated ectopic gene-expression patterns in medullary thymic epithelial cells. *Nat. Immunol.* 16, 933–941.
- Bunch, H., Lawney, B.P., Lin, Y.-F., Asaithamby, A., Murshid, A., Wang, Y.E., et al., 2015. Transcriptional elongation requires DNA break-induced signalling. *Nat. Commun.* 6, 10191.
- Burnet, F.M., 1959. The Clonal Selection Theory of Acquired Immunity. University Press.
- Burnet, F.M., 1962. The immunological significance of the thymus: an extension of the clonal selection theory of immunity. *Australas. Ann. Med.* 11, 79–91.
- Burzyn, D., Kuswanto, W., Kolodkin, D., Shadrach, J.L., Cerletti, M., Jang, Y., et al., 2013. A special population of regulatory T cells potentiates muscle repair. *Cell* 155, 1282–1295.
- Cantor, H., Hugenerger, J., McVay-Boudreau, L., Eardley, D.D., Kemp, J., Shen, F.W., et al., 1978. Immunoregulatory circuits among T-cell sets. Identification of a subpopulation of T-helper cells that induces feedback inhibition. *J. Exp. Med.* 148, 871–877.
- Cao, X., Cai, S.F., Fehniger, T.A., Song, J., Collins, L.I., Piwnica-Worms, D.R., et al., 2007. Granzyme B and perforin are important for regulatory T cell-mediated suppression of tumor clearance. *Immunity* 27, 635–646.
- Cebula, A., Seweryn, M., Rempala, G.A., Pabla, S.S., McIndoe, R.A., Denning, T.L., et al., 2013. Thymus-derived regulatory T cells contribute to tolerance to commensal microbiota. *Nature* 497, 258–262.
- Chaudhry, A., Rudra, D., Treuting, P., Samstein, R.M., Liang, Y., Kas, A., et al., 2009. CD4+ regulatory T cells control Th17 responses in a Stat3-dependent manner. *Science* 326 (5955), 986–991.
- Chen, W., Wahl, S.M., 2003. TGF-beta: the missing link in CD4+ CD25+ regulatory T cell-mediated immunosuppression. *Cytokine Growth Factor Rev.* 14, 85–89.
- Chen, W., Konkel, J.E., 2015. Development of thymic Foxp3(+) regulatory T cells: TGF-β matters. *Eur. J. Immunol.* 45, 958–965.
- Chen, W., Jin, W., Hardegen, N., Lei, K.-J., Li, L., Marinos, N., et al., 2003. Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J. Exp. Med.* 198, 1875–1886.
- Chinen, T., Kannan, A.K., Levine, A.G., Fan, X., Klein, U., Zheng, Y., et al., 2016. An essential role for the IL-2 receptor in Treg cell function. *Nat. Immunol.* 17, 1322–1333.
- Chuprin, A., Avin, A., Goldfarb, Y., Herzig, Y., Levi, B., Jacob, A., et al., 2015. The deacetylase Sirt1 is an essential regulator of Aire-mediated induction of central immunological tolerance. *Nat. Immunol.* 16, 737–745.
- Cipolletta, D., 2014. Adipose tissue-resident regulatory T cells: phenotypic specialization, functions and therapeutic potential. *Immunology* 142, 517–525.
- Claman, H.N., 1963. Tolerance to a protein antigen in adult mice and the effect of nonspecific factors. *J. Immunol.* 91, 833–839.
- Cohen, J.N., Guidi, C.J., Tewalt, E.F., Qiao, H., Rouhani, S.J., Ruddell, A., et al., 2010. Lymph node-resident lymphatic endothelial cells mediate peripheral tolerance via Aire-independent direct antigen presentation. *J. Exp. Med.* 207, 681–688.
- Collison, L.W., Workman, C.J., Kuo, T.T., Boyd, K., Wang, Y., Vignali, K.M., et al., 2007. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. *Nature* 450, 566–569.
- Collison, L.W., Chaturvedi, V., Henderson, A.L., Giacomini, P.R., Guy, C., Bankoti, J., et al., 2010. IL-35-mediated induction of a potent regulatory T cell population. *Nat. Immunol.* 11, 1093–1101.
- Cowan, J.E., Parnell, S.M., Nakamura, K., Caamano, J.H., Lane, P.J.L., Jenkinson, E.J., et al., 2013. The thymic medulla is required for Foxp3+ regulatory but not conventional CD4+ thymocyte development. *J. Exp. Med.* 210, 675–681.
- Cretney, E., Kallies, A., Nutt, S.L., 2013. Differentiation and function of Foxp3+ effector regulatory T cells. *Trends Immunol.* 34, 74–80.
- Curotto de Lafaille, M.A., Kutchukhidze, N., Shen, S., Ding, Y., Yee, H., Lafaille, J.J., 2008. Adaptive Foxp3+ regulatory T cell-dependent and -independent control of allergic inflammation. *Immunity* 29, 114–126.

- Daley, S.R., Hu, D.Y., Goodnow, C.C., 2013. Helios marks strongly autoreactive CD4+ T cells in two major waves of thymic deletion distinguished by induction of PD-1 or NF- $\kappa$ B. *J. Exp. Med.* 210, 269–285.
- Daley, S.R., Teh, C., Hu, D.Y., Strasser, A., Gray, D.H.D., 2017. Cell death and thymic tolerance. *Immunol. Rev.* 277, 9–20.
- Danan-Gotthold, M., Guyon, C., Giraud, M., Levanon, E.Y., Abramson, J., 2016. Extensive RNA editing and splicing increase immune self-representation diversity in medullary thymic epithelial cells. *Genome Biol.* 17, 219.
- Das, G., Augustine, M.M., Das, J., Bottomly, K., Ray, P., Ray, A., 2003. An important regulatory role for CD4+CD8 alpha alpha T cells in the intestinal epithelial layer in the prevention of inflammatory bowel disease. *Proc. Natl. Acad. Sci. U.S.A.* 100, 5324–5329.
- Davidson, T.S., Shevach, E.M., 2011. Polyclonal Treg cells modulate T effector cell trafficking. *Eur. J. Immunol.* 41, 2862–2870.
- Davis, M.M.M., 2015. Not-so-negative selection. *Immunity* 43, 833–835.
- Deaglio, S., Dwyer, K.M., Gao, W., Friedman, D., Usheva, A., Erat, A., et al., 2007. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. *J. Exp. Med.* 204, 1257–1265.
- de la Rosa, M., Rutz, S., Dorninger, H., Scheffold, A., 2004. Interleukin-2 is essential for CD4+CD25+ regulatory T cell function. *Eur. J. Immunol.* 34, 2480–2488.
- Derbinski, J., Schulte, A., Kyewski, B., Klein, L., 2001. Promiscuous gene expression in medullary thymic epithelial cells mirrors the peripheral self. *Nat. Immunol.* 2, 1032–1039.
- Derbinski, J., Pinto, S., Rösch, S., Hexel, K., Kyewski, B., 2008. Promiscuous gene expression patterns in single medullary thymic epithelial cells argue for a stochastic mechanism. *Proc. Natl. Acad. Sci. U.S.A.* 105, 657–662.
- DeVoss, J., Hou, Y., Johannes, K., Lu, W., Liou, G.I., Rinn, J., et al., 2006. Spontaneous autoimmunity prevented by thymic expression of a single self-antigen. *J. Exp. Med.* 203, 2727–2735.
- Dhainaut, M., Coquerelle, C., Uzureau, S., Deneoud, J., Acolty, V., Oldenhove, G., et al., 2015. Thymus-derived regulatory T cells restrain pro-inflammatory Th1 responses by downregulating CD70 on dendritic cells. *EMBO J.* 34, 1336–1348.
- Dhopakar, M.V., Steinman, R.M., Krasovskiy, J., Munz, C., Bhardwaj, N., 2001. Antigen-specific inhibition of effector T cell function in humans after injection of immature dendritic cells. *J. Exp. Med.* 193, 233–238.
- Dombrowski, Y., O'Hagan, T., Dittmer, M., Penalva, R., Mayoral, S.R., Bankhead, P., et al., 2017. Regulatory T cells promote myelin regeneration in the central nervous system. *Nat. Neurosci.* 20, 674–680.
- Dresser, D.W., 1961. Acquired immunological tolerance to a fraction of bovine gamma globulin. *Immunology* 4, 13–23.
- Dresser, D.W., 1962. Specific inhibition of antibody production. I. Protein-over loading paralysis. *Immunology* 5, 161–168.
- Dubois, P.M., Pihlgren, M., Tomkowiak, M., Van Mechelen, M., Marvel, J., 1998. Tolerant CD8 T cells induced by multiple injections of peptide antigen show impaired TCR signaling and altered proliferative responses in vitro and in vivo. *J. Immunol.* 161, 5260–5267.
- Duhen, T., Duhen, R., Lanzavecchia, A., Sallusto, F., Campbell, D.J., 2012. Functionally distinct subsets of human FOXP3+ Treg cells that phenotypically mirror effector Th cells. *Blood* 119, 4430–4440.
- Fahlén, L., Read, S., Gorelik, L., Hurst, S.D., Coffman, R.L., Flavell, R.A., et al., 2005. T cells that cannot respond to TGF-beta escape control by CD4(+)/CD25(+) regulatory T cells. *J. Exp. Med.* 201, 737–746.
- Fallarino, F., Grohmann, U., Hwang, K.W., Orabona, C., Vacca, C., Bianchi, R., et al., 2003. Modulation of tryptophan catabolism by regulatory T cells. *Nat. Immunol.* 4, 1206–1212.
- Fan, Y., Rudert, W.A., Grupillo, M., He, J., Sisino, G., Trucco, M., 2009. Thymus-specific deletion of insulin induces autoimmune diabetes. *EMBO J.* 28, 2812–2824.
- Feuerer, M., Hill, J.A., Mathis, D., Benoist, C., 2009. Foxp3+ regulatory T cells: differentiation, specification, subphenotypes. *Nat. Immunol.* 10, 689–695.
- Fletcher, A.L., Lukacs-Kornek, V., Reynoso, E.D., Pinner, S.E., Bellemare-Pelletier, A., Curry, M.S., et al., 2010. Lymph node fibroblastic reticular cells directly present peripheral tissue antigen under steady-state and inflammatory conditions. *J. Exp. Med.* 207, 689–697.
- Fontenot, J.D., Gavin, M.A., Rudensky, A.Y., 2003. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat. Immunol.* 4, 330–336.
- Fontenot, J.D., Dooley, J.L., Farr, A.G., Rudensky, A.Y., 2005. Developmental regulation of Foxp3 expression during ontogeny. *J. Exp. Med.* 202, 901–906.
- Freeman, G.J., Long, A.J., Iwai, Y., Bourque, K., Chernova, T., Nishimura, H., et al., 2000. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J. Exp. Med.* 192, 1027–1034.
- Friesen, T.J., Ji, Q., Fink, P.J., 2016. Recent thymic emigrants are tolerized in the absence of inflammation. *J. Exp. Med.* 213, 913–920.
- Frommer, F., Waisman, A., Andersson, J., Higgins, C., Elliott, J., 2010. B cells participate in thymic negative selection of murine auto-reactive CD4+ T cells. *PLoS One* 5, e15372.
- Fu, H., Kishore, M., Gittens, B., Wang, G., Coe, D., Komarowska, I., et al., 2014. Self-recognition of the endothelium enables regulatory T-cell trafficking and defines the kinetics of immune regulation. *Nat. Commun.* 5, 3436.
- Furusawa, Y., Obata, Y., Fukuda, S., Endo, T.A., Nakato, G., Takahashi, D., et al., 2013. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 504, 446–450.
- Galea, I., Bechmann, I., Perry, V.H., 2007. What is immune privilege (not)? *Trends Immunol.* 28, 12–18.
- Gallucci, S., Lolkema, M., Matzinger, P., 1999. Natural adjuvants: endogenous activators of dendritic cells. *Nat. Med.* 5, 1249–1255.
- Gardner, J.M., Devoss, J.J., Friedman, R.S., Wong, D.J., Tan, Y.X., Zhou, X., et al., 2008. Deletional tolerance mediated by extrathymic Aire-expressing cells. *Science* 321, 843–847.
- Gardner, J.M., Metzger, T.C., McMahon, E.J., Au-Yeung, B.B., Krawisz, A.K., Lu, W., et al., 2013. Extrathymic Aire-expressing cells are a distinct bone marrow-derived population that induce functional inactivation of CD4+ T cells. *Immunity* 39, 560–572.
- Garín, M.I., Chu, C.-C., Golshayan, D., Cernuda-Morollón, E., Wait, R., Lechler, R.I., 2007. Galectin-1: a key effector of regulation mediated by CD4+CD25+ T cells. *Blood* 109, 2058–2065.
- Garza, K.M., Agersborg, S.S., Baker, E., Tung, K.S., 2000. Persistence of physiological self antigen is required for the regulation of self tolerance. *J. Immunol.* 164, 3982–3989.
- Gascoigne, N.R., Palmer, E., 2011. Signaling in thymic selection. *Curr. Opin. Immunol.* 23, 207–212.

- Gershon, R.K., Kondo, K., 1970. Cell interactions in the induction of tolerance: the role of thymic lymphocytes. *Immunology* 18, 723–737.
- Gershon, R.K., Kondo, K., 1971. Infectious immunological tolerance. *Immunology* 21, 903–914.
- Gershon, R.K., Cohen, P., Hencin, R., Liehaber, S.A., 1972. Suppressor T cells. *J. Immunol.* 108, 586–590.
- Giraud, M., Yoshida, H., Abramson, J., Rahl, P.B., Young, R.A., Mathis, D., et al., 2012. Aire unleashes stalled RNA polymerase to induce ectopic gene expression in thymic epithelial cells. *Proc. Natl. Acad. Sci. U.S.A.* 109, 535–540.
- Godfrey, V.L., Wilkinson, J.E., Rinchik, E.M., Russell, L.B., 1991. Fatal lymphoreticular disease in the scurfy (sf) mouse requires T cells that mature in a sf thymic environment: potential model for thymic education. *Proc. Natl. Acad. Sci. U.S.A.* 88, 5528–5532.
- Gollwitzer, E.S., Saglani, S., Trompette, A., Yadava, K., Sherburn, R., McCoy, K.D., et al., 2014. Lung microbiota promotes tolerance to allergens in neonates via PD-L1. *Nat. Med.* 20, 642–647.
- Gondek, D.C., Lu, L.-F., Quezada, S.A., Sakaguchi, S., Noelle, R.J., 2005. Cutting edge: contact-mediated suppression by CD4 + CD25 + regulatory cells involves a granzyme B-dependent, perforin-independent mechanism. *J. Immunol.* 174, 1783–1786.
- Gratz, I.K., Campbell, D.J., 2014. Organ-specific and memory Treg cells: specificity, development, function, and maintenance. *Front. Immunol.* 5, 1–17.
- Grohmann, U., Orabona, C., Fallarino, F., Vacca, C., Calcinaro, F., Falorni, A., et al., 2002. CTLA-4-Ig regulates tryptophan catabolism in vivo. *Nat. Immunol.* 3, 1097–1101.
- Grossman, W.J., Verbsky, J.W., Tollefson, B.L., Kemper, C., Atkinson, J.P., Ley, T.J., 2004. Differential expression of granzymes A and B in human cytotoxic lymphocyte subsets and T regulatory cells. *Blood* 104, 2840–2848.
- Guha, M., Saare, M., Maslovskaja, J., Kisand, K., Liiv, I., Haljasorg, U., et al., 2017. DNA breaks and chromatin structural changes enhance the transcription of autoimmune regulator target genes. *J. Biol. Chem.* 292, 6542–6554. jbc. M116. 764704.
- Hadeiba, H., Lahl, K., Edalati, A., Oderup, C., Habtezion, A., Pachynski, R., et al., 2012. Plasmacytoid dendritic cells transport peripheral antigens to the thymus to promote central tolerance. *Immunity* 36, 438–450.
- Haribhai, D., Williams, J.B., Jia, S., Nickerson, D., Schmitt, E.G., Edwards, B., et al., 2011. A requisite role for induced regulatory T cells in tolerance based on expanding antigen receptor diversity. *Immunity* 35, 109–122.
- Hasek, M., Hraba, T., 1955. Immunological effects of experimental embryonal parabiosis. *Nature* 175, 764–765.
- Hauri-Hohl, M., Zuklys, S., Holländer, G.A., Ziegler, S.F., 2014. A regulatory role for TGF- $\beta$  signaling in the establishment and function of the thymic medulla. *Nat. Immunol.* 15, 554–561.
- Hawiger, D., Inaba, K., Dorsett, Y., Guo, M., Mahnke, K., Rivera, M., et al., 2001. Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo. *J. Exp. Med.* 194, 769–779.
- Hengartner, H., Odermat, B., Schneider, R., Schreyer, M., Wälle, G., MacDonald, H.R., et al., 1988. Deletion of self-reactive T cells before entry into the thymus medulla. *Nature* 336, 388–390.
- Hinterberger, M., Aichinger, M., da Costa, O.P., Voehringer, D., Hoffmann, R., Klein, L., 2010. Autonomous role of medullary thymic epithelial cells in central CD4(+) T cell tolerance. *Nat. Immunol.* 11, 512–519.
- Hinterberger, M., Wirnsberger, G., Klein, L., 2011. B7/CD28 in central tolerance: costimulation promotes maturation of regulatory T cell precursors and prevents their clonal deletion. *Front. Immunol.* 2, 1–12.
- Hori, S., Nomura, T., Sakaguchi, S., 2003. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 299 (5609), 1057–1061.
- Hsieh, C.-S., Liang, Y., Tyznik, A.J., Self, S.G., Liggitt, D., Rudensky, A.Y., 2004. Recognition of the peripheral self by naturally arising CD25 + CD4 + T cell receptors. *Immunity* 21, 267–277.
- Hsieh, C.-S., Lee, H.-M., Lio, C.-W.J., 2012. Selection of regulatory T cells in the thymus. *Nat. Rev. Immunol.* 12, 157–167.
- Hsu, P., Santner-Nanan, B., Hu, M., Skarratt, K., Lee, C.H., Stormon, M., et al., 2015. IL-10 potentiates differentiation of human induced regulatory T cells via STAT3 and Foxo1. *J. Immunol.* 195, 3665–3674.
- Hu, D.Y., Yap, J.Y., Wirasinha, R.C., Howard, D.R., Goodnow, C.C., Daley, S.R., 2016. A timeline demarcating two waves of clonal deletion and Foxp3 upregulation during thymocyte development. *Immunol. Cell Biol.* 94, 357–366.
- Ignatowicz, L., Kappler, J., Marrack, P., 1996. The repertoire of T cells shaped by a single MHC/peptide ligand. *Cell* 84, 521–529.
- Ishida, Y., Agata, Y., Shibahara, K., Honjo, T., 1992. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J.* 11, 3887–3895.
- Jameson, S.C., Hogquist, K.A., Bevan, M.J., 1995. Positive selection of thymocytes. *Annu. Rev. Immunol.* 13, 93–126.
- Janeway, C.A., 1992. The immune system evolved to discriminate infectious nonself from noninfectious self. *Immunol. Today* 13, 11–16.
- Jenkins, M.K., Pardoll, D.M., Mizuguchi, J., Quill, H., Schwartz, R.H., 1987. T-cell unresponsiveness in vivo and in vitro: fine specificity of induction and molecular characterization of the unresponsive state. *Immunol. Rev.* 95, 113–135.
- Johnson, J.G., Jenkins, M.K., 1993. Accessory cell-derived signals required for T cell activation. *Immunol. Res.* 12, 48–64.
- Jolicoeur, C., Hanahan, D., Smith, K.M., 1994. T-cell tolerance toward a transgenic beta-cell antigen and transcription of endogenous pancreatic genes in thymus. *Proc. Natl. Acad. Sci. U.S.A.* 91, 6707–6711.
- Jordan, M.S., Boesteanu, A., Reed, A.J., Petrone, A.L., Holenbeck, A.E., Lerman, M.A., et al., 2001. Thymic selection of CD4 + CD25 + regulatory T cells induced by an agonist self-peptide. *Nat. Immunol.* 2, 301–306.
- Josefowicz, S.Z., Lu, L.-F., Rudensky, A.Y., 2012a. Regulatory T cells: mechanisms of differentiation and function. *Annu. Rev. Immunol.* 30, 531–564.
- Josefowicz, S.Z., Niec, R.E., Kim, H.Y., Treuting, P., Chinen, T., Zheng, Y., et al., 2012b. Extrathymically generated regulatory T cells control mucosal Th2 inflammation. *Nature* 482, 395–399.
- Kabat, E.A., Wolf, A., Bezer, A.E., 1946. Rapid production of acute disseminated encephalomyelitis in rhesus monkeys by injection of brain tissue with adjuvants. *Science* 104 (2703), 362–363.
- Kalekar, L.A., Mueller, D.L., 2017. Relationship between CD4 regulatory T cells and anergy in vivo. *J. Immunol.* 198, 2527–2533.
- Kalekar, L.A., Schmiel, S.E., Nandiwada, S.L., Lam, W.Y., Barsness, L.O., Zhang, N., et al., 2016. CD4 + T cell anergy prevents autoimmunity and generates regulatory T cell precursors. *Nat. Immunol.* 17, 304–314.
- Kappler, J.W., Roehm, N., Marrack, P., 1987. T cell tolerance by clonal elimination in the thymus. *Cell* 49, 273–280.

- Keir, M.E., Liang, S.C., Guleria, I., Latchman, Y.E., Qipo, A., Albacker, L.A., et al., 2006. Tissue expression of PD-L1 mediates peripheral T cell tolerance. *J. Exp. Med.* 203, 883–895.
- Kendal, A.R., Waldmann, H., 2010. Infectious tolerance: therapeutic potential. *Curr. Opin. Immunol.* 22, 560–565.
- Khattri, R., Cox, T., Yasayko, S.-A., Ramsdell, F., 2003. An essential role for Scurfin in CD4 + CD25 + T regulatory cells. *Nat. Immunol.* 4, 337–342.
- Kim, J.M., Rasmussen, J.P., Rudensky, A.Y., 2007. Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. *Nat. Immunol.* 8, 191–197.
- Kim, K.S., Hong, S.-W., Han, D., Yi, J., Jung, J., Yang, B.-G., et al., 2016. Dietary antigens limit mucosal immunity by inducing regulatory T cells in the small intestine. *Science* 351 (6275), 858–863.
- Kisielow, P., Blüthmann, H., Staerz, U.D., Steinmetz, M., von Boehmer, H., 1988. Tolerance in T-cell-receptor transgenic mice involves deletion of nonmature CD4 + 8 + thymocytes. *Nature* 333, 742–746.
- Klein, L., Kyewski, B., Allen, P.M., Hogquist, K.A., 2014. Positive and negative selection of the T cell repertoire: what thymocytes see (and don't see). *Nat. Rev. Immunol.* 14, 377–391.
- Kobie, J.J., Shah, P.R., Yang, L., Rebhahn, J.A., Fowell, D.J., Mosmann, T.R., 2006. T regulatory and primed uncommitted CD4 T cells express CD73, which suppresses effector CD4 T cells by converting 5'-adenosine monophosphate to adenosine. *J. Immunol.* 177, 6780–6786.
- Koble, C., Kyewski, B., 2009. The thymic medulla: a unique microenvironment for intercellular self-antigen transfer. *J. Exp. Med.* 206, 1505–1513.
- Koch, M.A., Tucker-Heard, G., Perdue, N.R., Killebrew, J.R., Urdahl, K.B., Campbell, D.J., 2009. The transcription factor T-bet controls regulatory T cell homeostasis and function during type 1 inflammation. *Nat. Immunol.* 10, 595–602.
- Koh, A.S., Kuo, A.J., Park, S.Y., Cheung, P., Abramson, J., Bua, D., et al., 2008. Aire employs a histone-binding module to mediate immunological tolerance, linking chromatin regulation with organ-specific autoimmunity. *Proc. Natl. Acad. Sci. U.S.A.* 105, 15878–15883.
- Konkel, J.E., Jin, W., Abbatiello, B., Grainger, J.R., Chen, W., 2014. Thymocyte apoptosis drives the intrathymic generation of regulatory T cells. *Proc. Natl. Acad. Sci. U.S.A.* 111, E465–E473.
- Kretschmer, K., Apostolou, I., Hawiger, D., Khazaie, K., Nussenzweig, M.C., von Boehmer, H., 2005. Inducing and expanding regulatory T cell populations by foreign antigen. *Nat. Immunol.* 6, 1219–1227.
- Lafferty, K.J., Woolnough, J., 1977. The origin and mechanism of the allograft reaction. *Immunol. Rev.* 35, 231–262.
- Lafferty, K.J., Misko, I.S., Cooley, M.A., 1974. Allogeneic stimulation modulates the in vitro response of T cells to transplantation antigen. *Nature* 249, 275–276.
- Larkin, J., Rankin, A.L., Picca, C.C., Riley, M.P., Jenks, S.A., Sant, A.J., et al., 2008. CD4 + CD25 + regulatory T cell repertoire formation shaped by differential presentation of peptides from a self-antigen. *J. Immunol.* 180, 2149–2157.
- Lathrop, S.K., Bloom, S.M., Rao, S.M., Nutsch, K., Lio, C.-W., Santacruz, N., et al., 2011. Peripheral education of the immune system by colonic commensal microbiota. *Nature* 478, 250–254.
- Laufer, T.M., DeKoning, J., Markowitz, J.S., Lo, D., Glimcher, L.H., 1996. Unopposed positive selection and autoreactivity in mice expressing class II MHC only on thymic cortex. *Nature* 383, 81–85.
- Lee, H.M., Bautista, J.L., Scott-Browne, J., Mohan, J.F., Hsieh, C.S., 2012. A broad range of self-reactivity drives thymic regulatory T cell selection to limit responses to self. *Immunity* 37, 475–486.
- Lee, J.-W., Epardaud, M., Sun, J., Becker, J.E., Cheng, A.C., Yonekura, A., et al., 2007. Peripheral antigen display by lymph node stroma promotes T cell tolerance to intestinal self. *Nat. Immunol.* 8, 181–190.
- Lee, K.M., Chuang, E., Griffin, M., Khattri, R., Hong, D.K., Zhang, W., et al., 1998. Molecular basis of T cell inactivation by CTLA-4. *Science* 282, 2263–2266.
- Legoux, F.P., Lim, J.-B., Cauley, A.W., Dikiy, S., Ertelt, J., Mariani, T.J., et al., 2015. CD4 + T cell tolerance to tissue-restricted self antigens is mediated by antigen-specific regulatory T cells rather than deletion. *Immunity* 43, 896–908.
- Lenardo, M.J., 1996. Fas and the art of lymphocyte maintenance. *J. Exp. Med.* 183, 721–724.
- Leonard, J.D., Gilmore, D.C., Dileepan, T., Nawrocka, W.I., Chao, J.L., Schoenbach, M.H., et al., 2017. Identification of natural regulatory T cell epitopes reveals convergence on a dominant autoantigen. *Immunity* 47, 107–117.e8.
- Lerman, M.A., Larkin, J., Cozzo, C., Jordan, M.S., Caton, A.J., 2004. CD4 + CD25 + regulatory T cell repertoire formation in response to varying expression of a neo-self-antigen. *J. Immunol.* 173, 236–244.
- Levine, A.G., Arvey, A., Jin, W., Rudensky, A.Y., 2014. Continuous requirement for the TCR in regulatory T cell function. *Nat. Immunol.* 15, 1070–1078.
- Levine, A.G., Medoza, A., Hemmers, S., Moltedo, B., Niec, R.E., Schizas, M., et al., 2017. Stability and function of regulatory T cells expressing the transcription factor T-bet. *Nature* 546, 421–425.
- Liang, B., Workman, C., Lee, J., Chew, C., Dale, B.M., Colonna, L., et al., 2008. Regulatory T cells inhibit dendritic cells by lymphocyte activation gene-3 engagement of MHC class II. *J. Immunol.* 180, 5916–5926.
- Liberal, R., Grant, C.R., Holder, B.S., Ma, Y., Mieli-Vergani, G., Vergani, D., et al., 2012. The impaired immune regulation of autoimmune hepatitis is linked to a defective galectin-9/tim-3 pathway. *Hepatology* 56, 677–686.
- Liblau, R., Tournier-Lasserve, E., Maciazek, J., Dumas, G., Sifert, O., Hashim, G., et al., 1991. T cell response to myelin basic protein epitopes in multiple sclerosis patients and healthy subjects. *Eur. J. Immunol.* 21, 1391–1395.
- Linsk, R., Gottesman, M., Pernis, B., 1989. Are tissues a patch quilt of ectopic gene expression? *Science* 246 (4927), 261.
- Linsley, P.S., Clark, E.A., Ledbetter, J.A., 1990. T-cell antigen CD28 mediates adhesion with B cells by interacting with activation antigen B7/BB-1. *Proc. Natl. Acad. Sci. U.S.A.* 87, 5031–5035.
- Liston, A., Nutsch, K.M., Farr, A.G., Lund, J.M., Rasmussen, J.P., Koni, P.A., et al., 2008. Differentiation of regulatory Foxp3 + T cells in the thymic cortex. *Proc. Natl. Acad. Sci. U.S.A.* 105, 11903–11908.
- Liu, G.Y., Wraith, D.C., 1995. Affinity for class II MHC determines the extent to which soluble peptides tolerize autoreactive T cells in naive and primed adult mice—implications for autoimmunity. *Int. Immunol.* 7, 1255–1263.

- Magnusson, F.C., Liblau, R.S., von Boehmer, H., Pittet, M.J., Lee, J.-W., Turley, S.J., et al., 2008. Direct presentation of antigen by lymph node stromal cells protects against CD8 T-cell-mediated intestinal autoimmunity. *Gastroenterology* 134, 1028–1037.
- Mahmud, S.A., Manlove, L.S., Schmitz, H.M., Xing, Y., Wang, Y., Owen, D.L., et al., 2014. Costimulation via the tumor-necrosis factor receptor superfamily couples TCR signal strength to the thymic differentiation of regulatory T cells. *Nat. Immunol.* 15, 473–481.
- Malchow, S., Leventhal, D.S., Nishi, S., Fischer, B.I., Shen, L., Paner, G.P., et al., 2013. Aire-dependent thymic development of tumor-associated regulatory T cells. *Science* 339, 1219–1224.
- Malchow, S., Leventhal, D.S., Lee, V., Nishi, S., Socci, N.D., Savage, P.A., 2016. Aire enforces immune tolerance by directing autoreactive T cells into the regulatory T cell lineage. *Immunity* 44, 1102–1113.
- Maldonado, R.A., von Andrian, U.H., 2010. How tolerogenic dendritic cells induce regulatory T cells. *Adv. Immunol.* 108, 111–165.
- Malhotra, D., Fletcher, A.L., Astarita, J., Lukacs-Kornek, V., Tayalia, P., Gonzalez, S.F., et al., 2012. Transcriptional profiling of stroma from inflamed and resting lymph nodes defines immunological hallmarks. *Nat. Immunol.* 13, 499–510.
- Malhotra, D., Fletcher, A.L., Turley, S.J., 2013. Stromal and hematopoietic cells in secondary lymphoid organs: partners in immunity. *Immunol. Rev.* 251, 160–176.
- Market, E., Papavasiliou, F.N., 2003. V(D)J recombination and the evolution of the adaptive immune system. *PLoS Biol.* 1, e16.
- Marrack, P., Bender, J., Hildeman, D., Jordan, M., Mitchell, T., Murakami, M., et al., 2000. Homeostasis of alpha beta TCR + T cells. *Nat. Immunol.* 1, 107–111.
- Martinez, R.J., Zhang, N., Thomas, S.R., Nandiwada, S.L., Jenkins, M.K., Binstadt, B.A., et al., 2012. Arthritogenic self-reactive CD4 + T cells acquire an FR4hiCD73hi anergic state in the presence of Foxp3 + regulatory T cells. *J. Immunol.* 188, 170–181.
- Masopust, D., Vezys, V., Usherwood, E.J., Cauley, L.S., Olson, S., Marzo, A.L., et al., 2004. Activated primary and memory CD8 T cells migrate to nonlymphoid tissues regardless of site of activation or tissue of origin. *J. Immunol.* 172, 4875–4882.
- Mathis, D., Benoist, C., 2010. Levees of immunological tolerance. *Nat. Immunol.* 11, 3–6.
- Maynard, C.L., Harrington, L.E., Janowski, K.M., Oliver, J.R., Zindl, C.L., Rudensky, A.Y., et al., 2007. Regulatory T cells expressing interleukin 10 develop from Foxp3 + and Foxp3 – precursor cells in the absence of interleukin 10. *Nat. Immunol.* 8, 931–941.
- McCaughtry, T.M., Baldwin, T.A., Wilken, M.S., Hogquist, K.A., 2008. Clonal deletion of thymocytes can occur in the cortex with no involvement of the medulla. *J. Exp. Med.* 205, 2575–2584.
- Mempel, T.R., Henrickson, S.E., von Andrian, U.H., 2004. T-cell priming by dendritic cells in lymph nodes occurs in three distinct phases. *Nature* 427, 154–159.
- Meredith, M., Zemmour, D., Mathis, D., Benoist, C., 2015. Aire controls gene expression in the thymic epithelium with ordered stochasticity. *Nat. Immunol.* 16, 942–949.
- Mezrich, J.D., Fechner, J.H., Zhang, X., Johnson, B.P., Burlingham, W.J., Bradfield, C.A., 2010. An interaction between kynurenone and the aryl hydrocarbon receptor can generate regulatory T cells. *J. Immunol.* 185, 3190–3198.
- Miller, J.F., 1961. Immunological function of the thymus. *Lancet* 2, 748–749.
- Moran, A.E., Holzapfel, K.L., Xing, Y., Cunningham, N.R., Maltzman, J.S., Punt, J., et al., 2011. T cell receptor signal strength in Treg and iNKT cell development demonstrated by a novel fluorescent reporter mouse. *J. Exp. Med.* 208, 1279–1289.
- Morrissey, P.J., Charrier, K., Braddy, S., Liggitt, D., Watson, J.D., 1993. CD4 + T cells that express high levels of CD45RB induce wasting disease when transferred into congenic severe combined immunodeficient mice. Disease development is prevented by cotransfer of purified CD4 + T cells. *J. Exp. Med.* 178, 237–244.
- Mouri, Y., Nishijima, H., Kawano, H., Hirota, F., Sakaguchi, N., Morimoto, J., et al., 2014. NF-B-inducing kinase in thymic stroma establishes central tolerance by orchestrating cross-talk with not only thymocytes but also dendritic cells. *J. Immunol.* 193, 4356–4367.
- Mueller, D.L., 2010. Mechanisms maintaining peripheral tolerance. *Nat. Immunol.* 11, 21–27.
- Mueller, D.L., Jenkins, M.K., Schwartz, R.H., 1989. Clonal expansion versus functional clonal inactivation: a costimulatory signalling pathway determines the outcome of T cell antigen receptor occupancy. *Annu. Rev. Immunol.* 7, 445–480.
- Müller, M.R., Rao, A., 2010. NFAT, immunity and cancer: a transcription factor comes of age. *Nat. Rev. Immunol.* 10, 645–656.
- Murphy, K.M., Heimberger, A.B., Loh, D.Y., 1990. Induction by antigen of intrathymic apoptosis of CD4 + CD8 + TCRlo thymocytes in vivo. *Science* 250, 1720–1723.
- Nagamine, K., Peterson, P., Scott, H.S., Kudoh, J., Minoshima, S., Heino, M., et al., 1997. Positional cloning of the APECED gene. *Nat. Genet.* 17, 393–398.
- Nakamura, K., Kitani, A., Strober, W., 2001. Cell contact-dependent immunosuppression by CD4(+)CD25(+) regulatory T cells is mediated by cell surface-bound transforming growth factor beta. *J. Exp. Med.* 194, 629–644.
- Nedjic, J., Aichinger, M., Emmerich, J., Mizushima, N., Klein, L., 2008. Autophagy in thymic epithelium shapes the T-cell repertoire and is essential for tolerance. *Nature* 455, 396–400.
- Nichols, L.A., Chen, Y., Colella, T.A., Bennett, C.L., Clausen, B.E., Engelhard, V.H., 2007. Deletional self-tolerance to a melanocyte/melanoma antigen derived from tyrosinase is mediated by a radio-resistant cell in peripheral and mesenteric lymph nodes. *J. Immunol.* 179, 993–1003.
- Nishimura, H., Nose, M., Hiai, H., Minato, N., Honjo, T., 1999. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity* 11, 141–151.
- Nishizuka, Y., Sakakura, T., 1969. Thymus and reproduction: sex-linked dysgenesis of the gonad after neonatal thymectomy in mice. *Science* 166, 753–755.
- Nosbaum, A., Prevel, N., Truong, H.-A., Mehta, P., Ettinger, M., Scharschmidt, T.C., et al., 2016. Cutting edge: regulatory T cells facilitate cutaneous wound healing. *J. Immunol.* 196, 2010–2014.
- Ohnacht, C., Park, J.-H., Cording, S., Wing, J.B., Atarashi, K., Obata, Y., et al., 2015. The microbiota regulates type 2 immunity through ROR $\gamma$ t + T cells. *Science* 349 (6251), 989–993.
- Opitz, C.A., Litzenburger, U.M., Sahm, F., Ott, M., Tritschler, I., Trump, S., et al., 2011. An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. *Nature* 478, 197–203.

- Org, T., Chignola, F., Hetényi, C., Gaetani, M., Rebane, A., Liiv, I., et al., 2008. The autoimmune regulator PHD finger binds to non-methylated histone H3K4 to activate gene expression. *EMBO Rep.* 9, 370–376.
- Org, T., Rebane, A., Kisand, K., Laan, M., Haljasorg, U., Andreson, R., et al., 2009. AIRE activated tissue specific genes have histone modifications associated with inactive chromatin. *Hum. Mol. Genet.* 18, 4699–4710.
- Ostroukhova, M., Seguin-Devaux, C., Oriss, T.B., Dixon-McCarthy, B., Yang, L., Ameredes, B.T., et al., 2004. Tolerance induced by inhaled antigen involves CD4+ T cells expressing membrane-bound TGF- $\beta$  and FOXP3. *J. Clin. Invest.* 114, 28–38.
- Owen, I., Brdicková, N., Kohoutek, J., Vaupotic, T., Narat, M., Peterlin, B.M., 2007. AIRE recruits P-TEFb for transcriptional elongation of target genes in medullary thymic epithelial cells. *Mol. Cell. Biol.* 27, 8815–8823.
- Owen, R.D., 1945. Immunogenetic consequences of vascular anastomoses between bovine twins. *Science* 102 (2651), 400–401.
- Pacholczyk, R., Ignatowicz, H., Kraj, P., Ignatowicz, L., 2006. Origin and T cell receptor diversity of Foxp3+ CD4+ CD25+ T cells. *Immunity* 25, 249–259.
- Paiva, R.S., Lino, A.C., Bergman, M.-L., Caramalho, I., Sousa, A.E., Zelenay, S., et al., 2013. Recent thymic emigrants are the preferential precursors of regulatory T cells differentiated in the periphery. *Proc. Natl. Acad. Sci. U.S.A.* 110, 6494–6499.
- Palmer, E., 2003. Negative selection – clearing out the bad apples from the T-cell repertoire. *Nat. Rev. Immunol.* 3, 383–391.
- Pandiyar, P., Zheng, L., Lenardo, M.J., 2011. The molecular mechanisms of regulatory T cell immunosuppression. *Front Immunol.* 2, 60.
- Panduro, M., Benoist, C., Mathis, D., 2016. Tissue Tregs. *Annu. Rev. Immunol.* 34, 609–633.
- Pape, K.A., Merica, R., Mondino, A., Khoruts, A., Jenkins, M.K., 1998. Direct evidence that functionally impaired CD4+ T cells persist in vivo following induction of peripheral tolerance. *J. Immunol.* 160, 4719–4729.
- Perry, J.S.A., Lio, C.J., Kau, A.L., Nutsch, K., Yang, Z., Gordon, J.I., et al., 2014. Distinct contributions of Aire and antigen-presenting-cell subsets to the generation of self-tolerance in the thymus. *Immunity* 41, 414–426.
- Piazza, C., Gilardini Montani, M.S., Moretti, S., Cundari, E., Piccolella, E., 1997. Cutting edge: CD4+ T cells kill CD8+ T cells via Fas/Fas ligand-mediated apoptosis. *J. Immunol.* 158, 1503–1506.
- Picca, C.C., Oh, S., Panarey, L., Aitken, M., Basehoar, A., Caton, A.J., 2009. Thymocyte deletion can bias Treg formation toward low-abundance self-peptide. *Eur. J. Immunol.* 39, 3301–3306.
- Pinto, S., Michel, C., Schmidt-Glenewinkel, H., Harder, N., Rohr, K., Wild, S., et al., 2013. Overlapping gene coexpression patterns in human medullary thymic epithelial cells generate self-antigen diversity. *Proc. Natl. Acad. Sci. U.S.A.* 110, E3497–E3505.
- Pott, S., Lieb, J.D., 2014. What are super-enhancers? *Nat. Genet.* 47, 8–12.
- Powrie, F., Mason, D., 1990. OX-22high CD4+ T cells induce wasting disease with multiple organ pathology: prevention by the OX-22low subset. *J. Exp. Med.* 172, 1701–1708.
- Powrie, F., Leach, M.W., Mauze, S., Caddle, L.B., Coffman, R.L., 1993. Phenotypically distinct subsets of CD4+ T cells induce or protect from chronic intestinal inflammation in C. B-17 SCID mice. *Int. Immunopharmacol.* 5, 1461–1471.
- Pribyl, T.M., Campagnoni, C., Kampf, K., Handley, V.W., Campagnoni, A.T., 1996. The major myelin protein genes are expressed in the human thymus. *J. Neurosci. Res.* 45, 812–819.
- Puck, J.M., Sneller, M.C., 1997. ALPS: an autoimmune human lymphoproliferative syndrome associated with abnormal lymphocyte apoptosis. *Semin. Immunol.* 9, 77–84.
- Qureshi, O.S., Zheng, Y., Nakamura, K., Attridge, K., Manzotti, C., Schmidt, E.M., et al., 2011. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. *Science* 332, 600–603.
- Ren, X., Ye, F., Jiang, Z., Chu, Y., Xiong, S., Wang, Y., 2007. Involvement of cellular death in TRAIL/DR5-dependent suppression induced by CD4(+)/CD25(+) regulatory T cells. *Cell Death Differ.* 14, 2076–2084.
- Rudensky, A.Y., Gavin, M., Zheng, Y., 2006. FOXP3 and NFAT: partners in tolerance. *Cell* 126, 253–256.
- Sakaguchi, S., 1982. Study on cellular events in post-thymectomy autoimmune oophoritis in mice. II. Requirement of Lyt-1 cells in normal female mice for the prevention of oophoritis. *J. Exp. Med.* 156, 1577–1586.
- Sakaguchi, S., Takahashi, T., Nishizuka, Y., 1982. Study on cellular events in postthymectomy autoimmune oophoritis in mice. I. Requirement of Lyt-1 effector cells for oocytes damage after adoptive transfer. *J. Exp. Med.* 156, 1565–1576.
- Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M., Toda, M., 1995. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol.* 155, 1151–1164.
- Sakamoto, Y., Matsumoto, T., Mizunoe, Y., Haraoka, M., Sakumoto, M., Kumazawa, J., 1995. Testicular injury induces cell-mediated autoimmune response to testis. *J. Urol.* 153, 1316–1320.
- Samstein, R.M., Josefowicz, S.Z., Arvey, A., Treuting, P.M., Rudensky, A.Y., 2012. Extrathymic generation of regulatory T cells in placental mammals mitigates maternal-fetal conflict. *Cell* 150, 29–38.
- Sanchez Rodriguez, R., Pauli, M.L., Neuhaus, I.M., Yu, S.S., Arron, S.T., Harris, H.W., et al., 2014. Memory regulatory T cells reside in human skin. *J. Clin. Invest.* 124, 1027–1036.
- Sansom, S.N., Shikama-Dorn, N., Zhanybekova, S., Nusspaumer, G., Macaulay, I.C., Deadman, M.E., et al., 2014. Population and single-cell genomics reveal the Aire dependency, relief from Polycomb silencing, and distribution of self-antigen expression in thymic epithelia. *Genome Res.* 24, 1918–1931.
- Sarrabayrouse, G., Bossard, C., Chauvin, J.-M., Jarry, A., Meurette, G., Quévrain, E., et al., 2014. CD4CD8 $\alpha\alpha$  lymphocytes, a novel human regulatory T cell subset induced by colonic bacteria and deficient in patients with inflammatory bowel disease. *PLoS Biol.* 12, e1001833.
- Scharschmidt, T.C., Vasquez, K.S., Truong, H.-A., Gearty, S.V., Pauli, M.L., Nosbaum, A., et al., 2015. A wave of regulatory T cells into neonatal skin mediates tolerance to commensal microbes. *Immunity* 43, 1011–1021.
- Scharschmidt, T.C., Vasquez, K.S., Pauli, M.L., Leitner, E.G., Chu, K., Truong, H.-A., et al., 2017. Commensal microbes and hair follicle morphogenesis coordinately drive Treg migration into neonatal skin. *Cell Host Microbe* 21, 467–477.e5.
- Schiering, C., Krausgruber, T., Chomka, A., Fröhlich, A., Adelmann, K., Wohlfert, E.A., et al., 2014. The alarmin IL-33 promotes regulatory T-cell function in the intestine. *Nature* 513, 564–568.

- Sefik, E., Geva-Zatorsky, N., Oh, S., Konnikova, L., Zemmour, D., McGuire, A.M., et al., 2015. Individual intestinal symbionts induce a distinct population of ROR $\gamma^+$  regulatory T cells. *Science* 349, 993–997.
- Sha, W.C., Nelson, C.A., Newberry, R.D., Kranz, D.M., Russell, J.H., Loh, D.Y., 1988. Positive and negative selection of an antigen receptor on T cells in transgenic mice. *Nature* 336, 73–76.
- Simons, D.M., Picca, C.C., Oh, S., Perng, O.A., Aitken, M., Erikson, J., et al., 2010. How specificity for self-peptides shapes the development and function of regulatory T cells. *J. Leukoc. Biol.* 88, 1099–1107.
- Smith, H., Sakamoto, Y., Kasai, K., Tung, K.S., 1991. Effector and regulatory cells in autoimmune oophoritis elicited by neonatal thymectomy. *J. Immunol.* 147, 2928–2933.
- Smith, K.M., Olson, D.C., Hirose, R., Hanahan, D., 1997. Pancreatic gene expression in rare cells of thymic medulla: evidence for functional contribution to T cell tolerance. *Int. Immunol.* 9, 1355–1365.
- Smith, P.M., Howitt, M.R., Panikov, N., Michaud, M., Gallini, C.A., Bohlooly-Y, M., et al., 2013. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* 341 (6145), 569–573.
- Sospedra, M., Ferrer-Francesch, X., Domínguez, O., Juan, M., Foz-Sala, M., Pujol-Borrell, R., 1998. Transcription of a broad range of self-antigens in human thymus suggests a role for central mechanisms in tolerance toward peripheral antigens. *J. Immunol.* 161, 5918–5929.
- Soto-Nieves, N., Puga, I., Abe, B.T., Bandyopadhyay, S., Baine, I., Rao, A., et al., 2009. Transcriptional complexes formed by NFAT dimers regulate the induction of T cell tolerance. *J. Exp. Med.* 206, 867–876.
- Steinman, L., 2015. No quiet surrender: molecular guardians in multiple sclerosis brain. *J. Clin. Invest.* 125, 1371–1378.
- Stritesky, G.L., Jameson, S.C., Hogquist, K.A., 2012. Selection of self-reactive T cells in the thymus. *Annu. Rev. Immunol.* 30, 95–114.
- Stritesky, G.L., Xing, Y., Erickson, J.R., Kalekar, L.A., Wang, X., Mueller, D.L., et al., 2013. Murine thymic selection quantified using a unique method to capture deleted T cells. *Proc. Natl. Acad. Sci. U.S.A.* 110, 4679–4684.
- Sun, J.B., Olsson, T., Wang, W.Z., Xiao, B.G., Kostulas, V., Fredrikson, S., et al., 1991. Autoreactive T and B cells responding to myelin proteolipid protein in multiple sclerosis and controls. *Eur. J. Immunol.* 21, 1461–1468.
- Sun, J., Furio, L., Mecheri, R., van der Does, A.M., Lundeberg, E., Saveanu, L., et al., 2015. Pancreatic  $\beta$ -cells limit autoimmune diabetes via an immunoregulatory antimicrobial peptide expressed under the influence of the gut microbiota. *Immunity* 43, 304–317.
- Surh, C., Sprent, J., 1994. T-cell apoptosis detected in situ during positive and negative selection in the thymus. *Nature* 372, 100–103.
- Suri-Payer, E., Amar, A.Z., Thornton, A.M., Shevach, E.M., 1998. CD4 + CD25 + T cells inhibit both the induction and effector function of autoreactive T cells and represent a unique lineage of immunoregulatory cells. *J. Immunol.* 160, 1212–1218.
- Takaba, H., Morishita, Y., Tomofuji, Y., Danks, L., Nitta, T., Komatsu, N., et al., 2015. Fezf2 orchestrates a thymic program of self-antigen expression for immune tolerance. *Cell* 163, 975–987.
- Taniguchi, R.T., DeVoss, J.J., Moon, J.J., Sidney, J., Sette, A., Jenkins, M.K., et al., 2012. Detection of an autoreactive T-cell population within the polyclonal repertoire that undergoes distinct autoimmune regulator (Aire)-mediated selection. *Proc. Natl. Acad. Sci. U.S.A.* 109, 7847–7852.
- Tanoue, T., Atarashi, K., Honda, K., 2016. Development and maintenance of intestinal regulatory T cells. *Nat. Rev. Immunol.* 16, 295–309.
- Tivol, E.A., Borriello, F., Schweitzer, A.N., Lynch, W.P., Bluestone, J.A., Sharpe, A.H., 1995. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity* 3, 541–547.
- Tourne, S., Miyazaki, T., Oxenius, A., Klein, L., Fehr, T., Kyewski, B., et al., 1997. Selection of a broad repertoire of CD4 + T cells in H-2Ma0/0 mice. *Immunity* 7, 187–195.
- Traub, E., 1938. Immunization of guinea pigs against lymphocytic choriomeningitis with formalized tissue vaccines. *J. Exp. Med.* 68, 95–110.
- Tung, K.S., Smith, S., Teuscher, C., Cook, C., Anderson, R.E., 1987. Murine autoimmune oophoritis, epididymoorchitis, and gastritis induced by day 3 thymectomy. *Immunopathol. Am. J. Pathol.* 126, 293–302.
- Tykocinski, L.-O., Simenus, A., Rezavandy, E., Weiland, Y., Baddeley, D., Cremer, C., et al., 2010. Epigenetic regulation of promiscuous gene expression in thymic medullary epithelial cells. *Proc. Natl. Acad. Sci. U.S.A.* 107, 19426–19431.
- Vahl, J.C., Drees, C., Heger, K., Heink, S., Fischer, J.C., Nedjic, J., et al., 2014. Continuous T cell receptor signals maintain a functional regulatory T cell pool. *Immunity* 41, 722–736.
- van Meerwijk, J.P., Marguerat, S., Lees, R.K., Germain, R.N., Fowlkes, B.J., MacDonald, H.R., 1997. Quantitative impact of thymic clonal deletion on the T cell repertoire. *J. Exp. Med.* 185, 377–383.
- Vignali, D.A.A., Collison, L.W., Workman, C.J., 2008. How regulatory T cells work. *Nat. Immunol.* 9, 523–532.
- Villaseñor, J., Besse, W., Benoist, C., Mathis, D., 2008. Ectopic expression of peripheral-tissue antigens in the thymic epithelium: probabilistic, monoallelic, misinitiated. *Proc. Natl. Acad. Sci. U.S.A.* 105, 15854–15859.
- Walker, L.S.K., Abbas, A.K., 2002. The enemy within: keeping self-reactive T cells at bay in the periphery. *Nat. Rev. Immunol.* 2, 11–19.
- Walters, S.N., Webster, K.E., Daley, S., Grey, S.T., 2014. A role for intrathymic B cells in the generation of natural regulatory T cells. *J. Immunol.* 193, 170–176.
- Wang, F., Wan, L., Zhang, C., Zheng, X., Li, J., Chen, Z.K., 2009. Tim-3-Galectin-9 pathway involves the suppression induced by CD4 + CD25 + regulatory T cells. *Immunobiology* 214, 342–349.
- Wang, Y., Su, M.A., Wan, Y.Y., 2011. An essential role of the transcription factor GATA-3 for the function of regulatory T cells. *Immunity* 35, 337–348.
- Wang, R.-X., Yu, C.-R., Dambuza, I.M., Mahdi, R.M., Dolinska, M.B., Sergeev, Y.V., et al., 2014. Interleukin-35 induces regulatory B cells that suppress autoimmune disease. *Nat. Med.* 20, 633–641.
- Waterfield, M., Khan, I.S., Cortez, J.T., Fan, U., Metzger, T., Greer, A., et al., 2014. The transcriptional regulator Aire coopts the repressive ATF7ip-MBD1 complex for the induction of immunotolerance. *Nat. Immunol.* 15, 258–265.
- Waterhouse, P., Penninger, J.M., Timms, E., Wakeham, A., Shahinian, A., Lee, K.P., et al., 1995. Lymphoproliferative disorders with early lethality in mice deficient in Cta-4. *Science* 270, 985–988.
- Weber, S.E., Harbertson, J., Godebu, E., Mros, G.A., Padrick, R.C., Carson, B.D., et al., 2006. Adaptive islet-specific regulatory CD4 T cells control autoimmune diabetes and mediate the disappearance of pathogenic Th1 cells in vivo. *J. Immunol.* 176, 4730–4739.
- Wherry, E.J., Kurachi, M., 2015. Molecular and cellular insights into T cell exhaustion. *Nat. Rev. Immunol.* 15, 486–499.

- Wildin, R.S., 2002. Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. *J. Med. Genet.* 39, 537–545.
- Wing, K., Onishi, Y., Prieto-martin, P., Yamaguchi, T., Miyara, M., Fehervari, Z., et al., 2008. CTLA-4 control over Foxp3<sup>+</sup> regulatory T cell function. *Science* 322 (5899), 271–275.
- Wirnsberger, G., Hinterberger, M., Klein, L., 2011. Regulatory T-cell differentiation versus clonal deletion of autoreactive thymocytes. *Immunol. Cell Biol.* 89, 45–53.
- Wohlfert, E.A., Grainger, J.R., Bouladoux, N., Konkel, J.E., Oldenhove, G., Ribeiro, C.H., et al., 2011. GATA3 controls Foxp3<sup>+</sup> regulatory T cell fate during inflammation in mice. *J. Clin. Invest.* 121, 4503–4515.
- Woodland, D., Happ, M.P., Bill, J., Palmer, E., 1990. Requirement for cotolerogenic gene products in the clonal deletion of I-E reactive T cells. *Science* 247, 964–967.
- Wyss, L., Stadinski, B.D., King, C.G., Schallenberg, S., McCarthy, N.I., Lee, J.Y., et al., 2016. Affinity for self antigen selects Treg cells with distinct functional properties. *Nat. Immunol.* 17, 1093–1101.
- Xing, Y., Wang, X., Jameson, S.C., Hogquist, K.A., 2016. Late stages of T cell maturation in the thymus involve NF-κB and tonic type I interferon signaling. *Nat. Immunol.* 17, 565–573.
- Yamano, T., Nedjic, J., Hinterberger, M., Steinert, M., Koser, S., Pinto, S., et al., 2015. Thymic B cells are licensed to present self antigens for central T cell tolerance induction. *Immunity* 42, 1048–1061.
- Yang, B.-H., Hagemann, S., Mamareli, P., Lauer, U., Hoffmann, U., Beckstette, M., et al., 2016. Foxp3<sup>+</sup> T cells expressing ROR $\gamma$ t represent a stable regulatory T-cell effector lineage with enhanced suppressive capacity during intestinal inflammation. *Mucosal Immunol.* 9, 444–457.
- Yang, S., Fujikado, N., Kolodkin, D., Benoist, C., Mathis, D., 2015. Regulatory T cells generated early in life play a distinct role in maintaining self-tolerance. *Science* 348, 589–594.
- Yu, F., Sharma, S., Edwards, J., Feigenbaum, L., Zhu, J., 2015a. Dynamic expression of transcription factors T-bet and GATA-3 by regulatory T cells maintains immunotolerance. *Nat. Immunol.* 16, 197–206.
- Yu, W., Jiang, N., Ebert, P.J.R., Kidd, B.A., Müller, S., Lund, P.J., et al., 2015b. Clonal deletion prunes but does not eliminate self-specific  $\alpha\beta$  CD8<sup>+</sup> T lymphocytes. *Immunity* 42, 929–941.
- Zhang, L., Zhang, Z.-X., Yang, L., Young, K.J., DuTemple, B., 2000. Identification of a previously unknown antigen-specific regulatory T cell and its mechanism of suppression. *Nat. Med.* 6, 782–789.
- Zhao, D.-M., Thornton, A.M., DiPaolo, R.J., Shevach, E.M., 2006. Activated CD4<sup>+</sup>CD25<sup>+</sup> T cells selectively kill B lymphocytes. *Blood* 107, 3925–3932.
- Zheng, Y., Chaudhry, A., Kas, A., deRoos, P., Kim, J.M., Chu, T.-T., et al., 2009. Regulatory T-cell suppressor program co-opts transcription factor IRF4 to control Th2 responses. *Nature* 458, 351–356.

## Further Reading

- Bouillet, P., Purton, J.F., Godfrey, D.I., Zhang, L.-C., Coulter, L., Puthalakath, H., et al., 2002. BH3-only Bcl-2 family member Bim is required for apoptosis of autoreactive thymocytes. *Nature* 415, 922–926.
- Li, J., Park, J., Foss, D., Goldschneider, I., 2009. Thymus-homing peripheral dendritic cells constitute two of the three major subsets of dendritic cells in the steady-state thymus. *J. Exp. Med.* 206, 607–622.

## 6

# T Cells and Their Subsets in Autoimmunity

Patrick R. Burkett<sup>1,2,\*</sup>, Mathias Pawlak<sup>1,\*</sup>, Anneli Peters<sup>3,\*</sup> and  
Vijay K. Kuchroo<sup>1</sup>

<sup>1</sup>Evergrande Center for Immunologic Diseases, Harvard Medical School and Brigham and Women's Hospital, Boston, MA, United States <sup>2</sup>Pulmonary and Critical Care Division, Department of Medicine, Brigham and Women's Hospital, Boston, MA, United States <sup>3</sup>Max Planck Institute of Neurobiology, Martinsried, Germany

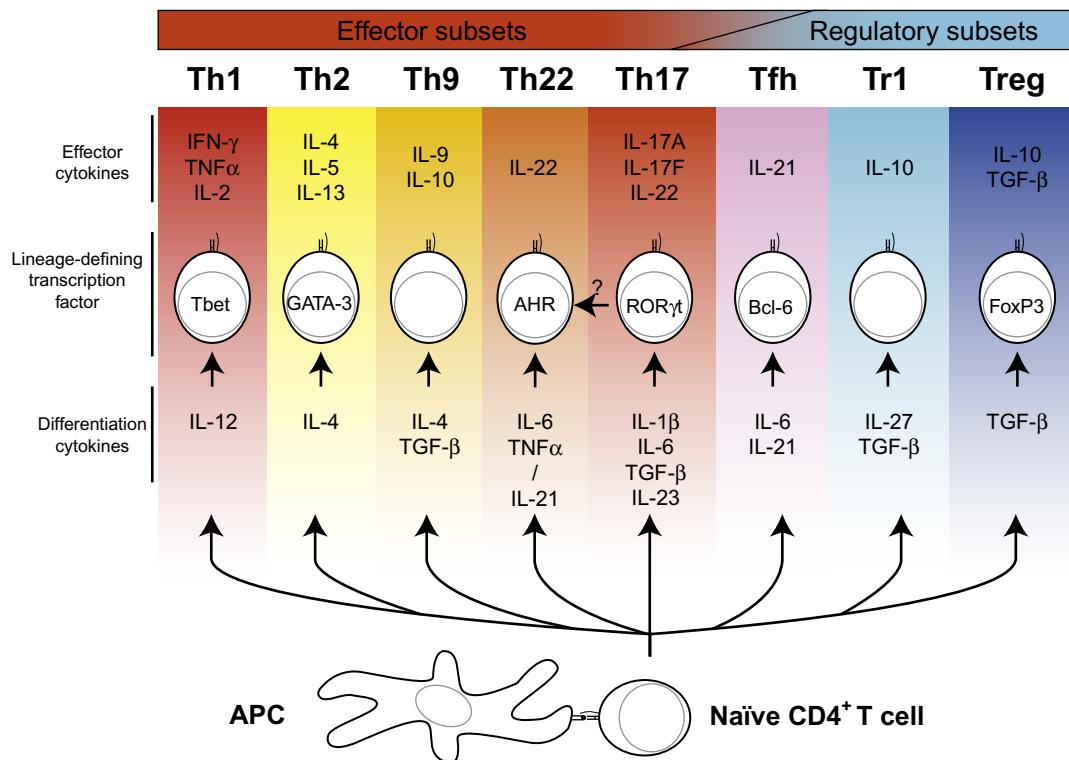
## O U T L I N E

Introduction	91	Regulatory CD4 <sup>+</sup> T Cells	99
T Helper 1 Cells	92	Type 1 Regulatory T Cells	102
T Helper 17 Cells	94	Follicular T Helper Cells	102
Identification	94	T Helper 2 Cells	104
Differentiation	94	T Helper 9 Cells	105
Pathogenicity	94	Concluding Remarks	105
Transcriptional Regulation	96	References	105
Environmental Cues and T Helper 17 Cell	97	Further Reading	116
Regulation in the Intestine	97		
Function	98		
T Helper 22 Cells	98		

## INTRODUCTION

The immune system has evolved to defend against a wide array of pathogens, and optimal immune function requires coordinated responses from both the innate and adaptive systems. While the innate immune system utilizes a relatively small number of invariant receptors specific for conserved microbial products, such as the cell wall components of bacteria, the adaptive immune system makes use of a nearly unlimited repertoire of receptors generated by random recombination of the T- and B-cell receptor loci. Although random recombination allows for incredible lymphocyte receptor diversity, it comes at the price of generating potentially autoreactive receptors. The risk for self-reactivity is in part mitigated by checkpoints during lymphocyte development that eliminate most of the self-reactive clones (referred to as central tolerance) as well as mechanisms that limit the ability of self-reactive clones to mount responses in the periphery (referred to as peripheral tolerance). However, the existence of lymphocyte-dependent autoimmune disease is a clear indication that these mechanisms are imperfect and some self-reactive lymphocytes are able to initiate destructive autoimmune inflammation.

\*These authors contributed equally to this work.



**FIGURE 6.1** Generation and function of Th subsets. Naïve CD4<sup>+</sup> T cells are capable of differentiating into various effector and regulatory subsets. Th subsets are shown, along with the cytokines critical for differentiation, lineage-defining transcription factors, and effector cytokines. *Th*, T helper.

Self-reactive CD4<sup>+</sup> T helper (Th) cells play a crucial role in the pathogenesis of many human autoimmune diseases. This is in part due to the unique ability of Th cells to undergo further differentiation into distinct subsets that are specialized at recruiting and coordinating different immune effector mechanisms, largely via the secretion of specific combinations of cytokines and chemokines (Fig. 6.1). The differentiation of naïve Th cells into distinct subsets is dependent both upon recognition of cognate antigen–MHC complexes as well as environmental cues, particularly cytokines, that drive different transcriptional modules. Although multiple factors are important for Th differentiation, in many cases a single transcription factor is crucial for initiating and stabilizing the expression of the transcriptional module that defines a given Th subset (Fig. 6.1). In addition, there is a complicated interplay between the different Th subsets, as factors that are produced by one subset may inhibit the differentiation of another. Thus, understanding the factors that drive differentiation of Th subsets and the effector mechanisms those Th subsets utilize to promote or inhibit tissue inflammation is important for understanding the mechanisms that drive induction of autoimmune disease.

## T HELPER 1 CELLS

In 1986 Tim Mosmann and Robert Coffman published a seminal paper describing the heterogeneity of effector CD4<sup>+</sup> T cells, which they divided into two distinct subsets, called Th1 and Th2 cells, so named because they were the first two to be described (Mosmann et al., 1986). Th1 cells play a critical role in inducing protective immune responses to extracellular pathogens such as *Mycobacterium tuberculosis* or intracellular pathogens such as *Listeria monocytogenes* (Mosmann and Coffman, 1989a,b; Neighbors et al., 2001; Geginat et al., 1998; Viegas et al., 2007; Wan and Flavell, 2009). In response to certain microbial stimuli, dendritic cells and macrophages produce cytokines, including interleukin (IL)-12, IL-18, and type 1 interferons (IFNs), that promote Th1 development by inducing the expression of the transcription factor T-bet, the key transcriptional regulator of Th1 cells (Nakanishi et al., 2001; Szabo et al., 2003; Wan and Flavell, 2009). While multiple cytokines are important for Th1 differentiation, IL-12 is particularly crucial. Thus, mice deficient for either IL-12 or the IL-12 receptor (IL-12R)

chain have impaired Th1 differentiation and are extremely susceptible to intracellular pathogens (Mosmann and Coffman, 1989a,b). Upon binding to its receptor, IL-12 promotes Th1 lineage commitment by activating STAT4, which then enhances production of IFN- $\gamma$ , the hallmark cytokine of Th1 cells (Oh and Ghosh, 2013; Yamane and Paul, 2013; Mucida and Cheroutre, 2010). IFN- $\gamma$  signals in an autocrine manner via activation of STAT1 to enhance T-bet expression, thus further promoting Th1 polarization. T-bet then maintains IFN- $\gamma$  production, in part by inducing chromatin remodeling of the IFN- $\gamma$  locus (Szabo et al., 2003; Oh and Ghosh, 2013; Yamane and Paul, 2013; Mucida and Cheroutre, 2010). Finally, IFN- $\gamma$  also inhibits the differentiation of naïve CD4 $^{+}$  T cells into other subsets, such as Th2 or Th17 cells (Schoenborn and Wilson, 2007). Thus Th1 differentiation utilizes a feed-forward loop, in which IFN- $\gamma$  is first induced and then acts to amplify Th1 differentiation and, at the same time, suppresses the differentiation of other T-cell subsets. Notably, T-bet also exerts a repressive function toward the type I IFN transcriptional program, further highlighting the complex transcriptional response mediated by T-bet (Iwata et al., 2017).

Pathways that are crucial to innate immune cell function were recently described to also play a vital role in the induction and function of human Th1 cells. For example, the inflammasome component NLRP3 and the complement protein C5 work in conjunction to stabilize Th1 cell differentiation and prevent alternative fates (Arbore et al., 2016). In addition, complement controls nutrient influx to regulate cellular metabolism during Th1 responses (Kolev et al., 2015). Similarly, recent studies indicate that aerobic glycolysis directs Th1 differentiation in the gut, highlighting the importance of metabolism in Th cell differentiation (Peng et al., 2016). Finally, retinoic acid signaling stabilizes the Th1 lineage and represses genes that are important for other Th lineages, such as Th17 cells (Brown et al., 2015). Therefore multiple molecular and metabolic pathways are responsible for promoting the unidirectional development of Th1 cells, both by supporting Th1 cell differentiation and also suppressing alternate cell fates.

The role of Th1 cells in inflammation and autoimmune pathology has been extensively studied and Th1 cells have been shown to play an important role in many organ-specific autoimmune diseases, including insulin-independent type 1 diabetes mellitus (IDDM), multiple sclerosis (MS), inflammatory bowel disease (IBD), and rheumatoid arthritis (RA) (Szabo et al., 2003). Prior to the discovery of Th17 cells, an imbalance between Th1/Th2 subsets was proposed to be a key driver of autoimmunity, with skewing toward a Th2 phenotype being considered protective, while Th1 predominance appeared to be pathogenic (Adorini et al., 1996a,b). For instance, animal studies revealed that transgenic expression of IL-4 on pancreatic islets protected nonobese diabetic mice from developing IDDM, while adoptive transfer of Th1 cell clones induced diabetes (Katz et al., 1995; Mueller et al., 1996). IFN- $\gamma$ -producing Th cells were often found within the target tissue in multiple other organ-specific human autoimmune diseases, thereby implicating Th1 cells in disease pathogenesis. Subsequent studies in animal models of human diseases have further validated the importance of Th1 cells in causing immunopathology (Szabo et al., 2003; Lafaille, 1998; Oppmann et al., 2000; Neurath et al., 2002; Kuchroo et al., 1993). Moreover, IL-18, another cytokine associated with the Th1 lineage, is important for the induction of colitis, as deletion of IL-18 specifically in the epithelium protected animals from disease development (Nowarski et al., 2015). However, how exactly IL-18 is involved in Th1 regulation and function remains unknown.

In an animal model of MS, experimental autoimmune encephalomyelitis (EAE), Th1 cells have been shown to induce disease and play an important role in propagating epitope spreading, whereby infiltrating Th1 cells in the CNS induce inflammation and demyelination causing the release of new, previously sequestered antigens (Katz-Levy et al., 2000; Mack et al., 2003; Yin et al., 2001). Newly released antigens are then picked up and presented by antigen presenting cells (APCs) to induce activation of additional autoreactive T cells (McMahon et al., 2005). It is unknown how myelin-specific T cells are initially activated, particularly as myelin-associated antigens are sequestered within the CNS behind the blood–brain barrier. One hypothesis is that a viral infection may induce the activation of T cells that cross-react with myelin components, a mechanism termed molecular mimicry (Rose, 2017). These cross-reactive T cells then attain a pathogenic effector phenotype, traffic to the target organ, and induce inflammation and tissue destruction, resulting in CNS autoimmunity (Schreiner et al., 2007; Chastain and Miller, 2012). Furthermore, IFN- $\gamma$  has been shown to enhance effector function of scavenger APCs which may promote molecular mimicry as well as epitope spreading by the autoreactive T cells and enhance autoimmune pathology (Schoenborn and Wilson, 2007; Smith and Miller, 2006; Karni et al., 2006). However, there is evidence that IFN- $\gamma$  may not be the key cytokine responsible for pathogenic effector functions of Th1 cells in many autoimmune conditions (Zhang et al., 2012; Trembleau et al., 2003; Chu et al., 2000; Matthys et al., 1998). In particular, the loss of IFN- $\gamma$  does not prevent the development of EAE; in fact, IFN- $\gamma$  deficient mice are even more susceptible to disease development (Ferber et al., 1996). These conflicting data could only be understood in context with the discovery of Th17 cells (see “T Helper 17 Cells” section).

## T HELPER 17 CELLS

### Identification

Although Th1 cells were thought to be the main drivers of organ-specific autoimmunity, animals lacking the Th1 signature cytokine IFN- $\gamma$  or other molecules involved in the Th1 differentiation pathway, including IFN- $\gamma$  R and STAT1, are in fact not resistant, but actually more susceptible to multiple autoimmune diseases including EAE, experimental autoimmune uveitis, and collagen-induced arthritis (CIA) (Ferber et al., 1996; Jones et al., 1997; Matthys et al., 1998). Even more surprising, mice deficient for the IL-12 chain p35 were also more susceptible to EAE, whereas loss of the other chain of IL-12, p40, conferred resistance to EAE. This conundrum was solved when it was shown that p40 does not only pair with p35 to form IL-12, but can also pair up with another cytokine chain, p19, to form a novel cytokine called IL-23 (Oppmann et al., 2000). In a seminal study, Cua and colleagues showed that loss of both p40 and p19 (IL-23) protected animals from EAE, whereas IL-12-p35-deficient animals, which lacked IL-12 and Th1 responses, remained susceptible to EAE (Cua et al., 2003; Langrish et al., 2005). These data provided the foundation for the hypothesis that IL-23, rather than IL-12, is crucial for the development of autoimmunity. It was later revealed that IL-23 is involved in the generation of a unique T-cell subset, named Th17 cells owing to their production of the effector cytokine IL-17 (Korn et al., 2009).

### Differentiation

A unique subset of IL-17-producing T cells, termed Th17 cells, was first predicted in 2005 (Bettelli and Kuchroo, 2005; McKenzie et al., 2006). Generation of Th17 cells was initially thought to be driven by IL-23, however, naïve T cells do not express the receptor for IL-23 (IL-23R); instead, Th17 differentiation requires the presence of the cytokines TGF- $\beta$  and IL-6 (Bettelli et al., 2006; Veldhoen et al., 2006; Mangan et al., 2006). Exposure of naïve T cells to TGF- $\beta$  alone leads to the expression of the transcription factor Foxp3 and differentiation into immunosuppressive regulatory T cells (Tregs). However, in the presence of the proinflammatory cytokine IL-6, which signals via STAT3, TGF- $\beta$ -signaling results in the induction of the Th17 lineage transcription factor ROR $\gamma$ t and promotes Th17 differentiation (Bettelli et al., 2006; Veldhoen et al., 2006). The molecular basis for the reciprocal relationship between Tregs and Th17 cells lies in the ability of ROR $\gamma$ t/ROR $\alpha$  and Foxp3 to physically bind to each other and antagonize each other's function (Zhou et al., 2008; Du et al., 2008). Thus, many factors including IL-2, IL-21, retinoic acid, and aryl hydrocarbon receptor (AHR) ligands, have been shown to have opposing effects on Tregs and Th17 cells and modulate immune responses by shifting the balance between Tregs and Th17 cells (Laurence et al., 2007; Liu et al., 2011; Mucida et al., 2007; Veldhoen et al., 2008a; Quintana et al., 2008). In addition, recent studies reported that HIF1 $\alpha$ , a metabolic sensor of hypoxia, enhances Th17 differentiation on a transcriptional level, while simultaneously attenuating Treg development by targeting Foxp3 for proteasomal degradation (Shi et al., 2011; Dang et al., 2011).

Aside from IL-6, and particularly in its absence, TGF- $\beta$  can also cooperate with IL-21 to induce Th17 differentiation, and since Th17 cells also produce IL-21, it promotes the self-amplification of Th17 cells in a feed-forward loop (Korn et al., 2007; Nurieva et al., 2007; Zhou et al., 2007). In addition, IL-1 $\beta$  can synergize with IL-6 to promote murine Th17 cell differentiation, in part by expanding the population via mammalian target of rapamycin (mTOR), and IL-1R1 signaling is crucial for the generation of pathogenic Th17 cells in vivo (Chung et al., 2009; Gulen et al., 2010; Sutton et al., 2006). In conjunction with TGF- $\beta$ , IL-6, IL-23, and IL-21, IL-1 $\beta$  has also been described as a critical differentiation factor for human Th17 cells (Manel et al., 2008; Yang et al., 2008; Volpe et al., 2008).

### Pathogenicity

Although initial in vitro and in vivo differentiation of naïve T cells into Th17 cells does not require IL-23, generation of pathogenic Th17 responses in vivo is impaired in both IL-23 and IL-23R deficient mice (Langrish et al., 2005; McGeachy et al., 2009; Awasthi et al., 2009; Lee et al., 2012). Furthermore, several studies have demonstrated genetic linkage of IL23R to susceptibility to human autoimmune diseases, including psoriasis, ankylosing spondylitis, and Crohn's disease (Duerr et al., 2006; Cargill et al., 2007; Liu et al., 2008; Burton et al., 2007), suggesting that IL-23 may be critical for the generation of autoimmune T-cell responses in humans. Expression of the IL-23R, which is composed of IL-12R $\beta$ 1 and the specific chain IL-23R (Parham et al., 2002), is induced by IL-6 and TGF- $\beta$  during Th17 differentiation and is further upregulated by IL-23 itself (Awasthi et al., 2009). Moreover,

recent data show that the canonical Notch signaling pathway drives IL-23R expression on Th17 cells via RBPJ and thereby controls Th17 pathogenicity (Meyer Zu Horste et al., 2016).

IL-23 induces pSTAT3 and thus reinforces the Th17 transcriptional program. In addition, IL-23 induces de novo production of the Th17 effector cytokine IL-22 (Liang et al., 2006; Zheng et al., 2007), which promotes epithelial growth and induces expression of acute-phase reactants and  $\beta$ -defensins, thus promoting barrier function (Wolk et al., 2010). IL-23 also drives re-expression of IL-7R $\alpha$  on Th17 cells (McGeachy et al., 2009), thus rendering Th17 cells responsive to IL-7, which may be important for terminal differentiation and the survival/expansion of pathogenic Th17 cells (Liu et al., 2010). Furthermore, IL-23R signaling activates the salt-sensitive kinase Sgk-1, which deactivates the transcriptional repressor Foxo1 and thereby allows ROR $\gamma$ t to transactivate target genes to a greater degree (Wu et al., 2013). The protein–protein interaction network that is induced by IL-23R signaling is not fully understood, but research into this pathway holds promise for understanding the mechanisms by which IL-23R signaling induces pathogenic Th17 cells.

In addition to expanding and stabilizing Th17 cells, IL-23 is essential for inducing pathogenic effector functions in Th17 cells. Adoptive transfer studies in EAE have shown that Th17 cells generated with TGF- $\beta$ 1/IL-6 in the absence of IL-23 are nonpathogenic despite their production of IL-17 (Ghoreschi et al., 2010; McGeachy et al., 2007; Lee et al., 2012). In contrast, Th17 cells generated in the presence of TGF- $\beta$ 1/IL-6/IL-23 or IL-1 $\beta$ /IL-6/IL-23 are capable of inducing severe autoimmune tissue inflammation (Ghoreschi et al., 2010; Jäger et al., 2009; Lee et al., 2012; McGeachy et al., 2007), indicating that abundance of TGF- $\beta$ 1 together with relative lack of IL-23 during differentiation favors generation of nonpathogenic Th17 cells, while the presence of IL-23 promotes the generation of pathogenic Th17 cells. Interestingly, the type of TGF- $\beta$  may be important for pathogenicity, since differentiation in the presence of or TGF- $\beta$ 3/IL-6 generates encephalitogenic Th17 cells (even in absence of IL-23), whereas differentiation with TGF- $\beta$ 1/IL-6 does not (Lee et al., 2012). In addition, other factors fine-tune the effector phenotype of Th17 cells; thus IL-1 $\beta$  enhances IL-23R expression via MyD88 and mTOR and thereby promotes responsiveness of Th17 cells to IL-23 (Chang et al., 2013; Gulen et al., 2012). Interestingly, it was shown that IL-1 $\beta$  is produced intrinsically by Th17 cells in an ASC-NLRP3 inflammasome-dependent manner (Martin et al., 2016).

While multiple cytokines modulate the Th17 effector phenotype, at the molecular level this phenotype is a result of transcription factors that are differentially expressed in pathogenic versus nonpathogenic Th17 cells. Transcriptional profiling, including at the single cell level, has identified pathogenic and nonpathogenic signature genes in Th17 cells, which determine the functional properties of Th17 cells (Lee et al., 2012; Yosef et al., 2013; Gaublomme et al., 2015). A prominent example for a pathogenic signature gene is *Csf2*, which encodes the cytokine GM-CSF. GM-CSF has been associated with Th17 pathogenicity during EAE in two independent studies, and IL-23 and IL-1 $\beta$  drive GM-CSF production in Th17 cells (El-Behi et al., 2011; Codarri et al., 2011). GM-CSF prompts APCs to produce proinflammatory cytokines including IL-6 and IL-23, which amplify Th17 responses, and it also attracts a wave of secondary cells, primarily macrophages, which further propagate tissue inflammation (El-Behi et al., 2011; Codarri et al., 2011). In contrast, important examples of nonpathogenic signature genes are *cMaf* and *Il10*. Consistent with the role of IL-23 in promoting pathogenicity, expression of c-Maf and IL-10 is diminished by IL-23 and enhanced by TGF- $\beta$ 1 (McGeachy et al., 2007; Ahern et al., 2010). IL-10 can limit autoimmune tissue inflammation by enabling Tregs to suppress Th17-induced inflammation (Chaudhry et al., 2011), and also by directly inhibiting the proliferation of Th17 cells (Huber et al., 2011). Finally, genes such as *Cd5l* are upregulated in nonpathogenic Th17 cells and prevent the development of a pathogenic phenotype by modulating cellular lipid metabolism (Wang et al., 2015).

The Th1 transcription factor T-bet, which controls the expression of IFN- $\gamma$  is also part of the pathogenic signature in Th17 cells. Although Th17 cells do not produce much IFN- $\gamma$  in vitro, IL-17 $^+$ IFN- $\gamma$  $^+$  double producers are commonly seen in target organs of various autoimmune disease models, including the CNS during EAE (Ivanov et al., 2006; Abromson-Leeman et al., 2009) and the colon during colitis (Hue et al., 2006; Kullberg et al., 2006). Whether these cells arise from Th1 or Th17 cells has been much debated, however, fate-mapping experiments now indicate that IL-17 $^+$ IFN- $\gamma$  $^+$  double producers are derived from Th17 cells in an IL-23 dependent fashion (Hirota et al., 2011). This plasticity can have important physiological function and has been shown to be important for IgA production in the intestinal epithelium (Hirota et al., 2013). In addition, Th17 cells generated in the absence of IL-23 lose expression of IL-17 relatively easily and switch over/de-differentiate into IFN- $\gamma$  producers (Shi et al., 2008; Lee et al., 2009; Bending et al., 2009; Jäger et al., 2009), while Th17 cells generated in the presence of IL-23 maintain stable IL-17 production but often coproduce IFN- $\gamma$  in the target organ (Mangan et al., 2006; McGeachy et al., 2009; Jäger et al., 2009; Liu et al., 2010). The co-production of the Th1 cytokine IFN- $\gamma$  by Th17 cells emphasizes that Th17 cells are potentially more plastic than other Th subsets. However, whether the production of IFN- $\gamma$  by Th17 cells is beneficial or detrimental for the development of tissue inflammation and

autoimmunity remains unclear. In humans, IL-17<sup>+</sup>IFN- $\gamma$ <sup>+</sup> double producers have also been described and have been termed Th1\* cells (Acosta-Rodriguez et al., 2007). Analogous to the variety of Th17 effector phenotypes described in mice, human Th17 cells also show different effector phenotypes depending on the microenvironment. For instance, *Candida albicans* infection generates an IL-1 $\beta$ -rich cytokine milieu that prompts Th17 cells to co-produce IL-17 and IFN- $\gamma$ , whereas *Staphylococcus aureus* infection primes Th17 cells to produce IL-17 together with IL-10 (Zielinski et al., 2012). A transcriptomic analysis of human Th17 cells has shown that Th17 cells induced by *C. albicans* are similar to pathogenic murine Th17 cells and Th17 cells with specificity for *S. aureus* are transcriptionally similar to murine nonpathogenic Th17 cells (Hu et al., 2017). These findings support the concept that various subtypes of Th17 cells are naturally derived to counteract various types of infections but may play different roles if they are directed against an autoantigen. While nonpathogenic Th17 cells are key regulators of tissue homeostasis, pathogenic Th17 cells are the drivers of autoimmune inflammation. Therefore, next-generation therapeutics would ideally include agents that target pathogenic Th17 cells while keeping nonpathogenic Th17 cells intact.

Evidence that IL-23 induces important effector functions in humans comes from clinical trials in Crohn's disease: blockade of the IL-12/23 p40 subunit via ustekinumab was beneficial, whereas neutralization of IL-17 only via secukinumab did not improve health, and in some patients even worsened disease activity (Hueber et al., 2012; Sandborn et al., 2012). These data emphasize that production of IL-17 cannot be used as a measure for the presence of pathogenic Th17 cells, which must be defined via many additional effector functions. This is especially true for the intestine, where Th17 cells play an important role in homeostasis.

## Transcriptional Regulation

Upon engagement with their respective receptors, IL-6, IL-21, and IL-23 all induce phosphorylation of STAT3 via Janus kinases, which is absolutely crucial for proper Th17 differentiation (Yang et al., 2007; Harris et al., 2007). Phosphorylated STAT3 dimerizes, translocates to the nucleus, and activates transcription of many Th17-specific genes including *Il17a*, *Il17f*, *Il21*, *Il23r*, and *Rorc*, which encodes for the Th17 lineage-specific transcription factor ROR $\gamma$ t. STAT3 also enables epigenetic changes that reinforce the Th17 transcriptional program and promote survival and proliferation (Chen et al., 2006; Durant et al., 2010).

ROR $\gamma$ t is the master transcription factor for Th17 cells and, together with the related ROR $\alpha$ , transactivates Th17 signature genes including *IL17a*, *IL17f*, *Il23r*, and the Th17-specific chemokine receptor *Ccr6* (Zhou and Littman, 2009; Hsu et al., 2008; Ciofani et al., 2012; Xiao et al., 2014). As expected for a lineage-defining transcription factor, expression of ROR $\gamma$ t is a prerequisite for Th17 differentiation in vitro and in vivo but is dispensable for the differentiation of other lineages (Bettelli et al., 2008).

To ensure optimal conditions for the transcriptional activity of STAT3 and ROR $\gamma$ t, other factors are needed. The transcription factors IRF4 and BATF mediate the effects of IL-21 on Th17 differentiation and self-amplification and are required for proper induction of ROR $\gamma$ t (Huber et al., 2008; Schraml et al., 2009; Brüstle et al., 2007). More recent studies demonstrated that BATF and IRF4, which are induced early upon TCR signaling, act as pioneer factors which bind to DNA and regulate chromatin accessibility, allowing for cooperative binding of pSTAT3 and ROR $\gamma$ t at Th17-specific loci. In addition, they facilitate recruitment of other enhancers and stabilizers of the Th17 transcriptional program, most importantly HIF1 $\alpha$  and the histone acetyltransferase p300 (Ciofani et al., 2012; Li et al., 2012; Vahedi et al., 2012).

Another factor that is involved in certain aspects of Th17 cell differentiation and regulation is c-Maf, which promotes the production of IL-21 and thereby facilitates the self-amplification of Th17 cells (Bauquet et al., 2009). However, transcriptional analysis showed that c-Maf expression supports the generation of nonpathogenic Th17 cells (Ghoreschi et al., 2010; Lee et al., 2012) by repressing several proinflammatory Th17 genes, such as *Il22*, and transactivating antiinflammatory genes such as *Il10* and *Ctla4* (Rutz et al., 2011; Ciofani et al., 2012). Thus c-Maf is an important modulator of Th17 pathogenicity and effector functions.

Recent studies examining transcriptional networks with genome-scale profiling techniques have revealed that besides the above-described factors, many others participate in Th17 differentiation and have helped to understand how and in which sequence different factors work together or inhibit each other (Ciofani et al., 2012; Yosef et al., 2013). In particular, network analysis has demonstrated that a positive transcriptional module promotes Th17 differentiation and, at the same time, suppresses the development of other T-cell subsets, while a negative transcriptional module has the opposite effect. These mutually antagonistic modules provide insight into the molecular mechanisms of how different T-cell subsets antagonize each other (Yosef et al., 2013).

## Environmental Cues and T Helper 17 Cell Regulation in the Intestine

It has long been noted that many autoimmune diseases display genetic risk loci that predispose for developing diseases, and the human leukocyte antigen locus has emerged as the most important site for single nucleotide polymorphisms (SNPs) conferring risk (Jones et al., 2006). In addition, genome-wide association studies have shown that genes and pathways in immune cell function, most notably in Th cell development, represent high-risk loci for developing autoimmune disease (International Multiple Sclerosis Genetics Consortium et al., 2011). However, these SNPs are relatively common, raising the question as to what additional driving forces lead to the clinical manifestations of disease. Moreover, a common aspect of autoimmune diseases is to have periods of clinical disease activity along with periods of intermittent disease remission. One such prominent example is the relapsing-remitting form of MS (Dendrou et al., 2015). While the factors that trigger relapse are mostly unknown, this represents a highly active area of research (Olsson et al., 2017).

A multitude of environmental factors have been implicated in the induction and progression of autoimmune diseases, including diet, infection, and toxins. For example, vitamin D has been shown to be important for relapse rate in MS patients and studies suggest that simple dietary supplementation of vitamin D may lower disease activity (Miclea et al., 2017). However, it is not known how vitamin D levels influence the development of particular Th subsets and whether this contributes to the observed beneficial effects.

The intestine is the main site for Th17 cell differentiation under homeostatic conditions and certain commensal bacteria play a major part in this process. In particular, SFB (segmented filamentous bacteria) have been shown to be critical inducers of intestinal Th17 cell differentiation and the TCRs of intestinal Th17 cells show specificity for SFB antigens (Huber et al., 2012; Ivanov et al., 2009; Yang et al., 2014). Under nonpathologic conditions, these gut-resident Th17 cells protect the organism from microbial invasion in the intestine, strengthen intestinal barrier functions and maintain tissue homeostasis. However, if not controlled or activated to attain a pathogenic phenotype, Th17 cells in the intestine may induce autoimmune tissue inflammation both directly in the intestine (Huber et al., 2012) as well as in distant organs. Indeed, recent studies demonstrated that Th17 differentiation triggered in the intestine by commensals can promote autoimmune tissue inflammation in the CNS during EAE and in the joints during arthritis (Berer et al., 2011; Wu et al., 2010). Moreover, the transfer of fecal material from MS patients is sufficient to increase susceptibility to central nervous system disease to animals (Berer et al., 2017). It is tempting to speculate that a particular commensal bacterial strain could be targeted to provide clinical benefit in treating autoimmunity. Indeed, a recent study suggested that proinflammatory Th17 cells can be redirected to the gut and tolerized (Esplugues et al., 2011). A similar approach could potentially be beneficial in the therapy for human autoimmune diseases.

Key cytokines of the Th17 lineage, in particular IL-17 and IL-22, signal to epithelial cells to control barrier function and dysregulation of these signals may promote the development of autoimmune tissue inflammation. This is mediated in part by serum amyloid A (Sano et al., 2015). Certain T helper cell lineages have been shown to possess significant plasticity meaning that they can start expressing signature cytokines of other lineages and that this plasticity may be part of a physiological program to switch off inflammation once the pathogen has been cleared. One prominent example is the conversion of Th17 cells into type 1 Tregs (Tr1) in the intestine during the resolution of inflammation (Gagliani et al., 2015). This plasticity could be potentially exploited for therapeutic purposes.

Indeed, depending on individual niches within the intestine, the localization and differentiation of particular Th lineages can be manipulated. For example, certain microbiotas influence the trafficking and lineage conversion of intestinal Tregs which, in turn, influences intestinal homeostasis and potentially influences autoimmunity (Sujino et al., 2016). Moreover, particular microbiota can influence the emergence of ROR $\gamma$ t<sup>+</sup> Tregs within the intestine, highlighting that transcription factors for one lineage can modulate the function of other lineages as well (Sefik et al., 2015). These ROR $\gamma$ t<sup>+</sup> Tregs play an important role in balancing protective and autoimmune inflammatory responses, especially type 2 immunity (Ohnmacht et al., 2015). The increased incidence of autoimmune disease in industrialized civilizations has correlated with marked changes in diet, including increased sodium intake. Correspondingly, it was suggested that dietary sodium intake may contribute to the emergence of pathogenic T-cell subsets, in particular Th17 cells (Wu et al., 2013; Kleinewietfeld et al., 2013). Recent data have supported the effect of sodium on the differentiation of Th17 cells but also emphasized that the clinical consequence of environmental cues such as sodium need to be investigated in greater detail to understand combinatorial action, as no patient is exposed to any sole environmental cue that may affect the progression of autoimmune disease (Hammer et al., 2017; Cortese et al., 2017). Environmental toxins have also been suspected to influence immune responses. For example, AHR promotes Th17 cell differentiation and is activated by binding

phenol-derived chemical compounds, including microbial metabolites and environmental toxins (Quintana et al., 2008; Rothhammer et al., 2016). Thus toxin-mediated AHR-activation influences Th17 cell differentiation, potentially contributing to autoimmune tissue inflammation (Veldhoen et al., 2008a). Finally, it has been recognized that there is seasonality to disease activity in patients with autoimmune disease, with the disease worsening during winter when the days are shorter. Recent work has highlighted that melatonin may influence the differentiation of Th17 cells and thus, confer a seasonal increase in disease-inducing pathogenic Th subsets (Farez et al., 2015). However, much more research and a deeper understanding are needed to define how environmental cues such as diet, infections, and toxins influence the development of pathogenic T cells that drive autoimmunity.

## Function

The Th17 signature cytokines IL-17A and IL-17F can form homo- or heterodimers and are partly redundant in their effector functions. They signal through a receptor complex composed of IL-17RA and IL-17RC, which is expressed on both hematopoietic and nonhematopoietic cells. IL-17 receptor signaling induces production of proinflammatory cytokines and chemokines, including IL-6, IL-1, TNF, CXCL1, CCL20, GCP-2, and IL-8, as well as antimicrobial peptides and matrix metalloproteinases. Thus Th17 cells promote tissue inflammation and neutrophil recruitment (Bettelli et al., 2008). Th17 cells are important for host defense against a variety of pathogens, most notably bacteria such as *Citrobacter*, *Klebsiella pneumoniae*, and *S. aureus*, as well as fungi such as *C. albicans* (Bettelli et al., 2008). In humans, the critical role of IL-17 and Th17 cells in host defense is evident in the susceptibility of patients with genetic defects in the *IL17RA*, *IL17F*, or *STAT3* genes to *C. albicans* and *S. aureus* infections (Cypowij et al., 2012).

Aside from host defense, Th17 cells have been primarily associated with autoimmune tissue inflammation. Thus, elevated levels of IL-17 were detected in several autoimmune diseases including MS (Matusevicius et al., 1999), RA (Aarvak et al., 1999), and psoriasis (Teunissen et al., 1998). In EAE, treatment with IL-17 neutralizing antibodies ameliorates disease (Hofstetter et al., 2005) and IL-17 deficient animals developed attenuated CIA and EAE (Nakae et al., 2003; Ishigame et al., 2009). Many clinical studies have been conducted to investigate the potential benefit of using humanized monoclonal antibodies to interfere with IL-12 and IL-23 signaling in various autoimmune diseases (Teng et al., 2015). Some of these antibodies, such as ustekinumab, target both IL-12 and IL-23 by binding to the common p40 subunit, therefore impacting both Th1 and Th17 cell responses, while other antibodies such as secukinumab specifically target IL-17A. These therapies are highly efficacious and approved for treatment in psoriasis, psoriatic arthritis, and ankylosing spondylitis (Papp et al., 2008; Leonardi et al., 2008; Langley et al., 2014; McInnes et al., 2013; Baeten et al., 2013; Poddubnyy et al., 2014). In MS, initial clinical trials showed that in vivo administration of secukinumab had a significant impact on lesion load as detected by MRI (Havrdova et al., 2016). However, the drug has not been further developed as a multitude of new therapies, including those that are orally available, have recently been approved for relapsing-remitting MS.

Other autoimmune diseases, including RA (Genovese et al., 2013) and Crohn's disease, have shown more mixed results. For example, secukinumab did not show benefit in Crohn's disease, whereas ustekinumab did (Hueber et al., 2012; Sandborn et al., 2012). However, these results may be complicated by the fact that the selected patients did not respond to the standard treatment for Crohn's disease, anti-TNF therapy and, therefore, may suffer from more treatment-refractory disease. Finally, ustekinumab did not show a benefit in relapsing-remitting MS (Segal et al., 2008); however, this does not mean that an IL-23p19-specific agent could not work in MS. Additional clinical trials are required to specifically address this issue. Considering that the underlying disease mechanisms may be quite different among separate autoimmune diseases, these results emphasize the need for further investigation of the underlying molecular pathways involved in disease pathogenesis.

## T HELPER 22 CELLS

As described above, the cytokine IL-22 can be produced by Th17 cells in an IL-23 dependent manner. However, the detection of IL-22 producing Th cells that do not co-express IL-17, IL-4, or IFN- $\gamma$  in human skin have lent support to the idea that Th22 cells may exist as an independent T-cell subset (Duhen et al., 2009; Eyerich et al., 2009; Trifari et al., 2009). In vitro, differentiation of Th22 cells is driven by IL-6 and TNF $\alpha$  for human cells, and by IL-21 for mouse cells via STAT3 (Yeste et al., 2014), and critically depends on the transcription factor AHR. Accordingly, exposure to AHR ligands, such as FICZ, increases IL-22 production. However, AHR is not a classic lineage-defining transcription factor, since it is also expressed in other subsets, especially in

Th17 cells, and does not inhibit the differentiation of other lineages. Moreover, although expression of ROR $\gamma$ t and other lineage transcription factors is low or absent in Th22 cells or clones isolated ex vivo, in vitro experiments suggest that ROR $\gamma$ t nevertheless promotes the initial differentiation of Th22 cells (Trifari et al., 2009; Yeste et al., 2014). Rather than an independent lineage, Th22 cells may, therefore, represent a specialized subtype that primarily derives from the Th17 lineage, but switches off many of the Th17-specific modules under the influence of different environmental cues and cytokines. Accordingly, fate-mapping studies have shown the heterogeneity and plasticity of Th22 cells in terms of cytokine and transcription factor expression (Ahlfors et al., 2014).

Regardless of their origin, it is clear that Th22 cells exist and participate in different immune reactions including autoimmune inflammatory processes. Thus, increased frequencies of Th22 cells have been described in psoriasis (Eyerich et al., 2009; Kagami et al., 2010), ankylosing spondylitis, RA (Zhang et al., 2012), Hashimoto's thyroiditis (Bai et al., 2014), and also in MS (Rolla et al., 2014). In psoriasis, Th22 cells expressing the skin-homing receptors CCR4 and CCR10 on their surface are clearly pathogenic and drive acanthosis and hyperkeratosis via excessive production of IL-22, which acts directly on keratinocytes (Zheng et al., 2007). The role of Th22 cells in MS is less well understood. Although IL-22-deficient mice develop EAE normally (Kreyberg et al., 2007), Th22 cells are increased in the CSF of MS patients just before relapse, suggesting that they may be involved in early pathogenic processes. Of note, Th22 cells in the CSF express the chemokine receptor CCR6, rather than CCR4 and CCR10, indicating that chemokine receptor expression in Th22 cells is dependent on the disease context (Rolla et al., 2014). In contrast to psoriasis, Th22 cells play a protective role in IBD, where IL-22 promotes barrier function and stem cell-mediated regeneration of the epithelium (Lindemans et al., 2015; Sugimoto et al., 2008; Zenewicz et al., 2008). Taken together, whether Th22 cells are pathogenic or protective is highly dependent on the disease context.

## REGULATORY CD4<sup>+</sup> T CELLS

While Th1 and Th17 CD4<sup>+</sup> T-cell subsets have been implicated in inducing autoimmune disease, CD4<sup>+</sup> Tregs represent a distinct Th cell subset that maintains self-tolerance and controls immune responses. The importance of T cells for controlling autoimmunity was first suggested by neonatal thymectomy experiments carried out by Nishizuka and Sakakura (1969), who found that thymectomy prior to day 7 of life led to the development of multiorgan autoimmune disease. Subsequent adoptive transfer studies found that transfer of purified populations of CD4<sup>+</sup> T cells (CD45RB<sup>hi</sup> or CD5<sup>lo</sup>) into lymphopenic rodents led to the development of T cell-mediated autoimmunity that could be mitigated by cotransfer of CD45RB<sup>lo</sup> or CD5<sup>hi</sup> CD4<sup>+</sup> T cells, suggesting the existence of a subset of CD4<sup>+</sup> T cells with regulatory properties (Powrie and Mason, 1990; Sakaguchi et al., 1985). Sakaguchi and colleagues (1995) further demonstrated that within the CD5<sup>hi</sup> CD45RB<sup>lo</sup> CD4<sup>+</sup> T cell population the CD25<sup>+</sup> subset was uniquely able to inhibit the development of organ-specific autoimmunity, whereas depletion of CD25<sup>+</sup> cells was sufficient to cause multiorgan autoimmune disease. Subsequently, it was demonstrated that CD4<sup>+</sup>CD25<sup>+</sup> T cells express Foxp3, the lineage-defining transcription factor for Tregs (Hori et al., 2003), and spontaneous mutations in Foxp3 lead to the development of severe, multiorgan autoimmunity in both mice (*scurfy*) and humans (immunodysregulation polyendocrinopathy enteropathy X-linked syndrome) (Bennett et al., 2001; Wildin et al., 2001; Khattri et al., 2003). Thus Foxp3-expressing CD4<sup>+</sup>CD25<sup>+</sup> Tregs are critical for maintaining peripheral self-tolerance in both mice and humans.

The number of genes directly regulated by Foxp3 is relatively small and does not encompass the majority of the genes that regulate Treg development, suggesting that other factors are equally critical to maintaining the Treg transcriptional program (Hill et al., 2007; Fu et al., 2012). In addition, naïve human Th cells can upregulate Foxp3 upon activation without acquiring regulatory function (Gavin et al., 2006). Fate-mapping experiments in mice have also demonstrated a population of "ex-Tregs" that once expressed Foxp3, but no longer do so and readily produce effector cytokines, including IFN- $\gamma$  and IL-17 (Zhou and Littman, 2009). Intriguingly, adoptive transfer of such "ex-Tregs" leads to rapid development of autoimmune disease, consistent with loss of regulatory and acquisition of effector function. Thus, while expression of Foxp3 is a defining characteristic of Tregs, Foxp3 expression alone is not sufficient for stable regulatory function. Several groups have recently shown that Tregs possess a unique epigenetic pattern of DNA methylation (Floess et al., 2007; Ohkura et al., 2012; Schmidl et al., 2009; Wei et al., 2009). Acquisition of this methylation pattern begins during thymic development and does not require Foxp3 expression, although it does help to maintain Foxp3 expression and regulatory function in vivo and in vitro (Ohkura et al., 2012). Therefore, stable Treg differentiation depends upon both Foxp3 expression and a unique pattern of DNA methylation.

Tregs can develop both in the thymus (tTregs, formerly natural, or nTregs) and in the periphery (pTregs, formerly induced, or iTregs). Both tTregs and pTregs express Foxp3 and CD25; however, tTregs preferentially express the cell surface receptor neuropilin-1 and the transcription factor Helios (Thornton et al., 2010; Weiss et al., 2012; Yadav et al., 2012). Both tTregs and pTregs suppress effector cell proliferation in vitro; however, they may play distinct roles in vivo. tTregs appear to primarily maintain peripheral tolerance to organ-specific antigens, while pTregs are thought to be important in maintaining tolerance to exogenous but innocuous antigens the immune system encounters at mucosal sites, or dampening antigen-specific immune responses.

The neonatal thymectomy studies of Nishizuka and Sakakura highlighted the importance of thymus-derived tTregs in preventing organ-specific autoimmunity and raised the question of what drives their development in the thymus. One factor appears to be some degree of self-reactivity. On a recombination activating gene (RAG)-deficient background, foreign antigen-specific TCR transgenic mice had no tTregs, whereas on a RAG-sufficient background, tTregs were present and showed evidence of endogenous TCR $\alpha$  chain rearrangement (Itoh et al., 1999). Moreover, ectopic thymic expression of cognate foreign antigen was sufficient to allow tTreg differentiation in RAG-deficient TCR transgenic mice, and the unique DNA methylation pattern seen in Tregs depended on TCR recognition of cognate peptide (Ohkura et al., 2012; Kawahata et al., 2002). In the absence of AIRE, which promotes ectopic thymic expression of tissue-restricted antigens, mice develop T cell-mediated multiorgan autoimmunity, associated with the expansion of certain effector CD4 T cell clones. Many of the TCRs expressed by tissue-infiltrating effector CD4 T cells in AIRE $^{-/-}$  mice are typically found in Tregs, suggesting that during thymic development recognition of autoantigens can promote Treg differentiation in addition to negative selection (Malchow et al., 2016). This appears to be in part due to a conserved noncoding element within the Foxp3 locus, CNS3, which promotes Foxp3 expression in developing thymocytes. In the absence of CNS3, the TCR repertoire of Tregs is altered, and mice lacking both CNS3 and AIRE develop severe early-onset autoimmune disease (Feng et al., 2015). These data, therefore, suggest a model in which recognition of self-peptide/MHC complexes triggers the upregulation of Foxp3 and promotes the development of tTregs.

While specificity for self-antigen appears to be a critical factor in tTreg development and differentiation in the thymus, pTreg differentiation is controlled by the molecular context in which T cells are activated. The cytokine TGF- $\beta$  has been shown to be important for the development of pTregs both in vivo and in vitro via activation of SMAD3, which, in turn, binds to the CNS1 regulatory element within the *Foxp3* gene and promotes pTreg differentiation in concert with other factors (Chen et al., 2003; Fantini et al., 2004; Kim and Leonard, 2007; Zheng et al., 2010). Interestingly, TGF- $\beta$  can also promote Th17 differentiation in conjunction with inflammatory cytokines such as IL-6 (Bettelli et al., 2006). Therefore, depending on the context in which the antigen is encountered, a T cell may either be driven to differentiate into a potentially proinflammatory Th17 cell or a tolerogenic pTreg.

Treg expression of CD25, which along with CD122 and the common  $\gamma$ -chain ( $\gamma_c$ ) makes up the high-affinity IL-2 receptor complex, highlights the importance of IL-2 in Treg biology. Because IL-2 was initially described as a growth factor for activated T cells, the finding that mice deficient in IL-2, CD25, or CD122 developed autoimmune and lymphoproliferative disorders was surprising (Kramer et al., 1995; Schorle et al., 1991; Suzuki et al., 1995). This apparent conundrum was resolved when mice deficient in IL-2 or its receptor components were found to have markedly reduced numbers of Foxp3 $+$  Tregs in the periphery (Almeida et al., 2002; Malek et al., 2002; Papiernik et al., 1998). Subsequent studies revealed a critical role for IL-2-mediated activation of STAT5 in supporting the peripheral survival and function of Tregs (Fontenot et al., 2005; Thornton et al., 2004; Chinen et al., 2016). Recent imaging-based analysis has revealed that discrete clusters of phospho-STAT5 $+$  Tregs are found in secondary lymphoid organs, and these Tregs demonstrate increased expression of molecules associated with suppressive function, such as CTLA-4 (Liu et al., 2015). Notably, STAT5 phosphorylation is dependent upon locally produced IL-2 from activated effector CD4 T cells, while Treg clustering is TCR dependent. Thus, low-level activation of autoreactive conventional CD4 T cells leads to the production of IL-2 that, in turn, directly promotes Treg function, suppressing potentially pathogenic effector responses.

There is increasing evidence that it may be possible to therapeutically manipulate IL-2/IL-2R signaling in vivo and promote Treg function in patients with immune-mediated disease. Administration of blocking antibodies to IL-2 or CD25 resulted in a marked decline in Treg numbers, whereas administration of IL-2 at low doses augmented Treg numbers (Grinberg-Bleyer et al., 2010; Setoguchi et al., 2005). Moreover, Tregs that express a constitutively active STAT5 transgene have increased suppressive function in vitro and decreased severity of EAE, consistent with enhanced Treg function in vivo (Chinen et al., 2016). Modulating IL-2 to enhance Treg function has recently been extended to early phase clinical trials of immune-mediated diseases. For instance, administration of low dose IL-2 led to increased Treg frequency and clinical improvement in graft versus host disease (GvHD), hepatitis C-associated vasculitis, and systemic lupus erythematosus (SLE)

(Koreth et al., 2011; Saadoun et al., 2011; He et al., 2016). Thus, IL-2 functions to support the peripheral homeostasis and function of Tregs and is amenable to therapeutic modulation in T cell–mediated inflammatory conditions.

The means by which Tregs control effector T-cell responses is an area of active inquiry. In vitro proliferation suppression assays have demonstrated that Tregs can control effector responses both by acting directly on the responding effector T cell as well as indirectly, by modifying APC function. Regardless of the mechanism of action, Tregs require cell–cell interaction in order to exert their suppressive function in vitro. Direct suppressive mechanisms in vitro include release of inhibitory cytokines, such as IL-10 and TGF- $\beta$ , restricting the bioavailability of IL-2, either by decreasing IL-2 transcription or by preferentially consuming IL-2, and direct granzyme-mediated cytolysis (Collison et al., 2007; Grossman et al., 2004; Pandiyan et al., 2007; Thornton and Shevach, 1998). In contrast, Tregs indirectly reduce effector T-cell proliferation by altering APC function, either by inhibiting APC maturation (such as via LAG-3/MHCII interactions or catabolizing ATP released on tissue damage via CD39) or by reducing expression of B7-1 or B7-2 (Borsig et al., 2007; Liang et al., 2008; Onishi et al., 2008). B7-1 and B7-2 downregulation is dependent upon Treg expression of CTLA-4, which binds B7-1 and B7-2 and physically removes them from the APC via transendocytosis (Qureshi et al., 2011; Wing et al., 2008). Thus, Tregs are able to utilize a variety of molecular mechanisms to both directly and indirectly regulate effector T-cell activation and expansion in vitro.

An intriguing finding is that while *Foxp3*-mutant *scurfy* mice uniformly develop a spontaneous autoimmune disease, the nature of the pathogenic effector response varies depending on the genetic background of the mice (Lin et al., 2005; Suscovich et al., 2012). Thus, modifier genes can exert significant influence on the ability of Tregs to control immune responses in vivo, suggesting that Tregs may utilize distinct molecular mechanisms to control distinct effector responses. The hypothesis that Treg control of different effector subsets requires additional factors has been bolstered by the finding that Tregs can be induced to express transcription factors associated with these subsets and expression of these transcription factors is important for proper in vivo regulation of the associated effector subset. For instance, Tregs upregulate T-bet in response to either IFN- $\gamma$  or IL-27, and T-bet, in turn, drives expression of CXCR3, facilitating Treg responsiveness to IFN- $\gamma$ -dependent chemokines, such as CXCL10 (Hall et al., 2012; Koch et al., 2009, 2012). Interestingly, while T-bet-deficient Tregs had normal in vitro suppressive activity (Bettelli et al., 2004), they failed to prevent lethal Th1-mediated autoimmune disease upon adoptive transfer into *scurfy* mice (Koch et al., 2009), suggesting that Treg-specific T-bet expression is crucial for proper regulation of Th1 responses in vivo. Similarly, expression of IRF4 or STAT3 appears to be critical for Tregs to properly regulate Th2 or Th17 responses in vivo, while ROR $\gamma$ t expression in colonic Tregs modulates the severity of intestinal inflammation (Chaudhry et al., 2009; Zheng et al., 2009; Sefik et al., 2015). These findings support the idea that Tregs possess both a core module of regulatory factors that are generally required for their suppressive function, including *Foxp3* and CTLA-4, as well as modules specific for control of distinct effector Th subsets (Wing and Sakaguchi, 2012).

In addition to suppressing effector T-cell responses, Tregs also play a role in tissue repair after injury. After sterile injury, a transcriptionally distinct population of muscle-resident Tregs proliferates and promotes muscle regeneration (Burzyn et al., 2013). This is mediated in part via expression of the EGFR-family member amphiregulin (Areg) on Tregs, which can be induced by alarmins, such as IL-33 (Kuswanto et al., 2016). Areg is also induced by alarmins on Tregs residing in other tissues, including the lung and the gastrointestinal tract (Arpaia et al., 2015). Importantly, while Treg-specific deletion of Areg did not alter the effector T-cell response to influenza, it did result in increased lung injury and hypoxemia after influenza infection, suggesting that the ability of Tregs to promote tissue repair may be distinct from their ability to suppress effector T-cell responses. Tregs have also been shown to promote tissue repair and regeneration via other mechanisms. For instance, after focal injury, CNS-resident Tregs express CCN3, which promotes oligodendrocyte differentiation and remyelination (Dombrowski et al., 2017), while acute lung injury induces Tregs to express keratinocyte growth factor, which promotes epithelial cell proliferation (Dial et al., 2017). Similarly, a population of Tregs in the skin localize to the hair follicle, where they promote the survival and function of hair follicle stem cells via expression of Jagged1, a Notch ligand (Ali et al., 2017). Thus, tissue resident Tregs not only promote immune homeostasis but also directly promote tissue repair.

The multiorgan autoimmunity seen in both mice and humans with spontaneous mutations in *Foxp3* shows that Tregs are vital in restraining potentially autoreactive Th cells and suggests that defects in Treg function may underlie the development of other autoimmune diseases. Supporting this, SNPs in multiple genes important for Treg function, including *Ctla4*, *Il2*, *Il2ra*, and *Il10*, have been found to be risk alleles in genome-wide association screens for a number of autoimmune diseases, such as IDDM, RA, IBD, and MS (Howson et al., 2012; Vandenbroucke, 2012). Moreover, in some autoimmune diseases there is evidence for the altered frequency of

Tregs, such as the finding that in IBD Treg frequency in the peripheral blood inversely correlates with disease activity (Saruta et al., 2007; Takahashi et al., 2006). Similarly, careful immunophenotyping of circulating Tregs allowed resting and activated Treg populations to be distinguished, and the population dynamics of these cells were altered in patients with active SLE (Miyara et al., 2009). Finally, impaired Treg-mediated suppression of effector T-cell proliferation has been demonstrated in some patients with MS, SLE, and IDDM (Alvarado-Sanchez et al., 2006; Ferraro et al., 2011; Kumar et al., 2006; Viglietta et al., 2004). Thus, alterations in Treg frequency and function are observed in human autoimmune disease, and pathways regulating Treg survival and function may represent promising therapeutic targets for the treatment of human autoimmune diseases. Indeed, clinical trials have been performed examining the efficacy of passive transfer of *in vitro* expanded Tregs into patients with type 1 diabetes (Bluestone et al., 2015), while similar trials are planned in other autoimmune diseases.

## TYPE 1 REGULATORY T CELLS

Tr1 cells are a recently identified Th subset that possesses a regulatory function in the absence of Foxp3 expression, in large part via production of IL-10. Tr1 cell differentiation is dependent upon IL-27, a heterodimeric cytokine composed of Ebi3 and p28, and in addition, Tr1 cell differentiation can be amplified by TGF- $\beta$  (Hall et al., 2012; Pot et al., 2011a). IL-27 induces activation of STAT1 and STAT3, which have been shown to be essential for Tr1 cell differentiation (Hall et al., 2012). Exogenous IL-27 also promotes transcription of c-Maf, IL-21, and inducible costimulator (ICOS), and, subsequently, IL-21 acts as an autocrine growth factor (Hall et al., 2012; Pot et al., 2009, 2011a,b) indicating that IL-27 and IL-21 cooperate in the generation of Tr1 cells. Other transcription factors implicated in Tr1 cell differentiation include AHR, HIF1 $\alpha$ , IRF1, and BATF, but no single lineage-defining transcription factor has yet been identified (Karwacz et al., 2017; Mascanfroni et al., 2015). While to date detailed functional and phenotypic characterization of Tr1 cells has been hampered by lack of specific markers that can reliably differentiate Tr1s from other IL-10 producing regulatory and effector T-cell subsets, recent work suggests that coexpression of LAG-3 and CD49b may serve to identify such cells (Gagliani et al., 2013). Finally, it is unclear whether Tr1 cells are only generated in the periphery or whether Tr1 cells are also generated through thymic selection, analogous to tTregs (Fujio et al., 2010).

Functionally, IL-27 treatment ameliorated EAE by increasing the frequency of IL-10 producing T cells and thereby suppressing IL-17-producing Th17 cells, suggesting it may be useful therapeutically (Kastelein et al., 2007). Furthermore, IL-27R $\alpha$  deficient mice were shown to develop exacerbated EAE (Batten et al., 2006). Intriguingly, recent clinical trials suggested that IFN- $\beta$ , which is widely used as a first-line treatment for MS, increased serum IL-27 levels (Sweeney et al., 2011). Some MS patients have been reported to have decreased IL-10 production (Ozenci et al., 1999); thus, the clinical efficacy of IFN- $\beta$  in MS has been attributed to the induction of IL-27, consequently promoting an increased frequency of IL-10 producing Tr1 which can then potentially control autopathogenic T effector cells (Guo et al., 2008; Ramgolam et al., 2009; Sweeney et al., 2011). Another area where regulatory cells are of high therapeutic value is organ transplants, such as pancreatic islet transplants in IDDM, where inadequate immunosuppression gives rise to graft rejection. A strong correlation has been shown between transplant acceptance and frequency of circulating tTregs (Berney et al., 2009), as well as IL-10 production by peripheral blood mononuclear cells (Huurman et al., 2009), suggesting that tTregs play an important role in maintaining long-term tolerance. However, generation, expansion, and stability of antigen-specific Tregs remain a major challenge (Sagoo et al., 2008). Thus, an alternative to tTreg therapy is the adoptive transfer of *in vitro* generated IL-10 producing Tr1 cells that can sustain long-term survival *in vivo*. Indeed, the transfer of antigen-specific Tr1 cells prevented islet graft rejection in mouse models of islet transplant (Gagliani et al., 2010). In addition, preliminary data indicate that adoptive transfer of host-specific Tr1 cells may prevent GvHD in leukemia patients who underwent stem cell transplant (Allan et al., 2008; Bacchetta et al., 2010; Hippen et al., 2011; Roncarolo et al., 2011) supporting the potential value of Tr1 cell therapy in generating tolerance.

## FOLLICULAR T HELPER CELLS

T cell-dependent antibody responses occur in germinal centers (GCs) of lymphoid follicles and require interaction between antigen-specific B and Th cells (MacLennan et al., 1997; Garside et al., 1998). Recent evidence has demonstrated that this interaction is mediated by a distinct Th subset, termed follicular Th (TFH) cells. These TFH cells are activated by antigen in the T cell zone of lymphoid tissues and then migrate specifically to the

outer edge of the B cell follicles in order to encounter antigen-specific B cells that have also migrated to this location (Vinuesa and Cook, 2011). The homing of the TFH cells to the GC is dependent on a two-step chemotactic process that begins with the downregulation of CCR7, the receptor for the chemokines CCL19 and CCL21, which are produced in the T cell zone of the lymphoid tissue (Ansel et al., 2000; Luther et al., 2000; Gunn et al., 1998), followed by the induction of CXCR5, which allows TFH cells to migrate into the GCs along a gradient of CXCL13 (Haynes et al., 2007; Breitfeld et al., 2000). The downregulation of CCR7 and upregulation of CXCR5 is driven by the B cell lymphoma 6 protein (Bcl6), the master transcription factor regulating TFH cell differentiation. Bcl6 is a transcriptional repressor and while its main function is to repress other Th cell fates, it promotes TFH differentiation by regulating multiple transcriptional pathways (Hatzis et al., 2015). Recently, a second transcription factor, Ascl2, was identified that governs the upregulation of CXCR5 independently from Bcl6 and that may act earlier in the induction of TFH cell fate and the migration to GCs (Liu et al., 2014). The stable induction of Bcl6 is also critically dependent on the physical interaction of T and B cells (Liu et al., 2012, 2013). In addition to CXCR5, TFH cells express the costimulatory molecule ICOS and interaction of ICOS with ICOS ligand on B cells promotes the differentiation of T cells to TFH cells (Ramiscal and Vinuesa, 2013; Bauquet et al., 2009). TFH cell development also requires expression of the signaling molecule SAP and SLAM, an adaptor protein, as these are crucial for the induction of Bcl6 (Ramiscal and Vinuesa, 2013). The deletion of Bcl6 prevents the generation of TFH cells and inhibits GC reactions, including affinity maturation, isotype switching, B cell proliferation, and generation of memory B cells and plasma cells (Nurieva et al., 2009a). The differentiation of TFH cells is mediated by IL-21 and IL-6, and TFH cells require STAT3 (Nurieva et al., 2008) and c-Maf for their generation (Bauquet et al., 2009). With the activation of Bcl6, TFH cells begin secreting their signature cytokine IL-21, which has been shown to be essential for B cell survival and promoting GC reactions (Nurieva et al., 2008, 2009b). While other Th subsets also produce IL-21, the amount of IL-21 produced by TFH cells is far greater than that produced by Th1 and Th2 cells (Chtanova et al., 2004). In addition, IL-4 and CD40L are other key signals that TFH cells provide in help for developing B cells (Crotty, 2014). Lef1 and Tcf1 have been shown to orchestrate transcriptional profiles important for TFH development upstream of Bcl6 (Choi et al., 2015). Recently, an unexpected role of the neurotransmitter dopamine has been identified in the development of GCs. Indeed, TFH cells can produce high amounts of this neurotransmitter and it is important for a proper GC reaction (Papa et al., 2017). Apart from the factors that promote TFH cell fate, it has been shown that Blimp-1 and STAT5 have important antagonistic roles (Johnston et al., 2009, 2012; Nurieva et al., 2012). Furthermore, IL-2 has been identified as a cytokine that inhibits TFH cell induction (Ballesteros-Tato et al., 2012). Importantly, proper positioning within the GC is crucial for developing TFH cells to be shielded from IL-2 exposure and the G protein-coupled receptor Ebi2 mediates this spatial organization (Li et al., 2016). Other surface molecules such as PD-1 and SLAMF6 are crucially involved in regulating the extent of the help that TFH cells provide to B cells. This fine-tuning is achieved by limiting the proliferation of TFH cells within the GC. It is now appreciated that uncontrolled help can contribute to abnormal responses and influence disease (Cubas et al., 2013).

Immune responses that drive differentiation of other Th subsets also give rise to distinct subtypes of TFH cells (King et al., 2008), although the lineage relationship of these specialized TFH cells to effector CD4 T-cell subsets is not yet clear. In vitro generated Th1 and Th2 cells have been shown to develop into TFH cells upon adoptive transfer, suggesting that effector CD4 T cells can acquire the TFH program, including upregulating Bcl6 and CXCR5. Similarly, recent evidence suggests that Th17 cells can give rise to TFH cells in the intestinal mucosa due to significant developmental plasticity and that these cells play a crucial part in IgA production of GC B cells (Hiota et al., 2013).

During the early stages of TFH cell development, some cells may have upregulated CXCR5 but not yet turned on Bcl6 (Crotty, 2011). With an entry into the follicle and upon interaction with B cells, Bcl6 expression increases, and TFH lineage commitment stabilizes, commensurate with downregulation of the Th1, Th2, and Th17 associated genes (Crotty, 2011). However, adoptive transfer of TFH cells from an IL-21-reporter mouse indicated that TFH cells can also give rise to effector CD4 T cells (Luthje et al., 2012), and in a mouse model of asthma, effector Th2 cell differentiation appears to require the initial induction of type 2 TFH cells (Ballesteros-Tato et al., 2016), suggesting that there is considerable plasticity between TFH cells and effector CD4 T cells. The plasticity of TFH cells with other effector CD4 T-cell subsets is likely crucial to appropriately coordinate humoral and cellular immune responses (Crotty, 2011). Collectively, TFH cells have emerged as a distinct Th subset that provides critical help to B cells and regulates GC reactions, and, as a result, ultimately controls humoral immune responses.

In addition to TFH cells, there is also a population of  $\text{Foxp3}^+$  CD4 T cells within the B cell follicle, and these have been termed T follicular regulatory (TFR) cells (Sage and Sharpe, 2016). Similar to TFH, TFRs also express CXCR5, PD-1, and Bcl6 (Chung et al., 2011; Linterman et al., 2011; Sage et al., 2013) but restrain, rather than

promote, GC reactions. TFR differentiation requires both CD28 and ICOS expression, while the expression of PD-1 and CTLA-4 appear to inhibit TFR differentiation, even though CTLA-4 expression also represents an important pathway by which TFRs suppress B cell responses (Sage et al., 2013, 2014; Wing et al., 2014). TFRs are able to suppress B cell responses both indirectly, for example by modulating TFH cell function, as well as directly, by regulating B cell metabolism, and TFR-mediated inhibition can be overcome by IL-21 (Sage et al., 2016). Importantly, the TCR repertoire of TFH cells becomes oligoclonal after immunization, whereas the TCR repertoire of TFRs shows more similarity to the Treg repertoire, suggesting that TFR function is either independent of the inciting antigen, or that these cells may preferentially inhibit autoreactive B cell responses (Maceiras et al., 2017). This is consistent with recent observations that ablation of TFRs did not impact antibody responses to influenza infection, but did allow the production of antinuclear antibodies following infection (Botta et al., 2017). Thus, TFRs are a population of specialized Tregs that modulate TFH and B cell function and may play a particularly important role in inhibiting autoreactive antibody responses.

A number of autoimmune diseases are characterized by the presence of self-reactive autoantibodies, and it has been suggested that GCs may drive pathogenic generation of autoantibodies. In animal models for SLE, for example, spontaneous generation of GCs correlated with an increased serum concentration of autoantibodies (Luzina et al., 2001; Hsu et al., 2008). Cognate help from TFH cells has been demonstrated to promote the survival, expansion, and differentiation of self-reactive B cells, which ultimately resulted in autoantibody production and promoted tissue injury (Vinuesa et al., 2005; Zhang et al., 2013). In addition, aberrant TFH cell function has been shown to promote the development of disease in animal models of SLE and RA and is characterized by GC expansion, increased IL-21, and TFH cell expansion in extrafollicular sites (Zhang et al., 2013). In human autoimmune diseases, expansion of circulating TFH cells in patients with juvenile dermatomyositis, RA, SLE, and Sjögren's syndrome has been observed to strongly correlate with increased plasmablasts and serum concentrations of anti-dsDNA and antinuclear antibodies (Zhang et al., 2013; Morita et al., 2011; Ma et al., 2012). These observations are highly suggestive of the role that aberrant TFH cells may play in the pathogenesis of autoimmunity, particularly in diseases characterized by autoreactive antibodies.

## T HELPER 2 CELLS

Initially described for their distinct pattern of cytokine expression and their ability to promote IgE class-switching (Mosmann et al., 1986), Th2 cells are critical for amplifying and sustaining inflammation marked by eosinophil recruitment and mast cell activation. Th2 responses may be either protective, in the case of host defense against helminths and other extracellular parasites, or detrimental and lead to the development of asthma and environmental allergies (Paul, 2010; Pulendran and Artis, 2012). Th2 differentiation is induced by IL-4, which, upon binding a heterodimeric receptor consisting of IL-4R $\alpha$  and the  $\gamma_c$ -chain, results in the phosphorylation and activation of STAT6 and, in turn, induces expression of GATA-3. GATA-3 then transactivates many Th2-specific cytokines, most notably IL-4, IL-5, and IL-13, all of which share a common genetic locus (Zheng and Flavell, 1997). Th2 development results in significant chromatin remodeling at the *Il4* gene locus, such that IL-4 production by established Th2 cells can be seen even after the conditional deletion of GATA-3 (Baguet and Bix, 2004; Zhu et al., 2004). The same molecular pathways that promote Th2 differentiation simultaneously counterregulate the development of other effector Th subsets, particularly Th1 cells. For instance, GATA-3 can downregulate the expression of Th1-promoting genes such as STAT4 and IL-12R $\beta$ 2 (Zheng and Flavell, 1997). In addition, the transcription factor c-Maf both transactivates the *Il4* locus and induces downregulation of IFN- $\gamma$  (Ho et al., 1996, 1998). Th2 differentiation is thus a powerful counterregulator of Th1 differentiation.

The role of Th2 cells in autoimmune disease is complex. They have been implicated in facilitating isotype switching in SLE (Singh et al., 2003) and high levels of IL-4 and IL-13 are commonly seen in patients with systemic sclerosis, although the role of these cytokines in the pathogenesis of this disease is still unclear (O'Reilly et al., 2012). In contrast, many inflammatory autoimmune diseases show marked bias toward Th1 and Th17 responses and prior infection with a Th2 skewing pathogen can ameliorate EAE in mice (Sewell et al., 2003). Moreover, many inflammatory autoimmune diseases have lower incidences in areas where parasitic infections are more common, and longitudinal studies of MS patients with asymptomatic intestinal parasitemia suggest that these patients have milder disease than uninfected controls (Correale and Farez, 2007). These observations have been extended to clinical trials assessing the efficacy and safety of administering ova from *Trichuris suis*, a pig nematode that is unable to productively infect humans, to patients with ulcerative colitis (UC). Early studies showed clinically significant improvement in over 40% of the patients with severe UC, compared to a placebo

response rate of 17% (Summers et al., 2005); however, subsequent larger studies have not shown clear benefit (Garg et al., 2014). The efficacy of *T. suis* ova in MS has also been studied, although clear data indicating clinical efficacy are presently lacking (Fleming et al., 2017; Voldsgaard et al., 2015). However, glatiramer acetate, an immunomodulatory agent approved for use in MS, appears to be efficacious in part due to skewing the immune response toward a Th2-biased response (Arnon and Aharoni, 2004). Thus, Th2 counterregulation of other Th effector subsets can be utilized clinically in inflammatory autoimmune disorders.

## T HELPER 9 CELLS

Th9 cells, a recently identified Th subset generated by activating naïve Th cells in the presence of TGF- $\beta$  and IL-4, produce both IL-9 and IL-10 (Dardalhon et al., 2008; Veldhoen et al., 2008b). The requirement for IL-4 in Th9 differentiation raises the question of whether Th9 cells represent a distinct lineage or are a subset of specialized Th2 cells, particularly given that both Th2 and Th9 cells can readily induce allergic airway inflammation and IL-9 is implicated in asthma (Chang et al., 2010; Staudt et al., 2010). Similar to Th2 cells, Th9 cells also require the transcription factors IRF4 and STAT6; however, IL-9 production is uniquely dependent upon the TGF- $\beta$ -induced transcription factor PU.1, which, although expressed by other Th subsets, is not required for Th2 differentiation (Chang et al., 2010; Goswami and Kaplan, 2012; Staudt et al., 2010). The transcription factor Foxo1 is highly expressed by Th9 cells and can directly promote expression of IL-9 in multiple CD4 T-cell subsets (Bi et al., 2017; Malik et al., 2017). Thus, multiple transcription factors contribute to the development of IL-9-producing CD4 T cells.

In vivo, Th9 cells have been implicated in a variety of settings. While some of these also involve Th2 responses, such as anti-helminth responses (Licona-Limon et al., 2013) or allergic lung inflammation (Wilhelm et al., 2011), Th9 cells were also shown to play roles in antitumor responses (Purwar et al., 2012; Vegrán et al., 2014), as well as in mouse models of autoimmunity, including EAE and colitis. While myelin-specific in vitro differentiated Th2 cells failed to induce EAE upon adoptive transfer, transfer of Th9 cells resulted in robust disease, suggesting that Th9 cells have distinct functional properties from Th2 cells (Jäger et al., 2009). PU.1 and IL-9 are both highly expressed in T cells from patients with UC, and mice lacking either PU.1 or IL-9 have ameliorated oxalozone-induced colitis, suggesting that Th9 cells may contribute to disease pathogenesis (Gerlach et al., 2014; Nalleweg et al., 2015). Notably, Th9 cells do show considerable plasticity after adoptive transfer (Dardalhon et al., 2008; Jäger et al., 2009), and IL-9 can be produced by other CD4 T-cell subsets. It is therefore not entirely clear whether Th9 cells are a truly terminally differentiated Th subset, or represent a distinct but transient functional state.

## CONCLUDING REMARKS

The functional diversity and plasticity of CD4 $^{+}$  T cells is crucial for successful host defense against diverse environmental pathogens, but can also go dangerously awry in the setting of autoimmune disease. Recent years have seen the identification of novel Th subsets as well as a remarkable growth in our understanding of the molecular mechanisms that underlie Th cell differentiation. In particular, the identification of Th17 cells and Tregs as distinct lineages has greatly enhanced our knowledge of the pathobiology of a variety of autoimmune disorders and allowed the identification and development of novel therapeutic approaches. Recent work investigating the complex transcriptional networks involved in Th17 differentiation clearly illustrates the dynamic tension between transcriptional modules that enhance Th17 cell differentiation and inhibit the development of other T-cell subsets, thus providing the molecular basis for counterregulation between Th subsets (Ciofani et al., 2012; Yosef et al., 2013). Although impairment of counterregulatory mechanisms may lead to autoimmune tissue inflammation, these same pathways are amenable to modulation and offer opportunities for the development of novel immunomodulatory therapies for autoimmune diseases.

## References

- Arvak, T., Chabaud, M., Miossec, P., Natvig, J.B., 1999. IL-17 is produced by some proinflammatory Th1/Th0 cells but not by Th2 cells. *J. Immunol.* 162, 1246–1251.  
Abromson-Leeman, S., Bronson, R.T., Dorf, M.E., 2009. Encephalitogenic T cells that stably express both T-bet and ROR gamma t consistently produce IFNgamma but have a spectrum of IL-17 profiles. *J. Neuroimmunol.* 215, 10–24.

- Acosta-Rodriguez, E.V., Rivino, L., Geginat, J., Jarrossay, D., Gattorno, M., Lanzavecchia, A., et al., 2007. Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. *Nat. Immunol.* 8, 639–646.
- Adorini, L., Gregori, S., Magram, J., Trembleau, S., 1996a. The role of IL-12 in the pathogenesis of Th1 cell-mediated autoimmune diseases. *Ann. N.Y. Acad. Sci.* 795, 208–215.
- Adorini, L., Guery, J.C., Trembleau, S., 1996b. Manipulation of the Th1/Th2 cell balance: an approach to treat human autoimmune diseases? *Autoimmunity* 23, 53–68.
- Ahern, P.P., Schiering, C., Buonocore, S., Mcgeachy, M.J., Cua, D.J., Maloy, K.J., et al., 2010. Interleukin-23 drives intestinal inflammation through direct activity on T cells. *Immunity* 33, 279–288.
- Ahlfors, H., Morrison, P.J., Duarte, J.H., Li, Y., Biro, J., Tolaini, M., et al., 2014. IL-22 fate reporter reveals origin and control of IL-22 production in homeostasis and infection. *J. Immunol.* 193, 4602–4613.
- Ali, N., Zirak, B., Rodriguez, R.S., Pauli, M.L., Truong, H.A., Lai, K., et al., 2017. Regulatory T cells in skin facilitate epithelial stem cell differentiation. *Cell* 169, 1119.e11–1129.e11.
- Allan, S.E., Broady, R., Gregori, S., Himmel, M.E., Locke, N., Roncarolo, M.G., et al., 2008. CD4+ T-regulatory cells: toward therapy for human diseases. *Immunol. Rev.* 223, 391–421.
- Almeida, A.R., Legrand, N., Papiernik, M., Freitas, A.A., 2002. Homeostasis of peripheral CD4+ T cells: IL-2R alpha and IL-2 shape a population of regulatory cells that controls CD4+ T cell numbers. *J. Immunol.* 169, 4850–4860.
- Alvarado-Sanchez, B., Hernandez-Castro, B., Portales-Perez, D., Baranda, L., Layseca-Espinosa, E., Abud-Mendoza, C., et al., 2006. Regulatory T cells in patients with systemic lupus erythematosus. *J. Autoimmun.* 27, 110–118.
- Ansel, K.M., Ngo, V.N., Hyman, P.L., Luther, S.A., Forster, R., Sedgwick, J.D., et al., 2000. A chemokine-driven positive feedback loop organizes lymphoid follicles. *Nature* 406, 309–314.
- Arbore, G., West, E.E., Spolski, R., Robertson, A.A.B., Klos, A., Rheinheimer, C., et al., 2016. T helper 1 immunity requires complement-driven NLRP3 inflammasome activity in CD4(+) T cells. *Science* 352, aad1210.
- Arnon, R., Aharoni, R., 2004. Mechanism of action of glatiramer acetate in multiple sclerosis and its potential for the development of new applications. *Proc. Natl. Acad. Sci. U.S.A.* 101 (Suppl. 2), 14593–14598.
- Arpaia, N., Green, J.A., Moltedo, B., Arvey, A., Hemmers, S., Yuan, S., et al., 2015. A distinct function of regulatory T cells in tissue protection. *Cell* 162, 1078–1089.
- Awasthi, A., Riol-Blanco, L., Jäger, A., Korn, T., Pot, C., Galileos, G., et al., 2009. Cutting edge: IL-23 receptor gfp reporter mice reveal distinct populations of IL-17-producing cells. *J. Immunol.* 182, 5904–5908.
- Bacchetta, R., Gregori, S., Serafini, G., Sartirana, C., Schulz, U., Zino, E., et al., 2010. Molecular and functional characterization of allogantigen-specific anergic T cells suitable for cell therapy. *Haematologica* 95, 2134–2143.
- Baeten, D., Baraliakos, X., Braun, J., Sieper, J., Emery, P., Van Der Heijde, D., et al., 2013. Anti-interleukin-17A monoclonal antibody secukinumab in treatment of ankylosing spondylitis: a randomised, double-blind, placebo-controlled trial. *Lancet* 382, 1705–1713.
- Baguet, A., Bix, M., 2004. Chromatin landscape dynamics of the Il4-Il13 locus during T helper 1 and 2 development. *Proc. Natl. Acad. Sci. U.S.A.* 101, 11410–11415.
- Bai, X., Sun, J., Wang, W., Shan, Z., Zheng, H., Li, Y., et al., 2014. Increased differentiation of Th22 cells in Hashimoto's thyroiditis. *Endocr. J.* 61, 1181–1190.
- Ballesteros-Tato, A., Leon, B., Graf, B.A., Moquin, A., Adams, P.S., Lund, F.E., et al., 2012. Interleukin-2 inhibits germinal center formation by limiting T follicular helper cell differentiation. *Immunity* 36, 847–856.
- Ballesteros-Tato, A., Randall, T.D., Lund, F.E., Spolski, R., Leonard, W.J., Leon, B., 2016. T follicular helper cell plasticity shapes pathogenic T helper 2 cell-mediated immunity to inhaled house dust mite. *Immunity* 44, 259–273.
- Batten, M., Li, J., Yi, S., Kljavin, N.M., Danilenko, D.M., Lucas, S., et al., 2006. Interleukin 27 limits autoimmune encephalomyelitis by suppressing the development of interleukin 17-producing T cells. *Nat. Immunol.* 7, 929–936.
- Bauquet, A.T., Jin, H., Paterson, A.M., Mitsdoerffer, M., Ho, I.C., Sharpe, A.H., et al., 2009. The costimulatory molecule ICOS regulates the expression of c-Maf and IL-21 in the development of follicular T helper cells and TH-17 cells. *Nat. Immunol.* 10, pp. 167–175.
- Bending, D., De La Pena, H., Veldhoen, M., Phillips, J.M., Uyttenhove, C., Stockinger, B., et al., 2009. Highly purified Th17 cells from BDC2.5NOD mice convert into Th1-like cells in NOD/SCID recipient mice. *J. Clin. Invest.* 119, 565–572.
- Bennett, C.L., Christie, J., Ramsdell, F., Brunkow, M.E., Ferguson, P.J., Whitesell, L., et al., 2001. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat. Genet.* 27, 20–21.
- Berer, K., Mues, M., Koutrolos, M., Rasbi, Z.A., Boziki, M., Johner, C., et al., 2011. Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. *Nature* 479, 538–541.
- Berer, K., Gerdes, L.A., Cekanaviciute, E., Jia, X., Xiao, L., Xia, Z., et al., 2017. Gut microbiota from multiple sclerosis patients enables spontaneous autoimmune encephalomyelitis in mice. *Proc. Natl. Acad. Sci. U.S.A.* 114, 10719–10724.
- Berney, T., Ferrari-Lacraz, S., Buhler, L., Oberholzer, J., Marangon, N., Philippe, J., et al., 2009. Long-term insulin-independence after allogeneic islet transplantation for type 1 diabetes: over the 10-year mark. *Am. J. Transplant.* 9, 419–423.
- Bettelli, E., Kuchroo, V.K., 2005. IL-12- and IL-23-induced T helper cell subsets: birds of the same feather flock together. *J. Exp. Med.* 201, 169–171.
- Bettelli, E., Sullivan, B., Szabo, S.J., Sobel, R.A., Glimcher, L.H., Kuchroo, V.K., 2004. Loss of T-bet, but not STAT1, prevents the development of experimental autoimmune encephalomyelitis. *J. Exp. Med.* 200, 79–87.
- Bettelli, E., Carrier, Y., Gao, W., Korn, T., Strom, T.B., Oukka, M., et al., 2006. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 441, 235–238.
- Bettelli, E., Korn, T., Oukka, M., Kuchroo, V.K., 2008. Induction and effector functions of T(H)17 cells. *Nature* 453, 1051–1057.
- Bi, E., Ma, X., Lu, Y., Yang, M., Wang, Q., Xue, G., et al., 2017. Foxo1 and Foxp1 play opposing roles in regulating the differentiation and anti-tumor activity of TH9 cells programmed by IL-7. *Sci. Signal.* 10.
- Bluestone, J.A., Buckner, J.H., Fitch, M., Gitelman, S.E., Gupta, S., Hellerstein, M.K., et al., 2015. Type 1 diabetes immunotherapy using polyclonal regulatory T cells. *Sci. Transl. Med.*, 7. p. 315ra189.

- Borsellino, G., Kleinewietfeld, M., Mitri, D.I., Sternjak, D., Diamantini, A., Giometto, A., et al., 2007. Expression of ectonucleotidase CD39 by Foxp3<sup>+</sup> Treg cells: hydrolysis of extracellular ATP and immune suppression. *Blood* 110, 1225–1232.
- Botta, D., Fuller, M.J., Marquez-Lago, T.T., Bachus, H., Bradley, J.E., Weinmann, A.S., et al., 2017. Dynamic regulation of T follicular regulatory cell responses by interleukin 2 during influenza infection. *Nat. Immunol.* 18, 1249–1260.
- Breitfeld, D., Ohl, L., Kremmer, E., Ellwart, J., Sallusto, F., Lipp, M., et al., 2000. Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. *J. Exp. Med.* 192, 1545–1552.
- Brown, C.C., Esterhazy, D., Sarde, A., London, M., Pullabhatla, V., Osma-Garcia, I., et al., 2015. Retinoic acid is essential for Th1 cell lineage stability and prevents transition to a Th17 cell program. *Immunity* 42, 499–511.
- Brüstle, A., Heink, S., Huber, M., Rosenplanter, C., Stadelmann, C., Yu, P., et al., 2007. The development of inflammatory T(H)-17 cells requires interferon-regulatory factor 4. *Nat. Immunol.* 8, 958–966.
- Burton, P.R., Clayton, D.G., Cardon, L.R., Craddock, N., Deloukas, P., Duncanson, A., et al., 2007. Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. *Nat. Genet.* 39, 1329–1337.
- Burzyn, D., Kuswanto, W., Kolodkin, D., Shadrach, J.L., Cerletti, M., Jang, Y., et al., 2013. A special population of regulatory T cells potentiates muscle repair. *Cell* 155, 1282–1295.
- Cargill, M., Schrodin, S.J., Chang, M., Garcia, V.E., Brandon, R., Callis, K.P., et al., 2007. A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. *Am. J. Hum. Genet.* 80, 273–290.
- Chang, H.C., Sehra, S., Goswami, R., Yao, W., Yu, Q., Stritesky, G.L., et al., 2010. The transcription factor PU.1 is required for the development of IL-9-producing T cells and allergic inflammation. *Nat. Immunol.* 11, 527–534.
- Chang, J., Burkett, P.R., Borges, C.M., Kuchroo, V.K., Turka, L.A., Chang, C.H., 2013. MyD88 is essential to sustain mTOR activation necessary to promote T helper 17 cell proliferation by linking IL-1 and IL-23 signaling. *Proc. Natl. Acad. Sci. U.S.A.* 110, 2270–2275.
- Chastain, E.M., Miller, S.D., 2012. Molecular mimicry as an inducing trigger for CNS autoimmune demyelinating disease. *Immunol. Rev.* 245, 227–238.
- Chaudhry, A., Rudra, D., Treuting, P., Samstein, R.M., Liang, Y., Kas, A., et al., 2009. CD4<sup>+</sup> regulatory T cells control TH17 responses in a Stat3-dependent manner. *Science* 326, 986–991.
- Chaudhry, A., Samstein, R.M., Treuting, P., Liang, Y., Pils, M.C., Heinrich, J.M., et al., 2011. Interleukin-10 signaling in regulatory T cells is required for suppression of Th17 cell-mediated inflammation. *Immunity* 34, 566–578.
- Chen, W., Jin, W., Hardegen, N., Lei, K.J., Li, L., Marinov, N., et al., 2003. Conversion of peripheral CD4<sup>+</sup>CD25<sup>-</sup> naive T cells to CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J. Exp. Med.* 198, 1875–1886.
- Chen, Z., Laurence, A., Kanno, Y., Pacher-Zavisin, M., Zhu, B.M., Tato, C., et al., 2006. Selective regulatory function of Socs3 in the formation of IL-17-secreting T cells. *Proc. Natl. Acad. Sci. U.S.A.* 103, 8137–8142.
- Chinen, T., Kannan, A.K., Levine, A.G., Fan, X., Klein, U., Zheng, Y., et al., 2016. An essential role for the IL-2 receptor in Treg cell function. *Nat. Immunol.* 17, pp. 1322–1333.
- Choi, Y.S., Gullicksrud, J.A., Xing, S., Zeng, Z., Shan, Q., Li, F., et al., 2015. LEF-1 and TCF-1 orchestrate T(FH) differentiation by regulating differentiation circuits upstream of the transcriptional repressor Bcl6. *Nat. Immunol.* 16, 980–990.
- Chtanova, T., Tangye, S.G., Newton, R., Frank, N., Hodge, M.R., Rolph, M.S., et al., 2004. T follicular helper cells express a distinctive transcriptional profile, reflecting their role as non-Th1/Th2 effector cells that provide help for B cells. *J. Immunol.* 173, 68–78.
- Chu, C.Q., Wittmer, S., Dalton, D.K., 2000. Failure to suppress the expansion of the activated CD4 T cell population in interferon gamma-deficient mice leads to exacerbation of experimental autoimmune encephalomyelitis. *J. Exp. Med.* 192, 123–128.
- Chung, Y., Chang, S.H., Martinez, G.J., Yang, X.O., Nurieva, R., Kang, H.S., et al., 2009. Critical regulation of early Th17 cell differentiation by interleukin-1 signaling. *Immunity* 30, 576–587.
- Chung, Y., Tanaka, S., Chu, F., Nurieva, R.I., Martinez, G.J., Rawal, S., et al., 2011. Follicular regulatory T cells expressing Foxp3 and Bcl-6 suppress germinal center reactions. *Nat. Med.* 17, pp. 983–988.
- Cifani, M., Madar, A., Galan, C., Sellars, M., Mace, K., Pauli, F., et al., 2012. A validated regulatory network for Th17 cell specification. *Cell* 151, 289–303.
- Codarri, L., Gyulveszsi, G., Tosevski, V., Hesske, L., Fontana, A., Magnenat, L., et al., 2011. RORgammat drives production of the cytokine GM-CSF in helper T cells, which is essential for the effector phase of autoimmune neuroinflammation. *Nat. Immunol.* 12, 560–567.
- Collison, L.W., Workman, C.J., Kuo, T.T., Boyd, K., Wang, Y., Vignali, K.M., et al., 2007. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. *Nature* 450, 566–569.
- Correale, J., Farez, M., 2007. Association between parasite infection and immune responses in multiple sclerosis. *Ann. Neurol.* 61, 97–108.
- Cortese, M., Yuan, C., Chitnis, T., Ascherio, A., Munger, K.L., 2017. No association between dietary sodium intake and the risk of multiple sclerosis. *Neurology* 89, 1322–1329.
- Crotty, S., 2011. Follicular helper CD4 T cells (TFH). *Annu. Rev. Immunol.* 29, 621–663.
- Crotty, S., 2014. T follicular helper cell differentiation, function, and roles in disease. *Immunity* 41, 529–542.
- Cua, D.J., Sherlock, J., Chen, Y., Murphy, C.A., Joyce, B., Seymour, B., et al., 2003. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* 421, 744–748.
- Cubas, R.A., Mudd, J.C., Savoye, A.L., Perreau, M., van Grevenynghe, J., Metcalf, T., et al., 2013. Inadequate T follicular cell help impairs B cell immunity during HIV infection. *Nat. Med.* 19, 494–499.
- Cypowij, S., Picard, C., Marodi, L., Casanova, J.L., Puel, A., 2012. Immunity to infection in IL-17-deficient mice and humans. *Eur. J. Immunol.* 42, 2246–2254.
- Dang, E.V., Barbi, J., Yang, H.Y., Jinasena, D., Yu, H., Zheng, Y., et al., 2011. Control of T(H)17/T(reg) balance by hypoxia-inducible factor 1. *Cell* 146, 772–784.
- Dardalhon, V., Awasthi, A., Kwon, H., Galileos, G., Gao, W., Sobel, R.A., et al., 2008. IL-4 inhibits TGF-beta-induced Foxp3<sup>+</sup> T cells and, together with TGF-beta, generates IL-9 + IL-10 + Foxp3<sup>(-)</sup> effector T cells. *Nat. Immunol.* 9, 1347–1355.
- Dendrou, C.A., Fugger, L., Fries, M.A., 2015. Immunopathology of multiple sclerosis. *Nat. Rev. Immunol.* 15, 545–558.

- Dial, C.F., Tune, M.K., Doerschuk, C.M., Mock, J.R., 2017. Foxp3<sup>+</sup> regulatory T cell expression of keratinocyte growth factor enhances lung epithelial proliferation. *Am. J. Respir. Cell Mol. Biol.* 57, 162–173.
- Dombrowski, Y., O'hagan, T., Dittmer, M., Penalva, R., Mayoral, S.R., Bankhead, P., et al., 2017. Regulatory T cells promote myelin regeneration in the central nervous system. *Nat. Neurosci.* 20, 674–680.
- Du, J., Huang, C., Zhou, B., Ziegler, S.F., 2008. Isoform-specific inhibition of ROR alpha-mediated transcriptional activation by human FOXP3. *J. Immunol.* 180, 4785–4792.
- Duerr, R.H., Taylor, K.D., Brant, S.R., Rioux, J.D., Silverberg, M.S., Daly, M.J., et al., 2006. A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *Science* 314, 1461–1463.
- Duhen, T., Geiger, R., Jarrossay, D., Lanzavecchia, A., Sallusto, F., 2009. Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells. *Nat. Immunol.* 10, 857–863.
- Durant, L., Watford, W.T., Ramos, H.L., Laurence, A., Vahedi, G., Wei, L., et al., 2010. Diverse targets of the transcription factor STAT3 contribute to T cell pathogenicity and homeostasis. *Immunity* 32, 605–615.
- El-Behi, M., Ceric, B., Dai, H., Yan, Y., Cullimore, M., Safavi, F., et al., 2011. The encephalitogenicity of T(H)17 cells is dependent on IL-1- and IL-23-induced production of the cytokine GM-CSF. *Nat. Immunol.* 12, 568–575.
- Esplugues, E., Huber, S., Gagliani, N., Hauser, A.E., Town, T., Wan, Y.Y., et al., 2011. Control of TH17 cells occurs in the small intestine. *Nature* 475, 514–518.
- Eyerich, S., Eyerich, K., Pennino, D., Carbone, T., Nasorri, F., Pallotta, S., et al., 2009. Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. *J. Clin. Invest.* 119, 3573–3585.
- Fantini, M.C., Becker, C., Monteleone, G., Pallone, F., Galle, P.R., Neurath, M.F., 2004. Cutting edge: TGF-beta induces a regulatory phenotype in CD4<sup>+</sup> CD25<sup>+</sup> T cells through Foxp3 induction and down-regulation of Smad7. *J. Immunol.* 172, 5149–5153.
- Farez, M.F., Mascanfroni, I.D., Mendez-Huergo, S.P., Yeste, A., Murugaiyan, G., Garo, L.P., et al., 2015. Melatonin contributes to the seasonality of multiple sclerosis relapses. *Cell* 162, 1338–1352.
- Feng, Y., van Der Veeken, J., Shugay, M., Putintseva, E.V., Osmanbeyoglu, H.U., Dikiy, S., et al., 2015. A mechanism for expansion of regulatory T-cell repertoire and its role in self-tolerance. *Nature* 528, 132–136.
- Ferber, I.A., Brocke, S., Taylor-Edwards, C., Ridgway, W., Dinisco, C., Steinman, L., et al., 1996. Mice with a disrupted IFN-gamma gene are susceptible to the induction of experimental autoimmune encephalomyelitis (EAE). *J. Immunol.* 156, 5–7.
- Ferraro, A., Soccia, C., Stabilini, A., Valle, A., Monti, P., Piemonti, L., et al., 2011. Expansion of Th17 cells and functional defects in T regulatory cells are key features of the pancreatic lymph nodes in patients with type 1 diabetes. *Diabetes* 60, 2903–2913.
- Fleming, J., Hernandez, G., Hartman, L., Maksimovic, J., Nace, S., Lawler, B., et al., 2017. Safety and efficacy of helminth treatment in relapsing-remitting multiple sclerosis: results of the HINT 2 clinical trial. *Mult. Scler.* 1352458517736377.
- Floess, S., Freyer, J., Siewert, C., Baron, U., Olek, S., Polansky, J., et al., 2007. Epigenetic control of the foxp3 locus in regulatory T cells. *PLoS Biol.* 5, e38.
- Fontenot, J.D., Rasmussen, J.P., Gavin, M.A., Rudensky, A.Y., 2005. A function for interleukin 2 in Foxp3-expressing regulatory T cells. *Nat. Immunol.* 6, 1142–1151.
- Fu, W., Ergun, A., Lu, T., Hill, J.A., Haxhinasto, S., Fassett, M.S., et al., 2012. A multiply redundant genetic switch 'locks in' the transcriptional signature of regulatory T cells. *Nat. Immunol.* 13, 972–980.
- Fujio, K., Okamura, T., Yamamoto, K., 2010. The family of IL-10-secreting CD4<sup>+</sup> T cells. *Adv. Immunol.* 105, 99–130.
- Gagliani, N., Jofra, T., Stabilini, A., Valle, A., Atkinson, M., Roncarolo, M.G., et al., 2010. Antigen-specific dependence of Tr1-cell therapy in preclinical models of islet transplant. *Diabetes* 59, 433–439.
- Gagliani, N., Magnani, C.F., Huber, S., Gianolini, M.E., Pala, M., Licona-Limon, P., et al., 2013. Coexpression of CD49b and LAG-3 identifies human and mouse T regulatory type 1 cells. *Nat. Med.* 19, 739–746.
- Gagliani, N., Amezcuia Vesely, M.C., Iseppon, A., Brockmann, L., Xu, H., Palm, N.W., et al., 2015. Th17 cells transdifferentiate into regulatory T cells during resolution of inflammation. *Nature*, 523. pp. 221–225.
- Garg, S.K., Croft, A.M., Bager, P., 2014. Helminth therapy (worms) for induction of remission in inflammatory bowel disease. *Cochrane Database Syst. Rev.* CD009400.
- Garside, P., Ingulli, E., Merica, R.R., Johnson, J.G., Noelle, R.J., Jenkins, M.K., 1998. Visualization of specific B and T lymphocyte interactions in the lymph node. *Science* 281, 96–99.
- Gaublomme, J.T., Yosef, N., Lee, Y., Gertner, R.S., Yang, L.V., Wu, C., et al., 2015. Single-cell genomics unveils critical regulators of Th17 cell pathogenicity. *Cell* 163, 1400–1412.
- Gavin, M.A., Torgerson, T.R., Houston, E., Deroos, P., Ho, W.Y., Stray-Pedersen, A., et al., 2006. Single-cell analysis of normal and FOXP3<sup>-</sup> mutant human T cells: FOXP3 expression without regulatory T cell development. *Proc. Natl. Acad. Sci. U.S.A.* 103, 6659–6664.
- Geginat, G., Lalic, M., Kretschmar, M., Goebel, W., Hof, H., Palm, D., et al., 1998. Th1 cells specific for a secreted protein of *Listeria monocytogenes* are protective in vivo. *J. Immunol.* 160, 6046–6055.
- Genovese, M.C., Durez, P., Richards, H.B., Supronik, J., Dokoupilova, E., Mazurov, V., et al., 2013. Efficacy and safety of secukinumab in patients with rheumatoid arthritis: a phase II, dose-finding, double-blind, randomised, placebo controlled study. *Ann. Rheum. Dis.* 72, 863–869.
- Gerlach, K., Hwang, Y., Nikolaev, A., Atreya, R., Dornhoff, H., Steiner, S., et al., 2014. TH9 cells that express the transcription factor PU.1 drive T cell-mediated colitis via IL-9 receptor signaling in intestinal epithelial cells. *Nat. Immunol.* 15, 676–686.
- Ghoreschi, K., Laurence, A., Yang, X.P., Tato, C.M., Mcgeachy, M.J., Konkel, J.E., et al., 2010. Generation of pathogenic T(H)17 cells in the absence of TGF-beta signalling. *Nature* 467, 967–971.
- Goswami, R., Kaplan, M.H., 2012. Gcn5 is required for PU.1-dependent IL-9 induction in Th9 cells. *J. Immunol.* 189, 3026–3033.
- Grinberg-Bleyer, Y., Baeyens, A., You, S., Elhage, R., Fourcade, G., Gregoire, S., et al., 2010. IL-2 reverses established type 1 diabetes in NOD mice by a local effect on pancreatic regulatory T cells. *J. Exp. Med.* 207, 1871–1878.
- Grossman, W.J., Verbsky, J.W., Barchet, W., Colonna, M., Atkinson, J.P., Ley, T.J., 2004. Human T regulatory cells can use the perforin pathway to cause autologous target cell death. *Immunity* 21, 589–601.

- Gulen, M.F., Kang, Z., Bulek, K., Youzhong, W., Kim, T.W., Chen, Y., et al., 2010. The receptor SIGIRR suppresses Th17 cell proliferation via inhibition of the interleukin-1 receptor pathway and mTOR kinase activation. *Immunity* 32, 54–66.
- Gulen, M.F., Bulek, K., Xiao, H., Yu, M., Gao, J., Sun, L., et al., 2012. Inactivation of the enzyme GSK3alpha by the kinase IKK $\beta$  promotes AKT-mTOR signaling pathway that mediates interleukin-1-induced Th17 cell maintenance. *Immunity* 37, 800–812.
- Gunn, M.D., Tangemann, K., Tam, C., Cyster, J.G., Rosen, S.D., Williams, L.T., 1998. A chemokine expressed in lymphoid high endothelial venules promotes the adhesion and chemotaxis of naive T lymphocytes. *Proc. Natl. Acad. Sci. U.S.A.* 95, 258–263.
- Guo, B., Chang, E.Y., Cheng, G., 2008. The type I IFN induction pathway constrains Th17-mediated autoimmune inflammation in mice. *J. Clin. Invest.* 118, 1680–1690.
- Hall, A.O., Silver, J.S., Hunter, C.A., 2012. The immunobiology of IL-27. *Adv. Immunol.* 115, 1–44.
- Hammer, A., Schliep, A., Jorg, S., Haghikia, A., Gold, R., Kleinewietfeld, M., et al., 2017. Impact of combined sodium chloride and saturated long-chain fatty acid challenge on the differentiation of T helper cells in neuroinflammation. *J. Neuroinflammation* 14, 184.
- Harris, T.J., Grossi, J.F., Yen, H.R., Xin, H., Kortylewski, M., Albesiano, E., et al., 2007. Cutting edge: an in vivo requirement for STAT3 signaling in TH17 development and TH17-dependent autoimmunity. *J. Immunol.* 179, 4313–4317.
- Hatzi, K., Nance, J.P., Kroenke, M.A., Bothwell, M., Haddad, E.K., Melnick, A., et al., 2015. BCL6 orchestrates Tfh cell differentiation via multiple distinct mechanisms. *J. Exp. Med.* 212, 539–553.
- Havrdova, E., Belova, A., Goloborodko, A., Tisserant, A., Wright, A., Wallstroem, E., et al., 2016. Activity of secukinumab, an anti-IL-17A antibody, on brain lesions in RRMS: results from a randomized, proof-of-concept study. *J. Neurol.* 263, 1287–1295.
- Haynes, N.M., Allen, C.D., Lesley, R., Ansel, K.M., Killeen, N., Cyster, J.G., 2007. Role of CXCR5 and CCR7 in follicular Th cell positioning and appearance of a programmed cell death gene-1high germinal center-associated subpopulation. *J. Immunol.* 179, 5099–5108.
- He, J., Zhang, X., Wei, Y., Sun, X., Chen, Y., Deng, J., et al., 2016. Low-dose interleukin-2 treatment selectively modulates CD4(+) T cell subsets in patients with systemic lupus erythematosus. *Nat. Med.* 22, 991–993.
- Hill, J.A., Feuerer, M., Tash, K., Haxhinasto, S., Perez, J., Melamed, R., et al., 2007. Foxp3 transcription-factor-dependent and -independent regulation of the regulatory T cell transcriptional signature. *Immunity* 27, 786–800.
- Hippen, K.L., Merkel, S.C., Schirm, D.K., Sieben, C.M., Sumstad, D., Kadidlo, D.M., et al., 2011. Massive ex vivo expansion of human natural regulatory T cells (Tregs) with minimal loss of in vivo functional activity. *Sci. Transl. Med.* 3, 83ra41.
- Hirota, K., Duarte, J.H., Veldhoen, M., Hornsby, E., Li, Y., Cua, D.J., et al., 2011. Fate mapping of IL-17-producing T cells in inflammatory responses. *Nat. Immunol.* 12, 255–263.
- Hirota, K., Turner, J.E., Villa, M., Duarte, J.H., Demengeot, J., Steinmetz, O.M., et al., 2013. Plasticity of Th17 cells in Peyer's patches is responsible for the induction of T cell-dependent IgA responses. *Nat. Immunol.* 14, 372–379.
- Ho, I.C., Hodge, M.R., Rooney, J.W., Glimcher, L.H., 1996. The proto-oncogene c-maf is responsible for tissue-specific expression of interleukin-4. *Cell* 85, 973–983.
- Ho, I.C., Lo, D., Glimcher, L.H., 1998. c-maf promotes T helper cell type 2 (Th2) and attenuates Th1 differentiation by both interleukin 4-dependent and -independent mechanisms. *J. Exp. Med.* 188, 1859–1866.
- Hofstetter, H.H., Ibrahim, S.M., Koczan, D., Kruse, N., Weishaupt, A., Toyka, K.V., et al., 2005. Therapeutic efficacy of IL-17 neutralization in murine experimental autoimmune encephalomyelitis. *Cell Immunol.* 237, 123–130.
- Hori, S., Nomura, T., Sakaguchi, S., 2003. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 299, 1057–1061.
- Howson, J.M., Cooper, J.D., Smyth, D.J., Walker, N.M., Stevens, H., She, J.X., et al., 2012. Evidence of gene-gene interaction and age-at-diagnosis effects in type 1 diabetes. *Diabetes* 61, 3012–3017.
- Hsu, H.C., Yang, P., Wang, J., Wu, Q., Myers, R., Chen, J., et al., 2008. Interleukin 17-producing T helper cells and interleukin 17 orchestrate autoreactive germinal center development in autoimmune BXD2 mice. *Nat. Immunol.* 9, 166–175.
- Hu, D., Notarbartolo, S., Croonenborghs, T., Patel, B., Cialic, R., Yang, T.H., et al., 2017. Transcriptional signature of human pro-inflammatory TH17 cells identifies reduced IL10 gene expression in multiple sclerosis. *Nat. Commun.* 8, 1600.
- Huber, M., Brustle, A., Reinhard, K., Guralnik, A., Walter, G., Mahiny, A., et al., 2008. IRF4 is essential for IL-21-mediated induction, amplification, and stabilization of the Th17 phenotype. *Proc. Natl. Acad. Sci. U.S.A.* 105, 20846–20851.
- Huber, S., Gagliani, N., Esplugues, E., O'connor, W., Huber, J.R., Chaudhry, F.J., et al., 2011. Th17 cells express interleukin-10 receptor and are controlled by Foxp3 and Foxp3+ regulatory CD4+ T cells in an interleukin-10-dependent manner. *Immunity* 34, 554–565.
- Huber, S., Gagliani, N., Flavell, R.A., 2012. Life, death, and miracles: Th17 cells in the intestine. *Eur. J. Immunol.* 42, 2238–2245.
- Hue, S., Ahern, P., Buonocore, S., Kullberg, M.C., Cua, D.J., Mckenzie, B.S., et al., 2006. Interleukin-23 drives innate and T cell-mediated intestinal inflammation. *J. Exp. Med.* 203, 2473–2483.
- Hueber, W., Sands, B.E., Lewitzky, S., Vandemeulebroecke, M., Reinisch, W., Higgins, P.D., et al., 2012. Secukinumab, a human anti-IL-17A monoclonal antibody, for moderate to severe Crohn's disease: unexpected results of a randomised, double-blind placebo-controlled trial. *Gut* 61, 1693–1700.
- Huurman, V.A., Velthuis, J.H., Hilbrands, R., Tree, T.I., Gillard, P., van Der Meer-Prins, P., et al., 2009. Allograft-specific cytokine profiles associate with clinical outcome after islet cell transplantation. *Am. J. Transplant.* 9, 382–388.
- International Multiple Sclerosis Genetics Consortium, Wellcome Trust Case Control Consortium 2, Sawcer, S., Hellenthal, G., Pirinen, M., Spencer, C.C., et al., 2011. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature*, 476, pp. 214–219.
- Ishigame, H., Kakuta, S., Nagai, T., Kadoki, M., Nambu, A., Komiya, Y., et al., 2009. Differential roles of interleukin-17A and -17F in host defense against mucosal bacterial infection and allergic responses. *Immunity* 30, 108–119.
- Itoh, M., Takahashi, T., Sakaguchi, N., Kuniyasu, Y., Shimizu, J., Otsuka, F., et al., 1999. Thymus and autoimmunity: production of CD25+CD4+ naturally anergic and suppressive T cells as a key function of the thymus in maintaining immunologic self-tolerance. *J. Immunol.* 162, 5317–5326.
- Ivanov, I.I., Mckenzie, B.S., Zhou, L., Tadokoro, C.E., Lepelley, A., Lafaille, J.J., et al., 2006. The orphan nuclear receptor ROR $\gamma$ T directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell* 126, 1121–1133.

- Ivanov, I.I., Atarashi, K., Manel, N., Brodie, E.L., Shima, T., Karaoz, U., et al., 2009. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 139, 485–498.
- Iwata, S., Mikami, Y., Sun, H.W., Brooks, S.R., Jankovic, D., Hirahara, K., et al., 2017. The transcription factor T-bet limits amplification of type I IFN transcriptome and circuitry in T helper 1 cells. *Immunity* 46, 983.e4–991.e4.
- Jäger, A., Dardalhon, V., Sobel, R.A., Bettelli, E., Kuchroo, V.K., 2009. Th1, Th17, and Th9 effector cells induce experimental autoimmune encephalomyelitis with different pathological phenotypes. *J. Immunol.* 183, 7169–7177.
- Johnston, R.J., Poholek, A.C., Ditoro, D., Yusuf, I., Eto, D., Barnett, B., et al., 2009. Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. *Science* 325, 1006–1010.
- Johnston, R.J., Choi, Y.S., Diamond, J.A., Yang, J.A., Crotty, S., 2012. STAT5 is a potent negative regulator of TFH cell differentiation. *J. Exp. Med.* 209, 243–250.
- Jones, L.S., Rizzo, L.V., Agarwal, R.K., Tarrant, T.K., Chan, C.C., Wiggert, B., et al., 1997. IFN-gamma-deficient mice develop experimental autoimmune uveitis in the context of a deviant effector response. *J. Immunol.* 158, 5997–6005.
- Jones, E.Y., Fugger, L., Strominger, J.L., Siebold, C., 2006. MHC class II proteins and disease: a structural perspective. *Nat. Rev. Immunol.* 6, 271–282.
- Kagami, S., Rizzo, H.L., Lee, J.J., Koguchi, Y., Blauvelt, A., 2010. Circulating Th17, Th22, and Th1 cells are increased in psoriasis. *J. Invest. Dermatol.* 130, 1373–1383.
- Karni, A., Abraham, M., Monsonego, A., Cai, G., Freeman, G.J., Hafler, D., et al., 2006. Innate immunity in multiple sclerosis: myeloid dendritic cells in secondary progressive multiple sclerosis are activated and drive a proinflammatory immune response. *J. Immunol.*, 177. pp. 4196–4202.
- Karwacz, K., Miraldi, E.R., Pokrovskii, M., Madi, A., Yosef, N., Wortman, I., et al., 2017. Critical role of IRF1 and BATF in forming chromatin landscape during type 1 regulatory cell differentiation. *Nat. Immunol.* 18, 412–421.
- Kastlein, R.A., Hunter, C.A., Cua, D.J., 2007. Discovery and biology of IL-23 and IL-27: related but functionally distinct regulators of inflammation. *Annu. Rev. Immunol.* 25, 221–242.
- Katz, J.D., Benoist, C., Mathis, D., 1995. T helper cell subsets in insulin-dependent diabetes. *Science* 268, 1185–1188.
- Katz-Levy, Y., Neville, K.L., Padilla, J., Rahbe, S., Begolka, W.S., Girvin, A.M., et al., 2000. Temporal development of autoreactive Th1 responses and endogenous presentation of self myelin epitopes by central nervous system-resident APCs in Theiler's virus-infected mice. *J. Immunol.* 165, 5304–5314.
- Kawahata, K., Misaki, Y., Yamauchi, M., Tsunekawa, S., Setoguchi, K., Miyazaki, J., et al., 2002. Generation of CD4(+)CD25(+) regulatory T cells from autoreactive T cells simultaneously with their negative selection in the thymus and from nonautoreactive T cells by endogenous TCR expression. *J. Immunol.* 168, 4399–4405.
- Khattri, R., Cox, T., Yasayko, S.A., Ramsdell, F., 2003. An essential role for Scurfin in CD4 + CD25 + T regulatory cells. *Nat. Immunol.* 4, 337–342.
- Kim, H.P., Leonard, W.J., 2007. CREB/ATF-dependent T cell receptor-induced FoxP3 gene expression: a role for DNA methylation. *J. Exp. Med.* 204, 1543–1551.
- King, C., Tangye, S.G., Mackay, C.R., 2008. T follicular helper (TFH) cells in normal and dysregulated immune responses. *Annu. Rev. Immunol.* 26, 741–766.
- Kleinewietfeld, M., Manzel, A., Titze, J., Kvakan, H., Yosef, N., Linker, R.A., et al., 2013. Sodium chloride drives autoimmune disease by the induction of pathogenic TH17 cells. *Nature* 496, 518–522.
- Koch, M.A., Tucker-Heard, G., Perdue, N.R., Killebrew, J.R., Urdahl, K.B., Campbell, D.J., 2009. The transcription factor T-bet controls regulatory T cell homeostasis and function during type 1 inflammation. *Nat. Immunol.* 10, 595–602.
- Koch, M.A., Thomas, K.R., Perdue, N.R., Smigiel, K.S., Srivastava, S., Campbell, D.J., 2012. T-bet(+) Treg cells undergo abortive Th1 cell differentiation due to impaired expression of IL-12 receptor beta2. *Immunity* 37, 501–510.
- Kolev, M., Dimeloe, S., Friec, L.E., Navarini, G., Arbore, A., Povoleri, G., et al., 2015. Complement regulates nutrient influx and metabolic reprogramming during Th1 cell responses. *Immunity* 42, 1033–1047.
- Koreth, J., Matsuoka, K., Kim, H.T., McDonough, S.M., Bindra, B., Alyea III, E.P., et al., 2011. Interleukin-2 and regulatory T cells in graft-versus-host disease. *N. Engl. J. Med.* 365, 2055–2066.
- Korn, T., Bettelli, E., Gao, W., Awasthi, A., Jäger, A., Strom, T.B., et al., 2007. IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. *Nature* 448, 484–487.
- Korn, T., Bettelli, E., Oukka, M., Kuchroo, V.K., 2009. IL-17 and Th17 cells. *Annu. Rev. Immunol.* 27, 485–517.
- Kramer, S., Schimpl, A., Hunig, T., 1995. Immunopathology of interleukin (IL) 2-deficient mice: thymus dependence and suppression by thymus-dependent cells with an intact IL-2 gene. *J. Exp. Med.* 182, 1769–1776.
- Kreymborg, K., Etzensperger, R., Dumoutier, L., Haak, S., Rebollo, A., Buch, T., et al., 2007. IL-22 is expressed by Th17 cells in an IL-23-dependent fashion, but not required for the development of autoimmune encephalomyelitis. *J. Immunol.* 179, 8098–8104.
- Kuchroo, V.K., Martin, C.A., Greer, J.M., Ju, S.T., Sobel, R.A., Dorf, M.E., 1993. Cytokines and adhesion molecules contribute to the ability of myelin proteolipid protein-specific T cell clones to mediate experimental allergic encephalomyelitis. *J. Immunol.* 151, 4371–4382.
- Kullberg, M.C., Jankovic, D., Feng, C.G., Hue, S., Gorelick, P.L., McKenzie, B.S., et al., 2006. IL-23 plays a key role in *Helicobacter hepaticus*-induced T cell-dependent colitis. *J. Exp. Med.* 203, 2485–2494.
- Kumar, M., Putzki, N., Limmroth, V., Remus, R., Lindemann, M., Knop, D., et al., 2006. CD4 + CD25 + FoxP3 + T lymphocytes fail to suppress myelin basic protein-induced proliferation in patients with multiple sclerosis. *J. Neuroimmunol.* 180, 178–184.
- Kuswanto, W., Burzyn, D., Panduro, M., Wang, K.K., Jang, Y.C., Wagers, A.J., et al., 2016. Poor repair of skeletal muscle in aging mice reflects a defect in local, interleukin-33-dependent accumulation of regulatory T cells. *Immunity* 44, 355–367.
- Lafaille, J.J., 1998. The role of helper T cell subsets in autoimmune diseases. *Cytokine Growth Factor Rev.* 9, 139–151.
- Langley, R.G., Elewski, B.E., Lebwohl, M., Reich, K., Griffiths, C.E., Papp, K., et al., 2014. Secukinumab in plaque psoriasis—results of two phase 3 trials. *N. Engl. J. Med.* 371, 326–338.

- Langrish, C.L., Chen, Y., Blumenschein, W.M., Mattson, J., Basham, B., Sedgwick, J.D., et al., 2005. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J. Exp. Med.* 201, 233–240.
- Laurence, A., Tato, C.M., Davidson, T.S., Kanno, Y., Chen, Z., Yao, Z., et al., 2007. Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation. *Immunity* 26, 371–381.
- Lee, Y.K., Turner, H., Maynard, C.L., Oliver, J.R., Chen, D., Elson, C.O., et al., 2009. Late developmental plasticity in the T helper 17 lineage. *Immunity* 30, 92–107.
- Lee, Y., Awasthi, A., Yosef, N., Quintana, F.J., Xiao, S., Peters, A., et al., 2012. Induction and molecular signature of pathogenic T(H)17 cells. *Nat. Immunol.* 13, 991–999.
- Leonardi, C.L., Kimball, A.B., Papp, K.A., Yeilding, N., Guzzo, C., Wang, Y., et al., 2008. Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 76-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 1). *Lancet* 371, 1665–1674.
- Li, P., Spolski, R., Liao, W., Wang, L., Murphy, T.L., Murphy, K.M., et al., 2012. BATF-JUN is critical for IRF4-mediated transcription in T cells. *Nature* 490, 543–546.
- Li, J., Lu, E., Yi, T., Cyster, J.G., 2016. EBI2 augments Tfh cell fate by promoting interaction with IL-2-quenching dendritic cells. *Nature* 533, 110–114.
- Liang, S.C., Tan, X.Y., Luxenberg, D.P., Karim, R., Dunussi-Joannopoulos, K., Collins, M., et al., 2006. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J. Exp. Med.* 203, 2271–2279.
- Liang, B., Workman, C., Lee, J., Chew, C., Dale, B.M., Colonna, L., et al., 2008. Regulatory T cells inhibit dendritic cells by lymphocyte activation gene-3 engagement of MHC class II. *J. Immunol.* 180, 5916–5926.
- Licona-Limon, P., Henao-Mejia, J., Temann, A.U., Gagliani, N., Licona-Limon, I., Ishigame, H., et al., 2013. Th9 cells drive host immunity against gastrointestinal worm infection. *Immunity* 39, 744–757.
- Lin, W., Truong, N., Grossman, W.J., Haribhai, D., Williams, C.B., Wang, J., et al., 2005. Allergic dysregulation and hyperimmunoglobulinemia E in Foxp3 mutant mice. *J. Allergy Clin. Immunol.* 116, 1106–1115.
- Lindemans, C.A., Calafiore, M., Mertelsmann, A.M., O'connor, M.H., Dudakov, J.A., Jenq, R.R., et al., 2015. Interleukin-22 promotes intestinal-stem-cell-mediated epithelial regeneration. *Nature* 528, 560–564.
- Linterman, M.A., Pierson, W., Lee, S.K., Kallies, A., Kawamoto, S., Rayner, T.F., et al., 2011. Foxp3+ follicular regulatory T cells control the germinal center response. *Nat. Med.* 17, 975–982.
- Liu, Y., Helms, C., Liao, W., Zaba, L.C., Duan, S., Gardner, J., et al., 2008. A genome-wide association study of psoriasis and psoriatic arthritis identifies new disease loci. *PLoS Genet.* 4, e1000041.
- Liu, X., Leung, S., Wang, C., Tan, Z., Wang, J., Guo, T.B., et al., 2010. Crucial role of interleukin-7 in T helper type 17 survival and expansion in autoimmune disease. *Nat. Med.* 16, 191–197.
- Liu, S.M., Lee, D.H., Sullivan, J.M., Chung, D., Jager, A., Shum, B.O., et al., 2011. Differential IL-21 signaling in APCs leads to disparate Th17 differentiation in diabetes-susceptible NOD and diabetes-resistant NOD.Idd3 mice. *J. Clin. Invest.* 121, 4303–4310.
- Liu, X., Yan, X., Zhong, B., Nurieva, R.I., Wang, A., Wang, X., et al., 2012. Bcl6 expression specifies the T follicular helper cell program in vivo. *J. Exp. Med.* 209, 1841–1852. S1–24.
- Liu, X., Nurieva, R.I., Dong, C., 2013. Transcriptional regulation of follicular T-helper (Tfh) cells. *Immunol. Rev.* 252, 139–145.
- Liu, X., Chen, X., Zhong, B., Wang, A., Wang, X., Chu, F., et al., 2014. Transcription factor achaete-scute homologue 2 initiates follicular T-helper-cell development. *Nature* 507, 513–518.
- Liu, Z., Gerner, M.Y., Van Panhuys, N., Levine, A.G., Rudensky, A.Y., Germain, R.N., 2015. Immune homeostasis enforced by co-localized effector and regulatory T cells. *Nature* 528, 225–230.
- Luther, S.A., Tang, H.L., Hyman, P.L., Farr, A.G., Cyster, J.G., 2000. Coexpression of the chemokines ELC and SLC by T zone stromal cells and deletion of the ELC gene in the plt=plt mouse. *Proc. Natl. Acad. Sci. U.S.A.* 97, 12694–12699.
- Luthje, K., Kallies, A., Shimohakamada, Y., Belz, G.T., Light, A., Tarlinton, D.M., et al., 2012. The development and fate of follicular helper T cells defined by an IL-21 reporter mouse. *Nat. Immunol.* 13, 491–498.
- Luzina, I.G., Atamas, S.P., Storrer, C.E., Dasilva, L.C., Kelsoe, G., Papadimitriou, J.C., et al., 2001. Spontaneous formation of germinal centers in autoimmune mice. *J. Leukoc. Biol.* 70, 578–584.
- Ma, J., Zhu, C., Ma, B., Tian, J., Baidoo, S.E., Mao, C., et al., 2012. Increased frequency of circulating follicular helper T cells in patients with rheumatoid arthritis. *Clin. Dev. Immunol.* 2012, 827480.
- Maceiras, A.R., Almeida, S.C.P., Mariotti-Ferrandiz, E., Chaara, W., Jebbawi, F., Six, A., et al., 2017. T follicular helper and T follicular regulatory cells have different TCR specificity. *Nat. Commun.* 8, 15067.
- Mack, C.L., Vanderlugt-Castaneda, C.L., Neville, K.L., Miller, S.D., 2003. Microglia are activated to become competent antigen presenting and effector cells in the inflammatory environment of the Theiler's virus model of multiple sclerosis. *J. Neuroimmunol.* 144, 68–79.
- MacLennan, I.C., Gulbranson-Judge, A., Toellner, K.M., Casamayor-Palleja, M., Chan, E., Sze, D.M., et al., 1997. The changing preference of T and B cells for partners as T-dependent antibody responses develop. *Immunol. Rev.* 156, 53–66.
- Malchow, S., Leventhal, D.S., Lee, V., Nishi, S., Socci, N.D., Savage, P.A., 2016. Aire enforces immune tolerance by directing autoreactive T cells into the regulatory T cell lineage. *Immunity* 44, 1102–1113.
- Malek, T.R., Yu, A., Vincek, V., Scibelli, P., Kong, L., 2002. CD4 regulatory T cells prevent lethal autoimmunity in IL-2R $\beta$ -deficient mice. Implications for the nonredundant function of IL-2. *Immunity* 17, 167–178.
- Malik, S., Sadhu, S., Elesela, S., Pandey, R.P., Chawla, A.S., Sharma, D., et al., 2017. Transcription factor Foxo1 is essential for IL-9 induction in T helper cells. *Nat. Commun.* 8, 815.
- Manel, N., Unutmaz, D., Littman, D.R., 2008. The differentiation of human T(H)-17 cells requires transforming growth factor-beta and induction of the nuclear receptor ROR $\gamma$ mat. *Nat. Immunol.* 9, 641–649.
- Mangan, P.R., Harrington, L.E., O'quinn, D.B., Helms, W.S., Bullard, D.C., Elson, C.O., et al., 2006. Transforming growth factor- $\beta$  induces development of Th17 lineage. *Nature* 441, 231–234.

- Martin, B.N., Wang, C., Zhang, C.J., Kang, Z., Gulen, M.F., Zepp, J.A., et al., 2016. T cell-intrinsic ASC critically promotes T(H)17-mediated experimental autoimmune encephalomyelitis. *Nat. Immunol.* 17, 583–592.
- Mascanfroni, I.D., Takenaka, M.C., Yeste, A., Patel, B., Wu, Y., Kenison, J.E., et al., 2015. Metabolic control of type 1 regulatory T cell differentiation by AHR and HIF1-alpha. *Nat. Med.* 21, 638–646.
- Matthys, P., Vermeire, K., Mitera, T., Heremans, H., Huang, S., Billiau, A., 1998. Anti-IL-12 antibody prevents the development and progression of collagen-induced arthritis in IFN-gamma receptor-deficient mice. *Eur. J. Immunol.* 28, 2143–2151.
- Matusevicius, D., Kivisakk, P., He, B., Kostulas, N., Ozenci, V., Fredrikson, S., et al., 1999. Interleukin-17 mRNA expression in blood and CSF mononuclear cells is augmented in multiple sclerosis. *Mult. Scler.* 5, 101–104.
- Mcgeachy, M.J., Bak-Jensen, K.S., Chen, Y., Tato, C.M., Blumenschein, W., Mcclanahan, T., et al., 2007. TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. *Nat. Immunol.* 8, 1390–1397.
- Mcgeachy, M.J., Chen, Y., Tato, C.M., Laurence, A., Joyce-Shaikh, B., Blumenschein, W.M., et al., 2009. The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells in vivo. *Nat. Immunol.* 10, 314–324.
- McInnes, I.B., Kavanaugh, A., Gottlieb, A.B., Puig, L., Rahman, P., Ritchlin, C., et al., 2013. Efficacy and safety of ustekinumab in patients with active psoriatic arthritis: 1 year results of the phase 3, multicentre, double-blind, placebo-controlled PSUMMIT 1 trial. *Lancet* 382, 780–789.
- Mckenzie, B.S., Kastelein, R.A., Cua, D.J., 2006. Understanding the IL-23-IL-17 immune pathway. *Trends Immunol.* 27, 17–23.
- Mcmahon, E.J., Bailey, S.L., Castenada, C.V., Waldner, H., Miller, S.D., 2005. Epitope spreading initiates in the CNS in two mouse models of multiple sclerosis. *Nat. Med.* 11, 335–339.
- Meyer Zu Horste, G., Wu, C., Wang, C., Cong, L., Pawlak, M., Lee, Y., et al., 2016. RBPJ controls development of pathogenic Th17 cells by regulating IL-23 receptor expression. *Cell Rep.* 16, 392–404.
- Miclea, A., Miclea, M., Pistor, M., Hoepner, A., Chan, A., Hoepner, R., 2017. Vitamin D supplementation differentially affects seasonal multiple sclerosis disease activity. *Brain Behav.* 7, e00761.
- Miyara, M., Amoura, Z., Gorochov, G., 2009. Human lupus, fewer Treg cells indeed: comment on the article by Venigalla et al. *Arthritis Rheum.* 60, 630.
- Morita, R., Schmitt, N., Bentebibel, S.E., Ranganathan, R., Bourdery, L., Zurawski, G., et al., 2011. Human blood CXCR5(+)CD4(+) T cells are counterparts of T follicular cells and contain specific subsets that differentially support antibody secretion. *Immunity* 34, 108–121.
- Mosmann, T.R., Coffman, R.L., 1989a. Heterogeneity of cytokine secretion patterns and functions of helper T cells. *Adv. Immunol.* 46, 111–147.
- Mosmann, T.R., Coffman, R.L., 1989b. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol.* 7, 145–173.
- Mosmann, T.R., Cherwinski, H., Bond, M.W., Giedlin, M.A., Coffman, R.L., 1986. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* 136, 2348–2357.
- Mucida, D., Cheroutre, H., 2010. The many face-lifts of CD4 T helper cells. *Adv. Immunol.* 107, 139–152.
- Mucida, D., Park, Y., Kim, G., Turovskaia, O., Scott, I., Kronenberg, M., et al., 2007. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science* 317, 256–260.
- Mueller, R., Krahl, T., Sarvetnick, N., 1996. Pancreatic expression of interleukin-4 abrogates insulitis and autoimmune diabetes in nonobese diabetic (NOD) mice. *J. Exp. Med.* 184, 1093–1099.
- Nakae, S., Nambu, A., Sudo, K., Iwakura, Y., 2003. Suppression of immune induction of collagen-induced arthritis in IL-17-deficient mice. *J. Immunol.* 171, 6173–6177.
- Nakanishi, K., Yoshimoto, T., Tsutsui, H., Okamura, H., 2001. Interleukin-18 regulates both Th1 and Th2 responses. *Annu. Rev. Immunol.* 19, 423–474.
- Nalleweg, N., Chiriac, M.T., Podstawa, E., Lehmann, C., Rau, T.T., Atreya, R., et al., 2015. IL-9 and its receptor are predominantly involved in the pathogenesis of UC. *Gut* 64, 743–755.
- Neighbors, M., Xu, X., Barrat, F.J., Ruuls, S.R., Churakova, T., Debets, R., et al., 2001. A critical role for interleukin 18 in primary and memory effector responses to *Listeria monocytogenes* that extends beyond its effects on Interferon gamma production. *J. Exp. Med.* 194, 343–354.
- Neurath, M.F., Finotto, S., Glimcher, L.H., 2002. The role of Th1/Th2 polarization in mucosal immunity. *Nat. Med.* 8, 567–573.
- Nishizuka, Y., Sakakura, T., 1969. Thymus and reproduction: sex-linked dysgenesis of the gonad after neonatal thymectomy in mice. *Science* 166, 753–755.
- Nowarski, R., Jackson, R., Gagliani, N., De Zoete, M.R., Palm, N.W., Bailis, W., et al., 2015. Epithelial IL-18 equilibrium controls barrier function in colitis. *Cell* 163, 1444–1456.
- Nurieva, R., Yang, X.O., Martinez, G., Zhang, Y., Panopoulos, A.D., Ma, L., et al., 2007. Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. *Nature* 448, 480–483.
- Nurieva, R.I., Chung, Y., Hwang, D., Yang, X.O., Kang, H.S., Ma, L., et al., 2008. Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. *Immunity* 29, 138–149.
- Nurieva, R.I., Chung, Y., Martinez, G.J., Yang, X.O., Tanaka, S., Matskevitch, T.D., et al., 2009a. Bcl6 mediates the development of T follicular helper cells. *Science* 325, 1001–1005.
- Nurieva, R.I., Liu, X., Dong, C., 2009b. Yin-Yang of costimulation: crucial controls of immune tolerance and function. *Immunol. Rev.* 229, 88–100.
- Nurieva, R.I., Podd, A., Chen, Y., Alekseev, A.M., Yu, M., Qi, X., et al., 2012. STAT5 protein negatively regulates T follicular helper (Tfh) cell generation and function. *J. Biol. Chem.* 287, 11234–11239.
- Oh, H., Ghosh, S., 2013. NF-kappaB: roles and regulation in different CD4(+) T-cell subsets. *Immunol. Rev.* 252, 41–51.
- Ohkura, N., Hamaguchi, M., Morikawa, H., Sugimura, K., Tanaka, A., Ito, Y., et al., 2012. T cell receptor stimulation-induced epigenetic changes and Foxp3 expression are independent and complementary events required for Treg cell development. *Immunity* 37, 785–799.
- Ohnacht, C., Park, J.H., Cording, S., Wing, J.B., Atarashi, K., Obata, Y., et al., 2015. MUCOSAL IMMUNOLOGY. The microbiota regulates type 2 immunity through RORgammat(+) T cells. *Science* 349, 989–993.

- Olsson, T., Barcellos, L.F., Alfredsson, L., 2017. Interactions between genetic, lifestyle and environmental risk factors for multiple sclerosis. *Nat. Rev. Neurol.* 13, 25–36.
- Onishi, Y., Fehervari, Z., Yamaguchi, T., Sakaguchi, S., 2008. Foxp3<sup>+</sup> natural regulatory T cells preferentially form aggregates on dendritic cells in vitro and actively inhibit their maturation. *Proc. Natl. Acad. Sci. U.S.A.* 105, 10113–10118.
- Oppmann, B., Lesley, R., Blom, B., Timans, J.C., Xu, Y., Hunte, B., et al., 2000. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity* 13, 715–725.
- Ozenci, V., Kouwenhoven, M., Huang, Y.M., Xiao, B., Kivisakk, P., Fredrikson, S., et al., 1999. Multiple sclerosis: levels of interleukin-10-secreting blood mononuclear cells are low in untreated patients but augmented during interferon-beta-1b treatment. *Scand. J. Immunol.* 49, 554–561.
- O'Reilly, S., Hugle, T., Van Laar, J.M., 2012. T cells in systemic sclerosis: a reappraisal. *Rheumatology (Oxford)* 51, 1540–1549.
- Pandian, P., Zheng, L., Ishihara, S., Reed, J., Lenardo, M.J., 2007. CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> regulatory T cells induce cytokine deprivation-mediated apoptosis of effector CD4<sup>+</sup> T cells. *Nat. Immunol.* 8, 1353–1362.
- Papa, I., Saliba, D., Poncini, M., Bustamante, S., Canete, P.F., Gonzalez-Figueroa, P., et al., 2017. TFH-derived dopamine accelerates productive synapses in germinal centres. *Nature* 547, 318–323.
- Papiernik, M., De Moraes, M.L., Pontoux, C., Vasseur, F., Penit, C., 1998. Regulatory CD4 T cells: expression of IL-2R alpha chain, resistance to clonal deletion and IL-2 dependency. *Int. Immunol.* 10, 371–378.
- Papp, K.A., Langley, R.G., Lebwohl, M., Krueger, G.G., Szapary, P., Yeilding, N., et al., 2008. Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 52-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 2). *Lancet* 371, 1675–1684.
- Parham, C., Chirica, M., Timans, J., Vaisberg, E., Travis, M., Cheung, J., et al., 2002. A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R. *J. Immunol.* 168, 5699–5708.
- Paul, W.E., 2010. What determines Th2 differentiation, in vitro and in vivo? *Immunol. Cell Biol.* 88, 236–239.
- Peng, M., Yin, N., Chhangawala, S., Xu, K., Leslie, C.S., Li, M.O., 2016. Aerobic glycolysis promotes T helper 1 cell differentiation through an epigenetic mechanism. *Science* 354, 481–484.
- Poddubnyy, D., Hermann, K.G., Callhoff, J., Listing, J., Sieper, J., 2014. Ustekinumab for the treatment of patients with active ankylosing spondylitis: results of a 28-week, prospective, open-label, proof-of-concept study (TOPAS). *Ann. Rheum. Dis.* 73, 817–823.
- Pot, C., Jin, H., Awasthi, A., Liu, S.M., Lai, C.Y., Madan, R., et al., 2009. Cutting edge: IL-27 induces the transcription factor c-Maf, cytokine IL-21, and the costimulatory receptor ICOS that coordinately act together to promote differentiation of IL-10-producing Tr1 cells. *J. Immunol.* 183, 797–801.
- Pot, C., Apetoh, L., Awasthi, A., Kuchroo, V.K., 2011a. Induction of regulatory Tr1 cells and inhibition of T(H)17 cells by IL-27. *Semin. Immunol.* 23, 438–445.
- Pot, C., Apetoh, L., Kuchroo, V.K., 2011b. Type 1 regulatory T cells (Tr1) in autoimmunity. *Semin. Immunol.* 23, 202–208.
- Powrie, F., Mason, D., 1990. OX-22high CD4<sup>+</sup> T cells induce wasting disease with multiple organ pathology: prevention by the OX-22low subset. *J. Exp. Med.* 172, 1701–1708.
- Pulendran, B., Artis, D., 2012. New paradigms in type 2 immunity. *Science* 337, 431–435.
- Purwar, R., Schlapbach, C., Xiao, S., Kang, H.S., Elyaman, W., Jiang, X., et al., 2012. Robust tumor immunity to melanoma mediated by interleukin-9-producing T cells. *Nat. Med.* 18, 1248–1253.
- Quintana, F.J., Basso, A.S., Iglesias, A.H., Korn, T., Farez, M.F., Bettelli, E., et al., 2008. Control of T(reg) and T(H)17 cell differentiation by the aryl hydrocarbon receptor. *Nature* 453, 65–71.
- Qureshi, O.S., Zheng, Y., Nakamura, K., Attridge, K., Manzotti, C., Schmidt, E.M., et al., 2011. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. *Science* 332, 600–603.
- Ramgolam, V.S., Sha, Y., Jin, J., Zhang, X., Markovic-Plese, S., 2009. IFN-beta inhibits human Th17 cell differentiation. *J. Immunol.* 183, 5418–5427.
- Ramiscal, R.R., Vinuesa, C.G., 2013. T-cell subsets in the germinal center. *Immunol. Rev.* 252, 146–155.
- Rolla, S., Bardina, V., De Mercanti, S., Quagliino, P., De Palma, R., Gned, D., et al., 2014. Th22 cells are expanded in multiple sclerosis and are resistant to IFN-beta. *J. Leukoc. Biol.* 96, 1155–1164.
- Roncarolo, M.G., Gregori, S., Lucarelli, B., Ciceri, F., Bacchetta, R., 2011. Clinical tolerance in allogeneic hematopoietic stem cell transplantation. *Immunol. Rev.* 241, 145–163.
- Rose, N.R., 2017. Negative selection, epitope mimicry and autoimmunity. *Curr. Opin. Immunol.* 49, 51–55.
- Rothhammer, V., Mascanfroni, I.D., Bunse, L., Takenaka, M.C., Kenison, J.E., Mayo, L., et al., 2016. Type I interferons and microbial metabolites of tryptophan modulate astrocyte activity and central nervous system inflammation via the aryl hydrocarbon receptor. *Nat. Med.* 22, 586–597.
- Rutz, S., Noubade, R., Eidenschenk, C., Ota, N., Zeng, W., Zheng, Y., et al., 2011. Transcription factor c-Maf mediates the TGF-beta-dependent suppression of IL-22 production in T(H)17 cells. *Nat. Immunol.* 12, 1238–1245.
- Saadoun, D., Rosenzwajg, M., Joly, F., Six, A., Carrat, F., Thibault, V., et al., 2011. Regulatory T-cell responses to low-dose interleukin-2 in HCV-induced vasculitis. *N. Engl. J. Med.* 365, 2067–2077.
- Sage, P.T., Sharpe, A.H., 2016. T follicular regulatory cells. *Immunol. Rev.* 271, 246–259.
- Sage, P.T., Francisco, L.M., Carman, C.V., Sharpe, A.H., 2013. The receptor PD-1 controls follicular regulatory T cells in the lymph nodes and blood. *Nat. Immunol.* 14, 152–161.
- Sage, P.T., Paterson, A.M., Lovitch, S.B., Sharpe, A.H., 2014. The coinhibitory receptor CTLA-4 controls B cell responses by modulating T follicular helper, T follicular regulatory, and T regulatory cells. *Immunity* 41, 1026–1039.
- Sage, P.T., Ron-Harel, N., Juneja, V.R., Sen, D.R., Maleri, S., Sungnak, W., et al., 2016. Suppression by TFR cells leads to durable and selective inhibition of B cell effector function. *Nat. Immunol.* 17, 1436–1446.
- Sagoo, P., Lombardi, G., Lechner, R.I., 2008. Regulatory T cells as therapeutic cells. *Curr. Opin. Organ Transplant.* 13, 645–653.

- Sakaguchi, S., Fukuma, K., Kuribayashi, K., Masuda, T., 1985. Organ-specific autoimmune diseases induced in mice by elimination of T cell subset. I. Evidence for the active participation of T cells in natural self-tolerance; deficit of a T cell subset as a possible cause of autoimmune disease. *J. Exp. Med.* 161, 72–87.
- Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M., Toda, M., 1995. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol.* 155, 1151–1164.
- Sandborn, W.J., Gasink, C., Gao, L.L., Blank, M.A., Johanns, J., Guzzo, C., et al., 2012. Ustekinumab induction and maintenance therapy in refractory Crohn's disease. *N. Engl. J. Med.* 367, 1519–1528.
- Sano, T., Huang, W., Hall, J.A., Yang, Y., Chen, A., Gavzy, S.J., et al., 2015. An IL-23R/IL-22 circuit regulates epithelial serum amyloid A to promote local effector Th17 responses. *Cell* 163, 381–393.
- Saruta, M., Yu, Q.T., Fleshner, P.R., Mantel, P.Y., Schmidt-Weber, C.B., Banham, A.H., et al., 2007. Characterization of FOXP3<sup>+</sup> CD4<sup>+</sup> regulatory T cells in Crohn's disease. *Clin. Immunol.* 125, 281–290.
- Schmidl, C., Klug, M., Boeld, T.J., Andreesen, R., Hoffmann, P., Edinger, M., et al., 2009. Lineage-specific DNA methylation in T cells correlates with histone methylation and enhancer activity. *Genome Res.* 19, 1165–1174.
- Schoenborn, J.R., Wilson, C.B., 2007. Regulation of interferon-gamma during innate and adaptive immune responses. *Adv. Immunol.* 96, 41–101.
- Schorle, H., Holtschke, T., Hunig, T., Schimpl, A., Horak, I., 1991. Development and function of T cells in mice rendered interleukin-2 deficient by gene targeting. *Nature* 352, 621–624.
- Schraml, B.U., Hildner, K., Ise, W., Lee, W.L., Smith, W.A., Solomon, B., et al., 2009. The AP-1 transcription factor Batf controls T(H)17 differentiation. *Nature* 460, 405–409.
- Schreiner, B., Bailey, S.L., Miller, S.D., 2007. T-cell response dynamics in animal models of multiple sclerosis: implications for immunotherapies. *Expert Rev. Clin. Immunol.* 3, 57–72.
- Sefik, E., Geva-Zatorsky, N., Oh, S., Konnikova, L., Zemmour, D., McGuire, A.M., et al., 2015. MUCOSAL IMMUNOLOGY. Individual intestinal symbionts induce a distinct population of ROR $\gamma$ (+) regulatory T cells. *Science* 349, 993–997.
- Segal, B.M., Constantinescu, C.S., Raychaudhuri, A., Kim, L., Fidelus-Gort, R., Kasper, L.H., et al., 2008. Repeated subcutaneous injections of IL12/23 p40 neutralising antibody, ustekinumab, in patients with relapsing-remitting multiple sclerosis: a phase II, double-blind, placebo-controlled, randomised, dose-ranging study. *Lancet Neurol.* 7, 796–804.
- Setoguchi, R., Hori, S., Takahashi, T., Sakaguchi, S., 2005. Homeostatic maintenance of natural Foxp3(+) CD25(+) CD4(+) regulatory T cells by interleukin (IL)-2 and induction of autoimmune disease by IL-2 neutralization. *J. Exp. Med.* 201, 723–735.
- Sewell, D., Qing, Z., Reinke, E., Elliot, D., Weinstock, J., Sandor, M., et al., 2003. Immunomodulation of experimental autoimmune encephalomyelitis by helminth ova immunization. *Int. Immunol.* 15, 59–69.
- Shi, G., Cox, C.A., Vistica, B.P., Tan, C., Wawrousek, E.F., Gery, I., 2008. Phenotype switching by inflammation-inducing polarized Th17 cells, but not by Th1 cells. *J. Immunol.* 181, 7205–7213.
- Shi, L.Z., Wang, R., Huang, G., Vogel, P., Neale, G., Green, D.R., et al., 2011. HIF1alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. *J. Exp. Med.* 208, 1367–1376.
- Singh, R.R., Saxena, V., Zang, S., Li, L., Finkelman, F.D., Witte, D.P., et al., 2003. Differential contribution of IL-4 and STAT6 vs STAT4 to the development of lupus nephritis. *J. Immunol.* 170, 4818–4825.
- Smith, C.E., Miller, S.D., 2006. Multi-peptide coupled-cell tolerance ameliorates ongoing relapsing EAE associated with multiple pathogenic autoreactivities. *J. Autoimmun.* 27, 218–231.
- Staudt, V., Bothur, E., Klein, M., Lingnau, K., Reuter, S., Grebe, N., et al., 2010. Interferon-regulatory factor 4 is essential for the developmental program of T helper 9 cells. *Immunity* 33, 192–202.
- Sugimoto, K., Ogawa, A., Mizoguchi, E., Shimomura, Y., Andoh, A., Bhan, A.K., et al., 2008. IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. *J. Clin. Invest.* 118, 534–544.
- Sujino, T., London, M., Hoytema van Konijnenburg, D.P., Rendon, T., Buch, T., Silva, H.M., et al., 2016. Tissue adaptation of regulatory and intraepithelial CD4(+) T cells controls gut inflammation. *Science* 352, 1581–1586.
- Summers, R.W., Elliott, D.E., Urban Jr, J.F., Thompson, R.A., Weinstock, J.V., 2005. *Trichuris suis* therapy for active ulcerative colitis: a randomized controlled trial. *Gastroenterology* 128, 825–832.
- Suscovich, T.J., Perdue, N.R., Campbell, D.J., 2012. Type-1 immunity drives early lethality in scurfy mice. *Eur. J. Immunol.* 42, 2305–2310.
- Sutton, C., Brereton, C., Keogh, B., Mills, K.H., Lavelle, E.C., 2006. A crucial role for interleukin (IL)-1 in the induction of IL-17-producing T cells that mediate autoimmune encephalomyelitis. *J. Exp. Med.* 203, 1685–1691.
- Suzuki, H., Kundig, T.M., Furlonger, C., Wakeham, A., Timms, E., Matsuyama, T., et al., 1995. Deregulated T cell activation and autoimmunity in mice lacking interleukin-2 receptor beta. *Science* 268, 1472–1476.
- Sweeney, C.M., Lonergan, R., Basdeo, S.A., Kinsella, K., Dungan, L.S., Higgins, S.C., et al., 2011. IL-27 mediates the response to IFN-beta therapy in multiple sclerosis patients by inhibiting Th17 cells. *Brain Behav. Immun.* 25, 1170–1181.
- Szabo, S.J., Sullivan, B.M., Peng, S.L., Glimcher, L.H., 2003. Molecular mechanisms regulating Th1 immune responses. *Annu. Rev. Immunol.* 21, 713–758.
- Takahashi, M., Nakamura, K., Honda, K., Kitamura, Y., Mizutani, T., Araki, Y., et al., 2006. An inverse correlation of human peripheral blood regulatory T cell frequency with the disease activity of ulcerative colitis. *Dig. Dis. Sci.* 51, 677–686.
- Teng, M.W., Bowman, E.P., McElwee, J.J., Smyth, M.J., Casanova, J.L., Cooper, A.M., et al., 2015. IL-12 and IL-23 cytokines: from discovery to targeted therapies for immune-mediated inflammatory diseases. *Nat. Med.* 21, 719–729.
- Teunissen, M.B., Koomen, C.W., De Waal Malefydt, R., Wierenga, E.A., Bos, J.D., 1998. Interleukin-17 and interferon-gamma synergize in the enhancement of proinflammatory cytokine production by human keratinocytes. *J. Invest. Dermatol.* 111, 645–649.
- Thornton, A.M., Shevach, E.M., 1998. CD4<sup>+</sup> CD25<sup>+</sup> immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. *J. Exp. Med.* 188, 287–296.

- Thornton, A.M., Donovan, E.E., Piccirillo, C.A., Shevach, E.M., 2004. Cutting edge: IL-2 is critically required for the in vitro activation of CD4 + CD25 + T cell suppressor function. *J. Immunol.* 172, 6519–6523.
- Thornton, A.M., Korty, P.E., Tran, D.Q., Wohlfert, E.A., Murray, P.E., Belkaid, Y., et al., 2010. Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced Foxp3 + T regulatory cells. *J. Immunol.* 184, 3433–3441.
- Trembleau, S., Penna, G., Gregori, S., Giarratana, N., Adorini, L., 2003. IL-12 administration accelerates autoimmune diabetes in both wild-type and IFN-gamma-deficient nonobese diabetic mice, revealing pathogenic and protective effects of IL-12-induced IFN-gamma. *J. Immunol.* 170, 5491–5501.
- Trifari, S., Kaplan, C.D., Tran, E.H., Crellin, N.K., Spits, H., 2009. Identification of a human helper T cell population that has abundant production of interleukin 22 and is distinct from T(H)-17, T(H)1 and T(H)2 cells. *Nat. Immunol.* 10, 864–871.
- Vahedi, G., Takahashi, H., Nakayamada, S., Sun, H.W., Sartorelli, V., Kanno, Y., et al., 2012. STATs shape the active enhancer landscape of T cell populations. *Cell* 151, 981–993.
- Vandenebbeck, K., 2012. Cytokine gene polymorphisms and human autoimmune disease in the era of genome-wide association studies. *J. Interferon Cytokine Res.* 32, 139–151.
- Vegran, F., Berger, H., Boidot, R., Mignot, G., Bruchard, M., Dosset, M., et al., 2014. The transcription factor IRF1 dictates the IL-21-dependent anticancer functions of TH9 cells. *Nat. Immunol.* 15, 758–766.
- Veldhoen, M., Hocking, R.J., Atkins, C.J., Locksley, R.M., Stockinger, B., 2006. TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* 24, 179–189.
- Veldhoen, M., Hirota, K., Westendorf, A.M., Buer, J., Dumoutier, L., Renaud, J.C., et al., 2008a. The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. *Nature* 453, 106–109.
- Veldhoen, M., Uyttenhove, C., van Snick, J., Helmy, H., Westendorf, A., Buer, J., et al., 2008b. Transforming growth factor-beta ‘reprograms’ the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset. *Nat. Immunol.* 9, 1341–1346.
- Viegas, M.S., Carmo, D.O., Silva, A., Seco, T., Serra, F., Lacerda, V., et al., 2007. CD38 plays a role in effective containment of mycobacteria within granulomata and polarization of Th1 immune responses against *Mycobacterium avium*. *Microbes Infect.* 9, 847–854.
- Viglietta, V., Baecher-Allan, C., Weiner, H.L., Hafler, D.A., 2004. Loss of functional suppression by CD4 + CD25 + regulatory T cells in patients with multiple sclerosis. *J. Exp. Med.* 199, 971–979.
- Vinuesa, C.G., Cook, M.C., 2011. Blood relatives of follicular helper T cells. *Immunity* 34, 10–12.
- Vinuesa, C.G., Cook, M.C., Angelucci, C., Athanasopoulos, V., Rui, L., Hill, K.M., et al., 2005. A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. *Nature* 435, 452–458.
- Voldsgaard, A., Bager, P., Garde, E., Akeson, P., Leffers, A.M., Madsen, C.G., et al., 2015. *Trichuris suis* ova therapy in relapsing multiple sclerosis is safe but without signals of beneficial effect. *Mult. Scler.* 21, 1723–1729.
- Volpe, E., Servant, N., Zollinger, R., Bogiatzi, S.I., Hupe, P., Barillot, E., et al., 2008. A critical function for transforming growth factor-beta, interleukin 23 and proinflammatory cytokines in driving and modulating human T(H)-17 responses. *Nat. Immunol.* 9, 650–657.
- Wan, Y.Y., Flavell, R.A., 2009. How diverse—CD4 effector T cells and their functions. *J. Mol. Cell Biol.* 1, 20–36.
- Wang, C., Yosef, N., Gaublomme, J., Wu, C., Lee, Y., Clish, C.B., et al., 2015. CD5L/AIM regulates lipid biosynthesis and restrains Th17 cell pathogenicity. *Cell* 163, 1413–1427.
- Wei, G., Wei, L., Zhu, J., Zang, C., Hu-Li, J., Yao, Z., et al., 2009. Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating CD4 + T cells. *Immunity* 30, 155–167.
- Weiss, J.M., Bilate, A.M., Gobert, M., Ding, Y., Curotto De Lafaille, M.A., Parkhurst, C.N., et al., 2012. Neuropilin 1 is expressed on thymus-derived natural regulatory T cells, but not mucosa-generated induced Foxp3 + T reg cells. *J. Exp. Med.* 209, 1723–1742. S1.
- Wildin, R.S., Ramsdell, F., Peake, J., Faravelli, F., Casanova, J.L., Buiist, N., et al., 2001. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat. Genet.* 27, 18–20.
- Wilhelm, C., Hirota, K., Stieglitz, B., Van Snick, J., Tolaini, M., Lahli, K., et al., 2011. An IL-9 fate reporter demonstrates the induction of an innate IL-9 response in lung inflammation. *Nat. Immunol.* 12, 1071–1077.
- Wing, J.B., Sakaguchi, S., 2012. Multiple treg suppressive modules and their adaptability. *Front. Immunol.* 3, 178.
- Wing, J.B., Ise, W., Kurosaki, T., Sakaguchi, S., 2014. Regulatory T cells control antigen-specific expansion of Tfh cell number and humoral immune responses via the coreceptor CTLA-4. *Immunity* 41, 1013–1025.
- Wing, K., Onishi, Y., Prieto-Martin, P., Yamaguchi, T., Miyara, M., Fehervari, Z., et al., 2008. CTLA-4 control over Foxp3 + regulatory T cell function. *Science* 322, 271–275.
- Wolk, K., Witte, E., Witte, K., Warszawska, K., Sabat, R., 2010. Biology of interleukin-22. *Semin. Immunopathol.* 32, 17–31.
- Wu, H.J., Ivanov, I.I., Darce, J., Hattori, K., Shima, T., Umesaki, Y., et al., 2010. Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. *Immunity* 32, 815–827.
- Wu, C., Yosef, N., Thalhamer, T., Zhu, C., Xiao, S., Kishi, Y., et al., 2013. Induction of pathogenic TH17 cells by inducible salt-sensing kinase SGK1. *Nature* 496, 513–517.
- Xiao, S., Yosef, N., Yang, J., Wang, Y., Zhou, L., Zhu, C., et al., 2014. Small-molecule RORgammat antagonists inhibit T helper 17 cell transcriptional network by divergent mechanisms. *Immunity* 40, 477–489.
- Yadav, M., Louvet, C., Davini, D., Gardner, J.M., Martinez-Llordella, M., Bailey-Bucktrout, S., et al., 2012. Neuropilin-1 distinguishes natural and inducible regulatory T cells among regulatory T cell subsets in vivo. *J. Exp. Med.* 209, 1713–1722. S1–19.
- Yamane, H., Paul, W.E., 2013. Early signaling events that underlie fate decisions of naive CD4(+) T cells toward distinct T-helper cell subsets. *Immunol. Rev.* 252, 12–23.
- Yang, X.O., Panopoulos, A.D., Nurieva, R., Chang, S.H., Wang, D., Watowich, S.S., et al., 2007. STAT3 regulates cytokine-mediated generation of inflammatory helper T cells. *J. Biol. Chem.* 282, 9358–9363.
- Yang, L., Anderson, D.E., Baecher-Allan, C., Hastings, W.D., Bettelli, E., Oukka, M., et al., 2008. IL-21 and TGF-beta are required for differentiation of human TH17 cells. *Nature* 454, 350–352.
- Yang, Y., Torchinsky, M.B., Gobert, M., Xiong, H., Xu, M., Linehan, J.L., et al., 2014. Focused specificity of intestinal TH17 cells towards commensal bacterial antigens. *Nature* 510, 152–156.

- Yeste, A., Mascanfroni, I.D., Nadeau, M., Burns, E.J., Tukpah, A.M., Santiago, A., et al., 2014. IL-21 induces IL-22 production in CD4 + T cells. *Nat. Commun.* 5, 3753.
- Yin, L., Yu, M., Edling, A.E., Kawczak, J.A., Mathisen, P.M., Nanavati, T., et al., 2001. Pre-emptive targeting of the epitope spreading cascade with genetically modified regulatory T cells during autoimmune demyelinating disease. *J. Immunol.* 167, 6105–6112.
- Yosef, N., Shalek, A.K., Gaublomme, J.T., Jin, H., Lee, Y., Awasthi, A., et al., 2013. Dynamic regulatory network controlling TH17 cell differentiation. *Nature* 496, 461–468.
- Zenewicz, L.A., Yancopoulos, G.D., Valenzuela, D.M., Murphy, A.J., Stevens, S., Flavell, R.A., 2008. Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease. *Immunity* 29, 947–957.
- Zhang, L., Li, Y.G., Li, Y.H., Qi, L., Liu, X.G., Yuan, C.Z., et al., 2012. Increased frequencies of Th22 cells as well as Th17 cells in the peripheral blood of patients with ankylosing spondylitis and rheumatoid arthritis. *PLoS One* 7, e31000.
- Zhang, X., Ing, S., Fraser, A., Chen, M., Khan, O., Zakem, J., et al., 2013. Follicular helper T cells: new insights into mechanisms of autoimmune diseases. *Ochsner J.* 13, 131–139.
- Zheng, W., Flavell, R.A., 1997. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell* 89, 587–596.
- Zheng, Y., Danilenko, D.M., Valdez, P., Kasman, I., Eastham-Anderson, J., Wu, J., et al., 2007. Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. *Nature* 445, 648–651.
- Zheng, Y., Chaudhry, A., Kas, A., Deroos, P., Kim, J.M., Chu, T.T., et al., 2009. Regulatory T-cell suppressor program co-opts transcription factor IRF4 to control T(H)2 responses. *Nature* 458, 351–356.
- Zheng, Y., Josefowicz, S., Chaudhry, A., Peng, X.P., Forbush, K., Rudensky, A.Y., 2010. Role of conserved non-coding DNA elements in the Foxp3 gene in regulatory T-cell fate. *Nature* 463, 808–812.
- Zhou, L., Littman, D.R., 2009. Transcriptional regulatory networks in Th17 cell differentiation. *Curr. Opin. Immunol.* 21, 146–152.
- Zhou, L., Ivanov, I.I., Spolski, R., Min, R., Shenderov, K., Egawa, T., et al., 2007. IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat. Immunol.* 8, 967–974.
- Zhou, L., Lopes, J.E., Chong, M.M., Ivanov, I.I., Min, R., Victora, G.D., et al., 2008. TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammat function. *Nature* 453, 236–240.
- Zhu, J., Min, B., Hu-Li, J., Watson, C.J., Grinberg, A., Wang, Q., et al., 2004. Conditional deletion of Gata3 shows its essential function in T(H)1-T(H)2 responses. *Nat. Immunol.* 5, 1157–1165.
- Zielinski, C.E., Mele, F., Aschenbrenner, D., Jarrossay, D., Ronchi, F., Gattorno, M., et al., 2012. Pathogen-induced human TH17 cells produce IFN-gamma or IL-10 and are regulated by IL-1beta. *Nature* 484, 514–518.

## Further Reading

- Kim, S.J., Schatzle, S., Ahmed, S.S., Haap, W., Jang, S.H., Gregersen, P.K., et al., 2017. Increased cathepsin S in Prdm1 – / – dendritic cells alters the TFH cell repertoire and contributes to lupus. *Nat. Immunol.* 18, 1016–1024.

# The Role of Invariant Natural Killer T Cells in Autoimmune Diseases

Gerhard Wingender<sup>1,2</sup> and Mitchell Kronenberg<sup>3</sup>

<sup>1</sup>Izmir International Biomedicine and Genome Institute, Balcova/Izmir, Turkey <sup>2</sup>Izmir Biomedicine and Genome Center (IBG), Dokuz Eylul University, Balcova/Izmir, Turkey <sup>3</sup>La Jolla Institute for Immunology (LJI), San Diego, CA, United States

## O U T L I N E

<b>The Curious Case of Invariant Natural Killer T Cells</b>	
The Many Names of Natural Killer T Cells	117
The Many Faces of Invariant Natural Killer T Cells	117
The Many Effector Functions of Invariant Natural Killer T Cells	118
The many kinds of Invariant Natural Killer T Cells	119
Technical Problems and the Species Divide	121
The Species Divide	123
<b>The Janus-Like Character of Invariant Natural Killer T Cells in Autoimmunity</b>	123
Too Much of a Good Thing: Detrimental Roles of Invariant Natural Killer T Cells	125
Missed So Sadly: Beneficial Roles of Invariant Natural Killer T Cells	130
Good or Bad Actors?	133
The Far End of the Question?	134
What Activates Invariant Natural Killer T Cells During Autoimmune Responses?	134
How Do Invariant Natural Killer T Cells Influence Autoimmune Responses?	135
Conclusion	136
Acknowledgments	136
Abbreviations	136
References	137

## THE CURIOUS CASE OF INVARIANT NATURAL KILLER T CELLS

### The Many Names of Natural Killer T Cells

Natural killer T (NKT) cells are a unique subset of T lymphocytes found in mice, humans, and other mammals that phenotypically and functionally resemble NK cells as well as T cells, and thereby exhibiting features of the innate as well as the adaptive immune system (Bendelac et al., 2007; Brennan et al., 2013; Cerundolo et al., 2009; Godfrey and Kronenberg, 2004; Godfrey et al., 2010; Kinjo and Kronenberg, 2005; Kronenberg, 2005; Kronenberg and Gapin, 2002; Salio et al., 2014; Tupin et al., 2007; Van Kaer et al., 2015; Yoshiga et al., 2011).

They were originally defined by coexpression of a T-cell antigen receptor (TCR) and NK cell receptors, especially NK1.1 (NKR-P1C) in certain mouse strains (Watanabe et al., 1995) and CD56 or CD161 (NKR-P1A) in

human (Doherty et al., 1999; Exley et al., 1998; Lanier et al., 1994). This classification, however, is an oversimplification, as conventional T cells can express NK receptors, especially after activation (Assarsson et al., 2000; Hammond et al., 1999; McMahon et al., 2002; Slifka et al., 2000) and the expression of NK markers by NKT cells varies with their maturity, activation state, and in mice, with the genetic background (Bendelac et al., 2007; Kronenberg, 2005). Although the term "NKT cell" is still sometimes used in this broad sense, nowadays it usually refers to T cells that express an  $\alpha\beta$  TCR that is reactive to antigens presented by CD1d, a nonpolymorphic major histocompatibility complex (MHC) class I-like antigen-presenting molecule. CD1d reactive  $\alpha\beta$  T cells are often divided into Type I and II NKT cells, based on the TCR they express (for reviews see: Bendelac et al., 2007; Brennan et al., 2013; Godfrey and Kronenberg, 2004; Godfrey et al., 2010; Kinjo and Kronenberg, 2005; Kronenberg, 2005; Salio et al., 2014; Tupin et al., 2007; Van Kaer et al., 2015).

Type I NKT cells are the largest and best-studied fraction of NKT cells and they carry a canonical V $\alpha$ 14 to J $\alpha$ 18 TCR rearrangement (V $\alpha$ 14*i*, *Trav11-Traj18*) in mice and an orthologous V $\alpha$ 24-J $\alpha$ 18 TCR chain (V $\alpha$ 24*i*, *TRAV10-TRAJ18*) in humans. This invariant TCR (*i*TCR)  $\alpha$  chain pairs predominantly with V $\beta$ 8, V $\beta$ 7, or V $\beta$ 2 in mice, and almost exclusively with V $\beta$ 11 in human, although these  $\beta$  chains have highly diverse rearrangements to J $\beta$  segments (Matsuda et al., 2001; Ronet et al., 2001). Considering the  $\beta$  chain variability, and the near complete absence of variability in the  $\alpha$  chain, in reality these cells have a semi-*i*TCR. However, they are typically referred to as invariant NKT (*i*NKT) cells, a designation we will use herein. The specificity of *i*NKT cells is remarkably conserved over at least 50 million years of evolution, since the divergence of hominids and rodents. In fact, there is a surprising degree of interspecies cross-reactivity, with mouse *i*NKT cells recognizing human CD1d and vice versa (Brossay and Kronenberg, 1999; Brossay et al., 1998; Kronenberg, 2005). This conservation suggests an important function for these cells in the mammalian immune system.

In contrast to *i*NKT cells, type II NKT cells, also termed variant NKT (*v*NKT) cells, encompass all other CD1d reactive T lymphocytes that utilize a more variable TCR. However, there is a degree of oligoclonality in these cells, with enrichment for some TCRs such as V $\alpha$ 3.2 and V $\alpha$ 8 (Park et al., 2001). The specificity of these cells is largely unknown, although some recognize sulfatide, a self-glycolipid antigen (Blomqvist et al., 2009; Jahng et al., 2004), and some phospholipids (Tatituri et al., 2013).

Sometimes, especially in older literature, a third group (type III) of NKT cells is listed. This group is more heterogeneous, as it subsumes all non-CD1d reactive T lymphocytes that express NK receptors (Kronenberg and Gapin, 2002; Taniguchi et al., 2003; Wingender et al., 2006). Furthermore, it should be noted that several other nonconventional T-cell subsets have been defined based on their TCR  $\alpha$  chain repertoire. Mucosal-associated invariant T (MAIT) cells express a fixed V $\alpha$ 19-J $\alpha$ 33 (*Trav1 Traj33*) TCR in mice, and a homologous V $\alpha$ 7.2-J $\alpha$ 33 (*TRAV1–2 TRAJ33*) in humans (Le Bourhis et al., 2010; Shimamura and Huang, 2002; Treiner et al., 2003). They are reactive to MR1, a highly conserved MHC class I homolog (Le Bourhis et al., 2011; Treiner et al., 2003). A third subset of cells utilizing a semi-*i*TCR is "Germline-Encoded, Mycolyl lipid-reactive" (GEM) T cells that express V $\alpha$ 7.2-J $\alpha$ 9 (*TRAV1–2 TRAJ9*) and are restricted by CD1b, which is found in humans but not in mice (Van Rhijn et al., 2013). Finally, V $\alpha$ 10 expressing NKT cells have been described in mice (Uldrich et al., 2011). These cells are also CD1d reactive and have a specificity similar to *i*NKT cells, although the complementarity determining regions (CDR3) of their  $\alpha$  chains are not invariant (Uldrich et al., 2011).

The focus of this review is exclusively on *i*NKT cells in rodents, nonhuman-primates, and humans and their roles in autoimmune disease pathogenesis and prevention.

## The Many Faces of Invariant Natural Killer T Cells

### **Phenotype**

Even as they differentiate in the thymus (for reviews see Constantinides and Bendelac, 2013; Engel and Kronenberg, 2014; Hu et al., 2011), *i*NKT cells begin to express a pattern of surface markers (CD69 $^+$ , CD44 $^{\text{high}}$ , CD11a $^{\text{high}}$ , CD62L $^{\text{low}}$ , CD122 $^+$ ) typically associated with activated or memory T cells (Bendelac et al., 1997; Godfrey et al., 2000; Kronenberg, 2005; Kronenberg and Gapin, 2002; Sprent and Surh, 2002). This effector/memory phenotype is also displayed by *i*NKT cells that are derived from umbilical cord blood (D'Andrea et al., 2000; van Der Vliet et al., 2000) or from germ-free animals (Park et al., 2000; Wingender et al., 2012b), and they maintain this pattern as mature cells.

In addition, these cells constitutively express for example detectable mRNA for IL-4 and IFN $\gamma$  (Matsuda et al., 2003; Stetson et al., 2003), although these transcripts increase dramatically following activation. The steady-state

transcripts have potential biologic effects. For example, the IL-4 produced by thymic *i*NKT cells contributes to the differentiation of a population of mature thymic CD8<sup>+</sup> T cells that is characterized by an activated or memory phenotype and by the expression of the transcription factor EOMES (Lee et al., 2013). Taken together, these data suggest that *i*NKT cells undergo a strong antigenic stimulation during their differentiation, consistent with the hypothesis that true TCR agonists mediate their positive selection (Kronenberg, 2005). However, two reports indicated that peripheral TCR-CD1d interactions aid the full maturation and reactivity of *i*NKT cells (McNab et al., 2005; Wingender et al., 2012b).

### **Distribution**

Vα14*i* NKT cells have the highest representation within the total T-cell population in the liver and the bone marrow (>10%), followed by significant numbers in thymus, peripheral blood, and spleen (1%–3%), whereas they are relatively rare in all other organs (<0.5%), including sites where conventional T lymphocytes are numerous, such as the lymph nodes and intestinal tissue (Bendelac et al., 2007; Kronenberg, 2005). Interestingly, in humans the frequency of *i*NKT cells is generally much lower, although a high degree of interindividual variability in peripheral blood mononuclear cell (PBMC) has been reported (Gumperz et al., 2002; Kita et al., 2002; Prussin and Foster, 1997; van der Vliet et al., 2001). Although the reason for this variability is unknown, nonetheless, the frequency of *i*NKT cells in the PBMC of an individual appears to be stable over time (Lee et al., 2002a; Wither et al., 2008). *i*NKT cells are also relatively infrequent, compared to mice, in human intrahepatic lymphocytes (Kenna et al., 2003; Kita et al., 2002), but evidence indicates that they are more abundant in adipose tissue (Lynch et al., 2009) and also to some extent in peritoneal fluid (Wingender et al., 2012a).

## The Many Effector Functions of Invariant Natural Killer T Cells

### **Activation**

*i*NKT cells recognize glycolipid antigens presented by CD1d (Bendelac, 1995a,b; Kawano et al., 1997; Zeng et al., 1997), and one of the best-studied antigens is the glycolipid α-galactosylceramide (αGalCer). αGalCer is a synthetic glycosphingolipid, a category of glycolipids that contains ceramide as the lipid portion. Glycosphingolipids are natural compounds found in many organisms, including mammals. αGalCer was originally purified from a marine sponge and was optimized by medicinal chemistry to yield an exceptionally strong agonist (Morita et al., 1995). αGalCer contains an unusual α-anomeric linkage of the sugar to the ceramide lipid, unlike mammalian glycosphingolipids (Morita et al., 1995), and this α-linkage is crucial for its great stimulatory capacity (Bendelac et al., 1997; Kawano et al., 1997). Numerous synthetic derivatives of αGalCer have been studied (Birkholz et al., 2015b; East et al., 2014), and natural antigens that can stimulate the majority of *i*NKT cells have been characterized as well from several bacteria, including glycosphingolipids from *Sphingomonas* spp. (Kinjo et al., 2005; Mattner et al., 2005), diacylglycerols from *Borrelia burgdorferi*, the causative agent of Lyme disease (Kinjo et al., 2006), diacylglycerols from *Streptococcus pneumoniae* and group B *Streptococcus* (Kinjo et al., 2011), cholesteryl α-glucosides from *Helicobacter pylori* (Chang et al., 2011; Ito et al., 2013), as well as asperamide B from the fungi *Aspergillus fumigatus* (Albacker et al., 2013; Godfrey et al., 2013). Antigens are found in pathogens such as *S. pneumoniae* and *B. burgdorferi*, environmental bacteria such as *Sphingomonas* sp., and in bacteria that are commensal organisms in the intestine such as *Bacteroides fragilis* (An et al., 2014; Wieland Brown et al., 2013). The biochemistry of lipid antigen binding to CD1d and recognition of the antigen CD1d complex by the TCR is very well understood as a result of biophysical and X-ray crystallographic studies. This topic has been reviewed elsewhere (Rossjohn et al., 2012; Zajonc and Girardi, 2015).

Activation of *i*NKT cells by TCR-mediated recognition of antigen bound to CD1d has been referred to as direct or antigen-dependent activation (Kinjo and Kronenberg, 2005; Parekh et al., 2005). It is known, however, that memory T cells can be activated by cytokines alone in a TCR-independent manner (Berg and Forman, 2006). Considering their constitutively activated phenotype, it is, therefore, not surprising that a similar cytokine-dependent activation was reported for *i*NKT cells as well (Nagarajan and Kronenberg, 2007; Tyznik et al., 2008). This so-called indirect or antigen-independent activation (Kinjo and Kronenberg, 2005; Parekh et al., 2005) can be induced by several proinflammatory cytokines, such as IFNα, IFNβ, IL-12, and IL-18, alone (Biron and Brossay, 2001; Leite-de-Moraes et al., 1999) or, more effectively, in combination (Dao et al., 1998; Holzapfel et al., 2014; Leite-de-Moraes et al., 1999, 2001; Ogasawara et al., 1998). Dendritic cells (DCs) and macrophages produce these

cytokines following engagement of Toll-like receptors or other stimuli for the innate immune system, like early after bacterial or viral infections.

One has to keep in mind, however, that the direct, TCR-driven and the indirect, cytokine-driven pathways of iNKT cell activation are not exclusive. The innate cell-derived cytokines IL-12 and IFN $\alpha/\beta$  have been reported to augment CD1d-dependent iNKT cell activation (Brigl et al., 2003; Mallevaey et al., 2006; Mattner et al., 2005; Paget et al., 2007), which was especially crucial for stimulation with presumably weak antigens.

Furthermore, in some cases, it has been shown that iNKT cells require the recognition of endogenous or self-ligands presented by CD1d for activation in a cytokine-dependent context (Brigl et al., 2003). This is exemplified by the activation of iNKT cells following *Salmonella* infection, which is not only dependent on IL-12 but also on CD1d (Brigl et al., 2003; Mallevaey et al., 2006; Mattner et al., 2005). As *Salmonella* is not known to express antigens that activate iNKT cells, these data demonstrate that self-antigens presented by CD1d acted in concert with IL-12 to activate iNKT cells. In line with the idea of recognition of “self” by iNKT cells is the observation that mature iNKT cells can under some circumstances display auto-reactivity (Hegde et al., 2010; Salio and Cerundolo, 2009). In addition, data with tumor-derived glycolipids suggest (Behar et al., 1999; Gumperz et al., 2000; Metelitsa et al., 2003; Metelitsa, 2011; Rauch et al., 2003; Wu et al., 2003) such self-antigens could provide an “altered-self” for iNKT cell activation.

The structure of such self-antigens important for the activation of mature iNKT cells is still debated, and there could be several types, but currently the best candidates are lysophospholipids (Fox et al., 2009), several types of glycosphingolipids including isoglobotrihexosylceramide (Zhou et al., 2004) and a still uncharacterized mammalian glycosphingolipid with an  $\alpha$ -linked sugar (Brennan et al., 2014; Kain et al., 2014). A lysophosphatidylethanolamine, with an ether bond or plasmalogen, has been suggested to be important for iNKT cell positive selection (Facciotti et al., 2012).

### Cytokine Production

In accordance with their constitutively activated phenotype is the remarkable ability of iNKT cells to rapidly (<1 hour) exert effector functions such as cytokine production and cytotoxicity (Wingender et al., 2010). After antigenic, TCR-driven activation, they rapidly produce copious amounts of various cytokines such as T helper type 1 (Th1, e.g., IFN $\gamma$ , TNF) and Th2 cytokines (e.g., IL-4, IL-5, IL-13), with subsets capable of producing IL-9, IL-10, IL-17A, and IL-22 (see the “The many kinds of invariant natural killer T cells” section). This explosive production of cytokines is more similar to innate immune cells or a memory T-cell response, as a conventional naïve T lymphocyte would take days of stimulation to produce these cytokines. In contrast to naïve T cells, iNKT cells are somewhat resistant to cytokine polarization (Matsuda et al., 2003) and the response toward a strong TCR agonist is a mixed IFN $\gamma$ /IL-4 or Th0-response (Sullivan et al., 2010).

Contrary to this manifold array of cytokines following TCR-driven activation, the indirect, cytokine-driven activation, for example, by IL-12 and IL-18, leads exclusively to the production of IFN $\gamma$  by the stimulated iNKT cells (Nagarajan and Kronenberg, 2007; Tyznik et al., 2008). Similarly, IL-1 $\beta$  and IL-23 stimulate the IL-17A producing subset to secrete IL-17A (Doisne et al., 2011; Moreira-Teixeira et al., 2011; Price et al., 2012) and some iNKT cells are stimulated by IL-25 to produce IL-13 (Terashima et al., 2008).

### Down-Stream Effects

Due to their cytokine production, iNKT cells have a pronounced impact on other lymphocytes, amplifying responses of DCs (Chen et al., 2005; Kitamura et al., 1999; Kojo et al., 2005), macrophages (Denney et al., 2012; Flesch et al., 1997), neutrophils (De Santo et al., 2010; Emoto et al., 2010; Li et al., 2007; Michel et al., 2007; Wintermeyer et al., 2009), NK cells (Eberl and MacDonald, 2000; Smyth et al., 2000a,b), and B and T cells. However, in some cases the interaction has been shown to be in part cell–cell contact dependent (Baev et al., 2008; Caielli et al., 2010; Novak et al., 2005; Yang et al., 2011).

Moreover, activated iNKT cells can skew or polarize the character of the entire immune response either toward a Th1- (Cui et al., 1999; Denkers et al., 1996; Gonzalez-Aseguinolaza et al., 2002; Kitamura et al., 1999) or a Th2- (Bendelac et al., 1996; Burdin et al., 1999; Hong et al., 2001; Sharif et al., 2001; Singh et al., 1999; Yoshimoto et al., 1995a, 1995b) direction. This can be promoted by the stimulating antigen utilized, with some synthetic glycolipids, such as C-glycoside (Schmieg et al., 2003), leading to a Th1 bias and others such as OCH (Miyamoto et al., 2001) and C:20 (Yu et al., 2005), leading to a Th2-bias (Birkholz et al., 2015; East et al., 2014). Interestingly, this bias is not reflected in the initial iNKT cell cytokine response, which remains Th0 (Sullivan et al., 2010), but instead reflects the interactions between networks of cells including the presentation of the antigen on antigen-presenting cells (APCs) (Arora et al., 2011; Im et al., 2009). For

example, Th1 skewing of the cytokine response reflects enhanced IFN $\gamma$  production by NK cells (Birkholz et al., 2015b; East et al., 2014).

Consequently, by orchestrating the ensuing immune response, with regard to its strength and properties, iNKT cells can act as a bridge between innate and adaptive immune responses. Therefore it is not surprising that iNKT cells have been reported to be crucially involved in the early phases of a dazzling variety of different immune reactions, ranging from self-tolerance and autoimmunity to include responses to pathogens and tumors.

## The many kinds of Invariant Natural Killer T Cells

There is increasing evidence that the functionally diverse roles of iNKT cells are partly due to functional iNKT cells subsets with distinct homing properties and cytokine profiles. Depending on the subset and the conditions, these may be preprogrammed in the thymus or induced in the periphery under the influence of particular stimulatory conditions. At first, iNKT cells subsets were defined by the expression of surface molecules, such as CD4 (Gumperz et al., 2002; Kim et al., 2002; Lee et al., 2002a), NK1.1 (McNab et al., 2007), IL17RB (Terashima et al., 2008), Ly49 receptors (Hammond et al., 1999; Skold and Cardell, 2000), and CD49b/DX5 (Hammond et al., 1999; Pellicci et al., 2005), among others.

However, with recent advances it is now feasible, at least in the mouse, to attempt to define some iNKT cell subsets based on transcription factor usage (Table 7.1): (1) NKT1 cells are  $Tbet^{high}$ , PLZF $^{low}$ , GATA3 $^{low}$ , and ROR $\gamma t^{neg}$  and are characterized by a Th1–cytokine pattern (IFN $\gamma$  > IL-4) (Lee et al., 2013). (2) NKT2 cells are PLZF $^{high}$ , GATA3 $^{high}$ ,  $Tbet^{low}$ , ROR $\gamma t^{low}$ , and produce cytokines with a Th2–cytokine pattern (IL-4 > IFN $\gamma$ ) (Lee et al., 2013). (3) NKT17 cells are ROR $\gamma t^{pos}$ , GATA3 $^{high}$ , PLZF $^{int}$ , and  $Tbet^{low}$  and are characterized by IL-17A production (Coquet et al., 2008; Doisne et al., 2009, 2011; Lee et al., 2013; Michel et al., 2007, 2008; Milpied et al., 2011; Pichavant et al., 2008). These subsets can be identified in the thymus and, therefore, may be considered preprogrammed, and their transcriptomes differ by hundreds of genes, despite their similar specificities (Engel et al., 2016; Georgiev et al., 2016; Lee et al., 2016). (4) NKT10 cells are defined by their production of IL-10. They too can be detected in the thymus, albeit, at very low frequency. However, NKT10 cells greatly expand after antigenic stimulation with Th1-biasing iNKT cell antigens in vivo, and they are long lived (Birkholz et al., 2015a; Lynch et al., 2015; Sag et al., 2014; Wingender et al., 2015a,b). It was suggested that NKT10 cells are characterized by the transcription factor E4BP4 (Lynch et al., 2015; Vieth et al., 2016). (5) NKT<sub>FH</sub> cells are induced by strong antigenic stimulation, are short-lived, and are characterized by the expression of the transcription factor Bcl6 and the surface markers CD185 (CXCR5) and CD279 (PD-1) (Chang et al., 2012; King et al., 2012; Toni et al., 2012). (6) FoxP3 $^+$  iNKT cells acquire FoxP3 expression following strong antigenic stimulation in the presence of high amounts of TGF $\beta$  (Moreira-Teixeira et al., 2011, 2012). Furthermore, iNKT cells capable of producing IL-9 (Kim and Chung, 2013; Monteiro et al., 2015) or IL-22 (Paget et al., 2012; Raifer et al., 2012) have been described, however, it is not clear to date if they constitute independent iNKT cell subsets.

In contrast, in humans iNKT cell subsets are not yet as well defined. However, expression of CD4 has been correlated with functional differences. Following antigenic stimulation the cytokine production of CD4 $^+$  V $\alpha$ 24*i* NKT cells follows a Th0-pattern, whereas CD4 $^{neg}$  iNKT cells are biased toward a Th1-pattern (Gumperz et al., 2002; Lee et al., 2002a). Furthermore, an IL-17 producing subset of human iNKT cells exists, but unlike in mice, it requires exposure to inflammatory cytokines as well as TCR activation for IL-17 production (Moreira-Teixeira et al., 2011).

Altogether, these data demonstrate that iNKT cells are heterogeneous. Because the prevalence of functional iNKT cell subsets may differ between inbred mouse strains or individuals, some of the current controversies regarding the roles of iNKT cells could depend on subset prevalence or selective activation of subsets under certain conditions.

## TECHNICAL PROBLEMS AND THE SPECIES DIVIDE

Several technical problems complicate the interpretation of the reported human data on iNKT cells and autoimmunity that we summarize below. Human iNKT cells can be identified by CD1d/ $\alpha$ GalCer tetramers, which may be the most inclusive and accurate method. A clonotype specific antibody that recognizes the CDR3 $\alpha$  region formed by the invariant V $\alpha$ 24-J $\alpha$ 18 rearrangement also is available (clone 6B11) (Montoya et al., 2007), but may miss some human  $\alpha$ GalCer and CD1d reactive cells that do not use V $\alpha$ 24 (Brigl et al., 2006; Constantinides et al.,

**TABLE 7.1** Features of CD1d Reactive NKT Cell Subsets

Name	Invariant ( <i>i</i> )NKT								Variable ( <i>v</i> )NKT	
Alternative names	V $\alpha$ 14 <i>i</i> NKT type I or classical NKT cells								V $\alpha$ 24 <i>i</i> NKT type I or classical NKT cells	
Species	Mouse								Human	
TCR repertoire	Invariant V $\alpha$ 14-J $\alpha$ 18 ( <i>Trav11-Traj18</i> )								Invariant V $\alpha$ 24-J $\alpha$ 18 ( <i>TRAV10-TRAJ18</i> )	
Antigens	$\alpha$ GalCer and others								$\alpha$ GalCer and others	
Positive selection	DP thymocytes								DP thymocytes	
Subset	NKT1	NKT2	NKT17	NKT10	NKT <sub>FH</sub>	FoxP3 <sup>+</sup>	CD4 <sup>pos</sup>	CD4 <sup>neg</sup>	Sulfatide-reactive	nd
Differentiation requirement: thymus	IL-15	Stronger TCR signal	nd	nd	—	—	nd	nd	nd	nd
Differentiation requirement: periphery	nd	nd	nd	Strong or repeated TCR trigger	Strong TCR trigger	Strong TCR trigger + TGF $\beta$	nd	nd	nd	nd
Defining transcription factor	Tbet <sup>high</sup> PLZF <sup>low</sup>	GATA3 <sup>high</sup> PLZF <sup>high</sup>	ROR $\gamma$ t <sup>pos</sup> PLZF <sup>int</sup>	E4BP4 <sup>pos?</sup>	Bcl6 <sup>pos</sup>	FoxP3 <sup>pos</sup>	nd	nd	nd	nd
Peripheral phenotype	CD4 or DN, NK1.1 <sup>+</sup> , CD122 <sup>+</sup> , CD27 <sup>+</sup>	CD4 <sup>+</sup> NK1.1 <sup>neg</sup> , CD122 <sup>neg</sup> CD27 <sup>+</sup>	DN NK1.1 <sup>neg</sup> CD122 <sup>neg</sup> CD27 <sup>+/−</sup> , CD103 <sup>+</sup> CD121a <sup>+</sup> , CD138 <sup>+</sup> , CD196 <sup>+</sup>	CD4 <sup>+</sup> CD49d <sup>+</sup> , CD127 <sup>+</sup> , CD185 <sup>+</sup> CD200 <sup>+</sup> , CD279 <sup>+</sup> , CD304 <sup>+</sup> , FR4 <sup>+</sup>	CD4 <sup>+</sup> , NK1.1 <sup>neg</sup> , CD127 <sup>low</sup> , CD185 <sup>+</sup> , CD279 <sup>+</sup>	CD4 <sup>+/−</sup> NK1.1 <sup>neg</sup> , CD25 <sup>+</sup> CD103 <sup>+</sup> , GITR <sup>+</sup>	CD4 <sup>+</sup> , CD56 <sup>+/−</sup> , CD161 <sup>+/−</sup>	DN or CD8 $\alpha\alpha^{+/-}$ , CD56 <sup>+/−</sup> CD161 <sup>+/−</sup>	CD4 <sup>+</sup> or DN, NK1.1 <sup>+</sup>	CD4 <sup>+</sup> or DN, NK1.1 <sup>+/−</sup>
Prominent location	Liver, thymus, spleen (red pulp), bone marrow	Liver, thymus, spleen (white pulp), lung	LN, skin, lung	Adipose tissue (long lived)	Spleen, LN (short-lived)	Intestine	Liver, thymus, spleen, bone marrow	Liver, thymus, spleen, bone marrow	Liver, spleen	Liver, spleen
Key cytokine/function	IFN $\gamma$ > IL-4	IL-4 > IFN $\gamma$	IL-17A	IL-10	IL-21	IL-10?, TGF $\beta$ ?	Th0/Th2	Th1	nd	nd

The table does not include the V $\alpha$ 10<sup>+</sup> NKT cells or other nonconventional T cells. *nd*, Not determined or not clear; *DP*, double positive; *TCR*, T-cell antigen receptor; *LN*, lymph node.

2011; Gadola et al., 2002; López-Sagasta et al., 2012). Although a large variability in the frequency of such V $\alpha$ 24<sup>neg</sup> CD1d/ $\alpha$ GalCer-reactive T cells was found within in vitro expanded cells lines (Brigl et al., 2006), their frequency in fresh PBMCs was estimated to be around  $1 \times 10^{-5}$  among total CD3<sup>+</sup> cells (Constantinides et al., 2011). Interestingly, the TCRs expressed by these cells are related to the canonical TCR, as they express V $\beta$ 11 and the predominant V $\alpha$ -segments they express (V $\alpha$ 3.1 and V $\alpha$ 10.1) are rearranged to the same J $\alpha$ 18 expressed by V $\alpha$ 24<sup>i</sup> NKT cells (Brigl et al., 2006; Constantinides et al., 2011; Gadola et al., 2002). The combination of V $\alpha$ 24 and V $\beta$ 11 antibodies also is sometimes used, but this might leave out some V $\alpha$ 24-negative *i*NKT cells, as well as including some non-*i*NKT cells. However, frequently less accurate methods to define *i*NKT cells are used, including (1) V $\alpha$ 24-specific antibodies alone, (2)  $\alpha\beta$ TCR and expression of one of several NK cell markers (e.g., CD161, CD56, CD16), (3) CD4<sup>-</sup>CD8<sup>-</sup> (DN)  $\alpha\beta$ T cells, and (4) RT-PCR for the *i*TCR, which does not allow for a direct assessment of cell frequency. Altogether, this means that different human studies are difficult to compare, as different populations may have been investigated. Here we will focus mainly on reports using CD1d/ $\alpha$ GalCer tetramers, the 6B11 antibody, or V $\alpha$ 24<sup>+</sup>V $\beta$ 11<sup>+</sup> double positive cells, which are now the standards. Furthermore, the depths of the characterization of *i*NKT cells in humans remains limited, especially regarding functional subsets, as analysis is usually confined to CD4 expression, one of the NK receptors, IFN $\gamma$  and IL-4. However, a “global” look at *i*NKT cells might miss important phenotypic and functional changes by a so far overlooked subset, which might be relevant in a particular disease. Moreover, most information about human *i*NKT cells is, for apparent reasons, derived from PBMCs. How human *i*NKT cells in peripheral blood relate to tissue *i*NKT cells is largely unknown. For mice it has been reported that *i*NKT cells in blood poorly reflect the cells in other tissues regarding frequency, phenotype, and function (Berzins et al., 2004). Furthermore, when the frequency or function of *i*NKT cells during autoimmune diseases was reported, this was usually done at a single time in an individual, rather than longitudinally, which could be misleading in cases where the importance of *i*NKT cells changes importance at different stages during disease progression. Finally, some problems were noted with one commonly used *i*NKT cell-deficient animals. The J $\alpha$ 18<sup>-/-</sup> (*Traj18*<sup>-/-</sup>) mouse line, originally described by Cui et al. (1999) (called J $\alpha$ 281<sup>-/-</sup> therein) and used since then by many researchers, carries a neomycin-resistance gene and it was shown that this gene suppresses a substantial amount of rearrangements leading to the absence of approximately 60% of the  $\alpha\beta$ TCR diversity in these mice (Bedel et al., 2012). This raised the question if the results obtained with this J $\alpha$ 18<sup>-/-</sup> mouse line were due to the absence of *i*NKT cells or the effects on the TCR repertoire in conventional T cells. Since then, four new J $\alpha$ 18<sup>-/-</sup> mouse lines with an undisturbed  $\alpha\beta$ TCR repertoire have been described (Chandra et al., 2015; Dashtsoodol et al., 2016; Ren et al., 2017; Zhang et al., 2016), and so far, the data obtained with those mice replicated earlier findings on the importance of *i*NKT cells.

## The Species Divide

Most of the work on *i*NKT cells has been done in mouse models. Apart from the quip that “mice are not humans,” and the obvious fact that the genetic background in human subjects is much more varied than in the few inbred mice strains commonly used, there are a few other aspects worth noting that complicate the translation of findings from mice to humans. A striking difference is in the relative distribution of *i*NKT cells in different organs. In mice the highest frequency of *i*NKT cells is found in liver, followed in descending order by adipose tissue, thymus/spleen/bone marrow/PBMCs, and lymph nodes. In humans, *i*NKT cells are abundant in adipose tissue, but not very abundant in liver/peritoneum and generally lower in frequency than in the commonly studied inbred mouse strains. In addition, in humans MAIT and type II NKT cells are more frequent than in mice and they contain additional nonconventional T lymphocytes reactive to CD1a, CD1b, and CD1c (Bendelac et al., 2007; Kronenberg, 2005; Kronenberg and Gapin, 2002). So, whereas in many cases the role of *i*NKT cells in animal models of autoimmunity is well established, controversy to varying degrees remains with regard to their role in human autoimmunity.

## THE JANUS-LIKE CHARACTER OF INVARIANT NATURAL KILLER T CELLS IN AUTOIMMUNITY

In line with the diversity of *i*NKT cell cytokine and effector responses, and their involvement in shaping immune responses, it is not surprising that *i*NKT cells have also been reported to participate in autoimmune disease pathogenesis (for earlier reviews on this topic see: Berzins and Ritchie, 2014; Berzins et al., 2011; Di Pietro

and Falcone, 2014; Novak and Lehuen, 2011; Simoni et al., 2012). Despite conflicting data, here we distinguish those autoimmune diseases in which *i*NKT cells mostly seem to promote pathogenesis in the majority of reports from those in which they tend to prevent it. Given the vast literature in the field, not all details can be described here, but rather we summarize the major findings, emphasizing the underlying mechanisms and focusing on recent developments.

**TABLE 7.2** Detrimental Involvement of Human *i*NKT Cells in Selected Autoimmune Diseases

Disease	<i>i</i> NKT cell frequency <sup>a</sup>	<i>i</i> NKT cell cytokines	Effects of treatment	Etc.
Atherosclerosis	<ul style="list-style-type: none"> <li>Decreased in PBMCs: Kyriakis et al. (2010)</li> <li>Present in the atherosclerotic plaques: Kyriakis et al. (2010)</li> </ul>	<i>i</i> NKT cell lines derived from atherosclerotic plaques were more sensitive to antigen stimulation than lines derived from PBMCs (Kyriakis et al., 2010)		CD1d expression is enhanced in the atherosclerotic plaques (Melián et al., 1999; Bobryshev and Lord, 2005; Chan et al., 2005; Kyriakis et al., 2010) [1]
Asthma	<ul style="list-style-type: none"> <li>Decreased in PBMCs?           <ul style="list-style-type: none"> <li>Yes: Ikegami et al. (2009), Sen et al. (2005), Koh et al. (2010), Yanming et al. (2012)</li> <li>No: Magnan et al. (2000), Akbari et al. (2006), Koh et al. (2010), Koh et al. (2012), Shim and Koh (2014)</li> </ul> </li> <li>Present in the BALF of asthmatic patients: Akbari et al. (2006), Pham-Thi et al. (2006), Thomas et al. (2006), Bratke et al. (2007), Heron et al. (2007), Mutualithas et al. (2007), Thomas et al. (2007), Vijayanand et al. (2007), Matangkasombut et al. (2009), Brooks et al. (2010)</li> <li>Present in the lung of asthmatic patients: Akbari et al. (2006), Vijayanand et al. (2007), Reynolds et al. (2009)</li> </ul>	<ul style="list-style-type: none"> <li>Th2-bias by <i>i</i>NKT cells from PBMCs?           <ul style="list-style-type: none"> <li>Yes: Yan-ming et al. (2012), Shim and Koh (2014)</li> <li>No: Sen et al. (2005).</li> </ul> </li> <li><i>i</i>NKT cells from BALF of asthmatic patients produced IL-4 and IL-13, but hardly any IFN<math>\gamma</math> (Akbari et al., 2006)</li> </ul>	<ul style="list-style-type: none"> <li>The frequency of <i>i</i>NKT cells in the lung increases with the severity of symptoms (Akbari et al., 2006; Hamzaoui et al., 2006; Matangkasombut et al., 2009; Reynolds et al., 2009; Koh and Shim, 2010)</li> <li><i>i</i>NKT cell antigenic content in the house dust directly correlated with asthma at age 7 (Chandra et al., 2018)</li> </ul>	
Colitis	<ul style="list-style-type: none"> <li>Decrease in PBMCs: van der Vliet et al. (2001), Grose et al. (2007b)</li> <li>Less Vo24 mRNA in the intestine of colitis patients: Grose et al. (2007b)</li> </ul>			
Dermatitis	<ul style="list-style-type: none"> <li>Decreased in PBMCs: Oishi et al. (2000), Takahashi et al. (2003), Ilhan et al. (2007), Gyimesi et al. (2011)[2]</li> </ul>	Skin <i>i</i> NKT cells in patient lesions produce IFN $\gamma$ and IL-4 (Gober et al., 2008; Simon et al., 2009; Kono et al., 2014)		

(Continued)

**TABLE 7.2** (Continued)

Disease	iNKT cell frequency <sup>a</sup>	iNKT cell cytokines	Effects of treatment	Etc.
	<ul style="list-style-type: none"> <li>Increased in the skin: Gober et al. (2008), Simon et al. (2009), Wu et al. (2010), Balato et al. (2012)</li> </ul>			
Primary biliary cirrhosis	<ul style="list-style-type: none"> <li>Increased in PBMCs and the liver: Kita et al. (2002) [3]</li> </ul>			Livers contain higher IFN $\gamma$ mRNA levels (Omenetti et al., 2009)
Psoriasis	<ul style="list-style-type: none"> <li>Decreased in PBMCs: van der Vliet et al. (2004)</li> <li>Increased in psoriatic plaques: Zhao et al. (2008) [4]</li> </ul>		IFN $\gamma$ pretreated keratinocytes upregulated CD1d and could induce IFN $\gamma$ , but not IL-4, production by iNKT cell lines (Bonish et al., 2000)	CD1d is upregulated in the inflamed skin (Bonish et al., 2000; Gober et al., 2008; Zhao et al., 2008)
Rheumatoid arthritis	<ul style="list-style-type: none"> <li>Decreased in PBMCs: van der Vliet et al. (2004), Kojo et al. (2001), Parietti et al. (2010), Tudhope et al. (2010), Mansour et al. (2015)</li> <li>Higher in synovium than PBMCs: Linsen et al. (2005), Gutowska et al. (2014), Zhao et al. (2018)</li> </ul>	Cytokine response of <ul style="list-style-type: none"> <li>PBMCs derived iNKT cell lines: less cytokines and/or Th1 bias (Kojo et al., 2001; Linsen et al., 2005; Gutowska-Owsiaik et al., 2014; Jin et al., 2015; Mansour et al., 2015)</li> <li>Synovial derived iNKT cell lines: no Th1 bias (Linsen et al., 2005)</li> <li>Synovial iNKT cells (ex vivo): less IFN<math>\gamma</math> (Zhao et al., 2018)</li> </ul>	iNKT cell frequency increased in PBMCs following $\alpha$ CD20 antibody (rituximab) treatment (Parietti et al., 2010, positive correlation with clinical outcome) and methotrexate therapy (Tudhope et al., 2010, no correlation with clinical outcome)	RA patients have decreased levels of soluble CD1d in the serum (Kojo et al., 2003; Segawa et al., 2009)
Systemic sclerosis	<ul style="list-style-type: none"> <li>Decreased in PBMCs: Kojo et al. (2001), van der Vliet et al. (2004)</li> </ul>	Th1 bias in iNKT cell lines derived from PBMCs (Kojo et al., 2001)		

<sup>a</sup>The table only lists reports in which iNKT cells were identified by CD1d/ $\alpha$ GalCer tetramers or 6B11 antibodies or the combination of Vo24 $^+$ V311 $^+$ . Comments: [1] NKT-cell like cells (CD3 $^+$ CD161 $^+$ ) were found in atherosclerotic plaques (Bobryshev and Lord, 2005; Chan et al., 2005). [2] Differing results in Magnan et al. (2000), Prell et al. (2003), and Wu et al. (2010). [3] NKT-like (CD3 $^+$ CD57 $^+$ ) cells accumulated in the bile ducts of PBC patients (Harada et al., 2003). [4] NKT-like cells (Vo24 $^+$  CD56/CD161 $^+$ ) were found in psoriatic plaques and their frequency directly correlated with the length of the rete ridge, a measure of the skin inflammation (Kono et al., 2014).

PBMC, Peripheral blood mononuclear cells; RA, rheumatoid arthritis.

## Too Much of a Good Thing: Detrimental Roles of Invariant Natural Killer T Cells

For diseases in this section, the data are most consistent with roles for iNKT cells in initiating and/or aggravating pathogenesis (Table 7.2).

### Atherosclerosis

Atherosclerosis is a chronic inflammatory disease within the vessels, driven by responses to accumulated lipids in the arterial intima. A contribution of the adaptive as well as the innate immune system, and an autoimmune component, have become apparent (earlier reviews on the role of iNKT cell in atherosclerosis: Braun et al., 2010b; Getz and Reardon, 2017; Grundtman and Wick, 2011; van Puijvelde and Kuiper, 2017).

In PBMCs of human patients, iNKT cell numbers were decreased (Kyriakakis et al., 2010). Furthermore, iNKT (Kyriakakis et al., 2010) and NKT-like cells (CD3 $^+$ CD161 $^+$ ) (Bobryshev and Lord, 2005; Chan et al., 2005) were found in atherosclerotic plaques, where they represented up to 3% of the lymphocytes. Of note, CD1d expression was enhanced in the atherosclerotic plaques (Bobryshev and Lord, 2000, 2005; Chan et al., 2005; Kyriakakis et al., 2010; Melián et al., 1999). iNKT cell lines derived from atherosclerotic plaques produced proinflammatory cytokines following  $\alpha$ GalCer stimulation and did this with an approximately 10-fold higher antigenic sensitivity than PBMC-derived iNKT cell lines (Kyriakakis et al., 2010).

In mouse models of atherosclerosis, iNKT cells also could be found in the atherosclerotic lesions (Aslanian et al., 2005; Nakai, 2004; Tupin et al., 2004), and data with reporter mice suggest that CD186 (CXCR6) signaling might be required for this accumulation (Galkina et al., 2007). Interestingly, when serum lipids from atherosclerosis-prone LDL-receptor deficient mice were fed to DCs, this led to a CD1d-dependent stimulation of iNKT cell hybridoma cells (Vanderlaan et al., 2007), and inhibition of CD1d presentation with DPPE-PEG<sub>350</sub> ameliorated the development of atherosclerosis in vivo (Li et al., 2016). iNKT cell-deficient mice developed a greatly ameliorated disease (Aslanian et al., 2005; Major et al., 2004; Nakai, 2004; Rogers et al., 2008; Ström et al., 2007; To et al., 2009; Tupin et al., 2004) and transfer of CD4<sup>+</sup> iNKT cells could transfer susceptibility (Li et al., 2015; To et al., 2009; Vanderlaan et al., 2007). The reduced plaque formation in iNKT cell-deficient animals correlated with an approximately 90% decrease in IFN $\gamma$  mRNA in the atherosclerotic lesions (Rogers et al., 2008). Consequently, activation of iNKT cells either by antigens (Major et al., 2004; Nakai, 2004; Tupin et al., 2004) or with lipopolysaccharide (LPS) (Andoh et al., 2013; Ostos et al., 2002) aggravated disease, and increased iNKT cell numbers within the atherosclerotic lesions (Nakai, 2004; Tupin et al., 2004). This is in line with the observation that microbial infections enhance the development of atherosclerosis in both human and animal models (Hansson et al., 2006). Importantly, the proatherosclerotic effect of the transferred CD4<sup>+</sup> iNKT cells was independent of T-, B-, and NK cells, as well as independent of IL-4, IL-21, and IFN $\gamma$  (Li et al., 2015), rather the expression of cytotoxic proteins (granzyme B, perforin) by the CD4<sup>+</sup> iNKT cells was required for the proatherosclerotic effect (Li et al., 2015). A similar mechanism has been suggested for NK cells (Selathurai et al., 2014) and CD8<sup>+</sup> T cells (Kyaw et al., 2013). Nonetheless, one study reported a protective effect of iNKT cell stimulation on atherosclerosis (van Puijvelde et al., 2009). Finally, it is important to keep in mind that several proteins involved in lipid metabolism have been shown also to be involved in CD1d lipid antigen loading, including apoE (Elzen et al., 2005), sphingolipid activator protein (Zhou et al., 2004), and microsomal triglyceride transfer protein (Brozovic et al., 2004). For example, apoE can bind  $\alpha$ GalCer and aid in its uptake (Allan et al., 2009; Elzen et al., 2005), and two studies suggested that iNKT cells in apoE-deficient mice were hypo-responsive (Braun et al., 2010a; Soh et al., 2016).

### Asthma

Asthma is multifactorial disease that could in some cases have an autoimmune component. Several studies have implicated V $\alpha$ 24i NKT cells in the pathogenesis of asthma in humans (earlier iNKT cell reviews see: DeKruyff et al., 2014; Rijavec et al., 2011; Thomas et al., 2010; Umetsu and DeKruyff, 2010). A decreased frequency in PBMCs from asthmatic patients, at least in the CD4<sup>+</sup> subset of iNKT cells, has been reported by some (Ikegami et al., 2009; Koh et al., 2010; Yan-ming et al., 2012), but not by others (Akbari et al., 2006; Antunes et al., 2018; Koh and Shim, 2010; Koh et al., 2012; Magnan et al., 2000; Shim and Koh, 2014). Interestingly, upper respiratory tract infections, which often exacerbates asthma, were correlated with a decrease of iNKT cells in PBMC (Koh et al., 2012), suggesting potential recruitment to the lung. In line with this notion, iNKT cells are almost undetectable in the airways of healthy controls, either in lung biopsies (Akbari et al., 2006; Reynolds et al., 2009; Vijayanand et al., 2007) or in the bronchoalveolar lavage fluid (BALF) (Akbari et al., 2006; Matangkasombut et al., 2009; Thomas et al., 2007) but can be detected in samples from asthmatic patients. However, controversy remains about the magnitude of this increase within the lungs of asthmatics. For BALF only two studies reported a frequency of more than 2.5% of iNKT cells within total T cells (Akbari et al., 2006; Matangkasombut et al., 2009), with most studies reporting frequencies between 0.6% and 2.5% (Bratke et al., 2007; Brooks et al., 2010; Heron et al., 2007; Matangkasombut et al., 2009; Mutualithas et al., 2007; Pham-Thi et al., 2006; Thomas et al., 2007, 2006; Vijayanand et al., 2007). For lung biopsies the values ranged from 1.7% (Vijayanand et al., 2007) to 9.8% (Reynolds et al., 2009) to over 50% (Akbari et al., 2006). Importantly, it was noted that the frequency of iNKT cells in the lung increases with the severity of symptoms (Akbari et al., 2006; Hamzaoui et al., 2006; Koh and Shim, 2010; Matangkasombut et al., 2009; Reynolds et al., 2009). A prospective cohort study did not find a correlation between the iNKT cell frequency in PBMCs of children at age 1 and asthma at age 7 (Chandra et al., 2018). However, a correlation with the iNKT cell antigenic content in the house dust was noted. Young children growing up in homes with more iNKT cell antigens were less likely to develop asthma (Chandra et al., 2018), perhaps reflecting increased microbial load in those homes and consistent with the hygiene hypothesis. Most forms of asthma are associated with Th2 cytokine profile, but conflicting data were reported regarding the presence of a Th2 skewed cytokine production by restimulated iNKT cells from PBMC of asthma patients (Th2-bias? Yes: Shim and Koh, 2014; Yan-ming et al., 2012, no: Sen et al., 2005). However, antigen-stimulated iNKT cells from BALF of asthmatic patients produced IL-4 and IL-13, but hardly any IFN $\gamma$  (Akbari et al., 2006), in line with the hypothesized Th2-bias.

In mouse airway inflammation models, the evidence is strong for a major pathogenic role for *i*NKT cells in airway hypersensitivity. The potency of *i*NKT cells in airway inflammation is illustrated by the fact that airway challenge with *i*NKT cell glycolipid antigens alone, such as  $\alpha$ GalCer, induces many of the pathological features of asthma, even in MHC class II-deficient mice that lack conventional CD4 $^{+}$  T cells (Meyer et al., 2006). This suggests that asthma might be induced directly by airway exposures to *i*NKT cell antigens present in inspired air. Clearly, weak environmental *i*NKT cell antigens found in house dust could act as potent adjuvants in the sensitization to a model antigen (Wingender et al., 2011). Even in the absence of known *i*NKT cells antigens, several groups have found that *i*NKT cells are required in mouse models of asthma initiated by ovalbumin sensitization (Akbari et al., 2003; Kim et al., 2009, 2004; Lisbonne et al., 2003), ragweed (Bilenki et al., 2004), or ozone (Pichavant et al., 2008). This is best illustrated by the clearly reduced airway inflammation in *i*NKT cell-deficient animals in several models, and the restoration of susceptibility by *i*NKT cell transfer (Akbari et al., 2003; Cui et al., 1999; He et al., 2017; Kim et al., 2012; Lisbonne et al., 2003; Nie et al., 2015; Pichavant et al., 2008; Scheuplein et al., 2015; Wingender et al., 2011). In the allergen-induced model of airway hypersensitivity, secretion of IL-4 and IL-13 by *i*NKT cells has been implicated (Akbari et al., 2003; Meyer et al., 2006; Terashima et al., 2008), while the ozone-induced model depended upon IL-17A as well as IL-4 and IL-13 (Pichavant et al., 2008). Germ-free mice were reported to have an increased frequency of lung *i*NKT cells and enhanced asthma susceptibility, and both could be reversed by early life exposure to normal microbiota (Olszak et al., 2012). Furthermore, antigenic stimulation of lung *i*NKT cells early in life (2 weeks of age) expanded CD38 $^{+}$  *i*NKT cells that predominantly produced IFN $\gamma$  and were protective against airway hypersensitivity (Chang et al., 2011; Chuang et al., 2018). This suggests that different *i*NKT cell subsets play opposing roles during asthma.

Three studies in macaques were in line with the findings in mouse models in showing that a glycolipid antigen for *i*NKT cells could induce hypersensitivity and that an allergen challenge could lead to *i*NKT cell infiltration into the lung (Ayanoglu et al., 2011; Matangkasombut et al., 2008; Nambiar et al., 2015).

### **Inflammatory Bowel Disease/Colitis**

Inflammatory bowel disease (IBD) denotes a spectrum of chronic disorders of different parts of the gastrointestinal tract. The inflammatory response is triggered by antigens derived from the gut lumen, which may follow a perturbation of the epithelial layer, caused either through genetic alterations or the administration of exogenous agents. Several reviews on the role of *i*NKT cells in colitis models have been published (Liao et al., 2013; Wingender and Kronenberg, 2008; Zeissig and Blumberg, 2014). Some types of intestinal bacteria are probiotic, in that they prevent inflammation of the intestine. It is therefore of note that *i*NKT cells can influence the microbial colonization and the composition of intestinal bacteria (Nieuwenhuis et al., 2009). Furthermore, the activation state of *i*NKT cells, and their influence on the mucosal immune response, is highly altered in germ-free mice (Olszak et al., 2012; Wingender et al., 2012b).

Ulcerative colitis is an inflammation of the superficial layers of the colon that has been attributed in some studies to an exaggerated Th2 response. *i*NKT cells were decreased in the PBMCs of ulcerative colitis patients (Grose et al., 2007b; van der Vliet et al., 2001). In addition, there was a reduction in V $\alpha$ 24 mRNA in the intestine of colitis patients (Grose et al., 2007b), but the majority of these V $\alpha$ 24 $^{+}$  cells might not be *i*NKT cells, as judged by V $\beta$ 11 expression (O'Keeffe et al., 2004). Interestingly, in ulcerative colitis patients, it was found that *v*NKT cells, rather than *i*NKT cells, were the major CD1d-dependent producers of the deleterious IL-13 (Fuss et al., 2004). This is in contrast to results obtained using the oxazolone-induced mouse model of colitis, where it was shown that the pathogenic IL-13 was derived from *i*NKT cells (Heller et al., 2002). In this model, *i*NKT cells from lamina propria had increased CD199/CCR9 expression and chemotaxis to CCL25, which mediate intestinal homing (Zhu et al., 2014). Furthermore, interfering with *i*NKT cell activation prevented disease development (Brozovic et al., 2004; Camelo et al., 2012; Heller et al., 2002; Rosen et al., 2013; Schiechl et al., 2011).

### **Primary Biliary Cirrhosis**

Primary biliary cirrhosis (PBC) is characterized by portal inflammation and immune-mediated chronic destruction of intrahepatic small bile duct epithelial cells (i.e., cholangitis) (for earlier *i*NKT cell reviews see: Camelo et al., 2012; Mattner, 2013; Uibo et al., 2012).

*i*NKT cells are increased in PBMCs as well as in the liver of PBC patients (Kita et al., 2002), and these livers contain higher IFN $\gamma$  levels (Omenetti et al., 2009). The increase of *i*NKT cells in the liver coincided with an increase of CD1d expression levels on epithelial cells of the small bile duct of PBC patients in most studies (yes: Kita et al., 2002; Tsuneyama et al., 1998; no: Schrumpf et al., 2015). The cross-reactivity of antibodies to several

bacteria with the lipidated mitochondrial protein PDC-E2 has been implicated in the genesis of PBC (Bogdanos and Vergani, 2009; Bogdanos et al., 2004; Padgett et al., 2005; Selmi et al., 2003; Yanagisawa et al., 2011).

One bacterium in particular that is the target of these cross-reactive antibodies from PBC patients is *Novosphingobium aromaticivorans* (Olafsson et al., 2004; Padgett et al., 2005; Selmi et al., 2003). This bacterium is of interest because of its relation to Sphingomonas bacteria, which bear known iNKT cell antigens (Kinjo et al., 2005; Mattner et al., 2005). Most importantly, *N. aromaticivorans* can induce a PBC-like disease in a mouse model in an iNKT cell-dependent fashion (Mattner et al., 2008; Mohammed et al., 2011), although other bacteria, without known iNKT cell antigen, could cause inflammation as well (Wang et al., 2014). In line with a crucial role for iNKT cells in initiating PBC-like disease in mice is the observation that stimulation of iNKT cells can exacerbate disease (Chang et al., 2015; Chuang et al., 2008; Wu et al., 2011), whereas in iNKT cell-deficient mice disease was ameliorated (Berntsen et al., 2018; Chang et al., 2015).

### Rheumatoid Arthritis

In rheumatoid arthritis (RA) auto-reactive Th1 and Th17 T cells cause inflammation of small and large synovial joints, leading to joint deformation and loss of movement (for earlier iNKT cell reviews see: Chen et al., 2016; Drennan et al., 2010; Sowden and Ng, 2012).

Human RA patients display reduced iNKT cell frequencies in PBMCs (Kojo et al., 2001; Mansour et al., 2015; Parietti et al., 2010; Tudhope et al., 2010; van der Vliet et al., 2004). Furthermore, iNKT cells were detected in the rheumatoid synovium (Gutowska-Owsiaik et al., 2014; Linsen et al., 2005; Maeda et al., 1999; Zhao et al., 2018), with two studies showing an increased frequency in the synovium compared to the PBMCs (Gutowska-Owsiaik et al., 2014; Zhao et al., 2018). Although the iNKT cell frequency in PBMCs did not correlate with clinical disease severity in most studies (no: Parietti et al., 2010; Tudhope et al., 2010; yes: Mansour et al., 2015), it was noted that the frequency increased following treatment (Parietti et al., 2010; Tudhope et al., 2010). iNKT cell lines derived from RA patient's PBMCs expanded less in vitro following  $\alpha$ GalCer stimulation (Jin et al., 2015; Kojo et al., 2001; Linsen et al., 2005; Mansour et al., 2015; Tudhope et al., 2010) and produced reduced cytokines, although with a Th1 bias (Gutowska-Owsiaik et al., 2014; Jin et al., 2015; Kojo et al., 2001; Linsen et al., 2005; Mansour et al., 2015), when compared to control lines. In contrast to PBMCs, no impairment of iNKT cell expansion and function was observed in synovial derived iNKT cell lines (Linsen et al., 2005). However, when synovial iNKT cells from RA patients were analyzed directly ex vivo they produced less IFN $\gamma$  than the iNKT cells from matched PBMCs (Zhao et al., 2018). Interestingly, the iNKT cell lines derived from patient's PBMCs were particularly depleted of iNKT cells with a high-affinity TCR, and those that remained lost their Th0-cytokine pattern in favor for a Th1- or Th2 pattern (Mansour et al., 2015). Furthermore, even though the expression levels of CD1d on PBMCs might not be altered (no change: Kojo et al., 2003; Mansour et al., 2015; higher on DCs: Jacques et al., 2010), it was reported that RA patients have decreased levels of soluble CD1d in the serum (Kojo et al., 2003; Segawa et al., 2009).

Without delineating the details of the different mouse models for RA that have been studied, the role of iNKT cells appears to depend on the dominant iNKT cell subset: NKT1 cells being protective and NKT17 cells being pathogenic. The frequency of iNKT cells increased with disease progression (Chiba et al., 2005; Postigo et al., 2012; Zhao et al., 2018), they upregulated activation markers (Jung et al., 2009; Kim et al., 2006; Miellot-Gafsou et al., 2010; Zhao et al., 2018), and they were recruited to the joints (Kim et al., 2005; Park et al., 2010; Zhao et al., 2018), where they could be activated directly by aggregated antibodies (Kim et al., 2005). Furthermore, the percentage of IL-17A $^+$  NKT17 cells increased with disease progression (Jung et al., 2009; Zhao et al., 2018) with a concomitant decrease of IFN $\gamma$  + NKT1 cells (Zhao et al., 2018). Injection of blocking  $\alpha$ CD1d-antibodies ameliorated arthritis (Chiba et al., 2005; Miellot-Gafsou et al., 2010) and in iNKT cell-deficient mice the disease was less severe ( $\text{J}\alpha 18^{-/-}$ : Chiba et al., 2005; Ohnishi et al., 2005; Yoshiga et al., 2008; CD1d $^{-/-}$ : Jung et al., 2009; Kim et al., 2006, 2005; Ohnishi et al., 2005; Teige et al., 2010; Zhao et al., 2018). Such diseased iNKT cell-deficient animals had higher IL-10 production by collagen-specific T cells (Chiba et al., 2005; Jung et al., 2009) and the levels of proinflammatory cytokines tended to be lower (Chiba et al., 2005; Jung et al., 2009; Ohnishi et al., 2005; Yoshiga et al., 2008). In addition, they had reduced antigen-specific antibody titers (Jung et al., 2009; Ohnishi et al., 2005), with an elevated IgG1/IgG2a ratio (Chiba et al., 2005), suggesting a Th2 deviation. Furthermore, injection of iNKT cell antigens generally ameliorated disease (Chiba et al., 2004; Coppieters et al., 2007; Jin et al., 2015; Kaieda et al., 2007; Miellot et al., 2005; Oleinika et al., 2018; Takagi et al., 2006; Yoshiga et al., 2008, 2011; Zhao et al., 2018). The disease amelioration could be attributed to the impairment of the pathogenic Th17 response, rather than to a Th2 cytokine deviation (Chiba et al., 2004; Jung et al., 2009; Kaieda et al., 2007; Yoshiga et al., 2011). In line with this, several studies showed that IFN $\gamma$  is protective in different RA mouse models

(Coppieeters et al., 2007; Jin et al., 2015; Kaieda et al., 2007; Kim et al., 2005; Oleinika et al., 2018; Yoshiga et al., 2011; Zhao et al., 2018). Although IL-4 and/or IL-10 were implicated as well by some studies (Chiba et al., 2004; Kaieda et al., 2007; Kim et al., 2005; Miellot et al., 2005), one study reported a pathogenic role of IFN $\gamma$  (Teige et al., 2010). Two studies reported on a required interaction of iNKT cells with other cells for the protective effect of iNKT cells. Both studies showed that CD1d expression by DCs (Jung et al., 2010) or Bregs (Oleinika et al., 2018) is necessary to ameliorate arthritis. Importantly, one study showed that the Th1-biasing iNKT cell antigen 7DW8-5 was therapeutically active and could ameliorate arthritis after the onset of disease (Zhao et al., 2018). This could not be achieved with  $\alpha$ GalCer, which, although highly potent, is a less Th1-biasing antigen (Zhao et al., 2018). Intriguingly, it has been suggested that V $\alpha$ 14i NKT cells might recognize a mouse collagen II-derived peptide (mCII<sub>707–721</sub>) in a CD1d-dependent manner (Liu et al., 2011). This report is one of the very few that indicate that peptides, in addition to lipids, can be presented by CD1d (Castano et al., 1995; Girardi et al., 2016; Lee et al., 1998; Tangri et al., 1998; Zeng et al., 1997). Further studies will be required to understand the mechanism of peptide binding into the hydrophobic CD1d groove and the relevance of such peptides for iNKT cell function.

### **Skin Disorders**

Psoriasis is a local inflammation of the skin driven by a mixed Th1/Th17 T cell response (Lowes et al., 2008; Zaba et al., 2009) leading to abnormal proliferation and differentiation of keratinocytes (for earlier iNKT cell reviews see Balato et al., 2009; Peternel and Kastelan, 2009; Tobin et al., 2011). One report showed a decreased frequency of iNKT cells in PBMCs from psoriasis patients (van der Vliet et al., 2001). For the skin, there are several reports of T cells expressing NK cell markers in the psoriatic skin lesions (Bonish et al., 2000; Cameron et al., 2002; Curry et al., 2003; Gilhar et al., 2002; Koreck et al., 2002; Langewouters et al., 2007; Nickoloff, 2000), but only two studies reported an increase of NKT-like ( $V\alpha 24^+ CD56/CD161^+$ ) (Kono et al., 2014) or iNKT cells (Zhao et al., 2008) in psoriatic plaques. Interestingly, the frequency of these NKT-like cells directly correlated with the length of the rete ridge, a measure of the skin inflammation, and they produced more IFN $\gamma$  than IL-4 (Kono et al., 2014). Furthermore, CD1d expression was upregulated in the inflamed skin (Bonish et al., 2000; Gober et al., 2008; Zhao et al., 2008). Specifically, IFN $\gamma$  pretreated keratinocytes up-regulate CD1d and could induce IFN $\gamma$ , but not IL-4, production by iNKT cell lines (Bonish et al., 2000).

In systemic sclerosis iNKT cells from patient's PBMCs have been reported to be decreased (Kojo et al., 2001) and to display a Th1-bias (Kojo et al., 2001).

Similarly, in contact dermatitis iNKT cells from patient's PBMCs, especially the DN subset, were decreased in most (Gyimesi et al., 2011; Ilhan et al., 2007; Oishi et al., 2000; Takahashi et al., 2003), but not all (Magnan et al., 2000; Prell et al., 2003; Wu et al., 2010) reports. Whereas skin from control subjects did not contain detectable iNKT cells, they could be found in patient skin biopsies (Balato et al., 2012; Gober et al., 2008; Simon et al., 2009; Wu et al., 2010), consistent with a possible migration from the blood to the site of inflammation. iNKT cells, all CD4 $^+$ , were found within the dermal infiltrating cells and made up 6%–9% of the T cells, although none were found in the epidermis (Gober et al., 2008; Simon et al., 2009). After the stimulation of PBMC iNKT cells, two reports noted a Th2-bias (IL-4 > IFN $\gamma$ ) of the cytokine response in patient iNKT cells (Gyimesi et al., 2011; Takahashi et al., 2003), whereas one report observed a Th1 bias (Oishi et al., 2000). Furthermore, production of IFN $\gamma$  and IL-4 by iNKT cells was also detected within the affected skin of patients (Gober et al., 2008; Kono et al., 2014; Simon et al., 2009). Interestingly, thymic stromal lymphopoietin (TSLP), a cytokine that is produced by activated keratinocytes (Soumelis et al., 2002) and is elevated in patient lesions (Nakamura et al., 2008; Wu et al., 2010), can bias cytokine production by activated iNKT cells toward a Th2 pattern (IL-4, IL-13) (Jariwala et al., 2011; Wu et al., 2010).

In animal models of contact hypersensitivity (CHS), the majority of studies demonstrated a pathogenic role for iNKT cells (Askenase et al., 2011; Campos et al., 2003, 2006a,b; Curzytek et al., 2015; Dey et al., 2011; Eguchi et al., 2013; Nieuwenhuis et al., 2005; Shigematsu et al., 2014; Shimizuhira et al., 2014). However, two reports each suggested a beneficial (Fjelbye et al., 2015; Goubier et al., 2013) or no (Elkhal et al., 2006; Majewska-Szczepanik et al., 2013) role. This discrepancy is potentially due to differences in the protocols utilized, the genetic background, or the microbiota of the mice studied. iNKT cells accumulated in the affected skin (Campos et al., 2006a; Eguchi et al., 2013; Shigematsu et al., 2014) and the draining lymph node (Shimizuhira et al., 2014) in some, but not all (Goubier et al., 2013; Nieuwenhuis et al., 2005), studies. Interestingly, topical application of a CD1d-binding nonstimulatory glycolipid reduced the severity of the CHS response, likely by blocking the presentation of antigenic lipids by CD1d (Nieuwenhuis et al., 2005). The pathogenic role of iNKT cells was attributed to their rapid production of IL-4 (Campos et al., 2003, 2006b).

**TABLE 7.3** Beneficial Involvement of Human iNKT Cells in Selected Autoimmune Diseases

Disease	iNKT cell frequency <sup>a</sup>	iNKT cell cytokines	Effects of treatment	Etc.
Diabetes mellitus	Frequency in PBMCs: <ul style="list-style-type: none"> <li>Decreased: Wilson et al. (1998), Kukreja et al. (2002), Kis et al. (2007), Montoya et al. (2007)</li> <li>Unchanged: Lee et al. (2002b), Michalek et al. (2006), Tsutsumi et al. (2006), Oling et al. (2007), Zhang et al. (2011), Beristain-Covarrubias (2015), Tocheva et al. (2017)</li> <li>Increased: Oikawa et al. (2002)</li> </ul>	<ul style="list-style-type: none"> <li>The cytokine production from PBMC-derived iNKT cell (lines) was: <ul style="list-style-type: none"> <li>Less and/or Th1 bias in Wilson et al. (1998), Wilson et al. (2000), Kukreja et al. (2002), Kis et al. (2007), Li et al. (2014), Beristain-Covarrubias (2017)</li> <li>No difference: Lee et al. (2002b), Roman-Gonzalez et al. (2009), Tocheva et al. (2017)</li> </ul> </li> <li>Less IL-4 production by pancreatic lymph node derived iNKT cell lines (Kent et al., 2005)</li> </ul>		
Crohn's disease	<ul style="list-style-type: none"> <li>Decreased in PBMCs:</li> <li>van der Vliet et al. (2001), Grose et al. (2007b)</li> <li>Vα24 mRNA and the numbers of Vα24<sup>+</sup> T cells are reduced in the intestine: Grose et al. (2007b)</li> </ul>			
Multiple sclerosis	Frequency in PBMCs: <ul style="list-style-type: none"> <li>Decreased: Illés et al. (2000), van der Vliet et al. (2001), Araki et al. (2003), Demoulin et al. (2003)</li> <li>Unchanged: Gigli et al. (2007), O'Keeffe et al. (2008), De Biasi et al. (2016)</li> </ul>	The cytokine production from PBMC derived <ul style="list-style-type: none"> <li>iNKT cell lines displayed: <ul style="list-style-type: none"> <li>A Th2-bias: Araki et al. (2003), O'Keeffe et al. (2008).</li> <li>No difference: Gigli et al. (2007).</li> <li>A Th1/17-bias: Gausling et al. (2001)</li> </ul> </li> <li>iNKT cells (ex vivo) displayed a Th1/17-bias: De Biasi et al. (2016)</li> </ul>	iNKT cell frequency increased in PBMCs from patients under treatment (IFNβ, Gigli et al., 2007) or patients in remission (Araki et al., 2003)	The frequency of iNKT cells in PBMCs was especially decreased in patients under remission (Illés et al., 2000; Araki et al., 2003)
Systemic lupus erythematosus	<ul style="list-style-type: none"> <li>Decreased in PBMCs: Sumida et al. (1995, 1998), Kojo et al. (2001), van der Vliet et al. (2001), Wither et al. (2008), Wong et al. (2009), Parietti et al. (2010), Cho et al. (2011), Yu and Wang (2011), Bosma et al. (2012), Cho et al. (2014), Shen et al. (2015), Smith et al. (2016)</li> </ul>	iNKT cell lines (Kojo et al., 2001; Cho et al., 2011; Yu and Wang, 2011; Bosma et al., 2012; Shen et al., 2015) or fresh iNKT cells from PBMCs (Bosma et al., 2012; Shen et al., 2015) produced less cytokines and/or displayed a Th1-bias	The frequency of iNKT cells in PBMCs increased following therapy (Oishi et al., 2001; Bosma et al., 2012)	The frequency of iNKT cells in PBMCs correlated with <ol style="list-style-type: none"> <li>Disease severity (Parietti et al., 2010; Cho et al., 2011) [1]</li> <li>Serum levels of auto-reactive IgGs (Green et al., 2007; Wither et al., 2008)</li> </ol>

<sup>a</sup>The table only lists reports in which iNKT cells were identified by CD1d/αGalCer tetramers or 6B11 antibodies or the combination of Vα24<sup>+</sup>Vβ11<sup>+</sup>. Comments: [1] Differing results by Wither et al. (2008).

PBMC, Peripheral blood mononuclear cells.

## Missed So Sadly: Beneficial Roles of Invariant Natural Killer T Cells

Here we outline cases where the current consensus points to a beneficial role of iNKT cells in preventing disease development (Table 7.3).

### Type 1 Diabetes

Type 1 diabetes (T1D) mellitus, also called insulin-dependent diabetes mellitus, is a Th1 autoimmune disease caused by the selective destruction of insulin-producing  $\beta$ -cells in the islets of Langerhans in the pancreas, leading ultimately to the loss of glucose homeostasis (for earlier *iNKT* cell reviews see [Ghazarian et al., 2014](#); [Magalhaes et al., 2015](#); [Tard et al., 2015](#)).

Conflicting data on the frequency and function of *iNKT* cells from patient's PBMC have been reported. Whereas some reports observed lower *iNKT* cell frequencies, at least of the CD4 $^{+}$  cells ([Kis et al., 2007](#); [Kukreja et al., 2002](#); [Montoya et al., 2007](#); [Wilson et al., 1998](#)), others found equal ([Beristain-Covarrubias et al., 2015](#); [Lee et al., 2002b](#); [Michalek et al., 2006](#); [Oling et al., 2007](#); [Tocheva et al., 2017](#); [Tsutsumi et al., 2006](#); [Zhang et al., 2011](#)) or even more *iNKT* cells ([Oikawa et al., 2002](#)) in patient's PBMCs compared to healthy controls. Furthermore, whereas some authors noted reduced cytokine production of *iNKT* cell (lines) and/or a Th1 bias ([Beristain-Covarrubias et al., 2015](#); [Kis et al., 2007](#); [Kukreja et al., 2002](#); [Li et al., 2014](#); [Wilson et al., 1998, 2000](#)), others did not find such differences ([Lee et al., 2002b](#); [Roman-Gonzalez et al., 2009](#); [Tocheva et al., 2017](#)). Importantly, one study analyzed *iNKT* cell lines derived from the draining pancreatic lymph node and observed reduced IL-4 production following antigen stimulation ([Kent et al., 2005](#)).

Nonobese diabetic (NOD) mice develop spontaneous T1D and have been studied extensively. *iNKT* cells in NOD mice are reduced in frequency ([Berzins et al., 2004](#); [Chen et al., 2011](#)) and produce less cytokines following  $\alpha$ GalCer ([Falcone et al., 1999](#); [Gombert et al., 1996](#); [Hong et al., 2001](#); [Lehuen et al., 1998](#); [Li et al., 2008](#); [Sharif et al., 2001](#)) or stimulation of the innate immune system ([Falcone et al., 1999](#)). Increasing the *iNKT* cell frequency in NOD mice, either by cell transfer ([Baxter et al., 1997](#); [Beaudoin et al., 2002](#); [Cain et al., 2006](#); [Chen et al., 2005](#); [Hammond et al., 1998](#); [Lehuen et al., 1998](#); [Simoni et al., 2011](#)) or by over-expression of the *iTCR* ([Beaudoin et al., 2014](#); [Lehuen et al., 1998](#)) or by over-expression of CD1d on pancreatic island cells ([Falcone et al., 2004](#)), prevented or ameliorated symptoms. Consequently, *iNKT* cell-deficient NOD mice showed increased frequency and earlier onset of disease ([Fletcher and Baxter, 2009](#); [Naumov et al., 2001](#); [Shi et al., 2001](#); [Wang et al., 2001](#)), and similar results were obtained after antibody-mediated depletion of *iNKT* cells ([Scheuplein et al., 2015](#)). Furthermore, the repetitive injection of  $\alpha$ GalCer ameliorated disease progression, even when treatment was started after the onset of insulitis ([Hong et al., 2001](#); [Naumov et al., 2001](#); [Sharif et al., 2001](#); [Wang et al., 2001](#)). Other  $\alpha$ GalCer derivatives were similarly effective ([Forestier et al., 2007](#); [Ly et al., 2009](#); [Mizuno et al., 2004](#)). Such antigenic stimulation led to a significant increase of *iNKT* cells in the pancreatic island ([Beaudoin et al., 2014](#)). The original explanation for the therapeutic effect of  $\alpha$ GalCer was the shift to a Th2 response ([Hong et al., 2001](#); [Naumov et al., 2001](#); [Sharif et al., 2001](#)), but not all studies observed such a shift ([Beaudoin et al., 2002](#); [Shi et al., 2001](#); [Wang et al., 2001](#)), and work with congenic NOD lines demonstrated that such a shift is not required for protection ([Jordan et al., 2007](#); [Rocha-Campos et al., 2006](#)). Furthermore, the results from a number of publications are not in agreement as to which cytokine is essential for the protection from diabetes. IL-10 ([Hammond et al., 1998](#); [Hong et al., 2001](#); [Naumov et al., 2001](#); [Sharif et al., 2001](#)) and/or IL-4 ([Hong et al., 2001](#); [Naumov et al., 2001](#); [Sharif et al., 2001](#)) have been suggested to be important, but not in every case ([Beaudoin et al., 2002](#); [Chen et al., 2006](#); [Mi et al., 2004](#); [Novak et al., 2005](#)), and even IFN $\gamma$  elicited by *iNKT* cell activation was implicated as protective in one study ([Cain et al., 2006](#)). Besides the production of protective cytokines by *iNKT* cells, their lack of production of pathogenic cytokines seems to play a role as well. It was reported that *iNKT* cells in NOD mice, although reduced in total numbers, contained an increased frequency of pathogenic NKT17 cells ([De Giorgi et al., 2018](#); [Di Pietro et al., 2016](#); [Li et al., 2014](#); [Simoni et al., 2011](#)). The repeated injection of  $\alpha$ GalCer suppressed the IL-17A production by *iNKT* cells ([Simoni et al., 2011](#)), and this ([Simoni et al., 2011](#)) or the neutralization of IL-17A in vivo ([Emamaulee et al., 2009](#)) ameliorated the disease in the NOD mice. Besides such direct effects, it was shown that activated *iNKT* cells can also interact and influence T cells and DCs in multiple ways, improving disease progression indirectly. Activated *iNKT* cells were reported to impair the differentiation of pathogenic islet-specific CD4 $^{+}$  T cells, leading to T cell anergy ([Beaudoin et al., 2002](#); [Hugues et al., 2002](#); [Novak et al., 2005](#)). This was dependent on cell-cell contact between the *iNKT* and CD4 $^{+}$  T cells ([Novak et al., 2005](#)) and independent of CD4 $^{+}$  T cell CD1d expression ([Novak et al., 2007](#)). In regard to regulatory T (Treg) cells,  $\alpha$ GalCer injection did not necessarily increase their frequency in the NOD mice (no change: [Forestier et al., 2007](#); [Ly et al., 2006](#); [Sharif et al., 2001](#); increase: [Beaudoin et al., 2014](#); [Li et al., 2008](#)). Nonetheless, a protective role for Tregs activity in this NOD/ $\alpha$ GalCer model has been implicated by some ([Beaudoin et al., 2014](#); [Diana et al., 2011](#); [Ly et al., 2006](#)), but not all ([Beaudoin et al., 2002](#); [Cain et al., 2006](#)) studies. Tolerogenic ([Chuang et al., 2011](#)) or inhibitory ([Beaudoin et al., 2014](#)) effects on CD8 $^{+}$  T cells have been reported too. Furthermore, *iNKT* cells in NOD mice treated with  $\alpha$ GalCer could affect several DC populations leading to a tolerogenic outcome ([Beaudoin](#)

et al., 2014; Chen et al., 2005; Diana et al., 2011; Driver et al., 2010; Naumov et al., 2001; Saxena et al., 2007). Interestingly, this also required both cell–cell contact (Baev et al., 2008; Caielli et al., 2010) and CD1d expression by the DCs (Caielli et al., 2010). These tolerogenic DC were reported to induce Tregs (Beaudoin et al., 2014; Diana et al., 2011) and migrate to the pancreatic lymph node to tolerize T cells locally (Chen et al., 2005). The diverse outcomes in the NOD mouse could reflect the different experimental systems used, or differences in the microbiota associated with the respective animal facilities (De Giorgi et al., 2018; Okada et al., 2010).

### **Inflammatory Bowel Disease/Crohn's Disease**

Crohn's disease is characterized by a chronic and discontinuous inflammation deep in the tissues of both the small and large intestines, with high levels of Th1 cytokines. iNKT cells are reduced in PBMCs of affected patients (Grose et al., 2007b; van der Vliet et al., 2001). In addition, V $\alpha$ 24 mRNA and the numbers of V $\alpha$ 24 $^{+}$  cells were reduced in the intestine (Grose et al., 2007b).

In Th1 cytokine-mediated mouse colitis models (induced by TNBS or DSS), the iNKT cell frequency increased in the colon (Burrello et al., 2018). Stimulation of iNKT cells could ameliorate disease in most studies (yes: Numata et al., 2005; Saubermann et al., 2000; Ueno et al., 2005, no: Selvanantham et al., 2016). Furthermore, iNKT cell-deficient animals showed exacerbated diseases in most studies [yes: Selvanantham et al., 2016 (CD1d $^{-/-}$ ), Kim and Chung, 2013, and Montbarbon et al., 2013 (J $\alpha$ 18 $^{-/-}$ ); no: Huang et al., 2016 (CD1d $^{-/-}$ ), Shen et al., 2018 (J $\alpha$ 18 $^{-/-}$ )]. However, it is important to keep in mind that CD1d-deficient (Nieuwenhuis et al., 2009; Sáez de Guinoa et al., 2018; Selvanantham et al., 2016) and J $\alpha$ 18-deficient (Shen et al., 2018) mice were found to host an altered gut microbiota, which was proinflammatory upon transfer into wild-type animals (Selvanantham et al., 2016), highlighting again the mutual influence of the gut microbiota and iNKT cells (Hapil and Wingender, 2018; Wingender, 2016; Zeissig and Blumberg, 2014). Together, these data are consistent with the conclusion that iNKT cell activation shifted the immune response from the deleterious Th1 cytokine pattern to a protective Th2 response (Numata et al., 2005; Saubermann et al., 2000; Ueno et al., 2005).

### **Multiple Sclerosis/Experimental Autoimmune Encephalomyelitis**

Multiple sclerosis (MS) is a chronic, Th1/Th17 T-cell driven inflammatory disease directed against myelin antigens in the central nervous system (CNS), leading to progressive paralysis (for earlier iNKT cell reviews, see Bianchini et al., 2017; Van Kaer et al., 2015).

The frequency of iNKT cells was reduced in patient's PBMCs in most (Araki et al., 2003; Demoulin et al., 2003; Illés et al., 2000; van der Vliet et al., 2001), but not in all the studies (De Biasi et al., 2016; Gigli et al., 2007; O'Keeffe et al., 2008). This reduction was especially prominent in patients under remission (Araki et al., 2003; Illés et al., 2000). Furthermore, when iNKT cell lines derived from patient's PBMCs were analyzed for IFN $\gamma$  and IL-4 production, no difference (Gately et al., 2013; Gigli et al., 2007), a Th1 bias (Gausling et al., 2001), or a Th2-bias of the iNKT cell lines (Araki et al., 2003; O'Keeffe et al., 2008), was observed. When iNKT cells were stimulated directly ex vivo a mixed Th1/Th17 cytokine bias was observed in MS patients (De Biasi et al., 2016). Interestingly, MS patients under treatment (IFN $\beta$ , Gigli et al., 2007) or patients in remission (Araki et al., 2003) displayed an increased frequency of iNKT cells in PBMCs and increased cytokine production by them. Furthermore, myelin-derived antigens for iNKT cells have been proposed, but not characterized (Gately et al., 2013).

MS-like features can be replicated in mice by immunization with CNS-specific peptides, a model called experimental autoimmune encephalomyelitis (EAE). Most (Denney et al., 2012; Jahng et al., 2001; Oh and Chung, 2011; Teige et al., 2004), but not all studies (Furlan et al., 2003; Singh et al., 2001; Waddell et al., 2015) reported that iNKT cell-deficient animals display exacerbated disease. In addition, an increased iNKT cell frequency, due to expression of a V $\alpha$ 14*i* transgene, ameliorated disease (Mars et al., 2008, 2002). Importantly, activation of iNKT cells with  $\alpha$ GalCer (Denney et al., 2012; Furlan et al., 2003; Jahng et al., 2001; Parekh et al., 2004; Qian et al., 2010; Singh et al., 2001; Waddell et al., 2015) or Th2-biasing glycolipids related to  $\alpha$ GalCer (Miyamoto et al., 2001; Shiozaki et al., 2013; Zhang et al., 2008) protected mice against EAE. Protection correlated with a Th1 to Th2 cytokine diversion (Furlan et al., 2003; Jahng et al., 2001; Miyamoto et al., 2001; Oh and Chung, 2011; Pal et al., 2001; Singh et al., 2001; Zhang et al., 2008) and a reduction of the Th17 T cell response (Mars et al., 2009; Oh and Chung, 2011; Waddell et al., 2015; Yokote et al., 2008). However, which iNKT cell cytokines are important for these changes is not clear. IL-4 and/or IL-10 have been suggested (Jahng et al., 2001; Miyamoto et al., 2001; Oh and Chung, 2011; Sag et al., 2014; Singh et al., 2001), but some data contradict this (Furlan et al., 2003; Mars et al., 2002; Waddell et al., 2015). Similarly, divergent results have been reported a role for IFN $\gamma$  (Pro: Furlan et al., 2003; Mars et al., 2009; Con: Jahng et al., 2001; Oh and Chung, 2011). Although iNKT cells can enter the CNS

(Jahng et al., 2001; Mars et al., 2008; Oh and Chung, 2011), this was not seen in all studies (Denney et al., 2012; Waddell et al., 2015), and it is not certain if this is required for protection. Likewise, the importance of CD1d, beyond the primary iNKT cell stimulation, has been disputed (Mars et al., 2008; Wiethe et al., 2007). Finally, other immune cells were implicated in the  $\alpha$ GalCer-induced protection against EAE, such as M2 macrophages (Denney et al., 2012) or myeloid-derived suppressor cells (MDSC) (Parekh et al., 2013) infiltrating into the CNS, or tolerogenic DCs (Kojo et al., 2005; Wiethe et al., 2007).

### **Systemic Lupus Erythematosus**

Systemic lupus erythematosus (SLE) is characterized by the production and deposition of auto-antibodies against multiple nuclear antigens, leading to chronic inflammation of various tissues. The autoantigens are thought to derive from a defective clearance of apoptotic cells. According to one current hypothesis, iNKT cells suppress auto-reactive B cells in a CD1d-dependent fashion (for earlier iNKT cell reviews see Chen et al., 2015; Chuang et al., 2012).

iNKT cells from patient's PBMCs were reduced in frequency (Bosma et al., 2012; Cho et al., 2014, 2011; Kojo et al., 2001; Parietti et al., 2010; Shen et al., 2014; Smith et al., 2016; Sumida et al., 1998, 1995; van der Vliet et al., 2001; Wither et al., 2008; Wong et al., 2009; Yu and Wang, 2011) and proliferated less after stimulation in vitro with  $\alpha$ GalCer (Cho et al., 2011; Kojo et al., 2001; Yu and Wang, 2011). Furthermore, resulting iNKT cell lines (Bosma et al., 2012; Cho et al., 2011; Kojo et al., 2001; Shen et al., 2014; Yu and Wang, 2011), or fresh iNKT cells from PBMCs (Bosma et al., 2012; Shen et al., 2014), produced fewer cytokines than controls and/or displayed a Th1 bias. The frequency of iNKT cells in PBMCs correlated in most studies with disease severity (yes: Cho et al., 2011; Parietti et al., 2010; no: Wither et al., 2008) and serum levels of auto-reactive IgGs (Green et al., 2007; Wither et al., 2008). Interestingly, the frequency of iNKT cells in peripheral blood increased following therapy (Bosma et al., 2012; Oishi et al., 2001). Whereas CD1d expression levels on APCs from patient's PBMCs and healthy subjects did not differ, a decrease of CD1d surface expression was observed on patient B cells (Cho et al., 2011), and it was suggested that B cells in SLE patients were responsible for most of the iNKT cell defects (Bosma et al., 2012). Interestingly, the incubation of patient iNKT cells with patient B cells from PBMCs augmented the production of auto-antibodies by the B cells in a CD1d- and CD40-dependent fashion (Shen et al., 2014). Similar findings were reported in animal models (Takahashi and Strober, 2008; Zeng et al., 2003).

SLE in mice is studied either with mouse lines genetically prone to develop lupus-like diseases spontaneously or in induced models by injection of either the hydrocarbon oil pristane, LPS, or apoptotic bodies in selected mouse strains. Most data are in line with the idea of a protective role of iNKT cells. Crossing lupus-prone mouse lines onto an iNKT cell-deficient background led to exacerbated disease (Chan et al., 2001; Yang et al., 2004, 2007), although this was more pronounced for some organs than others (Chan et al., 2001; Yang et al., 2004). Interestingly, aged  $J\alpha 18^{-/-}$  mice by themselves have been shown to develop lupus-like symptoms (Sireci et al., 2007). Similarly, in the induced lupus models iNKT cell deficiency exacerbated symptoms (Wermeling et al., 2010; Yang et al., 2011, 2003). However, the effect of antigenic stimulation of iNKT cells led to conflicting outcomes in the different models. In the (NZB/NZW)F1 mouse model of spontaneous SLE, injection of  $\alpha$ GalCer into young mice was beneficial, but detrimental in aged animals (Major et al., 2006; Uchida et al., 2018; Yang et al., 2007; Zeng et al., 2003). In line with a switch to a pathogenic role later in the disease is the observation that iNKT cell numbers increased with lupus progression in these (NZB/NZW)F1 mice (Forestier et al., 2005; Morshed et al., 2002; Yang et al., 2007). Furthermore, in aged mice iNKT cells become hyperactive (Forestier et al., 2005) and interfering with their activation by means of blocking glycolipids (Morshed et al., 2009) or anti-CD1d-antibodies (Takahashi and Strober, 2008; Zeng et al., 2000, 2003) ameliorated disease. However, one study reported a reduced cytokine production by iNKT cells from (NZB/NZW)F1 mice at all ages (Yang et al., 2013). Not surprisingly, strain differences are also important, as the effects of  $\alpha$ GalCer treatment in the spontaneous models affected lupus nephritis in one mouse line, but not the other (Forestier et al., 2005; Zeng et al., 2003), and likewise, a strain dependence was observed for the induced models (Singh et al., 2005).

### **Good or Bad Actors?**

In some autoimmune diseases, iNKT cell number and/or function are altered, but it is much less certain if they play a role in pathogenesis or in regulation/prevention of disease. We consider three examples here.

Sjögren's syndrome is mediated by autoimmune destruction of exocrine glands. iNKT cells in patient's PBMCs were reduced in numbers in most studies (yes: Guggino et al., 2016; Kojo et al., 2001; van der Vliet et al., 2001;

no: Papp et al., 2016), produced less cytokines (Guggino et al., 2016; Kojo et al., 2001), and tended to show a Th1 cytokine bias following antigen stimulation (Kojo et al., 2001) compared to control cells. Interestingly, no *i*NKT cells were detected in the salivary glands of patients (Guggino et al., 2016).

Celiac disease is a chronic inflammation of the small intestine driven by the adaptive immune system and triggered by dietary gluten (Stepniak and Koning, 2006). As there is to date no animal model, data on the role of *i*NKT cells are still very limited. In PBMCs from celiac patients a decrease of *i*NKT cells has been reported in most studies (yes: Bernardo et al., 2008; Cseh et al., 2011; Dunne et al., 2013; Grose et al., 2007a, 2008; no: van der Vliet et al., 2001). In addition, fewer  $V\alpha 24^+$  T cells were found in the intestine (duodenum) of celiac patients (Calleja et al., 2011; Grose et al., 2007a, 2008). However, when the analysis focused on *i*NKT cells, the results were conflicting, with one study finding an increased (Montalvillo et al., 2015) and one a decreased (Dunne et al., 2013) frequency of *i*NKT cells in the duodenum of adult patients. Interestingly, an inverse reduction was observed in patients depending on the age: *i*NKT cells were reduced in the PBMCs of adult but not pediatric patients, whereas they were reduced in the duodenum only in pediatric but not in adult patients (Dunne et al., 2013). Furthermore, it has been suggested that patient *i*NKT cells produced less cytokine following in vitro stimulation (Grose et al., 2007a, 2008). In patients the adherence to a gluten-free diet normalized symptoms, and interestingly, this improvement correlated with a normalization of the *i*NKT cell numbers in PBMCs (Bernardo et al., 2008; Calleja et al., 2011; Cseh et al., 2011).

Experimental autoimmune uveitis (EAU) is a chronic inflammatory eye disease that can be induced in animals by immunization with retinal antigens and which serves as a model for human autoimmune uveitis (Kielczewski and Caspi, 2015). During EAU up to 7% of the eye-infiltrating mouse lymphocytes were *i*NKT cells (Oh et al., 2010) and in most studies *i*NKT cell-deficient mice showed an exacerbated course of disease (protective: Oh et al., 2010; Satoh et al., 2016; no difference: Grajewski et al., 2008). Furthermore, stimulation of *i*NKT cells at the time of the immunization ameliorated the disease (Grajewski et al., 2008; Satoh et al., 2016), presumably due to the action of IFN $\gamma$  (Grajewski et al., 2008; Satoh et al., 2016). In line with the role of IFN $\gamma$  was the observation that a Th1-biasing *i*NKT cell antigen (C-Glycoside) was most potent at protecting mice during EAU (Grajewski et al., 2008), although a Th2-biasing antigen was effective too (Grajewski et al., 2008; Satoh et al., 2016). Interestingly, though, the production of IFN $\gamma$  by *i*NKT cells themselves was not necessary, as *i*NKT cells deficient for IFN $\gamma$ , IL-4, or IL-10 were equally protective (Oh et al., 2010). From a therapeutic viewpoint, however, it is important to note that the stimulation of *i*NKT cells at the onset of the disease exacerbated EAU (Satoh et al., 2016). Together, it suggests that *i*NKT cells are beneficial for EAU early in the disease, but detrimental at later stages.

## THE FAR END OF THE QUESTION?

What emerges from this summary is that unlike some other T lymphocyte subsets, for example, Foxp3 $^+$  Tregs, *i*NKT cells have diverse effects on autoimmune disease pathogenesis. How can we account for this, considering their limited TCR diversity and essentially clonal specificity? Two main questions capture the potential involvement of *i*NKT cells during autoimmune diseases: (1) what draws *i*NKT cells in, and (2) what effects do they initiate?

### What Activates Invariant Natural Killer T Cells During Autoimmune Responses?

It is likely that *i*NKT cells need some form of activation to be involved in disease progression and like conventional T cells this could be modulated by differences in signal 1 (TCR), signal 2 (costimulation), and signal 3 (cytokines).

- Signal 1 (TCR): Beyond the inherent auto-reactivity of *i*NKT cells, CD1d-mediated activation of *i*NKT cells could conceivably be augmented in inflammatory settings by several means: (1) up-regulation of CD1d on APCs; (2) the increased presentation of stimulatory self-antigens, for example, following metabolic changes in activated APCs; (3) the presentation of stimulatory neo-antigen; and (4) so far unknown foreign antigens, for example, from commensals or opportunistic pathogens. Furthermore, heterogeneity in the outcome of *i*NKT cell activation could arise from the fact that different glycolipids can elicit divergent cytokine responses, and also, different *i*NKT cell subsets respond differently to the same antigen.
- Signal 2 (costimulation): Besides the “classical” means of *i*NKT cell activation, additional pathways of activation have been described, some of which are known to be important for T cell costimulation. Well-

known costimulatory molecules such as CD28 (Hayakawa et al., 2001; Kawano et al., 1997; Wang et al., 2009) and CD154 (CD40L) (Hayakawa et al., 2001; Kawano et al., 1997) can affect *i*NKT cell activation but so can other members of the TNF super family such as 4-1BB (Kim et al., 2008) and OX40 (Diana et al., 2009). The Ig superfamily molecule CD279 (PD-1), known as a coinhibitory molecule, plays a role in modulating *i*NKT cells in mouse asthma models (Akbari et al., 2010). In addition, there are other receptors that can modulate *i*NKT cell stimulation, including NK receptors (Arase et al., 1996; Exley et al., 1998; Ortaldo et al., 2006), Fc $\gamma$ RIII during arthritis (Kim et al., 2006), adenosine receptors (Lappas et al., 2006), and  $\beta$ -adrenergic receptors (Wong et al., 2011). Some of these, such as the NK receptors, can act independently of TCR stimulation, while others apparently act in concert with the TCR. Although there is still limited information, one might expect that different *i*NKT cell subsets likely express different sets of these receptors and will, therefore, respond differently. As these effects are cell–cell contact dependent, they are also dependent on the migratory behavior of *i*NKT cells to allow them to interact with a given APC.

- Signal 3 (cytokines): Activation of *i*NKT cells by cytokine combinations, such as IL-12/IL-18, IL-1 $\beta$ /IL-23, IL-25, and TGF $\beta$ , have been outlined above. Additional cytokines have been suggested to modulate *i*NKT cell responses, including IL-2 (Sakuishi et al., 2007), IL-7 (Hameg et al., 1999), IL-15 (Li et al., 2006), IL-33 (Bourgeois et al., 2009; Smithgall et al., 2008), GM-CSF (Crough et al., 2004), the prostaglandin D<sub>2</sub> (PGD2) (Torres et al., 2008), and TSLP (Jariwala et al., 2011; Wu et al., 2010). Many of these cytokines can be secreted in affected tissues during autoimmune-mediated inflammation. Therefore different combinations of local cytokines could induce diverse responses from *i*NKT cell subsets that express the appropriate cytokine receptors.

However, many other signals could be relevant as well, and given the gender-bias for many autoimmune diseases, sex hormones are one important aspect in this context. *i*NKT cells were reported to be more frequent in females than in males in some (Kee et al., 2012; Montoya et al., 2007; Sandberg et al., 2003) but not all (Bernin et al., 2016; Jing et al., 2007; Snyder-Cappione et al., 2010) studies. Furthermore, it was suggested that sex hormones could modulate the cytokine production by *i*NKT cells (Gourdy et al., 2005; Lotter et al., 2013).

Therefore the *i*NKT cell–mediated influence on the immune response depends on the means by which a particular *i*NKT cell gets activated. This reflects a complex “information input” that depends on the subset characteristics of the *i*NKT cell, the particulars of the local cytokine milieu, and the type and activation status of the APC they interact with.

## How Do Invariant Natural Killer T Cells Influence Autoimmune Responses?

The different “inputs” causing the activation of *i*NKT cells lead to different “outputs” or *i*NKT cell responses that will affect autoimmune disease pathogenesis in different ways. Three aspects will be highlighted here.

- Cytokine bias: The bias toward Th2 cytokines and a reduction of Th1/Th17 responses imparted by *i*NKT cells seems to be most often proposed as the mechanism for the effects of *i*NKT cells in autoimmune diseases. This is in line with the observations that *i*NKT cells are an important contributor to early IL-4 production in some contexts and that Th2-biasing antigens can have superior effects than  $\alpha$ GalCer in some cases. *i*NKT cells can impact the Th1/Th2 balance via their cytokine production, whereby IL-4 and IL-13 promote Th2; IFN $\gamma$  and TNF promote a Th1 response; IL-10 and TGF $\beta$  suppress pathogenic Th1 responses; and under some circumstances even IFN $\gamma$  has been shown to contribute to the development of Th2 responses (Bocek et al., 2004). However, newer data paint a more complex picture and the Th2-bias is clearly not sufficient to explain all observations. Importantly, beyond the Th1/2 paradigm Th17 responses can be pathogenic (Weaver and Hatton, 2009; Weaver et al., 2007) and *i*NKT cells have shown to be effective at suppressing Th17 responses. Furthermore, other *i*NKT cell–derived soluble factors can play a role, dependent on the particular disease, its stage, and many other circumstances.
- Direct regulation of cells: Besides a general cytokine bias, more direct *i*NKT cell driven mechanisms have been suggested. NKT10 cell–derived IL-10 is immunoinhibitory (Sag et al., 2014; Lynch et al., 2015). Furthermore, IFN $\gamma$  can aid in the induction of anergy or apoptosis in antigen-specific Th1 T cells and this was suggested to be important in diabetes (Beaudoin et al., 2002; Hugues et al., 2002; Novak et al., 2005). In addition, IL-4, IL-10, or GM-CSF can promote the differentiation/recruitment of myeloid cells with tolerogenic properties, such as DCs (Kojo et al., 2005; Naumov et al., 2001; Wang et al., 2008), MDSCs (Huang et al., 2014; Parekh et al., 2013), macrophages (Denney et al., 2012; Lynch et al., 2015), and neutrophils (De Santo et al., 2010). In addition,

*i*NKT cells can under some circumstances aid in the induction of regulatory T cells, either via tolerogenic DCs, pDCs, or via cytokines such as IL-2, IL-10, and TGF $\beta$  (Beaudoin et al., 2014; Diana et al., 2011; Kojo et al., 2005; La Cava et al., 2006; Liu et al., 2005; Ly et al., 2006; Lynch et al., 2015). Furthermore, cell–cell contact-dependent mechanisms are important, either via costimulatory molecules or via retrograde CD1d-signaling (Olszak et al., 2014; Sáez de Guinoa et al., 2018; Yue et al., 2009).

- *i*NKT cell subsets: Due to the increased awareness of *i*NKT cells subsets, at least in the mice, it becomes increasingly clear that different *i*NKT cell subsets can play different roles during autoimmune responses. NKT1 cells are protective, for example, in asthma (Chuang et al., 2018) and RA (Zhao et al., 2018), whereas NKT17 are pathogenic, for example, in diabetes (Simoni et al., 2011) and RA (Zhao et al., 2018). In addition, the timing of *i*NKT cell action might be critical, as exemplified in RA, where a switch from protective NKT1 cells to pathogenic NKT17 cells is seen with disease progression (Zhao et al., 2018). Therefore the impact of *i*NKT cell subsets can be beneficial or detrimental at different stages of the disease. Furthermore, NKT10 cells have been shown to be beneficial during EAE (Sag et al., 2014) and in the adipose tissue (Lynch et al., 2015; Sag et al., 2014), indicating that organ-specific preferences for *i*NKT cells could play a role as well.

The diverse means by which distinct *i*NKT cell subsets are activated, and the different responses they make, are of course not mutually exclusive. This complexity surely contributes to some of the contradictory results on the role of *i*NKT cells in particular autoimmune diseases.

## CONCLUSION

As outlined here, *i*NKT cells have been shown to be crucially involved in a wide range of autoimmune responses. Whereas it seems at first puzzling that a relatively small population has so many effects, one should keep in mind that even if present at less than a 1% frequency, *i*NKT cells are as abundant as typical CD4 $^+$  T cell memory populations, and they can exert their pivotal role due to their clonal specificity and explosive cytokine production. However, despite the vast literature on the role of *i*NKT cells in autoimmune diseases, much remains to be learned.

One important question, of course, is the extent to which the knowledge gained from animal models can be applied to humans. Although data from human patients are still relatively scarce, in several contexts they do suggest roles for *i*NKT cells that are comparable to the ones observed in mice. This is indeed very promising. Furthermore, we expect that soon functionally distinct *i*NKT cell subsets will be better characterized in humans, which most likely will help to readdress more specifically the role of *i*NKT cells in autoimmune diseases. A related issue is the potential of stimulating or inhibiting *i*NKT cells for therapeutic purposes. Despite the fact that  $\alpha$ GalCer is used already in clinical trials for cancer, and other glycolipids and approaches for manipulating *i*NKT cells are in trial or are being planned, the therapeutic potential of *i*NKT cells remains to be validated. The therapeutic potential is great, however, because basically all individuals have *i*NKT cells that respond to the same antigens in a comparable fashion.

## Acknowledgments

The work is supported by grants from NIH (AI 71922, AI 105215) (MK), TÜBITAK (116Z272, 117Z216), and EMBO (IG3073) (GW).

## ABBREVIATIONS

$\alpha$ GalCer	$\alpha$ -galactosylceramide
APC	antigen-presenting cell
BALF	bronchoalveolar lavage fluid
Bcl6	B-cell lymphoma 6 protein
c	canonical
CDR	complementarity determining regions
CHS	contact hypersensitivity
CNS	central nervous system
CXCR	chemokine (C-X-C motif) receptor
DC	dendritic cell
DN	double negative (CD4 $^-$ CD8 $^-$ )
DP	double positive (CD4 $^+$ CD8 $^+$ )

DSS	dextran sodium sulfate
EAE	experimental autoimmune encephalomyelitis
FoxP3	forkhead box P3
GATA3	GATA binding protein 3
I	invariant
IFN	interferon
Ig	immunoglobulin
IL	interleukin
LN	lymph node
int	intermediate
LPS	lipopolysaccharide
MAIT	mucosal-associated invariant T cell
MS	multiple sclerosis
NK	natural killer
NKT	natural killer T
NKT <sub>FH</sub>	follicular helper NKT cells
NOD	nonobese diabetic
PBC	primary biliary cirrhosis
PBMC	peripheral blood mononuclear cells
PD-1	programmed cell death 1
PGD2	prostaglandin D <sub>2</sub>
PLZF	promyelocytic leukemia zinc finger
RA	rheumatoid arthritis
ROR $\gamma$ t	RAR-related orphan receptor gamma
SLE	systemic lupus erythematosus
T1D	type 1 diabetes
Tbet	T-cell-specific T-box transcription factor
TCR	T cell antigen receptor
tg	transgenic
TGF	transforming growth factor
Th	T helper type
TNBS	trinitrobenzene sulfonic acid
Treg	regulatory T
TSLP	thymic stromal lymphopoietin
v	variable repertoire
V	antigen receptor variable region

## References

- Akbari, O., Stock, P., Meyer, E., Kronenberg, M., Sidobre, S., Nakayama, T., et al., 2003. Essential role of NKT cells producing IL-4 and IL-13 in the development of allergen-induced airway hyperreactivity. *Nat. Med.* 9, 582–588.
- Akbari, O., Faul, J.L., Hoyte, E.G., Berry, G.J., Wahlström, J., Kronenberg, M., et al., 2006. CD4 + invariant T-cell-receptor + natural killer T cells in bronchial asthma. *N. Engl. J. Med.* 354, 1117–1129.
- Akbari, O., Stock, P., Singh, A.K., Lombardi, V., Lee, W.-L., Freeman, G.J., et al., 2010. PD-L1 and PD-L2 modulate airway inflammation and iNKT-cell-dependent airway hyperreactivity in opposing directions. *Mucosal Immunol.* 3, 81–91.
- Albacker, L.A., Chaudhary, V., Chang, Y.-J., Kim, H.Y., Chuang, Y.-T., Pichavant, M., et al., 2013. Invariant natural killer T cells recognize a fungal glycosphingolipid that can induce airway hyperreactivity. *Nat. Med.* 19, 1297–1304.
- Allan, L.L., Hoefl, K., Zheng, D.J., Chung, B.K., Kozak, F.K., Tan, R., et al., 2009. Apolipoprotein-mediated lipid antigen presentation in B cells provides a pathway for innate help by NKT cells. *Blood* 114, 2411–2416.
- An, D., Oh, S.F., Olszak, T., Neves, J.F., Avci, F.Y., Erturk-Hasdemir, D., et al., 2014. Sphingolipids from a symbiotic microbe regulate homeostasis of host intestinal natural killer T cells. *Cell* 156, 123–133.
- Andoh, Y., Ogura, H., Satoh, M., Shimano, K., Okuno, H., Fujii, S., et al., 2013. Natural killer T cells are required for lipopolysaccharide-mediated enhancement of atherosclerosis in apolipoprotein E-deficient mice. *Immunobiology* 218, 561–569.
- Antunes, L., de Souza, A.P.D., de Araújo, P.D., Pinto, L.A., Jones, M.H., Stein, R.T., et al., 2018. iNKT cells are increased in children with severe therapy-resistant asthma. *Allergol. Immunopathol.* 46, 175–180.
- Araki, M., Kondo, T., Gumperz, J.E., Brenner, M.B., Miyake, S., Yamamura, T., 2003. Th2 bias of CD4 + NKT cells derived from multiple sclerosis in remission. *Int. Immunol.* 15, 279–288.
- Arase, H., Arase, N., Saito, T., 1996. Interferon gamma production by natural killer (NK) cells and NK1.1 + T cells upon NKR-P1 cross-linking. *J. Exp. Med.* 183, 2391–2396.
- Arora, P., Venkataswamy, M.M., Baena, A., Bricard, G., Li, Q., Veerapen, N., et al., 2011. A rapid fluorescence-based assay for classification of iNKT cell activating glycolipids. *J. Am. Chem. Soc.* 133, 5198–5201.
- Askenase, P.W., Majewska-Szczepanik, M., Kerfoot, S., Szczepanik, M., 2011. Participation of iNKT cells in the early and late components of Tc1-mediated DNFB contact sensitivity: cooperative role of  $\gamma\delta$ -T cells. *Scand. J. Immunol.* 73, 465–477.
- Aslanian, A.M., Chapman, H.A., Charo, I.F., 2005. Transient role for CD1d-restricted natural killer T cells in the formation of atherosclerotic lesions. *Arterioscler. Thromb. Vasc. Biol.* 25, 628–632.

- Assarsson, E., Kambayashi, T., Sandberg, J.K., Hong, S., Taniguchi, M., Van Kaer, L., et al., 2000. CD8+ T cells rapidly acquire NK1.1 and NK cell-associated molecules upon stimulation in vitro and in vivo. *J. Immunol.* 165, 3673–3679.
- Ayanoglu, G., Desai, B., Fick, R.B., Grein, J., de Waal Malefyt, R., Mattson, J., et al., 2011. Modelling asthma in macaques: longitudinal changes in cellular and molecular markers. *Eur. Respir. J.* 37, 541–552.
- Baev, D.V., Caielli, S., Ronchi, F., Coccia, M., Facciotti, F., Nichols, K.E., et al., 2008. Impaired SLAM-SLAM homotypic interaction between invariant NKT cells and dendritic cells affects differentiation of IL-4/IL-10-secreting NKT2 cells in nonobese diabetic mice. *J. Immunol.* 181, 869–877.
- Balato, A., Unutmaz, D., Gaspari, A.A., 2009. Natural killer T cells: an unconventional T-cell subset with diverse effector and regulatory functions. *J. Invest. Dermatol.* 129, 1628–1642.
- Balato, A., Zhao, Y., Harberts, E., Groleau, P., Liu, J., Fishlevich, R., et al., 2012. CD1d-dependent, iNKT-cell cytotoxicity against keratinocytes in allergic contact dermatitis. *Exp. Dermatol.* 21, 915–920.
- Baxter, A.G., Kinder, S.J., Hammond, K.J., Scollay, R., Godfrey, D.I., 1997. Association between alphabetaTCR + CD4-CD8- T-cell deficiency and IDDM in NOD/Lt mice. *Diabetes* 46, 572–582.
- Beaudoin, L., Laloux, V., Novak, J., Lucas, B., Lehuen, A., 2002. NKT cells inhibit the onset of diabetes by impairing the development of pathogenic T cells specific for pancreatic beta cells. *Immunity* 17, 725–736.
- Beaudoin, L., Diana, J., Ghazarian, L., Simoni, Y., Boitard, C., Lehuen, A., 2014. Plasmacytoid dendritic cells license regulatory T cells, upon iNKT-cell stimulation, to prevent autoimmune diabetes. *Eur. J. Immunol.* 44, 1454–1466.
- Bedel, R., Matsuda, J.L., Brigl, M., White, J., Kappler, J., Marrack, P., et al., 2012. Lower TCR repertoire diversity in *Traj18*-deficient mice. *Nat. Immunol.* 13, 705–706.
- Behar, S.M., Podrebarac, T.A., Roy, C.J., Wang, C.R., Brenner, M.B., 1999. Diverse TCRs recognize murine CD1. *J. Immunol.* 162, 161–167.
- Bendelac, A., 1995a. CD1: presenting unusual antigens to unusual T lymphocytes. *Science* 269, 185–186.
- Bendelac, A., 1995b. Positive selection of mouse NK1+ T cells by CD1-expressing cortical thymocytes. *J. Exp. Med.* 182, 2091–2096.
- Bendelac, A., Hunziker, R.D., Lantz, O., 1996. Increased interleukin 4 and immunoglobulin E production in transgenic mice overexpressing NK1 T cells. *J. Exp. Med.* 184, 1285–1293.
- Bendelac, A., Rivera, M.N., Park, S.H., Roark, J.H., 1997. Mouse CD1-specific NK1 T cells: development, specificity, and function. *Annu. Rev. Immunol.* 15, 535–562.
- Bendelac, A., Savage, P.B., Teyton, L., 2007. The biology of NKT cells. *Annu. Rev. Immunol.* 25, 297–336.
- Berg, R.E., Forman, J., 2006. The role of CD8 T cells in innate immunity and in antigen non-specific protection. *Curr. Opin. Immunol.* 18, 338–343.
- Beristain-Covarrubias, N., Canche-Pool, E., Gomez-Diaz, R., Sanchez-Torres, L.E., Ortiz-Navarrete, V., 2015. Reduced iNKT cells numbers in type 1 diabetes patients and their first-degree relatives. *Immun. Inflamm. Dis.* 3, 411–419.
- Bernardo, D., van Hoogstraten, I.M.W., Verbeek, W.H.M., Peña, A.S., Mearin, M.L., Arranz, E., et al., 2008. Decreased circulating iNKT cell numbers in refractory coeliac disease. *Clin. Immunol.* 126, 172–179.
- Beristain-Covarrubias, N., Canche-Pool, E.B., Ramirez-Velazquez, C., Barragan-Galvez, J.C., Gomez-Diaz, R.A., Ortiz-Navarrete, V., 2017. Class I-restricted T cell-associated molecule Is a marker for IFN- $\gamma$ -producing iNKT cells in healthy subjects and patients with type 1 diabetes. *J Interferon Cytokine Res* 37, 39–49.
- Bernin, H., Fehling, H., Marggraff, C., Tannich, E., Lotter, H., 2016. The cytokine profile of human NKT cells and PBMCs is dependent on donor sex and stimulus. *Med. Microbiol. Immunol.* 205, 321–332.
- Berntsen, N.L., Fosby, B., Tan, C., Reims, H.M., Ogaard, J., Jiang, X., et al., 2018. Natural killer T cells mediate inflammation in the bile ducts. *Mucosal Immunol.* 11, 1582–1590.
- Berzins, S.P., Ritchie, D.S., 2014. Natural killer T cells: drivers or passengers in preventing human disease? *Nat. Rev. Immunol.* 14, 640–646.
- Berzins, S.P., Kyriassoudis, K., Pellicci, D.G., Hammond, K.J., Sidobre, S., Baxter, A., et al., 2004. Systemic NKT cell deficiency in NOD mice is not detected in peripheral blood: implications for human studies. *Immunol. Cell Biol.* 82, 247–252.
- Berzins, S.P., Smyth, M.J., Baxter, A.G., 2011. Presumed guilty: natural killer T cell defects and human disease. *Nat. Rev. Immunol.* 11, 131–142.
- Bianchini, E., De Biasi, S., Simone, A.M., Ferraro, D., Sola, P., Cossarizza, A., et al., 2017. Invariant natural killer T cells and mucosal-associated invariant T cells in multiple sclerosis. *Immunol. Lett.* 183, 1–7.
- Bilenki, L., Yang, J., Fan, Y., Wang, S., Yang, X., 2004. Natural killer T cells contribute to airway eosinophilic inflammation induced by ragweed through enhanced IL-4 and eotaxin production. *Eur. J. Immunol.* 34, 345–354.
- Birkholz, A.M., Girardi, E., Wingender, G., Khurana, A., Wang, J., Zhao, M., et al., 2015a. A Novel Glycolipid Antigen for NKT Cells That Preferentially Induces IFN- $\gamma$  Production. *J. Immunol.* 195, 924–933.
- Birkholz, A.M., Howell, A.R., Kronenberg, M., 2015b. The alpha and omega of galactosylceramides in T cell immune function. *J. Biol. Chem.* 290, 15365–15370.
- Biron, C.A., Brossay, L., 2001. NK cells and NKT cells in innate defense against viral infections. *Curr. Opin. Immunol.* 13, 458–464.
- Blomqvist, M., Rhost, S., Teneberg, S., Lofbom, L., Osterbye, T., Brigl, M., et al., 2009. Multiple tissue-specific isoforms of sulfatide activate CD1d-restricted type II NKT cells. *Eur. J. Immunol.* 39, 1726–1735.
- Bobryshev, Y.V., Lord, R.S., 2000. CD1 expression and the nature of CD1-expressing cells in human atherosclerotic plaques. *Am. J. Pathol.* 156, 1477–1478.
- Bobryshev, Y.V., Lord, R.S.A., 2005. Co-accumulation of dendritic cells and natural killer T cells within rupture-prone regions in human atherosclerotic plaques. *J. Histochem. Cytochem.* 53, 781–785.
- Bocek Jr., P., Foucras, G., Paul, W.E., 2004. Interferon  $\gamma$  enhances both in vitro and in vivo priming of CD4+ T cells for IL-4 production. *J. Exp. Med.* 199, 1619–1630.
- Bogdanos, D.P., Vergani, D., 2009. Bacteria and primary biliary cirrhosis. *Clin. Rev. Allergy Immunol.* 36, 30–39.
- Bogdanos, D.-P., Baum, H., Grasso, A., Okamoto, M., Butler, P., Ma, Y., et al., 2004. Microbial mimics are major targets of crossreactivity with human pyruvate dehydrogenase in primary biliary cirrhosis. *J. Hepatol.* 40, 31–39.
- Bonish, B., Jullien, D., Dutronc, Y., Huang, B.B., Modlin, R., Spada, F.M., et al., 2000. Overexpression of CD1d by keratinocytes in psoriasis and CD1d-dependent IFN-gamma production by NK-T cells. *J. Immunol.* 165, 4076–4085.

- Bosma, A., Abdel-Gadir, A., Isenberg, D.A., Jury, E.C., Mauri, C., 2012. Lipid-antigen presentation by CD1d(+) B cells is essential for the maintenance of invariant natural killer T cells. *Immunity* 36, 477–490.
- Bourgeois, E., Van, L.P., Samson, M., Diem, S., Barra, A., Roga, S., et al., 2009. The pro-Th2 cytokine IL-33 directly interacts with invariant NKT and NK cells to induce IFN- $\gamma$  production. *Eur. J. Immunol.* 39, 1046–1055.
- Bratke, K., Julius, P., Virchow, J.C., 2007. Invariant natural killer T cells in obstructive pulmonary diseases. *N. Engl. J. Med.* 357, 194. author reply 194–5.
- Braun, N.A., Mendez-Fernandez, Y.V., Covarrubias, R., Porcelli, S.A., Savage, P.B., Yagita, H., et al., 2010a. Development of spontaneous anergy in invariant natural killer T cells in a mouse model of dyslipidemia. *Arterioscler. Thromb. Vasc. Biol.* 30, 1758–1765.
- Braun, N.A., Covarrubias, R., Major, A.S., 2010b. Natural killer T cells and atherosclerosis: form and function meet pathogenesis. *J. Innate Immun.* 2, 316–324.
- Brennan, P.J., Brigl, M., Brenner, M.B., 2013. Invariant natural killer T cells: an innate activation scheme linked to diverse effector functions. *Nat. Rev. Immunol.* 13, 101–117.
- Brennan, P.J., Tatituri, R.V.V., Heiss, C., Watts, G.F.M., Hsu, F.-F., Veerapen, N., et al., 2014. Activation of iNKT cells by a distinct constituent of the endogenous glucosylceramide fraction. *Proc. Natl. Acad. Sci. U.S.A.* 111, 13433–13438.
- Brigl, M., Bry, L., Kent, S.C., Gumperz, J.E., Brenner, M.B., 2003. Mechanism of CD1d-restricted natural killer T cell activation during microbial infection. *Nat. Immunol.* 4, 1230–1237.
- Brigl, M., van den Elzen, P., Chen, X., Meyers, J.H., Wu, D., Wong, C.-H., et al., 2006. Conserved and heterogeneous lipid antigen specificities of CD1d-restricted NKT cell receptors. *J. Immunol.* 176, 3625–3634.
- Brooks, C.R., Weinkove, R., Hermans, I.F., van Dalen, C.J., Douwes, J., 2010. Invariant natural killer T cells and asthma: immunologic reality or methodologic artifact? *J. Allergy Clin. Immunol.* 126, 882–885.
- Brossay, L., Kronenberg, M., 1999. Highly conserved antigen-presenting function of CD1d molecules. *Immunogenetics* 50, 146–151.
- Brossay, L., Chioda, M., Burdin, N., Koezuka, Y., Casorati, G., Dellabona, P., et al., 1998. CD1d-mediated recognition of an alpha-galactosylceramide by natural killer T cells is highly conserved through mammalian evolution. *J. Exp. Med.* 188, 1521–1528.
- Brozovic, S., Nagaishi, T., Yoshida, M., Betz, S., Salas, A., Chen, D., et al., 2004. CD1d function is regulated by microsomal triglyceride transfer protein. *Nat. Med.* 10, 535–539.
- Burdin, N., Brossay, L., Kronenberg, M., 1999. Immunization with alpha-galactosylceramide polarizes CD1-reactive NK T cells towards Th2 cytokine synthesis. *Eur. J. Immunol.* 29, 2014–2025.
- Burrello, C., Garavaglia, F., Cribiù, F.M., Ercoli, G., Bosari, S., Caprioli, F., et al., 2018. Short-term oral antibiotics treatment promotes inflammatory activation of colonic invariant natural killer T and conventional CD4+ T cells. *Front. Med. (Lausanne)* 5, 21.
- Caielli, S., Conforti-Andreoni, C., Di Pietro, C., Usuelli, V., Badami, E., Malosio, M.L., et al., 2010. On/Off TLR signaling decides proinflammatory or tolerogenic dendritic cell maturation upon CD1d-mediated interaction with invariant NKT cells. *J. Immunol.* 185, 7317–7329.
- Cain, J.A., Smith, J.A., Ondr, J.K., Wang, B., Katz, J.D., 2006. NKT cells and IFN-gamma establish the regulatory environment for the control of diabetogenic T cells in the nonobese diabetic mouse. *J. Immunol.* 176, 1645–1654.
- Calleja, S., Vivas, S., Santiuste, M., Arias, L., Hernando, M., Nistal, E., et al., 2011. Dynamics of non-conventional intraepithelial lymphocytes \text{NK}, NKT, and  $\gamma\delta$ T\text{c}el celiac disease: relationship with age, diet, and histopathology. *Dig. Dis. Sci.* 56, 2042–2049.
- Camelo, A., Barlow, J.L., Drynan, L.F., Neill, D.R., Ballantyne, S.J., Wong, S.H., et al., 2012. Blocking IL-25 signalling protects against gut inflammation in a type-2 model of colitis by suppressing nuocyte and NKT derived IL-13. *J. Gastroenterol.* 47, 1198–1211.
- Cameron, A.L., Kirby, B., Fei, W., Griffiths, C.E.M., 2002. Natural killer and natural killer-T cells in psoriasis. *Arch. Dermatol. Res.* 294, 363–369.
- Campos, R.A., Szczepanik, M., Itakura, A., Akahira-Azuma, M., Sidobre, S., Kronenberg, M., et al., 2003. Cutaneous immunization rapidly activates liver invariant Valpha14 NKT cells stimulating B-1 B cells to initiate T cell recruitment for elicitation of contact sensitivity. *J. Exp. Med.* 198, 1785–1796.
- Campos, R.A., Szczepanik, M., Itakura, A., Lisbonne, M., Dey, N., Leite-de-Moraes, M.C., et al., 2006a. Interleukin-4-dependent innate collaboration between iNKT cells and B-1 B cells controls adaptative contact sensitivity. *Immunology* 117, 536–547.
- Campos, R.A., Szczepanik, M., Lisbonne, M., Itakura, A., Leite-de-Moraes, M., Askenase, P.W., 2006b. Invariant NKT cells rapidly activated via immunization with diverse contact antigens collaborate in vitro with B-1 cells to initiate contact sensitivity. *J. Immunol.* 177, 3686–3694.
- Castano, A.R., Tangri, S., Miller, J.E., Holcombe, H.R., Jackson, M.R., Huse, W.D., et al., 1995. Peptide binding and presentation by mouse CD1. *Science* 269, 223–226.
- Cerundolo, V., Silk, J.D., Masri, S.H., Salio, M., 2009. Harnessing invariant NKT cells in vaccination strategies. *Nat. Rev. Immunol.* 9, 28–38.
- Chan, O.T., Paliwal, V., McNiff, J.M., Park, S.H., Bendelac, A., Shlomchik, M.J., 2001. Deficiency in beta(2)-microglobulin, but not CD1, accelerates spontaneous lupus skin disease while inhibiting nephritis in MRL-Fas(lpr) mice: an example of disease regulation at the organ level. *J. Immunol.* 167, 2985–2990.
- Chan, W.L., Pejnovic, N., Hamilton, H., Liew, T.V., Popadic, D., Poggi, A., et al., 2005. Atherosclerotic abdominal aortic aneurysm and the interaction between autologous human plaque-derived vascular smooth muscle cells, type 1 NKT, and helper T cells. *Circ. Res.* 96, 675–683.
- Chandra, S., Zhao, M., Budelsky, A., de Mingo Pulido, A., Day, J., Fu, Z., et al., 2015. A new mouse strain for the analysis of invariant NKT cell function. *Nat. Immunol.* 16, 799–800.
- Chandra, S., Wingender, G., Greenbaum, J.A., Khurana, A., Gholami, A.M., Ganesan, A.-P., et al., 2018. Development of asthma in inner-city children: possible roles of MAIT cells and variation in the home environment. *J. Immunol.* 200, 1995–2003.
- Chang, Y.-J., Kim, H.Y., Albacker, L.A., Lee, H.-H., Baumgarth, N., Akira, S., et al., 2011. Influenza infection in suckling mice expands an NKT cell subset that protects against airway hyperreactivity. *J. Clin. Invest.* 121, 57–69.
- Chang, P.-P., Barral, P., Fitch, J., Pratama, A., Ma, C.S., Kallies, A., et al., 2012. Identification of Bcl-6-dependent follicular helper NKT cells that provide cognate help for B cell responses. *Nat. Immunol.* 13, 35–43.
- Chang, C.-H., Chen, Y.-C., Zhang, W., Leung, P.S.C., Gershwin, M.E., Chuang, Y.-H., 2015. Innate immunity drives the initiation of a murine model of primary biliary cirrhosis. *PLoS One* 10, e0121320.

- Chen, Y.-G., Choisy-Rossi, C.-M., Holl, T.M., Chapman, H.D., Besra, G.S., Porcelli, S.A., et al., 2005. Activated NKT cells inhibit autoimmune diabetes through tolerogenic recruitment of dendritic cells to pancreatic lymph nodes. *J. Immunol.* 174, 1196–1204.
- Chen, Y.-G., Chen, J., Osborne, M.A., Chapman, H.D., Besra, G.S., Porcelli, S.A., et al., 2006. CD38 is required for the peripheral survival of immunotolerogenic CD4+ invariant NK T cells in nonobese diabetic mice. *J. Immunol.* 177, 2939–2947.
- Chen, Y.G., Tsaih, S.-W., Serreze, D.V., 2011. Genetic control of murine invariant natural killer T-cell development dynamically differs dependent on the examined tissue type. *Genes Immun.* 13, 164–174.
- Chen, J., Wu, M., Wang, J., Li, X., 2015. Immunoregulation of NKT cells in systemic lupus erythematosus. *J. Immunol. Res.* 2015, 206731.
- Chen, J., Yang, J., Qiao, Y., Li, X., 2016. Understanding the regulatory roles of natural killer T cells in rheumatoid arthritis: T helper cell differentiation dependent or independent? *Scand. J. Immunol.* 84, 197–203.
- Chiba, A., Oki, S., Miyamoto, K., Hashimoto, H., Yamamura, T., Miyake, S., 2004. Suppression of collagen-induced arthritis by natural killer T cell activation with OCH, a sphingosine-truncated analog of alpha-galactosylceramide. *Arthritis Rheum.* 50, 305–313.
- Chiba, A., Kaijeda, S., Oki, S., Yamamura, T., Miyake, S., 2005. The involvement of V(α)14 natural killer T cells in the pathogenesis of arthritis in murine models. *Arthritis Rheum.* 52, 1941–1948.
- Cho, Y.-N., Kee, S.-J., Lee, S.-J., Seo, S.-R., Kim, T.-J., Lee, S.-S., et al., 2011. Numerical and functional deficiencies of natural killer T cells in systemic lupus erythematosus: their deficiency related to disease activity. *Rheumatology (Oxford)* 50, 1054–1063.
- Cho, Y.-N., Kee, S.-J., Kim, T.-J., Jin, H.M., Kim, M.-J., Jung, H.-J., et al., 2014. Mucosal-associated invariant T cell deficiency in systemic lupus erythematosus. *J. Immunol.* 193, 3891–3901.
- Chuang, Y.-H., Lian, Z.-X., Yang, G.-X., Shu, S.-A., Moritoki, Y., Ridgway, W.M., et al., 2008. Natural killer T cells exacerbate liver injury in a transforming growth factor beta receptor II dominant-negative mouse model of primary biliary cirrhosis. *Hepatology* 47, 571–580.
- Chuang, Y.-P., Lin, Y.-C., Sytwu, H.-K., 2011. α-Galactosylceramide ameliorates autoimmune diabetes in non-obese diabetic mice through a suppressive effect mediated by CD8+ T cells. *Immunol. Lett.* 138, 54–62.
- Chuang, Y.-P., Wang, C.-H., Wang, N.-C., Chang, D.-M., Sytwu, H.-K., 2012. Modulatory function of invariant natural killer T cells in systemic lupus erythematosus. *Clin. Dev. Immunol.* 2012, 478429.
- Chuang, Y.-T., Leung, K., Chang, Y.-J., DeKruyff, R.H., Savage, P.B., Cruse, R., et al., 2018. A natural killer T-cell subset that protects against airway hyperreactivity. *J. Allergy Clin. Immunol.* doi:10.1016/j.jaci.2018.03.022.
- Constantinides, M.G., Picard, D., Savage, A.K., Bendelac, A., 2011. A naive-like population of human CD1d-restricted T cells expressing intermediate levels of promyelocytic leukemia zinc finger. *J. Immunol.* 187, 309–315.
- Constantinides, M.G., Bendelac, A., 2013. Transcriptional regulation of the NKT cell lineage. *Curr. Opin. Immunol.* 25, 161–167.
- Coppiepers, K., Van Beneden, K., Jacques, P., Dewint, P., Vervloet, A., Vander Cruyssen, B., et al., 2007. A single early activation of invariant NK T cells confers long-term protection against collagen-induced arthritis in a ligand-specific manner. *J. Immunol.* 179, 2300–2309.
- Coquet, J.M., Chakravarti, S., Kyriakisoudis, K., McNab, F.W., Pitt, L.A., McKenzie, B.S., et al., 2008. Diverse cytokine production by NKT cell subsets and identification of an IL-17-producing CD4-NK1.1- NKT cell population. *Proc. Natl. Acad. Sci. U.S.A.* 105, 11287–11292.
- Crough, T., Nieda, M., Nicol, A.J., 2004. Granulocyte colony-stimulating factor modulates alpha-galactosylceramide-responsive human Valpha24 + Vbeta11 + NKT cells. *J. Immunol.* 173, 4960–4966.
- Cseh, A., Vasarhelyi, B., Szalay, B., Molnar, K., Nagy-Szakal, D., Treszl, A., et al., 2011. Immune phenotype of children with newly diagnosed and gluten-free diet-treated celiac disease. *Dig. Dis. Sci.* 56, 792–798.
- Cui, J., Watanabe, N., Kawano, T., Yamashita, M., Kamata, T., Shimizu, C., et al., 1999. Inhibition of T helper cell type 2 cell differentiation and immunoglobulin E response by ligand-activated Valpha14 natural killer T cells. *J. Exp. Med.* 190, 783–792.
- Curry, J.L., Qin, J.-Z., Robinson, J., Nickoloff, B.J., 2003. Reactivity of resident immunocytes in normal and prepsoriatic skin using an ex vivo skin-explant model system. *Arch. Pathol. Lab. Med.* 127, 289–296.
- Curzytek, K., Kubera, M., Majewska-Szczepanik, M., Szczepanik, M., Ptak, W., Duda, W., et al., 2015. Inhibitory effect of antidepressant drugs on contact hypersensitivity reaction is connected with their suppressive effect on NKT and CD8(+) T cells but not on TCR delta T cells. *Int. Immunopharmacol.* 28, 1091–1096.
- D'Andrea, A., Goux, D., de Lalla, C., Koezuka, Y., Montagna, D., Moretta, A., et al., 2000. Neonatal invariant Valpha24 + NKT lymphocytes are activated memory cells. *Eur. J. Immunol.* 30, 1544–1550.
- Dao, T., Mehal, W.Z., Crispe, I.N., 1998. IL-18 augments perforin-dependent cytotoxicity of liver NK-T cells. *J. Immunol.* 161, 2217–2222.
- Dashtsoodol, N., Shigeura, T., Ozawa, R., Harada, M., Kojo, S., Watanabe, T., et al., 2016. Generation of novel Traj18-deficient mice lacking Vα14 natural killer T cells with an undisturbed T cell receptor α-chain repertoire. *PLoS One* 11, e0153347.
- De Biasi, S., Simone, A.M., Nasi, M., Bianchini, E., Ferraro, D., Vitetta, F., et al., 2016. iNKT cells in secondary progressive multiple sclerosis patients display pro-inflammatory profiles. *Front. Immunol.* 7, 1900.
- De Giorgi, L., Sorini, C., Cosorich, I., Ferrarese, R., Canducci, F., Falcone, M., 2018. Increased iNKT17 cell frequency in the intestine of non-obese diabetic mice correlates with high bacteroidales and low clostridiales abundance. *Front. Immunol.* 9, 1752.
- De Santo, C., Arscott, R., Booth, S., Karydis, I., Jones, M., Asher, R., et al., 2010. Invariant NKT cells modulate the suppressive activity of IL-10-secreting neutrophils differentiated with serum amyloid A. *Nat. Publ. Group* 11, 1039–1046.
- DeKruyff, R.H., Yu, S., Kim, H.Y., Umetsu, D.T., 2014. Innate immunity in the lung regulates the development of asthma. *Immunol. Rev.* 260, 235–248.
- Demoulin, T., Gachelin, G., Bequet, D., Dormont, D., 2003. A biased Valpha24 + T-cell repertoire leads to circulating NKT-cell defects in a multiple sclerosis patient at the onset of his disease. *Immunol. Lett.* 90, 223–228.
- Denkers, E.Y., Scharton-Kersten, T., Barbieri, S., Caspar, P., Sher, A., 1996. A role for CD4+ NK1.1+ T lymphocytes as major histocompatibility complex class II independent helper cells in the generation of CD8+ effector function against intracellular infection. *J. Exp. Med.* 184, 131–139.
- Denney, L., Kok, W.L., Cole, S.L., Sanderson, S., McMichael, A.J., Ho, L.-P., 2012. Activation of invariant NKT cells in early phase of experimental autoimmune encephalomyelitis results in differentiation of Ly6Chi inflammatory monocyte to M2 macrophages and improved outcome. *J. Immunol.* 189, 551–557.
- Dey, N., Szczepanik, M., Lau, K., Majewska-Szczepanik, M., Askenase, P.W., 2011. Stimulatory lipids accumulate in the mouse liver within 30 min of contact sensitization to facilitate the activation of naïve iNKT cells in a CD1d-dependent fashion. *Scand. J. Immunol.* 74, 52–61.

- Di Pietro, C., Falcone, M., 2014. The role of invariant NKT cells in organ-specific autoimmunity. *Front. Biosci. (Landmark Ed)* 19, 1240–1250.
- Di Pietro, C., De Giorgi, L., Cosorich, I., Sorini, C., Fedeli, M., Falcone, M., 2016. MicroRNA-133b regulation of Th-POK expression and dendritic cell signals affect NKT17 cell differentiation in the thymus. *J. Immunol.* 197, 3271–3280.
- Diana, J., Griseri, T., Lagaye, S., Beaudoin, L., Autrusseau, E., Gautron, A.S., et al., 2009. NKT cell-plasmacytoid dendritic cell cooperation via OX40 controls viral infection in a tissue-specific manner. *Immunity* 30, 289–299.
- Diana, J., Brezar, V., Beaudoin, L., Dalod, M., Mellor, A., Tafuri, A., et al., 2011. Viral infection prevents diabetes by inducing regulatory T cells through NKT cell–plasmacytoid dendritic cell interplay. *J. Exp. Med.* 208, 729–745.
- Doherty, D.G., Norris, S., Madrigal-Estebas, L., McEntee, G., Traynor, O., Hegarty, J.E., et al., 1999. The human liver contains multiple populations of NK cells, T cells, and CD3 + CD56 + natural T cells with distinct cytotoxic activities and Th1, Th2, and Th0 cytokine secretion patterns. *J. Immunol.* 163, 2314–2321.
- Doisne, J.M., Becourt, C., Amniai, L., Duarte, N., Le Luduec, J.B., Eberl, G., et al., 2009. Skin and peripheral lymph node invariant NKT cells are mainly retinoic acid receptor-related orphan receptor ( $\gamma$ )t + and respond preferentially under inflammatory conditions. *J. Immunol.* 183, 2142–2149.
- Doisne, J.-M., Soulard, V., Becourt, C., Amniai, L., Henrot, P., Havenar-Daughton, C., et al., 2011. Cutting edge: crucial role of IL-1 and IL-23 in the innate IL-17 response of peripheral lymph node NK1.1- invariant NKT cells to bacteria. *J. Immunol.* 186, 662–666.
- Drennan, M.B., Aspeslagh, S., Elewaut, D., 2010. Invariant natural killer T cells in rheumatic disease: a joint dilemma. *Nat. Rev. Rheumatol.* 6, 90–98.
- Driver, J.P., Scheuplein, F., Chen, Y.-G., Grier, A.E., Wilson, S.B., Serreze, D.V., 2010. Invariant natural killer T-cell control of type 1 diabetes: a dendritic cell genetic decision of a silver bullet or Russian roulette. *Diabetes* 59, 423–432.
- Dunne, M.R., Elliott, L., Hussey, S., Mahmud, N., Kelly, J., Doherty, D.G., et al., 2013. Persistent changes in circulating and intestinal gamma-delta T cell subsets, invariant natural killer T cells and mucosal-associated invariant T cells in children and adults with coeliac disease. *PLoS One* 8, e76008.
- East, J.E., Kennedy, A.J., Webb, T.J., 2014. Raising the roof: the preferential pharmacological stimulation of Th1 and Th2 responses mediated by NKT cells. *Med. Res. Rev.* 34, 45–76.
- Eberl, G., MacDonald, H.R., 2000. Selective induction of NK cell proliferation and cytotoxicity by activated NKT cells. *Eur. J. Immunol.* 30, 985–992.
- Eguchi, T., Kumagai, K., Kobayashi, H., Shigematsu, H., Kitaura, K., Suzuki, S., et al., 2013. Accumulation of invariant NKT cells into inflamed skin in a novel murine model of nickel allergy. *Cell Immunol.* 284, 163–171.
- Elkhal, A., Pichavant, M., He, R., Scott, J., Meyer, E., Goya, S., et al., 2006. CD1d restricted natural killer T cells are not required for allergic skin inflammation. *J. Allergy Clin. Immunol.* 118, 1363–1368.
- Elzen, P.V.D., Garg, S., León, L., Bríg, M., Leadbetter, E.A., Gumperz, J.E., et al., 2005. Apolipoprotein-mediated pathways of lipid antigen presentation. *Nature* 437, 906–910.
- Emamalilee, J.A., Davis, J., Merani, S., Toso, C., Elliott, J.F., Thiesen, A., et al., 2009. Inhibition of Th17 cells regulates autoimmune diabetes in NOD Mice. *Diabetes* 58, 1302–1311.
- Emoto, M., Emoto, Y., Yoshizawa, I., Kita, E., Shimizu, T., Hurwitz, R., et al., 2010. Alpha-GalCer ameliorates listeriosis by accelerating infiltration of Gr-1 + cells into the liver. *Eur. J. Immunol.* 40, 1328–1341.
- Engel, I., Kronenberg, M., 2014. Transcriptional control of the development and function of Valpha14i NKT cells. *Curr. Top Microbiol. Immunol.* 381, 51–81.
- Engel, I., Seumois, G.E.G., Chavez, L., Samaniego-Castruita, D., White, B., Chawla, A., et al., 2016. Innate-like functions of natural killer T cell subsets result from highly divergent gene programs. *Nat. Immunol.* 1–15.
- Exley, M., Porcelli, S., Furman, M., Garcia, J., Balk, S., 1998. CD161 (NKR-P1A) costimulation of CD1d-dependent activation of human T cells expressing invariant V alpha 24 J alpha Q T cell receptor alpha chains. *J. Exp. Med.* 188, 867–876.
- Facciotti, F., Ramanjaneyulu, G.S., Lepore, M., Sansano, S., Cavallari, M., Kistowska, M., et al., 2012. Peroxisome-derived lipids are self antigens that stimulate invariant natural killer T cells in the thymus. *Nat. Immunol.* 13, 474–480.
- Falcone, M., Yeung, B., Tucker, L., Rodriguez, E., Sarvetnick, N., 1999. A defect in interleukin 12-induced activation and interferon gamma secretion of peripheral natural killer T cells in nonobese diabetic mice suggests new pathogenic mechanisms for insulin-dependent diabetes mellitus. *J. Exp. Med.* 190, 963–972.
- Falcone, M., Facciotti, F., Ghidoli, N., Monti, P., Olivieri, S., Zaccagnino, L., et al., 2004. Up-regulation of CD1d expression restores the immunoregulatory function of NKT cells and prevents autoimmune diabetes in nonobese diabetic mice. *J. Immunol.* 172, 5908–5916.
- Fjelbye, J., Antvorskov, J.C., Buschard, K., Issazadeh-Navikas, S., Engkilde, K., 2015. CD1d knockout mice exhibit aggravated contact hypersensitivity responses due to reduced interleukin-10 production predominantly by regulatory B cells. *Exp. Dermatol.* 24, 853–856.
- Flesch, I.E., Wandersee, A., Kaufmann, S.H., 1997. IL-4 secretion by CD4 + NK1 + T cells induces monocyte chemoattractant protein-1 in early listeriosis. *J. Immunol.* 159, 7–10.
- Fletcher, M.T., Baxter, A.G., 2009. Clinical application of NKT cell biology in type I (autoimmune) diabetes mellitus. *Immunol. Cell Biol.* 87, 315–323.
- Forestier, C., Molano, A., Im, J.S., Dutronc, Y., Diamond, B., Davidson, A., et al., 2005. Expansion and hyperactivity of CD1d-restricted NKT cells during the progression of systemic lupus erythematosus in (New Zealand Black × New Zealand White)F1 mice. *J. Immunol.* 175, 763–770.
- Forestier, C., Takaki, T., Molano, A., Im, J.S., Baine, I., Jerud, E.S., et al., 2007. Improved outcomes in NOD mice treated with a novel Th2 cytokine-biasing NKT cell activator. *J. Immunol.* 178, 1415–1425.
- Fox, L.M., Cox, D.G., Lockridge, J.L., Wang, X., Chen, X., Scharf, L., et al., 2009. Recognition of lyso-phospholipids by human natural killer T lymphocytes. *PLoS Biol.* 7, e1000228.
- Furlan, R., Bergami, A., Cantarella, D., Brambilla, E., Taniguchi, M., Dellabona, P., et al., 2003. Activation of invariant NKT cells by alphaGalCer administration protects mice from MOG35-55-induced EAE: critical roles for administration route and IFN-gamma. *Eur. J. Immunol.* 33, 1830–1838.

- Fuss, I.J., Heller, F., Boirivant, M., Leon, F., Yoshida, M., Fichtner-Feigl, S., et al., 2004. Nonclassical CD1d-restricted NK T cells that produce IL-13 characterize an atypical Th2 response in ulcerative colitis. *J. Clin. Invest.* 113, 1490–1497.
- Gadola, S.D., Dulphy, N., Salio, M., Cerundolo, V., 2002. Valpha24-JalphaQ-independent, CD1d-restricted recognition of alpha-galactosylceramide by human CD4(+) and CD8alpha/beta(+) T lymphocytes. *J. Immunol.* 168, 5514–5520.
- Galkina, E., Harry, B.L., Ludwig, A., Liehn, E.A., Sanders, J.M., Bruce, A., et al., 2007. CXCR6 promotes atherosclerosis by supporting T-cell homing, interferon- $\gamma$  production, and macrophage accumulation in the aortic wall. *Circulation* 116, 1801–1811.
- Gately, C.M., Podbielska, M., Counihan, T., Hennessy, M., Leahy, T., Moran, A.P., et al., 2013. *J. Neuroimmunol.* 259, 1–7.
- Gausling, R., Trollmo, C., Hafler, D.A., 2001. Decreases in interleukin-4 secretion by invariant CD4 – CD8 – Vo24J $\alpha$ Q T cells in peripheral blood of patients with relapsing–remitting multiple sclerosis. *Clin. Immunol.* 98, 11–17.
- Georgiev, H., Ravens, I., Benarafa, C., Ruster, R.F.O., Bernhardt, G.U.N., 2016. Distinct gene expression patterns correlate with developmental and functional traits of iNKT subsets. *Nat. Commun.* 7, 1–24.
- Getz, G.S., Reardon, C.A., 2017. Natural killer T cells in atherosclerosis. *Nat. Publ. Group* 14, 304–314.
- Ghazarian, L., Simoni, Y., Magalhaes, I., Lehuen, A., 2014. Invariant NKT cell development: focus on NOD mice. *Curr. Opin. Immunol.* 27, 83–88.
- Gigli, G., Caielli, S., Cutuli, D., Falcone, M., 2007. Innate immunity modulates autoimmunity: type 1 interferon-beta treatment in multiple sclerosis promotes growth and function of regulatory invariant natural killer T cells through dendritic cell maturation. *Immunology* 122, 409–417.
- Gilhar, A., Ullmann, Y., Kerner, H., Assy, B., Shalaginov, R., Serafimovich, S., et al., 2002. Psoriasis is mediated by a cutaneous defect triggered by activated immunocytes: induction of psoriasis by cells with natural killer receptors. *J. Invest. Dermatol.* 119, 384–391.
- Girardi, E., Wang, J., Zajonc, D.M., 2016. Structure of an  $\alpha$ -helical peptide and lipopeptide bound to the nonclassical major histocompatibility complex (MHC) class I molecule CD1d. *J. Biol. Chem.* 291, 10677–10683.
- Gober, M.D., Fishelevich, R., Zhao, Y., Unutmaz, D., Gaspari, A.A., 2008. Human natural killer T cells infiltrate into the skin at elicitation sites of allergic contact dermatitis. *J. Invest. Dermatol.* 128, 1460–1469.
- Godfrey, D.I., Hammond, K.J., Poulton, L.D., Smyth, M.J., Baxter, A.G., 2000. NKT cells: facts, functions and fallacies. *Immunol. Today* 21, 573–583.
- Godfrey, D.I., Kronenberg, M., 2004. Going both ways: immune regulation via CD1d-dependent NKT cells. *J. Clin. Invest.* 114, 1379–1388.
- Godfrey, D.I., Stankovic, S., Baxter, A.G., 2010. Raising the NKT cell family. *Nat. Immunol.* 11, 197–206.
- Godfrey, D.I., Pellicci, D.G., Rossjohn, J., 2013. NKT cells: the smoking gun in fungal-induced asthma? *Nat. Med.* 19, 1210–1211.
- Gombert, J.M., Herbelin, A., Tancrede-Bohin, E., Dy, M., Carnaud, C., Bach, J.F., 1996. Early quantitative and functional deficiency of NK1.1-like thymocytes in the NOD mouse. *Eur. J. Immunol.* 26, 2989–2998.
- Gonzalez-Aseguinolaza, G., Van Kaer, L., Bergmann, C.C., Wilson, J.M., Schmieg, J., Kronenberg, M., et al., 2002. Natural killer T cell ligand alpha-galactosylceramide enhances protective immunity induced by malaria vaccines. *J. Exp. Med.* 195, 617–624.
- Goubier, A., Vocanson, M., Macari, C., Poyet, G., Herbelin, A., Nicolas, J.-F., et al., 2013. Invariant NKT cells suppress CD8(+) T-cell-mediated allergic contact dermatitis independently of regulatory CD4(+) T cells. *J. Invest. Dermatol.* 133, 980–987.
- Gourdy, P., Araujo, L.M., Zhu, R., Garmy-Susini, B., Diem, S., Laurell, H., et al., 2005. Relevance of sexual dimorphism to regulatory T cells: estradiol promotes IFN-gamma production by invariant natural killer T cells. *Blood* 105, 2415–2420.
- Grajewski, R.S., Hansen, A.M., Agarwal, R.K., Kronenberg, M., Sidobre, S., Su, S.B., et al., 2008. Activation of invariant NKT cells ameliorates experimental ocular autoimmunity by a mechanism involving innate IFN-gamma production and dampening of the adaptive Th1 and Th17 responses. *J. Immunol.* 181, 4791–4797.
- Green, M.R.J., Kennell, A.S.M., Larche, M.J., Seifert, M.H., Isenberg, D.A., Salaman, M.R., 2007. Natural killer T cells in families of patients with systemic lupus erythematosus: their possible role in regulation of IgG production. *Arthritis Rheum.* 56, 303–310.
- Grose, R.H., Cummins, A.G., Thompson, F.M., 2007a. Deficiency of invariant natural killer T cells in coeliac disease. *Gut* 56, 790–795.
- Grose, R.H., Thompson, F.M., Baxter, A.G., Pellicci, D.G., Cummins, A.G., 2007b. Deficiency of invariant NK T cells in Crohn's disease and ulcerative colitis. *Dig. Dis. Sci.* 52, 1415–1422.
- Grose, R.H., Thompson, F.M., Cummins, A.G., 2008. Deficiency of 6B11+ invariant NK T-cells in celiac disease. *Dig. Dis. Sci.* 53, 1846–1851.
- Grundtman, C., Wick, G., 2011. The autoimmune concept of atherosclerosis. *Curr. Opin. Lipidol.* 22, 327–334.
- Guggino, G., Ciccia, F., Raimondo, S., Giardina, G., Alessandro, R., Dieli, F., et al., 2016. Invariant NKT cells are expanded in peripheral blood but are undetectable in salivary glands of patients with primary Sjogren's syndrome. *Clin. Exp. Rheumatol.* 34, 25–31.
- Gumperz, J.E., Roy, C., Makowska, A., Lum, D., Sugita, M., Podrebarac, T., et al., 2000. Murine CD1d-restricted T cell recognition of cellular lipids. *Immunity* 12, 211–221.
- Gumperz, J.E., Miyake, S., Yamamura, T., Brenner, M.B., 2002. Functionally distinct subsets of CD1d-restricted natural killer T cells revealed by CD1d tetramer staining. *J. Exp. Med.* 195, 625–636.
- Gutowska-Owsia, D., Birchall, M.A., Moots, R.J., Christmas, S.E., Pazmany, L., 2014. Proliferatory defect of invariant population and accumulation of non-invariant CD1d-restricted natural killer T cells in the joints of RA patients. *Mod. Rheumatol.* 24, 434–442.
- Gyimesi, E., Nagy, G., Remenyik, E., Sipka, S., Zeher, M., Biro, T., et al., 2011. Altered peripheral invariant natural killer T cells in atopic dermatitis. *J. Clin. Immunol.* 31, 864–872.
- Hameg, A., Gouarin, C., Gombert, J.M., Hong, S., Van Kaer, L., Bach, J.F., et al., 1999. IL-7 up-regulates IL-4 production by splenic NK1.1+ and NK1.1-MHC class I-like/CD1-dependent CD4+ T cells. *J. Immunol.* 162, 7067–7074.
- Hammond, K.J., Poulton, L.D., Palmisano, L.J., Silveira, P.A., Godfrey, D.I., Baxter, A.G., 1998. alpha/beta-T cell receptor (TCR) + CD4-CD8-(NKT) thymocytes prevent insulin-dependent diabetes mellitus in nonobese diabetic (NOD)/Lt mice by the influence of interleukin (IL)-4 and/or IL-10. *J. Exp. Med.* 187, 1047–1056.
- Hammond, K.J., Pelikan, S.B., Crowe, N.Y., Randle-Barrett, E., Nakayama, T., Taniguchi, M., et al., 1999. NKT cells are phenotypically and functionally diverse. *Eur. J. Immunol.* 29, 3768–3781.
- Hamzaoui, A., Rouhou, S.C., Grairi, H., Abid, H., Ammar, J., Chelbi, H., et al., 2006. NKT cells in the induced sputum of severe asthmatics. *Mediators Inflamm.* 2006, 71214.
- Hansson, G.K., Robertson, A.-K.L., Söderberg-Nauclér, C., 2006. Inflammation and atherosclerosis. *Annu. Rev. Pathol.* 1, 297–329.
- Hapil, F.Z., Wingender, G., 2018. The interaction between invariant Natural Killer T cells and the mucosal microbiota. *Immunology* 46, 562.

- Harada, K., Isse, K., Tsuneyama, K., Ohta, H., Nakanuma, Y., 2003. Accumulating CD57 + CD3 + natural killer T cells are related to intrahepatic bile duct lesions in primary biliary cirrhosis. *Liver Int.* 23, 94–100.
- Hayakawa, Y., Takeda, K., Yagita, H., Van Kaer, L., Saiki, I., Okumura, K., 2001. Differential regulation of Th1 and Th2 functions of NKT cells by CD28 and CD40 costimulatory pathways. *J. Immunol.* 166, 6012–6018.
- He, Q., Liu, L., Yang, Q., Wang, A., Chen, S., Li, R., et al., 2017. Invariant natural killer T cells promote immunogenic maturation of lung dendritic cells in mouse models of asthma. *Am. J. Physiol. Lung Cell Mol. Physiol.* 313, L973–L990.
- Hegde, S., Fox, L., Wang, X., Gumperz, J.E., 2010. Autoreactive natural killer T cells: promoting immune protection and immune tolerance through varied interactions with myeloid antigen-presenting cells. *Immunology* 130, 471–483.
- Heller, F., Fuss, I.J., Nieuwenhuis, E.E., Blumberg, R.S., Strober, W., 2002. Oxazolone colitis, a Th2 colitis model resembling ulcerative colitis, is mediated by IL-13-producing NK-T cells. *Immunity* 17, 629–638.
- Heron, M., Claessen, A.M.E., Grutters, J.C., 2007. Invariant natural killer T cells in obstructive pulmonary diseases. *N. Engl. J. Med.* 357, 194. author reply 194–5.
- Holzapfel, K.L., Tyznik, A.J., Kronenberg, M., Hogquist, K.A., 2014. Antigen-dependent versus -independent activation of invariant NKT cells during infection. *J. Immunol.* 192, 5490–5498.
- Hong, S., Wilson, M.T., Serizawa, I., Wu, L., Singh, N., Naidenko, O.V., et al., 2001. The natural killer T-cell ligand alpha-galactosylceramide prevents autoimmune diabetes in non-obese diabetic mice. *Nat. Med.* 7, 1052–1056.
- Hu, T., Gimferrer, I., Alberola-Illa, J., 2011. Control of early stages in invariant natural killer T-cell development. *Immunology* 134, 1–7.
- Huang, J.-R., Tsai, Y.-C., Chang, Y.-J., Wu, J.-C., Hung, J.-T., Lin, K.-H., et al., 2014.  $\alpha$ -Galactosylceramide but not phenyl-glycolipids induced NKT cell anergy and IL-33-mediated myeloid-derived suppressor cell accumulation via upregulation of egr2/3. *J. Immunol.* 192, 1972–1981.
- Huang, E., Liu, R., Lu, Z., Liu, J., Liu, X., Zhang, D., et al., 2016. NKT cells mediate the recruitment of neutrophils by stimulating epithelial chemokine secretion during colitis. *Biochem. Biophys. Res. Commun.* 474, 252–258.
- Hugues, S., Mougnéau, E., Ferlin, W., Jeske, D., Hofman, P., Homann, D., et al., 2002. Tolerance to islet antigens and prevention from diabetes induced by limited apoptosis of pancreatic beta cells. *Immunity* 16, 169–181.
- Ikegami, Y., Yokoyama, A., Haruta, Y., Hiyama, K., Kohno, N., 2009. Circulating natural killer T cells in patients with asthma. *J. Asthma* 41, 877–882.
- Ilhan, F., Kandi, B., Akbulut, H., Turgut, D., Cicek, D., 2007. Atopic dermatitis and Valpha24 + natural killer T cells. *Skinmed* 6, 218–220.
- Illés, Z., Kondo, T., Newcombe, J., Oka, N., Tabira, T., Yamamura, T., 2000. Differential expression of NK T cell V alpha 24J alpha Q invariant TCR chain in the lesions of multiple sclerosis and chronic inflammatory demyelinating polyneuropathy. *J. Immunol.* 164, 4375–4381.
- Im, J.S., Arora, P., Bricard, G., Molano, A., Venkataswamy, M.M., Baine, I., et al., 2009. Kinetics and cellular site of glycolipid loading control the outcome of natural killer T cell activation. *Immunity* 30, 888–898.
- Ito, Y., Vela, J.L., Matsumura, F., Hoshino, H., Tyznik, A., Lee, H., et al., 2013. *Helicobacter pylori* cholestryl  $\alpha$ -glucosides contribute to its pathogenicity and immune response by natural killer T cells. *PLoS One* 8, e78191.
- Jacques, P., Venken, K., Van Beneden, K., Hammad, H., Seeuws, S., Drennan, M.B., et al., 2010. Invariant natural killer T cells are natural regulators of murine spondylarthritis. *Arthritis Rheum.* 62, 988–999.
- Jahng, A.W., Maricic, I., Pedersen, B., Burdin, N., Naidenko, O., Kronenberg, M., et al., 2001. Activation of natural killer T cells potentiates or prevents experimental autoimmune encephalomyelitis. *J. Exp. Med.* 194, 1789–1799.
- Jahng, A., Maricic, I., Aguilera, C., Cardell, S., Halder, R.C., Kumar, V., 2004. Prevention of autoimmunity by targeting a distinct, noninvariant CD1d-reactive T cell population reactive to sulfatide. *J. Exp. Med.* 199, 947–957.
- Jariwala, S.P., Abrams, E., Benson, A., Fodeman, J., Zheng, T., 2011. The role of thymic stromal lymphopoietin in the immunopathogenesis of atopic dermatitis. *Clin. Exp. Allergy* 41, 1515–1520.
- Jin, H.M., Kee, S.-J., Cho, Y.-N., Kang, J.-H., Kim, M.-J., Jung, H.-J., et al., 2015. Dysregulated osteoclastogenesis is related to natural killer T cell dysfunction in rheumatoid arthritis. *Arthritis Rheumatol.* 67, 2639–2650.
- Jing, Y., Gravenstein, S., Rao Chaganty, N., Chen, N., Lyerly, K.H., Joyce, S., et al., 2007. Aging is associated with a rapid decline in frequency, alterations in subset composition, and enhanced Th2 response in CD1d-restricted NKT cells from human peripheral blood. *Exp. Gerontol.* 42, 719–732.
- Jordan, M.A., Fletcher, J.M., Pellicci, D., Baxter, A.G., 2007. Slamf1, the NKT cell control gene Nkt1. *J. Immunol.* 178, 1618–1627.
- Jung, S., Shin, H.S., Hong, C., Lee, H., Park, Y.K., Shin, J.H., et al., 2009. Natural killer T cells promote collagen-induced arthritis in DBA/1 mice. *Biochem. Biophys. Res. Commun.* 390, 399–403.
- Jung, S., Park, Y.-K., Shin, J.H., Lee, H., Kim, S.-Y., Lee, G.R., et al., 2010. The requirement of natural killer T-cells in tolerogenic APCs-mediated suppression of collagen-induced arthritis. *Exp. Mol. Med.* 42, 547–554.
- Kaeda, S., Tomi, C., Oki, S., Yamamura, T., Miyake, S., 2007. Activation of invariant natural killer T cells by synthetic glycolipid ligands suppresses autoantibody-induced arthritis. *Arthritis Rheum.* 56, 1836–1845.
- Kain, L., Webb, B., Anderson, B.L., Deng, S., Holt, M., Constanzo, A., et al., 2014. The identification of the endogenous ligands of natural killer T cells reveals the presence of mammalian a-linked glycosylceramides. *Immunity* 41, 543–554.
- Kawano, T., Cui, J., Koezuka, Y., Toura, I., Kaneko, Y., Motoki, K., et al., 1997. CD1d-restricted and TCR-mediated activation of valpha14 NKT cells by glycosylceramides. *Science* 278, 1626–1629.
- Kee, S.-J., Park, Y.-W., Cho, Y.-N., Jin, H.M., Kim, M.-J., Lee, S.-J., et al., 2012. Age- and gender-related differences in circulating natural killer T cells and their subset levels in healthy Korean adults. *Hum. Immunol.* 73, 1011–1016.
- Kenna, T., Golden-Mason, L., Porcelli, S.A., Koezuka, Y., Hegarty, J.E., O'Farrelly, C., et al., 2003. NKT cells from normal and tumor-bearing human livers are phenotypically and functionally distinct from murine NKT cells. *J. Immunol.* 171, 1775–1779.
- Kent, S.C., Chen, Y., Clemmings, S.M., Viglietta, V., Kenyon, N.S., Ricordi, C., et al., 2005. Loss of IL-4 secretion from human type 1a diabetic pancreatic draining lymph node NKT cells. *J. Immunol.* 175, 4458–4464.
- Kielczewski, J.L., Caspi, R.R., 2015. Animal Models of Autoimmune Uveitis. In *Essentials in Ophthalmology*. Springer International Publishing, Cham, pp. 85–100.

- Kim, H.S., Chung, D.H., 2013. IL-9-producing invariant NKT cells protect against DSS-induced colitis in an IL-4-dependent manner. *Mucosal Immunol.* 6, 347–357.
- Kim, C.H., Butcher, E.C., Johnston, B., 2002. Distinct subsets of human Valpha24-invariant NKT cells: cytokine responses and chemokine receptor expression. *Trends Immunol.* 23, 516–519.
- Kim, H.Y., Kim, S., Chung, D.H., 2006. FcgammaRIII engagement provides activating signals to NKT cells in antibody-induced joint inflammation. *J. Clin. Invest.* 116, 2484–2492.
- Kim, J.-O., Kim, D.-H., Chang, W.-S., Hong, C., Park, S.-H., Kim, S., et al., 2004. Asthma is induced by intranasal coadministration of allergen and natural killer T-cell ligand in a mouse model. *J. Allergy Clin. Immunol.* 114, 1332–1338.
- Kim, H.Y., Kim, H.J., Min, H.S., Kim, S., Park, W.S., Park, S.H., et al., 2005. NKT cells promote antibody-induced joint inflammation by suppressing transforming growth factor beta1 production. *J. Exp. Med.* 201, 41–47.
- Kim, D.-H., Chang, W.-S., Lee, Y.-S., Lee, K.-A., Kim, Y.-K., Kwon, B.S., et al., 2008. 4-1BB engagement costimulates NKT cell activation and exacerbates NKT cell ligand-induced airway hyperresponsiveness and inflammation. *J. Immunol.* 180, 2062–2068.
- Kim, H.Y., Pichavant, M., Matangkasombut, P., Koh, Y.I., Savage, P.B., DeKruyff, R.H., et al., 2009. The development of airway hyperreactivity in T-bet-deficient mice requires CD1d-restricted NKT cells. *J. Immunol.* 182, 3252–3261.
- Kim, H.Y., Chang, Y.-J., Subramanian, S., Lee, H.-H., Albacker, L.A., Matangkasombut, P., et al., 2012. Innate lymphoid cells responding to IL-33 mediate airway hyperreactivity independently of adaptive immunity. *J. Allergy Clin. Immunol.* 129, 216–27.e1–6.
- King, I.L., Fortier, A., Tighe, M., Dibble, J., Watts, G.F.M., Veerapen, N., et al., 2012. Invariant natural killer T cells direct B cell responses to cognate lipid antigen in an IL-21-dependent manner. *Nat. Immunol.* 13, 44–50.
- Kinjo, Y., Kronenberg, M., 2005. V alpha14 i NKT cells are innate lymphocytes that participate in the immune response to diverse microbes. *J. Clin. Immunol.* 25, 522–533.
- Kinjo, Y., Wu, D., Kim, G., Xing, G.W., Poles, M.A., Ho, D.D., et al., 2005. Recognition of bacterial glycosphingolipids by natural killer T cells. *Nature* 434, 520–525.
- Kinjo, Y., Tupin, E., Wu, D., Fujio, M., Garcia-Navarro, R., Benhnia, M.R.-E.-I., et al., 2006. Natural killer T cells recognize diacylglycerol antigens from pathogenic bacteria. *Nat. Immunol.* 7, 978–986.
- Kinjo, Y., Illarionov, P., Vela, J.L., Pei, B., Girardi, E., Li, X., et al., 2011. Invariant natural killer T cells recognize glycolipids from pathogenic Gram-positive bacteria. *Nat. Immunol.* 12, 966–974.
- Kis, J., Engelmann, P., Farkas, K., Richman, G., Eck, S., Lolley, J., et al., 2007. Reduced CD4+ subset and Th1 bias of the human iNKT cells in Type 1 diabetes mellitus. *J. Leukoc. Biol.* 81, 654–662.
- Kita, H., Naidenko, O.V., Kronenberg, M., Ansari, A.A., Rogers, P., He, X.-S., et al., 2002. Quantitation and phenotypic analysis of natural killer T cells in primary biliary cirrhosis using a human CD1d tetramer. *Gastroenterology* 123, 1031–1043.
- Kitamura, H., Iwakabe, K., Yahata, T., Nishimura, S., Ohta, A., Ohmi, Y., et al., 1999. The natural killer T (NKT) cell ligand alpha-galactosylceramide demonstrates its immunopotentiating effect by inducing interleukin (IL)-12 production by dendritic cells and IL-12 receptor expression on NKT cells. *J. Exp. Med.* 189, 1121–1128.
- Koh, Y.I., Shim, J.U., Wi, J.O., Han, E.R., Jin, N.C., Oh, S.H., et al., 2010. Inverse association of peripheral blood CD4. *Hum. Immunol.* 71, 186–191.
- Koh, Y.-I., Shim, J.-U., 2010. Association between sputum natural killer T cells and eosinophilic airway inflammation in human asthma. *Int. Arch. Allergy Immunol.* 153, 239–248.
- Koh, Y.-I., Shim, J.-U., Wi, J., Kwon, Y.E., 2012. The role of natural killer T cells in the pathogenesis of acute exacerbation of human asthma. *Int. Arch. Allergy Immunol.* 158, 131–141.
- Kojo, S., Adachi, Y., Keino, H., Taniguchi, M., Sumida, T., 2001. Dysfunction of T cell receptor AV24AJ18+, BV11+ double-negative regulatory natural killer T cells in autoimmune diseases. *Arthritis Rheum.* 44, 1127–1138.
- Kojo, S., Tsutsumi, A., Goto, D., Sumida, T., 2003. Low expression levels of soluble CD1d gene in patients with rheumatoid arthritis. *J. Rheumatol.* 30, 2524–2528.
- Kojo, S., Seino, K.-I., Harada, M., Watarai, H., Wakao, H., Uchida, T., et al., 2005. Induction of regulatory properties in dendritic cells by Valpha14 NKT cells. *J. Immunol.* 175, 3648–3655.
- Kono, F., Honda, T., Aini, W., Manabe, T., Haga, H., Tsuruyama, T., 2014. Interferon- $\gamma$ /CCR5 expression in invariant natural killer T cells and CCL5 expression in capillary veins of dermal papillae correlate with development of psoriasis vulgaris. *Br. J. Dermatol.* 170, 1048–1055.
- Koreck, A., Surányi, A., Szönyi, B.J., Farkas, A., Bata-Csörgö, Z., Kemény, L., et al., 2002. CD3+CD56+ NK T cells are significantly decreased in the peripheral blood of patients with psoriasis. *Clin. Exp. Immunol.* 127, 176–182.
- Kronenberg, M., 2005. Toward an understanding of NKT cell biology: progress and paradoxes. *Annu. Rev. Immunol.* 23, 877–900.
- Kronenberg, M., Gapin, L., 2002. The unconventional lifestyle of NKT cells. *Nat. Rev. Immunol.* 2, 557–568.
- Kukreja, A., Cost, G., Marker, J., Zhang, C., Sun, Z., Lin-Su, K., et al., 2002. Multiple immuno-regulatory defects in type-1 diabetes. *J. Clin. Invest.* 109, 131–140.
- Kyaw, T., Winship, A., Tay, C., Kanellakis, P., Hosseini, H., Cao, A., et al., 2013. Cytotoxic and proinflammatory CD8+ T lymphocytes promote development of vulnerable atherosclerotic plaques in ApoE-deficient mice. *Circulation* 127, 1028–1039.
- Kyriakis, E., Cavallari, M., Andert, J., Philippova, M., Koella, C., Bochkov, V., et al., 2010. Invariant natural killer T cells: linking inflammation and neovascularization in human atherosclerosis. *Eur. J. Immunol.* 40, 3268–3279.
- La Cava, A., Van Kaer, L., Fu-Dong-Shi, 2006. CD4+CD25+ Tregs and NKT cells: regulators regulating regulators. *Trends Immunol.* 27, 322–327.
- Langewouters, A.M.G., Bovenschen, H.J., De jong, E.M.G.J., Van Erp, P.E.J., van de Kerkhof, P.C.M., 2007. The effect of topical corticosteroids in combination with alefacept on circulating T-cell subsets in psoriasis. *J. Dermatol. Treat.* 18, 279–285.
- Lanier, L.L., Chang, C., Phillips, J.H., 1994. Human NKR-P1A. A disulfide-linked homodimer of the C-type lectin superfamily expressed by a subset of NK and T lymphocytes. *J. Immunol.* 153, 2417–2428.
- Lappas, C.M., Day, Y.J., Marshall, M.A., Engelhard, V.H., Linden, J., 2006. Adenosine A2A receptor activation reduces hepatic ischemia reperfusion injury by inhibiting CD1d-dependent NKT cell activation. *J. Exp. Med.* 203, 2639–2648.

- Le Bourhis, L., Martin, E., Péguyillet, I., Guihot, A., Froux, N., Coré, M., et al., 2010. Antimicrobial activity of mucosal-associated invariant T cells. *Nat. Publ. Group* 11, 701–708.
- Le Bourhis, L., Guerri, L., Dusseaux, M., Martin, E., Soudais, C., Lantz, O., 2011. Mucosal-associated invariant T cells: unconventional development and function. *Trends Immunol.* 32, 212–218.
- Lee, D.J., Abeyratne, A., Carson, D.A., Corr, M., 1998. Induction of an antigen-specific, CD1-restricted cytotoxic T lymphocyte response *In vivo*. *J. Exp. Med.* 187, 433–438.
- Lee, P.T., Benlagha, K., Teyton, L., Bendelac, A., 2002a. Distinct functional lineages of human V(alpha)24 natural killer T cells. *J. Exp. Med.* 195, 637–641.
- Lee, P.T., Putnam, A., Benlagha, K., Teyton, L., Gottlieb, P.A., Bendelac, A., 2002b. Testing the NKT cell hypothesis of human IDDM pathogenesis. *J. Clin. Invest.* 110, 793–800.
- Lee, Y.J., Holzapfel, K.L., Zhu, J., Jameson, S.C., Hogquist, K.A., 2013. Steady-state production of IL-4 modulates immunity in mouse strains and is determined by lineage diversity of iNKT cells. *Nat. Publ. Group* 14, 1146–1154.
- Lee, Y.J., Starrett, G.J., Lee, S.T., Yang, R., Henzler, C.M., Jameson, S.C., et al., 2016. Lineage-specific effector signatures of invariant NKT cells are shared amongst  $\gamma\delta$  T, innate lymphoid, and Th cells. *J. Immunol.* 197, 1460–1470.
- Lehuen, A., Lantz, O., Beaudoin, L., Laloux, V., Carnaud, C., Bendelac, A., et al., 1998. Overexpression of natural killer T cells protects Valpha14/Jalpha281 transgenic nonobese diabetic mice against diabetes. *J. Exp. Med.* 188, 1831–1839.
- Leite-de-Moraes, M.C., Hameg, A., Arnould, A., Machavoine, F., Koezuka, Y., Schneider, E., et al., 1999. A distinct IL-18-induced pathway to fully activate NK T lymphocytes independently from TCR engagement. *J. Immunol.* 163, 5871–5876.
- Leite-de-Moraes, M.C., Hameg, A., Pacilio, M., Koezuka, Y., Taniguchi, M., Van Kaer, L., et al., 2001. IL-18 enhances IL-4 production by ligand-activated NKT lymphocytes: a pro-Th2 effect of IL-18 exerted through NKT cells. *J. Immunol.* 166, 945–951.
- Li, B., Sun, R., Wei, H., Gao, B., Tian, Z., 2006. Interleukin-15 prevents concanavalin A-induced liver injury in mice via NKT cell-dependent mechanism. *Hepatology* 43, 1211–1219.
- Li, L., Huang, L., Sung, S.-S.J., Lobo, P.I., Brown, M.G., Gregg, R.K., et al., 2007. NKT cell activation mediates neutrophil IFN-gamma production and renal ischemia-reperfusion injury. *J. Immunol.* 178, 5899–5911.
- Li, W., Ji, F., Zhang, Y., Wang, Y., Yang, N., Ge, H., et al., 2008. Cooperation of invariant NKT cells and CD4 + CD25 + T regulatory cells in prevention of autoimmune diabetes in non-obese diabetic mice treated with alpha-galactosylceramide. *Acta Biochim. Biophys. Sin. (Shanghai)* 40, 381–390.
- Li, S., Joseph, C., Becourt, C., Klibi, J., Luce, S., Dubois-Laforgue, D., et al., 2014. Potential role of IL-17-producing iNKT cells in type 1 diabetes. *PLoS One* 9, e96151.
- Li, Y., To, K., Kanellakis, P., Hosseini, H., Deswaerte, V., Tipping, P., et al., 2015. CD4 + natural killer T cells potently augment aortic root atherosclerosis by perforin- and granzyme B-dependent cytotoxicity. *Circ. Res.* 116, 245–254.
- Li, Y., Kanellakis, P., Hosseini, H., Cao, A., Deswaerte, V., Tipping, P., et al., 2016. A CD1d-dependent lipid antagonist to NKT cells ameliorates atherosclerosis in ApoE-/- mice by reducing lesion necrosis and inflammation. *Cardiovasc. Res.* 109, 305–317.
- Liao, C.-M., Zimmer, M.I., Wang, C.-R., 2013. The functions of type I and type II natural killer T cells in inflammatory bowel diseases. *Inflamm. Bowel. Dis.* 19, 1330–1338.
- Linsen, L., Thewissen, M., Baeten, K., Somers, V., Geusens, P., Raus, J., et al., 2005. Peripheral blood but not synovial fluid natural killer T cells are biased towards a Th1-like phenotype in rheumatoid arthritis. *Arthritis Res. Ther.* 7, R493–R502.
- Lisbonne, M., Diem, S., de Castro Keller, A., Lefort, J., Araujo, L.M., Hachem, P., et al., 2003. Cutting edge: invariant V alpha 14 NKT cells are required for allergen-induced airway inflammation and hyperreactivity in an experimental asthma model. *J. Immunol.* 171, 1637–1641.
- Liu, R., La Cava, A., Bai, X.-F., Jee, Y., Price, M., Campagnolo, D.I., et al., 2005. Cooperation of invariant NKT cells and CD4 + CD25 + T regulatory cells in the prevention of autoimmune myasthenia. *J. Immunol.* 175, 7898–7904.
- Liu, Y., Teige, A., Mondoc, E., Ibrahim, S., Holmdahl, R., Issazadeh-Navikas, S., 2011. Endogenous collagen peptide activation of CD1d-restricted NKT cells ameliorates tissue-specific inflammation in mice. *J. Clin. Invest.* 121, 249–264.
- Lotter, H., Helk, E., Bernin, H., Jacobs, T., Prehn, C., Adamski, J., et al., 2013. Testosterone increases susceptibility to amebic liver abscess in mice and mediates inhibition of IFN $\gamma$  secretion in natural killer T cells. *PLoS One* 8, e55694.
- Lowes, M.A., Kikuchi, T., Fuentes-Duculan, J., Cardinale, I., Zaba, L.C., Haider, A.S., et al., 2008. Psoriasis vulgaris lesions contain discrete populations of Th1 and Th17 T cells. *J. Invest. Dermatol.* 128, 1207–1211.
- López-Sagasta, J., Kung, J.E., Savage, P.B., Gumperz, J., Adams, E.J., 2012. The molecular basis for recognition of CD1d/ $\alpha$ -galactosylceramide by a human non-V $\alpha$ 24 T cell receptor. *PLoS Biol.* 10, e1001412.
- Ly, D., Mi, Q.-S., Hussain, S., Delovitch, T.L., 2006. Protection from type 1 diabetes by invariant NK T cells requires the activity of CD4 + CD25 + regulatory T cells. *J. Immunol.* 177, 3695–3704.
- Ly, D., Tohn, R., Rubin, B., Blumenfeld, H., Besra, G.S., Veerapen, N., et al., 2009. An  $\alpha$ -galactosylceramide C20:2 N-acyl variant enhances anti-inflammatory and regulatory T cell-independent responses that prevent type 1 diabetes. *Clin. Exp. Immunol.* 160, 185–198.
- Lynch, L., O'Farrell, Shea, D., Winter, D.C., Geoghegan, J., Doherty, D.G., O'Farrell, Farrell, C., 2009. Invariant NKT cells and CD1d(+) cells amass in human omentum and are depleted in patients with cancer and obesity. *Eur. J. Immunol.* 39, 1893–1901.
- Lynch, L., Michelet, X., Zhang, S., Brennan, P.J., Moseman, A., Lester, C., et al., 2015. Regulatory iNKT cells lack expression of the transcription factor PLZF and control the homeostasis of T(reg) cells and macrophages in adipose tissue. *Nat. Immunol.* 16, 85–95.
- Maeda, T., Keino, H., Asahara, H., Taniguchi, M., Nishioka, K., Sumida, T., 1999. Decreased TCR AV24AJ18 + double-negative T cells in rheumatoid synovium. *Rheumatology (Oxford)* 38, 186–188.
- Magalhaes, I., Kifayat, B., Lehuen, A., 2015. iNKT and MAIT cell alterations in diabetes. *Front Immunol* 6, 341.
- Magnan, A., Mély, L., Prato, S., Vervloet, D., Romagne, F., Camilla, C., et al., 2000. Relationships between natural T cells, atopy, IgE levels, and IL-4 production. *Allergy* 55, 286–290.
- Majewska-Szczepanik, M., Paust, S., Andrian, von, U.H., Askenase, P.W., Szczepanik, M., 2013. Natural killer cell-mediated contact sensitivity develops rapidly and depends on interferon-alpha, interferon-gamma and interleukin-12. *Immunology* 140, 98–110.

- Major, A.S., Wilson, M.T., McCaleb, J.L., Ru Su, Y., Stanic, A.K., Joyce, S., et al., 2004. Quantitative and qualitative differences in proatherogenic NKT cells in apolipoprotein E-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* 24, 2351–2357.
- Major, A.S., Singh, R.R., Joyce, S., Van Kaer, L., 2006. The role of invariant natural killer T cells in lupus and atherogenesis. *Immunol. Res.* 34, 49–66.
- Mallevaey, T., Zanetta, J.P., Faveeuw, C., Fontaine, J., Maes, E., Platt, F., et al., 2006. Activation of invariant NKT cells by the helminth parasite *schistosoma mansoni*. *J. Immunol.* 176, 2476–2485.
- Mansour, S., Tocheva, A.S., Sanderson, J.P., Goulston, L.M., Platten, H., Serhal, L., et al., 2015. Structural and functional changes of the invariant NKT clonal repertoire in early rheumatoid arthritis. *J. Immunol.* 195, 5582–5591.
- Mars, L.T., Laloux, V., Goude, K., Desbois, S., Saoudi, A., Van Kaer, L., et al., 2002. Cutting edge: V alpha 14-J alpha 281 NKT cells naturally regulate experimental autoimmune encephalomyelitis in nonobese diabetic mice. *J. Immunol.* 168, 6007–6011.
- Mars, L.T., Gautron, A.-S., Novak, J., Beaudoin, L., Diana, J., Liblau, R.S., et al., 2008. Invariant NKT cells regulate experimental autoimmune encephalomyelitis and infiltrate the central nervous system in a CD1d-independent manner. *J. Immunol.* 181, 2321–2329.
- Mars, L.T., Araujo, L., Kerschen, P., Diem, S., Bourgeois, E., Van, L.P., et al., 2009. Invariant NKT cells inhibit development of the Th17 lineage. *Proc. Natl. Acad. Sci. U.S.A.* 106, 6238–6243.
- Matangkasombut, P., Pichavant, M., Yasumi, T., Hendricks, C., Savage, P.B., DeKruyff, R.H., et al., 2008. Direct activation of natural killer T cells induces airway hyperreactivity in nonhuman primates. *J. Allergy Clin. Immunol.* 121, 1287–1289.
- Matangkasombut, P., Marigowda, G., Ervine, A., Idris, L., Pichavant, M., Kim, H.Y., et al., 2009. Natural killer T cells in the lungs of patients with asthma. *J. Allergy Clin. Immunol.* 123, 1181–1185.e1181.
- Matsuda, J.L., Gapin, L., Fazilleau, N., Warren, K., Naidenko, O.V., Kronenberg, M., 2001. Natural killer T cells reactive to a single glycolipid exhibit a highly diverse T cell receptor beta repertoire and small clone size. *Proc. Natl. Acad. Sci. U.S.A.* 98, 12636–12641.
- Matsuda, J.L., Gapin, L., Baron, J.L., Sidobre, S., Stetson, D.B., Mohrs, M., et al., 2003. Mouse V alpha 14i natural killer T cells are resistant to cytokine polarization in vivo. *Proc. Natl. Acad. Sci. U.S.A.* 100, 8395–8400.
- Mattner, J., 2013. Natural killer T (NKT) cells in autoimmune hepatitis. *Curr. Opin. Immunol.* 25, 697–703.
- Mattner, J., Debord, K.L., Ismail, N., Goff, R.D., Cantu, C., Zhou, D., et al., 2005. Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. *Nature* 434, 525–529.
- Mattner, J., Savage, P.B., Leung, P., Oertelt, S.S., Wang, V., Trivedi, O., et al., 2008. Liver autoimmunity triggered by microbial activation of natural killer T cells. *Cell Host Microbe* 3, 304–315.
- McMahon, C.W., Zajac, A.J., Jamieson, A.M., Corral, L., Hammer, G.E., Ahmed, R., et al., 2002. Viral and bacterial infections induce expression of multiple NK cell receptors in responding CD8(+) T cells. *J. Immunol.* 169, 1444–1452.
- McNab, F.W., Berzins, S.P., Pellicci, D.G., Kyriakis, K., Field, K., Smyth, M.J., et al., 2005. The influence of CD1d in postselection NKT cell maturation and homeostasis. *J. Immunol.* 175, 3762–3768.
- McNab, F.W., Pellicci, D.G., Field, K., Besra, G., Smyth, M.J., Godfrey, D.I., et al., 2007. Peripheral NK1.1 NKT cells are mature and functionally distinct from their thymic counterparts. *J. Immunol.* 179, 6630–6637.
- Melián, A., Geng, Y.J., Sukhova, G.K., Libby, P., Porcelli, S.A., 1999. CD1 expression in human atherosclerosis. A potential mechanism for T cell activation by foam cells. *Am. J. Pathol.* 155, 775–786.
- Metelitsa, L.S., 2011. Anti-tumor potential of type-I NKT cells against CD1d-positive and CD1d-negative tumors in humans. *Clin. Immunol.* 140, 119–129.
- Metelitsa, L.S., Weinberg, K.I., Emanuel, P.D., Seeger, R.C., 2003. Expression of CD1d by myelomonocytic leukemias provides a target for cytotoxic NKT cells. *Leukemia* 17, 1068–1077.
- Meyer, E.H., Goya, S., Akbari, O., Berry, G.J., Savage, P.B., Kronenberg, M., et al., 2006. Glycolipid activation of invariant T cell receptor + NK T cells is sufficient to induce airway hyperreactivity independent of conventional CD4+ T cells. *Proc. Natl. Acad. Sci. U.S.A.* 103, 2782–2787.
- Mi, Q.-S., Ly, D., Zucker, P., McGarry, M., Delovitch, T.L., 2004. Interleukin-4 but not interleukin-10 protects against spontaneous and recurrent type 1 diabetes by activated CD1d-restricted invariant natural killer T-cells. *Diabetes* 53, 1303–1310.
- Michalek, J., Vrabelova, Z., Hrotkova, Z., Kyr, M., Pejchlova, M., Kolouskova, S., et al., 2006. Immune regulatory T cells in siblings of children suffering from type 1 diabetes mellitus. *Scand. J. Immunol.* 64, 531–535.
- Michel, M.L., Keller, A.C., Paget, C., Fujio, M., Trottein, F., Savage, P.B., et al., 2007. Identification of an IL-17-producing NK1.1neg iNKT cell population involved in airway neutrophilia. *J. Exp. Med.* 204, 995–1001.
- Michel, M.-L., Mendes-da-Cruz, D., Keller, A.C., Lochner, M., Schneider, E., Dy, M., et al., 2008. Critical role of ROR $\gamma$ t in a new thymic pathway leading to IL-17-producing invariant NKT cell differentiation. *Proc. Natl. Acad. Sci. U.S.A.* 105, 19845–19850.
- Miellet, A., Zhu, R., Diem, S., Boissier, M.-C., Herbelin, A., Bessis, N., 2005. Activation of invariant NK T cells protects against experimental rheumatoid arthritis by an IL-10-dependent pathway. *Eur. J. Immunol.* 35, 3704–3713.
- Miellet-Gafsou, A., Biton, J., Bourgeois, E., Herbelin, A., Boissier, M.-C., Bessis, N., 2010. Early activation of invariant natural killer T cells in a rheumatoid arthritis model and application to disease treatment. *Immunology* 130, 296–306.
- Milpied, P., Massot, B., Renand, A., Diem, S., Herbelin, A., Leite-De-Moraes, M., et al., 2011. IL-17-producing invariant NKT cells in lymphoid organs are recent thymic emigrants identified by neuropilin-1 expression. *Blood* 118, 2993–3002.
- Miyamoto, K., Miyake, S., Yamamura, T., 2001. A synthetic glycolipid prevents autoimmune encephalomyelitis by inducing TH2 bias of natural killer T cells. *Nature* 413, 531–534.
- Mizuno, M., Masumura, M., Tomi, C., Chiba, A., Oki, S., Yamamura, T., et al., 2004. Synthetic glycolipid OCH prevents insulitis and diabetes in NOD mice. *J. Autoimmun.* 23, 293–300.
- Mohammed, J.P., Fusakio, M.E., Rainbow, D.B., Moule, C., Fraser, H.I., Clark, J., et al., 2011. Identification of Cd101 as a susceptibility gene for *Novosphingobium aromaticivorans*-induced liver autoimmunity. *J. Immunol.* 187, 337–349.
- Montalvillo, E., Bernardo, D., Martinez-Abad, B., Allegretti, Y., Fernandez-Salazar, L., Calvo, C., et al., 2015. Increased intraepithelial Valpha24 invariant NKT cells in the celiac duodenum. *Nutrients* 7, 8960–8976.

- Montbarbon, M., Pichavant, M., Langlois, A., Erdual, E., Maggiotto, F., Neut, C., et al., 2013. Colonic inflammation in mice is improved by cigarette smoke through iNKT cells recruitment. *PLoS One* 8, e62208.
- Monteiro, M., Agua-Doce, A., Almeida, C.F., Fonseca-Pereira, D., Veiga-Fernandes, H., Graca, L., 2015. IL-9 expression by invariant NKT cells is not imprinted during thymic development. *J. Immunol.* 195, 3463–3471.
- Montoya, C.J., Pollard, D., Martinson, J., Kumari, K., Wasserfall, C., Mulder, C.B., et al., 2007. Characterization of human invariant natural killer T subsets in health and disease using a novel invariant natural killer T cell-clonotypic monoclonal antibody, 6B11. *Immunology* 122, 1–14.
- Moreira-Teixeira, L., Resende, M., Coffre, M., Devergne, O., Herbeuval, J.P., Hermine, O., et al., 2011. Proinflammatory environment dictates the IL-17-producing capacity of human invariant NKT cells. *J. Immunol.* 186, 5758–5765.
- Moreira-Teixeira, L., Resende, M., Devergne, O., Herbeuval, J.P., Hermine, O., Schneider, E., et al., 2012. Rapamycin combined with TGF-beta converts human invariant NKT cells into suppressive Foxp3+ regulatory cells. *J. Immunol.* 188, 624–631.
- Morita, M., Motoki, K., Akimoto, K., Natori, T., Sakai, T., Sawa, E., et al., 1995. Structure-activity relationship of alpha-galactosylceramides against B16-bearing mice. *J. Med. Chem.* 38, 2176–2187.
- Morshed, S.R.M., Mannoor, K., Halder, R.C., Kawamura, H., Bannai, M., Sekikawa, H., et al., 2002. Tissue-specific expansion of NKT and CD5 + B cells at the onset of autoimmune disease in (NZBxNZW)F1 mice. *Eur. J. Immunol.* 32, 2551–2561.
- Morshed, S.R., Takahashi, T., Savage, P.B., Kambham, N., Strober, S., 2009. Beta-galactosylceramide alters invariant natural killer T cell function and is effective treatment for lupus. *Clin. Immunol.* 132, 321–333.
- Mutalithas, K., Croudace, J., Guillen, C., Siddiqui, S., Thickett, D., Wardlaw, A., et al., 2007. Bronchoalveolar lavage invariant natural killer T cells are not increased in asthma. *J. Allergy Clin. Immunol.* 119, 1274–1276.
- Nagarajan, N.A., Kronenberg, M., 2007. Invariant NKT cells amplify the innate immune response to lipopolysaccharide. *J. Immunol.* 178, 2706–2713.
- Nakai, Y., 2004. Natural killer T cells accelerate atherogenesis in mice. *Blood* 104, 2051–2059.
- Nakamura, K., Tsuchida, T., Tsunemi, Y., Saeki, H., Tamaki, K., 2008. Serum thymic stromal lymphopoietin levels are not elevated in patients with atopic dermatitis. *J. Dermatol.* 35, 546–547.
- Nambiar, J., Clarke, A.W., Shim, D., Mabon, D., Tian, C., Windloch, K., et al., 2015. Potent neutralizing anti-CD1d antibody reduces lung cytokine release in primate asthma model. *MAbs* 7, 638–650.
- Naumov, Y.N., Bahjat, K.S., Gausling, R., Abraham, R., Exley, M.A., Koezuka, Y., et al., 2001. Activation of CD1d-restricted T cells protects NOD mice from developing diabetes by regulating dendritic cell subsets. *Proc. Natl. Acad. Sci. U.S.A.* 98, 13838–13843.
- Nickoloff, B.J., 2000. Characterization of lymphocyte-dependent angiogenesis using a SCID mouse: human skin model of psoriasis. *J. Invest. Dermatol.* 5, 67–73.
- Nie, H., Yang, Q., Zhang, G., Wang, A., He, Q., Liu, M., et al., 2015. Invariant NKT cells act as an adjuvant to enhance Th2 inflammatory response in an OVA-induced mouse model of asthma. *PLoS One* 10, e0119901.
- Nieuwenhuis, E.E.S., Gillessen, S., Schepers, R.J., Exley, M.A., Taniguchi, M., Balk, S.P., et al., 2005. CD1d and CD1d-restricted iNKT-cells play a pivotal role in contact hypersensitivity. *Exp. Dermatol.* 14, 250–258.
- Nieuwenhuis, E.E.S., Matsumoto, T., Lindenbergh, D., Willemsen, R., Kaser, A., Simons-Oosterhuis, Y., et al., 2009. Cd1d-dependent regulation of bacterial colonization in the intestine of mice. *J. Clin. Invest.* 119, 1241–1250.
- Novak, J., Lehuen, A., 2011. Mechanism of regulation of autoimmunity by iNKT cells. *Cytokine* 53, 263–270.
- Novak, J., Beaudoin, L., Griseri, T., Lehuen, A., 2005. Inhibition of T cell differentiation into effectors by NKT cells requires cell contacts. *J. Immunol.* 174, 1954–1961.
- Novak, J., Beaudoin, L., Park, S., Griseri, T., Teyton, L., Bendelac, A., et al., 2007. Prevention of type 1 diabetes by invariant NKT cells is independent of peripheral CD1d expression. *J. Immunol.* 178, 1332–1340.
- Numata, Y., Tazuma, S., Ueno, Y., Nishioka, T., Hyogo, H., Chayama, K., 2005. Therapeutic effect of repeated natural killer T cell stimulation in mouse cholangitis complicated by colitis. *Dig. Dis. Sci.* 50, 1844–1851.
- O'Keeffe, J., Doherty, D.G., Kenna, T., Sheahan, K., O'Donoghue, D.P., Hyland, J.M., et al., 2004. Diverse populations of T cells with NK cell receptors accumulate in the human intestine in health and in colorectal cancer. *Eur. J. Immunol.* 34, 2110–2119.
- O'Keeffe, J., Gately, C.M., Counihan, T., Hennessy, M., Leahy, T., Moran, A.P., et al., 2008. T-cells expressing natural killer (NK) receptors are altered in multiple sclerosis and responses to alpha-galactosylceramide are impaired. *J. Neurol. Sci.* 275, 22–28.
- Ogasawara, K., Takeda, K., Hashimoto, W., Satoh, M., Okuyama, R., Yanai, N., et al., 1998. Involvement of NK1+ T cells and their IFN-gamma production in the generalized Shwartzman reaction. *J. Immunol.* 160, 3522–3527.
- Oh, S.J., Chung, D.H., 2011. Invariant NKT cells producing IL-4 or IL-10, but not IFN-gamma, inhibit the Th1 response in experimental autoimmune encephalomyelitis, whereas none of these cells inhibits the Th17 response. *J. Immunol.* 186, 6815–6821.
- Oh, K., Byoun, O.-J., Ham, D.-I., Kim, Y.S., Lee, D.-S., 2010. Invariant NKT cells regulate experimental autoimmune uveitis through inhibition of Th17 differentiation. *Eur. J. Immunol.* 41, 392–402.
- Ohnishi, Y., Tsutsumi, A., Goto, D., Itoh, S., Matsumoto, I., Taniguchi, M., et al., 2005. TCR Valpha14 natural killer T cells function as effector T cells in mice with collagen-induced arthritis. *Clin. Exp. Immunol.* 141, 47–53.
- Oikawa, Y., Shimada, A., Yamada, S., Motohashi, Y., Nakagawa, Y., Irie, J.-I., et al., 2002. High frequency of valpha24(+) vbeta11(+) T-cells observed in type 1 diabetes. *Diabetes Care* 25, 1818–1823.
- Oishi, Y., Sakamoto, A., Kurasawa, K., Nakajima, H., Nakao, A., Nakagawa, N., et al., 2000. CD4-CD8- T cells bearing invariant Valpha24JalphaQ TCR alpha-chain are decreased in patients with atopic diseases. *Clin. Exp. Immunol.* 119, 404–411.
- Oishi, Y., Sumida, T., Sakamoto, A., Kita, Y., Kurasawa, K., Nawata, Y., et al., 2001. Selective reduction and recovery of invariant Valpha24JalphaQ T cell receptor T cells in correlation with disease activity in patients with systemic lupus erythematosus. *J. Rheumatol.* 28, 275–283.
- Okada, H., Kuhn, C., Feillet, H., Bach, J.F., 2010. The "hygiene hypothesis" for autoimmune and allergic diseases: an update. *Clin. Exp. Immunol.* 160, 1–9.

- Olafsson, S., Gudjonsson, H., Selmi, C., Amano, K., Invernizzi, P., Podda, M., et al., 2004. Antimitochondrial antibodies and reactivity to *N. aromaticivorans* proteins in Icelandic patients with primary biliary cirrhosis and their relatives. *Am. J. Gastroenterol.* 99, 2143–2146.
- Oleinika, K., Rosser, E.C., Matei, D.E., Nistala, K., Bosma, A., Drozdov, I., et al., 2018. CD1d-dependent immune suppression mediated by regulatory B cells through modulations of iNKT cells. *Nature Commun.* 9, 1–58.
- Oling, V., Marttila, J., Knip, M., Simell, O., Ilonen, J., 2007. Circulating CD4 + CD25 high regulatory T cells and natural killer T cells in children with newly diagnosed type 1 diabetes or with diabetes-associated autoantibodies. *Ann. N. Y. Acad. Sci.* 1107, 363–372.
- Olszak, T., An, D., Zeissig, S., Vera, M.P., Richter, J., Franke, A., et al., 2012. Microbial exposure during early life has persistent effects on natural killer T cell function. *Science* 336, 489–493.
- Olszak, T., Neves, J.F., Dowds, C.M., Baker, K., Glickman, J., Davidson, N.O., et al., 2014. Protective mucosal immunity mediated by epithelial CD1d and IL-10. *Nature* 509, 497–502.
- Omenetti, A., Syn, W.-K., Jung, Y., Francis, H., Porrello, A., Witek, R.P., et al., 2009. Repair-related activation of hedgehog signaling promotes cholangiocyte chemokine production. *Hepatology* 50, 518–527.
- Ortaldo, J.R., Winkler-Pickett, R., Wigginton, J., Horner, M., Bere, E.W., Mason, A.T., et al., 2006. Regulation of ITAM-positive receptors: role of IL-12 and IL-18. *Blood* 107, 1468–1475.
- Ostos, M.A., Recalde, D., Zakin, M.M., Scott-Algara, D., 2002. Implication of natural killer T cells in atherosclerosis development during a LPS-induced chronic inflammation. *FEBS Lett.* 519, 23–29.
- Padgett, K.A., Selmi, C., Kenny, T.P., Leung, P.S.C., Balkwill, D.L., Ansari, A.A., et al., 2005. Phylogenetic and immunological definition of four lipoylated proteins from *Novosphingobium aromaticivorans*, implications for primary biliary cirrhosis. *J. Autoimmun.* 24, 209–219.
- Paget, C., Mallevaey, T., Speak, A.O., Torres, D., Fontaine, J., Sheehan, K.C., et al., 2007. Activation of invariant NKT cells by toll-like receptor 9-stimulated dendritic cells requires type I interferon and charged glycosphingolipids. *Immunity* 27, 597–609.
- Paget, C., Ivanov, S., Fontaine, J., Renneson, J., Blanc, F., Pichavant, M., et al., 2012. Interleukin-22 Is Produced by Invariant Natural Killer T Lymphocytes during Influenza A Virus Infection: potential role in protection against lung epithelial damages. *J. Biol. Chem.* 287, 8816–8829.
- Pal, E., Tabira, T., Kawano, T., Taniguchi, M., Miyake, S., Yamamura, T., 2001. Costimulation-dependent modulation of experimental autoimmune encephalomyelitis by ligand stimulation of V alpha 14 NK T cells. *J. Immunol.* 166, 662–668.
- Papp, G., Gyimesi, E., Szabó, K., Zöld, É., Zeher, M., 2016. Increased IL-21 expression induces granzyme B in peripheral CD5 + B cells as a potential counter-regulatory effect in primary Sjögren's syndrome. *Mediators Inflamm.* 2016, 1–8.
- Parekh, V.V., Singh, A.K., Wilson, M.T., Olivares-Villagomez, D., Bezbradica, J.S., Inazawa, H., et al., 2004. Quantitative and qualitative differences in the in vivo response of NKT cells to distinct alpha- and beta-anomeric glycolipids. *J. Immunol.* 173, 3693–3706.
- Parekh, V.V., Wilson, M.T., Van Kaer, L., 2005. iNKT-cell responses to glycolipids. *Crit. Rev. Immunol.* 25, 183–213.
- Parekh, V.V., Wu, L., Olivares-Villagomez, D., Wilson, K.T., Van Kaer, L., 2013. Activated invariant NKT cells control central nervous system autoimmunity in a mechanism that involves myeloid-derived suppressor cells. *J. Immunol.* 190, 1948–1960.
- Parietti, V., Chifflot, H., Sibilia, J., Muller, S., Monneaux, F., 2010. Rituximab treatment overcomes reduction of regulatory iNKT cells in patients with rheumatoid arthritis. *Clin. Immunol.* 134, 331–339.
- Park, S.H., Benlagha, K., Lee, D., Balish, E., Bendelac, A., 2000. Unaltered phenotype, tissue distribution and function of Valpha14(+) NKT cells in germ-free mice. *Eur. J. Immunol.* 30, 620–625.
- Park, S.H., Weiss, A., Benlagha, K., Kyin, T., Teyton, L., Bendelac, A., 2001. The mouse CD1d-restricted repertoire is dominated by a few auto-reactive T cell receptor families. *J. Exp. Med.* 193, 893–904.
- Park, Y., Kim, H.S., Ahn, J.Y., Yun, D., Cho, M.L., Hong, S., et al., 2010. IL-12p35 promotes antibody-induced joint inflammation by activating NKT cells and suppressing TGF-beta. *J. Immunol.* 185, 1476–1484.
- Pellicci, D.G., Hammond, K.J.L., Coquet, J., Kyriakisoudis, K., Brooks, A.G., Kedzierska, K., et al., 2005. DX5/CD49b-positive T cells are not synonymous with CD1d-dependent NKT cells. *J. Immunol.* 175, 4416–4425.
- Peternel, S., Kastelan, M., 2009. Immunopathogenesis of psoriasis: focus on natural killer T cells. *J. Eur. Acad. Dermatol. Venereol.* 23, 1123–1127.
- Pham-Thi, N., de Blic, J., Leite-de-Moraes, M.C., 2006. Invariant natural killer T cells in bronchial asthma. *N. Engl. J. Med.* 354, 2613–2616. author reply 2613–6.
- Pichavant, M., Goya, S., Meyer, E.H., Johnston, R.A., Kim, H.Y., Matangkasombut, P., et al., 2008. Ozone exposure in a mouse model induces airway hyperreactivity that requires the presence of natural killer T cells and IL-17. *J. Exp. Med.* 205, 385–393.
- Postigo, J., Iglesias, M., Cerezo-Wallis, D., Rosal-Vela, A., Garcia-Rodriguez, S., Zubiaur, M., et al., 2012. Mice deficient in CD38 develop an attenuated form of collagen type II-induced arthritis. *PLoS One* 7, e33534.
- Prell, C., Konstantopoulos, N., Heinzelmann, B., Frankenberger, B., Reinhardt, D., Schendel, D.J., et al., 2003. Frequency of Valpha24 + CD161 + natural killer T cells and invariant TCRAV24-AJ18 transcripts in atopic and non-atopic individuals. *Immunobiology* 208, 367–380.
- Price, A.E., Reinhardt, R.L., Liang, H.-E., Locksley, R.M., 2012. Marking and quantifying IL-17A-producing cells in vivo. *PLoS One* 7, e39750.
- Prussin, C., Foster, B., 1997. TCR V alpha 24 and V beta 11 coexpression defines a human NK1 T cell analog containing a unique Th0 subpopulation. *J. Immunol.* 159, 5862–5870.
- Qian, G., Qin, X., Zang, Y.Q., Ge, B., Guo, T.B., Wan, B., et al., 2010. High doses of alpha-galactosylceramide potentiate experimental autoimmune encephalomyelitis by directly enhancing Th17 response. *Nat. Publ. Group* 20, 480–491.
- Raifer, H., Mahiny, A.J., Bollig, N., Petermann, F., Hellhund, A., Kellner, K., et al., 2012. Unlike  $\alpha\beta$  T cells,  $\gamma\delta$  T cells, LTi cells and NKT cells do not require IRF4 for the production of IL-17A and IL-22. *Eur. J. Immunol.* 42, 3189–3201.
- Rauch, J., Gumperz, J., Robinson, C., Skold, M., Roy, C., Young, D.C., et al., 2003. Structural features of the acyl chain determine self-phospholipid antigen recognition by a CD1d-restricted invariant NKT (iNKT) cell. *J. Biol. Chem.* 278, 47508–47515.
- Ren, Y., Sekine-Kondo, E., Shibata, R., Kato-Itoh, M., Umino, A., Yanagida, A., et al., 2017. A novel mouse model of iNKT cell-deficiency generated by CRISPR/Cas9 reveals a pathogenic role of iNKT cells in metabolic disease. *Sci. Rep.* 7, 1–10.

- Reynolds, C., Barkans, J., Clark, P., Kariyawasam, H., Altmann, D., Kay, B., et al., 2009. Natural killer T cells in bronchial biopsies from human allergen challenge model of allergic asthma. *J. Allergy Clin. Immunol.* 124, 860–862. author reply 862.
- Rijavec, M., Volarevic, S., Osolnik, K., Kosnik, M., Korosec, P., 2011. Natural killer T cells in pulmonary disorders. *Respir. Med.* 105 (Suppl 1), S20–S25.
- Rocha-Campos, A.-C., Melki, R., Zhu, R., Deruytter, N., Damotte, D., Dy, M., et al., 2006. Genetic and functional analysis of the Nkt1 locus using congenic NOD mice: improved Valpha14-NKT cell performance but failure to protect against type 1 diabetes. *Diabetes* 55, 1163–1170.
- Rogers, L., Burchat, S., Gage, J., Hasu, M., Thabet, M., Wilcox, L., et al., 2008. Deficiency of invariant V $\sim$ 14 natural killer T cells decreases atherosclerosis in LDL receptor null mice. *Cardiovasc. Res.* 78, 167–174.
- Roman-Gonzalez, A., Moreno, M.E., Alfaro, J.M., Uribe, F., Latorre-Sierra, G., Rugeles, M.T., et al., 2009. Frequency and function of circulating invariant NKT cells in autoimmune diabetes mellitus and thyroid diseases in Colombian patients. *Hum. Immunol.* 70, 262–268.
- Ronet, C., Mempel, M., Thieblemont, N., Lehen, A., Kourilsky, P., Gachelin, G., 2001. Role of the complementarity-determining region 3 (CDR3) of the TCR-beta chains associated with the V alpha 14 semi-invariant TCR alpha-chain in the selection of CD4 $+$  NK T Cells. *J. Immunol.* 166, 1755–1762.
- Rosen, M.J., Chaturvedi, R., Washington, M.K., Kuhnhein, L.A., Moore, P.D., Coggshall, S.S., et al., 2013. STAT6 deficiency ameliorates severity of oxazolone colitis by decreasing expression of claudin-2 and Th2-inducing cytokines. *J. Immunol.* 190, 1849–1858.
- Rossjohn, J., Pellicci, D.G., Patel, O., Gapin, L., Godfrey, D.I., 2012. Recognition of CD1d-restricted antigens by natural killer T cells. *Nat. Rev. Immunol.* 12, 845–857.
- Sag, D., Krause, P., Hedrick, C.C., Kronenberg, M., Wingender, G., 2014. IL-10-producing NKT10 cells are a distinct regulatory invariant NKT cell subset. *J. Clin. Invest.* 124, 3725–3740.
- Sakuishi, K., Oki, S., Araki, M., Porcelli, S.A., Miyake, S., Yamamura, T., 2007. Invariant NKT cells biased for IL-5 production act as crucial regulators of inflammation. *J. Immunol.* 179, 3452–3462.
- Salio, M., Cerundolo, V., 2009. Linking inflammation to natural killer T cell activation. *PLoS Biol.* 7, e1000226.
- Salio, M., Silk, J.D., Yvonne Jones, E., Cerundolo, V., 2014. Biology of CD1- and MR1-restricted T cells. *Annu. Rev. Immunol.* 32, 323–366.
- Sandberg, J.K., Bhardwaj, N., Nixon, D.F., 2003. Dominant effector memory characteristics, capacity for dynamic adaptive expansion, and sex bias in the innate Valpha24 NKT cell compartment. *Eur. J. Immunol.* 33, 588–596.
- Satoh, M., Namba, K.-I., Kitaichi, N., Endo, N., Kitamei, H., Iwata, D., et al., 2016. Invariant natural killer T cells play dual roles in the development of experimental autoimmune uveoretinitis. *Exp. Eye Res.* 153, 79–89.
- Saubermann, L.J., Beck, P., de Jong, Y.P., Pitman, R.S., Ryan, M.S., Kim, H.S., et al., 2000. Activation of natural killer T cells by alpha-galactosylceramide in the presence of CD1d provides protection against colitis in mice. *Gastroenterology* 119, 119–128.
- Saxena, V., Ondr, J.K., Magnusen, A.F., Munn, D.H., Katz, J.D., 2007. The countervailing actions of myeloid and plasmacytoid dendritic cells control autoimmune diabetes in the nonobese diabetic mouse. *J. Immunol.* 179, 5041–5053.
- Sáez de Guinoa, J., Jimeno, R., Gaya, M., Kipling, D., Garzón, M.J., Dunn Walters, D., et al., 2018. CD1d-mediated lipid presentation by CD11c $+$  cells regulates intestinal homeostasis. *EMBO J.* 37, e97537.
- Scheuplein, F., Lamont, D.J., Poynter, M.E., Boyson, J.E., Serreze, D., Lundblad, L.K.A., et al., 2015. Mouse invariant monoclonal antibody NKT14: a novel tool to manipulate iNKT cell function in vivo. *PLoS One* 10, e0140729.
- Schiechl, G., Bauer, B., Fuss, I., Lang, S.A., Moser, C., Ruemmele, P., et al., 2011. Tumor development in murine ulcerative colitis depends on MyD88 signaling of colonic F4/80 $+$  CD11b(high)Gr1(low) macrophages. *J. Clin. Invest.* 121, 1692–1708.
- Schmieg, J., Yang, G., Franck, R.W., Tsuji, M., 2003. Superior protection against malaria and melanoma metastases by a C-glycoside analogue of the natural killer T cell ligand alpha-Galactosylceramide. *J. Exp. Med.* 198, 1631–1641.
- Schrumpf, E., Tan, C., Karlsen, T.H., Sponheim, J., Bjorkstrom, N.K., Sundnes, O., et al., 2015. The biliary epithelium presents antigens to and activates natural killer T cells. *Hepatology* 62, 1249–1259.
- Segawa, S., Goto, D., Yoshiga, Y., Hayashi, T., Matsumoto, I., Ito, S., et al., 2009. Low levels of soluble CD1d protein alters NKT cell function in patients with rheumatoid arthritis. *Int. J. Mol. Med.* 24, 481–486.
- Selathurai, A., Deswaerde, V., Kanellakis, P., Tipping, P., Toh, B.-H., Bobik, A., et al., 2014. Natural killer (NK) cells augment atherosclerosis by cytotoxic-dependent mechanisms. *Cardiovasc. Res.* 102, 128–137.
- Selmi, C., Balkwill, D.L., Invernizzi, P., Ansari, A.A., Coppel, R.L., Podda, M., et al., 2003. Patients with primary biliary cirrhosis react against a ubiquitous xenobiotic-metabolizing bacterium. *Hepatology* 38, 1250–1257.
- Selvanantham, T., Lin, Q., Guo, C.X., Surendra, A., Fieve, S., Escalante, N.K., et al., 2016. NKT cell-deficient mice harbor an altered microbiota that fuels intestinal inflammation during chemically induced colitis. *J. Immunol.* 197, 4464–4472.
- Sen, Y., Yongyi, B., Yuling, H., Luokun, X., Li, H., Jie, X., et al., 2005. V alpha 24-invariant NKT cells from patients with allergic asthma express CCR9 at high frequency and induce Th2 bias of CD3 $+$  T cells upon CD226 engagement. *J. Immunol.* 175, 4914–4926.
- Sharif, S., Arreaza, G.A., Zucker, P., Mi, Q.S., Sondhi, J., Naidenko, O.V., et al., 2001. Activation of natural killer T cells by alpha-galactosylceramide treatment prevents the onset and recurrence of autoimmune Type 1 diabetes. *Nat. Med.* 7, 1057–1062.
- Shen, L., Zhang, H., Caimol, M., Benike, C.J., Chakravarty, E.F., Strober, S., et al., 2014. Invariant natural killer T cells in lupus patients promote IgG and IgG autoantibody production. *Eur. J. Immunol.* 45, 612–623.
- Shen, S., Prame Kumar, K., Stanley, D., Moore, R.J., Van, T.T.H., Wen, S.W., et al., 2018. Invariant natural killer T cells shape the gut microbiota and regulate neutrophil recruitment and function during intestinal inflammation. *Front. Immunol.* 9, 999.
- Shi, F.D., Flodstrom, M., Balasa, B., Kim, S.H., Van Gunst, K., Strominger, J.L., et al., 2001. Germ line deletion of the CD1 locus exacerbates diabetes in the NOD mouse. *Proc. Natl. Acad. Sci. U.S.A.* 98, 6777–6782.
- Shigematsu, H., Kumagai, K., Kobayashi, H., Eguchi, T., Kitaura, K., Suzuki, S., et al., 2014. Accumulation of metal-specific T cells in inflamed skin in a novel murine model of chromium-induced allergic contact dermatitis. *PLoS One* 9, e85983.
- Shim, J.-U., Koh, Y.-I., 2014. Increased Th2-like invariant natural killer T cells in peripheral blood from patients with asthma. *Allergy Asthma Immunol. Res.* 6, 444.

- Shimamura, M., Huang, Y.-Y., 2002. Presence of a novel subset of NKT cells bearing an invariant V(alpha)19.1-J(alpha)26 TCR alpha chain. *FEBS Lett.* 516, 97–100.
- Shimizuhira, C., Otsuka, A., Honda, T., Kitoh, A., Egawa, G., Nakajima, S., et al., 2014. Natural killer T cells are essential for the development of contact hypersensitivity in BALB/c mice. *J. Invest. Dermatol.* 134, 2709–2718.
- Shiozaki, M., Tashiro, T., Koshino, H., Shigeura, T., Watarai, H., Taniguchi, M., et al., 2013. Synthesis and biological activity of hydroxylated analogues of KRN7000 ( $\alpha$ -galactosylceramide). *Carbohydr. Res.* 370, 46–66.
- Simon, D., Kozlowski, E., Simon, H., 2009. Natural killer T cells expressing IFN-gamma and IL-4 in lesional skin of atopic eczema. *Allergy* 64, 1681–1684.
- Simoni, Y., Gautron, A.-S., Beaudoin, L., Bui, L.-C., Michel, M.-L., Coumoul, X., et al., 2011. NOD mice contain an elevated frequency of iNKT17 cells that exacerbate diabetes. *Eur. J. Immunol.* 41, 3574–3585.
- Simoni, Y., Diana, J., Ghazarian, L., Beaudoin, L., Lehuen, A., 2012. Therapeutic manipulation of natural killer (NK) T cells in autoimmunity: are we close to reality? *Clin. Exp. Immunol.* 171, 8–19.
- Singh, N., Hong, S., Scherer, D.C., Serizawa, I., Burdin, N., Kronenberg, M., et al., 1999. Cutting edge: activation of NK T cells by CD1d and alpha-galactosylceramide directs conventional T cells to the acquisition of a Th2 phenotype. *J. Immunol.* 163, 2373–2377.
- Singh, A.K., Wilson, M.T., Hong, S., Olivares-Villagomez, D., Du, C., Stanic, A.K., et al., 2001. Natural killer T cell activation protects mice against experimental autoimmune encephalomyelitis. *J. Exp. Med.* 194, 1801–1811.
- Singh, A.K., Yang, J.-Q., Parekh, V.V., Wei, J., Wang, C.-R., Joyce, S., et al., 2005. The natural killer T cell ligand alpha-galactosylceramide prevents or promotes pristane-induced lupus in mice. *Eur. J. Immunol.* 35, 1143–1154.
- Sireci, G., Russo, D., Dieli, F., Porcelli, S.A., Taniguchi, M., La Manna, M.P., et al., 2007. Immunoregulatory role of Jalpha281 T cells in aged mice developing lupus-like nephritis. *Eur. J. Immunol.* 37, 425–433.
- Skold, M., Cardell, S., 2000. Differential regulation of Ly49 expression on CD4+ and CD4-CD8- (double negative) NK1.1+ T cells. *Eur. J. Immunol.* 30, 2488–2496.
- Slifka, M.K., Pagarigan, R.R., Whitton, J.L., 2000. NK markers are expressed on a high percentage of virus-specific CD8+ and CD4+ T cells. *J. Immunol.* 164, 2009–2015.
- Smith, E., Croca, S., Waddington, K.E., Sofat, R., Griffin, M., Nicolaides, A., et al., 2016. Cross-talk between iNKT cells and monocytes triggers an atheroprotective immune response in SLE patients with asymptomatic plaque. *Sci. Immunol.* 1, pii: eaah4081.
- Smithgall, M.D., Comeau, M.R., Park Yoon, B.R., Kaufman, D., Armitage, R., Smith, D.E., 2008. IL-33 amplifies both Th1- and Th2-type responses through its activity on human basophils, allergen-reactive Th2 cells, iNKT and NK cells. *Int. Immunopharmacol.* 20, 1019–1030.
- Smyth, M.J., Taniguchi, M., Street, S.E., 2000a. The anti-tumor activity of IL-12: mechanisms of innate immunity that are model and dose dependent. *J. Immunol.* 165, 2665–2670.
- Smyth, M.J., Thia, K.Y., Street, S.E., Cretney, E., Trapani, J.A., Taniguchi, M., et al., 2000b. Differential tumor surveillance by natural killer (NK) and NKT cells. *J. Exp. Med.* 191, 661–668.
- Snyder-Cappione, J.E., Tincati, C., Eccles-James, I.G., Cappione, A.J., Ndhlovu, L.C., Koth, L.L., et al., 2010. A comprehensive ex vivo functional analysis of human NKT cells reveals production of MIP1- $\alpha$  and MIP1- $\beta$ , a lack of IL-17, and a Th1-bias in males. *PLoS One* 5, e15412.
- Soh, S.Y., Faveeuw, C., Thiam, C.H., Khoo, L.H.B., Yeo, K.P., Lim, S.Y., et al., 2016. NKT cell hyporesponsiveness leads to unrestrained accumulation of marginal zone B cells in hypercholesterolemic apolipoprotein E-deficient mice. *J. Immunol.* 197, 3894–3904.
- Soumelis, V., Reche, P.A., Kanzler, H., Yuan, W., Edward, G., Homey, B., et al., 2002. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat. Immunol.* 3, 673–680.
- Sowden, E., Ng, W.F., 2012. Invariant natural killer T cells in rheumatoid arthritis and other inflammatory arthritides. *Rheum. Arthritis Etiol.* doi:10.5772/28269.
- Sprent, J., Surh, C.D., 2002. T cell memory. *Annu. Rev. Immunol.* 20, 551–579.
- Stepniak, D., Koning, F., 2006. Celiac disease—sandwiched between innate and adaptive immunity. *Hum. Immunol.* 67, 460–468.
- Stetson, D.B., Mohrs, M., Reinhardt, R.L., Baron, J.L., Wang, Z.-E., Gapin, L., et al., 2003. Constitutive cytokine mRNAs mark natural killer (NK) and NK T cells poised for rapid effector function. *J. Exp. Med.* 198, 1069–1076.
- Ström, A., Wigren, M., Hultgårdh-Nilsson, A., Saxena, A., Gomez, M.F., Cardell, S., et al., 2007. Involvement of the CD1d-natural killer T cell pathway in neointima formation after vascular injury. *Circ. Res.* 101, e83–e89.
- Sullivan, B.A., Nagarajan, N.A., Wingender, G., Wang, J., Scott, I., Tsuji, M., et al., 2010. Mechanisms for glycolipid antigen-driven cytokine polarization by Valpha14i NKT cells. *J. Immunol.* 184, 141–153.
- Sumida, T., Sakamoto, A., Murata, H., Makino, Y., Takahashi, H., Yoshida, S., et al., 1995. Selective reduction of T cells bearing invariant V alpha 24J alpha Q antigen receptor in patients with systemic sclerosis. *J. Exp. Med.* 182, 1163–1168.
- Sumida, T., Maeda, T., Taniguchi, M., Nishioka, K., Stohl, W., 1998. TCR AV24 gene expression in double negative T cells in systemic lupus erythematosus. *Lupus* 7, 565–568.
- Takagi, D., Iwabuchi, K., Maeda, M., Nakamaru, Y., Furuta, Y., Fukuda, S., et al., 2006. Natural killer T cells ameliorate antibody-induced arthritis in macrophage migration inhibitory factor transgenic mice. *Int. J. Mol. Med.* 18, 829–836.
- Takahashi, T., Strober, S., 2008. Natural killer T cells and innate immune B cells from lupus-prone NZB/W mice interact to generate IgM and IgG autoantibodies. *Eur. J. Immunol.* 38, 156–165.
- Takahashi, T., Nakamura, K., Chiba, S., Kanda, Y., Tamaki, K., Hirai, H., 2003. V alpha 24+ natural killer T cells are markedly decreased in atopic dermatitis patients. *Hum. Immunol.* 64, 586–592.
- Tangri, S., Brossay, L., Burdin, N., Lee, D.J., Corr, M., Kronenberg, M., 1998. Presentation of peptide antigens by mouse CD1 requires endosomal localization and protein antigen processing. *Proc. Natl. Acad. Sci. U.S.A.* 95, 14314–14319.
- Taniguchi, M., Harada, M., Kojo, S., Nakayama, T., Wakao, H., 2003. The regulatory role of Valpha14 NKT cells in innate and acquired immune response. *Annu. Rev. Immunol.* 21, 483–513.
- Tard, C., Rouxel, O., Lehuen, A., 2015. Regulatory role of natural killer T cells in diabetes. *Biomed. J.* 38, 484–495.
- Tatituri, R.V.V., Watts, G.F.M., Bhowruth, V., Barton, N., Rothchild, A., Hsu, F.-F., et al., 2013. Recognition of microbial and mammalian phospholipid antigens by NKT cells with diverse TCRs. *Proc. Natl. Acad. Sci. U.S.A.* 110, 1827–1832.

- Teige, A., Teige, I., Lavasani, S., Bockermann, R., Mondoc, E., Holmdahl, R., et al., 2004. CD1-dependent regulation of chronic central nervous system inflammation in experimental autoimmune encephalomyelitis. *J. Immunol.* 172, 186–194.
- Teige, A., Bockermann, R., Hasan, M., Olofsson, K.E., Liu, Y., Issazadeh-Navikas, S., 2010. CD1d-dependent NKT cells play a protective role in acute and chronic arthritis models by ameliorating antigen-specific Th1 responses. *J. Immunol.* 185, 345–356.
- Terashima, A., Watarai, H., Inoue, S., Sekine, E., Nakagawa, R., Hase, K., et al., 2008. A novel subset of mouse NKT cells bearing the IL-17 receptor B responds to IL-25 and contributes to airway hyperreactivity. *J. Exp. Med.* 205, 2727–2733.
- Thomas, S.Y., Lilly, C.M., Luster, A.D., 2006. Invariant natural killer T cells in bronchial asthma. *N. Engl. J. Med.* 354, 2613–2616. author reply 2613–6.
- Thomas, S.Y., Banerji, A., Medoff, B.D., Lilly, C.M., Luster, A.D., 2007. Multiple chemokine receptors, including CCR6 and CXCR3, regulate antigen-induced T cell homing to the human asthmatic airway. *J. Immunol.* 179, 1901–1912.
- Thomas, S.Y., Chyung, Y.H., Luster, A.D., 2010. Natural killer T cells are not the predominant T cell in asthma and likely modulate, not cause, asthma. *J. Allergy Clin. Immunol.* 125, 980–984.
- To, K., Agrotis, A., Besra, G., Bobik, A., Toh, B.H., 2009. NKT cell subsets mediate differential proatherogenic effects in ApoE-/ mice. *Arterioscler. Thromb. Vasc. Biol.* 29, 671–677.
- Tobin, A.-M., Lynch, L., Kirby, B., O'Farrelly, C., 2011. Natural killer cells in psoriasis. *J. Innate Immun.* 3, 403–410.
- Tocheva, A.S., Mansour, S., Holt, T.G.H., Jones, S., Chancellor, A., Sanderson, J.P., et al., 2017. The clonal invariant NKT cell repertoire in people with type 1 diabetes is characterized by a loss of clones expressing high-affinity TCRs. *J. Immunol.* 198, 1452–1459.
- Tonti, E., Fedeli, M., Napolitano, A., Iannacone, M., Andrian, von, U.H., et al., 2012. Follicular helper NKT cells induce limited B cell responses and germinal center formation in the absence of CD4(+) T cell help. *J. Immunol.* 188, 3217–3222.
- Torres, D., Paget, C., Fontaine, J., Mallevaey, T., Matsuoka, T., Maruyama, T., et al., 2008. Prostaglandin D2 inhibits the production of IFN-gamma by invariant NK T cells: consequences in the control of B16 melanoma. *J. Immunol.* 180, 783–792.
- Treiner, E., Duban, L., Bahram, S., Radosavljevic, M., Wanner, V., Tilloy, F., et al., 2003. Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. *Nature* 422, 164–169.
- Tsuneyama, K., Yasoshima, M., Harada, K., Hiramatsu, K., Gershwin, M.E., Nakanuma, Y., 1998. Increased CD1d expression on small bile duct epithelium and epithelioid granuloma in livers in primary biliary cirrhosis. *Hepatology* 28, 620–623.
- Tsutsumi, Y., Jie, X., Ihara, K., Nomura, A., Kanemitsu, S., Takada, H., et al., 2006. Phenotypic and genetic analyses of T-cell-mediated immunoregulation in patients with Type 1 diabetes. *Diabet. Med.* 23, 1145–1150.
- Tudhope, S.J., Delwig, von, A., Falconer, J., Pratt, A., Woolridge, T., et al., 2010. Profound invariant natural killer T-cell deficiency in inflammatory arthritis. *Ann. Rheum. Dis.* 69, 1873–1879.
- Tupin, E., Nicoletti, A., Elhage, R., Rudling, M., Ljunggren, H.-G., Hansson, G.K., et al., 2004. CD1d-dependent activation of NKT cells aggravates atherosclerosis. *J. Exp. Med.* 199, 417–422.
- Tupin, E., Kinjo, Y., Kronenberg, M., 2007. The unique role of natural killer T cells in the response to microorganisms. *Nat. Rev. Microbiol.* 5, 405–417.
- Tyznik, A.J., Tupin, E., Nagarajan, N.A., Her, M.J., Benedict, C.A., Kronenberg, M., 2008. Cutting edge: the mechanism of invariant NKT cell responses to viral danger signals. *J. Immunol.* 181, 4452–4456.
- Uchida, T., Nakashima, H., Yamagata, A., Ito, S., Ishikiriyama, T., Nakashima, M., et al., 2018. Repeated administration of alpha-galactosylceramide ameliorates experimental lupus nephritis in mice. *Sci. Rep.* 8, 8225.
- Ueno, Y., Tanaka, S., Sumii, M., Miyake, S., Tazuma, S., Taniguchi, M., et al., 2005. Single dose of OCH improves mucosal T helper type 1/T helper type 2 cytokine balance and prevents experimental colitis in the presence of valpha14 natural killer T cells in mice. *Inflamm. Bowel Dis.* 11, 35–41.
- Uibo, R., Kisand, K., Yang, C.-Y., Gershwin, M.E., 2012. Primary biliary cirrhosis: a multi-faced interactive disease involving genetics, environment and the immune response. *Apmis* 120, 857–871.
- Uldrich, A.P., Patel, O., Cameron, G., Pellicci, D.G., Day, E.B., Sullivan, L.C., et al., 2011. A semi-invariant  $\text{V}\alpha 10 +$  T cell antigen receptor defines a population of natural killer T cells with distinct glycolipid antigen-recognition properties. *Nat. Immunol.* 12, 616–623.
- Umetsu, D.T., DeKruyff, R.H., 2010. Natural killer T cells are important in the pathogenesis of asthma: The many pathways to asthma. *J. Allergy Clin. Immunol.* 125, 975–979.
- van Der Vliet, H.J., Nishi, N., de Gruijl, T.D., Blomberg, von, B.M., van den Eertwegh, A.J., et al., 2000. Human natural killer T cells acquire a memory-activated phenotype before birth. *Blood* 95, 2440–2442.
- van der Vliet, H.J.J., Blomberg, von, B.M.E., Nishi, N., Reijm, M., Voskuyl, A.E., et al., 2001. Circulating  $\text{V}\alpha 24 + \text{V}\beta 11 +$  NKT cell numbers are decreased in a wide variety of diseases that are characterized by autoreactive tissue damage. *Clin. Immunol.* 100, 144–148.
- van der Vliet, H.J.J., Molling, J.W., Blomberg, von, B.M.E., Nishi, N., Kolgen, W., et al., 2004. The immunoregulatory role of CD1d-restricted natural killer T cells in disease. *Clin. Immunol.* 112, 8–23.
- Van Kaer, L., Parekh, V.V., Wu, L., 2015. The response of CD1d-restricted invariant NKT cells to microbial pathogens and their products. *Front. Immunol.* 6, 177.
- van Puijvelde, G.H.M., Kuiper, J., 2017. NKT cells in cardiovascular diseases. *Eur. J. Pharmacol.* 816, 47–57.
- van Puijvelde, G.H.M., van Wanrooij, E.J.A., Hauer, A.D., de Vos, P., van Berkel, T.J.C., Kuiper, J., 2009. Effect of natural killer T cell activation on initiation of atherosclerosis. *Thromb Haemost.* 102, 223–230.
- Van Rhijn, I., Kasmar, A., de Jong, A., Gras, S., Bhati, M., Doorenspleet, M.E., et al., 2013. A conserved human T cell population targets mycobacterial antigens presented by CD1b. *Nat. Publ. Group* 14, 706–713.
- Vanderlaan, P.A., Reardon, C.A., Sagiv, Y., Blachowicz, L., Lukens, J., Nissenbaum, M., et al., 2007. Characterization of the natural killer T-cell response in an adoptive transfer model of atherosclerosis. *Am. J. Pathol.* 170, 1100–1107.
- Vieth, J.A., Das, J., Ranaivoson, F.M., Comolatti, D., Denzin, L.K., Sant'Angelo, D.B., 2016. TCR $\alpha$ -TCR $\beta$  pairing controls recognition of CD1d and directs the development of adipose NKT cells. *Nat. Immunol.* 18, 36–44.
- Vijayanand, P., Seumois, G., Pickard, C., Powell, R.M., Angco, G., Sammut, D., et al., 2007. Invariant natural killer T cells in asthma and chronic obstructive pulmonary disease. *N. Engl. J. Med.* 356, 1410–1422.

- Waddell, A., Zhao, J., Cantorna, M.T., 2015. NKT cells can help mediate the protective effects of 1,25-dihydroxyvitamin D3 in experimental autoimmune encephalomyelitis in mice. *Int. Immunol.* 27, 237–244.
- Wang, B., Geng, Y.B., Wang, C.R., 2001. CD1-restricted NK T cells protect nonobese diabetic mice from developing diabetes. *J. Exp. Med.* 194, 313–320.
- Wang, J., Cho, S., Ueno, A., Cheng, L., Xu, B.-Y., Desrosiers, M.D., et al., 2008. Ligand-dependent induction of noninflammatory dendritic cells by anergic invariant NKT cells minimizes autoimmune inflammation. *J. Immunol.* 181, 2438–2445.
- Wang, J., Cheng, L., Wondimu, Z., Swain, M., Santamaria, P., Yang, Y., 2009. Cutting edge: CD28 engagement releases antigen-activated invariant NKT cells from the inhibitory effects of PD-1. *J. Immunol.* 182, 6644–6647.
- Wang, J.J., Yang, G.X., Zhang, W.C., Lu, L., Tsuneyama, K., Kronenberg, M., et al., 2014. *Escherichia coli* infection induces autoimmune cholangitis and anti-mitochondrial antibodies in non-obese diabetic (NOD) B6 (Idd10/Idd18) mice. *Clin. Exp. Immunol.* 175, 192–201.
- Watanabe, H., Miyaji, C., Kawachi, Y., Imai, T., Ohtsuka, K., Iwanage, T., et al., 1995. Relationships between intermediate TCR cells and NK1.1+ T cells in various immune organs. NK1.1+ T cells are present within a population of intermediate TCR cells. *J. Immunol.* 155, 2972–2983.
- Weaver, C.T., Hatton, R.D., 2009. Interplay between the TH17 and TReg cell lineages: a (co-)evolutionary perspective. *Nat. Rev. Immunol.* 9, 883–889.
- Weaver, C.T., Hatton, R.D., Mangan, P.R., Harrington, L.E., 2007. IL-17 family cytokines and the expanding diversity of effector T cell lineages. *Annu. Rev. Immunol.* 25, 821–852.
- Wermeling, F., Lind, S.M., Jordó, E.D., Cardell, S.L., Karlsson, M.C.I., 2010. Invariant NKT cells limit activation of autoreactive CD1d-positive B cells. *J. Exp. Med.* 207, 943–952.
- Wieland Brown, L.C., Penaranda, C., Kashyap, P.C., Williams, B.B., Clardy, J., Kronenberg, M., et al., 2013. Production of  $\alpha$ -galactosylceramide by a prominent member of the human gut microbiota. *PLoS Biol.* 11, e1001610.
- Wiethe, C., Schiemann, M., Busch, D., Haeberle, L., Kopf, M., Schuler, G., et al., 2007. Interdependency of MHC class II/self-peptide and CD1d/self-glycolipid presentation by TNF-matured dendritic cells for protection from autoimmunity. *J. Immunol.* 178, 4908–4916.
- Wilson, S.B., Kent, S.C., Patton, K.T., Orban, T., Jackson, R.A., Exley, M., et al., 1998. Extreme Th1 bias of invariant Valpha24JalphaQ T cells in type 1 diabetes. *Nature* 391, 177–181.
- Wilson, S.B., Kent, S.C., Horton, H.F., Hill, A.A., Bollyky, P.L., Hafler, D.A., et al., 2000. Multiple differences in gene expression in regulatory Valpha 24Jalpha Q T cells from identical twins discordant for type I diabetes. *Proc. Natl. Acad. Sci. U.S.A.* 97, 7411–7416.
- Wingender, G., 2016. From the Deep Sea to Everywhere: Environmental Antigens for iNKT Cells. *Arch. Immunol. Ther. Exp. (Warsz)* 64, 291–298.
- Wingender, G., Kronenberg, M., 2008. Role of NKT cells in the digestive system. IV. The role of canonical natural killer T cells in mucosal immunity and inflammation. *Am. J. Physiol. Gastrointest. Liver Physiol.* 294, G1–G8.
- Wingender, G., Berg, M., Jüngerkes, F., Diehl, L., Sullivan, B.A., Kronenberg, M., et al., 2006. Immediate antigen-specific effector functions by TCR-transgenic CD8+ NKT cells. *Eur. J. Immunol.* 36, 570–582.
- Wingender, G., Krebs, P., Beutler, B., Kronenberg, M., 2010. Antigen-specific cytotoxicity by invariant NKT cells in vivo is CD95/CD178-dependent and is correlated with antigenic potency. *J. Immunol.* 185, 2721–2729.
- Wingender, G., Rogers, P., Batzer, G., Lee, M.S., Bai, D., Pei, B., et al., 2011. Invariant NKT cells are required for airway inflammation induced by environmental antigens. *J. Exp. Med.* 208, 1151–1162.
- Wingender, G., Hiss, M., Engel, I., Peukert, K., Ley, K., Haller, H., et al., 2012a. Neutrophilic granulocytes modulate invariant NKT cell function in mice and humans. *J. Immunol.* 188, 3000–3008.
- Wingender, G., Stepniak, D., Krebs, P., Lin, L., McBride, S., Wei, B., et al., 2012b. Intestinal microbes affect phenotypes and functions of invariant natural killer T cells in mice. *Gastroenterology* 143, 418–428.
- Wingender, G., Birkholz, A.M., Sag, D., Farber, E., Chitale, S., Howell, A.R., et al., 2015a. Selective conditions are required for the induction of invariant NKT cell hyporesponsiveness by antigenic stimulation. *J. Immunol.* 195, 3838–3848.
- Wingender, G., Sag, D., Kronenberg, M., 2015b. NKT10 cells: a novel iNKT cell subset. *Oncotarget* 6, 26552–26553.
- Wintermeyer, P., Cheng, C.W., Gehring, S., Hoffman, B.L., Holub, M., Brossay, L., et al., 2009. Invariant natural killer T cells suppress the neutrophil inflammatory response in a mouse model of cholestatic liver damage. *Gastroenterology* 136, 1048–1059.e2.
- Wither, J., Cai, Y.-C., Lim, S., McKenzie, T., Roslin, N., Claudio, J.O., et al., 2008. Reduced proportions of natural killer T cells are present in the relatives of lupus patients and are associated with autoimmunity. *Arthritis Res. Ther.* 10, R108.
- Wong, P.T.Y., Wong, C.K., Tam, L.S., Li, E.K., Chen, D.P., Lam, C.W.K., 2009. Decreased expression of T lymphocyte co-stimulatory molecule CD26 on invariant natural killer T cells in systemic lupus erythematosus. *Immunol. Invest.* 38, 350–364.
- Wong, C.H.Y., Jenne, C.N., Lee, W.-Y., Léger, C., Kubes, P., 2011. Functional innervation of hepatic iNKT cells is immunosuppressive following stroke. *Science* 334, 101–105.
- Wu, D.Y., Segal, N.H., Sidobre, S., Kronenberg, M., Chapman, P.B., 2003. Cross-presentation of disialoganglioside GD3 to natural killer T cells. *J. Exp. Med.* 198, 173–181.
- Wu, W.H., Park, C.O., Oh, S.H., Kim, H.J., Kwon, Y.S., Bae, B.G., et al., 2010. Thymic stromal lymphopoietin-activated invariant natural killer T cells trigger an innate allergic immune response in atopic dermatitis. *J. Allergy Clin. Immunol.* 126, 290–299., 299.e1–4.
- Wu, S.-J., Yang, Y.-H., Tsuneyama, K., Leung, P.S.C., Illarionov, P., Gershwin, M.E., et al., 2011. Innate immunity and primary biliary cirrhosis: activated invariant natural killer T cells exacerbate murine autoimmune cholangitis and fibrosis. *Hepatology* 53, 915–925.
- Yan-ming, L., Lan-fang, C., Chen, L., Ya-qin, L., Wei, C., Wen-ming, Z., 2012. The effect of specific immunotherapy on natural killer T cells in peripheral blood of house dust mite-sensitized children with asthma. *Clin. Dev. Immunol.* 2012, 148262.
- Yanagisawa, N., Haruta, I., Kikuchi, K., Shibata, N., Yagi, J., 2011. Are dysregulated inflammatory responses to commensal bacteria involved in the pathogenesis of hepatobiliary-pancreatic autoimmune disease? An analysis using mice models of primary biliary cirrhosis and autoimmune pancreatitis. *ISRN Gastroenterol.* 2011, 513514.
- Yang, J.-Q., Singh, A.K., Wilson, M.T., Satoh, M., Stanic, A.K., Park, J.-J., et al., 2003. Immunoregulatory role of CD1d in the hydrocarbon oil-induced model of lupus nephritis. *J. Immunol.* 171, 2142–2153.

- Yang, J.-Q., Chun, T., Liu, H., Hong, S., Bui, H., Van Kaer, L., et al., 2004. CD1d deficiency exacerbates inflammatory dermatitis in MRL-lpr/lpr mice. *Eur. J. Immunol.* 34, 1723–1732.
- Yang, J.-Q., Wen, X., Liu, H., Folayan, G., Dong, X., Zhou, M., et al., 2007. Examining the role of CD1d and natural killer T cells in the development of nephritis in a genetically susceptible lupus model. *Arthritis Rheum.* 56, 1219–1233.
- Yang, J.-Q., Wen, X., Kim, P.J., Singh, R.R., 2011. Invariant NKT cells inhibit autoreactive B cells in a contact- and CD1d-dependent manner. *J. Immunol.* 186, 1512–1520.
- Yang, J.Q., Kim, P.J., Halder, R.C., Singh, R.R., 2013. Intrinsic hyporesponsiveness of invariant natural killer T cells precedes the onset of lupus. *Clin. Exp. Immunol.* 173, 18–27.
- Yokote, H., Miyake, S., Croxford, J.L., Oki, S., Mizusawa, H., Yamamura, T., 2008. NKT cell-dependent amelioration of a mouse model of multiple sclerosis by altering gut flora. *Am. J. Pathol.* 173, 1714–1723.
- Yoshiga, Y., Goto, D., Segawa, S., Ohnishi, Y., Matsumoto, I., Ito, S., et al., 2008. Invariant NKT cells produce IL-17 through IL-23-dependent and -independent pathways with potential modulation of Th17 response in collagen-induced arthritis. *Int. J. Mol. Med.* 22, 369–374.
- Yoshiga, Y., Goto, D., Segawa, S., Horikoshi, M., Hayashi, T., Matsumoto, I., et al., 2011. Activation of natural killer T cells by  $\alpha$ -carba-GalCer (RCA1-56), a novel synthetic glycolipid ligand, suppresses murine collagen-induced arthritis. *Clin. Exp. Immunol.* 164, 236–247.
- Yoshimoto, T., Bendelac, A., Hu-Li, J., Paul, W.E., 1995a. Defective IgE production by SJL mice is linked to the absence of CD4+, NK1.1+ T cells that promptly produce interleukin 4. *Proc. Natl. Acad. Sci. U.S.A.* 92, 11931–11934.
- Yoshimoto, T., Bendelac, A., Watson, C., Hu-Li, J., Paul, W.E., 1995b. Role of NK1.1+ T cells in a TH2 response and in immunoglobulin E production. *Science* 270, 1845–1847.
- Yu, X.-M., Wang, X.-F., 2011. The in vitro proliferation and cytokine production of Valpha24+ Vbeta11+ natural killer T cells in patients with systemic lupus erythematosus. *Chin Med J (Engl)* 124, 61–65.
- Yu, K.O.A., Im, J.S., Molano, A., Dutronc, Y., Illarionov, P.A., Forestier, C., et al., 2005. Modulation of CD1d-restricted NKT cell responses by using N-acyl variants of alpha-galactosylceramides. *Proc. Natl. Acad. Sci. U.S.A.* 102, 3383–3388.
- Yue, S.C., Nowak, M., Shaulov-Kask, A., Wang, R., Yue, D., Balk, S.P., et al., 2009. Direct CD1d-mediated stimulation of APC IL-12 production and protective immune response to virus infection in vivo. *J. Immunol.* 184, 268–276.
- Zaba, L.C., Fuentes-Duculan, J., Eungdamrong, N.J., Abello, M.V., Novitskaya, I., Pierson, K.C., et al., 2009. Psoriasis is characterized by accumulation of immunostimulatory and Th1/Th17 cell-polarizing myeloid dendritic cells. *J. Invest. Dermatol.* 129, 79–88.
- Zajonc, D.M., Girardi, E., 2015. Recognition of microbial glycolipids by natural killer T cells. *Front. Immunol.* 6, 523.
- Zeissig, S., Blumberg, R.S., 2014. Commensal microbial regulation of natural killer T cells at the frontiers of the mucosal immune system. *FEBS Lett.* 588, 4188–4194.
- Zeng, D., Lee, M.K., Tung, J., Brendolan, A., Strober, S., 2000. Cutting edge: a role for CD1 in the pathogenesis of lupus in NZB/NZW mice. *J. Immunol.* 164, 5000–5004.
- Zeng, D., Liu, Y., Sidobre, S., Kronenberg, M., Strober, S., 2003. Activation of natural killer T cells in NZB/W mice induces Th1-type immune responses exacerbating lupus. *J. Clin. Invest.* 112, 1211–1222.
- Zeng, Z., Castano, A.R., Segelke, B.W., Stura, E.A., Peterson, P.A., Wilson, I.A., 1997. Crystal structure of mouse CD1: An MHC-like fold with a large hydrophobic binding groove. *Science* 277, 339–345.
- Zhang, W., Zheng, X., Xia, C., Perali, R.S., Yao, Q., Liu, Y., et al., 2008.  $\alpha$ -Lactosylceramide as a novel “sugar-capped” CD1d ligand for natural killer T cells: biased cytokine profile and therapeutic activities. *Chembiochem* 9, 1423–1430.
- Zhang, Q., Xiao, H.P., Cui, H.Y., Sugawara, I., 2011. Significant increase in natural-killer T cells in patients with tuberculosis complicated by type 2 diabetes mellitus. *J. Int. Med. Res.* 39, 105–111.
- Zhang, J., Bedel, R., Krovi, S.H., Tuttle, K.D., Zhang, B., Gross, J., et al., 2016. Mutation of the Traj18 gene segment using TALENs to generate natural killer T cell deficient mice. *Sci. Rep.* 6, 1–10.
- Zhao, Y., Fishelevich, R., Petrali, J.P., Zheng, L., Anatolievna, M.A., Deng, A., et al., 2008. Activation of keratinocyte protein kinase C zeta in psoriasis plaques. *J. Invest. Dermatol.* 128, 2190–2197.
- Zhao, M., Svensson, M.N.D., Venken, K., Chawla, A., Liang, S., Engel, I., et al., 2018. Altered thymic differentiation and modulation of arthritis by invariant NKT cells expressing mutant ZAP70. *Nat. Commun.* 9, 1–28.
- Zhou, D., Mattner, J., Cantu, C., Schrantz, N., Yin, N., Gao, Y., et al., 2004. Lysosomal glycosphingolipid recognition by NKT cells. *Science* 306, 1786–1789.
- Zhu, S., Bing, Y., Wang, X., Yu, Q., Wang, Y., Xu, S., et al., 2014. CCL25/CCR9 interactions regulate the function of iNKT cells in oxazolone-induced colitis in mice. *PLoS One* 9, e100167.

# B-Cell Development: How to Become One of the Chosen Ones

Fritz Melchers<sup>1,2</sup>

<sup>1</sup>Max Planck Institute for Infection Biology, Berlin, Germany <sup>2</sup>Deutsches Rheuma-Forschungszentrum, Berlin, Germany

## OUTLINE

Introduction—What Has to be Generated in B-Cell Development to Make it to Maturity?	155	The Second, Eventually Autoantigen-Sensitive, Phase of B-Cell Development to sIgM <sup>+</sup> Immature B Cells	161
Follicular B Cells	156	The First Checkpoint for the Emerging B-Cell Repertoire—Probing the Fitness for a Good BCR	162
Intraepithelial B Cells	156	Expression of IgL Chains	163
Two Types of Memory B Cells	157	The Second Checkpoint: Sites and Mechanisms of Selection of Newly Generated sIgM <sup>+</sup> B Cells	164
B Lymphopoiesis Before Ig Repertoire Generation—Development of Progenitor and Precursor Cells	158	Future Approaches to Understanding Central B-Cell Tolerance	166
Development in Waves During Ontogeny and in Niches Throughout Life	158	Acknowledgments	167
Cellular Environments of the First Phase of Early, Antigen-Independent B-Cell Development	159	References	167
Early Commitments to Antigen-Independent B-Cell Development	160	Further Reading	170

## INTRODUCTION—WHAT HAS TO BE GENERATED IN B-CELL DEVELOPMENT TO MAKE IT TO MATURITY?

One B lymphocyte makes one antibody, which it displays on the cell surface in  $10^4$ – $10^5$  copies as antigen-recognizing B-cell receptors (BCRs). This central dogma of immunology (Jerne, 1955; Burnet, 1959) is observed in 98%–99% of all B cells for the immunoglobulin (Ig) heavy (IgH) chain locus, and in 95%–97% of all Ig light (IgL) chain loci. Hence, in the majority of all B cells only one of the two IgHs, and only one of the four (in the mouse) or six (in humans) IgL loci are expressed. This choice of only one IgH and one IgL allele for the expression of protein is called “allelic exclusion” (for reviews see Melchers and Rolink, 1999, 2006).

The repertoires of different antigen-specific BCRs can maximally contain the number of Ig-producing B lymphocyte-lineage cells that produce one IgH and one IgL chain. From the cellular dynamics of B-cell development one can try to estimate how many B lineage cells are generated in the lifetime of a mouse or a human; how

many of these B lineage cells make it to a successful, Ig-producing B cell; and how many of these Ig<sup>+</sup> B cells make it into the pools of peripheral, mature, antigen-reactive B cells. The antibody repertoire which is generated from progenitor cells by V(D)J rearrangements in the IgH and IgL chain gene loci is so diverse that many of the developing B cells can recognize autoantigens, and some are even polyreactive to several antigens (Zhou et al., 2007). At the first immature stage of B-cell development, these autoreactive B cells are eliminated (Lederberg, 1959) in the primary lymphoid organ in a process termed “negative selection” (during adult life in bone marrow), at cellular sites yet to be discovered, before they can reach the peripheral sites of mature B cells. Pools of mature B lymphocytes are found at different sites in the body in different numbers and at different stages of differentiation from newly generated cells to antigen-experienced memory cells and antibody-secreting plasma cells. In a 6–8-month-old inbred strain of mice, such as C57BL/6, such mature cells are predominantly found at three types of sites of the body that accommodate either B1, marginal zone (MZ) or B2 B cells.

## FOLLICULAR B CELLS

In the follicular regions of spleen and lymph nodes, B cells aggregate in “B cell-rich” areas and find themselves surrounded, in the neighborhood of follicular helper T lymphocytes (King, 2009), ready to react to foreign antigen, but—in the absence of foreign antigen—over 98% are in a nonstimulated, resting, G<sub>0</sub> cell cycle state. Clearly, they are not stimulated by autoantigens present in their neighborhood. Newly generated B cells are short lived, with half-lives of less than a week. Experience by exposure to stimulation (e.g., by antigen) induces a longer half-life, in some cases of weeks and months. T cell-dependent stimulation in expanding germinal centers (Klein and Dalla-Favera 2008) at the interphase between T and B cell-rich regions induces not only longevity, but also hypermutations of V regions of IgH and L chain genes at rates of  $10^{-3}$  per base pair per division of the proliferating B cells, so that every cell division generates a somatic mutant in the variable regions of IgH and IgL chain genes. Hypermutating B cells in the dark zone of germinal centers divide at least once every 18 hours and maybe even more rapidly, for example, with cell cycle times of 6 hours, a finding that is paradoxical if DNA replication during the S phase of the cell cycle takes 8 hours and needs further investigation (Gitlin et al., 2015).

Therefore this proliferative expansion generates at least 1000 V region-mutant B cells from one antigen-stimulated B cell in a week. It is not the subject of this review but should be noted that such hypermutations are likely to generate long-lived, autoantigen-reactive mature B cells that need to be silenced, not to elicit an autoaggressive response. It is also clear that the antigen-driven expansion of mutant B cells increasingly contributes to the total B-cell repertoires as life goes on.

T cell-dependent stimulation of follicular B cells also induces IgH-class switching and B-cell differentiation to Ig-secreting plasma cells, followed by the migration of resting, long-lived, hypermutated, and Ig class-switched “memory” B cells and Ig-secreting plasma cells (Shapiro-Shelef and Calame, 2005) out of the follicular regions of spleen and lymph nodes, maybe even out of the secondary lymphoid organ and into specific sites (“niches”) within spleen and bone marrow (Tokoyoda et al., 2010; Weill et al., 2013). It has been estimated that a 6–8-week-old C57BL/6 mouse has around  $5 \times 10^8$  of these B2 cells, but a more detailed quantitation of the sizes of these different B-cell subpopulations with age is missing for mice and humans.

## INTRAEPITHELIAL B CELLS

In the gut-associated (and lung-associated) epithelia B lymphocytes are located at two distinct sites: in the gut they are found in follicular structures, surrounded by T lymphocytes not unlike in spleen and lymph nodes, near flat epithelial cells which are suspected to allow permeation of food- and commensal bacteria-derived antigens from the lumen into these lymphoid cell aggregations. Separate from these aggregations, single B lymphocytes and Ig (predominantly M and A)-secreting plasma cells are dispersed in the lamina propria of the gut, from where their secreted IgA can traverse the epithelium and the mucous membranes into the lumen to interact with antigens of food and with commensal bacteria. Many of these so-called B1 lymphocytes (Baumgarth, 2011; Montecino-Rodriguez and Dorshkind, 2012) appear somehow activated (as they are slightly larger and, occasionally, enter cell cycle), and it has been suggested that these B cells are reactive, or at least cross reactive, to autoantigens and such “quasi-autoantigens” as food and commensal bacteria. B1 cells need the spleen to develop (Wardemann et al., 2002). It is, therefore, possible that these B1 cells are positively selected by autoantigens by a

low-affinity reaction of their BCRs with these autoantigens present at these sites. Many of them can respond to antigens in the absence of helper T cells and, hence, to T cell-independent antigens. Many of them can activate activation-induced cytidine deaminase (Muramatsu et al., 2000; Revy et al., 2000) for Ig class switch recombination, again apparently without the help of T cells, but this class switch is almost entirely to IgA, and their capacity to hypermutate their V regions is very limited. It is not clear whether both populations of B cells in the gut, those organized in aggregates and surrounded by T cells near flat epithelium, and the others, the single B cells in the lamina propria, are B1-type cells.

Furthermore, and in contrast to conventional, B2-type B cells, B1 cells are able to repopulate their compartments after adoptive transfers and show strong clonal persistence in vivo. Thus they can be considered to have “stem cell”–like properties, for example, the capacity of migration to the right sites (e.g., gut) and the property of long-term (LT) self-renewal. Many of the B lymphocytes at these sites can be characterized as B1-type cells. In a 6–8-week-old C57BL/6 mouse around  $5 \times 10^8$  cells may be found, that is, as many as there are conventional B2-type cells in follicular regions of spleen and lymph nodes, but these numbers vary considerably with the health of the animal. B1 cells are considered to be the main source of so-called natural antibodies (Hooijkaas et al., 1984; Bos et al., 1989). Again, a more precise quantitative analysis of the sizes of these B1 compartments with time is missing.

All these B-cell sites are connected by blood and lymph vessels. Hence, when B lymphocytes are first generated in primary lymphoid organs, they enter the peripheral immune system via the blood, mainly through the central artery in the spleen from where they distribute themselves via the MZ to the different locations. Some MZ B cells probably continue their travel to the gut- and lung-associated lymphoid regions where many of them establish themselves as B1-type B cells. Other MZ B cells begin to migrate continuously from the MZ into the follicular regions of spleen and back (Arnon et al., 2013). Eventually, they also emigrate from the MZ to peripheral lymphoid organs, such as lymph nodes. In the peripheral lymphoid organs, B cells can become experienced by antigenic stimulation, then circulate back, and reappear in the MZ of the spleen. This may, on rare and ill-understood circumstances, even allow them to change their preimposed preference to circulate back to the peripheral lymphoid site from whence they came and redistribute themselves into another site in the system. For a site-specific distribution into B1-, MZ-, or B2-rich areas they need to be directed by specific chemokines, but it remains unclear whether (low affinity) recognition by autoantigens influences their choice of the B1 compartments, and whether ignorance (lack of any affinity) of autoantigens allows their aggregation in follicular B2 zones in spleen and lymph nodes. In addition, autoantigen recognition at nonlymphoid sites (e.g., in the articular synovial fluid of joints during active phases of rheumatoid arthritis) may contribute to the formation of lymphoid follicular structures capable of performing germinal center reactions at unusual, normally nonlymphoid sites in the body.

## TWO TYPES OF MEMORY B CELLS

The distinction between B1 and B2 cells, particularly striking in the way memory, is maintained in antibody-producing B cells. Longevity in the B1 compartment appears to be connected to the continuous presence of the antigen and to its stimulation to maintain an antigen-specific, long-lived, modestly activated state. Whenever such B1 cells show LT repopulation potential upon transplantation, with longevity and persistence in the areas of the gut-associated lymphoid tissues, a continued low-level antigenic stimulation via the BCR is suspected to be the selecting and survival-inducing signaling force. Food- or commensal bacterially derived antigens, maybe with low-level cross-reactivity with autoantigens, could be such BCR-mediated selecting forces. It is suspected that this cross-reactivity to autoantigens is beneficial to positively selecting and expanding the first line of B cells that encounter microbes which colonize the body after birth.

Memory B2 (classical) B cells and long-lived plasma cells have properties of LT repopulating hematopoietic stem cells (LT-phHSCs) (Wagers and Weissman 2004; Luckey et al., 2006; Eliasson and Jönsson, 2010; Lesinski et al., 2012). They can migrate to, and become resident in, “niches” of spleen and bone marrow as LT resting cells for long, in some cases life-long, periods of time. They retain this property when they are transplanted. We expect memory B2 cells not to rely on continuous low-level stimulation via BCRs for survival. Their LT resting persistence should be independent of the presence and continuous stimulation by an antigen. Antigen-dependent restimulation mobilizes these resting B2 cells to leave their “niches,” and it remains to be seen how antigen-specific this mobilization really is, that is, whether memory cells specific for other, unrelated antigens are comobilized. Once the mobilized B2 memory cells have arrived at a peripheral lymphoid organ (e.g., a lymph node), the

rechallenging antigen will activate the antigen-specific memory B2 cells to a memory-type B-cell response, while those mobilized memory cells that are not specific for the challenging antigen may remain resting and may return to their sites of rest in spleen and bone marrow.

Once the peripheral, mature, B lymphoid sites in the body have been formed and filled, a constant number of cells appear to maintain a given size by (largely unknown) mechanisms of homeostasis. Since the large majority of B cells are expected to turn over within days, weeks, or months, it follows that these B-cell populations must be continuously replenished during life. However, the relative, and even the absolute, sizes of different B-cell compartments change with age.

The description of the different B-cell compartments, restricted to a few inbred strains of mice at a restricted time in their life, is far more detailed than that of humans—not to mention other species such as other mammals, fish, or birds. B1, MZ-, and conventional B2 compartments, comparable but not necessarily identical to a mouse, have been found and defined in humans (Descatoire et al., 2011). Very generally summarized, humans with a 1000-fold higher body size than mice have a thousand times more B cells with similar sites of cellular organization of similar subpopulations with similar functions. However, we lack a more systematic comparison with age and immunological experience of the two species, as these are studied for different reasons—mice, because of the tremendous experimental opportunities to study and interfere with its immune system, and humans, often because of self-centered, health-minded interests. The limitations of the description of B-cell development that follows should be seen in this light.

## B LYMPHOPOIESIS BEFORE IG REPERTOIRE GENERATION—DEVELOPMENT OF PROGENITOR AND PRECURSOR CELLS

Lymphocyte development occurs in two phases. In the first phase, cells are developed in sufficient numbers to the stage at which they first express an antigen receptor. Obviously, this phase is independent of any influence by antigen, because the antigen receptors have not yet been made. Developing hematopoietic cells on their way to B lineage cells enter a series of decisions, which are influenced by their interactions with an inductive microenvironment. Once they are committed to the B lineage cells they begin the second phase, when they begin to express BCRs.

## DEVELOPMENT IN WAVES DURING ONTOGENY AND IN NICHES THROUGHOUT LIFE

Three waves of hematopoietic cell developments colonize the mouse embryo (Cumano et al., 2001; Godin and Cumano, 2002; Ling and Dzierzak, 2002). The first wave (primitive hematopoiesis) begins at embryonic day (E) 7.5 in yolk sac, that is, extra embryonically. This wave, initially, does not develop B lymphocytes, but only fetal-type hemoglobin-expressing erythrocytes, megakaryocytes, platelets, and special types of myeloid cells—the latter with unusual longevity (Irion et al., 2010; Schulz et al., 2012). However, they develop B1-type B cells, when they can enter the developing fetal liver from day 12 of embryonic development. The second and third waves of (now definitive) hematopoiesis originate intraembryonically at E10.5 from the aorta-gonad-mesonephros area (Medvinsky and Dzierzak, 1996), from where undifferentiated pHSCs (Ohmura et al., 2001; Cumano and Godin, 2007; Yokomizo et al., 2011) migrate through embryonic blood to developing rudiments of fetal liver, omentum, thymus, and bone marrow. B cells develop in fetal liver, omentum, and bone marrow, probably in a process involving the transmigration of pHSCs from inside the blood vessels through vascular endothelium, to further differentiate on the other side in contact with mesenchymal and epithelial microenvironments (Tsuneto et al., 2013; Kajikhina et al., 2016). Since B1 cell development in fetal liver occurs in one wave between E12.5 and birth of a mouse, this organ never establishes a niche for LT residing pHSCs.

By contrast, bone and its marrow—which generates B1 and B2 cells throughout life—is capable of developing “niches” of LT resting pHSCs in the neighborhood of, and in close contact with, nonhematopoietic stromal cells. It secures a continuous, life-long generation of new hematopoietic cells, hence B lymphocytes also (Lu et al., 2011). The niches for pHSCs appear localized at subendosteal sites near intrabone surfaces that allow the LT residence of pHSCs with the capacity to LT reconstitute a lethally irradiated host with all lineages of hematopoietic cells, including B lymphocytes. LT-pHSCs exist in different states of the cell cycle. In one state, they are activated into the cell cycle, that is, they divide. One of the two asymmetrically dividing daughter pHSCs keep their stem

cell status, hence they self-renew, while the other daughter cell differentiates to short-term (ST) repopulating HSCs that retain hematopoietic pluripotency, that is, capacity to differentiate to multipotent myeloid/lymphoid progenitors (MPP), to common myeloid progenitors, and to common lymphoid progenitors (CLP), from which B lineage cells develop (Akashi et al., 1999).

In the other state, they are resting in a G<sub>0</sub> state of the cell cycle and consume low levels of energy in a subosteal, hypoxic microenvironment. From this resting state, a small number of cells can be mobilized (stem cell mobilization) to enter the cycling state. However, cycling LT-pHSCs might also revert back to a LT resting state. Bone marrow microenvironments appear uniquely specialized to harbor resting, long-time residing cell types, such as LT repopulating pHSCs, memory lymphocytes, and plasma cells. Among all LT-pHSCs in the resting cell population, only a minor subfraction of cells is “mobilized” at any time to enter asymmetric cell divisions and, because of this, only a part of all LT resting pHSCs participate in hematopoiesis at any given time. This might allow the hematopoietic cell system to continuously refresh its pHSC pools by new, unused pHSCs, thereby preserving its genetic integrity and protecting itself from adverse somatic mutations in actively proliferating pHSCs—mutations that could contribute to breaking tolerance to autoantigens. LT-pHSC can also leave the bone marrow, circulate through blood in the periphery (Pierce et al., 2017), maybe pick up bacteria from centers of infections (Tornack et al., 2017), and reenter the bone marrow. On the other hand, if cycling LT-pHSCs can revert back to the G<sub>0</sub> LT resting state, their somatically mutated genetic constitution might increase the chances for abnormal hematopoiesis and B lymphopoiesis with age (Rossi et al., 2007).

## CELLULAR ENVIRONMENTS OF THE FIRST PHASE OF EARLY, ANTIGEN-INDEPENDENT B-CELL DEVELOPMENT

Early embryonic phases of the first wave of B-cell development in the fetal liver of the mouse can be studied *in situ* in histological sections as the organ develops with time. Attracted by the chemokine ligands CXCL10 and CXCL12, produced by nonhematopoietic vascular endothelial cells and mesenchymally derived stromal cells, CXCR3 and CXCR4-expressing pHSCs and other early progenitors transit from embryonic blood through vascular endothelium into the developing fetal liver and migrate inside to mesenchymally derived stromal cells. These nonhematopoietic cells provide the hematopoietic and B-lymphopoietic progenitors with interleukin-7 (IL-7), the cytokine which is mandatory to interact with IL-7 receptors (IL-7 R) on the hematopoietic progenitors to induce the early, antigen-independent stages of B1 cell development (Tsuneto et al., 2013, Kajikhina et al., 2016). Thymic stromal lymphopoietin has been implied as an alternative stimulating cytokine in fetal liver (Montecino-Rodriguez et al., 2006).

Microenvironmental influences of these early phases of B-cell development during the second continuous wave of B-cell development in developing bone are mostly yet to be discovered, but it appears reasonable to expect that, by similar modes, pHSCs will have to transit from embryonic blood into the environment of developing bone and marrow, and that IL-7, again, is the major force that drives the early phases of B-cell development. Notably, the role of IL7 is less clear in human B-cell development. While fetal liver generates almost exclusively B1a cells, bone marrow develops B1- and B2-type B lymphocytes, but the B1 cells, called B1b, differ from the embryonically developed B1a cells. Transplantation of E13.5 fetal liver pHSC-like cells, upon transplantation, repopulate the host preferentially with B1 cells, while transplantations of pHSCs and progenitors from adult mice repopulate the host preferentially with B2 lymphocytes. Bone marrow retains this capacity, though the numbers of pHSCs decrease with increasing age by at least 100-fold in 1 year of the life of a mouse.

B1a cells generated in fetal liver also differ in some properties from B1b and B2 cells generated in bone marrow. Since the enzyme terminal deoxynucleotidyl transferase (TdT)—which inserts N-region nucleotides at VDJ joints during Ig gene rearrangements—is not expressed in fetal liver (but only in bone marrow) the repertoires of newly generated BCRs are less diverse. How much that influences the capacities of these early embryonic repertoires to recognize and distinguish foreign from autoantigens is still not clear. B1a cells from fetal liver express CD5, a cell surface marker indicating cell activation, while most bone marrow-derived B1b cells do not. Furthermore, micro-RNAs play regulatory roles in the development of fetal liver-derived B1a and bone marrow-derived B cells. Thus the gene Lin28b is selectively expressed in hematopoietic progenitors of fetal liver, but not of adult bone marrow. It downregulates the expression of a family of miRNAs, the let7 miRNA cluster. Ectopic expression of Lin28 in bone marrow-derived pHSCs and progenitors reprograms B-cell development, so

that B1a cells are generated. These results argue for the existence of two B-cell lineages, B1a and B2 (including, maybe, B1b), which are developed as two consecutive layers of B-cell repertoires (Tung et al., 2006; Montecino-Rodriguez and Dorshkind, 2012).

## EARLY COMMITMENTS TO ANTIGEN-INDEPENDENT B-CELL DEVELOPMENT

The initiation and maintenance of the development of B lymphoid lineage cells from pHSCs, MPP, CLP, and pro-/pre-B cells to pre-BI cells, before the generation of sIgM + immature and mature B cells, is controlled externally by a microenvironment of nonhematopoietic (and maybe also hematopoietic) cells that provide cell contacts, cytokines, and chemokines for their mutual attraction, proliferation, and differentiation. ST-pHSCs are induced from LT-pHSCs by the action of FLT3 ligand, acting on flt3, to become MPPs, and after IL-7 has induced IL-7R-expressing progenitors to enter the lymphoid pathway of differentiation. Inside the hematopoietic cells signal transducing and gene transcription-controlling factors regulate these processes of proliferation, cell survival, and differentiation. Three transcription factors, that is, E2A, EBF, and Pax5 (Bain et al., 1994; Zhuang et al., 1994; Sigvardsson et al., 1997; Kee and Murre, 1998; Roessler and Grosschedl, 2006; Kwon et al., 2008), control B-cell development from CLP. By contrast, little is known how microenvironments influence the choices of a multipotent progenitor to become myeloid or lymphoid and how an oligopotent common lymphoid progenitor is influenced to become either a natural killer (NK) lineage, T lineage, or B lineage cell. While these decisions should have no influence on the repertoires of IgH and IgL chains that are generated, they may well be important for the efficiencies with which B cells are provided among the mature pools of cells. Over- or underproduction of B lineage cells could well have an influence on the functions of these B-cell pools.

The molecular details of the transcriptional activation of B-cell development have been clarified in remarkable detail (Decker et al., 2009; Ebert et al., 2011; Medvedovic et al., 2011; Revilla-I-Domingo et al., 2012; Vilagos et al., 2012). First, E2A begins to be transcribed as early in hematopoiesis as in pHSCs. In these cells the promotor of Pax5 remains silenced while its enhancer is activated. One developmental stage later, in MPP, E2A directly activates the expression of early B-cell factor (EBF). This remains the pattern of gene activation, until the Pax5 promoter is activated in pro-/pre-B cells. This allows EBF to bind to the Pax5 promoter, and PU.1, IRF-4, IRF-8, and nFkB to bind to the activated Pax5 enhancer. EBF-complexed promoter and PU.1/IRF-4/IRF-8/nFkB-complexed enhancer form a supercomplex that allows the expression of Pax5. Pax5 then induces a large number of genes involved in the early and late steps of B-cell development, while downregulating the expression of genes involved in myelopoiesis (Schebesta et al., 2007). Finally, Pax5 expression is terminated when differentiation to Ig-secreting plasma cells is induced (Shapiro-Shelef and Calame, 2005; Delogu et al., 2006; Ochiai et al., 2013).

Targeted deletions of the genes encoding these transcription factors result in blocking of B-cell development at different developmental stages. E2A-deficient progenitor B cells have not yet entered DH to JH rearrangements at the IgH chain locus (Ikawa et al., 2004), whereas EBF-deficient and Pax5-deficient progenitors have done so, the latter on both alleles (Rolink et al., 1999). Both EBF- and Pax5-deficient CLP-like pro-/pre-B cells from bone marrow can be established as stable cell lines proliferating for long periods of time, such as pre-BI cell lines (Rolink et al., 1991), identifiable as clones of cells by their individual sets of DHJH-rearranged IgH chain alleles on stromal cells (providing stem cell factor, the ligand of c-kit, and IL-7), added in sufficient quantities *in vitro* by the recombinant cytokine.

The Pax5-deficient progenitors are remarkably flexible cells. Clones of these cells, genetically marked by individual sets of DHJH/DHJH rearrangements at their IgH chain alleles, respond to different cell contacts and cytokines by different differentiation programs. Thus *in vitro* they can be induced to macrophages by macrophage colony-stimulating factor (M-CSF), to dendritic cells by granulocyte-M-CSF (GM-CSF) and M-CSF, to granulocytes by GM-CSF and G-CSF, to NK cells by IL-2 and IL-15, to osteoclasts by TRANCE expressed on mesenchymally derived stromal cells, and to thymocytes by mesenchymally derived stromal cells expressing the NOTCH ligand DELTA-1 in the presence of FLT3 ligand and IL-7, while their development to B cells—which is induced by stromal cells in the presence of FLT3 ligand and IL-7 in Pax5<sup>+/+</sup> cells—is blocked by the Pax5 deficiency (Nutt et al., 1999; Rolink et al., 1999; Höflinger et al., 2004; Radtke et al., 2004).

Removal of the cytokine IL-7 *in vitro*, or transplantation into suitable recipient mice *in vivo*, induces the differentiation to VHDJH/VLJL-rearranged, sIgM + immature B cells—however only of wild-type—but not of EBF- or Pax5-deficient cells. *In vivo* mature B1 cells (Hayakawa et al., 1985) develop from fetal liver as well as bone marrow-derived wild-type cell lines, detectable in spleen and peritoneum of recombination activating gene

(RAG)-deficient recipient mice. From fetal liver cell lines, B1a cells develop, while bone marrow-derived cells generate B1b cells (Hayakawa et al., 1985; Végh et al., 2010).

In the presence of T cells, as in JH-T mice, or in RAG-deficient mice cotransplanted with CD4<sup>+</sup> (and CD4<sup>+</sup>25<sup>+</sup>) T cells), B2-type follicular B cells are also formed, mainly in the spleen. These wild type-, fetal liver-, or bone marrow-derived Pax5-expressing cells do not repopulate the bone marrow. By contrast, Pax5-deficient pro-/pre-B cells do migrate and reside after transplantation to bone marrow (Schaniel et al., 2002a,b), and this homing to bone marrow, also a characteristic of earlier cellular stages of hematopoiesis (including pHSCs), is controlled by miR221 (Knoll et al., 2013). The expression of externally added, that is, retrovirally transduced Pax5 induces B-cell differentiation to CD19<sup>+</sup> pre-BI cells, but not beyond, since the Pax5-deficient pro-/pre-B-cell lines express an endogenously VH(7183)DHJH-rearranged IgH chain that apparently affects allelic exclusion (Simmons et al., 2012). It is still not clear whether such premature IgH chain rearrangements also occur during normal B-cell development *in vivo*, and, if so, how such cells overcome the arrest due to allelic exclusion—maybe by VH gene replacements.

The LT proliferating pro-/pre-B and pre-BI cell lines have been successfully used in experiments which have investigated the transgenic expression of genes active in B-cell development and their functions *in vivo*, such as graded Pax5 expression in Pax5<sup>2/2</sup> cells to biphenotypic myeloid/lymphoid cells, or miR221 expression in pre-BI cells, inducing cell migration to bone marrow, or the synergistic transforming activities of two oncogenes, one, pim-1, inhibiting apoptosis and the other, c-myc, inducing proliferation of pre-B cells to form pre-B lymphomas (Bouquet and Melchers, 2012; Wolf et al., 2016, 2017). Unfortunately, such transduced expression of individual antibody genes has not yet been achieved, because the IgH chain gene cannot be expressed at the appropriate stage of B-cell development but is induced too early, blocking normal B-cell development due to allelic exclusion.

## THE SECOND, EVENTUALLY AUTOANTIGEN-SENSITIVE, PHASE OF B-CELL DEVELOPMENT TO SIGM<sup>+</sup> IMMATURE B CELLS

In the second phase of B-cell development, Ig genes are rearranged, Ig H and L chains are made, deposited as pre-BCRs and BCRs on the cell surface and molecularly linked to intracellular signaling pathways. V(D)J rearrangements at the Ig loci occur stepwise at different cellular stages of B-cell development. The emerging repertoires of IgH and L chains are screened successively at two checkpoints, at the first for their fitness by pre-BCR formation and at the second for autoantigen recognition after BCR formation.

After IL-7 has induced IL-7R-expressing lymphoid/myeloid progenitors to enter the lymphoid pathway of differentiation, the rearrangement-active genes RAG1 and 2 and TdT (the latter only in bone marrow, not in fetal liver), as well as the genes encoding the Ig membrane anchoring molecules Ig alpha and beta, and the components of the surrogate light chain (SLC), Vpre-B, and lambda5, become expressed in CLPs. Vpre-B and lambda5 associate noncovalently to form an IgL chain-like structure in pro-/pre-B cells.

As soon as IgH chains become expressed, this SLC can form, between the IgL chain constant region domain-like lambda5 and the first cμH domain, S–S-bonded Ig (BCR)-like structures, called pre-BCRs (Melchers, 2005).

In SLC a carboxy-terminal peptide of Vpre-B and an amino-terminal peptide of lambda5 protrude at the location of the third complementarity-determining region (CDR) of a normal VL domain. These non-Ig parts control the turnover of pre-BCRs with opposite effects on the cell surface: Vpre-B stabilizes surface deposition, while lambda5 downregulates pre-BCR by internalization. As pre-BCR signaling depends on the quantity of molecules on the surface, they appear to modulate pre-BCR signaling in opposite ways (Knoll et al., 2012).

When CLPs enter the B lymphoid pathway they open the IgH chain loci for DH to JH rearrangements and become c-kit flt31CD19-pro-/pre-B cells, not yet expressing Pax5. Pax5<sup>-/-</sup> pro-/pre-B cells appear arrested in B-cell development at that stage.

In pro-/pre-B cells of mice, but not of humans, B-cell development with DHJH rearrangements in reading frame 2 results in the expression of a DJ-cμ protein that can assemble with SLC to form a VH-deficient pre-BCR-like molecule on the surface of such cells. This appears to result in negative signaling to induce the deletion of these pre-BCR-like expressing cells (Haasner et al., 1994). This state of negative selection may extend into that phase of development when VH to DHJH rearrangements are initiated. In Pax5<sup>-/-</sup> pro-/pre-B-cell lines we have found VHDHJH-rearranged IgH loci, expressing IgH chains containing VH segments within the 7183-VH cluster (most frequently the VH81X segment), which are located at the 3' end of the clusters of VH segments in the IgH locus, but which cannot pair with SLC, thereby apparently evading this negative signaling by a pre-BRR (Wolf, I. and Melchers, F., unpublished). If IgH chains were made that could pair with SLC, such IgH chain loci might

enter VH replacement reactions to generate an SLC nonpairing H chain to evade this negative selection (Nakajima et al., 2009). It has been estimated that, in the mouse, between 1% and 7% of all VHDHJH-rearranged IgH loci result from such editing of VH replacements (Davila et al., 2007). It is not known whether a possible specificity of the VH to autoantigens influences this negative selection, but an autoreactive (transgenic) IgH chain has been shown to induce VH editing in pro-/pre-B cells without having to form a functional pre-BCR (Nakajima et al., 2009).

At the chromosomal level of organization the IgH locus is controlled by contractions of large regions (Feeney et al., 2011; Guo et al., 2011a,b). In cells not yet expressing Pax5 the parts of the regions of the IgH locus containing DH segments are brought into proximity to the JH region for rearrangements.

Once these Pax5-negative cells have undergone DH to JH rearrangements, DH proximal VH regions (VH7183 and Q52) can also be brought near to the DHJH-rearranged regions, and rearranged, even before Pax5 is expressed. Strikingly, one VH segment, VH81x, is rearranged as the first VH segment most frequently. When  $\mu$ -chain gene rearrangements are made in bone marrow with N-region insertions, the vast majority of these  $\mu$ H chains cannot pair with SLC. Hence, in the adult, the B2 repertoire is selected against the inclusion of these VH81x-expressing pre-B and B cells. By contrast, VH81x-containing  $\mu$ H chains, produced in the fetal liver without N-region insertions, can pair with SLC and appear in the peripheral, mature B1a cell repertoire. This “useless” function of the earliest VDJ rearrangements for the developing adult B-cell repertoire in bone marrow has long been an enigma. However, the recent discovery, the IgH locus is further subdivided for additional control of VH rearrangements, suggests a more differential usage of VH segments for the total process of VDJ rearrangements at the IgH locus. In the chromosomal region of the IgH locus between the DH and the JH segments, a CTCF-cohesin binding site is located that further regulates the order of VH to DHJH rearrangements. Deletion of this site on the IgH locus increases proximal (VH7183, especially VH81x) over distal (VH J558) rearrangements (Giallourakis et al., 2010). As the 3'-located VH segments have been found to be used preferentially, as B cells begin VHDHJH rearrangements, we can speculate that the VH regions of the IgH locus are opened in a stepwise fashion. In the first step, accessibility of the locus may be more important than productivity for  $\mu$ H chains.

## THE FIRST CHECKPOINT FOR THE EMERGING B-CELL REPERTOIRE—PROBING THE FITNESS FOR A GOOD BCR

Once the locus is open at all VH regions, distal VH rearrangements induced by Pax5 can be made. They are induced when Pax5-negative flt3<sup>+</sup>CD19-pro-/pre-B cells differentiate to Pax5-expressing flt3<sup>-</sup>CD19<sup>+</sup> pre-BI cells (Ebert et al., 2011). At this first checkpoint the emerging repertoire of  $\mu$ H chains is probed for fitness of pairing with SLC. Pre-B cells expressing an SLC-fitting  $\mu$ H chain can display the resulting pre-BCR on the surface. Cross-linking of the pre-BCRs by ligands provided either by the microenvironment or by the pre-B cells themselves induce proliferation of large pre-BII cells, signal downregulation of the rearrangement machinery (RAG1 and 2) and of SLC expression, thereby limiting the supply of SLCs for pre-BCR formation and, hence, limiting pre-BCR-induced proliferation. The VH repertoires of early pre-B cells before SLC-mediated proliferative expansion and of large pre-BII cells during proliferative expansion have been found to use VH segments at different frequencies, suggesting that the fitness for SLC pairing may be different for different VH families. This VH repertoire shift is delayed in SLC-deficient bone marrow (ten Boekel et al., 1997).

Only between 20% and 50% of all IgH chains can pair to form a pre-BCR on the surface of pre-BII cells, and they do so with varying degrees of fitness (ten Boekel et al., 1997, 1998; Kawano et al., 2006). The better they fit, the longer they proliferate. Cross-linking of pre-BCRs through positively charged arginine residues in the non-Ig portion of  $\lambda$ 5, possibly mediated by repetitive negative charges on molecules such as nucleic acids and other molecules on stromal cells, or by molecules expressed in pre-B cells themselves (Bradl et al., 2003; Ohnishi and Melchers, 2003), initiates pre-BCR signaling. Some  $\mu$ H chains have been found that enter proliferation as large pre-BII cells even in the absence of a functional SLC. They carry charged amino acids (arginines, lysines) in the CDR3 regions of their VH domains, not unlike the carriage of non-Ig parts of SLC. It suggests that the charged amino acids in CDR3s can function in place of SLC to signal the proliferative expansion of pre-BII cells. It remains to be seen what terminates this proliferation that is normally limited by SLC expression.

Pre-BII cells also close the second, often DHJH-rearranged IgH allele for further rearrangements, thereby securing allelic exclusion at the IgH locus. Since triple-deficient V<sub>pre-B1</sub><sup>-/-</sup>, V<sub>pre-B2</sub><sup>-/-</sup>,  $\lambda$ 5<sup>-/-</sup> B lineage cells still show allelic exclusion of the expression of their IgH loci, pre-BCRs cannot be involved in signaling for allelic exclusion, leaving it open just how the successful expression of an IgH chain mediates allelic exclusion in such

situations (Shimizu et al., 2002). Since double productively VHDHJH-rearranged B lineage cells have been found in numbers expected from a random process of in- and out-of-frame rearrangements at the IgH alleles, and since in all these cases only one of the two productively rearranged alleles could form a pairing IgH chain (ten Boekel et al., 1998), it remains to be seen just which is the SLC analogous partner to sense the pairing capacity of a newly made IgH chain.

Those heavy chains unfit to pair with SLCs do not induce proliferation of pre-BII cells through pre-BCRs. In situations where pre-BCRs cannot be formed, such nonproliferating pre-BII cells nevertheless proceed toward differentiation (Grawunder et al., 1993, 1995; Rolink et al., 1996). However, because such pre-BCR-defective pre-B cells do not proliferate, their contribution to the developing B-cell compartment should be at least 20- to 40-fold lower than that of their pre-BCR-expressing counterparts (Rolink et al., 1993).

In conclusion, the pre-BCRs do not use the classical CDRs of the VH domain of their IgH chains to bind ligands that could induce proliferation. Therefore the newly generated VH domain repertoire is not screened for antigen, that is, autoantigen binding, but merely for fitness to pair, first with SLC and eventually with conventional IgL chains. In this way, there is an exclusion of unfit H chains that may have other unwanted deleterious properties, such as the formation of self-aggregating immune complexes that might confer the danger of glomerulonephritis and vasculitis (Melchers, 2005).

## EXPRESSION OF IGL CHAINS

When large pre-BII cells have terminated their proliferative expansion, they become resting, small pre-BII cells, just as the SLC nonpairing, nonproliferating pre-BII cells do. The IgL loci are opened, RAG1 and 2 become reexpressed, and VL-to-JL rearrangements are initiated, in the mouse five times more frequently at the kL locus than the  $\lambda$ L loci, whereas in the human equally frequently at the kL and  $\lambda$ L loci. Only 10% of all potentially rearrangeable Vk segments, spread over the entire Vk region, are used in these first rearrangements, and they use the most 5'-located Jk most frequently. Vk segments, which are located in both orientations in the locus, are equally frequently rearranged by deletions and inversions. Two-thirds of the emerging in-frame rearranged immature sIgM<sup>+</sup> B cells are immediately selected, such that further IgL loci rearrangements are terminated by downregulation of the RAG genes, the second kL allele remains in germline, nonrearranged form, and all  $\lambda$ L loci remain nonrearranged (Melchers et al., 2007). The immature B-cell is likely to sense the production and surface deposition of a BCR, probably by "tonic" signaling and without recognition by an autoantigen binding to the VH/VL domains of the BCRs.

In the remaining pre-BII cells secondary and even subsequent rearrangements occur, probably for two reasons. One reason is that two of three VL-to-JL rearrangements end up out of frame, and hence do not allow translation into IgL chains. Hence, the cell remains in the pre-BII stage, does not express BCRs, keeps the RAG genes expressed, and all IgL alleles open for secondary and subsequent rearrangements, until it either manages a productive rearrangement to produce an IgL chain and, therefore, a BCR and becomes an immature sIgM<sup>+</sup> B cell, or until it runs out of opportunities to rearrange, when all JL segments have been used up.

The other reason is that either after the first or after any subsequent productive VL-to-JL rearrangement, the resulting BCR is autoantigen reactive. This immature autoreactive B-cell is then recognized at the second checkpoint by autoantigens existing in bone marrow and presented by a specialized microenvironment. It has been found that such autoantigen recognition keeps RAG expression upregulated, giving the immature B-cell the opportunity to "edit" its BCR by secondary and subsequent VL-to-JL rearrangements, in an attempt to abrogate the recognition of autoantigen and enter the resting, immature sIgM<sup>+</sup> B-cell compartment, ready for exit from the bone marrow to the periphery, through blood via the central artery into the spleen. Hence, "edited" B cells contain signs of secondary rearrangements, as circles of DNA containing the excised intervening sequences between the VL and the JL segment used in the rearrangement, and by an increased frequency of V $\lambda$  to J $\lambda$ -rearranged alleles.

In human B-cell development, most of the polyreactive cells (Wardemann and Nussenzweig, 2007) and some, but not all, autoreactive cells are lost during the transition from pre-B2 to immature B cells. In patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis, however, these autoreactive and polyreactive BCRs are not lost at this checkpoint (Witsch et al., 2006), pointing to a role of this checkpoint in preventing autoimmune disease. By contrast, Köhler et al. (2008) found a subset of polyreactive pre-BCRs, expressed in pre-B2-like cells, to induce proliferation in vitro, suggesting positive selection by polyreactive pre-BCRs. However, other

experiments indicate that pre-BCR cross-linking in fetal liver organ culture does not boost pre-B-cell proliferation ([Ceredig et al., 1998](#)).

During the process of light chain editing, evolutionarily selected “editor” Ig light chains with low iso-electric points can “neutralize” the DNA-binding properties of certain heavy chains. Such IgL chains may preclude diseases, for example, SLE that is mediated by antibody–DNA complexes ([Li et al., 2001](#)). Continued VL-to-JL rearrangement can also lead to the expression of two or even more different light chains in a single immature B cell ([Doyle et al., 2006; Khan et al., 2008](#)). Immature B cells with one autoreactive and one nonautoreactive BCR can become unreactive to autoantigen because of dilution of the autoreactive with the nonautoreactive BCR. Dual-IgL-expressing B cells can enter the mature B-cell pool while remaining potentially autoreactive. Light chain replacement can also lead to polyreactivity ([Witsch et al., 2006](#)). Interestingly, some human B cells coexpress Vpre-B and conventional light chains together with  $\mu$  chains containing CDR3 regions enriched in positively charged and/or aromatic amino acids. Two-thirds of these cells are autoreactive and appear to have escaped central tolerance. Although human Vpre-B, which has an isoelectric point of 5.67, may act as an “editor” by neutralizing positively charged CDR3 regions, mouse Vpre-B proteins have an isoelectric point of 9.37 and thus may not be able to perform this function ([Meffre et al., 2004](#)).

## THE SECOND CHECKPOINT: SITES AND MECHANISMS OF SELECTION OF NEWLY GENERATED SIGM<sup>+</sup> B CELLS

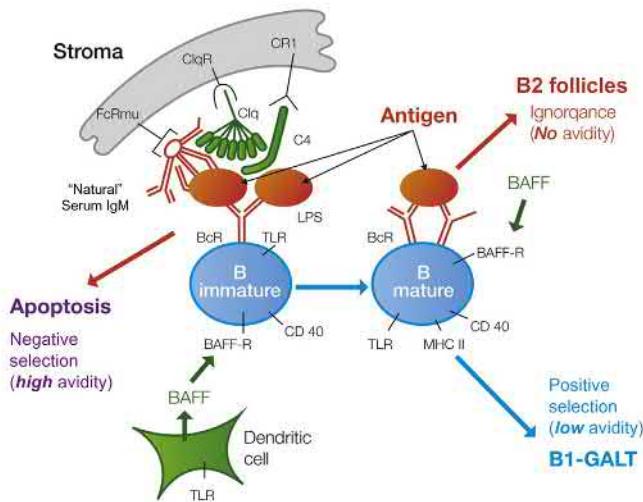
While VH repertoires expressed in immature B cells in the bone marrow and in immature and mature B cells in the spleen are not significantly different, almost 90% of the newly formed immature B cells never leave the bone marrow ([Rolink et al., 1999](#)). They have half-lives of less than a week, probably for two reasons: (1) they might have an intrinsic cellular program to enter apoptosis, unless selected to transit out of bone marrow into the periphery, (2) and they might be subject to autoantigen-induced mechanisms of cellular deletion. In essence, a 6–8-week-old mouse continuously generates from around 10<sup>4</sup> pHSCs over  $2.5 \times 10^6$  pre-BI cells, and  $5 \times 10^7$  small pre-BII  $2 \times 10^7$  immature sIgM<sup>+</sup> B cells, into a pool of around  $10^8$  such immature B cells, only to discard  $9 \times 10^7$ , that is, the vast majority of them, mostly in fear of autoreactivity. The invention of nature to generate a diversity of recognition by V(D)J rearrangements must have been an accident in evolution that was developed as the adaptive branch of the immune system to be advantageous for the defense of the individual against unexpected aggressors, yet a dangerous weapon that could destroy this individual. No wonder, then, that most of the BCRs on immature B cells ever made have to be eliminated.

In this establishment of “central” tolerance in B-cell repertoires by negative selection, neither the cellular microenvironments in the bone marrow nor the molecular mechanisms of deletion are well known. This is all the more remarkable since the processes of negative selection to establish central tolerance for the T-cell repertoires in the thymus have been so well investigated. While the studies in the thymus have been aided by the realization that autoantigens should be presented by major histocompatibility complex (MHC) I and II molecules, nothing comparable is evident from the genetics of B-cell development that would guide the search for the autoantigen-presenting modes that establish central B-cell tolerance. However, the rapidly expanding cases of genetically identified immunodeficiencies leading to early blockages of B-cell development should help to clarify the means whereby central B-cell tolerance is established. Thus it has been realized for some time that the complement components C1q, C4, serum amyloid protein, complement receptor-2, or secreted natural serum IgM could be involved, as deficiencies of any one of these lead to SLE.

Two models for the selection of the emerging B-cell repertoires have been proposed. In one model ([Carroll, 2004](#)), the maturing B cells are protected from the stimulatory influence of autoantigens released from apoptosing, blebbing cells in the primary lymphoid organ because macrophages expressing complement receptors (CIqR for CIq, CRI for C4) efficiently take up and, thereby, remove apoptosing cells bound by natural serum IgM and complexed with CIq and C4b. This model, however, does not explain how the developing repertoire of immature B cells is purged of autoreactive cells, nor does it take into account that immature B cells are sensitive to induction of apoptosis rather than to proliferation and development of Ig-secreting plasma cells and memory B cells.

In contrast, the second model ([Melchers and Rolink, 2006](#)) ([Fig. 8.1](#), redrawn from Figure 2 in [von Boehmer and Melchers, 2010](#)) proposes that autoantigens from dying cells are presented to the emerging repertoire of B cells by stromal cells in the primary lymphoid organ, that is, bone marrow. The presenting cells are expected to express receptors for natural serum IgM that can bind via its Fc to IgM-Fc receptors. IgM via its variable regions could bind to autoantigens, possibly even in solution, then fix CIq and create a bridge between the CIqR, CRI,

### Selection of the primary B cell repertoires in bone marrow



**FIGURE 8.1** Proposed site for autoantigen-mediated selection of immature B cells. A hypothetical microenvironmental cell, nonhematopoietic?, follicular dendritic?, expresses Fc-μ-receptors, Clq receptors, and CR1. Note that several cells close to each other could also express these different receptors. Fc-μ-R bind natural IgM antibodies, which bind Clq when complexed with autoantigen. That allows binding of Clq to ClqR. C4 bridges the immune complexes with CR1. Newly generated immature sIgM<sup>+</sup> B cells bind autoantigens with different avidities. High avidity binding induces apoptosis (negative selection), possibly aided by signaling from unknown cytokines. Low avidity binding induces survival and selection into B1 pools (not aided by BAFF). No avidity (ignorance) induces BAFF-mediated transfer into the periphery of mature follicular B2 cell pools (via T1 and T2 intermediates) expressing MHC II and CD40. CR1, Complement receptor 1. Source: Adapted from von Boehmer, H., Melchers, F., 2010. Checkpoints in lymphocyte development and autoimmune disease. *Nat. Immunol.* 11, 14–20, and redrawn by Justin Hewlett, MNHS Multimedia Unit, Monash University.

and FcR on the presenting cells. In this way autoreactive BCR on the immature B cell would then be brought together by Clq, C4, serum IgM, and autoantigens. Depending on the strength of the interaction of the BCRs with autoantigens, and possibly also on the nature of the presenting stromal cell, this autoantigen-induced signaling can have different outcomes. It can lead to apoptosis of the B cell, if the avidity of autoantigen-BCR binding is high, leading to a negative selection of the B-cell repertoire. Such negative selection has been documented in a variety of experimental settings. If BCR downregulation and reexpression of a secondary BCR due to editing of a new L chain, thus leading to a new, nonautoreactive BCR, occurs fast enough, this apoptosis may be avoided. Since approximately 90% of the  $2 \times 10^7$  sIgM<sup>+</sup> immature B cells that are made each day in the bone marrow of a mouse never arrive at the immature sIgM<sup>+</sup> B-cell pool of the spleen, it is likely that this negative selection engages the vast majority of the newly generated immature sIgM<sup>+</sup> B-cell repertoires in bone marrow, and before their transit to spleen (Rolink et al., 1999).

Alternatively, the interaction of the newly generated immature B cells can lead to positive selection, maybe into the B1b compartment, if the avidity of BCR-mediated interaction is low. Those B cells expressing BCRs with no avidity to autoantigens present in bone marrow will be “ignored” and will be allowed to exit to the periphery, as long as they show their “passport” specifying an unrecognized BCR on their surface. It is truly remarkable that despite this extensive effort to avoid autoimmunity, the peripheral, mature B cells continue to be an autoimmune threat. Needless to point out, we still do not know how the adaptive immune system “tolerates” BCRs, TCRs, and Igs, when they are first made.

Further, it remains equally remarkable just how many cells are made, and then discarded, to generate the few that are finally allowed to leave the bone marrow to face the world of foreign antigens. This fate of the majority of all newly generated B cells reminds me of a fable, told by France (1893), of a young Persian king, who asked his academicians to write the history of mankind—as this article is written to describe the history of B cells. Their initial rendition was carried to the king on 20 camels with 500 books each. The king complained that he would not have the time to read it all and asked for a shorter version. Even the shorter versions were always too long to be read by the king in his remaining lifetime. Finally, a lone academician arrived with only one book, but the king responded by saying: “So I will die without knowing human history.” To which the academician replied, “I can summarize it for you in three words: ‘ils naquinent, ils souffrent, ils moururent’ (They are born, they suffer, they die).”

## FUTURE APPROACHES TO UNDERSTANDING CENTRAL B-CELL TOLERANCE

A better understanding of the modes of central tolerance induced by apoptosis, editing, anergy, and ignorance still requires an identification of all of the relevant antigen-presenting cell populations. A strikingly similar model has been proposed for the establishment of peripheral B-cell tolerance, that is, negative selection of autoreactive B cells, which are randomly, hence accidentally, generated by somatic hypermutation and class switching of a follicular helper T cell–driven immune response within germinal centers (Vinuesa et al., 2010). In this model the hypermutated, autoantigen-recognizing IgG takes the role of the natural IgM antibody proposed in our model (above) for the establishment of central tolerance of B cells.

In contrast to T-cell development, in which only MHC-restricted, that is, self MHC-I- or II-plus autoantigen peptide-recognizing, T cells that recognize self MHC-I or MHC-II are allowed to exit the thymus and enter the periphery, B cells just need to show surface-bound IgM that does not recognize any autoantigen in bone marrow and which has been “ignored” by the selecting environment to gain exit from the bone marrow. Once in the periphery, particularly the spleen, hardly any further cell loss is detected during the transition from immature (including transitional T1 and T2 B cells) to mature B cells in the spleen. In humans there is a further reduction of the percentage of autoreactive cells (from 40% to 20%) across this transition, whereas the percentage of poly-reactive cells remains at the low level (6%) already achieved during the transition from pre-B to immature B cells (Wardemann and Nussenzweig, 2007). Patients with, say, SLE or rheumatoid arthritis fail to establish recessive tolerance even at this second checkpoint, indicating that failure of several mechanisms for establishing tolerance likely contributes to these diseases (Yurasov et al., 2005). The genetics of autoimmune diseases and immunodeficiencies are now investigated by full genome analyses (Mackay et al., 1999; Rolink et al., 2002; Kumar et al., 2006; Deane et al., 2007; Liu et al., 2007; Xie et al., 2007; Shlomchik, 2008). It is predictable that an ever-increasing number of mutant molecules involved in (often also epigenetic) regulation of gene expression, of signal transductions from a variety of receptors involved in the interactions of B lineage cells with their inducing microenvironments, controlling proliferation, differentiation, migration, and survival on their way to mature B cells will be found to change the normal course of B-cell development.

Finally, it is unclear how the affinity for antigen of a BCR decides what it signals to either the immature or later the mature B cell. Of relevance here, a monoclonal, autoreactive, dsDNA-binding, SLE-propagating, hypermutated IgG autoantibody was isolated from an SLE patient. The corresponding VH and VL domains of the IgH and IgL chain genes were then “back-mutated” to the germline-encoded VH/VL sequence and expressed as an antibody (Schroeder et al., 2013). In this back-mutated form, the antibody remarkably failed to bind to dsDNA any longer. The corresponding immature B-cell expressing this antibody as a BCR was evidently not negatively selected by dsDNA, which is abundantly present in bone marrow throughout life. How, then, did the mature BCR1 B-cell ever become autoreactive? Possibly the cell entered a germinal center reaction, hypermutated, and that a dsDNA-binding somatic mutant B-cell then evaded negative selection in the process of peripheral tolerance induction—but who or what triggered it?

It is fully possible that we do not understand how a cell surface-bound BCR establishes contact with the autoantigen (in bone marrow) or antigen (in the periphery), possibly presented (as occurs for T cells in thymus and periphery) by antigen-presenting cells. To quote Huppa and Davis (Huppa et al., 2010; Huppa and Davis, 2013) on their studies of the interaction of T cells with antigen-presenting cells in immunological synapses, “There is increasing evidence that the molecular dynamics of receptor-ligand interactions are not only dependent on the intrinsic properties of the binding partners but also become transformed by cell biological parameters such as the geometrical constraints within the immune synapse, mechanical forces, and local molecular crowding. To appreciate the complete picture, we think a multidisciplinary approach is imperative, which includes genetics, biochemistry, and structure determination and also biophysical analyses and the latest molecular imaging techniques.” It, therefore, seems not unreasonable to expect that similar dynamics of antigen recognition by BCRs on immature and on mature B cells, and of costimulatory or coinhibitory surface-bound ligands and receptors will become evident, which will lead to redefinitions of the distinction between “self” and “nonsel” in B-cell repertoires at different stages of development.

All these examples should make it clear that we are merely at the very beginning of a vision of the control of autoimmunity, or better, the lack of it, in the genetic variants of nearly  $7 \times 10^9$  human individuals with a functional immune system. Moreover, it will not only be the genetics of B cells, but also their environment, that will be found to exert control. Kurt Tucholsky (1890–1935), a German journalist and satirical writer, unwillingly but farsightedly defined what appears to become so important to understand about the functioning of the immune

system of lymphocytes in their environments: "Ein Loch ist da, wo etwas nicht ist. Das Merkwürdigste an einem Loch ist der Rand. Er gehört noch zum Etwas, sieht aber beständig in das Nichts, eine Grenzwache der Materie. Das Nichts hat keine Grenzwache: während den Molekülen am Rande schwindlig wird, weil sie das Loch sehen, wird den Molekülen des Lochs 'fest-lig'?" (Zur soziologischen Psychologie der Löcher, 1931). [Translated: "A hole is there where something is not. The most remarkable thing about the hole is its rim. It belongs to the something, but looks into the nothing, as a border control of the material. The nothing has no border control, while the molecules at the rim of the hole get dizzy, because they see the hole, do the molecules of the hole get 'stabilized'?" (The Sociological Psychology of the Holes, 1931)].

## Acknowledgments

It should be clear to the reader that my description of B-cell development is highly personal, often biased, and incomplete in the quotation of relevant literature, particularly of opposing views. I apologize to all those whom I might have disappointed or even offended. During my attempts to assemble it, I was often ready to give up. Only the patient persistence of the editors has brought this chapter to its present status. To once more quote Anatole France (as I was alerted to him by one of the editors, I.M.), "My article is less than 500 books in size, and more than the final format of the one sentence quoted above, in the hope that the king does have the time to read it." I thank my long-time scientific friends, especially Jan Andersson and Ton Rolink at the no-longer-existing Basel Institute for Immunology, for the never-ending discussions of the scientific subject that has united us for so many years. Finally, I thank the Max Planck Society and its Institute for Infection Biology in Berlin for the many years of support as a Max Planck Fellow, well beyond the limits of my official retirement from the Basel Institute for Immunology.

The second edition is left largely unchanged.

## References

- Akashi, K., Kondo, M., Cheshier, S., Shizuru, J., Gandy, K., Domen, J., et al., 1999. Lymphoid development from stem cells and the common lymphocyte progenitors. *Cold Spring Harb. Symp. Quant. Biol.* 64, 1–12.
- Arnon, T.I., Horton, R.M., Grigorova, I.L., Cyster, J.G., 2013. Visualization of splenic marginal zone B-cell shuttling and follicular B-cell egress. *Nature* 493, 684–688.
- Bain, G., Maandag, E.C., Izon, D.J., Amsen, D., Kruisbeek, A.M., Weintraub, B.C., et al., 1994. E2A proteins are required for proper B cell development and initiation of immunoglobulin gene rearrangements. *Cell* 79, 885–892.
- Baumgarth, N., 2011. The double life of a B-1 cell: self-reactivity selects for protective effector functions. *Nat. Rev. Immunol.* 11, 34–46.
- Bos, N.A., Kimura, H., Meeuwesen, C.G., De Visser, H., Hazenberg, M.P., Wostmann, B.S., et al., 1989. Serum immunoglobulin levels and naturally occurring antibodies against carbohydrate antigens in germ-free BALB/c mice fed chemically defined ultrafiltered diet. *Eur. J. Immunol.* 19, 2335–2339.
- Bouquet, C., Melchers, F., 2012. Pim1 and Myc reversibly transform murine precursor B lymphocytes but not mature B lymphocytes. *Eur. J. Immunol.* 42, 522–532.
- Bradl, H., Wittmann, J., Milius, D., Vettermann, C., Jäck, H.M., 2003. Interaction of murine precursor B cell receptor with stroma cells is controlled by the unique tail of lambda 5 and stroma cell-associated heparan sulfate. *J. Immunol.* 171, 2338–2348.
- Burnet, F.M., 1959. The Clonal Selection Theory of Acquired Immunity. Cambridge Univ. Press, London.
- Carroll, M.C., 2004. A protective role for innate immunity in systemic lupus erythematosus. *Nat. Rev. Immunol.* 4, 825–831.
- Ceredig, R., ten Boekel, E., Rolink, A., Melchers, F., Andersson, J., 1998. Fetal liver organ cultures allow the proliferative expansion of pre-B receptor-expressing pre B-II cells and the differentiation of immature and mature B cells in vitro. *Int. Immunol.* 10, 49–59.
- Cumano, A., Godin, I., 2007. Ontogeny of the hematopoietic system. *Annu. Rev. Immunol.* 25, 745–785.
- Cumano, A., Ferraz, J.C., Klaine, M., Di Santo, J.P., Godin, I., 2001. Intraembryonic, but not yolk sac hematopoietic precursors, isolated before circulation, provide long-term multilineage reconstitution. *Immunity* 15, 477–485.
- Davila, M., Liu, F., Cowell, L.G., Lieberman, A.E., Heikamp, E., Patel, A., et al., 2007. Multiple, conserved cryptic recombination signals in VH gene segments, detection of cleavage products only in pro B cells. *J. Exp. Med.* 204, 3195–3208.
- Deane, J.A., Pisitkun, P., Barrett, R.S., Feigenbaum, L., Town, T., Ward, J.M., et al., 2007. Control of toll-like receptor 7 expression is essential to restrict autoimmunity and dendritic cell proliferation. *Immunity* 27, 801–810.
- Decker, T., Pasca di Magliano, M., McManus, S., Sun, Q., Bonifer, C., Tagoh, H., et al., 2009. Stepwise activation of enhancer and promoter regions of the B cell commitment gene Pax5 in early lymphopoiesis. *Immunity* 30, 508–520.
- Delogu, A., Schebesta, A., Sun, Q., Aschenbrenner, K., Perlot, T., Busslinger, M., 2006. Gene repression by Pax5 in B cells is essential for blood cell homeostasis and is reversed in plasma cells. *Immunity* 24, 269–281.
- Descatoire, M., Weill, J.C., Reynaud, C.A., Weller, S., 2011. A human equivalent of mouse B-1 cells? *J. Exp. Med.* 208, 2563–2564.
- Doyle, C.M., Han, J., Weigert, M.G., Prak, E.T., 2006. Consequences of receptor editing at the lambda locus, multireactivity and light chain secretion. *Proc. Natl. Acad. Sci. U.S.A.* 103, 11264–11269.
- Ebert, A., McManus, S., Tagoh, H., Medvedovic, J., Salvagiotti, G., Novatchkova, M., et al., 2011. The distal V(H) gene cluster of the IgH locus contains distinct regulatory elements with Pax5 transcription factor-dependent activity in pro-B cells. *Immunity* 34, 175–187.
- Eliasson, P., Jönsson, J.I., 2010. The hematopoietic stem cell niche: low in oxygen but a nice place to be. *J. Cell Physiol.* 222, 17–22.
- Feeney, A.J., 2011. Epigenetic regulation of antigen receptor gene rearrangement. *Curr. Opin. Immunol.* 23, 171–177.
- France, A., 1893. Les Opinions de M. Jerome Coignard. In: L'Histoire (Chapter XVI). (Ed.), Calmann-Lévy, Paris, 256 p.
- Giallourakis, C.C., Franklin, A., Guo, C., Cheng, H.L., Yoon, H.S., Gallagher, M., et al., 2010. Elements between the IgH variable (V) and diversity (D) clusters influence antisense transcription and lineage-specific V(D)J recombination. *Proc. Natl. Acad. Sci. U.S.A.* 107, 22207–22212.

- Gitlin, A.D., Mayer, C.T., Oliveira, T.Y., Shulman, Z., Jones, M.J., Koren, A., et al., 2015. Humoral immunity. T cell help controls the speed of the cell cycle in germinal center B cells. *Science* 349 (6248), 643–646. Available from: <https://doi.org/10.1126/science.aac4919>. Epub 2015 Jul 16.
- Godin, I., Cumano, A., 2002. The hare and the tortoise: an embryonic haematopoietic race. *Nat. Rev. Immunol.* 2, 593–604.
- Grawunder, U., Haasner, D., Melchers, F., Rolink, A., 1993. Rearrangement and expression of kappa light chain genes can occur without mu heavy chain expression during differentiation of pre-B cells. *Int. Immunol.* 5, 1609–1618.
- Grawunder, U., Leu, T.M., Schatz, D.G., Werner, A., Rolink, A.G., Melchers, F., et al., 1995. Down-regulation of RAG1 and RAG2 gene expression in preB cells after functional immunoglobulin heavy chain rearrangement. *Immunity* 3, 601–608.
- Guo, C., Alt, F.W., Giallourakis, C., 2011a. PAIRing for distal IgH locus V(D)J recombination. *Immunity* 34, 139–141.
- Guo, C., Yoon, H.S., Franklin, A., Jain, S., Ebert, A., Cheng, H.L., et al., 2011b. CTCF-binding elements mediate control of V(D) J recombination. *Nature* 477, 424–430.
- Haasner, D., Rolink, A., Melchers, F., 1994. Influence of surrogate L chain on DHJH-reading frame 2 suppression in mouse precursor B cells. *Int. Immunol.* 6, 21–30.
- Hayakawa, K., Hardy, R.R., Herzenberg, L.A., Herzenberg, L.A., 1985. Progenitors for Ly-1 B cells are distinct from progenitors for other B cells. *J. Exp. Med.* 161, 1554–1568.
- Höflinger, S., Kesavan, K., Fuxa, M., Hutter, C., Heavey, B., Radtke, F., et al., 2004. Analysis of Notch1 function by in vitro T cell differentiation of Pax5 mutant lymphoid progenitors. *J. Immunol.* 173, 3935–3944.
- Hooijkaas, H., Benner, R., Pleasants, J.R., Wostmann, B.S., 1984. Isotypes and specificities of immunoglobulins produced by germ-free mice fed chemically defined ultrafiltered “antigen-free” diet. *Eur. J. Immunol.* 14, 1127–1130.
- Huppa, J.B., Davis, M.M., 2013. The interdisciplinary science of T-cell recognition. *Adv. Immunol.* 119, 1–50.
- Huppa, J.B., Axmann, M., Mörtelmaier, M.A., Lillemeyer, B.F., Newell, E.W., Brameshuber, M., et al., 2010. TCR-peptide-MHC interactions in situ show accelerated kinetics and increased affinity. *Nature* 463, 963–967.
- Ikawa, T., Kawamoto, H., Wright, L.Y., Murre, C., 2004. Long-term cultured E2A-deficient hematopoietic progenitor cells are pluripotent. *Immunity* 20, 349–360.
- Irion, S., Clarke, R.L., Luche, H., Kim, I., Morrison, S.J., Fehling, H.J., et al., 2010. Temporal specification of blood progenitors from mouse embryonic stem cells and induced pluripotent stem cells. *Development* 137, 2829–2839.
- Jerne, N.K., 1955. The natural-selection theory of antibody formation. *Proc. Natl. Acad. Sci. U.S.A.* 41, 849–857.
- Kajikhina, K., Tsuneto, M., Melchers, F., 2016. B-lymphopoiesis in fetal liver, guided by chemokines. *Adv. Immunol.* 132, 71–89. Available from: <https://doi.org/10.1016/bs.ai.2016.07.002>. Epub 2016 Aug 5. Review.
- Kawano, Y., Yoshikawa, S., Minegishi, Y., Karasuyama, H., 2006. Pre-B cell receptor assesses the quality of IgH chains and tunes the pre-B cell repertoire by delivering differential signals. *J. Immunol.* 177, 2242–2249.
- Kee, B.L., Murre, C.J., 1998. Induction of early B cell factor (EBF) and multiple B lineage genes by the basic helix-loop helix transcription factor E12. *Exp. Med.* 188, 699–713.
- Khan, S.N., Witsch, E.J., Goodman, N.G., Panigrahi, A.K., Chen, C., Jiang, Y., et al., 2008. Editing and escape from editing in anti-DNA B cells. *Proc. Natl. Acad. Sci. U.S.A.* 105, 3861–3866.
- King, C., 2009. New insights into the differentiation and function of T follicular helper cells. *Nat. Rev. Immunol.* 9, 757–766.
- Klein, U., Dalla-Favera, R., 2008. Germinal centres, role in B-cell physiology and malignancy. *Nat. Rev. Immunol.* 8, 22–33.
- Knoll, M., Yanagisawa, Y., Simmons, S., Engels, N., Wienands, J., Melchers, F., et al., 2012. The non-Ig parts of the VpreB and λ5 proteins of the surrogate light chain play opposite roles in the surface representation of the precursor B cell receptor. *J. Immunol.* 188, 6010–6017.
- Knoll, M., Simmons, S., Bouquet, C., Grün, J.R., Melchers, F., 2013. miR-221 redirects precursor B cells to the BM and regulates their residence. *Eur. J. Immunol.* Available from: <https://doi.org/10.1002/eji.201343367>.
- Köhler, F., Hug, E., Eschbach, C., Meixlperger, S., Hobeka, E., Kofer, J., et al., 2008. Autoreactive B cell receptors mimic autonomous pre-B cell receptor signaling and induce proliferation of early B cells. *Immunity* 29, 912–921.
- Kumar, K.R., Li, L., Yan, M., Bhaskarabhatla, M., Mobley, A.B., Nguyen, C., et al., 2006. Regulation of B cell tolerance by the lupus susceptibility gene Ly108. *Science* 312, 1665–1669.
- Kwon, K., Hutter, C., Sun, Q., Bilic, I., Cobaleda, C., Malin, S., et al., 2008. Instructive role of the transcription factor E2A in early B lymphopoiesis and germinal center B cell development. *Immunity* 28, 751–762.
- Lederberg, J., 1959. Genes and antibodies: do antigens bear instructions for antibody specificity or do they select cell lines that arise by mutation? *Science* 129, 1649–1653.
- Lesinski, D.A., Heinz, N., Pilat-Carotta, S., Rudolph, C., Jacobs, R., Schlegelberger, B., et al., 2012. Serum- and stromal cell-free hypoxic generation of embryonic stem cell-derived hematopoietic cells in vitro, capable of multilineage repopulation of immunocompetent mice. *Stem Cells Transl. Med.* 1, 581–591.
- Editors and editing of anti-DNA receptors. In: Li, H., Jiang, Y., Prak, E.L., Radic, M., Weigert, M. (Eds.), *Immunity*, 15. pp. 947–957.
- Ling, K.W., Dzierzak, E., 2002. Ontogeny and genetics of the hemato/lymphopoietic system. *Curr. Opin. Immunol.* 14, 186–191.
- Liu, Y., Li, L., Kumar, K.R., Xie, C., Lightfoot, S., Zhou, X.J., et al., 2007. Lupus susceptibility genes may breach tolerance to DNA by impairing receptor editing of nuclear antigen-reactive B cells. *J. Immunol.* 179, 1340–1352.
- Lu, R., Neff, N.F., Quake, S.R., Weissman, I.L., 2011. Tracking single hematopoietic stem cells in vivo using high-throughput sequencing in conjunction with viral genetic barcoding. *Nat. Biotechnol.* 29, 928–933.
- Luckey, C.J., Bhattacharya, D., Goldrath, A.W., Weissman, I.L., Benoit, C., Mathis, D., 2006. Memory T and memory B cells share a transcriptional program of self-renewal with long-term hematopoietic stem cells. *Proc. Natl. Acad. Sci. U.S.A.* 103, 3304–3309.
- Mackay, F., Woodcock, S.A., Lawton, P., Ambrose, C., Baetscher, M., Schneider, P., et al., 1999. Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. *J. Exp. Med.* 190, 1697–1710.
- Medvedovic, J., Ebert, A., Tagoh, H., Busslinger, M., 2011. Pax5: a master regulator of B cell development and leukemogenesis. *Adv. Immunol.* 111, 179–206.
- Medvinsky, A., Dzierzak, E., 1996. Definitive hematopoiesis is autonomously initiated by the AGM region. *Cell* 86, 897–906.

- Meffre, E., Schaefer, A., Wardemann, H., Wilson, P., Davis, E., Nussenzweig, M.C., 2004. Surrogate light chain expressing human peripheral B cells produce self-reactive antibodies. *J. Exp. Med.* 199, 145–150.
- Melchers, F., 2005. The pre-B-cell receptor, selector of fitting immuno-globulin heavy chains for the B-cell repertoire. *Nat. Rev. Immunol.* 5, 578–584.
- Melchers, F., Rolink, A., 1999. B lymphocyte development and biology. In: Paul, W.E. (Ed.), *Fundamental Immunology*, fourth ed Lippincott-Raven, Philadelphia, PA, pp. 183–224.
- Melchers, F., Rolink, A.R., 2006. B cell tolerance—how to make it and how to break it. *Curr. Top. Microbiol. Immunol.* 305, 1–23.
- Melchers, F., Yamagami, T., Rolink, A., Andersson, J., 2007. Rules for the rearrangement events at the L chain gene loci of the mouse. *Adv. Exp. Med. Biol.* 596, 63–70.
- Montecino-Rodriguez, E., Dorshkind, K., 2012. B-1 B cell development in the fetus and adult. *Immunity* 36, 13–21.
- Montecino-Rodriguez, E., Leathers, H., Dorshkind, K., 2006. Identification of a B-1 B cell-specified progenitor. *Nat. Immunol.* 7, 293–301.
- Muramatsu, M., Kinoshita, K., Fagarasan, S., Yamada, S., Shinkai, Y., Honjo, T., 2000. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell* 102, 553–563.
- Nakajima, P.B., Kiefer, K., Price, A., Bosma, G.C., Bosma, M.J., 2009. Two distinct populations of H chain-edited B cells show differential surrogate L chain dependence. *J. Immunol.* 182, 3583–3596.
- Nutt, S.L., Heavey, B., Rolink, A.G., Busslinger, M., 1999. Commitment to the B-lymphoid lineage depends on the transcription factor Pax5. *Nature* 401, 556–562.
- Ochiai, K., Maienschein-Cline, M., Simonetti, G., Chen, J., Rosenthal, R., Brink, R., et al., 2013. Transcriptional regulation of germinal center B and plasma cell fates by dynamical control of IRF4. *Immunity* 38, 918–929.
- Ohmura, K., Kawamoto, H., Lu, M., Ikawa, T., Ozaki, S., Nakao, K., et al., 2001. Immature multipotent hemopoietic progenitors lacking long-term bone marrow-reconstituting activity in the aorta-gonad-mesonephros region of murine day 10 fetuses. *J. Immunol.* 166, 3290–3296.
- Ohnishi, K., Melchers, F., 2003. The nonimmunoglobulin portion of lambda5 mediates cell-autonomous pre-B cell receptor signaling. *Nat. Immunol.* 4, 849–856.
- Pierce, H., Zhang, D., Magnon, C., Lucas, D., Christin, J.R., Huggins, M., et al., 2017. Cholinergic signals from the CNS regulate G-CSF-mediated HSC mobilization from bone marrow via a glucocorticoid signaling relay. *Cell Stem Cell* 20 (5), 648–658.e4. doi: 10.1016/j.stem.2017.01.002.
- Radtke, F., Wilson, A., MacDonald, H.R., 2004. Notch signaling in T- and B-cell development. *Curr. Opin. Immunol.* 16, 174–179.
- Revilla-I-Domingo, R., Bilic, I., Vilagos, B., Tagoh, H., Ebert, A., et al., 2012. The B-cell identity factor Pax5 regulates distinct transcriptional programmes in early and late B lymphopoiesis. *EMBO J.* 31, 3130–3146.
- Revy, P., Muto, T., Levy, Y., Geissmann, F., Plebani, A., Sanal, O., et al., 2000. Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the hyper-IgM syndrome (HIGM2). *Cell* 102, 565–575.
- Roessler, S., Grosschedl, R., 2006. Role of transcription factors in commitment and differentiation of early B lymphoid cells. *Semin. Immunol.* 18, 12–19.
- Rolink, A., Kudo, A., Karasuyama, H., Kikuchi, Y., Melchers, F., 1991. Long-term proliferating early pre B cell lines and clones with the potential to develop to surface Ig-positive, mitogen reactive B cells in vitro and in vivo. *EMBO J.* 10, 327–336.
- Rolink, A., Karasuyama, H., Grawunder, U., Haasner, D., Kudo, A., Melchers, F., 1993. B cell development in mice with a defective lambda 5 gene. *Eur. J. Immunol.* 23, 1284–1288.
- Rolink, A., Melchers, F., Andersson, J., 1996. The SCID but not the RAG-2 gene product is required for S mu-S epsilon heavy chain class switching. *Immunity* 5, 319–330.
- Rolink, A.G., Nutt, S.L., Melchers, F., Busslinger, M., 1999. Long-term in vivo reconstitution of T-cell development by Pax5-deficient B-cell progenitors. *Nature* 401, 603–606.
- Rolink, A.G., Tschopp, J., Schneider, P., Melchers, F., 2002. BAFF is a survival and maturation factor for mouse B cells. *Eur. J. Immunol.* 32, 2004–2010.
- Rossi, D.J., Seita, J., Czechowicz, A., Bhattacharya, D., Bryder, D., Weissman, I.L., 2007. Hematopoietic stem cell quiescence attenuates DNA damage response and permits DNA damage accumulation during aging. *Cell Cycle* 6, 2371–2376.
- Schaniel, C., Bruno, L., Melchers, F., Rolink, A.G., 2002a. Multiple hematopoietic cell lineages develop in vivo from transplanted Pax5-deficient pre-B I cell clones. *Blood* 99, 472–478.
- Schaniel, C., Gottar, M., Roosnek, E., Melchers, F., Rolink, A.G., 2002b. Extensive in vivo self-renewal, long-term reconstitution capacity, and hematopoietic multipotency of Pax5-deficient precursor B-cell clones. *Blood* 99, 2760–2766.
- Schebesta, A., McManus, S., Salvagiotto, G., Delogu, A., Busslinger, G.A., Busslinger, M., 2007. Transcription factor Pax5 activates the chromatin of key genes involved in B cell signaling, adhesion, migration, and immune function. *Immunity* 27, 49–63.
- Schroeder, K., Herrmann, M., Winkler, T.H., 2013. The role of somatic hypermutation in the generation of pathogenic antibodies in SLE. *Autoimmunity* 46, 121–127.
- Schulz, C., Gomez Perdiguer, E., Chorro, L., Szabo-Rogers, H., Cagnard, N., Kierdorf, K., et al., 2012. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* 336, 86–90.
- Shapiro-Shelef, M., Calame, K., 2005. Regulation of plasma-cell development. *Nat. Rev. Immunol.* 5, 230–242.
- Shimizu, T., Mundt, C., Licence, S., Melchers, F., Mårtensson, I.L., 2002. VpreB1/VpreB2/lambda 5 triple-deficient mice show impaired B cell development but functional allelic exclusion of the IgH locus. *J. Immunol.* 168, 6286–6293.
- Shlomchik, M.J., 2008. Sites and stages of autoreactive B cell activation and regulation. *Immunity* 28, 18–28.
- Sigvardsson, M., O'Riordan, M., Grosschedl, R., 1997. EBF and E47 collaborate to induce expression of the endogenous immunoglobulin surrogate light chain genes. *Immunity* 7, 25–36.
- Simmons, S., Knoll, M., Drewell, C., Wolf, I., Mollenkopf, H.J., Bouquet, C., et al., 2012. Biphenotypic B-lymphoid/myeloid cells expressing low levels of Pax5, potential targets of BAL development. *Blood* 120, 3688–3698.
- ten Boekel, E., Melchers, F., Rolink, A.G., 1997. Changes in the V(H) gene repertoire of developing precursor B lymphocytes in mouse bone marrow mediated by the pre-B cell receptor. *Immunity* 7, 357–368.

- ten Boekel, E., Melchers, F., Rolink, A.G., 1998. Precursor B cells showing H chain allelic inclusion display allelic exclusion at the level of pre-B cell receptor surface expression. *Immunity* 8, 199–207.
- Tokoyoda, K., Hauser, A.E., Nakayama, T., Radbruch, A., 2010. Organization of immunological memory by bone marrow stroma. *Nat. Rev. Immunol.* 10, 193–200.
- Tornack, J., Reece, S.T., Bauer, W.M., Vogelzang, A., Bandermann, S., Zedler, U., et al., 2017. Human and mouse hematopoietic stem cells are a depot for dormant mycobacterium tuberculosis. *PLoS One* 12 (1), e0169119. doi: 10.1371/journal.pone.0169119.eCollection 2017.
- Tsuneto, M., Tokoyoda, K., Kajikhina, E., Hauser, A.E., Hara, T., Tanilchi, S., et al., 2013. B cell progenitors and precursors change their microenvironment in fetal liver during early development. *Stem Cells* doi . Available from: <https://doi.org/10.1002/stem.1421>.
- Tung, J.W., Mrazek, M.D., Yang, Y., Herzenberg, L.A., Herzenberg, L.A., 2006. Phenotypically distinct B cell development pathways map to the three B cell lineages in the mouse. *Proc. Natl. Acad. Sci. U.S.A.* 103, 6293–6298.
- Vegh, P., Winckler, J., Melchers, F., 2010. Long-term “in vitro” proliferating mouse hematopoietic progenitor cell lines. *Immunol. Lett.* 130, 32–35.
- Vilagos, B., Hoffmann, M., Souabni, A., Sun, Q., Werner, B., Medvedovic, J., et al., 2012. Essential role of EBF1 in the generation and function of distinct mature B cell types. *J. Exp. Med.* 209, 775–792.
- Vinuesa, C.G., Linterman, M.A., Goodnow, C.C., Randall, K.L., 2010. T cells and follicular dendritic cells in germinal center B-cell formation and selection. *Immunol. Rev.* 237, 72–89.
- von Boehmer, H., Melchers, F., 2010. Checkpoints in lymphocyte development and autoimmune disease. *Nat. Immunol.* 11, 14–20.
- Wagers, A.J., Weissman, I.L., 2004. Plasticity of adult stem cells. *Cell* 116, 639–648.
- Wardemann, H., Nussenzweig, M.C., 2007. B-cell self-tolerance in humans. *Adv. Immunol.* 95, 83–110.
- Wardemann, H., Boehm, T., Dear, N., Carsetti, R., 2002. B-1a B cells that link the innate and adaptive immune responses are lacking in the absence of the spleen. *J. Exp. Med.* 195, 771–780.
- Weill, J.C., Le Gallou, S., Hao, Y., Reynaud, C.A., 2013. Multiple players in mouse B cell memory. *Curr. Opin. Immunol.* 25, 334–338.
- Witsch, E.J., Cao, H., Fukuyama, H., Weigert, M., 2006. Light chain editing generates polyreactive antibodies in chronic graft-versus-host reaction. *J. Exp. Med.* 203, 1761–1772.
- Wolf, I., Bouquet, C., Melchers, F., 2016. cDNA-library testing identifies transforming genes cooperating with c-myc in mouse pre-B cells. *Eur. J. Immunol.* 46 (11), 2555–2565. Available from: <https://doi.org/10.1002/eji.201646419>. Epub 2016 Sep 14.
- Wolf, I., Bouquet, C., Naumann, F., Melchers, F., 2017. Generation of precursor, immature, and mature murine B1-cell lines from c-myc/bcl-xL-overexpressing pre-B1 cells. *Eur. J. Immunol.* 47 (5), 911–920. Available from: <https://doi.org/10.1002/eji.201746937>. Epub 2017 Apr 6.
- Xie, C., Patel, R., Wu, T., Zhu, J., Henry, T., Bhaskarabhatla, M., et al., 2007. PI3K/AKT/mTOR hypersignaling in autoimmune lymphoproliferative disease engendered by the epistatic interplay of Sle1b and FASlpr. *Int. Immunopharmacol.* 19, 509–522.
- Yokomizo, T., Ng, C.E., Osato, M., Dzierzak, E., 2011. Three-dimensional imaging of whole midgestation murine embryos shows an intravascular localization for all hematopoietic clusters. *Blood* 117, 6132–6134.
- Yurasov, S., Wardemann, H., Hammersen, J., Tsuji, M., Meffre, E., Pascual, V., et al., 2005. Defective B cell tolerance checkpoints in systemic lupus erythematosus. *J. Exp. Med.* 201, 703–711.
- Zhou, Z.H., Tzioufas, A.G., Notkins, A.L., 2007. Properties and function of polyreactive antibodies and polyreactive antigen-binding B cells. *J. Autoimmun.* 29, 219–228.
- Zhuang, Y., Soriano, P., Weintraub, H., 1994. The helix-loop-helix gene E2A is required for B cell formation. *Cell* 79, 875–884.

## Further Reading

- Bos, N.A., Meeuwsen, C.G., 1989. B cell repertoire in adult antigen-free and conventional neonatal BALB/c mice. I. Preferential utilization of the CH-proximal VH gene family PC7183. *Eur. J. Immunol.* 19, 1811–1815.
- Nat Gerdes, T., Wabl, M., 2004. Autoreactivity and allelic inclusion in a B cell nuclear transfer mouse. *Immunology* 5, 1282–1287.
- Pillai, S., Cariappa, A., Pirnie, S.P., 2009. Esterases and autoimmunity: the sialic acid acetylesterease pathway and the regulation of peripheral B cell tolerance. *Trends Immunol.* 30, 488–493.
- Tucholsky, K., 1931. Zwischen Gestern und Morgen. Eine Auswahl aus seinen Schriften und Gedichten. Herausgegeben von Mary Gerold-Tucholsky Rowohld Taschenbuch Verlag, 60. Auflage 2012, 42–43.

# B Cell Activation and B Cell Tolerance

Kristine Oleinika, Claudia Mauri and Paul A. Blair

Centre for Rheumatology, Division of Medicine, University College London, London, United Kingdom

## OUTLINE

<b>B Cell Activation</b>			
Antigen-Driven B Cell Activation	171	B Cell Anergy	179
Amplification and Modulation of B Cell Activation Signals	172	Characteristics of Anergic B Cells	179
Antigen Processing Following B Cell Activation	173		
<b>Optimal B Cell Activation Requires Interaction With T Helper Cells</b>		<b>B Cell–Activating Factor and Tonic Signals</b>	
Surface Molecules in B Cell–T Cell Interactions	173	<b>Modulate B Cell Tolerance</b>	180
Cytokines Involved in B Cell–T Cell Interactions	174	B Cell–Activating Factor in B Cell Development and Survival	180
<b>Maturation and Activation of B Cells Occur in Lymphoid Organs</b>		Tonic Signaling in B Cell Development and Survival	180
Location of B Cell Activation	174	B Cell–Activating Factor, Tonic Signaling, and Autoimmunity	180
B Cell Activation and the Germinal Center Response	175		
The Germinal Center	176	<b>Regulatory T Cells</b>	181
<b>T Cell–Independent Antibody Responses</b>			
<b>B Cell Tolerance: Traditional and New Concepts</b>		<b>Antibody-Independent Activity of B Cells in Tolerance</b>	
Mechanisms of B Cell Central Tolerance	177	Antigen Presentation by B Cells	181
Receptor Editing and Clonal Deletion	177	Cytokine Production by B Cells	182
Defective Receptor Editing and Clonal Deletion Can Promote Autoimmunity	178	Regulatory B Cells	182
	178	Future Directions	183
		<b>References</b>	184
		<b>Further Reading</b>	187

## B CELL ACTIVATION

B cells form an important part of the immune system by helping the host to fight pathogens or their products, and most successful vaccination strategies rely on an antibody response by B lymphocytes (Plotkin, 2010). Surveillance for foreign antigens requires B cells to (1) gain access to areas where antigen is available (migration); (2) recognize and bind to antigen (antigen specificity); (3) increase antigen-specific cell numbers (proliferation); and (4) give rise to *plasma cells* that secrete antibodies to neutralize or opsonize antigen and *memory cells* that “remember” previous antigen encounter and aid rapid and robust recognition of the same antigen during future infections (differentiation).

The process controlling this series of events is referred to as B cell activation. T cell–dependent (TD) activation of B cells occurs in two distinct phases. First, B cells are triggered by antigen and, second, antigen-specific B cells interact with activated T helper (Th) cells with the same antigen specificity.

## Antigen-Driven B Cell Activation

Like T cells, B cells express an antigen receptor, the B cell receptor (BCR), that is highly variable between different B cells (basic features of the BCR are given in [Box 9.1](#)). Engagement of the BCR by antigen initiates two interdependent processes, signaling and internalization of the antigen-receptor complex. Mature B cells that have not been previously activated by their specific antigen are referred to as “naïve” and express surface immunoglobulin (Ig) D and IgM (Ig isotypes are discussed further below). Nanoclusters of BCRs comprising IgD and IgM molecules are organized in regions of the cell membrane with distinct lipid and protein compositions ([Mattila et al., 2013](#)). Three alternative models have been proposed for how antigen-binding results in the initial signaling events downstream of the BCR. First, the conformational change model, which proposes that an antigen-induced change in the shape of the Ig is transmitted across the membrane allowing access to molecules that initiate BCR signaling ([Harwood and Batista, 2010](#)). Second, the dissociation model, which proposes that in naïve B cells, BCRs exist in inhibitory nanoclusters, which prevent access to the signaling molecules, and that antigen binding leads to the reorganization of these domains in such a way that the BCRs are dissociated, allowing signaling to occur. Third, the kinetic segregation/collision coupling model proposes that BCRs in resting B cells are associated with inhibitory coreceptors and segregated from activating coreceptors. Upon the binding and close contact with membrane-associated antigen, bulky inhibitory receptors are excluded from the close contact sites between the BCR and membrane, allowing signaling to occur. To date, there is evidence for the involvement of all three models in BCR signaling. All three models rely on the signal transduction ability of CD79a(Ig $\alpha$ )/CD79b(Ig $\beta$ ) dimers, which are molecules that are noncovalently linked to the membrane-bound Ig molecules. Membrane-bound Ig molecules contain no intrinsic signaling capability, which is provided by CD79 dimers. The conserved signaling motifs [immunoreceptor tyrosine-based activation motifs (ITAM)] contained within the cytosolic tails of CD79a/CD79b are tyrosine phosphorylated by Src-family tyrosine kinases (importantly, Lyn and Syk). These events cause further activation of intracellular signaling molecules and transcription of genes involved in the regulation of B cell activation ([Harwood and Batista, 2010](#)).

### BOX 9.1

#### BASIC FEATURES OF THE B CELL RECEPTOR

- B cells recognize specific antigens through antigen receptors, B cell receptors (BCRs), that are expressed early in B cell development following the rearrangement of immunoglobulin (Ig) genes.
- The BCR is a membrane-bound version of monomeric Ig (antibody) and comprises two identical heavy chains and light chains that are noncovalently associated with CD79 dimers that provide the signaling capacity of the BCR.
- In health, B cells should only express one copy of heavy and light chain variable region genes, and thus one specificity of BCRs.
- The BCR recognizes unprocessed antigen based on the complementarity of shape and charge between the antigen and the variable, antigen-binding domains of the BCR.
- Diversity in BCR specificities is achieved through the stochastic recombination of the genes that encode the highly variable antigen-binding domains of Ig heavy and light chains.
- Rearrangement of Ig genes to bring heavy chain variable region genes into proximity with different heavy chain constant region genes determines the isotype of antibodies the B cells produce. These can be either IgM, IgG, IgA, or IgE.
- The nature of the constant region of the BCR, and the isotype of the antibody produced, can change over the course of an immune response, allowing the B cell to make the appropriate antibody response for the particular invading pathogen.

## Amplification and Modulation of B Cell Activation Signals

A number of B cell surface coreceptors are involved in amplification and modulation of BCR-mediated signals. These fine-tune the threshold for B cell triggering and thus balance activation and tolerance. Among these molecules are CD19, CD81, and CD21, which comprise the BCR-coreceptor complex. CD19 is the only molecule with a signal transduction domain in this coreceptor complex. CD81 immobilizes CD19 in nanoclusters at the plasma membrane (Mattila et al., 2013). CD21 (also referred to as complement receptor 2) binds to iC3b, C3dg, or C3d, which are components of the complement pathway (Carroll, 1998; Harwood and Batista, 2010). Via CD21, the BCR-coreceptor complex can bind to complement components deposited on microbial antigens at the same time as the BCR binds to epitopes on the antigen. Thus CD21 allows the cross-linking of the BCR and CD19 and enhances BCR activation and subsequent signal transduction in response to antigen. In support of their importance to BCR signaling, CD19 or CD21 deficiency leads to an inability to mount responses to TD antigens (Ahearn et al., 1996). The importance of complement-mediated activation of B cells is shown in mice with a disrupted CD21 gene, these mice display a reduced number of B cells in the spleen, a defect that is reversed upon reconstitution with bone marrow from CD21-expressing wild-type mice (Ahearn et al., 1996). Similarly, mice lacking CD21 expression are unable to generate antigen-specific plasma cells or to maintain antibody production in response to viral infection, as CD21-mediated costimulation is required to induce the transcriptional regulators Blimp-1 and XBP-1, molecules driving plasma cell differentiation (Gatto et al., 2005).

B cells also express a variety of surface molecules containing immunoreceptor tyrosine-based inhibitory motifs (ITIM). For example, the ligation of the inhibitory coreceptor CD22 causes phosphorylation of the ITIM and, subsequently, recruitment of the SH2 domain-containing phosphatase 1 (SHP-1), which negatively regulates signaling through the BCR (Doody et al., 1995) by dephosphorylating BCR-activated protein tyrosine kinases and their downstream targets. In addition to SHP-1, ITIMs recruit such regulators as SH2 domain-containing inositol 5'-phosphatase 1 (SHIP-1) and phosphatase and tensin homolog (PTEN). These are particularly important in establishing thresholds for B cell activation and maintenance of anergy, a state of antigen-specific hyporesponsiveness. Indeed, a number of studies have shown that aberrant negative circuits can lead to a breach in tolerance and, consequently, autoimmunity (discussed further below).

## Antigen Processing Following B Cell Activation

B cells process antigen through a series of orderly steps. BCR-mediated antigen capture and internalization is the initial event in the process that culminates in antigen presentation to T cells. Internalized antigens are directed to the endo/lysosomes, where the antigen is degraded. Importantly, these are sites of proteolysis that generate peptide fragments to be loaded onto major histocompatibility complex (MHC) class II molecules and transported to the cell membrane for presentation to Th cells. The intracellular trafficking of BCR–antigen complexes is mediated via the reorganization of actin cytoskeleton and involves the synergistic action of small proteins that promote cell contraction, for example, myosin II and chaperone molecules that guide the BCR–antigen complexes to lysosomes (Vascotto et al., 2007). BCR-mediated endocytosis initiates signaling cascades that enhance the synthesis of molecules involved in B–T cell interaction. Among these are MHC class II molecules and increased expression of the costimulatory molecule 86 (B7–2) (Rodriguez-Pinto and Moreno, 2005). CD86 binds CD28 on T cells and is critical for their activation. Any defects in the molecules or mechanisms involved in the transport of antigen or its degradation, or in the loading onto MHC class II, result in impaired antigen presentation to T cells and consequently abnormal B cell activation.

## OPTIMAL B CELL ACTIVATION REQUIRES INTERACTION WITH T HELPER CELLS

T cell help for B cells manifests through both cognate interactions and secretion of cytokines (Guy and Hodes, 1989). During cognate interactions, B cells present peptides in MHC class II–antigen complexes for T cell recognition, and both cells provide reciprocal activating signals in a process known as costimulation. Of note, this interaction requires specificity of B and T cells for the same, or linked, antigen, but each cell may recognize a different epitope of those antigens. For example, the BCR can recognize an exposed structure on a pathogen surface in its native conformation, while the T cell can respond to a processed peptide fragment of internal pathogen molecule that is internalized along with the molecule recognized by the BCR. In addition, the BCR can recognize multiple

types of molecules, for instance, proteins, lipids, and carbohydrates in their native structure, while the T cell receptor can only recognize processed peptides presented by MHC molecules. This ability to recognize different, but associated, parts of the same pathogen is referred to as linked recognition.

## Surface Molecules in B Cell–T Cell Interactions

CD40, which is constitutively expressed on the surface of B cells, has a critical role in sustaining B cell activation and enhancement of their antigen-presenting capacity. CD40 interacts with CD154, which is expressed on activated T cells. The engagement of CD40 by CD154 leads to a further upregulation of CD86 on B cells, which in turn binds to CD28 on T cells, resulting in feed-forward activation of T cells.

The interaction between CD40 and CD154 is also important for class-switch recombination (CSR) (discussed later), the clinical relevance of which became apparent when defects in the CD154 gene were found to be associated with the development of X-linked hyper IgM syndrome. These patients are unable to switch the isotype of secreted antibodies, resulting in a higher proportion of IgM production at the expense of IgG, IgA, and IgE. As a result of the inability to produce class-switched antibodies, patients with X-linked hyper IgM syndrome also have increased susceptibility to common infections, in particular to opportunistic pathogens (Noelle, 1996). Blocking the CD40–CD154 pathway, and thus immune activation, using an anti-CD154 monoclonal antibody has been shown to be effective in reducing the severity of autoimmune responses, such as experimental collagen-induced arthritis in rodents (Durie et al., 1993) and immune thrombocytopenia in humans (Kuwana et al., 2004), further reinforcing the importance of this pathway in B cell activation. More recently it has also been shown that a CD40 blocking antibody (CFZ533, in phase II clinical trials), which inhibits activation through CD40/CD154 interactions, has beneficial effects for patients with primary Sjögren's syndrome (NCT02291029).

## Cytokines Involved in B Cell–T Cell Interactions

Activation of costimulatory pathways not only stabilizes and strengthens the communication between B and T cells but also leads to the transcription of several genes by both B and T cells, including the upregulation of cytokine receptors on the surface of B lymphocytes. A number of cytokines produced by activated T cells are important in guiding B cell activation. For example, T cell IL-2 promotes B cell proliferation, while IL-4 and IL-10 are known to drive B cell differentiation (Mingari et al., 1984; Rush and Hodgkin, 2001). Cytokines also instruct the isotype of Ig in CSR. For example, IL-4 drives IgG1 and IgE production, while TGF- $\beta$  drives IgG2b and IgA and IFN- $\gamma$  drives IgG2a and IgG3 in mice (McHeyzer-Williams and McHeyzer-Williams, 2005).

Understanding the costimulation pathways involved in the activation of B cells and their further maturation to antibody-producing plasma cells has led to the development of therapeutic agents to prevent the development of autoreactive antibodies and to treat autoimmune diseases. For example, abatacept, a CTLA4-Ig fusion protein, was developed to block costimulation of T cells by B cells, and other antigen-presenting cells, and its safety and therapeutic efficacy have been demonstrated in the treatment of rheumatoid arthritis (Genovese et al., 2012).

## MATURATION AND ACTIVATION OF B CELLS OCCUR IN LYMPHOID ORGANS

The previous section described how activation of B cells requires highly synchronized processes initiated by the recognition of antigen and by the interaction with T cells. But where do these interactions take place? In the bone marrow, B cells carrying an antigen receptor develop from pluripotent hematopoietic stem cells. B cells that have successfully expressed a functional BCR and that have passed central tolerance checkpoints (discussed next) migrate to the periphery where they progress through distinct developmental stages. Finally, upon antigen-driven activation, they can differentiate into long-lived plasma cells and memory B cells.

## Location of B Cell Activation

Mature naïve B cells reside mainly in primary lymphoid follicles in lymph nodes, spleen, and mucosa-associated lymphoid tissue. In addition to B cells, these areas are enriched with follicular dendritic cells (FDC), which are cells of mesenchymal origin that help organize the lymphoid architecture and play critical roles in B cell activation and affinity maturation. FDCs express a variety of cell surface receptors, including Fc receptors

CD23 and CD32, which bind the constant (nonvariable) region of IgS and complement receptors CD21 and CD35. These cells can, therefore, capture and retain antigen in native conformation on their surface, generating what are referred to as antigen depots. These antigen deposits are important in the development of an already initiated immune response; however, initial B cell encounter with antigen may rely on several methods. First, small soluble antigens may gain access to the follicles directly from afferent lymph vessels and be accessible to B cells as free diffusing antigen. Second, particulate opsonized antigen can be presented to B cells as they migrate through the follicles, either on the surface of specialized macrophages located in the subcapsular sinus of lymph nodes or on the surfaces of FDCs. Third, dendritic cells may migrate into lymph nodes carrying unprocessed intact antigen that antigen-specific B cells are able to remove from their cell surface (Cyster, 2010, 135).

B cell migration through the lymph nodes is guided by the release of chemokines by FDCs, and marginal and fibroblastic reticular cells (Cyster, 2010). Mature B cells express the chemokine receptors CXCR5, CCR7, and Epstein–Barr virus-induced gene 2 (EBI2), which is a G protein-coupled receptor that binds oxysterols (Hannedouche et al., 2011). Integration of signals from CXCR5 and CCR7 allows B cells to navigate the lymphoid structures guided by stromal cell chemokine gradients of CXCL13 in the B cell follicle and CCL19/21 in the T cell zone, respectively. Importantly, CCR7 and EBI2 are upregulated upon B cell activation. The upregulation of CCR7 expression causes increased responsiveness to CC19/21, enabling the B cells to migrate to the B–T cell border where they interact with cognate T cells. B cell migration is a dynamic process and B cells that have not been primed (not encountered their specific antigen) within around 24 hours of arriving in the lymphoid tissue recirculate back to the blood in search of cognate antigen in other peripheral lymphoid tissues (Gonzalez et al., 2011).

## B Cell Activation and the Germinal Center Response

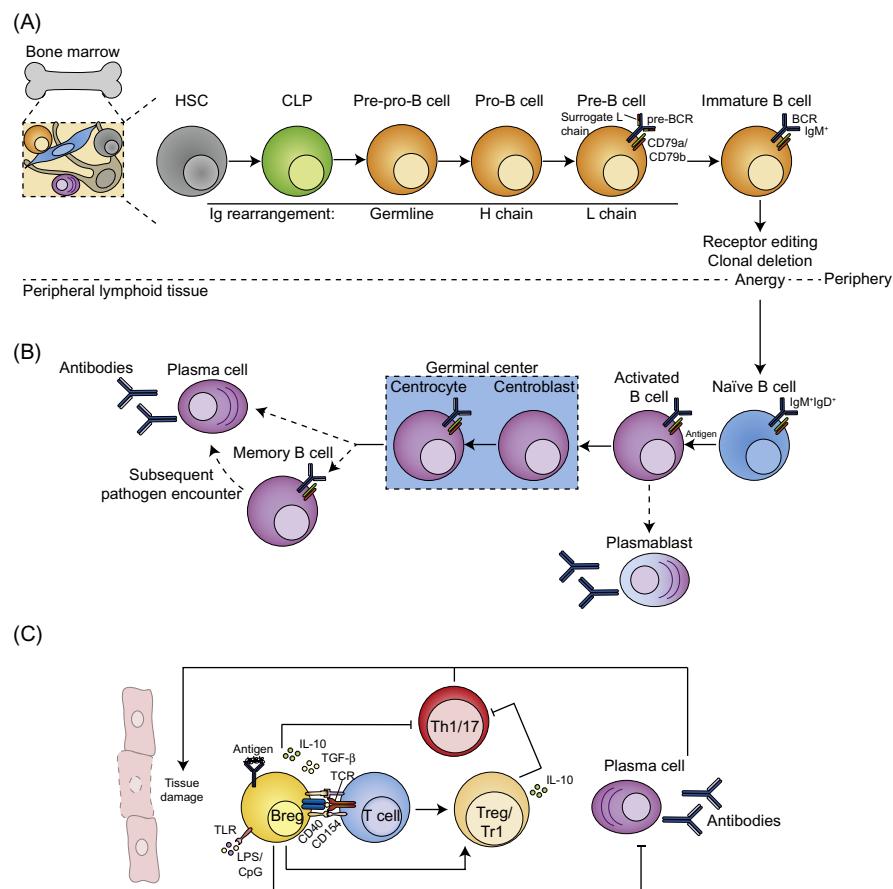
The initial interaction with T cells at the B–T cell border can result in two alternative outcomes for B cells. First, B cells can differentiate into short-lived antibody-secreting cells, known as plasmablasts, which provide an immediate IgM antibody response to control the spread of infection. Second, some of the antigen-activated B cells migrate into the primary lymphoid follicle and form germinal centers. Germinal centers comprise dividing B cells, FDCs, and a small proportion of antigen-specific T cells that provide help to B cells (Cyster, 2010). Several important events related to activation occur in germinal centers, including *somatic hypermutation* (SHM), a process altering the sequences of the variable (V)-regions of Ig genes; *affinity maturation*, the selection of the B cells with high antigen specificity; and CSR, whereby the selected B cells change the class of antibodies they secrete to exert their distinct functions.

During SHM, site-directed point mutations are induced in the V regions of the Ig genes of activated B cells, resulting in the expression of BCRs with potentially altered specificities. This process is facilitated by the enzyme activation-induced cytidine deaminase (AID). The affinity of the newly generated BCRs is then tested by competition with other B cells for the ability to bind and remove antigens held by FDCs. If SHM results in a BCR with an increased affinity for the original antigen, the B cell expressing this BCR will outcompete antigen-specific B cells with lower BCR–antigen affinities to bind antigen held by FDCs, which it can subsequently present to in cognate interactions to Tfh cells. Tfh then provides survival signals to these B cells, through both CD154–CD40 interactions and by provision of IL-21, a cytokine that drives B cell proliferation and retention in the germinal centers. This process, called affinity maturation, will lead to a pool of antigen-specific B cells secreting higher affinity antibodies than those secreted by the initial antigen-specific B-cell pool and will thus result in the generation of B cells better equipped to combat the infection (Di Noia and Neuberger, 2007).

CSR refers to the irreversible recombination events that change the class of the BCR and antibody expressed by the B cell from IgM/IgD initially to IgG, IgA, or IgE (Muramatsu et al., 2000). Each of these classes of the antibody has distinct effector functions and the particular class of antibody that results will be driven by the nature of the immune response and resultant cytokine microenvironment. Intrachromosomal recombination of the Ig genes causes IgM and IgD heavy chain genes to be excised from the Ig locus and the constant region for another Ig heavy chain to be joined to the heavy chain Ig variable region. The variable region of the Ig heavy chain remains unchanged. Thus in the germinal center reaction, B lymphocytes can alter their IgS from low-affinity IgM/IgD to a high-affinity IgG/IgA/IgE/IgM class (Di Noia and Neuberger, 2007). Like SHM, CSR requires AID, and AID deficiency results in the development of hyper IgM syndrome type 2. These immunodeficient patients are characterized by the absence of Ig CSR, the lack of Ig SHM, and lymph node hyperplasia caused by the presence of enlarged germinal centers (Revy et al., 2000). The germinal center reaction prepares the B cell for production of a long-term targeted response to a foreign antigen.

## The Germinal Center

Histologically, the germinal centers can be divided into two main zones: the dark zone and the light zone. Within the dark zone of the germinal centers, antigen-activated B cells mature to form large rapidly proliferating cells called centroblasts (Fig. 9.1B). It is at this stage and in this location that centroblasts diversify their IgV genes by SHM. Centroblasts move out from the densely packed dark zone and enter the light zone (FDC area) where they differentiate into noncycling centrocytes. Although most centrocytes die in situ, those receiving appropriate help from surrounding T cells will survive and can either undergo further rounds of SHM or differentiate into memory B cells or plasma cells. The outcome of the germinal center reaction is the production of (1) plasma cells



**FIGURE 9.1** Following the journey of B cells from the bone marrow to the periphery. (A) B cell development and tolerance. In the bone marrow, B cells develop from common lymphoid progenitors that originate from HSC. The stochastic rearrangement of Ig genes results in expression of the BCR. In the bone marrow, the B cell is tested for self-reactivity. If the B cell recognizes self-antigen, its developmental progress can be halted and the BCR may be edited to produce a nonself-reactive version, this is known as receptor editing. If receptor editing cannot remove the autoreactivity, then the B cell will generally either apoptose (clonal deletion) or enter a hyporesponsive state (anergy). B cells exit the bone marrow as immature transitional cells and complete their maturation to IgM<sup>+</sup>IgD<sup>+</sup> mature naive B cells in peripheral lymphoid organs, in particular the spleen. B cells may also enter a state of anergy after they leave the bone marrow if they are subject to prolonged exposure to soluble antigen exposure in the absence of costimulation by cognate T cells. (B) B cell activation. Mature naive B cells recirculate around the peripheral lymphoid tissues (lymph nodes and spleen) via the blood and lymphatic systems. In peripheral lymphoid tissues, B cells follow chemoattractant gradients to migrate to areas of these tissues where they can interact with antigen-bearing cells and T cells. Interaction with antigen results in B cell activation. If the antigen-activated B cell receives T cell help, or sufficient T cell-independent stimulation, the B cell will begin the process of differentiation into terminal effector cells such as plasmablasts, plasma cells, and memory B cells. Some B cells will give rise to short-lived plasmablasts initially largely producing IgM, while others will enter germinal centers in an attempt to refine specificity and affinity of their BCRs for the activating antigen. These B cells will differentiate into long-lived memory B cells and plasma cells. (C) Suppression of an autoreactive response. Additional regulatory systems exist to control the potential damage that maturation of autoreactive B cells may have on the immune system. In response to activation, some B cells may differentiate into Bregs. Bregs limit tissue damage in certain autoimmune conditions by dampening inflammatory responses. This is achieved by the secretion of antiinflammatory cytokines, such as IL-10 and TGF- $\beta$ , and/or via inhibition of the development of proinflammatory T cells (T helper 1/T helper 17), and/or by promoting the development of Tregs, which in turn inhibit the production of autoantibody. HSC, Hematopoietic stem cells; Ig, immunoglobulin; BCR, B cell receptor; Bregs, regulatory B cells; IL, interleukin; TGF, transforming growth factor; Tregs, regulatory T cells.

that secrete high-affinity antigen-specific IgGs and (2) memory B cells that protect against any subsequent encounter with the same antigen. Memory B cells and plasma cells are long-lived cells, surviving for many years or potentially for the lifetime of the host (Klein and Dalla-Favera, 2008). The potential of B cells to differentiate from naïve B cells into plasma cells, memory, and germinal center B cells was addressed by Taylor et al. They showed that while some naïve B cells only gave rise to one of these effector cell types, others, through clonal expansion, were able to differentiate into all three. This ability to acquire all three fates is dependent on the B cell's ability to resist apoptosis and affinity for antigen in the germinal center reaction (Taylor et al., 2015).

## T CELL-INDEPENDENT ANTIBODY RESPONSES

B cell activation and antibody production that require T cell help, as described above, are known as TD responses. An Ig response that occurs in the absence of T cell help is referred to as T cell independent (TI). TI responses can be induced by structures shared by many common pathogens, for instance, lipopolysaccharides that form the outer surface membrane of Gram-negative bacteria or repetitive polysaccharides of bacterial cell wall of Gram-positive bacteria. TI responses can be further classified into types 1 and 2. Type 1 responses (TI-1) are elicited by the antigen engaging both the BCR and pattern-sensing Toll-like receptors (TLRs), which are molecules involved in innate immune recognition and regulation. TLRs recognize highly conserved molecular patterns on microorganisms. B cells can express multiple TLRs, and while in naïve B cells TLR expression is low, it is enhanced in response to BCR engagement. In humans, for example, naïve B cells express low levels of TLR7 and TLR9, but these receptors are upregulated in memory B cells (Bernasconi, 2003, 133). Thus optimal response to TI type 1 antigens requires concomitant engagement of the BCR and TLR. Type 2 responses (TI-2) are initiated by BCR engagement by multivalent ligands that are able to simultaneously cross-link multiple BCRs and deliver sufficiently strong signals in the absence of coreceptor engagement. An example of a multivalent ligand is the repetitive structures of the streptococcal cell wall. In both cases, the cumulative signal strength resulting from either engagement of BCR and TLR, or BCR cross-linking, is sufficient to overcome the requirement for T cell help. Nevertheless, in general in the absence of T cell help, there is a lack of memory, SHM, and CSR.

## B CELL TOLERANCE: TRADITIONAL AND NEW CONCEPTS

The immune system is highly efficient at defending the host against continuous exposure to diverse foreign antigens whilst halting the maturation or activation of B cells that recognize self-antigens—autoreactive B cells. This phenomenon is known as B cell tolerance. Defects in tolerance contribute to the development of autoimmune diseases wherein various organs in the body come under misguided attack from its own defense system. The profound discrepancy between the frequency of autoreactive cells generated in the thymus or bone marrow, and the rarity of autoimmune diseases, highlights the efficacy of tolerance mechanisms for purging or controlling autoreactive lymphocytes. Tolerance acquired during the development of B cells in the bone marrow constitutes “central tolerance,” whereas mechanisms halting the maturation or activation of autoreactive B cells in the peripheral lymphoid tissues constitute “peripheral tolerance.” In humans it is estimated that 55%–75% of the IgGs expressed by early immature B cells are autoreactive (Wardemann et al., 2003); however, multiple checkpoints throughout B cell development usually prevent mature B cells from producing autoantibodies.

The number of mechanisms described to contribute to tolerance is expanding, but classically, central tolerance comprises BCR editing, clonal deletion, and anergy (Nemazee, 2017). More recently, sialic acid-binding Ig-like lectin (Siglec)-dependent induction of tolerance has been described to be important (Duong et al., 2010), as has the induction of regulatory B cells (Bregs) as active suppressors of immune responses (Mauri and Bosma, 2012).

### Mechanisms of B Cell Central Tolerance

First, if a B cell expresses an autoreactive BCR, particularly at an early stage of development, it can alter its BCR genes in order to express a nonautoreactive version, a process called “receptor editing.” Second, if receptor editing fails, autoreactive B cells can be removed while still in the bone marrow by a process termed “clonal deletion.” Third, autoreactive B cells that nevertheless escape to the periphery may acquire tolerance by entering a state of specific hyporesponsiveness to antigen, termed “anergy” (Fig. 9.1A).

## Receptor Editing and Clonal Deletion

The generation of adaptive immune responses relies on the stochastic rearrangement of antigen receptor genes to produce a diverse repertoire of antigen receptor specificities. Successful in frame V, diversity (D), and joining (J) gene segment, recombination leads to the expression of a BCR, followed by suppression of RAG 1 and 2 expression to prevent further rearrangements (Nussenzweig et al., 1987). Following the productive rearrangement of the Ig genes on one allele, the Ig genes on the other allele are silenced and thus not expressed. This phenomenon is called allelic exclusion.

However, if these rearrangements produce an autoreactive BCR, the B cells may undergo receptor editing. Receptor editing is the continued rearrangement of the V–J genes encoding the variable regions of Ig light chains, or less commonly the V–(D)J genes encoding the heavy chain variable loci, which results in the expression of a BCR with a new specificity (Gay et al., 1993). This has been reported to be the main mechanism of B cell central tolerance toward high avidity membrane-bound antigens (Halverson et al., 2004). If the rearrangement of the light chain genes results in the expression of a functional BCR that does not bind self-antigen present in the bone marrow, then B cells survive and leave for the periphery. Receptor editing usually occurs early during B cell development and predominantly, although not exclusively, in the bone marrow. Secondary rearrangements may also occur in the periphery, although their importance in maintaining tolerance remains to be fully defined. RAG genes are reexpressed in germinal centers, and it has been demonstrated in mice that mature follicular B cells that are autoreactive/anti-dsDNA specific can undergo receptor editing in germinal centers, following antigen activation, which removes dsDNA specificity (Rice et al., 2005).

Receptor editing allows for several attempts at altering the specificity of the Ig; however, if receptor editing in the bone marrow fails to produce B cells with functional BCRs that are not autoreactive, then the B cells may be eliminated by apoptosis, a process termed “clonal deletion” (Nemazee and Burki, 1989; Hartley et al., 1993). Immature B cells are susceptible to apoptosis by surface Ig cross-linking. Clonal deletion was originally thought to be the major mechanism of B cell central tolerance to high avidity membrane-bound or multivalent self-antigens. However, later studies have suggested that receptor editing is the primary mechanism for removing autoreactive B cells and clonal deletion acts to maintain tolerance when receptor editing fails (Pelanda and Torres, 2012). For instance, Gay et al. generated mice that were transgenic for Ig heavy and light chain genes that encoded a BCR that was specific for dsDNA, an autoantigen in murine and human lupus. As these genes were already rearranged, they would be the first heavy and light chains to be transcribed and, in the absence of receptor editing, all B cells should have been clonally deleted due to their autoreactive BCRs. However, the adult mice had a normal number of mature B cells due to the fact that, while all their B cells expressed the heavy chain transgene, all mature B cells expressed an endogenous light chain, demonstrating that receptor editing had taken place to produce a nonautoreactive BCR (Gay et al., 1993).

## Defective Receptor Editing and Clonal Deletion Can Promote Autoimmunity

Although receptor editing is the main process contributing to the removal of autoreactive B cells, this system is not perfect, and both insufficient and excessive editing can have potentially adverse consequences on autoreactivity (Verkoczy et al., 2004; Yachimovich-Cohen et al., 2003). The Ig heavy chain often has the dominant contribution to BCR specificity and if the heavy chain is autoreactive, the rearrangement of the light chain may not fully remove the autoreactivity (Luning Prak et al., 2011). In addition, light chain rearrangement does not always lead to the abrogation of expression of the initial light chain and when the B cells reach the periphery, the initial autoreactive light chain may be reexpressed (Li et al., 2002b).

Defective receptor editing has been associated with autoimmunity in both mice and humans. In models of SLE (Clark et al., 2013; Lamoureux et al., 2007), type 1 diabetes mellitus (Henry-Bonami et al., 2013) and Sjögren's syndrome (Meng et al., 2012) as well as in patients with SLE (Yurasov et al., 2005) defects in receptor editing and early B cell tolerance have been reported. It has been hypothesized that inefficient receptor editing in autoimmune-prone mice might allow the escape to the periphery of B cells with two types of Ig heavy or light chains. These dual-reactive B cells are rare in healthy mice but present at increased levels in autoimmune strains and are frequently more autoreactive than B cells expressing only one heavy and light chain (Fournier et al., 2012). Excessive receptor editing may also contribute to pathogenesis through the generation of B cells with autoreactive BCRs. For example, lupus-prone NZB/NZW F1 mice that develop disease utilize secondary receptor arrangements to generate high-affinity antibodies from low-affinity precursor clones (Yachimovich-Cohen et al., 2003). In addition, lupus-prone MRL/lpr.56R mice that are transgenic for an

autoreactive Ig heavy chain have increased production of autoantibodies compared to nontransgenic MRL/lpr mice. However, the majority of the autoantibodies in the transgenic mouse do not use the transgenic Ig heavy chain, suggesting that excessive receptor editing has led to the generation of autoreactive BCRs (Li et al., 2002a). These findings indicate that even small perturbations in the efficacy of selection mechanisms could potentially result in the breach of tolerance.

## B Cell Anergy

Despite the general efficiency of receptor editing and clonal deletion, B cells carrying self-reactive BCRs may still escape central tolerance and reach the periphery. Here, elimination of autoreactive B lymphocytes can be achieved by inducing a state of specific hyporesponsiveness to antigen, known as anergy, in the autoreactive B cells. Typically, anergy is a consequence of prolonged exposure to antigen and is achieved through B cell functional inactivation (Cambier et al., 2007). Anergic B cells are unable to interact effectively with helper T cells and do not participate in immune responses against their cognate antigen. It was initially demonstrated that B cell tolerance to antigens could arise through anergy rather than clonal deletion (Nossal and Pike, 1980), as follows. Treatment of newborn mice with a soluble molecule that would generate an immune response in adult mice, fluorescein-human gamma globulin (Flu-HGG), resulted in tolerance to this molecule when the mice reached maturity, even though they maintained normal numbers of Flu-HGG-specific B cells. Subsequently, Hartley et al. (1991) demonstrated that it is the nature of the antigen, rather than the affinity of BCR–antigen interactions, that is responsible for whether tolerance comes from clonal deletion or anergy. Hartley et al. generated two transgenic mouse models both carrying BCRs that were specific for hen egg lysozyme (HEL); the first expressed membrane-bound HEL as well as the transgenic BCR, and the second expressed soluble HEL as well as the transgenic BCR. The results demonstrated that clonal deletion of the HEL-specific transgenic B cells occurred when mice expressed membrane-bound HEL, whereas clonal anergy occurred in mice expressing soluble HEL. Thus in immature B cells, binding to high avidity multivalent membrane-bound antigen predominantly leads to receptor editing, or clonal deletion, while binding to low avidity monovalent, or oligovalent, soluble antigen mainly leads to anergy. More recently, it has been demonstrated that there is a spectrum of B cell hyporesponsiveness following B cell encounter with self-antigen; the signal strength of the interaction with self-antigen directs the degree of B cell responsiveness to subsequent antigen encounter. Thus an anergic B cell can exist somewhere along a continuum of possible anergy states (Zilberman et al., 2012).

## Characteristics of Anergic B Cells

Anergic B cells are characterized by reduced expression of BCR on their surface. This low expression of the BCR is an important feature of anergic B cells as the impaired transduction of positive signals via CD79 prevents autoreactive B cell signaling and activation. If B cells are unable to downregulate their BCR in response to self-antigen, autoimmunity may develop. For instance, double deficiency in the E3 ubiquitin ligases Cbl and Cblb leads to the inability to downregulate surface BCR and development of lupus-like disease (Kitaura et al., 2007). Anti-CD79 antibody has been shown to ameliorate disease in lupus-prone MRL/lpr mice (Li et al., 2008) as well as in a murine model of rheumatoid arthritis (Hardy et al., 2014). The latter study demonstrated that anti-CD79 antibody treatment modulated BCR signal strength and induced an anergic state in B cells, suggesting induction of anergy as an alternative strategy to B cell–depletion therapy in the treatment of autoimmune diseases (Hardy et al., 2014). Another characteristic of anergic B cells is the increased recruitment of negative signaling regulators, including the SHP-1 and SHIP-1, to the BCR complex. Increased SHP-1 and SHIP-1 expression can inhibit BCR-mediated B-cell activation by the dephosphorylation of signaling molecules downstream of the BCR and CD79. Defects in SHP-1 or SHIP-1 are associated with increased autoantibody production (Huang et al., 2003). B cell–specific deletion of SHIP-1 causes loss of anergy and lupus-like disease in mice (O'Neill et al., 2011). Resistance to apoptotic stimuli and increased autoantibody production in the absence of B cell PTEN have also been demonstrated (Suzuki et al., 2003). The short half-life of anergic B cells is a further characteristic that adds an additional brake to B cell autoreactivity. Anergic B cells have an estimated half-life of 3–4 days in contrast to nonanergic naïve B cells with a half-life of around 4–5 weeks (Fulcher and Basten, 1994).

## B CELL–ACTIVATING FACTOR AND TONIC SIGNALS MODULATE B CELL TOLERANCE

### B Cell–Activating Factor in B Cell Development and Survival

B cell development, survival, and differentiation depend on the presence of several key chemical mediators, among which B cell–activating factor (BAFF), also known as B lymphocyte stimulator, is critical. The importance of BAFF in B cell maturation was demonstrated by work from the Mackay group, they showed that B cells in BAFF- and BAFF-receptor (BAFF-R)-deficient mice were developmentally arrested at the immature stage of B cell development (Mackay et al., 2002). BAFF is a trimeric member of the tumor necrosis factor (TNF) family and it signals through BAFF-R, B cell maturation antigen (BCMA), and transmembrane activator and calcium modulator and cyclophilin-ligand interactor (TACI) (Mackay and Schneider, 2009). These receptors are differentially expressed at each stage of B cell development and differentiation. For example, BAFF-R is expressed from the immature B cell stage and increases in expression as they mature; however, it is absent on germinal center B cells and long-lived plasma cells in the bone marrow. Antibody-producing cells express BCMA, while TACI is expressed on switched memory B cells amongst others. BAFF itself is produced by stromal and myeloid lineage cells, as well as lymphocytes under certain conditions (Mackay and Schneider, 2009).

### Tonic Signaling in B Cell Development and Survival

Tonic signaling is important for the development of immature B cells in the bone marrow and for the maintenance and survival of mature B cells in the periphery. Tonic signaling refers to the process of low-level constitutive signaling in the resting state in the absence of full and robust B cell activation. In B cells, this basal signaling is thought to occur through the BCR. Whether endogenous ligand expression plays a role in tonic signaling, or whether BCR expression without ligation is sufficient for tonic signaling, remains unclear (Cariappa and Pillai, 2002; Cancro and Kearney, 2004; Su et al., 2004). Nevertheless, evidence suggests that while early in B cell development, tonic signaling may be antigen independent, mature B cell survival in the periphery may require low-level antigen stimulation. In mature naïve B lymphocytes, cells with low-level self-reactivity have a competitive survival advantage over B cell clones that do not (Rosado and Freitas, 1998).

In the steady state, the stochastic phosphorylation of ITAMs in CD79 is held in dynamic equilibrium with negative signals through the phosphatase activity of CD45 and inhibitory receptors such as CD5, CD22, and CD72. Src kinases that are activated by tonic BCR signals also phosphorylate inhibitory ITIMs on molecules such as CD22. These, in turn, recruit protein tyrosine phosphatases that dephosphorylate the ITAMs on CD79 to inhibit BCR signals.

A failure of tonic signaling at an immature B cell stage can lead to a developmental regression that can restart the process of Ig gene recombination (Tze et al., 2005). Indeed, during development in the bone marrow, the inability to express BCR or its signaling components leads to ongoing light chain rearrangement (Pelanda, 2014). Thus the tonic signaling that results from successful BCR expression is needed for the B cell to move on to the next developmental stage. The requirement for tonic signaling in mature B cell peripheral persistence is demonstrated by the rapid loss of mature B cells upon inducible BCR ablation (Lam et al., 1997; Kraus et al., 2004). It has been subsequently demonstrated that mature naïve B cell survival in the absence of BCR expression can be rescued through the expression of PI3K, which makes up for the lack of tonic signaling through the BCR (Srinivasan et al., 2009).

Tonic signaling positively regulates the expression of BAFF-R, and the combination of tonic signaling and BAFF/BAFF-R signaling drives B cell maturation (Rowland et al., 2013). The requirement for both BAFF-R expression and tonic signaling for B cell maturation provides a checkpoint for preventing the escape of autoreactive B cells into the periphery as binding of autoantigen by autoreactive B cells leads to the internalization of the BCR and thus a loss of both tonic signaling and BAFF-R expression (Pelanda and Torres, 2012; Rowland et al., 2010).

### B Cell–Activating Factor, Tonic Signaling, and Autoimmunity

A role of BAFF in autoimmunity was observed in BAFF-transgenic mice that developed an autoimmune-like syndrome, where the B cells from BAFF-transgenic mice secreted unusually high amounts of rheumatoid factor

antibodies and the mice developed lupus-like disease (Mackay et al., 1999). BAFF overexpression rescued autoreactive B cells from deletion in the periphery, but not in the bone marrow, and favored their migration to secondary lymphoid tissues for maturation (Thien et al., 2004). Patients with autoimmune diseases such as Sjögren's syndrome and SLE have high levels of BAFF, which may contribute to altered differentiation of B cells (Pillai et al., 2011), and this was the rationale for clinical application of the anti-BAFF antibody belimumab in the treatment of SLE (Navarra et al., 2011). It is currently the only biologic treatment approved for SLE. Thus whereas BAFF signaling is critical in normal B cell development, when dysregulated, it can provoke a breakdown in B-cell tolerance leading to systemic autoimmunity.

Defects or deficiencies in tonic signaling can lead to autoimmunity. A breach of tolerance can occur due to incomplete allelic exclusion of light chain genes, whereby dual-specificity BCRs are expressed, that is, development of B cells that express both autoreactive and nonautoreactive BCRs. If the ratio of autoreactive to nonautoreactive BCRs is such that the majority of signaling is tonic signaling from the nonautoreactive BCR, rather than stronger self-antigen-mediated signaling through the autoreactive BCR, this may allow the maturation of these bispecific B cells (Pelanda and Torres, 2012). In such cases, the autoreactive BCR is generally expressed first, and allelic inclusion occurs as a consequence of incomplete silencing.

Deficiencies in the molecules that negatively regulate tonic, and antigen-dependent, BCR signaling can also lead to autoimmunity. CD22-deficient mice produce high-affinity anti-dsDNA antibodies suggesting that defects in CD22 function may support B cell–mediated autoimmunity (O'Keefe et al., 1999). The combined deficiency of CD22 and its ligand Siglec-G causes spontaneous autoimmunity in mice and thus targeting this pathway could be useful in the treatment of autoimmunity (Muller and Nitschke, 2014).

Fc $\gamma$ RIIb, an inhibitory Fc receptor, which also contains an ITIM on its intracellular domain, plays a role in regulating BCR signals, through the recruitment of tyrosine phosphatases. Mice deficient in this receptor develop a spontaneous autoimmune disease characterized by fatal glomerulonephritis and accumulation of anti-dsDNA-producing plasma cells (Fukuyama et al., 2005). In humans, polymorphisms in the Fc $\gamma$ RIIb gene, which encode receptors with reduced regulatory activity, have been associated with SLE (Niederer et al., 2010). In lupus-prone mice, overexpression of Fc $\gamma$ RIIb restored B cell tolerance (McGaha et al., 2005).

## REGULATORY T CELLS

B cell peripheral tolerance is also maintained by regulatory T cell (Treg) suppression of autoantibody production by B cells. Tregs can suppress maturation of the humoral response either indirectly by suppressing the activation of Tfh cells that would support B cell autoantibody production (Fields et al., 2005) or directly by physically removing cognate Tfh help (Sage et al., 2016). Direct induction of B cell apoptosis by Tregs in a B cell PD-1-dependent manner has also been described (Gotot et al., 2012). It is likely that additional checkpoints for Tregs in the suppression of B cell responses are yet to be uncovered.

## ANTIBODY-INDEPENDENT ACTIVITY OF B CELLS IN TOLERANCE

As discussed earlier, B cell tolerance is shaped during the development of B lymphocytes, by receptor editing, clonal deletion, and anergy. However, evidence is mounting for an antibody-independent role for B cells in the maintenance and breakdown of tolerance. In part driven by the success of B cell–depletion therapies even in cases where autoantibody levels remain unaffected, it is now recognized that B cells have antibody-independent effects on disease. Other B cell roles in the maintenance and breakdown of tolerance include the antigen-presenting role of B cells and their production of both pro- and antiinflammatory cytokines.

### Antigen Presentation by B Cells

B cells act as antigen-presenting cells, priming T cells and enhancing their activation. It has been demonstrated that B cell expression of MHC class II is required for the development of autoimmunity in a murine model of multiple sclerosis, and this occurs in an antibody-independent manner (Molnarfi et al., 2013). In a small cohort of patients with the autoimmune disease pemphigus vulgaris who had been treated with rituximab, the depletion of B cells was accompanied not only by the reduction in autoantibody levels but also a

decline in the autoantigen desmoglein 3-specific Th1 and Th2 cell frequencies. At the same time, the total CD4+ T cell count as well as irrelevant antigen-specific T cell frequencies were maintained (Eming et al., 2008). Similar observations have been made in rheumatoid arthritis patients, where the synovial tissue following rituximab treatment was shown to have reduced numbers of Th17 cells, as well as reduced expression of the associated transcription factor ROR- $\gamma$ t and the cytokine IL-22. These reductions correlated with better clinical outcome (van de Veerdonk et al., 2011).

## Cytokine Production by B Cells

Most of our knowledge about B cell cytokine production in the regulation of immune responses come from animal models (Shen and Fillatreau, 2015). It is now recognized that B cells can produce a polarized array of cytokines depending on their primary encounter with antigen and T cells. Therefore, similar to T cells, B cells can be categorized based on the cytokines that they produce.

It was first recognized in mice that under certain conditions, B cells could be polarized to secrete IFN- $\gamma$  or IL-4 (Harris et al., 2000). Naive B cells differentiated in the presence of Th1 cells release IFN- $\gamma$  and IL-12. In contrast, naïve B cells that differentiate in the presence of Th2 cells predominantly produce IL-2, IL-4, and IL-6 (Harris et al., 2000). IL-17-producing B cells have been reported to have a physiological role in response to *Trypanosoma cruzi* infection (Bermejo et al., 2013). Using both murine and human primary B cells, Rawlings et al. demonstrated that B cell–specific IL-17 deficiency led to inability to control infection, increased liver damage, and reduced survival compared to control animals (Bermejo et al., 2013). Murine B cells have also been shown to contribute to protective immunity in *Salmonella enterica* serovar *Typhimurium* infection through IL-6- and IFN- $\gamma$ -mediated Th1 programming (Barr et al., 2010), as well as to enhance Th1 responses to *Toxoplasma gondii* through the provision of TNF- $\alpha$  (Menard et al., 2007). Thus B cells can produce a variety of cytokines and contribute to the protective immunity during infection. The contribution of the cytokines released by B cells in the pathogenesis of autoimmune disease is yet to be clearly defined, but recent findings have shown that IL-6-producing B cells promote Th17 responses and contribute to the pathogenesis of experimental autoimmune encephalomyelitis and to multiple sclerosis (Barr et al., 2012). Further, proinflammatory GM-CSF-producing memory B cells are enriched in multiple sclerosis patients compared to healthy controls, which was normalized following B cell–depletion therapy (Li et al., 2015).

## Regulatory B Cells

B cells can also produce antiinflammatory cytokines IL-10 and TGF- $\beta$ , known to restrain the excessive inflammation associated with autoimmunity. Over the last decade, numerous studies have confirmed the importance of IL-10-producing Bregs in the maintenance of tolerance (Mauri and Bosma, 2012). Studies utilizing chimeric mice, whose B cells alone are IL-10 deficient, have revealed that B cell–derived IL-10 limits the severity of inflammation in several autoimmune disorders (Carter et al., 2011; Fillatreau et al., 2002). In the absence of IL-10-producing B cells, chimeric mice present a reduction in the number of T regulatory 1 and Foxp3 $^{+}$  Tregs, and an increase in inflammatory Th1 and Th17 cells compared to mice with IL-10 sufficient B cells. In mice, TGF- $\beta$ -mediated inhibition of CD8 $^{+}$  T cell responses by lipopolysaccharide (LPS)-activated B cells has also been demonstrated (Parekh et al., 2003). Thus Bregs contribute to the maintenance of peripheral tolerance by the production of the potent antiinflammatory cytokines IL-10 and/or TGF $\beta$ , directly by inhibiting the differentiation of Th1, Th17, and CD8 $^{+}$  T cells and indirectly by converting targeted effector T cells into Tregs (Carter et al., 2011).

BCR activation, which is crucial in dictating the specificity of antibody responses and the function of B cells as antigen-presenting cells, has been shown to play a role in Breg function. Research has revealed that exposure to an autoantigen leads to an increased IL-10 production by Bregs and an enhanced ability to suppress autoimmune responses (Mauri et al., 2003). Similarly, in the field of transplantation, it has been reported that TIM-1 $^{+}$  Bregs prolonged pancreatic islet allograft survival, but only when the TIM-1 $^{+}$  B cells are specific for the MHC expressed by the transplanted islet cells, suggesting a role for specific BCR signaling (Ding et al., 2011). Other signals that induce IL-10-producing Bregs have also been uncovered, including CD40 ligation and cytokines including IL-1 and IL-6 (Rosser et al., 2014), IL-21 (Yoshizaki et al., 2012), and IL-35 (Wang et al., 2014).

Bregs also play a role in the maintenance of tolerance in healthy individuals. While murine Bregs are functionally better characterized, human IL-10-producing B cells have been postulated to arise at multiple stages of B cell development and can be detected within populations that phenotypically resemble CD24<sup>hi</sup>CD38<sup>hi</sup> immature B cells, CD24<sup>+</sup>CD27<sup>+</sup> memory B cells, and CD24<sup>-</sup>CD38<sup>hi</sup>CD27<sup>+</sup> plasmablasts. Immature B cells isolated from healthy volunteers produce a higher amount of IL-10 than any other human peripheral blood B cell subset and can suppress proinflammatory cytokine production by anti-CD3-activated CD4<sup>+</sup> T cells in vitro. Interestingly, in patients with SLE, isolated Bregs failed to upregulate IL-10 in response to CD40 engagement and failed to suppress the production of proinflammatory cytokines by T cells (Blair et al., 2010). Accordingly rituximab, a B cell-depleting anti-CD20 monoclonal antibody, which has proved successful for treating some autoimmune conditions, may function not only by depleting autoantibody-producing B cells but also by resetting the balance between pathogenic and Bregs following B cell repopulation (Bosma et al., 2012; Manjarrez-Orduno et al., 2009). Interestingly, while CD24<sup>hi</sup>CD38<sup>hi</sup> Bregs are either numerically or functionally deficient in autoimmunity, CD24<sup>hi</sup>CD27<sup>+</sup> Bregs are expanded in patients with autoimmune disease (Iwata et al., 2011).

The immune system, thus, has several checkpoints to curtail the escape of autoreactive B cells to the periphery, as well as their activation, and hence to prevent autoimmunity. Among these checkpoints, as described earlier, are receptor editing, clonal deletion, and anergy. However, it is becoming clearer that in the periphery, tolerance is maintained also by regulatory subsets of cells, including Bregs and Tregs, that control the production of inflammatory cytokines and limit autoreactive responses (Fig. 9.1C).

## Future Directions

Although there are a number of potential models for the first steps of B cell activation, it is still unclear how antigen binding to the BCR initiates activation. Better understanding this process may lead to the development of therapies that would be beneficial for the treatment of autoimmunity. For example antigen-specific immobilization of BCR or its signaling components within certain cell surface domains, or inhibiting the segregation of stimulatory and inhibitory coreceptors, could prevent the activation of autoreactive B cells. Since B cell role in antigen presentation requires antigen internalization via BCR following binding, this may also lead to down-modulation of B cell antigen presentation to autoreactive T cells.

Whether defects in particular mechanisms of B cell tolerance result in clinically different autoimmune diseases remains unanswered. In particular, it is yet to be determined exactly how B cells bearing two different BCRs are both generated and able to escape central tolerance and whether the persistence of these cells does actually contribute to the development of autoimmunity. For instance, could defects in receptor editing that lead to the expression of two distinct BCRs by mature B cells, one autoreactive and one reactive to a microbial antigen, be important in generating autoantibody to autoantigens that the B cells might otherwise not see in the bone marrow? Thus B cells expressing dual BCRs may be activated by the microbial antigen to produce autoreactive antibody and contribute to the pathogenesis of autoimmune diseases, even though the relevant autoantigen would not generally be subject to immune surveillance (i.e., nuclear antigens following microbe-induced inflammatory response)? Or will dual-specific B cells only be generated to self-antigen available in the early stages of development?

Do evolutionary pressures to maintain diversity in B cell specificity have the consequence of also maintaining some autoreactivity within the mature B cell compartment? It has been recently demonstrated that self-reactive B-cell clones participate in responses to foreign antigens that are structurally similar to self-antigens. In this case, self-reactive clone anergy was reversed through a challenge with high-density foreign antigen, but the BCR hypermutation that occurred resulted in increased recognition of the foreign antigen and decreased self-reactivity (Burnett et al., 2018). Thus perhaps maintaining a degree of autoreactivity allows for a larger diversity in B cell antigen reactivity and is of benefit to the host as it allows defense against a wider array of pathogens.

Similarly, it is not currently clear what effect the presence of a constantly renewing niche of anergic B cells in healthy humans has on B cell–driven immune responses (Palanichamy et al., 2009). Assuming a large proportion of these anergic B cells are autoreactive, they may compete with nonanergic B cells for autoantigen, and thus their presence could act as a sink for autoantigen, sequestering it from nonanergic autoreactive B cells and preventing the activation of autoreactive T cells. Defects in the generation of anergic B cells may thus lead to autoreactive T cell–mediated autoimmunity. Or conversely, an excessively large anergic B cell pool containing polyreactive as well as autoreactive B cell specificities may prevent nonanergic pathogen-specific B cells from activating T cells.

## References

- Ahearn, J.M., Fischer, M.B., Croix, D., Goerg, S., Ma, M., Xia, J., et al., 1996. Disruption of the Cr2 locus results in a reduction in B-1a cells and in an impaired B cell response to T-dependent antigen. *Immunity* 4, 251–262.
- Barr, T.A., Brown, S., Mastroeni, P., Gray, D., 2010. TLR and B cell receptor signals to B cells differentially program primary and memory Th1 responses to *Salmonella enterica*. *J. Immunol.* 185 (5), 2783–2789.
- Barr, T.A., Shen, P., Brown, S., Lampropoulou, V., Roch, T., Lawrie, S., et al., 2012. B cell depletion therapy ameliorates autoimmune disease through ablation of IL-6-producing B cells. *J. Exp. Med.* 209, 1001–1010.
- Bermejo, D.A., Jackson, S.W., Gorosito-Serran, M., Acosta-Rodriguez, E.V., Amezcuá-Vesely, M.C., Sather, B.D., et al., 2013. *Trypanosoma cruzi* trans-sialidase initiates a program independent of the transcription factors ROR $\gamma$ T and Ahr that leads to IL-17 production by activated B cells. *Nature Immun* 14, 514–522.
- Bernasconi, N.L., Onai, N., Lanzavecchia, A., 2003. A role for Toll-like receptors in acquired immunity: up-regulation of TLR9 by BCR triggering in naive B cells and constitutive expression in memory B cells. *Blood* 101 (11), 4500–4504.
- Blair, P.A., Norena, L.Y., Flores-Borja, F., Rawlings, D.J., Isenberg, D.A., Ehrenstein, M.R., et al., 2010. CD19(+)/CD24(hi)/CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic lupus erythematosus patients. *Immunity* 32, 129–140.
- Bosma, A., Abdel-Gadir, A., Isenberg, D.A., Jury, E.C., Mauri, C., 2012. Lipid-antigen presentation by CD1d(+) B cells is essential for the maintenance of invariant natural killer T cells. *Immunity* 36, 477–490.
- Burnett, D.L., Langley, D.B., Schofield, P., Hermes, J.R., Chan, T.D., Jackson, J., et al., 2018. Germinal center antibody mutation trajectories are determined by rapid self/foreign discrimination. *Science* 360 (6385), 223–226.
- Cambier, J.C., Gauld, S.B., Merrell, K.T., Vilen, B.J., 2007. B-cell anergy: from transgenic models to naturally occurring anergic B cells? *Nat. Rev. Immun* 7 (8), 633–643.
- Cancro, M.P., Kearney, A.E., 2004. B cell positive selection: road map to the primary repertoire? *J. Immunol.* 173, 15–19.
- Cariappa, A., Pillai, S., 2002. Antigen-dependent B-cell development. *Curr. Opin. Immunol.* 14, 241–249.
- Carroll, M.C., 1998. CD21/CD35 in B cell activation. *Semin. Immunol.* 10, 279–286.
- Carter, N.A., Vasconcellos, R., Rosser, E.C., Tulone, C., Munoz-Suano, A., Kamanaka, M., et al., 2011. Mice lacking endogenous IL-10-producing regulatory B cells develop exacerbated disease and present with an increased frequency of Th1/Th17 but a decrease in regulatory T cells. *J. Immunol.* 186, 5569–5579.
- Clark, A.G., Fan, Q., Brady, G.F., Mackin, K.M., Coffman, E.D., Weston, M.L., et al., 2013. Regulation of basement membrane-reactive B cells in BXSB, (NZBxNZWF1, NZB, and MRL/lpr lupus mice. *Autoimmunity*, 46 (3), 188–204.
- Cyster, J.G., 2010. B cell follicles and antigen encounters of the third kind. *Nat. Immunol.* 11, 989–996.
- Ding, Q., Yeung, M., Camirand, G., Zeng, Q., Akiba, H., Yagita, H., et al., 2011. Regulatory B cells are identified by expression of TIM-1 and can be induced through TIM-1 ligation to promote tolerance in mice. *J. Clin. Invest.* 121, 3645–3656.
- Di Noia, J.M., Neuberger, M.S., 2007. Molecular mechanisms of antibody somatic hypermutation. *Annu. Rev. Biochem.* 76, 1–22.
- Doody, G.M., Justement, L.B., Delibrias, C.C., Matthews, R.J., Lin, J., Thomas, M.L., et al., 1995. A role in B cell activation for CD22 and the protein tyrosine phosphatase SHP. *Science* 269, 242–244.
- Duong, B.H., Tian, H., Ota, T., Completo, G., Han, S., Vela, J.L., et al., 2010. Decoration of T-independent antigen with ligands for CD22 and Siglec-G can suppress immunity and induce B cell tolerance in vivo. *J. Exper. Med.* 207 (1), 173–187.
- Durie, F.H., Fava, R.A., Foy, T.M., Aruffo, A., Ledbetter, J.A., Noelle, R.J., 1993. Prevention of collagen-induced arthritis with an antibody to gp39, the ligand for CD40. *Science* 261, 1328–1330.
- Eming, R., Nagel, A., Wolff-Franke, S., Podstawa, E., Debus, D., Hertl, M., 2008. Rituximab exerts a dual effect in pemphigus vulgaris. *J. Inves. Dermatol.* 128 (12), 2850–2858.
- Fields, M.L., Hondowicz, B.D., Metzgar, M.H., Nish, S.A., Wharton, G.N., Picca, C.C., et al., 2005. CD4 + CD25 + regulatory T cells inhibit the maturation but not the initiation of an autoantibody response. *J. Immunol.* 175, 4255–4264.
- Fillatreau, S., Sweeney, C.H., McGeachy, M.J., Gray, D., Anderton, S.M., 2002. B cells regulate autoimmunity by provision of IL-10. *Nat. Immunol.* 3, 944–950.
- Fournier, E.M., Velez, M.G., Leahy, K., Swanson, C.L., Rubtsov, A.V., Torres, R.M., et al., 2012. Dual-reactive B cells are autoreactive and highly enriched in the plasmablast and memory B cell subsets of autoimmune mice. *J. Exp. Med.* 209, 1797–1812.
- Fukuyama, H., Nimmerjahn, F., Ravetch, J.V., 2005. The inhibitory Fc $\gamma$  receptor modulates autoimmunity by limiting the accumulation of immunoglobulin G + anti-DNA plasma cells. *Nat. Immunol.* 6, 99–106.
- Fulcher, D.A., Basten, A., 1994. Reduced life span of anergic self-reactive B cells in a double-transgenic model. *J. Exp. Med.* 179, 125–134.
- Gatto, D., Pfister, T., Jegerlehner, A., Martin, S.W., Kopf, M., Bachmann, M.F., 2005. Complement receptors regulate differentiation of bone marrow plasma cell precursors expressing transcription factors Blimp-1 and XBP-1. *J. Exp. Med.* 201, 993–1005.
- Gay, D., Saunders, T., Camper, S., Weigert, M., 1993. Receptor editing: an approach by autoreactive B cells to escape tolerance. *J. Exp. Med.* 177, 999–1008.
- Genovese, M.C., Schiff, M., Luggen, M., Le Bars, M., Aranda, R., Elegbe, A., et al., 2012. Long-term safety and efficacy of abatacept through 5 years of treatment in patients with rheumatoid arthritis and an inadequate response to tumor necrosis factor inhibitor therapy. *J. Rheumatol.* 39, 1546–1554.
- Gonzalez, S.F., Degen, S.E., Pitcher, L.A., Woodruff, M., Heesters, B.A., Carroll, M.C., 2011. Trafficking of B cell antigen in lymph nodes. *Annu. Rev. Immunol.* 29, 215–233.
- Gotot, J., Gottschalk, C., Leopold, S., Knolle, P.A., Yagita, H., Kurts, C., et al., 2012. Regulatory T cells use programmed death 1 ligands to directly suppress autoreactive B cells in vivo. *Proc Natl Acad Sci U S A* 109 (26), 10468–10473.
- Guy, R., Hodes, R.J., 1989. Antigen-specific, MHC-restricted B cell activation by cell-free Th2 cell products. Synergy between antigen-specific helper factors and IL-4. *J. Immunol.* 143, 1433–1440.
- Halverson, R., Torres, R.M., Pelanda, R., 2004. Receptor editing is the main mechanism of B cell tolerance toward membrane antigens. *Nat. Immunol.* 5, 645–650.

- Hannoudouche, S., Zhang, J., Yi, T., Shen, W., Nguyen, D., Pereira, J.P., et al., 2011. Oxysterols direct immune cell migration via EBI2. *Nature* 475, 524–527.
- Hardy, I.R., Ainceriz, N., Rousseau, F., Seefeldt, M.B., Hatterer, E., Irla, M., et al., 2014. Anti-CD79 Antibody Induces B Cell Anergy That Protects against Autoimmunity. *J Immunol.* 192 (4), 1641–1650.
- Harris, D.P., Haynes, L., Sayles, P.C., Duso, D.K., Eaton, S.M., Lepak, N.M., et al., 2000. Reciprocal regulation of polarized cytokine production by effector B and T cells. *Nat. Immunol.* 1, 475–482.
- Hartley, S.B., Crosbie, J., Brink, R., Kantor, A.B., Basten, A., Goodnow, C.C., 1991. Elimination from peripheral lymphoid tissues of self-reactive B lymphocytes recognizing membrane-bound antigens. *Nature* 353, 765–769.
- Hartley, S.B., Cooke, M.P., Fulcher, D.A., Harris, A.W., Cory, S., Basten, A., et al., 1993. Elimination of self-reactive B lymphocytes proceeds in two stages: arrested development and cell death. *Cell* 72, 325–335.
- Harwood, N.E., Batista, F.D., 2010. Early events in B cell activation. *Annu. Rev. Immunol.* 28, 185–210.
- Henry-Bonami, R.A., Williams, J.M., Rachakonda, A.B., Karamali, M., Kendall, P.L., Thomas, J.W., 2013. B lymphocyte “original sin” in the bone marrow enhances islet autoreactivity in type 1 diabetes-prone nonobese diabetic mice. *J Immunol.* 190 (12), 5992–6003.
- Huang, Z.Y., Hunter, S., Kim, M.K., Indik, Z.K., Schreiber, A.D., 2003. The effect of phosphatases SHP-1 and SHIP-1 on signaling by the ITIM- and ITAM-containing Fc $\gamma$ RIIB and Fc $\gamma$ RIIA. *J. Leukoc. Biol.* 73, 823–829.
- Iwata, Y., Matsushita, T., Horikawa, M., Dilillo, D.J., Yanaba, K., Venturi, G.M., et al., 2011. Characterization of a rare IL-10-competent B-cell subset in humans that parallels mouse regulatory B10 cells. *Blood* 117 (2), 530–541.
- Kitaura, Y., Jang, I.K., Wang, Y., Han, Y.C., Inazu, T., Cadera, E.J., et al., 2007. Control of the B cell-intrinsic tolerance programs by ubiquitin ligases Cbl and Cbl-b. *Immunity*. 26 (5), 567–578.
- Klein, U., Dalla-Favera, R., 2008. Germinal centres: role in B cell physiology and malignancy. *Nat. Rev. Immunol.* 8, 22–33.
- Kraus, M., Alimzhanov, M.B., Rajewsky, N., Rajewsky, K., 2004. Survival of resting mature B lymphocytes depends on BCR signaling via the Ig alpha/beta heterodimer. *Cell* 117, 787–800.
- Kuwana, M., Nomura, S., Fujimura, K., Nagasawa, T., Muto, Y., Kurata, Y., et al., 2004. Effect of a single injection of humanized anti-CD154 monoclonal antibody on the platelet-specific autoimmune response in patients with immune thrombocytopenic purpura. *Blood* 103, 1229–1236.
- Lam, K.P., Kuhn, R., Rajewsky, K., 1997. In vivo ablation of surface immunoglobulin on mature B cells by inducible gene targeting results in rapid cell death. *Cell* 90, 1073–1083.
- Lamoureux, J.L., Watson, L.C., Cherrier, M., Skog, P., Nemazee, D., Feeney, A.J., 2007. Reduced receptor editing in lupus-prone MRL/lpr mice. *J. Exp. Med.* 204, 2853–2864.
- Li, Y., Li, H., Ni, D., Weigert, M., 2002a. Anti-DNA B cells in MRL/lpr mice show altered differentiation and editing pattern. *J. Exp. Med.* 196, 1543–1552.
- Li, Y., Li, H., Weigert, M., 2002b. Autoreactive B cells in the marginal zone that express dual receptors. *J. Exp. Med.* 195, 181–188.
- Luning Prak, E.T., Monestier, M., Eisenberg, R.A., 2011. B cell receptor editing in tolerance and autoimmunity. *Ann. N. Y. Acad. Sci.* 1217, 96–121.
- Li, Y., Chen, F., Putt, M., Koo, Y.K., Madaio, M., Cambier, J.C., et al., 2008. B cell depletion with anti-CD79 mAbs ameliorates autoimmune disease in MRL/lpr mice. *J. Immunol.* 181 (5), 2961–2972.
- Li, R., Rezk, A., Miyazaki, Y., Hilgenberg, E., Touil, H., Shen, P., et al., 2015. Proinflammatory GM-CSF-producing B cells in multiple sclerosis and B cell depletion therapy. *Sci. Transl. Med.* 7 (310), 310–366.
- Mackay, F., Schneider, P., 2009. Cracking the BAFF code. *Nat. Rev. Immunol.* 9 (7), 491–502.
- Mackay, F., Woodcock, S.A., Lawton, P., Ambrose, C., Baetscher, M., Schneider, P., et al., 1999. Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. *J. Exp. Med.* 190, 1697–1710.
- Mackay, I.R., Groom, J., Mackay, C.R., 2002. Levels of BAFF in serum in primary biliary cirrhosis and autoimmune diabetes. *Autoimmunity* 35, 551–553.
- Manjarrez-Orduno, N., Quach, T.D., Sanz, I., 2009. B cells and immunological tolerance. *J. Invest. Dermatol.* 129, 278–288.
- Mattila, P.K., Feest, C., Depoil, D., Treanor, B., Montaner, B., Otipoby, K.L., et al., 2013. The actin and tetraspanin networks organize receptor nanoclusters to regulate B cell receptor-mediated signaling. *Immunity* 38, 461–474.
- Mauri, C., Bosma, A., 2012. Immune regulatory function of B cells. *Annu. Rev. Immunol.* 30, 221–241.
- Mauri, C., Gray, D., Mushtaq, N., Londei, M., 2003. Prevention of arthritis by interleukin 10-producing B cells. *J. Exp. Med.* 197, 489–501.
- McGaha, T.L., Sorrentino, B., Ravetch, J.V., 2005. Restoration of tolerance in lupus by targeted inhibitory receptor expression. *Science* 307, 590–593.
- McHeyzer-Williams, L.J., McHeyzer-Williams, M.G., 2005. Antigen-specific memory B cell development. *Ann. Rev. Immunol.* 23, 487–513.
- Menard, L.C., Minns, L.A., Darche, S., Mielcarz, D.W., Foureau, D.M., Roos, D., et al., 2007. B cells amplify IFN-gamma production by T cells via a TNF-alpha-mediated mechanism. *J. Immunol.* 179 (7), 4857–4866.
- Meng, W., Li, Y., Xue, E., Satoh, M., Peck, A.B., Cohen, P.L., et al., 2012. B-cell tolerance defects in the B6. Aec1/2 mouse model of Sjogren's syndrome. *J. Clinical Immunol* 32 (3), 551–564.
- Mingari, M.C., Gerosa, F., Carra, G., Accolla, R.S., Moretta, A., Zubler, R.H., et al., 1984. Human interleukin-2 promotes proliferation of activated B cells via surface receptors similar to those of activated T cells. *Nature* 312, 641–643.
- Molnarfi, N., Schulze-Topphoff, U., Weber, M.S., Patarroyo, J.C., Prod'homme, T., Varrin-Doyer, M., et al., 2013. MHC class II-dependent B cell APC function is required for induction of CNS autoimmunity independent of myelin-specific antibodies. *J. Exper. Med.* 210 (13), 2921–2937.
- Muller, J., Nitschke, L., 2014. The role of CD22 and Siglec-G in B-cell tolerance and autoimmune disease. *Nat. Rev. Rheum* 10 (7), 422–428.
- Muramatsu, M., Kinoshita, K., Fagarasan, S., Yamada, S., Shinkai, Y., Honjo, T., 2000. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell* 102, 553–563.
- Navarra, S.V., Guzman, R.M., Gallacher, A.E., Hall, S., Levy, R.A., Jimenez, R.E., et al., 2011. Efficacy and safety of belimumab in patients with active systemic lupus erythematosus: a randomised, placebo-controlled, phase 3 trial. *Lancet* 377, 721–731.

- Nemazee, D., 2017. Mechanisms of central tolerance for B cells. *Nat. Rev. Immunol.* 17, 281–294.
- Nemazee, D.A., Burki, K., 1989. Clonal deletion of B lymphocytes in a transgenic mouse bearing anti-MHC class I antibody genes. *Nature* 337, 562–566.
- Niederer, H.A., Clatworthy, M.R., Willcocks, L.C., Smith, K.G., 2010. FcgammaRIIB, FcgammaRIIB, and systemic lupus erythematosus. *Ann. N. Y. Acad. Sci.* 1183, 69–88.
- Noelle, R.J., 1996. CD40 and its ligand in host defense. *Immunity* 4, 415–419.
- Nossal, G.J., Pike, B.L., 1980. Clonal anergy: persistence in tolerant mice of antigen-binding B lymphocytes incapable of responding to antigen or mitogen. *Proc. Natl. Acad. Sci. U.S.A.* 77, 1602–1606.
- Nussenzweig, M.C., Shaw, A.C., Sinn, E., Danner, D.B., Holmes, K.L., Morse III, H.C., et al., 1987. Allelic exclusion in transgenic mice that express the membrane form of immunoglobulin mu. *Science* 236, 816–819.
- O'Neill, S.K., Getahun, A., Gauld, S.B., Merrell, K.T., Tamir, I., Smith, M.J., et al., 2011. Monophosphorylation of CD79a and CD79b ITAM motifs initiates a SHIP-1 phosphatase-mediated inhibitory signaling cascade required for B cell anergy. *Immunity* 35 (5), 746–756.
- O'Keefe, T.L., Williams, G.T., Batista, F.D., Neuberger, M.S., 1999. Deficiency in CD22, a B cell-specific inhibitory receptor, is sufficient to predispose to development of high affinity autoantibodies. *J. Exp. Med.* 189, 1307–1313.
- Palanichamy, A., Barnard, J., Zheng, B., Owen, T., Quach, T., Wei, C., et al., 2009. Novel human transitional B cell populations revealed by B cell depletion therapy. *J. Immunol.* 182, 5982–5993.
- Parekh, V.V., Prasad, D.V., Banerjee, P.P., Joshi, B.N., Kumar, A., Mishra, G.C., 2003. B cells activated by lipopolysaccharide, but not by anti-Ig and anti-CD40 antibody, induce anergy in CD8+ T cells: role of TGF-beta 1. *J. Immunol.* 170 (12), 5897–5911.
- Pelanda, R., 2014. Dual immunoglobulin light chain B cells: Trojan horses of autoimmunity? *Curr. Opin. Immunol.* 27, 53–59.
- Pelanda, R., Torres, R.M., 2012. Central B cell tolerance: where selection begins. *Cold Spring Harb. Perspect. Biol.* 4, a007146.
- Pillai, S., Mattoo, H., Cariappa, A., 2011. B cells and autoimmunity. *Curr. Opin. Immunol.* 23, 721–731.
- Plotkin, S.A., 2010. Correlates of protection induced by vaccination. *Clin. Vaccine Immunol.* 17, 1055–1065.
- Revy, P., Muto, T., Levy, Y., Geissmann, F., Plebani, A., Sanal, O., et al., 2000. Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the Hyper-IgM syndrome (HIGM2). *Cell* 102, 565–575.
- Rice, J.S., Newman, J., Wang, C., Michael, D.J., Diamond, B., 2005. Receptor editing in peripheral B cell tolerance. *Proc. Natl. Acad. Sci. U.S.A.* 102, 1608–1613.
- Rodriguez-Pinto, D., Moreno, J., 2005. B cells can prime naive CD4+ T cells in vivo in the absence of other professional antigen-presenting cells in a CD154-CD40-dependent manner. *Eur. J. Immunol.* 35, 1097–1105.
- Rosado, M.M., Freitas, A.A., 1998. The role of the B cell receptor V region in peripheral B cell survival. *Eur. J. Immunol.* 28, 2685–2693.
- Rosser, E.C., Oleinika, K., Tonon, S., Doyle, R., Bosma, A., Carter, N.A., et al., 2014. Regulatory B cells are induced by gut microbiota-driven interleukin-1beta and interleukin-6 production. *Nat. Med.* 20 (11), 1334–1339.
- Rowland, S.L., Leahy, K.F., Halverson, R., Torres, R.M., Pelanda, R., 2010. BAFF receptor signaling aids the differentiation of immature B cells into transitional B cells following tonic BCR signaling. *J. Immunol.* 185, 4570–4581.
- Rowland, S.L., Tuttle, K., Torres, R.M., Pelanda, R., 2013. Antigen and cytokine receptor signals guide the development of the naive mature B cell repertoire. *Immunol. Res.* 55 (1–3), 231–240.
- Rush, J.S., Hodgkin, P.D., 2001. B cells activated via CD40 and IL-4 undergo a division burst but require continued stimulation to maintain division, survival and differentiation. *Eur. J. Immunol.* 31, 1150–1159.
- Sage, P.T., Ron-Harel, N., Juneja, V.R., Sen, D.R., Maleri, S., Sungnak, W., et al., 2016. Suppression by TFR cells leads to durable and selective inhibition of B cell effector function. *Nat. Immunol.* 17 (12), 1436–1446.
- Shen, P., Fillatreau, S., 2015. Antibody-independent functions of B cells: a focus on cytokines. *Nat. Rev. Immunol.* 15 (7), 441–451.
- Srinivasan, L., Sasaki, Y., Calado, D.P., Zhang, B.C., Paik, J.H., Depinho, R.A., et al., 2009. PI3 kinase signals BCR-dependent mature B cell survival. *Cell* 139, 573–586.
- Su, T.T., Guo, B., Wei, B., Braun, J., Rawlings, D.J., 2004. Signaling in transitional type 2B cells is critical for peripheral B cell development. *Immunol. Rev.* 197, 161–178.
- Suzuki, A., Kaisho, T., Ohishi, M., Tsukio-Yamaguchi, M., Tsubata, T., Koni, P.A., et al., 2003. Critical roles of Pten in B cell homeostasis and immunoglobulin class switch recombination. *J. Exper. Med.* 197 (5), 657–667.
- Taylor, J.J., Pape, K.A., Steach, H.R., Jenkins, M.K., 2015. Humoral immunity. Apoptosis and antigen affinity limit effector cell differentiation of a single naive B cell. *Science* 347, 784–787.
- Thien, M., Phan, T.G., Gardam, S., Amesbury, M., Basten, A., Mackay, F., et al., 2004. Excess BAFF rescues self-reactive B cells from peripheral deletion and allows them to enter forbidden follicular and marginal zone niches. *Immunity* 20, 785–798.
- Tze, L.E., Schram, B.R., Lam, K.P., Hogquist, K.A., Hippen, K.L., Liu, J., et al., 2005. Basal immunoglobulin signaling actively maintains developmental stage in immature B cells. *PLoS Biol.* 3, e82.
- van de Veerdonk, F.L., Lauwerys, B., Marijnissen, R.J., Timmermans, K., Di Padova, F., Koenders, M.I., et al., 2011. The anti-CD20 antibody rituximab reduces the Th17 cell response. *Arthritis and Rheumatism* 63 (6), 1507–1516.
- Vascotto, F., Lankar, D., Faure-Andre, G., Vargas, P., Diaz, J., Le roux, D., et al., 2007. The actin-based motor protein myosin II regulates MHC class II trafficking and BCR-driven antigen presentation. *J. Cell Biol.* 176, 1007–1019.
- Verkoczy, L.K., Martensson, A.S., Nemazee, D., 2004. The scope of receptor editing and its association with autoimmunity. *Curr. Opin. Immunol.* 16 (6), 808–814.
- Wang, H., Feng, J., Qi, C.F., Li, Z., Morse III, H.C., Clarke, S.H., 2007. Transitional B cells lose their ability to receptor edit but retain their potential for positive and negative selection. *J. Immunol.* 179, 7544–7552.
- Wang, R.X., Yu, C.R., Dambuza, I.M., Mahdi, R.M., Dolinska, M.B., Sergeev, Y.V., et al., 2014. Interleukin-35 induces regulatory B cells that suppress autoimmune disease. *Nat. Med.* 20 (6), 633–641.
- Wardemann, H., Yurasov, S., Schaefer, A., Young, J.W., Meffre, E., Nussenzweig, M.C., 2003. Predominant autoantibody production by early human B cell precursors. *Science* 301, 1374–1377.

- Yachimovich-Cohen, N., Fischel, R., Bachar, N., Yarkoni, Y., Eilat, D., 2003. Autoimmune NZB/NZW F1 mice utilize B cell receptor editing for generating high-affinity anti-dsDNA autoantibodies from low-affinity precursors. *Eur J Immunol* 33 (9), 2469–2478.
- Yoshizaki, A., Miyagaki, T., DiLillo, D.J., Matsushita, T., Horikawa, M., Kountikov, E.I., et al., 2012. Regulatory B cells control T-cell autoimmunity through IL-21-dependent cognate interactions. *Nature* 491 (7423), 264–268.
- Yurasov, S., Wardemann, H., Hammersen, J., Tsuji, M., Meffre, E., et al., 2005. Defective B cell tolerance checkpoints in systemic lupus erythematosus. *J. Exp. Med.* 201 (5), 703–711.
- Zikherman, J., Parameswaran, R., Weiss, A., 2012. Endogenous antigen tunes the responsiveness of naive B cells but not T cells. *Nature* 489, 160–164.

## Further Reading

- Celhar, T., Magalhaes, R., Fairhurst, A.M., 2012. TLR7 and TLR9 in SLE: when sensing self goes wrong. *Immunol. Res.* 53, 58–77.
- Green, N.M., Marshak-Rothstein, A., 2011. Toll-like receptor driven B cell activation in the induction of systemic autoimmunity. *Semin. Immunol.* 23, 106–112.
- Meffre, E., Nussenzweig, M.C., 2002. Deletion of immunoglobulin  $\beta$  in developing B cells leads to cell death. *Proc. Natl. Acad. Sci. U.S.A.* 99, 11334–11339.

## 10

# Macrophages and Autoimmunity

Luisa Martinez-Pomares<sup>1</sup> and Siamon Gordon<sup>2,3</sup>

<sup>1</sup>School of Life Sciences, University of Nottingham, Nottingham, United Kingdom <sup>2</sup>Graduate Institute of Biomedical Sciences, College of Medicine, Chang Gung University, Taoyuan City, Taiwan <sup>3</sup>Sir William Dunn School of Pathology, Oxford University, Oxford, United Kingdom

## OUTLINE

Introduction	191	Toll-Like Receptors	199
General Characteristics of Macrophages	192	Scavenger Receptors	200
Heterogeneity of Tissue Macrophages Under Steady-State Conditions	192	Lectin Receptors	200
Macrophage Heterogeneity During Inflammation	193	Cytosolic Pattern Recognition Receptors	202
Opsonic and Nonopsonic Receptors in Macrophages	196	The Phagocytic Process	203
Fc Receptors	196	Clearance of Apoptotic Cells by Macrophages	204
Pattern Recognition Receptors	198	Concluding Remarks; Macrophages and Autoimmunity	205
		References	206

## INTRODUCTION

Macrophages are part of the innate immune system and play an important role in the maintenance of tissue homeostasis as well as initiation and regulation of inflammation. The versatility of these cells is linked to their ability to sample and react to their environment in a tissue-dependent manner, exploit their arsenal of degrading enzymes to effectively eliminate endocytic and phagocytic cargo, and secrete a wide range of mediators that influence the behavior of immune and nonimmune cells in a paracrine and endocrine manner (Taylor et al., 2005).

The strong link between particular human leukocyte antigen (HLA) haplotypes and autoimmune diseases highlights the important role of adaptive immunity in autoimmune diseases (Gough and Simmonds, 2007). Dendritic cells instruct T and B lymphocytes to undergo specific activation profiles in the context of selected cytokine environments by displaying at the cell-surface antigens in the form of major histocompatibility complex (MHC)-peptide complexes alongside costimulatory signals (Audiger et al., 2017). Thus dendritic cells enjoy central stage in autoimmunity research aiming to understand how self-antigens are presented in an immunogenic fashion to autoreactive T cells that escape negative selection in the thymus (Audiger et al., 2017). Dendritic cells and macrophages are both mononuclear myeloid cells and share numerous characteristics including a highly developed endocytic compartment, expression of a wide range of sensing receptors termed pattern recognition receptors (PRRs), and ability to migrate and respond to their environment. Both cells are professional antigen presenting cells but, from a functional point of view, dendritic cells are endowed with the capacity to activate

naïve T cells (Guerder et al., 2013). Macrophages, in contrast, interact with activated T cells during inflammation and, as such, act as effector and regulatory cells during T cell–mediated inflammatory responses. The contribution of macrophages to T cell–mediated immunity in autoimmunity can be further shaped through interaction with self-reactive antibodies recognized by receptors for their Fc portion (Fc receptors, FcRs) (Clynes et al., 1999; Swanson and Hoppe, 2004; Takai, 2002).

Distinction between autoimmune and autoinflammatory diseases is based on the identification of self-reactive T and B cells, which are lacking in autoinflammatory diseases (Manthiram et al., 2017). Nevertheless, it is plausible that dysregulated antigen presentation takes place in the context of genetic abnormalities in intracellular sensing pathways of macrophages linked to autoinflammatory diseases. In this context, the lower threshold of macrophage activation and associated inflammation could provide the setting for the release and modification of self-antigens that can then be internalized and presented by activated dendritic cells.

This chapter will first provide a general overview of macrophage biology focusing on macrophage heterogeneity, receptors used by macrophages to survey their environment, and the phagocytic process. Because of the importance of self-recognition in the induction of autoimmune diseases, special mention will be made of endogenous activators and inhibitors of macrophage receptors and the process of apoptotic cell clearance.

## GENERAL CHARACTERISTICS OF MACROPHAGES

Macrophages are cells of hematopoietic origin first identified by Élie Metchnikoff more than a century ago as mediators of cell-mediated immunity in the form of phagocytosis (Gordon, 2016). Macrophages reside in all tissues under steady-state conditions and adopt tissue-specific characteristics. Tissue adaptation is underpinned by epigenetic modifications and translates into distinct transcriptome profiles (Davies et al., 2013). Macrophages display a highly developed endosomal compartment which enables effective endocytosis and phagocytosis. In addition, macrophages secrete a wide array of soluble molecules encompassing cytokines and chemokines, complement components, lipids, and growth factors. Macrophages can also control amino acid availability through the action of indoleamine 2,3-dioxygenase (Munn et al., 1998, 1999; Ravishankar et al., 2012) and arginase (Pesce et al., 2009) that degrades tryptophan and L-arginine, respectively, with important consequences on dampening T cell proliferation, and contributes to bone remodeling, erythropoiesis, brain development, lung homeostasis, iron recycling (Soares and Hamza, 2016), and thermogenesis (Gordon and Martinez-Pomares, 2017).

## HETEROGENEITY OF TISSUE MACROPHAGES UNDER STEADY-STATE CONDITIONS

Macrophages are intrinsic components of all organs where they contribute to tissue homeostasis and surveillance (Okabe and Medzhitov, 2016). Macrophage phenotype in tissues is associated with distinct gene expression patterns controlled epigenetically and largely dictated by the tissue environment rather than ontogeny (Lavin et al., 2014). Hence, under steady-state conditions, tissue macrophages display a variety of phenotypic adaptations linked to their anatomical location and contribution to tissue function. For instance, alveolar macrophages contribute to surfactant homeostasis (Gordon and Martinez-Pomares, 2017), while splenic red pulp macrophages eliminate old erythrocytes and degrade heme groups, hence contributing to iron recycling (Gordon and Martinez-Pomares, 2017). Microglia in the central nervous system promote neuronal development and are involved in synaptic remodeling, and bone-marrow macrophages support hematopoiesis (Gordon and Martinez-Pomares, 2017). Phagocytosis of circulating blood cells further increases the heterogeneity of macrophages within tissues (Noelia et al., 2017). In spite of these differences, all tissue macrophages share an important role in immuno-surveillance which places them as central initiators of inflammation (Haldar and Murphy, 2014). The threshold of activation and sensing mechanisms can vary substantially amongst different macrophage populations (Gordon and Martinez-Pomares, 2017).

The model proposing a monocyte-derived origin of tissue macrophages (van Furth et al., 1972) dominated the field of macrophage differentiation for decades. In 2011 a seminal work by Jenkins et al. (2011) demonstrated that in the context of T helper (Th)2-dominated inflammation, numbers of macrophages increased through cellular replication rather than monocyte recruitment. These findings challenged the idea of macrophages as long lived, terminally differentiated cells with limited ability for self-replication; self-replication has been observed in tissue macrophages under steady-state conditions and additional populations of inflammatory macrophages

(Amano et al., 2014) with early reports showing replication of human alveolar macrophages under inflammatory conditions (Bitterman et al., 1984).

Fate-mapping techniques have enabled the establishment of the embryonic origin of most tissue macrophages. Tissue macrophages are seeded during embryonic development and derived from the yolk sac and/or fetal liver. Once definitive hematopoiesis initiates in the bone marrow, hematopoietic stem cells (HSC) contribute to the replenishment of tissue macrophages through their differentiation into circulating monocytes (Epelman et al., 2014; Ginhoux and Guilliams, 2016; Haldar and Murphy, 2014; Sieweke and Allen, 2013; Wynn et al., 2013). The contribution of monocytes to the maintenance of tissue macrophage populations varies among organs and depends on the self-renewal capacity of resident macrophages and accessibility to blood of the particular organ. Tissues with rapid macrophage turnover that rely on monocyte recruitment and differentiation to maintain tissue macrophage numbers include skin dermis and intestine; macrophages in both locations have an estimated half-life of 4–6 weeks and are not capable of self-maintenance (Ginhoux and Guilliams, 2016). Tissues with low access to blood such as epidermis, lung, and brain (Langerhans cells, alveolar macrophages, and microglia, respectively) depend on self-renewal of resident macrophages. In between these extremes, organs, such as spleen and kidney, contain variable percentages of fetal- and monocyte-derived macrophages. Guilliams and Scott (Guilliams and Scott, 2017; van de Laar et al., 2016) recently proposed that niche availability determines the contribution of recruited monocytes to the maintenance of macrophages in tissues and suggests a mechanism of “quorum sensing” by tissue macrophages based on availability of macrophage colony stimulating factor (M-CSF, CSF-1). Under-stated conditions where there is a drastic reduction in the number of tissue macrophages, these will be replenished through an increased replication of resident macrophages, as well as increased recruitment of monocytes that differentiate into macrophages. The population of “monocyte-derived tissue macrophages” will be maintained through self-renewal alongside their fetal-origin counterparts.

In adulthood, all monocytes are generated from definitive HSC from monocyte–macrophage–dendritic cell progenitors, and their production is completely M-CSF-dependent (Ginhoux and Jung, 2014). Two populations of monocytes have been described in mouse, LY6C<sup>hi</sup>CX<sub>3</sub>CR1<sup>mid</sup>CCR2<sup>+</sup>CD62L<sup>+</sup>CD43<sup>low</sup> and LY6C<sup>low</sup>CX3CR1<sup>hi</sup>CCR2<sup>-</sup>CD62L<sup>-</sup>CD43<sup>hi</sup>, which broadly correspond to the CD14<sup>+</sup> and CD14<sup>low</sup> CD16<sup>+</sup> monocyte populations found in humans (Epelman et al., 2014; Halder and Murphy, 2014). LyC6<sup>high</sup> monocytes constitutively migrate into tissues in a CD62L-dependent manner without undergoing differentiation. After antigen acquisition in the peripheral tissue, LyC6<sup>high</sup> monocytes migrate through lymphatic vessels to draining lymph nodes (Jakubzick et al., 2013). The LyC6<sup>-</sup> monocytes (CD14<sup>low</sup> CD16<sup>+</sup> in humans) probably derive from the LyC6<sup>high</sup> cells and likely represent terminally differentiated “blood-resident macrophages.” LyC6<sup>-</sup> monocytes survey the vasculature and contribute to the maintenance of endothelial integrity.

## MACROPHAGE HETEROGENEITY DURING INFLAMMATION

Macrophages exemplify the “modus operandi” of the innate immune system—they display a wide range of systems to sense their environment and respond fast to disturbance(s) of the homeostatic status, while using their substantial endocytic and phagocytic capabilities to contain the damage. The range of “sensing systems” expressed, the threshold of activation, and the type of effector functions can vary dramatically among different macrophage populations. These variations occur among macrophages in different tissues under steady-state conditions, as discussed above, as well as among macrophages within the same organ but exposed to different inflammatory environments. The stage of the inflammatory process (initiation vs resolution) and the predominant cytokine milieu—proinflammatory (Th1-, Th2-, or Th17-dominated) or antiinflammatory (Treg-dominated)—will have major consequences on macrophage phenotype. This heterogeneity described as a “spectrum of macrophage activation” encompasses both proinflammatory (M1-like) and proresolving (M2-like) macrophages which are considered the extremes of a continuum of macrophage phenotypes (Mosser and Edwards, 2008; Murray et al., 2014).

The macrophage polarization concept was originally developed based on the distinct effects of the prototypical Th1 and Th2 cytokines, IFN- $\gamma$ /TNF- $\alpha$  and IL-4/IL-13, respectively, on the phenotype of cultured macrophages (Gordon, 2003; Gordon and Martinez, 2010; Murray, 2017; Murray et al., 2014; Stein et al., 1992). IFN- $\gamma$ -treated macrophages are microbicidal for intracellular pathogens and produce high levels of radical oxygen species (ROS) and, particularly in mice, nitrogen oxide (NO). ROS are generated through the action of the NADPH oxidase NOX2 and NO through the action of iNOS that processes L-arginine into NO and citrulline. M1 macrophages also produce proinflammatory cytokines such as IL-12, IL-6, and TNF- $\alpha$  alongside low IL-10 and the chemokines CXCL9, 10, and 11 and CCL2, 3, 4, and 5. IL-4/IL-13-treated macrophages display high expression of selected

surface markers such as lectin [mannose receptor (MR)], CD163 and scavenger receptors, and process L-arginine through the action of arginase into ornithine and urea. Ornithine can be further processed into proline which is thought to promote collagen synthesis and, in turn, fibrosis. Cytokines produced by M2-like macrophages include IL-1Ra, IL-10, and chemokines CCL17, 18, 22, and CCL24 (Biswas et al., 2013; Mantovani et al., 2004).

Metabolic changes are also characteristic of macrophage polarization (Langston et al., 2017; O'Neill and Pearce, 2016). M1 macrophages change their metabolism toward aerobic glycolysis, termed “the Warburg effect,” and fatty acid synthesis. In M1 macrophages, the Krebs cycle is broken at two places, one after citrate and the other after succinate. Citrate accumulation promotes the synthesis of NO, ROS, and prostaglandins as well as itaconic acid which has antibacterial properties and has been shown to limit the viability of *Salmonella typhimurium* and *Mycobacterium tuberculosis*. Succinate accumulation promotes the synthesis of IL-1 $\beta$  through activation of the transcription factor HIF1 $\alpha$ , which leads to sustained transcription of pro-IL-1 $\beta$ . It has also been shown that glycolysis is required for the activation of the NLRP3 inflammasome which will further promote IL-1 $\beta$  synthesis.

In contrast, the metabolism of M2 macrophages is based on oxidative phosphorylation. The Krebs cycle is intact, and there is an increased amino sugar and nucleotide sugar metabolism leading to high levels of UDP-GlcNAc, UDP-glucose, and UDP-glucuronate (Jha et al., 2015), as well as transcriptional upregulation of steps in the N-glycan pathway (Jha et al., 2015) which suggest the importance of protein glycosylation in these cells. Liu et al. recently showed that  $\alpha$ -ketoglutarate produced through glutaminolysis promotes M2 activation while restricting M1 activation. Interestingly,  $\alpha$ -ketoglutarate also promotes endotoxin tolerance after M1 activation (Liu et al., 2017).

The antiinflammatory cytokine IL-10 has been shown to counteract the switch to glycolysis and glucose consumption induced by lipopolysaccharide (LPS) in macrophages (Ip et al., 2017). IL-10 promotes the induction of autophagy and inhibits the master regulator of metabolism mTOR through the action of the negative regulator DDIT4. Importantly, IL-10 appears to work in an autocrine manner, since IL-10 deficient macrophages displayed an altered metabolic profile after LPS activation (Ip et al., 2017) supporting a self-regulatory circuit during macrophage activation.

Metabolic differences between M1 and M2 macrophages could be related to their different involvement during immune responses. M1 macrophages are required in an acute fashion, and their energy needs are provided by a poorly efficient but fast metabolic setting. M2 macrophage metabolism would be better suited for long-term responses such as those taking place during parasitic infections which are dominated by Th2 immunity (Gause et al., 2013). Iron metabolism is also affected by M1/M2 polarization with M1 shown to sequester iron, an important determinant of microbial growth, while an M2 phenotype has been shown to increase iron availability (Cairo et al., 2011; Soares and Hamza, 2016).

Additional changes associated with macrophage polarization include alterations in the endocytic compartment (Montaner et al., 1999). Phagocytosis in macrophages, unlike neutrophils, follows the endocytic pathway, and differences between M1 and M2 phagolysosomes have been identified (Levin et al., 2016). In M1 macrophages, as in neutrophils, there is a production of large quantities of ROS because of high activity of the NADPH oxidase NOX2, and the luminal pH remains neutral or alkaline because of reduced activity of the V-ATPase (a proton pump) and a large rate of proton consumption through dismutation of superoxide to H<sub>2</sub>O<sub>2</sub>. M2 phagosomes, in contrast, generate less ROS because of reduced NOX2 activity and reach lower pH values. The lower pH provides the optimal environment for the activation of hydrolases present in the lysosomes improving the degradative capacity of the phagosomal compartment.

Although it is relatively safe to link “proinflammatory phenotype” and “M1 activation,” the M2 phenotype is broader and encompasses cells that contribute to Th2-mediated inflammation, such as those induced in response to allergens and parasites (Gause et al., 2013) as well as fibrotic diseases (Vannella and Wynn, 2017). In addition, the term M2 also includes cells with immunosuppressive characteristics that promote resolution of inflammation. Some authors distinguish three types of M2 macrophages: M2a (IL-4/IL-13 induced), M2b (induced by immunocomplexes and LPS), and M2c macrophages (induced by IL-10, TGF- $\beta$ , and/or glucocorticoids) (Roszer, 2015). The existence of M2d macrophages, obtained in response to IL-6 and adenosine (Roszer, 2015), has also been described. Means used by macrophages to inhibit immune responses include sequestration of essential amino acids such as L-arginine and tryptophan (see above), expression of PD-L1 (Fangchao et al., 2017), as well as the production of the antiinflammatory cytokines IL-10 and TGF- $\beta$ . Macrophages also contribute to the synthesis of proresolving lipids (Basil and Levy, 2016; Serhan, 2014) which could contribute to the resolution phase of inflammation (Freire-de-Lima et al., 2006). The acquisition of an M2-like phenotype (M2b) in mouse macrophages upon treatment with endotoxin and immunocomplexes (Edwards et al., 2006) was unexpected as both stimuli in separation are considered proinflammatory. Thus a better understanding of the “decision-making process”

undertaken by macrophages exposed to independent signals is essential to better design tools for the manipulation of macrophages in the context of autoimmune diseases. Members of the collectin family surfactant protein A and C1q have been identified as local tissue amplifiers of IL-4R $\alpha$ -mediated macrophage activation in lung and liver, respectively, through engagement of their receptor myosin 18A (Minutti et al., 2017).

Environmental factors shown to reduce the effector functions of macrophages include the expression of CD200 (Minas and Liversidge, 2006) or CD47 (Sosale et al., 2015) by surrounding cells. CD200 interacts with the inhibitory CD200R receptor expressed by macrophages, resulting in an increased cellular activation threshold. The inhibitory receptor SIRP-1 $\alpha$  inhibits phagocytic uptake upon engagement of the “don’t eat me” signal CD47.

Inflammatory processes differ substantially depending on the target organ and nature and extent of the stimulatory agent(s), but a shared feature is the activation of the vascular endothelium. Endothelial cells respond to the presence of proinflammatory cytokines such as TNF- $\alpha$  and IL-1 $\beta$  produced by resident macrophages upon engagement of PRRs, by sequentially upregulating expression of P and E-selectins and adhesion molecules that facilitate the recruitment of neutrophils and inflammatory monocytes (Soehnlein and Lindbom, 2010). Macrophages also produce eicosanoids derived from the processing of arachidonic acid through the action of cyclooxygenases (to generate prostaglandins) or lipoxygenase (to produce leukotrienes) (Funk, 2001). LyC6<sup>high</sup> monocytes migrate into tissues during inflammation and readily differentiate to macrophages (Epelman et al., 2014). Activation of endothelium increases vascular permeability leading to edema and early recruitment of neutrophils. Granule contents in neutrophils (cathepsin G, LL-37, and azurocidin) engage formyl-peptide receptors in monocytes and favor recruitment of lyC6<sup>high</sup> monocytes (Soehnlein and Lindbom, 2010; Soehnlein et al., 2008). Increase in the number and activation of macrophages in inflamed tissue is probably due to the recruitment and differentiation of LyC6<sup>high</sup> monocytes as well as replication and activation of resident macrophages.

Cross talk between neutrophils and macrophages has important consequences for the outcome of the inflammatory process. The arsenal of degradative products encapsulated within neutrophil granules, if released, has the potential to liquefy tissues, and timely removal of these cells by macrophages is essential for successful resolution. Furthermore, neutrophils when highly activated are capable of releasing their nuclear content to form extracellular neutrophil extracellular traps (NETs) (Papayannopoulos and Zychlinsky, 2009) that contribute to pathogen trapping and killing. Of note, two of the best characterized autoantigens targeted by antineutrophil cytoplasmic antibodies (ANCA) are the neutrophil products myeloperoxidase and the serine protease protease-3 (Thieblemont et al., 2016) which highlights the importance of reducing the availability of these products to minimize self-recognition. In addition, myeloperoxidase can be internalized by macrophages through the MR (CD206) (Shepherd and Hoidal, 1990) and trigger their activation leading to increased inflammation in the context of autoimmunity (Lefkowitz et al., 1999). Similarly, the antimicrobial peptide LL-37 has been proposed as an autoantigen in psoriasis (Lande et al., 2014) and neutrophil proteases that have been shown to mediate IL-1 $\beta$  processing independently of caspase 1 (Guma et al., 2009; Karmakar et al., 2012; Schreiber et al., 2012). More recently, monocytes, which also contain myeloperoxidase and protease-3 within lysosomes and at the plasma membrane after activation, have also been implicated in the pathogenesis of ANCA-associated vasculitides (Brunini et al., 2016).

The specific aspects of the interaction between macrophages and neutrophils determine the extent of neutrophil activation and neutrophil half-life. Although, in general, neutrophils are considered short lived, a concept recently challenged (Kolaczkowska and Kubes, 2013), their viability can be extended during inflammation (Soehnlein and Lindbom, 2010). Similarly, production of granulocyte-macrophage (GM)-CSF by natural killer cells in response to IL-15, produced by inflammatory LyC6<sup>high</sup> monocytes, improves neutrophil activation and their effectiveness in controlling *Candida albicans* infection (Dominguez-Andres et al., 2017). These findings further highlight the important cross talk between monocytes/macrophages and neutrophils during inflammation.

Th17 responses have taken a center stage in multiple autoimmune diseases (Bedoya et al., 2013; Bettelli et al., 2007). The paradigm of Th17-driven inflammation implicates high neutrophil involvement and, accordingly, Th17 cells are essential for the control of infections by extracellular bacteria and fungi (Bedoya et al., 2013; Bettelli et al., 2007). Th17 cells produce IL-17A and IL-17F together with GM-CSF and IL-22, among others, and are considered as the third type of effector T cells (Annunziato et al., 2015). Similarly to M1 and M2 macrophages, respectively, associated with Th1- and Th2-dominated immunity, macrophages also contribute to IL-17-mediated inflammation (Barin et al., 2012) with “M17 macrophages” displaying a proinflammatory phenotype including increased transcription of chemokines CCL2, CCL8, CCL20, CXCL1, CXCL2, and CXCL6 and cell-surface receptors CD14 and CD163 with substantial overlap with M1 cells (Erbel et al., 2014). The paradigm of a simple Th cell expressing a particular subset of signature cytokines and master regulators does not conform to the reality of T cells retaining a high degree of plasticity after differentiation (Hirahara et al., 2013; Nakayamada et al., 2012). Thus a “Th17-component” has been found in Th2-dominated inflammatory responses with negative

consequences on disease severity (Akdis et al., 2012; Bedoya et al., 2013), and Th17 cells expressing both IL-17A and IFN- $\gamma$  have been described in several autoimmune diseases (Boniface et al., 2010; Duhen et al., 2013; Kebir et al., 2009). Conditions leading to Th17 differentiation closely resemble those described for Treg cells (Diller et al., 2016), and IL-17A and IL-10 coproducing Th17 cells have been observed (McGeachy et al., 2007). Conversion of Th17 cells into Treg cells during inflammation (Gagliani et al., 2015) has been described which suggests that this conversion and the associated changes in cytokine environment will contribute to the resolution of inflammation. Consequently, processes hampering this shift in phenotype could lead to chronicity.

Timely uptake of apoptotic neutrophils by macrophages is essential for the resolution of inflammation because (1) it minimizes the potential for secondary neutrophil necrosis which will cause tissue damage and (2) it promotes production of proresolving lipid mediators as part of the lipid switch that takes place (Basil and Levy, 2016; Serhan and Savill, 2005) and the acquisition by macrophages of a “wound healing” phenotype characterized by production of IL-10 and TGF- $\beta$  (see below). The presence of both IL-10 and IL-17A at the site of inflammation could promote resolution with one process involving the improved uptake of apoptotic neutrophils by macrophages. IL-17A in combination with IL-10 improves uptake of apoptotic neutrophils through the apoptotic cell receptor MerTK and acquisition of an immunosuppressive phenotype by macrophages (Zizzo and Cohen, 2013). IFN- $\gamma$ , on the other hand, inhibits this process and promotes upregulation of CD95, FASL, which would make them more susceptible to apoptosis (Zizzo and Cohen, 2013) hampering resolution.

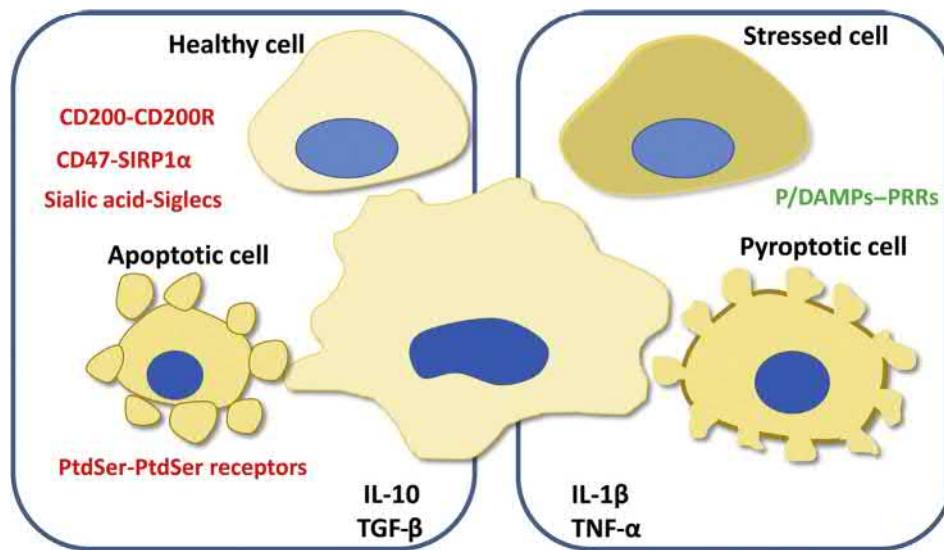
Thus even though macrophage heterogeneity represents a major challenge for researchers trying to understand the contribution of these cells to autoimmune diseases, it also provides the opportunity to harness macrophage malleability through macrophage-targeted therapeutics directed toward pathways that control their regulatory potential.

## OPSONIC AND NONOPSONIC RECEPTORS IN MACROPHAGES

The soluble and cellular arms of the innate immune system intertwine during an immune response through the action of opsonic receptors. Opsonic recognition refers to the ability of myeloid cells to display enhanced recognition of antibody and/or complement-coated material through the action of opsonic receptors. Opsonic receptors include receptors for the constant portion of antibodies and protein fragments derived from active C3. Vignesh et al. (2017) provide a recent overview of the contribution of complement to autoimmune diseases. Nonopsonic recognition refers to the ability of myeloid cells to directly detect and, in some instances, internalize a range of ligands through the action of PRRs (Boller and Felix, 2009; Vance et al., 2009). Recognition ascertains the physiological status of tissues by detecting the presence in the extracellular milieu of endogenous molecules normally sequestered within cells that are released through tissue damage or cellular activation (damage-associated molecular patterns, DAMPs). In addition, PRRs detect the presence of microbial compounds, such as endotoxin, peptidoglycan, and flagellin. In most cases, these microbial molecules are shared between pathogens and commensals, and thus the term microbe-associated molecular patterns, MAMPs, instead of pathogen-associated molecular patterns, PAMPs, has also been proposed (Boller and Felix, 2009). Activation of antimicrobial immunity relies on the detection of MAMPs in the context of DAMPs, that is, an induction of an immune response requires the presence of virulence factors that compromise cell viability alongside MAMPs (Blader and Sander, 2012; Nathan and Ding, 2010; Vance et al., 2009) (Fig. 10.1).

### Fc Receptors

Receptors for the constant region of antibodies (FcR) enable macrophages to bind and internalize antigen-antibody complexes and are also responsible for antibody-dependent cell-mediated cytotoxicity and release of cytokines and cytotoxic molecules (Nimmerjahn and Ravetch, 2008; Takai, 2002). Receptors specific for the IgG Fc fragment are termed Fc $\gamma$ Rs. Many laboratories have shown that Fc $\gamma$ Rs are responsible for IgG effector function with lack of these receptors leading to protection against the development of autoimmune diseases. Intriguingly, although Fc $\gamma$ Rs are widely expressed in phagocytes, the activity of IgG in vivo in a model of IgG-dependent platelet removal and B cell depletion appears to depend on the presence of the Ly6C<sup>lo</sup>CX<sub>3</sub>CR1<sup>high</sup>CD11c<sup>int</sup> monocyte subset (Biburger et al., 2011) which suggests that the cell population responsible for antibody-mediated pathology might be disease dependent.



**FIGURE 10.1** Macrophages as sensors of the health status of tissues. Environmental factors in addition to cellular origin drive tissue specialization of macrophages. Within tissues, in addition to contributing to organ function, macrophages ascertain the health status of surrounding cells with different set of receptors dominating interactions in healthy and pathological conditions. Healthy/apoptotic cells will minimize macrophage activation through the action of inhibitory receptor such as CD200R and SIRP-1 $\alpha$ , and the presence of antiinflammatory cytokines produced upon uptake of apoptotic cells. Under pathological conditions, cells-stressed/damaged by infection, injury or metabolic dysfunction will trigger PRRs in macrophages leading to activation and production of proinflammatory cytokines. Ligands for PRRs can be of self (DAMPs) and microbial origin (M/PAMPs). DAMPs, Damage-associated molecular patterns; M/PAMPs, microbe/pathogen-associated molecular patterns; PRRs, pattern recognition receptors.

Two major types of Fc $\gamma$ Rs have been described, type I and type II. Engagement to each type and, in turn, effector function depends on the glycosylation of the IgG molecule (Biermann et al., 2016; Bournazos et al., 2016). N-linked glycosylation at the Fc portion of the IgG antibody occurs at Asn297 which is located in a hydrophobic cleft formed by the two CH2-proximal hinge regions. Glycosylation at Asn297 is required for maintenance of a conformation permissive for interaction of the IgG molecule with Fc $\gamma$ Rs. Binding to type I Fc $\gamma$ Rs is favored by the presence of the heptasaccharide (GlcNAc2-Man3-GlcNAc2); the addition of branched fucose, and/or galactose, and sialic acid, in particular, promotes binding to the type II FcRs by exposing a region at the CH2–CH3 interface that represents the binding site for these receptors. Type II FcRs belong to the C-type lectins and Siglec families of lectin receptors (see next). The presence of sialic acid induces a closed conformation within the Fc region that inhibits binding to the type I Fc $\gamma$ Rs and promotes lectin-mediated binding (Biermann et al., 2016; Bournazos et al., 2016). IgG, modified by glycans with  $\alpha$ 2,6-linked terminal sialic acid, displays immunosuppressive properties and is responsible for the therapeutic effect of intravenous immunoglobulin therapy (Schwab et al., 2014; Schwab and Nimmerjahn, 2014). There is substantial heterogeneity in Fc glycosylation under steady-state conditions and differential IgG glycosylation; in particular, increased agalactosyl IgG-G0 is associated with disease activity in patients suffering from rheumatoid arthritis, Crohn's disease, juvenile onset arthritis, and systemic lupus erythematosus (SLE) complicated by Sjogren's syndrome. In IgA nephropathy, lack of terminal galactose reduces uptake by the asialoglycoprotein receptor in the liver, which results in its deposition in the kidney (Biermann et al., 2016). Type II Fc $\gamma$ Rs will be discussed below.

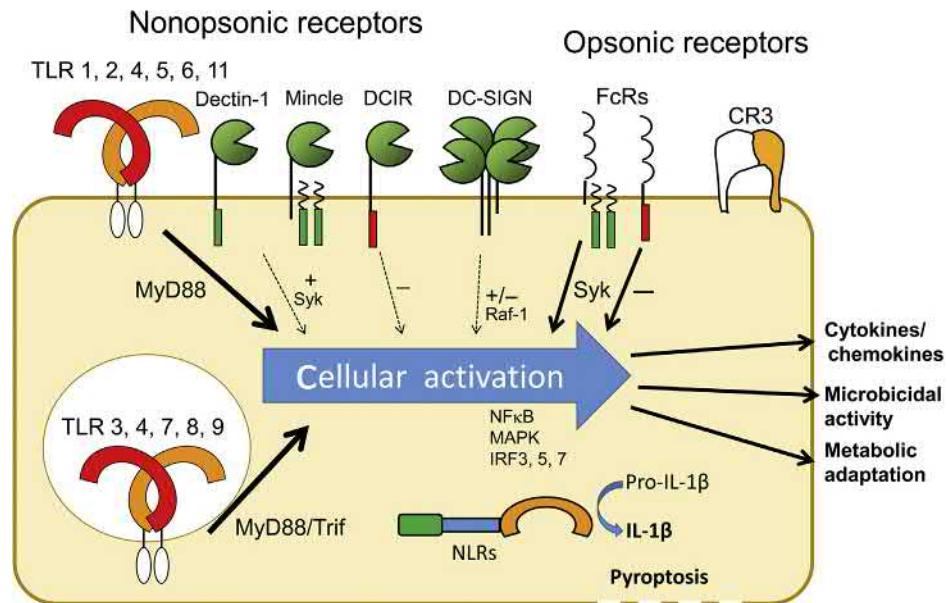
Type I Fc $\gamma$ Rs belong to the Ig superfamily and can act as activating or inhibitory receptors based on the presence of immunoreceptor tyrosine-based activation motif (ITAM) or immunoreceptor tyrosine-based inhibitory motif (ITIM) motifs that transduce activating or inhibitory signals, respectively (Bournazos et al., 2016). In humans, activating Fc $\gamma$ Rs include Fc $\gamma$ RI, Fc $\gamma$ RIIa, Fc $\gamma$ RIIc, and Fc $\gamma$ RIIIa; Fc $\gamma$ RIIb is an inhibitory receptor, and Fc $\gamma$ RIIb is glycosylphosphatidylinositol (GPI)-linked and abundantly expressed by neutrophils. With the exception of the Fc $\gamma$ RI, which binds IgG with high affinity, the rest of the Fc $\gamma$ R receptors display low affinity for monomeric IgG and preferentially recognize antibody-coated targets or immunocomplexes because of an increased avidity. Activating Fc $\gamma$ Rs trigger responses through phosphorylation of ITAM motifs in the common Fc $\gamma$  chain associated with Fc $\gamma$ RI and Fc $\gamma$ RIIIa or located at the C-terminus of Fc $\gamma$ RIIA and Fc $\gamma$ RIIC by Src kinases and recruitment and activation of Syk kinase (Mocsai et al., 2010). NK cells only express low-affinity activating Fc $\gamma$ Rs, and B cells only express inhibitory Fc $\gamma$ R. In contrast, macrophages and dendritic cells express multiple Fc $\gamma$ Rs per

cell, and the balance between responses originated by the activating and inhibitory Fc $\gamma$ Rs will determine the biological outcome (Boruchov et al., 2005). Fc $\gamma$ R activation will lead to endocytosis and phagocytosis of the antibody-antigen complex (Swanson and Hoppe, 2004) and cellular activation. As mentioned above, coengagement of FcR using immunocomplexes and Toll-like receptor (TLR)-4 using endotoxin in nonpolarized macrophages induces a M2-like phenotype referred to as M2b characterized by the production of IL-10, IL-6, and IL-1 and increased phagocytosis and migratory capacity (Edwards et al., 2006). Fc $\gamma$ RIIb-deficient mice are more susceptible to immune-complex-induced shock and arthritis, and this phenotype correlates with a lower activation threshold in macrophages when stimulated with immune complexes (Clynes et al., 1999). On the other hand, these animals are more resistant to pneumococcal peritonitis which emphasizes the important balance set by activating and inhibitory signals in antibody-mediated processes (Clatworthy and Smith, 2004).

## PATTERN RECOGNITION RECEPTORS

PRRs can be broadly classified into “canonical” PRRs, mostly involved in triggering signaling events such as TLRs and nucleotide binding and oligomerization domain (NOD)-like receptors (NLRs), as well as “noncanonical” PRRs, such as scavenger receptors and lectin receptors (Takeuchi and Akira, 2010), which mediate cellular uptake through promotion of endocytosis and phagocytosis. “Noncanonical” PRRs, in general, do not transduce signaling events in isolation but can modulate the signaling triggered by opsonic receptors and canonical PRRs. Macrophages, together with dendritic cells, display a wide range of PRRs and cellular responses result from the integration of the multiple signals triggered through simultaneous or sequential engagement of different PRRs (Fig. 10.2).

Recognition of DAMPs and MAMPs by PRRs takes place at the cell surface, endosomes, and cytoplasm. A vast array of molecular structures including lipids, proteins, lipoproteins, carbohydrates, and nucleic acids is sensed by these receptors. Interestingly, nucleic acid recognition is restricted to the endosomal compartment and cytosol, and this is considered a safeguard mechanism to minimize recognition of self (Crowl et al., 2017; Schlee and Hartmann, 2016). Macrophages respond to PRR engagement by internalizing the triggering agent through



**FIGURE 10.2** Signal integration during macrophage activation. The engagement of opsonic and nonopsonic receptors in macrophages will substantially affect their phenotype leading to changes in their microbicidal and metabolic activity and production of inflammatory mediators. Dominating activating responses such as those triggered by acute engagement of TLRs and activating Fc receptors can be modulated through coengagement of additional PRRs such as lectin receptors that can be activating or inhibitory. Cellular activation can also be influenced by the length and level of stimulation and can lead to tolerance (lack of response) (Collins and Carmody, 2015; Rajaiah et al., 2015) or “training” (primed for enhanced response) (Netea et al., 2011, 2016; van der Meer et al., 2015). PRRs, pattern recognition receptors; TLRs, Toll-like receptors.

endocytosis or phagocytosis and producing immune mediators, growth factors, and complement components. PRRs engagement in macrophages can contribute to the induction and perpetuation of inflammation in the context of autoimmunity.

## Toll-Like Receptors

The family of TLRs (Kawai and Akira, 2010; O'Neill et al., 2013) possess an N-terminal leucine-rich domain (LRR) shaped as a solenoid that mediates ligand binding, a transmembrane domain, and a cytosolic region containing a Toll/interleukin-1 receptor (TIR) domain that in most cases (TLR1, TLR2, TLR4, TLR5, and TLR6) engages the MyD88-mediated signaling machinery shared with the receptors for IL-1 and IL-18 (Gay et al., 2014; O'Neill and Bowie, 2007). In addition, two TLRs (TLR3 and TLR4) trigger MyD88-independent signaling through the adaptor Toll–IL-1 receptor domain-containing adaptor inducing IFN- $\beta$  (TRIF) (O'Neill and Bowie, 2007). TLR4, the TLR responsible for LPS sensing in combination with MD2 and CD14 (Bryant et al., 2010), signals through both MyD88 and TRIF. Unlike MyD88-mediated signaling which promotes the release of proinflammatory cytokines through the action of the transcription factor NF $\kappa$ B and activation of MAP kinases, the TRIF-mediated pathway triggers delayed NF $\kappa$ B activation and, most importantly, production of type I interferons (Kawai and Akira, 2010; O'Neill and Bowie, 2007). Type 1 interferons act in an autocrine and paracrine fashion and induce the synthesis of interferon stimulating genes through binding to their receptor (Novick et al., 1994). TLRs transduce signals through the formation of homo- (TLR3, TLR4, TLR5, TLR7, TLR8, and TLR9) or heterodimers (TLR1/TLR2 and TLR2/TLR6) which bring two TIR domains into proximity providing a functional platform for the formation of the relevant signalosome. The GPI-linked protein CD14 was originally recognized as an important adaptor for the recognition of endotoxin by TLR4 (Fitzgerald et al., 2004) but is now acknowledged as a facilitator of ligand recognition by several TLRs (Finberg and Kurt-Jones, 2006). CD14 has been shown to promote endocytosis of TLR4 after LPS recognition (Zanoni et al., 2011) with this involvement in TLR4-endocytosis being ligand dependent (Rajaiah et al., 2015). Traffic of TLR4 to endosomes recruits TRIF and leads to activation of interferon-regulatory factor (IRF)3. The endosome is considered a unique site for IFN-induction due to the intracellular localization of TRAF-3 which is restricted to the intracellular compartment (Barton and Kagan, 2009).

In the context of autoimmune diseases, TLRs have been linked to the pathogenesis of rheumatoid arthritis, SLE, inflammatory bowel disease, type I diabetes, and multiple sclerosis. TLR dysregulation could act by lowering the threshold of activation of macrophages which will have consequences both during the initiation and progression of disease by generating a positive feedback loop. TLR activation is tightly controlled by negative regulators, including inhibitors of the NF $\kappa$ B pathway (Afonina et al., 2017), and there are strong links between variants in these molecules and autoimmunity. Mice with deficiency in some of these constitutive inhibitors develop spontaneous autoimmune and inflammatory diseases which suggest that they are important for controlling TLR activation in response to endogenous ligands or microbiota (Hamerman et al., 2016). For instance, A20 is a deubiquitinase that cleaves K63-linked ubiquitin from TRAF6 which impairs, among others, TLR-mediated NF $\kappa$ B signaling. Polymorphisms in A20 have been associated with increased risk of autoimmune and inflammatory diseases including RA, SLE, psoriasis, Crohn's disease, and type 1 diabetes (Vereecke et al., 2009). A20 is widely expressed in multiple cell types, but conditional deletion of A20 in macrophages and neutrophils causes the development of spontaneous polyarticular arthritis that resembles RA indicating that A20 in these cells is important for the control of disease (Matmati et al., 2011). TLR10 which is exclusively expressed in humans has been recently characterized as a negative regulator of TLR signaling (Jiang et al., 2016). Thus TLR10 can be added to the extensive list of inhibitors of TLR signaling (Liew et al., 2005).

TLRs are best known for their ability to interact with MAMPs/PAMPs, but substantial evidence supports their involvement in the recognition of DAMPs (Piccinini and Midwood, 2010). Under conditions where there is substantial tissue destruction, local and systemic levels of DAMPs are increased. For instance, heat shock proteins, the nuclear protein HMGB1, host DNA, fibrinogen, FNEDA, and tenascin-C are observed in synovium of RA patients compared to normal synovium and synovium from osteoarthritis patients. Hence, the presence of DAMPs could have a negative effect on the pathophysiology of autoimmune diseases as both triggers of a tissue repair response that, under particular conditions, could lead to activation of self-reactive B and T cells, or as amplifiers of a pathological inflammatory response driven by autoantibodies and self-reactive T cells.

Interestingly, engagement of the same TLR (TLR4) by MAMPs (LPS) or DAMPs (C-tenascin) triggers distinct patterns of activation (Piccinini et al., 2016) which suggest that although TLRs are activated during sterile inflammation, the outcome differs from that obtained in response to infection.

Endosomal TLRs recognize nucleic acids in the form of double-stranded DNA (TLR9), double-stranded RNA (TLR3), and single-stranded RNA (TLRs 7 and 8) (Kawai and Akira, 2010; O'Neill and Bowie, 2007). Endosomal localization will restrict access of endogenous nucleic acids to these sensors (Crowl et al., 2017), but this protective compartmentalization can be lost in the presence of anti-DNA antibodies (Barrat et al., 2005) or deficiency in DNA degradation after uptake of apoptotic cells (see below) which will promote self-recognition and chronic inflammation.

## Scavenger Receptors

The macrophage scavenger receptors are a large family of structurally diverse receptors, which recognize a range of ligands including modified low-density lipoproteins (LDLs), selected polyanionic ligands, and microbial structures (Pluddemann et al., 2007). The Class A scavenger receptor I (SR-AI) was the first to be identified in studies by Brown and Goldstein who investigated the accumulation of cholesterol from LDL in atherosclerotic plaques. Other Class A scavenger receptors expressed on macrophages include two alternative splice variants of the SR-AI gene, SR-AII, and SR-AIII and the distinct macrophage receptor with collagenous structure (MARCO). The receptors are all trimeric type II transmembrane glycoproteins generally consisting of several domains including an  $\alpha$  helical coiled-coil domain, collagenous domain, and C-terminal cysteine-rich domain, although MARCO lacks the coiled-coil domain and has a longer collagenous domain. For SR-AI and SR-AII, the collagenous domain has been shown to be the ligand-binding domain, whereas for MARCO, this may lie within the cysteine-rich domain. SR-A is expressed on tissue macrophages and selected endothelia, but not resting monocytes, while MARCO is constitutively expressed on subpopulations of tissue macrophages (resident peritoneal, spleen marginal zone, and medullary lymph node macrophages). For Class B, scavenger receptors CD36 and SR-B, ligand binding is likely mediated via the central part of the extracellular loop structure and these receptors recognize not only modified LDL but also native lipoproteins (VLDL, LDL, and HDL) and play an important role in cholesterol transport, metabolism, and homeostasis. Other scavenger receptors expressed by macrophages include CD68 and FEEL-1 (predominantly expressed intracellularly), SREC, and SR-PSOX. SREC receptors have been shown to bind molecular chaperones (e.g., calreticulin, gp96, and heat shock protein 70), playing a role in the transport of ligands to the MHC Class-I antigen presentation pathway (Berwin et al., 2004). SR-PSOX is the chemokine ligand for a G-protein coupled CXC chemokine receptor 6 expressed on activated T cells and NKT cells, supporting the adhesion of these cells to DCs (Shimaoka et al., 2004).

Class A scavenger receptors SR-A and MARCO recognize oxidized and acetylated LDL and play a role in vascular disease by mediating uptake of these modified lipoproteins, leading to the formation of lipid-laden foam cells in atherosclerotic lesions (Krieger and Herz, 1994). SR-A and CD36 have also been implicated in the pathology of Alzheimer's disease, where microglia bind  $\beta$ -amyloid fibrils via SR-A and CD36, which stimulates the production of cytotoxic reactive oxygen molecules (Santiago-Garcia et al., 2001). Macrophages bind and endocytose-advanced glycation end products (AGE) via several receptors including the scavenger receptors SR-AII and CD36, and the AGE receptors 1, 2, and 3 (Lu et al., 2004). AGE are found in many tissues during oxidative stress and inflammation, such as atherosclerotic lesions of arterial walls and kidneys of diabetic patients with chronic renal failure (Nagai et al., 2000), as well as during ageing. Dietary AGE (e.g., from processed food) have also been linked to chronic kidney injury, for example, by suppressing AGER1 and inducing oxidative stress and inflammatory responses, thus contributing to pathogenesis and complications of diabetes mellitus (Vlassara and Striker, 2011).

## Lectin Receptors

Carbohydrates displayed by secreted and cell-surface molecules have major implications for their half-life and sensing of the tissue health status (Rabinovich et al., 2012; Schnaar, 2016). Accordingly, alteration in the normal processing of complex N-linked sugars through deletion of the  $\alpha$ -mannosidase-II leads to the accumulation of mannose-bearing ligands that causes the development of systemic autoimmunity with the characteristics similar to human SLE (Green et al., 2007). Carbohydrate-binding (lectin) receptors expressed by mononuclear myeloid cells mediate ligand uptake and cell adhesion. Consequences of carbohydrate recognition will be strongly influenced by the nature of the ligand as number, and distribution of sugar units will impact on the avidity of these low-affinity interactions.

Important lectin receptors expressed by macrophages belong to two main families: C-type lectin receptors (CLRs) (Dambuza and Brown, 2015; Drickamer and Taylor, 2015) and sialic acid binding Ig-like lectins (Siglecs)

([Macaulay et al., 2014](#)). CLRs contain one or more domains with a fold initially described in the mannose-binding lectin which initiates lectin pathway of complement activation. This chapter will focus on members of the Group 2 of CLRs that include dendritic cell-specific intercellular adhesion molecule (ICAM)-3 grabbing nonintegrin (DC-SIGN, CD209) ([Garcia-Vallejo and van Kooyk, 2013](#)), Dectin-2 (CLEC4N, CLEC6A), DCIR (CLEC4A), macrophage inducible C-type lectin (Mincle, CLEC4E), and myeloid inhibitory C-type lectin-like receptor (MICL, CLEC12A, and CLEC4D) ([Dambuza and Brown, 2015; Graham and Brown, 2009](#)), Group 5 that includes Dectin-1 (CLEC7A) ([Dambuza and Brown, 2015](#)), and Group 6 that includes the MR (CD206) ([Martinez-Pomares, 2012](#)).

DC-SIGN was originally described as a dendritic cell-specific marker but has now been documented in multiple macrophage populations that might represent subsets with an alternative phenotype related to the positive effect of IL-4 on DC-SIGN expression. DC-SIGN is a type II membrane protein that assembles as a tetramer and projects the CRDs 35 nm away from the membrane. DC-SIGN can bind fucosylated glycans, such as the blood-type Lewis antigens ( $\text{Le}^a$ ,  $\text{Le}^b$ ,  $\text{Le}^X$ ,  $\text{Le}^Y$ , and sulfo- $\text{Le}^a$ ) and high-mannose structures ([Garcia-Vallejo and van Kooyk, 2013](#)). DC-SIGN is both an uptake and adhesion receptor. Ligands for DC-SIGN include pathogens (HIV, Ebola virus, *M. tuberculosis*, *C. albicans*, *Schistosoma mansoni*, and *Helicobacter pylori*) as well as endogenous ICAM-2, ICAM-3, macrophage receptor 1, carcinoembryonic antigen, and, as mentioned above when describing type II FcRs, the Fc portion of immunoglobulins. DC-SIGN signaling in dendritic cells shows an inability to trigger signaling in isolation but rather modulates signaling triggers by “canonical PRRs” such as TLRs. Interestingly, the nature of the DC-SIGN ligand (mannosylated vs fucosylated) influences signaling outcome ([Gringhuis et al., 2009](#)), but in general, DC-SIGN coengagement is perceived to blunt cellular activation. In agreement with this concept, DC-SIGN expressing macrophages have been involved in the induction of transplantation tolerance ([Conde et al., 2015](#)).

Dectin-1 recognizes  $\beta$ -1,3-glucans present in the cell wall of many fungal species ([Brown et al., 2002; Taylor et al., 2002](#)) and has been implicated in antifungal immunity. Dectin-1 deficiency in humans can lead to susceptibility to certain fungal infections including chronic mucocutaneous candidiasis and recurrent vulvovaginal candidiasis ([Plato et al., 2015](#)). Dectin-1 also recognizes uncharacterized ligand(s) on *M. tuberculosis* and T cells as well as the self-molecules vimentin ([Thiagarajan et al., 2013](#)) and galectin-9 ([Daley et al., 2017](#)). Dectin-1 triggers cellular activation, including the production of ROS and cytokine production through direct recruitment of the Syk/caspase and recruitment domain (CARD)9 pathway via a hemi-TAM/ITAM like motif leading to NF $\kappa$ B activation ([Mocsai et al., 2010](#)). Dectin-1 has also been shown to induce TLR9 recruitment to endosomes containing  $\beta$ -1,3 glucan ([Khan et al., 2016](#)) suggesting control of nucleic acid recognition. Surprisingly, the interaction between Dectin-1 and its endogenous ligand Galectin-9, identified in the context of pancreatic carcinoma, has been shown to lead to the acquisition of a tolerogenic phenotype by macrophages. Lack of dectin-1 expression is protective against pancreatic carcinoma and induces immunogenic reprogramming of tumor-associated macrophages promoting tumor clearance ([Daley et al., 2017](#)). These results suggest an unexpected role for Dectin-1 in sterile inflammation under conditions of high galectin-9 expression.

Mincle (CLEC4E) engages Syk indirectly through association with the ITAM-containing adaptor FcR $\gamma$ . In addition to being involved in recognition of fungi ([Yamasaki et al., 2009](#)) and mycobacteria ([Ishikawa et al., 2009](#)), Mincle has also been implicated in the induction of sterile inflammation by recognizing SAP130 released by damaged cells ([Yamasaki et al., 2008](#)) which can be linked to its involvement in the inflammatory response in response to ischemic stroke ([Suzuki et al., 2013](#)) and traumatic brain injury ([de Rivero Vaccari et al., 2015](#)).

MICL (CLEC12A, CLL-1) recognizes uric acid crystals in necrotic cells ([Neumann et al., 2014](#)) and plays an important role in controlling sterile inflammation *in vivo* ([Neumann et al., 2014](#)). Its inhibitory activity is granted by the presence of an ITIM in its intracellular domain. MICL is required for the control of inflammation in a model of collagen antibody-induced arthritis ([Redelinghuys et al., 2016](#)), and although polymorphisms in MICL have not been associated with RA in humans, antibodies against MICL have been identified in a subset of RA patients ([Redelinghuys et al., 2016](#)).

The MR (CD206), unlike previous CLRs, is a type I membrane molecule and contains eight CTLDs. In addition, MR contains two additional domains, a cysteine-rich domain that binds sulfated glycans present in hormones produced by the anterior pituitary and a fibronectin type II domain that recognizes collagen ([Martinez-Pomares, 2012](#)). MR expression by tissue macrophages has been linked to phagocytic activity ([Noelia et al., 2017](#)) and, during inflammation, to M2 promoting conditions ([Martinez-Pomares, 2012](#)). The CTLD region in MR recognizes both microbial and endogenous ligands, including thyroid autoantigens ([Chazenbalk et al., 2005; Martinez-Pomares, 2012](#)) and the ANCA target myeloperoxidase ([Shepherd and Hoidal, 1990](#)). Safeguards against the endocytic activity of MR promoting presentation of self-antigens to the acquired immune system are provided by the lack of signaling motifs at its intracellular region, the restricted expression of MR in dendritic cells *in situ* and

the effective role played by MR expressed by macrophages and nonlymphatic endothelia in clearing potential autoantigens (Martinez-Pomares, 2012). In addition, a potential MR involvement in autoimmunity has not been supported by gene-association studies, although MR has been implicated in the uptake of agalactosyl IgG (Dong et al., 1999).

Siglecs are cell-surface transmembrane receptors comprising 2–17 Ig domains that include a V-set domain at the N-terminus that mediates binding to sialic acid (Macaulay et al., 2014). Most Siglecs contain an ITIM motif at the C-terminus that transduces negative signals through the recruitment of tyrosine phosphatases such as SHP1 and SHP2. Siglecs 14, 15, and 16 engage DAP-12 and are predicted to act as activating receptors through the recruitment of Syk. The first characterized Siglec, Siglec 1 is CD169, sialoadhesin, which lacks any obvious signaling motifs at the C-terminus. It contains 17 Ig domains and is selectively expressed by macrophages. In particular, metallophilic macrophages within the marginal zone of spleen express high levels of CD169 and have been implicated in tolerance to apoptotic cells (Ravishankar et al., 2014). Glycans terminating in sialic acid are considered as markers of self that can be exploited by pathogens and tumors, and it is fitting that most Siglecs transduce inhibitory signals. Indeed, sialylated antigens have been shown to induce a tolerogenic response when incubated with human dendritic cells (Perdicchio et al., 2016).

## Cytosolic Pattern Recognition Receptors

Cytosolic recognition of DAMPs and PAMPs is achieved, among others, through the action of PRRs belonging to three families: NLRs, PYHIN receptors and retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), and cGAS.

All NLRs contain an N-terminal domain that mediates interaction with signaling adaptors, an intermediate NOD domain that is responsible for oligomerization and a C-terminal LRR. Members of the NLR family include NOD1 and NOD2 which have the basic structure CARD–NOD–LRR and CARD(X2)–NOD–LRR (Caruso et al., 2014). NOD1 and NOD2 signal through the kinase RICK (also called RIPK2) to trigger NF $\kappa$ B activation in response to the presence of peptidoglycan, a component of the bacterial cell wall. NOD1 has been involved in autophagy and in the detection of changes in RHO family GTPases caused by bacterial invasion of host cells (Keestra et al., 2013). NOD2 mutations have been associated with the development of Crohn's disease which could be linked to the ability of NOD1 and NOD2 to direct autophagy by recruiting ATG16L1 to the site of bacterial entry (Travassos et al., 2010).

Other members of the NLR family, together with the pyrin (PY)-HIN family member absent in melanoma 2 (AIM2) and PY, can nucleate multimeric structures termed inflammasomes that act as scaffolds for the recruitment and activation of caspase 1 (Guo et al., 2015; Rathinam et al., 2012; Schroder and Tschopp, 2010; Vanaja et al., 2015; von Moltke et al., 2013). Inflammasome-forming NLRs include NLR-family CARD domain-containing protein 4 (NLRC4), NOD, LRR, and PY domain-containing protein 1 (NLRP1), NLRP3, and NLRP6. The adaptor apoptosis-associated speck-like protein containing a CARD domain (ASC) which contains a CARD and a PY domain is essential for caspase 1 recruitment in the case of PY-containing NLRs. ASC requirement for CARD-containing NLRs, such as NLRC4 and NLRP1, is more complex with an ASC-independent pathway leading to cell death mediated by procaspase 1 and an ASC-dependent pathway promoting autoproteolytic processing of caspase 1 and efficient cytokine production (Broz et al., 2010). Caspase 1 is an inflammatory caspase that, similarly to the LPS-sensing human caspases 4 and 5 (Vigano et al., 2015) and mouse caspase 11, mediates processing of IL-1 $\beta$  and IL-18 and induction of a proinflammatory form of cell death termed pyroptosis (Blander, 2014; Man and Kanneganti, 2016), although both processes can occur independently (Broz et al., 2010). These caspases cleave gasdermin D; the resulting 31 kDa N-terminal fragment of gasdermin D, released from the repressor C-terminus, inserts itself and forms holes in the plasma membrane (Kovacs and Miao, 2017) leading to pyroptosis and release of IL-1 $\beta$  and IL-18 alongside DAMPs. Thus pyroptosis, unlike apoptosis, is an inflammatory form of cell death (Bergsbaken et al., 2009; Man and Kanneganti, 2016). Most of the NLR members known to trigger inflammasome formation act as sensors of infection and specific triggers for some of them have been identified. For instance, NLRP1 is activated by a lethal factor, a protease from *Bacillus anthracis*, and NLRC4 responds to bacterial proteins, which are directly recognized by NLR-family apoptosis inhibitory proteins (NAIPs) which are the ligands for NLRC4 (von Moltke et al., 2013). In mice, NAIP1 binds the bacterial type III secretory system (T3SS) needle protein, NAIP2 binds the bacterial T3SS rod protein, and both NAIP5 and NAIP6 bind bacterial flagellin. In humans, only one NAIP protein has been characterized, and it was found to bind only the T3SS needle protein. AIM2, which contains HIN200 DNA binding motifs and a PY domain, recognizes cytosolic dsDNA above 80 base pairs.

The molecular basis for NLRP1, NLRP3, and PY activation is more elusive, and a model is emerging by which these PRRs act as sensors of altered homeostasis (Liston and Masters, 2017). This model would explain their broad reactivity, in particular, in the case of NLRP3 that can be activated by infectious agents in addition to sterile products such as crystals, saturated fatty acids, ROS, and damaged mitochondria (Guo et al., 2015; Rathinam et al., 2012; Schroder and Tschopp, 2010; Vanaja et al., 2015; von Moltke et al., 2013). Upon inflammasome activation, ASC redistributes and forms aggregates (specks) that can be released from cells. Specks can cleavage extracellular pro-IL-1 $\beta$  and activate caspase 1 in macrophages when internalized in a process termed “inflammasome spreading” (Baroja-Mazo et al., 2014; Franklin et al., 2014). In addition to the substantial body of literature supporting inflammasome involvement in the development of autoinflammatory diseases (Liston and Masters, 2017), links between pyroptosis and SLE have been proposed (Magna and Pisetsky, 2015) and between polymorphisms in NLRP1 and Alzheimer’s disease (Pontillo et al., 2012). Blocking of IL-1, particularly IL-1 $\beta$ , is now standard therapy for many autoinflammatory diseases, such as rheumatoid arthritis, osteoarthritis, Blau syndrome, familial Mediterranean fever, and type 2 diabetes, among others (Dinarello, 2011).

The RLRs (RIG-I and melanoma differentiation associated factor 5, MDA-5) (Takeuchi and Akira, 2008; Yoneyama et al., 2015) contain a DExD/H-box RNA helicase domain and a C-terminal domain in addition to a CARD domain that mediates interaction with the adaptor molecule IFN- $\beta$  promoter stimulator-1, also called MAVS, which is located at the outer membrane of mitochondria. Upon interaction, there is recruitment of a signaling complex leading to the induced expression of IFN- $\beta$ , IRF3-target genes, and NF $\kappa$ B target genes. Both RIG-I and MDA-5 detect cytosolic RNA and are involved in viral recognition.

cGAS is a sensor of cytosolic DNA that binds and catalyzes the synthesis of cyclic GMP–AMP (cGAMP). cGAMP then binds to and activates STING, a transmembrane adapter protein on the endoplasmic reticulum that triggers the TBK1/IRF3 pathway. Broadly, the cGAS-STING pathway is involved in the response to infection by DNA viruses and retroviruses (Crowl et al., 2017).

## THE PHAGOCYTIC PROCESS

Macrophages exploit their impressive phagocytic capacity under steady-state conditions to (1) facilitate tissue differentiation, as exemplified by the role of microglia during neuronal development, (2) remove effete cells from the circulation, as in the case of splenic red pulp macrophage-mediated removal of aged red blood cells, and (3) facilitation of cell differentiation, as in the case of phagocytosis of extruded nuclei during erythrocyte generation. The same phagocytic capacity enables macrophages to minimize inflammation by timely removal of cells undergoing programmed cell death in healthy tissues and apoptotic neutrophils during the resolution phase of inflammation. The phagocytic process in macrophages also contributes to host defense by enabling the elimination of infectious agents. Receptors that trigger phagocytosis are numerous, and although there is overlapping receptor expression among macrophages in different tissues, selective expression also occurs depending on the population of macrophages under consideration and their activation state.

Autophagy is a mechanism which macrophages can use to capture cytoplasmic components in a double-membrane vacuole for degradation in lysosomes. Autophagy is induced by different families of receptors (e.g., TLRs and NLRs) and is not only a host defense mechanism against intracellular pathogens but can also be used as a source of nutrients during starvation. Alterations in the functions of autophagy proteins can lead to enhanced susceptibility to disease and have also been implicated in chronic inflammatory and autoimmune disease processes. For example, mutations in regulators of autophagy have been linked to Crohn’s disease (Levine et al., 2011). The contribution of autophagy to autoimmunity has been recently reviewed (Wu and Adamopoulos, 2017).

Phagocytosis refers to the uptake of particles above 0.5  $\mu$ m by cells into vesicles termed phagosomes (Flannagan et al., 2009; Groves et al., 2008; Stuart and Ezekowitz, 2005). It is initiated by engagement of surface receptors that undergo lateral clustering leading to extension of pseudopodia. Pseudopodia expand and surround the particle and fuse at the distal tip leaving the particle inside the phagocytic vesicle, which is then brought into the cell. Phagosomes undergo maturation that finalizes in the formation of a phagolysosome through fusion with lysosomes. Detection and capture of material are enhanced by the ability of macrophages to produce membrane protrusions called “ruffling.” Receptor phosphorylation is important, and exclusion of inhibitory phosphatases such as CD45 and CD148 from the phagocytic cup has been described. “Tonic signaling” by Syk and SFKs in macrophages maintains a dynamic actin meshwork that facilitates lateral clustering upon receptor engagement. Phagosome maturation occurs through endosome fusion and recycling (fission) events that sequentially change

the nature of the phagosome making it a degradative environment (Groves et al., 2008; Vieira et al., 2002). Thus maturing phagosomes transition from an early phase characterized by the presence of Rab 5 to a late phase through the fusion with late endosomes that expresses Rab7. In early phagosomes, Rab5 facilitates the production of PtdIns3P through the action of the PI3K Vp34, and Rab5 and PtdIns3P collaborate in the recruitment of early endosomal marker 1. Although cargo is targeted for degradation, some components are transported to the plasma membrane or the transgolgi network through retrograde transport for recycling. There are inward vesiculation and formation of intraluminal vesicles (ILVs) that are targeted for degradation, both cargo and membrane.

Late phagosomes are more acidic because they acquire increased copies of the proton-pumping V-ATPase with enrichment of ILVs, loss of PtIns3P, and appearance of PtIns-4P. Late phagosomes fuse with lysosomes to form phagolysosomes. Phagolysosomes contain lysosomal-associated membrane proteins-1 and -2 which are essential for the following maturation stages and microbial killing. The pH in phagolysosomes can reach 4.5–5 in some cell types, and acidification itself appears to promote phagosome maturation.

Phagosome acidification impedes microbial growth by affecting their metabolism, and it also facilitates the action of hydrolytic enzymes (Flannagan et al., 2009). Formation of ROS is mediated through the action of the NOX2 NADPH oxidase that transfers electrons from cytosolic NADPH to molecular oxygen releasing O<sub>2</sub><sup>-</sup> into the lumen. O<sub>2</sub><sup>-</sup> can be further processed through the action of dismutase to generate H<sub>2</sub>O<sub>2</sub>, which can react with O<sub>2</sub><sup>-</sup> to generate hydroxyl radicals and singlet oxygen. H<sub>2</sub>O<sub>2</sub> can be converted by myeloperoxidase into hypochlorous acid and chloramines. These ROS can kill microbes. Proteins within the phagosomes can act by preventing the growth of the organisms by limiting the availability of nutrients as in the case of lactoferrin (which sequesters iron) and NRAMP1 which extrudes divalent cations such as Zn<sup>2+</sup>, Fe<sup>2+</sup>, and Mn<sup>2+</sup> from the lumen. Other proteins include defensins, and enzymes capable of degrading carbohydrates (lysozyme, β-hexosaminidase), lipids (lysosomal phospholipase A2), and proteins (cathepsins, carboxypeptidases, and aminopeptidases).

Phagosome resolution is poorly characterized but is required for a return to hemostasis. This process will have major implications for the limitation of autoimmunity upon uptake of cellular material. Phagosome resolution requires disposal of nucleic acids, which is achieved through the action of DNaseII, which is delivered to phagosomes through lysosome fusion. Resulting nucleosides need to be transported to the cytosol. The amino acids resulting from protein degradation can serve as nutrients and are transported to the cytosol through solute carriers. Lipases enable digestion of lipids extracted from membranes by lipid transfer proteins and glycoproteins present in the phagosome membrane prevent digestion of its membrane (Levin et al., 2016).

## CLEARANCE OF APOPTOTIC CELLS BY MACROPHAGES

Apoptosis was discovered in 1965 by Lockshin and Williams who coined the term “programmed cell death” after observing that some cells were destined to die in the silkworm model. Kerr et al. in 1972 found in human tissues a form of cell death characterized by the cellular and nuclear condensation and fragmentation, termed apoptosis (Nagata and Tanaka, 2017). Single gene variations in genes involved in apoptotic cell clearance result in autoimmunity in mice (Petersen et al., 2017).

The mechanism of apoptosis induction has been well characterized, and two induction pathways have been identified (Nagata and Tanaka, 2017). The extrinsic pathway is triggered by the engagement of death receptors such as FAS (CD95). Binding of Fas cell surface death receptor (FAS) Ligand to FAS induces a conformational change in the FAS trimer that leads to the formation of a complex (death-inducing signaling complex) that includes the FAS-associated death domain protein and procaspase 8. Procaspsase 8 is processed into mature caspase 8 which in turn processes caspase 3. The intrinsic pathway is initiated when a developmental signal or genetic damage activates a member of the B cell lymphoma-2, BCL-2, family. In response to activation of BCL-2 proteins, mitochondria release components including cytochrome *c*. Cytochrome *c* forms a complex with procaspsase 9 and apoptotic protease-activating factor-1, leading to the generation of mature caspase 9 and processing of caspase 3. Caspase 3 can cleave more than 500 substrates to complete the apoptosis process. Defects in the apoptotic process have been intimately linked to autoimmunity as in the case of loss of function of the FAS–FAS Ligand pathway (Nagata and Tanaka, 2017).

Efferocytosis refers to the clearance of apoptotic cells; macrophages are the main phagocytic cells responsible for their uptake. Apoptotic cell uptake by macrophages should be considered antiinflammatory rather than “immunologically silent” (Blander, 2017; Nagata and Tanaka, 2017). Efficient clearance of apoptotic cells is required to avoid the release of intracellular cellular components through secondary necrosis. Exposed phosphatidyl serine (PtdSer) is an early sign of apoptosis. In healthy cells, PtdSer is maintained at the inner

side of the plasma membrane through an active process mediated by phospholipid-transporting ATPases (ATP11A and ATP11C). During early apoptosis, the asymmetry of the plasma membrane is lost by lack of function of the phospholipid-transporting ATPases, aided by action of a phospholipid scramblase (XK-related protein 8) that is activated by caspase 3. Apoptotic cells release “find me signals” that include nucleotides (ATP and UTP) as well as the chemokine fractalkine (CX3CL1) and the lipids lysophosphatidylcholine and sphingosine-1 phosphate. These signals could also improve the process of apoptotic cell recognition capacity of neighboring cells by increasing expression of receptors for apoptotic cells.

The recognition of apoptosis by macrophages is centered on, but not limited to, the recognition of PtdSer. Other motifs present on apoptotic cells include a modified form of ICAM-3, oxidized LDL, calreticulin (recognized by CD91), annexin1, cell surface-bound thrombospondin (recognized by CD36), and complement C1q (recognized by scavenger receptors SCARF1 and LAIR1, CD305). There are also changes in surface protein charge and glycosylation (Arandjelovic and Ravichandran, 2015; Blander, 2017; Nagata and Tanaka, 2017).

Receptors involved in direct PtdSer recognition include the following molecules: brain angiogenesis inhibitor 1, a G-protein coupled adhesion receptor that has been also implicated in inhibition of angiogenesis, and myoblast fusion among others; TIM-1, 3, and 4 (T cell-immunoglobulin domain containing); Stabilin-2 and RAGE as well as scavenger receptors CD36 and Class A scavenger receptor (Arandjelovic and Ravichandran, 2015). Indirect PtdSer receptors include the TAM receptors MerTK, Tyro3, and Axl and the integrins  $\alpha v\beta 3$  or  $\alpha v\beta 5$  which recognize MFG-E8, Gas-6, or Protein S that constitute the PtdSer receptors. TAM receptors are tyrosine kinases that activate Src and PI3K and phospholipase PLC. Tim-4 does not signal directly but acts as a tethering receptor. Mutations in TAM receptors and integrin  $\alpha v$ , Sacrf1, and C1q lead to the development of spontaneous autoimmunity (Arandjelovic and Ravichandran, 2015).

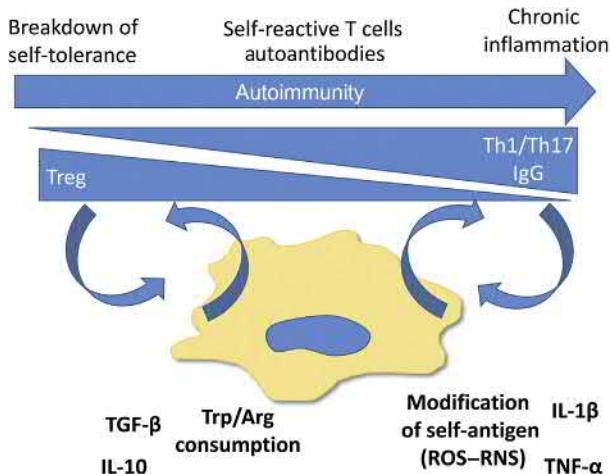
The cargo resulting from efferocytosis needs to be processed. This poses a metabolic burden on the macrophage and regulation is essential to avoid activation of PRRs by DAMPs. For instance, lack of DNaseII expression by macrophages leads to autoimmunity through activation of TLR-independent cytosolic nucleic acid-sensing PRRs (Baum et al., 2015; Gao et al., 2015). Works by Cummings et al. and Uderhardt et al. have highlighted the important roles of specific populations of myeloid cells in the maintenance of self-tolerance upon uptake of apoptotic cells. Cummings et al. (2016) identified a dendritic cell subset and two macrophage populations that mediate uptake of apoptotic intestinal epithelial cells and adopt an immunosuppressive gene expression signature. Uderhardt et al. established the important role of 12/15-lipoxygenase expressed by resident macrophages in the generation of oxidation products of phosphatidylethanolamine that interfere with the uptake of apoptotic cells by inflammatory LyC6<sup>high</sup> monocytes. Oxidized phosphatidylethanolamine at the membrane of resident macrophages sequesters the soluble PtdSer receptor MFG-E8 which mediates uptake of apoptotic cells by inflammatory monocytes. Loss of 12/15-lipoxygenase leads to uptake of apoptotic cells by inflammatory monocytes and lupus-like autoimmune disease (Uderhardt et al., 2012).

## CONCLUDING REMARKS; MACROPHAGES AND AUTOIMMUNITY

Macrophages as the “gatekeepers” of inflammation are, consequently, major players in the initiation and maintenance of chronic inflammatory conditions and, in turn, the induction and perpetuation of autoimmune diseases. Macrophage contribution to autoimmunity stems from its ability to sense their environment through detection of PAMPs and DAMPs by PRRs, degradative capacity, illustrated in this chapter by the uptake and processing of apoptotic cargo under homeostatic and inflammatory conditions, production of immunomodulators, and potential to cause damage.

Evidence for a primary pathogenic role in autoimmunity will depend on implicating molecules that are myeloid, if not macrophage restricted, or disease models in which myeloid-selective gene knockout, for example, of FAS, excludes primary lymphoid cell functions. The efficacy of anti-TNF and IL-1 $\beta$  treatment indicates that primarily macrophage-derived inflammatory products are major factors in tissue damage, even if part of a more complex immune network. Host proteins modified by myeloid-induced oxidative injury or amino acid modification, for example, by citrullination (Kinloch et al., 2008), can give rise to autoantigenicity (Nguyen and James, 2016).

In order to progress, technical challenges have to be overcome; in humans, improved cell phenotyping and population analysis in tissues, for example, by laser capture microscopy and immunocytochemistry, single cell analysis, before, early, as well as late in the development of autoimmune diseases. Analysis of macrophage-expressed gene polymorphisms in disease-prone individuals should emphasize negative as well as positive controls of macrophage growth, recruitment, activation, and turnover. The identification of biomarkers on readily



**FIGURE 10.3** Macrophages and autoimmunity. Macrophages play central roles in homeostasis and inflammation through cross talk with cellular and soluble components of the immune system. In the context of autoimmune diseases, macrophages will respond to antiinflammatory cytokines produced by Tregs and restrict T cell proliferation through consumption of arginine and tryptophan and promote resolution. On the other hand, immune complexes and cytokines produced by Th17 and Th1 cells which are dominant in autoimmune diseases will promote macrophage activation. Activated macrophages can further promote chronicity by causing tissue damage and producing proinflammatory cytokines.

accessible blood monocytes could improve diagnosis and be used to follow the natural history of systemic autoimmune diseases; a recent example has indicated a correlation between monocyte subsets and Th17 responses (Rossol et al., 2012). In mouse models, better methods are required for selective and efficient macrophage depletion, silencing, and conditional genetic manipulation, distinct from dendritic and other myeloid cells.

The balance of activation and inhibition of tissue macrophage phenotypes determines the outcome of many autoimmune diseases and provides targets for treatment (Fig. 10.3). While the role of macrophage activities and products in diseases such as rheumatoid arthritis and type 1 diabetes is established (Navegantes et al., 2017), their possible contribution to systemic autoimmune diseases such as scleroderma, for example, is obscure. The relevance of mouse models to human disease is often problematic. Above all, the predilection of many autoimmune diseases to affect selected tissues such as eyes, joints, and endocrine organs remains obscure. The presence of resident macrophages and possibly organ-specific stromal cells (McGettrick et al., 2012) at these sites and their response to many types of local injury make them attractive targets for further investigation.

## References

- Afonina, I.S., Zhong, Z., Karin, M., Beyaert, R., 2017. Limiting inflammation—the negative regulation of NF- $\kappa$ B and the NLRP3 inflammasome. *Nat. Immunol.* 18, 861–869.
- Akdis, M., Palomares, O., van de Veen, W., van Splunter, M., Akdis, C.A., 2012. TH17 and TH22 cells: a confusion of antimicrobial response with tissue inflammation versus protection. *J. Allergy Clin. Immunol.* 129, 1438–1449. quiz1450–1431.
- Amano, S.U., Cohen, J.L., Vangala, P., Tencerova, M., Nicoloro, S.M., Yawer, J.C., et al., 2014. Local proliferation of macrophages contributes to obesity-associated adipose tissue inflammation. *Cell Metab.* 19, 162–171.
- Annunziato, F., Romagnani, C., Romagnani, S., 2015. The 3 major types of innate and adaptive cell-mediated effector immunity. *J. Allergy Clin. Immunol.* 135, 626–635.
- Arandjelovic, S., Ravichandran, K.S., 2015. Phagocytosis of apoptotic cells in homeostasis. *Nat. Immunol.* 16, 907–917.
- Audiger, C., Rahman, M.J., Yun, T.J., Tarbell, K.V., Lesage, S., 2017. The importance of dendritic cells in maintaining immune tolerance. *J. Immunol.* 198, 2223–2231.
- Barin, J.G., Baldeviano, G.C., Talor, M.V., Wu, L., Ong, S., Quader, F., et al., 2012. Macrophages participate in IL-17-mediated inflammation. *Eur. J. Immunol.* 42, 726–736.
- Baroja-Mazo, A., Martin-Sanchez, F., Gomez, A.I., Martinez, C.M., Amores-Iniesta, J., Compan, V., et al., 2014. The NLRP3 inflammasome is released as a particulate danger signal that amplifies the inflammatory response. *Nat. Immunol.* 15, 738–748.
- Barrat, F.J., Meeker, T., Gregorio, J., Chan, J.H., Uematsu, S., Akira, S., et al., 2005. Nucleic acids of mammalian origin can act as endogenous ligands for Toll-like receptors and may promote systemic lupus erythematosus. *J. Exp. Med.* 202, 1131–1139.
- Barton, G.M., Kagan, J.C., 2009. A cell biological view of Toll-like receptor function: regulation through compartmentalization. *Nat. Rev. Immunol.* 9, 535–542.
- Basil, M.C., Levy, B.D., 2016. Specialized pro-resolving mediators: endogenous regulators of infection and inflammation. *Nat. Rev. Immunol.* 16, 51–67.
- Baum, R., Sharma, S., Carpenter, S., Li, Q.Z., Bustos, P., Fitzgerald, K.A., et al., 2015. Cutting edge: AIM2 and endosomal TLRs differentially regulate arthritis and autoantibody production in DNase II-deficient mice. *J. Immunol.* 194, 873–877.
- Bedoya, S.K., Lam, B., Lau, K., Larkin 3rd, J., 2013. Th17 cells in immunity and autoimmunity. *Clin. Dev. Immunol.* 2013, 986789.
- Bergsbaken, T., Fink, S.L., Cookson, B.T., 2009. Pyroptosis: host cell death and inflammation. *Nat. Rev. Microbiol.* 7, 99–109.
- Berwin, B., Delneste, Y., Lovingood, R.V., Post, S.R., Pizzo, S.V., 2004. SREC-I, a type F scavenger receptor, is an endocytic receptor for calreticulin. *J. Biol. Chem.* 279, 51250–51257.
- Bettelli, E., Oukka, M., Kuchroo, V.K., 2007. T(H)-17 cells in the circle of immunity and autoimmunity. *Nat. Immunol.* 8, 345–350.

- Biburger, M., Aschermann, S., Schwab, I., Lux, A., Albert, H., Danzer, H., et al., 2011. Monocyte subsets responsible for immunoglobulin G-dependent effector functions in vivo. *Immunity* 35, 932–944.
- Biermann, M.H., Griffante, G., Podolska, M.J., Boeltz, S., Sturmer, J., Munoz, L.E., et al., 2016. Sweet but dangerous – the role of immunoglobulin G glycosylation in autoimmunity and inflammation. *Lupus* 25, 934–942.
- Biswas, S.K., Allavena, P., Mantovani, A., 2013. Tumor-associated macrophages: functional diversity, clinical significance, and open questions. *Semin. Immunopathol.* 35, 585–600.
- Bitterman, P.B., Saltzman, L.E., Adelberg, S., Ferrans, V.J., Crystal, R.G., 1984. Alveolar macrophage replication. One mechanism for the expansion of the mononuclear phagocyte population in the chronically inflamed lung. *J. Clin. Invest.* 74, 460–469.
- Blander, J.M., 2014. A long-awaited merger of the pathways mediating host defence and programmed cell death. *Nat. Rev. Immunol.* 14, 601–618.
- Blander, J.M., 2017. The many ways tissue phagocytes respond to dying cells. *Immunol. Rev.* 277, 158–173.
- Blander, J.M., Sander, L.E., 2012. Beyond pattern recognition: five immune checkpoints for scaling the microbial threat. *Nat. Rev. Immunol.* 12, 215–225.
- Boller, T., Felix, G., 2009. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annu. Rev. Plant Biol.* 60, 379–406.
- Boniface, K., Blumenschein, W.M., Brovont-Porth, K., McGeachy, M.J., Basham, B., Desai, B., et al., 2010. Human Th17 cells comprise heterogeneous subsets including IFN-gamma-producing cells with distinct properties from the Th1 lineage. *J. Immunol.* 185, 679–687.
- Boruchov, A.M., Heller, G., Veri, M.C., Bonvini, E., Ravetch, J.V., Young, J.W., 2005. Activating and inhibitory IgG Fc receptors on human DCs mediate opposing functions. *J. Clin. Invest.* 115, 2914–2923.
- Bournazos, S., Wang, T.T., Ravetch, J.V., 2016. The role and function of Fc $\gamma$  receptors on myeloid cells. *Microbiol. Spectr.* 4. Available from: <http://dx.doi.org/10.1128/microbiolspec.MCHD-0045-2016>.
- Brown, G.D., Taylor, P.R., Reid, D.M., Willment, J.A., Williams, D.L., Martinez-Pomares, L., et al., 2002. Dectin-1 is a major beta-glucan receptor on macrophages. *J. Exp. Med.* 196, 407–412.
- Broz, P., von Moltke, J., Jones, J.W., Vance, R.E., Monack, D.M., 2010. Differential requirement for Caspase-1 autoproteolysis in pathogen-induced cell death and cytokine processing. *Cell Host Microbe* 8, 471–483.
- Brunini, F., Page, T.H., Gallieni, M., Pusey, C.D., 2016. The role of monocytes in ANCA-associated vasculitides. *Autoimmun. Rev.* 15, 1046–1053.
- Bryant, C.E., Spring, D.R., Gangloff, M., Gay, N.J., 2010. The molecular basis of the host response to lipopolysaccharide. *Nat. Rev. Microbiol.* 8, 8–14.
- Cairo, G., Recalcati, S., Mantovani, A., Locati, M., 2011. Iron trafficking and metabolism in macrophages: contribution to the polarized phenotype. *Trends Immunol.* 32, 241–247.
- Caruso, R., Warner, N., Inohara, N., Nunez, G., 2014. NOD1 and NOD2: signaling, host defense, and inflammatory disease. *Immunity* 41, 898–908.
- Chazenbalk, G.D., Pichurin, P.N., Guo, J., Rapoport, B., McLachlan, S.M., 2005. Interactions between the mannose receptor and thyroid autoantigens. *Clin. Exp. Immunol.* 139, 216–224.
- Clatworthy, M.R., Smith, K.G., 2004. FcgammaRIIb balances efficient pathogen clearance and the cytokine-mediated consequences of sepsis. *J. Exp. Med.* 199, 717–723.
- Clynes, R., Maizes, J.S., Guinamard, R., Ono, M., Takai, T., Ravetch, J.V., 1999. Modulation of immune complex-induced inflammation in vivo by the coordinate expression of activation and inhibitory Fc receptors. *J. Exp. Med.* 189, 179–185.
- Collins, P.E., Carmody, R.J., 2015. The regulation of endotoxin tolerance and its impact on macrophage activation. *Crit. Rev. Immunol.* 35, 293–323.
- Conde, P., Rodriguez, M., van der Touw, W., Jimenez, A., Burns, M., Miller, J., et al., 2015. DC-SIGN(+) macrophages control the induction of transplantation tolerance. *Immunity* 42, 1143–1158.
- Crowl, J.T., Gray, E.E., Pestal, K., Volkman, H.E., Stetson, D.B., 2017. Intracellular nucleic acid detection in autoimmunity. *Annu. Rev. Immunol.* 35, 313–336.
- Cummings, R.J., Barbet, G., Bongers, G., Hartmann, B.M., Gettler, K., Muniz, L., et al., 2016. Different tissue phagocytes sample apoptotic cells to direct distinct homeostasis programs. *Nature* 539, 565–569.
- Daley, D., Mani, V.R., Mohan, N., Akkad, N., Ochi, A., Heindel, D.W., et al., 2017. Dectin 1 activation on macrophages by galectin 9 promotes pancreatic carcinoma and peritumoral immune tolerance. *Nat. Med.* 23, 556–567.
- Dambuza, I.M., Brown, G.D., 2015. C-type lectins in immunity: recent developments. *Curr. Opin. Immunol.* 32, 21–27.
- Davies, L.C., Jenkins, S.J., Allen, J.E., Taylor, P.R., 2013. Tissue-resident macrophages. *Nat. Immunol.* 14, 986–995.
- de Rivero Vaccari, J.C., Brand 3rd, F.J., Berti, A.F., Alonso, O.F., Bullock, M.R., de Rivero Vaccari, J.P., 2015. Mincle signaling in the innate immune response after traumatic brain injury. *J. Neurotrauma* 32, 228–236.
- Diller, M.L., Kudchadkar, R.R., Delman, K.A., Lawson, D.H., Ford, M.L., 2016. Balancing inflammation: the link between Th17 and regulatory T cells. *Mediators Inflamm.* 2016, 6309219.
- Dinarello, C.A., 2011. Blocking interleukin-1beta in acute and chronic autoinflammatory diseases. *J. Intern. Med.* 269, 16–28.
- Dominguez-Andres, J., Feo-Lucas, L., Minguito de la Escalera, M., Gonzalez, L., Lopez-Bravo, M., Ardavin, C., 2017. Inflammatory Ly6Chigh monocytes protect against candidiasis through IL-15-driven NK cell/neutrophil activation. *Immunity* 46, 1059–1072. e1054.
- Dong, X., Storkus, W.J., Salter, R.D., 1999. Binding and uptake of agalactosyl IgG by mannose receptor on macrophages and dendritic cells. *J. Immunol.* 163, 5427–5434.
- Drickamer, K., Taylor, M.E., 2015. Recent insights into structures and functions of C-type lectins in the immune system. *Curr. Opin. Struct. Biol.* 34, 26–34.
- Duhen, R., Glatigny, S., Arbelaez, C.A., Blair, T.C., Oukka, M., Bettelli, E., 2013. Cutting edge: the pathogenicity of IFN-gamma-producing Th17 cells is independent of T-bet. *J. Immunol.* 190, 4478–4482.

- Edwards, J.P., Zhang, X., Frauwirth, K.A., Mosser, D.M., 2006. Biochemical and functional characterization of three activated macrophage populations. *J. Leukoc. Biol.* 80, 1298–1307.
- Epelman, S., Lavine, K.J., Randolph, G.J., 2014. Origin and functions of tissue macrophages. *Immunity* 41, 21–35.
- Erbel, C., Akhavanpoor, M., Okuyucu, D., Wangler, S., Dietz, A., Zhao, L., et al., 2014. IL-17A influences essential functions of the monocyte/macrophage lineage and is involved in advanced murine and human atherosclerosis. *J. Immunol.* 193, 4344–4355.
- Fangchao, Y., Wenfeng, Z., Di, M., Jianping, G., 2017. Kupffer cell in the immune activation and tolerance toward HBV/HCV infection. *Adv. Clin. Exp. Med.* 26, 739–745.
- Finberg, R.W., Kurt-Jones, E.A., 2006. CD14: chaperone or matchmaker? *Immunity* 24, 127–129.
- Fitzgerald, K.A., Rowe, D.C., Golenbock, D.T., 2004. Endotoxin recognition and signal transduction by the TLR4/MD2-complex. *Microbes Infect.* 6, 1361–1367.
- Flannagan, R.S., Cosio, G., Grinstein, S., 2009. Antimicrobial mechanisms of phagocytes and bacterial evasion strategies. *Nat. Rev. Microbiol.* 7, 355–366.
- Franklin, B.S., Bossaller, L., De Nardo, D., Ratter, J.M., Stutz, A., Engels, G., et al., 2014. The adaptor ASC has extracellular and ‘prionoid’ activities that propagate inflammation. *Nat. Immunol.* 15, 727–737.
- Freire-de-Lima, C.G., Xiao, Y.Q., Gardai, S.J., Bratton, D.L., Schiemann, W.P., Henson, P.M., 2006. Apoptotic cells, through transforming growth factor-beta, coordinately induce anti-inflammatory and suppress pro-inflammatory eicosanoid and NO synthesis in murine macrophages. *J. Biol. Chem.* 281, 38376–38384.
- Funk, C.D., 2001. Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science* 294, 1871–1875.
- Gagliani, N., Amezcuia Vesely, M.C., Iseppon, A., Brockmann, L., Xu, H., Palm, N.W., et al., 2015. Th17 cells transdifferentiate into regulatory T cells during resolution of inflammation. *Nature* 523, 221–225.
- Gao, D., Li, T., Li, X.D., Chen, X., Li, Q.Z., Wight-Carter, M., et al., 2015. Activation of cyclic GMP-AMP synthase by self-DNA causes autoimmune diseases. *Proc. Natl. Acad. Sci. U.S.A.* 112, E5699–E5705.
- Garcia-Vallejo, J.J., van Kooyk, Y., 2013. The physiological role of DC-SIGN: a tale of mice and men. *Trends Immunol.* 34, 482–486.
- Gause, W.C., Wynn, T.A., Allen, J.E., 2013. Type 2 immunity and wound healing: evolutionary refinement of adaptive immunity by helminths. *Nat. Rev. Immunol.* 13, 607–614.
- Gay, N.J., Symmons, M.F., Gangloff, M., Bryant, C.E., 2014. Assembly and localization of Toll-like receptor signalling complexes. *Nat. Rev. Immunol.* 14, 546–558.
- Ginhoux, F., Guilliams, M., 2016. Tissue-resident macrophage ontogeny and homeostasis. *Immunity* 44, 439–449.
- Ginhoux, F., Jung, S., 2014. Monocytes and macrophages: developmental pathways and tissue homeostasis. *Nat. Rev. Immunol.* 14, 392–404.
- Gordon, S., 2003. Alternative activation of macrophages. *Nat. Rev. Immunol.* 3, 23–35.
- Gordon, S., 2016. Phagocytosis: the legacy of Metchnikoff. *Cell* 166, 1065–1068.
- Gordon, S., Martinez-Pomares, L., 2017. Physiological roles of macrophages. *Pflugers Arch.* 469, 365–374.
- Gordon, S., Martinez, F.O., 2010. Alternative activation of macrophages: mechanism and functions. *Immunity* 32, 593–604.
- Gough, S.C., Simmonds, M.J., 2007. The HLA region and autoimmune disease: associations and mechanisms of action. *Curr. Genomics* 8, 453–465.
- Graham, L.M., Brown, G.D., 2009. The Dectin-2 family of C-type lectins in immunity and homeostasis. *Cytokine* 48, 148–155.
- Green, R.S., Stone, E.L., Tenno, M., Lehtonen, E., Farquhar, M.G., Marth, J.D., 2007. Mammalian N-glycan branching protects against innate immune self-recognition and inflammation in autoimmune disease pathogenesis. *Immunity* 27, 308–320.
- Gringhuis, S.I., den Dunnen, J., Litjens, M., van der Vlist, M., Geijtenbeek, T.B., 2009. Carbohydrate-specific signaling through the DC-SIGN signalosome tailors immunity to *Mycobacterium tuberculosis*, HIV-1 and *Helicobacter pylori*. *Nat. Immunol.* 10, 1081–1088.
- Groves, E., Dart, A.E., Covarelli, V., Caron, E., 2008. Molecular mechanisms of phagocytic uptake in mammalian cells. *Cell. Mol. Life Sci.* 65, 1957–1976.
- Guerder, S., Joncker, N., Mahiddine, K., Serre, L., 2013. Dendritic cells in tolerance and autoimmune diabetes. *Curr. Opin. Immunol.* 25, 670–675.
- Guilliams, M., Scott, C.L., 2017. Does niche competition determine the origin of tissue-resident macrophages? *Nat. Rev. Immunol.* 17, 451–460.
- Guma, M., Ronacher, L., Liu-Bryan, R., Takai, S., Karin, M., Corr, M., 2009. Caspase 1-independent activation of interleukin-1beta in neutrophil-predominant inflammation. *Arthritis Rheum.* 60, 3642–3650.
- Guo, H., Callaway, J.B., Ting, J.P., 2015. Inflammasomes: mechanism of action, role in disease, and therapeutics. *Nat. Med.* 21, 677–687.
- Haldar, M., Murphy, K.M., 2014. Origin, development, and homeostasis of tissue-resident macrophages. *Immunol. Rev.* 262, 25–35.
- Hamerman, J.A., Pottle, J., Ni, M., He, Y., Zhang, Z.Y., Buckner, J.H., 2016. Negative regulation of TLR signaling in myeloid cells—implications for autoimmune diseases. *Immunol. Rev.* 269, 212–227.
- Hirahara, K., Poholek, A., Vahedi, G., Laurence, A., Kanno, Y., Milner, J.D., et al., 2013. Mechanisms underlying helper T-cell plasticity: implications for immune-mediated disease. *J. Allergy Clin. Immunol.* 131, 1276–1287.
- Ip, W.K.E., Hoshi, N., Shouval, D.S., Snapper, S., Medzhitov, R., 2017. Anti-inflammatory effect of IL-10 mediated by metabolic reprogramming of macrophages. *Science* 356, 513–519.
- Ishikawa, E., Ishikawa, T., Morita, Y.S., Toyonaga, K., Yamada, H., Takeuchi, O., et al., 2009. Direct recognition of the mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle. *J. Exp. Med.* 206, 2879–2888.
- Jakubzick, C., Gautier, E.L., Gibbons, S.L., Sojka, D.K., Schlitzer, A., Johnson, T.E., et al., 2013. Minimal differentiation of classical monocytes as they survey steady-state tissues and transport antigen to lymph nodes. *Immunity* 39, 599–610.
- Jenkins, S.J., Ruckerl, D., Cook, P.C., Jones, L.H., Finkelman, F.D., van Rooijen, N., et al., 2011. Local macrophage proliferation, rather than recruitment from the blood, is a signature of TH2 inflammation. *Science* 332, 1284–1288.
- Jha, A.K., Huang, S.C., Sergushichev, A., Lampropoulou, V., Ivanova, Y., Loginicheva, E., et al., 2015. Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. *Immunity* 42, 419–430.
- Jiang, S., Li, X., Hess, N.J., Guan, Y., Tapping, R.I., 2016. TLR10 is a negative regulator of both MyD88-dependent and -independent TLR signaling. *J. Immunol.* 196, 3834–3841.

- Karmakar, M., Sun, Y., Hise, A.G., Rietsch, A., Pearlman, E., 2012. Cutting edge: IL-1beta processing during *Pseudomonas aeruginosa* infection is mediated by neutrophil serine proteases and is independent of NLRC4 and caspase-1. *J. Immunol.* 189, 4231–4235.
- Kawai, T., Akira, S., 2010. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat. Immunol.* 11, 373–384.
- Kebir, H., Ifergan, I., Alvarez, J.I., Bernard, M., Poirier, J., Arbour, N., et al., 2009. Preferential recruitment of interferon-gamma-expressing TH17 cells in multiple sclerosis. *Ann. Neurol.* 66, 390–402.
- Keestra, A.M., Winter, M.G., Auburger, J.J., Frassle, S.P., Xavier, M.N., Winter, S.E., et al., 2013. Manipulation of small Rho GTPases is a pathogen-induced process detected by NOD1. *Nature* 496, 233–237.
- Khan, N.S., Kasperkowitz, P.V., Timmons, A.K., Mansour, M.K., Tam, J.M., Seward, M.W., et al., 2016. Dectin-1 controls TLR9 trafficking to phagosomes containing beta-1,3 glucan. *J. Immunol.* 196, 2249–2261.
- Kinloch, A., Lundberg, K., Wait, R., Wegner, N., Lim, N.H., Zendman, A.J., et al., 2008. Synovial fluid is a site of citrullination of autoantigens in inflammatory arthritis. *Arthritis Rheum.* 58, 2287–2295.
- Kolaczkowska, E., Kubis, P., 2013. Neutrophil recruitment and function in health and inflammation. *Nat. Rev. Immunol.* 13, 159–175.
- Kovacs, S.B., Miao, E.A., 2017. Gasdermins: effectors of pyroptosis. *Trends Cell Biol.* 27, 673–684.
- Krieger, M., Herz, J., 1994. Structures and functions of multiligand lipoprotein receptors: macrophage scavenger receptors and LDL receptor-related protein (LRP). *Annu. Rev. Biochem.* 63, 601–637.
- Lande, R., Botti, E., Jandus, C., Dojcinovic, D., Fanelli, G., Conrad, C., et al., 2014. The antimicrobial peptide LL37 is a T-cell autoantigen in psoriasis. *Nat. Commun.* 5, 5621.
- Langston, P.K., Shibata, M., Horng, T., 2017. Metabolism supports macrophage activation. *Front. Immunol.* 8, 61.
- Lavin, Y., Winter, D., Blecher-Gonen, R., David, E., Keren-Shaul, H., Merad, M., et al., 2014. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell* 159, 1312–1326.
- Lefkowitz, D.L., Gelderman, M.P., Fuhrmann, S.R., Graham, S., Starnes III, J.D., Lefkowitz, A., et al., 1999. Neutrophilic myeloperoxidase-macrophage interactions perpetuate chronic inflammation associated with experimental arthritis. *Clin. Immunol.* 91, 145–155.
- Levin, R., Grinstein, S., Canton, J., 2016. The life cycle of phagosomes: formation, maturation, and resolution. *Immunol. Rev.* 273, 156–179.
- Levine, B., Mizushima, N., Virgin, H.W., 2011. Autophagy in immunity and inflammation. *Nature* 469, 323–335.
- Liew, F.Y., Xu, D., Brint, E.K., O'Neill, L.A., 2005. Negative regulation of toll-like receptor-mediated immune responses. *Nat. Rev. Immunol.* 5, 446–458.
- Liston, A., Masters, S.L., 2017. Homeostasis-altering molecular processes as mechanisms of inflammasome activation. *Nat. Rev. Immunol.* 17, 208–214.
- Liu, P.S., Wang, H., Li, X., Chao, T., Teav, T., Christen, S., et al., 2017. Alpha-ketoglutarate orchestrates macrophage activation through metabolic and epigenetic reprogramming. *Nat. Immunol.* 18, 985–994.
- Lu, C., He, J.C., Cai, W., Liu, H., Zhu, L., Vlassara, H., 2004. Advanced glycation endproduct (AGE) receptor 1 is a negative regulator of the inflammatory response to AGE in mesangial cells. *Proc. Natl. Acad. Sci. U.S.A.* 101, 11767–11772.
- Macauley, M.S., Crocker, P.R., Paulson, J.C., 2014. Siglec-mediated regulation of immune cell function in disease. *Nat. Rev. Immunol.* 14, 653–666.
- Magna, M., Pisetsky, D.S., 2015. The role of cell death in the pathogenesis of SLE: is pyroptosis the missing link? *Scand. J. Immunol.* 82, 218–224.
- Man, S.M., Kanneganti, T.D., 2016. Converging roles of caspases in inflammasome activation, cell death and innate immunity. *Nat. Rev. Immunol.* 16, 7–21.
- Manthiram, K., Zhou, Q., Aksentijevich, I., Kastner, D.L., 2017. The monogenic autoinflammatory diseases define new pathways in human innate immunity and inflammation. *Nat. Immunol.* 18, 832–842.
- Mantovani, A., Sica, A., Sozzani, S., Allavena, P., Vecchi, A., Locati, M., 2004. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol.* 25, 677–686.
- Martinez-Pomares, L., 2012. The mannose receptor. *J. Leukoc. Biol.* 92, 1177–1186.
- Matmati, M., Jacques, P., Maelfait, J., Verheugen, E., Kool, M., Sze, M., et al., 2011. A20 (TNFAIP3) deficiency in myeloid cells triggers erosive polyarthritides resembling rheumatoid arthritis. *Nat. Genet.* 43, 908–912.
- McGeachy, M.J., Bak-Jensen, K.S., Chen, Y., Tato, C.M., Blumenschein, W., McClanahan, T., et al., 2007. TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. *Nat. Immunol.* 8, 1390–1397.
- McGettrick, H.M., Butler, L.M., Buckley, C.D., Rainger, G.E., Nash, G.B., 2012. Tissue stroma as a regulator of leukocyte recruitment in inflammation. *J. Leukoc. Biol.* 91, 385–400.
- Minas, K., Liversidge, J., 2006. Is the CD200/CD200 receptor interaction more than just a myeloid cell inhibitory signal? *Crit. Rev. Immunol.* 26, 213–230.
- Minutti, C.M., Jackson-Jones, L.H., Garcia-Fojeda, B., Knipper, J.A., Sutherland, T.E., Logan, N., et al., 2017. Local amplifiers of IL-4Ralpha-mediated macrophage activation promote repair in lung and liver. *Science* 356, 1076–1080.
- Mocsai, A., Ruland, J., Tybulewicz, V.L., 2010. The SYK tyrosine kinase: a crucial player in diverse biological functions. *Nat. Rev. Immunol.* 10, 387–402.
- Montaner, L.J., da Silva, R.P., Sun, J., Sutterwala, S., Hollinshead, M., Vaux, D., et al., 1999. Type 1 and type 2 cytokine regulation of macrophage endocytosis: differential activation by IL-4/IL-13 as opposed to IFN-gamma or IL-10. *J. Immunol.* 162, 4606–4613.
- Mosser, D.M., Edwards, J.P., 2008. Exploring the full spectrum of macrophage activation. *Nat. Rev. Immunol.* 8, 958–969.
- Munn, D.H., Zhou, M., Attwood, J.T., Bondarev, I., Conway, S.J., Marshall, B., et al., 1998. Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science* 281, 1191–1193.
- Munn, D.H., Shafizadeh, E., Attwood, J.T., Bondarev, I., Pashine, A., Mellor, A.L., 1999. Inhibition of T cell proliferation by macrophage tryptophan catabolism. *J. Exp. Med.* 189, 1363–1372.
- Murray, P.J., 2017. Macrophage Polarization. *Annu. Rev. Physiol.* 79, 541–566.

- Murray, P.J., Allen, J.E., Biswas, S.K., Fisher, E.A., Gilroy, D.W., Goerdt, S., et al., 2014. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity* 41, 14–20.
- Nagai, R., Matsumoto, K., Ling, X., Suzuki, H., Araki, T., Horiuchi, S., 2000. Glycolaldehyde, a reactive intermediate for advanced glycation end products, plays an important role in the generation of an active ligand for the macrophage scavenger receptor. *Diabetes* 49, 1714–1723.
- Nagata, S., Tanaka, M., 2017. Programmed cell death and the immune system. *Nat. Rev. Immunol.* 17, 333–340.
- Nakayamada, S., Takahashi, H., Kanno, Y., O’Shea, J.J., 2012. Helper T cell diversity and plasticity. *Curr. Opin. Immunol.* 24, 297–302.
- Nathan, C., Ding, A., 2010. Nonresolving inflammation. *Cell* 140, 871–882.
- Navegantes, K.C., de Souza Gomes, R., Pereira, P.A.T., Czaikoski, P.G., Azevedo, C.H.M., Monteiro, M.C., 2017. Immune modulation of some autoimmune diseases: the critical role of macrophages and neutrophils in the innate and adaptive immunity. *J. Transl. Med.* 15, 36.
- Netea, M.G., Quintin, J., van der Meer, J.W., 2011. Trained immunity: a memory for innate host defense. *Cell Host Microbe* 9, 355–361.
- Netea, M.G., Joosten, L.A., Latz, E., Mills, K.H., Natoli, G., Stunnenberg, H.G., et al., 2016. Trained immunity: a program of innate immune memory in health and disease. *Science* 352, aaf1098.
- Neumann, K., Castineiras-Vilarino, M., Hockendorf, U., Hanneschlager, N., Lemeer, S., Kupka, D., et al., 2014. Clec12a is an inhibitory receptor for uric acid crystals that regulates inflammation in response to cell death. *Immunity* 40, 389–399.
- Nguyen, H., James, E.A., 2016. Immune recognition of citrullinated epitopes. *Immunology* 149, 131–138.
- Nimmerjahn, F., Ravetch, J.V., 2008. Fc $\gamma$  receptors as regulators of immune responses. *Nat. Rev. Immunol.* 8, 34–47.
- Noelia, A.G., Quintana, J.A., Garcia-Silva, S., Mazariegos, M., Gonzalez de la Aleja, A., Nicolas-Avila, J.A., et al., 2017. Phagocytosis imprints heterogeneity in tissue-resident macrophages. *J. Exp. Med.* 214, 1281–1296.
- Novick, D., Cohen, B., Rubinstein, M., 1994. The human interferon alpha/beta receptor: characterization and molecular cloning. *Cell* 77, 391–400.
- Okabe, Y., Medzhitov, R., 2016. Tissue biology perspective on macrophages. *Nat. Immunol.* 17, 9–17.
- O’Neill, L.A., Bowie, A.G., 2007. The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. *Nat. Rev. Immunol.* 7, 353–364.
- O’Neill, L.A., Pearce, E.J., 2016. Immunometabolism governs dendritic cell and macrophage function. *J. Exp. Med.* 213, 15–23.
- O’Neill, L.A., Golenbock, D., Bowie, A.G., 2013. The history of Toll-like receptors – redefining innate immunity. *Nat. Rev. Immunol.* 13, 453–460.
- Papayannopoulos, V., Zychlinsky, A., 2009. NETs: a new strategy for using old weapons. *Trends Immunol.* 30, 513–521.
- Perdicchio, M., Ilarregui, J.M., Verstege, M.I., Cornelissen, L.A., Schetters, S.T., Engels, S., et al., 2016. Sialic acid-modified antigens impose tolerance via inhibition of T-cell proliferation and de novo induction of regulatory T cells. *Proc. Natl. Acad. Sci. U.S.A.* 113, 3329–3334.
- Pesce, J.T., Ramalingam, T.R., Mentink-Kane, M.M., Wilson, M.S., El Kasmi, K.C., Smith, A.M., et al., 2009. Arginase-1-expressing macrophages suppress Th2 cytokine-driven inflammation and fibrosis. *PLoS Pathog.* 5, e1000371.
- Petersen, F., Yue, X., Riemekasten, G., Yu, X., 2017. Dysregulated homeostasis of target tissues or autoantigens – a novel principle in autoimmunity. *Autoimmun. Rev.* 16, 602–611.
- Piccinini, A.M., Midwood, K.S., 2010. DAMPening inflammation by modulating TLR signalling. *Mediators Inflamm.* 2010, pii: 672395.
- Piccinini, A.M., Zuliani-Alvarez, L., Lim, J.M., Midwood, K.S., 2016. Distinct microenvironmental cues stimulate divergent TLR4-mediated signaling pathways in macrophages. *Sci. Signal.* 9, ra86.
- Plato, A., Hardison, S.E., Brown, G.D., 2015. Pattern recognition receptors in antifungal immunity. *Semin. Immunopathol.* 37, 97–106.
- Pluddemann, A., Neyen, C., Gordon, S., 2007. Macrophage scavenger receptors and host-derived ligands. *Methods* 43, 207–217.
- Pontillo, A., Catamo, E., Arosio, B., Mari, D., Crovella, S., 2012. NALP1/NLRP1 genetic variants are associated with Alzheimer disease. *Alzheimer Dis. Assoc. Disord.* 26, 277–281.
- Rabinovich, G.A., van Kooyk, Y., Cobb, B.A., 2012. Glycobiology of immune responses. *Ann. N. Y. Acad. Sci.* 1253, 1–15.
- Rajaiah, R., Perkins, D.J., Ireland, D.D., Vogel, S.N., 2015. CD14 dependence of TLR4 endocytosis and TRIF signaling displays ligand specificity and is dissociable in endotoxin tolerance. *Proc. Natl. Acad. Sci. U.S.A.* 112, 8391–8396.
- Rathinam, V.A., Vanaja, S.K., Fitzgerald, K.A., 2012. Regulation of inflammasome signaling. *Nat. Immunol.* 13, 333–342.
- Ravishankar, B., Liu, H., Shinde, R., Chandler, P., Baban, B., Tanaka, M., et al., 2012. Tolerance to apoptotic cells is regulated by indoleamine 2,3-dioxygenase. *Proc. Natl. Acad. Sci. U.S.A.* 109, 3909–3914.
- Ravishankar, B., Shinde, R., Liu, H., Chaudhary, K., Bradley, J., Lemos, H.P., et al., 2014. Marginal zone CD169+ macrophages coordinate apoptotic cell-driven cellular recruitment and tolerance. *Proc. Natl. Acad. Sci. U.S.A.* 111, 4215–4220.
- Redelinghuys, P., Whitehead, L., Augello, A., Drummond, R.A., Levesque, J.M., Vautier, S., et al., 2016. MICL controls inflammation in rheumatoid arthritis. *Ann. Rheum. Dis.* 75, 1386–1391.
- Rossol, M., Kraus, S., Pierer, M., Baerwald, C., Wagner, U., 2012. The CD14(bright) CD16+ monocyte subset is expanded in rheumatoid arthritis and promotes expansion of the Th17 cell population. *Arthritis Rheum.* 64, 671–677.
- Roszer, T., 2015. Understanding the mysterious M2 macrophage through activation markers and effector mechanisms. *Mediators Inflamm.* 2015, 816460.
- Santiago-Garcia, J., Mas-Oliva, J., Innerarity, T.L., Pitas, R.E., 2001. Secreted forms of the amyloid-beta precursor protein are ligands for the class A scavenger receptor. *J. Biol. Chem.* 276, 30655–30661.
- Schlee, M., Hartmann, G., 2016. Discriminating self from non-self in nucleic acid sensing. *Nat. Rev. Immunol.* 16, 566–580.
- Schnaar, R.L., 2016. Glycobiology simplified: diverse roles of glycan recognition in inflammation. *J. Leukoc. Biol.* 99, 825–838.
- Schreiber, A., Pham, C.T., Hu, Y., Schneider, W., Luft, F.C., Kettritz, R., 2012. Neutrophil serine proteases promote IL-1 $\beta$  generation and injury in necrotizing crescentic glomerulonephritis. *J. Am. Soc. Nephrol.* 23, 470–482.
- Schroder, K., Tschopp, J., 2010. The inflammasomes. *Cell* 140, 821–832.
- Schwab, I., Nimmerjahn, F., 2014. Role of sialylation in the anti-inflammatory activity of intravenous immunoglobulin – F(ab') $(2)$  versus Fc sialylation. *Clin. Exp. Immunol.* 178 (Suppl. 1), 97–99.

- Schwab, I., Mihai, S., Seeling, M., Kasperkiewicz, M., Ludwig, R.J., Nimmerjahn, F., 2014. Broad requirement for terminal sialic acid residues and Fc $\gamma$ RIIB for the preventive and therapeutic activity of intravenous immunoglobulins in vivo. *Eur. J. Immunol.* 44, 1444–1453.
- Serhan, C.N., 2014. Pro-resolving lipid mediators are leads for resolution physiology. *Nature* 510, 92–101.
- Serhan, C.N., Savill, J., 2005. Resolution of inflammation: the beginning programs the end. *Nat. Immunol.* 6, 1191–1197.
- Shepherd, V.L., Hoidal, J.R., 1990. Clearance of neutrophil-derived myeloperoxidase by the macrophage mannose receptor. *Am. J. Respir. Cell Mol. Biol.* 2, 335–340.
- Shimaoka, T., Nakayama, T., Fukumoto, N., Kume, N., Takahashi, S., Yamaguchi, J., et al., 2004. Cell surface-anchored SR-PSOX/CXC chemokine ligand 16 mediates firm adhesion of CXC chemokine receptor 6-expressing cells. *J. Leukoc. Biol.* 75, 267–274.
- Sieweke, M.H., Allen, J.E., 2013. Beyond stem cells: self-renewal of differentiated macrophages. *Science* 342, 1242974.
- Soares, M.P., Hamza, I., 2016. Macrophages and iron metabolism. *Immunity* 44, 492–504.
- Soehnlein, O., Lindbom, L., 2010. Phagocyte partnership during the onset and resolution of inflammation. *Nat. Rev. Immunol.* 10, 427–439.
- Soehnlein, O., Zernecke, A., Eriksson, E.E., Rothfuchs, A.G., Pham, C.T., Herwald, H., et al., 2008. Neutrophil secretion products pave the way for inflammatory monocytes. *Blood* 112, 1461–1471.
- Sosale, N.G., Spinler, K.R., Alvey, C., Discher, D.E., 2015. Macrophage engulfment of a cell or nanoparticle is regulated by unavoidable opsonization, a species-specific ‘Marker of Self’ CD47, and target physical properties. *Curr. Opin. Immunol.* 35, 107–112.
- Stein, M., Keshav, S., Harris, N., Gordon, S., 1992. Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. *J. Exp. Med.* 176, 287–292.
- Stuart, L.M., Ezekowitz, R.A., 2005. Phagocytosis: elegant complexity. *Immunity* 22, 539–550.
- Suzuki, Y., Nakano, Y., Mishiro, K., Takagi, T., Tsuruma, K., Nakamura, M., et al., 2013. Involvement of Mincle and Syk in the changes to innate immunity after ischemic stroke. *Sci. Rep.* 3, 3177.
- Swanson, J.A., Hoppe, A.D., 2004. The coordination of signaling during Fc receptor-mediated phagocytosis. *J. Leukoc. Biol.* 76, 1093–1103.
- Takai, T., 2002. Roles of Fc receptors in autoimmunity. *Nat. Rev. Immunol.* 2, 580–592.
- Takeuchi, O., Akira, S., 2008. MDA5/RIG-I and virus recognition. *Curr. Opin. Immunol.* 20, 17–22.
- Takeuchi, O., Akira, S., 2010. Pattern recognition receptors and inflammation. *Cell* 140, 805–820.
- Taylor, P.R., Brown, G.D., Reid, D.M., Willment, J.A., Martinez-Pomares, L., Gordon, S., et al., 2002. The beta-glucan receptor, dectin-1, is predominantly expressed on the surface of cells of the monocyte/macrophage and neutrophil lineages. *J. Immunol.* 169, 3876–3882.
- Taylor, P.R., Martinez-Pomares, L., Stacey, M., Lin, H.H., Brown, G.D., Gordon, S., 2005. Macrophage receptors and immune recognition. *Annu. Rev. Immunol.* 23, 901–944.
- Thiagarajan, P.S., Yakubenko, V.P., Elsori, D.H., Yadav, S.P., Willard, B., Tan, C.D., et al., 2013. Vimentin is an endogenous ligand for the pattern recognition receptor Dectin-1. *Cardiovasc. Res.* 99, 494–504.
- Thieblemont, N., Wright, H.L., Edwards, S.W., Witko-Sarsat, V., 2016. Human neutrophils in auto-immunity. *Semin. Immunol.* 28, 159–173.
- Travassos, L.H., Carneiro, L.A., Ramjeet, M., Hussey, S., Kim, Y.G., Magalhaes, J.G., et al., 2010. Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. *Nat. Immunol.* 11, 55–62.
- Uderhardt, S., Herrmann, M., Oskolkova, O.V., Aschermann, S., Bicker, W., Ipseiz, N., et al., 2012. 12/15-lipoxygenase orchestrates the clearance of apoptotic cells and maintains immunologic tolerance. *Immunity* 36, 834–846.
- van de Laar, L., Saelens, W., De Prijck, S., Martens, L., Scott, C.L., Van Isterdael, G., et al., 2016. Yolk sac macrophages, fetal liver, and adult monocytes can colonize an empty niche and develop into functional tissue-resident macrophages. *Immunity* 44, 755–768.
- van der Meer, J.W., Joosten, L.A., Riksen, N., Netea, M.G., 2015. Trained immunity: a smart way to enhance innate immune defence. *Mol. Immunol.* 68, 40–44.
- van Furth, R., Cohn, Z.A., Hirsch, J.G., Humphrey, J.H., Spector, W.G., Langevoort, H.T., 1972. The mononuclear phagocyte system: a new classification of macrophages, monocytes, and their precursor cells. *Bull. World Health Organ.* 46, 845–852.
- Vanaja, S.K., Rathinam, V.A., Fitzgerald, K.A., 2015. Mechanisms of inflammasome activation: recent advances and novel insights. *Trends Cell Biol.* 25, 308–315.
- Vance, R.E., Isberg, R.R., Portnoy, D.A., 2009. Patterns of pathogenesis: discrimination of pathogenic and nonpathogenic microbes by the innate immune system. *Cell Host Microbe* 6, 10–21.
- Vannella, K.M., Wynn, T.A., 2017. Mechanisms of organ injury and repair by macrophages. *Annu. Rev. Physiol.* 79, 593–617.
- Vereecke, L., Beyaert, R., van Loo, G., 2009. The ubiquitin-editing enzyme A20 (TNFAIP3) is a central regulator of immunopathology. *Trends Immunol.* 30, 383–391.
- Vieira, O.V., Botelho, R.J., Grinstein, S., 2002. Phagosome maturation: aging gracefully. *Biochem. J.* 366, 689–704.
- Vigano, E., Diamond, C.E., Spreafico, R., Balachander, A., Sobota, R.M., Mortellaro, A., 2015. Human caspase-4 and caspase-5 regulate the one-step non-canonical inflammasome activation in monocytes. *Nat. Commun.* 6, 8761.
- Vignesh, P., Rawat, A., Sharma, M., Singh, S., 2017. Complement in autoimmune diseases. *Clin. Chim. Acta* 465, 123–130.
- Vlassara, H., Striker, G.E., 2011. AGE restriction in diabetes mellitus: a paradigm shift. *Nat. Rev. Endocrinol.* 7, 526–539.
- von Moltke, J., Ayres, J.S., Kofoed, E.M., Chavarria-Smith, J., Vance, R.E., 2013. Recognition of bacteria by inflammasomes. *Annu. Rev. Immunol.* 31, 73–106.
- Wu, D.J., Adamopoulos, I.E., 2017. Autophagy and autoimmunity. *Clin. Immunol.* 176, 55–62.
- Wynn, T.A., Chawla, A., Pollard, J.W., 2013. Macrophage biology in development, homeostasis and disease. *Nature* 496, 445–455.
- Yamasaki, S., Ishikawa, E., Sakuma, M., Hara, H., Ogata, K., Saito, T., 2008. Mincle is an ITAM-coupled activating receptor that senses damaged cells. *Nat. Immunol.* 9, 1179–1188.
- Yamasaki, S., Matsumoto, M., Takeuchi, O., Matsuzawa, T., Ishikawa, E., Sakuma, M., et al., 2009. C-type lectin Mincle is an activating receptor for pathogenic fungus, *Malassezia*. *Proc. Natl. Acad. Sci. U.S.A.* 106, 1897–1902.

- Yoneyama, M., Onomoto, K., Jogi, M., Akaboshi, T., Fujita, T., 2015. Viral RNA detection by RIG-I-like receptors. *Curr. Opin. Immunol.* 32, 48–53.
- Zanoni, I., Ostuni, R., Marek, L.R., Barresi, S., Barbalat, R., Barton, G.M., et al., 2011. CD14 controls the LPS-induced endocytosis of Toll-like receptor 4. *Cell* 147, 868–880.
- Zizzo, G., Cohen, P.L., 2013. IL-17 stimulates differentiation of human anti-inflammatory macrophages and phagocytosis of apoptotic neutrophils in response to IL-10 and glucocorticoids. *J. Immunol.* 190, 5237–5246.

## 11

# Dendritic Cells in Autoimmune Disease

Kristin V. Tarbell<sup>1,2</sup> and M. Jubayer Rahman<sup>1,3</sup>

<sup>1</sup>Immune Tolerance Section, NIDDK, NIH, Bethesda, MD, United States <sup>2</sup>Amgen Discovery Research, South San Francisco, CA, United States <sup>3</sup>NHLBI, NIH, Bethesda, MD, United States

## O U T L I N E

<p>Introduction</p> <p>Antigen Uptake, Processing, and Presentation by Dendritic Cells</p> <ul style="list-style-type: none"> <li>Migration</li> </ul> <p>Pattern Recognition Receptors and Dendritic Cell Activation</p> <ul style="list-style-type: none"> <li>Alignment of Mouse and Human Dendritic Cell Subsets by Function and Development</li> </ul> <p>Development of Dendritic Cell Subsets</p> <p>Dendritic Cell Subset Phenotypes</p> <ul style="list-style-type: none"> <li>Conventional Dendritic Cell 1</li> <li>Conventional Dendritic Cell 2</li> <li>Plasmacytoid Dendritic Cells</li> <li>Tissue-Specific Dendritic Cells</li> </ul>	<p>213</p> <p>214</p> <p>214</p> <p>214</p> <p>215</p> <p>216</p> <p>216</p> <p>216</p> <p>216</p> <p>216</p> <p>217</p>	<p>Dendritic Cell Subsets and Tolerance</p> <p>Dendritic Cells and Autoimmune Disease</p> <ul style="list-style-type: none"> <li>Systemic Lupus Erythematosus</li> <li>Inflammatory Bowel Disease—Crohn's Disease and Ulcerative Colitis</li> <li>Psoriasis and Psoriatic Arthritis</li> <li>Type 1 Diabetes</li> </ul> <p>Targeting of Dendritic Cells in Autoimmune Disease</p> <p>Conclusion and Future Prospects</p> <p>Acknowledgments</p> <p>References</p>	<p>218</p> <p>219</p> <p>219</p> <p>219</p> <p>220</p> <p>220</p> <p>221</p> <p>222</p> <p>222</p> <p>223</p>
--	--	---	---

## INTRODUCTION

Dendritic cells (DCs) are highly specialized leukocytes with the potent ability to uptake, process, and present antigens (Ags) to the T cells. DCs are found in lymphoid and non lymphoid tissues, and display heterogeneity in both functional subsets and maturation state. In the 1970s Steinman et al. first identified DCs (Steinman and Cohn, 1973) as a separate antigen presenting population with high major histocompatibility complex (MHC) expression and strong induction of mixed lymphocyte reaction (Steinman and Witmer, 1978; Steinman et al., 1979). After 40 years of research on DCs, in 2011, the Nobel Prize in Physiology or Medicine was awarded to Bruce A. Beutler and Jules A. Hoffmann for their discoveries in innate immune recognition and to Ralph M. Steinman for his discovery of the functional role of DCs and their importance in vaccination. As outlined in this chapter, DCs play a role for both T-cell activation and tolerance induction and as such are central modulators of immune responses. Dysregulation of DC function can contribute to autoimmunity, and correcting these defects has the potential to treat autoimmune disease.

## ANTIGEN UPTAKE, PROCESSING, AND PRESENTATION BY DENDRITIC CELLS

DCs take up diverse antigenic material (e.g., soluble and particulate, self, and foreign) by a variety of processes including phagocytosis, pinocytosis, and receptor-mediated endocytosis. In contrast to other phagocytic cells such as macrophages that rapidly and completely degrade phagocytosed material, DCs conserve antigenic material for a prolonged period, allowing continuous processing and presentation of antigen. Certain DCs are equipped with receptors for recognition of apoptotic or necrotic cells (Sancho et al., 2009; Zhang et al., 2012). Some critical DC functions are dependent on the maturation process that is induced by innate immune activation and lead to the expression of proinflammatory cytokines and costimulatory molecules. Mature DCs are specialized for robust T-cell stimulation. Immature or steady-state DCs are especially active in antigen uptake. Maturation shuts off some uptake processes, but mature DCs can still capture soluble antigens (Platt et al., 2010). Receptor-mediated endocytosis and phagocytosis are two key processes for mature DCs to capture antigens. Captured antigens are transported to late endosomes or lysosomes and loaded on MHC class II (MHCII) for presentation. Therefore DCs can continue to acquire new antigens for activation of T cells under inflammatory condition (Drutman and Trombetta, 2010).

There are several antigen processing routes in DC (Trombetta and Mellman, 2005; Wilson and Villadangos, 2005). Exogenous antigens are usually processed in endocytic vesicles, leading to appropriate peptides being loaded onto MHCII molecules. The MHCII associated antigens then presented to CD4 T cells will include peptides from exogenous foreign antigens, along with peptides from self, such as recycling DC surface molecules. When conventional DCs (cDCs) are activated, the MHCII peptide complexes shift to the cell surface and are no longer recycled, so for their limited lifespan, the mature DC presents a “snapshot” of the antigenic environment at the time of activation.

DCs, along with most other cells, produce, as a by-product of protein synthesis, peptides that are loaded onto MHC class I (MHCI) for presentation to CD8 T cells. Such endogenously derived antigens will include viral antigens if the DC is infected with a virus. Some DCs have the additional ability to take up and shuttle exogenous antigens into the MHCI presentation pathway. Only particular DC subtypes are proficient for this specialized function, termed “cross-presentation,” and this capacity is induced as a late step in their development (Heath et al., 2004; Shortman and Heath, 2010). Cross-presentation of exogenous antigens, including material from dead cells, is important for the generation of cytotoxic T-cell responses to intracellular pathogens that do not infect the DCs themselves (Heath and Carbone, 2001). The pathways of cross-presentation are still being determined but involve the movement of antigen from the endocytic vesicles through the cytosol into the endoplasmic reticulum to join the MHCI loading pathway (Mantegazza et al., 2013). Another way DCs may present exogenous antigens on MHCI is via a process termed “cross-dressing” in which either soluble peptides are directly loaded into cell surface MHCI or entire MHCI peptide complexes are transferred from other cells via membrane exchange or exosomes (Maravillas-Montero and Martínez-Cortés, 2017; Robbins and Morelli, 2014).

## Migration

Another attribute that distinguishes DCs from other antigen presenting cells (APC) types is the ability to more efficiently traffic from tissues, where antigen is first encountered and taken up, to the draining lymph node via the lymph. This trafficking is primarily mediated by CCR7 and allows optimal activation of naïve T cells that are mostly present in lymphoid tissues (Randolph et al., 2005). Recent studies have shown that specific DC subsets preferentially migrate to different parts of the T-cell zone to allow optimal contact with the relevant T cells. XC-chemokine receptor 1 (XCR1) + conventional dendritic cell 1 (cDC1) that activate CD8 + T cells preferentially migrate to CD8 T-cell areas, and DCIR2 + conventional dendritic cell 2 (cDC2) that are better at activating CD4 + T cells preferentially migrate to CD4 T-cell areas (Calabro et al., 2016; Gerner et al., 2015). Most other APC mainly presents antigen to T cells in an anatomical site close to where these cells picked up antigen. Because naïve T cells have very minimal trafficking to nonlymphoid organs, the initial priming of T cells (i.e., the first peripheral encounter with antigen) is mediated by DCs. Therefore the types of nonantigenic signals the DCs give during priming can greatly influence the type of T-cell responses that ensues.

## PATTERN RECOGNITION RECEPTORS AND DENDRITIC CELL ACTIVATION

All DCs express receptors that recognize microbial products or damaged cells or other altered aspects of the environment. These receptors, collectively called pattern recognition receptors (PRR), are not all expressed on all

DC subsets and indeed the differential expression of PRR is a major functional discriminator of DC subsets (Hochrein and O'Keeffe, 2008). In addition, DC subset expression patterns of particular PRR are not always the same in different species, which can confound the application of PRR stimulation results from animal models to human disease settings. Pathogen-associated molecular patterns directly interact with PRR on DCs, which is crucial for the induction of primary T-cell responses. PRRs exist on the plasma membrane, in the cytoplasm, and on endosomal membranes of cells. They belong to four major families: Toll-like receptors (TLR), Rig-like helicases (RLH), C-type lectin receptors, and nucleotide-binding domain, leucine-rich repeat containing (NLR) receptors. The engagement of PRR provides signals that are needed to activate DC to full immunogenic function. The signaling pathways downstream of PRR converge on the activation of the transcription factor family NF- $\kappa$ B, interferon (IFN) response factors, and mitogen-activated protein kinases, leading to the production of cytokines and the upregulation of costimulatory molecules and MHC molecules on the DC surface (Diebold, 2009; Kawai and Akira, 2007; Mohammad Hosseini et al., 2015). The PRRs recognize a variety of pathogen-derived or endogenous molecules. For recent reviews see TLR (Kawai and Akira, 2010, 2011; Kawasaki and Kawai, 2014), NLR (Davis et al., 2011; Elinav et al., 2011; Martinon and Tschopp, 2005), RLH (Barbalat et al., 2011; Loo and Gale, 2011), and C-type lectin receptors (Kerrigan et al., 2009; Osorio and Reis e Sousa, 2011; Robinson et al., 2006). Self-derived endogenous ligands can be increased in autoimmune-susceptible individuals and can contribute to disease pathogenesis. TLR signaling is important in DC-mediated activation of adaptive T cells by inducing proinflammatory cytokines and expression of costimulatory molecules. Considering TLRs are important in the context of linking innate and adaptive immune response, aggressive or dysregulation of TLR signaling may lead to autoimmune pathogenesis such as failure of maintaining self-tolerance. For example, PRR activation in autoimmune disease may involve the activation of discrete DC subsets exemplified by the activation of plasmacytoid DC (pDC) in patients with lupus (see next).

## Alignment of Mouse and Human Dendritic Cell Subsets by Function and Development

Recent work shows that the major subsets of DCs defined by both development and function are parallel between mouse and human. This has been appreciated only relatively recently because some of the surface markers that identify these subsets do differ between species, and because of single cell RNAseq that allows for a better understanding of cellular heterogeneity (Villani et al., 2017). This topic has been well reviewed elsewhere (Guilliams and van de Laar, 2015; Guilliams et al., 2014). Briefly, the mononuclear phagocytic system that includes DCs is broadly divided into three components defined by development: tissue-resident macrophages, monocyte-derived cells, and DCs. The bona fide DCs develop from a common precursor and are divided into cDCs and pDCs. cDCs are further divided into cDC1 that are capable of cross-presentation and activate CD8+ T cells and cDC2s that drive optimal CD4+ T-cell activation. Separately, some monocyte-derived cells can upregulate MHCII and share some functions with cDC2s but tend to be less migratory or capable of priming naïve T cells. The cells, sometimes termed moDCs, are upregulated with inflammation. Although these are the main DC subsets, the specific markers and functions of the DCs can vary depending on the tissue location of the DCs and the inflammatory state of the environment. Migratory DC collects antigen in peripheral tissues then migrates to lymph nodes for presentation to T cells. DCs from specific tissues can have specialized phenotypes. For example, in the gut, DCs have increased transforming growth factor- $\beta$  (TGF- $\beta$ ) and retinoic acid signals and are potent inducers of regulatory T cells (Tregs). The lymphoid tissue-resident DC collects antigen within the lymphoid organs, either directly or as antigen acquired from other cell types, including migratory DC.

It is also useful to distinguish the DC found in normal, healthy "steady state" from activated "inflammatory" DCs that are generated, sometimes in substantial numbers, in response to inflammation or infection. Inflammatory DCs can develop from monocytes that upregulate MHCII and are sometimes called moDCs. These moDCs share some markers with cDC2 and are modeled in vitro by culturing monocytes from bone marrow with cytokines including granulocyte-macrophage colony-stimulating factor (GM-CSF), although recent reports suggest that macrophage colony-stimulating factor (M-CSF) may actually be the crucial growth factor required by these DC (Greter et al., 2012). In the past, the term myeloid DC has been used to refer to different populations depending on the author, including cDC2, all cDCs, or monocyte-derived cells. Likewise, lymphoid DCs have been used to describe either pDCs or cDC1. These terms are less useful because of their inconsistent use and because they do not reflect ontogeny as we now understand.

## DEVELOPMENT OF DENDRITIC CELL SUBSETS

Generation of cDCs and pDCs starts in the bone marrow from common monocyte-DC precursors and differentiates into either common monocyte progenitors that develop into monocytes and monocyte-derived DCs, or common DC precursors (CDPs). CDPs initially give rise to pre-DCs that express Zbtb46 transcription factor and convert into cDCs that find the destination of lymphoid or nonlymphoid tissue via circulation. Other molecules that also discriminate cDCs from macrophages include Flt3, c-kit (the receptor for stem cell factor), and the chemokine receptor CCR7 (Förster et al., 1999; Maraskovsky et al., 1996; McKenna et al., 2000). Maintenance of cDCs needs Flt3 cytokine and their ability to respond to Flt3L. Flt3 is a key regulator of DC commitment in hematopoiesis and signals via pSTAT3. Both interleukin-2 (IL-2) and GM-CSF signal via pSTAT5 and can inhibit Flt3L-mediated DC development (Esashi et al., 2008; Lau-Kilby et al., 2011); for IL-2, this effect is mediated by an increase in apoptosis-inducing protein Bim, and a decrease in CD135, the receptor for Flt3L and pSTAT3 activation (Guerrero et al., 2014). Flt3 expression is present on pre-DCs but the expression is lost in precursors that become committed to non-DC populations such as monocytes. Monocyte-derived DCs are functionally distinct from cDCs and display different developmental paths that mainly occur in the context of inflammation (Cheong et al., 2010) and are dependent on GM-CSF.

### DENDRITIC CELL SUBSET PHENOTYPES

#### Conventional Dendritic Cell 1

CD8 $\alpha$  has been a useful marker in mice for identifying lymphoid-resident cDC1s, although its function on DCs is unknown and it is not expressed on human DC. More recently, XCR1 and Clec9a have been identified as markers on cDC1 in both mouse and human. XCR1 is a chemokine receptor for XCL1 expressed by activated T cells; the importance of XCR1 for DC migration is not clear but may be important for colocalization of DCs with T cells. Clec9a is a lectin receptor that allows efficient antigen uptake from dying cells and recognizes the filamentous form of actin (F actin), exposed when the membrane of a cell is disrupted (Sancho et al., 2009; Zhang et al., 2012). cDC1 as well as having the unifying DC function of presenting exogenous antigens on MHCII have the additional capacity for “cross-presentation,” that is, the ability to present exogenous antigen in the context of MHC I (Villadangos and Schnorrer, 2007). Accordingly, cDC1s are particularly efficient at inducing CD8 + T cells in response to exogenous antigens. Upon activation through PRR, cDC1s can produce high IL-12p70 production (Hochrein et al., 2001; Maldonado-López et al., 1999; Reis e Sousa et al., 1997) that can bias activated T cells to an inflammatory Th1 response. CD70 expression by cDC1 also provides an IL-12-independent path toward Th1 development (Soares et al., 2007). Mouse cDC1 expresses TLR3, TLR9 that recognize dsRNA or ssDNA, respectively, but in human TLR9 is restricted to pDCs. With the dual functions of cross-presentation and dead cell uptake, the cDC1s are perfectly equipped to protect against viral or bacterial antigens, but in an autoimmune setting could be particularly detrimental as they may be activated by self-nucleic acids taken up in dead cells and self-antigen presentation may lead to activation of cytotoxic T cells. In nonlymphoid tissue, the cDC1 subset expresses CD103, integrin marker ( $\alpha_E\beta_7$ ) (Bursch et al., 2007). CD103 + cDC, a minor but important migratory DC subset, was discovered in the skin dermis express langerin, a pathogen recognition molecule expressed not only at high levels in Langerhans cells but also at lower levels in some other DC (Kubo et al., 2009). Development of CD8 + or CD103 + cDC1s is orchestrated by the same transcription factors such as the inhibitor of DNA binding 2 (Id2), interferon regulatory factor 8 (IRF8), basic leucine zipper ATF-like 3 transcription factor (BATF3), and the nuclear factor IL-3 regulated (Mildner and Jung, 2014). IRF8 is known as the master regulator of CD8 + and CD103 + cDC development. IRF8 is necessary for the development of Id2-expressing DC precursors but BATF3 is actually induced in later stages of DC development.

#### Conventional Dendritic Cell 2

cDC2s, which are more abundant in lymphoid tissue than cDC1, can be identified by expression of DCIR2 or CD11b in mice and CD1c in human. Although cDC2s are not proficient for cross-presenting antigens, they show superior activity in inducing CD4 + T-cell responses, potentially due to higher expression of MHCII machinery (Dudziak et al., 2007; Lewis et al., 2011). The CD11b + cDCs express very high levels of intracellular Rig-like helicase receptors (RLR) that recognize dsRNA in the cytoplasm (Luber et al., 2010). The high expression of RLR and

some NLR suggests that the cDC2s are particularly primed to rapidly respond to intracellular viral and bacterial infection. cDC2s express high levels of chemokines including CC-chemokine ligand 5 (CCL5) (RANTES), CCL3 (MIP-1 $\alpha$ ), and CCL4 (MIP-1 $\beta$ ). Several transcription factors control cDC2 development, including RelB (Wu et al., 1998), NOTCH2 (Lewis et al., 2011), RBP-J (Caton et al., 2007), IRF2 (Ichikawa et al., 2004), and IRF4 (Suzuki et al., 2004). IRF4 controls MHCII presentation and migration. Since cDC2s are very heterogeneous in both mouse and human, lack of IRF4 and NOTCH2 only partially impairs their development and shows differential penetrance in different tissues (Lewis et al., 2011).

## Plasmacytoid Dendritic Cells

pDCs share with cDC the dependence on Flt3L for their development and the ability to prime T cells after activation (Kingston et al., 2009; Sapoznikov et al., 2007). The development and homeostasis of pDCs are controlled by the helix-loop-helix transcription factor E2-2 (also known as TCF4) (Cisse et al., 2008) that suppresses the expression of Id2 (Ghosh et al., 2010), a transcription factor critical for cDC development (Hacker et al., 2003). pDCs accumulate mainly in the blood and other lymphoid tissues. Because of the low expression of MHCII and other costimulatory molecules, pDCs are inefficient antigen presenting cells. pDCs express high levels of TLR7 and TLR9. If given specific PRR stimuli, they can induce some T-cell division, more than B cells or macrophages but typically in the order of 10-fold or less that of the cDC (Villadangos and Young, 2008). Unlike cDC, the pDCs continually present antigens on MHCII molecules once they are activated and as a result can continue to present new viral antigens during the course of infection (Villadangos and Young, 2008). The importance of this function of pDC during an ongoing infection is not yet elucidated. pDCs can contribute to tolerance induction via maintaining Treg activation and expansion, in part through indoleamine 2,3-dioxygenase (IDO) (Beaudoin et al., 2014; Lippens et al., 2016; Matta et al., 2010). pDCs can also function as an innate cell and are renowned for their high production of Type I interferons (IFN-I) upon recognition of foreign nucleic acids, including ssDNA via TLR9 (Gilliet et al., 2008; Kadokami et al., 2000). As a consequence of endoplasmic reticulum to lysosome internal trafficking of TLR9 and differential expression of molecules that are involved in the TLR9 signaling complex, such as high constitutive expression of IRF7, the pDCs are specialized to produce extremely high levels of IFN-I upon TLR9 ligation (Gilliet et al., 2008). Upon activation, pDCs can also produce proinflammatory cytokines and chemokines, including IL-6, IL-12, CXC-chemokine ligand 8 (CXCL8), CXCL10, CCL3, and CCL4. As a result, pDCs could influence on activating T cells by presenting antigens as well as contribute to B-cell activation, plasma cell generation, and antibody secretion through the production of IFNs-I, IL-6, B cell-activating factor, and a proliferation-inducing ligand (Swiecki and Colonna, 2015).

## Tissue-Specific Dendritic Cells

In addition to the general features of the main DC subsets described above, in particular tissues, DCs can obtain specialized functions and markers. For example, in the gut, DCs can express high levels of TGF- $\beta$  and retinoic acid that allows efficient induction of Foxp3 $^{+}$  Tregs both in the mucosal tissue and in the mesenteric draining lymph nodes (Coombes et al., 2007).

Because human skin is more accessible for study compared to other tissues, DCs from skin have undergone extensive characterization and serve as a model for the heterogeneity of tissue APCs. Skin is composed of two distinct layers, epidermis and dermis. In mice where langerin $^{+}$  migratory DCs are depleted, anti-DEC-205-mediated antigen-specific delivery to DCs is no longer able to induce antigen-specific Tregs in the spleen and skin-draining lymph nodes and results in a loss of immune tolerance (Bennett et al., 2005; Idoyaga et al., 2013). Importantly, langerin $^{+}$  migratory DCs may, in fact, uniquely contribute to the induction of Tregs and the maintenance of peripheral tolerance, as the specific depletion of langerin $^{+}$  DCs, has no effect on the initiation of anti-viral responses (Anandasabapathy et al., 2014). This latter finding suggests that specific DC subsets found in unique environments may have specialized roles.

Several subsets of DCs are found in the dermis including XCR1 $^{+}$  cDC1s, CD11b $^{+}$  cDC2s, and XCR1 $^{-}$ CD11b $^{-}$  double-negative cDCs (Malissen et al., 2014; Tan et al., 2015). Dermal cDCs express CD207 and could be either CD103 $^{+}$  or CD103 $^{-}$ . Dermal cDC1s carry antigens from skin commensals to cutaneous lymph nodes and induce IL-17-producing CD8T cells that migrate to the skin. These skin CD8 T cells are further activated by IL-1 cytokine released by the resident cDC2s or moDCs. T cells activated by this dermal DC-mediated antigen presentation are thought to provide skin protection from pathogens in an IL-17-dependent manner.

(Naik et al., 2015). pDCs are rare in healthy human skin but accumulate in inflamed tissue and facilitate disease pathogenesis in systemic lupus erythematosus (SLE) and psoriasis (Blanco et al., 2001; Nestle et al., 2005). Inflammatory myeloid DCs infiltrate both epidermis and dermis of psoriatic lesions and are proposed to play a major role in psoriasis pathogenesis by production of the inflammatory mediators, inducible nitric oxide synthase, and TNF- $\alpha$  (Lowes et al., 2005; Zaba et al., 2009).

## DENDRITIC CELL SUBSETS AND TOLERANCE

The process of central tolerance in the thymus eliminates potentially autoreactive thymocytes by negative selection and promotes T-cell differentiation into various Treg subsets via additional selection processes (Baldwin et al., 2004; Hogquist et al., 2005; Klein et al., 2014; Labrecque et al., 2011; Pobezinsky et al., 2012). Although thymic DC may serve the same sentinel role as in other tissues, their main role is likely to be in the selection of the antigen receptor repertoire of developing T cells, so ensuring tolerance to self (Ardavín, 1997; Audiger et al., 2017). Central tolerance is, in fact, highly dependent on the presentation of self-antigens to T cells by both thymic epithelial cells and DCs (Gallegos and Bevan, 2004; Guerri et al., 2013; Jenkinson et al., 1992). Early work showed that MHC expression on thymic bone marrow-derived APC contributes to central tolerance induction (van Meerwijk et al., 1997). In vitro organ culture showed the dominant role of DCs in central tolerance when compared with other APCs, like macrophages or B cells (Guerri et al., 2013). Three thymic DC subsets contribute to central tolerance, namely, resident DC ( $CD8\alpha^+ SIRP\alpha^-$ ), migratory DC ( $CD8\alpha^- CD11b^+ SIRP\alpha^+$ ), and pDC ( $CD11c^{int} CD45RA^{int}$ ) (Corcoran et al., 2003; Lahoud et al., 2006; Vremec et al., 2000; Wu and Shortman, 2005). Interestingly, and likely due to the experimental challenges associated with separating central and peripheral tolerance processes, the general outcome of a defect in DC-mediated central tolerance on the potential development of an autoimmune phenotype has yet to be clearly defined. DCs can induce tolerance by increasing IL-10, TGF- $\beta$ , IL-4, or IDO levels. Tolerogenic DCs express low levels of costimulatory molecules CD80, CD86, and CD40 (Steinman et al., 2003; Stoop et al., 2011). Together, these signals allow antigen presentation in a noninflammatory environment, leading to T-cell deletion, anergy, or induction of Tregs.

Although thymic selection eliminates most self-reactive T cells, some remain and must be kept in check with additional peripheral tolerance mechanisms to avoid autoimmunity. Several factors can alter DC-mediated tolerance including the extent of DC maturation and level and timing of antigen presentation. Thus understanding these conditions under which DC remains activated or immature informs modulation of tolerance in pathogenic settings (Ardouin et al., 2016; George et al., 2003; Wallet et al., 2005). In this context, DC-induced T-cell activation does not always correlate with common maturation/activation markers in part because Treg uses some of the same signals including CD80/CD86. Therefore directly assessing the signals given to T cells, including Tregs and the ability of DCs to elicit functional T-cell activation, ideally in vivo, is often needed to define tolerogenic and immunogenic DCs (Salomon et al., 2000; Yamazaki et al., 2003; Zheng et al., 2004; Ardouin et al., 2016). Tregs activated by DCs in vitro display strong autoimmune inhibition (Tarbell et al., 2004, 2007). Particular subsets have specialized roles for maintaining Tregs. Migratory DCs are potent inducers of peripheral Treg generation. Similarly, pDCs are also capable of migrating to lymph nodes and increase peripheral Treg generation (Ochando et al., 2006; Seth et al., 2011). DCs, whether in the steady state or in inflamed conditions, may have differential expression of a wide variety of molecules that together determine if the sum of these signals is sufficient to break or maintain self-tolerance. Vaccination with tolerogenic DC can prevent disease onset and limit disease severity in the collagen-induced arthritis mouse model, a commonly used model of rheumatoid arthritis. Likewise in non-obese diabetic (NOD) mice, transfer of tolerogenic DC or targeting autoantigens to tolerogenic DCs prevents or delays the onset of diabetes (Giannoukakis et al., 2008; Mukherjee and Dilorenzo, 2010; Mukhopadhyaya et al., 2008).

Steady-state DCs are exposed to commensal microorganisms and other tonic inflammatory signals that can induce the expression of maturation markers at low levels and become immunoregulatory and less migratory to the draining lymph nodes. DC migration is associated with significant change in the transcriptional level, suggesting that immunoregulatory function of steady-state DCs is an active process (Ardouin et al., 2016). Induction of inhibitory molecules including programmed cell death protein 1 (PD-1), TGF- $\beta$ , or IDO and downregulation of costimulatory molecules or cytokines are important correlates of regulatory DC function (Kaliński et al., 1997; Selenko-Gebauer et al., 2003). Although DCs under inflamed conditions can display increased effector T-cell activation and inflammatory cytokine production, these DCs can also be immunoregulatory. For example, there are different types of inflammatory signals that counter-regulate each other such as IL-1 and IFN-I. A DC activated

by the environment to increase IFN-I responses could downmodulate an IL-1-driven response (Rahman et al., 2016). Therefore IFN-I can be regulatory and block autoimmunity in some contexts (Kasper and Reder, 2014; Rahman et al., 2018; Tarbell and Egen, 2018; Trinchieri, 2010). Upon infection with *Listeria monocytogenes*, DCs suppress T-cell activity mainly by IL-10 and cyclooxygenase 2-mediated mechanisms (Popov et al., 2008; Schmidt et al., 2012). In chronic viral infection, DCs can become immunosuppressive, losing their surface expression of MHC I and MHC II and costimulatory molecules (Sevilla et al., 2004).

## DENDRITIC CELLS AND AUTOIMMUNE DISEASE

Alterations in central or peripheral tolerance can precipitate development of autoimmune pathology. DC subsets integrate many environmental signals and, in response to these signals, are capable of a wide spectrum of functions ranging from induction of thymic and peripheral tolerance to the induction of potent inflammatory responses. Any imbalances introduced in these DC responses, from complex genetic to environment factors, could lead to or enhance autoimmune disease. Although the role DCs play in orchestrating responses in each autoimmune disease can differ, these diseases do have overlapping pathogenic processes. In general, autoimmune diseases can be categorized into systemic (such as SLE) or tissue-specific [such as type 1 diabetes (T1D)]. In addition, when the targeted tissue is at an interface with the external environment, such as with psoriasis and colitis, signals from commensal organisms in those tissues can alter the balance between autoimmunity and tolerance. In the following sections, some examples are discussed.

### Systemic Lupus Erythematosus

In patients with SLE, autoreactive B- and T-cell responses target nuclear proteins and self-nucleic acids. Genetic influences, including human leukocyte antigen (HLA) loci, play a major role in determining susceptibility to SLE. Other susceptibility genes include many involved in proinflammatory cascades, for example, genes encoding Fc $\gamma$  receptors, TNFSF4, IRAK1, STAT4 (Delgado-Vega et al., 2010; Deng and Tsao, 2010; Sestak et al., 2011). Many of these genes are involved in innate immune pathways leading to production of IFN-I or responses to IFN-I. Patients with SLE displaying an increased IFN gene signature, and these IFN-I responses are pathogenic in the development of SLE (Rönnblom and Pascual, 2008; Ferreira et al., 2014; Obermoser et al., 2010).

IFN-I in SLE is predominantly produced by pDCs that respond to nucleic acid/autoantibody complexes via signaling through TLR7 and TLR9 (Means et al., 2005; Rönnblom and Pascual, 2008). Delivery of nucleic acid complexes to pDC in SLE depends on Fc receptor (FcR) that binds antinucleic acid immune complexes (Means et al., 2005) or nucleic acid complexes associated with neutrophils. Dying neutrophils in SLE patients release neutrophil extracellular traps; these neutrophils die from “netosis,” thereby releasing large amounts of DNA/DNA-binding antimicrobial protein complexes that allow increased uptake of DNA by pDC and subsequent IFN-I production by TLR9-induced activation (Garcia-Romo et al., 2011). pDCs from these patients are more prone to induce pathogenic T-cell responses (Chaussabel et al., 2008; Palucka et al., 2002). The number of circulating pDCs in patients with SLE is decreased and correlates with disease pathogenesis. Recruitment of pDCs into the draining lymph nodes or diseased tissues might contribute to SLE progression.

IFN-I production in SLE is seen as a major contributor to the etiology of this disease since it greatly enhances activation of DC, self-reactive B cells and T cells, and the production of many other proinflammatory cytokines. Indeed, this pathway has recently been the target of new drugs being tested in clinical trials, and blocking IFN-I signals can decrease SLE disease severity, especially in the individuals showing higher IFN-I-driven gene expression prior to treatment (Narain and Furie, 2016). The biggest effects on SLE pathogenesis have been observed with anifrolumab that blocks the IFN-I receptor, but this was also associated with higher adverse events associated with viral infections, specifically increases in herpes zoster and influenza infections. Therefore targeting innate pathways can be powerful for modulating autoimmune pathogenesis, but blocking these signals can also increase infections, narrowing the therapeutic window.

### Inflammatory Bowel Disease—Crohn’s Disease and Ulcerative Colitis

Inflammatory bowel diseases (IBD) ulcerative colitis (UC) and Crohn’s disease (CrD) likely manifest as a result of dysregulated immune responses to the intestinal microbiota, but the pathology is autoimmune in nature.

Mutations in NLR family members NOD2 and NLRP3 are highly associated with CrD (Cho, 2008; Villani et al., 2009). These PRRs are mainly expressed by DC and macrophages and emerging evidence suggests they play a crucial role in maintaining immunological homeostasis in the intestine (Strober, 2009; Strober et al., 2006; Zaki et al., 2011). NOD2 functions as a receptor for the bacterial cell wall component muramyl dipeptide. Triggering of NOD2 in human MoDC in vitro stimulates processing and presentation of bacterial antigens to CD41 T cells, so generating an antibacterial response due to an increased production of antibacterial IL-17 (van Beelen et al., 2007; Cooney et al., 2010). NLRP3 is activated by a wide variety of microbial agonists and drives IL-1 $\beta$  secretion that activates the antimicrobial functions of innate immune cells such as macrophages and DC and induces CD41 Th17 immune responses (Schroder et al., 2010). Thus abnormal signaling by DC in response to the intestinal microbiota, consequent to mutations in these PRR, likely contributes to the pathogenesis of CrD.

Further evidence derived from mice and humans suggests that an imbalance in intestinal DC subsets, distribution, and function plays a crucial role driving inflammation and disease pathogenesis (Ng et al., 2011; Varol et al., 2010). Depletion of DC in mouse models of colitis leads to increased or decreased disease severity depending on the time point, demonstrating a crucial role for DC in both the downregulation and exacerbation of intestinal inflammation. The balance between the functions of mouse intestinal CD103 and CX3CR1 DC subsets regulates immune homeostasis and controls inflammatory responses. CD103 DCs are migratory DC that reside in the lamina propria and transport microbial antigens to the lymph nodes where they play an essential role in the induction of peripheral Tregs and the generation of oral tolerance. In contrast, the levels of CX3CR1 DC are dramatically increased in mouse colitis models and augment the severity of disease. pDCs also accumulate in intestinal tissue in mouse colitis models (Karlis et al., 2004) and are contributors to the protective effects of GM-CSF treatment on colitis (Sainathan et al., 2008).

CD11c + MHCII + DC can be found in the lamina propria of the human colonic mucosa (Ng et al., 2010), but their phenotype, function, and degree of alignment with mouse DC subsets are poorly characterized. Increased numbers of activated cDC are found in inflamed tissue in CrD compared to noninflamed tissue, supporting a role for DC in disease pathogenesis (Ng et al., 2010). These DCs express CD40 and CD83, TLR2 and TLR4 and produce proinflammatory cytokines including IL-6, tumor necrosis factor (TNF), and IL-12. An increased number of pDC are also present in the colonic mucosa and mesenteric lymph nodes of patients with CrD and UC (Baumgart et al., 2011). However, pDC infiltration in the lamina propria is associated with a clinical response and remission in patients with CrD following granulocyte-colony stimulating factor (G-CSF) treatment (Mannon et al., 2009). Thus the contribution of pDC to IBD pathogenesis remains unclear.

## Psoriasis and Psoriatic Arthritis

Psoriasis is a common chronic inflammatory skin disorder associated with increased accumulation of natural killer cells, type 3 innate lymphoid cells, TH17 cells, and  $\gamma\delta$  T cells that drive production of inflammatory cytokines like IL-17 and IL-22 (Guttmann-Yassky et al., 2011). Approximately 2% of the US population has psoriasis, and up to 30% of those will develop psoriatic arthritis. The pathology of psoriasis is better understood than that of associated arthritis, but some evidence points to common antigens (Dolcino et al., 2015), suggesting a shared role for dysfunctional APCs. CD11c + DCs are greatly increased in number in the psoriatic skin, either mature or immature forms. Mature DCs often aggregate with the lesional infiltrating T cells. T cells within the lesions are highly proliferating and show a memory phenotype; therefore accumulated DCs within the lesions are thought to be acting as cellular nidus that contribute to the activation of T cell by presenting autoantigens (Bos et al., 1989; Chang et al., 1992). In this regards, it has been shown that depletion of DCs in CD11c-diphtheria toxin receptor-transgenic mice prevents disease onset suggesting the involvement of DCs in disease pathogenesis. pDCs are also increased in psoriatic plaques, produce large amounts of IFN-I. There are several molecules that have been recently tested against psoriasis treatment based on the fact that reducing the ability of DCs to interact with T cells may limit the pathogenic T-cell activity. Alefacept or Efalizumab, which both block the interaction of DCs with T cells by binding to CD2 or intercellular adhesion molecule 1, respectively, shows good clinical therapeutic efficacy.

## Type 1 Diabetes

T1D is an autoimmune disorder that results from the defective induction or maintenance of T-cell tolerance against islet  $\beta$ -cell self-antigens. There are multiple layers of immune cell activation during the progression

of disease. In this context, both genetic and environmental factors contribute to the development of T1D (Hotta-Iwamura and Tarbell, 2016). Genome-wide association studies and disease association studies have identified >50 polymorphic loci that lend susceptibility or resistance to insulin-dependent diabetes mellitus. In parallel, diabetes susceptibility regions known as insulin-dependent diabetes loci have been identified in the nonobese diabetic mouse, a model for human T1D, providing a better understanding of potential immunomodulatory factors in T1D risk (Hotta-Iwamura and Tarbell, 2016). Both innate and adaptive immune cells are activated in an inflammatory environment dominated by IL-1 and IFN-I. A chronic inflammatory environment establishes later, but before the onset of T1D. The immune equilibrium is disrupted by chronic stimulation with autoantigens and altered cytokine stimulation. DCs contribute to early diabetes pathogenesis (Calderon et al., 2011; Diana et al., 2013; Ferris et al., 2014), but because of their role in maintaining Tregs, increasing DCs at later stages of T1D can block disease (Darrasse-Jèze et al., 2009). DCs presenting self-antigen can increase self-specific Treg numbers, function, and activation, and these DC-driven Tregs are potent for inhibiting autoimmunity. Autoimmunity disrupts DC-mediated steady-state tolerance either through intrinsic genetic alterations or because of endogenous innate signals that induce some level of maturation (Rönnblom et al., 2006; Turley et al., 2003). Like SLE, one critical inflammatory signal for diabetes pathogenesis is IFN-I cytokine that increases in early life and remains high until the onset of disease. Early blockade of IFN-I signal inhibits diabetes pathogenesis (Carrero et al., 2013; Rahman et al., 2016; Sen et al., 2003), which suggests a role for IFN-I in breaking tolerance. However, when chronic inflammation establishes, the role of IFN-I appears to shift and blocking IFN- $\alpha$  receptor signals later actually accelerates diabetes development. This has been shown by the fact that despite increased chronic IFN-I exposure, DCs from older prediabetic NOD mice display impaired IFN-I responses (Rahman et al., 2016) due in part to down-modulation of IFN- $\alpha$  receptor (Sen et al., 2003; Weaver et al., 2001). Increased expression of PGE2 and one receptor for PGE2, EP4 in NOD, also down-modulates IFN signals, and blocking EP4 signals decreases lymphocytic infiltrate (Rahman et al.). Despite this decreased IFN-I response, APCs are still hyperactive due to the altered regulation of NF- $\kappa$ B activation that enhances antigen presentation to CD8 T cells (Blanco et al., 2008; Mayer-Barber and Yan, 2017). The role of DC subsets in different autoimmune conditions may differ. For example, both CD8 $\alpha^+$ DEC-205 $^+$  and CD11b $^+$ DCIR2 $^+$  DCs are tolerogenic in normal mice, but only the CD11b $^+$ DCIR2 $^+$  DCs are able to induce CD4 tolerance in NOD mice (Price and Tarbell, 2015; Price et al., 2014). CD8 $\alpha^+$ DEC-205 $^+$  DCs in NOD mice play a more pathogenic role as demonstrated in NOD Batf3 $^{-/-}$  mice that lack CD8 $\alpha^+$  T cell-mediated cross-presentation (Ferris et al., 2014). The progress of DC-based immunotherapy is currently being evaluated with DC generated from blood precursors and treated with antisense oligonucleotides in vitro (Giannoukakis et al., 2008). In vitro generated DCs are then injected intradermally at a site proximal to the pancreas and are expected to migrate to nearby lymph nodes and the pancreas.

## TARGETING OF DENDRITIC CELLS IN AUTOIMMUNE DISEASE

DCs are critical for maintaining a tolerogenic state and preventing the development of autoimmune disease. More importantly, increasing evidence also shows that, in an autoimmune environment, DCs can become inflammatory and override their tolerogenic state. Therefore solutions for manipulating DCs toward stable tolerogenic states for treatment of autoimmune diseases are desired approaches. For the goal of antigen-driven tolerance, one consideration is how best to induce dominant tolerance, namely, the ability of tolerance directed at a single antigen specificity to promote tolerance to other antigens expressed in the same tissue. Unfortunately, ex vivo manipulation of DC is logically impractical and needs to be tailored specifically for individual patients. Most preclinical studies and ex vivo human assays have used monocyte-derived DCs, but new methods for generation of Flt3 ligand-based human cDCs may broaden the types of DCs available (Lee et al., 2015). Delivering antigen to DC directly in vivo using antibodies specific for DC-associated molecules such as DEC-205 can overcome many of the current limitations of DC therapy (Tacken et al., 2007). This approach is currently in early phase clinical trials for cancer and is being explored in preclinical experimental models for autoimmune diseases. In the absence of adjuvant, targeting antigen to DC in vivo using antibodies to DEC-205 induces antigen-specific T-cell deletion and unresponsiveness to myelin oligodendrocyte glycoprotein (MOG) in a model of autoimmune experimental acute encephalomyelitis and to model antigens including ovalbumin (Bonifaz et al., 2002; Hawiger et al., 2004). Furthermore, DEC-205 targeting of autoantigens in the NOD and other mouse models of T1D prevents disease onset and progression (Bruder et al., 2005; Mukhopadhyaya et al., 2008). However, because autoimmune

individuals inherently have tolerance defects, “steady-state” DC tolerance may fail (Price and Tarbell, 2015). Therefore adding tolerogenic signals may be necessary.

An alternative approach being developed is microspheres carrying CD40, CD80, and CD86 antisense oligonucleotides. Vaccination with these microspheres can prevent and reverse the new onset of T1D in NOD mice, presumably via uptake by DC *in vivo* and the expansion of Tregs (Phillips et al., 2008). Targeting the innate functions of DC, such as cytokine production, is also likely to be helpful in autoimmune diseases. The example of SLE cited above is a key candidate for pDC targeting. Targeting strategies that block TLR7 and 9, for example, would extinguish the IFN-I production by pDC in response to self-nucleic acids in SLE. For any of these current or future treatments, defining reliable biomarkers is critical. Biomarkers could potentially identify the subset of patients who have particular tolerance defects and who may respond to a particular treatment. Baseline alterations in DC phenotype may be useful because the genetic and environmental modulators of DC function will vary among individuals with T1D. Biomarkers can also identify efficacy and responsiveness to specific modulations, and this type of marker will likely include T-cell readouts, but assessing the function and stability of tolerogenic DCs after treatment would also be informative.

## CONCLUSION AND FUTURE PROSPECTS

Through both antigen presentation functions and innate immune functions, DCs are critical for the initiation and maintenance of self-tolerance that is broken in autoimmune disease. But DCs are also central contributors of autoimmune pathology by giving autoreactive T-cell activation and differentiation signals. Because of this, modulation of DC has the potential to both increase tolerance induction and block the expansion of self-specific effector T cells. But increasing these tolerogenic effects can be difficult to do without at the same time exacerbating pathogenic effects of DCs. For example, DC-specific expression of some of the same phenotypic markers can both increase Tregs and autoreactive Teff. Conversely, decreasing DC maturation to avoid activation of pathogenic cells can also interfere with Treg homeostasis. Therefore to balance the effects on both Treg and Teff, the specific phenotype of the tolerogenic DC is critical.

New techniques such as single cell RNAseq that allow elucidation of the diversity of cell phenotype with small numbers of cells have allowed a better understanding of DC function in different contexts. From these and accompanying functional assays, the role of DC subsets in both mice and human is now better understood (Villani et al., 2017). In the future, more information of how DCs change in pathological contexts will further design of therapies that can modulate DCs for treatment of disease. Targeting self-antigen to DCs has the potential to treat autoimmunity but challenges remain. Importantly, key self-antigens need to be defined. Because T cells against many antigens facilitate autoimmune pathogenesis, targeting antigen must induce dominant tolerance.

Another approach is to adjust DCs to a tolerogenic phenotype without exogenous delivery of antigen and rely on endogenous self-antigen presentation. For this approach, the drawback is potential effects on immunity to infectious agents. Blocking TNF and other inflammatory cytokines have been efficacious for the treatment of arthritis and other chronic autoimmune conditions and work in part through effects on DCs (Khan et al., 2009). Surprisingly, 20 years of experience with this class of drug shows that, although a small (20%) increase in infections does occur, this is not a major side effect and does not often lead to patients stopping treatment (Minozzo et al., 2016). But only a minority of autoimmune patients are successfully treated with inhibitors of inflammatory cytokines. Specific infections, such as tuberculosis, for which TNF is critical show a larger increase under TNF blockade. New ways of pushing DCs toward tolerance are needed, but with more potent effects on blocking autoimmunity may come a larger inhibition of immunity to infection.

Combination approaches that deliver antigen to tolerogenic DCs along with increasing signals that encourage tolerance may have the best chance of success.

## Acknowledgments

This chapter is based on the DC chapter authored by Kristen Radford, Ken Shortman, and Meredith O’Keeffe that was included in the fifth edition of this book. This work was supported by the intramural programs of NIDDK and NHLBI.

## References

- Anandasabapathy, N., Feder, R., Mollah, S., Tse, S.-W., Longhi, M.P., Mehandru, S., et al., 2014. Classical Flt3L-dependent dendritic cells control immunity to protein vaccine. *J. Exp. Med.* 211, 1875–1891.
- Ardavín, C., 1997. Thymic dendritic cells. *Immunol. Today* 18, 350–361.
- Ardouin, L., Luche, H., Chelbi, R., Carpentier, S., Shawket, A., Montanana Sanchis, F., et al., 2016. Broad and largely concordant molecular changes characterize tolerogenic and immunogenic dendritic cell maturation in thymus and periphery. *Immunity* 45, 305–318.
- Audiger, C., Rahman, M.J., Yun, T.J., Tarbell, K.V., Lesage, S., 2017. The importance of dendritic cells in maintaining immune tolerance. *J. Immunol.* 198, 2223–2231.
- Baldwin, T.A., Hogquist, K.A., Jameson, S.C., 2004. The fourth way? Harnessing aggressive tendencies in the thymus. *J. Immunol.* 173, 6515–6520.
- Barbalat, R., Ewald, S.E., Mouchess, M.L., Barton, G.M., 2011. Nucleic acid recognition by the innate immune system. *Annu. Rev. Immunol.* 29, 185–214.
- Baumgart, D.C., Metzke, D., Guckelberger, O., Pascher, A., Grötzing, C., Przesdzing, I., et al., 2011. Aberrant plasmacytoid dendritic cell distribution and function in patients with Crohn's disease and ulcerative colitis. *Clin. Exp. Immunol.* 166, 46–54.
- Beaudoin, L., Diana, J., Ghazarian, L., Simoni, Y., Boitard, C., Lehuen, A., 2014. Plasmacytoid dendritic cells license regulatory T cells, upon iNKT-cell stimulation, to prevent autoimmune diabetes. *Eur. J. Immunol.* 44, 1454–1466.
- van Beelen, A.J., Zelinkova, Z., Taanman-Kueter, E.W., Muller, F.J., Hommes, D.W., Zaaij, S.A.J., et al., 2007. Stimulation of the intracellular bacterial sensor NOD2 programs dendritic cells to promote interleukin-17 production in human memory T cells. *Immunity* 27, 660–669.
- Bennett, C.L., van Rijn, E., Jung, S., Inaba, K., Steinman, R.M., Kapsenberg, M.L., et al., 2005. Inducible ablation of mouse Langerhans cells diminishes but fails to abrogate contact hypersensitivity. *J. Cell Biol.* 169, 569–576.
- Blanco, P., Palucka, A.K., Gill, M., Pascual, V., Banchereau, J., 2001. Induction of dendritic cell differentiation by IFN-alpha in systemic lupus erythematosus. *Science* 294, 1540–1543.
- Blanco, P., Palucka, A.K., Pascual, V., Banchereau, J., 2008. Dendritic cells and cytokines in human inflammatory and autoimmune diseases. *Cytokine Growth Factor Rev.* 19, 41–52.
- Bonifaz, L., Bonnyay, D., Mahnke, K., Rivera, M., Nussenzweig, M.C., Steinman, R.M., 2002. Efficient targeting of protein antigen to the dendritic cell receptor DEC-205 in the steady state leads to antigen presentation on major histocompatibility complex class I products and peripheral CD8+ T cell tolerance. *J. Exp. Med.* 196, 1627–1638.
- Bos, J.D., Hagenaars, C., Das, P.K., Krieg, S.R., Voorn, W.J., Kapsenberg, M.L., 1989. Predominance of "memory" T cells (CD4+, CDw29+) over "naive" T cells (CD4+, CD45R+) in both normal and diseased human skin. *Arch. Dermatol. Res.* 281, 24–30.
- Bruder, D., Westendorf, A.M., Hansen, W., Prettin, S., Gruber, A.D., Qian, Y., et al., 2005. On the edge of autoimmunity: T-cell stimulation by steady-state dendritic cells prevents autoimmune diabetes. *Diabetes* 54, 3395–3401.
- Bursch, L.S., Wang, L., Iggyarto, B., Kissenpennig, A., Malissen, B., Kaplan, D.H., et al., 2007. Identification of a novel population of Langerin+ dendritic cells. *J. Exp. Med.* 204, 3147–3156.
- Calabro, S., Liu, D., Gallman, A., Nascimento, M.S.L., Yu, Z., Zhang, T.-T., et al., 2016. Differential intrasplenic migration of dendritic cell subsets tailors adaptive immunity. *Cell Rep.* 16, 2472–2485.
- Calderon, B., Carrero, J.A., Miller, M.J., Unanue, E.R., 2011. Entry of diabetogenic T cells into islets induces changes that lead to amplification of the cellular response. *Proc. Natl. Acad. Sci. U.S.A.* 108, 1567–1572.
- Carrero, J.A., Calderon, B., Towfic, F., Artyomov, M.N., Unanue, E.R., 2013. Defining the transcriptional and cellular landscape of type 1 diabetes in the NOD mouse. *PLoS One* 8, e59701.
- Caton, M.L., Smith-Raska, M.R., Reizis, B., 2007. Notch-RBP-J signaling controls the homeostasis of CD8+ dendritic cells in the spleen. *J. Exp. Med.* 204, 1653–1664.
- Chang, E.Y., Hammerberg, C., Fisher, G., Baadsgaard, O., Ellis, C.N., Voorhees, J.J., et al., 1992. T-cell activation is potentiated by cytokines released by lesional psoriatic, but not normal, epidermis. *Arch. Dermatol.* 128, 1479–1485.
- Chaussabel, D., Quinn, C., Shen, J., Patel, P., Glaser, C., Baldwin, N., et al., 2008. A modular analysis framework for blood genomics studies: application to systemic lupus erythematosus. *Immunity* 29, 150–164.
- Cheong, C., Matos, I., Choi, J.-H., Dandamudi, D.B., Shrestha, E., Longhi, M.P., et al., 2010. Microbial stimulation fully differentiates monocytes to DC-SIGN/CD209(+) dendritic cells for immune T cell areas. *Cell* 143, 416–429.
- Cho, J.H., 2008. The genetics and immunopathogenesis of inflammatory bowel disease. *Nat. Rev. Immunol.* 8, 458–466.
- Cisse, B., Caton, M.L., Lehner, M., Maeda, T., Scheu, S., Locksley, R., et al., 2008. Transcription factor E2-2 is an essential and specific regulator of plasmacytoid dendritic cell development. *Cell* 135, 37–48.
- Coombes, J.L., Siddiqui, K.R.R., Arancibia-Cárcamo, C.V., Hall, J., Sun, C.-M., Belkaid, Y., et al., 2007. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J. Exp. Med.* 204, 1757–1764.
- Cooney, R., Baker, J., Brain, O., Danis, B., Pichulik, T., Allan, P., et al., 2010. NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation. *Nat. Med.* 16, 90–97.
- Corcoran, L., Ferrero, I., Vremec, D., Lucas, K., Waithman, J., O'Keeffe, M., et al., 2003. The lymphoid past of mouse plasmacytoid cells and thymic dendritic cells. *J. Immunol.* 170, 4926–4932.
- Darrasse-Jéze, G., Deroubaix, S., Mouquet, H., Victoria, G.D., Eisenreich, T., Yao, K., et al., 2009. Feedback control of regulatory T cell homeostasis by dendritic cells in vivo. *J. Exp. Med.* 206, 1853–1862.
- Davis, B.K., Wen, H., Ting, J.P.-Y., 2011. The inflammasome NLRs in immunity, inflammation, and associated diseases. *Annu. Rev. Immunol.* 29, 707–735.
- Delgado-Vega, A., Sánchez, E., Löfgren, S., Castillejo-López, C., Alarcón-Riquelme, M.E., 2010. Recent findings on genetics of systemic autoimmune diseases. *Curr. Opin. Immunol.* 22, 698–705.
- Deng, Y., Tsao, B.P., 2010. Genetic susceptibility to systemic lupus erythematosus in the genomic era. *Nat. Rev. Rheumatol.* 6, 683–692.

- Diana, J., Simoni, Y., Furio, L., Beaudoin, L., Agerberth, B., Barrat, F., et al., 2013. Crosstalk between neutrophils, B-1a cells and plasmacytoid dendritic cells initiates autoimmune diabetes. *Nat. Med.* 19, 65–73.
- Diebold, S.S., 2009. Activation of dendritic cells by toll-like receptors and C-type lectins. *Handb. Exp. Pharmacol.* 188, 3–30.
- Dolcino, M., Ottria, A., Barbieri, A., Patuzzo, G., Tinazzi, E., Argentino, G., et al., 2015. Gene expression profiling in peripheral blood cells and synovial membranes of patients with psoriatic arthritis. *PLoS One* 10, e0128262.
- Drutman, S.B., Trombetta, E.S., 2010. Dendritic cells continue to capture and present antigens after maturation in vivo. *J. Immunol.* 185, 2140–2146.
- Dudziak, D., Kamphorst, A.O., Heidkamp, G.F., Buchholz, V.R., Trumppfeller, C., Yamazaki, S., et al., 2007. Differential antigen processing by dendritic cell subsets in vivo. *Science* 315, 107–111.
- Elinav, E., Strowig, T., Henao-Mejia, J., Flavell, R.A., 2011. Regulation of the antimicrobial response by NLR proteins. *Immunity* 34, 665–679.
- Esashi, E., Wang, Y.-H., Perng, O., Qin, X.-F., Liu, Y.-J., Watowich, S.S., 2008. The signal transducer STAT5 inhibits plasmacytoid dendritic cell development by suppressing transcription factor IRF8. *Immunity* 28, 509–520.
- Ferreira, R.C., Guo, H., Coulson, R.M.R., Smyth, D.J., Pekalski, M.L., Burren, O.S., et al., 2014. A type I interferon transcriptional signature precedes autoimmunity in children genetically at risk for type 1 diabetes. *Diabetes* 63, 2538–2550.
- Ferris, S.T., Carrero, J.A., Mohan, J.F., Calderon, B., Murphy, K.M., Unanue, E.R., 2014. A minor subset of Batf3-dependent antigen-presenting cells in islets of Langerhans is essential for the development of autoimmune diabetes. *Immunity* 41, 657–669.
- Förster, R., Schubel, A., Breitfeld, D., Kremmer, E., Renner-Müller, I., Wolf, E., et al., 1999. CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell* 99, 23–33.
- Gallegos, A.M., Bevan, M.J., 2004. Central tolerance to tissue-specific antigens mediated by direct and indirect antigen presentation. *J. Exp. Med.* 200, 1039–1049.
- Garcia-Romo, G.S., Caielli, S., Vega, B., Connolly, J., Allantaz, F., Xu, Z., et al., 2011. Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. *Sci. Transl. Med.* 3, 73ra20.
- George, T.C., Bilsborough, J., Viney, J.L., Norment, A.M., 2003. High antigen dose and activated dendritic cells enable Th cells to escape regulatory T cell-mediated suppression in vitro. *Eur. J. Immunol.* 33, 502–511.
- Gerner, M.Y., Torabi-Parizi, P., Germain, R.N., 2015. Strategically localized dendritic cells promote rapid T cell responses to lymph-borne particulate antigens. *Immunity* 42, 172–185.
- Ghosh, H.S., Cisse, B., Bunin, A., Lewis, K.L., Reizis, B., 2010. Continuous expression of the transcription factor e2-2 maintains the cell fate of mature plasmacytoid dendritic cells. *Immunity* 33, 905–916.
- Giannoukakis, N., Phillips, B., Trucco, M., 2008. Toward a cure for type 1 diabetes mellitus: diabetes-suppressive dendritic cells and beyond. *Pediatr. Diabetes* 9, 4–13.
- Gilliet, M., Cao, W., Liu, Y.-J., 2008. Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases. *Nat. Rev. Immunol.* 8, 594–606.
- Greter, M., Helft, J., Chow, A., Hashimoto, D., Mortha, A., Agudo-Cantero, J., et al., 2012. GM-CSF controls nonlymphoid tissue dendritic cell homeostasis but is dispensable for the differentiation of inflammatory dendritic cells. *Immunity* 36, 1031–1046.
- Guerrero, A.D., Dong, M.B., Zhao, Y., Lau-Kilby, A., Tarbell, K.V., 2014. Interleukin-2-mediated inhibition of dendritic cell development correlates with decreased CD135 expression and increased monocyte/macrophage precursors. *Immunology* 143, 640–650.
- Guerri, L., Peguillet, I., Geraldo, Y., Nabti, S., Premel, V., Lantz, O., 2013. Analysis of APC types involved in CD4 tolerance and regulatory T cell generation using reaggregated thymic organ cultures. *J. Immunol.* 190, 2102–2110.
- Guilliams, M., van de Laar, L., 2015. A Hitchhiker's guide to myeloid cell subsets: practical implementation of a novel mononuclear phagocyte classification system. *Front. Immunol.* 6, 406.
- Guilliams, M., Ginhoux, F., Jakubzick, C., Naik, S.H., Onai, N., Schraml, B.U., et al., 2014. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nat. Rev. Immunol.* 14, 571–578.
- Guttman-Yassky, E., Nogales, K.E., Krueger, J.G., 2011. Contrasting pathogenesis of atopic dermatitis and psoriasis—part II: immune cell subsets and therapeutic concepts. *J. Allergy Clin. Immunol.* 127, 1420–1432.
- Hacker, C., Kirsch, R.D., Ju, X.-S., Hieronymus, T., Gust, T.C., Kuhl, C., et al., 2003. Transcriptional profiling identifies Id2 function in dendritic cell development. *Nat. Immunol.* 4, 380–386.
- Hawiger, D., Masilamani, R.F., Bettelli, E., Kuchroo, V.K., Nussenzweig, M.C., 2004. Immunological unresponsiveness characterized by increased expression of CD5 on peripheral T cells induced by dendritic cells in vivo. *Immunity* 20, 695–705.
- Heath, W.R., Carbone, F.R., 2001. Cross-presentation in viral immunity and self-tolerance. *Nat. Rev. Immunol.* 1, 126–134.
- Heath, W.R., Belz, G.T., Behrens, G.M.N., Smith, C.M., Forehan, S.P., Parish, I.A., et al., 2004. Cross-presentation, dendritic cell subsets, and the generation of immunity to cellular antigens. *Immunol. Rev.* 199, 9–26.
- Hochrein, H., O'Keeffe, M., 2008. Dendritic cell subsets and toll-like receptors. *Handb. Exp. Pharmacol.* 153–179.
- Hochrein, H., Shortman, K., Vremec, D., Scott, B., Hertzog, P., O'Keeffe, M., 2001. Differential production of IL-12, IFN-alpha, and IFN-gamma by mouse dendritic cell subsets. *J. Immunol.* 166, 5448–5455.
- Hogquist, K.A., Baldwin, T.A., Jameson, S.C., 2005. Central tolerance: learning self-control in the thymus. *Nat. Rev. Immunol.* 5, 772–782.
- Hotta-Iwamura, C., Tarbell, K.V., 2016. Type 1 diabetes genetic susceptibility and dendritic cell function: potential targets for treatment. *J. Leukoc. Biol.* 100, 65–80.
- Ichikawa, E., Hida, S., Omatsu, Y., Shimoyama, S., Takahara, K., Miyagawa, S., et al., 2004. Defective development of splenic and epidermal CD4+ dendritic cells in mice deficient for IFN regulatory factor-2. *Proc. Natl. Acad. Sci. U.S.A.* 101, 3909–3914.
- Idoyaga, J., Fiorese, C., Zbytnuik, L., Lubkin, A., Miller, J., Malissen, B., et al., 2013. Specialized role of migratory dendritic cells in peripheral tolerance induction. *J. Clin. Invest.* 123, 844–854.
- Jenkinson, E.J., Anderson, G., Owen, J.J., 1992. Studies on T cell maturation on defined thymic stromal cell populations in vitro. *J. Exp. Med.* 176, 845–853.
- Kadowaki, N., Antonenko, S., Lau, J.Y., Liu, Y.J., 2000. Natural interferon alpha/beta-producing cells link innate and adaptive immunity. *J. Exp. Med.* 192, 219–226.

- Kaliński, P., Hilkens, C.M., Snijders, A., Snijdewint, F.G., Kapsenberg, M.L., 1997. IL-12-deficient dendritic cells, generated in the presence of prostaglandin E2, promote type 2 cytokine production in maturing human naïve T helper cells. *J. Immunol.* 159, 28–35.
- Karlis, J., Penttila, I., Tran, T.B., Jones, B., Nobbs, S., Zola, H., et al., 2004. Characterization of colonic and mesenteric lymph node dendritic cell subpopulations in a murine adoptive transfer model of inflammatory bowel disease. *Inflamm. Bowel Dis.* 10, 834–847.
- Kasper, L.H., Reder, A.T., 2014. Immunomodulatory activity of interferon-beta. *Ann. Clin. Transl. Neurol.* 1, 622–631.
- Kawai, T., Akira, S., 2007. Signaling to NF-κappaB by Toll-like receptors. *Trends Mol. Med.* 13, 460–469.
- Kawai, T., Akira, S., 2010. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat. Immunol.* 11, 373–384.
- Kawai, T., Akira, S., 2011. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity* 34, 637–650.
- Kawasaki, T., Kawai, T., 2014. Toll-like receptor signaling pathways. *Front. Immunol.* 5, 461.
- Kerrigan, A.M., Dennehy, K.M., Mourão-Sá, D., Faro-Trindade, I., Willment, J.A., Taylor, P.R., et al., 2009. CLEC-2 is a phagocytic activation receptor expressed on murine peripheral blood neutrophils. *J. Immunol.* 182, 4150–4157.
- Khan, S., Greenberg, J.D., Bhardwaj, N., 2009. Dendritic cells as targets for therapy in rheumatoid arthritis. *Nat. Rev. Rheumatol.* 5, 566–571.
- Kingston, D., Schmid, M.A., Onai, N., Obata-Onai, A., Baumjohann, D., Manz, M.G., 2009. The concerted action of GM-CSF and Flt3-ligand on in vivo dendritic cell homeostasis. *Blood* 114, 835–843.
- Klein, L., Kyewski, B., Allen, P.M., Hogquist, K.A., 2014. Positive and negative selection of the T cell repertoire: what thymocytes see (and don't see). *Nat. Rev. Immunol.* 14, 377–391.
- Kubo, A., Nagao, K., Yokouchi, M., Sasaki, H., Amagai, M., 2009. External antigen uptake by Langerhans cells with reorganization of epidermal tight junction barriers. *J. Exp. Med.* 206, 2937–2946.
- Labrecque, N., Baldwin, T., Lesage, S., 2011. Molecular and genetic parameters defining T-cell clonal selection. *Immunol. Cell Biol.* 89, 16–26.
- Lahoud, M.H., Proietto, A.I., Gartlan, K.H., Kitsoulis, S., Curtis, J., Wettenhall, J., et al., 2006. Signal regulatory protein molecules are differentially expressed by CD8+ dendritic cells. *J. Immunol.* 177, 372–382.
- Lau-Kilby, A.W., Kretz, C.C., Pechhold, S., Price, J.D., Dorta, S., Ramos, H., et al., 2011. Interleukin-2 inhibits FMS-like tyrosine kinase 3 receptor ligand (flt3L)-dependent development and function of conventional and plasmacytoid dendritic cells. *Proc. Natl. Acad. Sci. U.S.A.* 108, 2408–2413.
- Lee, J., Breton, G., Oliveira, T.Y.K., Zhou, Y.J., Aljoufi, A., Puhr, S., et al., 2015. Restricted dendritic cell and monocyte progenitors in human cord blood and bone marrow. *J. Exp. Med.* 212, 385–399.
- Lewis, K.L., Caton, M.L., Bogunovic, M., Greter, M., Grajkowska, L.T., Ng, D., et al., 2011. Notch2 receptor signaling controls functional differentiation of dendritic cells in the spleen and intestine. *Immunity* 35, 780–791.
- Lippens, C., Duraes, F.V., Dubrot, J., Brighouse, D., Lacroix, M., Irla, M., et al., 2016. IDO-orchestrated crosstalk between pDCs and Tregs inhibits autoimmunity. *J. Autoimmun.* 75, 39–49.
- Loo, Y.-M., Gale, M., 2011. Immune signaling by RIG-I-like receptors. *Immunity* 34, 680–692.
- Lowes, M.A., Chamian, F., Abello, M.V., Fuentes-Duculan, J., Lin, S.-L., Nussbaum, R., et al., 2005. Increase in TNF-alpha and inducible nitric oxide synthase-expressing dendritic cells in psoriasis and reduction with efalizumab (anti-CD11a). *Proc. Natl. Acad. Sci. U.S.A.* 102, 19057–19062.
- Luber, C.A., Cox, J., Lauterbach, H., Fancke, B., Selbach, M., Tschoopp, J., et al., 2010. Quantitative proteomics reveals subset-specific viral recognition in dendritic cells. *Immunity* 32, 279–289.
- Maldonado-López, R., De Smedt, T., Michel, P., Godfroid, J., Pajak, B., Heirman, C., et al., 1999. CD8alpha+ and CD8alpha- subclasses of dendritic cells direct the development of distinct T helper cells in vivo. *J. Exp. Med.* 189, 587–592.
- Malissen, B., Tamoutounour, S., Henri, S., 2014. The origins and functions of dendritic cells and macrophages in the skin. *Nat. Rev. Immunol.* 14, 417–428.
- Mannon, P.J., Leon, F., Fuss, I.J., Walter, B.A., Begnami, M., Quezado, M., et al., 2009. Successful granulocyte-colony stimulating factor treatment of Crohn's disease is associated with the appearance of circulating interleukin-10-producing T cells and increased lamina propria plasmacytoid dendritic cells. *Clin. Exp. Immunol.* 155, 447–456.
- Mantegazza, A.R., Magalhaes, J.G., Amigorena, S., Marks, M.S., 2013. Presentation of phagocytosed antigens by MHC class I and II. *Traffic* 14, 135–152.
- Maraskovsky, E., Brasel, K., Teepe, M., Roux, E.R., Lyman, S.D., Shortman, K., et al., 1996. Dramatic increase in the numbers of functionally mature dendritic cells in Flt3 ligand-treated mice: multiple dendritic cell subpopulations identified. *J. Exp. Med.* 184, 1953–1962.
- Maravillas-Montero, J.L., Martínez-Cortés, I., 2017. Regulation of immune responses by exosomes derived from antigen presenting cells. *Rev. Alerg. Mex.* 64, 463–476.
- Martinon, F., Tschoopp, J., 2005. NLRs join TLRs as innate sensors of pathogens. *Trends Immunol.* 26, 447–454.
- Matta, B.M., Castellaneta, A., Thomson, A.W., 2010. Tolerogenic plasmacytoid DC. *Eur. J. Immunol.* 40, 2667–2676.
- Mayer-Barber, K.D., Yan, B., 2017. Clash of the cytokine titans: counter-regulation of interleukin-1 and type I interferon-mediated inflammatory responses. *Cell. Mol. Immunol.* 14, 22–35.
- McKenna, H.J., Stocking, K.L., Miller, R.E., Brasel, K., De Smedt, T., Maraskovsky, E., et al., 2000. Mice lacking flt3 ligand have deficient hematopoiesis affecting hematopoietic progenitor cells, dendritic cells, and natural killer cells. *Blood* 95, 3489–3497.
- Means, T.K., Latz, E., Hayashi, F., Murali, M.R., Golenbock, D.T., Luster, A.D., 2005. Human lupus autoantibody-DNA complexes activate DCs through cooperation of CD32 and TLR9. *J. Clin. Invest.* 115, 407–417.
- van Meerwijk, J.P., Marguerat, S., Lees, R.K., Germain, R.N., Fowlkes, B.J., MacDonald, H.R., 1997. Quantitative impact of thymic clonal deletion on the T cell repertoire. *J. Exp. Med.* 185, 377–383.
- Mildner, A., Jung, S., 2014. Development and function of dendritic cell subsets. *Immunity* 40, 642–656.
- Minozzi, S., Bonovas, S., Lytras, T., Pecoraro, V., González-Lorenzo, M., Bastiampillai, A.J., et al., 2016. Risk of infections using anti-TNF agents in rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis: a systematic review and meta-analysis. *Expert Opin. Drug Saf.* 15, 11–34.

- Mohammad Hosseini, A., Majidi, J., Baradaran, B., Yousefi, M., 2015. Toll-like receptors in the pathogenesis of autoimmune diseases. *Adv. Pharm. Bull.* 5, 605–614.
- Mukherjee, G., Dilorenzo, T.P., 2010. The immunotherapeutic potential of dendritic cells in type 1 diabetes. *Clin. Exp. Immunol.* 161, 197–207.
- Mukhopadhyaya, A., Hanafusa, T., Jarchum, I., Chen, Y.-G., Iwai, Y., Serreze, D.V., et al., 2008. Selective delivery of beta cell antigen to dendritic cells in vivo leads to deletion and tolerance of autoreactive CD8+ T cells in NOD mice. *Proc. Natl. Acad. Sci. U.S.A.* 105, 6374–6379.
- Naik, S., Bouladoux, N., Linehan, J.L., Han, S.-J., Harrison, O.J., Wilhelm, C., et al., 2015. Commensal-dendritic-cell interaction specifies a unique protective skin immune signature. *Nature* 520, 104–108.
- Narain, S., Furie, R., 2016. Update on clinical trials in systemic lupus erythematosus. *Curr. Opin. Rheumatol.* 28, 477–487.
- Nestle, F.O., Conrad, C., Tun-Kyi, A., Homey, B., Gombert, M., Boyman, O., et al., 2005. Plasmacytoid predendritic cells initiate psoriasis through interferon-alpha production. *J. Exp. Med.* 202, 135–143.
- Ng, S.C., Kamm, M.A., Stagg, A.J., Knight, S.C., 2010. Intestinal dendritic cells: their role in bacterial recognition, lymphocyte homing, and intestinal inflammation. *Inflamm. Bowel Dis.* 16, 1787–1807.
- Ng, S.C., Benjamin, J.L., McCarthy, N.E., Hedin, C.R.H., Koutsoumpas, A., Plamondon, S., et al., 2011. Relationship between human intestinal dendritic cells, gut microbiota, and disease activity in Crohn's disease. *Inflamm. Bowel Dis.* 17, 2027–2037.
- Obermoser, G., Sontheimer, R.D., Zelger, B., 2010. Overview of common, rare and atypical manifestations of cutaneous lupus erythematosus and histopathological correlates. *Lupus* 19, 1050–1070.
- Ochando, J.C., Homma, C., Yang, Y., Hidalgo, A., Garin, A., Tacke, F., et al., 2006. Alloantigen-presenting plasmacytoid dendritic cells mediate tolerance to vascularized grafts. *Nat. Immunol.* 7, 652–662.
- Osorio, F., Reis e Sousa, C., 2011. Myeloid C-type lectin receptors in pathogen recognition and host defense. *Immunity* 34, 651–664.
- Palucka, A.K., Banchereau, J., Blanco, P., Pascual, V., 2002. The interplay of dendritic cell subsets in systemic lupus erythematosus. *Immunol. Cell Biol.* 80, 484–488.
- Phillips, B., Nylander, K., Harnaha, J., Machen, J., Lakomy, R., Styche, A., et al., 2008. A microsphere-based vaccine prevents and reverses new-onset autoimmune diabetes. *Diabetes* 57, 1544–1555.
- Platt, C.D., Ma, J.K., Chalouni, C., Ebersold, M., Bou-Reslan, H., Carano, R.A.D., et al., 2010. Mature dendritic cells use endocytic receptors to capture and present antigens. *Proc. Natl. Acad. Sci. U.S.A.* 107, 4287–4292.
- Pobezinsky, L.A., Angelov, G.S., Tai, X., Jeurling, S., Van Laethem, F., Feigenbaum, L., et al., 2012. Clonal deletion and the fate of autoreactive thymocytes that survive negative selection. *Nat. Immunol.* 13, 569–578.
- Popov, A., Driesen, J., Abdullah, Z., Wickenhauser, C., Beyer, M., Debey-Pascher, S., et al., 2008. Infection of myeloid dendritic cells with *Listeria monocytogenes* leads to the suppression of T cell function by multiple inhibitory mechanisms. *J. Immunol.* 181, 4976–4988.
- Price, J.D., Tarbell, K.V., 2015. The role of dendritic cell subsets and innate immunity in the pathogenesis of type 1 diabetes and other autoimmune diseases. *Front. Immunol.* 6, 288.
- Price, J.D., Beauchamp, N.M., Rahir, G., Zhao, Y., Rieger, C.C., Lau-Kilby, A.W., et al., 2014. CD8+ dendritic cell-mediated tolerance of auto-reactive CD4+ T cells is deficient in NOD mice and can be corrected by blocking CD40L. *J. Leukoc. Biol.* 95, 325–336.
- Rahman, M.J., Rodrigues, K.B., Quiel, J.A., Liu, Y., Bhargava, V., Zhao, Y., et al., 2018. Restoration of the type I IFN-IL-1 balance through targeted blockade of PTGER4 inhibits autoimmunity in non-obese diabetic mice. *JCI Insight* 3, pii: 97843.
- Rahman, M.J., Rahir, G., Dong, M.B., Zhao, Y., Rodrigues, K.B., Hotta-Iwamura, C., et al., 2016. Despite increased Type 1 IFN, autoimmune nonobese diabetic mice display impaired dendritic cell response to CpG and decreased nuclear localization of IFN-activated STAT1. *J. Immunol.* 196, 2031–2040.
- Randolph, G.J., Angeli, V., Swartz, M.A., 2005. Dendritic-cell trafficking to lymph nodes through lymphatic vessels. *Nat. Rev. Immunol.* 5, 617–628.
- Reis e Sousa, C., Hieny, S., Scharton-Kersten, T., Jankovic, D., Charest, H., Germain, R.N., et al., 1997. In vivo microbial stimulation induces rapid CD40 ligand-independent production of interleukin 12 by dendritic cells and their redistribution to T cell areas. *J. Exp. Med.* 186, 1819–1829.
- Robbins, P.D., Morelli, A.E., 2014. Regulation of immune responses by extracellular vesicles. *Nat. Rev. Immunol.* 14, 195–208.
- Robinson, M.J., Sancho, D., Slack, E.C., LeibundGut-Landmann, S., Reis e Sousa, C., 2006. Myeloid C-type lectins in innate immunity. *Nat. Immunol.* 7, 1258–1265.
- Rönnblom, L., Pascual, V., 2008. The innate immune system in SLE: type I interferons and dendritic cells. *Lupus* 17, 394–399.
- Rönnblom, L., Eloranta, M.-L., Alm, G.V., 2006. The type I interferon system in systemic lupus erythematosus. *Arthritis Rheum.* 54, 408–420.
- Sainathan, S.K., Hanna, E.M., Gong, Q., Bishnupuri, K.S., Luo, Q., Colonna, M., et al., 2008. Granulocyte macrophage colony-stimulating factor ameliorates DSS-induced experimental colitis. *Inflamm. Bowel Dis.* 14, 88–99.
- Salomon, B., Lenschow, D.J., Rhee, L., Ashourian, N., Singh, B., Sharpe, A., et al., 2000. B7/CD28 costimulation is essential for the homeostasis of the CD4+ CD25+ immunoregulatory T cells that control autoimmune diabetes. *Immunity* 12, 431–440.
- Sancho, D., Joffre, O.P., Keller, A.M., Rogers, N.C., Martínez, D., Hernanz-Falcón, P., et al., 2009. Identification of a dendritic cell receptor that couples sensing of necrosis to immunity. *Nature* 458, 899–903.
- Sapoznikov, A., Fischer, J.A.A., Zaft, T., Krauthgamer, R., Dzinek, A., Jung, S., 2007. Organ-dependent in vivo priming of naive CD4+, but not CD8+, T cells by plasmacytoid dendritic cells. *J. Exp. Med.* 204, 1923–1933.
- Schmidt, S.V., Nino-Castro, A.C., Schultze, J.L., 2012. Regulatory dendritic cells: there is more than just immune activation. *Front. Immunol.* 3, 274.
- Schroder, K., Zhou, R., Tschopp, J., 2010. The NLRP3 inflammasome: a sensor for metabolic danger? *Science* 327, 296–300.
- Selenko-Gebauer, N., Majdic, O., Szekeres, A., Höfler, G., Guthann, E., Korthäuer, U., et al., 2003. B7-H1 (programmed death-1 ligand) on dendritic cells is involved in the induction and maintenance of T cell anergy. *J. Immunol.* 170, 3637–3644.
- Sen, P., Bhattacharyya, S., Wallet, M., Wong, C.P., Poligone, B., Sen, M., et al., 2003. NF-kappa B hyperactivation has differential effects on the APC function of nonobese diabetic mouse macrophages. *J. Immunol.* 170, 1770–1780.
- Sestak, A.L., Fürnrohr, B.G., Harley, J.B., Merrill, J.T., Namjou, B., 2011. The genetics of systemic lupus erythematosus and implications for targeted therapy. *Ann. Rheum. Dis.* 70 (Suppl. 1), i37–i43.

- Seth, S., Oberdörfer, L., Hyde, R., Hoff, K., Thies, V., Worbs, T., et al., 2011. CCR7 essentially contributes to the homing of plasmacytoid dendritic cells to lymph nodes under steady-state as well as inflammatory conditions. *J. Immunol.* 186, 3364–3372.
- Sevilla, N., McGavern, D.B., Teng, C., Kunz, S., Oldstone, M.B.A., 2004. Viral targeting of hematopoietic progenitors and inhibition of DC maturation as a dual strategy for immune subversion. *J. Clin. Invest.* 113, 737–745.
- Shortman, K., Heath, W.R., 2010. The CD8+ dendritic cell subset. *Immunol. Rev.* 234, 18–31.
- Soares, H., Waechter, H., Glaichenhaus, N., Mougnéau, E., Yagita, H., Mizenina, O., et al., 2007. A subset of dendritic cells induces CD4+ T cells to produce IFN-gamma by an IL-12-independent but CD70-dependent mechanism in vivo. *J. Exp. Med.* 204, 1095–1106.
- Steinman, R.M., Cohn, Z.A., 1973. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J. Exp. Med.* 137, 1142–1162.
- Steinman, R.M., Witmer, M.D., 1978. Lymphoid dendritic cells are potent stimulators of the primary mixed leukocyte reaction in mice. *Proc. Natl. Acad. Sci. U.S.A.* 75, 5132–5136.
- Steinman, R.M., Kaplan, G., Witmer, M.D., Cohn, Z.A., 1979. Identification of a novel cell type in peripheral lymphoid organs of mice. V. Purification of spleen dendritic cells, new surface markers, and maintenance in vitro. *J. Exp. Med.* 149, 1–16.
- Steinman, R.M., Hawiger, D., Nussenzweig, M.C., 2003. Tolerogenic dendritic cells. *Annu. Rev. Immunol.* 21, 685–711.
- Stoop, J.N., Robinson, J.H., Hilkens, C.M.U., 2011. Developing tolerogenic dendritic cell therapy for rheumatoid arthritis: what can we learn from mouse models? *Ann. Rheum. Dis.* 70, 1526–1533.
- Strober, W., 2009. The multifaceted influence of the mucosal microflora on mucosal dendritic cell responses. *Immunity* 31, 377–388.
- Strober, W., Murray, P.J., Kitani, A., Watanabe, T., 2006. Signalling pathways and molecular interactions of NOD1 and NOD2. *Nat. Rev. Immunol.* 6, 9–20.
- Suzuki, S., Honma, K., Matsuyama, T., Suzuki, K., Toriyama, K., Akitoyo, I., et al., 2004. Critical roles of interferon regulatory factor 4 in CD11b high CD8alpha-dendritic cell development. *Proc. Natl. Acad. Sci. U.S.A.* 101, 8981–8986.
- Swiecki, M., Colonna, M., 2015. The multifaceted biology of plasmacytoid dendritic cells. *Nat. Rev. Immunol.* 15, 471–485.
- Tacken, P.J., de Vries, I.J.M., Torensma, R., Fidgor, C.G., 2007. Dendritic-cell immunotherapy: from ex vivo loading to in vivo targeting. *Nat. Rev. Immunol.* 7, 790–802.
- Tan, S.-Y., Roediger, B., Weninger, W., 2015. The role of chemokines in cutaneous immunosurveillance. *Immunol. Cell Biol.* 93, 337–346.
- Tarbell, K.V., Egen, J.G., 2018. Breaking self-tolerance during autoimmunity and cancer immunity: myeloid cells and type I interferon response regulation. *J. Leukoc. Biol.* Available from: <https://doi.org/10.1002/JLB.3MIR1017-400R>.
- Tarbell, K.V., Yamazaki, S., Olson, K., Toy, P., Steinman, R.M., 2004. CD25+ CD4+ T cells, expanded with dendritic cells presenting a single autoantigenic peptide, suppress autoimmune diabetes. *J. Exp. Med.* 199, 1467–1477.
- Tarbell, K.V., Petit, L., Zuo, X., Toy, P., Luo, X., Mqadmi, A., et al., 2007. Dendritic cell-expanded, islet-specific CD4+ CD25+ CD62L+ regulatory T cells restore normoglycemia in diabetic NOD mice. *J. Exp. Med.* 204, 191–201.
- Trinchieri, G., 2010. Type I interferon: friend or foe? *J. Exp. Med.* 207, 2053–2063.
- Trombetta, E.S., Mellman, I., 2005. Cell biology of antigen processing in vitro and in vivo. *Annu. Rev. Immunol.* 23, 975–1028.
- Turley, S., Poirot, L., Hattori, M., Benoist, C., Mathis, D., 2003. Physiological beta cell death triggers priming of self-reactive T cells by dendritic cells in a type-1 diabetes model. *J. Exp. Med.* 198, 1527–1537.
- Varol, C., Zigmond, E., Jung, S., 2010. Securing the immune tightrope: mononuclear phagocytes in the intestinal lamina propria. *Nat. Rev. Immunol.* 10, 415–426.
- Villadangos, J.A., Schnorrer, P., 2007. Intrinsic and cooperative antigen-presenting functions of dendritic-cell subsets in vivo. *Nat. Rev. Immunol.* 7, 543–555.
- Villadangos, J.A., Young, L., 2008. Antigen-presentation properties of plasmacytoid dendritic cells. *Immunity* 29, 352–361.
- Villani, A.-C., Lemire, M., Fortin, G., Louis, E., Silverberg, M.S., Collette, C., et al., 2009. Common variants in the NLRP3 region contribute to Crohn's disease susceptibility. *Nat. Genet.* 41, 71–76.
- Villani, A.-C., Satija, R., Reynolds, G., Sarkizova, S., Shekhar, K., Fletcher, J., et al., 2017. Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. *Science* 356, pii: eaah4573.
- Vremec, D., Pooley, J., Hochrein, H., Wu, L., Shortman, K., 2000. CD4 and CD8 expression by dendritic cell subtypes in mouse thymus and spleen. *J. Immunol.* 164, 2978–2986.
- Wallet, M.A., Sen, P., Tisch, R., 2005. Immunoregulation of dendritic cells. *Clin. Med. Res.* 3, 166–175.
- Weaver, D.J., Poligone, B., Bui, T., Abdel-Motal, U.M., Baldwin, A.S., Tisch, R., 2001. Dendritic cells from nonobese diabetic mice exhibit a defect in NF-kappa B regulation due to a hyperactive I kappa B kinase. *J. Immunol.* 167, 1461–1468.
- Wilson, N.S., Villadangos, J.A., 2005. Regulation of antigen presentation and cross-presentation in the dendritic cell network: facts, hypothesis, and immunological implications. *Adv. Immunol.* 86, 241–305.
- Wu, L., Shortman, K., 2005. Heterogeneity of thymic dendritic cells. *Semin. Immunol.* 17, 304–312.
- Wu, L., D'Amico, A., Winkel, K.D., Suter, M., Lo, D., Shortman, K., 1998. RelB is essential for the development of myeloid-related CD8alpha-dendritic cells but not of lymphoid-related CD8alpha+ dendritic cells. *Immunity* 9, 839–847.
- Yamazaki, S., Iyoda, T., Tarbell, K., Olson, K., Velinzon, K., Inaba, K., et al., 2003. Direct expansion of functional CD25+ CD4+ regulatory T cells by antigen-processing dendritic cells. *J. Exp. Med.* 198, 235–247.
- Zaba, L.C., Krueger, J.G., Lowes, M.A., 2009. Resident and "inflammatory" dendritic cells in human skin. *J. Invest. Dermatol.* 129, 302–308.
- Zaki, M.H., Lamkanfi, M., Kanneganti, T.-D., 2011. The Nlrp3 inflammasome: contributions to intestinal homeostasis. *Trends Immunol.* 32, 171–179.
- Zhang, J.-G., Czabotar, P.E., Policheni, A.N., Caminschi, I., Wan, S.S., Kitsoulis, S., et al., 2012. The dendritic cell receptor Clec9A binds damaged cells via exposed actin filaments. *Immunity* 36, 646–657.
- Zheng, Y., Manzotti, C.N., Liu, M., Burke, F., Mead, K.I., Sansom, D.M., 2004. CD86 and CD80 differentially modulate the suppressive function of human regulatory T cells. *J. Immunol.* 172, 2778–2784.

## 12

# Natural Killer Cells

Yenan T. Bryceson<sup>1</sup>, Niklas K. Björkström<sup>1,2</sup>, Jenny Mjösberg<sup>1</sup> and Hans-Gustaf Ljunggren<sup>1</sup>

<sup>1</sup>Department of Medicine, Center for Infectious Medicine, Karolinska Institute, Karolinska University Hospital, Stockholm, Sweden <sup>2</sup>Liver Immunology Laboratory, Department of Medicine, Karolinska Institute, Karolinska University Hospital, Stockholm, Sweden

## OUTLINE

Introduction to Natural Killer Cells	229	Natural Killer Cell Cytolytic Granule Exocytosis	234
Natural Killer Cell Development and Differentiation	230	Natural Killer Cell Chemokine and Cytokine Production	235
Phenotype and Tissue Localization	231	Natural Killer Cells and Human Autoimmunity	235
Functional Responses by Natural Killer Cells	232	Defective Control of other Immune Cells Links	
Natural Killer Cell Receptor Signaling and Effector Functions	232	Natural Killer Cells to Autoimmune Diseases	236
Natural Killer Cell Contact and Adhesion to Target Cells	233	Genetic Association Studies Revealing Links Between Natural Killer Cells and Autoimmune Diseases	237
Natural Killer Cell Lytic Granule Polarization and Maturation	234	Conclusions	238
		References	238

## INTRODUCTION TO NATURAL KILLER CELLS

Natural killer (NK) cells were described in the 1970s as large granular lymphocytes exhibiting “natural cytotoxicity” against several types of tumor cells (Kiessling et al., 1975). Significant progress has been made since then in the dissection of additional functional traits of NK cells (See chapter 18). By their cytotoxic potential, they can kill many more types of cells than tumor cells, for example, virus-infected cells and several types of activated immune cells (Vivier et al., 2008). To carry out these functions, and to discriminate target cells from nonactivated “normal” cells, NK cells are equipped with a molecular detection system that includes a variety of cell surface–activating and inhibitory receptors (Lanier, 2005). Activating NK cell receptors can detect the presence of ligands on cells in “distress,” such as stress-induced self-ligands (Bryceson et al., 2006a). In parallel, NK cells use inhibitory receptors to monitor the presence of constitutively expressed self-major histocompatibility complex (MHC) class I molecules (Long, 2008); MHC class I expressing cells are normally spared while cells lacking these molecules can be targeted by NK cells, a recognition strategy originally referred to as “missing-self” recognition (Ljunggren and Kärre, 1990). NK cells are also critical components of the innate immune response, because of their ability to produce a variety of cytokines and chemokines (Vivier et al., 2008). Thus NK cells may drive, shape, and regulate the activities of other immune cells and by these means

affect the development of adaptive immune responses. NK cells respond to initial cues from myeloid cells including myeloid-derived dendritic cells (DCs), as well as macrophages and plasmacytoid DC. This response is possible because NK cells express receptors for several myeloid-derived cytokines (Caligiuri, 2008; Vivier et al., 2008). Thus secondary to triggering of myeloid cells by pattern recognition receptors, NK cells can relay and amplify key cytokine signals. Perhaps the best characterized cytokine produced by NK cells is interferon (IFN)- $\gamma$ . However, NK cells also secrete a number of other additional cytokines and other factors, including tumor necrosis factor (TNF)- $\alpha$  and immunoregulatory cytokines such as interleukin (IL)-5, IL-10, IL-13, the growth factor GM-CSF, and chemokines CCL3 (MIP-1 $\alpha$ ), CCL4 (MIP-1 $\beta$ ), CCL5 (RANTES), and CXCL8 (IL-8). The ability to respond to a variety of cytokines suggests that the local microenvironment in which NK cells exist may shape and modulate their function (Caligiuri, 2008; Vivier et al., 2008). Consistent with their functions as innate sentinels, NK cells are spread throughout lymphoid and nonlymphoid tissues. In many tissues, they represent a minor fraction of total lymphocytes. However, in other tissues such as the liver and pregnant uterus they are abundant (Vivier et al., 2008).

Although important aspects of NK cell biology have been revealed using mice as a model organism, it should be noted that many central NK cell receptors are rapidly evolving in evolutionary terms. Thus significant differences between the mouse and human immune systems exist, particularly with respect to NK cells (Mestas and Hughes, 2004). Our review is focused on the phenotype and function of human NK cells, highlighting recent advances in understanding their role in autoimmune diseases.

## NATURAL KILLER CELL DEVELOPMENT AND DIFFERENTIATION

NK cells belong to the hematopoietic system. They are derived from CD34 $^{+}$  hematopoietic precursor cells (HPCs). In transcriptional terms, NK cells are most closely related to cytotoxic T lymphocytes (Sun and Lanier, 2011) and in humans typically defined as CD3 CD56 $^{+}$  lymphocytes. According to a newly proposed nomenclature, NK cells have been categorized as belonging to the family of innate lymphoid cells (ILCs) and more specifically the group 1 ILCs (Spits et al., 2013). In addition to differential transcriptional regulation, NK cells are distinguishable from other ILCs as NK cells do not express CD127. Furthermore, ILCs lack cytotoxic function as well as several functional NK cell markers including NKG2A and NKG2D. However, the exact relationships and plasticity between the NK cells and other ILCs remain to be further elucidated, especially in humans (Spits et al., 2013).

During fetal development, NK cells are among the first immune cells to appear. It is generally accepted that most NK cells develop in the bone marrow, based on evidence from early studies of bone-marrow ablation in mice (Haller and Wigzell, 1977). Bone marrow–derived human CD34 $^{+}$  HPCs can also be manipulated to differentiate *in vitro* into NK cells (Miller et al., 1992). In this setting, NK cell development is dependent on bone-marrow stroma (Miller et al., 1994). Furthermore, it requires IL-2 and/or IL-15 (Miller et al. 1992, 1994) and is potentiated by flt3-ligands (Yu et al., 1998). One important question is, do NK cell precursors, formed in the bone marrow, traffic to distinct peripheral anatomical locations for their final stages of development? Several lines of research support this notion; for example, immune precursor cells with NK cell developmental potential are selectively enriched in human secondary lymphoid tissues (SLT), such as lymph nodes and tonsil (Freud et al., 2005). These CD34 $^{+}$ CD45RA $^{+}$  cells represent more than 95% of all HPCs in SLT, but are infrequent in bone marrow and blood.

Based on this, and other insights, a continuous SLT NK cell developmental pathway has been proposed (Freud et al., 2006; Freud and Caligiuri, 2006). In this pathway, “stage 1” NK cells (pro-NK cells) are characterized by a CD34 $^{+}$ CD117 CD94 phenotype, the lack of expression and transcription of CD122 (IL-2/15 receptor  $\beta$ -chain), and unresponsiveness to IL-15. Upon stimulation with flt3-ligand, IL-3, and IL-7, possibly in a stroma cell-dependent manner, these pro-NK cells acquire a “stage 2” NK cell (pre-NK cells) phenotype characterized by upregulation of CD122 that provides responsiveness to IL-15 (Freud et al., 2006). CD34 $^{+}$  CD117 $^{+}$  CD94 pre-NK cells are not fully committed to the NK cell lineage and can also give rise to other immune cells such as T cells and myeloid DCs. Furthermore, they lack characteristic NK cell functions, such as cytotoxicity and the capacity to produce IFN- $\gamma$ . However, with IL-15 stimulation, pre-NK cells develop into “stage 3” NK cells [immature NK (iNK) cells] characterized by loss of CD34 and a step-wise acquisition of CD56 together with a functional commitment to the NK cell lineage. Finally, these iNK cells progress into “stage 4” NK cells characterized by bright expression of CD56 (CD56 $^{\text{bright}}$  NK cells). This progression is marked by the acquisition of the inhibitory receptor CD94/NKG2A and such NK cell-associated activating receptors as NKp46 and NKG2D,

as well as by the capacity to produce IFN- $\gamma$  and to release perforin and granzyme-containing granules (Freud et al., 2006). NK cell expression of killer cell immunoglobulin-like receptors (KIRs) for self-MHC class I confers NK cells with effector potential in a yet molecularly poorly defined process termed “education” or “licensing” (Kim et al., 2005; Anfossi et al., 2006; Elliott and Yokoyama, 2011). At present, many open questions remain with respect to how NK cell function relates to development and to what extent NK cell differentiation can be regarded as linear or branched.

Key transcription factors that regulate NK cell development and differentiation have recently been characterized (Klose et al., 2012; Cichocki et al., 2013). STAT5B has been implicated in the early stages of NK cell differentiation, alongside E4BP4 that induces ID2 and GATA3. Without any one of these transcription factors, NK cell development is grossly impaired with a block at the pre-NK to iNK stage. Similarly, but in an apparently parallel pathway, TOX is required for the pre-NK to iNK transition. TBX21 (T-bet) is also required for early NK cell development, while expression of EOMES is required for sustenance of NK cells in a mature stage. Importantly, NK cell selective deficiencies have been associated with autosomal dominant mutations in *GATA2* and *MCM4* (Gineau et al., 2012; Mace et al., 2013). Much more information is needed to get a better view of how transcription factors program NK cell development and differentiation.

## PHENOTYPE AND TISSUE LOCALIZATION

In humans, “mature” NK cells are divided into CD56<sup>bright</sup> and CD56<sup>dim</sup> subsets, defined by expression levels of CD56 (Lanier et al., 1986; Jacobs et al., 1992; Cooper et al., 2001; Caligiuri, 2008). These two subsets are present at varying proportions in different compartments of the human body. In blood, bone marrow, and spleen, CD56<sup>dim</sup> NK cells dominate, representing around 90% of all NK cells. On the other hand, in tonsils and other SLT, CD56<sup>bright</sup> NK cells dominate. The liver, in contrast, contains nearly equal proportions of CD56<sup>dim</sup> and CD56<sup>bright</sup> NK cells. The CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cell subsets differ in phenotype and function. In brief, CD56<sup>bright</sup> NK cells are negative for, or express only low levels of, the low-affinity Fc-receptor CD16, express few inhibitory KIRs, or cytotoxic molecules such as perforin and different granzymes (Cooper et al., 2001). On the other hand, CD56<sup>bright</sup> NK cells uniformly express the inhibitory CD94/NKG2A receptor, as well as CD62L and the high-affinity IL-2 receptor  $\alpha$  chain (CD25). CD56<sup>dim</sup> NK cells display a variegated expression of KIRs, NKG2A, and CD62L, express high levels of CD16 on their surface, and contain intracellular granules with large amounts of perforin and granzymes (Cooper et al., 2001). With respect to function, CD56<sup>bright</sup> NK cells were generally believed to produce large amounts of cytokines but have little capacity to perform cytotoxicity, whereas CD56<sup>dim</sup> NK cells were believed to be efficient killers but poor at producing cytokines (Fehniger et al., 2003; Ferlazzo et al., 2004a,b; Morandi et al., 2006; Burt et al., 2009). However, recent work suggests that CD56<sup>bright</sup> NK cells also can mediate some cytotoxicity and produce cytokines in response to exogenous cytokine stimuli, whereas CD56<sup>dim</sup> NK cells both have efficient capacities for performing cytotoxicity and producing cytokines upon specific target-cell recognition (Fauriat et al., 2010).

CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cell subsets might represent two sequential stages of NK cell differentiation, with CD56<sup>bright</sup> cells being less mature (Lanier et al., 1986; Caligiuri, 2008). Several lines of (sometimes indirect) evidence support this hypothesis. First, CD56<sup>bright</sup> NK cells appear earlier in immune reconstitution settings than do CD56<sup>dim</sup> NK cells, and the former can represent up to 70%–80% of all peripheral blood NK cells a few months after hematopoietic stem-cell transplantation (Jacobs et al., 1992; Shilling et al., 2003). Second, CD56<sup>bright</sup> NK cells have a more immature phenotype compared to CD56<sup>dim</sup> NK cells with, for instance, expression of CD117 (c-kit) (Matos et al., 1993), which is typically expressed by immature HPCs. CD56<sup>bright</sup> NK cells also display longer telomeres (25) and, morphologically, constitute both granular and agranular cells, whereas all CD56<sup>dim</sup> NK cells are granular (Romagnani et al., 2007). Furthermore, in humanized mice, purified CD56<sup>bright</sup> NK cells were recently shown to differentiate into CD56<sup>dim</sup> NK cells (Huntington et al., 2009). Hence, a substantial amount of evidence suggests CD56<sup>bright</sup> (stage 4) NK cells to be precursors of CD56<sup>dim</sup> (stage 5) NK cells. Until recently, CD56<sup>dim</sup> NK cells were considered an “end stage” of human NK cell differentiation. However, recent data have demonstrated that even CD56<sup>dim</sup> NK cells undergo a continuous differentiation process, the latter characterized by a gradual shift from NKG2A<sup>+</sup> CD62L<sup>+</sup> CD57 KIR cells toward more terminally differentiated NKG2A CD62L CD57<sup>+</sup> KIR<sup>+</sup> cells (Björkström et al., 2010).

Besides CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cells, which constitute the majority of NK cells in human peripheral blood and tissues at variable relative frequencies, a distinct population of CD56<sup>superbright</sup> NK cells has been characterized in the endometrium which becomes the dominant lymphocyte population in the decidua of pregnant

women (Manaster and Mandelboim 2010). Such uterine NK cells may represent relatively iNK cells and display a unique phenotype, expressing the tetraspanin CD9, high frequencies of activation markers CD69 and HLA-DR, as well as inhibitory NKG2A and KIR. Interestingly, they do not express CD16 or CD57.

## FUNCTIONAL RESPONSES BY NATURAL KILLER CELLS

NK cells and cytotoxic T cells (CTLs) are thought to share mechanisms for target-cell elimination. Cytotoxicity by both NK cells and CTLs relies on directed release of their lytic granule contents. These lytic granules are specialized secretory lysosomes that contain perforin, granzymes, and Fas ligand that all contribute to target-cell killing (Dustin and Long, 2010; Griffiths et al., 2010). Imaging studies comparing target-cell recognition by NK cells and CTLs in vitro and in vivo suggest some noteworthy distinctions. In vitro, CTLs rapidly establish cytoskeletal polarity, whereas NK cells are more tentative in their interactions with target cells (Wulfing et al., 2003). In vivo, CTLs form stable contacts with tumor cells expressing cognate antigen, whereas NK cells establish mainly dynamic contacts (Deguine et al., 2010). These observations suggest differences in the molecular machinery underlying NK cell and CTL recognition and elimination of target cells.

Recent studies of NK cell responses have also highlighted considerable heterogeneity among human peripheral blood NK cells, including hierarchies that govern the strength of activation stimuli needed for induction of specific responses (Bryceson et al., 2005, 2009, 2011). For example, induction of inside-out signals for leukocyte functional antigen (LFA)-1-mediated adhesion exhibits a markedly low threshold for activation. Induction of chemokines such as MIP-1 $\beta$  requires stronger activating stimuli, whereas degranulation and production of cytokines such as TNF- $\alpha$  and IFN- $\gamma$  display the most stringent requirements for induction (Bryceson et al., 2011). Some of the functional heterogeneity in different NK cell populations can be accounted for by differences in cellular differentiation (Björkström et al., 2010; Juelke et al., 2010; Lopez-Verges et al., 2010). For example, relatively immature CD56<sup>bright</sup> NK cells excel at cytokine production in response to exogenous cytokines such as IL-2, IL-12, IL-15, and IL-18. The same cells, however, express low levels of perforin and are, consequentially, less cytotoxic than CD56<sup>dim</sup> NK cells and do not produce cytokines as readily in response to target-cell recognition. In contrast, relatively more differentiated (NKG2A<sup>+</sup> CD62L<sup>+</sup> CD57 KIR) CD56<sup>dim</sup> NK cells produce significant amounts of IFN- $\gamma$  in response to exogenous cytokines, express higher levels of perforin, and have a stronger ability to mediate cytotoxicity. They can also produce ample amounts of cytokines in response to target-cell recognition. Finally, highly differentiated (NKG2A CD62L CD57<sup>+</sup> KIR<sup>+</sup>) CD56<sup>dim</sup> NK cells express high levels of perforin and display potent cytotoxic capacity. These cells are also strong producers of cytokines in response to target-cell recognition. However, the response of these NK cells to exogenous cytokines is comparatively blunted.

In parallel to the continuous maturation process, recognition of self-MHC class I molecules by inhibitory receptors potentiates the NK cell's ability to respond functionally, a process referred to as "education" or "licensing" (Kim et al., 2005; Anfossi et al., 2006; Elliott and Yokoyama, 2011). Superimposing differentiation and education processes on NK cell functionality do not, however, fully explain the heterogeneity in NK cell responses. Studies have revealed that the thresholds for effector responses are highly dynamic. For example, different molecular pathways are likely used depending on activation by different cytokines and triggering of different (combinations of) cellular receptors and gives rise to differences in response rates (Bryceson et al., 2009, 2011).

## NATURAL KILLER CELL RECEPTOR SIGNALING AND EFFECTOR FUNCTIONS

A multitude of activating NK cell receptors that belong to different receptor families have been described (Table 12.1). They contain highly divergent cytoplasmic signaling domains. The signaling pathways orchestrated by some activating NK cell receptors are known, whereas others are significantly less well characterized (Bryceson et al., 2006a; Watzl and Long, 2010). In contrast, structurally distinct inhibitory NK cell receptors all contain immunoreceptor tyrosine-based inhibition motifs (ITIMs). The signaling by such motifs has been extensively studied and is mainly mediated through activation of tyrosine phosphatases such as SHP-1 as well as the tyrosine kinase c-Abl (Long, 2008; Peterson and Long, 2008). Some inhibitory receptors engage other negative regulators such as SHIP, an inositol 5-phosphatase, and the Src family tyrosine kinase CSK. Taken together, today's knowledge suggests that potent NK cell effector functions such as cytotoxicity and

**TABLE 12.1** Specificity and Signaling of Human Natural Killer (NK) Cell Receptors

Receptor	Signaling	Cellular ligand	Function
FcgRIIIa (CD16)	Activation (ITAM)	IgG	Elimination of ADCC
NKp30 (CD337)	Coactivation (ITAM)	B7-H6	NK cell-myeloid cell cross-talk, tumor recognition
NKp44 (CD336)	Activation (ITAM)	?	?
NKp46 (CD335)	Coactivation (ITAM)	?	Surveillance of mitotic cells
KIR (CD158a, b, etc.)	Activation (ITAM)	HLA class I	?
CD94/NKG2C (CD159c)	Activation (ITAM)	HLA-E	?
NKG2D (CD314)	Coactivation (YxNM)	ULBP, MICA, MICB	Surveillance of tumor cells and genotoxic stress
NKp80	? (hemi-ITAM)	AICL	NK cell-myeloid cell cross-talk
DNAM-1 (CD226)	Coactivation	CD112, CD155	Surveillance of tissue integrity
2B4 (CD244)	Coactivation (ITSM)	CD48	Interaction with hematopoietic cells
CRACC (CD319)	Coactivation (ITSM)	CRACC (CD319)	Interaction with hematopoietic cells
NTB-A	Coactivation (ITSM)	NTB-A	Interaction with hematopoietic cells
CD2	Coactivation	CD58	Interaction with hematopoietic and endothelial cells
KIR2DL4 (CD158d)	?	HLA-G (soluble)	Trophoblast-induced vascular remodeling
LFA-1 (CD11a/CD18)	Granule polarization	ICAM	Recruitment and activation during inflammation, efficient cytotoxicity
KIR (CD158)	Inhibition (ITIM)	HLA class I alleles	Assess loss of MHC class I alleles
LIR1, LILR1 (CD85j)	Inhibition (ITIM)	HLA class I	Assess loss of MHC class I expression
CD94/NKG2A (CD159a)	Inhibition (ITIM)	HLA-E	Gauge MHC class I expression
KLRG1	Inhibition (ITIM)	E-cadherin	Assess loss of tissue integrity
NKR-P1 (CD161)	Inhibition (ITIM)	LLT1	?
LAIR-1 (CD305)	Inhibition (ITIM)	Collagen	Control activation in extracellular matrix
Siglec-7 (CD328)	Inhibition (ITIM)	Sialic acid	?
Siglec-9 (CD329)	Inhibition (ITIM)	Sialic acid	?
IRP60 (CD300a)	Inhibition (ITIM)	?	?

ADCC, Antibody coated cells; ITIM, immunoreceptor tyrosine-based inhibition motif; MHC, major histocompatibility complex; NK, natural killer.

cytokine production require dynamic integration of signals derived from multiple receptors (Liu et al., 2009). The following sections will focus on advances in our understanding of the molecular processes underlying NK cell effector responses.

## Natural Killer Cell Contact and Adhesion to Target Cells

A first step in NK cell responses to pathogen-infected target cells or tumor cells involves recruitment of NK cells to the site of inflammation/infection or to the tumor. NK cells express several chemokine receptors. The mechanism of NK cell trafficking to inflamed tissues is fairly well understood and has recently been reviewed elsewhere (Gregoire et al., 2007). During inflammation, NK cells can be recruited by chemokines that bind the CCR2, CCR5, CXCR3, and CX3CR1 chemokine receptors. However, the degree to which NK cells constitutively traffic in normal tissues is relatively less well understood. How NK cells detect and discriminate target cells has recently been studied quite extensively. The initial contact between NK cells and target cells may involve any of a number of different receptors, leading to adhesion mediated by the integrin LFA-1. Activating receptors, including CD16, 2B4, NKG2D, DNAM-1, as well as LFA-1 itself, can rapidly induce necessary inside-out signals for the activation of LFA-1 in freshly isolated human NK cells, promoting the initial signals required for adhesion (Bryceson et al., 2009). As opposed to activating receptors, inhibitory receptor signals can abrogate adhesion to

target cells (Burshtyn et al., 2000; Bryceson et al., 2009). The signals for induction of inside-out activation of LFA-1 are not well defined. As the activating NK cell receptors have distinct cytoplasmic domains, their signaling cascades differ. Live cell imaging of NK cells on supported planar lipid bilayers carrying ligands for LFA-1, NKG2D, 2B4, and CD16 has shown that, indeed, these receptors induce quite diverse behaviors by NK cells (Liu et al., 2009). In regards to signals for inside-out activation of LFA-1, phosphorylation and activation of VAV1 have been postulated to be a common denominator of signaling pathways downstream of activating receptors including LFA-1 itself. Concomitantly, VAV1 phosphorylation provides a point at which inhibitory receptor signals can oppose signals from activating receptors. Specifically, phosphorylated VAV1 is a substrate of SHP-1 that is recruited by inhibitory NK cell receptors and acts to prevent NK activation (Stebbins et al., 2003). Several other signaling proteins, including WASP, Cdc42, Ras, Rap1, CrkL, and HS1, have been implicated in signaling for adhesion.

### Natural Killer Cell Lytic Granule Polarization and Maturation

Besides adhesion, LFA-1 has a key role in promoting perforin-containing granule polarization toward the target cell, facilitating efficient cytotoxicity (Barber et al., 2004; Bryceson et al., 2005). Moreover, such granule polarization toward the immune synapse is the result of two different molecular processes. First, granules rapidly converge in dynein-dependent, minus-end directed motion to the microtubule-organizing center (MTOC) (Mentlik et al., 2010). In the NK cell line YTS, antibody-mediated blockade of LFA-1 impairs granule convergence at the MTOC upon target-cell contact, suggesting that LFA-1-mediated signals facilitate this process (Mentlik et al., 2010). Second, following convergence of the granules and within minutes, the MTOC and granules polarize toward the interaction site in an LFA-1-dependent manner (Bryceson et al., 2005; Mentlik et al., 2010). In regards to the signaling pathways involved in LFA-1-mediated polarization, several key proteins have been identified. Talin, binding to the cytoplasmic tail of LFA-1, is required for the recruitment of WASP, Arp2/3, vinculin, and actin (Mace et al., 2009, 2010). Furthermore, WASP is required for the accumulation of F-actin at the synapse. Moreover, silencing of WASP interacting protein (WIP) in the human NK cell line YTS impaired lytic granule polarization but had no effect on the formation of conjugates with target cells (Krzewski et al., 2008). The second messenger, diacylglycerol, was required for MTOC polarization and cytotoxicity by CTL (Quann et al., 2009); however, the extent to which diacylglycerol regulates LFA-1-mediated MTOC polarization in NK cells remains to be established. Recent evidence indicated that signaling for granule polarization by LFA-1 in IL-2-expanded human NK cells also involved CD3 $\zeta$ -chain phosphorylation, SYK recruitment and activation, and PLC- $\gamma$  activation (March and Long 2011). Activation of PLC- $\gamma$  resulted in hydrolysis of phosphatidylinositol (4,5)-bisphosphate (PIP2) to generate the second messengers, diacylglycerol and inositol (1,4,5)-trisphosphate (IP3). Downstream of LFA-1, PLC- $\gamma$  activation led to PKC activation, and pharmacological inhibition of PLC- $\gamma$  abrogated granule polarization (March and Long 2011). Upon recognition of susceptible target cells, the tyrosine kinase PYK2 was also recruited to the immune synapse, and transfection of dominant negative PYK2 blocked MTOC and paxillin movement to the synapse (Sancho et al., 2000). In summary, a number of studies suggest pathways involving talin, WASP, WIP, PYK, JNK, and paxillin, as well as CD3 $\zeta$ , SYK, and PLC- $\gamma$  for granule polarization.

### Natural Killer Cell Cytolytic Granule Exocytosis

Vesicle exocytosis is a requirement for NK cell cytotoxicity (Bryceson et al., 2006b). Studies of knockout mice have demonstrated an essential role for PLC- $\gamma$  in granule exocytosis (Tassi et al., 2005; Caraux et al., 2006). Following the activation of PLC- $\gamma$ , IP3 can trigger cytoplasmic release of Ca<sup>2+</sup> from the endoplasmic reticulum (ER). Engagement of CD16 is sufficient to induce robust intracellular Ca<sup>2+</sup> mobilization, whereas several other receptors do not. Rather, coactivation receptors trigger intracellular Ca<sup>2+</sup> mobilization when engaged in specific pair-wise combinations (Bryceson et al., 2006b). Additional data suggest that the molecular basis for such coactivation involves complementary phosphorylation of SLP-76 as well as overcoming a threshold for activation of VAV1 that is set by the ubiquitin ligase c-Cbl (Kim et al., 2010). PLC- $\gamma$  activation leads to IP3-mediated Ca<sup>2+</sup> release from the ER, which ultimately results in depletion of intracellular Ca<sup>2+</sup> stores and induction of store-operated Ca<sup>2+</sup> entry (SOCE). NK cells from patients with mutations in either STIM1 or ORAI1 display defective degranulation, demonstrating a requirement for ORAI1 channel-mediated SOCE for lytic granule exocytosis (Maul-Pavicic et al., 2011). Notably, the ORAI1 deficiency does not affect signals for adhesion or granule

polarization (Maul-Pavicic et al., 2011). Further downstream, several proteins required for NK cell exocytosis have been identified through studies of patients with hyperinflammatory syndromes caused by defective lymphocyte cytotoxicity. These proteins include Rab27a that facilitates terminal trafficking of lytic granules to sites of exocytosis, Munc13-4 that primes lytic granule exocytosis, as well as syntaxin-11 and Munc18-2 that facilitate membrane fusion (Stinchcombe et al., 2001; Feldmann et al., 2003; Bryceson et al., 2007; Wood et al., 2011). Furthermore, results suggest that distinct endosomal compartments fuse prior to lytic granule fusion with the plasma membrane, facilitating the exocytic process (Menager et al., 2007; Wood et al., 2009). Finally, for efficient cytotoxicity, lytic granules must traverse the actin-rich immunological synapse to enable exocytosis. The adenosine triphosphate-dependent actin motor protein myosin IIA has been shown to associate with lytic granules in NK cells and play a role in mediating lytic granule traversal for exocytosis (Sanborn et al., 2009).

## Natural Killer Cell Chemokine and Cytokine Production

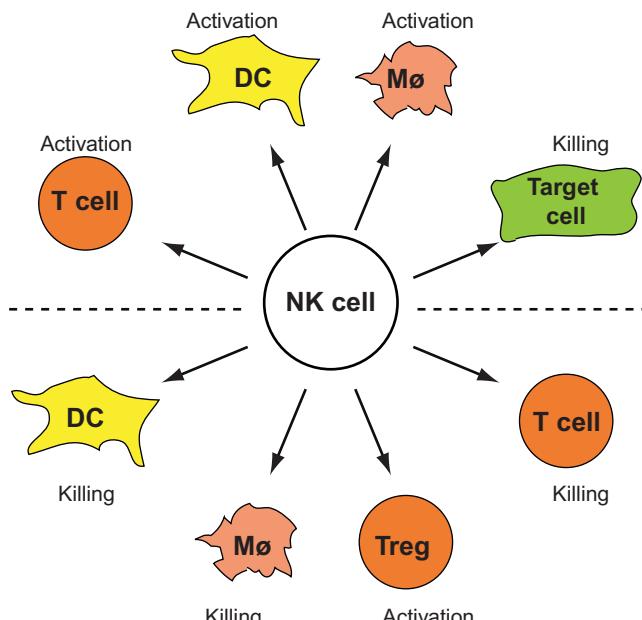
Target-cell recognition by freshly isolated human NK cells induces a set of chemokines, including CCL3, CCL4, CCL5, as well as the cytokines TNF- $\alpha$  and IFN- $\gamma$  (Fauriat et al., 2010). Chemokines are induced within 1 hour of stimulation, whereas secretion occurs several hours after activation. Importantly, experiments varying the signaling input for NK cell activation have revealed a hierarchy in requirements for the induction of chemokines and cytokines, with chemokines induced by weakly activating signals, degranulation induced by intermediate levels of activating stimuli, and cytokines requiring the strongest activation. This hierarchy is reflected in the requirements for induction of different effector responses. PLC- $\gamma$  is required for all responses (Tassi et al., 2005; Caraux et al., 2006). A deficiency in SOCE, as seen in NK cells from humans with autosomal recessive STIM1 and ORAI1 mutations, results in defective degranulation and cytokine production induced upon target-cell recognition but only partially impairs chemokine production (Maul-Pavicic et al., 2011). Notably, NK cells from PI3K p110 $\delta$ -deficient mice displayed selectively impaired cytokine production, whereas knockout of both p110 $\delta$  and p110 $\gamma$  was required to impair cytotoxicity (Kim et al., 2007; Tassi et al., 2007). Moreover, PKC $\theta$ -deficient mice had defects in IFN- $\gamma$  transcription and secretion due to impaired JNK, AP-1, and NFAT activation (Tassi et al., 2008). Yet, NK cell-mediated cytotoxicity was not impaired in PKC $\theta$ -deficient mice. Curiously, a Rap1b deficiency in mice selectively deters NK cell chemokine and cytokine production, but not cytotoxicity (Awasthi et al., 2010). Thus a few proteins, including PI3K p110 $\delta$  and PKC $\theta$ , may be specifically required for transcription of cytokine genes. Further studies are required to understand how these proteins are integrated in the signaling pathways for NK cell activation and how the engagement of different activating receptor controls their function.

## NATURAL KILLER CELLS AND HUMAN AUTOIMMUNITY

Classically, autoimmune diseases typify syndromes caused by an inappropriate activation of cells of the adaptive immune system, that is, T cells and B cells, resulting in cell-specific, organ-specific, or systemic tissue damage. Typical examples of such diseases include multiple sclerosis, rheumatoid arthritis, and insulin-dependent type 1 diabetes. Autoimmune diseases often display sexual bias. In this respect, several studies indicate somewhat higher NK cell numbers and activity in men than in women, but these differences often do not reach statistical significance with studies of relatively small sample sizes. Autoimmune diseases have been thought to be initiated in steps, including release of self-antigens from the target organ, a priming step in secondary lymphoid organs, and finally immune cell homing to the target organ/tissue and subsequent tissue destruction (Ji et al., 1999). NK cells can likely act at all these steps, that is, as mediators of initial target damage leading to release of self-antigens, at the level of T-cell priming in secondary lymphoid organs, and at the level of the target organ as immunomodulatory or effector cells (Pazmany, 2005; Shi and van Kaer, 2006; Perricone et al., 2008; Flodström-Tullberg et al., 2009) (Fig. 12.1).

Most research results pointing toward a role for NK cells in human autoimmune diseases are, obviously, correlative. Associations between NK cell activity and autoimmune conditions appear in several reports, including those describing studies finding altered numbers, phenotypes, and functions of NK cells (Shi and van Kaer, 2006; Pazmany, 2005; Perricone et al., 2008; Flodström-Tullberg et al., 2009). Some older studies are, however, difficult to interpret since they fail to distinguish NK cells from regulatory NKT cells or T cells expressing NK cell-associated receptors. An additional problem is determining if abnormalities in NK cell populations are a cause and/or an effect of a given disease state. Finally, most studies rely on assessment of NK cells from

### Promotion of autoimmune disease



### Protection from autoimmune disease

**FIGURE 12.1** NK cells may affect autoimmune diseases in many different ways, for example by promoting or preventing functions of other cells, or by killing them. The figure illustrates possible interactions between NK cells and other cells as well as their outcomes. NK, Natural killer. Source: Figure modified from Flodström-Tullberg, M., Bryceson, Y.T., Shi, F.D., Höglund, P.H., Ljunggren, H.G., 2009. Natural killer cells in human autoimmunity. *Curr. Opin. Immunol.* 21, 634–640.

peripheral blood, not from appropriate secondary lymphoid organs or affected tissues. Despite these shortcomings a substantial amount of evidence supports a direct involvement of NK cells in human autoimmunity.

### Defective Control of other Immune Cells Links Natural Killer Cells to Autoimmune Diseases

The impaired NK cell cytotoxic function frequently observed in some humans with autoimmune diseases and/or other inflammatory conditions highlights the role of NK cells in controlling other cells, for example, macrophages. Macrophage activation syndrome (MAS) is associated with several rheumatic diseases, most commonly with systemic juvenile idiopathic arthritis (sjIA) ([Villanueva et al., 2005](#); [Kelly and Ramanan, 2007](#)) (Chapter 37: The Autoimmune Myopathies). Clinically, this syndrome closely resembles familial hemophagocytic lymphohistiocytosis (FHL), a life-threatening genetic disorder in which NK cell function is absent or depressed ([Bryceson and Ljunggren, 2007](#); [Wood et al., 2011](#)). As in FHL, patients with MAS have profoundly decreased NK cell cytotoxic activity ([Ravelli et al., 2012](#)). Therefore impaired NK cell cytotoxicity might be, at least in part, causative of MAS. Supporting this notion, an sjIA patient with low NK cell cytotoxic function was found to harbor compound heterozygous splice-site and missense mutations in UNC13D, a gene associated with FHL ([Hazen et al., 2008](#)). Another link between NK cells and macrophages comes from studies showing that CD56<sup>bright</sup> NK cells accumulate in certain inflammatory lesions and, in the appropriate cytokine environment, can engage with monocytes in a reciprocal activation fashion, thereby amplifying the inflammatory response. Moreover, CD56<sup>bright</sup> NK cells can trigger the differentiation of monocytes into DCs ([Zhang et al., 2007](#)). Such positive feedback loops could well promote the pathogenesis of chronic inflammatory conditions such as rheumatoid arthritis ([Dalbeth et al., 2004](#)). Moreover, naturally occurring mutations in genes that cause a defect in NK cells are found in patients with abnormalities in TAP-peptide transporters, who typically suffer from chronic infections in childhood and autoimmune manifestations later in life. Notably, although interactions with macrophages are particularly relevant to organ-specific autoimmunity, NK cells also modulate innate and adaptive immunity by interacting with many other cell types, for example, other lymphocytes including effector

and regulatory T cells (Carnaud et al., 1999; Assarsson et al., 2004; Ferlazzo et al., 2004a,b; Martin-Fontecha et al., 2004; Ghiringhelli et al., 2006; Shanker et al., 2007).

A specific point at which NK cells cytotoxicity can act to modify immune responses is through killing of activated T cells. Interestingly, upon cytomegalovirus infection in mice, NK cells can kill activated CD4<sup>+</sup> T cells, thereby affecting CD8<sup>+</sup> T-cell function and exhaustion (Waggoner et al., 2011). In a mouse model of arthritis, blockade of the NK cell receptor NKG2A facilitated NK cell elimination of pathogenic CD4<sup>+</sup> T cells, arresting disease progression (Leavenworth et al., 2011). Such NK cell–mediated modulation of immune responses may well have implications for human autoimmunity. Moreover, NK cells may limit responses through modes other than cellular cytotoxicity. In studies of lupus, at least in vitro, NK cells can potentiate IFN- $\alpha$  production by plasmacytoid DC by up to a thousand-fold. IFN- $\alpha$  is a key cytokine involved in driving the pathogenesis of the disease (Eloranta et al., 2009). The NK cell–mediated enhancement of IFN- $\alpha$  production by plasmacytoid DC is contact dependent and partially relies on secretion of the chemokine CCL4 (MIP-1 $\beta$ ) by NK cells (Hagberg et al., 2011). Thus NK cell immunoregulatory interactions may also exacerbate autoimmune responses.

Although less is known in contexts of autoimmunity, roles for NK cells as inducers of organ-specific and systemic tissue damage have also recently been suggested in certain acute and chronic viral infections. For instance, during flares of chronic hepatitis B virus infection, NK cells have been implicated to mediate liver damage via the apoptosis-inducing molecule TRAIL (Dunn et al., 2007). During acute human hantavirus infection, a hemorrhagic fever-inducing virus infection with high mortality, NK cells are highly activated and damage arises to the vasculature system long after the virus has been cleared from the body (Björkström et al., 2011).

## Genetic Association Studies Revealing Links Between Natural Killer Cells and Autoimmune Diseases

Linkage analysis in several autoimmune disease conditions has implicated various NK cell receptors in pathogenesis (Kulkarni et al., 2008). For example, certain *KIR* receptor genes, the products of which are expressed on NK cells and some T-cell subsets, have been associated with the development of autoimmune diseases. *KIR* genes are polymorphic and the *KIR* gene complex is polygenic, encoding varying numbers of inhibitory and activating receptors. Interactions of the independently segregating *KIR* and HLA loci are important for NK cell education/licensing and target-cell recognition (Jonsson and Yokoyama, 2009). The existence of *KIR*–HLA genotypes that tune NK cells toward activation are favorable in some infectious diseases but might also predispose to autoimmunity (Kulkarni et al., 2008). A number of associations predisposing to autoimmune diseases have been identified in which activating *KIR* or *KIR*/HLA genotypes are accompanied by lack of inhibition. For example, scleroderma, a disease of tissue fibrosis, inflammation, and vascular injury, has been linked to the presence of activating *KIR2DS2*, in the absence of a corresponding inhibitory *KIR2DL2* (Momot et al., 2004). Likewise, the risk of psoriatic arthritis is enhanced in patients carrying *KIR2DS1* and/or *KIR2DS2*, and this effect is augmented in the absence of ligands for the inhibitory *KIR2DL1* and *KIR2DL2/3*, respectively (Nelson et al., 2004). Similarly, for rheumatoid arthritis, increased risk is associated with a presence of the *KIR2DS2* gene (Yen et al., 2001). Type 1 diabetes is associated with the expression of *KIR2DS2* HLA ligand pairs in an environment of weak inhibitory interactions (van der Slik et al., 2003, 2007). In primary sclerosing cholangitis, a claimed autoimmune liver disease characterized by progressive destruction of the biliary tract, homozygosity for HLA-C1, a ligand for *KIR2DL3*, is associated with increased susceptibility for disease (Karlsen et al., 2007; Hov et al., 2010). Finally, ankylosing spondylitis shows a strong relationship to HLA-B27, a ligand for *KIR3DL2*; NK cells from patients with this disease have predominant *KIR3DL2* expression and show an activated phenotype as measured by CD38 expression (Chan et al., 2005). Thus the interplay between human NK cell inhibitory and activating receptor-MHC class I ligand pairs has provided significant evidence with respect to the involvement of NK cells in human autoimmunity. Noteworthy, however, the function of *KIR* may in some instances also be exerted at the level of autoimmune T cells, for example, through augmentation of low-affinity activation signals (Björkström et al., 2012).

In addition to receptors that bind MHC class I molecules, a number of other receptors that bind stress-induced ligands or regulate interactions with other immune cells may dictate NK cell specificity. Inappropriate expression of activating receptor NKG2D ligands can lead to detrimental NK cell activation, which, in turn, may trigger or exacerbate autoimmunity. The best characterized NKG2D ligands are the MICA/B cellular stress-induced molecules that act as danger signals to alert NK cells and CD8T lymphocytes (Bauer et al., 1999). Acquisition of MICA/B ligands on the surfaces of potential target cells may cause the breakdown of tolerance. MICA/B molecules, when inappropriately expressed, may thus trigger or worsen an autoimmune response. The involvement

of NKG2D and its ligands in autoimmunity was first revealed in patients with rheumatoid arthritis (Groh et al., 2003). It has also been demonstrated that MICA/B are overexpressed on gut epithelium during active celiac disease (Hüe et al., 2004). However, in situations of MICA/B upregulation, these molecules may be targets not only for NK cells but also for T cells, as demonstrated in several studies (Meresse et al., 2004). Polymorphisms of human MICA have been associated with a variety of autoimmune disorders. One such example is patients with primary sclerosing cholangitis in whom several studies have identified the *MICA\*008* allele in association with increased disease susceptibility (Norris et al., 2001; Wiencke et al., 2001). The functional implications of such polymorphism are, however, far from clear, and *MICA* associations frequently reflect a linkage disequilibrium with *HLA-B* alleles (Field et al., 2008). Still to be established is whether mutations in the NKG2D gene (*KLRK1*) might underlie some autoimmune diseases. Recently, however, a single-nucleotide polymorphism (SNP) in *CD226*, conferring an amino acid substitution in the DNAM-1 receptor, was linked to the development of multiple sclerosis, rheumatoid arthritis, type 1 diabetes, and autoimmune thyroid disease (Hafler et al., 2009). In addition, two intronic SNPs in *CD244*, that likely increase 2B4 receptor surface expression, have been connected with the development of rheumatoid arthritis in a Japanese cohort (Suzuki et al., 2008). However, in a mouse model of lupus, *Cd244*-deficiency exacerbated autoantibody production in an NK cell-independent manner (Brown et al., 2011). Together, these data implicate activating NK cell receptors in susceptibility to multiple autoimmune diseases. We should note, however, that despite the often-used term “NK cell receptors,” these receptors are also expressed on other lymphocyte populations, and it remains to be established to what degree genetic associations are conferred by alterations specifically in NK cell function.

## CONCLUSIONS

With the exception of genetic linkage analysis, relatively few studies have been undertaken to explore the role of NK cells in human autoimmunity compared to other diseases where NK cells are more directly implicated. Much knowledge on the role of NK cells in autoimmunity still comes from studies in experimental animal models, and caution must be taken before translating those results directly to human conditions. The emerging view, however, is that NK cells may function as important regulatory cells during the priming phase of adaptive immune responses and as regulatory and effector cells at the sites of target organ during inflammation. NK cell control of macrophage activation may be one important checkpoint at the level of the target organ in the development of autoimmunity. The ability of NK cells to influence adaptive immune responses and to control immune cell homeostasis in humans clearly deserves further attention. Improved comprehension of how NK cells affect numerous autoimmune conditions may furthermore pave the way for new immunotherapeutic approaches toward alleviating or preventing such diseases.

## References

- Anfossi, N., Andre, P., Guia, S., Falk, C.S., Roetynck, S., Stewart, C.A., et al., 2006. Human NK cell education by inhibitory receptors for MHC class I. *Immunity* 25, 331–342.
- Assarsson, E., Kambayashi, T., Schatzle, J.D., Cramer, S.O., von Bonin, A., Jensen, P.E., et al., 2004. NK cells stimulate proliferation of T and NK cells through 2B4/CD48 interactions. *J. Immunol.* 173, 174–180.
- Awasthi, A., Samarakoon, A., Chu, H., Kamalakkannan, R., Quilliam, L.A., Chrzanowska-Wodnicka, M., et al., 2010. Rap1b facilitates NK cell functions via IQGAP1-mediated signalosomes. *J. Exp. Med.* 207, 1923–1938.
- Barber, D.F., Faure, M., Long, E.O., 2004. LFA-1 contributes an early signal for NK cell cytotoxicity. *J. Immunol.* 173, 3653–3659.
- Bauer, S., Groh, V., Wu, J., Steinle, A., Phillips, J.H., Lanier, L.L., et al., 1999. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science* 285, 727–729.
- Björkström, N.K., Riese, P., Heuts, F., Andersson, S., Fauriat, C., Ivarsson, M.A., et al., 2010. Expression patterns of NKG2A, KIR, and CD57 define a process of CD56dim NK cell differentiation uncoupled from NK cell education. *Blood* 116, 3853–3864.
- Björkström, N.K., Lindgren, T., Stoltz, M., Fauriat, C., Braun, M., Evander, M., et al., 2011. Rapid expansion and long-term persistence of elevated NK cell numbers in humans infected with hantavirus. *J. Exp. Med.* 208, 13–21.
- Björkström, N.K., Beziat, V., Chihocki, F., Liu, L.L., Levine, J., Larsson, S., et al., 2012. CD8T cells express randomly selected KIRs with distinct specificities compared with NK cells. *Blood* 120, 3455–3465.
- Brown, D.R., Calpe, S., Keszei, M., Wang, N., McArdel, S., Terhorst, C., et al., 2011. Cutting edge: an NK cell-independent role for Slamf4 in controlling humoral autoimmunity. *J. Immunol.* 187, 21–25.
- Bryceson, Y.T., Ljunggren, H.G., 2007. Lymphocyte effector functions: armed for destruction? *Curr. Opin. Immunol.* 19, 337–338.
- Bryceson, Y.T., March, M.E., Barber, D.F., Ljunggren, H.G., Long, E.O., 2005. Cytolytic granule polarization and degranulation controlled by different receptors in resting NK cells. *J. Exp. Med.* 202, 1001–1012.

- Bryceson, Y.T., March, M.E., Ljunggren, H.G., Long, E.O., 2006a. Activation, coactivation, and costimulation of resting human natural killer cells. *Immunol. Rev.* 214, 73–91.
- Bryceson, Y.T., March, M.E., Ljunggren, H.G., Long, E.O., 2006b. Synergy among receptors on resting NK cells for the activation of natural cytotoxicity and cytokine secretion. *Blood* 107, 159–166.
- Bryceson, Y.T., Rudd, E., Zheng, C., Edner, J., Ma, D., Wood, S.M., et al., 2007. Defective cytotoxic lymphocyte degranulation in syntaxin-11 deficient familial hemophagocytic lymphohistiocytosis 4 (FHL4) patients. *Blood* 110, 1906–1915.
- Bryceson, Y.T., Ljunggren, H.G., Long, E.O., 2009. Minimal requirement for induction of natural cytotoxicity and intersection of activation signals by inhibitory receptors. *Blood* 114, 2657–2666.
- Bryceson, Y.T., Chiang, S.C., Darmanin, S., Fauriat, C., Schlums, H., Theorell, J., et al., 2011. Molecular mechanisms of natural killer cell activation. *J. Innate Immun.* 3, 216–226.
- Burshtyn, D.N., Shin, J., Stebbins, C., Long, E.O., 2000. Adhesion to target cells is disrupted by the killer cell inhibitory receptor. *Curr. Biol.* 10, 777–780.
- Burt, B.M., Plitas, G., Zhao, Z., Bamboat, Z.M., Nguyen, H.M., Dupont, B., et al., 2009. The lytic potential of human liver NK cells is restricted by their limited expression of inhibitory killer Ig-like receptors. *J. Immunol.* 183, 1789–1796.
- Caligiuri, M.A., 2008. Human natural killer cells. *Blood* 112, 461–469.
- Caraux, A., Kim, N., Bell, S.E., Zompi, S., Ranson, T., Lesjean-Pottier, S., et al., 2006. Phospholipase C-gamma2 is essential for NK cell cytotoxicity and innate immunity to malignant and virally infected cells. *Blood* 107, 994–1002.
- Carnaud, C., Lee, D., Donnars, O., Park, S.H., Beavis, A., Koezuka, Y., et al., 1999. Cutting edge: Cross-talk between cells of the innate immune system: NKT cells rapidly activate NK cells. *J. Immunol.* 163, 4647–4650.
- Chan, A.T., Kollnberger, S.D., Wedderburn, L.R., Bowness, P., 2005. Expansion and enhanced survival of natural killer cells expressing the killer immunoglobulin-like receptor KIR3DL2 in spondylarthritis. *Arthritis Rheum.* 52, 3586–3595.
- Cichocki, F., Miller, J.S., Anderson, S.K., Bryceson, Y.T., 2013. Epigenetic regulation of NK cell differentiation and effector functions. *Front. Immunol.* 4, 55.
- Cooper, M.A., Fehniger, T.A., Turner, S.C., Chen, K.S., Ghaheri, B.A., Ghayur, T., et al., 2001. Human natural killer cells: a unique innate immunoregulatory role for the CD56 (bright) subset. *Blood* 97, 3146–3151.
- Dalbeth, N., Gundle, R., Davies, R.J., Lee, Y.C., McMichael, A.J., Callan, M.F., 2004. CD56bright NK cells are enriched at inflammatory sites and can engage with monocytes in a reciprocal program of activation. *J. Immunol.* 173, 6418–6426.
- Deguine, J., Breart, B., Lemaitre, F., Di Santo, J.P., Bousso, P., 2010. Intravital imaging reveals distinct dynamics for natural killer and CD8(1) T cells during tumor regression. *Immunity* 33, 632–644.
- Dunn, C., Brunetto, M., Reynolds, G., Christophides, T., Kennedy, P.T., Lampertico, P., et al., 2007. Cytokines induced during chronic hepatitis B virus infection promote a pathway for NK cell-mediated liver damage. *J. Exp. Med.* 204, 667–680.
- Dustin, M.L., Long, E.O., 2010. Cytotoxic immunological synapses. *Immunol. Rev.* 235, 24–34.
- Elliott, J.M., Yokoyama, W.M., 2011. Unifying concepts of MHC-dependent natural killer education. *Trends Immunol.* 32, 364–372.
- Eloranta, M.L., Lövgren, T., Finke, D., Mathsson, L., Rönnelid, J., Kastner, B., et al., 2009. Regulation of the interferon-alpha production induced by RNA-containing immune complexes in plasmacytoid dendritic cells. *Arthritis Rheum.* 60, 2418–2427.
- Fauriat, C., Long, E.O., Ljunggren, H.G., Bryceson, Y.T., 2010. Regulation of human NK-cell cytokine and chemokine production by target cell recognition. *Blood* 115, 2167–2176.
- Fehniger, T.A., Cooper, M.A., Nuovo, G.J., Cella, M., Facchetti, F., Colonna, M., et al., 2003. CD56bright natural killer cells are present in human lymph nodes and are activated by T cell-derived IL-2: a potential new link between adaptive and innate immunity. *Blood* 101, 3052–3057.
- Feldmann, J., Callebaut, I., Raposo, G., Certain, S., Bacq, D., Dumont, C., et al., 2003. Munc13-4 is essential for cytolytic granules fusion and is mutated in a form of familial hemophagocytic lymphohistiocytosis (FHL3). *Cell* 115, 461–473.
- Ferlazzo, G., Pack, M., Thomas, D., Paludan, C., Schmid, D., Strowig, T., et al., 2004a. Distinct roles of IL-12 and IL-15 in human natural killer cell activation by dendritic cells from secondary lymphoid organs. *Proc. Natl. Acad. Sci. U.S.A.* 101, 16606–16611.
- Ferlazzo, G., Thomas, D., Lin, S.L., Goodman, K., Morandi, B., Muller, W.A., et al., 2004b. The abundant NK cells in human secondary lymphoid tissues require activation to express killer cell Ig-like receptors and become cytolytic. *J. Immunol.* 172, 1455–1462.
- Field, S.F., Nejentsev, S., Walker, N.M., Howson, J.M., Godfrey, L.M., Jolley, J.D., et al., 2008. Sequencing-based genotyping and association analysis of the MICA and MICB genes in type 1 diabetes. *Diabetes* 57, 1753–1756.
- Flodström-Tullberg, M., Bryceson, Y.T., Shi, F.D., Höglund, P.H., Ljunggren, H.G., 2009. Natural killer cells in human autoimmunity. *Curr. Opin. Immunol.* 21, 634–640.
- Freud, A.G., Caligiuri, M.A., 2006. Human natural killer cell development. *Immunol. Rev.* 214, 56–72.
- Freud, A.G., Becknell, B., Roychowdhury, S., Mao, H.C., Ferketich, A.K., Nuovo, G.J., et al., 2005. A human CD34(1) subset resides in lymph nodes and differentiates into CD56bright natural killer cells. *Immunity* 22, 295–304.
- Freud, A.G., Yokohama, A., Becknell, B., Lee, M.T., Mao, H.C., Ferketich, A.K., et al., 2006. Evidence for discrete stages of human natural killer cell differentiation in vivo. *J. Exp. Med.* 203, 1033–1043.
- Ghiringhelli, F., Ménard, C., Martin, F., Zitvogel, L., 2006. The role of regulatory T cells in the control of natural killer cells: relevance during tumor progression. *Immunol. Rev.* 214, 229–238.
- Gineau, L., Cognet, C., Kara, N., Lach, F.P., Dunne, J., Veturi, U., et al., 2012. Partial MCM4 deficiency in patients with growth retardation, adrenal insufficiency, and natural killer cell deficiency. *J. Clin. Invest.* 122, 821–832.
- Gregoire, C., Chasson, L., Luci, C., Tomasello, E., Geissmann, F., Vivier, E., et al., 2007. The trafficking of natural killer cells. *Immunol. Rev.* 220, 169–182.
- Griffiths, G.M., Tsun, A., Stinchcombe, J.C., 2010. The immunological synapse: a focal point for endocytosis and exocytosis. *J. Cell Biol.* 189, 399–406.
- Groh, V., Bruhl, A., El-Gabalawy, H., Nelson, J.L., Spies, T., 2003. Stimulation of T cell autoreactivity by anomalous expression of NKG2D and its MIC ligands in rheumatoid arthritis. *Proc. Natl. Acad. Sci. U.S.A.* 100, 9452–9457.

- Hafler, J.P., Maier, L.M., Cooper, J.D., Plagnol, V., Hinks, A., Simmonds, M.J., et al., 2009. CD226 Gly307Ser association with multiple autoimmune diseases. *Genes Immun.* 10, 5–10.
- Hagberg, J.I., Berggren, O., Leonard, D., Weber, G., Bryceson, Y.T., Alm, G.V., et al., 2011. IFN- $\alpha$  production by plasmacytoid dendritic cells stimulated with RNA-containing immune complexes is promoted by NK cells via MIP-1 $\beta$  and LFA-1. *J. Immunol.* 186, 5085–5094.
- Haller, O., Wigzell, H., 1977. Suppression of natural killer cell activity with radioactive strontium: effector cells are marrow dependent. *J. Immunol.* 118, 1503–1506.
- Hazen, M.M., Woodward, A.L., Hofmann, I., Degar, B.A., Grom, A., Filipovich, A.H., et al., 2008. Mutations of the hemophagocytic lymphohistiocytosis-associated gene UNC13D in a patient with systemic juvenile idiopathic arthritis. *Arthritis Rheum.* 58, 567–570.
- Hov, J.R., Lleo, A., Selmi, C., Woldseth, B., Fabris, L., Strazzabosco, M., et al., 2010. Genetic associations in Italian primary sclerosing cholangitis: heterogeneity across Europe defines a critical role for HLA-C. *J. Hepatol.* 52, 712–717.
- Hüe, S., Mention, J.J., Monteiro, R.C., Zhang, S., Cellier, C., Schmitz, J., et al., 2004. A direct role for NKG2D/MICA interaction in villous atrophy during celiac disease. *Immunity* 21, 367–377.
- Huntington, N.D., Legrand, N., Alves, N.L., Jaron, B., Weijer, K., Plet, A., et al., 2009. IL-15 trans-presentation promotes human NK cell development and differentiation in vivo. *J. Exp. Med.* 206, 25–34.
- Jacobs, R., Stoll, M., Stratmann, G., Leo, R., Link, H., Schmidt, R.E., 1992. CD16–CD56+ natural killer cells after bone marrow transplantation. *Blood* 79, 3239–3244.
- Ji, H., Korganow, A.S., Mangialao, S., Höglund, P., André, I., Lühder, F., et al., 1999. Different modes of pathogenesis in T-cell-dependent auto-immunity: clues from two TCR transgenic systems. *Immunol. Rev.* 169, 139–146.
- Jonsson, A.H., Yokoyama, W.M., 2009. Natural killer cell tolerance licensing and other mechanisms. *Adv. Immunol.* 101, 27–79.
- Juelke, K., Killig, M., Luetke-Eversloh, M., Parente, E., Gruen, J., Morandi, B., et al., 2010. CD62L expression identifies a unique subset of poly-functional CD56dim NK cells. *Blood* 116, 1299–1307.
- Karlsen, T.H., Boberg, K.M., Olsson, M., Sun, J.Y., Senitzer, D., Bergquist, A., et al., 2007. *J. Hepatol.* 46, 899–906.
- Kelly, A., Ramanan, A.V., 2007. Recognition and management of macrophage activation syndrome in juvenile arthritis. *Curr. Opin. Rheumatol.* 19, 477–481.
- Kiessling, R., Klein, E., Wigzell, H., 1975. “Natural” killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. *Eur. J. Immunol.* 5, 112–117.
- Kim, H.S., Das, A., Gross, C.C., Bryceson, Y.T., Long, E.O., 2010. Synergistic signals for natural cytotoxicity are required to overcome inhibition by c-Cbl ubiquitin ligase. *Immunity* 32, 175–186.
- Kim, N., Saudemont, A., Webb, L., Camps, M., Rucke, T., Hirsch, E., et al., 2007. The p110delta catalytic isoform of PI3K is a key player in NK-cell development and cytokine secretion. *Blood* 110, 3202–3208.
- Kim, S., Poursine-Laurent, J., Truscott, S.M., Lybarger, L., Song, Y.J., Yang, L., et al., 2005. Licensing of natural killer cells by host major histocompatibility complex class I molecules. *Nature* 436, 709–713.
- Klose, C.S., Hoyler, T., Kiss, E.A., Tanriver, Y., Diefenbach, A., 2012. Transcriptional control of innate lymphocyte fate decisions. *Curr. Opin. Immunol.* 24, 290–296.
- Krzewski, K., Chen, X., Strominger, J.L., 2008. WIP is essential for lytic granule polarization and NK cell cytotoxicity. *Proc. Natl. Acad. Sci. U.S.A.* 105, 2568–2573.
- Kulkarni, S., Martin, M.P., Carrington, M., 2008. The Yin and Yang of HLA and KIR in human disease. *Semin. Immunol.* 20, 343–352.
- Lanier, L.L., 2005. NK cell recognition. *Ann. Rev. Immunol.* 23, 225–274.
- Lanier, L.L., Le, A.M., Civin, C.I., Loken, M.R., Phillips, J.H., 1986. The relationship of CD16 (Leu-11) and Leu-19 (NKH-1) antigen expression on human peripheral blood NK cells and cytotoxic T lymphocytes. *J. Immunol.* 136, 4480–4486.
- Leavenworth, J.W., Wang, X., Wenander, C.S., Spee, P., Cantor, H., 2011. Mobilization of natural killer cells inhibits development of collagen-induced arthritis. *Proc. Natl. Acad. Sci. U.S.A.* 108, 14584–14589.
- Liu, D., Bryceson, Y.T., Meckel, T., Vasiliver-Shamis, G., Dustin, M.L., Long, E.O., 2009. Integrin-dependent organization and bidirectional vesicular traffic at cytotoxic immune synapses. *Immunity* 31, 99–109.
- Ljunggren, H.G., Kärre, K., 1990. In search of the “missing self”: MHC molecules and NK cell recognition. *Immunol. Today* 11, 237–244.
- Long, E.O., 2008. Negative signaling by inhibitory receptors: the NK cell paradigm. *Immunol. Rev.* 224, 70–84.
- Lopez-Verges, S., Milush, J.M., Pandey, S., York, V.A., Arakawa-Hoyt, J., Pircher, H., et al., 2010. CD57 defines a functionally distinct population of mature NK cells in the human CD56dimCD16+ NK-cell subset. *Blood* 116, 3865–3874.
- Mace, E.M., Monkley, S.J., Critchley, D.R., Takei, F., 2009. A dual role for talin in NK cell cytotoxicity: activation of LFA-1-mediated cell adhesion and polarization of NK cells. *J. Immunol.* 182, 948–956.
- Mace, E.M., Zhang, J., Siminovitch, K.A., Takei, F., 2010. Elucidation of the integrin LFA-1-mediated signaling pathway of actin polarization in natural killer cells. *Blood* 116, 1272–1279.
- Mace, E.M., Hsu, A.P., Monaco-Shawver, L., Makedonas, G., Rosen, J.B., Dropulic, L., et al., 2013. Mutations in GATA2 cause human NK cell deficiency with specific loss of the CD56(bright) subset. *Blood* 121, 2669–2677.
- Manaster, I., Mandelboim, O., 2010. The unique properties of uterine NK cells. *Am. J. Reprod. Immunol.* 63, 434–444.
- March, M.E., Long, E.O., 2011. b2 Integrin induces TCRz-Syk-phospholipase C-g phosphorylation and paxillin-dependent granule polarization in human NK cells. *J. Immunol.* 186, 2998–3005.
- Martin-Fontecha, A., Thomsen, L.L., Brett, S., Gerard, C., Lipp, M., Lanzavecchia, A., et al., 2004. Induced recruitment of NK cells to lymph nodes provides IFN-gamma for Th1 priming. *Nat. Immunol.* 5, 1260–1265.
- Matos, M.E., Schnier, G.S., Beecher, M.S., Ashman, L.K., William, D.E., Caligiuri, M.A., 1993. Expression of a functional c-kit receptor on a subset of natural killer cells. *J. Exp. Med.* 178, 1079–1084.
- Maul-Pavlic, A., Chiang, S.C.C., Rensing-Ehl, A., Jessen, B., Fauriat, C., Wood, S.M., et al., 2011. ORAI1-mediated calcium influx is required for human cytotoxic lymphocyte degranulation and target cell lysis. *Proc. Natl. Acad. Sci. U.S.A.* 108, 3324–3329.
- Menager, M.M., Menasche, G., Romao, M., Knapnougel, P., Ho, C.H., Garfa, M., et al., 2007. Secretory cytotoxic granule maturation and exocytosis require the effector protein hMunc13-4. *Nat. Immunol.* 8, 257–267.

- Mentlik, A.N., Sanborn, K.B., Holzbaur, E.L., Orange, J.S., 2010. Rapid lytic granule convergence to the MTOC in natural killer cells is dependent on dynein but not cytolytic commitment. *Mol. Biol. Cell* 21, 2241–2256.
- Meresse, B., Chen, Z., Ciszewski, C., Tretiakova, M., Bhagat, G., Krausz, T.N., et al., 2004. Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease. *Immunity* 21, 357–366.
- Mestas, J., Hughes, C.C., 2004. Of mice and not men: differences between mouse and human immunology. *J. Immunol.* 172, 2731–2738.
- Miller, J.S., Verfaillie, C., McGlave, P., 1992. The generation of human natural killer cells from CD34 + /DR – primitive progenitors in long-term bone marrow culture. *Blood* 80, 2182–2187.
- Miller, J.S., Alley, K.A., McGlave, P., 1994. Differentiation of natural killer (NK) cells from human primitive marrow progenitors in a stroma-based long-term culture system: identification of a CD34 1 7 1 NK progenitor. *Blood* 83, 2594–2601.
- Momot, T., Koch, S., Hunzelmann, N., Krieg, T., Ulbricht, K., Schmidt, R.E., et al., 2004. Association of killer cell immunoglobulin-like receptors with scleroderma. *Arthritis Rheum.* 50, 1561–1565.
- Morandi, B., Bougras, G., Muller, W.A., Ferlazzo, G., Münz, C., 2006. NK cells of human secondary lymphoid tissues enhance T cell polarization via IFN-gamma secretion. *Eur. J. Immunol.* 36, 2394–2400.
- Nelson, G.W., Martin, M.P., Gladman, D., Wade, J., Trowsdale, J., Carrington, M., 2004. Cutting edge: heterozygote advantage in autoimmune disease: hierarchy of protection/susceptibility conferred by HLA and killer Ig-like receptor combinations in psoriatic arthritis. *J. Immunol.* 173, 4273–4276.
- Norris, S., Kondeatis, E., Collins, R., Satsangi, J., Clare, M., Chapman, R., et al., 2001. Mapping the MHC-encoded susceptibility and resistance in primary sclerosing cholangitis: the role of MICA polymorphism. *Gastroenterology* 120, 1475–1482.
- Pazmany, L., 2005. Do NK cells regulate human autoimmunity? *Cytokine* 32, 76–80.
- Perricone, R., Perricone, C., De Carolis, C., Shoenfeld, Y., 2008. NK cells in autoimmunity: a two-edged weapon of the immune system. *Autoimmun. Rev.* 7, 384–390.
- Peterson, M.E., Long, E.O., 2008. Inhibitory receptor signaling via tyrosine phosphorylation of the adaptor Crk. *Immunity* 29, 578–588.
- Quann, E.J., Merino, E., Furuta, T., Huse, M., 2009. Localized diacyl-glycerol drives the polarization of the microtubule-organizing center in T cells. *Nat. Immunol.* 10, 627–635.
- Ravelli, A., Grom, A.A., Behrens, E.M., Cron, R.Q., 2012. Macrophage activation syndrome as part of systemic juvenile idiopathic arthritis: diagnosis, genetics, pathophysiology and treatment. *Genes Immun.* 13, 289–298.
- Romagnani, C., Juelke, K., Falco, M., Morandi, B., D'Agostino, A., Costa, R., et al., 2007. CD56brightCD16-killer Ig-like receptor-NK cells display longer telomeres and acquire features of CD56dim NK cells upon activation. *J. Immunol.* 178, 4947–4955.
- Sanborn, K.B., Rak, G.D., Maru, S.Y., Demers, K., Difeo, A., Martignetti, J.A., et al., 2009. Myosin IIA associates with NK cell lytic granules to enable their interaction with F-actin and function at the immunological synapse. *J. Immunol.* 182, 6969–6984.
- Sancho, D., Nieto, M., Llano, M., Rodriguez-Fernandez, J.L., Tejedor, R., Avraham, S., et al., 2000. The tyrosine kinase PYK-2/RAFTK regulates natural killer (NK) cell cytotoxic response, and is translocated and activated upon specific target cell recognition and killing. *J. Cell Biol.* 149, 1249–1262.
- Shanker, A., Verdeil, G., Buferne, M., Inderberg-Suso, E.M., Puthier, D., Joly, F., et al., 2007. CD8T cell help for innate antitumor immunity. *J. Immunol.* 179, 6651–6662.
- Shi, F.D., van Kaer, L., 2006. Reciprocal regulation between natural killer cells and autoreactive T cells. *Nat. Rev. Immunol.* 6, 751–760.
- Shilling, H.G., McQueen, K.L., Cheng, N.W., Shizuru, J.A., Negrin, R.S., Parham, P., 2003. Reconstitution of NK cell receptor repertoire following HLA-matched hematopoietic cell transplantation. *Blood* 101, 3730–3740.
- van der Slik, A.R., Koeleman, B.P., Verduijn, W., Bruining, G.J., Roep, B.O., Giphart, M.J., 2003. KIR in type 1 diabetes: disparate distribution of activating and inhibitory natural killer cell receptors in patients versus HLA-matched control subjects. *Diabetes* 52, 2639–2642.
- van der Slik, A.R., Alizadeh, B.Z., Koeleman, B.P., Roep, B.O., Giphart, M.J., 2007. Modelling KIR-HLA genotype disparities in type 1 diabetes. *Tissue Antigens* 1, 101–105.
- Spits, H., Artis, D., Colonna, M., Diefenbach, A., Di Santo, J.P., Eberl, G., et al., 2013. Innate lymphoid cells – a proposal for uniform nomenclature. *Nat. Rev. Immunol.* 13, 145–149.
- Stebbins, C.C., Watzl, C., Billadeau, D.D., Leibson, P.J., Burshtyn, D.N., Long, E.O., 2003. Vav1 dephosphorylation by the tyrosine phosphatase SHP-1 as a mechanism for inhibition of cellular cytotoxicity. *Mol. Cell. Biol.* 23, 6291–6299.
- Stinchcombe, J.C., Barral, D.C., Mules, E.H., Booth, S., Hume, A.N., Machesky, L.M., et al., 2001. Rab27a is required for regulated secretion in cytotoxic T lymphocytes. *J. Cell Biol.* 152, 825–834.
- Sun, J.C., Lanier, L.L., 2011. NK cell development, homeostasis and function: parallels with CD8 + T cells. *Nat. Rev. Immunol.* 11, 645–657.
- Suzuki, A., Yamada, R., Kochi, Y., Sawada, T., Okada, Y., Matsuda, K., et al., 2008. Functional SNPs in CD244 increase the risk of rheumatoid arthritis in a Japanese population. *Nat. Genet.* 40, 1224–1229.
- Tassi, I., Presti, R., Kim, S., Yokoyama, W.M., Gilfillan, S., Colonna, M., 2005. Phospholipase C-γ2 is a critical signaling mediator for murine NK cell activating receptors. *J. Immunol.* 175, 749–754.
- Tassi, I., Celli, M., Gilfillan, S., Turnbull, I., Diacovo, T.G., Penninger, J.M., et al., 2007. p110gamma and p110delta phosphoinositide 3-kinase signaling pathways synergize to control development and functions of murine NK cells. *Immunity* 27, 214–227.
- Tassi, I., Celli, M., Presti, R., Colucci, A., Gilfillan, S., Littman, D.R., et al., 2008. NK cell-activating receptors require PKC-theta for sustained signaling, transcriptional activation, and IFN-gamma secretion. *Blood* 112, 4109–4116.
- Villanueva, J., Lee, S., Giannini, E.H., Graham, T.B., Passo, M.H., Filipovich, A., et al., 2005. Natural killer cell dysfunction is a distinguishing feature of systemic onset juvenile rheumatoid arthritis and macrophage activation syndrome. *Arthritis Res. Ther.* 7, R30–R37.
- Vivier, E., Tomasello, E., Baratin, M., Waltzer, T., Ugolini, S., 2008. Functions of natural killer cells. *Nat. Immunol.* 9, 503–510.
- Waggoner, S.N., Cornberg, M., Selin, L.K., Welsh, R.M., 2011. Natural killer cells act as rheostats modulating antiviral T cells. *Nature* 481, 394–398.
- Watzl, C., Long, E.O., 2010. Signal transduction during activation and inhibition of natural killer cells. *Curr. Protoc. Immunol.* (Chapter 11: Unit 11 19B).

- Wiencke, K., Spurkland, A., Schrumpf, E., Boberg, K.M., 2001. Primary sclerosing cholangitis is associated to an extended B8-DR3 haplo-type including particular MICA and MICB alleles. *Hepatology* 34, 625–630.
- Wood, S.M., Meeths, M., Chiang, S.C., Bechensteen, A.G., Boelens, J.J., Heilmann, C., et al., 2009. Different NK cell-activating receptors preferentially recruit Rab27a or Munc13-4 to perforin-containing granules for cytotoxicity. *Blood* 114, 4117–4127.
- Wood, S.M., Ljunggren, H.G., Bryceson, Y.T., 2011. Insights into NK cell biology from human genetics and disease associations. *Cell. Mol. Life Sci.* 68, 3479–3493.
- Wulfing, C., Purtic, B., Klem, J., Schatzle, J.D., 2003. Stepwise cyto-skeletal polarization as a series of checkpoints in innate but not adaptive cytolytic killing. *Proc. Natl. Acad. Sci. U.S.A.* 100, 7767–7772.
- Yen, J.H., Moore, B.E., Nakajima, T., Scholl, D., Schaid, D.J., Weyand, C.M., et al., 2001. Major histocompatibility complex class I-recognizing receptors are disease risk genes in rheumatoid arthritis. *J. Exp. Med.* 193, 1159–1167.

## 13

# Granulocytes: Neutrophils, Basophils, Eosinophils

Xavier Bosch<sup>1</sup> and Manuel Ramos-Casals<sup>2</sup>

<sup>1</sup>Department of Internal Medicine, Hospital Clinic, Institute of Biomedical Research August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain

<sup>2</sup>Department of Autoimmune Diseases, Laboratory of Autoimmune Diseases Josep Font, Hospital Clinic, CELLEX-Institute of Biomedical Research August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain

## OUTLINE

<b>Neutrophils</b>	<b>243</b>	<i>Basophils and IgE Antibodies in Autoimmune Diseases:</i>	253
Basic Biological Principles and Role in Immunity	243	Therapeutic Implications	252
Neutrophils and Neutrophil Extracellular Traps	244	<b>Eosinophils</b>	253
Neutrophils in Systemic Autoimmune Diseases: Pathogenic Role	245	Basic Biological Principles and Role in Immunity	253
Neutrophils in Systemic Autoimmune Diseases: Therapeutic Implications	250	Eosinophils in Autoimmune Diseases: Pathogenic Role	253
<b>Basophils</b>	<b>250</b>	Eosinophils in Eosinophilic Granulomatosis With Polyangiitis: Therapeutic Implications	254
Basic Biological Principles and Role in Immunity	250	<b>Conclusions</b>	255
Basophils and IgE Antibodies in Autoimmune Diseases: Pathogenic Role	251	<b>References</b>	255

## NEUTROPHILS

### Basic Biological Principles and Role in Immunity

Neutrophils, the most copious circulating white blood cells, are short lived, terminally differentiated cells that emanate from the bone marrow to target pathogens (Amulic et al., 2012; Kolaczkowska and Kubes, 2013). They attack pathogens using different mechanisms including phagocytosis, degranulation, and release of granular antimicrobial peptides such as neutrophil elastase (NE), myeloperoxidase (MPO), and matrix metalloproteinases (MMPs), and degradation through the synthesis of reactive oxygen species (ROS) within phagolysosomes (Gupta and Kaplan, 2016). When they meet a microorganism, neutrophils undergo a respiratory burst, a vital phenomenon in host defense, with activation of the complex nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and subsequent synthesis of superoxide (Kobayashi and DeLeo, 2009). Superoxide and its ensuing products, hypochlorous acid and hydrogen peroxide, have key antimicrobial roles (Kobayashi and DeLeo, 2009). Mitochondria (Fossati et al., 2003) and peroxisomes (Fransen et al., 2012) are other important sources of ROS in neutrophils.

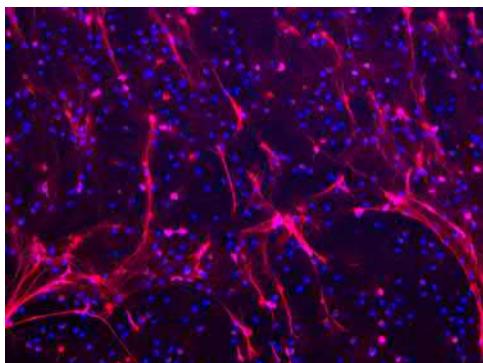
Besides their central role in the innate immune response, neutrophils regulate the adaptive immune response (Mantovani et al., 2011). In lymphoid organs, neutrophils engage with B and T cells and antigen-presenting cells (Scapini et al., 2008; Huard et al., 2008; Tvinnereim et al., 2004; Fazio et al., 2014; Megiovanni et al., 2006; Yang et al., 2010). The uptake of apoptotic neutrophils by dendritic cells (DCs) may increase dendritic antigen presentation (Megiovanni et al., 2006; Yang et al., 2010). Neutrophils can stimulate and inhibit T-cell responses and synthesize cytokines essential for B-cell development including, among others, the B-cell activating factor (BAFF) (Scapini et al., 2008) and the proliferation-inducing ligand A proliferation-inducing ligand (APRIL) (a molecule intimately related to BAFF) (Huard et al., 2008). In the spleen, neutrophils can act as B-cell helpers on a T-cell independent basis (Puga et al., 2011).

## Neutrophils and Neutrophil Extracellular Traps

A different form of defense used by neutrophils is the extrusion of a network of chromatin coated with proteases, histones, and cytosolic proteins in a process called neutrophil extracellular trap (NET) formation (Brinkmann et al., 2004; Jorch and Kubes, 2017; Kenny et al., 2017). NETs were discovered in 1996 as a form of cell death different from apoptosis or necrosis (Takei et al., 1996), and NETosis defines the process of NET production and release by neutrophils (Jorch and Kubes, 2017). This process plays an important role in the innate immune response by allowing neutrophils to immobilize and capture viruses, bacteria, or fungi (Gupta and Kaplan, 2016; Jorch and Kubes, 2017). NETs are induced by many microorganisms, by different autoantibodies, cytokines, and immune complexes (ICs), and, *in vitro*, by phorbol 12-myristate 13-acetate (PMA) or calcium ionophores (Fig. 13.1) (Lood et al., 2016). Although the molecular mechanisms leading to NETosis are not completely characterized, histone citrullination and ROS production appear to be essential (Brinkmann et al., 2004; Wang et al., 2009).

The term “NETosis” was first used to indicate neutrophils’ death as they discharged NETs. However, microorganism-derived stimulation of neutrophils can in some cases cause a fast and vital form of NETosis, where neutrophils can still accomplish their phagocytic role after producing NETs (Jorch and Kubes, 2017). The type of NETosis (i.e., inducing vital NETosis or resulting in cell death) seems largely determined by the type of stimulus. Suicidal NETosis is induced by diverse stimuli, such as autoantibodies or PMA, and occurs within hours of stimulation. In suicidal NETosis, production of ROS and activation of protein-arginine deiminase 4 (PAD4) occur through NADPH oxidase activation and results in chromatin decondensation, translocation of MPO and NE into the nucleus to boost additional chromatin unfolding, and nuclear membrane disruption. After chromatin is released into the cytosol, it becomes decorated with cytosolic and granular proteins and, eventually, disruption of the plasma membrane leads to NET release and neutrophil death (Jorch and Kubes, 2017; Yipp and Kubes, 2013). By contrast, vital NETosis is induced within minutes by *Staphylococcus aureus* through both Toll-like receptor (TLR) 2 (TLR2) ligands and complement receptors, or by *Escherichia coli* via TLR4 or TLR4-activated platelets. The subsequent activation of PAD4 leads to chromatin decondensation. As in suicidal NETosis, NE is translocated into the nucleus to boost further chromatin unfolding and nuclear membrane disruption. However, protein-decorated chromatin is discharged through vesicles, and the neutrophil remains alive for other functions such as phagocytosis (Jorch and Kubes, 2017; Pilsczek et al., 2010; Yipp et al., 2012).

Besides infection, NET-associated proteins may have a role in other diseases, including, but not restricted to, autoimmune diseases (Jorch and Kubes, 2017; Thieblemont et al., 2016). Several autoantibodies can promote NET



**FIGURE 13.1** Neutrophil extracellular traps. Human control neutrophils underwent NET formation upon stimulation with lipopolysaccharide. Visualization of NETs by staining of nuclear material (4',6-diamidino-2-phenylindole, blue) and neutrophil elastase (green). Original magnification,  $\times 40$ . NET, Neutrophil extracellular trap. Source: Modified from Kaplan, M.J., 2013. Role of neutrophils in systemic autoimmune diseases. *Arthritis Res. Ther.* 15 (5), 219. Licensed under the Creative Commons Attribution License (CC BY). Copyright: Kaplan M.J.

release and molecules exteriorized through NETosis [e.g., MPO and double-stranded DNA (dsDNA)] are known autoantigens in systemic autoimmune diseases. Neutrophils from subjects with some autoimmune diseases are more likely than those from healthy controls or subjects without autoimmune diseases to undergo NETosis. The association between NETosis and autoantibodies may promote a cycle by which autoantigen release by NETs results in autoantibody formation, which, in turn, boosts the release of antigens (Gupta and Kaplan, 2016).

## Neutrophils in Systemic Autoimmune Diseases: Pathogenic Role

### ***Systemic Lupus Erythematosus***

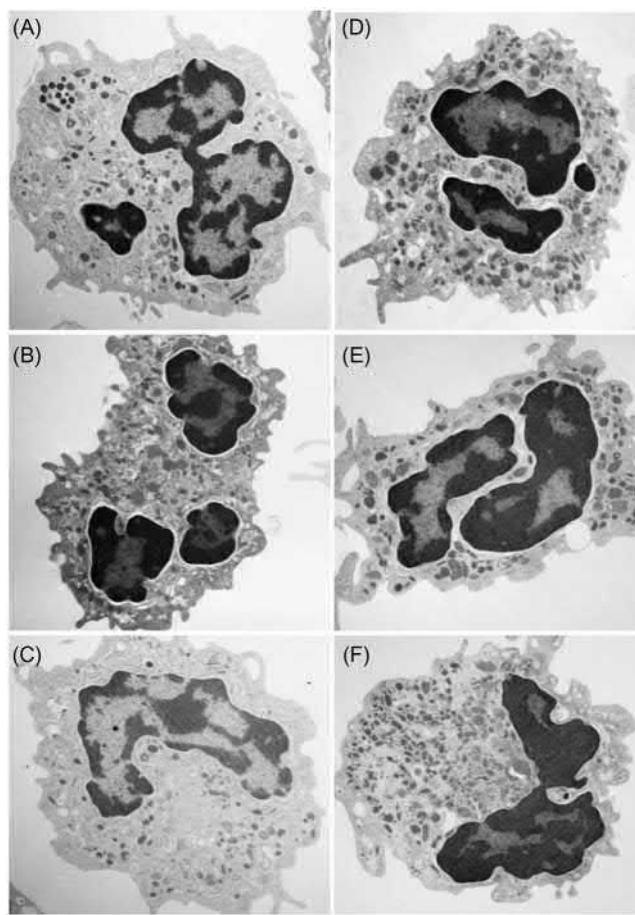
#### DYSREGULATED NEUTROPHIL PHENOTYPE AND FUNCTION AND PROINFLAMMATORY ROLE OF NEUTROPHIL PROTEASES IN SYSTEMIC LUPUS ERYTHEMATOSUS

Besides a dysregulation of B and T cells, neutrophils from patients with systemic lupus erythematosus (SLE) display various abnormalities in their phenotype and function such as a defective phagocytic ability (Brandt and Hedberg, 1969; Cairns et al., 2001), failure to be cleared by the apoptotic pathway mediated by C1q/calreticulin and CD91 (Donnelly et al., 2006), increased aggregation (Denny et al., 2010; Abramson et al., 1983), abnormal oxidative activity, increased numbers of circulating low-density granulocytes (LDGs), and an increased apoptosis that may result in neutropenia [which is observed in 20%–40% of SLE patients (Carli et al., 2015)] (Denny et al., 2010; Abramson et al., 1983; Courtney et al., 1999; Bennett et al., 2003).

The reduced expression of L-selectin (or CD62L) and C5a receptor and the normal expression of CD11B (or integrin  $\alpha$ -M) (Furebring et al., 2002; Bengtsson et al., 2014) in neutrophils from SLE patients indicates an altered rather than activated phenotype (Thieblemont et al., 2016). LDGs deserve a special consideration. These cells are a subgroup of pathogenic granulocytes that synthesize more cytokines with proinflammatory properties [e.g., type I interferons (IFNs)] than regular-density granulocytes and are toxic to endothelial cells (ECs) (Gupta and Kaplan, 2016; Denny et al., 2010; Villanueva et al., 2011). This subset of granulocytes has an activated phenotype and, though their nuclear morphology is similar to that of immature cells, they express surface markers of mature cells (Fig. 13.2) (Denny et al., 2010; Bennett et al., 2003; Kaplan, 2011). Studies have shown that circulating levels of LDGs in SLE correlate with levels of dsDNA antibodies and disease activity scores (Midgley and Beresford, 2016). Sera from SLE patients exhibit increased concentrations of defensins released by LDGs and/or neutrophils (Sthoeger et al., 2009; Vordenbäumen et al., 2010) and messenger RNA for several bactericidal proteins and alarmins [e.g., cathepsin G (CTSG), NE, and proteinase 3 (PR3)] are significantly overexpressed in LDGs from SLE patients compared with regular-density granulocytes from SLE patients and from healthy controls (Villanueva et al., 2011; Lyons et al., 2010; Kim et al., 2014).

The process that leads to autoimmunity in SLE may involve an increase in apoptotic neutrophils (which have a greater expression of autoantigens) and their impaired elimination by phagocytes (Potter et al., 2003). Autoimmunity pathogenesis also involves a decrease in ROS production by granulocytes, which has been associated with the severity of disease (Bengtsson et al., 2014). The lower production of ROS is accompanied by a lower number of circulating neutrophils with absent CD10 and reduced CD16 expression (CD10 $^-$ /CD16 $^{\text{low}}$ ) newly released from the bone marrow (Bengtsson et al., 2014). Neutrophil depletion in SLE leads to spontaneous activation of autoimmune B cells and natural killer cells *in vivo* (Huang et al., 2015a).

Chronic activation of plasmacytoid DCs (pDCs) by circulating ICs and the subsequent secretion of type I IFNs is recognized as an initial trigger of autoimmunity in SLE (Thieblemont et al., 2016). Animal models suggest that the production of tumor necrosis factor (TNF) damages selectively erythroid precursors via TLR7-driven neutrophil activation, causing the anemia frequently seen in SLE patients (Zhuang et al., 2014; Papadaki et al., 2002; Santiago-Raber et al., 2003). Immune cells, including myeloid cells, stimulate ribonucleoprotein (RNP)-containing ICs, which requires activation of both TLR and Fc receptors (FcRs) (Lau et al., 2005; Clynes et al., 1998). TLRs including TLR2, 4, 5, 7, 8, and 9 are implicated in the pathogenesis of SLE (Geering et al., 2013; Wu et al., 2015b), with TLR7 being essential for the recognition of RNP-associated autoantigens, and TLR9 being involved in DNA or RNA-associated autoantigen detection. Subsets of human neutrophil express all the members of the TLR family, except for TLR3, allowing them to start immune responses after recognition of endogenous or exogenous ligands (Geering et al., 2013; Huebener et al., 2015). The pathogenesis of SLE also comprises the release of high mobility group box 1 protein which, after binding DNA and anti-DNA autoantibodies through its receptor for advanced glycation endproducts, may prompt the recruitment of neutrophils, contributing to kidney damage in anti-DNA autoantibody-induced lupus nephritis (Thieblemont et al., 2016).



**FIGURE 13.2** Morphology of a low-density granulocyte under transmission electron microscopy in a patient with systemic lupus erythematosus. (A–C) show a normal-density granulocyte and (D–F) display a low-density granulocyte with different types of cytoplasmic granules. Dark (heterochromatic) and lighter (euchromatic) areas are well defined in both types of granulocytes. Whereas the normal-density granulocyte has nuclear lobes, there are less lobulated nuclei in the low-density granulocyte. Source: Permission to reproduce this figure was obtained from Springer-Verlag [Carmona-Rivera, C., Kaplan, M.J., 2013. *Semin. Immunopathol.* 35 (4), 455–463].

#### SYSTEMIC LUPUS ERYTHEMATOSUS AND NETOSIS

It has been suggested that NETs and NET-associated proteins may be potential inducers of autoantibody production in SLE (Jorch and Kubes, 2017), including anti-RNP and anti-DNA antibodies (Gupta and Kaplan, 2016). In vitro studies with type I IFN-primed neutrophils from SLE patients have shown that RNP-containing ICs, commonly present in lupus, can induce NETosis (Garcia-Romo et al., 2011). Moreover, LDGs display an enhanced spontaneous NETosis ex vivo (Villanueva et al., 2011). In contrast to their regular density or healthy counterparts, LDGs from SLE patients have an enhanced capability to produce NETs at baseline in vitro that remains unaffected in the presence of the NET inducer PMA, suggesting that LDGs might be extremely stimulated in vivo (Gupta and Kaplan, 2016; Villanueva et al., 2011). In contrast to NETs generated by neutrophils from healthy controls, NETs from SLE patients have greater levels of autoantigens and immunostimulatory molecules such as dsDNA, LL-37 (a cathelicidin antibacterial peptide that is a key mediator of pDC activation), and MMP-9 (Garcia-Romo et al., 2011; Lande et al., 2011; Leffler et al., 2012; Carmona-Rivera et al., 2015).

NETs are not appropriately removed from the circulation in a significant number of SLE patients because of the oxidation of nucleic acids externalized by NETs, complement activation within NETs (Lood et al., 2016; Leffler et al., 2012; Gehrke et al., 2013), and the existence of DNase 1 inhibitors or of anti-NET antibodies, which hinder the DNase 1 access to NETs (Hakkim et al., 2010).

The autoimmune response in patients with SLE can be further triggered by NETs exposing cathelicidin–DNA complexes, which enhance the synthesis of type I IFNs by pDCs via endosomal TLR activation (Garcia-Romo et al., 2011; Lande et al., 2011). Type I IFNs in myeloid cells can be stimulated by oxidized nucleic acids in NETs

through the activation of the stimulator of IFN genes (STING) pathway (Lood et al., 2016). In lipopolysaccharide-primed macrophages, cathelicidin can stimulate the nucleotide-binding domain leucine-rich repeat-containing receptor P3 (NLRP3) inflammasome via interaction with the purinergic receptor X7 (P2X7) and stimulation of potassium efflux, leading to interleukin (IL)-1 and IL-18 release. In turn, IL-1 and IL-18 promote NETosis, thus amplifying the inflammatory pathways at tissue and organ sites (Kahlenberg et al., 2013).

It is of note that mitochondria are a source of ROS and mitochondrial DNA participates in inflammatory responses (White et al., 2014; Oka et al., 2012; Shimada et al., 2012). NETs produced by LDGs are enriched in oxidized mitochondrial DNA and mitochondrial ROS can prompt NETosis upon RNP-containing ICs stimulation both in vitro and in LDGs in SLE subjects ex vivo (Lood et al., 2016). NETs might also contribute to the occurrence of early cardiovascular disease in patients with SLE (Barnado et al., 2016). Neutrophils and NETs in SLE can enhance the apoptosis of ECs in vitro (Villanueva et al., 2011), and SLE patients exhibit a dysfunction and an accelerated apoptosis of ECs (Rajagopalan et al., 2004) that leads to a loss of nitric oxide release, impaired vasodilation, and potentiation of atherosclerosis and thrombotic events (Rajagopalan et al., 2004; Smith et al., 2014).

### ***Antineutrophil Cytoplasmic Autoantibody-Associated Vasculitides***

#### **NEUTROPHIL-INDUCED VASCULITIC ORGAN DAMAGE**

The antineutrophil cytoplasmic autoantibody (ANCA)-associated vasculitides (AAVs) are characterized by a necrotizing inflammation of small vessels and comprise three clinical entities: (1) microscopic polyangiitis, (2) granulomatosis with polyangiitis (formerly known as Wegener's granulomatosis), and (3) eosinophilic granulomatosis with polyangiitis (EGPA) (formerly known as Churg–Strauss syndrome). The vascular lesions differ depending on the size of affected vessels, their localization, and the pathogenic mechanism involved (Thieblemont et al., 2016).

The neutrophil enzymes MPO and PR3 are the main autoantigens recognized by ANCAs. MPO is solely found in neutrophil azurophilic granules and has the exclusive property to produce chlorinated oxidants including chloramines and hypochlorous acid (Nauseef, 2007), the latter exerting toxic effects on microorganisms and host cells. Proteinase 3 is expressed by both neutrophils and monocytes and is localized in the neutrophil azurophilic granules along with its neutrophil serine protease family homologs NE, CTSG, and azurocidin (Campanelli et al., 1990). Upon phagocytosis of microorganisms, PR3 carries out its microbial function after being secreted into the phagolysosome. Animal models have revealed that PR3, NE, and CTSG have proinflammatory activities (Pham, 2006). Both MPO and PR3 share proinflammatory properties and can modulate the inflammatory process acting on a synergistic basis (Witko-Sarsat et al., 2000).

Several in vitro and in vivo studies have shown that ANCA can be pathogenic. In short, priming of neutrophils causes their accumulation at the inflammatory sites in medium- and small-sized vessels and capillaries (mainly pulmonary and glomerular). Membrane expression of MPO and PR3 is enhanced by such priming and both enzymes can bind ANCA to prompt the activation of neutrophils. TNF-primed neutrophils incubated with sera containing anti-MPO or anti-PR3 ANCAs can release granular proteins and produce superoxide anion (Jennette and Falk, 2014; Falk et al., 1990). The pathophysiological mechanisms sustaining the antigenic specificity of AAV (Csernok et al., 2006) have been described in genome-wide association studies (Lyons et al., 2012).

#### **NEUTROPHIL EXTRACELLULAR TRAPS AND ANTINEUTROPHIL CYTOPLASMIC AUTOANTIBODY-ASSOCIATED VASCULITIDES**

The formation of NETs comprising DNA ejected by dying neutrophils and granule-derived cationic proteins, including MPO and PR3, could participate in the pathophysiology of AAV (Kessenbrock et al., 2009). Neutrophils from AAV patients have an enhanced ability to generate NETs in vitro (Kessenbrock et al., 2009; Tang et al., 2015). Serum levels of NET remnants, such as MPO–DNA complexes (Kessenbrock et al., 2009), are increased in AAV patients and correlate positively with AAV disease activity and neutrophil count and inversely with ANCA levels (Söderberg et al., 2015). NETs and NET-associated molecules have been detected in areas of fibrinoid necrosis and in the arterial walls of renal biopsies from AAV patients with necrotizing glomerulonephritis (Kessenbrock et al., 2009; Yoshida et al., 2013). NETs can present MPO and PR3 to DCs (Sangaletti et al., 2012), which then incorporate NET material and induce ANCAs against MPO, PR3, and dsDNA when transferred to mice, leading to autoimmune vasculitis. Serum from AAV patients can induce NETosis and inhibit NET degradation (Nakazawa et al., 2014). By using whole blood from AAV patients, Grayson et al. (2015) observed a gene expression signature which overlapped with an LDG signature formerly detected in SLE patients (Villanueva et al., 2011). Increased transcripts for PR3 identified in circulating mononuclear cells from AAV patients were

associated with higher disease activity scores and a lack of therapeutic response (Grayson et al., 2015). Furthermore, neutrophils and LDGs from patients underwent an increased spontaneous NETosis in culture, with NETs staining positive for MPO and PR3 (Grayson et al., 2015).

Finally, it is well known that thrombosis is a relatively frequent consequence of ANCA-associated blood vessel inflammation (Rao et al., 2015) and that increased NET formation may cause thrombus formation [i.e., histones within NETs can bind platelets and blood coagulants (Xu et al., 2009; Fuchs et al., 2010)]. Thus the role of NETs in thrombosis in AAV patients might have clinical significance (von Scheven et al., 2003). A study in patients with acute myocardial infarction demonstrated an interaction of neutrophils with thrombin-activated platelets at the plaque rupture site, with local NETosis and activation of tissue factor, a protein that triggers coagulation and thrombin formation and that is expressed in leukocytes and the endothelium (Stakos et al., 2015). Activated tissue factor has also been detected in patients with AAV (Kambas et al., 2014). Specifically, ANCA-induced NETs produced by C5a-primed neutrophils cause enhanced inflammation and thrombosis in AAV by promoting the expression of tissue factor (Kambas et al., 2014; Huang et al., 2015b).

### **Rheumatoid Arthritis**

#### **PROINFLAMMATORY EFFECTS OF NEUTROPHIL PROTEASES AND REACTIVE OXYGEN SPECIES IN RHEUMATOID ARTHRITIS**

Rheumatoid arthritis is characterized by a dysregulation of innate and adaptive immune responses, with an increased production of inflammatory cytokines (including IL-1, 6, and 17, TNF- $\alpha$ , and granulocyte/macrophage-colony stimulating factor) and a defective tolerance to self-antigens, most notably citrullinated peptides (Wright et al., 2014; McInnes et al., 2016; Burmester et al., 2014; McInnes and Schett, 2011). The inflammatory pannus characteristic of rheumatoid arthritis contains activated synovial fibroblasts, lymphocytes, macrophages, and neutrophils (Thieblemont et al., 2016).

Circulating neutrophils of patients with rheumatoid arthritis have an activated phenotype, with an abnormal regulation of apoptosis (Wright et al., 2011; Weinmann et al., 2007), activation of transcription factors (Wright et al., 2011; Turrel-Davin et al., 2010), increased chemotactic and phagocytic ability (Talbot et al., 2015; de Siqueira et al., 2015), and FcRs upregulation, which leads to ROS production by ICs (including rheumatoid factor) (Rollet-Labelle et al., 2013; Watson et al., 1993; Quayle et al., 1997). In addition, synovial neutrophils release collagen-degrading proteases, cytotoxic ROS, cytokines, and chemokines (Wright et al., 2014). ICs at the synovial fluid induce degranulation and release of ROS into it (Robinson et al., 1992), and activated neutrophils adhere to synovial tissue ICs, leading to a direct degranulation onto the joint surface (Wright et al., 2014; Pilling and Abramson, 1995). Synovial fluid from patients with rheumatoid arthritis contains high levels of NE, PR3, CTSG as well as neutrophil MMP-8 and MMP-9, lipocalin, and gelatinase (Wright et al., 2014; Sopata et al., 1995; Wong et al., 2009; Momohara et al., 1997; Nzeusseu Toukap et al., 2014; Katano et al., 2009). These neutrophil proteases can split collagen within its matrix and digest hyaluronic acid (Pham, 2006; Van den Steen et al., 2002; Lefrançais et al., 2012; Hurst et al., 2001).

#### **NEUTROPHIL EXTRACELLULAR TRAPS AND NETOSIS IN RHEUMATOID ARTHRITIS**

NETs and suicidal NETosis may participate in the pathogenesis of rheumatoid arthritis (Jorch and Kubes, 2017). Neutrophils from patients with rheumatoid arthritis generate more NETs and higher levels of ROS than those from healthy subjects (Jorch and Kubes, 2017).

Citrullinated autoantigens are crucial autoantigens in rheumatoid arthritis (Khandpur et al., 2013). The presence of extracellular citrullinated autoantigens in the joints of rheumatoid arthritis patients (van Beers et al., 2013) may be explained by an increased NETosis and, indeed, NETs are a source of extracellular citrullinated autoantigens (van Beers et al., 2013), which can trigger inflammatory responses in synovial fibroblasts of affected patients, with release of proinflammatory cytokines, adhesion molecules, and chemokines (Khandpur et al., 2013; Pratesi et al., 2014).

NETs have been detected in rheumatoid nodules, synovial tissue, and skin of patients with rheumatoid arthritis. A significant correlation has also been observed between the proportion of circulating netting neutrophils and serum levels of autoantibodies to citrullinated protein antigen (ACPA) (i.e., highly specific autoantibodies targeting citrullinated proteins), erythrocyte sedimentation rate, C-reactive protein, and IL-17 (Khandpur et al., 2013). Markedly, autoantibodies including ACPA and inflammatory cytokines (e.g., IL-17 and TNF- $\alpha$ ) can induce NETosis in patients with rheumatoid arthritis (Khandpur et al., 2013).

### **Primary Sjögren Syndrome**

A few research studies have shed light on the potential implication of neutrophils in the etiopathogenesis of primary Sjögren syndrome. Autoantibodies to the La/SSB complex (anti-SSB antibodies) are frequently detected in patients with this disease (van Venrooij et al., 1991). These antibodies appear in up to 87% of patients with Sjögren's syndrome and are commonly associated with extraglandular manifestations such as purpura, vasculitis, neurological involvement, leucopenia, and lymphopenia (Hsieh et al., 2003).

The La or Sjögren syndrome antigen B (La/SSB) is an RNP reported to be involved in RNA biogenesis, maturation of RNA polymerase III transcripts, translation of viral and cellular messenger RNAs, and to influence telomere homeostasis (Wu et al., 2015a). Hsieh et al. (2003) reported that La/SSB is expressed in the surface membrane and nucleus of human neutrophils and that anti-SSB antibody concomitantly increases the synthesis of IL-8 and the apoptosis of neutrophils, with release of La/SSB after cell death.

In a study aimed to investigate the effect of La/SSB on human neutrophils, Wu et al. (2015a) reported that human La/SSB together with SSB-anti-SSB ICs induces IL-8 production from normal neutrophils, suggesting that this antigen might act as an endogenous dangerous molecule to enhance the expression of the IL-8 gene in human neutrophils.

Other reported data pointing at a possible role of neutrophils in the pathogenesis of primary Sjögren syndrome include (1) neutrophils are activated in this disorder (Torsteinsdóttir et al., 1998), (2) neutropenia is observed in one-third of cases and is strongly associated with anti-SSB antibodies (Brito-Zerón et al., 2009), and (3) the expression of La/SSB may be upregulated in inflamed tissues such as the labial salivary glands (van Woerkom et al., 2004).

### **Systemic Sclerosis**

Barnes et al. (2011) reported that serum from patients with systemic sclerosis can induce in vitro an activation and apoptosis of ECs, in a process dependent on the presence of neutrophils and mediated by IL-6. By studying the ROS generation ex vivo of neutrophils from patients with systemic sclerosis compared with neutrophils from control subjects, Barnes et al. (2012) subsequently reported that neutrophils from systemic sclerosis patients can be primed for ROS production. Since endothelial dysfunction appears to be fundamental in the pathogenesis of systemic sclerosis (Fleming and Schwartz, 2008), the authors suggested that binding of neutrophils to activated endothelium may induce local generation of ROS, sustaining endothelial dysfunction and facilitating fibrosis in systemic sclerosis (Barnes et al., 2012).

In a modified murine model that mimicked human systemic sclerosis with skin lesions and lung fibrosis, Liang et al. (2015) detected the presence of neutrophils in the skin of the murine model but not in the skin of control mice. Also, an enhanced expression of chemokine (C-X-C motif) ligand 2 (CXCL2), which is chemotactic for neutrophils, was observed in the skin lesions of mice with systemic sclerosis both at initial and later stages, suggesting that neutrophils could play a role in the progression of the disease (Liang et al., 2015).

### **Antiphospholipid Antibody Syndrome**

Patients with antiphospholipid antibody syndrome have autoantibodies against phospholipids and surface proteins (Barnado et al., 2016). Antiphospholipid and anti-β2-glycoprotein I (β2GPI) antibodies may promote thrombosis by activating ECs, platelets, and monocytes (Gupta and Kaplan, 2016). While NETs can activate the coagulation cascade and are important components of venous and arterial thrombi (Gupta and Kaplan, 2016; Fuchs et al., 2010; Massberg et al., 2010; Brill et al., 2012), neutrophils and NETosis have recently been implicated in the pathogenesis of antiphospholipid antibody syndrome. Specifically, the antiphospholipid antibody syndrome autoantigen β2GPI can stimulate NETosis in vitro when interacting with neutrophils through a ROS- and TLR4 signaling-dependent process (Yalavarthi et al., 2015). A positive correlation has also been observed between serum levels of anti-β2GPI antibodies and circulating levels of NETs (Yalavarthi et al., 2015).

Like SLE, sera of patients with antiphospholipid antibody syndrome have a decreased capacity to degrade NETs and this defect seems to correlate with the presence of higher concentrations of antibodies against both NETs and neutrophil remnants (Leffler et al., 2014). Likewise, as in SLE, serum and plasma from patients with antiphospholipid antibody syndrome have increased levels of NET remnants and cell-free DNA. Neutrophils from these patients can also produce spontaneous NETs ex vivo (Yalavarthi et al., 2015), and their sera and IgG are able to stimulate NET release from neutrophils of control individuals (Yalavarthi et al., 2015).

### **Polymyositis and Dermatomyositis**

A study by Lu et al. (2017) showed that, compared with healthy subjects, patients with early polymyositis and dermatomyositis had an increased LL-37 expression localized to neutrophils in muscle tissue samples. Neutrophils in the skin of symptomatic and nonsymptomatic patients with myositis also expressed LL-37, suggesting that unaffected skin of patients with both polymyositis and dermatomyositis contained an increased proportion of neutrophils with LL-37 expression. Moreover, compared with skin samples of the same patients or with muscle tissue samples of healthy controls, muscle tissue samples of patients with early polymyositis and dermatomyositis had a higher proportion of LL-37-positive cells (Lu et al., 2017). Interestingly, LL-37 muscular expression correlated with serum creatine phosphokinase levels, suggesting that, upon stimulation, LL-37-positive neutrophils could trigger inflammation, with subsequent necrosis of muscle fibers (Lu et al., 2017).

In another study in patients with polymyositis and dermatomyositis with and without interstitial lung disease, Zhang et al. reported that the DNase I activity and NET degradation were reduced in both groups of patients. However, those with interstitial lung disease displayed the lowest NET degradation in vitro as a result of a significant decrease in DNase I activity (Zhang et al., 2014). Besides, compared with healthy individuals, patients with polymyositis and dermatomyositis had a markedly higher capacity for inducing NETs, as supported by the presence of raised concentrations of plasma LL-37 and circulating cell-free DNA in them (Zhang et al., 2014).

### **Neutrophils in Systemic Autoimmune Diseases: Therapeutic Implications**

Undeniably, NETs are a promising therapeutic target in SLE and possibly other autoimmune disorders. As mentioned above, NETs release ROS as a defense against microorganisms. Treatment of neutrophils with ROS scavengers, specifically N-acetyl cysteine, blocks NET formation and ROS release in vitro (Patel et al., 2010). Two small studies reported an improved outcome of patients with SLE treated with ROS scavengers (Garcia et al., 2013; Lai et al., 2012). However, the production of ROS does not inhibit vital NETosis, which might lead to further autoimmune development (Jorch and Kubes, 2017). Treatment with the specific scavenger MitoTEMPO for mitochondrial ROS production in vivo in a mouse model of lupus reduced type I IFN responses and disease severity with reduced spontaneous NETosis by neutrophils from the bone marrow (Lood et al., 2016). An endogenous NET inhibitor that does not suppress the production of ROS (signal inhibitory receptor on leukocytes-1) was recently shown to block the release of NETs from neutrophils upon stimulation with plasma from SLE patients (Van Avondt et al., 2013).

Inhibition of PAD4, which suppresses NETosis without affecting the oxidative burst, represents another promising therapy for inhibiting NETosis (Jorch and Kubes, 2017; Barnado et al., 2016). Although the most frequently used NET inhibitor is DNase, it does not suppress NET production but rather dismantles its structure (Jorch and Kubes, 2017). Clinical studies have shown that DNase therapy has no toxicity. In mouse models of lupus, the administration of DNase led to disrupted NETs and decreased disease severity (Macanovic et al., 1996). Whereas DNase1 can dismantle DNA and is an important degrader of NETs, it has a trivial effect on elastase, histones, and other components with the potential to attach to vessel walls, thus causing ongoing inflammation (Kolaczkowska et al., 2015).

Both IL-17 and TNF induce NETosis in neutrophils from patients with rheumatoid arthritis. The administration of specific antibodies that block these cytokines can lead to less NETosis and an improved clinical progression (Khandpur et al., 2013). However, since it is not known whether it is a NETosis-specific mechanism, similar findings might be observed with the inhibition of other proinflammatory mediators (Jorch and Kubes, 2017). Last, MPO may participate in NET production, and an MPO inhibitor (PF1355) was reported to reduce vasculitis in mouse models (Zheng et al., 2015).

## **BASOPHILS**

### **Basic Biological Principles and Role in Immunity**

Basophils represent less than 1% of circulating leukocytes and are an important source of IL-4, pointing at their potential role in T helper (Th) 2 (Th2)-type immune responses including antiparasitic immunity and allergy (Karasuyama and Yamanishi, 2014; Karasuyama et al., 2011). Basophils can also be central immunomodulatory cells in other disorders, particularly in autoimmune diseases (Dijkstra and Meyer-Bahlburg, 2017; Denzel et al., 2008; Rodriguez Gomez et al., 2010).

Basophils modulate B-cell differentiation into antibody-producing plasma cells and produce numerous mediators after activation or during degranulation. In addition to IL-4, these mediators include leukotrienes, other ILs (3, 6, 8, and 13), vascular endothelial growth factor, histamine, heparin, and macrophage inflammatory protein (MIP)-1 $\alpha$  (MIP-1 $\alpha$ ) (Dahinden et al., 1989; Schroeder et al., 2009; Jeon et al., 2015; Clark et al., 1976; Li et al., 1996a,b; de Paulis et al., 2006). Basophils express in their surface different markers that stimulate neighboring cells such as CD40L, CD62L, OX40L (a ligand for CD134), and the receptor activator of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) ligand (Gauchat et al., 1993; Charles et al., 2010; Di et al., 2015; Poli et al., 2015). Basophils can respond to several mediators because of their expression of receptors for TLR ligands, cytokines (Komiya et al., 2006; Suurmond et al., 2014), thymic stromal lymphopoietin (Giacomin et al., 2012), IgE (Fc $\epsilon$ RI  $\alpha$ ) (Ishizaka and Ishizaka, 1977), and IgG (Fc $\gamma$ RIIa/IIb) (Cassard et al., 2012), thus easing antigen-specific basophil responses. Therefore basophils are well resourced to react to an extensive variety of signals, and to modulate the functions of other cells, especially B cells (Mack et al., 2005). Importantly, the hematopoiesis of basophils seems to be influenced by serum IgE concentrations (Hill et al., 2012) and, in fact, increased IgE concentrations correlate with increased numbers of circulating basophils in both humans and mice (Hill et al., 2012; Cahenzli et al., 2013).

## Basophils and IgE Antibodies in Autoimmune Diseases: Pathogenic Role

### ***Basophils and IgE Antibodies in Systemic Lupus Erythematosus***

The potential pathogenic relevance of basophils in autoimmune diseases is mainly attributable to their influence on Th2 polarization by producing IL-13, IL-4, and thymic stromal lymphopoietin, and to their effects on B-cell activation, differentiation, survival, and promotion of autoantibody production through IL-4, IL-6, and BAFF (Sharma and Bayry, 2015).

A breakthrough in the pathogenic role of basophils in SLE came in 2010 when Rivera et al. (Charles et al., 2010) demonstrated that basophils promote lupus nephritis in mice in an IgE- and IL-4-dependent manner. In particular, Rivera's team showed that ICs along with IgE antibodies with specificity for dsDNA and nuclear components activated basophils in *Lyn*<sup>-/-</sup> mice, triggering their homing to lymphoid organs (Charles et al., 2010). The tyrosine protein kinase Lyn regulates negatively B-cell functions, and mice lacking Lyn (*Lyn*<sup>-/-</sup> mice) develop a late lupus-like nephritis with glomerular deposition of ICs consisting of IgA, IgG, IgM, and C3. Rivera et al. demonstrated that IL-4 and BAFF produced by activated basophils promoted the polarization of Th2 cells, with B-cell activation and differentiation and eventual antibody production. Also, while patients with active SLE had increased titers of autoreactive IgE and activated circulating basophils, *in vivo* reduction of basophils led to a substantial improvement of the autoimmune and pathological signatures (Charles et al., 2010).

Despite the new findings, however, an inherent weakness in the *Lyn*<sup>-/-</sup> model of lupus nephritis is that these mice display early atopy-like allergic inflammation (Sharma and Bayry, 2015). To distinguish allergic inflammation from autoimmune inflammatory, Rivera et al. further investigated the effect of the IgE–basophil network on lupus pathogenesis. The investigators used two different strains of mice deficient for the inhibitory IgG Fc receptor Fc $\gamma$ RIIb, namely, *Fc $\gamma$ RIIb*<sup>-/-</sup> mice and *Fc $\gamma$ RIIb*<sup>-/-</sup>. *Yaa* mice [i.e., mice with a chromosomal translocation of the *Y-linked autoimmune acceleration locus* (*Yaa*)], and demonstrated that IgE creates an inflammatory environment by providing signals for innate immune cells' migration to secondary lymphoid organs (Dema et al., 2014). Specifically, both strains of mice developed a lupus-like disease, though with a more aggressive disease phenotype with raised levels of dsDNA-specific IgE and accelerated development of organ pathology in *Fc $\gamma$ RIIb*<sup>-/-</sup>. *Yaa* mice. In a further experiment, *Fc $\gamma$ RIIb*<sup>-/-</sup>. *Yaa* mice with IgE deficiency had a delayed onset of disease, a significant reduction in the number of B and plasma cells, and a reduced autoantibody production compared with their IgE-generating counterparts. A reduced amount of DCs, neutrophils, monocytes, and eosinophils was also observed in the secondary lymphoid organs of IgE-deficient mice, indicating that these innate cells expressed the  $\alpha$ -subunit of the IgE receptor, Fc $\epsilon$ RI (Dema et al., 2014).

More recently, Henault et al. (2016) reported that IgE can trigger IFN production, intensifying the self-destructive autoimmune responses. The researchers found that IgE antibodies specific for dsDNA activate pDCs in human SLE, resulting in the secretion of considerable amounts of IFN- $\alpha$ . In addition, serum levels of dsDNA-specific IgE correlated with the severity of disease and potentiated the functions of pDC by triggering phagocytosis through Fc $\epsilon$ RI with subsequent TLR9-mediated DNA sensing in phagosomes (Henault et al., 2016).

### **Basophils and IgE Antibodies in Bullous Pemphigoid**

The etiopathogenesis of bullous pemphigoid (BP), the most frequent pemphigoid disease (around 80% of cases), involves the activation of mast cells in the skin in an IgE autoantibody-mediated process (Sanjuan et al., 2016). The best studied autoantigen in BP is the hemidesmosomal protein BP180. Autoantibodies of the IgE class specific for BP180 have been reported in up to 90% of patients with this skin disease (Dimson et al., 2003; Messingham et al., 2014). Basophils from patients with BP, but not from healthy subjects, can undergo a degranulation in vitro on a BP180-induced cross-linking (Dimson et al., 2003). Fairley et al. (2007) demonstrated that, compared with injection of IgE from control subjects, injection of IgE purified from patients with BP into human skin grafted onto athymic nude mice produced the lesions that are typically observed in the early phase of human BP (i.e., urticarial plaques with dermal edema, eosinophilic inflammation, and mast cell activation and degranulation). Altogether, these findings suggest a pathogenic role of IgE autoantibodies in patients with BP through an Fc $\epsilon$ RI-induced degranulation of basophils and mast cells.

### **IgE Antibodies in Other Autoimmune Disorders**

Although several studies have reported the occurrence of increased levels of IgE autoantibodies (and total IgE) in patients with other autoimmune conditions (Table 13.1), their potential pathogenic significance has not been established.

### **Basophils and IgE Antibodies in Autoimmune Diseases: Therapeutic Implications**

The strongest evidence of the pathogenic implication of IgE autoantibodies in autoimmune diseases came from clinical trials investigating the effectiveness of omalizumab in patients with bullous pemphigous (Sanjuan et al., 2016). Administration of omalizumab, an anti-IgE monoclonal antibody that prevents the interaction of IgE with Fc $\epsilon$ RI, led to reduced urticarial plaques, decreased itching, and a reduction in eosinophilic inflammation (Fairley et al., 2009; Yu et al., 2014). Notably, improvement was achieved in spite of high concentrations of IgG autoantibodies with similar autoantigen specificity, which suggests that low concentrations of IgE autoantibodies can be enough to contribute to the disease process, almost certainly as a consequence of their interaction with Fc $\epsilon$ RI.

Based on the high prevalence of IgE autoantibodies in patients with SLE and the outstanding research by Rivera et al. (Charles et al., 2010; Dema et al., 2014) and Henault et al. (2016), these autoantibodies may well be involved in SLE autoimmunity. In this respect, an ongoing trial is investigating the effectiveness of omalizumab in SLE patients (NCT01716312) (Sanjuan et al., 2016).

Despite the biological differences between human and mouse basophils and the different pathways involved in the activation of basophils, the role of these cells in the etiopathogenesis of autoimmune disorders means that the investigation and development of therapies to target basophils should be actively pursued. The design of targeting therapies should consider the nature of the stimuli (cytokines, antibodies, and TLRs) that prompt basophil activation (Sharma and Bayry, 2015).

**TABLE 13.1** Autoimmune Diseases With Increased Levels of IgE Autoantibodies and Total IgE

Anti-IgE autoantibodies in systemic sclerosis (Kaufman et al., 1989)

Anti-SSA IgE autoantibodies in mothers with fetal loss (Sekigawa et al., 2008)

IgE autoantibodies against retinal S antigen in uveitis (Muiño et al., 1999)

Granulocyte- and organ-specific antinuclear IgE autoantibodies in rheumatoid arthritis (Permin and Wiik, 1978)

IgE reactive with myelin-derived peptides in multiple sclerosis (Mikol et al., 2006)

IgE autoantibodies against thyroid peroxidase in Hashimoto thyroiditis and Graves' disease (Guo et al., 1997; Sato et al., 1999)

IgE autoantibodies against dsDNA (Ishizaka and Ishizaka, 1977; Chang et al., 2015) and thyroglobulin and thyroperoxidase (Chang et al., 2015; Concha et al., 2004; Altrichter et al., 2008) in chronic idiopathic urticaria<sup>a</sup>

<sup>a</sup>Although chronic idiopathic urticaria is not considered a characteristic autoimmune disease, there is conclusive evidence for autoimmunity in some patients. dsDNA, Double-stranded DNA; SSA, Sjögren's-syndrome-related antigen A.

Modified from Sanjuan, M.A., Sagar, D., Kolbeck, R., 2016. Role of IgE in autoimmunity. *J. Allergy Clin. Immunol.* 137 (6), 1651–1661.

## EOSINOPHILS

### Basic Biological Principles and Role in Immunity

Eosinophils are multifunctional granulocytes with granular proteins including eosinophil cationic protein, major basic protein, eosinophil peroxidase, and eosinophil-derived neurotoxin. Eosinophils degranulate upon activation by exocytosis or by piecemeal degranulation with secretion of individual granule contents without disruption of the cell membrane (Khoury et al., 2014). In healthy subjects, over 90% of eosinophils reside in tissues, mostly in the gastrointestinal tract, lymph nodes, spleen, thymus, uterus, and mammary glands. However, in disease conditions, eosinophils can move along chemokine gradients to inflammation sites. Migration of mature eosinophils into circulation is mainly regulated by IL-5 (Khoury et al., 2014), with eosinophils being the main source of this IL. Of note, IL-5 plays a central role in their proliferation and maturation of eosinophil progenitors in the bone marrow, their migration across ECs at tissue sites, and the prevention of eosinophil apoptosis (Khoury et al., 2014; Varricchi et al., 2016; Broughton et al., 2015; Shahabuddin et al., 2000; Ochiai et al., 1997).

Several mediators are involved in directing circulating eosinophils to bind with integrins and selectins with subsequent migration into tissues. These mediators comprise the eotaxins [including eotaxin-1 or chemokine (C-C motif) ligand 11 (CCL11), eotaxin-2 or CCL24, and eotaxin-3 or CCL26], CCL5 (or RANTES), CCL28, and other factors such as the platelet activating factor and C5a (Hogan et al., 2008; Moqbel and Lacy, 2000; Walsh, 2001). Different inhibitory receptors regulate the survival and activation of eosinophils including the sialic acid-binding Ig-like lectin 8 (SIGLEC8), killer activating receptors, CD300A, Fc $\gamma$ RIIb, and potassium inwardly rectifying channel (Varricchi et al., 2016a).

Eosinophils have different functions including the production of numerous cytokines, growth factors, and chemokines that mediate allergic inflammation as well as thrombosis and fibrosis (Khoury et al., 2014). Besides expressing class II molecules of the major histocompatibility complex and costimulatory molecules such as CD80 or CD86 proteins, eosinophils stimulate T cells and process antigens (Varricchi et al., 2016a). Eosinophils prime B cells and promote the activation of DCs through CpG DNA and eosinophil-derived neurotoxin (Munitz et al., 2006). Following allergen sensitization, eosinophils regulate, together with DCs, the recruitment of Th2 cells by producing CCL22 and CCL17 (Munitz et al., 2006). Eosinophils can also promote the differentiation of T follicular helper cells via IL-6 production (Varricchi et al., 2016b).

### Eosinophils in Autoimmune Diseases: Pathogenic Role

#### ***Eosinophils in Eosinophilic Granulomatosis With Polyangiitis***

EGPA is characterized by three overlapping clinical stages that evolve at different intervals: (1) asthma, (2) tissue and blood eosinophilia, and (3) necrotizing small-vessel vasculitis with eosinophilic granulomas (Khoury et al., 2014; Churg and Strauss, 1951; Lanham et al., 1984). The pathogenic implication of eosinophils in EGPA remains to be fully explained. However, there is evidence showing that eosinophils are implicated in all three stages.

Eosinophils are likely implicated in the initial EGPA stage. The presence in sputum of increased numbers of eosinophils and increased levels of eosinophil granule proteins are characteristic of the first EGPA stage (Nair et al., 2013). Likewise, eosinophils are present in the second EGPA stage, characterized by blood and tissue eosinophilia. The typical pathologic finding of the third EGPA stage, with eosinophilic tissue infiltration, eosinophilic vasculitis, and granulomas with a central area of eosinophilic necrosis surrounded by multinucleated giant cells and palisading histiocytes, reflects the major role of eosinophils in this disease (Lie, 1990; Katzenstein, 2000).

Although ANCAAs can be detected in 30%–40% of patients with EGPA (Sablé-Fourtassou et al., 2005; Sinico et al., 2005), there are no reported data indicating that these autoantibodies are pathogenic in this disease.

The etiopathogenesis of EGPA is mainly inferred from clinical studies. On account of the initial allergic inflammation presentation and the increase in circulating levels of Th2-type cytokines that promote the recruitment, activation, and delayed apoptosis of eosinophils (Kiene et al., 2001; Jakiela et al., 2012), EGPA has been classically regarded as a Th2-mediated disease (Khoury et al., 2014).

The chemokine CCL17 (or thymus and activation-regulated chemokine) recruits Th2 cells and is associated with active EGPA (Khoury et al., 2012). Eosinophils in EGPA also produce IL-25, which enhances the production of Th2-type cytokines (Khoury et al., 2014; Varricchi et al., 2016a). Serum levels of IL-25 in EGPA correlate with disease activity and with levels of eosinophils (Terrier et al., 2010), suggesting that interactions between the

innate and adaptive immune systems may contribute to EGPA pathogenesis. It is of note that IL-5 seems to be upregulated in active EGPA (Varricchi et al., 2016a; Jakielka et al., 2012). There are other cytokines and chemokines produced by eosinophils and by other cell types that may be involved in EGPA pathogenesis (Table 13.2).

Some authors have proposed that serum concentrations of different cytokines, chemokines, and surface receptors that participate in the activation and recruitment of eosinophils should be used as biomarkers of disease activity in EGPA (Khoury et al., 2014). For instance, serum levels of eotaxin-3, an eotactic chemokine produced by endothelial and epithelial cells which may contribute to the tissue influx of eosinophils (Zwerina et al., 2011), correlate with eosinophil and total IgE levels in patients with EGPA but not with those in subjects with other eosinophilic conditions and vasculitis (Polzer et al., 2008).

### **Eosinophils in Other Vasculitis**

Eosinophilia, both peripheral and at tissue sites, has been reported in other AAV, specifically in granulomatosis with polyangiitis, albeit at mild or moderate levels (Potter et al., 1999; Schnabel et al., 2000). Patients with Kawasaki disease have been reported to have mild peripheral eosinophilia with substantial eosinophilic infiltrates in the epicardial microvasculature (Terai et al., 2002). Peripheral and tissue eosinophilia can be a prominent feature in drug-induced vasculitis (Mullick et al., 1979). However, there is no reported evidence about the role of eosinophils in the etiopathogenesis of these diseases.

### **Eosinophils in Eosinophilic Granulomatosis With Polyangiitis: Therapeutic Implications**

Treatments that modulate the production, survival, or recruitment of eosinophils might be effective in EGPA. For example, corticosteroids, which are the therapeutic cornerstone of EGPA, decrease the number of circulating and tissue eosinophils by promoting cell death and clearance (Fulkerson and Rothenberg, 2013). As mentioned, the eosinophil-specific cytokine IL-5 has crucial roles in the differentiation, maturation, migration, and survival of eosinophils (Gupta and Kaplan, 2016) and appears to be upregulated in active EGPA (Jakielka et al., 2012). Several agents currently used for the treatment of EGPA can reduce the levels of IL-5 [e.g., corticosteroids (Druilhe et al., 2003) and rituximab (Pepper et al., 2008)] (Khoury et al., 2014; Varricchi et al., 2016a). Preliminary data from two studies using mepolizumab, a humanized anti-IL-5 antibody, as a remission maintenance treatment in patients with EGPA indicate that it is safe agent that enables corticosteroid tapering, even though the lung function remains unmodified (Kim et al., 2010; Herrmann et al., 2012). Moreover, a pilot study in EGPA

**TABLE 13.2** Chemokines and Cytokines Implicated in the Pathogenesis of Eosinophilic Granulomatosis With Polyangiitis

#### **CHEMOKINES**

CCL11 (eotaxin-1) (Polzer et al., 2008)

CCL24 (eotaxin-2) (Polzer et al., 2008)

CCL26 (eotaxin-3) (Polzer et al., 2008; Zwerina et al., 2011)

CCL17 (Khoury et al., 2012; Dallos et al., 2010)

IL-8 (Khoury et al., 2012)

#### **CYTOKINES**

IL-5 (Jakielka et al., 2012)

IL-25 (Terrier et al., 2010)

sIL-2R (Khoury et al., 2012; Schmitt et al., 1998)<sup>a</sup>

IL-4 (Kiene et al., 2001)<sup>b</sup>

IL-13 (Kiene et al., 2001)<sup>b</sup>

<sup>a</sup>Cytokine receptor.

<sup>b</sup>Th2-type cytokines.

CCL11, (C-C motif) ligand 11; IL, interleukin; sIL-2R, soluble IL-2 receptor.

Modified from Khoury, P., Grayson, P.C., Klion, A.D., 2014. Eosinophils in vasculitis: characteristics and roles in pathogenesis. *Nat. Rev. Rheumatol.* 10 (8), 474–483.

patients showed that omalizumab, a recombinant humanized anti-IgE monoclonal antibody, had corticosteroid-sparing properties and caused a decrease in the number of circulating eosinophils and an improved lung function (Detoraki et al., 2016).

## CONCLUSIONS

The potential role of granulocytes in the pathogenesis of systemic autoimmune diseases is being actively investigated. The altered phenotype and function of neutrophils in SLE, illustrated by an abnormal oxidative activity, an increased apoptosis with an impaired elimination of apoptotic cells, and an increased number of circulating LDGs which are toxic to ECs, could be partly responsible for the etiopathogenesis of this disease. Research studies with neutrophils and LDGs from SLE patients have also shown that NETs, which are not properly cleared from the circulation in these patients, and enhanced NETosis can exacerbate the autoimmune responses with eventual organ damage. As such, the potential of therapies targeting NETs in SLE and possibly other autoimmune disorders is enormous. The relevance of neutrophils in the pathophysiology of AAV has been extensively investigated over the last 25 years. More recent evidence indicates that NETs composed of DNA expelled by dying neutrophils and granule-derived proteins, including the autoantigens MPO and PR3, could contribute to the pathogenesis of AAV. Of note, neutrophils and LDGs from patients with AAV can undergo an increased spontaneous NETosis and serum from these patients can induce NETosis and inhibit the degradation of NETs. Both circulating and synovial neutrophils of patients with rheumatoid arthritis play an active pathogenic role through the production of cytotoxic ROS and collagen-degrading proteases. Like SLE and AAV, NETs and NETosis seem to be implicated in the etiopathogenesis of rheumatoid arthritis. Remarkably, NETs in rheumatoid arthritis are a source of extracellular citrullinated autoantigens, which can trigger inflammatory responses in synovial fibroblasts, with release of proinflammatory cytokines and chemokines. Neutrophils and NETosis have been involved in the pathogenesis of antiphospholipid antibody syndrome. When interacting with neutrophils *in vitro*, the autoantigen  $\beta$ 2GPI can stimulate NETosis through a TLR4- and ROS-signaling-dependent process. Also, while neutrophils from patients with antiphospholipid antibody syndrome can produce spontaneous NETs *ex vivo*, serum levels of anti- $\beta$ 2GPI antibodies correlate positively with circulating levels of NETs. A few studies have investigated the role of neutrophils in other autoimmune diseases including primary Sjögren syndrome, systemic sclerosis, and polymyositis/dermatomyositis. However, their potential pathogenic relevance is less conclusive.

Regarding basophils, in addition to their role in allergy and antiparasitic immunity, they have a key immunomodulatory role in some autoimmune diseases due to their influence on Th2 polarization and their effects on B-cell activation, differentiation, survival, and promotion of autoantibody production. Studies have shown a close correlation between increased numbers of circulating basophils and increased serum levels of IgE. The pathogenic potential of these cells in SLE has mainly been investigated in murine models, where basophils not only promote lupus nephritis in an IgE- and IL-4-dependent manner but the IgE–basophil network creates an inflammatory environment by delivering effective signals for the migration of innate immune cells to secondary lymphoid organs. Basophils and IgE also play an active role in the pathophysiology of BP. Indeed, there is a need to design specific therapies to target basophils.

Finally, the involvement of eosinophils in the etiopathogenesis of EGPA remains to be clarified. There is some evidence that eosinophils participate in the three stages of EGPA and that chemokines and cytokines produced by eosinophils, and by many other cell types, may contribute to its pathogenesis. Importantly, IL-5 plays a fundamental role in the production, recruitment, and survival of eosinophils and seems to be upregulated in active EGPA. Consequently, therapies aimed to decrease the levels of IL-5, especially anti-IL-5 antibodies, are being actively investigated with promising preliminary results.

## References

- Abramson, S.B., Given, W.P., Edelson, H.S., Weissmann, G., 1983. Neutrophil aggregation induced by sera from patients with active systemic lupus erythematosus. *Arthritis Rheum.* 26 (5), 630–636.
- Altrichter, S., Kriehuber, E., Moser, J., Valenta, R., Kopp, T., Stingl, G., 2008. Serum IgE autoantibodies target keratinocytes in patients with atopic dermatitis. *J. Invest. Dermatol.* 128 (9), 2232–2239.
- Amulic, B., Cazalet, C., Hayes, G.L., Metzler, K.D., Zychlinsky, A., 2012. Neutrophil function: from mechanisms to disease. *Annu. Rev. Immunol.* 30, 459–489.

- Barnado, A., Crofford, L.J., Oates, J.C., 2016. At the bedside: neutrophil extracellular traps (NETs) as targets for biomarkers and therapies in autoimmune diseases. *J. Leukoc. Biol.* 99 (2), 265–278.
- Barnes, T.C., Spiller, D.G., Anderson, M.E., Edwards, S.W., Moots, R.J., 2011. Endothelial activation and apoptosis mediated by neutrophil-dependent interleukin 6 trans-signalling: a novel target for systemic sclerosis? *Ann. Rheum. Dis.* 70 (2), 366–372.
- Barnes, T.C., Anderson, M.E., Edwards, S.W., Moots, R.J., 2012. Neutrophil-derived reactive oxygen species in SSc. *Rheumatology (Oxford)* 51 (7), 1166–1169.
- Bengtsson, A.A., Pettersson, Å., Wichert, S., Gullstrand, B., Hansson, M., Hellmark, T., et al., 2014. Low production of reactive oxygen species in granulocytes is associated with organ damage in systemic lupus erythematosus. *Arthritis Res. Ther.* 16 (3), R120.
- Bennett, L., Palucka, A.K., Arce, E., Cantrell, V., Borvak, J., Banchereau, J., et al., 2003. Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. *J. Exp. Med.* 197 (6), 711–723.
- Brandt, L., Hedberg, H., 1969. Impaired phagocytosis by peripheral blood granulocytes in systemic lupus erythematosus. *Scand. J. Haematol.* 6 (5), 348–353.
- Brill, A., Fuchs, T.A., Savchenko, A.S., Thomas, G.M., Martinod, K., De Meyer, S.F., et al., 2012. Neutrophil extracellular traps promote deep vein thrombosis in mice. *J. Thromb. Haemost.* 10 (1), 136–144.
- Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D.S., et al., 2004. Neutrophil extracellular traps kill bacteria. *Science* 303 (5663), 1532–1535.
- Brito-Zerón, P., Soria, N., Muñoz, S., Bové, A., Akasbi, M., Belenguer, R., et al., 2009. Prevalence and clinical relevance of autoimmune neutropenia in patients with primary Sjögren's syndrome. *Semin. Arthritis Rheum.* 38 (5), 389–395.
- Broughton, S.E., Nero, T.L., Dhagat, U., Kan, W.L., Hercus, T.R., Tvorogov, D., et al., 2015. The  $\beta$ c receptor family—structural insights and their functional implications. *Cytokine* 74 (2), 247–258.
- Burmester, G.R., Feist, E., Dörner, T., 2014. Emerging cell and cytokine targets in rheumatoid arthritis. *Nat. Rev. Rheumatol.* 10 (2), 77–88.
- Cahenzli, J., Köller, Y., Wyss, M., Geuking, M.B., McCoy, K.D., 2013. Intestinal microbial diversity during early-life colonization shapes long-term IgE levels. *Cell Host Microbe* 14 (5), 559–570.
- Cairns, A.P., Crockard, A.D., McConnell, J.R., Courtney, P.A., Bell, A.L., 2001. Reduced expression of CD44 on monocytes and neutrophils in systemic lupus erythematosus: relations with apoptotic neutrophils and disease activity. *Ann. Rheum. Dis.* 60 (10), 950–955.
- Campanelli, D., Melchior, M., Fu, Y., Nakata, M., Shuman, H., Nathan, C., et al., 1990. Cloning of cDNA for proteinase 3: a serine protease, antibiotic, and autoantigen from human neutrophils. *J. Exp. Med.* 172 (6), 1709–1715.
- Carli, L., Tani, C., Vagnani, S., Signorini, V., Mosca, M., 2015. Leukopenia, lymphopenia, and neutropenia in systemic lupus erythematosus: prevalence and clinical impact—a systematic literature review. *Semin. Arthritis Rheum.* 45 (2), 190–194.
- Carmona-Rivera, C., Zhao, W., Yalavarthi, S., Kaplan, M.J., 2015. Neutrophil extracellular traps induce endothelial dysfunction in systemic lupus erythematosus through the activation of matrix metalloproteinase-2. *Ann. Rheum. Dis.* 74 (7), 1417–1424.
- Cassard, L., Jönsson, F., Arnaud, S., Daëron, M., 2012. Fc $\gamma$  receptors inhibit mouse and human basophil activation. *J. Immunol.* 189 (6), 2995–3006.
- Chang, T.W., Chen, C., Lin, C.J., Metz, M., Church, M.K., Maurer, M., 2015. The potential pharmacologic mechanisms of omalizumab in patients with chronic spontaneous urticaria. *J. Allergy Clin. Immunol.* 135 (2), 337–342.
- Charles, N., Hardwick, D., Daugas, E., Illei, G.G., Rivera, J., 2010. Basophils and the T helper 2 environment can promote the development of lupus nephritis. *Nat. Med.* 16 (6), 701–707.
- Churg, J., Strauss, L., 1951. Allergic granulomatosis, allergic angiitis, and periarteritis nodosa. *Am. J. Pathol.* 27 (2), 277–301.
- Clark, R.A., Gallin, J.I., Kaplan, A.P., 1976. Mediator release from basophil granulocytes in chronic myelogenous leukemia. *J. Allergy Clin. Immunol.* 58 (6), 623–634.
- Clynes, R., Dumitru, C., Ravetch, J.V., 1998. Uncoupling of immune complex formation and kidney damage in autoimmune glomerulonephritis. *Science* 279 (5353), 1052–1054.
- Concha, L.B., Chang, C.C., Szema, A.M., Dattwyler, R.J., Carlson, H.E., 2004. IgE antithyroid antibodies in patients with Hashimoto's disease and chronic urticaria. *Allergy Asthma Proc.* 25 (5), 293–296.
- Courtney, P.A., Crockard, A.D., Williamson, K., Irvine, A.E., Kennedy, R.J., Bell, A.L., 1999. Increased apoptotic peripheral blood neutrophils in systemic lupus erythematosus: relations with disease activity, antibodies to double stranded DNA, and neutropenia. *Ann. Rheum. Dis.* 58 (5), 309–314.
- Csernok, E., Lamprecht, P., Gross, W.L., 2006. Diagnostic significance of ANCA in vasculitis. *Nat. Clin. Pract. Rheumatol.* 2 (4), 174–175.
- Dahinden, C.A., Kurimoto, J., Baggolini, M., Dewald, B., Walz, A., 1989. Histamine and sulfidoleukotriene release from human basophils: different effects of antigen, anti-IgE, C5a, f-Met-Leu-Phe and the novel neutrophil-activating peptide NAF. *Int. Arch. Allergy Appl. Immunol.* 90 (2), 113–118.
- Dallos, T., Heiland, G.R., Strehl, J., Karonitsch, T., Gross, W.L., Moosig, F., et al., 2010. CCL17/thymus and activation-related chemokine in Churg-Strauss syndrome. *Arthritis Rheum.* 62 (11), 3496–3503.
- de Paulis, A., Prevete, N., Fiorentino, I., Rossi, F.W., Staibano, S., Montuori, N., et al., 2006. Expression and functions of the vascular endothelial growth factors and their receptors in human basophils. *J. Immunol.* 177 (10), 7322–7331.
- de Siqueira, M.B., da Mota, L.M., Couto, S.C., Muniz-Junqueira, M.I., 2015. Enhanced neutrophil phagocytic capacity in rheumatoid arthritis related to the autoantibodies rheumatoid factor and anti-cyclic citrullinated peptides. *BMC Musculoskelet. Disord.* 16, 159.
- Dema, B., Charles, N., Pellefigues, C., Ricks, T.K., Suzuki, R., Jiang, C., et al., 2014. Immunoglobulin E plays an immunoregulatory role in lupus. *J. Exp. Med.* 211 (11), 2159–2168.
- Denny, M.F., Yalavarthi, S., Zhao, W., Thacker, S.G., Anderson, M., Sandy, A.R., et al., 2010. A distinct subset of proinflammatory neutrophils isolated from patients with systemic lupus erythematosus induces vascular damage and synthesizes type I IFNs. *J. Immunol.* 184 (6), 3284–3297.
- Denzel, A., Maus, U.A., Rodriguez Gomez, M., Moll, C., Niedermeier, M., Winter, C., et al., 2008. Basophils enhance immunological memory responses. *Nat. Immunol.* 9 (7), 733–742.

- Detoraki, A., Di Capua, L., Varricchi, G., Genovese, A., Marone, G., Spadaro, G., 2016. Omalizumab in patients with eosinophilic granulomatosis with polyangiitis: a 36-month follow-up study. *J. Asthma* 53 (2), 201–206.
- Di, C., Lin, X., Zhang, Y., Zhong, W., Yuan, Y., Zhou, T., et al., 2015. Basophil-associated OX40 ligand participates in the initiation of Th2 responses during airway inflammation. *J. Biol. Chem.* 290 (20), 12523–12536.
- Dijkstra, D., Meyer-Bahlburg, A., 2017. Human basophils modulate plasma cell differentiation and maturation. *J. Immunol.* 198 (1), 229–238.
- Dimson, O.G., Giudice, G.J., Fu, C.L., Van den Berghe, F., Warren, S.J., Janson, M.M., et al., 2003. Identification of a potential effector function for IgE autoantibodies in the organ-specific autoimmune disease bullous pemphigoid. *J. Invest. Dermatol.* 120 (5), 784–788.
- Donnelly, S., Roake, W., Brown, S., Young, P., Naik, H., Wordsworth, P., et al., 2006. Impaired recognition of apoptotic neutrophils by the C1q/calreticulin and CD91 pathway in systemic lupus erythematosus. *Arthritis Rheum.* 54 (5), 1543–1556.
- Druilhe, A., Létuvé, S., Pretolani, M., 2003. Glucocorticoid-induced apoptosis in human eosinophils: mechanisms of action. *Apoptosis* 8 (5), 481–495.
- Fairley, J.A., Burnett, C.T., Fu, C.L., Larson, D.L., Fleming, M.G., Giudice, G.J., 2007. A pathogenic role for IgE in autoimmunity: bullous pemphigoid IgE reproduces the early phase of lesion development in human skin grafted to nu/nu mice. *J. Invest. Dermatol.* 127 (11), 2605–2611.
- Fairley, J.A., Baum, C.L., Brandt, D.S., Messingham, K.A., 2009. Pathogenicity of IgE in autoimmunity: successful treatment of bullous pemphigoid with omalizumab. *J. Allergy Clin. Immunol.* 123 (3), 704–705.
- Falk, R.J., Terrell, R.S., Charles, L.A., Jennette, J.C., 1990. Anti-neutrophil cytoplasmic autoantibodies induce neutrophils to degranulate and produce oxygen radicals in vitro. *Proc. Natl. Acad. Sci. U.S.A.* 87 (11), 4115–4119.
- Fazio, J., Kalyan, S., Wesch, D., Kabelitz, D., 2014. Inhibition of human  $\gamma\delta$  T cell proliferation and effector functions by neutrophil serine proteases. *Scand. J. Immunol.* 80 (6), 381–389.
- Fleming, J.N., Schwartz, S.M., 2008. The pathology of scleroderma vascular disease. *Rheum. Dis. Clin. North Am.* 34 (1), 41–55.
- Fossati, G., Moulding, D.A., Spiller, D.G., Moots, R.J., White, M.R., Edwards, S.W., 2003. The mitochondrial network of human neutrophils: role in chemotaxis, phagocytosis, respiratory burst activation, and commitment to apoptosis. *J. Immunol.* 170 (4), 1964–1972.
- Fransen, M., Nordgren, M., Wang, B., Apanasets, O., 2012. Role of peroxisomes in ROS/RNS-metabolism: implications for human disease. *Biochim. Biophys. Acta* 1822 (9), 1363–1373.
- Fuchs, T.A., Brill, A., Duerschmied, D., Schatzberg, D., Monestier, M., Myers, D.D., et al., 2010. Extracellular DNA traps promote thrombosis. *Proc. Natl. Acad. Sci. U.S.A.* 107 (36), 15880–15885.
- Fulkerson, P.C., Rothenberg, M.E., 2013. Targeting eosinophils in allergy, inflammation and beyond. *Nat. Rev. Drug Discov.* 12 (2), 117–129.
- Furebring, M., Håkansson, L.D., Venge, P., Nilsson, B., Sjölin, J., 2002. Expression of the C5a receptor (CD88) on granulocytes and monocytes in patients with severe sepsis. *Crit. Care.* 6 (4), 363–370.
- Garcia, R.J., Francis, L., Dawood, M., Lai, Z.W., Faraone, S.V., Perl, A., 2013. Attention deficit and hyperactivity disorder scores are elevated and respond to N-acetylcysteine treatment in patients with systemic lupus erythematosus. *Arthritis Rheum.* 65 (5), 1313–1318.
- Garcia-Romo, G.S., Caielli, S., Vega, B., Connolly, J., Allantaz, F., Xu, Z., et al., 2011. Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. *Sci. Transl. Med.* 3 (73), 73ra20.
- Gauchat, J.F., Henchoz, S., Mazzei, G., Aubry, J.P., Brunner, T., Blasey, H., et al., 1993. Induction of human IgE synthesis in B cells by mast cells and basophils. *Nature* 365 (6444), 340–343.
- Geering, B., Stoeckle, C., Conus, S., Simon, H.U., 2013. Living and dying for inflammation: neutrophils, eosinophils, basophils. *Trends Immunol.* 34 (8), 398–409.
- Gehrke, N., Mertens, C., Zillinger, T., Wenzel, J., Bald, T., Zahn, S., et al., 2013. Oxidative damage of DNA confers resistance to cytosolic nucleic acid TREX1 degradation and potentiates STING-dependent immune sensing. *Immunity* 39 (3), 482–495.
- Giacomin, P.R., Siracusa, M.C., Walsh, K.P., Grencis, R.K., Kubo, M., Comeau, M.R., et al., 2012. Thymic stromal lymphopoietin-dependent basophils promote Th2 cytokine responses following intestinal helminth infection. *J. Immunol.* 189 (9), 4371–4378.
- Grayson, P.C., Carmona-Rivera, C., Xu, L., Lim, N., Gao, Z., Asare, A.L., et al., 2015. Neutrophil-related gene expression and low-density granulocytes associated with disease activity and response to treatment in antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheumatol.* 67 (7), 1922–1932.
- Guo, J., Rapoport, B., McLachlan, S.M., 1997. Thyroid peroxidase autoantibodies of IgE class in thyroid autoimmunity. *Clin. Immunol. Immunopathol.* 82 (2), 157–162.
- Gupta, S., Kaplan, M.J., 2016. The role of neutrophils and NETosis in autoimmune and renal diseases. *Nat. Rev. Nephrol.* 12 (7), 402–413.
- Hakkim, A., Fürnrohr, B.G., Amann, K., Laube, B., Abed, U.A., Brinkmann, V., et al., 2010. Impairment of neutrophil extracellular trap degradation is associated with lupus nephritis. *Proc. Natl. Acad. Sci. U.S.A.* 107 (21), 9813–9818.
- Henault, J., Riggs, J.M., Karnell, J.L., Liarski, V.M., Li, J., Shirinian, L., et al., 2016. Self-reactive IgE exacerbates interferon responses associated with autoimmunity. *Nat. Immunol.* 17 (2), 196–203.
- Herrmann, K., Gross, W.L., Moosig, F., 2012. Extended follow-up after stopping mepolizumab in relapsing/refractory Churg-Strauss syndrome. *Clin. Exp. Rheumatol.* 30 (1 Suppl 70), S62–S65.
- Hill, D.A., Siracusa, M.C., Abt, M.C., Kim, B.S., Kobuley, D., Kubo, M., et al., 2012. Commensal bacteria-derived signals regulate basophil hematopoiesis and allergic inflammation. *Nat. Med.* 18 (4), 538–546.
- Hogan, S.P., Rosenberg, H.F., Moqbel, R., Phipps, S., Foster, P.S., Lacy, P., et al., 2008. Eosinophils: biological properties and role in health and disease. *Clin. Exp. Allergy* 38 (5), 709–750.
- Hsieh, S.C., Yu, H.S., Lin, W.W., Sun, K.H., Tsai, C.Y., Huang, D.F., et al., 2003. Anti-SSB/La is one of the antineutrophil autoantibodies responsible for neutropenia and functional impairment of polymorphonuclear neutrophils in patients with systemic lupus erythematosus. *Clin. Exp. Immunol.* 131 (3), 506–516.
- Huang, X., Li, J., Dorta-Estremera, S., Di Domizio, J., Anthony, S.M., Watowich, S.S., et al., 2015a. Neutrophils regulate humoral autoimmunity by restricting interferon- $\gamma$  production via the generation of reactive oxygen species. *Cell Rep.* 12 (7), 1120–1132.

- Huang, Y.M., Wang, H., Wang, C., Chen, M., Zhao, M.H., 2015b. Promotion of hypercoagulability in antineutrophil cytoplasmic antibody-associated vasculitis by C5a-induced tissue factor-expressing microparticles and neutrophil extracellular traps. *Arthritis Rheumatol.* 67 (10), 2780–2790.
- Huard, B., McKee, T., Bosshard, C., Durual, S., Matthes, T., Myit, S., et al., 2008. APRIL secreted by neutrophils binds to heparan sulfate proteoglycans to create plasma cell niches in human mucosa. *J. Clin. Invest.* 118 (8), 2887–2895.
- Huebener, P., Pradere, J.P., Hernandez, C., Gwak, G.Y., Caviglia, J.M., Mu, X., et al., 2015. The HMGB1/RAGE axis triggers neutrophil-mediated injury amplification following necrosis. *J. Clin. Invest.* 125 (2), 539–550.
- Hurst, S.M., Wilkinson, T.S., McLoughlin, R.M., Jones, S., Horiuchi, S., Yamamoto, N., et al., 2001. IL-6 and its soluble receptor orchestrate a temporal switch in the pattern of leukocyte recruitment seen during acute inflammation. *Immunity* 14 (6), 705–714.
- Ishizaka, T., Ishizaka, K., 1977. Immunological events at the surface of basophil granulocytes and mast cells which induce degranulation. *Scand. J. Respir. Dis. Suppl.* 98, 13–22.
- Jakiela, B., Szczeklik, W., Plutecka, H., Sokolowska, B., Mastalerz, L., Sanak, M., et al., 2012. Increased production of IL-5 and dominant Th2-type response in airways of Churg-Strauss syndrome patients. *Rheumatology (Oxford)* 51 (10), 1887–1893.
- Jennette, J.C., Falk, R.J., 2014. Pathogenesis of antineutrophil cytoplasmic autoantibody-mediated disease. *Nat. Rev. Rheumatol.* 10 (8), 463–473.
- Jeon, J.H., Ahn, K.B., Kim, S.K., Im, J., Yun, C.H., Han, S.H., 2015. Bacterial flagellin induces IL-6 expression in human basophils. *Mol. Immunol.* 65 (1), 168–176.
- Jorch, S.K., Kubes, P., 2017. An emerging role for neutrophil extracellular traps in noninfectious disease. *Nat. Med.* 23 (3), 279–287.
- Kahlenberg, J.M., Carmona-Rivera, C., Smith, C.K., Kaplan, M.J., 2013. Neutrophil extracellular trap-associated protein activation of the NLRP3 inflammasome is enhanced in lupus macrophages. *J. Immunol.* 190 (3), 1217–1226.
- Kambas, K., Chrysanthopoulou, A., Vassilopoulos, D., Apostolidou, E., Skendros, P., Girod, A., et al., 2014. Tissue factor expression in neutrophil extracellular traps and neutrophil derived microparticles in antineutrophil cytoplasmic antibody associated vasculitis may promote thromboinflammation and the thrombophilic state associated with the disease. *Ann. Rheum. Dis.* 73 (10), 1854–1863.
- Kaplan, M.J., 2011. Neutrophils in the pathogenesis and manifestations of SLE. *Nat. Rev. Rheumatol.* 7 (12), 691–699.
- Karasuyama, H., Yamanishi, Y., 2014. Basophils have emerged as a key player in immunity. *Curr. Opin. Immunol.* 31, 1–7.
- Karasuyama, H., Mukai, K., Obata, K., Tsujimura, Y., Wada, T., 2011. Nonredundant roles of basophils in immunity. *Annu. Rev. Immunol.* 29, 45–69.
- Katano, M., Okamoto, K., Arito, M., Kawakami, Y., Kurokawa, M.S., Suematsu, N., et al., 2009. Implication of granulocyte-macrophage colony-stimulating factor induced neutrophil gelatinase-associated lipocalin in pathogenesis of rheumatoid arthritis revealed by proteome analysis. *Arthritis Res. Ther.* 11 (1), R3.
- Katzenstein, A.L., 2000. Diagnostic features and differential diagnosis of Churg-Strauss syndrome in the lung. A review. *Am. J. Clin. Pathol.* 114 (5), 767–772.
- Kaufman, L.D., Gruber, B.L., Marchese, M.J., Seibold, J.R., 1989. Anti-IgE autoantibodies in systemic sclerosis (scleroderma). *Ann. Rheum. Dis.* 48 (3), 201–205.
- Kenny, E.F., Herzig, A., Krüger, R., Muth, A., Mondal, S., Thompson, P.R., et al., 2017. Diverse stimuli engage different neutrophil extracellular trap pathways. *eLife* 6, pii:e24437.
- Kessenbrock, K., Krumbholz, M., Schönermark, U., Back, W., Gross, W.L., Werb, Z., et al., 2009. Netting neutrophils in autoimmune small-vessel vasculitis. *Nat. Med.* 15 (6), 623–625.
- Khandpur, R., Carmona-Rivera, C., Vivekanandan-Giri, A., Gizinski, A., Yalavarthi, S., Knight, J.S., et al., 2013. NETs are a source of citrullinated autoantigens and stimulate inflammatory responses in rheumatoid arthritis. *Sci. Transl. Med.* 5 (178), 178ra40.
- Khoury, P., Zagallo, P., Talar-Williams, C., Santos, C.S., Dinerman, E., Holland, N.C., et al., 2012. Serum biomarkers are similar in Churg-Strauss syndrome and hypereosinophilic syndrome. *Allergy* 67 (9), 1149–1156.
- Khoury, P., Grayson, P.C., Klion, A.D., 2014. Eosinophils in vasculitis: characteristics and roles in pathogenesis. *Nat. Rev. Rheumatol.* 10 (8), 474–483.
- Kiene, M., Csernok, E., Müller, A., Metzler, C., Trabandt, A., Gross, W.L., 2001. Elevated interleukin-4 and interleukin-13 production by T cell lines from patients with Churg-Strauss syndrome. *Arthritis Rheum.* 44 (2), 469–473.
- Kim, S., Marigowda, G., Oren, E., Israel, E., Wechsler, M.E., 2010. Mepolizumab as a steroid-sparing treatment option in patients with Churg-Strauss syndrome. *J. Allergy Clin. Immunol.* 125 (6), 1336–1343.
- Kim, J.E., Park, S.J., Shin, J.I., 2014. The role of interleukin-17 in the associations between systemic lupus erythematosus and ANCA-associated vasculitis. *Rheumatol. Int.* 34 (5), 709–710.
- Kobayashi, S.D., DeLeo, F.R., 2009. Role of neutrophils in innate immunity: a systems biology-level approach. *Wiley Interdiscip. Rev. Syst. Biol. Med.* 1 (3), 309–333.
- Kolaczkowska, E., Kubes, P., 2013. Neutrophil recruitment and function in health and inflammation. *Nat. Rev. Immunol.* 13 (3), 159–175.
- Kolaczkowska, E., Jenne, C.N., Surewaard, B.G., Thanabalsurair, A., Lee, W.Y., Sanz, M.J., et al., 2015. Molecular mechanisms of NET formation and degradation revealed by intravital imaging in the liver vasculature. *Nat. Commun.* 6, 6673.
- Komiya, A., Nagase, H., Okugawa, S., Ota, Y., Suzukawa, M., Kawakami, A., et al., 2006. Expression and function of Toll-like receptors in human basophils. *Int. Arch. Allergy Immunol.* 140 (Suppl 1), 23–27.
- Lai, Z.W., Hanczko, R., Bonilla, E., Caza, T.N., Clair, B., Bartos, A., et al., 2012. N-Acetylcysteine reduces disease activity by blocking mammalian target of rapamycin in T cells from systemic lupus erythematosus patients: a randomized, double-blind, placebo-controlled trial. *Arthritis Rheum.* 64 (9), 2937–2946.
- Lande, R., Ganguly, D., Facchinetto, V., Frasca, L., Conrad, C., Gregorio, J., et al., 2011. Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus. *Sci. Transl. Med.* 3 (73), 73ra19.
- Lanham, J.G., Elkorn, K.B., Pusey, C.D., Hughes, G.R., 1984. Systemic vasculitis with asthma and eosinophilia: a clinical approach to the Churg-Strauss syndrome. *Medicine (Baltimore)* 63 (2), 65–81.

- Lau, C.M., Broughton, C., Tabor, A.S., Akira, S., Flavell, R.A., Mamula, M.J., et al., 2005. RNA-associated autoantigens activate B cells by combined B cell antigen receptor/Toll-like receptor 7 engagement. *J. Exp. Med.* 202 (9), 1171–1177.
- Leffler, J., Martin, M., Gullstrand, B., Tydén, H., Lood, C., Truedsson, L., et al., 2012. Neutrophil extracellular traps that are not degraded in systemic lupus erythematosus activate complement exacerbating the disease. *J. Immunol.* 188 (7), 3522–3531.
- Leffler, J., Stojanovich, L., Shoenfeld, Y., Bogdanovic, G., Hesselstrand, R., Blom, A.M., 2014. Degradation of neutrophil extracellular traps is decreased in patients with antiphospholipid syndrome. *Clin. Exp. Rheumatol.* 32 (1), 66–70.
- Lefrançais, E., Roga, S., Gautier, V., Gonzalez-de-Peredo, A., Monserrat, B., Girard, J.P., et al., 2012. IL-33 is processed into mature bioactive forms by neutrophil elastase and cathepsin G. *Proc. Natl. Acad. Sci. U.S.A.* 109 (5), 1673–1678.
- Li, H., Sim, T.C., Alam, R., 1996a. IL-13 released by and localized in human basophils. *J. Immunol.* 156 (12), 4833–4838.
- Li, H., Sim, T.C., Grant, J.A., Alam, R., 1996b. The production of macrophage inflammatory protein-1 alpha by human basophils. *J. Immunol.* 157 (3), 1207–1212.
- Liang, M., Lv, J., Zou, L., Yang, W., Xiong, Y., Chen, X., et al., 2015. A modified murine model of systemic sclerosis: bleomycin given by pump infusion induced skin and pulmonary inflammation and fibrosis. *Lab. Invest.* 95 (3), 342–350.
- Lie, J.T., 1990. Illustrated histopathologic classification criteria for selected vasculitis syndromes. American College of Rheumatology Subcommittee on Classification of Vasculitis. *Arthritis Rheum.* 33 (8), 1074–1087.
- Lood, C., Blanco, L.P., Purmalek, M.M., Carmona-Rivera, C., De Ravin, S.S., Smith, C.K., et al., 2016. Neutrophil extracellular traps enriched in oxidized mitochondrial DNA are interferogenic and contribute to lupus-like disease. *Nat. Med.* 22 (2), 146–153.
- Lu, X., Tang, Q., Lindh, M., Dastmalchi, M., Alexanderson, H., Popovic Silverfeldt, K., et al., 2017. The host defense peptide LL-37 a possible inducer of the type I interferon system in patients with polymyositis and dermatomyositis. *J. Autoimmun.* 78, 46–56.
- Lyons, P.A., McKinney, E.F., Rayner, T.F., Hatton, A., Woffendin, H.B., Koukoulaki, M., et al., 2010. Novel expression signatures identified by transcriptional analysis of separated leucocyte subsets in systemic lupus erythematosus and vasculitis. *Ann. Rheum. Dis.* 69 (6), 1208–1213.
- Lyons, P.A., Rayner, T.F., Trivedi, S., Holle, J.U., Watts, R.A., Jayne, D.R., et al., 2012. Genetically distinct subsets within ANCA-associated vasculitis. *N. Engl. J. Med.* 367 (3), 214–223.
- Macanovic, M., Sinicropi, D., Shak, S., Baughman, S., Thiru, S., Lachmann, P.J., 1996. The treatment of systemic lupus erythematosus (SLE) in NZB/W F1 hybrid mice: studies with recombinant murine DNase and with dexamethasone. *Clin. Exp. Immunol.* 106 (2), 243–252.
- Mack, M., Schneider, M.A., Moll, C., Cihak, J., Brühl, H., Ellwart, J.W., et al., 2005. Identification of antigen-capturing cells as basophils. *J. Immunol.* 174 (2), 735–741.
- Mantovani, A., Cassatella, M.A., Costantini, C., Jaillon, S., 2011. Neutrophils in the activation and regulation of innate and adaptive immunity. *Nat. Rev. Immunol.* 11 (8), 519–531.
- Massberg, S., Grahl, L., von Bruehl, M.L., Manukyan, D., Pfeiler, S., Goosmann, C., et al., 2010. Reciprocal coupling of coagulation and innate immunity via neutrophil serine proteases. *Nat. Med.* 16 (8), 887–896.
- McInnes, I.B., Schett, G., 2011. The pathogenesis of rheumatoid arthritis. *N. Engl. J. Med.* 365 (23), 2205–2219.
- McInnes, I.B., Buckley, C.D., Isaacs, J.D., 2016. Cytokines in rheumatoid arthritis—shaping the immunological landscape. *Nat. Rev. Rheumatol.* 12 (1), 63–68.
- Megiovanni, A.M., Sanchez, F., Robledo-Sarmiento, M., Morel, C., Gluckman, J.C., Boudaly, S., 2006. Polymorphonuclear neutrophils deliver activation signals and antigenic molecules to dendritic cells: a new link between leukocytes upstream of T lymphocytes. *J. Leukoc. Biol.* 79 (5), 977–988.
- Messingham, K.A., Holahan, H.M., Fairley, J.A., 2014. Unraveling the significance of IgE autoantibodies in organ-specific autoimmunity: lessons learned from bullous pemphigoid. *Immunol. Res.* 59 (1-3), 273–278.
- Midgley, A., Beresford, M.W., 2016. Increased expression of low density granulocytes in juvenile-onset systemic lupus erythematosus patients correlates with disease activity. *Lupus* 25 (4), 407–411.
- Mikol, D.D., Ditlow, C., Usatin, D., Biswas, P., Kalbfleisch, J., Milner, A., et al., 2006. Serum IgE reactive against small myelin protein-derived peptides is increased in multiple sclerosis patients. *J. Neuroimmunol.* 180 (1-2), 40–49.
- Momohara, S., Kashiwazaki, S., Inoue, K., Saito, S., Nakagawa, T., 1997. Elastase from polymorphonuclear leukocyte in articular cartilage and synovial fluids of patients with rheumatoid arthritis. *Clin. Rheumatol.* 16 (2), 133–140.
- Moqbel, R., Lacy, P., 2000. New concepts in effector functions of eosinophil cytokines. *Clin. Exp. Allergy* 30 (12), 1667–1671.
- Muiño, J.C., Juárez, C.P., Luna, J.D., Castro, C.C., Wolff, E.G., Ferrero, M., et al., 1999. The importance of specific IgG and IgE autoantibodies to retinal S antigen, total serum IgE, and sCD23 levels in autoimmune and infectious uveitis. *J. Clin. Immunol.* 19 (4), 215–222.
- Mullick, F.G., McAllister Jr, H.A., Wagner, B.M., Fenoglio Jr, J.J., 1979. Drug related vasculitis. Clinicopathologic correlations in 30 patients. *Hum. Pathol.* 10 (3), 313–325.
- Munitz, A., Bachelet, I., Eliashar, R., Moretta, A., Moretta, L., Levi-Schaffer, F., 2006. The inhibitory receptor IRP60 (CD300a) suppresses the effects of IL-5, GM-CSF, and eotaxin on human peripheral blood eosinophils. *Blood* 107 (5), 1996–2003.
- Nair, P., Ochkur, S.I., Protheroe, C., Radford, K., Efthimiadis, A., Lee, N.A., et al., 2013. Eosinophil peroxidase in sputum represents a unique biomarker of airway eosinophilia. *Allergy* 68 (9), 1177–1184.
- Nakazawa, D., Shida, H., Tomaru, U., Yoshida, M., Nishio, S., Atsumi, T., et al., 2014. Enhanced formation and disordered regulation of NETs in myeloperoxidase-ANCA-associated microscopic polyangiitis. *J. Am. Soc. Nephrol.* 25 (5), 990–997.
- Nauseef, W.M., 2007. How human neutrophils kill and degrade microbes: an integrated view. *Immunol. Rev.* 219, 88–102.
- Nzeusseu Toukap, A., Delporte, C., Noyon, C., Franck, T., Rousseau, A., Serteyn, D., et al., 2014. Myeloperoxidase and its products in synovial fluid of patients with treated or untreated rheumatoid arthritis. *Free Radic. Res.* 48 (4), 461–465.
- Ochiai, K., Kagami, M., Matsumura, R., Tomioka, H., 1997. IL-5 but not interferon-gamma (IFN-gamma) inhibits eosinophil apoptosis by up-regulation of bcl-2 expression. *Clin. Exp. Immunol.* 107 (1), 198–204.
- Okada, T., Hikoso, S., Yamaguchi, O., Taneike, M., Takeda, T., Tamai, T., et al., 2012. Mitochondrial DNA that escapes from autophagy causes inflammation and heart failure. *Nature* 485 (7397), 251–255.

- Papadaki, H.A., Kritikos, H.D., Valatas, V., Boumpas, D.T., Eliopoulos, G.D., 2002. Anemia of chronic disease in rheumatoid arthritis is associated with increased apoptosis of bone marrow erythroid cells: improvement following anti-tumor necrosis factor-alpha antibody therapy. *Blood* 100 (2), 474–482.
- Patel, S., Kumar, S., Jyoti, A., Srinag, B.S., Keshari, R.S., Saluja, R., et al., 2010. Nitric oxide donors release extracellular traps from human neutrophils by augmenting free radical generation. *Nitric Oxide* 22 (3), 226–234.
- Pepper, R.J., Fabre, M.A., Pavesio, C., Gaskin, G., Jones, R.B., Jayne, D., et al., 2008. Rituximab is effective in the treatment of refractory Churg-Strauss syndrome and is associated with diminished T-cell interleukin-5 production. *Rheumatology (Oxford)* 47 (7), 1104–1105.
- Permin, H., Wiik, A., 1978. The prevalence of IgE antinuclear antibodies in rheumatoid arthritis and systemic lupus erythematosus. *Acta Pathol. Microbiol. Scand. C* 86C (5), 245–249.
- Pham, C.T., 2006. Neutrophil serine proteases: specific regulators of inflammation. *Nat. Rev. Immunol.* 6 (7), 541–550.
- Pillinger, M.H., Abramson, S.B., 1995. The neutrophil in rheumatoid arthritis. *Rheum. Dis. Clin. North Am.* 21 (3), 691–714.
- Pilsczek, F.H., Salina, D., Poon, K.K., Fahey, C., Yipp, B.G., Sibley, C.D., et al., 2010. A novel mechanism of rapid nuclear neutrophil extracellular trap formation in response to *Staphylococcus aureus*. *J. Immunol.* 185 (12), 7413–7425.
- Poli, C., Martin, J.C., Braudeau, C., Bériou, G., Hémont, C., Charrier, C., et al., 2015. Receptor activating NF- $\kappa$ B ligand (RANKL) is a constitutive intracellular protein in resting human basophils and is strongly induced on their surface by interleukin 3. *Immunobiology* 220 (5), 692–700.
- Polzer, K., Karonitsch, T., Neumann, T., Eger, G., Haberler, C., Soleiman, A., et al., 2008. Eotaxin-3 is involved in Churg-Strauss syndrome—a serum marker closely correlating with disease activity. *Rheumatology (Oxford)* 47 (6), 804–808.
- Potter, M.B., Fincher, R.K., Finger, D.R., 1999. Eosinophilia in Wegener's granulomatosis. *Chest* 116 (5), 1480–1483.
- Potter, P.K., Cortes-Hernandez, J., Quartier, P., Botto, M., Walport, M.J., 2003. Lupus-prone mice have an abnormal response to thioglycolate and an impaired clearance of apoptotic cells. *J. Immunol.* 170 (6), 3223–3232.
- Pratesi, F., Dioni, I., Tommasi, C., Alcaro, M.C., Paolini, I., Barbetti, F., et al., 2014. Antibodies from patients with rheumatoid arthritis target citrullinated histone 4 contained in neutrophils extracellular traps. *Ann. Rheum. Dis.* 73 (7), 1414–1422.
- Puga, I., Cols, M., Barra, C.M., He, B., Cassis, L., Gentile, M., et al., 2011. B cell-helper neutrophils stimulate the diversification and production of immunoglobulin in the marginal zone of the spleen. *Nat. Immunol.* 13 (2), 170–180.
- Quayle, J.A., Watson, F., Bucknall, R.C., Edwards, S.W., 1997. Neutrophils from the synovial fluid of patients with rheumatoid arthritis express the high affinity immunoglobulin G receptor, Fc gamma RI (CD64): role of immune complexes and cytokines in induction of receptor expression. *Immunology* 91 (2), 266–273.
- Rajagopalan, S., Somers, E.C., Brook, R.D., Kehrer, C., Pfenninger, D., Lewis, E., et al., 2004. Endothelial cell apoptosis in systemic lupus erythematosus: a common pathway for abnormal vascular function and thrombosis propensity. *Blood* 103 (10), 3677–3683.
- Rao, A.N., Kazzaz, N.M., Knight, J.S., 2015. Do neutrophil extracellular traps contribute to the heightened risk of thrombosis in inflammatory diseases? *World J. Cardiol.* 7 (12), 829–842.
- Robinson, J., Watson, F., Bucknall, R.C., Edwards, S.W., 1992. Activation of neutrophil reactive-oxidant production by synovial fluid from patients with inflammatory joint disease. Soluble and insoluble immunoglobulin aggregates activate different pathways in primed and unprimed cells. *Biochem. J.* 286 (Pt 2), 345–351.
- Rodriguez Gomez, M., Talke, Y., Goebel, N., Hermann, F., Reich, B., Mack, M., 2010. Basophils support the survival of plasma cells in mice. *J. Immunol.* 185 (12), 7180–7185.
- Rollet-Labellé, E., Vaillancourt, M., Marois, L., Newkirk, M.M., Poubelle, P.E., Naccache, P.H., 2013. Cross-linking of IgGs bound on circulating neutrophils leads to an activation of endothelial cells: possible role of rheumatoid factors in rheumatoid arthritis-associated vascular dysfunction. *J. Inflamm. (Lond)* 10 (1), 27.
- Sablé-Fourtassou, R., Cohen, P., Mahr, A., Pagnoux, C., Mouthon, L., Jayne, D., et al., 2005. Antineutrophil cytoplasmic antibodies and the Churg-Strauss syndrome. *Ann. Intern. Med.* 143 (9), 632–638.
- Sangaletti, S., Tripodo, C., Chiodoni, C., Guarnotta, C., Cappetti, B., Casalini, P., et al., 2012. Neutrophil extracellular traps mediate transfer of cytoplasmic neutrophil antigens to myeloid dendritic cells toward ANCA induction and associated autoimmunity. *Blood* 120 (15), 3007–3018.
- Sanjuan, M.A., Sagar, D., Kolbeck, R., 2016. Role of IgE in autoimmunity. *J. Allergy Clin. Immunol.* 137 (6), 1651–1661.
- Santiago-Raber, M.L., Baccala, R., Haraldsson, K.M., Choubey, D., Stewart, T.A., Kono, D.H., et al., 2003. Type-I interferon receptor deficiency reduces lupus-like disease in NZB mice. *J. Exp. Med.* 197 (6), 777–788.
- Sato, A., Takemura, Y., Yamada, T., Ohtsuka, H., Sakai, H., Miyahara, Y., et al., 1999. A possible role of immunoglobulin E in patients with hyperthyroid Graves' disease. *J. Clin. Endocrinol. Metab.* 84 (10), 3602–3605.
- Scapini, P., Bazzoni, F., Cassatella, M.A., 2008. Regulation of B-cell-activating factor (BAFF)/B lymphocyte stimulator (BLyS) expression in human neutrophils. *Immunol. Lett.* 116 (1), 1–6.
- Schmitt, W.H., Csnerok, E., Kobayashi, S., Klinkeborg, A., Reinhold-Keller, E., Gross, W.L., 1998. Churg-Strauss syndrome: serum markers of lymphocyte activation and endothelial damage. *Arthritis Rheum.* 41 (3), 445–452.
- Schnabel, A., Csnerok, E., Braun, J., Gross, W.L., 2000. Activation of neutrophils, eosinophils, and lymphocytes in the lower respiratory tract in Wegener's granulomatosis. *Am. J. Respir. Crit. Care Med.* 161 (2 Pt 1), 399–405.
- Schroeder, J.T., Chichester, K.L., Bieneman, A.P., 2009. Human basophils secrete IL-3: evidence of autocrine priming for phenotypic and functional responses in allergic disease. *J. Immunol.* 182 (4), 2432–2438.
- Sekigawa, I., Kaneda, K., Kaneko, H., Takasaki, Y., Takamori, K., Ogawa, H., 2008. Detection of serum IgE class anti-SSA antibodies in mothers with foetal loss. *Rheumatol. Int.* 28 (7), 623–626.
- Shahabuddin, S., Ponath, P., Schleimer, R.P., 2000. Migration of eosinophils across endothelial cell monolayers: interactions among IL-5, endothelial-activating cytokines, and C-C chemokines. *J. Immunol.* 164 (7), 3847–3854.
- Sharma, M., Bayry, J., 2015. Autoimmunity: basophils in autoimmune and inflammatory diseases. *Nat. Rev. Rheumatol.* 11 (3), 129–131.
- Shimada, K., Crother, T.R., Karlin, J., Dagvadorj, J., Chiba, N., Chen, S., et al., 2012. Oxidized mitochondrial DNA activates the NLRP3 inflammasome during apoptosis. *Immunity* 36 (3), 401–414.

- Sinico, R.A., Di Toma, L., Maggiore, U., Bottero, P., Radice, A., Tosoni, C., et al., 2005. Prevalence and clinical significance of antineutrophil cytoplasmic antibodies in Churg-Strauss syndrome. *Arthritis Rheum.* 52 (9), 2926–2935.
- Smith, C.K., Vivekanandan-Giri, A., Tang, C., Knight, J.S., Mathew, A., Padilla, R.L., et al., 2014. Neutrophil extracellular trap-derived enzymes oxidize high-density lipoprotein: an additional proatherogenic mechanism in systemic lupus erythematosus. *Arthritis Rheumatol.* 66 (9), 2532–2544.
- Söderberg, D., Kurz, T., Motamedi, A., Hellmark, T., Eriksson, P., Segelmark, M., 2015. Increased levels of neutrophil extracellular trap remnants in the circulation of patients with small vessel vasculitis, but an inverse correlation to anti-neutrophil cytoplasmic antibodies during remission. *Rheumatology (Oxford)* 54 (11), 2085–2094.
- Sopata, I., Wize, J., Filipowicz-Sosnowska, A., Stanisawska-Biernat, E., Brzezińska, B., Maśliński, S., 1995. Neutrophil gelatinase levels in plasma and synovial fluid of patients with rheumatic diseases. *Rheumatol. Int.* 15 (1), 9–14.
- Stakos, D.A., Kambas, K., Konstantinidis, T., Mitroulis, I., Apostolidou, E., Arekaki, S., et al., 2015. Expression of functional tissue factor by neutrophil extracellular traps in culprit artery of acute myocardial infarction. *Eur. Heart J.* 36 (22), 1405–1414.
- Sthoeger, Z.M., Bezalel, S., Chapnik, N., Asher, I., Froy, O., 2009. High alpha-defensin levels in patients with systemic lupus erythematosus. *Immunology* 127 (1), 116–122.
- Suurmond, J., Stoop, J.N., Rivellese, F., Bakker, A.M., Huizinga, T.W., Toes, R.E., 2014. Activation of human basophils by combined Toll-like receptor- and FcεRI-triggering can promote Th2 skewing of naive T helper cells. *Eur. J. Immunol.* 44 (2), 386–396.
- Takei, H., Araki, A., Watanabe, H., Ichinose, A., Sendo, F., 1996. Rapid killing of human neutrophils by the potent activator phorbol 12-myristate 13-acetate (PMA) accompanied by changes different from typical apoptosis or necrosis. *J. Leukoc. Biol.* 59 (2), 229–240.
- Talbot, J., Bianchini, F.J., Nascimento, D.C., Oliveira, R.D., Souto, F.O., Pinto, L.G., et al., 2015. CCR2 expression in neutrophils plays a critical role in their migration into the joints in rheumatoid arthritis. *Arthritis Rheumatol.* 67 (7), 1751–1759.
- Tang, S., Zhang, Y., Yin, S.W., Gao, X.J., Shi, W.W., Wang, Y., et al., 2015. Neutrophil extracellular trap formation is associated with autophagy-related signalling in ANCA-associated vasculitis. *Clin. Exp. Immunol.* 180 (3), 408–418.
- Terai, M., Yasukawa, K., Honda, T., Jibiki, T., Hirano, K., Sato, J., et al., 2002. Peripheral blood eosinophilia and eosinophil accumulation in coronary microvessels in acute Kawasaki disease. *Pediatr. Infect. Dis. J.* 21 (8), 777–781.
- Terrier, B., Bièche, I., Maisonobe, T., Laurendeau, I., Rosenzwajg, M., Kahn, J.E., et al., 2010. Interleukin-25: a cytokine linking eosinophils and adaptive immunity in Churg-Strauss syndrome. *Blood* 116 (22), 4523–4531.
- Thieblemont, N., Wright, H.L., Edwards, S.W., Witko-Sarsat, V., 2016. Human neutrophils in auto-immunity. *Semin. Immunol.* 28 (2), 159–173.
- Torsteinsdóttir, I., Gudbjörnsson, B., Håkansson, L., 1998. Enhanced neutrophil and eosinophil adhesion in patients with primary Sjögren's syndrome. *Clin. Exp. Rheumatol.* 16 (3), 255–262.
- Turrel-Davin, F., Tournadre, A., Pachot, A., Arnaud, B., Cazalis, M.A., Mougin, B., et al., 2010. FoxO3a involved in neutrophil and T cell survival is overexpressed in rheumatoid blood and synovial tissue. *Ann. Rheum. Dis.* 69 (4), 755–760.
- Tvinnereim, A.R., Hamilton, S.E., Harty, J.T., 2004. Neutrophil involvement in cross-priming CD8+ T cell responses to bacterial antigens. *J. Immunol.* 173 (3), 1994–2002.
- Van Avondt, K., Fritsch-Stork, R., Derkzen, R.H., Meyaard, L., 2013. Ligation of signal inhibitory receptor on leukocytes-1 suppresses the release of neutrophil extracellular traps in systemic lupus erythematosus. *PLoS One* 8 (10), e78459.
- van Beers, J.J., Schwarte, C.M., Stammen-Vogelzangs, J., Oosterink, E., Božić, B., Pruijn, G.J., 2013. The rheumatoid arthritis synovial fluid citrullinome reveals novel citrullinated epitopes in apolipoprotein E, myeloid nuclear differentiation antigen, and β-actin. *Arthritis Rheum.* 65 (1), 69–80.
- Van den Steen, P.E., Proost, P., Grillet, B., Brand, D.D., Kang, A.H., Van Damme, J., et al., 2002. Cleavage of denatured natural collagen type II by neutrophil gelatinase B reveals enzyme specificity, post-translational modifications in the substrate, and the formation of remnant epitopes in rheumatoid arthritis. *FASEB J.* 16 (3), 379–389.
- Varricchi, G., Bagnasco, D., Borriello, F., Heffler, E., Canonica, G.W., 2016a. Interleukin-5 pathway inhibition in the treatment of eosinophilic respiratory disorders: evidence and unmet needs. *Curr. Opin. Allergy Clin. Immunol.* 16 (2), 186–200.
- Varricchi, G., Harker, J., Borriello, F., Marone, G., Durham, S.R., Shamji, M.H., 2016b. T follicular helper (Tfh) cells in normal immune responses and in allergic disorders. *Allergy* 71 (8), 1086–1094.
- van Venrooij, W.J., Charles, P., Maini, R.N., 1991. The consensus workshops for the detection of autoantibodies to intracellular antigens in rheumatic diseases. *J. Immunol. Methods* 140 (2), 181–189.
- van Woerkom, J.M., Geertzema, J.G., Nikkels, P.G., Kruize, A.A., Smeenk, R.J., Vroom, T.M., 2004. Expression of Ro/SS-A and La/SS-B determined by immunohistochemistry in healthy, inflamed and autoimmune diseased human tissues: a generalized phenomenon. *Clin. Exp. Rheumatol.* 22 (3), 285–292.
- Villanueva, E., Yalavarthi, S., Berthier, C.C., Hodgin, J.B., Khandpur, R., Lin, A.M., et al., 2011. Netting neutrophils induce endothelial damage, infiltrate tissues, and expose immunostimulatory molecules in systemic lupus erythematosus. *J. Immunol.* 187 (1), 538–552.
- von Scheven, E., Lu, T.T., Emery, H.M., Elder, M.E., Wara, D.W., 2003. Thrombosis and pediatric Wegener's granulomatosis: acquired and genetic risk factors for hypercoagulability. *Arthritis Rheum.* 49 (6), 862–865.
- Vordenbäumen, S., Fischer-Betz, R., Timm, D., Sander, O., Chehab, G., Richter, J., et al., 2010. Elevated levels of human beta-defensin 2 and human neutrophil peptides in systemic lupus erythematosus. *Lupus* 19 (14), 1648–1653.
- Walsh, G.M., 2001. Eosinophil granule proteins and their role in disease. *Curr. Opin. Hematol.* 8 (1), 28–33.
- Wang, Y., Li, M., Stadler, S., Correll, S., Li, P., Wang, D., et al., 2009. Histone hypercitrullination mediates chromatin decondensation and neutrophil extracellular trap formation. *J. Cell Biol.* 184 (2), 205–213.
- Watson, F., Robinson, J.J., Phelan, M., Bucknall, R.C., Edwards, S.W., 1993. Receptor expression in synovial fluid neutrophils from patients with rheumatoid arthritis. *Ann. Rheum. Dis.* 52 (5), 354–359.
- Weinmann, P., Moura, R.A., Caetano-Lopes, J.R., Pereira, P.A., Canhão, H., Queiroz, M.V., et al., 2007. Delayed neutrophil apoptosis in very early rheumatoid arthritis patients is abrogated by methotrexate therapy. *Clin. Exp. Rheumatol.* 25 (6), 885–887.

- White, M.J., McArthur, K., Metcalf, D., Lane, R.M., Cambier, J.C., Herold, M.J., et al., 2014. Apoptotic caspases suppress mtDNA-induced STING-mediated type I IFN production. *Cell* 159 (7), 1549–1562.
- Witko-Sarsat, V., Rieu, P., Descamps-Latscha, B., Lesavre, P., Halbwachs-Mecarelli, L., 2000. Neutrophils: molecules, functions and pathophysiological aspects. *Lab. Invest.* 80 (5), 617–653.
- Wong, S.H., Francis, N., Chahal, H., Raza, K., Salmon, M., Scheel-Toellner, D., et al., 2009. Lactoferrin is a survival factor for neutrophils in rheumatoid synovial fluid. *Rheumatology (Oxford)* 48 (1), 39–44.
- Wright, H.L., Chikura, B., Bucknall, R.C., Moots, R.J., Edwards, S.W., 2011. Changes in expression of membrane TNF, NF- $\kappa$ B activation and neutrophil apoptosis during active and resolved inflammation. *Ann. Rheum. Dis.* 70 (3), 537–543.
- Wright, H.L., Moots, R.J., Edwards, S.W., 2014. The multifactorial role of neutrophils in rheumatoid arthritis. *Nat. Rev. Rheumatol.* 10 (10), 593–601.
- Wu, C.H., Li, K.J., Yu, C.L., Tsai, C.Y., Hsieh, S.C., 2015a. Sjögren's syndrome antigen B acts as an endogenous danger molecule to induce interleukin-8 gene expression in polymorphonuclear neutrophils. *PLoS One* 10 (4), e0125501.
- Wu, Y.W., Tang, W., Zuo, J.P., 2015b. Toll-like receptors: potential targets for lupus treatment. *Acta Pharmacol. Sin.* 36 (12), 1395–1407.
- Xu, J., Zhang, X., Pelayo, R., Monestier, M., Ammollo, C.T., Semeraro, F., et al., 2009. Extracellular histones are major mediators of death in sepsis. *Nat. Med.* 15 (11), 1318–1321.
- Yalavarthi, S., Gould, T.J., Rao, A.N., Mazza, L.F., Morris, A.E., Núñez-Álvarez, C., et al., 2015. Release of neutrophil extracellular traps by neutrophils stimulated with antiphospholipid antibodies: a newly identified mechanism of thrombosis in the antiphospholipid syndrome. *Arthritis Rheumatol.* 67 (11), 2990–3003.
- Yang, C.W., Strong, B.S., Miller, M.J., Unanue, E.R., 2010. Neutrophils influence the level of antigen presentation during the immune response to protein antigens in adjuvants. *J. Immunol.* 185 (5), 2927–2934.
- Yipp, B.G., Kubes, P., 2013. NETosis: how vital is it? *Blood* 122 (16), 2784–2794.
- Yipp, B.G., Petri, B., Salina, D., Jenne, C.N., Scott, B.N., Zbytnuik, L.D., et al., 2012. Infection-induced NETosis is a dynamic process involving neutrophil multitasking in vivo. *Nat. Med.* 18 (9), 1386–1393.
- Yoshida, M., Sasaki, M., Sugisaki, K., Yamaguchi, Y., Yamada, M., 2013. Neutrophil extracellular trap components in fibrinoid necrosis of the kidney with myeloperoxidase-ANCA-associated vasculitis. *Clin. Kidney J.* 6 (3), 308–312.
- Yu, K.K., Crew, A.B., Messingham, K.A., Fairley, J.A., Woodley, D.T., 2014. Omalizumab therapy for bullous pemphigoid. *J. Am. Acad. Dermatol.* 71 (3), 468–474.
- Zhang, S., Shu, X., Tian, X., Chen, F., Lu, X., Wang, G., 2014. Enhanced formation and impaired degradation of neutrophil extracellular traps in dermatomyositis and polymyositis: a potential contributor to interstitial lung disease complications. *Clin. Exp. Immunol.* 177 (1), 134–141.
- Zheng, W., Warner, R., Ruggeri, R., Su, C., Cortes, C., Skoura, A., et al., 2015. PF-1355, a mechanism-based myeloperoxidase inhibitor, prevents immune complex vasculitis and anti-glomerular basement membrane glomerulonephritis. *J. Pharmacol. Exp. Ther.* 353 (2), 288–298.
- Zhuang, H., Han, S., Xu, Y., Li, Y., Wang, H., Yang, L.J., et al., 2014. Toll-like receptor 7-stimulated tumor necrosis factor  $\alpha$  causes bone marrow damage in systemic lupus erythematosus. *Arthritis Rheumatol.* 66 (1), 140–151.
- Zwerina, J., Bach, C., Martorana, D., Jatzwauk, M., Hegasy, G., Moosig, F., et al., 2011. Eotaxin-3 in Churg-Strauss syndrome: a clinical and immunogenetic study. *Rheumatology (Oxford)* 50 (10), 1823–1827.

# The Roles and Contributions of the Complement System in the Pathophysiology of Autoimmune Diseases

Wilhelm J. Schwaeble, Youssif M. Ali and Robert B. Sim

Faculty of Medicine, Department of Infection, Immunity and Inflammation, University of Leicester,  
Leicester, United Kingdom

## OUTLINE

<b>The Complement System and Complement Activation Pathways</b>			
The Classical Pathway	263	Membrane-Bound Regulators	267
The Lectin Pathway	264	The Biological Effects of Complement Activation	267
The Alternative Pathway	264	Complement Involvement in the Pathophysiology of Diverse Autoimmune Diseases	268
The Membrane Attack Complex	266	References	271
<b>Control of Complement Activation</b>	266		
Fluid Phase Regulators	266		

## THE COMPLEMENT SYSTEM AND COMPLEMENT ACTIVATION PATHWAYS

In the 1890s Jules Bordet observed that bactericidal activity of serum essentially requires two components, one which is present prior to immunization and heat-labile that he named Alexin (from Greek *alexein*: to ward off) and the other component, which is heat-stable and generated by immunization. He demonstrated in 1898 that the same basic mechanisms that compose the bacteriolytic activity of immune sera are responsible for the hemolytic activity of serum toward erythrocytes of other species, a methodology that was used to analyze the biological activities of complement for decades to come. The prevailing name “complement” for this bacteriolytic plasma component was coined by Paul Ehrlich at about the same time to underline that this component was essential for “amboceptors” (the name he proposed for antibodies) to lyse cells and thereby “complement” their function (Dunkelberger and Song, 2010; Schmalstieg and Goldman, 2010). Up to the present day, the bactericidal (or hemolytic) activity of complement mediated through the formation of a lytic membrane attack complex (MAC) (formed following the activation of the terminal complement components C5b–C9) is still the most widely known biological activity of the complement system.

Complement is composed of a very complex system of zymogen precursor components, their substrates, fluid phase or cell surface resident regulators/cofactors, and receptors for complement activation products comprising altogether more than 40 components (Whaley and Schwaeble, 1997; Carroll and Sim, 2011). Cell surface resident receptors for complement activation products continuously sense the activation state of the

complement system and coordinate cellular responses of both the innate and the adaptive immune system. As such, complement activation is involved in the initiation and maintenance of numerous inflammatory reactions and inappropriate control of complement activation can predispose to a wide spectrum of infectious or inflammatory pathologies.

Although the bactericidal or hemolytic activity of complement elicited through the formation of MACs is the most popular and widely known function of complement, it is only effective against some bacterial species, while complement-mediated opsonization which targets bacteria, viruses, yeast, host cellular and bacterial debris and eukaryotic parasites for phagocytosis and elimination through the various phagocytes of the reticulo-endothelial system (via their receptors either for C3b, iC3b, C3d, C4b, or C1q/MBL/ficolins). The cellular receptors on phagocytes responsible for the enhanced uptake of opsonized particles and cells include complement receptors type 1 (CR1 also known as CD35), type 3 (CR3, alias of CD11b/CD18), and type 4 (CR4 CD11c/CD18) (Wagner and Frank, 2010). Phagocytes are directed to the location of complement activation by chemotaxis sensing the release of the potent complement chemotactic factor and anaphylatoxin C5a and the anaphylatoxin C3a, through the receptors C3aR and C5aR (alias CD88) (Van Beek et al., 2003; Wallis, 2007). Complement anaphylatoxins C3a and C5a can also increase vascular permeability aiding the transmigration and extravasation of leukocytes into tissue (Williams, 1983). Various complement deficiencies result in a defective recruitment of leukocytes to the site of damage or infection.

Complement activation products also facilitate transport of antigens to the lymphoid follicles and critical interactions between follicular dendritic cells and B cells in order to promote both B-cell memory and the production of specific antibodies (Barrington et al., 2002; Le Fric and Kemper, 2009; Gonzalez et al., 2010). Moreover, complement activation also modulates T-cell responses by coordinating the necessary interactions between antigen-presenting cells and T cells (Zhou et al., 2006; Le Fric and Kemper, 2009; Kwan et al., 2012). The complement system is activated via three activation pathways which all converge through the formation of C3 and C5 convertase complexes and the subsequent formation of the MAC.

## The Classical Pathway

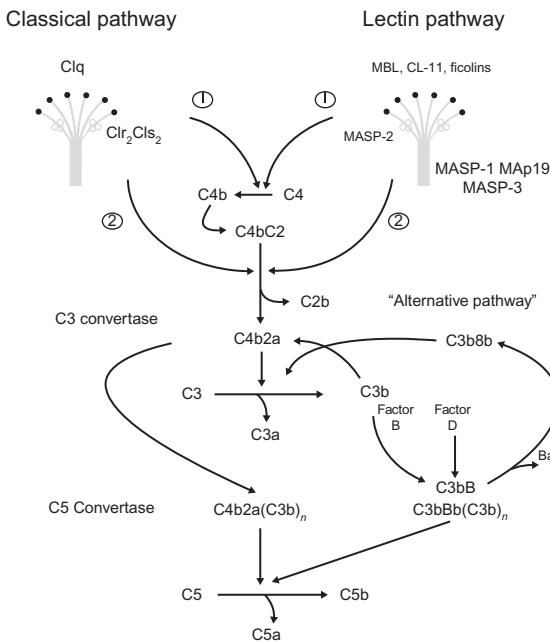
The first component of the complement C1 is composed of a multimolecular initiation complex that triggers complement activation.

The 790 kDa C1 complex consists of a recognition protein, C1q, and a heterotetramer of C1r and C1s zymogens to form the C1q:C1s:C1r2:C1s complex (Fig. 14.1). C1q is composed of six identical subunits joined together through their collagen-like stalks that end in globular heads. Each subunit consists of three homologous polypeptide chains (Arlaud et al., 2002). Classical pathway activation is initiated either by direct binding of C1q to a target (e.g., bacterial) surface or indirectly by binding of C1q to antibodies deposited on the target (immune complexes). IgM, IgG1, IgG2, and IgG3 bind C1q (Arlaud et al., 2002). Binding of C1q to complement activators leads to a conformational change in the collagenous region of C1q which in turn leads to the autoactivation of C1r, which cleaves its only substrate, C1s. C1s in turn cleaves C4 into C4a and C4b and then cleaves C2 bound to the C4b, resulting in the formation of the classical pathway C3 convertase (C4b2a) (Arlaud et al., 2002; Carroll and Sim, 2011).

C4b2a is a protease which activates C3 to form C3a and C3b. C3b, like C4b, can bind covalently to the complement activator, and hundreds of molecules of C3b can be deposited in close proximity to the C3 convertase complex. C3b can bind directly to C4b2a, forming the classical pathway C5 convertaseC4b2a3b, in which C4b and C3b form a binding site for C5, orienting it for cleavage by C2a.

## The Lectin Pathway

In evolutionary terms the lectin pathway (LP) of complement activation seems to be the oldest of the three (Dodds, 2002; Wallis, 2007). The LP is initiated by the binding of a multimolecular LP activation complex, similar in structure to the classical pathway C1, to pathogen-associated molecular patterns, mainly carbohydrate structures present on microorganisms, or damage-associated molecular patterns on damaged host tissue. The LP recognition molecules are mannose-binding lectin (MBL), an MBL-like collectin (CL) called CL-11, and ficolins. Like C1q, they are made up of subunits with globular heads and collagenous stalks. Activation is initiated by the binding of the globular heads to carbohydrate structures present on microorganisms or aberrant glycocalyx patterns on apoptotic, necrotic, malignant, or oxygen-deprived cells (Fujita, 2002; Schwaebel et al., 2002).



**FIGURE 14.1** Flow diagram of the three activation pathways of complement. Activation step 1 depicts the C1s and MASP-2-mediated cleavage of complement C4 to C4a and C4b, activation step 2 depicts the subsequent C1s and MASP-2-mediated cleavage of C4bC2 to form the C3 convertase of the classical and lectin pathway C4b2a. With the conversion of C5 into C5a and C5b, all enzymatic steps of complement activation are completed. Both the classical as well as the alternative pathways are thought to correspond with the alternative pathway activation loop through provision of C3b.

Rodents have at least four circulating LP recognition molecules, with differing, but overlapping, carbohydrate specificities; two mannan-binding lectins (MBL-A and MBL-C), CL-11, and ficolin A (Fnca) (Schwaebel et al., 2011; Ali et al. 2012). A second murine ficolin, Fnrb, associated with monocyte and macrophage cell surfaces does not activate complement in mice but may act as an LP recognition molecule in rats (Girija et al., 2011). Humans have a single MBL (the product of *MBL2*; *MBL1* is a pseudogene), CL-11 (CL kidney 1, CL-K1), and three ficolins, FCN1 (M-ficolin), FCN2 (L-ficolin), and FCN3 (H-ficolin) (Fujita, 2002; Liu et al., 2005; Hansen et al., 2010). These recognition molecules form complexes with the serine proteases called MASP-1, -2, and -3 (MBL-associated serine proteases 1, 2, and 3). These are homologous to C1r and C1s, and form homodimers (and perhaps some heterodimers). The serine proteases in the CP and LP activation complexes all interact with a highly conserved binding motif within the collagenous region of the recognition subunit, that is, C1q for the CP and MBL, ficolins or CL-11 for the LP (Wallis et al., 2004). Each recognition molecule can bind one protease dimer. The recognition molecules also interact with MAp19 and MAp44 (alias MBL/ficolin-associated protein 1), which are nonenzymatic, truncated alternative splice products of the *MASP2* and *MASP1/3* genes, respectively. Both truncated gene products lack the serine protease domain and may regulate LP activation by competing for the binding of MASPs to the carbohydrate recognition molecules (Thiel et al., 1997; Stover et al., 1999; Takahashi et al., 1999; Schwaebel et al., 2002; Iwaki et al., 2006; Degn et al., 2009; Skjoedt et al., 2010). Of the three MASPs, only MASP-2 is required and essential to form the LP C3 and C5 convertases (C4b2a and C4b2a3b) (Thiel et al., 1997; Vorup-Jensen et al., 2000; Rossi et al., 2001; Schwaebel et al., 2011).

Like C1s, activated MASP-2 cleaves C4 and C4b-bound C2, generating C4b2a. Neither MASP-1 nor MASP-3 can cleave C4 (Schwaebel et al., 2011). The function of MASP-1 is still uncertain. It may facilitate LP activation by either direct cleavage of complex-bound MASP-2 or cleavage of C4b-bound C2 (Takahashi et al., 2008; Kocsis et al., 2010; Schwaebel et al., 2011). Recent work demonstrated that MASP-1 (and possibly MASP-3) plays a key role in the maturation and initiation of the alternative activation pathway (Takahashi et al., 2008; Iwaki et al., 2011). The LP is probably mainly activated by charge-neutral sugar structures and clusters of acetyl moieties, whereas in the classical pathway, C1q recognizes mainly charge clusters. The LP can also respond to immunoglobulins deposited on targets, by binding to glycans on some human IgG, IgA glycoforms, and to the glycans on mouse (but not human) IgM (Carroll and Sim, 2011).

## The Alternative Pathway

Factor B, factor D, and properdin (factor P) are specific components of the alternative pathway of complement activation. Unlike the classical and LP (which are initiated via specific recognition proteins such as C1q or MBL), the alternative pathway is initiated through a spontaneous steady-state hydrolysis of C3 to form C3 ( $H_2O$ ) which in turn binds to factor B to form a  $C3(H_2O)B$  zymogen complex. In this complex, factor B is cleaved by factor D releasing a Ba fragment while Bb remains attached to the complex. The newly formed complex  $C3(H_2O)Bb$  is a C3 convertase enzyme and cleaves C3 into C3a and C3b. Once C3b is generated, it will bind covalently to the surface of pathogens where it can bind to another molecule of factor B and form a new alternative pathway C3 convertase  $C3bBb$  (Carroll and Sim, 2011). The alternative pathway also acts as an amplification loop where C3b generated by either the classical or the LP binds to factor B to generate the convertase  $C3bBb$  (Schwaebel and Reid, 1999). The  $C3bBb$  is homologous to C4b2a (C3 is a homolog of C4, and factor B of C2) and like C4b2a will switch its substrate specificity from cleaving C3 to cleaving C5 upon binding of C3b to the convertase, forming  $C3bBb3b$ , the C5 convertase.

## The Membrane Attack Complex

The C5 convertases C4b2a3b and  $C3bBb3b$  cleave C5 into C5b and C5a. C5b then binds to C6, C7, and C8 to form a C5b-8 complex that can bind to cell surfaces and initiate cell lysis by inserting into the lipid bilayer. Multiple C9 molecules can bind to C5b-8, accelerating lysis and forming the MAC, or C5b-9 (Podack et al., 1982).

## CONTROL OF COMPLEMENT ACTIVATION

The complement system is tightly regulated to avoid run-away activation of the enzymatic cascade that would otherwise lead to excess host tissue damage and inflammation. Key events at the center of the cascade are carefully controlled by five closely related complement control proteins, all of which are encoded by genes located in the regulator of complement activation cluster on chromosome 1q32 in man. Complement regulatory components include membrane-bound regulators and fluid phase regulators (Kirschfink and Mollnes, 2003; Carroll and Sim, 2011).

Two host membrane proteins, CR1 and membrane cofactor protein (MCP or CD46), and two fluid-phase regulators, factor H and C4-binding protein (C4bp), act as cofactors for factor I, which inactivates C3b and C4b, preventing formation of the C3 and C5 convertases. C4bp also shortens the half-life of the classical pathway C3 and C5 convertases, while factor H has the same effect on the alternative pathway C3 and C5 convertases. CR1 and another host membrane protein, decay accelerating factor (DAF), act on both the classical, and alternative pathway convertases. A truncated splice variant of factor H, factor H-like protein 1, is also found in serum and has regulatory activity similar to that of factor H (Schwaebel et al., 1987). Prevention of factor H binding results in an increased half-life of C3b containing convertase complexes and overshooting activation of the alternative pathway leading to FHR-C3 glomerulopathies (C3GN) (Ramaglia et al., 2012).

## Fluid Phase Regulators

C1 inhibitor, also known as Serpin G1, is a serine protease inhibitor which irreversibly inhibits C1r, C1s, MASP-1, and MASP-2 by forming a covalent complex with the protease active site (Chen et al., 1998; Presanis et al., 2004; Cicardi et al., 2005). Besides complement regulation Serpin G1 can also inactivate serine proteases of the coagulation cascade (FXIa and FXIIa), and of the contact system (kallikrein) (Cicardi et al., 2005; Wagner and Frank, 2010).

Factor I is a protease and a fluid phase regulator of all three pathways. It converts hemolytically active C3b into hemolytically inactive iC3b, in a cofactor-dependent manner. In the fluid phase, factor H, an abundant plasma component, binds to free and complex-bound C3b to allow factor I to convert C3b to iC3b. A further conversion of iC3b to C3c and C3dg by factor I is also cofactor dependent. In a similar fashion, factor I inactivates C4b in the presence of the cofactor C4-binding protein (C4BP) (Seya et al., 1995). Binding of C4BP to C4b inhibits C4b-C2a binding, preventing the formation of the C3 convertase (Blom et al., 2004). Subsequent factor I-dependent conversion of C4b generates the fragments iC4b, C4c, and C4d. Conversion of C3b to iC3b is

important in another function: iC3b is a powerful opsonin, recognized by CR3 and CR4, while C3b is a less effective opsonin, recognized by CR1.

Factor H is an important alternative pathway regulator, the main activity of which, besides mediating C3b inactivation by factor I, is to destabilize and also accelerate the decay of the alternative pathway C3 convertase (C3bBb) and C5 convertase (C3bBb3b) (Whaley and Ruddy, 1976; Weiler et al., 1976). Factor H destabilizes the C3 convertase by competitive binding to C3b, which dislodges Bb from the convertase (C3bBb). Factor H is important for the discrimination of self from nonself-cells and therefore preventing autoimmunity. Binding of factor H, for example on sialic acid or glycosaminoglycans of host cells, inhibits alternative pathway activation on the surface of host cells (Pangburn et al., 2000). Carboxypeptidase N is an inactivating regulator of the C3a and C5a anaphylatoxins (Bokisch and Muller-Eberhard, 1970). Clusterin and S protein are regulators for the terminal activation cascade of complement. They bind to C5b-7 complex and prevent the insertion of C8 and C9 leading to the inhibition of the MAC formation (Jenne and Tschopp, 1989; Wagner and Frank, 2010).

Properdin is the only known and essential positive regulator of the complement system. It is essential for alternative pathway activation as properdin-depleted sera lack the ability to activate the alternative pathway (Schwaebel and Reid, 1999). Properdin is produced by monocytes/macrophages, peripheral T lymphocytes, and granulocytes (i.e., neutrophils) (Wirthmueller et al., 1997) and acts as a positive regulator by stabilizing the alternative pathway C3 and C5 convertases (Schwaebel and Reid, 1999; Stover et al., 2008).

## Membrane-Bound Regulators

Complement activation is also controlled by at least four characterized membrane-bound proteins or receptors, which protect host cells from attack by complement. These comprise the decay-accelerating factor (DAF, also known as CD55), the CR1 (also known as CD35), the MCP (also known as CD46), and CD59 (also known as protectin) (Wagner and Frank, 2010). Like factor H, DAF regulates complement activation by accelerating the decay (dissociation of the convertases) of the C3 convertases (C3b2a and C3bBb) (Lublin and Atkinson, 1989). CR1 and MCP act as cofactors for the cleavage of C3b and C4b by factor I (Wagner and Frank, 2010). CD59 is a regulator of the MAC complex. CD59 binds the nascent MAC complex (C5b-8) inhibiting C9 polymerization and subsequent pore formation on the surface of a cell (Lehto et al., 1997; Farkas et al., 2002). DAF, MCP, and CD59 are found on most cell types, but CR1 has more restricted distribution (erythrocytes, lymphocytes, dendritic cells, and kidney podocytes).

## THE BIOLOGICAL EFFECTS OF COMPLEMENT ACTIVATION

Complement activation leads to a multitude of biological activities including opsonization, initiation of a proinflammatory response, immune complex clearance, and direct killing of cells via the MAC. Opsonization of pathogens is mediated by the major opsonin C3b or iC3b and C4b to a lesser extent. C3b coats the surface of microorganisms and enhances their phagocytosis by leukocyte via binding to CR1. iC3b binds to CR3 and CR4. L-Ficolin, MBL, and C1q have been reported to initiate phagocytosis directly by binding to pathogens and enhancing phagocytosis by binding to CL receptors on the surface of the phagocytes (Jack et al., 2001; Aoyagi et al., 2005). In other instances, complement can mediate direct killing of bacteria, especially Gram-negative bacteria via the formation of the MAC which form pores in the cell membrane leading to cell lysis (Nauta et al., 2004). During complement activation, proinflammatory cleavage products anaphylatoxins C4a, C5a, and C3a are released. The release of anaphylatoxins increases vascular permeability and formation of inflammatory exudates. These inflammatory exudates enhance the recruitment of inflammatory mediators and inflammatory cells to the site of injury and efficient elimination of invading pathogens or other inflammatory factors. Increased vascular permeability leads to extravasation of leukocytes to the site of inflammation, which helps in clearing invading pathogens. C5a acts as a potent chemotactic factor that stimulates leukocyte migration. In addition, C5a was found to increase the synthesis of other chemotactic agents such as eicosanoids and chemokines. Schindler et al. (1990) reported that C5a stimulates the expression of interleukin-1 and tumor necrosis factor. Complement also plays a major role in the clearance of apoptotic and necrotic cells in addition to immune complexes and microorganisms. The globular head of C1q binds to the surface of apoptotic cells and facilitates the uptake of the cells by macrophages (Taylor et al., 2000). In addition, opsonization of apoptotic cells with iC3b leads to recognition of

these cells by CR3 and CR4 on the surface of phagocytes with subsequent engulfment of these cells (Mevorach et al., 1998).

Complement also inhibits the precipitation of immune complexes and enhances their solubility by binding of C1q, C3b, and C4b. This binding inhibits further increase in the aggregation of the immune complex. These complexes bind to CR1 on the surface of erythrocytes which transfer them into the liver and the spleen where they are cleared from the circulation by the resident macrophages (Manderson et al., 2004).

## COMPLEMENT INVOLVEMENT IN THE PATHOPHYSIOLOGY OF DIVERSE AUTOIMMUNE DISEASES

As a powerful effector system of both the innate and the adaptive immune response, uncontrolled complement activation can lead to the loss of antimicrobial immune protection, but can also initiate, feed, and perpetuate inflammatory conditions leading to tissue destruction and autoimmune disease. Uncontrolled, dysregulated complement activation can be caused by inherited or acquired deficiencies of complement components as well as through gain of function or loss of function mutations in complement genes or genes encoding enzymes that further process complement components. An example for the latter is the X-linked phosphatidylinositol glycan class A (PIG-A) gene encoding an enzyme necessary for the synthesis of *N*-acetylglucosaminyl-phosphatidol, an intermediate required for the membrane anchoring of the membrane protective complement regulatory components DAF (i.e., DAF or CD55) and protectin (i.e., CD59) which are attached to the outer cell surface through glycosyl-phosphatidylinositol anchoring. A rare inherited or acquired deficiency of the PIG-A leads to the loss of the membrane localization of CD55 and CD59, which in turn renders in particular erythrocytes susceptible to autologous complement lysis cells and presents as a hematological disorder named paroxysmal nocturnal hemoglobinuria (PNH) or Marchiafava–Micheli syndrome. In most cases, PNH is a consequence of a nonmalignant clonal expansion of one or more hematopoietic stem cells with an acquired somatic mutation in the PIG-A gene. PNH clinically presents as a chronic hemolytic anemia with both intravascular and extravascular lysis of erythrocytes leading to hemoglobin in the urine and a predisposition of thrombosis. The severity of disease is predicted by the degree of loss of CD55 and CD59 anchoring on blood cells, which can vary between partial loss (PNH type II) and total loss (PNH type III). The condition is potentially life-threatening due to the high risk of thrombotic disease and is presently treated with high-dose applications of a recombinant humanized anti-C5 monoclonal antibody, eculizumab, which prevents C5 activation and MAC formation (Risitano, 2013). This treatment effectively reduces the intravascular lysis, but anemia with the need for blood transfusion persists due to the continuous opsonization of PNH erythrocytes by complement C3 activation products, which makes these erythrocytes susceptible to opsonophagocytosis and extravascular hemolysis. A more effective treatment would be to block complement activation further upstream to avoid C3 deposition. In PNH plasma the direct antiglobulin test (or direct Coombs' test) is usually negative, as the hemolysis of PNH is not caused by antibodies. This sets PNH apart from another autoimmune disorder, called autoimmune hemolytic anemia (AIHA), where antibody-mediated complement activation lyses erythrocytes. In most cases of AIHA the autoantibody is of the IgM class. Treating AIHA with red blood cell transfusions is often ineffective, since the autoantibodies attach to recipient and donor cells alike. Therapeutic application of C1-esterase inhibitor concentrate was recently shown to protect from complement-induced red blood cell destruction and may offer a safe and effective treatment of AIHA (Wouters et al., 2013).

An extremely strong association has been established between deficiencies of the classical pathway of complement activation and systemic lupus erythematosus (SLE).

The first description of an acquired deficiency of the first component of complement in the serum of an SLE patient was published more than 40 years ago showing the presence of anti-C1q autoantibodies by gel diffusion chromatography (Agnello et al., 1971). The hereditary deficiency of C1q and the predisposition for autoimmune SLE or SLE-like disease represents one of the most powerful associations with over 90% of C1q-deficient individuals developing severe SLE. The disease manifestations such as rash, glomerulonephritis, and central nervous system (CNS) disease can vary, but the severity of disease invariably progresses and requires extensive medical care (Walport et al., 1998). A recent report described the successful long-term replacement of C1q through fresh frozen plasma infusions in a young C1q-deficient patient for over 9 years (Mehta et al., 2010). Bone-marrow transplantation was successfully conducted replacing the bone marrow of a C1q-deficient mouse with bone marrow of a syngenic WT mouse (Cortes-Hernandez et al., 2004), restoring normal C1q levels. Interestingly, both

inherited or acquired deficiency of each of the classical pathway components C1, C4, C2, and C3 predisposes to lupus-like autoimmune disease (Carroll, 2004; Pradhan et al., 2012).

This strongly implies a critical role of the classical pathway in the maintenance of immune tolerance and prevention of autoimmune disease. The present understanding points to the conclusion that the classical pathway fulfills an important scavenger function in removing immune complexes as well as cellular debris and that in the absence of this scavenger system the clearance of debris is significantly impaired which in turn results in continuous presentation of autoantigens (Arlaud et al., 2002).

A rare but invariably severe autoimmune pathology is caused by an IgG autoantibody directed against the alternative pathway C3, convertase C3bBb. This autoantibody binds to C3bBb and protects this complex from decay by factor H and subsequent inactivation by factor I and thereby increases the half-life of the alternative pathway C3 convertase leading to a continuous activation of the alternative pathway and secondary hypocomplementemia. This perpetual activation of the alternative pathway clinically presents as a form of membranoproliferative glomerulonephritis (i.e., mesangiocapillary glomerulonephritis type II), which earned this autoantibody the name nephritic factor (NeF). Patients suffering from this rare autoimmune disease frequently show abnormalities in the distribution of their adipose tissue, named partial lipodystrophy. A recent report provided a plausible explanation showing that NeF can also stabilize the C3 convertase on the surface of fat cells and lyse fat cells through perpetual generation of MACs (Mathieson et al., 1993). An even rarer variant of NeF presents as an autoantibody directed against the classical/LP C3 convertase (i.e., C4b2a), called C4 NeF, which stabilizes this convertase causing hypocomplementemia by continuous consumption of C3 (Miller et al., 2012). The majority of the C4-NeF cases were identified in a cohort of lupus nephritis patients. Dysregulated alternative pathway activation caused by deficiencies of the alternative pathway regulators factor H, factor I, MCP, and the factor H-related genes (FHR5 and FHR1) predisposes to C3GN. The predisposing mutations in the factor H-related genes lead to the formation of FHR dimers which effectively bind and compete with factor H for the binding to C3b. Prevention of factor H binding results in an increased half-life of C3b-containing convertase complexes and overshooting activation of the alternative pathway (Ramaglia et al., 2012).

The commonest cause of C3GN is complement factor H-related 5 (CFHR5) nephropathy, which is endemic in Greek Cypriots. Other C3GNs include membranoproliferative glomerulonephritis 1 (MPGN1), fMPGN3, and dense deposit disease, a rare form of glomerulonephritis characterized by the presence of electron dense deposits within the glomerular basement membrane with intense deposition of C3 fragments (De Cordoba et al., 2012).

Hemolytic uremic syndrome (HUS) is a life-threatening condition often following infections with Shiga toxin-producing *Escherichia coli* strains (STEC-HUS; EHEC-HUS). It presents as Coombs' test-negative microangiopathic anemia with severe diarrhea. An atypical form of HUS (aHUS) does not present with diarrhea. It has a poor long-term prognosis as it is recurrent and results in a fatality rate of up to 30%. aHUS can develop in individuals with inherited or acquired predispositions for poorly controlled complement activation, either through mutations in the complement regulatory components factor H, or MCP, factor I or through gain-of-function mutations in factor B and complement C3 (Goicoechea de Jorge et al., 2007), or have autoantibodies against factor H or disease promoting mutations in factor H-related proteins CFHR1 and CFHR5 (see above). Another thrombotic microangiopathy called thrombotic thrombocytopenic purpura (TTP) is pathologically very similar to HUS as it presents with an autoantibody-induced consumption of complement and thrombi in the microcirculation of many organs, including kidney, brain, heart, lung, liver, and gut (Noris et al., 2012). A majority of TTP patients present with acquired autoantibodies that inhibit a plasma metalloprotease called ADAMTS13 that cleave von Willebrand factor. Inherited forms of TTP are caused by ADAMTS13 gene deficiencies.

In 2005 several independent reports identified a loss-of-function mutation (CHFY402H) in the complement regulatory component factor H to be a major predisposing factor for age-related macular degeneration (AMD), a degenerative disorder of the retinal pigment epithelium (RPE) and the most common cause of blindness in the Western world (Klein et al., 2005). Interestingly, retinal drusen, the hallmark of AMD, stain positive for complement activation products, indicating that complement activation is involved in drusen formation. In vitro models of complement activation on retinal pigmented epithelial monolayers have demonstrated that oxidative stress significantly induced complement activation on the surface of these cells and initiated complement activation in a LP-dependent fashion (Joseph et al., 2013). The sublytic deposition of complement on RPE required the presence of MASP-2 to initiate complement activation, while reduced functional activity of alternative pathway regulators may be critical for the disease process to establish and therefore predispose for the severity and the time of onset of AMD pathology. Most recent work also identified that the loss-of-function mutation (CHFY402H) of factor H also as a high risk polymorphism for other diseases with autoimmune characteristics such as ocular sarcoidosis, atherosclerosis, and a subgroup of Alzheimer patients (i.e., ApoE4 risk allele carriers) (Thompson et al., 2013).

Complement has also been identified as a critical player in the pathophysiology of diverse autoimmune diseases of the CNS caused by autoantibody-mediated destruction of cells where activation of complement by either the classical or the LP plays a critical role. These diseases include myasthenia gravis (MG) (Romi et al., 2005), Guillain Barré syndrome (GBS) (Kaida and Kusunoki, 2009), and neuromyelitis optica (NMO) (Asgari et al., 2013). MG is caused by an autoantibody directed against the acetylcholine receptor, which strongly activates complement and blocks signal transduction at the motor neuron synapse to muscle cells causing muscle weakness and loss of muscle control. In an animal model of MG, experimental autoimmune MG was shown that complement activation is required for the disease to develop, since the disease cannot be induced in absence of C3, C4, or C5, and the application of complement inhibitors ameliorates the severity of established disease (Tuzun and Christadoss 2013). In GBS, antiganglioside autoantibodies trigger complement activation leading to a complement-dependent disruption of voltage-gated sodium channel clusters, while in NMO, antiaquaporin-4 autoantibodies trigger complement activation through the classical and/or the lectin activation pathway.

In other autoimmune pathologies of the CNS, such as multiple sclerosis (MS) (a condition caused by autoimmune destruction of the myelin sheets around the axon of neurons), motor neuron disease (MND) (describing a group of neurological disorders selectively affecting motor neurons), and Alzheimer's disease (AD) [which was recently defined as an autoimmune disorder triggered by herpes simplex (Wozniak et al., 2007)]; the involvement of complement in the pathophysiology is somewhat more complex. In experimental autoimmune encephalomyelitis (EAE) a mouse model for MS, the alternative complement activation pathway is essential for the priming of microglial cells. Mice deficient in the C3 convertase regulator complement receptor 1-related protein y (Crry) show an accelerated onset of EAE signs and early microglial priming, but no disease develops in mice deficient in both Crry and C3 and Crry and factor B (Donev et al., 2013). Classical pathway deficiency, however, does not protect from developing EAE. Therapeutic inhibition of factor B in a mouse model does not prevent the onset of EAE but ameliorates the severity of disease once it is established (Hu et al., 2013). In MND, local complement biosynthesis is increased and therapeutic inhibition of the complement anaphylatoxin C5a reduces inflammatory pathology and survival in a rat model of amyotrophic lateral sclerosis, but at present too little is known to define a definite role of complement in this devastating autoimmune pathology (Woodruff et al., 2008).

In AD the classical pathway recognition component C1q colocalizes with the amyloid- $\beta$  depositions in the disease lesion areas. In a mouse model of AD, C1q-deficient mice show a faster progression of AD neurodegeneration, and most recent work demonstrated that C1q is neuroprotective against amyloid- $\beta$  toxicity (Benoit et al., 2013).

Rheumatoid arthritis (RA) is a highly disabling systemic autoimmune disease characterized by a persistent inflammation of the synovial membrane lining the joint, with associated infiltration of macrophages, granulocytes, T cells, and B cells. Local complement activation is critically involved in the onset and maintenance of this inflammatory disease with a preponderant role of the alternative activation pathway in a mouse model (Dimitrova et al., 2012). Deficiencies of C5 and C6 also protect from RA indicating that the terminal activation cascade essentially contributes to the severity of disease while deficiency of an early component such as C2 is a predisposing factor (Jonsson et al., 2005).

Complement-mediated cytotoxicity plays a critical role in the pathophysiology of vitiligo, a common depigmenting autoimmune disorder. Autoimmune antibodies lyse melanocytes through both complement activation and antibody-dependent cellular cytotoxicity (Norris et al., 1988).

Pernicious anemia (PA) is the most common cause of vitamin B12 deficiency. PA patients have complement activating autoantibodies against parietal cells in serum and gastric fluid, but since the antigen H<sup>+</sup>/K<sup>+</sup>-ATPase is not expressed on the surface of the target cell, cytotoxicity in PA is unlikely to be complement mediated (De Aizpurua et al., 1983).

Complement does not appear to be involved in the pathophysiology of primary Sjögren's syndrome. It is likely that complement consumption in Sjögren's syndrome patients is caused by concomitant and secondary systemic disease (Lindgren et al., 1993).

Hashimoto's thyroiditis (HT) and Graves' disease (GD) share many common features including the presence of autoantibodies against thyroperoxidase and thyroid stimulating hormone (TSH) receptor. In GD a subset of TSH receptor antibodies stimulates this receptor and causes the phenotype of hyperthyroidism, the hallmark of GD. The role of the other autoantibodies, however, remains unclear. In HT, immune complexes on the thyroid follicular membrane are often associated with complement deposition indicating a role of complement in the tissue destruction. Previous studies claim that thyrocytes are usually resistant to direct complement lysis as these cells are expressing high levels of membrane-associated complement regulators such as CD59 (Tandon et al., 1992). Nonlytic activation of complement generates soluble MACs during the acute phase of HT

(Weetman et al., 1989). A more recent study claims that thyroperoxidase on the surface of thyrocytes can also initiate complement activation through the binding of C4 and C4b to a complement control module in the ectodomain of thyroperoxidase leading to direct complement-mediated killing of thyrocytes in vitro (Blanchin et al., 2003). This proposed activation mechanism, however, has not been confirmed.

Ischemia/reperfusion injury (IRI) is a complement-mediated postinflammatory condition leading to major tissue loss. IRI was first thought to be mediated through the classical pathway, since it is less pronounced in IgM deficiency, but more recent work identified that it is predominantly driven by the LP of complement in a MASP-2-dependent fashion (Schwaeble et al., 2011). In experimental models of stroke and myocardial infarction, therapeutic inhibition of MASP-2 reduces infarct sizes by up to 40% and may offer a promising new avenue of treatment to reduce morbidity and mortality in these major ischemic pathologies.

## References

- Agnello, V., Koffler, D., Eisenberg, J.W., et al., 1971. C1q precipitins in the sera of patients with systemic lupus erythematosus and other hypocomplementemic states: characterization of high and low molecular weight types. *J. Exp. Med.* 134, 228–241.
- Ali, Y.M., Lynch, N.J., Haleem, K.S., Fujita, T., Endo, Y., Hansen, S., et al., 2012. The lectin pathway of complement activation is a critical component of the innate immune response to pneumococcal infection. *PLoS Pathog.* 8, e1002793.
- Aoyagi, Y., Adderson, E.E., Min, J.G., Matsushita, M., Fujita, T., Takahashi, S., et al., 2005. Role of L-ficolin/mannose-binding lectin-associated serine protease complexes in the opsonophagocytosis of type III group B streptococci. *J. Immunol.* 174, 418–425.
- Arlaud, G.J., Gaboriaud, C., Thielen, N.M., Budayova-Spano, M., Rossi, V., Fontecilla-Camps, J.C., 2002. Structural biology of the C1 complex of complement unveils the mechanisms of its activation and proteolytic activity. *Mol. Immunol.* 39, 383–394.
- Asgari, N., Khoroshi, R., Lillevang, S.T., Owens, T., 2013. Complement-dependent pathogenicity of brain-specific antibodies in cerebrospinal fluid. *J. Neuroimmunol.* 254, 76–82.
- Barrington, R.A., Pozdnyakova, O., Zafari, M.R., Benjamin, C.D., Carroll, M.C., 2002. B lymphocyte memory: role of stromal cell complement and Fc $\gamma$ RIIB receptors. *J. Exp. Med.* 196, 1189–1199.
- Van Beek, J., Elward, K., Gasque, P., 2003. Activation of complement in the central nervous system: roles in neurodegeneration and neuroprotection. *Ann. N. Y. Acad. Sci.* 992, 56–71.
- Benoit, M.E., Hernandez, M.X., Dinh, M.L., Benavente, F., Vasquez, O., Tenner, A.J., 2013. C1q-induced LRP1B and GPR6 proteins expressed early in Alzheimer disease mouse models, are essential for the C1q-mediated protection against amyloid-beta neurotoxicity. *J. Biol. Chem.* 288, 654–665.
- Blanchin, S., Estienne, V., Durand-Gorde, J.M., Carayon, P., Ruf, J., 2003. Complement activation by direct C4 binding to thyroperoxidase in Hashimoto's thyroiditis. *Endocrinology* 144, 5422–5429.
- Blom, A.M., Villoutreix, B.O., Dahlback, B., 2004. Complement inhibitor C4b-binding protein—friend or foe in the innate immune system? *Mol. Immunol.* 40 (18), 1333–1346.
- Bokisch, V.A., Muller-Eberhard, H.J., 1970. Anaphylatoxin inactivator of human plasma: its isolation and characterization as a carboxypeptidase. *J. Clin. Invest.* 49 (12), 2427–2436.
- Carroll, M.C., 2004. A protective role for innate immunity in systemic lupus erythematosus. *Nat. Rev. Immunol.* 4 (10), 825–831.
- Carroll, M.V., Sim, R.B., 2011. Complement in health and disease. *Adv. Drug Deliv. Rev.* 63 (12), 965–975.
- Chen, C.H., Lam, C.F., Boackle, R.J., 1998. C1 inhibitor removes the entire C1qr2s2 complex from anti-C1Q monoclonal antibodies with low binding affinities. *Immunology* 95, 648–654.
- Cicardi, M., Zingale, L., Zanichelli, A., Pappalardo, E., Cicardi, B., 2005. C1 inhibitor: molecular and clinical aspects. *Springer Semin. Immunopathol.* 27, 286–298.
- De Aizpurua, H.J., Cosgrove, L.J., Ungar, B., Toh, B.H., 1983. Autoantibodies cytotoxic to gastric parietal cells in serum of patients with pernicious anemia. *New Engl. J. Med.* 309, 625–629.
- De Cordoba, S.R., Tortajada, A., Harris, C.L., Morgan, B.P., 2012. Complement dysregulation and disease: from genes and proteins to diagnostics and drugs. *Immunobiology* 217, 1034–1046.
- Cortes-Hernandez, J., Fossati-Jimack, L., Petry, F., Loos, M., Izui, S., Walport, M.J., et al., 2004. Restoration of C1q levels by bone marrow transplantation attenuates autoimmune disease associated with C1q deficiency in mice. *Eur. J. Immunol.* 34, 3713–3722.
- Degen, S.E., Hansen, A.G., Steffensen, R., Jacobsen, C., Jensenius, J.C., Thiel, S., 2009. MAP44, a human protein associated with pattern recognition molecules of the complement system and regulating the lectin pathway of complement activation. *J. Immunol. (Baltimore)*. 183, 7371–7378.
- Dimitrova, P., Ivanovska, N., Belenska, L., Milanova, V., Schwaeble, W., Stover, C., 2012. Abrogated RANKL expression in properdin-deficient mice is associated with better outcome from collagen-antibody-induced arthritis. *Arthritis Res. Ther.* 14, R173.
- Dodds, A.W., 2002. Which came first, the lectin/classical pathway or the alternative pathway of complement? *Immunobiology* 205 (4–5), 340–354.
- Dunkelberger, J.R., Song, W.C., 2010. Complement and its role in innate and adaptive immune responses. *Cell Res.* 20, 34–50.
- Farkas, I., Baranyi, L., Ishikawa, Y., Okada, N., Bohata, C., Budai, D., et al., 2002. CD59 blocks not only the insertion of C9 into MAC but inhibits ion channel formation by homologous C5b-8 as well as C5b-9. *J. Physiol.* 539 (Pt 2), 537–545.
- Fujita, T., 2002. Evolution of the lectin-complement pathway and its role in innate immunity. *Nat. Rev. Immunol.* 2, 346–353.
- Girija, U.V., Mitchell, D.A., Roscher, S., Wallis, R., 2011. Carbohydrate recognition and complement activation by rat ficolin-B. *Eur. J. Immunol.* 41, 214–223.
- Goicoechea De Jorge, E., Harris, C.L., Esparza-Gordillo, J., Carreras, L., Arranz, E.A., Garrido, C.A., et al., 2007. Gain-of-function mutations in complement factor B are associated with atypical hemolytic uremic syndrome. *Proc. Natl. Acad. Sci. U.S.A.* 104, 240–245.

- Hansen, S., Selman, L., Palaniyar, N., Ziegler, K., Brandt, J., Kliem, A., et al., 2010. Collectin 11 (CL-11, CL-K1) is a MASP-1/3-associated plasma collectin with microbial-binding activity. *J. Immunol.* (Baltimore, Md.: 1950) 185 (10), 6096–6104.
- Hu, X., Holers, V.M., Thurman, J.M., Schoeb, T.R., Ramos, T.N., Barnum, S.R., 2013. Therapeutic inhibition of the alternative complement pathway attenuates chronic EAE. *Mol. Immunol.* 54 (3–4), 302–308.
- Iwaki, D., Kanno, K., Takahashi, M., Endo, Y., Lynch, N.J., Schwaebel, W.J., et al., 2006. Small mannose-binding lectin-associated protein plays a regulatory role in the lectin complement pathway. *J. Immunol.* (Baltimore, Md.: 1950) 177 (12), 8626–8632.
- Iwaki, D., Kanno, K., Takahashi, M., Endo, Y., Matsushita, M., Fujita, T., 2011. The role of mannose-binding lectin-associated serine protease-3 in activation of the alternative complement pathway. *J. Immunol.* 187, 3751–3758.
- Jack, D.L., Klein, N.J., Turner, M.W., 2001. Mannose-binding lectin: targeting the microbial world for complement attack and opsonophagocytosis. *Immunol. Rev.* 180, 86–99.
- Jenne, D.E., Tschopp, J., 1989. Molecular structure and functional characterization of a human complement cytotoxicity inhibitor found in blood and seminal plasma: identity to sulfated glycoprotein 2, a constituent of rat testis fluid. *Proc. Natl. Acad. Sci. U.S.A.* 86 (18), 7123–7127.
- Jonsson, G., Truedsson, L., Sturfelt, G., Oxelius, V.A., Braconier, J.H., Sjoholm, A.G., 2005. Hereditary C2 deficiency in Sweden: frequent occurrence of invasive infection, atherosclerosis, and rheumatic disease. *Medicine* 84, 23–34.
- Joseph, K., Kulik, L., Coughlin, B., Kunchithapautham, K., Bandyopadhyay, M., Thiel, S., et al., 2013. Oxidative stress sensitizes RPE cells to complement-mediated injury in a natural antibody-, lectin pathway- and phospholipid epitope-dependent manner. *J. Biol. Chem.* 288, 12753–12765.
- Kaida, K., Kusunoki, S., 2009. Guillain–Barre syndrome: update on immunobiology and treatment. *Exp. Rev. Neurother.* 9, 1307–1319.
- Kirschfink, M., Mollnes, T.E., 2003. Modern complement analysis. *Clin. Diagn. Lab. Immunol.* 10, 982–989.
- Klein, R.J., Zeiss, C., Chew, E.Y., Tsai, J.Y., Sackler, R.S., Haynes, C., et al., 2005. Complement factor H polymorphism in age-related macular degeneration. *Science* (New York, N.Y.) 308 (5720), 385–389.
- Kocsis, A., Kekesi, K.A., Szasz, R., Vegh, B.M., Balczer, J., Dobo, J., et al., 2010. Selective inhibition of the lectin pathway of complement with phage display selected peptides against mannose-binding lectin-associated serine protease (MASP)-1 and -2: significant contribution of MASP-1 to lectin pathway activation. *J. Immunol.* (Baltimore, Md.: 1950) 185, 4169–4178.
- Kwan, W.H., Van Der Touw, W., Heeger, P.S., 2012. Complement regulation of T cell immunity. *Immunol. Res.* 54 (1–3), 247–253.
- Le Friec, G., Kemper, C., 2009. Complement: coming full circle. *Arch. Immunol. Ther. Exp.* 57, 393–407.
- Lehto, T., Morgan, B.P., Meri, S., 1997. Binding of human and rat CD59 to the terminal complement complexes. *Immunology* 90, 121–128.
- Lindgren, S., Hansen, B., Sjoholm, A.G., Manthorpe, R., 1993. Complement activation in patients with primary Sjögren's syndrome: an indicator of systemic disease. *Autoimmunity* 16, 297–300.
- Liu, Y., Endo, Y., Iwaki, D., Nakata, M., Matsushita, M., Wada, I., et al., 2005. Human M-ficolin is a secretory protein that activates the lectin complement pathway. *J. Immunol.* (Baltimore, Md.: 1950) 175, 3150–3156.
- Manderson, A.P., Botto, M., Walport, M.J., 2004. The role of complement in the development of systemic lupus erythematosus. *Annu. Rev. Immunol.* 22, 431–456.
- Mathieson, P.W., Wurzner, R., Oliveria, D.B., Lachmann, P.J., Peters, D.K., 1993. Complement-mediated adipocyte lysis by nephritic factor sera. *J. Exp. Med.* 177, 1827–1831.
- Mehta, P., Norsworthy, P.J., Hall, A.E., Kelly, S.J., Walport, M.J., Botto, M., et al., 2010. SLE with C1q deficiency treated with fresh frozen plasma: a 10-year experience. *Rheumatology* (Oxford, England) 49, 823–824.
- Mevorach, D., Mascarenhas, J.O., Gershov, D., Elkon, K.B., 1998. Complement-dependent clearance of apoptotic cells by human macrophages. *J. Exp. Med.* 188 (12), 2313–2320.
- Miller, E.C., Chase, N.M., Densen, P., Hintermeyer, M.K., Casper, J.T., Atkinson, J.P., 2012. Autoantibody stabilization of the classical pathway C3 convertase leading to C3 deficiency and Neisserial sepsis: C4 nephritic factor revisited. *Clin. Immunol.* (Orlando, FL) 145, 241–250.
- Nauta, A.J., Castellano, G., Xu, W., Wolzman, A.M., Borrias, M.C., Daha, M.R., et al., 2004. Opsonization with C1q and mannose-binding lectin targets apoptotic cells to dendritic cells. *J. Immunol.* (Baltimore, Md.: 1950) 173, 3044–3050.
- Noris, M., Mescia, F., Remuzzi, G., 2012. STEC-HUS, atypical HUS and TTP are all diseases of complement activation. *Nat. Rev. Nephrol.* 8 (11), 622–633.
- Norris, D.A., Kissinger, R.M., Naughton, G.M., Bystryn, J.C., 1988. Evidence for immunologic mechanisms in human vitiligo: patients' sera induce damage to human melanocytes in vitro by complement-mediated damage and antibody-dependent cellular cytotoxicity. *J. Invest. Dermatol.* 90, 783–789.
- Podack, E.R., Muller-Eberhard, H.J., Horst, H., Hoppe, W., 1982. Membrane attach complex of complement (MAC): three-dimensional analysis of MAC-phospholipid vesicle recombinants. *J. Immunol.* (Baltimore, Md.: 1950) 128, 2353–2357.
- Pradhan, V., Rajadhyaksha, A., Mahant, G., Surve, P., Patwardhan, M., Dighe, S., et al., 2012. Anti-C1q antibodies and their association with complement components in Indian systemic lupus erythematosus patients. *Indian J. Nephrol.* 22, 353–357.
- Presanis, J.S., Hajela, K., Ambrus, G., Gal, P., Sim, R.B., 2004. Differential substrate and inhibitor profiles for human MASP-1 and MASP-2. *Mol. Immunol.* 40 (13), 921–929.
- Ramaglia, V., Hughes, T.R., Donev, R.M., Ruseva, M.M., Wu, X., Huitinga, I., et al., 2012. C3-dependent mechanism of microglial priming relevant to multiple sclerosis. *Proc. Natl. Acad. Sci. U.S.A.* 109, 965–970.
- Risitano, A.M., 2013. Paroxysmal nocturnal hemoglobinuria and the complement system: recent insights and novel anticomplement strategies. *Adv. Exp. Med. Biol.* 735, 155–172.
- Romi, F., Kristoffersen, E.K., Aarli, J.A., Gilhus, N.E., 2005. The role of complement in myasthenia gravis: serological evidence of complement consumption in vivo. *J. Neuroimmunol.* 158 (1–2), 191–194.
- Rossi, V., Cseh, S., Bally, I., Thielen, N.M., Jensenius, J.C., Arlaud, G.J., 2001. Substrate specificities of recombinant mannan-binding lectin-associated serine proteases-1 and -2. *J. Biol. Chem.* 276 (44), 40880–40887.
- Schindler, R., Lonnemann, G., Shaldon, S., Koch, K.M., Dinarello, C.A., 1990. Transcription, not synthesis, of interleukin-1 and tumor necrosis factor by complement. *Kidney Int.* 37, 85–93.
- Schmalstieg Jr., F.C., Goldman, A.S., 2010. Birth of the science of immunology. *J. Med. Biogr.* 18, 88–98.

- Schwaebel, W., Dahl, M.R., Thiel, S., Stover, C., Jensenius, J.C., 2002. The mannan-binding lectin-associated serine proteases (MASPs) and MAp19: four components of the lectin pathway activation complex encoded by two genes. *Immunobiology* 205 (4–5), 455–466.
- Schwaebel, W.J., Reid, K.B., 1999. Does properdin crosslink the cellular and the humoral immune response? *Immunol. Today* 20, 17–21.
- Schwaebel, W.J., Lynch, N.J., Clark, J.E., Marber, M., Samani, N.J., Ali, Y.M., et al., 2011. Targeting of mannan-binding lectin-associated serine protease-2 confers protection from myocardial and gastrointestinal ischemia/reperfusion injury. *Proc. Natl. Acad. Sci. U.S.A.* 108 (18), 7523–7528.
- Schwaebel, W., Zwirner, J., Schulz, T.F., Linke, R.P., Dierich, M.P., Weiss, E.H., 1987. Human complement factor H: expression of an additional truncated gene product of 43 kDa in human liver. *Eur. J. Immunol.* 17 (10), 1485–1489.
- Seya, T., Nakamura, K., Masaki, T., Ichihara-Itoh, C., Matsumoto, M., Nagasawa, S., 1995. Human factor H and C4b-binding protein serve as factor I-cofactors both encompassing inactivation of C3b and C4b. *Mol. Immunol.* 32, 355–360.
- Skjoedt, M.O., Hummelshoj, T., Palarasah, Y., Honore, C., Koch, C., Skjodt, K., et al., 2010. A novel mannose-binding lectin/ficolin-associated protein is highly expressed in heart and skeletal muscle tissues and inhibits complement activation. *J. Biol. Chem.* 285 (11), 8234–8243.
- Stover, C.M., Luckett, J.C., Echtenacher, B., Dupont, A., Figgitt, S.E., Brown, J., et al., 2008. Properdin plays a protective role in polymicrobial septic peritonitis. *J. Immunol. (Baltimore, Md.: 1950)* 180, 3313–3318.
- Stover, C.M., Thiel, S., Thelen, M., Lynch, N.J., Vorup-Jensen, T., Jensenius, J.C., et al., 1999. Two constituents of the initiation complex of the mannan-binding lectin activation pathway of complement are encoded by a single structural gene. *J. Immunol. (Baltimore, Md.: 1950)* 162, 3481–3490.
- Takahashi, M., Endo, Y., Fujita, T., Matsushita, M., 1999. A truncated form of mannose-binding lectin-associated serine protease (MASP)-2 expressed by alternative polyadenylation is a component of the lectin complement pathway. *Int. Immunol.* 11, 859–863.
- Takahashi, M., Iwaki, D., Kanno, K., Ishida, Y., Xiong, J., Matsushita, M., et al., 2008. Mannose-binding lectin (MBL)-associated serine protease (MASP)-1 contributes to activation of the lectin complement pathway. *J. Immunol. (Baltimore, Md.: 1950)* 180, 6132–6138.
- Tandon, N., Morgan, B.P., Weetman, A.P., 1992. Expression and function of membrane attack complex inhibitory proteins on thyroid follicular cells. *Immunology* 75, 372–377.
- Taylor, P.R., Carugati, A., Fadok, V.A., Cook, H.T., Andrews, M., Carroll, M.C., et al., 2000. A hierarchical role for classical pathway complement proteins in the clearance of apoptotic cells in vivo. *J. Exp. Med.* 192, 359–366.
- Thiel, S., Vorup-Jensen, T., Stover, C.M., Schwaebel, W., Laursen, S.B., Poulsen, K., et al., 1997. A second serine protease associated with mannan-binding lectin that activates complement. *Nature* 386 (6624), 506–510.
- Thompson, I.A., Liu, B., Sen, H.N., Jiao, X., Katamay, R., Li, Z., et al., 2013. Association of complement factor H tyrosine 402 histidine genotype with posterior involvement in sarcoid-related uveitis. *Am. J. Ophthalmol.* 155, 1068–1074.
- Tuzun, E., Christadoss, P., 2013. Complement associated pathogenic mechanisms in myasthenia gravis. *Autoimmun. Rev.* 12, 904–911.
- Vorup-Jensen, T., Petersen, S.V., Hansen, A.G., Poulsen, K., Schwaebel, W., Sim, R.B., et al., 2000. Distinct pathways of mannan-binding lectin (MBL)- and C1-complex autoactivation revealed by reconstitution of MBL with recombinant MBL-associated serine protease-2. *J. Immunol. (Baltimore, Md.: 1950)* 165, 2093–2100.
- Wagner, E., Frank, M.M., 2010. Therapeutic potential of complement modulation. *Nat. Rev. Drug Discov.* 9, 43–56.
- Wallis, R., 2007. Interactions between mannose-binding lectin and MASP during complement activation by the lectin pathway. *Immunobiology* 212 (4–5), 289–299.
- Wallis, R., Shaw, J.M., Uitdehaag, J., Chen, C.B., Torgersen, D., Drickamer, K., 2004. Localization of the serine protease-binding sites in the collagen-like domain of mannose-binding protein: indirect effects of naturally occurring mutations on protease binding and activation. *J. Biol. Chem.* 279 (14), 14065–14073.
- Walport, M.J., Davies, K.A., Botto, M., 1998. C1q and systemic lupus erythematosus. *Immunobiology* 199, 265–285.
- Weetman, A.P., Cohen, S.B., Oleesky, D.A., Morgan, B.P., 1989. Terminal complement complexes and C1/C1 inhibitor complexes in autoimmune thyroid disease. *Clin. Exp. Immunol.* 77, 25–30.
- Weiler, J.M., Daha, M.R., Austen, K.F., Fearon, D.T., 1976. Control of the amplification convertase of complement by the plasma protein beta1H. *Proc. Natl. Acad. Sci. U.S.A.* 73, 3268–3272.
- Whaley, K., Ruddy, S., 1976. Modulation of the alternative complement pathways by beta 1 H globulin. *J. Exp. Med.* 144, 1147–1163.
- Whaley, K., Schwaebel, W., 1997. Complement and complement deficiencies. *Semin. Liver Dis.* 17, 297–310.
- Williams, T.J., 1983. Vascular permeability changes induced by complement-derived peptides. *Agents Actions* 13 (5–6), 451–455.
- Wirthmueller, U., Dewald, B., Thelen, M., Schafer, M.K., Stover, C., Whaley, K., et al., 1997. Properdin, a positive regulator of complement activation, is released from secondary granules of stimulated peripheral blood neutrophils. *J. Immunol. (Baltimore, Md.: 1950)* 158, 4444–4451.
- Woodruff, T.M., Costantini, K.J., Crane, J.W., Atkin, J.D., Monk, P.N., Taylor, S.M., et al., 2008. The complement factor C5a contributes to pathology in a rat model of amyotrophic lateral sclerosis. *J. Immunol. (Baltimore, Md.: 1950)* 181 (12), 8727–8734.
- Wouters, D., Stephan, F., Strengers, P., De Haas, M., Brouwer, C., Hagenbeek, A., et al., 2013. C1-esterase inhibitor concentrate rescues erythrocytes from complement-mediated destruction in autoimmune hemolytic anemia. *Blood* 121, 1242–1244.
- Wozniak, M.A., Itzhaki, R.F., Shipley, S.J., Dobson, C.B., 2007. Herpes simplex virus infection causes cellular beta-amyloid accumulation and secretase upregulation. *Neurosci. Lett.* 429 (2–3), 95–100.
- Zhou, W., Patel, H., Li, K., Peng, Q., Villiers, M.B., Sacks, S.H., 2006. Macrophages from C3-deficient mice have impaired potency to stimulate alloreactive T cells. *Blood* 107, 2461–2469.

# Cytokines, Their Receptors and Signals

*Joost J. Oppenheim*

Cancer and Inflammation Program, National Cancer Institute, National Institutes of Health,  
Frederick, MD, United States

## OUTLINE

Historical Perspective	275	Tumor Necrosis Factor Receptor Family	284
Cytokines and Immunity	277	The Interleukin-1/Toll-Like Receptor Family of Receptors	285
Cytokine Receptor Subsets	278	Immunosuppressive Cytokines/Growth Factors	286
The Common $\gamma$ c Chain Subset	278	Chemokines	287
The $\beta$ c Utilizing Subset	279	Alarmins	287
The gp130 Utilizing Subset (Interleukin-6 Family)	280	Conclusion	287
Cytokines Sharing Either a p35 or p40 Ligand Chain	280	Acknowledgments	288
Th17 Cytokines and Receptors	281	References	288
Class II Cytokine Receptor Family	282		
Type I Interferons $\alpha$ and $\beta$	282		
Type II Interferon Gamma	282		
Type III Interferon Lambda	283		
Noninterferon Members	283		

## HISTORICAL PERSPECTIVE

Although cytokines are the intermediary intercellular protein signals of the immune system, overproduction of proinflammatory cytokines or deficient production of immunosuppressive cytokine mediators can be a direct cause of autoimmune conditions. Cytokines have been the focus of research for over 70 years, and over 650,000 articles have reported on cytokines. This chapter will highlight in brief only those cytokines contributing to autoimmune and autoinflammatory diseases. Fortunately, there are only 38,000 plus references to autoimmunity and only about 9000 relating cytokines to autoimmunity.

The history of cytokine studies was initiated by the report of Menkin (1944) who was the first to propose that "soluble pyrexins" might be responsible for fever by purifying them from inflammatory exudates. However, these pyrexins survived boiling and were contaminated by pyrogenic bacterial endotoxins. Bennett and Beeson (1953) were the first to extract endotoxin-free endogenous pyrogens from peripheral blood leukocytes. At the same time, nerve-growth factors were discovered (Levimontalcini and Hamburger, 1953). Thereafter, interferons with antiviral activities were distinguished from antibodies (Isaacs and Lindenmann, 1957).

Several reports initiated the studies of lymphocyte-derived factors. Mitogenic factors for lymphocytes were first detected in the supernatants of antigen or alloantigen-stimulated mixed leukocyte cultures

([Kasakura and Lowenstein, 1965](#)). This was rapidly followed by the detection of immunologically nonspecific macrophage migration inhibitory factors (MIF) in such supernatants ([Bloom and Bennett, 1966](#)). The MIF activity was subsequently also found to have macrophage activating activity ([Nathan et al., 1971](#)) that was subsequently attributed to interferon gamma (IFN $\gamma$ ). The historic MIF activity is distinct from the more recently discovered MIF, which is produced by the anterior pituitary and is present at detectable levels in normal human serum. This MIF is hormone-like and counters the immunosuppressive effects of corticosteroids as reviewed by [Santos et al. \(2001\)](#). These lymphocyte-derived factors were termed lymphokines ([Dumonde et al., 1969](#)). Subsequently, it was shown that nonlymphocytes such as macrophages could also produce a thymocyte and lymphocyte activating factor ([Gery et al., 1971](#)). It was subsequently observed that even fibroblast cell lines could produce factors promoting inflammatory reactions and the term “cytokines” was coined ([Cohen et al., 1974](#)). Cytokines serve as signals between every nucleated cell type and regulate the survival, growth, activation, differentiation, and suppression of both innate and adaptive immune responses involved in host defense and restoration of homeostasis. Although by 1978 over 100 cytokine activities had been reported, in 1979 at a meeting of “cytokinologists,” it was decided to call all monocyte/macrophage-derived cytokines interleukin-1 (IL-1) and the lymphocyte-derived ones interleukin-2 (IL-2) ([Mizel and Farrar, 1979](#)). This was based on the simplistic presumption that all the activities could be attributed to these two molecules. Today we are up to interleukin 38, and there are many more cytokines with idiosyncratic names. For example, over 40 chemotactic cytokines called “chemokines” have been discovered that promote the directional migration of many cell types.

Immunologists at the time were enamored of specific antibodies and initially considered these nonspecific factors to be “lymphodrek” and to have little merit. However, the advent of the molecular age led to the identification of these molecules as gene products with distinct functions, forever changing immunology research. The first cytokine to be cloned was IFN $\beta$ 1 ([Taniguchi et al., 1980](#)), followed by IFN $\alpha$ 1 ([Nagata et al., 1980](#)) and IFN $\gamma$  ([Gray et al., 1982](#)). IL-2 was the first interleukin to be cloned ([Taniguchi et al., 1983](#)). The era of “receptorology” was initiated when the first of the three chains of the receptor for IL-2 (IL-2 R $\alpha$ ) was identified and cloned ([Leonard et al., 1984](#)). We now have hundreds of cloned cytokines.

Thus, cytokines, such as hormones, are intercellular regulators of immune cell reactions, but unlike hormones, they are more prominent at local inflammatory sites rather than in the serum. Unlike hormones which are usually produced by glands, cytokines are produced at lower (nanomolar) concentrations by a great variety of cells and tissues. Cytokines, such as hormones, also interact with selective cell receptors inducing an intracellular signal transduction cascade culminating in gene activation. Cytokines usually have localized “paracrine” effects on neighboring cells or “autocrine” effects on the producing cells but less often have “endocrine” systemic effects of hormones. The appearance of elevated levels of cytokines in the serum actually has systemic effects such as pyrexia, hypotension, muscle aches and pains, and malaise and is responsible for the systemic symptoms of infections and injury. In the most severe inflammatory states, as in sepsis, this is called a “cytokine storm,” which can be lethal.

Development of technology to delete or modify gene expression yielded “knockout” mice lacking selected cytokine gene activities. This revealed surprising *in vivo* roles and redefined the functions of a number of cytokines. For example, tumor necrosis factor (TNF), which had cytotoxic antitumor activity in tissue culture ([Carswell et al., 1975](#)), was identified as a key proinflammatory host defense cytokine ([Nedospasov et al., 2003](#)). IL-2 based on its *in vitro* effects was considered a mitogenic factor, but knockout mice often developed lymphoproliferative autoimmune syndromes based on the absence of the stimulating effects of IL-2 on immunosuppressive T regulatory cells (Tregs) ([Hunig and Schimpl, 2003](#)). Thus, studies of knockout mice often revealed specialized and unique roles for many cytokines. Ironically, the advent of knockout mice highlighted the primacy of “*in vivo veritas*” and drove immunologists and molecular biologists from tissue culture studies back to investigating *in vivo* models.

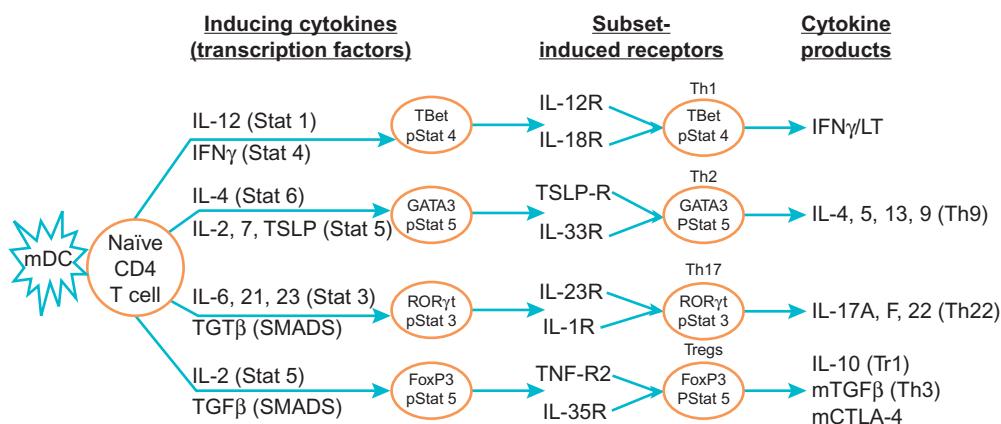
Today we are confronted by a plethora of cytokines, with apparent overlapping redundant activities, in part based on the sharing of receptors. The close interrelationship and apparent overlap in cytokine activities is accentuated by their capacity to induce one another and by their receptors to cross-talk and thus to “transactivate” each other. Thus, cytokines and their receptors operate in a linked network, and inhibition of one often downregulates the effects of other cytokines. It has become apparent over the past two decades that many cytokines mediate inflammatory responses aimed at eliminating invasive organisms and eliminating tissue debris generated by traumatic injuries. However, the identification of Tregs and their immunosuppressive mediators IL-10, transforming growth factor  $\beta$  (TGF $\beta$ ) and CTLA4 have identified a number of antiinflammatory cytokines that aim to restore homeostasis and mediate tissue repair. Thus, cytokines mediate the immunological balancing act between pro- and antiinflammatory reactions to pathogenic organisms and tissue damage. They also mediate the discrimination between self and nonself and can enhance or suppress inappropriate reactions to self.

## CYTOKINES AND IMMUNITY

The initial “innate” immune responses of cells are preprogrammed in the genome and are based on the induction of proinflammatory cytokine and chemokine production. These signals result in recruiting a rapid inflammatory response to invasive pathogenic organisms or tissue injury by phagocytic neutrophils followed by monocytes, macrophages, and dendritic cells (DCs). This cytokine-driven innate immune response is initiated by the interaction of exogenous “pathogen-associated molecular patterns” (PAMPs) or so-called stored endogenous “alarmins” with Toll-like receptors (TLRs), nucleotide-binding oligomerization domain 2 (NOD2), retinoic acid inducible gene (RIG)-like helicases, DNA sensors absent in melanoma2, or cell surface c-type lectin receptors present on or in many somatic cell types including inflammatory cells. These pattern recognition receptors enable host cells to respond to PAMPs and damaged self-proteins as reviewed in [Bellanti et al. \(2012\)](#).

The receptors on phagocytic DC internalize, digest, and process the antigens derived from PAMPs yielding peptide fragments that are then presented in conjunction with major histocompatibility complex (MHC) cell surface molecules on antigen presenting cells (APCs) to T lymphocytes. The presented MHC peptide complexes activate CD4 T cells that express the appropriate receptors to divide, multiply, and produce lymphokines, thus initiating the adaptive immune response with the capacity to mount specific reactions to invasive organisms and/or damaged cell products. The nature of the stimulant and cytokine response is crucial in determining the type of adaptive response that is generated. The activated CD41 T cells differentiate into subsets that exhibit Th1, Th2, Th17, or Treg cell responses as reviewed by [Zhu et al. \(2010\)](#). As shown in Fig. 15.1 ([O’Shea and Paul, 2010](#)), the Th1 lymphocytes producing IFN $\gamma$  favor the production of cellular immune delayed hypersensitivity reactions. Th2 lymphocyte-derived cytokines such as IL-4, 5, 9, and 13 favor humoral antibody-mediated immunity to parasites and allergic reactions. Furthermore, these cytokines reciprocally downregulate the other subset. Thus, IFN $\gamma$  suppresses the Th2 pathway and IL-4 and IL-13 suppress the Th1 pathway. The lymphocyte-derived Th17 type of cytokines (IL-17, IL-22, IL-23) favor acute inflammatory and autoimmune reactions. Treg cells consist of four subsets known as Th3, cell contact-dependent natural or induced Tregs, or Tr1 subsets which mediate their suppressive effects by expressing cell-associated TGF $\beta$ , CTLA4, and secreting IL-10, respectively. They are all engaged as feedback downregulators of inflammation and adaptive immunity and function to promote tissue repair by reducing inflammation and maintaining tolerance.

Lymphocyte subsets also express lineage-specific transcription factors called “master regulators,” such as Tbet, GATA3, ROR $\gamma$ t, and FoxP3 for Th 1, 2, 17, and Tregs, respectively, that are responsible for the production of the characteristic cytokines. The CD41 T cell commitment to the production of those cytokines in the case of Th1 and Th2 subsets is largely stable, but the Th17 and Treg subsets do show considerable plasticity and interconvertibility. Despite the fact that cytokines are mediators or “intermediaries,” they can play pivotal direct or indirect roles in the capacity of the immune system to distinguish between self and nonself. Overactivity of cytokines or of their receptors such as members of the IL-17 pathway that play a proinflammatory role can lead to excessive immune responses and decreases in the ability to differentiate between self and nonself. Thus, the IL-17 family of cytokines often mediates autoimmune reactions. Conversely, failure to produce antiinflammatory cytokines or defective



**FIGURE 15.1** Helper T cell “differentiation” and master regulators. Source: Adapted from O’Shea, J.J., Paul, W.E., 2010. Mechanisms underlying lineage commitment and plasticity of helper CD4 + T cells. *Science* 327, 1098–1102.

responses by cytokine receptors such as the IL-10 pathway can also result in autoimmune or autoinflammatory conditions, since this usually also results in upregulation and overproduction of proinflammatory cytokines.

The interaction of activated “matured” DC and naïve CD4 T lymphocytes in various cytokine milieus results in phosphorylation of lineage-specific transcription factors and master regulators as follows: The Th1 subset is induced by DC-derived IL-12, and this is markedly augmented by IL-18 and by a positive feedback loop involving IFN $\gamma$ . IL-4 derived from mast cells and basophils initiates the induction of the Th2 lineage. This can be augmented by IL-33 and thymic stromal-derived lymphopoietin (TSLP). TGF $\beta$  with the help of IL-6 and/or IL-21 induces Th17 lineage cells. This lineage is augmented by IL-1 and maintained by IL-23. TGF $\beta$  converts naïve CD41 cells to induced Tregs. Their survival requires IL-2, and they are expanded in numbers by cytokines such as TNF interacting with TNFR2. The “augmenting” cytokine ligands upregulate expression of selected cytokine receptors on the subsets enabling them to expand and maintain the lineage and its production of additional characteristic cytokine products.

The Th1 and Th2 lineages are largely stable, but the Th17 lineage can develop into dual Tbet and ROR $\gamma$ t expressing cells that produce both IL-17A and IFN $\gamma$ . Some of the Th2 subset can become IL-9 producing cells (Th9), while some of the Th17 cells or naïve CD41 T cells can go on to produce IL-22 and have been termed Th22. Some Tregs secrete the immunosuppressive IL-10 cytokine (Tr1 subset), while others express TGF $\beta$  on their cell membranes (Th3 subset) or membrane-associated CTLA4. The Treg subset is the most plastic and can develop into FoxP3 expressing cells that also express Tbet, GATA3, or ROR $\gamma$ t resulting in effector cells that are no longer suppressive but function as Th1, Th2, or Th17 cells.

Recently, “innate” lymphoid cells (ILCs) were identified that lacked T cell receptors and were therefore unable to participate in adaptive immune responses (Spits et al., 2013; Walker et al., 2013). Although small in numbers, these ILCs play important roles in lymphoid tissue development and the initiation of inflammation and throughout the duration of immune responses by promoting the transition to adaptive immunity and contributing to chronic inflammation based on their strategic location at mucosal barriers and in proximity to epithelial surfaces. The ILCs can be identified as belonging to three distinct groups. ILC1s that express Tbet and predominantly produce IFN $\gamma$  and contribute to cell-mediated Th1 protective immunity. The ILC1s are regulated differently and therefore distinct from cytotoxic natural killer (NK) cells. ILC2s express GATA3 and produce predominantly IL-5, IL-9, and IL-13, but also IL-4 and granulocyte macrophage colony-stimulating factor (GM-CSF). ILC2s not only protect against parasites and fungi but also promote allergic diseases and asthma. ILC3s express ROR $\gamma$ t and are a source of IL-17 and IL-22 and play a role in autoimmune diseases such as psoriasis and multiple sclerosis (MS). Some plasticities of these ILCs have been reported, and ILC3s can become ILC1 IFN $\gamma$  producers. They do not develop into T receptor-expressing T cells.

## CYTOKINE RECEPTOR SUBSETS

### The Common $\gamma$ c Chain Subset

Cytokines can be classified based on their structure, functions, gene locations, cells of origin, or targets, but such groupings lead to difficulties because of their pleomorphic overlapping effects. Although some cytokines exhibit homologous amino acid sequences, they can best be categorized based on their utilization of related members of receptor families which determines their target cells and effects.

The class I (hematopoietin) family of cytokines includes the common  $\gamma$ c utilizing subfamily of cytokines including IL-2, 4, 7, 9, 15, and 21 as well as the TSLP “stepbrother” of IL-7. Mice with defective  $\gamma$  common chain genes develop severe combined immune deficiency (SCID) syndrome and lack T and B cell immune host defenses against infectious agents as reviewed in Liao et al. (2011).

IL-2 was initially discovered as a mitogenic factor for T and B cells but subsequently also figured prominently in mediating the process of apoptosis (activation-induced cell death) of lymphocytes. IL-2 is produced by CD41 T cells and uses a receptor consisting of three chains IL-2R $\alpha$ ,  $\beta$ , and  $\gamma$  as reviewed by Akdis et al. (2011). The development of IL-2 deficient mice unexpectedly results in hyperplastic lymphadenopathy associated with autoimmune hemolytic anemia. This subsequently was attributed to the crucial role of IL-2 in maintaining the survival and expansion of CD251 (IL-2R $\alpha$ ) expressing Tregs. Scurvy mice based on defective Foxp3 genes also lack CD251 Tregs. Consequently, they have hyperactive CD41 T cells and overproduce proinflammatory cytokines. The homologous human autoimmune disease is known as “immune dysregulation, polyendocrinopathy, enteropathy, and X-linked.” Thus, the IL-2 function of supporting Tregs is crucial in preventing autoimmune disease.

IL-15 is structurally homologous to IL-2, but is produced by nonlymphoid cells (Di Sabatino et al., 2011). IL-15 interacts with a heterotrimeric receptor consisting of IL-2 15R $\beta$ ,  $\gamma$ C, and a unique IL-15R $\alpha$  chain. IL-15 is a more effective stimulant of NK cells, but less active in stimulating Treg cells than IL-2. Deletion of IL-15 or IL-15R $\alpha$  results in mice deficient in DC, NK cells, and DC81 memory T cells. Defective IL-15 production in mice has also been associated with increased susceptibility to infectious challenges. Conversely, IL-15 overproduction has been shown to play a crucial pathogenic role in a number of autoimmune diseases. Neutralizing antibodies to IL-15 have been shown to decrease inflammation in mouse models of rheumatoid arthritis (RA), psoriasis, and experimental autoimmune encephalomyelitis (EAE).

Another  $\gamma$ C cytokine is IL-7 which serves to develop and maintain a stable number of T, pre-B lymphocytes, and ILCs. This product of stromal cells thus maintains lymphocyte homeostasis by interacting with a receptor consisting of an IL-7 R $\alpha$  and the  $\gamma$ C chains. The absence of a functional IL-7 ligand or IL-7R $\alpha$  chain in mice and humans also yields a SCID phenotype, since IL-7 is required for the generation as well as maturation and survival of all T and B cells. Human polymorphisms of IL-7R resulting in excessive inflammatory and immune reactions are a risk factor for a variety of autoimmune diseases. Another gain of function mutation of IL-7R is associated with acute lymphoblastic leukemia (Mazzucchelli et al., 2012).

The deletion of IL-7 R $\alpha$  in mice also inhibits the functions of TSLP, which is produced by epithelial cells and uses a heterodimeric receptor consisting of TSLP-R and IL-7R $\alpha$  chains (Ziegler, 2010). TSLP is a potent inducer of Th2 responses and production of proallergic cytokines such as IL-4 and 13 by T and B cells, DC, mast cells, eosinophils, and macrophages. Mutations causing overproduction of TSLP result in mice with severe atopic dermatitis, and TSLP is necessary and sufficient to produce airway inflammatory disease in mice. Although they share a receptor chain, IL-7 functions to maintain the normal level of lymphocytes, while its stepbrother TSLP promotes Th2 immune responses.

IL-21 is a pleiotropic type I cytokine that is a product of CD41 T cells belonging to Th17 and T follicular helper (Tfh) subsets and NK T cells (Spolski and Leonard, 2010). The Tfh subset of cells has been reported to originate from naïve CD4 T cells by stimulation of Stat 5 and to express Bcl-6 as its master regulator. These T cells express CD41 CXCR51 ICOS1 markers and are located in germinal centers of lymph nodes. Tfh cells produce high levels of IL-21 and promote B cell antibody production and exhibit considerable plasticity. IL-21 is a potent inducer of proliferation and differentiation of CD41 and CD81 T cells, NK T cells, B cells, DC, macrophages, and epithelial cells including terminal differentiation and apoptosis. IL-21 interacts with the IL-21 R/ $\gamma$ C heterodimer. IL-21 stimulates a wide variety of target cells and contributes to the development of both innate and adaptive immunity. IL-21 does this in part by priming Th17 cells and by upregulating their IL-23R expression. IL-21 can also have feedback downregulatory effects by inducing Tr1 cells to produce IL-10 and by arresting DC in an immature state.

IL-21 along with TGF $\beta$  and IL-4 may also induce Th9 cells to produce IL-10. IL-21 stimulated B cells also are a rich source of IL-10. Despite these antiinflammatory effects, studies of IL-21 and IL-21R knockout mice show markedly reduced IL-17 production and reduced development and progression of EAE and systemic lupus erythematosus (SLE). Although IL-21 effects may be tempered by IL-10, overall IL-21 is a proinflammatory effector cytokine.

IL-9 is another pleiotropic cytokine that is produced by a distinct subset of T lymphocytes and mast cells and has therefore been termed a selective product of a unique Th9 pathway (Noelle and Nowak, 2010). However, it is unclear whether IL-9 is a product of stably differentiated cells or whether they exhibit plasticity. IL-9 can also be produced by the Th17 subset and can be coexpressed by IL-17A and by "redifferentiated" Tregs. TGF $\beta$  in the presence of IL-4 induces stat 6-dependent IL-9 production by GATA3 rather than by Fox3 expressing cells. The PU.1 transcription factor is also essential for the development of Th9 cells. IL-9 utilizes a heterodimeric receptor with IL-9R and  $\gamma$ C chains. IL-9 behaves like a Th2 cytokine. Mice deficient in IL-9 or IL-9R $\alpha$  are deficient in generating mast cells. In contrast, IL-9 induces mast cells to produce IL-1 $\beta$ , IL-5, IL-6, IL-9, IL-10, and IL-13. IL-9 is associated with allergic inflammation and immunity to extracellular parasites. Furthermore, adoptive transfer of Th9 polarized cells can promote the development of EAE and experimental autoimmune uveitis (EAU). Conversely, IL-9R $\alpha$  deficiency or neutralizing anti-IL-9 partially ameliorates EAE.

## The $\beta$ C Utilizing Subset

Another class I subset of cytokines, including GM-CSF, IL-3, and IL-5, interacts with heterodimeric receptors including a  $\beta$ C chain (Bellanti et al., 2012). In addition, each of these cytokine ligands uses a distinctive receptor chain. GM-CSF is produced not only by T cells and macrophages but also by endothelial cells and fibroblasts.

GM-CSF is a colony-stimulating factor that induces progenitor cells in the bone marrow to differentiate into granulocytic and myelocytic lineage cells. Transgenic mice overproducing GM-CSF developed increases in neutrophils, eosinophils, macrophages, and committed precursors of erythroid and megakaryocytes, which proved lethal because of the excessive inflammatory consequences. In vitro culture of monocytes with GM-CSF results in the development of DC, while in vivo local injections of GM-CSF or of tumor cells transfected to produce GM-CSF results in the in situ accumulation of DC and enhances tumor immunity.

Surprisingly, GM-CSF deficient mice exhibited normal hematopoiesis and myelopoiesis. Only infectious challenge revealed modest defects in granulocyte and macrophage responses. However, GM-CSF deficient mice do develop pulmonary alveolar proteinosis (PAP) and humans with PAP have high titers of anti- $\beta$ c antibodies or anti-GM-CSF. Since treatment with GM-CSF benefits such patients, PAP may be due to GM-CSF deficiency, which results in dysfunctional macrophages that fail to clear and metabolize surfactant lipoproteins. The GM-CSF deficient mice did accumulate lymphoid and mononuclear cells around their airways and pulmonary blood vessels. They also developed T cells deficient in IFN $\gamma$  production and cytotoxicity and decreases in B cell antibody production. GM-CSF knockout mice were resistant to lipopolysaccharide (LPS) challenge and resisted induction of autoimmune arthritis and EAE. Although the hematopoietic effects of GM-CSF appear to be redundant, GM-CSF has considerable proinflammatory cytokine functions.

IL-5, a Th2 cytokine produced by T cells and mast cells, is a critical regulator of eosinophil expansion in the bone marrow and peripheral blood and tissues in response to allergic and parasitic stimuli (Akdis et al., 2011). However, eosinophil migration is regulated by eotaxin (CCL11) and other chemokines and its development by hematopoietic growth factors. IL-5 deficient mice develop reduced numbers of mature functional eosinophils, but there is partial redundancy with other cytokines such as GM-CSF and IL-3.

IL-3 is a product of activated T cells that stimulates the proliferation, differentiation, and activation of multiple hematopoietic progenitor cells, mast cells, basophils, neutrophils, macrophages, eosinophils, and DC. IL-3 thus promotes antigen presentation to T cells and enhances macrophage cytotoxicity. IL-3 deficient mice, however, are normal, and its functions are redundant except for a reduced mast cell production in response to stem cell factor and parasitic challenge. They also produce fewer basophils in response to parasitic infections, and their contact hypersensitivity responses were also diminished.

### The gp130 Utilizing Subset (Interleukin-6 Family)

A third subfamily of class I cytokines utilizes a signal transducing gp130 receptor chain in common with a unique binding chain for each of the nine cytokine ligands in the family. They include IL-6, IL-11, IL-27, IL-31, leukemia inhibiting factor, oncostatin M, ciliary neurotrophic factor, cardiotropin-1, and cardiotropin-like cytokine (Akdis et al., 2011). Although these cytokines share one signaling chain, they have widely divergent functions depending on the cellular location of their unique binding chains.

IL-6 is one of the most important pleomorphic proinflammatory cytokines. IL-6 is produced not only by macrophages and activated T lymphocytes but also by fibroblasts, endothelial cells, and other somatic cells. IL-6 interacts with IL-6R $\alpha$ , which is restricted to certain tissues. However, IL-6R $\alpha$  is frequently shed and available in a soluble form that complexes IL-6. These complexes can trigger the widely expressed gp130 receptor and initiate signal transduction by JAK and STAT transcription factors. This enables IL-6 to activate T and B cells, macrophages, megakaryocytes, hepatocytes, endothelial cells, neuronal cells, and fibroblasts. This can result in local inflammation, but IL-6 often becomes available in the serum resulting in fever and acute phase responses. IL-6 is a potent inducer of B cell proliferation with the potential of developing plasma cell tumors (multiple myeloma) and growth of neuronal cells. Stimulation by the two other major proinflammatory cytokines, IL-1 and TNF, can induce IL-6 production and IL-6 can synergize with these cytokines. Consequently, IL-6 is a major exacerbator of autoimmune inflammatory diseases (Barr et al., 2012). Neutralizing anti-IL-6 antibodies have been used with therapeutic success to treat not only Castleman's disease, a form of plasma cell leukemia but also juvenile idiopathic arthritis and RA. Anti-IL-6 has been used successfully in some RA patients that were unresponsive to inhibitors of TNF.

### Cytokines Sharing Either a p35 or p40 Ligand Chain

The so-called IL-12 cytokine family consists of cytokines with an  $\alpha$  chain (p19, p28, or p35) and a  $\beta$  chain (p40 or EBI3) as follows: IL-12 (p35 and p40), IL-23 (p19 and p40), IL-27 (p28 and EBI3), and IL-35 (EBI3 and p35) (Akdis et al., 2011).

IL-12 is produced mostly not only by myeloid DC and macrophages but also by activated B lymphocytes and neutrophils. IL-12 utilizes a heterodimeric receptor formed by IL-12R $\beta$ 1 and IL-12R $\beta$ 2. IL-12R $\beta$ 2 appears to be the signaling component of the receptor on Th1 lymphocytes and NK cells that become tyrosine phosphorylated and activate Tyk2 and JAK2 and subsequently Stat 4. This enables IL-12 to play a crucial role in promoting Th1-dependent cytotoxicity of T cells and NK cells and their production of IFN $\gamma$ . IL-2 promotes this pathway by enhancing expression of the IL-12R, while IFN $\gamma$  promotes the production of more of the IL-12 cytokine, resulting in a positive loop. IL-12 has antiangiogenic effects by inducing IFN $\gamma$ , which in turn produces an angiostatic chemokine known as IP-10 or CXCL10. Mice defective in IL-12 p40 or IL-12R become much more susceptible to infections by intracellular pathogens such as mycobacteria and even nonpathogenic Bacille Calmette–Guerin organisms. Such knockout mice are much more resistant to the induction of autoimmunity, while administration of IL-12 exacerbates autoimmune conditions (Adorini, 2003). These results point out the important contribution of Th1 immune responses to various autoimmune states including EAE, EAU, RA, autoimmune myasthenia gravis, and autoimmune thyroiditis. Furthermore, an anti-IL-12 antibody has induced clinical responses and remissions in patients with Crohn's disease (Mannon et al., 2004).

IL-23 is a heterodimeric cytokine, which shares a p40 chain with IL-12. Like IL-12, IL-23 is produced by APCs such as DCs and macrophages and synovial fibroblasts. IL-23, however, has unique functions of stimulating committed Th17 memory cells that express the heterodimeric receptor for IL-23 consisting of IL-23R and IL-12 R $\beta$ 1. IL-23 serves to maintain Th17 responses by inducing the production of IL-17A, IL-17F, TNF, IL-6, GM-CSF, IL-22, and some chemokines (CXCL1 and CCL20). Knockout mice with deficiencies in either p19 or IL-23R develop less severe autoimmune inflammatory bowel disease (IBD) and EAE. This presumably is based on the reduced production of Th17 cytokines, which are important contributors to autoimmune inflammatory states. An anti-p40 antibody, which inhibits both IL-12 and IL-23, has therapeutic efficacy in psoriasis (Krueger et al., 2007).

IL-27 is reported to be a product of DC and macrophages as well as epithelial cells that interact with a receptor consisting of an IL-27R and the gp130 chains. Consequently, IL-27 belongs to both IL-6 and IL-12 subfamilies. IL-27 synergizes with IL-12 in activating T cells to produce IFN $\gamma$  with consequent Th1, antiangiogenic, and antitumor effects. IL-27 also suppresses the development of Th17 cells and can induce considerable IL-10 production by Tregs with antiinflammatory effects (Pot et al., 2011). IL-27 gene deletion results in mice with hyperreactive Th2 inflammatory responses to challenges with pathogenic parasitic organisms. Oral administration of IL-27 expressing lactococci by inducing mucosal IL-10 production suppresses IBD in mice. IL-27 also upregulates checkpoint expression by Tregs (Do et al., 2016). In addition, IL-27 has been shown to stimulate hematopoietic stem cells to produce myeloid progenitor cells (Hasegawa et al., 2016). Thus, the suppressive functions of IL-27 are more dominant.

IL-35 is reported to be a product of Treg cells and has suppressive effects on Th17 cells and CD4 $^1$  CD25 $^2$  T cells. IL-35 also can convert T cells to become FoxP3 Tregs, and this is known as "infectious tolerance." Furthermore, IL-35 is reported to stimulate the proliferation of Treg cells. IL-35 upregulates expression of multiple checkpoints by Tregs as well (Turnis et al., 2016). IL-35 also expands IL-35 producing B cells and protects from EAU. It is therefore said to be an immunosuppressive product contributing to the effects of Tregs. This cytokine has recently been reported to promote testicular immune privilege. Thus, IL-35 prevents autoimmunity but suppresses antitumor immunity (Hasegawa et al., 2016).

## Th17 Cytokines and Receptors

It was recently recognized that in addition to the Th1 IFN $\gamma$  cytokine, a family of IL-17 cytokines is a major contributor to autoimmune reactions; especially IL-17A and IL-17F, which show 50% homology (Iwakura et al., 2011). IL-17 is induced by the combination of TGF $\beta$  and IL-6 and is maintained by IL-23 and/or IL-1. IL-17 is produced mainly by Th17 CD4 and CD8 T cells, NK cells and by ILC3s. IL-17 activates neutrophils, T cells, APCs, and fibroblasts, endothelial cells, and stromal cells expressing various IL-17 receptors. IL-17A and IL-17F utilize IL-17RA and IL-17RD receptors, respectively, while IL-17B, C, and D use IL-17RB. IL-17 induces many other inflammatory cytokines, chemokines, and prostaglandins. Consequently, IL-17 is important in driving acute inflammatory as well as allergic and autoimmune responses. IL-17 actually has suppressive effects on the Th1 pathway and the production of IFN $\gamma$ . Nevertheless, T cells producing both IL-17 and IFN $\gamma$  have been detected. IL-17 promotes the production of proinflammatory cytokines such as IL-6, GM-CSF, G-CSF, IL-1 $\beta$ , TNF, IL-21, IL-22, IL-26, IL-11 various chemokines, and even TGF $\beta$ . Deletion of IL-17A and/or IL-17F or their receptors results in mice with markedly reduced resistance to infectious challenges. Airway hypersensitivity and T cell-dependent

antibody production are also considerably reduced. These IL-17A and F knockout mice develop less severe collagen-induced arthritis (CIA) and resist induction of EAE. Attempts to suppress autoimmune diseases in mouse models with neutralizing anti-IL-17 antibodies have not met with resounding success, perhaps because multiple redundant Th1 and Th2 cytokines all are involved in exacerbating the inflammation in these diseases. A humanized anti-IL-17 antibody has been effective in the treatment of RA ([Genovese et al., 2010](#)).

IL-25 (IL-17E) is produced by Th2 lymphocytes, mast cells, basophils, eosinophils, and epithelial cells ([Akdis et al., 2011](#)). IL-25 utilizes a receptor consisting of IL-17RA and IL-17RB chains. Receptor deleted mice fail to produce IL-5 or IL-13 in response to IL-25. Consequently, IL-25 is an inducer of Th2 and Th9 responses (IL-4, 5, and 13) and actually suppresses Th1 and IL-17 production. In particular, IL-25 is prominently involved in chronic inflammation of the gastrointestinal (GI) tract, including in IBD. IL-25 knockout mice show reduced eosinophil infiltration in allergic states due to the reduction in Th2 and Th9 responses.

## CLASS II CYTOKINE RECEPTOR FAMILY

The class II receptors are utilized by the interferon family (IFN- $\alpha$ ,  $\beta$ ,  $\gamma$ ) and also by IL-10, 19, 20, 22, 24, 26, 28, 29, and 30. The receptors consist of one ligand binding and one signal transducing chain. The interferons are the major host defense cytokines directed against viruses, bacteria, parasites, and fungi. They include IFN- $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\lambda$  (consisting of IL-28 and IL-29). The other family members have a diversity of functions.

### Type I Interferons $\alpha$ and $\beta$

There are actually 13 subtypes of IFN- $\alpha$  and two subtypes of IFN- $\beta$  ([Huber and Farrar, 2011](#)). IFN $\alpha/\beta$  both interact with the type I IFN receptor, which consists of two subunits, IFNAR1 and IFNAR2, and are widely expressed by all nucleated cells. The type I IFN- $\alpha/\beta$  are induced by a wide variety of exogenous and endogenous proinflammatory stimulants (especially viral infections) in many cell types. Plasmacytoid DCs are major producers of IFN- $\alpha$  and IFN- $\alpha$  is particularly elevated in patients with SLE and psoriasis. The fever and fatigue seen in active SLE are usually associated with high levels of type 1 IFN in the blood. Fibroblasts are a greater source of IFN- $\beta$ . Although many functions have been ascribed to IFN- $\alpha/\beta$ , mice knocked out for IFNAR1 exhibit predominantly reduced antiviral host defenses and some reduction in their responses to LPS and CSF-1. Null mutations of IFNAR genes also reduce SLE-like disease in mouse models ([Saadeh et al., 2016](#)). However, genetic polymorphisms resulting in the elevation of IFN- $\alpha$  predispose to the development of human SLE ([Niewold, 2011](#)). IFN- $\alpha/\beta$  are potent stimulators of the maturation of myeloid DC and thus promote adaptive immunity, but this is a redundant function. IFN- $\alpha$  is used in the treatment of hepatitis B and C. IFN- $\beta$  has been used to treat MS, but the mechanism by which IFN- $\beta$  suppresses symptoms and signs of MS may be based on suppression of IL-17 producing T cells. IFN- $\alpha/\beta$  therapy is associated with unpleasant flu-like side effects similar to those seen in SLE patients with elevated IFN $\alpha$  levels.

### Type II Interferon Gamma

IFN- $\gamma$  actually has minimal antiviral activity and is the major immunoregulatory product and coinducer of the Th1 cytokine pathway. IFN- $\gamma$  enhances autoimmunity ([Akdis et al., 2011](#)). It is selectively produced by T lymphocytes, NK cells, and ILC1 cells. There are reports claiming that IFN- $\gamma$  is also produced by macrophages, neutrophils, and B cells, but their contribution, if any, is minor. IFN- $\gamma$  utilizes a receptor consisting of IFNGR1 and IFNGR2 expressed by NK cells, macrophages, Th1 lymphocytes, CD81 CTL, and B cells. IFN- $\gamma$  has a major role in protecting against intracellular bacteria, based on its macrophage activating effects. Humans with gene defects in the IFN $\gamma$  pathway and mice lacking IFN- $\gamma$  or the IFNGR exhibit markedly reduced resistance to mycobacteria and other intracellular bacterial, fungal, and parasitic infections. Macrophage production of nitric oxide, TNF, IL-1, IL-12, and IL-6 is impaired. Unexpectedly, IFN- $\gamma$  knockout mice were not more resistant to the induction of EAE or uveitis, but more readily developed arthritis. Instead, IFN- $\gamma$  was found to be critical in suppressing chronic inflammation and restoring T cell homeostasis by promoting T cell apoptosis. Administration of IFN- $\gamma$  exacerbates MS. Chronic long-term exposure to low levels of IFN- $\gamma$  leads to a lupus-like autoimmune syndrome in mice ([Hodge et al., 2014](#)). IFN- $\gamma$  is used therapeutically to treat infections in patients with chronic granulomatous disease.

### Type III Interferon Lambda

The recently identified subgroup of IFN-λ 1, 2, and 3 are also termed as IL-29, IL-28A, and IL-28B, respectively ([Donnelly and Kotenko, 2010](#)). These cytokines utilize a heterodimeric receptor consisting of an IL-28R chain and IL-10R2 chain. The IFN-λs have both antiviral and immunostimulatory activities. Since the receptors for the IFN-λ are selectively expressed on epithelial cells, they may have fewer undesirable side effects than IFN-α and have demonstrated initial efficacy in the treatment of hepatitis. IL-28 may also have a protective role in EAE and is being investigated as a therapy for autoimmunity.

### Noninterferon Members

IL-10, another family member, has no antiviral activity but is an active immunomodulatory mediator that uses a heterodimeric receptor consisting of IL-10R1 and IL-10R2 chains ([Saraiva and O'Garra, 2010](#)). IL-10, a homodimeric cytokine, is produced by many cell types including Th1, Th2 lymphocytes, cytotoxic T cells, B lymphocytes, mast cells, monocytes, DCs, and the Tr1 subset of Tregs. IL-10 has many suppressive effects by inhibiting proinflammatory cytokine production and activities of T cells, NK cells, mononuclear phagocytes, and DC. However, IL-10 also has stimulatory effects particularly for B cells and promotes B cell proliferation, differentiation, and antibody production and induces isotype switching. Furthermore, by capturing and sequestering antigens, antibodies may, in turn, suppress cellular immune and inflammatory responses, thus further supporting the antiinflammatory role of IL-10.

Stat 3 is essential for the suppressive effects of IL-10 on cytokines since IL-10 fails to have such an effect on Stat 3 knockout mice. Stat 3 depleted mice become hypersensitive to endotoxic shock and develop chronic enterocolitis (IBD). The suppressive effects of IL-10 also involve the induction of inhibitory SOCS 1 and 3 signal transducers. IL-10 immunosuppressive effects, thus, protect the host from excessive inflammatory responses including allergic reactions. IL-10 knock-out mice show normal lymphocyte development and antibody responses, but they develop runting, anemia, and chronic colitis (IBD) due to excessive production of proinflammatory cytokines in response to enteric bacterial antigens. Polymorphisms reducing the effectiveness of IL-10 have resulted in a variety of human autoimmune diseases. IL-10 has a protective role in autoimmune diseases such as SLE, RA, and type 1 diabetes.

The other members of the class II cytokine receptor family, although members of the IL-10 family, have distinct nonimmunosuppressive functions ([Akdis et al., 2011](#)). These cytokines are also considered members of the IL-20 subfamily of cytokines and include IL-19, 20, 22, 24, and 26. They utilize common receptor subunits on target cells, thereby enhancing innate defense mechanisms and tissue repair processes at epithelial surfaces ([Rutz et al., 2014](#)). This is especially true of IL-22 ([Perusina Lanfranca et al., 2016](#)). IL-22 is usually considered a product of the Th17 pathways, although it is also induced by IL-9. IL-22 interacts with receptors consisting of IL-22R1 and IL-10R2. IL-22 is produced by nonlymphoid cells such as macrophages, fibroblasts, and mast cells. IL-22 is also produced by Th17, NK-22, and ILC22 cells and acts primarily on keratinocytes and epithelial lining cells, which express IL-22R1. IL-22 deficient and IL-22R-deficient mice have increased intestinal epithelial damage and become much more susceptible to *Citrobacter rodentium* infection. Thus, IL-22 plays a major role in the maintenance and repair of epithelial lining tissues. IL-22 levels are elevated in psoriasis and IBD. Prolonged expression of IL-22 can lead to chronic inflammation and cancer.

IL-19 shares the IL-20R receptor with IL-24 and IL-26. Since it augments IL-4, 5, 10, and 13 but decreases IFN $\gamma$  production, IL-19 favors Th2 responses. IL-19 is elevated and IL-19 polymorphisms have been associated with psoriasis. Conversely, IL-19 deficient mice more readily develop IBD with increased levels of Th1 cytokines such as IFN- $\gamma$  and also the elevation of IL-1 $\beta$ , IL-6, IL-12, TNF, and KC ([Leng et al., 2011](#)). Thus, IL-19 behaves as an immunosuppressive cytokine.

IL-20 is also elevated in psoriasis and RA, and IL-20 polymorphisms are associated with psoriasis. IL-20 is produced in particular by monocytes, DC, and synovial fibroblasts ([Leng et al., 2011](#)). IL-20 is proangiogenic and a keratinocyte stimulant. IL-20 can be considered a proinflammatory cytokine.

IL-24 promotes wound healing and is apoptotic for some tumor cells. IL-24 is elevated in melanomas and psoriasis. IL-26 is a proinflammatory cytokine, which is absent in mice and difficult to study. IL-26 is elevated in IBD and polymorphisms of IL-26 are protective of MS and RA.

Overall, this IL-20 subfamily is primarily produced by leukocytes and act on epithelial tissues. The IL-20 subfamily members synergize with other proinflammatory cytokines, thus exacerbating autoimmune and chronic inflammatory conditions. They have unique functions in protecting tissues and promoting regeneration and wound healing ([Rutz et al., 2014](#)).

## TUMOR NECROSIS FACTOR RECEPTOR FAMILY

The TNF family consists of 19 cytokines, which use 29 structurally related receptors that share intracellular signaling pathways (Aggarwal et al., 2012). TNF was discovered as a factor in serum that was cytotoxic for tumors (Carswell et al., 1975). TNF is produced by T and B cells, NK cells, and macrophages. Although it interacts with the widely expressed TNFR1 receptor that contains a death domain, TNF acts on immune, endothelial, and other cell types as a major proinflammatory cytokine that mediates acute and chronic inflammatory responses to bacterial infections. TNF stimulates the hypothalamic–pituitary axis to release numerous hormones, induces liver-derived acute phase proteins, activates endothelial cells to express adhesion molecules, and results in egress of TNF activated leukocytes out of blood vessels. Thus, systemic TNF can cause fever, myalgias, malaise, hypotension, vascular leakage, cachexia, osteoporosis, and other signs of inflammation. Nevertheless, studies of mice with deletion of TNF or TNFR1 receptors showed increased resistance to development of endotoxin shock, resistance to carcinogenesis, reduced resistance to infectious challenges, and partial resistance to induction of EAE. A number of polymorphisms of the human TNF gene resulting in excessive activity have been associated with a variety of autoimmune diseases. TNF promotes inflammation and exacerbates autoimmune disorders such as RA, ankylosing spondylitis, Crohn's disease, and psoriasis. This can be countered by treating patients with these disorders with inhibitors of TNF such as anti-TNF antibodies or soluble receptors for TNF. Such immunotherapies do reduce host resistance and can result in activating latent tuberculosis infections. Despite this TNF, the blockade has benefited millions of autoimmune patients.

The second receptor for TNF, TNFR2, is expressed mostly on T lymphocytes and NK cells. TNFR2 is predominantly expressed on all naturally occurring Tregs and activated-induced Tregs (iTregs). Consequently, ligand-induced signals from TNFR2 provide a major means of downregulating proinflammatory effects of TNF stimulation of TNFR1.

Lymphotoxin (LT) $\alpha$  is the closest relative of TNF and also utilizes TNFR1 and 2. LT $\beta$  also utilizes two other receptors (LT $\beta$ R and HVEM). The LT name also represents a misnomer based on their initial discovery as in vitro cytotoxic activities (Ruddle and Waksman, 1967). LT is produced by activated T cells, NK cells, mast cells, fibroblasts, and endothelial and epithelial cells. Studies of knockout mice yielded the surprising revelation that LT was crucial for the organogenesis and maintenance of peripheral lymphoid tissues. Mice deficient in LT had only rudimentary lymph nodes and Peyer's patches and a disorganized splenic lymphoid architecture. Although LT $\alpha$  also uses TNFR1 and TNFR2, the unique effects of LT are based on the fact that LT $\alpha$  forms heterotrimeric complexes with lymphocyte membrane-associated LT $\beta$ , which then act on LT- $\beta$ R. In contrast, LT $\alpha$  homotrimers by acting on TNFR1 can mimic the effects of TNF. Consequently, double TNF/LT $\alpha$  knockout mice become even more susceptible to bacterial challenge than single knockout mice. Although the antibody responses and immunoglobulin switching by such mice are defective, their cellular immune responses remain fairly intact based on redundant pathways.

LIGHT also binds to LT $\beta$ R as well as HVEM receptors. It is more active in tissue remodeling and protecting epithelium than in autoimmune processes. Although proinflammatory, TWEAK and its receptor Fn-14 when blocked have not as yet been shown to ameliorate any antiimmune conditions (Croft and Siegel, 2017). Some of the other members of the TNF superfamily contribute to autoimmune disorders. Humans with disabling heterozygous mutations in the “fragment apoptosis stimulating” (Fas/CD95) receptor develop a disease called autoimmune lymphoproliferative syndrome with lymphadenopathy, splenomegaly, and the production of autoantibodies (Lobito et al., 2011). This is based on failure to remove long-lived lymphocytes by the usual Fas-dependent apoptotic mechanism (Strasser et al., 2009). The homotrimeric FasL triggers the Fas receptor, which contains a death domain, and results in the elimination of senescent lymphocytes. In the absence of normal Fas or FasL functions, humans and mice develop B cell lymphomas and autoimmune syndromes based on the accumulation of senescent and defective lymphocytes.

Another family member, “TNF-related apoptosis-inducing ligand” (TRAIL), similarly acts on death domain expressing DR4 and DR5 receptors. In addition to mediating antitumor effects, mice with deletion of TRAIL or its receptors develop autoimmune diseases. Analogous to Fas deletion, the absence of TRAIL results in failure to eliminate senescent and defective lymphocytes by apoptosis.

A number of the TNF family of cytokines are potent stimulants of B cells and have been implicated in causing or exacerbating SLE. This is true of the B cell activating factor member of the TNF family known as BAFF or BLYS or BLVS (Vincent et al., 2012). BAFF is a product of activated T cells, macrophages, and DCs. BAFF

interacts with two receptors known as TACI and BR3 expressed by B and T cells. In addition, BAFF heterotrimerizes with another TNF family member known as APRIL, which interacts with TACI and BCMA receptors (Cancro et al., 2009). Mice with deletion of a functional BAFF show impaired development of B cells, while mice with defective APRIL expression show impaired development of more mature plasma cells. Conversely, transgenic mice that overexpress BAFF have B cell hyperplasia and develop a lupus-like phenotype. Both BAFF and APRIL are elevated in SLE patients and other autoimmune diseases. A monoclonal anti-BAFF antibody that depletes B cells and shows therapeutic benefit has therefore been approved for use in SLE.

In the 1990s, mice deficient in CD40, CD40L, and wild-type mice treated with blocking reagents were shown to have markedly reduced inflammatory reactions despite being subjected to CIA and prone to lupus-like disease (Croft and Siegel, 2017). Furthermore, patients with genetic polymorphisms resulting in overproduction of CD40L and CD40 have increased susceptibility to a number of autoimmune disorders. Unfortunately, therapies with antagonistic antibodies have had serious thromboembolic effects and other antagonists are being developed.

TL1A is the ligand for death receptor 3 (DR3) and their interactions have proinflammatory consequences and may contribute to rheumatic diseases. Their inhibition can benefit arthritic patients and perhaps other autoimmune conditions (Croft and Siegel, 2017). Glucocorticoid-induced TNF-related ligand and its interaction with the GITR receptor stimulate T and B cells and DCs and can contribute to arthritis. GITR deficient mice display a reduction in CIA.

The CD70 ligand on DC and B cells interacts with CD27 receptors on T lymphocytes. Blocking of these interactions with anti-CD70 reduces bone and cartilage erosion in mice subjected to CIA. CD70 and/or CD27 are elevated in a variety of autoimmune diseases. In contrast, stimulation of 4-1BB (TNFSR9) with a receptor agonist results in marked suppression of joint inflammation and bone resorption in CIA mice. Further 4-1BB deficiency exacerbates lupus-prone MRL/lpr mice. Thus, 4-1BB and its ligand 4-1BBL act as suppressors of inflammation.

## THE INTERLEUKIN-1/TOLL-LIKE RECEPTOR FAMILY OF RECEPTORS

The IL-1 family has expanded to 11 members, but the three initial members have been most thoroughly studied (Sims and Smith, 2010). They consist of agonistic IL-1 $\alpha$ , which is usually cell membrane associated, the soluble IL-1 $\beta$ , and the soluble IL-1 receptor antagonist (IL-1RA). These ligands interact with the ubiquitously expressed type I IL-1R, which transduces signals that result in proinflammatory effects similar to those of TNF, except they do not induce apoptosis. IL-1RA acts as an antagonist of IL-1 $\alpha$  and IL-1 $\beta$  by binding tightly to type I IL-1R and competes with the agonist ligands for this receptor. The type II IL-1R also binds IL-1 $\beta$  and IL-1 $\alpha$  with high affinity but fails to signal and is therefore considered a “decoy” receptor, which traps the ligands. Thus, IL-1 is downregulated by these two mechanisms plus the inhibitory effect of shed type II IL-1R that can also capture IL-1 $\alpha$  and IL-1 $\beta$ . Furthermore, IL-1 $\alpha$  and  $\beta$  are generated with prodomains. IL-1 $\beta$  and IL-18 are generally, but not always, cleaved by caspase-1. This enzyme is generated by the “inflammasome” pathway, which is activated by microbial-derived ligands that interact with NOD-like receptors. This provides another means of regulating the activity of IL-1 $\beta$  as evidenced by the fact that genetically heritable gain of function mutations in the inflammasome pathway result in autoinflammatory periodic fever syndromes such as Muckle–Wells syndrome, familial Mediterranean fever, hyper-IgD syndrome, familial cold autoinflammatory syndrome, and others (e.g., NOMID, CINCA). IL-1 $\alpha$  is usually cleaved by calpain and other enzymes, rather than by inflammasome-derived caspase-1. IL-1RA contains a classical signal peptide and is secreted by the endoplasmic reticulum and Golgi apparatus like most other cytokines. The other IL-1 family members all are biologically active as full-length molecules, but enzymatic processing at their N-terminal ends increases their activities. The IL-1 family members all interact with closely related receptors containing extracellular immunoglobulin domains, and they signal through cytoplasmic Toll/IL-1R domains using the MyD88 signal transduction pathway, similar to that of the TLR.

There is another IL-1 family inhibitory receptor, SIGIRR, expressed largely on epithelial cells that decrease the inflammatory responses to IL-1, IL-18, IL-33, IL-36 $\alpha$ ,  $\beta$ ,  $\gamma$ , and to TLR ligands. This is based on observations showing that SIGIRR deficient mice have enhanced responses to these ligands and develop more severe IBD, asthma, and colon cancer. Thus, SIGIRR also downregulates responses to most of the IL-1 family members.

Despite the plethora of IL-1 activities, deletion of IL-1 $\alpha$  and/or  $\beta$  or of the type I IL-1R has limited deleterious effects on mice with only a modest decrease in cellular and humoral immune responses and decreased resistance to infectious challenges. IL-1/IL-1R deleted mice exhibit greater resistance to the induction of autoimmune

diseases. In contrast, deletion of IL-1-RA yielded dramatic phenotypic effects with hyperinflammatory reactions due to overproduction of inflammatory cytokines, enhanced delayed-type hypersensitivity, humoral immunity, increased resistance to infection, and enhanced induction of autoimmune states including spontaneous development of arthritis. These findings have led to the development of IL-1RA (anakinra) and other inhibitors of L-1 as therapeutics. These antagonists are having dramatic benefits in patients with autoinflammatory diseases. They are also benefiting patients with gain of function mutations in TNFR1 (TRAPS syndrome), Behcet's syndrome, gout, and are being investigated for their utility in type 2 diabetes, congestive heart failure, and other conditions (Dinarello, 2011).

IL-18 also is a member of the IL-1 family that is expressed by a wide range of cell types as an inactive precursor that requires cleavage by caspase-1 generated by the inflammasome pathway. IL-18 interacts with widely expressed heterodimeric IL-18R in which the  $\alpha$  chain binds and the  $\beta$  chain signals in response to IL-18. The IL-18 ligand by itself is a weak stimulant of IFN $\gamma$  (Th1) or IL-13 (Th2) responses, but IL-18 synergizes with IL-12 to produce high levels of IFN $\gamma$  or with IL-2 to produce more IL-13. A neutralizing IL-18 binding protein (IL-18BP) has been isolated from the urine of Italian nuns, which binds and inhibits IL-18 with high affinity. IFN $\gamma$  is a potent inducer of IL-18BP, thus it functions as a negative feedback inhibitor.

IL-18 promotes the development of Th1 responses and functions of NK cells in host defense against bacterial infections. Consequently, IL-18 deficient mice show reduced resistance to parasitic, viral, and bacterial infections. IL-18 is usually elevated in autoimmune diseases and IL-18 deficient mice develop less severe CIA and resist induction of IBD. Moreover, polymorphisms in the promoter regions of the human IL-18 gene have been associated with RA.

IL-33 functions as a nuclear binding transcriptional factor as well as an extracellular cytokine, like IL-1 $\alpha$ . Full-length IL-33, like IL-1 $\alpha$ , is also active, but it can be cleaved by caspase-1 from a 30 kDa to an active 18 kDa cytokine. IL-33 interacts with the ST2 receptor expressed on T helper cells, DCs, and mast cells. Although initially reported as a potent inducer of Th2 responses, more recent data indicated that IL-33 also can be a potent inducer of Th1 polarized cytotoxic CD8 T cells, ILC2 cells, NK cells, and Tregs. A soluble shed form of ST2 has the capacity to bind and inhibit IL-33. Thus, administration of sST2 reduces inflammation in an asthma model in mice and IL-33 as well as sST2 levels are elevated in autoimmune disorders such as SLE, IBD, and RA. Mice with defective ST2 receptors have reduced Th2 responses, increased sensitivity to endotoxin challenge, and increased susceptibility to streptozotocin-induced diabetes (Liew et al., 2016).

The newest IL-1 family members IL-36 $\alpha$ ,  $\beta$ ,  $\gamma$ , IL-36Ra and the antagonist IL-38 all bind to IL-36Ra. Binding of the agonists results in the production of proinflammatory mediators, whereas the antagonistic IL-36Ra and IL-38 prevent the signaling via MyD88 and IRAK4 of NF- $\kappa$ B and mitogen-activated protein kinases (Hahn et al., 2017). In contrast, IL-37 is an inhibitory cytokine that belongs to the IL-18 subfamily and binds to IL-18R. IL-36, IL-37, and IL-38 are products of epithelial cells and infiltrating mononuclear cells. These cytokines are all involved in psoriasis, inflammatory arthritis, lupus, and IBD. In addition, IL-37 may play an important role in the suppression of allergic asthma by suppressing mast cells (Conti et al., 2017).

## IMMUNOSUPPRESSIVE CYTOKINES/GROWTH FACTORS

We have already discussed immunosuppressive cytokines that play pivotal roles in preventing exuberant inflammatory reactions against nonself and self-antigens such as IL-10, IL-25, IL-27, IL-35, and IL-37. The other cytokine that plays a major suppressive role is TGF $\beta$ . TGF $\beta$  belongs to a family of over 40 cytokines that utilize serine threonine kinase receptors. Tregs and the subset of Th3 cells, as well as platelets, macrophages, NK cells, epithelial cells, and glial cells are major sources of TGF $\beta$ . It is usually classified as a growth factor because it promotes wound healing and fibrosis. Concomitantly, TGF $\beta$  has antiproliferative and suppressive effects on T and B cells, macrophages, endothelial cells, hematopoiesis, and suppresses inflammatory reactions which interfere with wound healing. Tumor cells often produce considerable TGF $\beta$  and thus have antiinflammatory and antiimmune effects.

Deletion of TGF $\beta$  in mice is lethal for neonatal mice. They exhibit uncontrolled T cell activation with excessive production of cytokines and die of a "cytokine storm" resembling the effects of "septic endotoxic shock." Conversely, overexpression of TGF $\beta$  results in renal fibrosis and diabetes. TGF $\beta$  is crucial in the generation of Treg cells from naïve T cells and in preventing the maturation of DC, which are crucial in preventing the development of autoimmunity. We will not discuss the other growth factors because they are not known to have direct effects on autoimmune responses.

## CHEMOKINES

There are over 40 chemoattractant cytokines (chemokines) that interact with over 20 different Gi protein-coupled receptors (GiPCR). These chemokines play a major role in the trafficking of inflammatory and non-inflammatory cells and are responsible for organogenesis of lymphoid and other tissues. Chemokines generally have minimal immune cell activating effects. Deletion of various chemokine or chemokine receptor genes have diverse effects depending on the distribution of their receptors. Although elevated in autoimmune diseases, chemokines are not directly responsible for any autoimmune disorders. Nevertheless, as key mediators in the pathogenesis of inflammatory, autoimmune, and neoplastic disorders, polymorphisms of various chemokine genes have been associated with a variety of diseases (Colobran et al., 2007). For example, SDF1/CXCL-12 and IL-8/CXCL-8 polymorphisms have been associated with SLE (Sandling et al., 2011; Wu et al., 2012), while defective CCR5 expression has a protective effect on RA.

## ALARMINs

There are a number of preformed constitutively available molecules residing in cell granules, cytoplasm, or binding to chromatin that are released by cellular degranulation or necrotic cell death (Yang et al., 2009). These “alarmins” have chemotactic/recruiting activity and also activating effects on mononuclear phagocytes and on DCs resulting in cytokine production and promotion of antigen presentation and adaptive immunity. Thus, alarmins are proinflammatory and enhance both innate and adaptive immunity. Alarmins are produced by a wide variety of leukocytes and nonleukocytes and interact with some GiPCR as well as TLR to yield chemotactic and activating effects, respectively. Some alarmins such as histamine, IL-33, and eosinophil-derived neurotoxin polarize Th2 responses, while others such as HMGB1 and HMGN1 are potent polarizers of Th1 responses. Most other alarmins such as defensins, cathelicidins, IL-1 $\alpha$ , and granulysin promote both Th1 and Th2 responses. IL-1 $\alpha$  and IL-33 as well as HMGB1 and HMGN1 are dual function alarmins because they act as nuclear binding proteins as well as cytokines with proinflammatory recruiting and activating effects. Many of these alarmins have been experimentally deleted in mice. Deletion of various antimicrobial defensins and cathelicidin results in reduced resistance to bacterial challenges. Defects in NOD2 with consequent defensin deficiency are associated with enhanced susceptibility to IBD in humans. This NOD2 defect results in overreactive inflammatory responses to GI flora in the microbiome. Deletion of HMGB1 has embryonic lethal effects, while deletion of HMGN1 results in immunodeficient mice with reduced tumor immunity.

Studies of the role of alarmins in autoimmunity are limited. HMGB1 is released passively by necrotic or damaged cells or actively by immune cell activation. Thus, IFN $\gamma$ , TNF, IL-1, the inflammasome pathway, and endotoxin stimulate macrophages to release HMGB1. HMGB1 activates TLR4-MD-2 chain directly and by complexing to other molecules such as RNA, DNA, IL-1 $\beta$ , CXCL-12, and TIM-3 can activate a wide variety of receptors and signal transduction pathways. HMGB1 is, therefore, a very potent immune stimulant, which is fortunately inactivated by ROS resulting in oxidation of its cysteine groups at sites of inflammation. HMGB1 has been associated with the development of RA and is elevated in their synovial fluid (Bertheloot and Latz, 2017). The levels of HMGB1 have been found to be elevated in a number of autoimmune conditions and are correlated with the degree of inflammation. HMGB1 is present in the nucleosomes of SLE serum and autoantibodies to HMGB1 have been detected in SLE sera.

The S100 family of proteins has 25 members that like other alarmins lack a leader sequence and can therefore not be secreted but are released by cells. The calgranulins S100A8/A9 are largely present in the cytosol of neutrophils and monocytes and upon release can interact with RAGE and TLR4 to induce inflammatory and immune responses. They are found in joints inflamed by RA and in psoriatic lesions. Other family members such as S100A4, S100B, and S100A12 also contribute to RA. Despite all these observations, alarmins have not been implicated as directly causative of autoimmune disorders.

## CONCLUSION

Cytokines play important roles as either mediators or suppressors of inflammation in autoimmunity. They can, therefore, be targeted therapeutically with beneficial, although noncurative effects. This brief chapter is of necessity incomplete based on our ignorance and on the enormity of the topic. More cytokines and receptors

remain to be discovered, and some may play central roles in autoimmune processes. More detailed information concerning the role of cytokines in various autoimmune diseases is to be found in many of the chapters in this textbook.

## Acknowledgments

I am grateful to Drs. Scott Durum, Howard Young, and Dennis Klinman for their helpful constructive comments and for the secretarial help of Ms. Sharon Livingstone.

## References

- Adorini, L., 2003. IL-12 deficient mice. In: Fantuzzi, G. (Ed.), *Cytokine Knockouts*. Humana Press, Totowa, NJ, pp. 253–268.
- Aggarwal, B.B., Gupta, S.C., Kim, J.H., 2012. Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey. *Blood* 119, 651–665.
- Akdis, M., Burgler, S., Cramer, R., Eiwegger, T., Fujita, H., Gomez, E., et al., 2011. Interleukins, from 1 to 37, and interferon-gamma: receptors, functions, and roles in diseases. *J. Allergy Clin. Immunol.* 127, 701–721.e1-70.
- Barr, T.A., Shen, P., Brown, S., Lampropoulou, V., Roch, T., Lawrie, S., et al., 2012. B cell depletion therapy ameliorates autoimmune disease through ablation of IL-6-producing B cells. *J. Exp. Med.* 209, 1001–1010.
- Bellanti, J.A., Escobar-Gutierrez, A., Oppenheim, J.J., 2012. Cytokines, chemokines and the immune system. In: Bellanti, J.A. (Ed.), *Immunology IV Clinical Applications in Health and Disease*. I Care Press, Bethesda, MD, pp. 287–366.
- Bennett Jr., I.L., Beeson, P.B., 1953. Studies on the pathogenesis of fever. II. Characterization of fever-producing substances from polymorpho-nuclear leukocytes and from the fluid of sterile exudates. *J. Exp. Med.* 98, 493–508.
- Bertheloot, D., Latz, E., 2017. HMGB1, IL-1alpha, IL-33 and S100 proteins: dual-function alarmins. *Cell. Mol. Immunol.* 14, 43–64.
- Bloom, B.R., Bennett, B., 1966. Mechanism of a reaction in vitro associated with delayed-type hypersensitivity. *Science* 153, 80–82.
- Cancro, M.P., D'Cruz, D.P., Khamashta, M.A., 2009. The role of B lymphocyte stimulator (BLyS) in systemic lupus erythematosus. *J. Clin. Invest.* 119, 1066–1073.
- Carswell, E.A., Old, L.J., Kassel, R.L., Green, S., Fiore, N., Williamson, B., 1975. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc. Natl. Acad. Sci. U.S.A.* 72, 3666–3670.
- Cohen, S., Bigazzi, P.E., Yoshida, T., 1974. Commentary. Similarities of T cell function in cell-mediated immunity and antibody production. *Cell. Immunol.* 12, 150–159.
- Colobran, R., Pujol-Borrell, R., Armengol, M.P., Juan, M., 2007. The chemokine network. II. On how polymorphisms and alternative splicing increase the number of molecular species and configure intricate patterns of disease susceptibility. *Clin. Exp. Immunol.* 150, 1–12.
- Conti, P., Ronconi, G., Caraffa, A., Lessiani, G., Duraisamy, K., 2017. IL-37 a new IL-1 family member emerges as a key suppressor of asthma mediated by mast cells. *Immunol. Invest.* 46, 239–250.
- Croft, M., Siegel, R.M., 2017. Beyond TNF: TNF superfamily cytokines as targets for the treatment of rheumatic diseases. *Nat. Rev. Rheumatol.* 13, 217–233.
- Di Sabatino, A., Calarota, S.A., Vidali, F., Macdonald, T.T., Corazza, G.R., 2011. Role of IL-15 in immune-mediated and infectious diseases. *Cytokine Growth Factor Rev.* 22, 19–33.
- Dinarello, C.A., 2011. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood* 117, 3720–3732.
- Do, J.S., Visperas, A., Sanogo, Y.O., Bechtel, J.J., Dvorina, N., Kim, S., et al., 2016. An IL-27/Lag3 axis enhances Foxp3<sup>+</sup> regulatory T cell-suppressive function and therapeutic efficacy. *Mucosal Immunol.* 9, 137–145.
- Donnelly, R.P., Kotenko, S.V., 2010. Interferon-lambda: a new addition to an old family. *J. Interferon Cytokine Res.* 30, 555–564.
- Dumonde, D.C., Wolstencroft, R.A., Panayi, G.S., Matthew, M., Morley, J., Howson, W.T., 1969. "Lymphokines": non-antibody mediators of cellular immunity generated by lymphocyte activation. *Nature* 224, 38–42.
- Genovese, M.C., Van den Bosch, F., Roberson, S.A., Bojin, S., Biagini, I.M., Ryan, P., et al., 2010. LY2439821, a humanized anti-interleukin-17 monoclonal antibody, in the treatment of patients with rheumatoid arthritis: a phase I randomized, double-blind, placebo-controlled, proof-of-concept study. *Arthritis Rheum.* 62, 929–939.
- Gery, I., Gershon, R.K., Waksman, B.H., 1971. Potentiation of cultured mouse thymocyte responses by factors released by peripheral leucocytes. *J. Immunol.* 107, 1778–1780.
- Gray, P.W., Leung, D.W., Pennica, D., Yelverton, E., Najarian, R., Simonsen, C.C., et al., 1982. Expression of human immune interferon cDNA in *E. coli* and monkey cells. *Nature* 295, 503–508.
- Hahn, M., Frey, S., Hueber, A.J., 2017. The novel interleukin-1 cytokine family members in inflammatory diseases. *Curr. Opin. Rheumatol.* 29, 208–213.
- Hasegawa, H., Mizoguchi, I., Chiba, Y., Ohashi, M., Xu, M., Yoshimoto, T., 2016. Expanding diversity in molecular structures and functions of the IL-6/IL-12 heterodimeric cytokine family. *Front. Immunol.* 7, 479.
- Hodge, D.L., Berthet, C., Coppola, V., Kastenmuller, W., Buschman, M.D., Schaughency, P.M., et al., 2014. IFN-gamma AU-rich element removal promotes chronic IFN-gamma expression and autoimmunity in mice. *J. Autoimmun.* 53, 33–45.
- Huber, J.P., Farrar, J.D., 2011. Regulation of effector and memory T-cell functions by type I interferon. *Immunology* 132, 466–474.
- Hunig, T., Schimpl, A., 2003. A unique role for IL-2 in self-tolerance. In: Fantuzzi, G. (Ed.), *Cytokine Knockouts*. Humana Press, Totowa, NJ, pp. 135–150.
- Isaacs, A., Lindenmann, J., 1957. Virus interference. I. The interferon. *Proc. R. Soc. Lond. B Biol. Sci.* 147, 258–267.
- Iwakura, Y., Ishigame, H., Saijo, S., Nakae, S., 2011. Functional specialization of interleukin-17 family members. *Immunity* 34, 149–162.
- Kasakura, S., Lowenstein, L., 1965. A factor stimulating DNA synthesis derived from the medium of leukocyte cultures. *Nature* 208, 794–795.

- Krueger, G.G., Langley, R.G., Leonardi, C., Yeilding, N., Guzzo, C., Wang, Y., et al., 2007. A human interleukin-12/23 monoclonal antibody for the treatment of psoriasis. *N. Engl. J. Med.* 356, 580–592.
- Leng, R.X., Pan, H.F., Tao, J.H., Ye, D.Q., 2011. IL-19, IL-20 and IL-24: potential therapeutic targets for autoimmune diseases. *Expert Opin. Ther. Targets* 15, 119–126.
- Leonard, W.J., Depper, J.M., Crabtree, G.R., Rudikoff, S., Pumphrey, J., Robb, R.J., et al., 1984. Molecular cloning and expression of cDNAs for the human interleukin-2 receptor. *Nature* 311, 626–631.
- Levimontalcini, R., Hamburger, V., 1953. A diffusible agent of mouse sarcoma, producing hyperplasia of sympathetic-ganglia and hyperneurotization of viscera in the chick embryo. *J. Exp. Zool.* 123, 233–287.
- Liao, W., Lin, J.X., Leonard, W.J., 2011. IL-2 family cytokines: new insights into the complex roles of IL-2 as a broad regulator of T helper cell differentiation. *Curr. Opin. Immunol.* 23, 598–604.
- Liew, F.Y., Girard, J.P., Turnquist, H.R., 2016. Interleukin-33 in health and disease. *Nat. Rev. Immunol.* 16, 676–689.
- Lobito, A.A., Gabriel, T.L., Medema, J.P., Kimberley, F.C., 2011. Disease causing mutations in the TNF and TNFR superfamilies: focus on molecular mechanisms driving disease. *Trends Mol. Med.* 17, 494–505.
- Mannon, P.J., Fuss, I.J., Mayer, L., Elson, C.O., Sandborn, W.J., Present, D., et al., 2004. Anti-interleukin-12 antibody for active Crohn's disease. *N. Engl. J. Med.* 351, 2069–2079.
- Mazzucchelli, R.I., Riva, A., Durum, S.K., 2012. The human IL-7 receptor gene: deletions, polymorphisms and mutations. *Semin. Immunol.* 24, 225–230.
- Menkin, V., 1944. Chemical basis of fever. *Science* 100, 337–338.
- Mizel, S.B., Farrar, J.J., 1979. Revised nomenclature for antigen-nonspecific T-cell proliferation and helper factors. *Cell. Immunol.* 48, 433–436.
- Nagata, S., Taira, H., Hall, A., Johnsrud, L., Streuli, M., Ecsodi, J., et al., 1980. Synthesis in *E. coli* of a polypeptide with human leukocyte interferon activity. *Nature* 284, 316–320.
- Nathan, C.F., Karnovsky, M.L., David, J.R., 1971. Alterations of macrophage functions by mediators from lymphocytes. *J. Exp. Med.* 133, 1356–1376.
- Nedospasov, S.A., Grivennikov, S.I., Kuprash, D.V., 2003. Physiological roles of members of the TNF and TNF receptor families as revealed by knockout models. In: Fantuzzi, G. (Ed.), *Cytokine Knockouts*. Humana Press, Totowa, NJ, pp. 439–460.
- Niewold, T.B., 2011. Interferon alpha as a primary pathogenic factor in human lupus. *J. Interferon Cytokine Res.* 31, 887–892.
- Noelle, R.J., Nowak, E.C., 2010. Cellular sources and immune functions of interleukin-9. *Nat. Rev. Immunol.* 10, 683–687.
- O'Shea, J.J., Paul, W.E., 2010. Mechanisms underlying lineage commitment and plasticity of helper CD4 + T cells. *Science* 327, 1098–1102.
- Perusina Lanfranca, M., Lin, Y., Fang, J., Zou, W., Frankel, T., 2016. Biological and pathological activities of interleukin-22. *J. Mol. Med. (Berl.)* 94, 523–534.
- Pot, C., Apetoh, L., Awasthi, A., Kuchroo, V.K., 2011. Induction of regulatory Tr1 cells and inhibition of T(H)17 cells by IL-27. *Semin. Immunol.* 23, 438–445.
- Ruddle, N.H., Waksman, B.H., 1967. Cytotoxic effect of lymphocyte-antigen interaction in delayed hypersensitivity. *Science* 157, 1060–1062.
- Rutz, S., Wang, X., Ouyang, W., 2014. The IL-20 subfamily of cytokines—from host defence to tissue homeostasis. *Nat. Rev. Immunol.* 14, 783–795.
- Saadeh, D., Kurban, M., Abbas, O., 2016. Update on the role of plasmacytoid dendritic cells in inflammatory/autoimmune skin diseases. *Exp. Dermatol.* 25, 415–421.
- Sandling, J.K., Garnier, S., Sigurdsson, S., Wang, C., Nordmark, G., Gunnarsson, I., et al., 2011. A candidate gene study of the type I interferon pathway implicates IKBKE and IL8 as risk loci for SLE. *Eur. J. Hum. Genet.* 19, 479–484.
- Santos, L., Hall, P., Metz, C., Bucala, R., Morand, E.F., 2001. Role of macrophage migration inhibitory factor (MIF) in murine antigen-induced arthritis: interaction with glucocorticoids. *Clin. Exp. Immunol.* 123 (2), 309–314.
- Saraiva, M., O'Garra, A., 2010. The regulation of IL-10 production by immune cells. *Nat. Rev. Immunol.* 10, 170–181.
- Sims, J.E., Smith, D.E., 2010. The IL-1 family: regulators of immunity. *Nat. Rev. Immunol.* 10, 89–102.
- Spits, H., Artis, D., Colonna, M., Diefenbach, A., Di Santo, J.P., Eberl, G., et al., 2013. Innate lymphoid cells—a proposal for uniform nomenclature. *Nat. Rev. Immunol.* 13, 145–149.
- Spolski, R., Leonard, W.J., 2010. IL-21 is an immune activator that also mediates suppression via IL-10. *Crit. Rev. Immunol.* 30, 559–570.
- Strasser, A., Jost, P.J., Nagata, S., 2009. The many roles of FAS receptor signaling in the immune system. *Immunity* 30, 180–192.
- Taniguchi, T., Ohno, S., Fujii-Kuriyama, Y., Muramatsu, M., 1980. The nucleotide sequence of human fibroblast interferon cDNA. *Gene* 10, 11–15.
- Taniguchi, T., Matsui, H., Fujita, T., Takaoka, C., Kashima, N., Yoshimoto, R., et al., 1983. Structure and expression of a cloned cDNA for human interleukin-2. *Nature* 302, 305–310.
- Turnis, M.E., Sawant, D.V., Szymczak-Workman, A.L., Andrews, L.P., Delgoffe, G.M., Yano, H., et al., 2016. Interleukin-35 limits anti-tumor immunity. *Immunity* 44, 316–329.
- Vincent, F.B., Morand, E.F., Mackay, F., 2012. BAFF and innate immunity: new therapeutic targets for systemic lupus erythematosus. *Immunol. Cell Biol.* 90, 293–303.
- Walker, J.A., Barlow, J.L., McKenzie, A.N., 2013. Innate lymphoid cells—how did we miss them? *Nat. Rev. Immunol.* 13, 75–87.
- Wu, F.X., Luo, X.Y., Wu, L.J., Yang, M.H., Long, L., Liu, N.T., et al., 2012. Association of chemokine CXCL12-3'G801A polymorphism with systemic lupus erythematosus in a Han Chinese population. *Lupus* 21, 604–610.
- Yang, D., de la Rosa, G., Tewary, P., Oppenheim, J.J., 2009. Alarms link neutrophils and dendritic cells. *Trends Immunol.* 30, 531–537.
- Zhu, J., Yamane, H., Paul, W.E., 2010. Differentiation of effector CD4 T cell populations (\*). *Annu. Rev. Immunol.* 28, 445–489.
- Ziegler, S.F., 2010. The role of thymic stromal lymphopoietin (TSLP) in allergic disorders. *Curr. Opin. Immunol.* 22, 795–799.

## 16

# Cell Death and Autoimmune Disease

*Stefania Gallucci<sup>1</sup>, Roberto Caricchio<sup>1,2</sup> and Philip L. Cohen<sup>1,2</sup>*

<sup>1</sup>Department of Microbiology and Immunology, Lewis Katz School of Medicine at Temple University, Philadelphia, PA, United States

<sup>2</sup>Department of Medicine (Section of Rheumatology), Lewis Katz School of Medicine at Temple University, Philadelphia, PA, United States

## O U T L I N E

<b>Apoptosis</b>	<b>291</b>	<i>Find-Me Signals</i>	<b>295</b>
<b>Apoptosis in Autoimmunity</b>	<b>292</b>	<i>Eat-Me Signals and Their Receptors</i>	<b>296</b>
Defective Apoptosis	292	<i>Receptors for Necrotic Cells</i>	<b>297</b>
Excessive Apoptosis and Apoptotic Cells as Sources of Autoantigen	293	<i>Antiinflammatory Effects of Apoptotic Cells</i>	<b>298</b>
Apoptoses	293	<i>Immunostimulatory Effects of Necrotic Cells</i>	<b>298</b>
NETosis	294	<i>A Glimpse Into the Future</i>	<b>299</b>
<b>Necrosis</b>	<b>294</b>	<b>References</b>	<b>299</b>
<i>Necroptosis in Autoimmunity</i>	294		
<b>Clearance of Dead Cells</b>	<b>295</b>		

## APOPTOSIS

*Historical.* In 400 BC Hippocrates used the word “apoptosis” in the book of Mochlicon to describe the gangrene resulting from the treatment of fractures with bandages (Andre, 2003). Two millennia elapsed until the mid-19th century description of cell death by Vogt. Shortly after, Virchow and Flemming reported two types of cell death and demonstrated that they were consequences of mechanical or chemical damage (Diamantis et al., 2008). In the early 20th century, the seminal work in embryology of Perez and Glucksmann, among others, led to a detailed histological description of cellular death (Diamantis et al., 2008). In 1972, Kerr coined the word “apoptosis” independently from Hippocrates after a suggestion from a professor of Greek (Kerr et al., 1972). Kerr, Wyllie, and Currie described “shrinkage necrosis” as a defined pattern and sequence of events during cell death, leading to the recognition of apoptosis as an integral part of biological processes such as mitosis (Kerr et al., 1972). In the late 1970s, Sulston and Horvitz used *Caenorhabditis elegans*, a nematode in which a fixed percentage of somatic cells die predictably (Sulston and Horvitz, 1977), to dissect the pathways to cell death and to clone several genes named *CED* (*C. elegans* death). The *CED* genes were eventually shown to be highly preserved in mammals (Lockshin and Zakeri, 2001). Today, apoptosis and necrosis are considered fundamental parts of many physiological and pathological processes. Further understanding of the mechanisms of these processes is likely to lead to new targets for medical intervention.

*What do apoptotic cells look like?* Once apoptosis has been triggered, the cell starts to round up, mostly due to retraction of pseudopods and reduction of cellular and nuclear volume (pyknosis), accompanied by modification of cytoplasmic organelles and plasma membrane blebbing (Mills et al., 1999). There is preservation of plasma membrane integrity and exposure of phospholipids, a fundamental “eat-me” signal (Martin et al., 1995; Darzynkiewicz et al., 1992). The terminal (“executioner”) phase is characterized by nuclear fragmentation (karyorrhexis) and the cell’s disintegration into micronuclei surrounded by plasma membrane, known as apoptotic bodies and microparticles (Nagata, 2005). An important last step is the engulfment of the apoptotic cell by resident phagocytes (Wang et al., 2003).

*Molecular pathways leading to apoptosis.* Cells undergoing apoptosis utilize a programmed molecular cascade [reviewed by Kroemer et al. (2009) and Galluzzi et al. (2012)]. The process may be triggered via an engagement of cell surface death receptors by ligands in their external milieu (extrinsic apoptosis) or through biochemical changes within the cell itself (intrinsic apoptosis) (Riedl and Salvesen, 2007).

Initiation of *extrinsic apoptosis* involves the triggering of a *trans*-membrane receptor. In the presence of their ligands, such as FasL (CD178), TNF- $\alpha$ , or TNF-related apoptosis-inducing ligand (TRAIL) (Takahashi et al., 1994; Wiley et al., 1995), these death receptors [FAS (CD95), TNFR1 (CD120a), and TRAILR 1 and 2 (CD261 and CD262)] trimerize to form the “death-inducing signaling complex” (DISC) (Lavrik and Krammer, 2011). DISC formation depends on a conserved death domain (DD), which can recruit receptor-interacting protein (RIP) kinase 1 (Varfolomeev et al., 2005), FAS-associated protein with a DD (FADD) (Chinnaiyan et al., 1995), multiple isoforms of FADD-like IL-1 $\beta$ -converting enzyme)-inhibitory protein (c-FLIP), and procaspase-8. The triggering of TNFR1 may also lead to activation of NF $\kappa$ B or to necrotic death (see Necrotic Death, below). Once the DISC is formed, the activation of caspase-8 ensues (Scaffidi et al., 1999).

In apoptotic lymphocytes (also called type I cells) (Barnhart et al., 2003), caspase-8 activates caspase-3, which initiates the proteolytic cleavage of cytoplasmic and nuclear proteins, and the activation of caspase-activated DNase, an enzyme that cleaves internucleosomal chromatin (Enari et al., 1998).

*Intrinsic apoptosis* can be triggered by DNA damage, reactive oxygen species (ROS), and the accumulation of unfolded proteins in the endoplasmic reticulum (Galluzzi et al., 2012). The pathways that lead to cellular demise share mechanisms intrinsic to mitochondria. Antiapoptotic pathways (principally involving the *bcl-2* family of proteins) are simultaneously engaged, and the fate of a cell depends on which pathway prevails. In intrinsic apoptosis, membrane outer membrane polarization (MOMP) dramatically reduces ATP synthesis, thereby boosting ROS production (Aon et al., 2006). MOMP also triggers the release of cytochrome c (CYTC), diablo IAP binding mitochondrial protein (DIABLO), apoptosis-inducing factor (AIF), and endonuclease G (ENDOG) (Leibowitz and Yu, 2010). The assembly of the protein complex known as the apoptosome leads to the activation of caspase-9 and cleavage of caspase-3, with subsequent cytoplasmic demise and nuclear fragmentation (Bratton et al., 2001). In some circumstances, AIF and ENDOG translocate to the nucleus and initiate chromatin fragmentation in a caspase-independent manner (Lartigue et al., 2009). Therefore the mandatory involvement of mitochondria, the dispensable role of caspases and the bioenergetics, and metabolic catastrophe are major characteristics of this form of apoptosis.

There is cross talk between extrinsic and intrinsic pathways. For example, in apoptotic hepatocytes (also referred as type II cells) there is involvement of the mitochondrial pathway and caspase-8, instead of activating caspase-3, cleaves BH3-interacting domain death agonist, inducing MOMP and reduction of the transmembrane potential ( $\Delta\psi_m$ ) (Kroemer et al., 2007). The resulting release of CYTC triggers assembly of the apoptosome (CYTC, the cytoplasmic adaptor protein APAF1 and dATP), activation of caspase-9, and finally the initiation of the executioner phase by cleavage of caspase-3 (Bratton et al., 2001; Mills et al., 1999; Martin et al., 1995; Darzynkiewicz et al., 1992; Nagata, 2005; Wang et al., 2003).

## APOPTOSIS IN AUTOIMMUNITY

Abnormalities in the apoptotic process have been linked to a variety of autoimmune diseases both systemic such as systemic lupus erythematosus (SLE) and organ specific such as primary biliary cirrhosis (PBC).

### Defective Apoptosis

The inability to control the immune response via cell death can result in autoimmunity; for example, defects in the extrinsic pathway, such as the lack of *Fas* in MRL/lpr mice, render lymphocytes resistant to cell death with marked lymphadenopathy and lupus-like disease (Cohen and Eisenberg, 1991). MRL/lpr mice succumb

prematurely to severe lupus-like disease and accumulate large numbers of lymphocytes, mostly unusual T cells but also B cells (Kono and Theofilopoulos, 2000). Interestingly, the lack of Fas (or caspase-8) in dendritic cells also leads to lupus-like autoimmunity (Stranges et al., 2007; Chen et al., 2006). Human autoimmune lymphoproliferative syndrome, caused by mutations in the Fas death pathway, is a parallel condition (Drappa et al., 1996; Oliveira et al., 2010). The abnormal expression of apoptotic genes in the intrinsic pathway also results in autoimmunity. Overexpression of the antiapoptotic *bcl-2* gene or deletion of the proapoptotic *Bim* gene leads to accelerated autoimmunity, due to uncontrolled T and B-cell proliferation (Tischner et al., 2011; Strasser et al., 1991; Hutcheson et al., 2008). These findings demonstrate the tight control of autoimmunity by cell death.

## Excessive Apoptosis and Apoptotic Cells as Sources of Autoantigen

Apoptotic cell excess can result in systemic autoimmunity or in flares of preexisting disease, presumably by supplying immunogenic nuclear antigens. Injection of UV-irradiated apoptotic cells induces a rise in antinuclear antibodies in normal mice without glomerulonephritis (Mevorach et al., 1998). Injection of dendritic cells that had phagocytosed apoptotic cells accelerates disease onset in lupus-prone mice while inducing transient expression of autoantibodies in normal mice (Bondanza et al., 2003). These and other studies suggest that apoptotic cells may serve as a source of autoantigens.

Generally, an apoptotic cell is noninflammatory and induces tolerance; nevertheless, there are circumstances during which this type of cell death triggers inflammation and amplifies autoimmune response (Green et al., 2009). For example, the redistribution of nuclear contents into membrane blebs and microparticles allows opsonization by autoantibodies. Uptake via proinflammatory Fc receptors and autoantigen presentation may in turn amplify the autoimmune response (Frisoni et al., 2005). Further, during conditions of excessive apoptosis, dying cells may progress into secondary necrosis due to delayed clearance (Munoz et al., 2009). Secondary necrosis allows the release of proinflammatory “danger signals” (Wu et al., 2001).

Autoimmunity toward nuclear components such as chromatin and ribonucleoprotein complexes is a striking feature of SLE (Rahman and Isenberg, 2008). Yet, these autoantigens are generally protected by the nuclear and the cytoplasm membrane, which shield them from the adaptive immune system. Apoptotic cells redistribute their nuclear material into membrane blebs (Casciola-Rosen et al., 1994; Radic et al., 2004), and these apoptotic cell autoantigens, when engulfed by dendritic cells, may be presented to T lymphocytes (Frisoni et al., 2005).

The cellular components released from dead cells, notably nucleic acids, may further stimulate the autoimmune reaction (see anti-inflammatory effects of apoprotic cells, below). Indeed, lack of Toll-like receptors (TLR) 7 or TLR9, receptors for ssRNA and dsDNA, abolishes the production of autoantibodies in several models of lupus-like autoimmunity (Green and Marshak-Rothstein, 2011).

## Apoptoses

Modifications of antigens on apoptotic cells such as cleavage and posttranslational modifications (Rosen and Casciola-Rosen, 1997; Utz et al., 1997) lead to the generation of “apoptoses,” a term used to designate epitopes displayed on the surface of early or late apoptotic cells and derived from the translocation of intracellular autoantigens (Reed et al., 2007). Apoptoses are recognized by specific autoantibodies, leading to the formation of pathogenic immune complexes such as IgG-apoptotic cells (Cocca et al., 2002), IgG-blebs (Frisoni et al., 2005), or IgG-microparticles (Beyer and Pisetsky, 2010). The recognition of apoptoses by autoimmune IgG can also impair clearance of apoptotic cells by neighboring cells (Reed et al., 2008). PBC, Sjögren syndrome, neonatal lupus (NL), and SLE are major autoimmune diseases in which this concept has been demonstrated. In PBC, the E2 component of the pyruvate dehydrogenase complex (PDC-E2) is considered a major autoantigen, and biliary epithelial cells display PDC-E2 as an apoptote on blebs during cell death (Lleo et al., 2011). Importantly, PDC-E2 maintains its antigenicity after it translocates to the cell membrane and is recognized by antimitochondrial antibodies. Subsequently, these complexes activate the innate immune system in multiple ways, resulting in increased TNF- $\alpha$  production and autoantigen presentation (Lleo et al., 2009). Apoptoses have been also demonstrated in NL, a condition in which the passive transfer of anti-SS-A/SS-B (anti-Ro/anti-La) from the mother to the fetus induces congenital heart block (Buyon et al., 2009). Fetal cardiomyocytes expose these antigens during early and late phases of apoptosis, and they are recognized by anti-SS-A/SS-B, inducing further apoptosis (Tran et al., 2002). The apoptote of SS-A is normally masked by cytoplasmic Y RNAs, but during apoptosis, Y RNA is cleaved, and SS-A translocates to blebs and can be recognized by autoantibodies (Reed et al., 2010).

Lupus autoimmunity is arguably the most diverse; nevertheless, autoantibodies are usually directed toward chromatin and its individual components (i.e., DNA and histones), making the nucleosome, the basic unit of chromatin, the most frequent target (Arbuckle et al., 2003).

During apoptosis, the chromatin undergoes dramatic changes, with fragmentation into the nucleosomes and polynucleosomes (Nagata et al., 2003). These apoptope-bearing fragments also redistribute to the cell membrane into blebs and microparticles and may form complexes with antinuclear antibodies (Frisoni et al., 2005; Radic et al., 2004). These complexes may induce and sustain the autoimmune response and may activate TLR, the best known receptors that activate innate immunity (Marshak-Rothstein and Rifkin, 2007). Apoptotic blebbing requires Rho-associated coil-containing protein kinase 1 (ROCK1) (Orlando et al., 2006). Inhibition of ROCK1 inhibits the redistribution of nucleosomes and the binding of autoantibodies (Frisoni et al., 2005). Inhibition of apoptotic nuclear and chromatin fragmentation leads to milder lupus-like disease in inducible models but to worsened autoimmunity in genetically predisposed ones (Jog et al., 2012; Frisoni et al., 2007). The results suggest that apoptotic autoantigens might serve both as immunogens but also as tolerogens.

## NETosis

The formation and release of neutrophil extracellular traps (NETs) may play a key role in stimulating systemic autoimmunity [Knight and Kaplan, 2012]; see Chapter 14: The Roles and Contributions of the Complement System in the Pathophysiology of Autoimmune Diseases]. These extracellular structures are made of chromatin, histones, and microbicidal granular proteins, and are triggered in neutrophils in response to lipopolysaccharides, granulocyte–macrophage colony-stimulating factor, and IL-8 (Yousefi et al., 2008). NETosis is caspase-independent and depends on the production of NADPH oxidase and ROS.

NETs are triggered by granulomatous vasculitis-associated antineutrophil cytoplasmic antibodies and contain their target antigens proteinase-3 and myeloperoxidase (Kessenbrock et al., 2009). Excess NETosis may play a role in SLE (Knight and Kaplan, 2012). They contain histones and DNA, two major lupus autoantigens, and also trigger danger signals such as HMGB1 and proinflammatory cytokines such as IL-17 (Garcia-Romo et al., 2011). Moreover LL37, a neutrophil-derived antimicrobial peptide present in NETs, has been found in complexes with anti-DNA and DNA, and its presence triggers type I interferon (IFN-I) production by plasmacytoid dendritic cells (Garcia-Romo et al., 2011). Therefore in SLE, NETs may provide autoantigens and the milieu necessary to amplify the autoimmune response and tissue damage.

## NECROSIS

Swelling of the cytoplasm and intracellular organelles, early loss of membrane integrity, and moderate chromatin condensation are the typical morphological changes of a necrotic cell (Kroemer et al., 2009). Molecular steps regulating this process have led to the term programmed necrosis. Two major examples relevant to autoimmunity are described below.

*Necroptosis.* This necrotic molecular cascade is dependent on the activation of RIP-1 and/or its homologous RIP-3 (Degterev et al., 2008; Feng et al., 2007). The best described trigger of necroptosis is TNFR engagement. Trimerization of TNFR triggers the formation of DISC in which RIP-1/RIP-3 phosphorylation is inhibited by caspase-8; in this setting the molecular cascade proceeds toward apoptosis. In contrast, in the absence or inhibition of FADD or caspase-8, there is reciprocal phosphorylation of RIP-1 and RIP-3, which stimulates oxidative metabolism via activation of NADPH oxidase (NOX1), with production of ROS and consumption of ATP (He et al., 2009). The excess of ROS and decreased availability of ATP lead to energy collapse and necroptosis (Declercq et al., 2009).

### Necroptosis in Autoimmunity

Necroptosis plays a role in sterile inflammation such as ischemia/reperfusion injury and neurological disorders (Rosenbaum et al., 2010). Its inhibition benefits models of stroke, myocardial infarction, and Parkinson's disease (Vandenabeele et al., 2010). Nevertheless, necroptosis causes the release of damage-associated molecular patterns (DAMPs), danger signals derived from dead or damaged cells that activate the innate immune system. Necrotic cells stimulate proinflammatory cytokines in SLE and in inflammatory bowel disease

(Caricchio et al., 2003; Welz et al., 2011). In mice in which caspase-8 was conditionally eliminated, TNF- $\alpha$  induced Crohn's-like lesions with intestinal mucosal destruction and increased expression of RIP-3 kinase (Welz et al., 2011). The lesions were reversed by pretreatment with necrostatin-1, a RIP-1 inhibitor (Gunther et al., 2011). Similar lesions in patients with Crohn's disease, especially in Paneth cells, demonstrate necroptosis features, without caspase-3 activation and with increased RIP-3 expression (Gunther et al., 2011). This death pathway is likely to be relevant in both activating the immune system and inducing tissue damage, for example, in small-vessel vasculitis and glomerulonephritis.

*Parthanatos* {Par [poly(ADP-ribosylation] and *thanatos* (death)}. The poly(ADP-ribosylation) of nuclear proteins is an early response to DNA damage in eukaryotic cells (Schreiber et al., 2006). Upon DNA damage, often induced during inflammation by ROS, PARP-1 is selectively activated and uses NAD<sup>+</sup> to catalyze nuclear protein poly(ribosylation) (Luo and Kraus, 2012). If the repair is completed, cells survive, while if DNA damage is extensive, PARP-1 is overactivated, resulting in excessive consumption of NAD<sup>+</sup>. As the cell attempts to resynthesize this substrate, it depletes available ATP, resulting in a sudden reduction of cellular energy and necrosis (Bouchard et al., 2003). PARP-1 also induces a forward-loop by poly(ribosylation) of AIF which translocates to the nucleus and induces DNA damage and further PARP-1 activation (Moubarak et al., 2007). Interestingly, PARP-1 is also part of necroptosis and contributes to the energy collapse (Jouan-Lanhouet et al., 2012).

## Parthanatos in Autoimmunity

Parthanatos is characterized by the release of DAMPs, including HMGB1 (Ditsworth et al., 2007). PARP-1 activation and consequent necrosis participate in the pathogenesis of acute and chronic inflammation by facilitating NF $\kappa$ B activation and production of TNF- $\alpha$ , IL-6, and iNOS (Krishnakumar and Kraus, 2010). If PARP-1 is absent or inhibited, there is reduced NF $\kappa$ B activation and disease severity during septic shock, ischemia/reperfusion injury, or collagen-induced arthritis (Eliasson et al., 1997; Gonzalez-Rey et al., 2007; Soriano et al., 2002). Because necrotic lesions are often a key feature of immune-mediated nephropathies, parthanatos may be important in inducing tissue damage. The absence of PARP-1 protects from spontaneous and inducible mouse models of lupus nephritis by decreasing necrotic cell death and in situ production of TNF- $\alpha$ . As the protection only applies to males, females may use a different necrotic pathway, which does not require RIPK3 (Jog et al., 2009; Corradetti et al., 2016).

## CLEARANCE OF DEAD CELLS

One of the most important tasks of the immune system is the removal of the large number of cells that die every day (Ravichandran, 2011). Efferocytosis (DeCathelineau and Henson, 2003), from the latin root *effero* for "take to the grave," is the process of phagocytosis and digestion of dying cells that most cell types can perform, including epithelial cells and fibroblasts, although in mammals efferocytosis is also a primary function of "professional" phagocytes, such as macrophages. Efferocytosis removes apoptotic cells before they release harmful intracellular components and stimulates antiinflammatory factors to preserve a healthy noninflammatory environment (Erwig and Henson, 2007). Apoptotic cells are normally cleared with extreme efficiency and rapidity, so their detection *in vivo* is usually possible only when there are defects in their clearance (Scott et al., 2001). Failure to remove dead cells promptly has been shown to predispose to inflammation and autoimmunity in a variety of models and in human lupus, where uningested apoptotic cells are evident in lymphoid tissue (Baumann et al., 2002; Cohen and Caricchio, 2004). Deficient phagocytosis may lead to autoimmunity by allowing the apoptotic cells to undergo secondary necrosis and to release autoantigens and DAMPs-activating innate immune cells (Ren et al., 2003). In order to accomplish efficient efferocytosis, phagocytes need to be in close proximity to apoptotic cells and to be able to distinguish apoptotic cells from live or necrotic cells. These two functions are promoted by the find-me signals and the eat-me signals, respectively.

### Find-Me Signals

Macrophages and dendritic cells are recruited by "find-me" signals released by apoptotic cells that promote the directional migration of phagocytes by establishing a chemical gradient (Ravichandran, 2011; Nagata et al., 2010). Lysophosphatidylcholine (LPC) is released by dying cells upon activation of phospholipase A2 in a caspase-3-dependent way. LPC binds the G protein-coupled receptor G2A on phagocytes; mice lacking G2A

develop late-onset autoimmunity (Lauber et al., 2003). Another find-me signal is sphingosine 1-phosphate (S1P), an important sphingolipid involved in several biological processes, from lymphocyte trafficking to immunity and cancer (Orr Gandy and Obeid, 2012). S1P is released by apoptotic cells, and its inhibitor FTY720 is attracting interest in various autoimmune diseases such as multiple sclerosis and thyroiditis.

Another chemotactic factor is the soluble fragment of the chemokine fractalkine (CX3CL1), which is cleaved in a caspase-dependent way from the plasma membranes of apoptotic cells. Mice lacking fractalkine receptor CX3CR1 have defective migration of macrophages in germinal centers, where many B cells undergo apoptosis (Truman et al., 2008). The latest molecules proposed as find-me signals are ATP and UTP. These nucleotides are released by early apoptotic cells and recruit monocytes upon triggering P2Y2 receptors (Elliott et al., 2009); mice enzymatically depleted of nucleotides or lacking P2Y2 receptors show deficient phagocytosis and uncleared apoptotic cells. Early apoptotic cells employ the pannexin family of plasma membrane channels to secrete about 2% of the cellular content of ATP and UTP as a find-me signal, much less than the fraction of these nucleotides released by necrotic cells as DAMP upon the loss of plasma membrane integrity. Thus nucleotides either attract noninflammatory phagocytes or recruit neutrophils and activate dendritic cells, depending on the amount released. Apoptotic cells may also release “keep-out” signals, such as lactoferrin, to avoid the recruitment of proinflammatory cells (Bournazou et al., 2009).

## Eat-Me Signals and Their Receptors

*Phosphatidylserine.* Phagocytes engulf apoptotic cells because they can recognize specific “eat-me” signals expressed by the apoptotic cells. The anionic phospholipid phosphatidylserine is the best known eat-me signal (Fadok et al., 1992). Healthy cells maintain phosphatidylserine hidden in the inner leaflet of the plasma membrane, while apoptotic cells flip the phospholipid content of their membrane through the inhibition of translocases (flippases) and the activation of scramblases, and thereby expose phosphatidylserine in the outer leaflet (Nagata et al., 2010). Professional phagocytes and neighboring cells can recognize phosphatidylserine through a number of receptors and engage in phagocytosis. The exposure of phosphatidylserine in the outer membrane is a universal marker of early apoptosis, occurring within 1–2 hours from the apoptotic insult and is detected experimentally by staining with the soluble molecule annexin V (Ravichandran, 2011). The insertion of phosphatidylserine into the plasma membrane of erythrocytes leads to their phagocytosis by macrophages (Tanaka and Schroit, 1983), and the masking of phosphatidylserine with specific antibodies impairs the clearance of apoptotic cells in vitro and in vivo (Asano et al., 2004). It is still debated whether phosphatidylserine is per se sufficient to induce phagocytosis: indeed, contrary to what was observed in erythrocytes, living cells artificially exposing phosphatidylserine are not efficiently phagocytosed, suggesting that either other still unknown eat-me signals are necessary or that “don’t eat-me” signals, such as CD31 and CD47, protect living cells from removal (Ravichandran, 2011; Devitt and Marshall, 2011). Moreover, phosphatidylserine can be chemically modified during apoptosis, and indeed, some phosphatidylserine receptors preferentially bind oxidized molecules.

The binding of phagocytes to apoptotic cells requires cooperation with adhesion molecules for firmer binding, a process defined as “tethering and tickling” (Henson et al., 2001); this has been compared to the immunological synapse and therefore termed the “phagocytic synapse” (Devitt and Marshall, 2011). The molecules able to recognize phosphatidylserine can be divided into two categories: receptors on the plasma membrane of the phagocytes that directly recognize phosphatidylserine and those that instead recognize a bridge, that is, a soluble molecule that opsonizes apoptotic cells. The first category includes the members of the T-cell immunoglobulin and mucin (TIM) family. TIM4 is expressed by macrophages and dendritic cells, while Tim3 is expressed in the spleen by CD8a+ dendritic cells that are antigen presenting cells specialized in cross-priming. TIM receptors may allow dendritic cells to engulf dead cells and present their antigens for immunity. TIM4 deficient mice show the persistence of apoptotic bodies, hyperactivated T and B cells, and systemic autoimmunity (Rodriguez-Manzanet et al., 2010). Other possible direct phosphatidylserine receptors are the brain angiogenesis inhibitor 1, expressed in neurons, and Stabilin-2, expressed by endothelial cells in lymphoid organs (Nagata et al., 2010).

The second category of receptors for phosphatidylserine includes receptors that recognize soluble molecules bridging phagocytes and apoptotic cells. Milk fat globule EGF factor 8 (MFG-E8) is expressed by most phagocytes including tingible-body macrophages, by follicular dendritic cells in germinal centers, and by epidermal Langerhans cells. MFG-E8 binds phosphatidylserine on the apoptotic cells using its two factor-VIII-homologous

C-terminal C1 and C2 domains and to the integrins  $\alpha_v\beta_3$  or  $\alpha_v\beta_5$  on the phagocytes using an N-terminal EGF domain carrying an RGD motif (Arg–Gly–Asp) (Nagata et al., 2010). During an immune response, the mice deficient for MFG-E8 show accumulation of uncleared apoptotic cells in germinal centers, indicating a nonredundant role for MFG-E8 in the removal of apoptotic cells by tingible-body macrophages in vivo. MFG-E8-deficient mice also develop spontaneous lupus-like autoimmune disease, with anti-dsDNA and glomerulonephritis (Hanayama et al., 2004).

Gas-6 and protein S are two other examples of molecular bridges that opsonize apoptotic cells by recognizing phosphatidylserine and promote their phagocytosis. Gas-6 and protein S bind to members of the TAM family of tyrosine kinases: Tyro3, Axl, and Mer on phagocytes (Rothlin and Lemke, 2010). These kinases are involved in phagocytosis and in the modulation of the immune function of the phagocytes. Mer is expressed by macrophages, dendritic cells, natural killer (NK) cells, and NK T cells (Behrens et al., 2003). *Mer*-deficient mice show an accumulation of apoptotic cells upon a strong apoptotic stimulus and spontaneously develop SLE-like autoimmunity, characterized by anti-DNA, antichromatin, antiphospholipid antibodies, and a mild glomerulonephritis (Cohen et al., 2002). This autoimmune phenotype is more severe in mice lacking all three members of the TAMs, with more intense macrophage activation, swollen joints, and glomerular immune complex deposition (Lu and Lemke, 2001; Cohen and Caricchio, 2004). As seen in MFG-E8-deficient mice and other knockout mice (Cohen and Caricchio, 2004), *Mer*-deficient mice develop severe autoimmunity in the genetic background of the strain 129, while the phenotype is less striking on the C57BL/6 background, presumably reflecting the allelic polymorphisms carried by the strain 129 that promotes autoimmunity (Heidari et al., 2006).

*Other eat-me signals.* Many receptors, such as CD14, CD36, CD68, the LDL-receptor-related protein, the oxidized low-density lipoprotein recognizing receptors, and the scavenger receptors, bind dead cells independently from phosphatidylserine (Ravichandran, 2011). Natural IgM antibodies can bind phosphatidylcholine exposed by apoptotic cells and enhance the uptake of apoptotic cells by phagocytes by recruiting C1q and mannose-binding lectin. Studies suggest that these natural IgMs protect against autoimmunity and atherosclerosis by promoting the clearance of apoptotic cells and regulating immune activation (Gronwall et al., 2012). Some receptors for apoptotic cells are organ specific, such as surfactant A and D, which may be important in the lung: surfactant D-deficient mice spontaneously develop emphysema, possibly because the accumulation of intrapulmonary apoptotic cells promotes inflammation and destruction of the pulmonary parenchyma (Vandivier et al., 2002).

## Receptors for Necrotic Cells

The C-type lectins are a large family of proteins, and some of them are expressed by innate immune cells and recognize damaged and necrotic cells and pathogens (Sancho and Reis e Sousa, 2012). DNGR-1 is a C-type lectin that is expressed by CD8a<sup>+</sup> dendritic cells and binds a self-protein, the F-actin component of the cellular cytoskeleton that, such as phosphatidylserine, is normally hidden in the cytoplasm of healthy cells and is exposed extracellularly upon necrotic death. Triggering of DNGR-1 marks the phagocytosed cargo as necrotic and shuttles it for cross-presentation (Sancho and Reis e Sousa, 2012).

LOX-1 is another C-type lectin that recognizes molecules from injured or dead cells such as modified LDL and heat shock proteins and also facilitates other dead cell recognition receptors such as C-reactive protein (CRP) and C1q. LOX-1-deficient mice show delayed atherosclerosis and reduced ischemia-induced organ damage, possibly because of decreased inflammation (Sawamura et al., 2012).

Complement is an important player in the recognition of necrotic cells. C1q binds to cells in the late stage of apoptosis and possibly to primary necrotic cells and C1q-deficient mice develop a lupus-like disease, with anti-DNA and immune complex-dependent renal disease on the 129 genetic background (Mitchell et al., 2002). Furthermore, C1q deficiency in humans leads to lupus-like disease in nearly all homozygous individuals. Autoantibodies against C1q may also cause a functional deficiency of C1q and may block its binding to dead cells and thus exacerbate SLE disease (Cohen and Caricchio, 2004).

Another class of receptors involved in the recognition of dead cells, either apoptotic or necrotic, is the pentraxins (Bottazzi et al., 2010). The short pentraxins, CRP and serum amyloid P (SAP), are acute phase proteins induced by IL-6 in hepatocytes during inflammation, while the long pentraxin PTX3 is expressed by macrophages, myeloid dendritic cells, neutrophils, and some nonimmune cells in response to inflammatory cytokines and TLR ligands. CRP, SAP, and PTX3 bind pathogens and dead cells and promote their complement-mediated killing and phagocytosis. Mice deficient in pentraxins show defective clearance of dead cells and develop lupus (Muñoz et al., 2010).

## ANTIINFLAMMATORY EFFECTS OF APOPTOTIC CELLS

Apoptotic cells are normally considered antiinflammatory not only because they preserve their membrane integrity and so do not release DAMPs but also because they actively provide antiinflammatory signals to the phagocytes (Erwig and Henson, 2007). Apoptotic cells inhibit phagocyte production of proinflammatory cytokines such as TNF- $\alpha$  and IL-12 and increase the production of the antiinflammatory cytokines IL-10 (Voll et al., 1997), TGF-beta, prostaglandin E2, and platelet-activating factor (Fadok et al., 1998). Phagocytosis of the apoptotic cells polarizes macrophages toward the “alternatively activated” M2 differentiation state, which is reparative and nonphlogistic, through the production of the antiinflammatory cytokines TGF-beta and IL-10 (Sica and Mantovani, 2012). Many receptors may mediate this antiinflammatory effect of apoptotic cells.

Indeed, ligation of Mer by apoptotic cells can suppress the response of the innate immune cells to TLR stimulation and proinflammatory cytokines, through the autocrine production of IFNs-I and the induction of suppressors of cytokine signaling molecules, dampening the innate and adaptive immune response (Rothlin et al., 2007). Therefore the autoimmunity in *Mer*-deficient mice could be ascribed to an excess of autoantigens because of their impaired clearance, with these being presented immunogenically because of the DAMPs released by secondary necrotic cells and also because of the absence of the antiinflammatory function of Mer on innate immune cells (Rothlin and Lemke, 2010).

CR3, also known as CD11b or Mac-1, is expressed by macrophages and myeloid dendritic cells and, upon binding apoptotic cells opsonized by the complement component iC3b, it signals antiinflammatory effects in phagocytes (Behrens et al., 2007). CR3-deficient mice have neither defects in apoptotic cell clearance nor autoimmunity possibly due to the redundancy of antiinflammatory signals from apoptotic cells.

Some forms of apoptotic cells are instead proinflammatory, such as those killed by ultraviolet irradiation: such cells express IL-1 $\alpha$  (Caricchio et al., 2003) and activate dendritic cells (Rovere et al., 1998).

## IMMUNOSTIMULATORY EFFECTS OF NECROTIC CELLS

Necrotic cells activate dendritic cells and stimulate immune responses in vitro and in vivo (Gallucci et al., 1999; Matzinger, 2002) through release of endogenous “danger signals” or DAMPs (Miyake and Yamasaki, 2012). Among these signals, nucleic acids are crucial in lupus pathogenesis because they are important self-antigens, and, either in immune complexes with autoantibodies (Leadbetter et al., 2002) or carried by other chaperones (heat shock proteins, HMGB1 etc.), they activate innate immunity through TLR7 and TLR9 stimulation (Marshak-Rothstein and Rifkin, 2007).

The mechanisms whereby nucleic acids enter cells and trigger immune activation have attracted much attention (Gallucci and Maffei, 2017). It is clear that anti-DNA antibodies can form immune complexes with nucleic acids and subsequently bind to the activating Fc gamma receptor (Fc $\gamma$ RIIA, CD32), leading to their uptake and binding to TLR, provoking production of IFNs-I and cytokines (Bave et al., 2000).

Intracellular RNA, usually of viral origin, binds to cytosolic RNA receptors retinoic acid-inducible gene I and MDA5. After undergoing activation, these proteins bind to mitochondrial-associated adaptor protein (MAVS), which presents in high molecular aggregates in peripheral blood mononuclear cells of SLE patients, a form that provokes the stimulation of IFNs and other inflammatory cytokines and leads to inflammation and autoimmunity (Shao et al., 2016). Intracellular DNA is also recognized by sensor proteins, causing production of IFNs and other cytokines. The best studied is cGAS, which undergoes a conformational change after binding to double-stranded DNA with subsequent production of cyclic 2'-5' cGAMP. This molecule binds to stimulator of interferon genes (STING), with subsequent production of IFN-beta (Cai et al., 2014). Children with activating STING mutations develop a severe inflammatory and autoimmune syndrome (Jeremiah et al., 2014).

An important source of potentially inflammatory DNA is mitochondrial DNA (mtDNA), which can be released in the extracellular compartment by necrotic cells and during NETosis, and triggers immune activation by binding TLR9, or release in the cytoplasm to activate cGAS-STING, promoting autoimmunity (Lood et al., 2016). MtDNA may also be resistant to the action of TREX-1, an endonuclease that degrades single and double-stranded DNA (Gallucci and Maffei, 2017). Mutations of TREX-1 have been shown to result in a severe childhood autoimmune disease (Aicardi-Goutières disease), with antinuclear antibodies, chilblain-like skin lesions, and a variety of profound neurological defects (Crow and Manel, 2015). In this regard, the proinflammatory effects of

free nucleic acids released from dead and dying cells are counteracted by several nucleases (DNase I, RNase1L3, RNase1) that degrade RNA and DNA (Sisirak et al., 2016).

Necrotic cells also release the DAMPs heat shock proteins, uric acid, and degradation products of the extracellular matrix (ECM). These ECM products, such as hyaluronic acid, fibrinogen, and tenascin-C, are upregulated after tissue injury. Their levels are increased in rheumatoid arthritis (RA) tissues, and their administration induces joint inflammation in mice. Mice deficient in tenascin-C show rapid resolution of inflammation (Goh and Midwood, 2011), suggesting a pathogenetic role for ECM molecules in RA.

Finally, HMGB1 is a DNA-binding protein that stabilizes the nucleosome and regulates transcription (Andersson and Tracey, 2011). It is released by necrotic cells and activates innate immune cells by triggering RAGE, TLR2, and TLR4. Necrotic cells lacking HMGB1 are less stimulatory, and neutralizing antibodies ameliorate inflammation (Bianchi and Manfredi, 2007). HMGB1 may be involved in the pathogenesis of RA, SLE, experimental autoimmune encephalomyelitis, and autoimmune diabetes in nonobese diabetic (NOD) mice (Andersson and Tracey, 2011). Inhibition of HMGB1 ameliorates arthritis in animals, while its administration into the joints induces arthritis (Kokkola et al., 2003). In RA patients HMGB1 levels are increased in serum and synovial fluid and decreased with therapies that ameliorate joint inflammation (Zetterstrom et al., 2008). SLE patients have anti-HMGB1 autoantibodies, and their levels of serum HMGB1 correlate with disease activity; in animals the injection of HMGB1-nucleosome complexes induces lupus-like autoantibodies.

## A GLIMPSE INTO THE FUTURE

The last 20 years have been pivotal in the investigation of the role of apoptosis and the clearance of apoptotic cells in the pathogenesis of autoimmune diseases, while only very recently has necrosis been considered a form of programmed cell death with relevance to autoimmunity. Next-generation deep sequencing studies will indicate the genetic links between novel forms of cell death and disease and identify the subgroups of patients in the affected human population. An improved understanding of the fine mechanisms of cell death mediated by novel molecular cascades or programs is likely to lead to new therapeutic targets.

## References

- Andersson, U., Tracey, K.J., 2011. HMGB1 is a therapeutic target for sterile inflammation and infection. *Annu. Rev. Immunol.* 29, 139–162.
- Andre, N., 2003. Hippocrates of Cos and apoptosis. *Lancet* 361, 1306.
- Aon, M.A., Cortassa, S., Akar, F.G., O'Rourke, B., 2006. Mitochondrial criticality: a new concept at the turning point of life or death. *Biochim. Biophys. Acta* 1762, 232–240.
- Arbuckle, M.R., McClain, M.T., Rubertone, M.V., Scofield, R.H., Dennis, G.J., James, J.A., et al., 2003. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N. Engl. J. Med.* 349, 1526–1533.
- Asano, K., Miwa, M., Miwa, K., Hanayama, R., Nagase, H., Nagata, S., et al., 2004. Masking of phosphatidylserine inhibits apoptotic cell engulfment and induces autoantibody production in mice. *J. Exp. Med.* 200, 459–467.
- Barnhart, B.C., Alappat, E.C., Peter, M.E., 2003. The CD95 type I/type II model. *Semin. Immunol.* 15, 185–193.
- Baumann, I., Kolowos, W., Voll, R.E., Manger, B., Gaipl, U., Neuhuber, W.L., et al., 2002. Impaired uptake of apoptotic cells into tingible body macrophages in germinal centers of patients with systemic lupus erythematosus. *Arthritis Rheum.* 46, 191–201.
- Bave, U., Alm, G.V., Ronnblom, L., 2000. The combination of apoptotic U937 cells and lupus IgG is a potent IFN-alpha inducer. *J. Immunol.* 165, 3519–3526.
- Behrens, E.M., Gadue, P., Gong, S.Y., Garrett, S., Stein, P.L., Cohen, P.L., 2003. The mer receptor tyrosine kinase: expression and function suggest a role in innate immunity. *Eur. J. Immunol.* 33, 2160–2167.
- Behrens, E.M., Sriram, U., Shivers, D.K., Gallucci, M., Ma, Z., Finkel, T.H., et al., 2007. Complement receptor 3 ligation of dendritic cells suppresses their stimulatory capacity. *J. Immunol.* 178, 6268–6279.
- Beyer, C., Pisetsky, D.S., 2010. The role of microparticles in the pathogenesis of rheumatic diseases. *Nat. Rev. Rheumatol.* 6, 21–29.
- Bianchi, M.E., Manfredi, A.A., 2007. High-mobility group box 1 (HMGB1) protein at the crossroads between innate and adaptive immunity. *Immunol. Rev.* 220, 35–46.
- Bondanza, A., Zimmermann, V.S., Dell'Antonio, G., Dal Cin, E., Capobianco, A., Sabbadini, M.G., et al., 2003. Cutting edge: dissociation between autoimmune response and clinical disease after vaccination with dendritic cells. *J. Immunol.* 170, 24–27.
- Bottazzi, B., Doni, A., Garlanda, C., Mantovani, A., 2010. An integrated view of humoral innate immunity: pentraxins as a paradigm. *Annu. Rev. Immunol.* 28, 157–183.
- Bouchard, V.J., Rouleau, M., Poirier, G.G., 2003. PARP-1, a determinant of cell survival in response to DNA damage. *Exp. Hematol.* 31, 446–454.
- Bournazou, I., Pound, J.D., Duffin, R., Bournazos, S., Melville, L.A., Brown, S.B., et al., 2009. Apoptotic human cells inhibit migration of granulocytes via release of lactoferrin. *J. Clin. Invest.* 119, 20–32.

- Bratton, S.B., Walker, G., Srinivasula, S.M., Sun, X.M., Butterworth, M., Alnemri, E.S., et al., 2001. Recruitment, activation and retention of caspases-9 and -3 by Apaf-1 apoptosome and associated XIAP complexes. *EMBO J.* 20, 998–1009.
- Buyon, J.P., Clancy, R.M., Friedman, D.M., 2009. Cardiac manifestations of neonatal lupus erythematosus: guidelines to management, integrating clues from the bench and bedside. *Nat. Clin. Pract. Rheumatol.* 5, 139–148.
- Cai, X., Chiu, Y.H., Chen, Z.J., 2014. The cGAS-cGAMP-STING pathway of cytosolic DNA sensing and signaling. *Mol. Cell* 54, 289–296.
- Caricchio, R., Mcphie, L., Cohen, P.L., 2003. Ultraviolet B radiation-induced cell death: critical role of ultraviolet dose in inflammation and lupus autoantigen redistribution. *J. Immunol.* 171, 5778–5786.
- Casciola-Rosen, L.A., Anhalt, G., Rosen, A., 1994. Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *J. Exp. Med.* 179, 1317–1330.
- Chen, M., Wang, Y.H., Wang, Y., Huang, L., Sandoval, H., Liu, Y.J., et al., 2006. Dendritic cell apoptosis in the maintenance of immune tolerance. *Science* 311, 1160–1164.
- Chinnaiyan, A.M., O'rourke, K., Tewari, M., Dixit, V.M., 1995. FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. *Cell* 81, 505–512.
- Cocca, B.A., Cline, A.M., Radic, M.Z., 2002. Blebs and apoptotic bodies are B cell autoantigens. *J. Immunol.* 169, 159–166.
- Cohen, P.L., Caricchio, R., 2004. Genetic models for the clearance of apoptotic cells. *Rheum. Dis. Clin. North Am.* 30, 473–486. viii.
- Cohen, P.L., Eisenberg, R.A., 1991. Lpr and gld: single gene models of systemic autoimmunity and lymphoproliferative disease. *Annu. Rev. Immunol.* 9, 243–269.
- Cohen, P.L., Caricchio, R., Abraham, V., Camenisch, T.D., Jennette, J.C., Roubey, R.A., et al., 2002. Delayed apoptotic cell clearance and lupus-like autoimmunity in mice lacking the c-mer membrane tyrosine kinase. *J. Exp. Med.* 196, 135–140.
- Corradetti, C., Jog, N.R., Gallucci, S., Madaio, M., Balachandran, S., Caricchio, R., 2016. Immune-mediated nephropathy and systemic autoimmunity in mice does not require receptor interacting protein kinase 3 (RIPK3). *PLoS One* 11, e0163611.
- Crow, Y.J., Manel, N., 2015. Aicardi-Goutières syndrome and the type I interferonopathies. *Nat. Rev. Immunol.* 15, 429–440.
- Darzynkiewicz, Z., Bruno, S., Bino, D.E.L., Gorczyca, G., Hotz, W., Lassota, M.A., et al., 1992. Features of apoptotic cells measured by flow cytometry. *Cytometry* 13, 795–808.
- DeCathelineau, A.M., Henson, P.M., 2003. The final step in programmed cell death: phagocytes carry apoptotic cells to the grave. *Essays Biochem.* 39, 105–117.
- Declercq, W., Vanden Berghe, T., Vandenabeele, P., 2009. RIP kinases at the crossroads of cell death and survival. *Cell* 138, 229–232.
- Degterev, A., Hitomi, J., Germscheid, M., Ch'en, I.L., Korkina, O., Teng, X., et al., 2008. Identification of RIP1 kinase as a specific cellular target of necrostatins. *Nat. Chem. Biol.* 4, 313–321.
- Devitt, A., Marshall, L.J., 2011. The innate immune system and the clearance of apoptotic cells. *J. Leukoc. Biol.* 90, 447–457.
- Diamantis, A., Magiorkinis, E., Sakorafas, G.H., Androutsos, G., 2008. A brief history of apoptosis: from ancient to modern times. *Onkologie* 31, 702–706.
- Ditsworth, D., Zong, W.X., Thompson, C.B., 2007. Activation of poly(ADP)-ribose polymerase (PARP-1) induces release of the pro-inflammatory mediator HMGB1 from the nucleus. *J. Biol. Chem.* 282, 17845–17854.
- Drappa, J., Vaishnav, A.K., Sullivan, K.E., Chu, J.L., Elkorn, K.B., 1996. Fas gene mutations in the Canale-Smith syndrome, an inherited lymphoproliferative disorder associated with autoimmunity. *N. Engl. J. Med.* 335, 1643–1649.
- Eliasson, M.J., Sampei, K., Mandir, A.S., Hurn, P.D., Traystman, R.J., Bao, J., et al., 1997. Poly(ADP-ribose) polymerase gene disruption renders mice resistant to cerebral ischemia. *Nat. Med.* 3, 1089–1095.
- Elliott, M.R., Chekeni, F.B., Trampont, P.C., Lazarowski, E.R., Kadl, A., Walk, S.F., et al., 2009. Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. *Nature* 461, 282–286.
- Enari, M., Sakahira, H., Yokoyama, H., Okawa, K., Iwamatsu, A., Nagata, S., 1998. A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. *Nature* 391, 43–50.
- Erwig, L.P., Henson, P.M., 2007. Immunological consequences of apoptotic cell phagocytosis. *Am. J. Pathol.* 171, 2–8.
- Fadok, V.A., Voelker, D.R., Campbell, P.A., Cohen, J.J., Bratton, D.L., Henson, P.M., 1992. Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. *J. Immunol.* 148, 2207–2216.
- Fadok, V.A., Bratton, D.L., Konowal, A., Freed, P.W., Westcott, J.Y., Henson, P.M., 1998. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. *J. Clin. Invest.* 101, 890–898.
- Feng, S., Yang, Y., Mei, Y., Ma, L., Zhu, D.E., Hoti, N., et al., 2007. Cleavage of RIP3 inactivates its caspase-independent apoptosis pathway by removal of kinase domain. *Cell Signal.* 19, 2056–2067.
- Frisoni, L., Mcphie, L., Colonna, L., Sriram, U., Monestier, M., Gallucci, S., et al., 2005. Nuclear autoantigen translocation and autoantibody opsonization lead to increased dendritic cell phagocytosis and presentation of nuclear antigens: a novel pathogenic pathway for autoimmunity? *J. Immunol.* 175, 2692–2701.
- Frisoni, L., Mcphie, L., Kang, S.A., Monestier, M., Madaio, M., Satoh, M., et al., 2007. Lack of chromatin and nuclear fragmentation in vivo impairs the production of lupus anti-nuclear antibodies. *J. Immunol.* 179, 7959–7966.
- Gallucci, S., Maffei, M.E., 2017. DNA sensing across the tree of life. *Trends Immunol.* 38, 719–732.
- Gallucci, S., Lolkema, M., Matzinger, P., 1999. Natural adjuvants: endogenous activators of dendritic cells. *Nat. Med.* 5, 1249–1255.
- Galluzzi, L., Vitale, I., Abrams, J.M., Alnemri, E.S., Baehrecke, E.H., Blagosklonny, M.V., et al., 2012. Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. *Cell Death Differ.* 19, 107–120.
- Garcia-Romo, G.S., Caielli, S., Vega, B., Connolly, J., Allantaz, F., Xu, Z., et al., 2011. Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. *Sci. Transl. Med.* 3, 73ra20.
- Goh, F.G., Midwood, K.S., 2011. Intrinsic danger: activation of Toll-like receptors in rheumatoid arthritis. *Rheumatology (Oxford)* 51, 7–23.
- Gonzalez-Rey, E., Martinez-Romero, R., O'valle, F., Aguilar-Quesada, R., Conde, C., Delgado, M., et al., 2007. Therapeutic effect of a poly(ADP-ribose) polymerase-1 inhibitor on experimental arthritis by downregulating inflammation and Th1 response. *PLoS One* 2, e1071.

- Green, N.M., Marshak-Rothstein, A., 2011. Toll-like receptor driven B cell activation in the induction of systemic autoimmunity. *Semin. Immunol.* 23, 106–112.
- Green, D.R., Ferguson, T., Zitvogel, L., Kroemer, G., 2009. Immunogenic and tolerogenic cell death. *Nat. Rev. Immunol.* 9, 353–363.
- Gronwall, C., Vas, J., Silverman, G.J., 2012. Protective roles of natural IgM antibodies. *Front. Immunol.* 3, 66.
- Gunther, C., Martini, E., Wittkopf, N., Amann, K., Weigmann, B., Neumann, H., et al., 2011. Caspase-8 regulates TNF-alpha-induced epithelial necroptosis and terminal ileitis. *Nature* 477, 335–339.
- Hanayama, R., Tanaka, M., Miyasaka, K., Aozasa, K., Koike, M., Uchiyama, Y., et al., 2004. Autoimmune disease and impaired uptake of apoptotic cells in MFG-E8-deficient mice. *Science* 304, 1147–1150.
- He, S., Wang, L., Miao, L., Wang, T., Du, F., Zhao, L., et al., 2009. Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-alpha. *Cell* 137, 1100–1111.
- Heidari, Y., Bygrave, A.E., Rigby, R.J., Rose, K.L., Walport, M.J., Cook, H.T., et al., 2006. Identification of chromosome intervals from 129 and C57BL/6 mouse strains linked to the development of systemic lupus erythematosus. *Genes Immun.* 7, 592–599.
- Henson, P.M., Bratton, D.L., Fadok, V.A., 2001. The phosphatidylserine receptor: a crucial molecular switch? *Nat. Rev. Mol. Cell. Biol.* 2, 627–633.
- Hutcheson, J., Scatizzi, J.C., Siddiqui, A.M., Haines 3RD, G.K., Wu, T., Li, Q.Z., et al., 2008. Combined deficiency of proapoptotic regulators Bim and Fas results in the early onset of systemic autoimmunity. *Immunity* 28, 206–217.
- Jeremiah, N., Neven, B., Gentili, M., Callebaut, I., Maschalidi, S., Stolzenberg, M.C., et al., 2014. Inherited STING-activating mutation underlies a familial inflammatory syndrome with lupus-like manifestations. *J. Clin. Invest.* 124, 5516–5520.
- Jog, N.R., Dinnall, J.A., Gallucci, S., Madaio, M.P., Caricchio, R., 2009. Poly(ADP-ribose) polymerase-1 regulates the progression of autoimmune nephritis in males by inducing necrotic cell death and modulating inflammation. *J. Immunol.* 182, 7297–7306.
- Jog, N.R., Frisoni, L., Shi, Q., Monestier, M., Hernandez, S., Craft, J., et al., 2012. Caspase-activated DNase is required for maintenance of tolerance to lupus nuclear autoantigens. *Arthritis Rheum.* 64, 1247–1256.
- Jouan-Lanhoutet, S., Arshad, M.I., Piquet-Pellorce, C., Martin-Chouly, C., Le Moigne-Muller, G., Van Herreweghe, F., et al., 2012. TRAIL induces necroptosis involving RIPK1/RIPK3-dependent PARP-1 activation. *Cell Death Differ.* 19, 2003–2014.
- Kerr, J.F., Wyllie, A.H., Currie, A.R., 1972. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer* 26, 239–257.
- Kessenbrock, K., Krumbholz, M., Schonermarck, U., Back, W., Gross, W.L., Werb, Z., et al., 2009. Netting neutrophils in autoimmune small-vessel vasculitis. *Nat. Med.* 15, 623–625.
- Knight, J.S., Kaplan, M.J., 2012. Lupus neutrophils: 'NET' gain in understanding lupus pathogenesis. *Curr. Opin. Rheumatol.* 24, 441–450.
- Kokkola, R., Li, J., Sundberg, E., Aveberger, A.C., Palmblad, K., Yang, H., et al., 2003. Successful treatment of collagen-induced arthritis in mice and rats by targeting extracellular high mobility group box chromosomal protein 1 activity. *Arthritis Rheum.* 48, 2052–2058.
- Kono, D.H., Theofilopoulos, A.N., 2000. Genetics of systemic autoimmunity in mouse models of lupus. *Int. Rev. Immunol.* 19, 367–387.
- Krishnakumar, R., Kraus, W.L., 2010. The PARP side of the nucleus: molecular actions, physiological outcomes, and clinical targets. *Mol. Cell* 39, 8–24.
- Kroemer, G., Galluzzi, L., Brenner, C., 2007. Mitochondrial membrane permeabilization in cell death. *Physiol. Rev.* 87, 99–163.
- Kroemer, G., Galluzzi, L., Vandebaele, P., Abrams, J., Alnemri, E.S., Baehrecke, E.H., et al., 2009. Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009. *Cell Death Differ.* 16, 3–11.
- Lartigue, L., Kushnareva, Y., Seong, Y., Lin, H., Faustin, B., Newmeyer, D.D., 2009. Caspase-independent mitochondrial cell death results from loss of respiration, not cytotoxic protein release. *Mol. Biol. Cell* 20, 4871–4884.
- Lauber, K., Bohn, E., Krober, S.M., Xiao, Y.J., Blumenthal, S.G., Lindemann, R.K., et al., 2003. Apoptotic cells induce migration of phagocytes via caspase-3-mediated release of a lipid attraction signal. *Cell* 113, 717–730.
- Lavrik, I.N., Krammer, P.H., 2011. Regulation of CD95/Fas signaling at the DISC. *Cell Death Differ.* 19, 36–41.
- Leadbetter, E.A., Rifkin, I.R., Hohlbaum, A.M., Beaudette, B.C., Shlomchik, M.J., Marshak-Rothstein, A., 2002. Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. *Nature* 416, 603–607.
- Leibowitz, B., Yu, J., 2010. Mitochondrial signaling in cell death via the Bcl-2 family. *Cancer Biol. Ther.* 9, 417–422.
- Lleo, A., Selmi, C., Invernizzi, P., Podda, M., Coppel, R.L., Mackay, I.R., et al., 2009. Apoptoses and the biliary specificity of primary biliary cirrhosis. *Hepatology* 49, 871–879.
- Lleo, A., Shimoda, S., Ishibashi, H., Gershwin, M.E., 2011. Primary biliary cirrhosis and autoimmune hepatitis: apoptoses and epitopes. *J. Gastroenterol.* 46 (Suppl. 1), 29–38.
- Lockshin, R.A., Zakeri, Z., 2001. Programmed cell death and apoptosis: origins of the theory. *Nat. Rev. Mol. Cell Biol.* 2, 545–550.
- Lood, C., Blanco, L.P., Purmalek, M.M., Carmona-Rivera, C., Ravin, D.E., Smith, S.S., et al., 2016. Neutrophil extracellular traps enriched in oxidized mitochondrial DNA are interferogenic and contribute to lupus-like disease. *Nat. Med.* 22, 146–153.
- Lu, Q., Lemke, G., 2001. Homeostatic regulation of the immune system by receptor tyrosine kinases of the Tyro 3 family. *Science* 293, 306–311.
- Luo, X., Kraus, W.L., 2012. On PAR with PARP: cellular stress signaling through poly(ADP-ribose) and PARP-1. *Genes Dev.* 26, 417–432.
- Marshak-Rothstein, A., Rifkin, I.R., 2007. Immunologically active autoantigens: the role of toll-like receptors in the development of chronic inflammatory disease. *Annu. Rev. Immunol.* 25, 419–441.
- Martin, S.J., Reutelingsperger, C.P., McGahon, A.J., Rader, J.A., Van Schie, R.C., Laface, D.M., et al., 1995. Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl. *J. Exp. Med.* 182, 1545–1556.
- Matzinger, P., 2002. The danger model: a renewed sense of self. *Science* 296, 301–305.
- Mevorach, D., Zhou, J.L., Song, X., Elkon, K.B., 1998. Systemic exposure to irradiated apoptotic cells induces autoantibody production. *J. Exp. Med.* 188, 387–392.
- Mills, J.C., Stone, N.L., Pittman, R.N., 1999. Extranuclear apoptosis. The role of the cytoplasm in the execution phase. *J. Cell Biol.* 146, 703–708.

- Mitchell, D.A., Pickering, M.C., Warren, J., Fossati-Jimack, L., Cortes-Hernandez, J., Cook, H.T., et al., 2002. C1q deficiency and autoimmunity: the effects of genetic background on disease expression. *J. Immunol.* 168, 2538–2543.
- Miyake, Y., Yamasaki, S., 2012. Sensing necrotic cells. *Adv. Exp. Med. Biol.* 738, 144–152.
- Moubarak, R.S., Yuste, V.J., Artus, C., Bouharrou, A., Greer, P.A., Menissier-De Murcia, J., et al., 2007. Sequential activation of poly(ADP-Ribose) polymerase 1, calpains, and Bax is essential in apoptosis-inducing factor-mediated programmed necrosis. *Mol. Cell. Biol.* 27, 4844–4862.
- Munoz, L.E., Janko, C., Grossmayer, G.E., Frey, B., Voll, R.E., Kern, P., et al., 2009. Remnants of secondarily necrotic cells fuel inflammation in systemic lupus erythematosus. *Arthritis Rheum.* 60, 1733–1742.
- Munoz, L.E., Lauber, K., Schiller, M., Manfredi, A.A., Herrmann, M., 2010. The role of defective clearance of apoptotic cells in systemic autoimmunity. *Nat. Rev. Rheumatol.* 6, 280–289.
- Nagata, S., 2005. DNA degradation in development and programmed cell death. *Annu. Rev. Immunol.* 23, 853–875.
- Nagata, S., Nagase, H., Kawane, K., Mukae, N., Fukuyama, H., 2003. Degradation of chromosomal DNA during apoptosis. *Cell Death Differ.* 10, 108–116.
- Nagata, S., Hanayama, R., Kawane, K., 2010. Autoimmunity and the clearance of dead cells. *Cell* 140, 619–630.
- Oliveira, J.B., Bleesing, J.J., Dianzani, U., Fleisher, T.A., Jaffe, E.S., Lenardo, M.J., et al., 2010. Revised diagnostic criteria and classification for the autoimmune lymphoproliferative syndrome (ALPS): report from the 2009 NIH International Workshop. *Blood* 116, e35–e40.
- Orlando, K.A., Stone, N.L., Pittman, R.N., 2006. Rho kinase regulates fragmentation and phagocytosis of apoptotic cells. *Exp. Cell Res.* 312, 5–15.
- Orr Gandy, K.A., Obeid, L.M., 2012. Targeting the sphingosine kinase/sphingosine 1-phosphate pathway in disease: review of sphingosine kinase inhibitors. *Biochim. Biophys. Acta* 1831, 157–166.
- Radic, M., Marion, T., Monestier, M., 2004. Nucleosomes are exposed at the cell surface in apoptosis. *J. Immunol.* 172, 6692–6700.
- Rahman, A., Isenberg, D.A., 2008. Systemic lupus erythematosus. *N. Engl. J. Med.* 358, 929–939.
- Ravichandran, K.S., 2011. Beginnings of a good apoptotic meal: the find-me and eat-me signaling pathways. *Immunity* 35, 445–455.
- Reed, J.H., Neufing, P.J., Jackson, M.W., Clancy, R.M., Macardle, P.J., Buyon, J.P., et al., 2007. Different temporal expression of immunodominant Ro60/60 kDa-SSA and La/SSB apoptoses. *Clin. Exp. Immunol.* 148, 153–160.
- Reed, J.H., Jackson, M.W., Gordon, T.P., 2008. B cell apoptoses of the 60-kDa Ro/SSA and La/SSB autoantigens. *J. Autoimmun.* 31, 263–267.
- Reed, J.H., Jackson, M.W., Gordon, T.P., 2010. A Ro60 apoptote is cryptic on the intracellular autoantigen. *Lupus* 19, 107–108.
- Ren, Y., Tang, J., Mok, M.Y., Chan, A.W., Wu, A., Lau, C.S., 2003. Increased apoptotic neutrophils and macrophages and impaired macrophage phagocytic clearance of apoptotic neutrophils in systemic lupus erythematosus. *Arthritis Rheum.* 48, 2888–2897.
- Riedl, S.J., Salvesen, G.S., 2007. The apoptosome: signalling platform of cell death. *Nat. Rev. Mol. Cell Biol.* 8, 405–413.
- Rodriguez-Manzanet, R., Sanjuan, M.A., Wu, H.Y., Quintana, F.J., Xiao, S., Anderson, A.C., et al., 2010. T and B cell hyperactivity and autoimmunity associated with niche-specific defects in apoptotic body clearance in TIM-4-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* 107, 8706–8711.
- Rosen, A., Casciola-Rosen, L., 1997. Macromolecular substrates for the ICE-like proteases during apoptosis. *J. Cell. Biochem.* 64, 50–54.
- Rosenbaum, D.M., Degterev, A., David, J., Rosenbaum, P.S., Roth, S., Grotta, J.C., et al., 2010. Necroptosis, a novel form of caspase-independent cell death, contributes to neuronal damage in a retinal ischemia-reperfusion injury model. *J. Neurosci. Res.* 88, 1569–1576.
- Rothlin, C.V., Lemke, G., 2010. TAM receptor signaling and autoimmune disease. *Curr. Opin. Immunol.* 22, 740–746.
- Rothlin, C.V., Ghosh, S., Zuniga, E.I., Oldstone, M.B., Lemke, G., 2007. TAM receptors are pleiotropic inhibitors of the innate immune response. *Cell* 131, 1124–1136.
- Rovere, P., Vallinoto, C., Bondanza, A., Crosti, M.C., Rescigno, M., Ricciardi-Castagnoli, P., et al., 1998. Bystander apoptosis triggers dendritic cell maturation and antigen-presenting function. *J. Immunol.* 161, 4467–4471.
- Sancho, D., Reis e Sousa, C., 2012. Signaling by myeloid C-type lectin receptors in immunity and homeostasis. *Annu. Rev. Immunol.* 30, 491–529.
- Sawamura, T., Kakino, A., Fujita, Y., 2012. LOX-1: a multiligand receptor at the crossroads of response to danger signals. *Curr. Opin. Lipidol.* 23, 439–445.
- Scaffidi, C., Schmitz, I., Krammer, P.H., Peter, M.E., 1999. The role of c-FLIP in modulation of CD95-induced apoptosis. *J. Biol. Chem.* 274, 1541–1548.
- Schreiber, V., Dantzer, F., Ame, J.C., De Murcia, G., 2006. Poly(ADP-ribose): novel functions for an old molecule. *Nat. Rev. Mol. Cell Biol.* 7, 517–528.
- Scott, R.S., McMahon, E.J., Pop, S.M., Reap, E.A., Caricchio, R., Cohen, P.L., et al., 2001. Phagocytosis and clearance of apoptotic cells is mediated by MER. *Nature* 411, 207–211.
- Shao, W.H., Shu, D.H., Zhen, Y., Hilliard, B., Priest, S.O., Cesaroni, M., et al., 2016. Prion-like aggregation of mitochondrial antiviral signaling protein in lupus patients is associated with increased levels of type I interferon. *Arthritis Rheumatol.* 68, 2697–2707.
- Sica, A., Mantovani, A., 2012. Macrophage plasticity and polarization: in vivo veritas. *J. Clin. Invest.* 122, 787–795.
- Sisirak, V., Sally, B., D'agati, V., Martinez-Ortiz, W., Ozkarak, Z.B., David, J., et al., 2016. Digestion of chromatin in apoptotic cell microparticles prevents autoimmunity. *Cell* 166, 88–101.
- Soriano, F.G., Liaudet, L., Szabo, E., Virág, L., Mabley, J.G., Pacher, P., et al., 2002. Resistance to acute septic peritonitis in poly(ADP-ribose) polymerase-1-deficient mice. *Shock* 17, 286–292.
- Stranges, P.B., Watson, J., Cooper, C.J., Choisby-Rossi, C.M., Stonebraker, A.C., Beighton, R.A., et al., 2007. Elimination of antigen-presenting cells and autoreactive T cells by Fas contributes to prevention of autoimmunity. *Immunity* 26, 629–641.
- Strasser, A., Whittingham, S., Vaux, D.L., Bath, M.L., Adams, J.M., Cory, S., et al., 1991. Enforced BCL2 expression in B-lymphoid cells prolongs antibody responses and elicits autoimmune disease. *Proc. Natl. Acad. Sci. U.S.A.* 88, 8661–8665.
- Sulston, J.E., Horvitz, H.R., 1977. Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. *Dev. Biol.* 56, 110–156.
- Takahashi, T., Tanaka, M., Brannan, C.I., Jenkins, N.A., Copeland, N.G., Suda, T., et al., 1994. Generalized lymphoproliferative disease in mice, caused by a point mutation in the Fas ligand. *Cell* 76, 969–976.

- Tanaka, Y., Schroit, A.J., 1983. Insertion of fluorescent phosphatidylserine into the plasma membrane of red blood cells. Recognition by autologous macrophages. *J. Biol. Chem.* 258, 11335–11343.
- Tischner, D., Woess, C., Ottina, E., Villunger, A., 2011. Bcl-2-regulated cell death signalling in the prevention of autoimmunity. *Cell Death Dis.* 1, e48.
- Tran, H.B., Macardle, P.J., Hiscock, J., Cavill, D., Bradley, J., Buyon, J.P., et al., 2002. Anti-La/SSB antibodies transported across the placenta bind apoptotic cells in fetal organs targeted in neonatal lupus. *Arthritis Rheum.* 46, 1572–1579.
- Truman, L.A., Ford, C.A., Pasikowska, M., Pound, J.D., Wilkinson, S.J., Dumitriu, I.E., et al., 2008. CX3CL1/fractalkine is released from apoptotic lymphocytes to stimulate macrophage chemotaxis. *Blood* 112, 5026–5036.
- Utz, P.J., Hottelet, M., Schur, P.H., Anderson, P., 1997. Proteins phosphorylated during stress-induced apoptosis are common targets for autoantibody production in patients with systemic lupus erythematosus. *J. Exp. Med.* 185, 843–854.
- Vandenabeele, P., Galluzzi, L., Vanden Berghe, T., Kroemer, G., 2010. Molecular mechanisms of necroptosis: an ordered cellular explosion. *Nat. Rev. Mol. Cell Biol.* 11, 700–714.
- Vandivier, R.W., Ogden, C.A., Fadok, V.A., Hoffmann, P.R., Brown, K.K., Botto, M., et al., 2002. Role of surfactant proteins A, D, and C1q in the clearance of apoptotic cells in vivo and in vitro: calreticulin and CD91 as a common collectin receptor complex. *J. Immunol.* 169, 3978–3986.
- Varfolomeev, E., Maecker, H., Sharp, D., Lawrence, D., Renz, M., Vucic, D., et al., 2005. Molecular determinants of kinase pathway activation by Apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand. *J. Biol. Chem.* 280, 40599–40608.
- Voll, R.E., Herrmann, M., Roth, E.A., Stach, C., Kalden, J.R., Girkontaite, I., 1997. Immunosuppressive effects of apoptotic cells. *Nature* 390, 350–351.
- Wang, X., Wu, Y.C., Fadok, V.A., Lee, M.C., Gengyo-Ando, K., Cheng, L.C., et al., 2003. Cell corpse engulfment mediated by *C. elegans* phosphatidylserine receptor through CED-5 and CED-12. *Science* 302, 1563–1566.
- Welz, P.S., Wullaert, A., Vlantis, K., Kondylis, V., Fernandez-Majada, V., Ermolaeva, M., et al., 2011. FADD prevents RIP3-mediated epithelial cell necrosis and chronic intestinal inflammation. *Nature* 477, 330–334.
- Wiley, S.R., Schooley, K., Smolak, P.J., Din, W.S., Huang, C.P., Nicholl, J.K., et al., 1995. Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity* 3, 673–682.
- Wu, X., Molinaro, C., Johnson, N., Casiano, C.A., 2001. Secondary necrosis is a source of proteolytically modified forms of specific intracellular autoantigens: implications for systemic autoimmunity. *Arthritis Rheum.* 44, 2642–2652.
- Yousefi, S., Gold, J.A., Andina, N., Lee, J.J., Kelly, A.M., Kozlowski, E., et al., 2008. Catapult-like release of mitochondrial DNA by eosinophils contributes to antibacterial defense. *Nat. Med.* 14, 949–953.
- Zetterstrom, C.K., Jiang, W., Wahamaa, H., Ostberg, T., Aveberger, A.C., Schierbeck, H., et al., 2008. Pivotal advance: inhibition of HMGB1 nuclear translocation as a mechanism for the anti-rheumatic effects of gold sodium thiomalate. *J. Leukoc. Biol.* 83, 31–38.

## Autophagy in Autoimmunity

Christian W. Keller<sup>1</sup>, Christian Münz<sup>2</sup> and Jan D. Lünemann<sup>1</sup>

<sup>1</sup>Institute of Experimental Immunology, Laboratory of Neuroinflammation, University of Zurich, Zurich, Switzerland

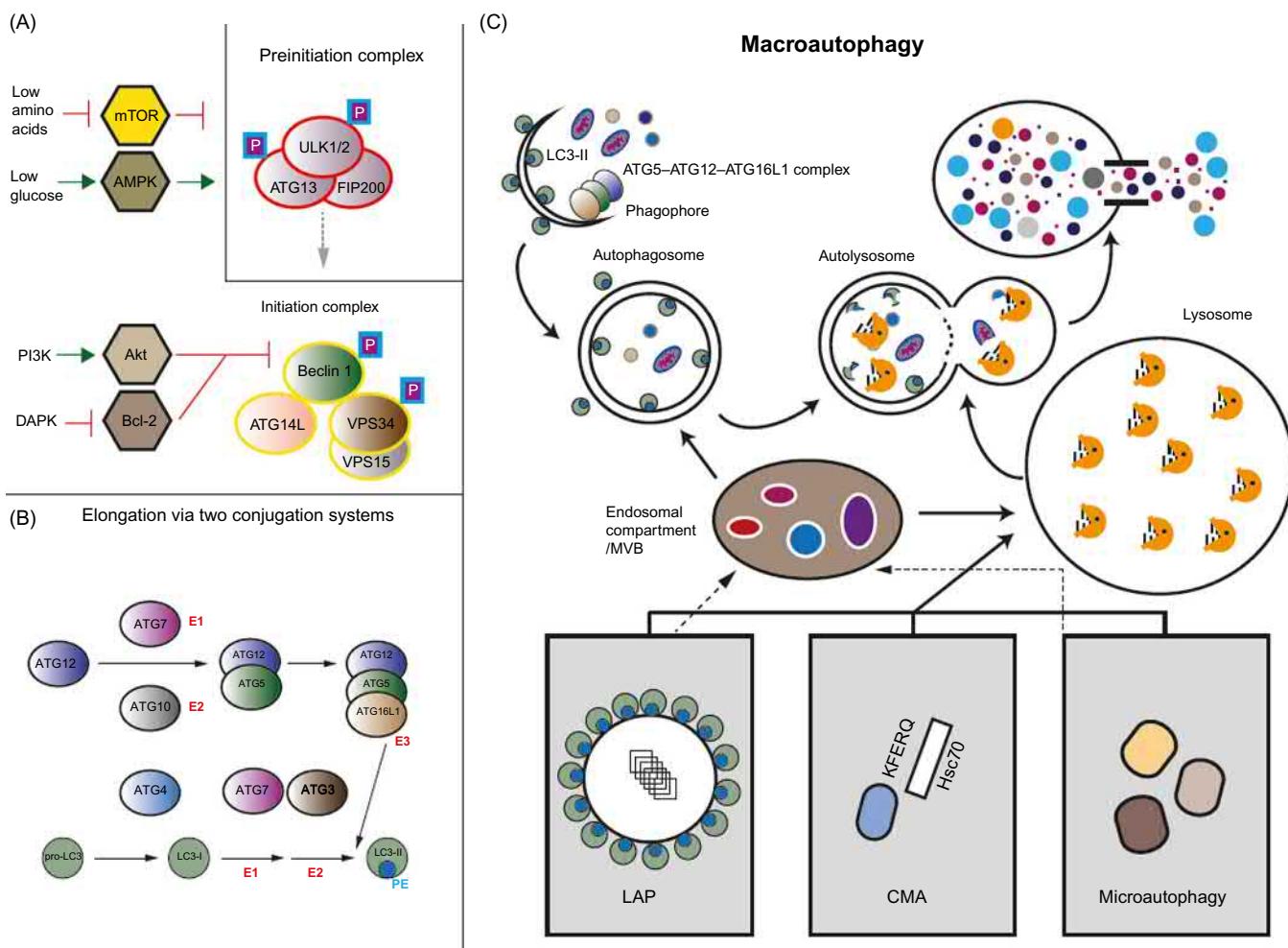
<sup>2</sup>Institute of Experimental Immunology, Laboratory of Viral Immunobiology, University of Zurich, Zurich, Switzerland

### OUTLINE

Autophagy Pathways	305	Autophagy Pathways During Antigen Presentation	310
Molecular Autophagy-Related Proteins			
Machinery of Macroautophagy	306	Autophagy in Tolerance and Autoimmunity	312
Noncanonical Autophagy Pathways	307	References	313
Autophagy in T- and B-Cell Development and Activation	308	Further Reading	317
Autophagy in Innate Immunity	309		

### AUTOPHAGY PATHWAYS

The term autophagy, derived from the Greek and meaning “eating of self,” was first coined by Christian de Duve in 1963 (de Reuck and Cameron, 1963; Klionsky, 2014), to explain earlier electron microscopic observations of mitochondrial degradation in double-membrane vesicles, a process that had been termed autolysis (Ashford and Porter, 1962), and finally, in 2016, the Nobel Prize in Physiology or Medicine was awarded to Yoshinori Ohsumi for his groundbreaking work in identifying the first set of autophagy-related genes in baker’s yeast (*Saccharomyces cerevisiae*) (Tsukada and Ohsumi, 1993), a discovery that ultimately led to a tremendous spark in the field of autophagy research. The identification of an evergrowing number of autophagy-related proteins (ATGs) in recent years has led to the characterization of several discrete autophagy pathways that all serve distinct objectives. Autophagy pathways may be subdivided into canonical and noncanonical pathways with canonical autophagy comprising macroautophagy (MA), microautophagy (MI), and chaperone-mediated autophagy (CMA) (Codogno et al., 2011). Here, we focus on MA, the most extensively studied among all autophagy pathways (Fig. 17.1). This pathway is highly conserved from yeast to mammalian cells and comprises a series of tightly regulated membrane reorganization events under the control of several ATGs ultimately leading to the formation of a double-membrane organelle called the autophagosome. De novo synthesis of this hallmark organelle is an exclusive morphological feature of MA and does not take place during MI and CMA. Three defined sequential processes pave the way to autophagogenesis: induction/nucleation, elongation, and closing/maturation. During the process of closing, the autophagosome sequesters and engulfs neighboring cytoplasmic content. These steps are then followed by fusion of the vesicle with the endolysosomal system and subsequent degradation of its cargo (Mizushima et al., 2011, 2002; Feng et al., 2014). The targeting of cytoplasmic MA substrates may be both unspecific or highly selective (e.g., mitophagy), mediated via several adaptor molecules and ATGs (Galluzzi et al., 2017; Farré and Subramani, 2016). Many mammalian tissues execute MA on a constitutive level at varying degrees and depending on the cell type, macroautophagic activity may be induced and/or modulated by



**FIGURE 17.1** Different autophagy pathways fusing with the endolysosomal compartment. (A) The preinitiation complex (ULK complex) is (among others) under the control of multisite phosphorylation through the two kinases mTOR and AMPK and activates the downstream initiation complex (PI3K complex) which is itself negatively and positively regulated by others. (B) The subsequent elongation reaction is highly dependent on two ubiquitin-like protein conjugation systems. (C) MA: the phagophore stems from PI3P-rich membrane sources. Cytoplasmic cargo is engulfed after recruitment of LC3 to the outer and inner leaflet of the arising autophagosome, and LC3 is removed from the outer membrane as the vesicle is closed. The completed autophagosome then fuses with endosomes and/or lysosomes. Also CMA, MI, and LAP may converge into the endolysosomal system. AMPK, 5' AMP-activated protein kinase; CMA, chaperone-mediated autophagy; LAP, LC3-associated phagocytosis; MA, macroautophagy; MI, microautophagy; mTOR, mechanistic target of rapamycin; MVB, multivesicular bodies; ULK, unc 51-like kinase.

several means (e.g., nutrient deprivation, transcriptional, and epigenetic regulation). MA is controlled both on a systemic and on a cellular level with the two antagonistic kinases 5' AMP-activated protein kinase and target of rapamycin complex 1 (TORC1) being the most central regulatory units of the cellular MA machinery so far identified (Kim et al., 2011; Egan et al., 2011; Efeyan et al., 2015).

### Molecular Autophagy-Related Proteins Machinery of Macroautophagy

Five functionally distinct, hierarchically organized, protein complexes orchestrate initiation and assembly of the autophagosome: the unc 51-like kinase (ULK) complex (1), the class III phosphatidylinositide 3-kinase (PI3K) complex (2), the ATG2/WD repeat domain phosphoinositide-interacting protein (WIPI) complex and the ATG9 cycling system (3), the ATG12-conjugation system (4), and the microtubule-associated protein 1 light chain 3 (LC3)-conjugation system (5) (Feng et al., 2014; Mizushima et al., 2011; Shibutani and Yoshimori, 2014) (Fig. 17.1). During the induction/nucleation step, a double-membrane structure called phagophore or isolation membrane twines around and sequesters designated parts of the cytoplasm. The subcellular source, which provides

membrane material for the phagophore, is still under debate. While some have reported that these parent membranes are predominantly derived from specialized endoplasmic reticulum (ER) domains (Axe et al., 2008; Ylä-Anttila et al., 2009; Hayashi-Nishino et al., 2009), others have suggested contributions from alternative sources such as mitochondria (Hailey et al., 2010), the plasma membrane (Ravikumar et al., 2010), or a novel compartment comprised ATG9<sup>+</sup> vesicles and tubules (Mari et al., 2010).

The ULK multiprotein complex consists of ULK1/2, focal adhesion kinase family–interacting protein of 200 kDa (FIP200), ATG13 and ATG101, is assembled upon an appropriate stimulus and then translocates to autophagosomal initiation sites on tubulovesicular areas consisting of ER and ATG9<sup>+</sup> vesicles (Ganley et al., 2009; Karanasios et al., 2016). Vacuolar protein sorting protein (Vps)34, Vps15 (also called p150), Beclin 1 (ortholog of Atg6), and Atg14L build the tetrameric core of the class III PI3K complex whose major function is to produce PI3P (Suzuki et al., 2016). Recruitment of the complex to autophagosomal initiation sites is mediated via the upstream ULK complex and ATG9. Downstream PI3P-binding partners can now be recruited to the newly generated PI3P-enriched membranes (Matsunaga et al., 2010; Itakura and Mizushima, 2010). These PI3P-enriched membrane sites that are generated via the PI3PK complex attract binding partners that are essential for the further assembly of the autophagosome such as WIPI1/2 and ATG2 (Itakura and Mizushima, 2010; Obara et al., 2008; Velikkakath et al., 2012). At the autophagosomal initiation site, the WIPI/ATG2 complex both facilitates recruitment of lipidated LC3 and prevents its ATG4-mediated deconjugation (Nair et al., 2010). ATG12, ATG7, ATG10, ATG5, and ATG16L1 are all members of the ubiquitin-like ATG12-conjugation system (Mizushima et al., 2011). The E1-like enzyme ATG7 activates ATG12, which is then transferred to E2-like enzyme ATG10. Next, ATG10 catalyzes the covalent conjugation of ATG12 to ATG5 and ATG16L1 can now bind to ATG5 to complete the complex (Geng and Klionsky, 2008). The ATG5–ATG12–ATG16L1 complex is recruited to the autophagosomal initiation site by WIPI2 (Dooley et al., 2014) and FIP200 (Nishimura et al., 2013) in an ATG16L1-dependent manner where it executes its function as an E3-like enzyme for the LC3-conjugation system. In addition, the ATG5–ATG12–ATG16L1 complex guides and determines LC3 to its subcellular destination (Fujita et al., 2008b). Although present on the outer membrane of the nascent phagophore, the ATG5–ATG12–ATG16L1 complex swiftly dissociates from there upon autophagosome closure (Mizushima et al., 2001). Members of the second conjugation system include the ubiquitin-like LC3, the hydrolase ATG4, E1-like ATG7, and E2-like ATG3. ATG4 cleaves off five C-terminal amino acids of the LC3 precursor form to expose a glycine residue (G120) (Hemelaar et al., 2003; Kabeya et al., 2004). The resulting LC3-I is lipidated with phosphatidylethanolamine at the previously exposed glycine residue by the concerted actions of ATG3, ATG7 (Kabeya et al., 2000; Ichimura et al., 2000; Tanida et al., 2004), and E3-like activity via ATG5–ATG12 conjugates (Geng and Klionsky, 2008; Fujita et al., 2008b). Lipidated LC3-II is initially present on the outer and inner membrane of the assembling autophagosome (Kabeya et al., 2000, 2004; Kimura et al., 2007). There it appears to facilitate the elongation of the phagophore as well as coordinate tethering and hemifusion of its membranes and the closing of the phagophore to the completed autophagosome (Nakatogawa et al., 2007; Fujita et al., 2008a; Weidberg et al., 2010; Sou et al., 2008). While LC3-II is recycled from the outer membrane after completion of the autophagosome by ATG4, LC3-II that is coupled to the inner membrane stays associated with the autophagosome and is degraded with this membrane by lysosomal hydrolysis.

## Noncanonical Autophagy Pathways

There are autophagy pathways that result in autophagosome formation but omit the usage of essential elements of the classical MA machinery; other autophagy modes may be autophagosome independent but utilize key components of the MA network. These and other entities may be categorized as noncanonical autophagy pathways (Codogno et al., 2011; Münz, 2015). Some recently characterized members of this group include LC3-associated phagocytosis (LAP), Beclin 1–independent autophagy, autophagy-associated unconventional protein secretion, and defective ribosomal products–containing autophagosome-rich blebs. Due to its strong link to antigen presentation and its reported function in the development of autoimmune disease phenotypes, we will confine ourselves to discussing LAP.

LAP is a process that links both phagocytosis of extracellular constituents and the autophagy molecular core machinery. While some key elements of the MA machinery are essential for LAP (e.g., ATG5, ATG7, LC3, Beclin 1), others are fully dispensable (e.g., the ULK complex including ULK1/2, FIP200, ATG13, ATG101). This pathway features a unique organelle, a single membrane LC3-decorated phagosome (also called LAPosome) and is initiated by ligation of a variety of extracellular receptors including Toll-like receptors (TLRs), fragment

crystallizable (Fc) receptors, C-type lectins, and phosphatidylserine (Ptd-L-Ser)-binding receptors (Romao et al., 2013; Sanjuan et al., 2007; Ma et al., 2012; Martinez et al., 2011; Henault et al., 2012). After binding of LAP-triggering surface receptors, a PI3PK complex, slightly different from the one active during MA, associates with the cytosolic membrane of the arising phagosome and generates PI3P, which, in concert with other factors such as Rubicon, mediates recruitment, stabilization, and activation of the NADPH oxidase (NOX)2 (Martinez et al., 2015). NOX2-derived reactive oxygen species together with LAPosomal PI3P then launch recruitment and activation of the two canonical MA conjugation systems for Atg12 and LC3 (see earlier), resulting in the deposition of lipidated LC3 on the outer LAPosomal membrane (Martinez et al., 2015). While several questions remain with regards to the differential fate of LAPosomes compared to conventional phagosomes, there is ample evidence that LAP in professional antigen-presenting cells (APCs) such as dendritic cells (DCs) enables the cell to retain antigenic material for sustained and more efficient major histocompatibility complex (MHC) class II antigen presentation (Lee et al., 2010; Romao et al., 2013; Ma et al., 2012).

## AUTOPHAGY IN T- AND B-CELL DEVELOPMENT AND ACTIVATION

Aside from its pivotal function in energy housekeeping, autophagy is increasingly recognized for actively participating in the development and maintenance of immune cells (Shibutani et al., 2015). Most of these immune cells originate from hematopoietic progenitor cells (HPCs) and this precursor cell population has been shown to be highly dependent on MA for its survival (Mortensen et al., 2011; Salemi et al., 2012). Not only were progenitors of multiple lineages reduced upon targeted deletion of the essential MA gene *Atg7* in HPCs but also severe myeloproliferation was observed in the affected mice (*Vav-Atg7<sup>-/-</sup>*) in vivo (Mortensen et al., 2011). Moreover, *Atg7*-deficient HPCs depicted defective mitochondrial quality control leading to accumulation of mitochondria with high membrane potential, increased production, and subsequent oxidative damage. These findings were corroborated by a report that shows that T cells lacking ATG5 and ATG7 are critically impaired in their mitochondrial maintenance (Stephenson et al., 2009). Again focusing on T lymphocytes, another study found that *Atg5*-deficient CD4<sup>+</sup> and CD8<sup>+</sup> T cells show normal initial development in the thymus. However, due to increased cell death and the incapacity to undergo efficient proliferation after T-cell receptor (TCR) stimulation in absence of the essential MA gene *Atg5*, these populations later on fail to repopulate the periphery (Pua et al., 2007). In mice with Beclin 1 deficiency in the hematopoietic lineage, T- and B-cell precursors are diminished in thymus and bone marrow, respectively, while peripheral T- and B-cell compartments are largely normal (Arsov et al., 2011). The susceptibility of lymphocyte precursors may result from the biphasic expression of Beclin 1 and, therefore, resulting macroautophagic activity during development, while nonactivated T and B cells seem to have low MA levels (Arsov et al., 2008). Upon activation, MA is upregulated in peripheral lymphocytes, including T cells (Arsov et al., 2008; Pua et al., 2007). T cell-specific conditional knockout of Beclin 1 (CD4-Cre Beclin 1<sup>f/f</sup>) also confirmed that cell-intrinsic absence of MA precludes peripheral homeostasis of T lymphocytes due to increased susceptibility to cell death upon activation (Kovacs et al., 2012). The requirement during T-cell activation, however, results at least in part from impaired Ca<sup>2+</sup> mobilization upon TCR engagement (Jia et al., 2011). While the Ca<sup>2+</sup> storing ER is enlarged, influx of this second messenger upon T-cell activation is impaired, resulting in reduced immune responses. Signal transmission from the TCR to NF-κB is in part mediated by the adaptor protein B-cell lymphoma/leukemia 10 (Bcl10), which is reflected by the failure of T cells to proliferate upon TCR ligation in absence of Bcl10 (Ruland et al., 2001). Targeted degradation of Bcl10 via selective MA has been identified as a feedback mechanism to avoid excessive TCR signaling and to limit NF-κB-dependent effector responses thereby broadening the concept of autophagy-mediated T-cell functions beyond survival fitness (Paul et al., 2012). Targeted deletion of yet another protein essential for MA, ATG16L1, in T cells leads to expansion of IL-13-secreting Th2 subsets and diminished numbers of intestinal T-regulatory cells (TREGs) resulting in the spontaneous development of intestinal inflammation (Kabat et al., 2016). Foxp<sup>+</sup>CD4<sup>+</sup> TREGs are specialized lymphocytes whose major function is to control and limit immune responses mounted against self- and foreign antigens in order to retain self-tolerance (Josefowicz et al., 2012). TREG-specific deletion of ATG5 and ATG7 results in lower frequencies and survival of this subset (Wei et al., 2016). TORC1 activity in TREGs has been reported to be crucial for integrating immunological signals from the TCR and IL-2 to functional fitness (Zeng et al., 2013). TREGs, which depict a higher autophagic activity as compared to naïve T cells, seem to utilize autophagy to restrain TCR-triggered TORC1 which leads to increased c-Myc expression and exuberant glycolytic metabolism in autophagy-deficient TREGs entailing compromised self-tolerance (Wei et al., 2016). A critical role has also been attributed to autophagy proteins during CD8<sup>+</sup> memory T-cell homeostasis. Targeted deletion of ATG7 in T

lymphocytes engenders weakened CD8<sup>+</sup> T cell–dependent recall responses upon infection with influenza and murine cytomegalovirus (MCMV) (Puleston et al., 2014). The age-related decline of CD8<sup>+</sup> T-cell memory function has been linked to reduced efficacy of vaccination in the elderly (Haq and McElhaney, 2014). In line with this, CD8<sup>+</sup> cells isolated from aged mice show lower levels of autophagic activity as compared to younger controls. By contrast, systemic pharmacological induction of autophagy partly restored memory CD8<sup>+</sup> T-cell function (Puleston et al., 2014). While in part contradicting previous findings (Pua et al., 2007), Xu et al. illustrate a more dynamic course of autophagic activity in T cells in that CD8<sup>+</sup> T lymphocytes initially downregulate autophagy following TCR ligation and during proliferation, followed by another upregulation of the process before the contraction phase. Mechanistically, loss of autophagy proteins in CD8<sup>+</sup> T cells results in dysfunctional mitochondrial fatty acid oxidation at the transition to the memory phase (Xu et al., 2014). Interestingly, there seems to be a differential requirement for autophagy pathways dependent on the T-cell subset (Kovacs et al., 2012). It is known that mice that lack the E3 ubiquitin ligase itchy E3 ubiquitin protein ligase (ITCH) are prone to develop hyperactive T cell–driven systemic autoimmunity (Fang et al., 2002). CMA has been reported to regulate the outcome of T-lymphocyte activation to the extent that regulators of calcineurin 1 and ITCH, both negative regulators of TCR signaling, serve as substrates for this autophagy pathway. CD4<sup>+</sup> T cells devoid of the essential CMA component LAMP-2A, exhibit a strong reduction in activation-induced proliferation showing that CMA-mediated degradation actively participates in the calibration of T-cell responses similar to MA-dependent clearance of Bcl10 (Valdor et al., 2014; Paul et al., 2012).

Invariant natural killer T cells (iNKT) comprise a conserved subset of unconventional T lymphocytes with a memory-like phenotype that recognizes glycolipids presented by CD1d molecules and in crosstalk with professional APCs, they facilitate and boost coordinated stimulation of innate and adaptive immune responses (Godfrey et al., 2015). During their thymic development and differentiation, iNKT cells upregulate autophagy in order to keep mitostasis. Consequently and in line with what has been described for other hematopoietic cells, the absence of ATG7 in iNKT cells leads to amassment of mitochondria and excessive generation of mitochondrial superoxide species followed by apoptosis. Hence, lack of autophagy in iNKT cells leads to an early arrest in thymic development accounting for the complete absence of mature iNKT cell in peripheral lymphoid organs (Salio et al., 2014). Similar to T-cell development, B cells require autophagy both during development and maintenance in the periphery. While in developing B cells the transition from pro- to pre-B cells is affected by the loss of MA (Arsov et al., 2011; Miller et al., 2008), among mature B cells, only the mostly innate B-1a subset is affected (Miller et al., 2008).

These data show that lymphocyte development, their activation, and effector functions are compromised upon disruption of autophagy pathways. This may for example result from half-live shortening or partial loss-of-function mutations in essential autophagy genes which may preclude affected cell population to efficiently mobilize nutrients, maintain cellular fitness, or adequately integrate extracellular signals. Further studies will be warranted to clarify differential roles of autophagy pathways in distinct lymphocyte subsets particularly in the context of T cell–driven autoimmunity.

## AUTOPHAGY IN INNATE IMMUNITY

As an innate immune response to viral and microbial pathogens, autophagy pathways participate in limiting pathogen replication in host cells. Bacteria and parasites that either escape from endosomes and replicate in the cytosol or condition the phagosome to serve as their replication niche—by preventing fusion with lysosomes—have been found to be delivered for lysosomal degradation via autophagy (Nakagawa et al., 2004; Gutierrez et al., 2004; Singh et al., 2006; Wild et al., 2011). For example, ruptured *Salmonella typhimurium*–containing vacuoles expose host glycans, which serve as ligands for the lectin and cytosolic danger receptor galectin 8. The recognition of these carbohydrate residues by galectin 8 entails recruitment of the autophagy receptor nuclear domain 10 protein 52 and the consecutive initiation of MA, thereby restricting *Salmonella* proliferation (Thurston et al., 2012). Furthermore, autophagy contributes to sealing endosomal membranes upon *S. typhimurium*'s type three secretion system-mediated damage (Kreibich et al., 2015). Some bacteria, such as *Listeria monocytogenes*, *Shigella flexneri*, and *Mycobacterium tuberculosis*, have already developed evasion strategies in order to circumvent autophagy-mediated clearance of pathogens (Ogawa et al., 2005; Yoshikawa et al., 2009; Kim et al., 2015). Innate immune responses to microbial antigens are initiated through stimulation of pattern-recognition receptors (PRRs) which include TLRs, nucleotide-binding and oligomerization domain-like receptors, retinoic acid–inducible gene I–like helicases, and a subset of C-type lectin receptors. These sensor molecules endow cells of the innate

immune system with the ability to recognize a large number of molecular patterns expressed by bacteria, viruses, or fungi. Recent data have revealed an intricate, mutual interplay between PRRs and autophagy pathways, whereby autophagy, on the one hand, facilitates PRR-mediated recognition of cognate ligands by fostering their physical interaction and, on the other, serves as an immune effector mechanism downstream of PRR stimulation. For example, several TLRs function as triggering receptors for initiating the noncanonical LAP pathway thereby linking members of the autophagy core machinery to phagocytosis (Sanjuan et al., 2007). Furthermore, cytosolic viral replication intermediates of single-stranded RNA viruses, recognized by PRRs, are delivered to endosomally located TLR7 through MA, which results in robust type I interferon (IFN)-dependent innate immune responses via plasmacytoid DCs (pDCs) (Lee et al., 2007). Also in pDCs, the LAP pathway has been implicated in channeling TLR9 to IFN regulatory transcription factor 7-signaling compartments upon Fc $\gamma$ R-mediated binding of immune complexes thereby coupling autophagy and pathogenic type I IFN secretion (Henault et al., 2012). During another noncanonical selective autophagy pathway in macrophages, TLR4 ligation leads to the nucleoporin 62 (p62)/sequestosome (SQSTM1)-dependent targeting of aggresome-like induced structures (Fujita et al., 2011). Also downstream of TLR4, p62/SQSTM1-dependent mitophagy restrains exuberant NACHT, leucine rich repeat (LRR), and pyrin domain (PYD) domain-containing protein (NLRP3)-inflammasome activity and consecutive release of proinflammatory mediators (Zhong et al., 2016). Extending previous findings, which highlight the role of autophagy pathways during antibacterial defense, another study using mice that are deficient in intestinal epithelial cell ATG5 shows that autophagy downstream of myeloid differentiation primary response 88 (MyD88)-signaling is crucial for impeding dissemination of *S. typhimurium* in vivo (Benjamin et al., 2013). PRR signaling and T<sub>h</sub>1 cytokines such as IFN $\gamma$  and members of the tumor necrosis factor family can induce or augment MA in several tissues, which might represent a feedback mechanism by which activated T cells augment MA under inflammatory conditions (Xu et al., 2007; Delgado et al., 2008; Orvedahl et al., 2007; Keller et al., 2011). Other groups of molecules such as T<sub>h</sub>2 cytokines (e.g., IL-4, IL-13) or regulatory cytokines (e.g., IL-10) have been shown to downregulate autophagy (Van Grol et al., 2010; Harris et al., 2007). The net effect of the exposure to a given cytokine, however, is likely to be dependent on multiple parameters such as coexposure to other mediators and the respective target tissue. Conversely, autophagy pathways may, through several mechanisms, also contribute to calibrating extracellular levels of cytokines as reported for the proinflammatory protein IL-1 $\beta$  (Dupont et al., 2011; Zhang et al., 2015). This cytokine very well exemplifies the complex reciprocal interrelationship of some soluble signal molecules with the autophagy machinery when keeping in mind that mitophagy limits NLRP3-mediated release of IL-1 $\beta$  (Zhong et al., 2016) or the fact that IL-1 $\beta$  itself may induce autophagy (Pilli et al., 2012).

## AUTOPHAGY PATHWAYS DURING ANTIGEN PRESENTATION

Although the exact molecular underpinnings remain unknown, it is widely accepted that certain genetic variants within the human leukocyte antigen locus render individuals more susceptible to autoimmunity (Matzarakis et al., 2017). In addition to regulating T-cell development and activation, autophagy pathways also participate in shaping of the T-cell repertoire during several antigen processing and presenting routes (Münz, 2016a,b). Conventional T cells recognize proteolytic fragments of proteins that are presented on MHC molecules. MHC class I molecules present primarily proteasomal substrates to CD8 $^{+}$  T cells, while MHC class II molecules present peptide ligands generated in the endolysosomal pathway to CD4 $^{+}$  T cells (Trombetta and Mellman, 2005). Autoimmunity is commonly triggered by a specific adaptive immune response that is mounted against self-antigens. Acknowledged degradation pathways which may facilitate MHC class II presentation of self-antigens include the classical MHC class II pathway, MA, and LAP (Münz, 2016a,b). Early work has already suggested presence of an endogenous MHC class II pathway that allows viral proteins to be presented to CD4 $^{+}$  T cells (Jacobson et al., 1989; Nuchtern et al., 1990) and analysis of the affinity-purified MHC class II peptidome revealed that about 30% of eluted natural ligands stem from endogenous protein sources (Rammensee et al., 1999; Dengjel et al., 2005) which gave rise to the assumption that a yet unidentified endolysosomal route delivers these ligands onto MHC molecules. Eventually, MA was recognized as a novel pathway that conveys endogenous cargo to endosomal MHC class II-containing compartments (MIICs) for subsequent recognition by CD4 $^{+}$  T cells (Nimmerjahn et al., 2003; Paludan et al., 2005; Schmid et al., 2007). In some professional APCs, autophagosomes frequently fuse with MIICs, and experimental delivery of antigens to autophagosomes by targeting the autophagosomal membrane via fusion with LC3 consequently leads to efficient recognition by cognate CD4 $^{+}$  T cells (Schmid et al., 2007; Comber et al., 2011; Aichinger et al., 2013; Jin et al., 2014; Coulon et al., 2016; Fonteneau

et al., 2016). Further evidence came from studies reporting that the two mammalian ATG8 homologs in higher eukaryotes, namely, LC3 and gamma-aminobutyric acid receptor-associated protein, are among the source proteins for natural MHC class II ligands (Dengjel et al., 2005; Suri et al., 2008). MHC class II presentation of endogenous cytosolic and nuclear antigens can be increased by 50% upon starvation, a strong inducer of MA, while starvation leaves loading of ligands from membrane-bound proteins unaffected (Dengjel et al., 2005). Several professional APCs, such as DCs and B cells, carry out MA on a constitutive level (Dengjel et al., 2005; Nimmerjahn et al., 2003; Paludan et al., 2005) and, as discussed previously, the process can be additionally upregulated (and downregulated) following immune stimulation via PRRs such as TLRs or inflammatory cytokines (Lee et al., 2007; Delgado et al., 2008; Xu et al., 2007; Van Grol et al., 2010; Harris et al., 2007). The proficiency to harness autophagy pathways for delivery of endogenous antigens to MHC class II appears differentially relevant depending on the specific APC population (Brazil et al., 1997).

Among the more in-depth characterized endogenous antigens to be delivered via MA to MIICs are the nuclear antigen 1 of the Epstein–Barr virus (EBNA1) and the bacterial transposon-derived neomycin phosphotransferase II (NeoR). EBNA1 is intracellularly processed for MHC class II presentation by Epstein–Barr virus–infected B cells (Munz et al., 2000). This processing is executed within lysosomes because EBNA1 accumulates in cytosolic vesicles upon inhibition of lysosomal proteolysis (Paludan et al., 2005). Furthermore, CD4<sup>+</sup> T-cell recognition of EBNA1 expressing B cells is compromised upon RNA silencing of essential MA genes (Leung et al., 2010; Paludan et al., 2005). Inhibition of nuclear import of EBNA1 increases MA-dependent MHC class II presentation of this antigen (Leung et al., 2010). NeoR, on the other hand, is a cytosolic antigen, whose processing for MHC class II presentation is sensitive to MA inhibition (Nimmerjahn et al., 2003). Vice versa, antigens that are fused to NeoR are more efficiently presented to CD4<sup>+</sup> T cells via MA (Comber et al., 2011). However, in contrast to EBNA1, nuclear import of NeoR does not compromise its presentation on MHC class II molecules after macroautophagic antigen processing (Riedel et al., 2008). Therefore nuclear localization by itself does not necessarily protect antigens from processing for MHC class II presentation via MA, suggesting that the subcompartmentalization of the antigen in the nucleus could dictate, if this antigen gains access to the cytosol after nuclear envelope dissociation during mitosis.

Ample data show that autophagy also contributes to extracellularly derived antigen processing and presentation. ATG5-deficient DCs process and present pulsed antigen less efficiently via MHC class II when compared to their wild-type counterparts (Lee et al., 2010). This defect correlates with decelerated phagosome maturation, possibly due to less efficient fusion with lysosomes. Lee et al. showed that the TLR-dependent phagocytosis of herpes simplex virus (HSV)-2 entails formation of LC3-decorated single-membraned organelles and while pharmacological intervention of MA via inhibition of TORC1 left MHC class II–mediated antigen presentation unperturbed, the targeted deletion of LAP-essential ATG5 in the DC compartment significantly abated HSV-2-specific CD4<sup>+</sup> T-cell responses indicative of a non-MA pathway linking phagocytosis and MHC class II presentation (Lee et al., 2010). While during canonical MA cytoplasmic cargo is sequestered into forming autophagosomes, LAP denotes a process by which extracellular constituents enter the cell through phagocytosis in a single-membraned phagosome that is subsequently decorated by LC3. This hallmark vesicle, also termed LAPosome, may deliver antigenic fragments to the endolysosomal system including MIICs, thereby prompting downstream T-cell responses (Münz, 2015; Martinez et al., 2015; Romao and Münz, 2014). There is evidence that in mouse macrophages, the decoration of phagosomes with LC3 leads to accelerated movement of these vesicles along microtubules and rapid fusion with the lysosomal compartment, which is in keeping with one of the primary tasks of macrophages being quick elimination of phagocytosed microorganisms or damaged cells (Florey et al., 2011; Sanjuan et al., 2007; Martinez et al., 2011; Ma et al., 2014). On the other hand and similar to what has been reported in mouse DCs (Lee et al., 2010), in human professional APCs, LAP is facilitated for sustained MHC class II antigen presentation in that antigenic cargo is retained in the endolysosomal system rather than promoting degradation of antigenic constituents (Romao et al., 2013). Decreased levels of lysosomal proteases and antigenic stabilization and containment are presumed mechanisms via which professional APCs such as DCs calibrate and maintain robust antigen presentation for prolonged stimulation of cognate T cells (Delamarre et al., 2005; Romao et al., 2013; Romao and Münz, 2014). Aside from the previously described role in pDCs, where LAP tunes TLR9-mediated release of IFN $\alpha$  in the context of immune complex binding to Fc $\gamma$ R (Henault et al., 2012), the process has also been implicated in aiding the clearance of *S. cerevisiae* and *Aspergillus fumigatus* (Sanjuan et al., 2007; Martinez et al., 2015).

Autophagy pathways may also participate in the delivery of distinct processing enzymes to phagosomes for increased antigenic peptide formation. In this view, it was reported that peptidylarginine deiminase, which citrullinates endocytosed proteins and thereby gives rise to autoantigens that are recognized in some autoimmune diseases, like rheumatoid arthritis, reaches phagosomes via MA (Ireland and Unanue, 2011). Thus the

support of extracellular antigen processing by the autophagy core machinery could result from LC3-assisted fusion with lysosomes or processing enzyme delivery to phagosomes by autophagosomes.

While an extensive body of evidence points toward a role of autophagy pathways during antigen processing toward MHC class II presentation, an increasing amount of studies also implicates autophagy proteins in MHC class I-mediated antigen presentation toward CD8<sup>+</sup> T cells. Along these lines, late during HSV infection of macrophages, it was observed that autophagosomes engulf viral capsids at the outer nuclear membrane (English et al., 2009). Furthermore, MHC class I presentation of an endogenous human cytomegalovirus latency-associated protein, pUL138, has been found to be facilitated via two distinct modes, one being the conventional transporter associated with antigen processing (TAP)-dependent pathway while the other, independent of TAP, relies on MA (Tey and Khanna, 2012).

The majority of MHC class I presented peptides are derived from the endogenous ligand pool. The term cross-presentation is commonly applied to subsume pathways that deliver exogenous peptides for MHC class I presentation to CD8<sup>+</sup> T cells. Autophagy pathways have so far been reported to modulate antigen cross-presentation in two distinct ways. First, antigen donor cells are capable of packaging antigen and subsequent exocytosis for cross-presentation onto MHC class I molecules more efficiently via MA (Li et al., 2008; Uhl et al., 2009). Second, cross-presenting cell-intrinsic roles for autophagy pathways have been delineated. Hereto, APCs may assemble endosomal cross-presenting compartments with the assistance of autophagy proteins for efficient cross-priming of antifungal CD8<sup>+</sup> T cells (De Luca et al., 2012). Interestingly, Mintern et al. have reported that for effective MHC class I-mediated presentation of exogenous antigen in mice, the autophagy machinery is particularly important in CD8<sup>+</sup> DCs, an APC population that is known for being the prime cross-presenting subset. However, this was only true for soluble antigen but not required for cell-associated or receptor-mediated endocytosed antigen (Mintern et al., 2015).

Nevertheless, the functions of autophagy proteins during antigen presentation to CD8<sup>+</sup> T cells seem to be far from unidirectional. It has been suggested that aggregated proteins are redirected toward proteasomal degradation and MHC class I antigen processing in the absence of MA (Wenger et al., 2012). Several reports document enhanced CD8<sup>+</sup> T-cell responses in the context of DC-inherent autophagy deficiency (Hubbard-Lucey et al., 2014; Lévy et al., 2015; Loi et al., 2016). A recent study broadened the concept of noncanonical autophagy pathways during antigen presentation to the MHC class I pathway by showing that absence of ATG5 in CD11c<sup>+</sup> DCs leads to impaired internalization of MHC class I molecules from the plasma membrane. The failure in internalization was due to reduced recruitment of adaptor-associated kinase 1 to cell surface-bound MHC class I. Persistence of MHC class I molecules on the surface of DCs consequently entailed enhanced CD8<sup>+</sup> T-cell responses upon influenza A and lymphocytic choriomeningitis (LCMV) infections *in vivo* (Loi et al., 2016). Finally, autophagy proteins assist in the recruitment of AP2 to the nonpolymorphic MHC class I-like molecule CD1d. Loss of the essential autophagy protein ATG5 in CD11c<sup>+</sup> DCs hampered clathrin-dependent internalization of CD1d molecules leading to the stabilization of stimulatory CD1d:glycolipid complexes and consecutively boosted iNKT cell activation (Keller et al., 2017). Thus ATGs seem to support antigen presentation of exogenous and endogenous antigens on MHC class II molecules, while they restrict classical and nonclassical MHC class I antigen presentation via enhanced internalization.

## AUTOPHAGY IN TOLERANCE AND AUTOIMMUNITY

The first *in vivo* situation that was identified to involve MA-mediated antigen processing for MHC class II presentation is thymic T cell selection. Recognition of self-antigen-derived epitopes presented by MHC class II molecules on thymic epithelial cells (TECs) is critical for the generation of a functional and self-tolerant CD4<sup>+</sup> T-cell repertoire. TECs shape the T-cell repertoire to recognize self-peptide/MHC complexes with low affinity by a process called positive selection and weed out T cells that react to these self-structures with high affinity by negative selection (Kyewski and Klein, 2006). Even so they express MHC class II molecules to execute these functions for CD4<sup>+</sup> T cells, their endocytic capacity is low, but autophagosomes fuse also in these cells frequently with MIICs (Kasai et al., 2009). Indeed, when positive selection of TCR transgenic T cells through MA-deficient thymi was investigated, only some T-cell specificities were correctly selected, while others were deleted and loss of MA in TECs resulted in severe colitis and multiorgan inflammation (Nedjic et al., 2008). While positive selection of CD8<sup>+</sup> T cells was found to be MA independent (Nedjic et al., 2008), MA in medullary TECs also participates in clonal deletion during thymic-negative selection by facilitating the direct presentation of endogenous self-antigens (Aichinger et al., 2013). These data suggest that MA processes certain self-ligands for MHC class II

presentations that are involved in positive and negative T-cell selection in the thymus and thereby contributes to the generation of a self-tolerant T-cell repertoire.

In addition to its function in central tolerance, MA in DCs has recently been identified as one potential mechanism that underlies  $\text{Foxp3}^+$  TREG-mediated immune suppression (Alissafi et al., 2017). In the aforementioned study,  $\text{Foxp3}^+$  TREGs were shown to impair autophagolysosome formation and antigen presentation capacities in DCs in a CTLA4-dependent manner. Autophagy-deficient DCs exhibited reduced immunogenic potential and failed to prime autoantigen-specific CD4 $^+$  T cells to mediate central nervous system autoimmunity (Alissafi et al., 2017). Along these lines, mice deficient for the essential autophagy protein ATG7 in DCs and other myeloid cells are reported to develop milder experimental autoimmune encephalomyelitis, a CD4 $^+$  T cell–driven autoimmune disease model, compared with control animals (Bhattacharya et al., 2014; Kanayama et al., 2016). These data suggest that the canonical autophagy pathway in DCs is a molecular target of  $\text{Foxp3}^+$  TREG-mediated suppression that can be harnessed to suppress autoimmune responses.

Aside from its function in inducing and maintaining central and peripheral T-cell tolerance, autophagy in myeloid cells has also been linked to autoimmune tissue inflammation through its role in the removal of apoptotic cell corpses. Rapid removal of apoptotic cell material is thought to be crucial for the prevention of tissue inflammation. Efficient elimination of apoptotic bodies during programmed cell death involves exposure of Ptd-L-Ser on the surface and release of lysophosphatidylcholine (LPC) by apoptotic cells. Apoptotic cells, which fail to express ATG5 or Beclin 1 genes, do not expose Ptd-L-Ser at the cell surface and produce lower levels of LPC compared to their wild-type counterpart (Qu et al., 2007). Defective clearance of apoptotic cells has long been suggested to drive autoimmunity in patients with systemic lupus erythematosus (SLE) and other autoimmune diseases (Bratton and Henson, 2005). Absence of LAP in myeloid cells by lysozyme M-Cre-mediated gene deletion, which targets macrophages, monocytes, some neutrophils, and cDCs, was recently shown to lead to development of a systemic autoinflammatory syndrome in mice with increased expression of IFN signature genes, occurrence of antididouble-stranded DNA and nuclear antibodies, and signs of kidney damage, commonly associated with SLE (Martinez et al., 2016). The aforementioned study showed that macrophages deficient in ATG7 or Rubicon, both required for ATG-dependent phagocytosis, did engulf, but not effectively clear, dying cells and produced proinflammatory cytokines, including IL-1 $\beta$  and IL-6, upon challenge with apoptotic cell material (Martinez et al., 2016). An increased propensity of ATG7 $^{-/-}$  macrophages to produce proinflammatory cytokines after engulfment of apoptotic cells has also been described in vitro (Martinez et al., 2011) indicating that defective ATG-dependent phagocytosis in macrophages can result in a failure to digest engulfed dying cells, leading to elevated inflammatory cytokine production and the development of a lupus-like syndrome.

Although the autophagy machinery has been implicated in almost every immunological-driven response, its role in the development of autoimmune diseases is still incompletely understood. Genetic deficiencies of essential autophagy proteins in myeloid cells have been shown to either ameliorate or drive autoimmune responses and disease phenotypes (Alissafi et al., 2017; Martinez et al., 2016). These seemingly contradictory effects suggest that autophagy protein functions are likely cell- and context-dependent. Future research will dissect the involvement of individual autophagy proteins and pathways in human autoimmune diseases and disease models. A clear and comprehensive concept of how the autophagy machinery couples to autoimmune disease development and progression appears to be required to predict the outcome of therapeutic interventions in this pathway.

## References

- Aichinger, M., et al., 2013. Macroautophagy substrates are loaded onto MHC class II of medullary thymic epithelial cells for central tolerance. *J. Exp. Med.* 210 (2), 287–300.
- Alissafi, T., et al., 2017. Tregs restrain dendritic cell autophagy to ameliorate autoimmunity. *J. Clin. Invest.* 127 (7), 2789–2804.
- Arsov, I., et al., 2008. BAC-mediated transgenic expression of fluorescent autophagic protein Beclin 1 reveals a role for Beclin 1 in lymphocyte development. *Cell Death Differ.* 15 (9), 1385–1395.
- Arsov, I., et al., 2011. A role for autophagic protein beclin 1 early in lymphocyte development. *J. Immunol.* 186 (4), 2201–2209.
- Ashford, T.P., Porter, K.R., 1962. Cytoplasmic components in hepatic cell lysosomes. *J. Cell. Biol.* 12 (1), 198–202.
- Axe, E.L., et al., 2008. Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. *J. Cell. Biol.* 182 (4), 685–701.
- Benjamin, J.L., et al., 2013. Intestinal epithelial autophagy is essential for host defense against invasive bacteria. *Cell Host Microbe.* 13 (6), 723–734.
- Bhattacharya, A., et al., 2014. Deficiency of autophagy in dendritic cells protects against experimental autoimmune encephalomyelitis. *J. Biol. Chem.* 289 (38), 26525–26532.
- Bratton, D.L., Henson, P.M., 2005. Autoimmunity and apoptosis: refusing to go quietly. *Nat. Med.* 11 (1), 26–27.

- Brazil, M.I., Weiss, S., Stockinger, B., 1997. Excessive degradation of intracellular protein in macrophages prevents presentation in the context of major histocompatibility complex class II molecules. *Eur. J. Immunol.* 27 (6), 1506–1514.
- Codogno, P., Mehrpour, M., Proikas-Cezanne, T., 2011. Canonical and non-canonical autophagy: variations on a common theme of self-eating? *Nat. Rev. Mol. Cell Biol.* 13 (1), 7–12.
- Comber, J.D., et al., 2011. Functional macroautophagy induction by influenza A virus without a contribution to major histocompatibility complex class II-restricted presentation. *J. Virol.* 85 (13), 6453–6463.
- Coulon, P.-G., et al., 2016. HIV-infected dendritic cells present endogenous MHC class II-restricted antigens to HIV-specific CD4+ T cells. *J. Immunol.* 197 (2), 517–532.
- Delamarre, L., Pack, M., Chang, H., Mellman, I., Trombetta, E.S., 2005. Differential lysosomal proteolysis in antigen-presenting cells determines antigen fate. *Science* 307 (5715), 1630–1634.
- Delgado, M.A., et al., 2008. Toll-like receptors control autophagy. *EMBO J.* 27 (7), 1110–1121.
- De Luca, A., et al., 2012. CD4(+) T cell vaccination overcomes defective cross-presentation of fungal antigens in a mouse model of chronic granulomatous disease. *J. Clin. Invest.* 122 (5), 1816–1831.
- Dengjel, J., et al., 2005. Autophagy promotes MHC class II presentation of peptides from intracellular source proteins. *Proc. Natl. Acad. Sci. U. S.A.* 102 (22), 7922–7927.
- de Reuck, A.V.S., Cameron, M.P. (Eds.), 1963. Ciba Foundation Symposium—Lysosomes. John Wiley & Sons, Ltd., Chichester, UK.
- Dooley, H.C., et al., 2014. WIPI2 links LC3 conjugation with PI3P, autophagosome formation, and pathogen clearance by recruiting Atg12-5-16L1. *Mol. Cell* 55 (2), 238–252.
- Dupont, N., et al., 2011. Autophagy-based unconventional secretory pathway for extracellular delivery of IL-1 $\beta$ . *EMBO J.* 30 (23), 4701–4711.
- Efeyan, A., Comb, W.C., Sabatini, D.M., 2015. Nutrient-sensing mechanisms and pathways. *Nature* 517 (7534), 302–310.
- Egan, D.F., Shackelford, D.B., Mihaylova, M.M., Gelino, S., Kohnz, R.A., Mair, W., 2011. Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science* 331 (6016), 456–461.
- English, L., et al., 2009. Autophagy enhances the presentation of endogenous viral antigens on MHC class I molecules during HSV-1 infection. *Nat. Immunol.* 10 (5), 480–487.
- Fang, D., et al., 2002. Dysregulation of T lymphocyte function in itchy mice: a role for Itch in TH2 differentiation. *Nat. Immunol.* 3 (3), 281–287.
- Farré, J.-C., Subramani, S., 2016. Mechanistic insights into selective autophagy pathways: lessons from yeast. *Nat. Rev. Mol. Cell Biol.* 17 (9), 537–552.
- Feng, Y., et al., 2014. The machinery of macroautophagy. *Cell Res.* 24 (1), 24–41.
- Florey, O., et al., 2011. Autophagy machinery mediates macroendocytic processing and entotic cell death by targeting single membranes. *Nat. Cell Biol.* 13 (11), 1335–1343.
- Fonteneau, J.F., et al., 2016. The tumor antigen NY-ESO-1 mediates direct recognition of melanoma cells by CD4+ T cells after intercellular antigen transfer. *J. Immunol.* 196 (1), 64–71.
- Fujita, N., Hayashi-Nishino, M., et al., 2008a. An Atg4B mutant hampers the lipidation of LC3 paralogues and causes defects in autophagosome closure. *Mol. Biol. Cell* 19 (11), 4651–4659.
- Fujita, N., Itoh, T., et al., 2008b. The Atg16L complex specifies the site of LC3 lipidation for membrane biogenesis in autophagy. *Mol. Biol. Cell* 19 (5), 2092–2100.
- Fujita, K.-I., et al., 2011. Nrf2-mediated induction of p62 controls Toll-like receptor-4-driven aggresome-like induced structure formation and autophagic degradation. *Proc. Natl. Acad. Sci. U.S.A.* 108 (4), 1427–1432.
- Galluzzi, L., et al., 2017. Molecular definitions of autophagy and related processes. *EMBO J.* 36 (13), 1811–1836.
- Ganley, I.G., et al., 2009. ULK1.ATG13.FIP200 complex mediates mTOR signaling and is essential for autophagy. *J. Biol. Chem.* 284 (18), 12297–12305.
- Geng, J., Klionsky, D.J., 2008. The Atg8 and Atg12 ubiquitin-like conjugation systems in macroautophagy. “Protein modifications: beyond the usual suspects” review series. *EMBO Rep.* 9 (9), 859–864.
- Godfrey, D.I., et al., 2015. The burgeoning family of unconventional T cells. *Nat. Immunol.* 16 (11), 1114–1123.
- Gutierrez, M.G., et al., 2004. Autophagy is a defense mechanism inhibiting BCG and *Mycobacterium tuberculosis* survival in infected macrophages. *Cell* 119 (6), 753–766.
- Hailey, D.W., et al., 2010. Mitochondria supply membranes for autophagosome biogenesis during starvation. *Cell* 141 (4), 656–667.
- Haq, K., McElhaney, J.E., 2014. Immunosenescence: influenza vaccination and the elderly. *Curr. Opin. Immunol.* 29, 38–42.
- Harris, J., et al., 2007. T helper 2 cytokines inhibit autophagic control of intracellular *Mycobacterium tuberculosis*. *Immunity* 27 (3), 505–517.
- Hayashi-Nishino, M., et al., 2009. A subdomain of the endoplasmic reticulum forms a cradle for autophagosome formation. *Nat. Cell Biol.* 11 (12), 1433–1437.
- Hemelaar, J., et al., 2003. A single protease, Apg4B, is specific for the autophagy-related ubiquitin-like proteins GATE-16, MAP1-LC3, GABARAP, and Apg8L. *J. Biol. Chem.* 278 (51), 51841–51850.
- Henault, J., et al., 2012. Noncanonical autophagy is required for type I interferon secretion in response to DNA-immune complexes. *Immunity* 37 (6), 986–997.
- Hubbard-Lucey, V.M., et al., 2014. Autophagy gene Atg16L1 prevents lethal T cell alloreactivity mediated by dendritic cells. *Immunity* 41 (4), 579–591.
- Ichimura, Y., et al., 2000. A ubiquitin-like system mediates protein lipidation. *Nature* 408 (6811), 488–492.
- Ireland, J.M., Unanue, E.R., 2011. Autophagy in antigen-presenting cells results in presentation of citrullinated peptides to CD4 T cells. *J. Exp. Med.* 208 (13), 2625–2632.
- Itakura, E., Mizushima, N., 2010. Characterization of autophagosome formation site by a hierarchical analysis of mammalian Atg proteins. *Autophagy* 6 (6), 764–776.
- Jacobson, S., et al., 1989. HLA class II-restricted presentation of cytoplasmic measles virus antigens to cytotoxic T cells. *J. Virol.* 63 (4), 1756–1762.

- Jia, W., et al., 2011. Autophagy regulates endoplasmic reticulum homeostasis and calcium mobilization in T lymphocytes. *J. Immunol.* 186 (3), 1564–1574.
- Jin, Y., et al., 2014. Regulation of SIV antigen-specific CD4+ T cellular immunity via autophagosome-mediated MHC II molecule-targeting antigen presentation in mice. *PLoS One* 9 (3), e93143.
- Josefowicz, S.Z., Lu, L.-F., Rudensky, A.Y., 2012. Regulatory T cells: mechanisms of differentiation and function. *Annu. Rev. Immunol.* 30 (1), 531–564.
- Kabat, A.M., et al., 2016. The autophagy gene Atg16l1 differentially regulates Treg and TH2 cells to control intestinal inflammation. *eLife* 5, e12444.
- Kabeya, Y., et al., 2000. LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. *EMBO J.* 19 (21), 5720–5728.
- Kabeya, Y., et al., 2004. LC3, GABARAP and GATE16 localize to autophagosomal membrane depending on form-II formation. *J. Cell. Sci.* 117 (Pt 13), 2805–2812.
- Kanayama, M., et al., 2016. Lung inflammation stalls Th17-cell migration en route to the central nervous system during the development of experimental autoimmune encephalomyelitis. *Int. Immunol.* 28 (9), 463–469.
- Karanasios, E., et al., 2016. Autophagy initiation by ULK complex assembly on ER tubulovesicular regions marked by ATG9 vesicles. *Nat. Commun.* 7, 12420.
- Kasai, M., Tanida, I., Ueno, T., Kominami, E., Seki, S., Ikeda, T., et al., 2009. Autophagic compartments gain access to the MHC class II compartments in thymic epithelium. *J. Immunol.* 183 (11), 7278–7285.
- Keller, C.W., et al., 2011. TNF-alpha induces macroautophagy and regulates MHC class II expression in human skeletal muscle cells. *J. Biol. Chem.* 286 (5), 3970–3980.
- Keller, C.W., et al., 2017. The autophagy machinery restrains iNKT cell activation through CD1D1 internalization. *Autophagy* 13, 1025–1036.
- Kim, J., et al., 2011. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat. Cell Biol.* 13 (2), 132–141.
- Kim, J.K., et al., 2015. MicroRNA-125a inhibits autophagy activation and antimicrobial responses during mycobacterial infection. *J. Immunol.* 194 (11), 5355–5365.
- Kimura, S., Noda, T., Yoshimori, T., 2007. Dissection of the autophagosome maturation process by a novel reporter protein, tandem fluorescent-tagged LC3. *Autophagy* 3 (5), 452–460.
- Klionsky, D.J., 2014. Autophagy revisited: a conversation with Christian de Duve. *Autophagy* 4 (6), 740–743.
- Kovacs, J.R., et al., 2012. Autophagy promotes T-cell survival through degradation of proteins of the cell death machinery. *Cell Death Differ.* 19 (1), 144–152.
- Kreibich, S., et al., 2015. Autophagy proteins promote repair of endosomal membranes damaged by the *Salmonella* type three secretion system 1. *Cell Host Microbe* 18 (5), 527–537.
- Kyewski, B., Klein, L., 2006. A central role for central tolerance. *Annu. Rev. Immunol.* 24, 571–606.
- Lee, H.K., Lund, J.M., Ramanathan, B., Mizushima, N., Iwasaki, A., 2007. Autophagy-dependent viral recognition by plasmacytoid dendritic cells. *Science* 315 (5817), 1398–1401.
- Lee, H.K., et al., 2010. In vivo requirement for Atg5 in antigen presentation by dendritic cells. *Immunity* 32 (2), 227–239.
- Leung, C.S., et al., 2010. Nuclear location of an endogenously expressed antigen, EBNA1, restricts access to macroautophagy and the range of CD4 epitope display. *Proc. Natl. Acad. Sci. U.S.A.* 107 (5), 2165–2170.
- Lévy, J., et al., 2015. Intestinal inhibition of Atg7 prevents tumour initiation through a microbiome-influenced immune response and suppresses tumour growth. *Nat. Cell Biol.* 17 (8), 1062–1073.
- Li, Y., et al., 2008. Efficient cross-presentation depends on autophagy in tumor cells. *Cancer Res.* 68 (17), 6889–6895.
- Loi, M., et al., 2016. Macroautophagy proteins control MHC class I levels on dendritic cells and shape anti-viral CD8(+) T cell responses. *Cell Rep.* 15 (5), 1076–1087.
- Ma, J., et al., 2012. Dectin-1-triggered recruitment of light chain 3 protein to phagosomes facilitates major histocompatibility complex class II presentation of fungal-derived antigens. *J. Biol. Chem.* 287 (41), 34149–34156.
- Ma, J., et al., 2014. Cutting edge: FYCO1 recruitment to dectin-1 phagosomes is accelerated by light chain 3 protein and regulates phagosome maturation and reactive oxygen production. *J. Immunol.* 192 (4), 1356–1360.
- Mari, M., et al., 2010. An Atg9-containing compartment that functions in the early steps of autophagosome biogenesis. *J. Cell. Biol.* 190 (6), 1005–1022.
- Martinez, J., et al., 2011. Microtubule-associated protein 1 light chain 3 alpha (LC3)-associated phagocytosis is required for the efficient clearance of dead cells. *Proc. Natl. Acad. Sci. U.S.A.* 108 (42), 17396–17401.
- Martinez, J., et al., 2015. Molecular characterization of LC3-associated phagocytosis reveals distinct roles for Rubicon, NOX2 and autophagy proteins. *Nat. Cell Biol.* 17 (7), 893–906.
- Martinez, J., et al., 2016. Noncanonical autophagy inhibits the autoinflammatory, lupus-like response to dying cells. *Nature* 533 (7601), 115–119.
- Matsunaga, K., et al., 2010. Autophagy requires endoplasmic reticulum targeting of the PI3-kinase complex via Atg14L. *J. Cell. Biol.* 190 (4), 511–521.
- Matzarakis, V., et al., 2017. The MHC locus and genetic susceptibility to autoimmune and infectious diseases. *Genome. Biol.* 18 (1), 76.
- Miller, B.C., et al., 2008. The autophagy gene ATG5 plays an essential role in B lymphocyte development. *Autophagy* 4 (3), 309–314.
- Mintern, J.D., et al., 2015. Differential use of autophagy by primary dendritic cells specialized in cross-presentation. *Autophagy* 11 (6), 906–917.
- Mizushima, N., et al., 2001. Dissection of autophagosome formation using Apg5-deficient mouse embryonic stem cells. *J. Cell. Biol.* 152 (4), 657–668.
- Mizushima, N., Ohsumi, Y., Yoshimori, T., 2002. Autophagosome formation in mammalian cells. *Cell Struct. Funct.* 27 (6), 421–429.
- Mizushima, N., Yoshimori, T., Ohsumi, Y., 2011. The role of Atg proteins in autophagosome formation. *Annu. Rev. Cell. Dev. Biol.* 27 (1), 107–132.

- Mortensen, M., et al., 2011. The autophagy protein Atg7 is essential for hematopoietic stem cell maintenance. *J. Exp. Med.* 208 (3), 455–467.
- Münz, C., 2015. Of LAP, CUPS, and DRibbles—unconventional use of autophagy proteins for MHC restricted antigen presentation. *Front. Immunol.* 6, 200.
- Münz, C., 2016a. Autophagy beyond intracellular MHC class II antigen presentation. *Trends Immunol.* 37 (11), 755–763.
- Münz, C., 2016b. Autophagy proteins in antigen processing for presentation on MHC molecules. *Immunol. Rev.* 272 (1), 17–27.
- Munz, C., et al., 2000. Human CD4(+) T lymphocytes consistently respond to the latent Epstein–Barr virus nuclear antigen EBNA1. *J. Exp. Med.* 191 (10), 1649–1660.
- Nair, U., et al., 2010. Roles of the lipid-binding motifs of Atg18 and Atg21 in the cytoplasm to vacuole targeting pathway and autophagy. *J. Biol. Chem.* 285 (15), 11476–11488.
- Nakagawa, I., et al., 2004. Autophagy defends cells against invading group A *Streptococcus*. *Science (New York, N.Y.)* 306 (5698), 1037–1040.
- Nakatogawa, H., Ichimura, Y., Ohsumi, Y., 2007. Atg8, a ubiquitin-like protein required for autophagosome formation, mediates membrane tethering and hemifusion. *Cell* 130 (1), 165–178.
- Nedjic, J., et al., 2008. Autophagy in thymic epithelium shapes the T-cell repertoire and is essential for tolerance. *Nature* 455 (7211), 396–400.
- Nimmerjahn, F., et al., 2003. Major histocompatibility complex class II-restricted presentation of a cytosolic antigen by autophagy. *Eur. J. Immunol.* 33 (5), 1250–1259.
- Nishimura, T., et al., 2013. FIP200 regulates targeting of Atg16L1 to the isolation membrane. *EMBO Rep.* 14 (3), 284–291.
- Nuchtern, J.G., Biddison, W.E., Klausner, R.D., 1990. Class II MHC molecules can use the endogenous pathway of antigen presentation. *Nature* 343 (6253), 74–76.
- Obara, K., et al., 2008. The Atg18-Atg2 complex is recruited to autophagic membranes via phosphatidylinositol 3-phosphate and exerts an essential function. *J. Biol. Chem.* 283 (35), 23972–23980.
- Ogawa, M., et al., 2005. Escape of intracellular *Shigella* from autophagy. *Science (New York, N.Y.)* 307 (5710), 727–731.
- Orvedahl, A., et al., 2007. HSV-1 ICP34.5 confers neurovirulence by targeting the Beclin 1 autophagy protein. *Cell Host Microbe.* 1 (1), 23–35.
- Paludan, C., Schmid, D., Landthaler, M., Vockerodt, M., Kube, D., Tuschl, T., et al., 2005. Endogenous MHC class II processing of a viral nuclear antigen after autophagy. *Science* 307 (5709), 593–596.
- Paul, S., et al., 2012. Selective autophagy of the adaptor protein Bcl10 modulates T cell receptor activation of NF- $\kappa$ B. *Immunity* 36 (6), 947–958.
- Pilli, M., et al., 2012. TBK-1 promotes autophagy-mediated antimicrobial defense by controlling autophagosome maturation. *Immunity* 37 (2), 223–234.
- Pua, H.H., et al., 2007. A critical role for the autophagy gene Atg5 in T cell survival and proliferation. *J. Exp. Med.* 204 (1), 25–31.
- Puleston, D.J., et al., 2014. Autophagy is a critical regulator of memory CD8(+) T cell formation. *eLife* 3, 2516.
- Qu, X., et al., 2007. Autophagy gene-dependent clearance of apoptotic cells during embryonic development. *Cell* 128 (5), 931–946.
- Rammensee, H., et al., 1999. SYFPEITHI: database for MHC ligands and peptide motifs. *Immunogenetics* 50 (3–4), 213–219.
- Ravikumar, B., et al., 2010. Plasma membrane contributes to the formation of pre-autophagosomal structures. *Nat. Cell Biol.* 12 (8), 747–757.
- Riedel, A., et al., 2008. Endogenous presentation of a nuclear antigen on MHC class II by autophagy in the absence of CRM1-mediated nuclear export. *Eur. J. Immunol.* 38 (8), 2090–2095.
- Romao, S., Münz, C., 2014. LC3-associated phagocytosis. *Autophagy* 10 (3), 526–528.
- Romao, S., et al., 2013. Autophagy proteins stabilize pathogen-containing phagosomes for prolonged MHC II antigen processing. *J. Cell. Biol.* 203 (5), 757–766.
- Ruland, J., et al., 2001. Bcl10 is a positive regulator of antigen receptor-induced activation of NF- $\kappa$ B and neural tube closure. *Cell* 104 (1), 33–42.
- Salemi, S., et al., 2012. Autophagy is required for self-renewal and differentiation of adult human stem cells. *Cell Res.* 22 (2), 432–435.
- Salio, M., et al., 2014. Essential role for autophagy during invariant NKT cell development. *Proc. Natl. Acad. Sci. U.S.A.* 111 (52), E5678–E5687.
- Sanjuan, M.A., et al., 2007. Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. *Nature* 450 (7173), 1253–1257.
- Schmid, D., Pypaert, M., Münz, C., 2007. Antigen-loading compartments for major histocompatibility complex class II molecules continuously receive input from autophagosomes. *Immunity* 26 (1), 79–92.
- Shibutani, S.T., Yoshimori, T., 2014. A current perspective of autophagosome biogenesis. *Cell Res.* 24 (1), 58–68.
- Shibutani, S.T., et al., 2015. Autophagy and autophagy-related proteins in the immune system. *Nat. Immunol.* 16 (10), 1014–1024.
- Singh, S.B., et al., 2006. Human IRGM induces autophagy to eliminate intracellular mycobacteria. *Science (New York, N.Y.)* 313 (5792), 1438–1441.
- Sou, Y.-S., et al., 2008. The Atg8 conjugation system is indispensable for proper development of autophagic isolation membranes in mice. *Mol. Biol. Cell* 19 (11), 4762–4775.
- Stephenson, L.M., et al., 2009. Identification of Atg5-dependent transcriptional changes and increases in mitochondrial mass in Atg5-deficient T lymphocytes. *Autophagy* 5 (5), 625–635.
- Suri, A., et al., 2008. First signature of islet beta-cell-derived naturally processed peptides selected by diabetogenic class II MHC molecules. *J. Immunol.* 180 (6), 3849–3856.
- Suzuki, H., et al., 2016. Structural biology of the core autophagy machinery. *Curr. Opin. Struct. Biol.* 43, 10–17.
- Tanida, I., et al., 2004. HsAtg4B/HsApg4B/autophagin-1 cleaves the carboxyl termini of three human Atg8 homologues and delipidates microtubule-associated protein light chain 3- and GABA<sub>A</sub> receptor-associated protein-phospholipid conjugates. *J. Biol. Chem.* 279 (35), 36268–36276.
- Tey, S.-K., Khanna, R., 2012. Autophagy mediates transporter associated with antigen processing-independent presentation of viral epitopes through MHC class I pathway. *Blood* 120 (5), 994–1004.
- Thurston, T.L.M., et al., 2012. Galectin 8 targets damaged vesicles for autophagy to defend cells against bacterial invasion. *Nature* 482 (7385), 1–6.
- Trombetta, E.S., Mellman, I., 2005. Cell biology of antigen processing in vitro and in vivo. *Annu. Rev. Immunol.* 23 (1), 975–1028.

- Tsukada, M., Ohsumi, Y., 1993. Isolation and characterization of autophagy-defective mutants of *Saccharomyces cerevisiae*. FEBS Lett. 333 (1–2), 169–174.
- Uhl, M., et al., 2009. Autophagy within the antigen donor cell facilitates efficient antigen cross-priming of virus-specific CD8+ T cells. Cell Death Differ. 16 (7), 991–1005.
- Valdor, R., et al., 2014. Chaperone-mediated autophagy regulates T cell responses through targeted degradation of negative regulators of T cell activation. Nat. Immunol. 15 (11), 1046–1054.
- Van Grol, J., et al., 2010. HIV-1 inhibits autophagy in bystander macrophage/monocytic cells through Src-Akt and STAT3. PLoS One 5 (7), e11733.
- Velikkakath, A.K.G., et al., 2012. Mammalian Atg2 proteins are essential for autophagosome formation and important for regulation of size and distribution of lipid droplets. Mol. Biol. Cell 23 (5), 896–909.
- Wei, J., et al., 2016. Autophagy enforces functional integrity of regulatory T cells by coupling environmental cues and metabolic homeostasis. Nat. Immunol. 17 (3), 277–285.
- Weidberg, H., et al., 2010. LC3 and GATE-16/GABARAP subfamilies are both essential yet act differently in autophagosome biogenesis. EMBO J. 29 (11), 1792–1802.
- Wenger, T., et al., 2012. Autophagy inhibition promotes defective neosynthesized proteins storage in ALIS, and induces redirection toward proteasome processing and MHCI-restricted presentation. Autophagy 8 (3), 350–363.
- Wild, P., et al., 2011. Phosphorylation of the autophagy receptor optineurin restricts *Salmonella* growth. Science (New York, N.Y.) 333 (6039), 228–233.
- Xu, Y., et al., 2007. Toll-like receptor 4 is a sensor for autophagy associated with innate immunity. Immunity 27 (1), 135–144.
- Xu, X., et al., 2014. Autophagy is essential for effector CD8(+) T cell survival and memory formation. Nat. Immunol. 15 (12), 1152–1161.
- Ylä-Anttila, P., et al., 2009. 3D tomography reveals connections between the phagophore and endoplasmic reticulum. Autophagy 5 (8), 1180–1185.
- Yoshikawa, Y., et al., 2009. *Listeria monocytogenes* ActA-mediated escape from autophagic recognition. Nat. Cell Biol. 11 (10), 1233–1240.
- Zeng, H., et al., 2013. mTORC1 couples immune signals and metabolic programming to establish T(reg)-cell function. Nature 499 (7459), 485–490.
- Zhang, M., et al., 2015. Translocation of interleukin-1 $\beta$  into a vesicle intermediate in autophagy-mediated secretion. eLife 4, 1463.
- Zhong, Z., et al., 2016. NF- $\kappa$ B restricts inflammasome activation via elimination of damaged mitochondria. Cell 164 (5), 896–910.

## Further Reading

- Klionsky, D.J., 2007. Autophagy: from phenomenology to molecular understanding in less than a decade. Nat. Rev. Mol. Cell Biol. 8 (11), 931–937.
- Mizushima, N., Klionsky, D.J., 2007. Protein turnover via autophagy: implications for metabolism. Annu. Rev. Nutr. 27 (1), 19–40.
- Mizushima, N., Komatsu, M., 2011. Autophagy: renovation of cells and tissues. Cell 147 (4), 728–741.
- Saitoh, T., et al., 2008. Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1 $\beta$  production. Nature 456 (7219), 264–268.

## Effector Mechanisms in Autoimmunity

Arian Laurence<sup>1</sup> and Martin Aringer<sup>2</sup>

<sup>1</sup>Department of Haematology, University College London Hospitals NHS Trust, London, United Kingdom

<sup>2</sup>University Medical Center Carl Gustav Carus, Dresden, Germany

### OUTLINE

Introduction	319	Natural Killer Cells and Cytotoxic T Cells	322
Autoantibodies	319	Effector T-Helper Cell–Mediated Autoimmune Disease	323
Direct Antibody-Mediated Disease	320	Innate Lymphoid Cells	324
Immune Complex Disease	321	Effector Cytokines and Their Targets	325
Complement Cascades	321	Conclusions	326
Macrophages	322	References	326
Neutrophils	322		
Mast Cells	322		

### INTRODUCTION

In the 1960s pathological reactions produced by an otherwise healthy immune system were subdivided into four groups on the basis of the effector mechanism initiated. More recent advances in our understanding of cellular immunology have led to less prominent use, and critique, of the Gell and Coombs classification system (Rajan, 2003), which cannot be upheld as such in the light of today's knowledge. Nevertheless, it remains a useful introductory way of characterizing some aspects of autoimmune effector mechanisms. Of the four types of "hypersensitivity reactions," type I represented "conventional" allergic and anaphylactic responses, while the other three contribute to *bona fide* autoimmune pathology.

### AUTOANTIBODIES

Autoantibodies are a hallmark of autoimmune disease (Tan, 1991). In fact, their presence is so common in autoimmunity that autoinflammatory disease, which does not involve specific immune recognition, is principally defined by the absence of autoantibodies (Ombrello and Kastner, 2011) (see Chapter 4: Innate and Adaptive Systems of Immunity). In most cases, autoantibodies are important for diagnostic purposes and may not directly influence the outcome of the disease. However, an increasing number of autoantibodies have been directly linked to inflammation, cell death, or functional problems. Antibody-mediated autoimmune disease is distinguished

**TABLE 18.1** Variable Effects of Well-Defined Autoantibodies

Antibody to	Disease	Function of antibody
Acetylcholine receptors	Myasthenia gravis	Receptor blockade
Aquaporin-4	Neuromyelitis optica	Cytotoxicity
Beta-2 glycoprotein I	Antiphospholipid syndrome	Activation of coagulation
Desmogleins	Pemphigus vulgaris	Binding function blocked
Factor VIII	Acquired hemophilia	Coagulation factor removal
Glycoprotein IIb/IIIa	Immune thrombocytopenic purpura	Thrombocyte clearance
Thyrotropin receptor	Graves' disease	Receptor stimulation

into two groups by the Gell Coombs classification system. Type II hypersensitivity reactions are mediated by a direct effect of the antibody binding its target antigen; examples of pathogenic autoantibody effects are listed in **Table 18.1**. By contrast, type III hypersensitivity reactions are mediated by the deposition of immune complexes in affected tissues, which will almost always result in inflammation, as discussed below.

## DIRECT ANTIBODY-MEDIATED DISEASE

Type II hypersensitivity reactions mainly lead to cell death and removal or disturb physiological functions (**Table 18.1**).

Autoantibodies leading to cell death or cell removal include those against erythrocyte membranes in hemolytic anemia, against various, mostly uncharacterized, antigens in the other systemic lupus erythematosus (SLE) cytopenias, or, presumably, against endothelial cell antigens in systemic sclerosis. These autoantibodies follow typical antibody function patterns that include antibody-dependent cell mediated cytotoxicity (ADCC) where the antibody recruits an immune (natural killer, NK) cell to induce cells to kill or phagocytose any target that is marked by an antibody. Alternatively, antibodies may destroy their target by recruiting components of the complement system. Essentially, all these antibodies are of particular IgG subclasses, and the specific IgG subclass is relevant for their properties. For example, IgG1 and IgG3 activate complement, while IgG2 is a poor complement activator, and IgG4 does not activate complement at all ([Daha et al., 2011](#)). The same classes that activate complement, that is, IgG1 and IgG3, also avidly bind the activating Fc receptor Fc $\gamma$ R1 ([Nimmerjahn and Ravetch, 2010](#)) and mediate ADCC.

Blood cells marked by antibodies will be phagocytosed in the spleen (and liver) and cleared ([Cines and Blanchette, 2002](#); [Gehrs and Friedberg, 2002](#)), while immobile cells, such as endothelial cells to which antibodies bind in systemic sclerosis, may be killed by cytotoxic cells ([Sgong et al., 1996](#)). While the former mechanism is usually obvious and can be easily proven, for example by recording clinical improvement following removal of autoantibodies ([American College of Rheumatology Ad Hoc Committee, 2004](#)), ADCC can often not be proven *in vivo* and is even difficult to prove in an *in vitro* situation. Nevertheless, the latter most probably is the more common process.

In addition to marking cells for ADCC or phagocytosis, autoantibodies can also cause significant pathology by functionally disturbing physiological processes. Important examples of this type of autoantibody-induced mechanism are antibodies to proteins and glycoproteins, such as to factor VIII ([Zeitler et al., 2013](#)) in acquired hemophilia or antibodies to  $\beta$ 2-glycoprotein I, which induce thrombotic events in the antiphospholipid antibody syndrome ([Giannakopoulos and Krilis, 2013](#)) (see Chapter 34: Rheumatoid Arthritis and Chapter 51: Multiple Sclerosis). Autoantibodies may engage cell receptors, such as the TSH receptor in Graves' disease ([Dalan and Leow, 2012](#)) triggering their activation and so leading to thyrotoxicosis or neuromuscular acetylcholine receptors in myasthenia gravis ([Levinson, 2013](#)) preventing their activation leading to muscle weakness. They may also target channel proteins, such as aquaporin-4, which is targeted in neuromyelitis optica/Devic's disease (see Chapter 57: Hepatitis and [Papadopoulos and Verkman, 2012](#)). Other examples of antibodies impairing functions are those to desmogleins in the autoimmune blistering disease pemphigus ([Stanley and Amagai, 2006](#)), or those mediating psychosis in SLE ([Fong and Thumboo, 2010](#)).

## IMMUNE COMPLEX DISEASE

Commonly, inflammatory pathology is not induced by IgG monomers, but by immune complexes consisting of autoantigen and autoantibodies resulting in what Gell and Coombs termed a type III hypersensitivity reaction. In this case, antibody classes other than IgG play a major role. In particular, this is relevant to the pentameric IgM and the dimeric IgA, due to their crosslinking capabilities. Immune complexes will strongly activate complement and will be recognized by Fc receptors, thus causing inflammation.

IgM and IgA rheumatoid factors (RFs) are characteristic for active seropositive rheumatoid arthritis. RFs bind to the Fc portion of IgG and in this way induce immune complexes, which likely play a major role in the pathophysiology of rheumatoid arthritis (RA). In fact, the complement system is clearly activated in rheumatic joints (Olmez et al., 1991). Moreover, beyond disease activity, high titer RFs are associated with incurring damage (Aletaha et al., 2012).

Immune complexes also play a major role in organ inflammation in systemic autoimmune diseases, and in SLE in particular. For example, immune complexes of double-stranded DNA (dsDNA) and anti-dsDNA antibodies lead to proliferative glomerulonephritis. These antibodies can directly be detected in glomeruli. However, rather than being deposited from the blood, they may form locally, by antibodies to nucleosomes being caught at the negatively charged glomerular basement membrane (Mjelle et al., 2011; Yung and Chan, 2008).

Similarly, antibodies to the nuclear antigen Ro/SS-A are associated with subacute cutaneous lupus erythematosus (Sontheimer, 2005), where, likewise, immune complexes can be directly detected in the affected skin. While anti-neutrophil cytoplasmic antibodies (ANCA)-associated vasculitides are not immune complex driven, lupus vasculitis is, as are RA vasculitis, cryoglobulinemic vasculitis, IgA vasculitis (Henoch–Schönlein disease), or hypocomplementemic urticarial vasculitis (Jennette et al., 2013).

## COMPLEMENT CASCADES

Immune complexes, as well as most antibodies binding to surfaces, activate the complement cascade via the classical pathway (see Chapter 15: Cytokines, their Receptors and Signals and Daha et al., 2011). Binding occurs via complement component C1, leading to cleavage of C4 and C2 (Walport, 2001). The activated larger cleavage products C4b and C2a form the convertase for C3, the central complement of the downstream complement cascade, which then leads to the activation of C5 and the membrane attack complex consisting of C5b, C6, 7, 8, and 9. In addition to formation of the membrane attack complex, C1q, C3b, and C4b bound to immune complexes facilitate phagocytosis, a process called opsonization. Mannose binding lectins form an initiating event similar to that of C1, which is not known to play a role in autoimmunity.

In contrast, the alternative complement pathway is commonly involved in autoimmune disease. This amplification pathway is started by cleavage of C3, upon which C3b has to bind to a membrane. Membrane bound C3b then binds to factor B, and together they activate the alternative C3 convertase C3bBb. Antibodies stabilizing the C3bBb complex, called nephritic factor, are associated with type II dense deposit membranoproliferative glomerulonephritis as well as with partial lipodystrophy (Walport, 2001).

Cells have mechanisms to defend against occasional deposition of C3b. CD59 (membrane inhibitor of reactive lysis) protects autologous cells against the membrane attack complex, and its deficiency leads to paroxysmal nocturnal hemoglobinuria (Yamashina et al., 1990), where erythrocytes are slowly but continuously destroyed by unrestrained activation of the alternate complement pathway. In addition, the inhibitory plasma protein factor H binds to C3b, leading to its inactivation by factor I (Walport, 2001). Two membrane proteins, namely CD35 (complement receptor type I) and CD46 (membrane cofactor protein), also play a role in complement inactivation. As another option, cells may be able to eject complement bound membrane by forming microparticles, albeit at the risk of inducing immune complex disease elsewhere (Pisetsky, 2012).

The classical complement pathway is checked by C1 inhibitor, the deficiency of which causes angioedema. This is related to yet another function of the complement system: The smaller fragments C3a, C5a, and C4a act as anaphylatoxins, inducing the chemotaxis and activation of leukocytes (Walport, 2001). This may play a role in the kidney disease of ANCA-associated vasculitides, since C5a receptor deficient animals are resistant to this disease (Schreiber et al., 2009).

Finally, complement may play a role in limiting immune complex disease by aiding the clearance of immune complexes—or of apoptotic cells. Inherited deficiencies of C1–C4 are all associated with an increased incidence of SLE (Aringer et al., 2013).

## MACROPHAGES

Monocytes and macrophages are the main cells to deal with immune complexes (see Chapter 11: Dendritic Cells in Autoimmune Disease). They are equipped with both Fc receptors and complement receptors, thus simultaneously recognizing immune complexes and C5a (Karsten and Kohl, 2012). Under such circumstances, removal of immune complexes (and attached particles) becomes a highly inflammatory process. Not only will these M1 macrophages then present antigen but they will also produce a variety of proinflammatory cytokines. Immune complex recognition leads to not only the release of TNF, (Aringer and Smolen, 2012) but also other cytokines, including IL-6, IL-1, IL-8, and GM-CSF (Jarvis et al., 1997). In this way, macrophages link between immune complex deposition and inflammation. Indeed, Fc receptor deficiency was illustrated by uncoupling of immune complex deposition from glomerulonephritis in a lupus mouse model (Clynes et al., 1998). In addition to their role as essential promoters of inflammation in autoimmune complex disease, macrophages are also an important effector in autoimmune thrombocytopenia and hemolytic anemia, where antibody-loaded cells are cleared in spleen and kidney (Cines and Blanchette, 2002; Gehrs and Friedberg, 2002).

## NEUTROPHILS

As the most common leukocytes, neutrophils are prototypical effector cells of the immune system (Amulic et al., 2012). In addition to phagocytosis and degranulation, NETosis has been more recently been investigated. NETosis is a form of “aggressive” neutrophil cell death that constrains and kills pathogens; in the end, it is the physiological background of what has long been known as pus. Neutrophil extracellular traps (NETs) consist of fibrous structures that contain decondensed chromatin, histones, and antimicrobial proteins (Amulic et al., 2012). NETosis may be importantly involved in autoimmune tissue inflammation, such as in SLE (Kaplan, 2011) or in ANCA-associated vasculitides (Kessenbrock et al., 2009). Neutrophils also produce cytokines, including IL-8, IL-1, IL-17, and tumour necrosis factor (TNF), as well as chemokines (Amulic et al., 2012; Hoshino et al., 2008).

Like macrophages, neutrophils carry both Fc and complement receptors and accordingly react to antibodies and immune complexes (Hoshino et al., 2008; Nemeth and Mocsai, 2012). In addition, neutrophils are activated by high concentrations of IL-8, which in lower concentrations acts as a chemoattractant (Amulic et al., 2012). Activated platelets can also activate neutrophils (Caudrillier et al., 2012). In other inflammatory situations, Toll-like and non obese diabetic (NOD)-like receptors recognizing pathogen-associated molecular patterns and danger-associated molecular patterns play a major role (Amulic et al., 2012).

## MAST CELLS

In addition to being the principal players in allergic or type I hypersensitivity reactions, there are indications that mast cells also act as effectors in autoimmune diseases. In addition to histamine and serotonin, mast cells produce the proinflammatory cytokines IL-6 and TNF (Wesolowski and Paumet, 2011). In RA, mast cells have been found within the synovitic joint (Kiener et al., 1998), and mast cell deficient mice are protected against arthritis in the K/BxN mouse model (Lee et al., 2002). Antibodies to citrullinated peptides, which are fairly specific for rheumatoid arthritis, also exist as IgE antibodies, that are associated with mast cell activation and synovial histamine release (Schuerwagh et al., 2010). Mast cells are also activated by IgG immune complexes, a process that is potentiated by the presence of the inflammatory cytokine, IL-33 (Kaieda et al., 2012).

## NATURAL KILLER CELLS AND CYTOTOXIC T CELLS

To protect the organism, virally infected and transformed cells are actively eliminated by NK cells and CD8 + cytotoxic T lymphocytes. Cytotoxicity is principally mediated via membrane bound ligands of death receptors, such as Fas ligand, and granzyme B (Afonina et al., 2010), all of which induce apoptosis in the target cell. Extracellular granzyme B in addition has proinflammatory effects in that it enhances IL-1 $\alpha$  activity by processing this cytokine (Afonina et al., 2011). Conversely, IFN $\gamma$  and TNF made by either cell type increase Fas (CD95) expression on target cells that facilitates their destruction (Bergman and D'Elios, 2010).

Cytotoxic T cells require priming by myeloid cell-dependent antigen presentation in order to be able to kill (Sigal et al., 1999) and recognize specific peptides presented by autologous major histocompatibility complex (MHC) I molecules. These cells are implicated in *Helicobacter pylori*–related gastric autoimmunity (Bergman and D’Elios, 2010), type I diabetes mellitus (Coppieters and von Herrath, 2011), and autoimmune CNS disease (Melzer et al., 2009).

In contrast to cytotoxic T lymphocytes, NK cells need no previous activation. NK cells have receptors that have evolved in parallel with the MHC class I proteins they recognize (Parham and Moffett, 2013). This points to their function in recognizing small changes in MHC class I, which may herald an important safety issue with the afflicted cell. In addition, NK cells kill cells targeted by autoantibodies through ADCC. Whereas circulating cells covered by autoantibodies are removed in spleen and liver, ADCC is the deadly effector pathway of IgG autoantibodies that bind noncirculating cells, such as endothelial cells in systemic sclerosis (Sgouros et al., 1996). This process may thus induce tissue damage anywhere in the body, but this will be difficult to prove *in vivo*.

## EFFECTOR T-HELPER CELL–MEDIATED AUTOIMMUNE DISEASE

Whereas CD8+ cytotoxic T cells act directly to damage target tissues, CD4+ helper T cells (Th cells) drive a specific immune attack by employing a variety of additional mechanisms. For any specific immune reaction, these cells are of the utmost importance and need to be addressed when investigating effector mechanisms in autoimmune disease.

*A priori*, the immune system does not know which kind of pathogens to expect next. Therefore, naïve T cells are entirely flexible. This flexibility changes for determination in the process of activation. Like cytotoxic T cells, naïve Th cells need antigen presentation by myeloid cells in order to be able to mature into effector Th cells. These two groups of T cells mediate delayed type (type IV) hypersensitivity responses; the 24–48-hour delay is due to the required period of antigen presentation. During this process of antigen presentation, the presence of cytokines secreted by the myeloid cell or other nearby immune cell will determine what lineage of effector Th cell will be generated. These include Th1 cells, Th2 cells, and Th17 cells (Reiner, 2007; Tato and O’Shea, 2006).

Th1 cells are important for the clearance of intracellular pathogens (Infante-Duarte and Kamradt, 1999). They are induced by activation in the presence of IL-12 and are characterized by the ability to secrete IFN- $\gamma$  (Table 18.2), which activates macrophages to kill such organisms and activates NK cells and cytotoxic T lymphocytes, as well as the ability to enhance MHC expression.

Th2 cells are essential for defending mucosal and epithelial barriers against worms and parasites (Maizels et al., 2012; Paul and Zhu, 2010). They are induced in the presence of IL-4 and are characterized by the ability to secrete IL-4, IL-13, and IL-5 when activated. IL-4 stimulates IgG class switching to IgG4 and IgE, and together with IL-13 alternatively activates macrophages toward the M2 phenotype. IL-9 mobilizes mast cells, and IL-5

**TABLE 18.2** Characterization of Important Effector Cells by Their Receptors and Their Mediators

Effector cell	Important receptors (receptor chains)	Important cytokines and mediators
Monocyte	Fc $\gamma$ RI, IIa, IIb; CR1, 3, 4, C5aR	TNF, IL-6, IL-8, GM-CSF
Macrophage	Fc $\gamma$ RI, IIa, IIb, RIIIa; CR1, 3, 4, C5aR	TNF, IL-6, IL-8, GM-CSF
Neutrophil	Fc $\gamma$ RI, IIa; CR1, C3aR, C4aR, C5aR	TNF, IL-1, IL-8
Mast cell	Fc $\epsilon$ R, Fc $\gamma$ RIII; C3aR, C4aR, C5aR	Histamine, serotonin, IL-6, TNF
NK cell	NCRs; Fc $\gamma$ RIIIa; CR3, 4; KIRs	IFN- $\gamma$ , TNF, GM-CSF, IL-8, granzyme B
Cytotoxic T cell	TCR; KIRs; CTLA-4	IFN- $\gamma$ , TNF, granzyme B, perforin
Th1	TCR; IL-12R ( $\beta$ 1 + $\beta$ 2), IL-18R	IFN- $\gamma$ , IL-2, TNF
Th2	TCR; IL-4R ( $\alpha$ + $\gamma$ c)	IL-4, IL-5, IL-9, IL-13, IL-25
Th17	TCR; IL-23R (IL-12R $\beta$ 1 + IL-23R)	IL-17, GM-CSF, TGF $\beta$ , TNF

Fc $\gamma$ RI = CD64, Fc $\gamma$ RIIa = CD32, Fc $\gamma$ RIIIa = CD16 activating Fc $\gamma$  receptors, Fc $\gamma$ RIIb inhibitory Fc $\gamma$  receptor; CR1 (complement receptor 1) = CD35, CR2 = CD21, CR3 = CD11b + CD18, CR4 = CD11c + CD18; NCRs, natural cytotoxicity receptors; KIRs, killer cell immunoglobulin-like receptors; TCR, T-cell receptor; NK, natural killer.

plays the same role for eosinophils. Together, IL-4, IL-13, and IL-5 increase mucus secretion, which helps eliminating pathogens such as helminthes and other parasites. The related, newly identified lineage, Th9 cells, mainly produce IL-9 but do not produce IL-4 (Dardalhon et al., 2008). They emerge in situations where both IL-4 and TGF $\beta$  are present.

Th17 cells are highly relevant for the defense against extracellular bacteria, such as *Staphylococcus* or *Klebsiella*, as well as against *Candida* (Annunziato et al., 2009; Peters et al., 2011). They are induced in the presence of TGF $\beta$ , IL-6, and IL-23 and are characterized by the secretion of IL-17 when activated, which leads to increased neutrophil formation via G-CSF and GM-CSF, and to increased neutrophil recruitment via IL-8 and other chemokines (Zuniga et al., 2013). In addition, IL-17 stimulates the production of defensins and other antimicrobial substances. While Th17 cells also produce IL-22 that induces expression of defensin proteins in target cells, there are cells that produce IL-22, but not IL-17, and are therefore termed Th22 cells (Duhen et al., 2009). This subset is particularly prevalent among skin homing cells.

After exposure, since the immune system tries to completely eliminate any foreign invader, this process may become protracted. It is therefore not surprising that the T-helper phenotype is largely stable. This is achieved by combining several mechanisms. First, lineage-specific transcription factors that drive differentiation are stably expressed. For Th1 cells, the critical transcription factor is T-bet, for Th2 cells, it is GATA-3, and for Th17 cells ROR $\gamma$ t.

The Th1 transcription factor T-bet is induced by Stat1 and Stat4 homodimers, activated by IFN $\gamma$  and IL-12, respectively. T-bet in turn promotes IFN $\gamma$  production and represses IL-4 production, among many other effects essential for the maturation of Th1 cells (Miller and Weinmann, 2010). The Th2 transcription factor GATA-3 is induced by homodimers of Stat6, the principal signaling molecule of IL-4 and IL-13. GATA-3 induces IL-4, IL-5, IL-13, and GATA-3 itself (Wei et al., 2011). ROR $\gamma$ t is induced by homodimers of Stat3, the principal signaling molecule of IL-6, IL-21, and IL-23 at the same time T-bet and IFN $\gamma$  production are inhibited, whether by (low concentrations of) TGF $\beta$  or by other means (Hirahara et al., 2010; Peters, Lee, and Kuchroo, 2011). ROR $\gamma$ t in a protein complex that includes STAT3 induces the expression of IL-17 and the IL-23R.

In addition, there is a second level of control, namely epigenetic regulation, whereby chromatin accessibility is regulated further restricting gene expression. Global DNA methylation mapping has demonstrated that the IFN $\gamma$  locus is open in Th1, but not Th2 or Th17 cells, the IL-4 locus exclusively open in Th2 cells, and the IL-17 locus open in Th17 cells only (Wei et al., 2009). Thus epigenetic modifications are one means by which cell differentiation is kept stable during T-cell expansion, or, less fortunately, in autoimmune disease or allergy.

Although there is much experimental evidence for the role of particular lineages of Th cells in protection from infection, the role of each lineage in driving autoimmune disease is less clear. Th2-generated cytokines, such as IL-13, are associated with type I hypersensitivity reactions including asthma in mouse models (Wills-Karp, 2004) and monoclonal antibodies against IL-13 are having some success in early clinical trials. By contrast, inhibition of IFN $\gamma$ , the principle Th1 cytokine, often leads to exacerbation of disease in mouse models of multiple sclerosis (extrinsic autoimmune encephalomyelitis) and acute graft versus host disease (Teng et al., 2015). This may be in part because IFN $\gamma$  is able to inhibit Th17 cell differentiation. Cytokines that drive Th17 differentiation including IL-1, IL-6, and IL-23 are linked with autoimmune disease in mouse models as is IL-17 itself (Teng et al., 2015). Furthermore, inhibitors of all these cytokines have been successful in the treatment of a number of autoimmune diseases.

## INNATE LYMPHOID CELLS

Innate lymphoid cells (ILCs) share many of the functional properties of T lymphocytes but notably lack T-cell receptors. Like T cells they are dependent on the common  $\gamma$  chain family of cytokines for development and survival. They are activated by similar cytokines that drive Th cell lineage polarization and in turn act by expressing cytokines that are associated with Th cell lineages. This activation is not dependent on an antigen receptor and is rapid. Thus ILC “hold the fort” in the initial stages of an infection until a T-cell lineage has expanded to resolve it. For every Th cell lineage, there is an equivalent ILC, with IFN $\gamma$  expressing group 1 ILCs that mirror Th1 cells, IL-4 and IL-13 expressing group 2 ILCs that mirror Th2 cells, and IL-17 and IL-22 expressing group 3 ILCs that mirror Th17 cells (Spits et al., 2013). The contribution of ILCs to autoimmune disease is not clear, although they are capable of recruiting inflammatory cells and driving acute inflammation in the same way as Th cells and any strategy designed to block a specific Th cell lineage will likely affect the equivalent ILC lineage. Elevated numbers of ILC1 and ILC3 cells have been identified in the synovium of patients with RA. ILC2 cells are present in lung tissue and have been linked with atopic disease and inflammatory bowel disease (Shikhagaie et al., 2017).

## EFFECTOR CYTOKINES AND THEIR TARGETS

Essentially, all of the immune cells discussed produce significant amounts of cytokines. These are essential for shaping the immune system at any given time. However, some of these cytokines also have important effects on other tissues (Table 18.3), thereby directly contributing to tissue pathology in autoimmune disease. The importance of individual cytokines is highlighted by the success of anticytokine antibodies in the treatment of a number of autoimmune diseases.

TNF is a particularly well-known example in this regard. TNF is being produced by many immune cells (Table 18.2), and by monocytes and macrophages in particular. Mice transgenic for, and thus overexpressing, human TNF display peripheral and sacroiliac joint pathology indistinguishable from RA and ankylosing spondylitis, respectively (Keffer et al., 1991; Redlich et al., 2004). TNF fosters the development of osteoclast precursors from monocytes and enhances osteoclast activity, which is essential in that osteoclasts are the only cells able to digest bone (Redlich et al., 2002). Conversely, pharmacological TNF blockade blocks radiographic progression in RA even when disease activity is not adequately controlled (Smolen et al., 2006). TNF also activates fibroblasts (Vasiliopoulos et al., 2007) and endothelial cells and leads to vascular leakage (Bradley, 2008).

Since both TNF and IL-1 signal via nuclear factor- $\kappa$ B (NF- $\kappa$ B) and mitogen-activated protein kinases, it is not surprising that IL-1 in part reduplicates and further enhances such effects, leading to pronounced inflammatory reactions (Dinarello et al., 2012). Unchecked IL-1 effects in infants lacking IL-1 receptor antagonist (IL-1RA) lead to a fatal, highly inflammatory disease with pronounced skin and bone involvement, which can be effectively treated with recombinant IL-1RA (Aksentijevich et al., 2009). Acting systemically, IL-1 is essential for inducing fever by switching on cyclooxygenase in hypothalamic cells. Acting locally, IL-1 may also be the key to the death of islet cells in diabetes (Dinarello et al., 2012).

IL-6 is the third highly inflammatory cytokine that is currently targeted in the clinic. While the full IL-6 receptor is only expressed on leukocytes and hepatocytes, all other cells are able to recruit IL-6 bound soluble IL-6 receptor to the gp130 chain they express (Rose-John et al., 2006). This process is termed transsignaling. Although IL-6 signals via STAT3, which is distinct from TNF and IL-1 signaling, its *in vivo* effects are less obviously different (Nishimoto and Kishimoto, 2006). Nevertheless, IL-6 has many unique actions including the induction of C-reactive protein and other acute phase reactants, the inhibition of iron uptake and use via hepcidin. IL-6 is important in the maturation of Th17 cells in both mice and humans. Finally, IL-6 is known to influence sleep and fatigue (Rohleider et al., 2012).

Like IL-1 and IL-6, IL-23 plays a role in Th17 and group 3 ILC differentiation via the activation of STAT3. It is of note that patients with mutations that lead to excessive STAT3 activation are associated with autoimmune disease (Vogel et al., 2015). Inhibition of IL-23 using the monoclonal antibodies ustekinumab and tildrakizumab

**TABLE 18.3** Important Examples of Cytokines Targeting Cells Outside the Immune System

Cytokine (signaling pathways)	Target cell	Effects
TNF (NF- $\kappa$ B, MAPK)	Monocytes	Osteoclast formation
	Osteoclasts	Bone resorption
	Fibroblasts	Activation
IL-1 (NF- $\kappa$ B, MAPK)	Hypothalamic cells	Fever
	Pancreatic islet cells	Cell death, diabetes
IL-6 (Stat3)	Osteoblasts	Reduced bone formation
	Hepatocytes	CRP and hepcidin production
IL-17 (NF- $\kappa$ B)	Epithelial cells	Chemokine production
	Osteoblasts	RANKL expression
TGF $\beta$ (Smads, MAPK)	Fibroblasts	Extracellular matrix deposition
	Podocytes	GBM thickening

*NF- $\kappa$ B*, Nuclear factor- $\kappa$ B; *MAPK*, mitogen-activated protein kinase; *Stat3*, signal transducer and activator of transcription 3; *GBM*, glomerular basement membrane; *CRP*, C-reactive protein.

has been successful in the treatment of psoriasis, psoriatic arthritis, and Crohn's disease. The receptors for both IL-6 and IL-23 require a family of protein tyrosine kinases known as Janus kinases (JAKs) in order to activate their target cells. Two JAK inhibitors, tofacitinib and baricitinib, have been licensed for the treatment of RA and many more are under development.

IL-17 receptors are found on a variety of nonimmune cells, including epithelial cells, endothelial cells, fibroblasts, and adipocytes (Pappu et al., 2011) as well as osteoblasts (Onishi and Gaffen, 2010). Signaling via NF- $\kappa$ B, albeit less effectively than TNF (Sonder et al., 2011), IL-17 induces epithelial cells to secrete chemokines, a process of great importance in psoriasis (Onishi and Gaffen, 2010), psoriatic arthritis, and ankylosing spondylitis, where IL-17 blockade works well (Miossec and Kolls, 2012). On osteoblasts, IL-17 is able to induce the expression of receptor activator of nuclear factor kappa-B ligand (RANKL), which stimulates osteoclast differentiation (Onishi and Gaffen, 2010). IL-17 may also reduce cartilage matrix synthesis by chondrocytes (Hu et al., 2011). By contrast, IL-17 blockade is detrimental in Crohn's disease.

TGF $\beta$  has a complex role, exerting both antiinflammatory and profibrotic effects within the immune system. It is able to inhibit the expression of a number of other inflammatory cytokines and is known to inhibit Th1 and Th2 polarization, and conversely, in the presence of IL-6, it is known to induce Th17 cells in both mice and humans and in the presence of IL-4 can induce IL-9 expressing Th9 cells. Furthermore, it acts on fibroblasts to produce extracellular matrix, and thus tissue fibrosis in many organs (Pohlers et al., 2009). TGF $\beta$  has therefore been suggested as a therapeutic target for systemic sclerosis (Varga and Pasche, 2009). Similarly, TGF $\beta$  may stimulate podocytes to produce extracellular matrix, leading to glomerular basal membrane thickening (Lee, 2012).

## CONCLUSIONS

As to be expected given the time the immune system has had to evolve in an environment where pathogens of various kinds threaten the organism, its effector mechanisms are impressive and have the potential to constrain all current and future pathogens. The adaptive immune system, in particular, has developed highly specialized cells and mechanisms for a differential response. Unfortunately, all of these cells and mechanisms can in some way damage the organism when the immune system makes the error of mistaking self for a dangerous intruder. While our understanding on the mechanisms involved has deepened, and while biologicals and apheresis methods have become more successful in arresting autoimmune disease while preserving immune function, effective nontoxic cures for many autoimmune diseases remain elusive. We hope that further advances in our understanding of the immune system will continue to improve the situation in years to come.

## References

- Afonina, I.S., Cullen, S.P., Martin, S.J., 2010. Cytotoxic and non-cytotoxic roles of the CTL/NK protease granzyme B. *Immunol. Rev.* 235 (1), 105–116. Available from: PM:20536558.
- Afonina, I.S., Tynan, G.A., Logue, S.E., Cullen, S.P., Bots, M., Luthi, A.U., et al., 2011. Granzyme B-dependent proteolysis acts as a switch to enhance the proinflammatory activity of IL-1alpha. *Mol. Cell* 44 (2), 265–278. Available from: PM:22017873.
- Aksentijevich, I., Masters, S.L., Ferguson, P.J., Dancey, P., Frenkel, J., van Royen-Kerkhoff, A., et al., 2009. An autoinflammatory disease with deficiency of the interleukin-1-receptor antagonist. *N. Engl. J. Med.* 360 (23), 2426–2437. Available from: PM:19494218.
- Aletaha, D., Alasti, F., Smolen, J.S., 2012. Rheumatoid factor determines structural progression of rheumatoid arthritis dependent and independent of disease activity. *Ann. Rheum. Dis* 72 (6), 875–880. Available from: PM:22798565 2013 Jun.
- American College of Rheumatology Ad Hoc Committee, 2004. The American College of Rheumatology response criteria for systemic lupus erythematosus clinical trials: measures of overall disease activity. *Arthritis Rheum.* 50 (11), 3418–3426. Available from: PM:15529383.
- Amulic, B., Cazalet, C., Hayes, G.L., Metzler, K.D., Zychlinsky, A., 2012. Neutrophil function: from mechanisms to disease. *Annu. Rev. Immunol.* 30, 459–489. Available from: PM:22224774.
- Annunziato, F., Cosmi, L., Liotta, F., Maggi, E., Romagnani, S., 2009. Type 17 T helper cells—origins, features and possible roles in rheumatic disease. *Nat. Rev. Rheumatol.* 5 (6), 325–331. Available from: PM:19434074.
- Aringer, M., Gunther, C., Lee-Kirsch, M.A., 2013. Innate immune processes in lupus erythematosus. *Clin. Immunol.* 147 (3), 216–222. Available from: PM:23290784.
- Aringer, M., Smolen, J.S., 2012. Therapeutic blockade of TNF in patients with SLE—promising or crazy? *Autoimmun. Rev.* 11 (5), 321–325.
- Bergman, M.P., D'Elios, M.M., 2010. Cytotoxic T cells in *H. pylori*-related gastric autoimmunity and gastric lymphoma. *J. Biomed. Biotechnol.* 2010, 104918. Available from: PM:20617132.
- Bradley, J.R., 2008. TNF-mediated inflammatory disease. *J. Pathol.* 214 (2), 149–160. Available from: PM:18161752.
- Caudrillier, A., Kessenbrock, K., Gilliss, B.M., Nguyen, J.X., Marques, M.B., Monestier, M., et al., 2012. Platelets induce neutrophil extracellular traps in transfusion-related acute lung injury. *J. Clin. Invest.* 122 (7), 2661–2671. Available from: PM:22684106.
- Cines, D.B., Blanchette, V.S., 2002. Immune thrombocytopenic purpura. *N. Engl. J. Med.* 346 (13), 995–1008. Available from: PM:11919310.

- Clynes, R., Dumitru, C., Ravetch, J.V., 1998. Uncoupling of immune complex formation and kidney damage in autoimmune glomerulonephritis. *Science* 279 (5353), 1052–1054. Available from: PM:9461440.
- Coppelters, K.T., von Herrath, M.G., 2011. Viruses and cytotoxic T lymphocytes in type 1 diabetes. *Clin. Rev. Allergy Immunol.* 41 (2), 169–178. Available from: PM:21181304.
- Daha, N.A., Banda, N.K., Roos, A., Beurskens, F.J., Bakker, J.M., Daha, M.R., et al., 2011. Complement activation by (auto-) antibodies. *Mol. Immunol.* 48 (14), 1656–1665. Available from: PM:21757235.
- Dalan, R., Leow, M.K., 2012. Immune manipulation for Graves' disease: re-exploring an unfulfilled promise with modern translational research. *Eur. J. Intern. Med.* 23 (8), 682–691. Available from: PM:22877994.
- Dardalhon, V., Awasthi, A., Kwon, H., Galileos, G., Gao, W., Sobel, R.A., et al., 2008. IL-4 inhibits TGF-beta-induced Foxp3+ T cells and, together with TGF-beta, generates IL-9 + IL-10 + Foxp3(-) effector T cells. *Nat. Immunol.* 9 (12), 1347–1355. Available from: PM:18997793.
- Dinarello, C.A., Simon, A., Van der Meer, J.W., 2012. Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. *Nat. Rev. Drug Discov.* 11 (8), 633–652. Available from: PM:22850787.
- Duhen, T., Geiger, R., Jarrossay, D., Lanzavecchia, A., Sallusto, F., 2009. Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells. *Nat. Immunol.* 10 (8), 857–863. Available from: PM:19578369.
- Fong, K.Y., Thumboo, J., 2010. Neuropsychiatric lupus: clinical challenges, brain-reactive autoantibodies and treatment strategies. *Lupus* 19 (12), 1399–1403. Available from: PM:20947548.
- Gehrs, B.C., Friedberg, R.C., 2002. Autoimmune hemolytic anemia. *Am. J. Hematol.* 69 (4), 258–271. Available from: PM:11921020.
- Giannakopoulos, B., Krilis, S.A., 2013. The pathogenesis of the antiphospholipid syndrome. *N. Engl. J. Med.* 368 (11), 1033–1044. Available from: PM:23484830.
- Hirahara, K., Ghoreshi, K., Laurence, A., Yang, X.P., Kanno, Y., O'Shea, J.J., 2010. Signal transduction pathways and transcriptional regulation in Th17 cell differentiation. *Cytokine Growth Factor Rev.* 21 (6), 425–434. Available from: PM:21084214.
- Hoshino, A., Nagao, T., Nagi-Miura, N., Ohno, N., Yasuhara, M., Yamamoto, K., et al., 2008. MPO-ANCA induces IL-17 production by activated neutrophils in vitro via classical complement pathway-dependent manner. *J. Autoimmun.* 31 (1), 79–89. Available from: PM:18501296.
- Hu, Y., Shen, F., Crellin, N.K., Ouyang, W., 2011. The IL-17 pathway as a major therapeutic target in autoimmune diseases. *Ann. N. Y. Acad. Sci.* 1217, 60–76. Available from: PM:21155836.
- Infante-Duarte, C., Kamradt, T., 1999. Th1/Th2 balance in infection. *Springer Semin. Immunopathol.* 21 (3), 317–338. Available from: PM:10666776.
- Jarvis, J.N., Wang, W., Moore, H.T., Zhao, L., Xu, C., 1997. In vitro induction of proinflammatory cytokine secretion by juvenile rheumatoid arthritis synovial fluid immune complexes. *Arthritis Rheum.* 40 (11), 2039–2046. Available from: PM:9365094.
- Jennette, J.C., Falk, R.J., Bacon, P.A., Basu, N., Cid, M.C., Ferrario, F., et al., 2013. 2012 revised International Chapel Hill consensus conference nomenclature of vasculitides. *Arthritis Rheum.* 65 (1), 1–11. Available from: PM:23045170.
- Kaijed, S., Wang, J.X., Shnayder, R., Fishgal, N., Hei, H., Lee, R.T., et al., 2012. Interleukin-33 primes mast cells for activation by IgG immune complexes. *PLoS One* 7 (10), e47252. Available from: PM:23071771.
- Kaplan, M.J., 2011. Neutrophils in the pathogenesis and manifestations of SLE. *Nat. Rev. Rheumatol.* 7 (12), 691–699. Available from: PM:21947176.
- Karsten, C.M., Kohl, J., 2012. The immunoglobulin, IgG Fc receptor and complement triangle in autoimmune diseases. *Immunobiology* 217 (11), 1067–1079. Available from: PM:22964232.
- Keffer, J., Probert, L., Cazlaris, H., Georgopoulos, S., Kaslaris, E., Kioussis, D., et al., 1991. Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. *EMBO J.* 10 (13), 4025–4031. Available from: PM:1721867.
- Kessenbrock, K., Krumbholz, M., Schonermarck, U., Back, W., Gross, W.L., Werb, Z., et al., 2009. Netting neutrophils in autoimmune small-vessel vasculitis. *Nat. Med.* 15 (6), 623–625. Available from: PM:19448636.
- Kiener, H.P., Baghestanian, M., Dominkus, M., Walchshofer, S., Ghannadan, M., Willheim, M., et al., 1998. Expression of the C5a receptor (CD88) on synovial mast cells in patients with rheumatoid arthritis. *Arthritis Rheum.* 41 (2), 233–245. Available from: PM:9485081.
- Lee, D.M., Friend, D.S., Gurish, M.F., Benoit, C., Mathis, D., Brenner, M.B., 2002. Mast cells: a cellular link between autoantibodies and inflammatory arthritis. *Science* 297 (5587), 1689–1692. Available from: PM:12215644.
- Lee, H.S., 2012. Mechanisms and consequences of TGF- $\beta$ s overexpression by podocytes in progressive podocyte disease. *Cell Tissue Res.* 347 (1), 129–140. Available from: PM:21541658.
- Levinson, A.I., 2013. Modeling the intrathymic pathogenesis of myasthenia gravis. *J. Neurol. Sci.* 333 (1-2), 60–67. Available from: PM:23332143 2013 Jan 16 ePub ahead of print.
- Maizels, R.M., Hewitson, J.P., Smith, K.A., 2012. Susceptibility and immunity to helminth parasites. *Curr. Opin. Immunol.* 24 (4), 459–466. Available from: PM:22795966.
- Melzer, N., Meuth, S.G., Wiendl, H., 2009. CD8+ T cells and neuronal damage: direct and collateral mechanisms of cytotoxicity and impaired electrical excitability. *FASEB J.* 23 (11), 3659–3673. Available from: PM:19567369.
- Miller, S.A., Weinmann, A.S., 2010. Molecular mechanisms by which T-bet regulates T-helper cell commitment. *Immunol. Rev.* 238 (1), 233–246. Available from: PM:20969596.
- Miossec, P., Kolls, J.K., 2012. Targeting IL-17 and TH17 cells in chronic inflammation. *Nat. Rev. Drug Discov.* 11 (10), 763–776. Available from: PM:23023676.
- Mjelle, J.E., Rekvig, O.P., Van Der Vlag, J., Fenton, K.A., 2011. Nephritogenic antibodies bind in glomeruli through interaction with exposed chromatin fragments and not with renal cross-reactive antigens. *Autoimmunity* 44 (5), 373–383. Available from: PM:21244336.
- Nemeth, T., Mocsai, A., 2012. The role of neutrophils in autoimmune diseases. *Immunol. Lett.* 143 (1), 9–19. Available from: PM:22342996.
- Nimmerjahn, F., Ravetch, J.V., 2010. Antibody-mediated modulation of immune responses. *Immunol. Rev.* 236, 265–275. Available from: PM:20636822.
- Nishimoto, N., Kishimoto, T., 2006. Interleukin 6: from bench to bedside. *Nat. Clin. Pract. Rheumatol.* 2 (11), 619–626. Available from: PM:17075601.

- Olmez, U., Garred, P., Mollnes, T.E., Harboe, M., Berntzen, H.B., Munthe, E., 1991. C3 activation products, C3 containing immune complexes, the terminal complement complex and native C9 in patients with rheumatoid arthritis. *Scand. J. Rheumatol.* 20 (3), 183–189. Available from: PM:2068540.
- Ombrello, M.J., Kastner, D.L., 2011. Autoinflammation in 2010: expanding clinical spectrum and broadening therapeutic horizons. *Nat. Rev. Rheumatol.* 7 (2), 82–84.
- Onishi, R.M., Gaffen, S.L., 2010. Interleukin-17 and its target genes: mechanisms of interleukin-17 function in disease. *Immunology* 129 (3), 311–321. Available from: PM:20409152.
- Papadopoulos, M.C., Verkman, A.S., 2012. Aquaporin 4 and neuromyelitis optica. *Lancet Neurol.* 11 (6), 535–544. Available from: PM:22608667.
- Pappu, R., Ramirez-Carrozzi, V., Sambandam, A., 2011. The interleukin-17 cytokine family: critical players in host defence and inflammatory diseases. *Immunology* 134 (1), 8–16. Available from: PM:21726218.
- Parham, P., Moffett, A., 2013. Variable NK cell receptors and their MHC class I ligands in immunity, reproduction and human evolution. *Nat. Rev. Immunol.* 13 (2), 133–144. Available from: PM:23334245.
- Paul, W.E., Zhu, J., 2010. How are T(H)2-type immune responses initiated and amplified? *Nat. Rev. Immunol.* 10 (4), 225–235. Available from: PM:20336151.
- Peters, A., Lee, Y., Kuchroo, V.K., 2011. The many faces of Th17 cells. *Curr. Opin. Immunol.* 23 (6), 702–706. Available from: PM:21899997.
- Pisetsky, D.S., 2012. Microparticles as autoantigens: making immune complexes big. *Arthritis Rheum.* 64 (4), 958–961. Available from: PM:22237935.
- Pohlers, D., Brenmoehl, J., Loffler, I., Muller, C.K., Leipner, C., Schultze-Mosgau, S., et al., 2009. TGF-beta and fibrosis in different organs - molecular pathway imprints. *Biochim. Biophys. Acta* 1792 (8), 746–756. Available from: PM:19539753.
- Rajan, T.V., 2003. The Gell-Coombs classification of hypersensitivity reactions: a re-interpretation. *Trends Immunol.* 24 (7), 376–379. Available from: PM:12860528.
- Redlich, K., Görtz, B., Hayer, S., Zwerina, J., Kollias, G., Steiner, G., et al., 2004. Overexpression of TNF causes bilateral sacroiliitis. *Arthritis Rheum.* 50 (3), 1001–1005. Ref Type: In Press.
- Redlich, K., Hayer, S., Ricci, R., David, J.P., Tohidast-Akrad, M., Kollias, G., et al., 2002. Osteoclasts are essential for TNF-alpha-mediated joint destruction. *J. Clin. Invest.* 110 (10), 1419–1427. Available from: PM:12438440.
- Reiner, S.L., 2007. Development in motion: helper T cells at work. *Cell* 129 (1), 33–36. Available from: PM:17418783.
- Rohleder, N., Aringer, M., Boentert, M., 2012. Role of interleukin-6 in stress, sleep, and fatigue. *Ann. N. Y. Acad. Sci.* 1261 (1), 88–96.
- Rose-John, S., Scheller, J., Elson, G., Jones, S.A., 2006. Interleukin-6 biology is coordinated by membrane-bound and soluble receptors: role in inflammation and cancer. *J. Leukoc. Biol.* 80 (2), 227–236. Available from: PM:16707558.
- Schreiber, A., Xiao, H., Jennette, J.C., Schneider, W., Luft, F.C., Kettritz, R., 2009. C5a receptor mediates neutrophil activation and ANCA-induced glomerulonephritis. *J. Am. Soc. Nephrol.* 20 (2), 289–298. Available from: PM:19073822.
- Schuerwegh, A.J., Ioan-Facsinay, A., Dorjee, A.L., Roos, J., Bajema, I.M., van der Voort, E.I., et al., 2010. Evidence for a functional role of IgE anticitrullinated protein antibodies in rheumatoid arthritis. *Proc. Natl. Acad. Sci. U. S. A.* 107 (6), 2586–2591. Available from: PM:20133791.
- Sgond, R., Gruschwitz, M.S., Dietrich, H., Recheis, H., Gershwin, M.E., Wick, G., 1996. Endothelial cell apoptosis is a primary pathogenetic event underlying skin lesions in avian and human scleroderma. *J. Clin. Invest.* 98 (3), 785–792.
- Shikagaia, M.M., et al., 2017. Innate lymphoid cells in autoimmunity: emerging regulators in rheumatic diseases. *Nat. Rev. Rheumatol.* 13 (3), 164–173. Available from: PM:28148916.
- Sigal, L.J., Crotty, S., Andino, R., Rock, K.L., 1999. Cytotoxic T-cell immunity to virus-infected non-haematopoietic cells requires presentation of exogenous antigen. *Nature* 398 (6722), 77–80. Available from: PM:10078533.
- Smolen, J.S., van der Heijde, D.M., St Clair, E.W., Emery, P., Bathon, J.M., Keystone, E., et al., 2006. Predictors of joint damage in patients with early rheumatoid arthritis treated with high-dose methotrexate with or without concomitant infliximab: results from the ASPIRE trial. *Arthritis Rheum.* 54 (3), 702–710. Available from: PM:16508926.
- Sonder, S.U., Saret, S., Tang, W., Sturdevant, D.E., Porcella, S.F., Siebenlist, U., 2011. IL-17-induced NF-kappaB activation via CIKS/Act1: physiologic significance and signaling mechanisms. *J. Biol. Chem.* 286 (15), 12881–12890. Available from: PM:21335551.
- Sontheimer, R.D., 2005. Subacute cutaneous lupus erythematosus: 25-year evolution of a prototypic subset (subphenotype) of lupus erythematosus defined by characteristic cutaneous, pathological, immunological, and genetic findings. *Autoimmun. Rev.* 4 (5), 253–263. Available from: PM:15990071.
- Spits, H., et al., 2013. Innate lymphoid cells—a proposal for uniform nomenclature. *Nat. Rev. Immunol.* 13 (2), 145–149. Available from: PM:23348417.
- Stanley, J.R., Amagai, M., 2006. Pemphigus, bullous impetigo, and the staphylococcal scalded-skin syndrome. *N. Engl. J. Med.* 355 (17), 1800–1810. Available from: PM:17065642.
- Tan, E.M., 1991. Autoantibodies in pathology and cell biology. *Cell* 67 (5), 841–842. Available from: PM:0001959131.
- Tato, C.M., O’Shea, J.J., 2006. Immunology: what does it mean to be just 17? *Nature* 441 (7090), 166–168. Available from: PM:16688162.
- Teng, M.W.L., et al., 2015. IL-12 and IL-23 cytokines: from discovery to targeted therapies for immune-mediated inflammatory diseases. *Nat. Med.* 21 (7), 719–729. Available from: PM:26121196.
- Varga, J., Pasche, B., 2009. Transforming growth factor beta as a therapeutic target in systemic sclerosis. *Nat. Rev. Rheumatol.* 5 (4), 200–206. Available from: PM:19337284.
- Vasilopoulos, Y., Gkretsi, V., Armaka, M., Aidinis, V., Kollias, G., 2007. Actin cytoskeleton dynamics linked to synovial fibroblast activation as a novel pathogenic principle in TNF-driven arthritis. *Ann. Rheum. Dis.* 66 (Suppl 3), iii23–iii28. Available from: PM:17934089.
- Vogel, T.P., Milner, J.D., Cooper, M.A., 2015. The Ying and Yang of STAT3 in human disease. *J. Clin. Immunol.* 35 (7), 615–623. Available from: PM:26280891.
- Walport, M.J., 2001. Complement. First of two parts. *N. Engl. J. Med.* 344 (14), 1058–1066. Available from: PM:11287977.
- Wei, G., Abraham, B.J., Yagi, R., Jothi, R., Cui, K., Sharma, S., et al., 2011. Genome-wide analyses of transcription factor GATA3-mediated gene regulation in distinct T cell types. *Immunity* 35 (2), 299–311. Available from: PM:21867929.

- Wei, G., Wei, L., Zhu, J., Zang, C., Hu-Li, J., Yao, Z., et al., 2009. Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating CD4 + T cells. *Immunity* 30 (1), 155–167. Available from: PM:19144320.
- Wesolowski, J., Paumet, F., 2011. The impact of bacterial infection on mast cell degranulation. *Immunol. Res.* 51 (2-3), 215–226. Available from: PM:22048902.
- Wills-Karp, M., 2004. Interleukin-13 in asthma pathogenesis. *Immunol. Rev.* 202 (1), 175–190. Available from: PM:15546393.
- Yamashina, M., Ueda, E., Kinoshita, T., Takami, T., Ojima, A., Ono, H., et al., 1990. Inherited complete deficiency of 20-kilodalton homologous restriction factor (CD59) as a cause of paroxysmal nocturnal hemoglobinuria. *N. Engl. J. Med.* 323 (17), 1184–1189. Available from: PM:1699124.
- Yung, S., Chan, T.M., 2008. Anti-DNA antibodies in the pathogenesis of lupus nephritis--the emerging mechanisms. *Autoimmun. Rev.* 7 (4), 317–321. Available from: PM:18295737.
- Zeitler, H., Goldmann, G., Marquardt, N., Ulrich-Merzenich, G., 2013. Long term outcome of patients with acquired hemophilia—a monocenter interim analysis of 82 patients. *Atheroscler. Suppl* 13 (1), 223–228.
- Zuniga, L.A., Jain, R., Haines, C., Cua, D.J., 2013. Th17 cell development: from the cradle to the grave. *Immunol. Rev.* 252 (1), 78–88. Available from: PM:23405896.

# Diet, the Gut Microbiome, and Autoimmune Diseases

Charles R. Mackay

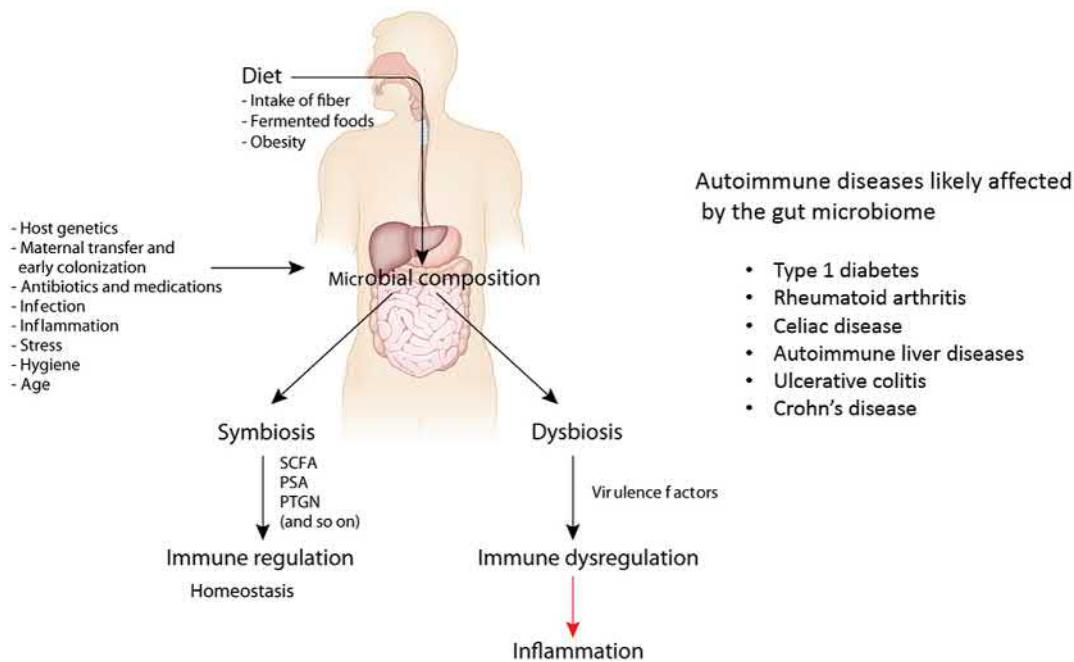
Department of Microbiology, Biomedicine Discovery Institute, Monash University, Clayton, VIC, Australia

## OUTLINE

Introduction	331	Mechanisms for Microbiome-Mediated Gut and Immune Homeostasis Metabolite-Sensing G-Protein Coupled Receptors	336
Evidence That Diet and the Gut Microbiome Associate With Human Autoimmune Diseases	332	Transcriptional and Epigenetic Effects	338
Diet and the Gut Microbiota	333	Other Important Metabolites: Tryptophan Catabolites, ω-3 Fatty Acids	338
Major Products of the Gut Microbiome—Short-Chain Fatty Acids, Mediate Gut Homeostasis, and Immune Tolerance	334	Concluding Remarks	339
Sites Other Than the Gut Where Dietary or Bacterial Metabolites May Influence the Immune Response	336	References	340

## INTRODUCTION

The incidence of many inflammatory conditions has increased dramatically in Western countries over the last decades. This includes not only certain autoimmune diseases, particularly type 1 diabetes (T1D), but also celiac disease, myasthenia gravis, Crohn's disease, and ulcerative colitis (Lerner et al., 2015; Molodecky et al., 2012). Moreover, other "Western lifestyle" diseases such as fatty liver disease, atherosclerosis, hypertension, Alzheimer's disease, and type 2 diabetes have a strong immunological connection, as well as connections to altered gut microbiota composition. The prevailing hypothesis for the increased incidence of inflammatory disease with Western lifestyle has been the hygiene hypothesis (Strachan, 1989, 2000), which proposed that declining family size and improvements in personal hygiene reduced opportunities for cross-infections in families, and this leads to dysregulated immune responses. This hypothesis has evolved, and now disruption of the beneficial gut commensal flora has been suggested as the driver of Western inflammatory diseases (Noverr and Huffnagle, 2004; Rook et al., 2003). In recent years, we have argued for diet and its effect on the microbiome, as an alternative or additional component (summarized in Fig. 19.1). Recent studies highlight the profound effect of diet on gut microbiota composition (Kau et al., 2011; Macia et al., 2015; Turnbaugh et al., 2006). In all likelihood, a variety of Western lifestyle factors combine to adversely affect the gut microbiota composition. This includes antibiotic use (including in the food chain), consumption of processed foods, insufficient intake of dietary fiber, and limited exposure to beneficial microbes, such as those encountered on a farm (Fig. 19.1).



**FIGURE 19.1** Diet, microbial composition, and regulation of the immune system. Diet and other environmental and host factors have a major effect on gut microbial composition. Our model would suggest that balanced microbial composition results in symbiosis; this provides regulation of immune and inflammatory responses through antiinflammatory and/or immunomodulatory products such as short chain fatty acids (SCFAs), polysaccharide A (PSA), and peptidoglycans (PTGN), which helps maintain homeostasis. Dysbiosis can lead to dysregulation of the immune system through lack of beneficial microbial products and changes in gut epithelial integrity, and altered immune tolerance (see below), which could leave the host susceptible to inflammation. Dysbiosis could occur through the consumption of a Western diet (insufficient fiber), as well as through changes induced by factors such as antibiotic use. Source: Adapted from Figure 1 of Maslowski, K.M., Mackay, C.R., 2011. *Diet, gut microbiota and immune responses*. *Nat. Immunol.* 12, 5–9.

Important mechanisms whereby dietary and bacterial metabolites interact with the immune system, or gut biology, and affect inflammation, have been uncovered. Many of the foodstuffs that have traditionally been considered healthy, such as dietary fiber, fish, and elements of the Mediterranean diet, can now be connected to cellular and molecular pathways that promote gut health and immune tolerance. These cellular and molecular studies, combined with epidemiology studies of autoimmune disease, make a compelling case that environment in the West is adding to the incidence of certain autoimmune diseases.

## EVIDENCE THAT DIET AND THE GUT MICROBIOME ASSOCIATE WITH HUMAN AUTOIMMUNE DISEASES

There are now many studies that show that an altered, dysbiosis-related microbiota composition associates with both autoimmune and nonautoimmune inflammatory diseases. Some examples include rheumatoid arthritis (Scher and Abramson, 2011; Van de Wiele et al., 2016), T1D (de Goffau et al., 2013; Endesfelder et al., 2014; Vaarala et al., 2008; Vatanen et al., 2016), Crohn's disease and ulcerative colitis (Huttenhower et al., 2014; Jostins et al., 2012a), celiac disease (Collado et al., 2009), systemic lupus erythematosus (SLE), and others (Vieira et al., 2014). There are considerably more references than those listed here. A central unanswered question is whether certain bacterial species promote disease, or whether the absence of protective commensal species does so. In all likelihood, a combination of these applies.

There have been comprehensive studies on the gut microbiota in human and mouse T1D. Variances in gut microbiota in children diagnosed with T1D, although conflicting, have been widely examined. Children who develop T1D have a less diverse gut microbiota with a decreased presence of *Firmicutes* phylum correlated with decreased fecal butyrate than children with no T1D that presented an increase in *Bacteroidetes* phylum (de Goffau et al., 2013; Giongo et al., 2011). In line with these findings, non-obese diabetic (NOD) mice deficient in the adaptor protein myeloid differentiation factor 88 (MyD88), important for the detection of microbial antigens, fail to

develop T1D under specific pathogen-free (SPF) conditions but in germ-free conditions lead to an exacerbated development of T1D (Wen et al., 2008). In addition, a study by Danska et al. demonstrated the role of the gut microbiota in the marked gender differences that characterize T1D in NOD mice (Markle et al., 2013). Similar to humans, male NOD mice display considerably delayed onset and reduced incidence of T1D. Remarkably, the female cohort gavaged with male gut microbiota were protected from T1D development, in comparison to female cohorts gavaged with a female gut microbiota or untreated, which displayed typical disease incidence (Markle et al., 2013). Treatment of NOD mice with probiotics coincides with the maintenance of  $\beta$ -cell function and prevention of T1D (Calcinaro et al., 2005), and probiotic treatment in genetically susceptible children for the prevention of T1D is currently the focus of the ongoing PRODIA study in Finland (Ljungberg et al., 2006). While these studies provide compelling evidence for the role of gut microbiota in modulating T1D development, the specific metabolites responsible for preventing or ameliorating the diabetic immune response remain to be identified.

## DIET AND THE GUT MICROBIOTA

The average American consumes  $\sim$ 16 g of fiber per day which is well below the recommended daily threshold of 25–38 g/day, and individuals in lower socioeconomic groups consume even less fiber (King et al., 2012). The Mediterranean diet, which is based on high consumption of vegetables, fruits, olive oil, and fish, has now gained scientific credibility, at least in the prevention of cardiovascular diseases, and also asthma (Berthon et al., 2013; Castro-Rodriguez et al., 2008; Estruch et al., 2013; Nagel et al., 2010). A dietary basis for asthma, allergies, and certain autoimmune diseases such as T1D fits with epidemiologic studies (Devereux, 2006; Eder et al., 2006). As a country undergoes development and transformation to the Western lifestyle, there is a rise in the prevalence of asthma and allergy (Eder et al., 2006) and certain autoimmune diseases.

An “obesogenic” diet is characterized by increased consumption of energy-dense, processed foods and reduced consumption of nutrient-rich foods such as fruits and vegetables. If diet does underpin many inflammatory diseases, then metabolites derived directly from the diet, or from commensal bacteria, are certainly involved. The current leading metabolites that likely play protective roles for autoimmune diseases (as well as other inflammatory diseases) are the short-chain fatty acids (SCFAs), omega-3 fatty acids, and tryptophan metabolites. There are well-characterized receptors, transcription factors, and epigenetic mechanisms that account for the anti-inflammatory, protolerogenic actions of bacterial metabolites.

Western diets shape the composition of the gut microbiota (De Filippo et al., 2010; Le Chatelier et al., 2013; Ou et al., 2013; Turnbaugh et al., 2008). The composition of the gut microbiota also relates to human disease (Clemente et al., 2012; Kau et al., 2011; Round and Mazmanian, 2009), through mostly poorly understood mechanisms. However, as stressed throughout this chapter, metabolites such as SCFAs, long-chain fatty acids, or tryptophan catabolites may play a major role in the prevention of inflammatory disease and likely underlie at least some of the microbiota-related associations with human autoimmune diseases. We have argued previously that the major metabolites produced by gut commensal bacteria are SCFAs. It is one of the important symbiotic relationships in vertebrate biology—the use of gut bacteria to digest dietary fiber to produce SCFAs. Vertebrates do not have the enzymes for breakdown of fiber, whereas commensal bacteria in the gut do. The more dietary fiber ingested, the higher the production of SCFAs. The microbiota composition of rural Africans is different to that of African-Americans, with a higher proportion of *Prevotella* (efficient at digesting fiber and producing SCFAs), a lower proportion of *Bacteroides*, and higher production of SCFAs such as butyrate (Ou et al., 2013). In one often quoted study (De Filippo et al., 2010), fecal microbiota of European children and rural African children (from Burkina Faso) were compared. African children showed a higher bacterial richness, and a significant enrichment in bacteria from the genus *Prevotella* and *Xylanibacter*, whereas these bacteria were completely absent from the microbiota from the European children. There were significantly more SCFAs in the feces of African versus European children, attributable to their higher consumption of fiber.

What are the features of the gut microbiota that might promote, or protect against, autoimmune and other diseases? Bacterial “richness” (diversity) is one feature. Individuals with a low bacterial richness ( $\sim$ 23% of the Danish population) tended to adiposity, insulin resistance, and dyslipidemia and a more pronounced inflammatory phenotype, compared with individuals with a high bacterial richness (Le Chatelier et al., 2013). Low bacterial diversity has already been associated with human inflammatory bowel disease (IBD) (Lepage et al., 2011; Manichanh et al., 2006). In our studies in mice, we further observed a close association between gut bacterial diversity and inflammation (colitis, food allergy, autoimmune diabetes). Mice on low-fiber diets produce

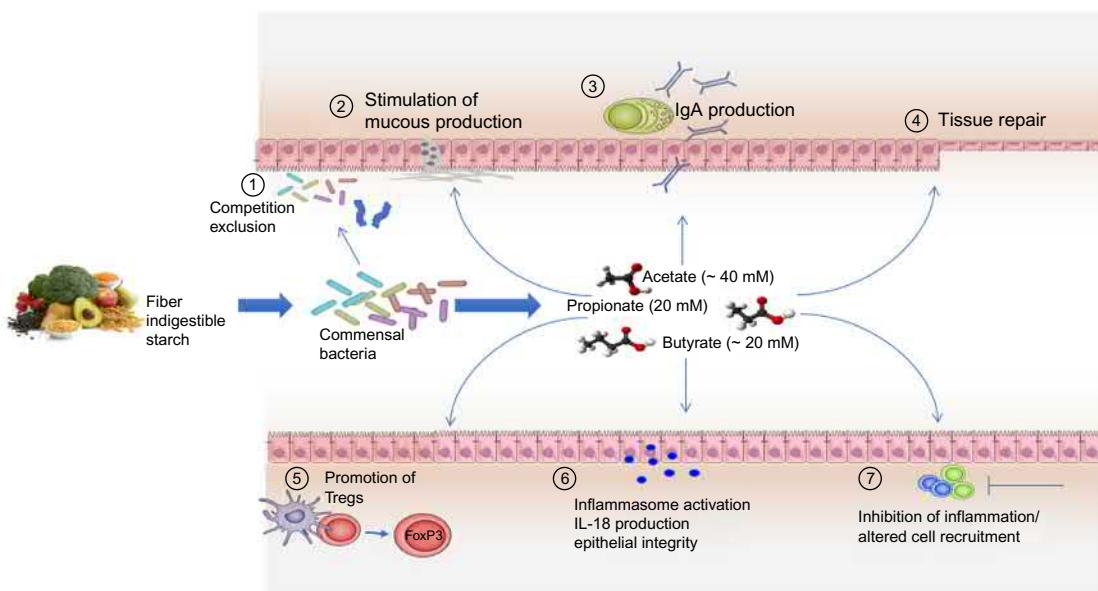
significantly less SCFA in the colon, presumably because of altered bacterial ecology. Such low-fiber fed mice show exacerbated inflammation in models of DSS colitis (Macia et al., 2015), food allergy (Tan et al., 2016), or *Citrobacter rodentium* infection (unpublished). To date, there is little information on the relative ability of different bacterial species to produce various metabolites. In general, *Clostridium* species, particularly clostridial cluster XIVa and IV, are the highest producers of butyrate, and these are also the bacterial species considered necessary for optimal colonic Treg development. All the features of a healthy microbiome have not been fully elucidated, although as discussed throughout this chapter, the capacity to produce SCFAs generally correlates with beneficial bacterial species. However, SCFAs are just one class of metabolite that may promote gut health, for instance, various metabolites associated with tryptophan catabolism promote gut homeostasis (discussed below). Coming years should see vastly improved bioinformatics tools that equate the presence of different bacterial species with enzymatic machinery, and actual capacity, for production of various metabolites.

## MAJOR PRODUCTS OF THE GUT MICROBIOME—SHORT-CHAIN FATTY ACIDS, MEDIATE GUT HOMEOSTASIS, AND IMMUNE TOLERANCE

Numerous metabolites may contribute to gut and immune homeostasis; however, SCFAs are probably the most important. Thus, deficiency of dietary fiber, the foodstuff that yields SCFAs, may underlie poor gut homeostasis and in addition contribute to the development of certain autoimmune diseases as well as asthma, allergies, and even cardiovascular disease and Alzheimer's disease (Maslowski and Mackay, 2011; McKenzie et al., 2017). Disrupted gut homeostasis likely precedes inflammatory conditions. For instance, compromised epithelial integrity may allow translocation of bacteria or their products from the gut lumen to peripheral tissues. Gut "leakiness" may also allow bacterial products such as lipopolysaccharide (LPS) to distribute systemically and potentiate immune cell stimulation. There is considerable evidence that poor gastrointestinal tract integrity plays a role in promoting the pathogenesis of autoimmune T1D (Vaarala et al., 2008). Moreover, obese mice display increased intestinal permeability, and endotoxemia (Cani et al., 2009). An obesogenic diet disrupts gut flora ecology and likely results in lower production of metabolites that maintain epithelial integrity and promote immune tolerance, such as SCFAs. Certain commensal bacteria promote gut epithelial integrity (Fukuda et al., 2011) or Treg function (Arpaia et al., 2013; Furusawa et al., 2013; Smith et al., 2013).

Some of the major mechanisms whereby SCFAs facilitate gut homeostasis and immune tolerance are illustrated in Fig. 19.2. The first relevant mechanism is "competitive exclusion" whereby high-fiber diets expand commensal bacteria, thus limiting access of pathogenic bacteria to gut epithelium. Insufficient intake of fiber or excessive intake of fat or carbohydrate could equally disrupt normal bacterial ecology and compromise competitive exclusion. SCFAs also promote the secretion of mucus by gut epithelial cells (Willemsen et al., 2003). In addition, high acetate-producing bacteria such as *Bacteroides thetaiotaomicron* promote goblet cell differentiation and expression of genes for mucus production (Wrzosek et al., 2013). The mucus barrier is one of the most important elements for physical separation of bacteria from the epithelial surface and is a critical contributor to gut homeostasis, and to immune tolerance (Shan et al., 2013). Interestingly, the inflammasome component Nalp6 is necessary for proper mucus secretion in the gut (Wlodarska et al., 2014), and we suspect that SCFA signaling through metabolite-sensing G-protein coupled receptors (GPCRs) activates Nalp6. In a recent paper, deficiency of dietary fiber had dramatic effects on colitis in the dextran sulfate sodium (DSS) model (Macia et al., 2015). There is evidence that SCFAs promote the secretion of IgA by B cells (Tan et al., 2016). IgA has a key role in maintaining the noninflammatory relationship between the host and the gut microbiota (Peterson et al., 2007) as well as microbiota composition (Round and Mazmanian, 2009). Another point at which SCFAs operate in the gut is in tissue repair (Fig. 19.2, point 3). The gut mucosa is prone to ulceration and like any other tissue relies on tissue repair. This may be particularly important in the GI tract as ulcers, physical damage, or actions of parasites would require constant wound healing processes.

Point 4 of Fig. 19.2 highlights the role of SCFAs in promoting Treg responses in the gut (Atarashi et al., 2013, 2011; Geuking et al., 2011, 2013). SCFAs promote the expansion of Tregs, which presumably facilitates immunological tolerance to food antigens and possibly some commensal bacteria. In one of the first studies, Atarashi et al. (2013) used human fecal samples and applied a sequential methodology to select bacterial species capable of inducing Tregs, when these species were used to colonize germ-free mice. The most potent inducers of Tregs fell within clusters IV, XIVa, and XVIII of *Clostridia*. Simple oral inoculation of *Clostridium* to mice during early life resulted in resistance to colitis and IgE responses (Atarashi et al., 2011), supporting the notion that some inflammatory diseases may have as their basis an unbalanced microbial ecology. The exact properties of



**FIGURE 19.2** Dietary fiber, SCFAs, and mechanisms for gut and immune homeostasis. There is now overwhelming evidence for the positive health benefits of high concentrations of SCFAs in the gut, as well as the high consumption of dietary fiber. Currently, the metabolite-sensor GPCRs responsible for the effects of fiber/SCFAs on gut health and immunity are not fully elucidated, although GPR43 and GPR109A and certain HDACs appear to be particularly important. The current knowledge on precise roles for these molecules is discussed in the main text. The seven major actions for fiber/SCFAs can be summarized as follows: (1) “competitive exclusion” whereby high-fiber diet expands commensal bacteria and limits pathogenic bacteria access to gut epithelium. (2) SCFAs promote the secretion of mucus by gut epithelial cells (Willemse et al., 2003). The mucus barrier is one of the most important elements for the physical separation of bacteria from the epithelial surface. (3) SCFAs promote the secretion of IgA by B cells. IgA has a key role in maintaining a mutualistic relationship between the host and the gut microbiota (Peterson et al., 2007) and SCFAs may influence IgA production. (4) SCFAs promote tissue repair and wound healing. This may be particularly important in the gastrointestinal tract as ulcers, physical damage, or actions of parasites would necessitate constant wound healing processes. (5) SCFAs promote Treg development in the gut, in a process that presumably facilitates immunological tolerance to food antigens and possibly some commensal bacteria (see text). (6) SCFAs (particularly acetate) enhance epithelial integrity (Fukuda et al., 2011) in a process dependent on inflammasome activation and IL-18 production. (7) SCFAs have well-established antiinflammatory effects (Maslowski et al., 2009; Tedelind et al., 2007) particularly inhibition of NF- $\kappa$ b. Source: This figure was adapted from Figure 1 from Thorburn, A.N., Macia, L., Mackay, C.R., 2014. Diet, metabolites, and “western-lifestyle” inflammatory diseases. *Immunity* 40, 833–842.

*Clostridia* that induce Tregs are unclear, although *Clostridia* species are one of the highest producers of the SCFA butyrate, and this may well explain the ability of *Clostridia* to promote Treg numbers. Indeed, recent reports show that SCFAs, particularly butyrate, directly influence numbers and function of inducible Tregs (Arpaia et al., 2013; Furusawa et al., 2013; Smith et al., 2013). Smith et al. (2013) showed that SCFAs delivered to germ-free mice regulated the size and function of the colonic Treg pool.

Point 5 in Fig. 19.2 depicts the effects of SCFAs on epithelial integrity. Acetate produced by a probiotic bacterial species *Bifidobacterium longum* was found to promote epithelial integrity and protect against infection by a pathogenic strain of *Escherichia coli* (Fukuda et al., 2011; Suzuki et al., 2008). However, to date, the best characterized molecular pathway for maintenance of epithelial integrity involves the inflammasome pathway, and production of the inflammasome-related cytokine IL-18 (Dupaul-Chicoine et al., 2010; Elinav et al., 2011; Normand et al., 2011; Zaki et al., 2010). Recently, membrane hyperpolarization, especially mediated by K<sup>+</sup> efflux, or Ca<sup>2+</sup> flux, was found to trigger Nalp3 inflammasome activation (Muñoz-Planillo et al., 2013). GPCR signaling is a common pathway for K<sup>+</sup> efflux, or Ca<sup>2+</sup> flux, and we showed that SCFA signaling through metabolite-sensing GPCRs such as GPR43 and GPR109A activated the Nalp3 inflammasome and production of IL-18 by colonic epithelium (Macia et al., 2015). In addition, we found that diets deficient in fiber, resembling a typical Western diet, enhanced colitis in the DSS model and in a colon cancer model whereas in mice a very high intake of dietary fiber showed a remarkable level of protection in DSS colitis, and colon cancer (Macia et al., 2015).

Point 6 in Fig. 19.2 depicts the antiinflammatory effects of SCFAs, for instance, on inflammatory cytokine production, or on leukocyte recruitment. The antiinflammatory effects of SCFAs have been documented now in a number of animal models of inflammation (Maslowski et al., 2009; Tan et al., 2014). SCFAs have been

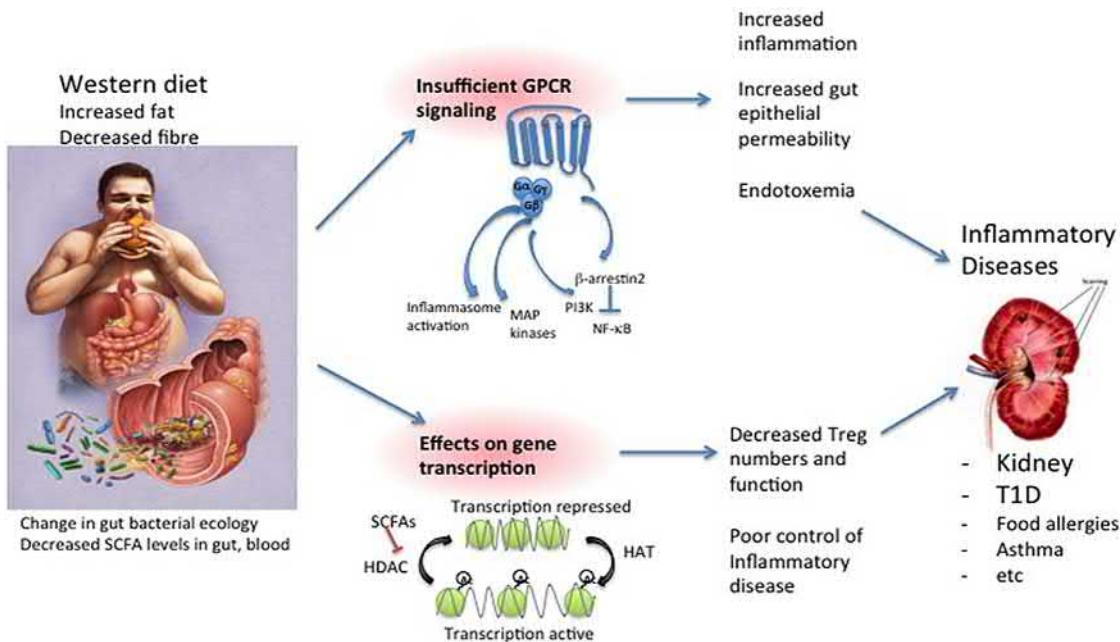
reported to reduce vascular cell adhesion molecule (VCAM) and Intercellular adhesion molecule (ICAM) expression, production of inflammatory chemokines, and inflammatory cytokines, tumor necrosis factor (TNF), Interleukin-6 (IL-6), and Interferon-gamma (IFN- $\gamma$ ) (Vinolo et al., 2011). The ability of SCFAs to inhibit nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) is well established, and this may depend on both histone deacetylase (HDAC) inhibition and activation of metabolite-sensing GPCRs, especially signaling through  $\beta$ -Arrestin2 (see below). Thus the actions of SCFAs in the gut shown in Fig. 19.2 are highly relevant to gut health and fit with many years of research that has demonstrated the benefits of either SCFAs or dietary fiber in gut inflammation. In addition to SCFAs, other metabolites show antiinflammatory properties, such as  $\omega$ -3 fatty acids which inhibit TNF or IL-6 production from macrophages (Oh et al., 2010). The antiinflammatory properties of many metabolites presumably reflect the need for constrained immune responses to bacterial or food antigens in the gut. The following sections outline the main molecular mechanisms whereby metabolites influence biological outcomes, which include “metabolite-sensing” GPCRs, and effects on gene transcription through HDAC inhibition, or agonism of specific transcription factors.

## SITES OTHER THAN THE GUT WHERE DIETARY OR BACTERIAL METABOLITES MAY INFLUENCE THE IMMUNE RESPONSE

Originally, the gut was considered the main site where dietary metabolites mediated their effects, such as on gut epithelial integrity, or mucosal immunity. However, several papers show that metabolites (particularly SCFAs) distributed systemically may affect the course of inflammatory responses, lung responses, and macrophage/dendritic cell (DC) differentiation in the bone marrow (Maslowski et al., 2009; Trompette et al., 2014). Nevertheless, the first major point is in the gut, particularly the lower colon, where fiber is fermented by commensal bacteria to produce large quantities of acetate, propionate, and butyrate, ~40, 20, and 20 mM, respectively (Tan et al., 2014). However, acetate is transported across the gut epithelium and reaches relatively high concentrations in the portal venous blood, up to 500  $\mu$ M (Marino et al., 2017). Many immune cells, particularly neutrophils, macrophages, and DCs, express high levels of metabolite-sensing GPCRs (see below). In the periphery, SCFAs influence inflammation through their antiinflammatory effects (Maslowski et al., 2009). The exacerbated inflammatory reactions observed in some germ-free mouse models (Herbst et al., 2011; Maslowski et al., 2009) likely relate to the absence of SCFAs in the gut, blood, or tissues. Another major point at which metabolites may affect biology is through their transport across the placenta to the developing fetus. Acetylation and deacetylation of histones as well as nonhistone proteins such as transcription factors represent one of the main mechanisms for epigenetic control of gene expression. The epigenetic effects of SCFAs in the fetus, acting through HDAC inhibition, represent one likely explanation for dietary links to asthma, and the developmental origin of human disease. For instance, SCFAs profoundly affect the expression and function of FoxP3. Similar considerations also apply to the supply of metabolites through breast milk. To date, the role of breast milk in protecting (or promoting) human disease is inconclusive. High fat feeding of mice during lactation predisposes adult offspring to obesity/and metabolic syndrome diseases (Vogt et al., 2014), and it is possible that quality of breast milk may also influence the development of autoimmune diseases.

## MECHANISMS FOR MICROBIOME-MEDIATED GUT AND IMMUNE HOMEOSTASIS METABOLITE-SENSING G-PROTEIN COUPLED RECEPTORS

Vertebrates have evolved several mechanisms to respond to dietary and bacterial metabolites (see Fig. 19.3). One is metabolite-sensing GPCRs which produce immediate biological responses to specific metabolites. Many of the common dietary and bacterial metabolites have GPCR sensors (Tan et al., 2017; Thorburn et al., 2014). During evolution, these metabolite sensors evolved to recognize metabolites produced either by mutualistic gut bacteria, or by nonbacterial digestion of foodstuffs. The reason why cells of the body, including immune cells, use these receptors to modify function is still uncertain but presumably relates to a need to sense the availability of nutrients. Many of the metabolite sensors were identified as late as the 1990s through large-scale sequencing efforts, and these sensors were deorphaned in the 2000s (reviewed in Macia et al., 2012; Tan et al., 2014). Probably the best characterized metabolite-sensing GPCRs are GPR43, GPR41, and GPR109A, which bind SCFAs. GPR43 and GPR109A appear to be important for gut homeostasis, and both are expressed by colonic epithelium, by inflammatory leukocytes such as neutrophils and macrophages, and by Treg cells. Hence, many of the actions



**FIGURE 19.3** Pathways whereby the gut microbiome may influence autoimmune disease. On the left is the role an altered gut microbiota, produced through poor diet or antibiotic use, may have on gut microbiota composition. Mechanisms are still uncertain but leaky gut and translocation of LPS or bacteria is a likely scenario. On the right are depicted molecular mechanisms whereby gut metabolites may promote gut homeostasis and protect against disease. The first is through immediate responses, mediated by metabolite-sensing GPCRs (top of figure) which signal through a variety of pathways, but in particular  $\beta$ -arrestin2 (which yields antiinflammatory effects), or through G-proteins such as G $\alpha$ i or G $\alpha$ q. The second mechanism whereby SCFAs affect biology is through effects on gene transcription. SCFAs inhibit the activity of HDACs, which deacetylate either histone proteins (bottom). Such inhibition of HDACs is a mechanism for regulation of gene transcription. Another mechanism not listed here is direct agonism of transcription factors such as AhR by certain metabolites.

described above for SCFAs in gut homeostasis can be ascribed to these two receptors. Deletion of either receptor in mice exacerbates DSS colitis (Maslowski et al., 2009; Singh et al., 2014). We have found that the beneficial effects of dietary fiber, in the DSS colitis model, depend on GPR43 and/or GPR109A (Macia et al. 2015). Moreover, the beneficial effect of acetate on epithelial integrity (Fukuda et al., 2011) probably operates through GPR43. In our studies, we see far fewer Th17 $^{+}$  T cells in the colon of *Gpr43* $^{-/-}$  mice during DSS colitis.

Regulation of colonic and possibly peripheral Treg numbers relates to metabolite-sensing receptors, including GPR43 and GPR109A (Singh et al., 2014; Smith et al., 2013). Our studies reveal that high-fiber feeding, or acetate, promotes colonic Treg numbers, whereas *Gpr43* $^{-/-}$  mice show reduced Treg numbers (Marino et al., 2017). Lack of GPR109A, a metabolite sensor for butyrate, was associated with fewer colonic Tregs due to the reduced ability of colonic macrophages and DCs to promote their development (Singh et al., 2014). Regardless of some discrepancies in these recent papers relating to the relative roles of acetate, propionate, and butyrate, SCFAs, the main metabolic products of commensal bacteria, are used as a mechanism for tolerance induction in the gut (Tan et al., 2016).

Other important metabolite-sensing GPCRs include GPR120 which recognizes long-chain fatty acids such as  $\omega$ -3 fatty acids (Oh et al., 2010) derived from foodstuffs including fish and olive oil, GPR91 as a receptor for succinate (a product of the citric acid cycle), GPR84 which is a receptor for medium chain fatty acids derived from milk in certain species, and GPR35 which is a receptor for metabolites of tryptophan catabolism, that is, kynurenic acid. All of the metabolite-sensing GPCRs are expressed by immune cells, and particularly by innate-type cells, although this knowledge has mostly been gleaned from transcript expression analyses. Another defining feature of metabolite-sensing GPCRs is their expression on tissues or cells relevant to metabolism, pancreatic islets, or white adipose tissue. Another common expression pattern is on intestinal epithelium, which probably relates to roles in the maintenance of epithelial integrity. The role of many of these receptors is poorly defined, and tools to study their expression and function await development.

Metabolite-sensing GPCRs have a large bearing on the control of inflammatory responses, particularly inflammatory bowel disease (Maslowski et al., 2009; Oh et al., 2010; Singh et al., 2014; Tan et al., 2016; Trompette et al., 2014). Indeed, most metabolite-sensing GPCRs including GPR43 and SCFAs, GPR120 and  $\omega$ -3 fatty acids,

GPR109A and its ligands, and possibly GPR35 and its ligands facilitate antiinflammatory effects (Tan et al., 2017). Concerning the SCFA receptors, *Gpr43*<sup>-/-</sup> mice showed exacerbated inflammation in models of airway hypersensitivity, DSS-induced colitis, and rheumatoid arthritis (Maslowski et al., 2009). Administration of acetate to mice in the drinking water protected against colitis in wild-type mice but not in *Gpr43*<sup>-/-</sup> mice suggesting that acetate mediates its antiinflammatory effects through GPR43. The antiinflammatory effects of SCFA are not limited to GPR43 as a recent study (Trompette et al., 2014) established a role for propionate and its receptor GPR41 in the generation of macrophage and DC precursors and in the seeding of the lungs by DCs that have high phagocytic capacity, but an impaired ability to promote Th2 cell responses.

An important question is how GPCR signaling by metabolite agonists produces antiinflammatory effects. Metabolite-sensing GPCRs signal in several ways to mediate biological effects, although this aspect remains unclear. There are several possible outcomes following engagement of a metabolite sensor with its agonist, including signaling through regular G-proteins such as G<sub>αi</sub> or G<sub>q</sub>, which usually lead to activation of MAP kinases, PI3 kinases, or mTOR. Interestingly, many metabolite-sensor GPCRs engage an alternative signaling pathway, mediated by β-Arrestin2 (Oh et al., 2010), which generally produces antiinflammatory effects, some of which relate to the inhibition of NF-κB. All of the major metabolite-sensor GPCRs signal through β-Arrestin2, but precise circumstances or cell types that preferentially use β-Arrestin2 have not yet been established. β-Arrestin2 directly interacts with IκBα (an inhibitor of NF-κB), so preventing the phosphorylation and degradation of IκBα (Gao et al., 2004).

## TRANSCRIPTIONAL AND EPIGENETIC EFFECTS

Acetylation and deacetylation of histones, as well as nonhistone proteins such as transcription factors, are a major mechanism for epigenetic regulation of gene expression (Fig. 19.3). Acetylation of histones, by histone acetyltransferases (HATs) or inhibition of HDACs, results in negatively charged histones which, when interacting with negatively charged DNA, loosen the chromatin structure resulting in a transcriptionally active conformation. Acetylation also promotes the activation, nuclear translocation, and DNA binding of transcription factors such as STAT3, NF-κB, FoxP3, N-FAT, and RUNX1. This promotes the expression of multiple genes, including those for proinflammatory cytokines (Wang et al., 2009). Acetylation of transcriptional factors can lead to structural changes that alter transcriptional factor binding to the promoter region, thereby initiating gene expression. In the case of NF-κB, acetylation of the RelA subunit prevents interaction with IκBα and results in poor formation and reduced transcriptional interaction (Chen et al., 2001). HDACs are a class of enzymes that catalyze the removal of acetyl groups from proteins, and ~1750 acetylated proteins have the potential to be affected by HDAC activity (Choudhary et al., 2009). SCFAs are natural inhibitors of HDAC enzymatic activity, and this may affect gut homeostasis, since HDAC3 deleted from intestinal epithelial cells severely disrupted gut homeostasis, in the DSS model (Alenghat et al., 2013). Butyrate is the most potent HDAC inhibitor, and acetate the least. However, SCFAs may also inhibit the transcription of individual HDAC genes, thereby influencing HDAC activity. Synthetic small molecule inhibitors of HDACs attenuate inflammation in animal models of arthritis, inflammatory bowel disease, asthma, diabetes, cardiovascular disease, and multiple sclerosis (Wang et al., 2009). In addition, the broad HDAC inhibitor trichostatin-A increases Treg gene expression and suppressive capacity (Tao et al., 2007) supporting involvement of SCFAs in Treg induction, and illustrating the potential of either natural or synthetic HDAC inhibitors to promote immunological tolerance, or antiinflammatory responses.

## OTHER IMPORTANT METABOLITES: TRYPTOPHAN CATABOLITES, ω-3 FATTY ACIDS

If Western diet and metabolites are related to the increase in incidence of some autoimmune diseases, then how much of this relates to the gut microbiota, versus metabolites derived directly from food digestion, such as ω-3 fatty acids, or tryptophan metabolites. Tryptophan, an essential amino acid, is found in foodstuffs such as red meat and fish, eggs, yogurt, and many vegetables. Tryptophan may be catabolized by microbial species, such as lactobacilli. One tryptophan metabolite produced by lactobacilli, indole-3-aldehyde, is an

Aryl hydrocarbon receptor (AhR) agonist (Zelante et al., 2013). Following agonist binding, AhR translocates to the nucleus, where it forms a heterodimer with AhR nuclear translocator. AhR-dependent gene expression includes genes involved in the production of mediators important for gut homeostasis including IL-22, antimicrobial factors, increased Th17 cell activity, and the maintenance of intraepithelial lymphocytes, and ROR $\gamma$ t<sup>+</sup> innate lymphoid cells (Li et al., 2011; Veldhoen and Brucklacher-Waldert, 2012). The absence of AhR in mice increases the severity of DSS colitis (Li et al., 2011) as well as *C. rodentium* infection (Kiss et al., 2011). However, various other metabolites bind AhR, including flavonoids and glucosinolates, which are abundant in plants. Tryptophan is degraded to kynurenin (an AhR agonist) by the immune-regulatory enzyme indoleamine 2,3-dioxygenase (IDO) and IDO activity is linked to suppression of T-cell responses, promotion of Tregs, and immune tolerance (King and Thomas, 2007). Moreover, a number of tryptophan metabolites including Kynurenic acid and niacin agonize metabolite-sensing GPCRs, such as GPR35 and GPR109A. GPR35 polymorphisms are a genetic risk factor for IBD (Jostins et al., 2012b). Dietary tryptophan can be metabolized by the gut microbiota into AhR agonists and in one study had an effect on astrocytes to limit CNS inflammation in the experimental autoimmune encephalomyelitis (EAE) model. Moreover, in individuals with MS, the circulating levels of AhR agonists were decreased (Rothhammer et al., 2016). Thus multiple elements of tryptophan catabolism may affect gut homeostasis, metabolite levels, and autoimmune disease.

The mere existence of metabolite-sensing GPCRs for dietary factors such as  $\omega$ -3 fatty acids (Oh et al., 2010; Toma et al., 2008) or tryptophan metabolites suggests nonbacterial metabolites are important for regulating immunity. It is possible that the health benefits of the Mediterranean diet relate, in part, to  $\omega$ -3 fatty acids, which show antiinflammatory effects in many animal models, as well as in clinical intervention studies (Simopoulos, 2002).

SCFAs may play a major role in the prevention of autoimmune disease and may underlie at least some microbiota-related associations with human disease. We have shown that SCFAs from the mother cross the placenta and protect against inflammatory asthma in offspring through epigenetic imprinting, mediating changes in gene transcription such as Foxp3 target genes important for tolerance/autoimmunity (Thorburn et al., 2015). SCFAs produced from bacterial fermentation of fiber not only promote Treg number and function in the colon (Furusawa et al., 2013; Smith et al., 2013) but also induce the promotion of extrathymic generation of Treg, via epigenetic effects (Arpaia et al., 2013). This, in turn, allows Treg to better control autoreactive lymphocytes and prevent the development of autoimmune disease (Bridle et al., 2013; Feuerer et al., 2009; Fontenot and Rudensky, 2005; Zhou et al., 2009). For instance, epigenetic alterations such as histone modifications of the FOXP3 locus are important for proper Foxp3 expression and the functional activity of Tregs (Zhang et al., 2013). Foxp3 also epigenetically modulates the transcriptional activity of target gene loci by altering DNA methylation, transcription factor associations, and histone modifications. These include the HATs Tip60 and p300 and the HDAC HDAC7 (Li et al., 2007; Tao et al., 2007). Tregs, driven by the Foxp3 transcription factor, are particularly important for limiting autoimmunity and chronic inflammation (Tao et al., 2007; Vignali et al., 2008).

## CONCLUDING REMARKS

New understanding in gut microbial ecology, metabolite biology, metabolite-sensing GPCRs, and transcriptional regulation by metabolites opens up an attractive new understanding of autoimmune diseases, as well as new avenues to prevent or treat autoimmune diseases—particularly those diseases that have a very strong environmental influence. Increases in the incidence of certain autoimmune disease likely relate to Western diet and lack of dietary fiber. It is also possible that antibiotic use by humans, or antibiotic presence in the food chain, adversely affects gut bacterial ecology which then flows through to higher incidences of some autoimmune diseases. Although it is early days, the implementation of diet and/or gut microbiota as a tool to prevent or treat autoimmune diseases is an exciting prospect that may impact greatly on human health. One of the advantages of a gut microbiota/prebiotic approach to human disease, including autoimmune disease, is that beneficial metabolites operate through numerous cellular and molecular pathways (Marino et al., 2017; Tan et al., 2016), they appear to be highly efficacious in most models tested, and they are natural products. For these reasons, the treatment (or prevention) of autoimmune diseases could undergo a transformation in the coming years.

## References

- Alenghat, T., Osborne, L.C., Saenz, S.A., Kobuley, D., Ziegler, C.G., Mullican, S.E., et al., 2013. Histone deacetylase 3 coordinates commensal-bacteria-dependent intestinal homeostasis. *Nature* 504, 153–157.
- Arpaia, N., Campbell, C., Fan, X., Dikiy, S., Van Der Veeken, J., Deroos, P., et al., 2013. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 504, 451–455.
- Atarashi, K., Tanoue, T., Shima, T., Imaoka, A., Kuwahara, T., Momose, Y., et al., 2011. Induction of colonic regulatory T cells by indigenous *Clostridium* species. *Science* 331, 337–341.
- Atarashi, K., Tanoue, T., Oshima, K., Suda, W., Nagano, Y., Nishikawa, H., et al., 2013. Treg induction by a rationally selected mixture of *Clostridia* strains from the human microbiota. *Nature* 500, 232–236.
- Berthon, B.S., Macdonald-Wicks, L.K., Gibson, P.G., Wood, L.G., 2013. Investigation of the association between dietary intake, disease severity and airway inflammation in asthma. *Respirology* 18, 447–454.
- Bridle, B.W., Chen, L., Lemay, C.G., Diallo, J.S., Pol, J., Nguyen, A., et al., 2013. HDAC inhibition suppresses primary immune responses, enhances secondary immune responses, and abrogates autoimmunity during tumor immunotherapy. *Mol. Ther.* 21, 887–894.
- Calcinaro, F., Dionisi, S., Marinaro, M., Candeloro, P., Bonato, V., Marzotti, S., et al., 2005. Oral probiotic administration induces interleukin-10 production and prevents spontaneous autoimmune diabetes in the non-obese diabetic mouse. *Diabetologia* 48, 1565–1575.
- Cani, P.D., Possemiers, S., Van de Wiele, T., Guiot, Y., Everard, A., Rottier, O., et al., 2009. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut* 58, 1091–1103.
- Castro-Rodriguez, J.A., Garcia-Marcos, L., Alfonseda Rojas, J.D., Valverde-Molina, J., Sanchez-Solis, M., 2008. Mediterranean diet as a protective factor for wheezing in preschool children. *J. Pediatr.* 152, 823–828, , 828.e1-2.
- Chen, L., Fischle, W., Verdin, E., Greene, W.C., 2001. Duration of nuclear NF-kappaB action regulated by reversible acetylation. *Science* 293, 1653–1657.
- Choudhary, C., Kumar, C., Gnad, F., Nielsen, M.L., Rehman, M., Walther, T.C., et al., 2009. Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science* 325, 834–840.
- Clemente, J.C., Ursell, L.K., Parfrey, L.W., Knight, R., 2012. The impact of the gut microbiota on human health: an integrative view. *Cell* 148, 1258–1270.
- Collado, M.C., Donat, E., Ribes-Koninckx, C., Calabuig, M., Sanz, Y., 2009. Specific duodenal and faecal bacterial groups associated with paediatric coeliac disease. *J. Clin. Pathol.* 62, 264–269.
- De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poulet, J.B., Massart, S., et al., 2010. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc. Natl. Acad. Sci. U.S.A.* 107, 14691–14696.
- Devereux, G., 2006. The increase in the prevalence of asthma and allergy: food for thought. *Nat. Rev. Immunol.* 6, 869–874.
- Dupaul-Chicoine, J., Yeretssian, G., Doiron, K., Bergstrom, K.S., McIntire, C.R., LeBlanc, P.M., et al., 2010. Control of intestinal homeostasis, colitis, and colitis-associated colorectal cancer by the inflammatory caspases. *Immunity* 32, 367–378.
- Eder, W., Ege, M.J., von Mutius, E., 2006. The asthma epidemic. *N. Engl. J. Med.* 355, 2226–2235.
- Elinav, E., Strowig, T., Kau, A.L., Henao-Mejia, J., Thaiss, C.A., Booth, C.J., et al., 2011. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. *Cell* 145, 745–757.
- Endesfelder, D., zu Castell, W., Ardissonne, A., Davis-Richardson, A.G., Achenbach, P., Hagen, M., et al., 2014. Compromised gut microbiota networks in children with anti-islet cell autoimmunity. *Diabetes* 63, 2006–2014.
- Estruch, R., Ros, E., Salas-Salvado, J., Covas, M.I., Corella, D., Aros, F., et al., 2013. Primary prevention of cardiovascular disease with a Mediterranean diet. *N. Engl. J. Med.* 368, 1279–1290.
- Feuerer, M., Herrero, L., Cipolletta, D., Naaz, A., Wong, J., Nayer, A., et al., 2009. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. *Nat. Med.* 15, 930–939.
- Fontenot, J.D., Rudensky, A.Y., 2005. A well adapted regulatory contrivance: regulatory T cell development and the forkhead family transcription factor Foxp3. *Nat. Immunol.* 6, 331–337.
- Fukuda, S., Toh, H., Hase, K., Oshima, K., Nakanishi, Y., Yoshimura, K., et al., 2011. Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature* 469, 543–547.
- Furusawa, Y., Obata, Y., Fukuda, S., Endo, T.A., Nakato, G., Takahashi, D., et al., 2013. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 504, 446–450.
- Gao, H., Sun, Y., Wu, Y., Luan, B., Wang, Y., Qu, B., et al., 2004. Identification of beta-arrestin2 as a G protein-coupled receptor-stimulated regulator of NF-kappaB pathways. *Mol. Cell* 14, 303–317.
- Geuking, M.B., Cahenzli, J., Lawson, M.A., Ng, D.C., Slack, E., Hapfelmeier, S., et al., 2011. Intestinal bacterial colonization induces mutualistic regulatory T cell responses. *Immunity* 34, 794–806.
- Geuking, M.B., McCoy, K.D., Macpherson, A.J., 2013. Metabolites from intestinal microbes shape Treg. *Cell Res.* 23, 1339–1340.
- Giongo, A., Gano, K.A., Crabb, D.B., Mukherjee, N., Novelo, L.L., Casella, G., et al., 2011. Toward defining the autoimmune microbiome for type 1 diabetes. *ISME J.* 5, 82–91.
- de Goffau, M.C., Luopajarvi, K., Knip, M., Ilonen, J., Ruohola, T., Harkonen, T., et al., 2013. Fecal microbiota composition differs between children with beta-cell autoimmunity and those without. *Diabetes* 62, 1238–1244.
- Herbst, T., Sichelstiel, A., Schar, C., Yadava, K., Burki, K., Cahenzli, J., et al., 2011. Dysregulation of allergic airway inflammation in the absence of microbial colonization. *Am. J. Respir. Crit. Care Med.* 184, 198–205.
- Huttenhower, C., Kostic, A.D., Xavier, R.J., 2014. Inflammatory bowel disease as a model for translating the microbiome. *Immunity* 40, 843–854.
- Jostins, L., Ripke, S., Weersma, R.K., Duerr, R.H., McGovern, D.P., Hui, K.Y., et al., 2012a. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* 491, 119–124.
- Jostins, L., Ripke, S., Weersma, R.K., Duerr, R.H., McGovern, D.P., Hui, K.Y., et al., 2012b. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* 491, 119–124.

- Kau, A.L., Ahern, P.P., Griffin, N.W., Goodman, A.L., Gordon, J.I., 2011. Human nutrition, the gut microbiome and the immune system. *Nature* 474, 327–336.
- King, D.E., Mainous 3rd, A.G., Lambourne, C.A., 2012. Trends in dietary fiber intake in the United States, 1999–2008. *J. Acad. Nutr. Diet.* 112, 642–648.
- King, N.J., Thomas, S.R., 2007. Molecules in focus: indoleamine 2,3-dioxygenase. *Int. J. Biochem. Cell Biol.* 39, 2167–2172.
- Kiss, E.A., Vonarbourg, C., Kopfmann, S., Hobeika, E., Finke, D., Esser, C., et al., 2011. Natural aryl hydrocarbon receptor ligands control organogenesis of intestinal lymphoid follicles. *Science* 334, 1561–1565.
- Le Chatelier, E., Nielsen, T., Qin, J., Prifti, E., Hildebrand, F., Falony, G., et al., 2013. Richness of human gut microbiome correlates with metabolic markers. *Nature* 500, 541–546.
- Lepage, P., Hasler, R., Spehlmann, M.E., Rehman, A., Zvirbliene, A., Begun, A., et al., 2011. Twin study indicates loss of interaction between microbiota and mucosa of patients with ulcerative colitis. *Gastroenterology* 141, 227–236.
- Lerner, A., Jeremias, P., Matthias, T., 2015. The world incidence and prevalence of autoimmune diseases is increasing. *Int. J. Celiac Dis.* 3, 151–155.
- Li, B., Samanta, A., Song, X., Iacono, K.T., Bembas, K., Tao, R., et al., 2007. FOXP3 interactions with histone acetyltransferase and class II histone deacetylases are required for repression. *Proc. Natl. Acad. Sci. U.S.A.* 104, 4571–4576.
- Li, Y., Innocentin, S., Withers, D.R., Roberts, N.A., Gallagher, A.R., Grigorieva, E.F., et al., 2011. Exogenous stimuli maintain intraepithelial lymphocytes via aryl hydrocarbon receptor activation. *Cell* 147, 629–640.
- Ljungberg, M., Korpela, R., Ilonen, J., Ludvigsson, J., Vaarala, O., 2006. Probiotics for the prevention of beta cell autoimmunity in children at genetic risk of type 1 diabetes – the PRODIA study. *Ann. N.Y. Acad. Sci.* 1079, 360–364.
- Macia, L., Thorburn, A.N., Binge, L.C., Marino, E., Rogers, K.E., Maslowski, K.M., et al., 2012. Microbial influences on epithelial integrity and immune function as a basis for inflammatory diseases. *Immunol. Rev.* 245, 164–176.
- Macia, L., Tan, J., Vieira, A.T., Leach, K., Stanley, D., Luong, S., et al., 2015. Metabolite-sensing receptors GPR43 and GPR109A facilitate dietary fibre-induced gut homeostasis through regulation of the inflammasome. *Nat. Commun.* 6, 6734.
- Manichanh, C., Rigottier-Gois, L., Bonnaud, E., Gloux, K., Pelletier, E., Frangeul, L., et al., 2006. Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. *Gut* 55, 205–211.
- Marino, E., Richards, J.L., McLeod, K.H., Stanley, D., Yap, Y.A., Knight, J., et al., 2017. Gut microbial metabolites limit the frequency of autoimmune T cells and protect against type 1 diabetes. *Nat. Immunol.* 18, 552–562.
- Markle, J.G.M., Frank, D.N., Mortin-Toth, S., Robertson, C.E., Feazel, L.M., Rolle-Kampczyk, U., et al., 2013. Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. *Science* 339, 1084–1088.
- Maslowski, K.M., Mackay, C.R., 2011. Diet, gut microbiota and immune responses. *Nat. Immunol.* 12, 5–9.
- Maslowski, K.M., Vieira, A.T., Ng, A., Kranich, J., Sierro, F., Yu, D., et al., 2009. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* 461, 1282–1286.
- McKenzie, C., Tan, J., Macia, L., Mackay, C.R., 2017. The nutrition-gut microbiome-physiology axis and allergic diseases. *Immunol. Rev.* 278, 277–295.
- Molodecky, N.A., Soon, I.S., Rabi, D.M., Ghali, W.A., Ferris, M., Chernoff, G., et al., 2012. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology* 142, 46–54.e42; quiz e30.
- Munoz-Planillo, R., Kuffa, P., Martinez-Colon, G., Smith, B.L., Rajendiran, T.M., Nunez, G., 2013. K(+) efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter. *Immunity* 38, 1142–1153.
- Nagel, G., Weinmayr, G., Kleiner, A., Garcia-Marcos, L., Strachan, D.P., 2010. Effect of diet on asthma and allergic sensitisation in the International Study on Allergies and Asthma in Childhood (ISAAC) Phase Two. *Thorax* 65, 516–522.
- Normand, S., Delanoye-Crespin, A., Bressenot, A., Huot, L., Grandjean, T., Peyrin-Biroulet, L., et al., 2011. Nod-like receptor pyrin domain-containing protein 6 (NLRP6) controls epithelial self-renewal and colorectal carcinogenesis upon injury. *Proc. Natl. Acad. Sci. U.S.A.* 108, 9601–9606.
- Noverr, M.C., Huffnagle, G.B., 2004. Does the microbiota regulate immune responses outside the gut? *Trends Microbiol.* 12, 562–568.
- Oh, D.Y., Talukdar, S., Bae, E.J., Imamura, T., Morinaga, H., Fan, W., et al., 2010. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell* 142, 687–698.
- Ou, J., Carbonero, F., Zoetendal, E.G., DeLany, J.P., Wang, M., Newton, K., et al., 2013. Diet, microbiota, and microbial metabolites in colon cancer risk in rural Africans and African Americans. *Am. J. Clin. Nutr.* 98, 111–120.
- Peterson, D.A., McNulty, N.P., Guruge, J.L., Gordon, J.I., 2007. IgA response to symbiotic bacteria as a mediator of gut homeostasis. *Cell Host Microbe* 2, 328–339.
- Rook, G.A., Martinelli, R., Brunet, L.R., 2003. Innate immune responses to mycobacteria and the downregulation of atopic responses. *Curr. Opin. Allergy Clin. Immunol.* 3, 337–342.
- Rothhammer, V., Mascagni, I.D., Bunse, L., Takenaka, M.C., Kenison, J.E., Mayo, L., et al., 2016. Type I interferons and microbial metabolites of tryptophan modulate astrocyte activity and central nervous system inflammation via the aryl hydrocarbon receptor. *Nat. Med.* 22, 586–597.
- Round, J.L., Mazmanian, S.K., 2009. The gut microbiota shapes intestinal immune responses during health and disease. *Nat. Rev. Immunol.* 9, 313–323.
- Scher, J.U., Abramson, S.B., 2011. The microbiome and rheumatoid arthritis. *Nat. Rev. Rheumatol.* 7, 569–578.
- Shan, M., Gentile, M., Yeiser, J.R., Walland, A.C., Bornstein, V.U., Chen, K., et al., 2013. Mucus enhances gut homeostasis and oral tolerance by delivering immunoregulatory signals. *Science* 342, 447–453.
- Simopoulos, A.P., 2002. Omega-3 fatty acids in inflammation and autoimmune diseases. *J. Am. Coll. Nutr.* 21, 495–505.
- Singh, N., Gurav, A., Sivaprakasam, S., Brady, E., Padia, R., Shi, H., et al., 2014. Activation of gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. *Immunity* 40, 128–139.
- Smith, P.M., Howitt, M.R., Panikov, N., Michaud, M., Gallini, C.A., Bohlooly, Y.M., et al., 2013. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* 341, 569–573.

- Strachan, D.P., 1989. Hay fever, hygiene, and household size. *BMJ* 299, 1259–1260.
- Strachan, D.P., 2000. Family size, infection and atopy: the first decade of the “hygiene hypothesis”. *Thorax* 55 (Suppl 1), S2–S10.
- Suzuki, T., Yoshida, S., Hara, H., 2008. Physiological concentrations of short-chain fatty acids immediately suppress colonic epithelial permeability. *Br. J. Nutr.* 100, 297–305.
- Tan, J., McKenzie, C., Potamitis, M., Thorburn, A.N., Mackay, C.R., Macia, L., 2014. The role of short-chain fatty acids in health and disease. *Adv. Immunol.* 121, 91–119.
- Tan, J., McKenzie, C.I., Vuillermin, P.J., Goverse, G., Vinuesa, C.G., Mebius, R.E., et al., 2016. Dietary fibre and bacterial SCFA enhance oral tolerance and protect against food allergy through diverse cellular pathways. *Cell Rep.* 15 (12), 2809–2824.
- Tan, J.K., McKenzie, C., Marino, E., Macia, L., Mackay, C.R., 2017. Metabolite-sensing G protein-coupled receptors-facilitators of diet-related immune regulation. *Annu. Rev. Immunol.* 35, 371–402.
- Tao, R., de Zoeten, E.F., Ozkaynak, E., Chen, C., Wang, L., Porrett, P.M., et al., 2007. Deacetylase inhibition promotes the generation and function of regulatory T cells. *Nat. Med.* 13, 1299–1307.
- Tedelind, S., Westberg, F., Kjerrulf, M., Vidal, A., 2007. Anti-inflammatory properties of the short-chain fatty acids acetate and propionate: a study with relevance to inflammatory bowel disease. *World J. Gastroenterol.* 13, 2826–2832.
- Thorburn, A.N., Macia, L., Mackay, C.R., 2014. Diet, metabolites, and “western-lifestyle” inflammatory diseases. *Immunity* 40, 833–842.
- Thorburn, A.N., McKenzie, C.I., Shen, S., Stanley, D., Macia, L., Mason, L.J., et al., 2015. Evidence that asthma is a developmental origin disease influenced by maternal diet and bacterial metabolites. *Nat. Commun.* 6, 7320.
- Toma, I., Kang, J.J., Sipos, A., Vargas, S., Bansal, E., Hanner, F., et al., 2008. Succinate receptor GPR91 provides a direct link between high glucose levels and renin release in murine and rabbit kidney. *J. Clin. Invest.* 118, 2526–2534.
- Trompette, A., Gollwitzer, E.S., Yadava, K., Sichelstiel, A.K., Sprenger, N., Ngom-Bru, C., et al., 2014. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat. Med.* 20, 159–166.
- Turnbaugh, P.J., Ley, R.E., Mahowald, M.A., Magrini, V., Mardis, E.R., Gordon, J.I., 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 444, 1027–1031.
- Turnbaugh, P.J., Backhed, F., Fulton, L., Gordon, J.I., 2008. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe* 3, 213–223.
- Vaarala, O., Atkinson, M.A., Neu, J., 2008. The “perfect storm” for type 1 diabetes: the complex interplay between intestinal microbiota, gut permeability, and mucosal immunity. *Diabetes* 57, 2555–2562.
- Van de Wiele, T., Van Praet, J.T., Marzorati, M., Drennan, M.B., Elewaut, D., 2016. How the microbiota shapes rheumatic diseases. *Nat. Rev. Rheumatol.* 12, 398–411.
- Vatanen, T., Kostic, A.D., d’Hennezel, E., Siljander, H., Franzosa, E.A., Yassour, M., et al., 2016. Variation in microbiome LPS immunogenicity contributes to autoimmunity in humans. *Cell* 165, 842–853.
- Veldhoen, M., Brucklacher-Waldert, V., 2012. Dietary influences on intestinal immunity. *Nat. Rev. Immunol.* 12, 696–708.
- Vieira, S.M., Pagovich, O.E., Kriegel, M.A., 2014. Diet, microbiota and autoimmune diseases. *Lupus* 23, 518–526.
- Vignali, D.A., Collison, L.W., Workman, C.J., 2008. How regulatory T cells work. *Nat. Rev. Immunol.* 8, 523–532.
- Vinolo, M.A., Rodrigues, H.G., Nachbar, R.T., Curi, R., 2011. Regulation of inflammation by short chain fatty acids. *Nutrients* 3, 858–876.
- Vogt, M.C., Paeger, L., Hess, S., Steculorum, S.M., Awazawa, M., Hampel, B., et al., 2014. Neonatal insulin action impairs hypothalamic neurocircuit formation in response to maternal high-fat feeding. *Cell* 156, 495–509.
- Wang, L., de Zoeten, E.F., Greene, M.I., Hancock, W.W., 2009. Immunomodulatory effects of deacetylase inhibitors: therapeutic targeting of FOXP3+ regulatory T cells. *Nat. Rev. Drug Discov.* 8, 969–981.
- Wen, L., Ley, R.E., Volchkov, P.Y., Stranges, P.B., Avanesyan, L., Stonebraker, A.C., et al., 2008. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. *Nature* 455, 1109–1113.
- Willemse, L.E., Koetsier, M.A., van Deventer, S.J., van Tol, E.A., 2003. Short chain fatty acids stimulate epithelial mucin 2 expression through differential effects on prostaglandin E(1) and E(2) production by intestinal myofibroblasts. *Gut* 52, 1442–1447.
- Włodarska, M., Thaiss, C.A., Nowarski, R., Henao-Mejía, J., Zhang, J.-P., Brown, E.M., et al., 2014. NLRP6 inflammasome regulates the intestinal host-microbial interface by orchestrating goblet cell-mediated mucus secretion. *Cell* 156, 1045–1059.
- Wrzosek, L., Miquel, S., Noordine, M.L., Bouet, S., Chevalier-Curt, M.J., Robert, V., et al., 2013. *Bacteroides thetaiotaomicron* and *Faecalibacterium prausnitzii* influence the production of mucus glycans and the development of goblet cells in the colonic epithelium of a gnotobiotic model rodent. *BMC Biol.* 11, 61.
- Zaki, M.H., Boyd, K.L., Vogel, P., Kastan, M.B., Lamkanfi, M., Kanneganti, T.D., 2010. The NLRP3 inflammasome protects against loss of epithelial integrity and mortality during experimental colitis. *Immunity* 32, 379–391.
- Zelante, T., Iannitti, R.G., Cunha, C., De Luca, A., Giovannini, G., Pieraccini, G., et al., 2013. Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. *Immunity* 39, 372–385.
- Zhang, Y., Maksimovic, J., Naselli, G., Qian, J., Chopin, M., Blewitt, M.E., et al., 2013. Genome-wide DNA methylation analysis identifies hypomethylated genes regulated by FOXP3 in human regulatory T cells. *Blood* 122, 2823–2836.
- Zhou, X., Bailey-Bucktrout, S.L., Jeker, L.T., Penaranda, C., Martinez-Llordella, M., Ashby, M., et al., 2009. Instability of the transcription factor Foxp3 leads to the generation of pathogenic memory T cells in vivo. *Nat. Immunol.* 10, 1000–1007.

# Noninfectious Environmental Agents and Autoimmunity

Adam Schiffenbauer<sup>1</sup> and Frederick W. Miller<sup>2</sup>

<sup>1</sup>Environmental Autoimmunity Group, Office of Clinical Research, National Institute of Environmental Health Sciences, National Institutes of Health, Bethesda, MD, United States <sup>2</sup>Environmental Autoimmunity Group, Office of Clinical Research, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC, United States

## OUTLINE

Introduction	345	Occupational Exposures	351
Evidence Supporting the Role of Environmental Agents in Autoimmune Disease	346	Others	352
Identifying and Defining Environmentally Associated Autoimmune Diseases	348	Possible Mechanisms by Which Environmental Agents May Induce Autoimmune Diseases	356
Noninfectious Agents Associated With Autoimmune Diseases	349	Overview and Future Directions	356
Drugs	349	Acknowledgments	358
		References	358

## INTRODUCTION

Although the mechanisms by which autoimmune diseases develop remain obscure, accumulating evidence suggests that these disorders result from environmental exposures in genetically susceptible individuals (Luppi et al., 1995; Cooper et al., 1999b; Miller, 1999; Gourley and Miller, 2007; Miller et al., 2012b; Pollard et al., 2010). Despite the great progress that has been made in understanding many major histocompatibility complex (MHC) and non-MHC genetic risk factors for autoimmune diseases (see Chapter 26: Genetic Predisposition to Autoimmune Diseases Conferred by the Major Histocompatibility Complex: Utility of Animal Models, and Chapter 28: Autoimmunity in Primary Immunodeficiency Disorders), relatively little information is currently available regarding the role that specific environmental agents play in the development and course of these disorders. This is partly a result of the lack of validated exposure biomarkers and environmental assessment tools, difficulties inherent in defining which of the many environmental exposures are related to autoimmune disease, little formal training in environmental medicine for physicians, environmental effects that are limited to specific genetic backgrounds, difficulty knowing the time frame from exposure to disease onset, the rarity of some autoimmune diseases (making well-powered studies difficult), and the relative lack of resources dedicated to this area. Thus for most autoimmune conditions, specific environmental triggers remain unknown. Just as there are likely to be multiple genes needed to induce autoimmune disease, multiple environmental exposures may occur

in a particular sequence or in tandem to provoke the chronic immune activation that leads to autoimmunity. It is also possible that environmental exposures might influence epigenetic modifications that are controlled by heritable but potentially reversible changes in DNA methylation and/or chromatin structure (Javierre et al., 2011; Meng et al., 2017). In this regard many lessons might be learned from studies of cancers. Like autoimmune diseases, cancers are multifactorial disorders in which multiple genetic and environmental risk factors must interact and may need to do so in a correct sequence, with occasional long latencies, before the development of disease (Sarasin, 2003). Thus in some cases, a change induced by one exposure might be necessary before a subsequent exposure can have its effect. Alternatively, mixtures of exposures, including possible infectious and noninfectious agents, perhaps occurring during critical windows when persons may be more susceptible to them (i.e., in utero, in childhood, during puberty, pregnancy, or lactation) may be necessary in order to overcome tolerance.

Other general principles from cancer research that might be relevant to autoimmunity include the pathogenetic heterogeneity of currently defined disorders, the likely low effect sizes from many environmental exposures, and the possible requirement for inducers, promoters, and sustainers of disease at different points in the pathogenetic process (Cooper et al., 2002b). This is particularly borne out by investigations, suggesting that immune activation and autoantibodies are antigen driven and precede the development of clinical disease by months to years (Dotta and Eisenbarth, 1989; Leslie et al., 2001; Arbuckle et al., 2003; Klareskog et al., 2004; Hirschfield and Gershwin, 2013; Miller et al., 1990). These findings suggest that certain environmental exposures might be necessary for tolerance to be overcome in genetically susceptible individuals, which induces autoantibody formation, while other agents might be necessary to promote the expression of clinical disease. While some environmental factors have a role in disease pathogenesis, other types of environmental factors appear to play a role in disease severity and presentation or alternatively may serve as protective factors (Deane, 2013). There is increasing evidence that some environmental factors, such as exercise, can reduce disease symptoms (Boehler et al., 2017) and potentially decrease inflammation, while other factors such as ultraviolet light may cause disease flares (Mamyrova et al., 2017). Thus the presence of multiple risk factors, combined with the relative absence of preventative factors, might allow for the development of autoimmune diseases.

If one defines environmental agents as everything outside the genome, the many exposures that have been suspected of being involved in the pathogenesis of autoimmunity may be divided into two general categories: infectious and noninfectious agents. This chapter will focus on the evidence for the role of noninfectious environmental agents in the pathogenesis of human autoimmune diseases. Infectious agents are reviewed in Chapter 19, Diet, the Gut Microbiome, and Autoimmune Diseases.

## EVIDENCE SUPPORTING THE ROLE OF ENVIRONMENTAL AGENTS IN AUTOIMMUNE DISEASE

Evidence for the role of environmental agents in autoimmune disease comes from a variety of investigative approaches (Box 20.1). Although many of these methods are indirect, anecdotal, or may be applicable only to single patients, taken together these diverse findings strongly support the contention that most autoimmune diseases have an important environmental component (Cooper et al., 1999a; Smyk et al., 2012). A principal line of evidence for the role of the environment is that for autoimmune diseases studied to date, there is generally less than 50% disease concordance in monozygotic twins (Cooper et al., 1999b; Leslie and Hawa, 1994; Bogdanos et al., 2012). These findings suggest that, if all the genetic risk factors for a given autoimmune disease were identified, without the incorporation of environmental or other factors, it would not be possible to predict disease onset more accurately than flipping a coin.

For certain agents, it can be relatively clear when a given exposure has induced a disease in an individual patient. The definition of an environmental disease in an individual can be accomplished by identifying a new clinical disorder, which develops soon after a novel exposure, resolves when the exposure is removed (dechallenge), and then recurs after reintroduction of the same exposure (rechallenge) (Miller et al., 2012a). This approach is most easily applied in cases of exposure to defined chemical entities, such as drugs, foods, and topical or inhaled toxicants. Unfortunately, many xenobiotics (compounds not naturally found in the body) cannot easily be removed from an organism after exposure; therefore for these agents, this approach is not usually helpful. Exposures in this category include inhaled silica, vaccines, fat-soluble oils, and collagen or silicone implants.

The nonrandom distribution in time and space of some autoimmune illnesses also implies that nongenetic factors are important in disease development (Moroni et al., 2012). Studies on these topics are preliminary and sometimes have not been reproduced, leading critics to posit that referral or other biases might explain some of the

**BOX 20.1****LINES OF EVIDENCE SUPPORTING THE ROLE OF ENVIRONMENTAL AGENTS IN THE DEVELOPMENT OF AUTOIMMUNE DISEASE**

1. Less than 50% disease concordance in monozygotic twins
2. Strong temporal associations between some environmental exposures and disease onset
3. Dechallenge (disease resolution or improvement in an individual after removal of the suspected agent)
4. Rechallenge (disease recurrence or worsening in an individual after reexposure to the suspected agent)
5. Seasonality in birth dates and disease onset
6. Geographic clustering in disease incidence and prevalence
7. Changes in the prevalence or incidence of disease over time and when genetically similar cohorts move to different geographic locations
8. Major genetic risk factors for autoimmune diseases are polymorphic genes that influence responses to environmental exposures
9. Strong biologic plausibility from animal models
10. Epidemiologic associations between particular exposures and certain diseases

findings. Nonetheless, intriguing investigations have suggested that certain autoimmune disorders have a seasonal onset or that there is a seasonal association with subsets of patients based on disease-specific autoantibodies. The diseases reported to have seasonal associations include autoimmune myositis (Leff et al., 1991; Miller, 1993; Sarkar et al., 2005), juvenile rheumatoid arthritis (Feldman et al., 1996), type 1 diabetes (Weinberg et al., 1984; Willis et al., 2002), narcolepsy (Han et al., 2011), antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (Schlesinger and Schlesinger, 2005), systemic lupus erythematosus, inflammatory bowel disease, autoimmune liver disease, and psoriasis (Watad et al., 2017). Furthermore, studies of type 1 diabetes, myositis, thyroid disease, autoimmune Addison's disease, and other diseases have shown significant associations with birth dates (Willis et al., 2002; Ursic-Bratina et al., 2001; Vegosen et al., 2007; Thvilum et al., 2017; Pazderska et al., 2016), implying that certain exposures at certain times of the year may alter the target tissues or immune systems of fetuses or neonates, resulting in later autoimmunity. Although infectious agents are often presumed to be the source of such seasonal or geographic associations, the immune system, like other organ systems, appears to have cyclic or rhythmic patterns (Haus and Smolensky, 1999; Terao et al., 2012), possibly related to light exposure and mediated by melatonin or other neurohormones (Nelson and Drazen, 2000). In addition, many occupational or other exposures are seasonal, including certain pesticides, chemicals in sunscreens, and some air or water pollutants, so it is possible that noninfectious agents could account for some of those data in ways that have not been accounted for. Geographic clustering or gradients in disease prevalence or incidence have also been found for some autoimmune diseases. These investigations have primarily shown associations with latitude, suggesting a role for ultraviolet radiation in (1) inducing disease, as may be the case for dermatomyositis and celiac disease (Okada et al., 2003; Love et al., 2009; Unalp-Arida et al., 2017); (2) altering age at onset, as may be the case for rheumatoid arthritis and multiple sclerosis (Latitude, 2017; Tao et al., 2016); (3) altering mortality, as may be the case in lupus (Grant, 2004); or (4) protecting from disease, as may be the case in multiple sclerosis and type 1 diabetes (Ponsonby et al., 2002). Measurable increases or decreases in the incidence or prevalence of disease over time imply a nongenetic etiology, given the slow rate of genetic change in a population. Although data are limited in this area, and several studies are possibly confounded as a result of improvements in the ability to diagnose some conditions over time, it appears that type 1 diabetes, multiple sclerosis, lupus, and myositis are becoming increasingly prevalent, whereas rheumatoid arthritis in adults and children may be decreasing in prevalence in some populations (Oddis et al., 1990; Onkamo et al., 1999; Uramoto et al., 1999; Cooper and Stroehla, 2003).

Changes in living standards and treatment approaches can also affect disease frequency, as in rheumatic heart disease, which is decreasing in prevalence (Johnson et al., 1975). Some authors believe that as our exposure to microbial agents has decreased in modern urban environments, the development of our immune systems has been altered, resulting in greater frequencies of autoimmune disorders, a concept known as the "hygiene

hypothesis" (Rook, 2012). Studies of genetically similar populations living under different conditions have been illuminating. For example, the incidences of both multiple sclerosis and type 1 diabetes changed as members of a population moved to new regions (Dahlquist, 1998; Noseworthy et al., 2000).

Epidemiologic studies linking specific exposures to autoimmunity are limited and usually consist of relatively small, often underpowered investigations, resulting in low effect sizes and large confidence limits. Much larger, well-designed, multicentered, and sometimes international studies, using appropriate controls and collecting adequate information to minimize confounding, will be needed in the future to more fully define the specific environmental risk factors for disease (Miller, 2012). In some cases it may be useful to assess novel observational study designs such as case crossover studies (Sun et al., 2016).

## IDENTIFYING AND DEFINING ENVIRONMENTALLY ASSOCIATED AUTOIMMUNE DISEASES

One limitation in making progress toward identifying and defining environmental triggers for autoimmune disorders has been the lack of consensus about the necessary and sufficient evidence needed to define a condition that is triggered by an environmental factor. Medical and legal issues that surround many environmental exposures have further complicated this area. A group of experts in the field—members of the American College of Rheumatology Environmentally Associated Rheumatic Disease Study Group—developed consensus on a general approach to overcome this problem (Miller et al., 2000) with revision and expansion based on an expert panel workshop (Miller et al., 2012a,b). In this scheme the overall process, from the identification of the first possible patient who developed a new disease after an exposure, to the refinement of classification criteria for the new disease, is divided into four stages (Table 20.1).

The *first stage* begins by identifying a single case, or a series of cases, suspected to result from a given exposure. Such cases need to meet certain criteria to ensure that a minimum number of attribution elements are present (Miller et al., 2012a). A total of at least four of eight possible attribution elements need to be present, including at least three of five primary elements.

The five primary elements are

1. temporal plausibility (taking into account the pharmacokinetics/pharmacodynamics of the agent, minimum induction time, and maximum latency);
2. exclusion of other likely causes for the syndrome;
3. dechallenge (if possible);
4. rechallenge (if appropriate); and
5. biologic plausibility.

The additional three secondary elements are

1. identification of prior reports of similar cases (analogy);
2. identification of prior reports of nearly identical cases (specificity); and
3. evidence for a dose–response effect.

**TABLE 20.1** Proposed Stages for Identifying and Defining New Environmentally Associated Autoimmune Diseases

Stage	Description	Nomenclature (example)
1. Proposing the association	Case reports, defined by ascertainment criteria, propose a possible association of a specific clinical syndrome with a given exposure	Syndrome following exposure (rheumatoid arthritis following hepatitis B vaccination)
2. Testing the association	After a number of such cases are reported, surveillance criteria are proposed and epidemiologic and laboratory studies test that hypothesis	Cardinal signs, symptoms, and labs but without the putative exposure (EMS)
3. Defining criteria for the condition	If studies above are positive, specific preliminary classification and other criteria are defined for that specific environmental disease	Exposure-associated disorder ( <i>L</i> -tryptophan-associated EMS)
4. Refining criteria for the condition	Criteria are reassessed and refined as additional data are obtained about the disease	Exposure-induced disorder (hydralazine-induced lupus-like disorder)

Modified from Miller, F.W., Pollard, K.M., Parks, C.G., Germolec, D.R., Leung, P.S., Selmi, C., et al., 2012a. Criteria for environmentally associated autoimmune diseases. *J. Autoimmun.* 39 (4), 253–258.

In addition to meeting those criteria, the study group suggested that complete information regarding the history and examination, laboratory reports, core demographic data, family history, prior infections or physiology-altering exposures, all prior diagnoses, and the type, route, dose, and duration of the exposure are detailed in the report.

The *second stage* involves testing the possible association. This should include epidemiologic studies that use surveillance criteria to evaluate the relationship between a given exposure and a given syndrome. In vitro, in vivo, and animal studies should also be conducted to assess the biologic effects of the agent and plausibility of the development of the syndrome. Other approaches—such as clinical, laboratory, or genetic risk factor studies—could determine in case-control settings if cases of environmental disease differ from those with similar diseases without the exposure or differ from subjects similarly exposed but who do not develop disease.

If data from the second stage produce convincing evidence that the association is real, then in the *third stage*, preliminary criteria for that environmentally associated disease are developed. Classification criteria will distinguish, with reasonable sensitivity and specificity, groups of patients with one disorder from those with closely related diseases. Expert committee consensus, mathematical algorithms, or other approaches could be used to develop such criteria. Symptom, sign, and laboratory criteria should be expressed in clinically sensible and practical formats with precise definitions of constituent elements. Diagnostic, prognostic, and outcome criteria, and disease activity and damage indexes should be considered when adequate data exist.

In the *fourth stage*, if new information is obtained and warranted a redefinition of the disease, the same processes used in the third stage are repeated. Although this proposed staging structure has limitations, in that the decisions as to when to progress to the next stage remain somewhat subjective, it nonetheless provides an overall framework to plan for future studies, and it allows the classification of the known environmental agents into groups with different levels of evidence for their association with specific syndromes. Most environmental agents suspected of being associated with autoimmune diseases today remain in stage 1 or 2. Similar approaches to assess the strength of associations with established diseases and in-clinic situations have also been proposed by an expert consensus approach (Miller et al., 2012a).

## NONINFECTIOUS AGENTS ASSOCIATED WITH AUTOIMMUNE DISEASES

### Drugs

Of all the noninfectious environmental exposures associated with autoimmunity, drugs are the best recognized and most often reported (Table 20.2). This is partly due to their widespread use and to more careful monitoring by clinicians, the strict regulatory oversight and adverse event reporting systems in many countries, and the ease of collecting dechallenge and rechallenge evidence to make associations in individual patients. Although several hundred drugs have been associated with autoimmune diseases, in case reports or case series, few of the publications have met the consensus criteria described above to allow the exclusion of confounding factors, and few drugs have been studied in epidemiologic investigations. Chemical agents have been the most frequently reported drugs that precede the development of autoimmunity, although the increasing use of biologic agents has resulted in more focus on them recently. The most commonly recognized drug-related syndromes are lupus-like disorders (Hess and Mongey, 1991). They are characterized by autoantibodies to histones and single-stranded DNA rather than autoantibodies to double-stranded DNA, as are found more often in nondrug-related lupus. Drug-related lupus also differs from nondrug-related lupus in that it has more frequent arthritis and less frequent neurologic and renal involvement, as well as possibly different genetic risk factors. Virtually all the 80 or more autoimmune diseases have been reported anecdotally to be associated with one or more drugs. In some cases drug-linked disorders differ from nondrug-related forms in clinical, serologic, or genetic features, but in other cases, they do not.

It is noteworthy that although some drugs appear to be associated with many autoimmune conditions (Table 20.2), there are no common structures, mechanisms of action, metabolites, or other features among them that consistently allow prediction of their toxicity or a current understanding of the pathogenesis of these syndromes. The challenge today is to decipher the genetic and other risk factors that interact with exposure to drugs and result in disease, so that those interactions can be predicted and disease prevented.

**TABLE 20.2** Selected Drugs Associated in Case Reports or Case Series With Autoimmune Disorders<sup>a</sup>

Drug	Associated autoimmune disorders
$\alpha$ -Methyldopa	Lupus-like syndrome, hemolytic anemia, thrombocytopenia
Allopurinol	Lupus-like syndrome, vasculitis
Aminoglutethimide	Sjögren's syndrome
Bleomycin	Scleroderma
Captopril	Lupus-like syndrome, vasculitis, membranous glomerulopathy, autoimmune thrombocytopenia
Chlorpromazine	Lupus-like syndrome, hemolytic anemia
D-Penicillamine	Lupus-like syndrome, myositis, hypothyroidism, Goodpasture's syndrome
Diphenylhydantoin	Linear IgA bullous disease
Erythromycins	Lupus-like syndrome, myositis, hepatitis
Estrogens	Lupus-like syndrome, myositis
Gold salts	Lupus-like syndrome, membranous glomerulopathy
Halothane	Hepatitis
Hydralazines	Lupus-like syndrome, vasculitis, hepatitis
Interferon-alpha	Lupus-like syndrome, antiphospholipid syndrome, arthritis, hemolytic anemia, thrombocytopenia, hepatitis, hypothyroidism
Interferon-gamma	Lupus-like syndrome, myositis, arthritis, hypothyroidism
Interleukin-2	Scleroderma, antiphospholipid syndrome, arthritis, hypothyroidism
Iodine	Hypothyroidism
Isoniazid	Lupus-like syndrome, arthritis, hepatitis, vasculitis, hypothyroidism
L-Tryptophan	EMS, scleroderma, myositis, neuropathies
Levamisole	Vasculitis
Lipid-lowering agents	Lupus-like syndrome, hepatitis, thrombocytopenia
Methyldopa	Lupus-like syndrome
Minocycline	Lupus-like syndrome
Penicillins	Anemia, lupus-like syndrome, hepatitis
Phenytoin	Scleroderma, lupus-like syndrome, hepatitis, thrombocytopenia
Procainamide	Lupus-like syndrome
Propylthiouracil	Lupus-like syndrome, ANCA-associated vasculitis, myositis
Quinidine	Lupus-like syndrome, arthritis, thrombocytopenia
Rifampin	Thrombocytopenia, vasculitis
Statins	Immune-mediated necrotizing myopathy
Sulfonamides	Lupus-like syndrome, vasculitis
Tetracyclines	Lupus-like syndrome, arthritis, vasculitis
TNF inhibitors	Lupus-like syndrome

<sup>a</sup>Reviewed in Love and Miller (1993), Bigazzi (1997), Mackay (1999), D'Cruz (2000), Hess (2002), Liu and Kaplowitz (2002), Chang and Gershwin (2011), Carmona-Rivera et al. (2017).

ANCA, Antineutrophil cytoplasmic antibodies; EMS, eosinophilia–myalgia syndrome; TNF, tumor necrosis factor.

## Occupational Exposures

Limited but growing epidemiologic and experimental data have linked several occupational exposures to autoimmune diseases (Table 20.3). The most studied of these include silica, solvents, pesticides, and ultraviolet radiation (Miller et al., 2012b; Westberg et al., 2009; Cooper et al., 2002a, 2010; Parks et al., 2011; Haupt-Jorgensen et al., 2017). One of the first rheumatic diseases associated with an occupational exposure was Caplan's syndrome, which is seropositive rheumatoid arthritis associated with a specific form of pneumoconiosis that develops in miners of anthracite coal and in persons exposed to silica and asbestos (Williams, 1991). The strongest occupational associations with autoimmune disease (i.e., a relative risk of 3.0 and higher) have been documented in investigations of silica dust and rheumatoid arthritis, lupus, scleroderma, and ANCA-associated glomerulonephritis (Parks et al., 1999; Khuder et al., 2002; Ilar et al., 2018). Weaker associations are seen, however, for solvent exposures (in scleroderma, undifferentiated multisystem rheumatic disease, and multiple sclerosis) and for farming or pesticide exposures (in rheumatoid arthritis, type 1 diabetes, and lupus). Vinyl chloride has been linked to the development of a scleroderma-like disease characterized by skin thickening, Raynaud's phenomenon, acroosteolysis, and pulmonary involvement. This observation, and the publication of several case reports, stimulated research into associations between scleroderma and other chlorinated solvents (e.g., trichloroethylene and trichloroethane) with varying results (Cooper et al., 2002a, 2009).

There are many difficulties in assessing the role of occupational exposures in disease. First, there are few biomarkers for such exposures and essentially none that allow an estimate of lifetime cumulative exposure. Second, there are few validated occupational exposure questionnaires, and those that do exist can be awkward, time

**TABLE 20.3** Occupational Exposures Associated With Autoimmune Diseases Via Epidemiologic Studies

Exposure	Disease	Summary of results
Silica	Scleroderma	Increased risk was found in occupational cohort studies; mixed results in several population-based case-control studies
	Rheumatoid arthritis	Threefold increased risk (or higher) in occupational cohort studies
	Lupus	10-Fold increased risk in multiple occupational cohort studies
	ANCA vasculitis	Fourfold increased risk in multiple case-control studies
Solvents	Scleroderma	Mixed results, but some evidence of two- to threefold increased risk with specific solvents (e.g., paint thinners and removers, trichloroethylene) and with "any" solvent
	Undifferentiated multisystem rheumatic diseases	Twofold increased risk with paint thinners and removers, mineral spirits; threefold increased risk with specific solvent-related occupations
	Rheumatoid arthritis	Weak or no association with specific solvents, but twofold increased risk among spray painters and lacquer workers
	Multiple sclerosis	Two- to threefold increased risk with solvent exposures in most studies
Farming	Lupus	Lupus has been associated with agricultural pesticide mixing and pesticide mixing has been associated with positive antinuclear antibody status
	Type 1 diabetes	Bakers and farm workers exposed to grain had a decreased risk of type 1 diabetes
	Rheumatoid arthritis	Specific pesticides as well as solvents and fertilizers have been shown to be risk factors, although livestock exposure has been protective
Ultraviolet radiation	Multiple sclerosis	Reduced risk (OR 0.74) of multiple sclerosis and mortality with increased occupational exposure to sunlight
	Dermatomyositis	Positive correlation of the proportion of dermatomyositis with global surface sunlight intensity in international and US studies

ANCA, Antineutrophil cytoplasmic antibodies; OR, odds ratio.

Data from Love, L.A., Weinberg, C.R., McConaughay, D.R., Oddis, C.V., Medsger, T.A., Jr., Reveille, J.D., et al., 2009. Ultraviolet radiation intensity predicts the relative distribution of dermatomyositis and anti-Mi-2 autoantibodies in women. *Arthritis Rheum* 60 (8), 2499–2504; Cooper, G.S., Miller, F.W., Germolec, D.R., 2002. Occupational exposures and autoimmune diseases. *Int. Immunopharmacol.* 2 (2–3), 303–313; Parks, C.G., Walitt, B.T., Pettinger, M., Chen, J.C., De Roos, A.J., Hunt, J., et al., 2011. Insecticide use and risk of rheumatoid arthritis and systemic lupus erythematosus in the Women's Health Initiative Observational Study. *Arthritis Care Res.* (Hoboken) 63 (2), 184–194; Cooper, G.S., Wither, J., Bernatsky, S., Claudio, J.O., Clarke, A., Rioux, J.D., et al., 2010. Occupational and environmental exposures and risk of systemic lupus erythematosus: silica, sunlight, solvents. *Rheumatology (Oxford)* 49 (11), 2172–2180; McCormic, Z.D., Khuder, S.S., Aryal, B.K., Ames, A.L., Khuder, S.A., 2010. Occupational silica exposure as a risk factor for scleroderma: a meta-analysis. *Int. Arch. Occup. Environ. Health* 83 (7), 763–769.

consuming, and costly to apply. Third, most studies in this area have been underpowered because of the rarity of the diseases, the likely effects of different genotypes, coexposures, and the occupations of interest, resulting in imprecise or inconsistent risk estimates. And finally, there is possible confounding from multiple concurrent exposures that makes it difficult to ascertain whether different effects can be attributed to different chemicals. Thus it may not be surprising that in some cases discrepancies exist in different investigations, making it difficult to assess the true overall risks of most occupational exposures.

## Others

A variety of other exposures with greatly different properties has been proposed to be associated with autoimmune diseases (Table 20.4). The evidence supporting these proposed associations ranges from case reports to epidemiologic studies, and many are speculative at this time. They are listed below by category into which they may be classified.

**TABLE 20.4** Other Exposures Proposed as Possible Risk or Protective Factors for Autoimmune Diseases

Exposure	Disease	Comments (Reference)
Foods (gluten)	Celiac disease	Celiac disease develops after ingestion of foods containing gluten and related proteins in some genetically susceptible persons (Alaedini and Green, 2005).
Alcohol	Rheumatoid arthritis	Alcohol has been shown to be protective for developing antibody positive rheumatoid arthritis (Scott et al., 2013).
Vitamin D	Rheumatoid arthritis, psoriasis, multiple sclerosis, systemic lupus erythematosus, and vitiligo	Seasonal variations in vitamin D are considered a possible reason for seasonal variations in disease flare and onset for many autoimmune diseases (Watad et al., 2017; Zhang et al., 2017).
Cigarette smoking	Rheumatoid arthritis	Studies suggest relative risks of 1.5–3 with a greater effect in seropositive disease and those with the HLA shared epitope (Krishnan, 2003; Stolt et al., 2003; Kallberg et al., 2011). Specific phenotypic features are also associated with smoking (Perricone et al., 2016).
	Myositis	Strongest association seen in connective tissue disease overlap myositis and Hungarian patients, in whom there is possible interaction with HLA DR3 (Lilleker et al., 2018; Schiffenbauer et al., 2018).
	Autoimmune thyroid disease	Meta-analyses suggest two to threefold increased risks of Graves' and Hashimoto's diseases (Vestergaard, 2002).
	Inflammatory bowel disease	Smoking increases risks for Crohn's disease but decreases risks for ulcerative colitis (Perricone et al., 2016).
	Psoriasis and psoriatic arthritis	There is a dose-response relationship between smoking and psoriasis and an increased risk of psoriasis in smokers that decreases with smoking cessation (Perricone et al., 2016).
	Behcet's disease	Smoking has been shown to improve oral and genital ulcers in Behcet's patients with the possibility of a gene-environment interaction (Perricone et al., 2016).
	Lupus	Studies show an increased risk of lupus in smoker OR 1.31; 95% CI, 1.02–1.70 with a stronger association found in cutaneous lupus (Perricone et al., 2016).
	Multiple sclerosis	Multiple sclerosis has an OR of 1.6 among current smokers and 1.2 among former smokers and has demonstrated a dose-response relationship (Perricone et al., 2016). There is also a possible gene-environment interaction.

(Continued)

**TABLE 20.4** (Continued)

Exposure	Disease	Comments (Reference)
Heavy metals	Multiple syndromes	"Pink disease" (acrodynia) and glomerulopathy from mercury toxicity; related syndromes with elements of autoimmunity from cadmium and gold salt toxicity; granulomatous pneumonitis from beryllium exposure; support for genetic risk factors in animal models (Bigazzi, 1994; Dally, 1997; Fontenot and Kotzin, 2003).
Microchimerism	Scleroderma, lupus, primary biliary cirrhosis, autoimmune thyroid disease	Fetal cells detected in maternal blood or target tissue specimens years after pregnancy—not all findings have been reproduced, possibly due to different methodologies with different sensitivities to detect rare microchimeric cells (Sarkar and Miller, 2004).
Collagen implants	Myositis	In one study, OR = 5.05; 95% CI, 2.31–9.59 for all forms of myositis (Cukier et al., 1993).
Silicone implants	Multiple syndromes	Most studies do not find associations with defined autoimmune diseases (Janowsky et al., 2000; Tugwell et al., 2001; Singh et al., 2017); rare or atypical multisystem rheumatic diseases and fibromyalgia remain inadequately studied (Singh et al., 2017; Brown et al., 1998; Brown, 2002).
Air pollution	Juvenile idiopathic arthritis (JIA) Juvenile dermatomyositis (JDM)	Small particulate matter has been associated with the development of JIA in several studies (Rider and Miller, 2017). Maternal exposure to inhaled carbon monoxide in the third trimester was associated with JDM (OR 12.21, CI 1.28–115.96) (Orione et al., 2014).
Microbiome	Lupus, Sjogren's syndrome, Behcet's disease, scleroderma, multiple sclerosis, and rheumatoid arthritis	Epidemiological data show associations of specific perturbations of the microbiome with diseased populations, particularly oral disease, but more work is needed to show a risk or protective effect (Talotta et al., 2017; Shahi et al., 2017; Maeda and Takeda, 2017).
Stress	Graves' disease Type 1 diabetes	Stressful life events in the 12 months preceding the diagnosis were significantly higher than controls (OR 5.6.3, CI 5.2.7–14.7) (Winsa et al., 1991); other diseases were poorly studied. Stressful life events, stress levels, and poorer coping skills were significantly associated with type 1 diabetes compared to controls (Faresjo, 2015).

## Foods

Foods are some of the better examples of environmental agents associated with autoimmune diseases. The best example is celiac disease, which is characterized by an immune response to ingested wheat gluten and related proteins of rye and barley, that leads to autoantibodies to transglutaminase resulting in inflammation, villous atrophy, and crypt hyperplasia in the intestine (Alaedini and Green, 2005). There is good evidence for gene–environment interaction in celiac disease, with human leukocyte antigens DQ2 and DQ8 being the major known genetic risk factors. Celiac disease is one of the few medical conditions for which dietary intervention is the main treatment modality, and a gluten-free diet markedly decreases symptoms in many individuals. Although there is less support for an autoimmune etiology of other food-associated inflammatory disorders, including the L-tryptophan-associated eosinophilia–myalgia syndrome (EMS) (Sullivan et al., 1996a,b) and the contaminated rapeseed oil–associated toxic oil syndrome (Gelpi et al., 2002), it is possible that some cases of these illnesses represent food-associated autoimmunity, given their frequent autoantibodies and immunogenetic associations (Okada et al., 2009). And it is possible that certain diets may also play a role in the development of some autoimmune diseases (Manzel et al., 2013).

## Vitamin D

Vitamin D has been found to have many impacts on the immune system. Often it is difficult to tell if vitamin D deficiency is a cause or consequence of autoimmunity. Seasonal variations in vitamin D and its interaction with the immune system have made it an exciting target for explaining the seasonal variations in disease onset

for autoimmune disease. Vitamin D deficiency has been implicated in rheumatoid arthritis, psoriasis, multiple sclerosis, systemic lupus erythematosus, and vitiligo (Watad et al., 2017; Zhang et al., 2017).

### Tobacco Smoke

Tobacco smoke has been associated epidemiologically with a higher risk of seropositive rheumatoid arthritis (Bang et al., 2010), all combined autoimmune thyroid disease, and Crohn's disease, in which a gene–environment interaction is clear, but inconsistent results were found in studies of smoking and systemic lupus erythematosus, multiple sclerosis, and Hashimoto's thyroiditis (Miller et al., 2012b; Perricone et al., 2016). Smoking has also been associated with specific phenotypes of the idiopathic inflammatory myopathies (Chinoy et al., 2012; Lilleker et al., 2018). There is also evidence that second-hand smoke is a risk factor for childhood lupus (Conde et al., 2018) and for disease severity in rheumatoid arthritis (Hamam and Gheita, 2017).

Conversely, smoking may be associated with a reduced risk of ulcerative colitis, an inflammatory bowel disease, implying that the complex mix of chemicals in tobacco smoke may have different effects in different backgrounds.

### Heavy Metals

Exposures to heavy metals, including mercury, cadmium, gold salts, and beryllium, have been associated with a variety of pathologic syndromes, some of which have features of autoimmunity. A study of communities in Amazonian Brazil with well-characterized exposures to mercury was the first to document immunologic changes, indicative of autoimmune dysfunction, in persons exposed to mercury (Silva et al., 2004). The overall clinical evidence of mercury impacting autoimmunity has not been clear (Crowe et al., 2017; Pigatto and Guzzi, 2010; Yeter et al., 2016; Somers et al., 2015), with low levels of mercury seen in patients with celiac disease, but elevated levels were associated with antinuclear antibody positivity and Kawasaki disease. In addition, many animal models have documented inflammatory and sometimes highly specific autoimmune responses to heavy metals, even at subtoxic doses, which appear to differ in different genetic backgrounds (Bagenstose et al., 1999a,b). The mechanisms for these many effects remain unclear, but possibilities include changing the response repertoire by direct and indirect means via changes in cytokine profiles, influencing expression of new antigens, new peptides, and/or antigen presentation by modifying the antigen-presenting complex (Rowley and Monestier, 2005), as has been reported for abacavir loading of novel self-peptides into human leukocyte antigen (HLA)-B\*57:01 (Norcross et al., 2012), or altering the Th17 response (Hemdan et al., 2013).

### Microchimerism

Microchimerism is the persistence of a low level of nonhost stem cells or their progeny in an individual. A possible role of microchimerism in the pathogenesis of some autoimmune diseases (systemic sclerosis, systemic lupus erythematosus, rheumatoid arthritis, primary biliary cirrhosis, autoimmune thyroid diseases, and juvenile myositis) but not all has been suggested (Sarkar and Miller, 2004; Nelson, 2012; Boddy et al., 2015). The initial impetus to explore this exposure was that many of the diseases associated with microchimerism have features that are shared with graft-versus-host disease, suggesting a possible mechanism. Although an appealing hypothesis, controversy in the area continues due to the lack of reproducible studies to date and the lack of proof of the role of microchimeric cells in the pathogenesis of these disorders. The possibility that cells of multiple origins and different genetic backgrounds may combine to result in functional organ systems, both in mothers and their offspring, requires a reevaluation of many current paradigms. It is possible that such chimeric mixtures play a role in autoimmunity, tissue repair, and other areas (Fugazzola et al., 2011). Further research using standardized, sensitive, and validated methods is needed to address the many questions that the early findings in this field have raised.

### Vaccines

Because vaccines are foreign proteins often injected with adjuvants into muscle to induce immune responses, it may not be surprising that immune-mediated adverse events have been reported after a wide variety of immunizations. Although a number of autoimmune diseases have been found to develop after vaccinations, only a few have been deemed to be associated with disease by the Advisory Committee on Immunization Practices (Robinson et al., 2017) and are now compensated by the National Vaccine Injury Compensation Program (<http://www.hrsa.gov/vaccinecompensation/index.html>). These include cases of chronic arthritis after rubella virus vaccine and thrombocytopenic purpura after measles vaccine. There remains significant controversy over other illnesses possibly caused by immunizations, but most epidemiologic studies in this area have not shown significant associations

(Wraith et al., 2003; De Martino et al., 2013). A report from the Institute of Medicine concluded that there was no increased risk of type 1 diabetes or Guillain–Barré syndrome from vaccination (Medicine Io et al., 2002).

### Implants

Bovine collagen implants are biomaterials used to correct dermal contour deformities. The use of bovine collagen implants in patients with a history of autoimmune diseases is contraindicated by the manufacturer due to concerns that they might induce adverse immune responses because anticollagen autoantibodies are present in some patients with multisystem rheumatic diseases. Few epidemiologic studies have been performed in this area, although one study evaluated the development of myositis in nine patients who received collagen implants (Cukier et al., 1993). Eight of the nine patients had a delayed-type hypersensitivity response at the test or treatment site, and five of six patients tested had elevated serum antibodies to collagen. Compared with the general population, the incidence of dermatomyositis or polymyositis among collagen-treated patients was significantly higher. There have also been case reports of autoimmune-like illness after such implants (Garcia-Domingo et al., 2000; Bonnet et al., 1996).

Silicone implants remain some of the most controversial environmental agents proposed to be associated with connective tissue disorders. Studies in this area have been hampered by the extensive litigation involved in adverse events following silicone breast implants and the lack of adequate regulatory review prior to their initial use. Most studies have not found associations with defined autoimmune diseases (Janowsky et al., 2000; Tugwell et al., 2001; Singh et al., 2017). However, there is ongoing research into whether there are atypical autoimmune disease manifestations associated with silicone implants (Colaris et al., 2017). Of interest, women who develop myositis after silicone implants appear to be an immunogenetically distinct subgroup with different allelic associations that are seen in women who develop myositis without implants (O'Hanlon et al., 2004).

### Stress

There is anecdotal evidence that stressful life events have preceded the development of many autoimmune diseases. A large population-based, case–control study of patients with Graves' disease showed that they had more negative life events in the 12 months preceding the diagnosis, and negative life-event scores were significantly higher than those of controls (Winsa et al., 1991; Vita et al., 2017; Wiersinga, 2016). There is also evidence for a link between stress and type 1 diabetes, celiac disease, lupus, juvenile idiopathic arthritis, alopecia areata, and vitiligo (Faresjo, 2015). Although the mechanisms for how stress may play a role in autoimmune disease remain unclear, it has been hypothesized that, under certain conditions, stress hormones may boost immune responses by inducing tumor necrosis factor (TNF)-alpha, interleukin (IL)-1, and IL-8, and by inhibiting transforming growth factor-beta production (Elenkov and Chrousos, 1999). Therefore conditions that are associated with significant changes in stress system activity may modulate the neuroendocrine–immune axis and perturb systemic cytokine balances, resulting in proinflammatory changes and disease induction.

### Air Pollution

Air pollution has often been considered a potential agent to dysregulate the immune system and incite autoimmune disease. This area of study is currently evolving, but some initial evidence indicates that air pollution might be a risk factor for systemic autoimmune disease, particularly juvenile idiopathic arthritis (Sun et al., 2016; Bernatsky et al., 2016). A Brazilian study showed an association between increased air pollutants and increased disease activity in patients with pediatric lupus (Fernandes et al., 2015).

### Exercise

The role of exercise in autoimmunity had garnered much attention. Animal models have demonstrated significant decreases in disease activity with the adoption of an exercise regimen (Aqel et al., 2017). In human studies lack of exercise has been associated with worse symptoms in rheumatoid arthritis, lupus, myositis (Pinto et al., 2017), and scleroderma (Azar et al., 2018). Some studies have shown the ability of exercise to help in disease management, such as in Sjogren's syndrome (Saraux et al., 2016) and myositis, although in lupus no benefit was seen (Abrahao et al., 2016; Thomas, 2013). Further studies are certainly needed to better understand the impact of exercise on autoimmune disease and to determine the optimal exercise regimens for patients.

### Microbiome

The microbiome represents a part of the environment that travels with individuals. It is being studied aggressively for its impact on autoimmune disease. Evidence has emerged suggesting a role for the microbiome in the

pathogenesis of lupus, Sjogren's syndrome, Behcet's disease, scleroderma (Talotta et al., 2017), multiple sclerosis (Shahi et al., 2017), and rheumatoid arthritis (Maeda and Takeda, 2017). This area poses an exciting target for inquiry as new tools are developed to better determine the microbiome and to fully assess the various geographic microbiota of humans (nasal, intestinal, skin, and others). Please see Chapter 21 for a more in-depth discussion on the microbiome.

## POSSIBLE MECHANISMS BY WHICH ENVIRONMENTAL AGENTS MAY INDUCE AUTOIMMUNE DISEASES

Although the mechanisms for the development of autoimmune diseases associated with exposures to noninfectious agents remain poorly understood, a variety of theories have postulated how xenobiotics might induce disease (Table 20.5). The wide range of these theories underscores the lack of understanding of mechanisms, even for the most carefully defined environmentally associated diseases, and suggests that different pathogenic mechanisms are likely at work in different syndromes.

Whatever specific mechanisms are involved in the development of an autoimmune disease, it has been suggested that an overall framework should include the concept of heterogeneity within the currently defined diseases. A working hypothesis that addresses this issue has been termed the "elemental disorder hypothesis," which posits that each autoimmune disease as currently recognized contains many elemental disorders (Gourley and Miller, 2007; Shamim and Miller, 2000; Schmidt, 2011). An elemental disorder is defined as a unique sign–symptom–laboratory complex (syndrome) that results from a distinct pathogenesis as a result of the interaction of the necessary and sufficient genetic and environmental risk factors. If this concept is true, elemental disorders are likely confounding most studies of disease today by inducing "comparisons of apples and oranges." If identified, elemental disorders should greatly increase the homogeneity of populations under study and thus decrease the numbers of individuals needed for genetic, environmental, and therapeutic studies. In the future identification of elemental disorders could allow for the prevention of some illnesses by avoidance of environmental risk factors, increasing exposure to protective factors, or via gene therapy to correct genetic risk factors.

## OVERVIEW AND FUTURE DIRECTIONS

The multifactorial nature of autoimmune diseases has inhibited our understanding of the mechanisms that initiate and sustain them. Autoimmune syndromes are believed to arise, however, from a complex and ill-understood interplay of predisposing genetic and environmental risk factors. Although progress is being made in defining the multiple genetic risk factors, we are in our infancy in identifying the environmental risk factors for autoimmune illnesses. By understanding the interactions of the elements that are necessary for disease to develop, we can prevent or treat autoimmune diseases in novel ways. Before that can be accomplished, however, important questions remain to be answered, such as: Which specific gene–environment interactions lead to which specific clinical syndromes? What are the pathogenic mechanisms involved? Is every autoimmune disease, as currently understood, actually composed of many subsets or "elemental disorders," each of which may be defined by a unique pathogenesis resulting from interactions of necessary and sufficient risk factors? Can selected autoimmune diseases be better treated, cured, or even prevented through answers to some of the above questions?

**TABLE 20.5** Possible Mechanisms by Which Environmental Agents May Induce Autoimmunity and Promote and Sustain Autoimmune Diseases (Possible Examples in Parentheses)<sup>a</sup>

1. Alteration of target tissue autoantigen structure (bystander drugs, heavy metals)
2. Upregulation or altered locations of normally sequestered autoantigens (UV radiation)
3. Cytotoxic, inhibitory, or stimulatory effects on components of the immune system (interferons, interleukins)
4. Molecular mimicry—structures shared between environmental agent and self (infectious agents)
5. Induction of epigenetic changes and subsequent gene activation or suppression (smoking)
6. Other effects and combinations of the above

<sup>a</sup>Initiators of autoimmunity may differ in action from promoters, sustainers, or agents that cause flares of autoimmune disease.

We live in an increasingly complex sea of xenobiotics, which complicates exposure assessments. More than 80,000 chemicals are registered for use in commerce in the United States, and an estimated 2000 new ones are introduced annually to be included in our foods, personal care products, drugs, household cleaners, and a host of industrial processes. The long-term effects of most of these chemicals on human health are unknown, yet we may be exposed to them during the manufacture, distribution, use, and disposal of products or as pollutants in our air, water, or soil. As a result, none of us knows the full range of environmental agents we are exposed to on a daily basis.

The concept of the exposome, which consists of all the exposures that an individual has experienced during their lifetime, has been proposed as a useful construct to focus attention on the critical need for more comprehensive environmental exposure assessments. Currently we are not able to simultaneously measure all the many important components of the exposome—including xenobiotics approved in commerce, the additional thousands of compounds not approved but to which we are exposed, plus stressful life events, cigarette smoke, or alcoholic drink compositions, drugs and dietary supplements, nanoparticles, biotoxins, cosmetics, hair dyes, ultraviolet and other radiation and climatic factors, and electric and magnetic fields—at any single time point in an individual, let alone over a lifetime. Much more work is needed to develop novel assessment tools that could evaluate these multiple exposures, either directly or indirectly, via their effects on gene expression or epigenetic modification, microRNA levels, or protein composition of various matrices in the body, both in real time and in summation over time.

Many challenges have prevented us from better understanding the environmental risk factors that might trigger autoimmune diseases in genetically susceptible individuals. These challenges include inadequate validated exposure assessment tools and bioassays; poor training in the evaluation of environmental exposures; the lack of population-based incidence, prevalence, demographic information, and databases or repositories for most diseases; inadequate funding; and the lack of accepted and standardized approaches for defining the minimal criteria for an environmentally triggered disease. Many coordinated initiatives may be useful in overcoming these obstacles and making more progress in the future (Box 20.2). Central to all these efforts are greater attention to and funding for understanding the essential environmental exposures that initiate, promote, or sustain autoimmune disorders. Such investments are likely to be very cost effective because they would have important clinical and financial implications for improving public health.

#### BOX 20.2

#### POSSIBLE APPROACHES TO ENHANCE IDENTIFICATION OF ENVIRONMENTAL RISK FACTORS FOR AUTOIMMUNE DISEASES

1. Foster national and international collaborations and coordination to integrate existing and newly developed clinical databases, registries, specimen repositories and other resources, and minimize duplication of effort
2. Develop and validate clinically useful standardized environmental exposure assessment tools
3. Develop and validate standardized biomarkers for environmental exposures
4. Increase support for well-designed, population-based and case-control hypothesis-testing studies for suspected environmental agents
5. Increase support for hypothesis-generating studies to identify new agents and syndromes
6. Collect systematic descriptive epidemiologic data for all autoimmune diseases and their major phenotypes as a baseline for future comparisons
7. Increase use of information technology and other novel approaches to enhance communications, coordinate efforts, and facilitate clinical studies
8. Improve coordination between animal model and epidemiologic studies
9. Develop novel mathematic, statistical, and bioinformatic approaches to enhance epidemiology studies
10. Define gene–gene, gene–environment, and environment–environment interactions
11. Establish an international coordinating committee to oversee and facilitate the above, encourage multidisciplinary research and prepare for and respond to epidemics of environmentally induced, immune-mediated diseases

## Acknowledgments

We thank Drs. Christine Parks, James Katz, and Peter Grayson for their useful comments and Lisa Maroski for technical assistance with the manuscript. This research was supported in part by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences.

## References

- Abrahao, M.I., Gomiero, A.B., Peccin, M.S., Grande, A.J., Trevisani, V.F., 2016. Cardiovascular training vs. resistance training for improving quality of life and physical function in patients with systemic lupus erythematosus: a randomized controlled trial. *Scand. J. Rheumatol.* 45 (3), 197–201.
- Alaedini, A., Green, P.H., 2005. Narrative review: celiac disease: understanding a complex autoimmune disorder. *Ann. Intern. Med.* 142 (4), 289–298.
- Aqel, S.I., Hampton, J.M., Bruss, M., Jones, K.T., Valiente, G.R., Wu, L.C., et al., 2017. Daily moderate exercise is beneficial and social stress is detrimental to disease pathology in murine lupus nephritis. *Front. Physiol.* 8, 236.
- Arbuckle, M.R., McClain, M.T., Rubertone, M.V., Scofield, R.H., Dennis, G.J., James, J.A., et al., 2003. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N. Engl. J. Med.* 349 (16), 1526–1533.
- Azar, M., Rice, D.B., Kwakkenbos, L., Carrier, M.E., Shrier, I., Bartlett, S.J., et al., 2018. Exercise habits and factors associated with exercise in systemic sclerosis: a Scleroderma Patient-centered Intervention Network (SPIN) cohort study. *Disabil. Rehabil.* 40 (17), 1997–2003.
- Bagenstose, L.M., Salgame, P., Monestier, M., 1999a. Cytokine regulation of a rodent model of mercuric chloride-induced autoimmunity. *Environ. Health Perspect.* 107 (Suppl 5), 807–810.
- Bagenstose, L.M., Salgame, P., Monestier, M., 1999b. Murine mercury-induced autoimmunity: a model of chemically related autoimmunity in humans. *Immunol. Res.* 20 (1), 67–78.
- Bang, S.Y., Lee, K.H., Cho, S.K., Lee, H.S., Lee, K.W., Bae, S.C., 2010. Smoking increases rheumatoid arthritis susceptibility in individuals carrying the HLA-DRB1 shared epitope, regardless of rheumatoid factor or anti-cyclic citrullinated peptide antibody status. *Arthritis Rheum.* 62 (2), 369–377.
- Bernatsky, S., Smargiassi, A., Barnabe, C., Svenson, L.W., Brand, A., Martin, R.V., et al., 2016. Fine particulate air pollution and systemic autoimmune rheumatic disease in two Canadian provinces. *Environ. Res.* 146, 85–91.
- Bigazzi, P.E., 1994. Autoimmunity and heavy metals. *Lupus* 3 (6), 449–453.
- Bigazzi, P.E., 1997. Autoimmunity caused by xenobiotics. *Toxicology* 119 (1), 1–21.
- Boddy, A.M., Fortunato, A., Wilson Sayres, M., Aktipis, A., 2015. Fetal microchimerism and maternal health: a review and evolutionary analysis of cooperation and conflict beyond the womb. *Bioessays* 37 (10), 1106–1118.
- Boehler, J.F., Hogarth, M.W., Barberio, M.D., Novak, J.S., Ghimbovschi, S., Brown, K.J., et al., 2017. Effect of endurance exercise on microRNAs in myositis skeletal muscle—a randomized controlled study. *PLoS One* 12 (8), e0183292.
- Bogdanos, D.P., Smyk, D.S., Rigopoulou, E.I., Mytilinaiou, M.G., Heneghan, M.A., Selmi, C., et al., 2012. Twin studies in autoimmune disease: genetics, gender and environment. *J. Autoimmun.* 38 (2–3), J156–J169.
- Bonnet, C., Charriere, G., Vaquier, J., Bertin, P., Vergne, P., Treves, R., 1996. Bovine collagen induced systemic symptoms: antibody formation against bovine and human collagen. *J. Rheumatol.* 23 (3), 545–547.
- Brown, S.L., 2002. Epidemiology of silicone-gel breast implants. *Epidemiology* 13 (3 Suppl), S34–S39.
- Brown, S.L., Langone, J.J., Brinton, L.A., 1998. Silicone breast implants and autoimmune disease. *J. Am. Med. Womens Assoc.* 53 (1), 21–24, 40.
- Carmona-Rivera, C., Purmalek, M.M., Moore, E., Waldman, M., Walter, P.J., Garrallo, H.M., et al., 2017. A role for muscarinic receptors in neutrophil extracellular trap formation and levamisole-induced autoimmunity. *JCI Insight* 2 (3), e89780.
- Chang, C., Gershwin, M.E., 2011. Drug-induced lupus erythematosus: incidence, management and prevention. *Drug Saf.* 34 (5), 357–374.
- Chinoy, H., Adimulam, S., Marriage, F., New, P., Vincze, M., Zilahi, E., et al., 2012. Interaction of HLA-DRB1\*03 and smoking for the development of anti-Jo-1 antibodies in adult idiopathic inflammatory myopathies: a European-wide case study. *Ann. Rheum. Dis.* 71 (6), 961–965.
- Colaris, M.J.L., de Boer, M., van der Hulst, R.R., Cohen Tervaert, J.W., 2017. Two hundreds cases of ASIA syndrome following silicone implants: a comparative study of 30 years and a review of current literature. *Immunol. Res.* 65 (1), 120–128.
- Conde, P.G., Farhat, L.C., Braga, A.L.F., Sallum, A.E.M., Farhat, S.C.L., Silva, C.A., 2018. Are prematurity and environmental factors determinants for developing childhood-onset systemic lupus erythematosus? *Mod. Rheumatol.* 28 (1), 156–160.
- Cooper, G.S., Stroehla, B.C., 2003. The epidemiology of autoimmune diseases. *Autoimmun. Rev.* 2 (3), 119–125.
- Cooper, G.S., Germolec, D., Heindel, J., Selgrade, M., 1999a. Linking environmental agents and autoimmune diseases. *Environ. Health Perspect.* 107 (Suppl 5), 659–660.
- Cooper, G.S., Miller, F.W., Pandey, J.P., 1999b. The role of genetic factors in autoimmune disease: implications for environmental research. *Environ. Health Perspect.* 107 (Suppl 5), 693–700.
- Cooper, G.S., Miller, F.W., Germolec, D.R., 2002a. Occupational exposures and autoimmune diseases. *Int. Immunopharmacol.* 2 (2–3), 303–313.
- Cooper, G.S., Dooley, M.A., Treadwell, E.L., St Clair, E.W., Gilkeson, G.S., 2002b. Risk factors for development of systemic lupus erythematosus: allergies, infections, and family history. *J. Clin. Epidemiol.* 55 (10), 982–989.
- Cooper, G.S., Makris, S.L., Nietert, P.J., Jinot, J., 2009. Evidence of autoimmune-related effects of trichloroethylene exposure from studies in mice and humans. *Environ. Health Perspect.* 117 (5), 696–702.
- Cooper, G.S., Wither, J., Bernatsky, S., Claudio, J.O., Clarke, A., Rioux, J.D., et al., 2010. Occupational and environmental exposures and risk of systemic lupus erythematosus: silica, sunlight, solvents. *Rheumatology (Oxford)* 49 (11), 2172–2180.
- Crowe, W., Allsopp, P.J., Watson, G.E., Magee, P.J., Strain, J.J., Armstrong, D.J., et al., 2017. Mercury as an environmental stimulus in the development of autoimmunity – a systematic review. *Autoimmun. Rev.* 16 (1), 72–80.

- Cukier, J., Beauchamp, R.A., Spindler, J.S., Spindler, S., Lorenzo, C., Trentham, D.E., 1993. Association between bovine collagen dermal implants and a dermatomyositis or a polymyositis-like syndrome. *Ann. Intern. Med.* 118, 920–928.
- Dahlquist, G., 1998. The aetiology of type 1 diabetes: an epidemiological perspective. *Acta Paediatr. Suppl.* 425, 5–10.
- Dally, A., 1997. The rise and fall of pink disease. *Soc. Hist. Med.* 10 (2), 291–304.
- D'Cruz, D., 2000. Autoimmune diseases associated with drugs, chemicals and environmental factors. *Toxicol. Lett.* 112–113, 421–432.
- Deane, K.D., 2013. Can rheumatoid arthritis be prevented? *Best Pract. Res. Clin. Rheumatol.* 27 (4), 467–485.
- De Martino, M., Chiappini, E., Galli, L., 2013. Vaccines and autoimmunity. *Int. J. Immunopathol. Pharmacol.* 26 (2), 283–290.
- Dotta, F., Eisenbarth, G.S., 1989. Type I diabetes mellitus: a predictable autoimmune disease with interindividual variation in the rate of beta cell destruction. *Clin. Immunol. Immunopathol.* 50 (1 Pt 2), S85–S95.
- Elenkov, I.J., Chrousos, G.P., 1999. Stress, cytokine patterns and susceptibility to disease. *Baillieres Best Pract. Res. Clin. Endocrinol. Metab.* 13 (4), 583–595.
- Faresjo, M., 2015. The link between psychological stress and autoimmune response in children. *Crit. Rev. Immunol.* 35 (2), 117–134.
- Feldman, B.M., Birdi, N., Boone, J.E., Dent, P.B., Duffy, C.M., Ellsworth, J.E., et al., 1996. Seasonal onset of systemic-onset juvenile rheumatoid arthritis. *J. Pediatr.* 129 (4), 513–518.
- Fernandes, E.C., Silva, C.A., Braga, A.L., Sallum, A.M., Campos, L.M., Farhat, S.C., 2015. Exposure to air pollutants and disease activity in juvenile-onset systemic lupus erythematosus patients. *Arthritis Care Res. (Hoboken)* 67 (11), 1609–1614.
- Fontenot, A.P., Kotzin, B.L., 2003. Chronic beryllium disease: immune-mediated destruction with implications for organ-specific autoimmunity. *Tissue Antigens* 62 (6), 449–458.
- Fugazzola, L., Cirello, V., Beck-Peccoz, P., 2011. Fetal microchimerism as an explanation of disease. *Nat. Rev. Endocrinol.* 7 (2), 89–97.
- Garcia-Domingo, M.J., Alijotas-Reig, J., Cistero-Bahima, A., Tresserra, F., Enrique, E., 2000. Disseminated and recurrent sarcoid-like granulomatous panniculitis due to bovine collagen injection. *J. Investig. Allergol. Clin. Immunol.* 10 (2), 107–109.
- Gelpi, E., de la Paz, M.P., Terracini, B., Abaitua, I., de la Camara, A.G., Kilbourne, E.M., et al., 2002. The Spanish toxic oil syndrome 20 years after its onset: a multidisciplinary review of scientific knowledge. *Environ. Health Perspect.* 110 (5), 457–464.
- Gourley, M., Miller, F.W., 2007. Mechanisms of disease: environmental factors in the pathogenesis of rheumatic disease. *Nat. Clin. Pract. Rheumatol.* 3 (3), 172–180.
- Grant, W.B., 2004. Solar UV-B radiation is linked to the geographic variation of mortality from systemic lupus erythematosus in the USA. *Lupus* 13 (4), 281–282.
- Hammam, N., Gheita, T.A., 2017. Impact of secondhand smoking on disease activity in women with rheumatoid arthritis. *Clin. Rheumatol.* 36, 2415–2420.
- Han, F., Lin, L., Warby, S.C., Faraco, J., Li, J., Dong, S.X., et al., 2011. Narcolepsy onset is seasonal and increased following the 2009 H1N1 pandemic in China. *Ann. Neurol.* 70 (3), 410–417.
- Haupt-Jorgensen, M., Nielsen, E., Engkilde, K., Lerche, M., Larsen, J., Buschard, K., 2017. Occupation with grain crops is associated with lower type 1 diabetes incidence: registry-based case-control study. *PLoS One* 12 (7), e0181143.
- Haus, E., Smolensky, M.H., 1999. Biologic rhythms in the immune system. *Chronobiol. Int.* 16 (5), 581–622.
- Hemdan, N.Y., Abu El-Saad, A.M., Sack, U., 2013. The role of T helper (TH)17 cells as a double-edged sword in the interplay of infection and autoimmunity with a focus on xenobiotic-induced immunomodulation. *Clin. Dev. Immunol.* 2013, 374769.
- Hess, E.V., 2002. Environmental chemicals and autoimmune disease: cause and effect. *Toxicology* 181–182, 65–70.
- Hess, E.V., Mongey, A.B., 1991. Drug-related lupus. *Bull. Rheum. Dis.* 40, 1–8.
- Hirschfield, G.M., Gershwin, M.E., 2013. The immunobiology and pathophysiology of primary biliary cirrhosis. *Annu. Rev. Pathol. Biol.* 8, 303–330.
- Ilar, A., Alfredsson, L., Wiebert, P., Klareskog, L., Bengtsson, C., 2018. Occupation and risk of developing rheumatoid arthritis: results from a population-based case-control study. *Arthritis Care Res. (Hoboken)* 70 (4), 499–509.
- Janowsky, E.C., Kupper, L.L., Hulka, B.S., 2000. Meta-analyses of the relation between silicone breast implants and the risk of connective-tissue diseases. *N. Engl. J. Med.* 342 (11), 781–790.
- Javierre, B.M., Hernando, H., Ballestar, E., 2011. Environmental triggers and epigenetic deregulation in autoimmune disease. *Discov. Med.* 12 (67), 535–545.
- Johnson, D.H., Rosenthal, A., Nadas, A.S., 1975. A forty-year review of bacterial endocarditis in infancy and childhood. *Circulation* 51 (4), 581–588.
- Kallberg, H., Ding, B., Padyukov, L., Bengtsson, C., Ronnelid, J., Klareskog, L., et al., 2011. Smoking is a major preventable risk factor for rheumatoid arthritis: estimations of risks after various exposures to cigarette smoke. *Ann. Rheum. Dis.* 70 (3), 508–511.
- Khader, S.A., Peshimam, A.Z., Agraharam, S., 2002. Environmental risk factors for rheumatoid arthritis. *Rev. Environ. Health* 17 (4), 307–315.
- Klareskog, L., Alfredsson, L., Rantapaa-Dahlqvist, S., Berglin, E., Stolt, P., Padyukov, L., 2004. What precedes development of rheumatoid arthritis? *Ann. Rheum. Dis.* 63 (Suppl 2), ii28–ii31.
- Krishnan, E., 2003. Smoking, gender and rheumatoid arthritis—epidemiological clues to etiology. Results from the behavioral risk factor surveillance system. *Joint Bone Spine* 70 (6), 496–502.
- GEO-RA Group, 2017. Latitude gradient influences the age of onset of rheumatoid arthritis: a worldwide survey. *Clin. Rheumatol.* 36, 485–497.
- Leff, R.L., Burgess, S.H., Miller, F.W., Love, L.A., Targoff, I.N., Dalakas, M.C., et al., 1991. Distinct seasonal patterns in the onset of adult idiopathic inflammatory myopathy in patients with anti-Jo-1 and anti-signal recognition particle autoantibodies. *Arthritis Rheum.* 34 (11), 1391–1396.
- Leslie, R.D., Hawa, M., 1994. Twin studies in auto-immune disease. *Acta Genet. Med. Gemellol.* 43, 71–81.
- Leslie, D., Lipsky, P., Notkins, A.L., 2001. Autoantibodies as predictors of disease. *J. Clin. Invest.* 108 (10), 1417–1422.
- Lilleker, J.B., Vencovsky, J., Wang, G., Wedderburn, L.R., Diederichsen, L.P., Schmidt, J., et al., 2018. The EuroMyositis registry: an international collaborative tool to facilitate myositis research. *Ann. Rheum. Dis.* 77 (1), 30–39.
- Liu, Z.X., Kaplowitz, N., 2002. Immune-mediated drug-induced liver disease. *Clin. Liver Dis.* 6 (3), 755–774.

- Love, L.A., Miller, F.W., 1993. Noninfectious environmental agents associated with myopathies. *Curr. Opin. Rheumatol.* 5 (6), 712–718.
- Love, L.A., Weinberg, C.R., McConaughay, D.R., Oddis, C.V., Medsger Jr., T.A., Reveille, J.D., et al., 2009. Ultraviolet radiation intensity predicts the relative distribution of dermatomyositis and anti-Mi-2 autoantibodies in women. *Arthritis Rheum.* 60 (8), 2499–2504.
- Luppi, P., Rossiello, M.R., Faas, S., Trucco, M., 1995. Genetic background and environment contribute synergistically to the onset of autoimmune diseases. *J. Mol. Med.* 73, 381–393.
- Mackay, I.R., 1999. Immunological perspectives on chronic hepatitis: virus infection, autoimmunity and xenobiotics. *Hepatogastroenterology* 46 (30), 3021–3033.
- Maeda, Y., Takeda, K., 2017. Role of gut microbiota in rheumatoid arthritis. *J. Clin. Med.* 6 (6), pii: E60.
- Mamyrova, G., Rider, L.G., Ehrlich, A., Jones, O., Pachman, L.M., Nickeson, R., et al., 2017. Environmental factors associated with disease flare in juvenile and adult dermatomyositis. *Rheumatology (Oxford)* 56 (8), 1342–1347.
- Manzel, A., Muller, D.N., Hafler, D.A., Erdman, S.E., Linker, R.A., Kleinewietfeld, M., 2013. Role of “Western Diet” in inflammatory autoimmune diseases. *Curr. Allergy Asthma Rep.* 14 (1), 404.
- McCormic, Z.D., Khuder, S.S., Aryal, B.K., Ames, A.L., Khuder, S.A., 2010. Occupational silica exposure as a risk factor for scleroderma: a meta-analysis. *Int. Arch. Occup. Environ. Health* 83 (7), 763–769.
- Institute of Medicine (US) Immunization Safety Review Committee, Stratton, K., Wilson, C.B., McCormick, M.C. (Eds.), 2002. *Immunization Safety Review: Multiple Immunizations and Immune Dysfunction*. The National Academies Press, Washington, DC, 152 p.
- Meng, W., Zhu, Z., Jiang, X., Too, C.L., Uebe, S., Jagodic, M., et al., 2017. DNA methylation mediates genotype and smoking interaction in the development of anti-citrullinated peptide antibody-positive rheumatoid arthritis. *Arthritis Res. Ther.* 19 (1), 71.
- Miller, F.W., 1993. In: Serrattice, G. (Ed.), *Seasonal, Geographic, Clinical and Immunogenetic Associations of the Myositis Specific Autoantibodies*. Francaise, Paris.
- Miller, F.W., 1999. Genetics of environmentally-associated rheumatic disease. In: Kaufman, L.D., Varga, J. (Eds.), *Rheumatic Diseases and the Environment*. Arnold Publishers, London, pp. 33–45.
- Miller, F.W., 2012. New approaches to the assessment and treatment of the idiopathic inflammatory myopathies. *Ann. Rheum. Dis.* 71 (Suppl 2), i82–i85.
- Miller, F.W., Twitty, S.A., Biswas, T., Plotz, P.H., 1990. Origin and regulation of a disease-specific autoantibody response. Antigenic epitopes, spectrotypic stability, and isotype restriction of anti-Jo-1 autoantibodies. *J. Clin. Invest.* 85 (2), 468–475.
- Miller, F.W., Hess, E.V., Clauw, D.J., Hertzman, P.A., Pincus, T., Silver, R.M., et al., 2000. Approaches for identifying and defining environmentally associated rheumatic disorders. *Arthritis Rheum.* 43 (2), 243–249.
- Miller, F.W., Pollard, K.M., Parks, C.G., Germolec, D.R., Leung, P.S., Selmi, C., et al., 2012a. Criteria for environmentally associated autoimmune diseases. *J. Autoimmun.* 39 (4), 253–258.
- Miller, F.W., Alfredsson, L., Costenbader, K.H., Kamen, D.L., Nelson, L.M., Norris, J.M., et al., 2012b. Epidemiology of environmental exposures and human autoimmune diseases: findings from a National Institute of Environmental Health Sciences Expert Panel Workshop. *J. Autoimmun.* 39 (4), 259–271.
- Moroni, L., Bianchi, I., Lleo, A., 2012. Geoepidemiology, gender and autoimmune disease. *Autoimmun. Rev.* 11 (6–7), A386–A392.
- Nelson, J.L., 2012. The otherness of self: microchimerism in health and disease. *Trends Immunol.* 33 (8), 421–427.
- Nelson, R.J., Drazen, D.L., 2000. Melatonin mediates seasonal changes in immune function. *Ann. N.Y. Acad. Sci.* 917, 404–415.
- Norcross, M.A., Luo, S., Lu, L., Boyne, M.T., Gomarteli, M., Rennels, A.D., et al., 2012. Abacavir induces loading of novel self-peptides into HLA-B\*57:01: an autoimmune model for HLA-associated drug hypersensitivity. *AIDS* 26 (11), F21–F29.
- Noseworthy, J.H., Lucchinetti, C., Rodriguez, M., Weinshenker, B.G., 2000. Multiple sclerosis. *N. Engl. J. Med.* 343 (13), 938–952.
- Oddis, C.V., Conte, C.G., Steen, V.D., Medsger Jr., T.A., 1990. Incidence of polymyositis-dermatomyositis: a 20-year study of hospital diagnosed cases in Allegheny County, PA 1963–1982. *J. Rheumatol.* 17 (10), 1329–1334.
- O’Hanlon, T., Koneru, B., Bayat, E., Love, L., Targoff, I., Malley, J., et al., 2004. Immunogenetic differences between Caucasian women with and those without silicone implants in whom myositis develops. *Arthritis Rheum.* 50 (11), 3646–3650.
- Okada, S., Weatherhead, E., Targoff, I.N., Wesley, R., Miller, F.W., 2003. Global surface ultraviolet radiation intensity may modulate the clinical and immunologic expression of autoimmune muscle disease. *Arthritis Rheum.* 48 (8), 2285–2293.
- Okada, S., Kamb, M.L., Pandey, J.P., Philen, R.M., Love, L.A., Miller, F.W., 2009. Immunogenetic risk and protective factors for the development of L-tryptophan-associated eosinophilia-myalgia syndrome and associated symptoms. *Arthritis Rheum.* 61 (10), 1305–1311.
- Onkamo, P., Vaananen, S., Karvonen, M., Tuomilehto, J., 1999. Worldwide increase in incidence of Type I diabetes—the analysis of the data on published incidence trends. *Diabetologia* 42 (12), 1395–1403.
- Orione, M.A., Silva, C.A., Sallum, A.M., Campos, L.M., Omori, C.H., Braga, A.L., et al., 2014. Risk factors for juvenile dermatomyositis: exposure to tobacco and air pollutants during pregnancy. *Arthritis Care Res. (Hoboken)* 66 (10), 1571–1575.
- Parks, C.G., Conrad, K., Cooper, G.S., 1999. Occupational exposure to crystalline silica and autoimmune disease. *Environ. Health Perspect.* 107 (Suppl 5), 793–802.
- Parks, C.G., Walitt, B.T., Pettinger, M., Chen, J.C., De Roos, A.J., Hunt, J., et al., 2011. Insecticide use and risk of rheumatoid arthritis and systemic lupus erythematosus in the Women’s Health Initiative Observational Study. *Arthritis Care Res. (Hoboken)* 63 (2), 184–194.
- Pazderska, A., Fichna, M., Mitchell, A.L., Napier, C.M., Gan, E., Ruchala, M., et al., 2016. Impact of month of birth on the risk of development of autoimmune Addison’s disease. *J. Clin. Endocrinol. Metab.* 101 (11), 4214–4218.
- Perricone, C., Versini, M., Ben-Ami, D., Gertel, S., Watad, A., Segel, M.J., et al., 2016. Smoke and autoimmunity: the fire behind the disease. *Autoimmun. Rev.* 15 (4), 354–374.
- Pigatto, P.D., Guzzi, G., 2010. Linking mercury amalgam to autoimmunity. *Trends Immunol.* 31 (2), 48–49.
- Pinto, A.J., Roschel, H., de Sa Pinto, A.L., Lima, F.R., Pereira, R.M.R., Silva, C.A., et al., 2017. Physical inactivity and sedentary behavior: overlooked risk factors in autoimmune rheumatic diseases? *Autoimmun. Rev.* 16 (7), 667–674.
- Pollard, K.M., Hultman, P., Kono, D.H., 2010. Toxicology of autoimmune diseases. *Chem. Res. Toxicol.* 23 (3), 455–466.
- Ponsonby, A.L., McMichael, A., van der Mei, I., 2002. Ultraviolet radiation and autoimmune disease: insights from epidemiological research. *Toxicology* 181–182, 71–78.

- Rider, L.G., Miller, F.W., 2017. Environmental factors in pediatric systemic autoimmune diseases. *Rheumatologist* 11 (3), 1–9.
- Robinson, C.L., Romero, J.R., Kempe, A., Pellegrini, C., 2017. Advisory Committee on Immunization Practices Recommended Immunization Schedule for Children and Adolescents Aged 18 Years or Younger—United States, 2017. *MMWR Morb. Mortal. Wkly. Rep.* 66 (5), 134–135.
- Rook, G.A., 2012. Hygiene hypothesis and autoimmune diseases. *Clin. Rev. Allergy Immunol.* 42 (1), 5–15.
- Rowley, B., Monestier, M., 2005. Mechanisms of heavy metal-induced autoimmunity. *Mol. Immunol.* 42 (7), 833–838.
- Sarasin, A., 2003. An overview of the mechanisms of mutagenesis and carcinogenesis. *Mutat. Res.* 544 (2–3), 99–106.
- Saraux, A., Pers, J.O., Devauchelle-Pensec, V., 2016. Treatment of primary Sjögren syndrome. *Nat. Rev. Rheumatol.* 12 (8), 456–471.
- Sarkar, K., Miller, F.W., 2004. Possible roles and determinants of microchimerism in autoimmune and other disorders. *Autoimmun. Rev.* 3 (6), 454–463.
- Sarkar, K., Weinberg, C.R., Oddis, C.V., Medsger Jr., T.A., Plotz, P.H., Reveille, J.D., et al., 2005. Seasonal influence on the onset of idiopathic inflammatory myopathies in serologically defined groups. *Arthritis Rheum.* 52 (8), 2433–2438.
- Schiffenbauer, A., Faghihi-Kashani, S., O'Hanlon, T.P., Flegel, W.A., Adams, S.D., Targoff, I.N., et al., 2018. The effect of cigarette smoking on the clinical and serological phenotypes of polymyositis and dermatomyositis. *Semin Arthritis Rheum.* Available from: <https://doi.org/10.1016/j.semarthrit.2018.02.003>.
- Schlesinger, N., Schlesinger, M., 2005. Seasonal variation of rheumatic diseases. *Discov. Med.* 5 (25), 64–69.
- Schmidt, C.W., 2011. Questions persist: environmental factors in autoimmune disease. *Environ. Health Perspect.* 119 (6), A249–A253.
- Scott, I.C., Tan, R., Stahl, D., Steer, S., Lewis, C.M., Cope, A.P., 2013. The protective effect of alcohol on developing rheumatoid arthritis: a systematic review and meta-analysis. *Rheumatology (Oxford)* 52 (5), 856–867.
- Shahi, S.K., Freedman, S.N., Mangalam, A.K., 2017. Gut microbiome in multiple sclerosis: the players involved and the roles they play. *Gut Microbes* 8 (6), 607–615.
- Shamim, E.A., Miller, F.W., 2000. Familial autoimmunity and the idiopathic inflammatory myopathies. *Curr. Rheumatol. Rep.* 2 (3), 201–211.
- Silva, I.A., Nyland, J.F., Gorman, A., Perisse, A., Ventura, A.M., Santos, E.C., et al., 2004. Mercury exposure, malaria, and serum antinuclear/antinucleolar antibodies in amazon populations in Brazil: a cross-sectional study. *Environ. Health* 3 (1), 11.
- Singh, N., Picha, G.J., Hardas, B., Schumacher, A., Murphy, D.K., 2017. Five-year safety data for more than 55,000 subjects following breast implantation: comparison of rare adverse event rates with silicone implants versus national norms and saline implants. *Plast. Reconstr. Surg.* 140 (4), 666–679.
- Smyk, D., Rigopoulou, E.I., Baum, H., Burroughs, A.K., Vergani, D., Bogdanos, D.P., 2012. Autoimmunity and environment: am I at risk? *Clin. Rev. Allergy Immunol.* 42 (2), 199–212.
- Somers, E.C., Ganser, M.A., Warren, J.S., Basu, N., Wang, L., Zick, S.M., et al., 2015. Mercury exposure and antinuclear antibodies among females of reproductive age in the United States: NHANES. *Environ. Health Perspect.* 123 (8), 792–798.
- Stolt, P., Bengtsson, C., Nordmark, B., Lindblad, S., Lundberg, I., Klarekog, L., et al., 2003. Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases. *Ann. Rheum. Dis.* 62 (9), 835–841.
- Sullivan, E.A., Staehling, N., Phalen, R.M., 1996a. Eosinophilia-myalgia syndrome among the non-L-tryptophan users and pre-epidemic cases [see comments]. *J. Rheumatol.* 23 (10), 1784–1787.
- Sullivan, E.A., Kamb, M.L., Jones, J.L., Meyer, P., Phalen, R.M., Falk, H., et al., 1996b. The natural history of eosinophilia-myalgia syndrome in a tryptophan-exposed cohort in South Carolina. *Arch. Intern. Med.* 156 (9), 973–979.
- Sun, G., Hazlewood, G., Bernatsky, S., Kaplan, G.G., Eksteen, B., Barnabe, C., 2016. Association between air pollution and the development of rheumatic disease: a systematic review. *Int. J. Rheumatol.* 2016, 5356307.
- Talotta, R., Atzeni, F., Ditto, M.C., Gerardi, M.C., Sarzi-Puttini, P., 2017. The microbiome in connective tissue diseases and vasculitides: an updated narrative review. *J. Immunol. Res.* 2017, 6836498.
- Tao, C., Simpson Jr., S., van der Mei, I., Blizzard, L., Havrdova, E., Horakova, D., et al., 2016. Higher latitude is significantly associated with an earlier age of disease onset in multiple sclerosis. *J. Neurol. Neurosurg. Psychiatry* 87 (12), 1343–1349.
- Terao, C., Ohmura, K., Yamamoto, K., Yukawa, N., Kawabata, D., Nojima, T., et al., 2012. Serum IgG levels demonstrate seasonal change in connective tissue diseases: a large-scale, 4-year analysis in Japanese. *Mod. Rheumatol.* 22 (3), 426–430.
- Thomas, J.L., 2013. Helpful or harmful? Potential effects of exercise on select inflammatory conditions. *Phys. Sportsmed.* 41 (4), 93–100.
- Thvilum, M., Brandt, F., Brix, T.H., Hegedus, L., 2017. Month of birth is associated with the subsequent diagnosis of autoimmune hypothyroidism. A nationwide Danish register-based study. *Clin. Endocrinol. (Oxf.)* 87, 832–837.
- Tugwell, P., Wells, G., Peterson, J., Welch, V., Page, J., Davison, C., et al., 2001. Do silicone breast implants cause rheumatologic disorders? A systematic review for a court-appointed national science panel. *Arthritis Rheum.* 44 (11), 2477–2484.
- Unalp-Arida, A., Ruhl, C.E., Choung, R.S., Brantner, T.L., Murray, J.A., 2017. Lower prevalence of celiac disease and gluten-related disorders in persons living in Southern vs Northern latitudes of the United States. *Gastroenterology* 152 (8), 1922–1932.e2.
- Uramoto, K.M., Michet Jr., C.J., Thumboo, J., Sunku, J., O'Fallon, W.M., Gabriel, S.E., 1999. Trends in the incidence and mortality of systemic lupus erythematosus, 1950–1992. *Arthritis Rheum.* 42 (1), 46–50.
- Ursic-Bratina, N., Battelino, T., Krzisnik, C., Laron-Kenet, T., Ashkenazi, I., Laron, Z., 2001. Seasonality of birth in children (0–14 years) with type 1 diabetes mellitus in Slovenia. *J. Pediatr. Endocrinol. Metab.* 14 (1), 47–52.
- Vegosen, L.J., Weinberg, C.R., O'Hanlon, T.P., Targoff, I.N., Miller, F.W., Rider, L.G., 2007. Seasonal birth patterns in myositis subgroups suggest an etiologic role of early environmental exposures. *Arthritis Rheum.* 56 (8), 2719–2728.
- Vestergaard, P., 2002. Smoking and thyroid disorders—a meta-analysis. *Eur. J. Endocrinol.* 146 (2), 153–161.
- Vita, R., Lapa, D., Trimarchi, F., Vita, G., Fallahi, P., Antonelli, A., et al., 2017. Certain HLA alleles are associated with stress-triggered Graves' disease and influence its course. *Endocrine* 55 (1), 93–100.
- Watad, A., Azrielant, S., Bragazzi, N.L., Sharif, K., David, P., Katz, I., et al., 2017. Seasonality and autoimmune diseases: the contribution of the four seasons to the mosaic of autoimmunity. *J. Autoimmun.* 82, 13–30.
- Weinberg, C.R., Dornan, T.L., Hansen, J.A., Raghu, P.K., Palmer, J.P., 1984. HLA-related heterogeneity in seasonal patterns of diagnosis in Type 1 (insulin-dependent) diabetes. *Diabetologia* 26 (3), 199–202.

- Westberg, M., Feychtig, M., Jonsson, F., Nise, G., Gustavsson, P., 2009. Occupational exposure to UV light and mortality from multiple sclerosis. *Am. J. Ind. Med.* 52 (5), 353–357.
- Wiersinga, W.M., 2016. Clinical relevance of environmental factors in the pathogenesis of autoimmune thyroid disease. *Endocrinol. Metab. (Seoul)* 31 (2), 213–222.
- Williams, W.J., 1991. Caplan's syndrome. *Br. J. Clin. Pract.* 45 (4), 285–288.
- Willis, J.A., Scott, R.S., Darlow, B.A., Lewy, H., Ashkenazi, I., Laron, Z., 2002. Seasonality of birth and onset of clinical disease in children and adolescents (0–19 years) with type 1 diabetes mellitus in Canterbury, New Zealand. *J. Pediatr. Endocrinol. Metab.* 15 (5), 645–647.
- Winsa, B., Adami, H.O., Bergstrom, R., Gamstedt, A., Dahlberg, P.A., Adamson, U., et al., 1991. Stressful life events and Graves' disease. *Lancet* 338 (8781), 1475–1479.
- Wraith, D.C., Goldman, M., Lambert, P.H., 2003. Vaccination and autoimmune disease: what is the evidence? *Lancet* 362 (9396), 1659–1666.
- Yeter, D., Portman, M.A., Aschner, M., Farina, M., Chan, W.C., Hsieh, K.S., et al., 2016. Ethnic Kawasaki disease risk associated with blood mercury and cadmium in U.S. children. *Int. J. Environ. Res. Public Health* 13 (1), pii: E101.
- Zhang, X., Wang, W., Li, Y., Wang, H., Liu, R., Zhu, L., 2017. Serum 25-hydroxyvitamin D status in Chinese children with vitiligo: a case-control study. *Clin. Pediatr. (Phila.)* 57, 802–805. 9922817734362.

# Microbial Infection as a Trigger of T-Cell Autoimmunity

Daniel R. Getts<sup>1</sup>, Alanna Spiteri<sup>2</sup>, Nicholas J.C. King<sup>2</sup> and Stephen D. Miller<sup>1</sup>

<sup>1</sup>Department of Microbiology-Immunology and Interdepartmental Immunobiology Center, Northwestern University Feinberg School of Medicine, Chicago, IL, United States <sup>2</sup>Faculty of Medicine and Health, The Discipline of Pathology, Bosch Institute, School of Medical Sciences, Charles Perkins Centre, The University of Sydney, Sydney, NSW, Australia

## O U T L I N E

<b>Introduction</b>	363	<b>How Do These Mechanisms Lead to Autoimmune Disease?</b>	370
<b>Infectious Triggering of Autoreactive T Cells</b>	364	<i>Autoimmunity Can Occur at a Site Distal to the Initiating Infection</i>	370
<b>Proposed Mechanisms Underlying Autoimmunity</b>	364	<b>Conclusions</b>	371
Molecular Mimicry	364	<b>Acknowledgments</b>	371
Bystander Activation of Autoreactive Cells and Epitope Spreading	367	<b>References</b>	371
Emerging Mechanisms of Infection-Induced Autoimmune Disease	369		
Reciprocal Relationships of Pathogen-Derived Mechanisms of Autoimmunity	369		

## INTRODUCTION

The recognition of self from nonself is an immune phenomenon that has evolved over time and is crucially important for host preservation. The detrimental impact of alterations in this fine balance includes the emergence of opportunistic infections when there are deficits in nonself-discrimination or, when a loss of self-recognition occurs, overt immune attack on host tissues resulting in immunopathology and autoimmunity. Failure in the checks and balances regulating self-/nonself-recognition has been mapped to numerous genetic loci, especially those associated with immune activation and regulation. However, genetic predisposition to autoimmunity does not always result in the development of autoimmune disease. In many cases, overt clinical symptoms manifest after viral or bacterial infections. The precise immune mechanisms underlying the induction of autoimmune disease after infection are not known, but it is clear that multiple pathways are involved. In addition, there are numerous pathogenic and/or opportunistic microbes that may trigger an autoimmune reaction. The multiplicity of immune pathways and myriad possible microbes that may trigger

autoimmunity make it difficult to identify with confidence, distinct immune response pathways, and the pathogens associated with any particular autoimmune disease. Notwithstanding, a number of pathogens have been implicated in triggering autoimmunity. Here, these will be reviewed, with a special emphasis on the underlying mechanisms by which microbe–host interactions may result in the breakdown of peripheral tolerance or a loss of the ability to regulate self-/nonself-discrimination that results in autoimmunity. Importantly, while viruses have received arguably the greatest level of attention in the literature and are a primary focus here, bacteria and other eukaryotic pathogenic organisms have also been implicated in the etiology of autoimmune diseases and likely use similar mechanisms.

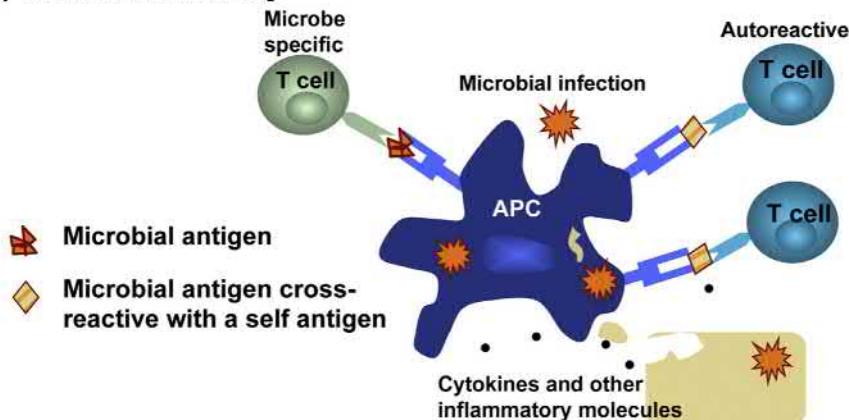
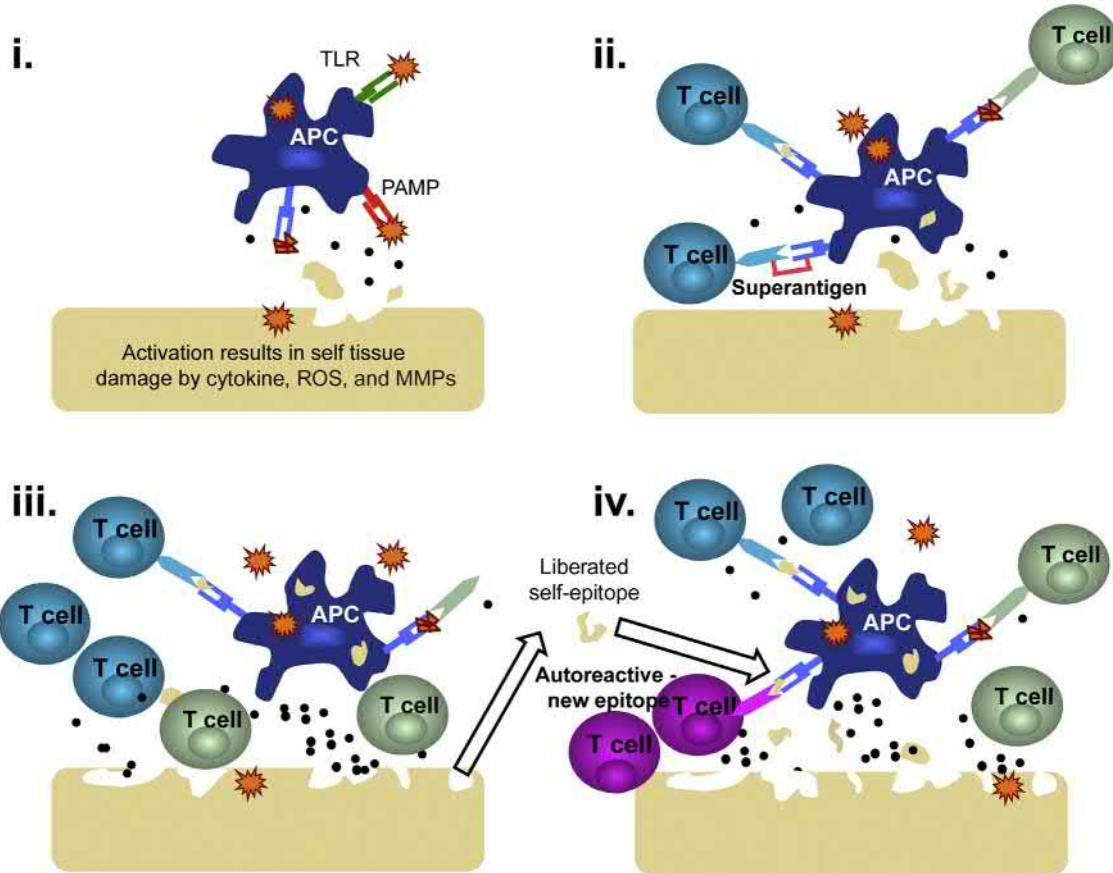
## INFECTIOUS TRIGGERING OF AUTOREACTIVE T CELLS

Pattern recognition receptors (PRR) are a broad family of proteins, expressed by numerous cells of the innate immune system, recognizing conserved molecular moieties commonly associated with pathogens. PRR include Toll-like receptors (TLR), nucleotide-binding and oligomerization domain (NOD)-like receptors, (RIG-I)-like helicases, and a subset of C-type lectin receptors (reviewed in [Ishii et al., 2008](#)). They recognize pattern-associated molecular patterns which are located within proteins, RNA and DNA. A successful early detection of pathogen infection is mediated by the innate immune cell recognition of microbes via PRR. The activation of these receptors results in the activation of a number of signal transduction cascades associated with inflammation, such as transcription and secretion of several cytokines, including type 1 interferons, costimulatory molecules, and chemokines. Together, these molecules serve to reduce the infection of parenchymal bystander cells, increase the recruitment of immune cells, and support the activation and expansion of adaptive T- and B-cell responses. These early responses to infection elicit a potent armory of weapons which, if not appropriately deployed and controlled, may result in bystander tissue damage and even death. This is convincingly shown both in murine models of viral infection, such as West Nile Virus (WNV) encephalitis, where fatality is immune-mediated ([Getts et al., 2014](#); [Terry et al., 2012](#)), and in humans in whom Ebola and SARS mortality has been associated with dysregulated immune responses to these viruses ([Basler, 2017](#); [Channappanavar and Perlman, 2017](#); [McElroy et al., 2015](#); [Younan et al., 2018](#)). In these examples, overexuberant immune responses result in damage to healthy tissue, culminating in immune pathology and mortality. Less striking examples that do not cause immediate mortality, but are nonetheless debilitating, include the development of myelin-specific autoimmunity in mice cerebrally infected with Theiler's Murine Encephalomyelitis Virus (TMEV) ([Munz et al., 2009](#)). In this example, autoimmunity results in the activation of myelin-specific T cells that migrate into the CNS and destroy myelinated axons. It has been demonstrated that the immune-mediated clearance of TMEV results in bystander damage and subsequent de novo induction of myelin-specific autoreactivity ([McMahon et al., 2005](#); [Miller et al., 1997](#)).

## PROPOSED MECHANISMS UNDERLYING AUTOIMMUNITY

### Molecular Mimicry

[Fujinami and Oldstone \(1985, 1989\)](#) first raised the idea of molecular mimicry, which proposed that a microbe-specific T-cell receptor (TCR) may cross-react with self-tissue-expressed antigens ([Fig. 21.1A](#)). As the TCR recognition domain cross-reacts with both viral and self-antigens, the T cell is unable to adequately discriminate between pathogenic challenge and self. As such, molecular mimicry is believed to be a significant virus-induced trigger of autoimmunity. While the ability for a TCR to recognize both self and nonself would at first glance appear to be an unfortunate random event associated with recombination-activating gene (RAG) recombination, the frequency of autoimmune disease suggests that other mechanisms are also involved. Certainly, similarities in shape and peptide charge distribution within the TCR binding domain have been described, both playing important roles in engagement of TCRs that exhibit significant promiscuity ([Gautam et al., 1994](#); [Gregersen et al., 2006](#); [Hammer et al., 1993](#); [Hemmer et al., 1999](#); [Lang et al., 2002](#); [Wucherpfennig et al., 1994](#); [Wucherpfennig and Strominger, 1995](#)). Furthermore, in vitro studies have shown that viral peptides can have significant homology with various self-peptides such that self and foreign antigens can cross-reactively stimulate autoreactive T cells ([Wucherpfennig and Strominger, 1995](#)).

**(A) Molecular mimicry****(B) Bystander activation and epitope spreading**

**FIGURE 21.1 Mechanisms of infection-induced autoimmunity .** (A) *Molecular mimicry* occurs through cross-reactive recognition between a microbial antigen/self MHC complex and a self-antigen/self MHC complex. (B) (i) Microbial PAMPs stimulate an immune response leading to tissue destruction via inflammatory mediators originating from cells of the innate immune system. (ii) During *bystander activation*, the engulfment of self-tissue debris leads to self-antigen presentation to autoreactive T cells (concomitant with presentation of virus antigen to virus-specific T cells) (see Chapter 64). Alternatively, an infection can lead to microbial *superantigen*-induced activation of a subset of T cells, some of which are specific for self-antigen. (iii) T-cell-mediated tissue destruction along with innate immune cell-derived inflammatory molecule-mediated destruction leads to release of endogenous self-epitopes from tissue. (iv) During *epitope spreading*, the response spreads to T cells specific for additional endogenous self-antigens.

MMP, matrix metalloproteinase; PAMP, pathogen-associated molecular pattern; ROS, reactive oxygen species; TLR, Toll-like receptor.

Numerous animal models support triggering of autoimmunity via molecular mimicry (Table 21.1). This triggering mechanism has been demonstrated in multiple pathogen-induced autoimmune models including *Acanthamoeba castellanii*-induced experimental autoimmune encephalomyelitis (EAE) in Swiss Jim Lambert (SJL) mice (Massilamany et al., 2010, 2011), herpes simplex virus (HSV) stromal keratitis, in which HSV infection leads to T-cell-mediated blindness in both humans and mice (Deshpande et al., 2001; Zhao et al., 1998), various diabetes models, autoimmune demyelinating disease associated with Semliki Forest Virus infection (Mokhtarian et al., 1999), autoimmune myocarditis associated with Coxsackievirus infection (Gauntt et al., 1995), and others (Lawson, 2000).

**TABLE 21.1** Selected Pathogen-Induced Murine Models of Human Autoimmune Disease

Relevant human disease	Mouse model/infectious agent	Proposed mechanism(s) of autoimmunity	Comment	References
MS	TMEV-IDD	Bystander activation/epitope spreading	Natural virus-induced autoimmune disease of mice	Miller et al. (1997)
	TMEV transgenically expressing PLP <sub>139–151</sub>	Molecular identity		Olson et al. (2001)
	TMEV transgenically expressing PLP <sub>139–151</sub> mimics	Molecular mimicry		Croxford et al. (2005, 2006)
	Coxsackievirus B4 transgenically expressing PLP <sub>139–151</sub>	Molecular identity	Infection can be at a site distant from where autoimmunity occurs	
	LCMV infection of mice expressing LCMV proteins in the CNS	Molecular identity		Evans et al. (1996)
	SFV infection	Molecular mimicry	Immunization with ACA-specific peptides NAD 108–120 results in EAE	Mokhtarian et al. (1999)
	<i>Acanthamoeba castellanii</i>	Molecular mimicry		Massilamany et al. (2011)
T1D	Coxsackie B4 virus infection	Bystander activation		Horwitz et al. (1998)
	LCMV infection of mice expressing LCMV protein in the pancreas	Molecular identity	TCR affinity for the LCMV peptide determines rapidity and severity of autoimmune disease	Ohashi et al. (1991), Oldstone et al. (1991), von Herrath et al. (1994)
	Pichinde virus infection of mice expressing LCMV protein in the pancreas	Molecular mimicry	Autoimmunity can only be accelerated, not initiated de novo, in this model	Christen et al. (2004)
Myocarditis	Mouse cytomegalovirus infection	Bystander activation or molecular mimicry	Circumstantial evidence points to molecular mimicry but does not exclude bystander activation	Fairweather et al. (2001), Lawson (2000), Lawson et al. (1992)
	Coxsackievirus B3 infection	Molecular mimicry/bystander activation		Fairweather et al. (2001), Gauntt et al. (1995), Lawson (2000)
Stromal keratitis	Corneal HSK	Molecular mimicry/bystander activation	Some controversy over which mechanism is responsible	Benoist and Mathis (2001), Deshpande et al. (2001), Zhao et al. (1998)
Celiac disease	Reovirus	Bystander activation	Argued to overcome regulatory mechanisms (Tregs) resulting in a loss of peripheral tolerance	Bouziat et al. (2017)

EAE, experimental autoimmune encephalomyelitis; HSV, herpes simplex virus; HSK, HSV infection–induced stromal keratitis; MS, multiple sclerosis; PLP, proteolipid protein; SFV, Semliki forest virus.

Other, albeit more synthetic models, that support molecular mimicry as a mechanism of autoimmunity development include transgenic models engineered to express tissue-specific self-associated epitopes. Infection results in the local production of inflammatory mediators, while microbially expressed "self"-antigens are processed and presented by host antigen-presenting cells in association with major histocompatibility complex molecules leading to T-cell activation. This results in the breakdown of peripheral tolerance mechanisms with activation and expansion of T cells specific for the "self"-transgene (Evans et al., 1996; Ohashi et al., 1991; Oldstone et al., 1991; von Herrath et al., 1994). The potential for molecular mimicry to cause autoimmunity has been confirmed in the TMEV-induced demyelinating disease (TMEV-IDD) model where the requirement for cerebral inoculation of TMEV to cause demyelinating disease was overcome by peripheral infection with TMEV engineered to express the immunodominant myelin proteolipid protein (PLP)139–151 epitope (Olson et al., 2001). Furthermore, infection with TMEV engineered to express peptides mimicking PLP<sub>139–151</sub> derived from infectious agents such as *Haemophilus influenzae* or murine hepatitis virus, respectively (Carrizosa et al., 1998), has also been shown to cause severe demyelinating disease similar to that induced by infection with TMEV containing PLP<sub>139–151</sub> itself (Croxford et al., 2006; Olson et al., 2001).

In humans, primary symptomatic Epstein–Barr virus (EBV) infection has been associated with an elevated risk of developing multiple sclerosis (MS) (Nielsen et al., 2007; Thacker et al., 2006). Patients with MS have circulating T cells specific for the EBV-encoded nuclear antigen 1 (EBNA1), the most consistently recognized EBV-derived CD4<sup>+</sup> T-cell antigen in healthy virus carriers, which may also recognize myelin antigens (Lunemann et al., 2008c). Interestingly, unlike EBNA1 monospecific TCR, myelin cross-reactive EBNA1 TCRs secrete less IFN-γ and interleukin-2 when activated, which may explain the persistence of these T-cell clones, as they are less susceptible to exhaustion and/or activation-induced cell death (Harari et al., 2007). Longitudinal studies have provided support for this hypothesis, with a long-term persistence of individual myelin-specific T-cell clones tracked over several years in the blood of EBV + ve patients with MS (Goebels et al., 2000; Meini et al., 1993; Muraro et al., 2003). Furthermore, the persistence of memory T cells that have self and viral reactivity has been observed in a number of other infectious diseases (Table 21.2).

## Bystander Activation of Autoreactive Cells and Epitope Spreading

The complex immune response associated with viral and bacterial containment and clearance unfortunately also results in the destruction of parenchymal tissues and subsequent liberation of other potential autoantigens. antigen-presenting cells (APCs) may uptake these antigens and either present them locally or in the secondary lymphoid tissues to autoreactive T and B cells. This process is loosely referred to as bystander activation, especially when it occurs locally within the infected tissue microenvironment (Enouz et al., 2012; Walker and Abbas, 2002; Zipris et al., 2005) (Fig. 21.1B). Another mechanism of bystander activation of T cells is associated with the ability of certain Vβ TCRs to be triggered nonspecifically by microbial-derived superantigens. If a superantigen-activated T-cell population expresses an autoreactive TCR, this may trigger or exacerbate autoimmunity (Wucherpfennig, 2001). In murine autoimmune models of MS (EAE), arthritis, and inflammatory bowel disease, superantigens have been shown to increase the severity of disease by initiating, or at least exacerbating, autoimmunity (Brocke et al., 1993; Cole and Griffiths, 1993; Dalwadi et al., 2001) (Table 21.1).

A further extension of bystander activation is the phenomenon known as epitope spreading. Epitope spreading occurs when novel TCR epitopes are liberated from tissues during the inflammatory process. Inflammatory tissue destruction associated with the response to an initiating epitope/antigen thus enables the presentation and activation of T cells specific for a new set of self-epitopes. These epitopes may be on the same protein molecule as the initiating epitope (intramolecular spreading) or to an epitope on a completely different protein (intermolecular spreading) (Fig. 21.1B). In either situation, the outcome is the activation and expansion of a novel set of autoreactive T cells. In the context of a pathogenic infection, an increase in the number of reactive T cells clones may be argued to be a positive event, as the immune repertoire focused on clearing infection is expanded. However, this expansion becomes problematic if the liberated epitopes are self-expressed proteins and result in a novel autoimmune reaction or potentiate an already active autoimmune response. The pathogenesis of many autoimmune diseases that are characterized by a relapse-remitting symptomatology, such as MS and type 1 diabetes (T1D), is potentially mediated by epitope spreading. While not confirmed in these human conditions, epitope spreading has been shown in EAE, a noninfectious model of MS (McRae et al., 1995; Yu et al., 1996), as well as in TMEV-IDD (Borrow et al., 1998; Katz-Levy et al., 1999, 2000; Miller et al., 1997) and in the

**TABLE 21.2** Virus Pathogens Implicated in Human Autoimmune Diseases

Pathogen	Autoimmune disease	Evidence	References
<b>RNA VIRUSES</b>			
Coxsackievirus	T1D	<ul style="list-style-type: none"> <li>Altered immune responses</li> <li>Enterovirus-positive beta cells detected in pancreata from T1D subjects</li> <li>Experimental infection causes T1D</li> </ul>	Horwitz et al. (1998, 2002), Jones and Crosby (1996), Ylipaasto et al. (2004)
Rubella virus	T1D	<ul style="list-style-type: none"> <li>Tropism for pancreatic beta cells</li> <li>Molecular mimicry</li> </ul>	Menser et al. (1978), Ou et al. (2000)
HTLV-1	HTLV-1-associated myelopathy	<ul style="list-style-type: none"> <li>Molecular mimicry</li> </ul>	Levin et al. (2002)
Measles virus	MS	<ul style="list-style-type: none"> <li>Infection can result in demyelination</li> <li>higher titers of virus-specific IgG and increased frequencies of virus-specific T cells in the CSF</li> </ul>	Jarius et al. (2009), Johnson et al. (1984), Link et al. (1992)
<b>DNA VIRUSES</b>			
HHV1/HSV1	Autoimmune stromal keratitis	<ul style="list-style-type: none"> <li>Molecular mimicry</li> </ul>	Zhao et al. (1998)
HHV4/EBV	MS	<ul style="list-style-type: none"> <li>Increased risk to develop MS after primary symptomatic infection</li> <li>Increased antibody responses in healthy individuals who will develop MS</li> <li>Increased seroprevalence</li> <li>Altered T cell and humoral immune responses</li> <li>Molecular mimicry</li> <li>Localization in diseased tissue</li> </ul>	Ascherio and Munch (2000), Levin et al. (2005), Lunemann et al. (2006, 2008b, 2008c), Nielsen et al. (2007), Serafini et al. (2007), Sundstrom et al. (2004), Thacker et al. (2006)
	RA	<ul style="list-style-type: none"> <li>Altered immune responses</li> <li>Higher viral loads in circulating blood cells</li> <li>Localization in diseased tissues</li> </ul>	Alspaugh et al. (1981), Balandraud et al. (2003), Lunemann et al. (2008a), Scotet et al. (1996), Tosato et al. (1981, 1984)
	SLE	<ul style="list-style-type: none"> <li>Increased seroprevalence</li> <li>Altered immune responses</li> <li>Increased viral load</li> <li>Molecular mimicry</li> </ul>	Gross et al. (2005), Kang et al. (2004), McClain et al. (2005)
HHV6	MS	<ul style="list-style-type: none"> <li>Localization in diseased tissue</li> <li>Heightened immune responses</li> </ul>	Challoner et al. (1995), Soldan et al. (1997)
Torque teno virus	MS	<ul style="list-style-type: none"> <li>Localization in diseased tissue</li> <li>Clonally expanded CSF-infiltrating T cells recognize virus-encoded antigen</li> </ul>	Sospedra et al. (2005)
Parvovirus B19	RA	<ul style="list-style-type: none"> <li>Phenotype of acute infection can mimic early RA</li> <li>Detection of viral DNA in synovial tissue</li> </ul>	Kozireva et al. (2008), Saal et al. (1992)
	SLE	<ul style="list-style-type: none"> <li>Phenotype of acute infection can mimic early SLE</li> <li>SLE patients increased frequency of virus carriers</li> </ul>	Seve et al. (2005)

EBV, Epstein–Barr virus; MS, multiple sclerosis; RA, Rheumatoid arthritis; SLE, systemic lupus erythematosus; T1D, Type 1 diabetes.

nonobese diabetic (NOD) mouse model of T1D (Kaufman et al., 1993; Prasad et al., 2012). In these models, the T-cell repertoire has been carefully examined, with epitope spreading clearly occurring in an orderly, directed, and hierarchical manner. More precisely, the initial immune response, usually triggered by an immunodominant epitope, sets in motion a series of cyclical episodes of immunopathology, each generated by specific responses to less and less dominant epitopes over time.

## Emerging Mechanisms of Infection-Induced Autoimmune Disease

Along with molecular mimicry and bystander activation, a recent study has proposed that microbial enhancement and/or maintenance of autoreactive T cells and APC signaling may additionally amplify or trigger autoimmunity. For examples, in an animal model of systemic lupus erythematosus, disease was induced by Cholera toxin B from *Vibrio cholerae* (Deng and Tsokos, 2008; James and Robertson, 2012).

Viruses such as EBV are able to immortalize B cells and assist their differentiation into long-lived memory B cells (Thorley-Lawson and Gross, 2004). Interestingly, infected memory B cells that usually do not express latent EBV proteins are resistant to cell death (Nanbo et al., 2002). The reservoir of EBV-infected B cells, observed in submeningeal aggregates of MS brains (Serafini et al., 2007), thus could potentially be preserved to present autoantigens, therefore promoting autoimmunity. Indeed, recent evidence in MS patients that depleting B cells with ocrelizumab (anti-CD20 monoclonal antibody) slows disease progression may support the role of B cells in disease.

Establishing a clear link between a particular microbial infection and a specific autoimmune disease is hampered by the ability of pathogens to evade host immune responses and/or produce a subclinical infection without clear clinical symptoms. This suggests that the investigation of other viral infections and changes in pathogen–host interactions should be considered. WNV, a pathogen that has spread significantly in the last decade (King et al., 2007) and, more recently, Zika virus (ZIKV), demonstrate numerous potential mechanisms through which immune responses to infections may result in or accelerate autoimmune diseases. However, with the exception of the higher incidence of Guillain–Barré syndrome associated with recent ZIKV infection (Brasil et al., 2016; do Rosario et al., 2016; Dos Santos et al., 2016; Parra et al., 2016), a direct link between flaviviruses and autoimmunity in humans remains to be established. This is complicated by the fact that the majority of infections by both WNV and ZIKV are asymptomatic. Moreover, the evidence of autoimmunity is likely to become clinically apparent only after a considerable period of subclinical autoimmune responses. WNV, while more efficiently replicating in cycling than quiescent cells (about 10-fold greater), paradoxically increases MHC expression to a much greater degree in quiescent cells (King et al., 2007). This decoy mechanism likely preferentially promotes immune-mediated killing of G0 cells that yield low virus outputs over infected proliferating cells, which better support virus replication. Nonetheless, increased MHC expression on infected cells which increases the avidity of interaction between T cells raises the possibility that WNV infection may activate poly-specific T cells which range from having a high affinity for MHC/virus peptide to those with significant cross-reactivity for self. Consequently, WNV may indirectly result in bystander self-reactive T-cell activation. This cross-reactivity of low affinity antiviral T cells with self has been demonstrated in a mouse model of WNV (King et al., 1989; Mullbacher et al., 1991). This may also be true of ZIKV, since this infection with this virus also increases MHC expression (Glasner et al., 2017).

A more recent study, investigating the role for viral infection in the etiology of celiac disease, has highlighted the potential for viral infection to potentially act as a trigger of disease. Infection of mice with reovirus at the same time as they were exposed to gliadin, the primary antigen associated with celiac disease, resulted in the emergence of gluten sensitivity as shown by the development of circulating antibody titers for gliadin, whereas animals exposed to gluten in the absence of infection did not develop antigliadin antibodies (Bouziat et al., 2017). Together, this highlights not only the potential role for infection in the emergence of celiac disease but also the underlying complexities as researchers attempt to identify host–pathogen relationships associated with autoimmunity.

## Reciprocal Relationships of Pathogen-Derived Mechanisms of Autoimmunity

It is important to recognize that the development of autoimmunity subsequent to infection is likely to result from numerous factors and that all of the mechanisms described above are interrelated, nonmutually exclusive, and dynamic, so the idea of microbial infection eliciting autoimmunity must be viewed not as a defined event that occurs via a particular mechanism, but as a process that can occur through many pathways simultaneously and/or sequentially. For example, epitope spreading can be initiated via molecular mimicry, as illustrated by the activation of PLP<sub>178–191</sub>-specific T cells in SJL mice in which autoimmunity was induced by infection with TMEV expressing PLP<sub>139–151</sub> (Olson et al., 2001), or following bystander damage (Miller et al., 1997). Molecular mimicry can initially activate autoreactive T cells that then expand and become pathogenic via bystander activation, or vice versa. As a result, it can be difficult to discriminate a specific cause from among the postulated mechanisms,

even in seemingly simple animal models (Benoist and Mathis, 2001; Deshpande et al., 2001; Fujinami and Oldstone, 1985; Fujinami et al., 2006).

The fact that the various mechanisms for infection-induced autoimmunity discussed here are nonmutually exclusive makes them both more complicated and more plausible as potential causes for human autoimmune disease. As the potentially multimechanistic nature of autoimmunity is considered, it is important to remember that an established autoimmune response can also have effects on pathogen-directed immune responses occurring in the same organ or elsewhere in the body.

## HOW DO THESE MECHANISMS LEAD TO AUTOIMMUNE DISEASE?

The development of autoimmune diseases is complex, but it is clear that there is a breakdown of the underlying mechanisms that regulate central and peripheral tolerance, resulting in the activation and effector function of autoreactive T cells. While T-cell development in the thymus results in the deletion of many self-reactive T cells, some clones escape deletion and enter into the periphery. Interestingly, the presence of these in the periphery does not necessarily result in autoimmune disease. Many patients may have circulating autoreactive T cells and never develop any signs of autoimmune disease. It is therefore argued that events, such as infection, are necessary to activate these T-cell clones. However, infection with the precise microbe is apparently required, as most common infections do not trigger autoimmunity, even when there are circulating autoreactive T cells. Therefore, microbes must exhibit certain physiochemical characteristics, many of which remain to be fully understood. Adding to the complexity, autoimmune disease may not be elicited even in the presence of both an appropriate infection and autoreactive immune cells. In these cases, autoreactive cells may need to be "primed" or previously activated and consequently exist at a higher precursor frequency (Hamilton-Williams et al., 2005). For instance in Pichinde virus infection in mice expressing the lymphocytic choriomeningitis virus (LCMV), nuclear protein (NP), under the rat insulin promoter (RIP) (RIP-LCMV-NP), a self-mimic-encoding Pichinde virus was not sufficient to initiate overt autoimmunity, but it was able to accelerate autoimmune disease that had already been established by infection with LCMV (Christen et al., 2004).

The development of autoimmune disease may also rely on the affinity of autoreactive TCRs for their cognate self-peptide/MHC complexes (Gronski et al., 2004). The speed in which autoimmune disease develops is decreased in RIP-LCMV mice expressing a self-antigen in the thymus during development (von Herrath et al., 1994). Thymic antigen expression is believed to reduce TCR affinity for the self-antigen. In T cells with a higher affinity for self-antigen, TLR interaction and subsequent signaling can be sufficient for the establishment of autoimmune diseases (Lang et al., 2005; von Herrath et al., 1994). However, since most T cells will have low affinity for self, studies in which TCR affinity for self-antigen is low may have greater relevance to the induction of human autoimmune disease.

Therefore, while the potential for the development of overt disease is dependent on the presence of autoreactive T cells, whether overt disease actually occurs may depend on various other coincident events. These include the number, avidity, and affinity of autoreactive T cells, and the presence of innate inflammatory signals required for the activation and differentiation of those T cells to a pathogenic phenotype. Despite the requirement for all these elements, it is apparent that these events do not necessarily need to happen at the same time or in the same place to elicit autoimmune disease.

### Autoimmunity Can Occur at a Site Distal to the Initiating Infection

In many animal models of infection-induced autoimmune disease, the immune response occurs exclusively in the infected organ. However, it has also been shown that autoimmunity can occur at sites temporally and/or spatially distal from the target organ in which the initiating infection occurred. Autoreactive responses that are limited to a particular organ in animals make a model that is much simpler to study. For example, mice expressing an LCMV protein in the CNS manifested autoimmune responses there, even though LCMV was peripherally inoculated and not detectable in the CNS (Evans et al., 1996). In humans, immune responses to an infection within a particular organ are usually not as easily associated with the development of autoimmunity, making definitive conclusions more difficult to draw. Thus, animal models that enable the study of this aspect of infection-induced autoimmune disease are few but in time may provide important new insights relevant to human disease.

## CONCLUSIONS

The pathogenesis of autoimmune diseases is extremely complex and multimechanistic in nature. Inherent genetic susceptibility clearly plays a major role in the generation of autoimmune disease. In addition, epidemiological and animal studies have shown that infection is an equally important factor in the generation of autoimmune disease. With recent evidence highlighting the importance of the gastrointestinal microflora for healthy immune regulation, future studies are likely to further investigate the potential role of the gut microbiome in the initiation and progression of autoimmune diseases. Numerous questions remain regarding how pathogenic challenge may disrupt immune regulation and trigger autoimmunity. In addition to molecular mimicry, bystander damage, epitope spreading, and infection-induced maintenance of signaling in APCs and autoreactive T cells, it is likely that other novel immune regulatory mechanisms yet to be described also contribute to clinical autoimmune disease.

## Acknowledgments

The authors thank the other members of the Miller Lab for advice and commentary.

## References

- Alspaugh, M.A., Henle, G., Lennette, E.T., Henle, W., 1981. Elevated levels of antibodies to Epstein-Barr virus antigens in sera and synovial fluids of patients with rheumatoid arthritis. *J. Clin. Invest.* 67, 1134–1140.
- Ascherio, A., Munch, M., 2000. Epstein-Barr virus and multiple sclerosis. *Epidemiology* 11, 220–224.
- Balandraud, N., Meynard, J.B., Auger, I., Sovran, H., Mugnier, B., Reviron, D., et al., 2003. Epstein-Barr virus load in the peripheral blood of patients with rheumatoid arthritis: accurate quantification using real-time polymerase chain reaction. *Arthritis Rheum.* 48, 1223–1228.
- Basler, C.F., 2017. Molecular pathogenesis of viral hemorrhagic fever. *Semin. Immunopathol.* 39, 551–561.
- Benoist, C., Mathis, D., 2001. Autoimmunity provoked by infection: how good is the case for T cell epitope mimicry? *Nat. Immunol.* 2, 797–801.
- Borrow, P., Welsh, C.J., Tonks, P., Dean, D., Blakemore, W.F., Nash, A.A., 1998. Investigation of the role of delayed-type-hypersensitivity responses to myelin in the pathogenesis of Theiler's virus-induced demyelinating disease. *Immunology* 93, 478–484.
- Bouziat, R., Hinterleitner, R., Brown, J.J., Stencel-Baerenwald, J.E., Ikizler, M., Mayassi, T., et al., 2017. Reovirus infection triggers inflammatory responses to dietary antigens and development of celiac disease. *Science* 356, 44–50.
- Brasil, P., Sequeira, P.C., Freitas, A.D., Zogbi, H.E., Calvet, G.A., de Souza, R.V., et al., 2016. Guillain-Barre syndrome associated with Zika virus infection. *Lancet* 387, 1482.
- Brocke, S., Gaur, A., Piercy, C., Gautam, A., Gijbels, K., Fathman, C.G., et al., 1993. Induction of relapsing paralysis in experimental autoimmune encephalomyelitis by bacterial superantigen. *Nature* 365, 642–644.
- Carrizosa, A.M., Nicholson, L.B., Farzan, M., Southwood, S., Sette, A., Sobel, R.A., et al., 1998. Expansion by self antigen is necessary for the induction of experimental autoimmune encephalomyelitis by T cells primed with a cross-reactive environmental antigen. *J. Immunol.* 161, 3307–3314.
- Challoner, P.B., Smith, K.T., Parker, J.D., MacLeod, D.L., Coulter, S.N., Rose, T.M., et al., 1995. Plaque-associated expression of human herpesvirus 6 in multiple sclerosis. *Proc. Natl. Acad. Sci. U.S.A.* 92, 7440–7444.
- Channappanavar, R., Perlman, S., 2017. Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. *Semin. Immunopathol.* 39, 529–539.
- Christen, U., Edelmann, K.H., McGavern, D.B., Wolfe, T., Coon, B., Teague, M.K., et al., 2004. A viral epitope that mimics a self antigen can accelerate but not initiate autoimmune diabetes. *J. Clin. Invest.* 114, 1290–1298.
- Cole, B.C., Griffiths, M.M., 1993. Triggering and exacerbation of autoimmune arthritis by the *Mycoplasma arthritidis* superantigen MAM. *Arthritis Rheum.* 36, 994–1002.
- Croxford, J.L., Anger, H.A., Miller, S.D., 2005. Viral delivery of an epitope from *Haemophilus influenzae* induces central nervous system autoimmune disease by molecular mimicry. *J. Immunol.* 174, 907–917.
- Croxford, J.L., Ercolini, A.M., Degutes, M., Miller, S.D., 2006. Structural requirements for initiation of cross-reactivity and CNS autoimmunity with a PLP139-151 mimic peptide derived from murine hepatitis virus. *Eur. J. Immunol.* 36, 2671–2680.
- Dalwadi, H., Wei, B., Kronenberg, M., Sutton, C.L., Braun, J., 2001. The Crohn's disease-associated bacterial protein I2 is a novel enteric T cell superantigen. *Immunity* 15, 149–158.
- Deng, G.M., Tsokos, G.C., 2008. Cholera toxin B accelerates disease progression in lupus-prone mice by promoting lipid raft aggregation. *J. Immunol.* 181, 4019–4026.
- Deshpande, S.P., Lee, S., Zheng, M., Song, B., Knipe, D., Kapp, J.A., et al., 2001. Herpes simplex virus-induced keratitis: evaluation of the role of molecular mimicry in lesion pathogenesis. *J. Virol.* 75, 3077–3088.
- do Rosario, M.S., de Jesus, P.A., Vasilakis, N., Farias, D.S., Novaes, M.A., Rodrigues, S.G., et al., 2016. Guillain-Barre syndrome after Zika virus infection in Brazil. *Am. J. Trop. Med. Hyg.* 95, 1157–1160.
- Dos Santos, T., Rodriguez, A., Almiron, M., Sanhueza, A., Ramon, P., de Oliveira, W.K., et al., 2016. Zika virus and the Guillain-Barre syndrome – case series from seven countries. *N. Engl. J. Med.* 375, 1598–1601.
- Enouz, S., Carrie, L., Merkler, D., Bevan, M.J., Zehn, D., 2012. Autoreactive T cells bypass negative selection and respond to self-antigen stimulation during infection. *J. Exp. Med.* 209, 1769–1779.

- Evans, C.F., Horwitz, M.S., Hobbs, M.V., Oldstone, M.B., 1996. Viral infection of transgenic mice expressing a viral protein in oligodendrocytes leads to chronic central nervous system autoimmune disease. *J. Exp. Med.* 184, 2371–2384.
- Fairweather, D., Kaya, Z., Shellam, G.R., Lawson, C.M., Rose, N.R., 2001. From infection to autoimmunity. *J. Autoimmun.* 16, 175–186.
- Fujinami, R.S., Oldstone, M.B., 1985. Amino acid homology between the encephalitogenic site of myelin basic protein and virus: mechanism for autoimmunity. *Science* 230, 1043–1045.
- Fujinami, R.S., Oldstone, M.B., 1989. Molecular mimicry as a mechanism for virus-induced autoimmunity. *Immunol. Res.* 8, 3–15.
- Fujinami, R.S., von Herrath, M.G., Christen, U., Whitton, J.L., 2006. Molecular mimicry, bystander activation, or viral persistence: infections and autoimmune disease. *Clin. Microbiol. Rev.* 19, 80–94.
- Gauntt, C.J., Arizpe, H.M., Higdon, A.L., Wood, H.J., Bowers, D.F., Rozek, M.M., et al., 1995. Molecular mimicry, anti-coxsackievirus B3 neutralizing monoclonal antibodies, and myocarditis. *J. Immunol.* 154, 2983–2995.
- Gautam, A.M., Lock, C.B., Smilek, D.E., Pearson, C.I., Steinman, L., McDevitt, H.O., 1994. Minimum structural requirements for peptide presentation by major histocompatibility complex class II molecules: implications in induction of autoimmunity. *Proc. Natl. Acad. Sci. U.S.A.* 91, 767–771.
- Getts, D.R., Terry, R.L., Getts, M.T., Deffrasnes, C., Muller, M., van Vredent, C., et al., 2014. Therapeutic inflammatory monocyte modulation using immune-modifying microparticles. *Sci. Transl. Med.* 6, 219ra217.
- Glasner, A., Oiknine-Djian, E., Weisblum, Y., Diab, M., Panet, A., Wolf, D.G., et al., 2017. Zika virus escapes NK cell detection by upregulating major histocompatibility complex class I molecules. *J. Virol.* 91, pii: e00785-17.
- Goebels, N., Hofstetter, H., Schmidt, S., Brunner, C., Wekerle, H., Hohlfeld, R., 2000. Repertoire dynamics of autoreactive T cells in multiple sclerosis patients and healthy subjects: epitope spreading versus clonal persistence. *Brain* 123, 508–518.
- Gregersen, J.W., Kranc, K.R., Ke, X., Svendsen, P., Madsen, L.S., Thomsen, A.R., et al., 2006. Functional epistasis on a common MHC haplotype associated with multiple sclerosis. *Nature* 443, 574–577.
- Gronski, M.A., Boulter, J.M., Moskophidis, D., Nguyen, L.T., Holmberg, K., Elford, A.R., et al., 2004. TCR affinity and negative regulation limit autoimmunity. *Nat. Med.* 10, 1234–1239.
- Gross, A.J., Hochberg, D., Rand, W.M., Thorley-Lawson, D.A., 2005. EBV and systemic lupus erythematosus: a new perspective. *J. Immunol.* 174, 6599–6607.
- Hamilton-Williams, E.E., Lang, A., Benke, D., Davey, G.M., Wiesmuller, K.H., Kurts, C., 2005. Cutting edge: TLR ligands are not sufficient to break cross-tolerance to self-antigens. *J. Immunol.* 174, 1159–1163.
- Hammer, J., Valsasnini, P., Tolba, K., Bolin, D., Higelin, J., Takacs, B., et al., 1993. Promiscuous and allele-specific anchors in HLA-DR-binding peptides. *Cell* 74, 197–203.
- Harari, A., Cellera, C., Enders, F.B., Kostler, J., Codarri, L., Tapia, G., et al., 2007. Skewed association of polyfunctional antigen-specific CD8 T cell populations with HLA-B genotype. *Proc. Natl. Acad. Sci. U.S.A.* 104, 16233–16238.
- Hemmer, B., Gran, B., Zhao, Y., Marques, A., Pascal, J., Tzou, A., et al., 1999. Identification of candidate T-cell epitopes and molecular mimics in chronic Lyme disease. *Nat. Med.* 5, 1375–1382.
- Horwitz, M.S., Bradley, L.M., Harbetson, J., Krahf, T., Lee, J., Sarvetnick, N., 1998. Diabetes induced by Coxsackie virus: initiation by bystander damage and not molecular mimicry. *Nat. Med.* 4, 781–786.
- Horwitz, M.S., Ilic, A., Fine, C., Rodriguez, E., Sarvetnick, N., 2002. Presented antigen from damaged pancreatic beta cells activates autoreactive T cells in virus-mediated autoimmune diabetes. *J. Clin. Invest.* 109, 79–87.
- Ishii, K.J., Koyama, S., Nakagawa, A., Coban, C., Akira, S., 2008. Host innate immune receptors and beyond: making sense of microbial infections. *Cell Host Microbe* 3, 352–363.
- James, J.A., Robertson, J.M., 2012. Lupus and Epstein-Barr. *Curr. Opin. Neurol.* 24, 383–388.
- Jarius, S., Eichhorn, P., Jacobi, C., Wildemann, B., Wick, M., Voltz, R., 2009. The intrathecal, polyspecific antiviral immune response: specific for MS or a general marker of CNS autoimmunity? *J. Neurol. Sci.* 280, 98–100.
- Johnson, R.T., Griffin, D.E., Hirsch, R.L., Wolinsky, J.S., Roedenbeck, S., Lindo de Soriano, I., et al., 1984. Measles encephalomyelitis—clinical and immunologic studies. *N. Engl. J. Med.* 310, 137–141.
- Jones, D.B., Crosby, I., 1996. Proliferative lymphocyte responses to virus antigens homologous to GAD65 in IDDM. *Diabetologia* 39, 1318–1324.
- Kang, I., Quan, T., Nolasco, H., Park, S.H., Hong, M.S., Crouch, J., et al., 2004. Defective control of latent Epstein-Barr virus infection in systemic lupus erythematosus. *J. Immunol.* 172, 1287–1294.
- Katz-Levy, Y., Neville, K.L., Girvin, A.M., Vanderlugt, C.L., Pope, J.G., Tan, L.J., et al., 1999. Endogenous presentation of self myelin epitopes by CNS-resident APCs in Theiler's virus-infected mice. *J. Clin. Invest.* 104, 599–610.
- Katz-Levy, Y., Neville, K.L., Padilla, J., Rahbe, S.M., Begolka, W.S., Girvin, A.M., et al., 2000. Temporal development of autoreactive Th1 responses and endogenous antigen presentation of self myelin epitopes by CNS-resident APCs in Theiler's virus-infected mice. *J. Immunol.* 165, 5304–5314.
- Kaufman, D.L., Clare-Salzler, M., Tian, J., Forsthober, T., Ting, G.S., Robinson, P., et al., 1993. Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. *Nature* 366, 69–72.
- King, N.J., Maxwell, L.E., Kesson, A.M., 1989. Induction of class I major histocompatibility complex antigen expression by West Nile virus on gamma interferon-refractory early murine trophoblast cells. *Proc. Natl. Acad. Sci. U.S.A.* 86, 911–915.
- King, N.J., Getts, D.R., Getts, M.T., Rana, S., Shrestha, B., Kesson, A.M., 2007. Immunopathology of flavivirus infections. *Immunol. Cell Biol.* 85, 33–42.
- Kozireva, S.V., Zestkova, J.V., Mikazane, H.J., Kadisa, A.L., Kakurina, N.A., Lejnieks, A.A., et al., 2008. Incidence and clinical significance of parvovirus B19 infection in patients with rheumatoid arthritis. *J. Rheumatol.* 35, 1265–1270.
- Lang, H.L., Jacobsen, H., Ikemizu, S., Andersson, C., Harlos, K., Madsen, L., et al., 2002. A functional and structural basis for TCR cross-reactivity in multiple sclerosis. *Nat. Immunol.* 3, 940–943.
- Lang, K.S., Recher, M., Junt, T., Navarini, A.A., Harris, N.L., Freigang, S., et al., 2005. Toll-like receptor engagement converts T-cell autoreactivity into overt autoimmune disease. *Nat. Med.* 11, 138–145.

- Lawson, C.M., 2000. Evidence for mimicry by viral antigens in animal models of autoimmune disease including myocarditis. *Cell Mol. Life Sci.* 57, 552–560.
- Lawson, C.M., O'Donoghue, H.L., Reed, W.D., 1992. Mouse cytomegalovirus infection induces antibodies which cross-react with virus and cardiac myosin: a model for the study of molecular mimicry in the pathogenesis of viral myocarditis. *Immunology* 75, 513–519.
- Levin, L.I., Munger, K.L., Rubertone, M.V., Peck, C.A., Lennette, E.T., Spiegelman, D., et al., 2005. Temporal relationship between elevation of Epstein-Barr virus antibody titers and initial onset of neurological symptoms in multiple sclerosis. *J. Am. Med. Assoc.* 293, 2496–2500.
- Levin, M.C., Lee, S.M., Kalume, F., Morcos, Y., Dohan Jr., F.C., et al., 2002. Autoimmunity due to molecular mimicry as a cause of neurological disease. *Nat. Med.* 8, 509–513.
- Link, H., Sun, J.B., Wang, Z., Xu, Z., Love, A., Fredrikson, S., et al., 1992. Virus-reactive and autoreactive T cells are accumulated in cerebrospinal fluid in multiple sclerosis. *J. Neuroimmunol.* 38, 63–73.
- Lunemann, J.D., Edwards, N., Muraro, P.A., Hayashi, S., Cohen, J.I., Munz, C., et al., 2006. Increased frequency and broadened specificity of latent EBV nuclear antigen-1-specific T cells in multiple sclerosis. *Brain* 129, 1493–1506.
- Lunemann, J.D., Frey, O., Eidner, T., Baier, M., Roberts, S., Sashihara, J., et al., 2008a. Increased frequency of EBV-specific effector memory CD8+ T cells correlates with higher viral load in rheumatoid arthritis. *J. Immunol.* 181, 991–1000.
- Lunemann, J.D., Huppke, P., Roberts, S., Bruck, W., Gartner, J., Munz, C., 2008b. Broadened and elevated humoral immune response to EBNA1 in pediatric multiple sclerosis. *Neurology* 71, 1033–1035.
- Lunemann, J.D., Jelcic, I., Roberts, S., Lutterotti, A., Tackenberg, B., Martin, R., et al., 2008c. EBNA1-specific T cells from patients with multiple sclerosis cross react with myelin antigens and co-produce IFN-gamma and IL-2. *J. Exp. Med.* 205, 1763–1773.
- Massilamany, C., Steffen, D., Reddy, J., 2010. An epitope from *Acanthamoeba castellanii* that cross-reacts with proteolipid protein 139-151-reactive T cells induces autoimmune encephalomyelitis in SJL mice. *J. Neuroimmunol.* 219, 17–24.
- Massilamany, C., Asojo, O.A., Gangaplara, A., Steffen, D., Reddy, J., 2011. Identification of a second mimicry epitope from *Acanthamoeba castellanii* that induces CNS autoimmunity by generating cross-reactive T cells for MBP 89-101 in SJL mice. *Int. Immunopharmacol.* 23, 729–739.
- McClain, M.T., Heinlen, L.D., Dennis, G.J., Roebuck, J., Harley, J.B., James, J.A., 2005. Early events in lupus humoral autoimmunity suggest initiation through molecular mimicry. *Nat. Med.* 11, 85–89.
- McElroy, A.K., Akondy, R.S., Davis, C.W., Ellebedy, A.H., Mehta, A.K., Kraft, C.S., et al., 2015. Human Ebola virus infection results in substantial immune activation. *Proc. Natl. Acad. Sci. U.S.A.* 112, 4719–4724.
- McMahon, E.J., Bailey, S.L., Castenada, C.V., Waldner, H., Miller, S.D., 2005. Epitope spreading initiates in the CNS in two mouse models of multiple sclerosis. *Nat. Med.* 11, 335–339.
- McRae, B.L., Vanderlugt, C.L., Dal Canto, M.C., Miller, S.D., 1995. Functional evidence for epitope spreading in the relapsing pathology of experimental autoimmune encephalomyelitis. *J. Exp. Med.* 182, 75–85.
- Meinl, E., Weber, F., Drexler, K., Morelle, C., Ott, M., Saruhan-Direskeneli, G., et al., 1993. Myelin basic protein-specific T lymphocyte repertoire in multiple sclerosis. Complexity of the response and dominance of nested epitopes due to recruitment of multiple T cell clones. *J. Clin. Invest.* 92, 2633–2643.
- Menser, M.A., Forrest, J.M., Bransby, R.D., 1978. Rubella infection and diabetes mellitus. *Lancet* 1, 57–60.
- Miller, S.D., Vanderlugt, C.L., Begolka, W.S., Pao, W., Yauch, R.L., Neville, K.L., et al., 1997. Persistent infection with Theiler's virus leads to CNS autoimmunity via epitope spreading. *Nat. Med.* 3, 1133–1136.
- Mokhtarian, F., Zhang, Z., Shi, Y., Gonzales, E., Sobel, R.A., 1999. Molecular mimicry between a viral peptide and a myelin oligodendrocyte glycoprotein peptide induces autoimmune demyelinating disease in mice. *J. Neuroimmunol.* 95, 43–54.
- Mullbacher, A., Hill, A.B., Blanden, R.V., Cowden, W.B., King, N.J., Hla, R.T., 1991. Alloreactive cytotoxic T cells recognize MHC class I antigen without peptide specificity. *J. Immunol.* 147, 1765–1772.
- Munz, C., Lunemann, J.D., Getts, M.T., Miller, S.D., 2009. Antiviral immune responses: triggers of or triggered by autoimmunity? *Nat. Rev. Immunol.* 9, 246–258.
- Muraro, P.A., Wandinger, K.P., Bielekova, B., Gran, B., Marques, A., Utz, U., et al., 2003. Molecular tracking of antigen-specific T cell clones in neurological immune-mediated disorders. *Brain* 126, 20–31.
- Nanbo, A., Inoue, K., Adachi-Takasawa, K., Takada, K., 2002. Epstein-Barr virus RNA confers resistance to interferon-alpha-induced apoptosis in Burkitt's lymphoma. *EMBO J.* 21, 954–965.
- Nielsen, T.R., Rostgaard, K., Nielsen, N.M., Koch-Henriksen, N., Haahr, S., Sorensen, P.S., et al., 2007. Multiple sclerosis after infectious mononucleosis. *Arch. Neurol.* 64, 72–75.
- Ohashi, P.S., Oehen, S., Buerki, K., Pircher, H., Ohashi, C.T., Odermatt, B., et al., 1991. Ablation of "tolerance" and induction of diabetes by virus infection in viral antigen transgenic mice. *Cell* 65, 305–317.
- Oldstone, M.B., Nerenberg, M., Southern, P., Price, J., Lewicki, H., 1991. Virus infection triggers insulin-dependent diabetes mellitus in a transgenic model: role of anti-self (virus) immune response. *Cell* 65, 319–331.
- Olson, J.K., Croxford, J.L., Calenoff, M., Dal Canto, M.C., Miller, S.D., 2001. A virus-induced molecular mimicry model of multiple sclerosis. *J. Clin. Invest.* 108, 311–318.
- Ou, D., Mitchell, L.A., Metzger, D.L., Gillam, S., Tingle, A.J., 2000. Cross-reactive rubella virus and glutamic acid decarboxylase (65 and 67) protein determinants recognised by T cells of patients with type I diabetes mellitus. *Diabetologia* 43, 750–762.
- Parra, B., Lizarazo, J., Jimenez-Arango, J.A., Zea-Vera, A.F., Gonzalez-Manrique, G., Vargas, J., et al., 2016. Guillain-Barre syndrome associated with Zika virus infection in Colombia. *N. Engl. J. Med.* 375, 1513–1523.
- Prasad, S., Kohm, A.P., McMahon, J.S., Luo, X., Miller, S.D., 2012. Pathogenesis of NOD diabetes is initiated by reactivity to the insulin B chain 9–23 epitope and involves functional epitope spreading. *J. Autoimmun.* 39, 347–353.
- Saal, J.G., Steidle, M., Einsele, H., Muller, C.A., Fritz, P., Zacher, J., 1992. Persistence of B19 parvovirus in synovial membranes of patients with rheumatoid arthritis. *Rheumatol. Int.* 12, 147–151.
- Scotet, E., David-Ameline, J., Peyrat, M.A., Moreau-Aubry, A., Pinczon, D., Lim, A., et al., 1996. T cell response to Epstein-Barr virus transactivators in chronic rheumatoid arthritis. *J. Exp. Med.* 184, 1791–1800.

- Serafini, B., Rosicarelli, B., Franciotta, D., Magliozi, R., Reynolds, R., Cinque, P., et al., 2007. Dysregulated Epstein-Barr virus infection in the multiple sclerosis brain. *J. Exp. Med.* 204, 2899–2912.
- Seve, P., Ferry, T., Koenig, M., Cathebras, P., Rousset, H., Broussolle, C., 2005. Lupus-like presentation of parvovirus B19 infection. *Semin. Arthritis Rheum.* 34, 642–648.
- Soldan, S.S., Berti, R., Salem, N., Secchiero, P., Flamand, L., Calabresi, P.A., et al., 1997. Association of human herpes virus 6 (HHV-6) with multiple sclerosis: increased IgM response to HHV-6 early antigen and detection of serum HHV-6 DNA. *Nat. Med.* 1394–1397.
- Sospedra, M., Zhao, Y., zur Hausen, H., Muraro, P.A., Hamashin, C., de Villiers, E.M., et al., 2005. Recognition of conserved amino acid motifs of common viruses and its role in autoimmunity. *PLoS Pathog.* 1, e41.
- Sundstrom, P., Juto, P., Wadell, G., Hallmans, G., Svenssonsson, A., Nystrom, L., et al., 2004. An altered immune response to Epstein-Barr virus in multiple sclerosis: a prospective study. *Neurology* 62, 2277–2282.
- Terry, R.L., Getts, D.R., Deffrasnes, C., van Vreden, C., Campbell, I.L., King, N.J., 2012. Inflammatory monocytes and the pathogenesis of viral encephalitis. *J. Neuroinflamm.* 9, 270.
- Thacker, E.L., Mirzaei, F., Ascherio, A., 2006. Infectious mononucleosis and risk for multiple sclerosis: a meta-analysis. *Ann. Neurol.* 59, 499–503.
- Thorley-Lawson, D.A., Gross, A., 2004. Persistence of the Epstein-Barr virus and the origins of associated lymphomas. *New Engl. J. Med.* 350, 1328–1337.
- Tosato, G., Steinberg, A.D., Blaese, R.M., 1981. Defective EBV-specific suppressor T-cell function in rheumatoid arthritis. *New Engl. J. Med.* 305, 1238–1243.
- Tosato, G., Steinberg, A.D., Yarchoan, R., Heilman, C.A., Pike, S.E., De Seau, V., et al., 1984. Abnormally elevated frequency of Epstein-Barr virus-infected B cells in the blood of patients with rheumatoid arthritis. *J. Clin. Invest.* 73, 1789–1795.
- von Herrath, M.G., Dockter, J., Oldstone, M.B., 1994. How virus induces a rapid or slow onset insulin-dependent diabetes mellitus in a transgenic model. *Immunity* 1, 231–242.
- Walker, L.S., Abbas, A.K., 2002. The enemy within: keeping self-reactive T cells at bay in the periphery. *Nat. Rev. Immunol.* 2, 11–19.
- Wucherpfennig, K.W., 2001. Mechanisms for the induction of autoimmunity by infectious agents. *J. Clin. Invest.* 108, 1097–1104.
- Wucherpfennig, K.W., Strominger, J.L., 1995. Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. *Cell* 80, 695–705.
- Wucherpfennig, K.W., Sette, A., Southwood, S., Oseroff, C., Matsui, M., Strominger, J.L., et al., 1994. Structural requirements for binding of an immunodominant myelin basic protein peptide to DR2 isotypes and for its recognition by human T cell clones. *J. Exp. Med.* 179, 279–290.
- Ylipaasto, P., Klingel, K., Lindberg, A.M., Otonkoski, T., Kandolf, R., Hovi, T., et al., 2004. Enterovirus infection in human pancreatic islet cells, islet tropism in vivo and receptor involvement in cultured islet beta cells. *Diabetologia* 47, 225–239.
- Younan, P., Iampietro, M., Bukreyev, A., 2018. Disabling of lymphocyte immune response by Ebola virus. *PLoS Pathog.* 14, e1006932.
- Yu, M., Johnson, J.M., Tuohy, V.K., 1996. A predictable sequential determinant spreading cascade invariably accompanies progression of experimental autoimmune encephalomyelitis: a basis for peptide-specific therapy after onset of clinical disease. *J. Exp. Med.* 183, 1777–1788.
- Zhao, Z.S., Granucci, F., Yeh, L., Schaffer, P.A., Cantor, H., 1998. Molecular mimicry by herpes simplex virus-type 1: autoimmune disease after viral infection. *Science* 279, 1344–1347.
- Zipris, D., Lien, E., Xie, J.X., Greiner, D.L., Mordes, J.P., Rossini, A.A., 2005. TLR activation synergizes with Kilham rat virus infection to induce diabetes in BBDR rats. *J. Immunol.* 174, 131–142.

# Autoimmune Diseases: The Role for Vaccines

S. Sohail Ahmed<sup>1</sup> and Lawrence Steinman<sup>2</sup>

<sup>1</sup>Translational Medicine, Galapagos GmbH, Basel, Switzerland <sup>2</sup>Neurology and Neuroscience, Stanford University School of Medicine, Stanford, CA, United States

## OUTLINE

Introduction	375	The Reality Facing Clinicians Currently	379
Concerns for Autoimmune Diseases in the Context of Vaccination	376	Certainty About Vaccines, Uncertainty About Compatibility of Administration in Certain Settings	379
Crossfire and Coincidence	377	Search and You Will Find	380
Example of an Animal Model Developed to Understand Acute Disseminated Encephalomyelitis Observed With Older Rabies Vaccine—The Experimental Autoimmune Encephalomyelitis Model	378	Conclusion	380
Practical Approach to Vaccination in Patients With Autoimmune Disease	379	References	381
		Further Reading	381

## INTRODUCTION

Vaccines are essential components of any public health program. Their beneficial effects have been largely recognized, and vaccination is generally considered the most cost-effective approach in preventive medicine. Therefore vaccines are used extensively, and coverage can reach over 90% in a given population. Since autoimmune diseases affect approximately 5%–10% of the population in Europe and North America, most people that develop an autoimmune disease are likely to be exposed to some vaccines at some time before or after the onset of the disease process. While earlier vaccines were mainly targeted for pediatric age-groups, the development of recent vaccines for adolescents [e.g., human papillomavirus vaccine (HPV)] and older individuals (e.g., pneumococcus and influenza) may increase the probability of coincidental associations of vaccination with autoimmune diseases.

Two major questions are of particular relevance to vaccination and autoimmune diseases. First, can vaccination trigger or enhance autoimmune responses? This frequently expressed concern is based either on a putative mimicry between vaccines and host antigens or on the fact that vaccination is associated with a transitory and variable activation of innate immunity. The use of adjuvants exploiting the immunostimulatory effect of Toll-like receptors (TLRs) has also triggered concerns related to their possible role in autoimmune disease development. Indeed, TLR-related pathways are being independently studied by investigators for their role in autoimmune disease modulation. Second, should patients with chronic autoimmune diseases be routinely vaccinated? Since

several infections are known to enhance disease activity in some autoimmune diseases, one can expect that their prevention, when feasible, would be beneficial. However, activation of innate immunity with some vaccines may also bear a theoretical risk of enhancing disease activity. Therefore, it is of importance to define guidelines regarding the use of essential vaccines in patients with autoimmune diseases. Such guidelines have been proposed by the American College of Rheumatology and the European League Against Rheumatism and form the basis of recommendations to be discussed in subsequent sections in this chapter.

## CONCERNS FOR AUTOIMMUNE DISEASES IN THE CONTEXT OF VACCINATION

Under the generic term of “vaccines,” there is a whole range of products that include complex live-attenuated microorganisms as well as purified proteins or polysaccharides. The common purpose of vaccination is to trigger a protective immune response in the host (similar to that seen with naturally acquired infection). Some vaccines are associated with a transient fever, and some vaccines commonly result in inflammation at the site of injection. Whether a vaccine can trigger or contribute to the initiation or worsening of autoimmune disease is a subject that is widely discussed.

Fear of adverse reactions versus the immense known benefit of approved vaccines continues to be a topic of lively debate. In the United States, the *National Childhood Vaccine Injury Act* of 1986 (42 U.S.C. §§ 300aa-1 to 300aa-34) was established under President Ronald Reagan as federal law (<https://www.nytimes.com/1986/11/15/us/reagan-signs-bill-on-drug-exports-and-payment-for-vaccine-injuries.html>) to provide a legal basis for contesting whether or not a particular individual may have suffered harm from a given vaccine. The fund is financed by a tax on vaccines and is administered by the Federal Courts in the United States. The legal standard is the “preponderance of evidence”—a standard that is accepted in civil litigation in the United States. The standard is often at variance with the vague standards established for scientific review where “statistical significance” is usually invoked.

Both in the previous decades for live or nonadjuvanted vaccines and more recently for adjuvanted vaccines, a major safety concern has centered on the possibility of potent stimulators of the immune response increasing the risk of developing an autoimmune disease. The old rabies vaccine that was produced using rabbit brain tissue was associated with the occasional (0.33/1000) development of immune-mediated encephalitis and antimyelin T-cell responses (Swaddiwudhipong et al., 1998). This is no longer observed with modern rabies vaccines produced with cell lines. Measles vaccination is also occasionally associated with a transitory immune thrombocytopenic purpura—like thrombocytopenia. However, this syndrome is observed 6–10 times more frequently after natural measles infection (Beeler et al., 1996; Wraith et al., 2003). The swine influenza vaccine that was used in 1976 was associated with a significant increase in the frequency of Guillain–Barré syndrome (incidence of 1 in 100,000) in the weeks following vaccination (Wraith et al., 2003), but this risk was significantly reduced with refinements in the manufacturing process of subsequent seasonal vaccines (risk of Guillain–Barré reduced to 1 in 1,000,000) (Schonberger et al., 1979; Chen et al., 2001).

In 2011, an epidemiological association of narcolepsy with the use of an AS03-adjuvanted H1N1 pandemic influenza vaccine was reported in Finland (National Narcolepsy Task Force Interim Report, 2011; Statement on Narcolepsy and Vaccination, 2011). The incidence of narcolepsy was 9.0 in the vaccinated as compared to 0.7/100,000 person years in the unvaccinated individuals, the rate ratio being 12.7. This H1N1 pandemic wave was shown in China to also be associated with a 3–4 rise of narcolepsy in the absence of vaccination (Han et al., 2011). A study published in 2015 may have established an immunologic link between the influenza virus and narcolepsy through antigenic molecular mimicry. Specifically, molecular mimicry was identified between a fragment of one of the influenza antigens (nucleoprotein) contained in the AS03-adjuvanted pandemic vaccine and a portion of the human brain, hypocretin receptor 2, that is responsible for promoting wakefulness (Ahmed et al., 2015). Using mass spectrometry, influenza nucleoprotein was demonstrated to be present in significant amounts in the AS03-adjuvanted inactivated split-virion pandemic vaccine compared to the MF59-adjuvanted inactivated subunit pandemic vaccine (that was not associated with narcolepsy and contained 73% less influenza nucleoprotein). The authors additionally demonstrated the presence of antibodies capable of cross-reacting with both influenza nucleoprotein and the hypocretin receptor that could, theoretically, disrupt wakefulness that is normally triggered by binding of hypocretin protein to the hypocretin receptor 2. These findings and the association of narcolepsy with influenza infection make the role of adjuvants, by themselves, in triggering autoimmune disease in genetically susceptible subjects less likely. However, both Guillain–Barré and narcolepsy should serve as reminders that vaccine antigen formulations need to be carefully selected to avoid potential autoimmune disease development in a percentage of the population receiving vaccines, and that disease development may, rarely, be potentiated by adjuvants in the presence of a cross-reactive antigen.

## CROSSFIRE AND COINCIDENCE

The associations described previously underscore the extent of our current capabilities in identifying risks associated with vaccines—that is, through statistical associations. While identifying causation would be ideal (e.g., a clear and unequivocal link between vaccination and rare adverse events, such as autoimmune disease), this is difficult given the individual, environmental, and temporal variables that occur during the window of immunization. While disease incidence in the setting of vaccination can be identified using statistical tools, this analysis can be confounded by the occurrence of “coincidental” associations. There is a background rate of these events that occur despite vaccination and a risk of such event occurring at the same time as the vaccine, by chance alone, which can confound the interpretation of vaccine safety.

One possible but not always a practical solution is to collect autoimmune disease prevalence and incidence data in a given population before vaccination to illustrate these coincidental associations. Such an approach was utilized prior to the large-scale introduction of the HPV vaccine where a cohort study of 214,896 female adolescents and 221,472 young adults was carried out to monitor the prevalence of autoimmune disease before vaccine introduction (Siegrist et al., 2007). This elegant approach collected data on the frequency of immune-mediated conditions leading to outpatient visits, the number of women hospitalized, and the most frequently diagnosed autoimmune disease. These data were then used to model temporal associations that would have occurred theoretically had the vaccine been used with 80% coverage. One can quickly appreciate how such population-based efforts enable one to identify, in advance, confounding issues affecting safety perception to avoid a negative impression of an inherently safe vaccine.

**Table 22.1** has been adapted primarily from autoimmune disease epidemiological data reported in a systematic review (Jacobson et al., 1997); in which, four additional categories were added from a study published in 2003 (Cooper and Stroehla, 2003). These combined data represent, to our knowledge, the most comprehensive and conservative estimates to date. **Table 22.1** intentionally focuses on the incidence of autoimmune diseases more commonly occurring in adults because the adult population is traditionally enrolled in first-in-human clinical trials with vaccines (the rates of autoimmunity in pediatric patients, though limited, suggest that they are quite different and reflect the contributions of time and environmental exposure to disease development). These autoimmune diseases, based on previously reported estimates of incidence (Jacobson et al., 1997; Cooper and Stroehla, 2003), would have been responsible for 204,789 new cases of autoimmune disease in a population >18 years of age in the United States based on the 2010 US Census (US Census Bureau, 2011). With this extrapolated

**TABLE 22.1** Autoimmune Disease Epidemiological Data Reported in a Systematic Review

Autoimmune disease <sup>a</sup>	Incidence <sup>a</sup> (per 100,000 persons per year)	Expected new diagnoses <sup>b</sup> (persons >18 years of age)
Adult rheumatoid arthritis	23.7	55,092
Thyroiditis (hypothyroidism)	21.8	50,675
Graves' disease (hyperthyroidism)	13.9	32,311
Type 1 diabetes (age 20 years)	8.1	18,829
Systemic lupus erythematosus	7.3	16,969
Sjögren disease	3.9	9065
Multiple sclerosis	3.2	7438
Primary systemic vasculitis	2.0	4649
Polymyositis/Dermatomyositis	1.8	4184
Systemic sclerosis	1.4	3254
Addison disease	0.6	1394
Myasthenia gravis	0.4	929
Total		204,789

<sup>a</sup>The table is obtained with permission from Ahmed, S., Plotkin, S.A., Black, S., Coffman, R.L., 2011. Assessing the safety of adjuvanted vaccines. *Sci. Transl. Med.* 3, 93rv2.

<sup>b</sup>Expected new diagnoses in the United States were extrapolated using the 2009 US Census data for population >18 years of age.

**TABLE 22.2** Incidence Data From Autoimmune Diseases

	Study sample size				
	N = 200	N = 1000	N = 2000	N = 3000	N = 10,000
Probability to observe at least one case <sup>a</sup>					
Autoimmune disease <sup>a</sup>					
Adult rheumatoid arthritis (%)	4.6	21.1	37.8	50.9	90.7
Thyroiditis (hypothyroidism) (%)	4.3	19.6	35.3	48.0	88.7
Graves' disease (hyperthyroidism) (%)	2.7	13.0	24.3	34.1	75.1
Type 1 diabetes (age >20 years) (%)	1.6	7.8	15.0	21.6	55.5
Systemic lupus erythematosus (%)	1.4	7.0	13.6	19.7	51.8
Sjögren disease (%)	0.8	3.8	7.5	11.0	32.3
Multiple sclerosis (%)	0.6	3.1	6.2	9.2	27.4
Primary systemic vasculitis (%)	0.4	2.0	3.9	5.8	18.1
Polymyositis/dermatomyositis (%)	0.4	1.8	3.5	5.3	16.5
Systemic sclerosis (%)	0.3	1.4	2.8	4.1	13.1
Addison disease (%)	0.1	0.6	1.2	1.8	5.8
Myasthenia gravis (%)	0.1	0.4	0.8	1.2	3.9
Total (%)	15.0	55.5	80.2	91.2	100.0

<sup>a</sup>The table is obtained with permission from Ahmed, S., Plotkin, S.A., Black, S., Coffman, R.L., 2011. Assessing the safety of adjuvanted vaccines. *Sci. Transl. Med.* 3, 93rv2.

table, one rapidly gains a perspective on the risk of coincidental association that can occur when an autoimmune disease is diagnosed in a subject immunized during a vaccine clinical trial (independent of the vaccine's effect). Table 22.2 uses the incidence data from these autoimmune diseases and calculates the probability of observing at least one case of autoimmune disease in clinical trials ranging from 200 to 10,000 subjects. As demonstrated, there is an increase in the probability of observing one patient with autoimmune disease when examining a trial with 200 subjects (15%) versus 3000 subjects (91%), which illustrates why coincidental associations occasionally occur during large phase III vaccine studies and even more during postlicensure monitoring of vaccination adverse events. Whether a given case of new onset autoimmune disease is "expected" statistically or due to an underlying predisposition to susceptibility is a recurrent issue. Sample sets, such as the Department of Defense serum repository, may help us to design studies to analyze who may be at substantial risk from a given immunization (Arbuckle et al., 2003).

### EXAMPLE OF AN ANIMAL MODEL DEVELOPED TO UNDERSTAND ACUTE DISSEMINATED ENCEPHALOMYELITIS OBSERVED WITH OLDER RABIES VACCINE—THE EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS MODEL

Animal models have played a major role in understanding how vaccines might induce damage. As mentioned in the Introduction, the old rabies vaccine that was produced using rabbit brain tissue was associated with the occasional (0.33/1000) development of immune-mediated encephalitis and antimyelin T-cell responses (Swaddiwudhipong et al., 1998). The model known as experimental allergic or experimental autoimmune encephalomyelitis (both now abbreviated EAE) was actually developed in the 1930s by Thomas Rivers and colleagues at the Rockefeller to help understand acute disseminated encephalomyelitis that was seen following rabies vaccine used at that time (Rivers et al., 1933). The EAE model comes in many forms these days and no longer requires adjuvant for induction. EAE has directly contributed to six new therapies for multiple sclerosis. It has provided a fertile test system for exploring how vaccines might induce neuroinflammatory damage in susceptible hosts (Steinman, 2003; Steinman and Zamvil, 2006).

## PRACTICAL APPROACH TO VACCINATION IN PATIENTS WITH AUTOIMMUNE DISEASE

What is frequently forgotten, but is commonly observed by clinicians managing patients with autoimmune diseases, is that natural infection, unlike vaccination, is a more *likely and proven risk factor* for triggering (flare) and augmenting severity of autoimmune diseases. For example, an influenza vaccine study with 69 patients with systemic lupus erythematosus (SLE) and 54 patients with rheumatoid arthritis demonstrated that every viral and bacterial infection (seen predominantly in the nonvaccinated cohort) resulted in worsening of the main disease (Stojanovich, 2006). Similarly, a case report described a severe flare of SLE in a patient infected with parvovirus B19 (Hemauer et al., 1999). A meta-analysis arrived at a similar conclusion that several vaccine-preventable infections occurred more often in patients with autoimmune disease, that vaccines were efficacious in these patients, and that there did not appear to be an increase in vaccination-related harm compared to nonvaccinated patients (van Assen et al., 2011). Influenza and other acute respiratory infections are also commonly associated with an increased frequency of relapses in patients with relapsing multiple sclerosis (Oikonen et al., 2011). This risk is markedly reduced in patients that received the seasonal influenza vaccine (De Keyser et al., 1998).

### THE REALITY FACING CLINICIANS CURRENTLY

The last 10 years have ushered in several new vaccines which rheumatologists caring for their patients should be familiar with. Some of these vaccines may be live-attenuated versus being inactivated (thus not capable of replication) and thus need to be considered carefully depending on the clinical interventions being considered for the patient. These vaccines include those for cholera (live oral), meningitis (quadrivalent conjugate and serogroup B recombinant), pneumococcus (conjugate), papillomavirus (alum and TLR4 adjuvanted), rotavirus, and, lastly, the DTaP vaccine (diphtheria, tetanus, and acellular pertussis). A shingles vaccine (containing varicella zoster virus (VZV) glycoprotein with AS01B T-cell boosting adjuvant) has been submitted in 2016 for evaluation by the FDA and has now been approved by the FDA (<https://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm581491.htm>). Yet despite evidence of the impact and benefits of vaccinations, clinicians are not adequately vaccinating their patients according to established guidelines. The most significant barrier to vaccination identified by the Centers for Disease Control is the *lack of knowledge* about vaccines among adult patients and providers (Kroger et al., 2011). This section will provide guidance to the practicing rheumatologist regarding the role for vaccines in the patients that they treat based on Vaccine Recommendation and Guidelines published by the Advisory Committee on Immunization Practices (ACIP) and detailed guidelines that have been proposed by the American College of Rheumatology and the European League Against Rheumatism (to be discussed next).

### CERTAINTY ABOUT VACCINES, UNCERTAINTY ABOUT COMPATIBILITY OF ADMINISTRATION IN CERTAIN SETTINGS

For those physicians that are aware of the vaccines currently available and recommended for their patients, questions are sometimes raised regarding what to do when patients are being treated with immunomodulators or the efficacy of vaccines in patients that are immunosuppressed. These concerns are based on the observation that immunosuppression resulting from primary or secondarily altered immunocompetence creates the greatest risk for infections in patients. Most patients being managed by rheumatologists are immunosuppressed by drugs prescribed to control their autoimmune disease and, thus, the immunodeficiency is a function of dose and type of therapy. A publication from the American College of Rheumatology Drug Safety Committee succinctly addresses this challenge and is the source of the recommendations highlighted in the following sections (Dao and Cush, 2012).

Distinguishing those vaccines that are live from those that are inactivated is critical when considering immunization of patients with autoimmune diseases on immunomodulatory treatments. Examples of live vaccines include the following: smallpox, adenovirus type 4 and 7, Bacille Calmette–Guérin, typhoid (oral), rotavirus, cholera, yellow fever, herpes zoster, influenza (live attenuated), varicella zoster, and measles–mumps–rubella. In the case of live vaccines, therapy with low doses of methotrexate and azathioprine are not considered sufficiently immunosuppressive, and corticosteroid therapy is not usually a contraindication in the following cases: duration of

treatment is less than 2 weeks, use of a dose less than 20 mg/day, duration of  $\geq 2$  weeks but with alternate-day treatment of short-acting formulations, doses that are physiologic, or the route of administration is topical or injected within joints, tendons, or bursae. However, the ACIP identifies patients receiving tumor necrosis factor (TNF) inhibitors or doses of prednisone greater than 20 mg/day (for longer than 2 weeks) to be sufficiently immunosuppressed that the use of live-attenuated vaccines is not recommended due to the concern for uncontrolled replication of the viral/bacterial microorganism in the host. If higher doses of steroids are used ( $>20$  mg/day for more than 2 weeks), it is recommended to wait a month after immunosuppression before a live vaccine is given as severe complications have been reported following vaccinations with live vaccines. Such concerns do not apply to inactivated vaccines (whether killed whole-organism, subunit, recombinant, polysaccharide, or toxoid vaccines). However, one should keep in mind that methotrexate + TNF inhibitors, anti-CD20 antibody, abatacept, and possibly azathioprine can reduce the immune response to these vaccines. Especially in the case of B-cell depleting biologic therapy, vaccines should be administered before the start of therapy. Examples of inactivated vaccines include the following: typhoid (polysaccharide), tetanus-diphtheria/acellular pertussis, hepatitis A, hepatitis B, human papilloma, influenza (A/B/H1N1), pneumococcal, polio, rabies, and meningococcal. The reader should be aware that some vaccines licensed for immunization and distribution in the United States (e.g., influenza and typhoid) have been developed by different manufacturers as distinct live or inactivated products. A complete list may be found at the following website: <http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM093833>.

## SEARCH AND YOU WILL FIND

Guidance has been published for the use of vaccination in patients with autoimmune diseases. This has been in part due to the increasing awareness of the importance of vaccines in healthy populations and, ironically, also due to the perceived risk with newer vaccines containing adjuvants. Furthermore, more studies are being conducted and published on the value of vaccinations in patients with autoimmune diseases. A recent review paper has succinctly summarized the epidemiology of vaccine-preventable infectious diseases and the efficacy and safety of vaccination to prevent these diseases in patients with autoimmune diseases (Westra et al., 2015). Influenza, pneumococcal disease, herpes zoster, and HPV infection are all more common (or cause complications more frequently) in these patients. Most vaccines are effective in preventing disease in patients with autoimmune disease despite their chronic use of immunomodulatory treatments with the exception of rituximab and abatacept that probably can suppress immune responses after vaccination (Westra et al., 2015). A summary of the 2010 EULAR recommendations for vaccination of adults with autoimmune disease is included in that review paper, and a more detailed publication outlining timing and specific notes for available vaccines was published in 2016 (Tanriöver et al., 2016). The reader is also referred to the Centers for Disease Control and Prevention web-based article (“What vaccines are recommended for you,” 2017) at the following website: <https://www.cdc.gov/vaccines/adults/rec-vac/index.html> for additional details.

## CONCLUSION

The primary focus of this chapter is to update clinicians treating patients with autoimmune disease with a concise overview of the challenges facing vaccine development and the best application of this health intervention for their patient population. Those familiar with autoimmune diseases and those involved with vaccine development may already be aware of the common thread to both fields—that is, the triggering of the immune response. However, for those not familiar with these specialties, this common thread has also led, sometimes, to unjustified speculations regarding the relationship between this disease state and this disease intervention.

While it is critical to have a high level of scrutiny for the benefit/risk ratio of any prophylactic or therapeutic intervention in human subjects, one needs to keep in mind that vaccines have been responsible for preventing more deaths than virtually any other medicinal product. The value and impact of vaccinations for human health are undeniable—this is the most cost-effective intervention in preventive medicine. Those clinicians not utilizing vaccines routinely in their practices are requested to familiarize themselves in detail regarding vaccines (those with and without adjuvants, live vs inactivated), the patient populations likely to benefit from such interventions (e.g., young girls and HPV vaccination), and their correct use in patients undergoing immunosuppressive treatments. It is hoped that this review has provided a concise overview that will serve as the basis for more detailed study and prepare the clinicians for the questions posed by their patients about the safety of vaccines.

## References

- Ahmed, S.S., Volkmarth, W., Duca, J., Corti, L., Pallaoro, M., Pezzicoli, A., et al., 2015. Antibodies to influenza nucleoprotein cross-react with human hypocretin receptor 2. *Sci. Transl. Med.* 7 (294), ra105.
- Arbuckle, M.R., McClain, M.T., Rubertone, M.V., Scofield, R.H., Dennis, G.J., James, J.A., et al., 2003. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N. Engl. J. Med.* 349, 1526–1533.
- Beeler, J., Varricchio, F., Wise, R., 1996. Thrombocytopenia after immunization with measles vaccines: review of the vaccine adverse events reporting system (1990 to 1994). *Pediatr. Infect. Dis. J.* 15, 88–90.
- Chen, R.T., Pless, R., Destefano, F., 2001. Epidemiology of autoimmune reactions induced by vaccination. *J. Autoimmun.* 16, 309–318.
- Cooper, G.S., Stroehla, B.C., 2003. The epidemiology of autoimmune diseases. *Autoimmun. Rev.* 2, 119–125.
- Dao, K., Cush, J.J., 2012. A vaccination primer for rheumatologists. *DSQ (Drug Saf. Q.)* 4 (1), 1–4.
- De Keyser, J., Zwanikken, C., Boon, M., 1998. Effects of influenza vaccination and influenza illness on exacerbations in multiple sclerosis. *J. Neurol. Sci.* 159 (1), 51–53.
- Han, F., Lin, L., Warby, S.C., Faraco, J., Li, J., Dong, S.X., et al., 2011. Narcolepsy onset is seasonal and increased following the 2009 H1N1 pandemic in China. *Ann. Neurol.* 70 (3), 410–417.
- Hemauer, A., Beckenlehner, K., Wolf, H., Lang, B., Modrow, S., 1999. Acute parvovirus B19 infection in connection with a flare of systemic lupus erythematoses in a female patient. *J. Clin. Virol.* 14, 73–77.
- Jacobson, D.L., Gange, S.J., Rose, N.R., Graham, N.M., 1997. Epidemiology and estimated population burden of selected autoimmune diseases in the United States. *Clin. Immunol. Immunopathol.* 84, 223–243.
- Kroger, A.T., Sumaya, C.V., Pickering, L.K., Atkinson, W.L., 2011. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *Morb. Mortal. Wkly. Rep.* 60, 1–60.
- National Narcolepsy Task Force Interim Report, 2011. National Institute for Health and Welfare, Helsinki.
- Oikonen, M., Laaksonen, M., Aalto, V., Iilonen, J., Salonen, R., Erälinna, J.P., et al., 2011. Temporal relationship between environmental influenza A and Epstein-Barr viral infections and high multiple sclerosis relapse occurrence. *Mult. Scler.* 17 (6), 672–680.
- Rivers, D.T., Sprunt, D.H., Berry, G.P., 1933. Observations on attempts to produce acute disseminated encephalomyelitis in monkeys. *J. Exp. Med.* 58 (1), 39–53.
- Schonberger, L.B., Bregman, D.J., Sullivan-Bolyai, J.Z., Keenlyside, R.A., Ziegler, D.W., Retallieu, H.F., et al., 1979. Guillain-Barre syndrome following vaccination in the National Influenza Immunization Program, United States, 1976–1977. *Am. J. Epidemiol.* 110, 105–123.
- Siegrist, C.A., Lewis, E.M., Eskola, J., Evans, S.J., Black, S.B., 2007. Human papilloma virus immunization in adolescent and young adults: a cohort study to illustrate what events might be mistaken for adverse reactions. *Pediatr. Infect. Dis. J.* 26, 979–984.
- Statement on Narcolepsy and Vaccination, 2011. Global Advisory Committee on Vaccine Safety, World Health Organization, Geneva.
- Steinman, L., 2003. Optic neuritis, a new variant of experimental encephalomyelitis, a durable model for all seasons, now in its seventieth year. *J. Exp. Med.* 197, 1065–1071.
- Steinman, L., Zamvil, S.S., 2006. How to successfully apply animal studies in experimental allergic encephalomyelitis to research on multiple sclerosis. *Ann. Neurol.* 60, 12–21.
- Stojanovich, L., 2006. Influenza vaccination of patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). *Clin. Dev. Immunol.* 13, 373–375.
- Swadiwuthipong, W., Weniger, B.G., Wattanasri, S., Warrell, M.J., 1998. A high rate of neurological complications following Semple anti-rabies vaccine. *Trans. R. Soc. Trop. Med. Hyg.* 82 (3), 472–475.
- Tanrıöver, M.D., Akar, S., Türkçapar, N., Karadağ, Ö., Ertenli, I., Kiraz, S., 2016. Vaccination recommendations for adult patients with rheumatic diseases. *Eur. J. Rheumatol.* 3 (1), 29–35.
- US Census Bureau, 2011. Statistical Abstract of the United States, Table 7. Resident Population by Sex and Age: 1980 to 2009. US Government Printing Office, Washington, DC, <<http://www.census.gov/compendia/statab/2011/tables/11s0007.pdf>>.
- van Assen, S., Elkayam, O., Agmon-Levin, N., Cervera, R., Doran, M.F., Dougados, M., et al., 2011. Vaccination in adult patients with autoimmune inflammatory rheumatic diseases: a systematic literature review for the European League Against Rheumatism evidence-based recommendations for vaccination in adult patients with auto-immune inflammatory rheumatic diseases. *Autoimmun. Rev.* 10, 341–352.
- Westra, J., Rondaan, C., van Assen, S., Bijl, M., 2015. Vaccination of patients with autoimmune inflammatory rheumatic diseases. *Nat. Rev. Rheumatol.* 11 (3), 134–145.
- Wraith, D.C., Goldman, M., Lambert, P.H., 2003. Vaccination and autoimmune disease: what is the evidence? *Lancet* 362, 1659–1666.

## Further Reading

- Krieg, A.M., Efler, S.M., Wittkopf, M., Al Adhami, M.J., Davis, H.L., 2004. Induction of systemic TH1-like innate immunity in normal volunteers following subcutaneous but not intravenous administration of CPG 7909, a synthetic B-class CpG oligodeoxynucleotide TLR9 agonist. *J. Immunother.* 27, 460–471.

# Genetic Predisposition, Humans

Margaret A. Jordan and Alan G. Baxter

Comparative Genomics Centre, College of Public Health, Medical & Veterinary Sciences, James Cook University,  
Townsville, QLD, Australia

## OUTLINE

<b>Introduction</b>	383	<b>Genetic Linkage Studies of Autoimmunity</b>	390
<b>Diseases of Interest</b>	384	<i>Linkage Studies of Type 1 Diabetes</i>	390
<i>Type 1 Diabetes</i>	384	<i>Linkage Studies in Multiple Sclerosis</i>	392
<i>Multiple Sclerosis</i>	384	<i>Linkage Studies in Systemic Lupus Erythematosus</i>	393
<i>Systemic Lupus Erythematosus</i>	384		
<b>Human Leukocyte Antigen and Other Candidate Genes</b>	385	<b>Genome-Wide Association Studies of Autoimmunity</b>	394
<i>Association of Type 1 Diabetes with Human Leukocyte Antigen and Other Candidate Genes</i>	385	<i>Genome-Wide Association Studies of Type 1 Diabetes</i>	395
<i>Association of Multiple Sclerosis with Human Leukocyte Antigen and Other Candidate Genes</i>	386	<i>Genome-Wide Association Studies of Multiple Sclerosis</i>	398
<i>Association of Systemic Lupus Erythematosus with Human Leukocyte Antigen and Other Candidate Genes</i>	387	<i>Genome-Wide Association Studies of Systemic Lupus Erythematosus</i>	403
<i>Mechanisms of Complement and Fc Associations with Autoimmunity</i>	389		
<i>Mechanisms of Human Leukocyte Antigen Association with Autoimmunity</i>	389	<b>Concluding Comments</b>	406
		<b>Acknowledgments</b>	408
		<b>References</b>	408

## INTRODUCTION

All common autoimmune diseases are complex genetic traits. That is, they result from a combination of many risk factors—genetic, environmental, and stochastic—each contributing a relatively small degree of risk. They are subject to genocopies (a genotype at one locus contributing to the risk of disease in a manner indistinguishable from that produced by another genotype and/or locus) and phenocopies (an environmental factor mimicking the effects of a susceptibility gene). A consequence of this is that the disease phenotype is only a weak predictor of the presence of a susceptibility allele. As this association underlies all current genetic approaches, the identification of susceptibility genes in complex disease traits is both practically and technologically challenging.

The main benefits of genetic studies of autoimmunity are to (1) provide informed genetic counseling; (2) develop improved models for risk prediction; and (3) develop detailed molecular models of etiology and pathogenesis. As a generalization, the first benefit arose from work prior to the 1990s; the second has, in part, been achieved already; and the third has become a major focus of the field over the last 5 years.

## DISEASES OF INTEREST

In any genetic study, clinical definition is critical since clinical heterogeneity associated with allelic differences weakens the statistical power. Here, we will concentrate primarily on progress in the genetics of type 1 (autoimmune) diabetes (T1D), multiple sclerosis (MS), and systemic lupus erythematosus (SLE).

### Type 1 Diabetes

T1D is an endocrine disease that results from the autoimmune destruction of the insulin-producing  $\beta$  cells in the pancreas, leading to a loss of insulin secretion and hyperglycemia, resulting in osmotic diuresis and the symptoms of polyuria, polydipsia, polyphagia, tiredness, and loss of weight. Potentially fatal complications include ketoacidosis and hyperglycemic coma. Progression from the preclinical stage of  $\beta$ -cell autoimmunity (insulitis) to established diabetes can take up to a decade (Johnston et al., 1989; Bonifacio et al., 1990). Diagnostically, the major confusion is with latent autoimmune diabetes of adults, which is of later onset, and type 2 diabetes, which does not have an autoimmune origin (and therefore lacks antiislet autoantibodies), but is common and of increasing prevalence.

Evidence for a genetic contribution to the risk of T1D includes increased prevalence of disease in first degree relatives (FDR; 2.5%–6.0% risk vs 0.1%–0.3% in the general population of Western countries) with a  $\lambda_s$  (recurrence-risk ratio of disease in siblings of patients) of about 15 (Tillil and Körberling, 1987; Pociot & McDermott, 2002). T1D has a high concordance in monozygotic (MZ) twins and intermediate concordance in dizygotic (DZ) twins, 27% and 3.8%, respectively (Hytinen et al., 2003) and is associated with a lifetime risk for the twin of an affected proband of 44% and 19%, respectively (Kumar et al., 1993). The prevalence of T1D varies markedly between countries and is increasing at a rate of about 3% per year (Onkamo et al., 1999), a change that is associated with alterations in autoantibody profiles and an accelerated onset of disease from the time of identification of autoantibodies. These trends are consistent with changing environmental effects on the pathogenesis of the disease (Ziegler et al., 2011; Long et al., 2012).

### Multiple Sclerosis

MS is a chronic and debilitating disease of the central nervous system (CNS) characterized by myelin loss and axonal degeneration, resulting in neurological symptoms and impairment. It primarily affects individuals of northern European descent with a prevalence of approximately 0.1%–0.2%; more females than males are affected, at a ratio of approximately 3:1. Clinically, MS can be divided into two major subtypes: relapsing–remitting MS (RRMS) and primary–progressive MS (PPMS). RRMS is characterized by relapses followed by periods of remission of variable duration during which there is complete or partial recovery. MS patients with RRMS may eventually progress to secondary–progressive MS (SPMS) characterized by steady decline without remit. PPMS, which affects approximately 5%–15% of all individuals with MS, differs from RRMS in that there are no periods of remission. Prior to genetic studies, there was a broad acceptance that RRMS and PPMS were likely to represent pathogenetically different diseases.

Familial aggregation of MS was noted as early as the 1890s and suggested that there was a genetic influence on disease development (Eichhorst, 1896). More recently, the proportion of MS patients who have a known blood relative with MS has been shown to be approximately 20% (Compston, 1991), reflecting a 3% risk in siblings of affected individuals and a  $\lambda_s$  of about 25 (Robertson et al., 1996). Twin studies have demonstrated a concordance of 25%–30% between MZ twin pairs and concordance similar to that of siblings in DZ twins (Ebers et al., 1986; Sadovnick and Baird, 1988; Sadovnick et al., 1993; Robertson et al., 1996; Carton et al., 1997; Montomoli et al., 2002).

### Systemic Lupus Erythematosus

SLE is a highly variable autoimmune disease diagnosed on the basis of a combination of clinical and laboratory criteria (American College of Rheumatology Ad Hoc Committee on Systemic Lupus Erythematosus Guidelines, 1999). It may be associated with hematologic, musculoskeletal, renal, or neuropsychiatric symptoms. It is generally characterized by the presence in plasma of antinuclear autoantibodies, primarily directed against molecules involved in transcription and translation, such as DNA, the U1 small nuclear ribonucleoprotein/Smith

(Sm) complex, and the Ro/SSA and La/SSB ribonucleoprotein autoantigens. The prevalence of SLE is around 40–50 cases per 100,000 people, primarily affecting women (F:M ratio is 9:1); the prevalence is much higher, and the course of disease much more pernicious, in Africans and Afro-Caribbean people living in Western countries and particularly in the Aborigines of northern Australia, where the prevalence reaches >90 per 100,000 (Stoll et al., 1996; Bossingham, 2003). SLE shows familial clustering, with approximately 1.5%–3.0% of FDR affected, but the variable and often mixed racial backgrounds of patients complicate the calculation of a  $\lambda_s$ , which lies somewhere between 5.0 and 30 (Block et al., 1975; Lawrence, Martins and Drake, 1987; Alarcón-Segovia et al., 2005). In twin studies, 24%–69% of MZ pairs and 2%–3% of DZ pairs were concordant (Block et al., 1975; Deapen et al., 1992).

## HUMAN LEUKOCYTE ANTIGEN AND OTHER CANDIDATE GENES

The major histocompatibility complex (MHC) was originally identified by murine allogeneic tumor transplantation (Gorer, 1937). Identification of the analogous gene complex in humans [termed the human leukocyte antigen (*HLA*) complex] was made when three groups described antibodies in sera from multitransfused patients or multiparous women that aggregated the leukocytes of many, but not all, donors (Dausset, 1958; van Rood et al., 1958; Payne and Rolfs, 1958). The collaborative dissection of the *HLA* complex is described elsewhere (Thorsby, 2009). After an association was reported between Hodgkin's disease and the 4c complex (subsequently known as *HLA-B*; Amiel, 1967), many diseases were tested for association with *HLA* alleles. The strongest *HLA* association with autoimmune disease found to date is with ankylosing spondylitis; 88%–96% of ankylosing spondylitis patients carry *HLA-B\*27* compared with 4%–8% of healthy controls (Brewerton et al., 1973; Schlosstein et al., 1973). *HLA-B\*27* carries a relative risk of ankylosing spondylitis of up to 100 (Arellano et al., 1984)—the highest genetic association for any autoimmune disease. The relative risk varies between populations and is much lower in African-Americans (Khan et al., 1977).

### Association of Type 1 Diabetes with Human Leukocyte Antigen and Other Candidate Genes

The *HLA* at chromosome 6p21 also shows association with T1D (locus termed *IDDM1*; Nerup et al., 1974; Bertrams, 1984; Todd, 1992; Cucca et al., 2001; Noble et al., 1996; Kelly et al., 2003; Devendra & Eisenbarth, 2003; Valdes, Erlich and Noble, 2005) and accounts for approximately 40% of the familial aggregation of the disease; the predisposing *HLA* Class II haplotypes, *HLA-DRB1\*04*, *DQB1\*03:02* (identified by serology as DR4) and *DRB1\*03:01*, *DQB1\*02:01* (DR3) are present in 95% of affected individuals. DR3/DR4 heterozygotes carry an absolute risk of T1D of approximately 5% (compared to a cumulative incidence of approximately 0.3% in Western communities), which rises to about 20% if a haploidentical sibling is affected (Noble et al., 1996; Valdes et al., 2001). DQ amino acid sequences directly correlate to the risk of T1D, and this association is largely dependent on the identity of residue 57 of the DQ $\beta$  chain, Asp being protective and Ala conferring susceptibility (Todd, Bell and McDevitt, 1987), and residue 52 of the DQ $\alpha$  chain (Arg confers susceptibility; Khalil et al., 1990). Khalil et al. (1990) reported that of 50 T1D patients, all expressed the DQ $\alpha$ -52Arg/DQ $\beta$ -57Ala susceptible heterodimer. Remarkably, the NOD mouse model of T1D expresses an A $\beta$  chain homologous to DQ $\beta$  with a substitution at position 57 (Todd, Bell and McDevitt, 1987).

### INS

Other T1D candidate genes were examined because of their biological relevance to disease. Insulin is primarily transcribed in the beta cells of pancreatic islets and is a major autoantigen in T1D (Kent et al., 2005). There are three common polymorphisms in strong linkage disequilibrium within the insulin (*INS*) gene on 11p15: (1) a variable number tandem repeat (VNTR) locus; (2) the rs3842753 A > C variant; and (3) the rs689 A > T variant within the Hph1 restriction endonuclease site 23 bp upstream of the insulin gene transcription start site. The VNTR is the best candidate because it contains binding sites for many transcription factors, including Myc-Associated Zinc Finger Protein (MAZ) (Kennedy, German and Rutter, 1995), while there is no obvious functional role for either of the other two variants (Barratt et al., 2004). The promoter of the insulin *INS* gene contains the VNTR locus, and the alleles with the shortest repeat sequences (Class I alleles; 26–63 repeats) are associated with T1D in *HLA-DR4*-expressing subjects [odds ratio (OR) 1.9; *IDDM2*; Bell et al., 1984; Julier et al., 1991; Bain et al., 1992; Bennett et al., 1995]. Class III alleles (the longest; 141–209 repeats) of the VNTR are associated with

marginally lower levels of insulin mRNA expression in pancreata (Bennett et al., 1995), but a two to threefold higher expression in fetal thymus (Vafiadis et al., 1997). These data are consistent with the hypothesis that protective alleles of the *INS* VNTR are responsible for increased thymic insulin expression driving more effective induction of central tolerance of insulin-reactive T cells. Although *INS* polymorphisms were associated with T1D in many Caucasian populations, the association was not seen in Chinese subjects because the frequency of the susceptible *INS* allele is ~95% in Chinese controls and close to 100% in Chinese T1D patients (Marron and She, unpublished data cited in Marron et al., 1997).

Consistent with its antigen-specific role in autoimmunity, variants in the *INS* gene are not associated with other autoimmune diseases, although they are associated with type 2 diabetes (Ng et al., 2014).

## **PTPN22**

Protein tyrosine phosphatases were thought to be good candidates for autoimmunity susceptibility genes, because they are involved in limiting T-cell activation by dephosphorylating T-cell receptor (TCR)-associated kinases and their substrates. *PTPN22*, on chromosome 1p13, encodes the lymphocyte-specific protein tyrosine phosphatase Lymphoid Phosphatase (LYP), which is a negative regulator of TCR signaling via the dephosphorylation of several proximal TCR signaling molecules, including the SRC family kinases LCK and FYN, ZAP70, and TCR $\zeta$ . A nonsynonymous variant, rs2476601 G > A, at position 1858 of *PTPN22* was reported to be associated with T1D in many populations (OR for the homozygous AA genotype was 1.7; Bottini et al., 2004; Onengut-Gumuscu et al., 2004; Smyth et al., 2004; Zheng and She, 2005; Steck et al., 2009, reviewed in Steck and Rewers, 2011). It encodes a missense substitution that changes an Arg to a Trp at position 620 causing the inability of LYP to bind its signaling molecule, CSK (Vang et al., 2005), resulting in increased phosphatase activity. T cells from T1D patients and healthy subjects carrying the rs2476601A allele show reduced production of interleukin (IL)-2 and other cytokines following TCR stimulation (Vang et al., 2005; Rieck et al., 2007). The rs2476601 G > A variant is also associated with Graves' disease (Steck et al., 2006), rheumatoid arthritis (RA) (Begovich et al., 2004), Crohn's disease (Franke et al., 2010), vitiligo (Jin et al., 2012), and SLE (Kyogoku et al., 2004).

TCR and immunoglobulin loci were also examined as candidates, without consistent evidence of involvement.

## **Association of Multiple Sclerosis with Human Leukocyte Antigen and Other Candidate Genes**

Association of MS with the HLA Class I alleles *HLA-A\*03* and *HLA-B\*07* (identified by serology as A3 and B7) were reported in 1972 (Jersild, Svegaard and Fog, 1972; Jersild and Fog, 1972; Naito et al., 1972). Further studies found that both of these associations were secondary to those of the Class II alleles *HLA-DRB1\*15:01* (DR2) and *HLA-DQB1\*06:02* (DQ6), which can be inherited together in strong linkage disequilibrium as the HLA-DR15 haplotype (*DRB5\*01:01*, *DRB1\*15:01*, *DQA1\*01:02*-*DQB1\*06:02*; Fogdell et al., 1995). This haplotype confers a relative risk of MS of between 2 and 4 (Francis et al., 1987a, 1987b). Approximately 30% of individuals with MS carry this extended haplotype compared to 15% in the normal population; its effect is dominant and shows evidence of gene dosage (Barcellos et al., 2006). The contribution of HLA to total (genetic) susceptibility to MS is estimated to be between 15% and 50% (Haines et al., 1996, 1998).

There is a very strong association between Epstein–Barr virus (EBV) infection and MS: EBV is unique in being the only virus known to have infected virtually all MS patients, in contrast to healthy subjects, of whom 85%–95% are infected (Sumaya et al., 1985; Myhr et al., 1998); EBV seronegativity has an OR of 0.06 (95% CI 0.03–0.13;  $P < 10^{-9}$ ; Ascherio and Munger, 2007). *HLA-DRB1\*15:01* appears to act synergistically as a risk factor with EBV exposure. The risk conferred by *HLA-DRB1\*15:01* was 2.9-fold higher in patients with a history of infectious mononucleosis and the risk of MS in *HLA-DRB1\*15:01* positive women increased ninefold in those with elevated anti-EBNA-1 (EBV) antibody titers (Nielsen et al., 2009; De Jager et al., 2008). A confounding problem is the potential effects of *DRB1\*15:012* on EBV susceptibility. EBV achieves penetration of the plasma membrane via the binding of its viral glycoprotein gp42 to HLA-DR (Li et al., 1997); the HLA-DR15 haplotype is associated with 15.7- and 8.3-fold higher expression of DQB1 and DRB1, respectively (Alcina et al., 2012), potentially conferring higher infection rates.

MS is also associated with childhood residence at higher geographic latitude (Acheson, Bachrach and Wright, 1960) and a significant component of this association appears to be mediated by low ultraviolet light exposure resulting in reduced 1,25(OH)<sub>2</sub> Vitamin D availability (Nieves et al., 1994; Munger et al., 2006). A functional vitamin D response element (VDRE) has been identified in the promoter region of *HLA-DRB1* (Ramagopalan et al., 2009), suggesting these alleles may be unusual in having vitamin D-regulated MHC Class II expression.

Class I alleles also affect susceptibility to MS: The *HLA-A\*03:01* allele increases the risk (OR 2.1), while *HLA-A\*02:01* and *B\*44* decrease it (OR 0.52 and 0.62, respectively; Fogdell-Hahn et al., 2000; Healy et al., 2010; Bergamaschi et al., 2010; Cree et al., 2010). The presence of *A\*02:01* reduces the relative risk of MS conferred by the HLA-DR15 haplotype from 3.6 to 1.5 (Fogdell-Hahn et al., 2000).

No associations between *HLA* genotypes and specific MS subtypes have been identified (Barcellos et al., 2006), although a significant correlation between the age-of-onset of MS and a variant tagging the HLA-DR15 haplotype has been reported [Masterman et al., 2000; International Multiple Sclerosis Genetics Consortium (IMSGC), 2011]; no associations with gender or disease severity were observed.

Other MS candidate genes that have been tested by association or transmission tests were selected largely on the basis of known or predicted roles in pathogenesis. In particular, genes encoding the T-cell receptor (TCR), IL-1 receptor (IL1R), interferons (IFNs)  $\alpha$ ,  $\beta$ , and  $\gamma$ , and CTLA4, as well as various cytokine/chemokine genes have been investigated with no consistent results.

## Association of Systemic Lupus Erythematosus with Human Leukocyte Antigen and Other Candidate Genes

*HLA-DRB1\*03:01* (identified by serology as DR3) is strongly associated with SLE (Freedman et al., 1993). In African-American patients, *HLA-DRB1\*03* was found in 62% of SLE patients, but only 20% of controls (relative risk of 6.41); in Caucasian patients the strength of the association is a little lower: 30% versus 13% (So et al., 1990). *DRB1\*03:01* is part of the 8.1 ancestral haplotype (*HLA-A\*01*, *C\*07*, *B\*08*, *DRB1\*03:01*, *DQA1\*05:01*, *DQB1\*02:01*), so named because it includes the Class I alleles *HLA-B\*8* (B8) and *HLA-A\*01* (A1). This haplotype therefore confers susceptibility to both SLE and T1D in Europeans, as does the  $H2^{g7}$  MHC haplotype in NOD mice (Price et al., 1999; Jordan et al., 2000). It is also associated with celiac disease (Ahmed et al., 1993), myasthenia gravis (Hammarström et al., 1975), chronic active (autoimmune) hepatitis (Strettell et al., 1997), and scleroderma (Kallenberg et al., 1981).

The Class II allele *HLA-DRB1\*15:01* [serotype DR15, a split of DR2, and usually associated with *DQB1\*06:02*, *DQA1\*0102* (serotype DQ6, a split of DQ1)] is also associated with SLE; it was found in 41% of African-American SLE patients and 18% of African-American healthy controls (relative risk of 3.03). Again, in Caucasian patients, the association is a little weaker: 21% versus 11% (So et al., 1990). The combined relative risk for SLE associated with expression of both haplotypes is 9.0 (Kachru et al., 1984).

### Tumor Necrosis Factor (TNF)

Interest in *TNF* genes in humans followed the finding of a polymorphism within the *Tnf* gene of SLE-prone (NZB × NZW)F1 hybrid mice (Jacob and McDevitt, 1988), in which the decreased expression of *Tnf* was associated with disease and TNF administration delayed nephritis. The same group applied serotyping and bioassays to determine the relevance of quantitative polymorphisms in *TNF* to SLE and lupus nephritis in patients, reporting that in SLE patients, the DR2-DQ1 serotype (inferred haplotype *DRB1\*05:01*, *DQB1\*06:02*, *DQA1\*01:02*) was associated with low levels of induced TNF expression and an increased incidence of nephritis. In contrast, SLE patients with the DR3 (*HLA-DRB1\*03:01*) and DR4 (*HLA-DRB1\*04:01*) serotypes had high TNF production and were not predisposed to nephritis (Jacob et al., 1990). One potential explanation for this association is the rs1800629 A > G variant at position -308 in the promoter of the *TNF* gene: The rs1800629G allele is associated with low TNF expression, whereas the A allele has high expression (Tan and Arnett, 1998; Hajeer and Hutchinson, 2001). This association with expression as well as the variant's association with disease has been controversial, in part due to attempts to generalize the original observation to SLE (as distinct from lupus nephritis) and in part due to racial heterogeneity (Rudwaleit et al., 1996). Where an association was found, often it could not be shown to be acting independently of *HLA-DRB1* alleles (Rudwaleit et al., 1996; Wilson et al., 1994).

### TNFSF4

Cunningham Graham et al. (2008) explored association between SLE and variants across *TNFRSF4* (OX40L) and its ligand *TNFSF4* (OX40). They genotyped by various methods parent-affected trios (a proband and both parents—in this case, one affected) from the United Kingdom (416 trios) and the United States (Minnesota; 263 trios) as well as 424 unrelated cases and 642 unaffected control samples from the British 1958 birth cohort. No evidence of linkage or association was identified at *TNFRSF4*. TDT analysis of the trios in both cohorts identified an overtransmitted haplotype tagged by multiple rare alleles, with rs12039904 C > T being the most significant in

a combined analysis ( $P = 1 \times 10^{-5}$ ). A similar significance was obtained by haplotype analysis. An association study was then performed on the independent UK case-control collection with the strongest association identified at rs844644 A>C ( $P = 6.8 \times 10^{-5}$ ), which lies just 2.8 kb distal to rs12039904 C>T and tags the undertransmitted haplotype. The linkage and association studies were combined using Fisher's combined probability test with which the significance at rs844644 A>C reached  $P = 6.8 \times 10^{-5}$ . Subsequent large Genome-Wide Association Studies (GWAS) confirmed this association at a genome-wide level of significance ( $P < 5 \times 10^{-8}$ ; see below).

The variant rs844644 A>C lies at 1q24, upstream of the 5' untranslated region (UTR) of *TNFSF4*, which encodes TNF Superfamily Member 4 (TNFSF4), the OX-40 Ligand. TNFSF4 is expressed broadly, including on the DC2 subset where it facilitates Th2 differentiation. Its receptor, OX-40 is expressed mainly on active CD4 T cells. The SLE-associated (minor) rs844644C allele was associated with increased TNFSF4 expression in EBV-transformed B-cell lines and peripheral blood leukocytes from SLE patients (Cunningham Graham et al., 2008).

#### **C4A, C4B**

One of the strongest associations with SLE within the HLA is with the C4 genes in the *HLA* Class III region (Christiansen et al., 1991). Both the major European DR3-associated haplotypes in SLE patients include the disruption of *C4A* or *C4B*. The 8.1 ancestral haplotype common in Western and Northern Europeans contains a null allele of the gene encoding *C4A* (*C4A\*Q0*; Fielder et al., 1983; So et al., 1990). In Caucasians the relative risk for SLE of carrying two *C4A\*Q0* alleles is 16.9 (Howard et al., 1986). Association studies of other *C4A* null alleles in African-American and Asian populations, where they are present on haplotypes other than the 8.1 ancestral haplotype, indicate that that *C4A* null alleles are SLE risk genes independently of *HLA-DRB1\*03:01*, showing a gene dose effect and conferring a relative risk of 2.7 in African-Americans (Howard et al., 1986; Yamada et al., 1990).

A null allele of the gene encoding *C4B* (*C4B\*Q0*) is present in the other major European DR3-associated ancestral haplotype (A30::DQ2, consisting of *HLA-A\*30:02*, *C\*05:01*, *B\*18*, *DRB1\*03:01*, *DQA1\*05:01*, *DQB1\*02:01*), which occurs with frequencies around 15% in Sardinia and Basque, and between 2% and 10% in Southern Europe. The *C4B\*Q0* allele is also relatively common in SLE patients (Fielder et al., 1983) and shows an association with SLE in Australian Aborigines (Christiansen et al., 1991), African-Americans (relative risk 2.0; Howard et al., 1986), and Spanish populations (relative risk 6.0; Naves et al., 1998), but not other Caucasians (Howard et al., 1986).

#### **C2**

The gene encoding the C2 complement component also lies within the Class III region of the HLA, between the *C4* genes and those encoding HSP70, TNF, LT $\alpha$ , and LT $\beta$ . Null alleles of *C2* are likewise associated with SLE, and 33% of Europeans with homozygous *C2* deficiency develop it (Walport, 1993). Homozygosity for null alleles is present in 0.4%–2% of cases compared to 0.01% of the general population; heterozygous *C2* deficiencies are present in 2.4%–5.8% of cases compared to 0.7%–1% of the general population (Sullivan et al., 1994; Lipsker et al., 2000). Patients with *C2* deficiency tend to experience photosensitivity and other forms of skin involvement; amongst *C2*-deficient patients without SLE, benign cutaneous (discoid) lupus is also common (Agnello, De Bracco and Kunkel, 1972; Provost, Arnett, Reichlin, 1983; Lipsker et al., 2000).

#### **C1Q**

Heredity deficiencies of other early complement components (C1q, C1r, C1s) are powerful but rare causes of a SLE-like syndrome. For example, 90% of people with C1q deficiency develop SLE. Onset can occur in childhood (even as early as 1 year of age), and the disease can be associated with very severe CNS and renal complications; of 30 patients, 22 died in childhood (Bowness et al., 1994).

#### **FCGR2A, FCGR3A, FCGR3B**

In the 1970s, reduced Fc-rosette formation of red blood cells from SLE patients was identified and attributed to saturation by circulating complexes (Nakai et al., 1977). While it was known that the Fc $\gamma$  receptors Fc $\gamma$ RI (CD64), Fc $\gamma$ RII (CD32), and Fc $\gamma$ RIII (CD16) contribute to the clearance of IgG and IgG-containing immune complexes, they were not examined as candidates until the 1990s. The C allele of the rs1801274 T>C variant in *FCGR2A* results in a His to Arg substitution at position 131 of Fc $\gamma$ RIIa and reduces the affinity by which Fc $\gamma$ RIIa binds IgG2 (Duits et al., 1995). This substitution also affects the clearance of IgG-coated erythrocytes and has been found to have an inconsistent association with SLE but not to nephritis (Duits et al., 1995; Dijstelbloem et al., 2000; Salmon et al., 1996).

Similarly, the T allele of the rs396991 G > T variant in *FCGR3A* results in a Val to Phe substitution at position 176 of Fc $\gamma$ RIIIa reducing, in 176Phe/Phe homozygotes, the binding of IgG1 and IgG3 by NK cells and monocytes, inhibiting NK cell activation, and activation-induced cell death (Wu et al., 1997). Homozygosity for this allele is present in a significantly higher proportion of SLE patients than controls (Wu et al., 1997; Koene et al., 1998) and is associated with lupus nephritis, arthritis, and serositis, or hematologic cytopenias, depending on the population (Salmon et al., 1999; Dijstelbloem et al., 2000).

Two alleles of *FCGR3B* vary in the encoded amino acid sequence of Fc $\gamma$ RIIb, affecting low-affinity IgG binding by neutrophils. The allele associated with decreased phagocytic activity (NA2) is associated with SLE (OR 1.9; Hatta et al., 1999). *FCGR3B* also shows copy number variation, and a low *FCGR3B* copy number, in particular complete Fc $\gamma$ RIIb deficiency, is strongly associated with SLE as well as with Wegener's granulomatosis (Fanciulli et al., 2007; McKinney and Merriman, 2012).

## **IRF5**

Increased production of type I IFNs and expression of IFN-inducible genes are commonly observed in SLE and appears to play a key role in the molecular pathogenesis of the disease (Baechler et al., 2003). Sigurdsson et al. (2005) performed linkage and association studies of 44 variants in 13 candidate genes involved in the type I IFN pathway on 679 Swedish, Finnish, and Icelandic patients with SLE, 798 unaffected family members, and 438 unrelated control individuals. Two genes were highly significantly associated with SLE: TYK2 (19p13) and *IRF5* (7q32). Remarkably, despite the relatively low power of the study, the *P* value for *IRF5* reached a genome-wide level of significance. This finding was quickly and robustly replicated (Graham et al., 2006). The most highly associated variant in the 7q32 region (rs2004640 T > G) lies in the splice junction of an alternate first exon of *IRF5*. The rs2004640T allele creates a 5' donor splice site allowing expression of several unique *IRF5* isoforms and is in linkage disequilibrium with a *cis*-acting variant associated with elevated expression of *IRF5*. *IRF5* is a transcription factor that regulates type I IFN gene expression. It is critical for the production of the proinflammatory cytokines TNF, IL-12, and IL-6 following Toll-like receptor (TLR) signaling (Takaoka et al., 2005), and it mediates type I IFN induction in response to some viruses (Barnes, Moore and Pitha, 2001). In a genome-wide association study of anti-dsDNA autoantibodies in SLE, *IRF5*, together with the *HLA*, *STAT4*, and *ITGAM*, was found to be significantly associated (Chung et al., 2011).

Other variants in this region are associated with ulcerative colitis (Anderson et al., 2011), Sjögren's syndrome (Lessard et al., 2013), systemic sclerosis (Mayes et al., 2014), RA (Okada et al., 2014), and primary biliary cirrhosis (Liu et al., 2012).

## Mechanisms of Complement and Fc Associations with Autoimmunity

The leading hypothesis explaining the association between SLE and deficiencies in FcR and complement is that these defects impair the catabolism of immune complexes by the mononuclear phagocytic system (Walport, 1993). Tracking of radio-labeled immune complexes revealed the following defects in SLE patients: (1) reduced immune complex uptake by the spleen; (2) accelerated clearance of complexes by the liver; and (3) release of those immune complexes from the liver back into circulation (Davies et al., 1992). In similar studies of a patient with C2 deficiency, a splenic uptake of immune complexes was found to be entirely complement dependent and could be restored by the transfusion of fresh frozen plasma (Davies et al., 1993). As C4A binds more effectively to amino groups than does C4B, it has been proposed that it is more efficient at clearing immune complexes (Schifferli et al., 1986). This may explain the stronger association of SLE with C4A deficiencies.

## Mechanisms of Human Leukocyte Antigen Association with Autoimmunity

The finding of a single ancestral haplotype (8.1) associated with multiple autoimmune diseases suggests a common mechanism mediating susceptibility. The analysis of HLA recombinant patients indicates that, for many autoimmune diseases, genetic association with the HLA can be primarily attributed to associations with the genes encoding specific peptide-presenting molecules, Class I or Class II (Thorsby, 1997). The hypothesis arising from this observation is that disease-associated HLA Class I and Class II alleles permit binding of disease-inducing peptides. The question of why one particular peptide of an autoantigen should be any more disease-inducing than another became the driving force behind a supplementary hypothesis: Molecular mimicry, in which it is proposed that bireactive TCRs permit the priming of T cells by a microbial peptide and effector activation by

autoantigens. The most plausible example of putative molecular mimicry is found in ankylosing spondylitis, which on microbiologic and serologic criteria is associated with *Klebsiella* infection (Ebringer, 1992). The amino acids 72–77 of the HLA Class I molecule B27 are identical to amino acids 188–193 of the nitrogenase of *Klebsiella pneumoniae* (Yu, Choo and Schaack, 1989).

Lang et al. (2002) reported bireactive TCRs of MS patients that bound both DRB5\*0101 presenting a peptide from the DNA polymerase of EBV as well as DRB1\*1501 presenting a myelin basic protein peptide. Crystal structure determination revealed a marked degree of structural equivalence at the surface presented for TCR recognition. In contrast, T cells from patients with SLE show no such cross-reactivity at the level of TCR/peptide/HLA. In addition to MS and SLE, DRB1\*1501 is positively associated with the autoimmune diseases Sjögren's syndrome (Guggenbuhl et al., 1998), Goodpasture syndrome (Fisher et al., 1997), and juvenile idiopathic arthritis (Garavito et al., 2004). Such a wide range of tissue specificities renders it most unlikely that a shared autoantigen is responsible, and there is little evidence of any etiological role for EBV in Goodpasture syndrome or juvenile idiopathic arthritis. The cross-reactivity identified in lupus autoantibodies can be explained by B-cell polyclonal activation, support of antibody production, and inhibition of apoptosis mediated by EBV, and these activities of themselves probably provide sufficient explanation for the association between EBV and SLE.

## GENETIC LINKAGE STUDIES OF AUTOIMMUNITY

The availability of dense maps of polymorphic genetic markers [microsatellites and single nucleotide polymorphisms (SNP)] revolutionized the localization of non-HLA-linked disease genes (Gyapay et al., 1994; Dietrich et al., 1992, 1996; Tsang et al., 2005). Linkage analyses rely on the disproportionate transmission of alleles to affected and unaffected progeny.

### Linkage Studies of Type 1 Diabetes

The first genome-wide scans for linkage to T1D were performed on large collections of T1D families with pairs of affected siblings (sib pairs) by microsatellite (variable number of tandem repeats) analysis in 1994 (Davies et al., 1994; Hashimoto et al., 1994; <http://www.immunobase.org>). These studies easily confirmed linkage to the HLA, both by their own statistical thresholds, as well as by those of Lander and Kruglyak (1995), which are set to a 5% probability per study of a single genomic region exceeding the significance threshold by chance (i.e., a logarithm of the odds (LOD) score >3.6). Davies et al. (1994) calculated that the HLA contributes about 42% of the familial clustering of T1D. Neither group showed evidence of linkage at *INS*.

### **CTLA4**

Owerbach and Gabbay (1995) performed a genome-wide linkage analysis in 162 type I diabetic families with an affected sibling pair and subset their data by the *INS* VNTR and HLA haplotypes. They identified an additional susceptibility locus on chromosome 2q31 near *HOXD8* (*IDDM7*; maximum LOD 4.8) in affected sib pairs lacking high-risk HLA-DR3/4 haplotypes and expressing homozygous high-risk class I VNTR alleles. This region is homologous to that on proximal mouse chromosome 1 where the *Idd5* T1D gene was subsequently identified in diabetes-prone NOD mice (Cornall et al., 1991) and contains the disease candidate genes *CTLA4* and *CD28*, which encode T-cell receptors involved in control of T-cell activation (Walunas et al., 1994).

The candidature of *CTLA4* was also supported by a subsequent linkage analysis in 48 Italian families, transmission disequilibrium testing in 187 Italian families (138 of which had only a single affected child) and 44 Spanish families, and a population-based case/control association study of 966 patients and 1058 controls from Belgium (Nisticò et al., 1996). Similar studies in British, Sardinian, and American families did not support *CTLA4* candidacy (Nisticò et al., 1996). A meta-analysis of 33 independent studies examined the variant rs231775 A > G/A > T, which causes a Thr-to-Ala substitution in the first exon; it showed an OR of 1.45 for the rs231775G allele; with a greater effect in cases with onset <20 years of age (OR 1.61; Kavvoura and Ioannidis, 2005). On the basis of *CTLA4* being 10 cM distal of the *IDDM7* linkage peak (*D2S152*), and the lack of disequilibrium between *D2S152* and *CTLA4* in the association study, the locus at 2q33 was designated *IDDM12* (Nisticò et al., 1996).

Ueda et al. (2003) examined expression levels of two major isoforms of *CTLA4* that are affected by the most T1D-associated variant rs3087243 G > A: the full-length sequence, and a soluble isoform (s-*CTLA4*) that lacks exon 3 (Oaks et al., 2000). The disease-associated rs3087243G allele was associated with a lower expression of

s-CTLA4 in a gene dose-dependent manner. Similarly, sequence-dependent variations in *Ctla4* isoforms were identified in T1D-susceptible NOD mice, and the differential expression of one appeared to mediate the allelic variation in T1D risk that maps to this chromosomal region (Oaks et al., 2000; Vijayakrishnan et al., 2004; Araki et al., 2009). CTLA4 is expressed constitutively on regulatory T (Treg) cells and is thought to, at least in part, mediate their immunosuppressive activities (Manzotti et al., 2002), as an interaction of CTLA4 with CD80 or CD86 inhibits human T-cell activation (Vandenborre et al., 1999). Soluble CTLA4 also has this activity (Oaks et al., 2000), and a knock-down transgene for s-CTLA4 exacerbated T1D in an NOD congenic strain that expresses the wild-type *Ctla4* allele (Gerold et al., 2011).

In addition to T1D, CTLA4 is associated with Graves' disease (Yanagawa et al., 1995; Kouki et al., 2000; Kotsa, Watson and Weetman, 1997), Hashimoto's autoimmune thyroiditis and autoimmune hypothyroidism (Kotsa, Watson and Weetman, 1997; Donner et al., 1997), Addison's disease (Blomhoff et al., 2004; Donner et al., 1997), MS (Harbo et al., 1999; Ligers et al., 1999) and alopecia areata (Petukhova et al., 2010), celiac disease (Dubois et al., 2010), and rheumatoid arthritis (Gonzalez-Escribano et al., 1999; Gregersen et al., 2009).

The 10p11-q11 region (designated *IDDM10* in unpublished data by Todd (1995); maximum LOD 2.03; Hashimoto et al., 1994) contains the putative candidate genes *GAD2* and *IL2RA* (*CD25*).

## **GAD2**

*GAD2* encodes the islet cell-specific (65 kDa) form of Glutamic Acid Decarboxylase (GAD65), a prevalent autoantigen in T1D. Association analysis of a highly polymorphic dinucleotide repeat linked to the gene did not support a significant role for *GAD2* (Wapelhorst et al., 1995).

## **IL2RA**

In contrast, *IL2RA* (10p15), which encodes the IL-2 receptor  $\alpha$  chain (IL2RA), was supported by a tag SNP approach and a large sample size (7457 cases and controls and 725 multiplex families; Vella et al., 2005). IL2RA plays a critical role in the development and maintenance of Treg and may play a role in Treg expression of CD62L, which is required for their entry into lymph nodes (Malek and Bayer, 2004). In association studies, T1D was associated with two independent groups of variants, spanning overlapping regions of 14 and 40 kb, encompassing the first intron of *IL2RA* and the 5' intragenic region (Lowe et al., 2007; Maier et al., 2009b). The T1D susceptibility genotypes were also associated with lower circulating levels of soluble IL2RA (s-IL2RA; Lowe et al., 2007). Dendrou et al. (2009) confirmed gene–phenotype correlation at the RNA level: Individuals with one or two protective G alleles at rs12722495 A > G showed a 27% increase in IL2RA levels on their CD4 $^{+}$ memory T cells when compared to homozygous susceptible individuals (AA) or to those with protective rs11594656A or rs2104286G alleles.

Allelic variation in *IL2RA* is also associated with MS (IMSGC et al., 2007; see below), RA (Stahl et al., 2010), juvenile idiopathic arthritis (Hinks et al., 2013), alopecia areata (Petukhova et al., 2010), primary sclerosing cholangitis (Liu et al., 2013), inflammatory bowel disease (Jostins et al., 2012), and Crohn's disease (Franke et al., 2010).

## **OTHER LOCI**

Other putative diabetes susceptibility loci initially identified using linkage studies were localized to chromosomes: 18q12-q21 (designated *IDDM6*; maximum LOD 3.7; Todd, 1995; Merriman et al., 1997), 6q27 (designated *IDDM8*; maximum LOD 3.4; Luo et al., 1995; Todd, 1995; Luo et al., 1996), 3q22-q25 [designated *IDDM9* (unpublished data, Todd, 1995), maximum LOD 2.4 in DR3/DR4 heterozygotes; Mein et al., 1998; Paterson, Rahman and Petronis, 1999], 14q24-q31 (designated *IDDM11*; maximum LOD 4.0, 4.6 in families without evidence of HLA linkage to T1D; Field et al., 1996), 2q34-q35 (designated *IDDM13*; maximum LOD 3.3; Morahan et al., 1996), and 6q2 (designated *IDDM15*; identified by an extension of identity-by-descent methods as adjacent to HLA,  $P < 5 \times 10^{-5}$ ; Delépine et al., 1997). The latter locus brings to a total four putative loci on chromosome 6q: *IDDM1/HLA*, *IDDM15*, *IDDM5*, and *IDDM8*, in that order from centromere to telomere over about 100 cM.

## **LINKAGE ANALYSES OF COMBINED DATASETS AND THE LIMITS OF LINKAGE ANALYSES**

By 1998, very large collections of families were being analyzed. Mein et al. (1998) studied 356 affected sib-pair families from the United Kingdom but found significant linkage only to three regions: (1) *IDDM1/HLA*; (2) *IDDM10/IL2RA* (10p13; maximum LOD 4.7); and (3) 16q22-24 (maximum LOD 3.4). Remarkably, most of the previously reported loci were excluded by exclusion mapping at a  $\lambda_s$  of 3 and a LOD of -2. Similarly, a two-staged analysis of 616 multiplex families from the United Kingdom and the United States identified only

*IDDM1/HLA* (maximum LOD 34.2) as significant by multipoint analysis, and a single previously unreported locus on 1q of suggestive significance (LOD 3.31; Concannon et al., 1998). The data were consistent with a locus distal from the *HLA*, at *IDDM15* (6q21) with a maximum LOD 3.8, but its proximity to the *HLA* required correction for linkage disequilibrium, resulting in an adjusted LOD of 2.27. On chromosome 2q, previous studies had proposed three loci (*IDDM7*, *IDM12*, and *IDDM13*) but Concannon et al. (1998) reported a maximum LOD in this region of 1.07, and little evidence for distinct loci. By multipoint analysis, even modest contributions ( $\lambda_s \geq 1.5$ ; LOD  $< -2$ ) to T1D could be excluded for *IDDM3*, *IDDM4*, *IDDM6*, *IDDM9*, and *IDDM10*. In an identity-by-descent (IBD) analysis of previously reported loci (other than *IDDM1/HLA* and *IDDM2/INS*), only *IDDM7/IDDM12/IDDM13* and *IDDM15* had LOD scores greater than 1; negligible support was found for six of the previously reported loci: *IDDM3*, *IDDM4*, *IDDM6*, *IDDM9*, *IDDM10*, and *IDDM11*.

To further increase the power of linkage analyses, multinational consortia were formed, allowing the analysis of combined datasets. The Type 1 Diabetes Genetics Consortium (T1DGC) was established for this purpose in 2002. Concannon et al. (2005) performed, under the auspices of the T1DGC, a combined linkage analysis of four datasets, three previously published (Concannon et al., 1998; Mein et al., 1998), providing a total sample of 1435 families with 1636 affected sib pairs. By multipoint linkage analysis, only the *HLA* was significant (*IDDM1*; LOD 116;  $\lambda_s$  of 3.35), and four other regions showed suggestive significance (i.e., uncorrected  $P < 7.4 \times 10^{-4}$ ): 2q31-33 (*IDDM7/IDDM12/CTLA4*; LOD 3.34;  $\lambda_s$  of 1.19), 6q21 (*IDDM15*; LOD 22.39;  $\lambda_s$  of 1.56), 10p14-q11 (*IDDM10/IL2RA*; LOD 3.21;  $\lambda_s$  of 1.12), 16q22-24 (LOD 2.64;  $\lambda_s$  of 1.19). LOD scores above 1 were found at *IDDM2/INS* and five other regions: 3p13-14, 9q33-34, 12q14-12, 16p12-q11, and 19p13. The 19p13 region contains the insulin receptor gene (*INSR*).

In 2009 T1DGC published a pedigree disequilibrium test (a family-based test of linkage disequilibrium applicable to larger-than-nuclear families; Martin et al., 2000) analysis of 2496 multiplex families (Concannon et al., 2009). Three of the most strongly associated (but not significant at a genome-wide level) loci had been previously identified (*INS*, *IFIH1*, and *CLEC16A*; the latter locus previously identified by genome-wide association study, discussed below). A fourth strongly associated variant was in the sixth intron of the gene *UBASH3A*, and this finding was validated by linkage analysis in 2214 parent-affected child trio families and association in panel of 7721 cases and 9679 control subjects (OR 1.10;  $P = 4.4 \times 10^{-12}$ ).

### UBASH3A

This gene encodes the protein ubiquitin-associated and SH3 domain containing A, which is expressed primarily in T cells. CRISPR/Cas9 manipulation of expression levels in Jurkat lymphoblastic T cells showed that its expression suppresses TCR-induced NF- $\kappa$ B signaling by inhibiting I $\kappa$ K $\alpha$ /b phosphorylation, I $\kappa$ B degradation, and NF- $\kappa$ B translocation to the nucleus. As a result, IL-2 secretion is inhibited (Ge et al., 2017). The T1D-associated *UBASH3A* rs11203203A (minor) allele is associated with increased expressions of *UBASH3A* and reduced *IL2* mRNA compared to subjects homozygous for the rs11203203G (major) allele (Ge et al., 2017).

The rs11203203 G > A variant is also associated with vitiligo (Jin et al., 2012) and rs1893592 A > C variant, which lies within an intron of *UBASH3A*, is associated with celiac disease (Trynka et al., 2011).

The following year (2009) T1DGC published the details of a linkage reanalysis of their collection of 2496 multiplex families applying a linear nonparametric linkage model that was less conservative in handling missing data (Kong and Cox, 1997). Only the *HLA* was significant at genome-wide significance levels (*IDDM1*; LOD 213), with significance at 6q21/*IDDM15*, resulting from an effect partly due to linkage disequilibrium with *HLA*. Suggestive linkage was found at *CTLA4* (*IDDM7/IDDM12*; LOD 3.28) and *INS* (*IDDM2*; LOD 3.16) and two regions on chromosome 19: 19p13 (*INSR*; LOD 2.84) and 19q13 (LOD 2.54; Concannon et al., 2009). The sample size of this study provided unprecedented power to detect linkage but provided little support for most loci previously implicated in T1D.

### Linkage Studies in Multiple Sclerosis

In contrast to the moderate progress in T1D, attempts to identify MS genes by linkage analysis of families such as affected sib-pair analysis or patient and unaffected parent trios were relatively unsuccessful. For example, Sawcer et al. (1996) studied 282 families using a staged approach but only obtained a maximized LOD score at the *HLA* of 2.8, compared to 8.0 for T1D (Davies et al., 1994). Only one other locus surpassed the statistical threshold for suggestive linkage, 17q22. In none of the other linkage studies did the *HLA* reach a significant maximized LOD score (Ebers et al., 1996; Kuokkanen et al., 1997; Haines et al., 1996; Coraddu et al., 2001;

Broadley et al., 2001; Ban et al., 2002). Ebers et al. (1996) reported linkage to a region just outside the *HLA* ( $\chi^2$  of 10.8 by transmission disequilibrium test), Kuokkanen et al. (1997) reported linkage (maximized LOD 3.6) at 17q22-24 and the Multiple Sclerosis Genetic Group reported suggestive linkage to *HLA-DR* and 7q21-22 (Haines et al., 1996). Several groups applied a modified threshold for suggestive significance (based on reduced marker density) and identified suggestive linkage at 1q31 (Coraddu et al., 2001), 2p13, 4q26-28 and 6q26 (Ban et al., 2002), 10q23 and 11p15 (Coraddu et al., 2001), and Xp11 (Ban et al., 2002).

The issue of why genome-wide linkage studies were relatively successful in T1D but not MS is an important one. Where the effective strength of putative loci could be determined, they were of similar orders between the two diseases. Patient heterogeneity is also an unlikely explanation, because the consolidation of sample sets occurred later for MS than it did for T1D; as a generalization, the MS results reflected samples collected within relatively restricted geographical areas. Similarly, while the power of several of the MS studies was rather low, others used sample sizes comparable to those used in many of the T1D studies. The most likely explanation lies in the relative heritability (the proportion of variation in risk within a community attributable to genetics) of the two diseases: that of T1D is about twice that of MS (Baxter, 1997; Baxter, 2007; Hawkes and Macgregor, 2009), an observation consistent with the identification of several major environmental factors contributing to the risk of MS including geographic latitude, cigarette smoking, and EBV infection.

## Linkage Studies in Systemic Lupus Erythematosus

Following the identification of linkage to distal chromosome 1 in the NZB/NZW and New Zealand mixed (NZM) mouse models of SLE, Tsao et al. (1997) examined seven markers in the syntenic region of human chromosome 1 (1q31-42) in 52 affected sib pairs and confirmed linkage to SLE.

In 1998 two major genome-wide linkage studies of SLE were published (Gaffney et al., 1998; Moser et al., 1998). Analysis of 105 families with at least two affected siblings identified just two loci surpassing the Lander and Kruglyak (1995) thresholds for significant linkage: 6p11-21 (just centromeric to the *HLA*; maximized LOD 3.9) and 16q13 (maximized LOD 3.6; Gaffney et al., 1998). Both these regions were confirmed in a subsequent reanalysis that included an additional 82 sib-pair families (maximized LODs of 4.1 and 3.9, respectively; Gaffney et al., 2000). Two other regions fulfilled the criteria for suggestive linkage: 14q21-23 (maximized LOD 2.8) and 20p12 (maximized LOD 2.6). A peak, albeit nonsignificant, was consistent with a linkage to 1q42, as reported in Tsao et al. (1997).

The other major analysis examined 94 African- and European-American multiplex extended pedigrees identifying four regions of suggestive linkage: 1q23 (maximized LOD 3.5), 13q32 (maximized LOD 2.5), 20q13 (maximized LOD 2.5), 1q31 (maximized LOD 2.0; Moser et al., 1998). Additional suggestive loci were identified only in African-American families—1q41 (maximized LOD 3.5), 11q14-23 (maximized LOD 2.1)—and only in European-American families—14q11 (maximized LOD 2.2), 4p15 (maximized LOD 2.2), 11q25 (maximized LOD 2.2), 2q32 (maximized LOD 2.1), 19q13 (maximized LOD 2.1), 6q26-27 (maximized LOD 2.0), and 12p13-11 (maximized LOD 2.0). No linkage to the *HLA* (6p21) was identified, probably reflecting the reduced power of genetic studies in mixed populations. The 1q23 locus is syntenic with the major murine chromosome 1 locus (Vyse and Kotzin, 1998) and contains the SLE candidate gene *FCGR2A*, and the 1q41 linkage is consistent with that found by both Tsao et al. (1997) and Gaffney et al. (1998).

## TNFR1, TNFR2, LTBR

The 12p13 locus (Moser et al., 1998) contains the genes *TNFR1* (*TNFRSF1A*) and *LTBR* (*TNFRSF3*), which encode TNF receptor 1 and the LT $\beta$  receptor, respectively. Similarly, the location of *TNFR2* (*TNFRSF1B*; encoding the TNF receptor 2) on 1p36 was sufficiently close to minor linkage peaks reported by Gaffney et al. (1998) and Shai et al. (1999) for Tsuchiya et al. (2000) to test and confirm the association of the nonsynonymous coding variant rs1061622 T > G with SLE in Japanese subjects. SLE is associated with serum concentrations of a soluble TNF receptor (s-TNFR; both type I and type II) sufficient to effectively inhibit the bioactivity of TNF (Aderka et al., 1993). Perhaps the clearest evidence of the disease's heterogeneity comes from an open-label trial of TNF blockade in SLE. While the majority of patients expressed increased titers of high-affinity autoantibodies to dsDNA, chromatin, and histones over the period of therapy, seven of nine patients with lupus nephritis experienced the stabilization of serum creatinine and a reduction in proteinuria of >50%, and all five patients with lupus arthritis underwent remission lasting for weeks after treatment was halted (Aringer et al., 2009).

In addition to the association of rs1061622 T>G with SLE, primary biliary cirrhosis (Liu et al., 2012), juvenile idiopathic arthritis (Hinks et al., 2013), MS (IMSGC et al., 2013), and ankylosing spondylitis [International Genetics of Ankylosing Spondylitis Consortium (IGAS) et al., 2013] are associated with different variants in this region.

### **Signal Transducer and Activator of Transcription 4**

A small genome-wide linkage study of RA identified a region on chromosome 2q33 with significant linkage (Amos et al., 2006). A subsequent high-resolution association study confirmed the involvement of this region in RA and implicated the gene *STAT4*, since the five most significantly associated variants all lay within the second intron of *STAT4* (Remmers et al., 2007). Signal Transducer and Activator of Transcription 4 (*STAT4*) is a cytokine-responsive transcription factor that mediates responses to IL-12 and plays a critical role in the differentiation of Th1 cells. The authors also genotyped 1039 SLE patients and 1248 controls at rs7574865 T>G, which lies within the third intron of the *STAT4* gene and was the variant most highly associated with RA. They found that the RA susceptibility rs7574865T (major) allele was also associated with SLE (Remmers et al., 2007). In SLE, this variant was strongly associated with anti-dsDNA AAB, particularly severe nephritis and early onset of disease (Taylor et al., 2008; Chung et al., 2011). The same risk allele also confers susceptibility to primary Sjögren's syndrome (Nordmark et al., 2009) and primary biliary cirrhosis (Liu et al., 2013).

Other variants within *STAT4* are associated with inflammatory bowel disease (Jostins et al., 2012), celiac disease (Trynka et al., 2011), juvenile idiopathic arthritis (Hinks et al., 2013), MS (IMSGC et al., 2013), and systemic sclerosis (Mayes et al., 2014).

### **RACIAL HETEROGENEITY IN SYSTEMIC LUPUS ERYTHEMATOSUS**

Since genome-wide linkage analyses had confirmed the importance of racial heterogeneity in SLE inheritance, Shai et al. (1999) analyzed 80 families with affected sib-pairs (43 Mexican-American families and 37 Caucasian families) and found no significant loci and only a single suggestive locus: 1q44. An analysis of the data by race made it clear that the linkage could be attributed only to the Mexican-American subgroup; there was no support for a gene in this region in the Caucasian families. Lindqvist et al. (2000) analyzed six Icelandic and eleven Swedish pedigrees with multiple affected members. The combined dataset showed significant linkage to 2q37 (LOD score 4.24). This region contains the *INPP5D* gene, which encodes an SH2-containing phosphatase which is recruited through tyrosine phosphorylation of FcgRIIB, CD19, or CD22 after the ligation of the B-cell receptor, and is thought to be important in FcgRIIB-mediated inhibition of B-cell activation. Suggestive linkage was identified at 4p15-13 and 19p13 when the Icelandic subjects were analyzed independently. The 4p15-13 locus is syntenic with the *Lmb2* murine lupus susceptibility gene. In most cases (14/16 markers), aggregating the data from the two countries eliminated any suggestion of linkage. In many subsequent studies, particularly in the genome-wide association studies that followed, experimental power was improved by restricting racial heterogeneity—generally to populations of European descent.

## **GENOME-WIDE ASSOCIATION STUDIES OF AUTOIMMUNITY**

Genome wide *linkage* analyses were limited in resolution by the recombination frequency observable over a few generations and limited in power by the relatively low number of multiplex families available. By the year 2000 a reasonable draft of the human genome sequence was completed, millions of SNP's had been deposited into public databases, and high-throughput technologies were under development for SNP genotyping. It was predicted that *case-control association studies* involving thousands of patients and population-based controls would provide far better resolution and power for the identification of disease-associated genes (Cardon and Bell, 2001). In contrast to linkage studies GWASs can detect alleles with much more modest effects on risk, if those alleles are relatively common and the sample size sufficiently large (Wang et al., 2005). In each region of the genome, preselected variants are chosen that are expected to represent the total genetic variation that is in linkage disequilibrium with the markers. Kruglyak (1999) estimated that in GWAS, linkage disequilibrium was unlikely to extend beyond an average distance of 3 kb in the general population; this implies that a minimum of 500,000 variants would be required for whole genome coverage; in practice, a marker density about twice that is used.

GWASs of autoimmune diseases were facilitated by the development of the ImmunoChip, an Illumina Infinium SNP genotyping microarray that interrogates approximately 200,000 SNPs and approximately 1000

small insertions–deletions putatively associated with at least one of 12 immune-mediated diseases: ankylosing spondylitis, autoimmune thyroid disease, celiac disease, Crohn's disease, juvenile idiopathic arthritis, MS, primary biliary cirrhosis, psoriasis, RA, SLE, T1D, and ulcerative colitis. This array particularly facilitated the direct comparison of risk- and protection-associated alleles between immune-mediated diseases (Parkes et al., 2013). The case/control association statistics from the ImmunoChip consortium have been integrated and curated on the ImmunoBase web-based resource ([www.immunobase.org](http://www.immunobase.org)), which contains complete association summary statistics and facilitates the direct comparison of these data for the ImmunoChip diseases at published genes, markers, and chromosomal regions.

## Genome-Wide Association Studies of Type 1 Diabetes

Smyth et al. (2006) performed a multilocus case–control association study of T1D using >6500 coding, nonsynonymous variants. Although not discussed in the paper, the rationale for studying nonsynonymous variants was the expectation that most alleles affecting common, complex diseases would alter the coding sequence, and therefore the causal variants might be amongst the markers selected (Weiss and Terwilliger, 2000). In an interim analysis of approximately 2000 cases and 1700 control samples, the most significantly associated variant was the previously published and confirmed rs2476601 G>A variant in *PTPN22* (Bottini et al., 2004). The authors referred to the next two most significantly T1D associated variants: one in *CAPSL*, which is adjacent to *IL17R* at 5p13, and the other on chromosome 2q24 in *IFIH1*.

### ***IFIH1***

The most strongly T1D-associated variant at 2q24, rs1990760 T>C, is a nonsynonymous variant in exon 15 of *IFIH1*, which encodes IFN Induced with Helicase C Domain 1 (IFIH1), a pattern recognition receptor for viral dsRNA. The T1D-associated rs1990760T (major) allele encodes an Ala to Thr substitution at position 946. The strength of the association with the rs1990760 T>C variant was increased by genotyping an additional 2471 cases and 4593 controls and by examining an independent collection of 2134 parent–child trios; the combined analysis for the locus reached a  $P = 1.42 \times 10^{-10}$  (Smyth et al., 2006).

The rs1990760T allele is also associated with rheumatoid arthritis (Stahl et al., 2010) and vitiligo (Jin et al., 2010) but is a resistance allele for Crohn's disease (Barrett et al., 2008). Other variants within the *IFIH1* haplotype block are associated with psoriasis (Genetic Analysis of Psoriasis Consortium & the Wellcome Trust Case Control Consortium 2 et al., 2010), inflammatory bowel disease (Jostins et al., 2012), and vitiligo (Jin et al., 2012).

Wellcome Trust Case Control Consortium et al. (2007) published a major genetic milestone: a GWAS of seven complex diseases (bipolar disorder, coronary artery disease, Crohn's disease, RA, T1D, and type 2 diabetes) with about 2000 cases per disease, and a shared group of approximately 3000 controls, typed at 500,568 variants using the Affymetrix GeneChip500k Mapping Array Set. Prior to the genome-wide analysis of the dataset, the authors examined the association at previously identified loci. For T1D, they confirmed association for *HLA*, *CTLA4*, *PTPN22*, *IL2RA*, and *IFIH1*; *INS* could not be tested because a suitable variant was not identified. It is sobering to note that the  $P$  values obtained for these “proof of principal” associations only surpassed genome-wide significance ( $P < 5 \times 10^{-8}$ ) for *HLA* and *PTPN22*. Even more concerning was the finding that, even after raising the significance threshold 500-fold, the only other previously reported genetic region identified was *IL2RA*. The study did, however, identify three new loci significantly associated with T1D: 12q13, 12q24, and 16p13. Two other loci identified by multilocus analysis were reported but were unlikely to be significant after correction for the additional hypotheses tested.

In a follow-up study, the Wellcome Trust Case Control Consortium (WTCCC) (Todd et al., 2007) genotyped an additional 4000 cases and 5000 controls (total 6000 affected, 6200 controls) and 2997 family trios, confirming genome-wide significance for 12q13 (*ERBB3*), 12q24 (gene *SH2B3*), 16p13 (*CLEC16A*), and 18p11 (*PTPN2*). Around the same time, Hakonarson et al. (2007) performed a GWAS on 563 patients and 1146 controls as well as 483 family trios of European ancestry. Significance was determined by  $\chi^2$  and transmission disequilibrium test, respectively, and the analyses combined using Fisher's combined probability test. Results for *HLA*, *PTPN22*, and *INS* surpassed a genome-wide level of significance ( $P < 5 \times 10^{-8}$ ), as well as at three variants associated with *CLEC16A* on 16p13. The latter associations were confirmed in a replication cohort of 939 affected trios. Two other chromosomal regions were identified in the discovery study, 1p31 and 7q21, but could not be validated with the replication cohort.

### **SH2B3**

The variant most strongly associated with T1D at 12q24, rs3184504 T>C, is a nonsynonymous variant that results in an Trp to Arg substitution at position 262 in exon 2 of *SH2B3*, which encodes SH2B Adaptor Protein 3 (SH2B3; previously named the lymphocyte adaptor protein), a negative regulator of lympho- and myelopoiesis as well as of cytokine signaling. *SH2B3* is highly expressed in lymphocytes (Velazquez et al., 2002), and *SH2B3* deletion is associated with acute lymphoblastic leukemia (Roberts et al., 2012). The T1D-associated rs3184504T (major) allele substitutes a Trp into position 262 in the Lymphocyte Adapter Protein (LNK)-family pleckstrin homology domain, resulting in reduced signaling (Li et al., 2000). As a consequence, this allele is associated with the increased proliferation of peripheral blood lymphocytes (Lavrikova et al., 2011), perhaps through its regulation of IL-7 receptor signaling (Cheng et al., 2016). *SH2B3* is also expressed in DC, and the DC of *Sh2b3*<sup>-/-</sup> knockout mice were increased in numbers and hyperresponsive to IL-15 and Granulocyte-Macrophage Colony-Stimulating Factor (GMCSF), expressed increased IL-12R $\beta$  and IFN $\gamma$ , and drove the differentiation of IFN $\gamma$ -producing CD4 T cells (Mori et al., 2014). Remarkably, *Sh2b3*<sup>-/-</sup> knockout DC could drive Th1 differentiation even in the presence of TGF- $\beta$ . In an extraordinary parallel to human T1D, diabetes-susceptible NOD mice, while not bearing a mutant allele of *Sh2b3* (Li et al., 2012), respond paradoxically to TGF- $\beta$ , by decreasing expression of *Sh2b3* instead of increasing it, consistent with the strain's Th1 polarization (Hisanaga-Oishi et al., 2014).

The *SH2B3* rs3184504 C>T variant is also associated with celiac disease (Trynka et al., 2011), juvenile idiopathic arthritis (Hinks et al., 2013), primary sclerosing cholangitis (Liu et al., 2013), and vitiligo (Jin et al., 2012). Other markers within the *SH2B3* haplotype block are associated with primary biliary cirrhosis (Liu et al., 2012), RA (Okada et al., 2014), and ankylosing spondylitis (IGAS et al., 2013).

### **ERBB3**

The most significantly T1D-associated variant in the 12q13 region, rs2292239 T>G, lies within an intron of *ERBB3*, in a major haplotype block that includes *SILV*, *SUOX*, *IKZF4*, *RPS26*, and *RPL41*; it is a quantitative trait locus (eQTL; i.e., its alleles are associated with differences in expression of protein coding genes) for *SUOX* and *RPS26* (Wang et al., 2010). *ERBB3* encodes the Erb-B2 Receptor Tyrosine Kinase 3 (ERBB3), a member of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases. Although it lacks a kinase domain, it can signal by forming heterodimers with kinase-active members of the EGFR family. The rs2292239 G>T variant is also associated with alopecia (Betz et al., 2015).

### **CLEC16A**

The most significantly T1D-associated variant at 16p13, rs12708716 A>G, lies within an intron of *CLEC16A*, which encodes C-type Lectin Domain Containing 16A (CLEC16A), a membrane-associated endosomal protein that interacts with the E3 ubiquitin ligase NRDPL. The rs12708716 A>G variant acts as an eQTL affecting the levels of expression of *CLEC16A* in human pancreatic  $\beta$  cells (Soleimanpour et al., 2014), *DEXI* and *SOCS1* in thymus, *DEXI* in monocytes and lymphoblastoid cell lines, and *CLEC16A* and *SOCS1* in CD4 T cells (Leikfoss et al., 2015). Individuals expressing the T1D-associated rs12708716A (major) allele have reduced islet *CLEC16A* expression and reduced insulin secretion (Soleimanpour et al., 2014). The proposed mechanism of action in beta cells is that reduced CLEC16A increases the expression of the NRDPL target PARKIN, which is a regulator of mitophagy (selective degradation of mitochondria by autophagy), causing a reduction in cellular adenosine triphosphate (ATP), which is required for insulin secretion (Soleimanpour et al., 2014). Remarkably, *PDX1*, a gene associated with both type 2 diabetes and monogenic diabetes of the young, regulates *CLEC16A*, and its reduced expression in these diseases recapitulates the mitophagy in  $\beta$  cells seen in T1D (Soleimanpour et al., 2015).

The rs12708716 A>G variant is also associated with susceptibility to MS (IMSGC, 2009; Hoppenbrouwers et al., 2009). Other variants within *CLEC16A* have been associated with primary biliary cirrhosis (Liu et al., 2012) and celiac disease (Dubois et al., 2010).

### **PTPN2**

The most significantly T1D-associated variant at 18p11, rs1893217 A>G, lies within an intron of *PTPN2*, which encodes Protein Tyrosine Phosphatase, Non-Receptor Type 2 (PTPN2). Upon stimulation by IFN $\gamma$  and other inflammatory cytokines, PTPN2 acts to reverse cytokine-induced STAT1 and Signal Transducer and Activator of Transcription 3 (STAT3) phosphorylation, inhibiting cytokine-induced, BAX-mediated apoptosis of pancreatic beta cells (Moore et al., 2009; Santin et al., 2011). Its effect on STAT3 also affects beta-cell endocrine function; *Ptpn2*<sup>-/-</sup> mice fed a high-fat diet have reduced glucose-stimulated insulin secretion and impaired

glucose tolerance (Xi et al., 2015). Within T cells, PTPN2 dephosphorylates and inactivates Src family kinases to regulate T-cell responses. Mice bearing a T-cell specific deficiency develop widespread inflammation and autoimmunity that was transferable with CD8 T cells (Wiede et al., 2011).

Having said this, it is not clear that this locus does affect PTPN2 function or expression; the rs1893217 A > G variant has not been found to be associated with differences in PTPN2 expression in genome-wide screens (GTEx Portal on 06/01/18), although Long et al. (2011) report that the rs1893217G T1D risk allele is associated with reduced PTPN2 expression and decreased phosphorylation of STAT5 in response to IL-2 receptor binding.

The rs1893217 A > G variant is also associated with celiac disease (Dubois et al., 2010) and Crohn's disease (Franke et al., 2010), and other variants in the same region are associated with juvenile idiopathic arthritis (Hinks et al., 2013) and RA (Okada et al., 2014).

In 2009 Hakonarson's group (Grant et al., 2009) reviewed their data published in 2007 (Hakonarson et al., 2007), selecting 982 markers with  $P < .5$  in both discovery cohorts for analysis in 636 independent nuclear families with 974 affected offspring. Six variants were selected that had reached the same threshold in, not only these three datasets but also, the WTCCC dataset (Todd et al., 2007). These markers were then tested by case-control association in a further independent sample of 1303 patients and 1673 controls, with two reaching significance in that validation study and surpassing a genome-wide significance in a meta-analysis that combined all five cohorts of samples: *BACH2* at 6q15 and *UBASH3A* at 21p22.

## BACH2

The most strongly T1D-associated variant at 6q15, rs597325 G > A, lies within an intron of *BACH2*, which encodes BTB Domain and CNC Homolog 2 (*BACH2*), a basic leucine zipper transcription factor that regulates the expression of *IL2*, *BCL2*, and *XBP1*. It acts as a repressor of *XBP1*, which itself encodes a transcription factor regulating a wide range of targets, including *CCL2*, *CXCL8*, *IL-6*, multiple heat shock proteins, and *NOS2* (TRRUST2; Han et al., 2017), acting to repress the differentiation of multiple effector linages of CD4 T cells (Roychoudhuri et al., 2013). *Bach2*<sup>-/-</sup> mice show a severe reduction in the numbers of FoxP3-expressing Treg cells and develop a progressive wasting disease associated with extensive pulmonary infiltration by macrophages and GATA3-expressing CD4 T cells. Expression of IL-13 and IL-4 is greatly increased with a little change in numbers of T-bet and IFN- $\gamma$  or IL-17 positive cells (Roychoudhuri et al., 2013; Kim et al., 2014). In the absence of *BACH2*, Tregs express reduced levels of FoxP3 and Bcl-2 (Kim et al., 2014), and in vitro, *BACH2* is required for the induction of FoxP3 and differentiation of induced Tregs (Kim et al., 2014). *BACH2* also appears to play a role in pancreatic beta cells, as INS-1E rat insulinoma cells transfected with *BACH2* siRNA to reduce its expression also decreased the expression of *BCL2* and *BCLXL* and increased Bcl-2 Interacting Mediator Of Cell Death (BIM) phosphorylation, exacerbating cytokine-induced apoptosis (Marroquí et al., 2014). Remarkably, *BACH2* inhibition also significantly diminished cytokine-induced *PTPN2* expression in beta-cell lines and dispersed beta cells (Marroquí et al., 2014).

Other variants within the same region have been associated with MS (IMSGC et al., 2013), celiac disease (Dubois et al., 2010), primary sclerosing cholangitis (Liu et al., 2013), Crohn's disease (Franke et al., 2010), and autoimmune thyroid disease (Cooper et al., 2012).

A combined meta-analysis incorporating cases from the WTCCC studies (Todd et al., 2007) and a combined British and US GWAS (Cooper et al., 2008) examined a total of 7514 case and 9045 reference samples (Barrett et al., 2009). By performing a meta-analysis of all three scans, they confirmed, at a genome-wide level of significance ( $P < 5 \times 10^{-8}$ ), association with 12 previously identified regions: 1p13 (gene of interest *PTPN22*), 2q24 (*IFIH1*), 2q33 (*CTLA4*), 4q27 (*IL2*), 6p21 (*HLA*), 10p15 (*IL2RA*), 11p15 (*INS*), 12q13 (*ERBB3*), 12q24 (*SH2B3*), 16p13 (*CLEC16A*), 18p11 (*PTPN2*), and 21p22 (*UBASH3A*) and obtained genome-wide significance for 18 other loci: 1q32 (*IL10*), 4p15, 6q22 (*C6orf173*), 7p15, 7q12 (*COBL*), 9p24 (*GLIS3*), 10q23 (*C10orf59*), 12p13 (*CD69*), 14q24, 14q32, 16p11(*IL27*), 16q23, 17q12 (*ORMDL3*), 17q21, 19q13, 20p13, 22q12, and Xq28.

## IL2

The chromosomal region 4q27 is of particular interest, because it is syntenic with the NOD mouse diabetes susceptibility gene *Idd3* (Denny et al., 1997). Within it, the variant most strongly associated with T1D is rs4505848 A > G, which lies within an intron of the gene *KIAA1109*, about 200 kb 3' of the *IL2* and *IL21* cytokine genes.

Variants within this haplotype block are associated with inflammatory bowel disease (Jostins et al., 2012), juvenile idiopathic arthritis (Hinks et al., 2013), alopecia areata (Petukhova et al., 2010), celiac disease (Dubois et al., 2010), and primary sclerosing cholangitis (Liu et al., 2013).

## **IL10**

The most significantly associated variant at 1q32, rs3024505 G > A, lies about 1 kb 3' of *IL10*, which encodes IL-10, an immunoregulatory cytokine produced by leukocytes, especially monocytes. IL-10 has pleiotropic effects on adaptive and innate immunity, suppressing cell-mediated immunity, while supporting B-cell survival, proliferation, and antibody production (Moore et al., 2001). Its ability to inhibit the production of inflammatory cytokines and chemokines, including IL-1 and TNF, and suppress the expression of MHC class II antigens and the costimulators CD80 (B7) and CD86 (B7.2), contribute to its antiinflammatory activities.

The T1D-associated rs3024505 C > T variant is also associated with SLE (Gateva et al., 2009), inflammatory bowel disease (Jostins et al., 2012), ulcerative colitis (Anderson et al., 2011), and Crohn's disease (Franke et al., 2010). While T1D is associated with the rs3024505C (major) allele, SLE, ulcerative colitis, and Crohn's disease are associated with the T allele.

## **CD69**

The most strongly associated variant at 12p13, rs4763879 G > A, lies within *CD69*'s first intron, which contains a *cis*-regulatory element (Vazquez et al., 2012). *CD69* is a member of the calcium-dependent lectin superfamily of type II transmembrane receptors that is induced on T cells when activated and mediates their costimulation. Significantly, *CD69* suppresses Sphingosine-1-Phosphate Receptor-1 (S1P1) function (Bankovich et al., 2010). S1P1 plays a critical role in lymphocyte recirculation; its pharmaceutical downmodulation by the agonist fingolimod sequesters lymphocytes in lymph nodes, inhibiting relapses of MS. MS is also associated with a variant in the 3' UTR of *CD69* (IMSGC et al., 2013).

Bradfield et al. (2011) extended the meta-analysis approach to combine their data with all publicly available genome-wide T1D and control data sets. The discovery cohort consisted of 6523 cases and 6648 controls genotyped by Illumina BeadChip and 3411 cases and 10,308 controls genotyped on Affymetrix arrays. Imputed data produced from each platform were analyzed separately and the *P*-values, betas (natural log of the OR) and standard errors at each locus for the two platforms combined using inverse-variance meta-analysis. This study had more than twice the power of Barrett's 2009 analysis to identify variants with a relative risk (RR) of 1.2 and approximately three times the power to identify those with a RR of 1.1. Of the 45 known T1D-associated autosomal variants, Bradfield et al. (2011) reached the threshold for genome-wide significance ( $P < 5 \times 10^{-8}$ ) for 11: *PTPN22*, *IL10*, *IFIH1*, *CTLA4*, *HLA*, *BACH2*, *GLIS3*, *IL2RA*, 12q13, *SH2B3*, and 16p13.

Excluding the known T1D-associated loci, an additional 52 independent variants with meta-analysis  $P < 1 \times 10^{-5}$  were tested in an independent replication cohort consisting of 1120 T1D-affected trios genotyped by Sequenom iPLEX and analyzed by the transmission disequilibrium test. For these loci the meta-analysis and the replication were combined using Fisher's combined probability test. Three additional loci surpassed a  $P < .05$  significance threshold in the replication set as well as met the significance threshold for genome-wide significance in the combined analysis: 13q22 (gene of interest *LMO7*), 2p23 (*EFR3B*), and 6q27.

## **Genome-Wide Association Studies of Multiple Sclerosis**

GWAS of MS soon followed those of T1D, and many studies were coordinated through the large collaborative network, the International MS Genetics Consortium (IMSGC). The first of these was published in 2007 (IMSGC et al., 2007); the discovery phase of the study involved 931 trios and interrogation of 334,923 variants. A total of 110 variants were then selected for a follow-up in a replication phase consisting of 2322 MS cases, 609 parent-case trios, 789 controls, and additional genotype data from two external control datasets, resulting in a combined analysis of 12,360 subjects. In addition to the *HLA*, two other regions were found to be strongly associated with MS risk: 5p13 (*IL7R*) and 10p15 (*IL2RA*).

## **IL7R**

The most significantly MS-associated variant in the 5p13 region, rs6897932 G > A/G > C, lies within exon 6 of *IL7R*, which encodes the IL 7 Receptor (*IL7R*). This receptor signals through CD132 (*IL2RG*), which is a common gamma chain shared by the receptors of various cytokines. It plays a critical role in V(D)J recombination, and null mutations cause a severe combined immunodeficiency. *IL7R* is alternately spliced and this variant affects gene expression; the MS-associated rs6897932G (major) allele encodes the amino acid Thr rather than Ile at amino acid position 244, disrupting an exonic splicing silencer, resulting in a twofold increase in the skipping of exon 6,

and a relative increase in the proportions of soluble, as distinct from membrane-bound, isoforms of the protein (Gregory et al., 2007).

Polymorphisms within *IL7R* are also associated with ulcerative colitis (Anderson et al., 2011), primary biliary cirrhosis (Mells et al., 2011), and T1D (Onengut-Gumuscu et al., 2015).

### **IL2RA**

The most strongly MS-associated variant at 10p15, rs2104286 A > G, lies in the first intron of *IL2RA*, while that most associated with T1D (rs12251307 C > T) lies about 20 kb upstream. Maier et al. (2009a) performed a careful association analysis of *IL2RA* alleles in a DNA collection of 9407 healthy controls, 2420 MS, and 6425 T1D subjects as well as 1303 MS parent/child trios. They observed significant complexity at this locus: the rs41295061C allele was associated with T1D but not MS; the rs11594656T allele was associated with susceptibility to T1D but protection from MS, and the rs2104286T allele conferred susceptibility to both diseases.

The presence of MS risk alleles at rs2104286 A > G and rs12722489 C > T were associated with increased soluble (s) IL2RA, explaining 15%–18% of variance in sIL2RA levels, and sIL2RA levels were particularly increased in very severe MS (Maier et al., 2009b). In contrast the T1D-associated alleles correlated with decreased sIL2RA expression (Lowe et al., 2007). Although sIL2RA inhibits IL-2 mediated signaling, it promotes T-cell activation and proliferation, presumably by reducing activation-induced cell death.

This region is also associated with Crohn's disease (Franke et al., 2010), primary sclerosing cholangitis (Liu et al., 2013), juvenile idiopathic arthritis (Hinks et al., 2013), RA (Stahl et al., 2010), and inflammatory bowel disease (Jostins et al., 2012).

Although not significant, moderate associations were also reported for an additional 13 variants, including those located at 1p13 (*CD58*), 1p22 (*EVI5*), and 16p13 (*CLEC16A*). It was not until replication studies were performed in independent cohorts of MS cases and controls that these genes could be confirmed as MS risk-associated variants (Weber et al., 2008; Hoppenbrouwers et al., 2008; Rubio et al., 2008).

### **CD58**

The most significantly MS-associated variant at 1p13, rs2300747 A > G, lies within the first intron of *CD58*, which encodes the costimulatory molecule LFA-3. The MS-protective rs2300747G (minor) allele is associated with a dose-dependent increase in *CD58* mRNA in lymphoblastic cell lines and peripheral blood mononuclear cell (PBMC) from MS subjects (De Jager et al., 2009a). The hypothesis that increased LFA-3 expression on circulating mononuclear cells is protective is supported by the finding that *CD58* mRNA expression is increased in MS subjects during clinical remission. One proposed mechanism of action is via the engagement of CD2 by LFA-3 upregulating the expression of transcription factor FoxP3, leading to the enhanced function of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells (De Jager et al., 2009b). This locus has not been significantly associated with other autoimmune diseases.

In 2009 a consortium from Australia and New Zealand performed an MS case/control GWAS involving 1618 MS cases in the initial discovery phase, and an independent cohort of 2256 MS cases and 2310 healthy controls in the replication phase [Australia and New Zealand Multiple Sclerosis Genetics Consortium (ANZgene), 2009]. This study confirmed the *HLA* at a genome-wide level of significance and identified one novel locus, at 12q13-14 (*CYP27B1*). It also reported evidence of association at 20q13, as well as at the previously identified loci *CD58*, *IL2RA*, and *IL7R* [Australia and New Zealand Multiple Sclerosis Genetics Consortium (ANZgene), 2009].

### **CYP27B1**

The most strongly MS-associated variant at 12q13-14, rs703842 A > G, lies within the 3' untranslated region of *METTL1*, about a 2 kb upstream of *CYP27B1*, which encodes the enzyme 25-hydroxy-vitamin D-1 alpha hydroxylase, an enzyme involved in vitamin D metabolism (Bailey et al., 2007). The variant acts as an eQTL for both *METTL1* and *CYP27B1* (GTEx Portal on 23/01/18). Vitamin D deficiency is a major risk factor for MS, and vitamin D plays an important role in immune function, including the regulation of expression of the MS-associated *HLA-DR15* (Ramagopalan et al., 2009). Other genes in this region include *CDK4*, also previously shown to play a role in immune function and autoimmune disease, and *AGAP2*, which participates in the prevention of neuronal apoptosis by enhancing PI3 kinase activity. *CYP27B1* has not been significantly associated with other autoimmune diseases.

### **CD40**

The most strongly MS-associated variant at 20q13, rs6074022 C > T, lies about 10 kb 5' of *CD40*, which encodes Tumor Necrosis Factor Receptor Superfamily Member 5 (CD40), a costimulatory protein found on antigen

presenting cells (APC). Its ligation by CD154 (CD40L) on CD4 T cells is required for the activation of APC and the subsequent initiation of cellular and humoral immunity; its deficiency can cause hyper-IgM syndrome type 3. The variant rs1883232 A > G is located at -1bp from the start of translation within the Kozak consensus sequence of *CD40*, and the MS resistance allele, rs1883232G, is associated with increased expression as well as susceptibility to RA (Eyre et al., 2012), Graves' disease and Crohn's disease (Jostins et al., 2012), while the MS susceptibility allele, rs1883232A, leads to lower *CD40* expression (Blanco-Kelly et al., 2010).

In the same year, De Jager et al. (2009a) published a meta-analysis that combined 895 subjects with MS from the IMSGC's original association study (IMSGC et al., 2007), 969 subjects from the GeneMSA consortium (Baranzini et al., 2009) and an additional unpublished set of 860 subjects, totaling 2624 cases and 7220 controls. This study confirmed, at a genome-wide level of significance, the *HLA* and *CD58*. It also identified chromosomal regions at 12p13 (gene of interest *TNFRSF1A*), 16q24 (*IRF8*), and 11q12 (*CD6*) as significantly associated. An additional region at 16p13 (*CLEC16A*) had been commented on in several previous underpowered studies and was of particular interest, because it had recently been identified as associated with T1D (Todd et al., 2007; see above). De Jager et al. (2009a) listed the locus as replicated with a  $P = 1.77 \times 10^{-7}$ , as well as *IL2RA* and *IL7R* with similar significance values.

### **TNFRSF1A**

The most strongly MS-associated variant at 12p13, rs1800693 T > C, lies at the splice junction at the 3' end of the sixth exon of *TNFRSF1A*, which encodes TNF receptor superfamily member 1A (TNFRSF1A). The risk allele, rs1800693C, generates a soluble form of TNFR1 that can block TNF signaling (Gregory et al., 2012), mimicking the effect of TNF-blocking drugs, which exacerbate MS (The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group, 1999). This is a common variant with modest effect (OR 1.2; De Jager et al., 2009a). In contrast the T allele, at rs4149584 C > G/C > T, is a nonsynonymous coding polymorphism of low frequency but with stronger effect (OR 1.6).

The MS-associated rs1800693C allele also confers susceptibility to primary biliary cirrhosis (Mells et al., 2011; Liu et al., 2012), and another variant in *TNFRSF1A* is associated with ankylosing spondylitis (IGAS et al., 2013); the region showed mild linkage to SLE (Moser et al., 1998).

### **IRF8**

The most significantly MS-associated variant at 16q24, rs17445836 G > A, lies approximately 60 kb 3' of *IRF8*, which encodes the transcription factor Interferon Regulatory Factor 8 (IRF8). IRF8 controls the expression of a wide range of immunologically related genes, including *IL10*, *IL12A*, *IL1B*, and *TLR3* and *TLR4* (TRRUST2; Han et al., 2017). Administration of exogenous IFN $\gamma$  exacerbates MS; *IRF8* is upregulated by IFN $\gamma$  and cooperatively enhances the IFN $\gamma$ -induced apoptosis of oligodendroglial progenitor cells (Horiuchi et al., 2011). *IRF8* is a key target of vitamin D, through binding the nuclear vitamin D receptor (Ramagopalan et al., 2010).

Variants in this region are associated with Sjögren's syndrome (Lessard et al., 2013), RA (Eyre et al., 2012), inflammatory bowel disease (Jostins et al., 2012), and primary biliary cirrhosis (Liu et al., 2012).

### **CD6**

The most significantly MS-associated variant at 11q12, rs17824933 C > A/C > G, lies in the first intron of *CD6*. *CD6* is a type 1 transmembrane glycoprotein found on T lymphocytes in association with the signaling components of the TCR. It acts as a costimulator of T-cell proliferation via its ligand, Activated Leukocyte Cell Adhesion Molecule (ALCAM; Zimmerman et al., 2006). The MS susceptibility (minor) allele, rs17824933G, is associated with a decreased expression of full-length *CD6* in T cells and an increase in a splice variant lacking exon 5, resulting in diminished proliferation during long-term activation of CD4 $^{+}$  T cells (Kofler et al., 2011). Nevertheless, the role of *CD6* in MS is not clear, as it can attenuate T-cell activation, possibly via the regulation of *CD5* tyrosine phosphorylation (Oliveira et al., 2012), and appears to play a role in leukocyte trafficking across the blood–brain barrier and into the CNS via its interactions with ALCAM on the endothelium transmigratory cups (Cayrol et al., 2008).

Variants in this region are also associated with inflammatory bowel disease (Jostins et al., 2012).

In 2011 a very large collaborative GWAS for MS was published by IMSGC et al. (2011). This study involved 22 collections of patient samples and controls from national blood services and other sources; 9772 MS samples and 17,376 control samples from throughout Europe, America, and Oceania were genotyped. A total of 57 genetic loci associated with MS in addition to the *HLA* were identified at a genome-wide level of significance, of which 28 had not been previously reported. Previously identified genes included *MMEL1*, *EVI5*, *CD58*, *IRF8*, *RGS1*,

*C1orf106, CBLB, TMEM39A, IL12A, IL7R, PTGER4, OLIG3, IL7, IL2RA, ZMIZ1, CD6, TNFRSF1A, CYP27B1, MPHOSPH9, CLEC16A, IRF8, STAT3, TYK2, and CD40.*

The 28 novel independent regions identified at a genome-wide level of significance were 1p21 (gene of interest *VCAM1*), 2p21, 2p13 (*PLEK*), 2q13 (*MERTK*), 2q37 (*SP140*), 3p24 (*EOMES*), 3q13 (*CD86*), 5q33 (*IL12B*), 6q15 (*BACH2*), 6q22 (*THEMIS*), 6q23 (*MYB*), 6q23.3 (*IL22RA2*), 6q25 (*TAGAP*), 7q36 (*ZNF746*), 8q24 (*MYC*), 8q24.2 (*PVT1*), 10q23 (*HHEX*), 12p13 (*CLECL1*), 14q24 (*ZFP36L1*), 14q24.3 (*BATF*), 14q31 (*GALC*), 18q21 (*MALT1*), 19p13 (*TNFSF14*), 19p13.11 (*MPV17L2*), 19q13 (*DKKL1*), 20q13 (*CYP24A1*), 22q11 (*MAPK1*), and 22q13 (*SCO2*).

Once again, outside of the *HLA*, all associations showed only modest ORs, between 1.1 and 1.3. The many of associated variants were located either near or within immune-system related genes, and a large proportion of the regions implicated had previously been associated with other autoimmune diseases.

## CD86

The most significantly MS-associated variant at 3q13, rs9282641 G > A, lies within the 5' UTR of *CD86*, which encodes CD86 (B7-2), a membrane receptor expressed by APC, that is the ligand for the T-cell costimulator CD28 and the immune checkpoint molecule CTLA4. *CD86* is expressed on macrophages within the brain lesions of MS patients (Windhagen et al., 1995), and inhibition with a CTLA4-Fc fusion protein prevented the onset of experimental autoimmune encephalomyelitis (EAE; an animal model of CNS autoimmunity) in the great majority of mice treated (Cross et al., 1995).

No other autoimmune diseases have been associated with polymorphisms in this gene to date.

## EOMES

The most significantly associated variant at 3p24, rs11129295 C > T, is approximately 24 kb 5' of *EOMES*, which is a member of the TBR1 (T-box Brain Protein 1) subfamily of T-box genes that encodes a transcription factor positively regulating the expression of IFN- $\gamma$  (TRRUST2; Han et al., 2017). The lymphocytes from MS patients produce elevated levels of IFN- $\gamma$  ex vivo (Hirsch, Panitch and Johnson, 1985) and treatment of MS patients with IFN $\gamma$  exacerbates disease (Panitch et al., 1987). *EOMES*-expressing CD4 T cells are increased in numbers in the peripheral blood and cerebrospinal fluid of patients with SPMS and T-cell specific gene deletion of *Eomes* in mice attenuates the severity of EAE (Raveney et al., 2015).

Another variant, 4 kb 5' of *EOMES*, rs3806624 G > A, is associated with RA (Okada et al., 2014).

## IL12B

The most significantly MS-associated variant at 5q33, rs2546890 A > G, lies 2.4 kb 5' of *IL12B*, in the second exon of an uncharacterized open reading frame (*LOC285626*). *IL12B* encodes the 40 kDa subunit of IL-12 and IL-23, cytokines have been strongly implicated in the pathogenesis of both MS and EAE (Langrish et al., 2005). The gene encoding the 35-kDa subunit of IL-12 (*IL12A* on 3q25) has also been implicated in MS by GWAS (IMSGC, 2010). IL-12 is expressed by activated DC, macrophages, and monocytes, and sustains IFN $\gamma$  production, which is essential for the differentiation of cytotoxic CD8 T cells and generation and maintenance of memory/effector Th1 cells. In contrast, IL-23 supports the differentiation of CD4 Th17 cells that produce IL-17, IL-17F, IL-6, and TNF (Thakker et al., 2007). Nevertheless, treatment of MS with ustekinumab, a neutralizing anti-IL-12 p40 mAb, did not reduce the gadolinium-enhancing T1-weighted lesions by magnetic resonance imaging (MRI; Segal et al., 2008).

Other variants in the region are associated with Crohn's disease (Franke et al., 2010), ulcerative colitis (Anderson et al., 2011), and ankylosing spondylitis (IGAS et al., 2013).

## CD5

The most strongly MS-associated variant at 11q12, rs650258 T > C, lies within the uncharacterized open reading frame *LOC105369325*, approximately 100 kb upstream of *CD5*, which encodes the type-I transmembrane glycoprotein CD5. The expression of CD5 is normally restricted to T cells and a subset of B (termed B-1) cells, on which it acts to mitigate antigen receptor signaling. Consistent with its role in downmodulating T-cell responses, in mice, targeted the deletion of *Cd5*, or its blockade with CD5-Fc fusion protein, decreased the severity of EAE by inducing activation-induced cell death in T cells (Axtell et al., 2004, 2006). The expression of CD5 on the B lymphocytes of patients with MS correlated positively with severity of disease activity as measured by the number of gadolinium-enhancing MRI lesions and inversely with disease duration (Seidi, Semra and Sharief, 2002). Increased expression is associated with increased risk that a patient presenting with their first demyelinating event (clinically isolated syndrome) will progress to MS (Villar et al., 2011).

Other variants in this region have been associated with RA (Eyre et al., 2012) and inflammatory bowel disease (Jostins et al., 2012).

### **Signal Transducer and Activator of Transcription 3**

The most significant MS-associated variant at 17q21, rs9891119 A > C, lies within an intron of, and is an eQTL for, *STAT3* (GTEx Portal on 11/01/18), which encodes STAT3. STAT3 is activated by phosphorylation in response to receptor ligation by cytokines such as IL-5, IL-6, and the IFNs and then translocates to the nucleus where it induces the transcription of a wide range of targets, including BCL-2-family members, chemokines, growth factors, cytokines, and other transcription factors. Lee et al. (2017) examined the cytokine dependencies of the generation of pathogenic T cells that could successfully transfer EAE in mice. They found that the induction of pathogenic Th1/Th2 CD4 T cells required IL-23 signaling mediated by a STAT3/STAT4 heterodimer. Mice with a targeted deletion of *Stat3* in CD4 T cells were completely resistant to EAE (Liu et al., 2008). CD4 memory T cells from MS patients had significantly higher levels of p-STAT3 and p-STAT4, and p-STAT3/p-STAT4 heterodimers were observed upon IL-23 signaling (Lee et al., 2017). Phospho-STAT3 was increased in CD4 and CD8 T cells and monocytes of patients in relapse, its expression strongly correlated with severity of disease (Frisullo et al., 2006), and it predicted the conversion of clinically isolated syndrome to MS (Frisullo et al., 2008). 1,25-Dihydroxyvitamin-D3 inhibits the IL-12-induced tyrosine phosphorylation of STAT3 and STAT4, resulting in decreased T-cell proliferation and, in mice, the inhibition of EAE (Muthian et al., 2006).

Other variants at 17q21 are associated with Crohn's disease (Franke et al., 2010), psoriasis (Tsoi et al., 2012), and inflammatory bowel disease (Jostins et al., 2012).

In 2013 the IMSGC used the ImmunoChip (i.e., limited to genomic regions of "immunological interest"—primarily loci implicated in autoimmunity) to genotype 14,498 subjects with MS and 24,091 healthy controls for the discovery phase and applied previous GWAS data from independent cohorts of 14,802 subjects with MS and 26,703 healthy controls for replication (IMSGC et al., 2013). They identified 48 new MS-associated susceptibility variants at a genome-wide level of significance ( $P < 5 \times 10^{-8}$ ), mapping to a total of 103 discrete loci outside the MHC. These new regions included 1p36 (gene of interest *PLEKHG5*), 1p22 (*BCL10* and *DDAH1*), 1p12 (*PHGDH*), 1q23 (*FCRL1* and *SLAMF7*), 2p23 (*CENPO*), 2p16 (*FLJ16341*), 2q32 (*STAT4*), 3p24, 3p22 (*CCR4*), 3p13 (*FOXP1*), 4q24 (*TET2*), 5q31 (*TCF7*), 5q31 (*NDFIP1*), 5q35 (*RGS14*), 6p23, 6p21 (*PXT1*), 7p22 (*CARD11*), 7p15 (*JAZF1*), 7p14 (*ELMO1*), 7p12 (*IKZF1*), 8q24, 10p11, 10q22 (*C10orf55*), 11p11 (*AGBL2*), 11q13 (*PRDX5*), 11q23 (*CXCR5* and *TREH*), 12p13 (*LTRB*), 13q32 (*MIR548AN*), 14q32 (*TRAF3*), 15q25 (*CTSH*), 15q26 (*IQGAP1*), 16p13 (*RMI2* and *CLEC16A*), 16p11 (*MAPK3*), 16q22 (*CDH3*), 16q23 (*WWOX* and *MAF*), 17q12 (*IKZF3*), 17q21 (*NPEPPS*), 19p13 (*SLC44A2* and *EPS15L1*), and 20q13 (*SLC9A8* and *SLC2A4RG*).

### **Signal Transducer and Activator of Transcription 4**

The most significantly MS-associated variant in the 2q32 region, rs9967792 T > C, lies within the third intron of *STAT4*, which encodes STAT4. This STAT-family member is activated through phosphorylation in response to receptor ligation by IL-12 and activation of the receptor-associated kinases JAK2 and TYK2; it is then translocated to the cell nucleus where it acts as a transcription factor for several genes involved in Th1 responses, including *IFNG*, *IL2RA*, *TBX21*, *PRF1* (TRRUST2; Han et al., 2017). The phosphorylation and activation of STAT4 in response to IL-12 is inhibited by TGF-β, which prevents the phosphorylation of JAK2 and TYK2 (Bright and Sriram, 1998).

Other variants in the same region have been associated with SLE (Armstrong et al., 2014), Sjögren's syndrome (Lessard et al., 2013), RA (Eyre et al., 2012), systemic sclerosis (Mayes et al., 2014), juvenile idiopathic arthritis (Hinks et al., 2013), primary biliary cirrhosis (Liu et al., 2012), celiac disease (Trynka et al., 2011), and inflammatory bowel disease (Jostins et al., 2012).

### **C-C Motif Chemokine Receptor 4**

The most significantly associated variant at 3p22, rs4679081 T > C, is located within an intron of an uncharacterized open reading frame, LOC105377022, approximately 6 kb 3' of *CCR4*. *CCR4* encodes the G-protein-coupled receptor C-C Motif Chemokine Receptor 4 (CCR4), which is a receptor for MIP-1, RANTES, CCL17, CCL22, and MCP-1. In a comparison of T cells from CSF and peripheral blood at the onset of MS relapse, *CCR4* expression was lower on the T cells from CSF (Misu et al., 2001). In C57BL/6 mice, targeted gene deletion of *Ccr4*, or treatment with a CCR4 antagonist starting before the induction of disease, resulted in reduced severity of EAE (Moriguchi et al., 2013, 2016).

Another variant in the same region, rs13314993 A > C, is associated with celiac disease (Dubois et al., 2010).

## Genome-Wide Association Studies of Systemic Lupus Erythematosus

In February 2008, three GWAS of SLE were published. The first was performed under the auspices of the International Consortium for Systemic Lupus Erythematosus Genetics et al. (2008). It was a phased study with an initial genome-wide analysis of 720 female SLE patients of European ancestry and 2337 controls, with the most significantly associated variants typed in an additional 1846 patients. This study confirmed, at a genome-wide level of significance, both the *HLA* and *IRF5*. It also found significant association at four other regions: 16p11 (gene of interest *ITGAM*), 11p15.5 (*PHRF1/IRF7*), 3p14.3 (*PXK*), and 1q25.1.

### **Integrin Alpha M**

The most strongly SLE-associated variant in the 16p11 region, rs9888739 C > T, lies within the 14th intron of *ITGAM*, which encodes the Integrin Alpha M (ITGAM) chain. Together with the beta 2 chain (ITGB2), ITGAM forms the leukocyte-specific integrin CD11b (MAC1, or Inactivated Complement Receptor 3). CD11b is important in the phagocytosis of complement- and antibody-associated aggregates, as well as the adherence and emigration from the bloodstream of neutrophils and monocytes via interactions with a diverse range of ligands, including ICAM-1 and ICAM-2, C3bi, and fibrinogen. In a replication study, the association of SLE with *ITGAM* was confirmed, but a variant with stronger association was identified, rs1143679 G > A, which encodes an Arg to His amino acid substitution at position 77 in the beta-propeller domain of CD11b, with subsequent consequences for MAC1 ligand binding (Nath et al., 2008). In a genome-wide association study of patients bearing anti-dsDNA in SLE, *ITGAM* together with the *HLA*, *STAT4*, and *IRF5*, was found to be significantly associated (Chung et al., 2011).

Another variant in the region, rs57348955 G > A, is associated with autoimmune thyroid disease (Cooper et al., 2012).

### **Plant Homeodomain (PHD) and Ring Finger Domains 1/ Interferon Regulatory Factor 7**

The most strongly SLE-associated variant at 11p15, rs4963128 T > C, lies within the fourth intron of and is an eQTL for, *PHRF1* (GTEx Portal on 11/01/18), which encodes Plant Homeodomain (PHD) and Ring Finger Domains 1 (PHRF1), a ubiquitin ligase that targets TGFB Induced Factor (TGIF) for degradation, promoting TGF-β's cytostatic effect (Ettahar et al., 2013). The 3' end of *PHRF1* almost immediately abuts the 3' end of the IFN response gene *IRF7*, which is in the reverse orientation. In a subsequent study (Bentham et al., 2015), the most significantly SLE-associated variant at 11p15 was rs12802200 C > A, which lies 45.6 kb 5' of, and is an eQTL for *IRF7* (GTEx Portal on 01/19/18). This gene encodes interferon regulatory factor 7 (IRF7), a transcription factor in lymphoid tissues that activates the transcription of a wide range of type I and type III IFNs, as well as CCL5 and CXCL10 (TRRUST2; Han et al., 2017). IRF7 is downregulated by the SLE susceptibility gene *TNFAIP*. A third SLE-associated variant in the region, rs1131665 T > C, is a nonsynonymous variant that encodes a Gln to Arg substitution at position 412 in IRF7. Gln is encoded by the (major) risk allele, which results in a twofold increase in IFN-stimulated transcriptional activity (Fu et al., 2011).

Genetic variation at this locus is associated with IFNα activity in SLE and titers of anti-Sm autoantibody (Salloum et al., 2010). This region has not been associated with other autoimmune diseases.

The second SLE GWAS study published early that year, Kozyrev et al. (2008) genotyped 279 patients and 515 healthy controls from Sweden using the Affymetrix 100k SNP array. Their analysis focused on nonsynonymous substitutions and they quickly settled on those they identified within the *BANK1* gene on 4q24.

### **B-Cell Scaffold Protein with Ankyrin Repeats 1**

Kozyrev et al. (2008) identified three functional variants in *BANK1*: rs10516487 G > A/G > T, encoding an Arg to His substitution at position 61; rs3733197 G > A encoding an Ala to Thr substitution at position 383; and rs17266594 T > C, which is located in a splice junction that affected the relative expression of full length and exon 2-deficient isoforms. They genotyped these variants in four additional cohorts from Argentina (255 cases, 337 controls), Germany (312 cases, 360 controls), Italy (279 cases, 279 controls), and Spain (702 cases, 446 controls), and the fourfold tables from each study were aggregated using a Mantel–Haenszel test. The most strongly associated marker was rs17266594 T > C ( $P = 4.74 \times 10^{-11}$ ), although none of the variants acted independently of the others, due to strong linkage disequilibrium between them. *BANK1* encodes B-cell Scaffold Protein with Ankyrin Repeats 1 (BANK1), which contributes to B-cell receptor-mediated signaling by affecting calcium mobilization and Lyn-mediated tyrosine phosphorylation of inositol 1,4,5-trisphosphate receptors. Variants within the gene have not been associated with other autoimmune diseases.

In the third SLE GWAS study published in early 2008, Hom et al. (2008) performed a GWAS on DNA samples from 1311 patients with SLE and 1783 controls, all North Americans of European descent. Strong confirmation at a genome-wide level of significance was found for *HLA*, *IRF5*, *ITGAM*, and *STAT4*, although there was a partial overlap of subjects included in the original report of *STAT4* association (Remmers et al., 2007). In addition, another region of genome-wide significance was identified, at 8p23 (*BLK*).

### **B-Lymphoid Tyrosine Kinase**

The most strongly SLE-associated variant at 8p23, rs13277113 G > A, is located in the promoter region of *BLK*, about 3 kb upstream of the transcription initiation site. This gene encodes B-Lymphoid Tyrosine Kinase (BLK), a Src tyrosine-kinase that has a role in B-cell receptor signaling and B-cell development, although *Blk*-deficient mice have no obvious phenotype (Texido et al., 2000). The rs13277113A SLE risk allele is associated with reduced *BLK* mRNA in transformed B-cell lines (Hom et al., 2008), but in primary human B lymphocytes, its *cis*-regulatory effects are restricted to naïve and transitional B cells; allelic variation has been shown to affect protein levels (Simpfendorfer et al., 2012). Another variant in this region, rs2736337 T > C, is associated with RA (Okada et al., 2014).

Graham et al. (2008) performed a GWAS on 431 SLE patients of European descent from the University of Minnesota SLE cohort and 2155 controls from publicly available datasets from the National Institute of Mental Health and the Wellcome Trust. Genotyping was performed using the Affymetrix Genome-Wide Human SNP Array 5.0. Three genomic regions met the threshold for genome-wide significance: *HLA*, *IRF5*, and 6q23 (*TNFAIP3*). Replication was performed in 740 independent trios, confirming association at 6q23. The weighted z scores from the two studies were summed and converted to *P* values to obtain a meta-analysis.

### **TNF Alpha-Induced Protein 3**

The most significant variant at 6q23 in the meta-analysis, rs5029939 C > G, is located in an intron of *TNFAIP3*, which encodes TNF Alpha-Induced Protein 3 (TNFAIP3), a zinc finger protein with ubiquitin-editing activity that functions as a negative regulator of NF-κB signaling by ubiquitin modification of the adaptor proteins RIP and TRAF6, in the TNF and TLR/IL1R signaling pathways respectively. In doing so it inhibits TNF-mediated apoptosis. The expression of TNFAIP3 is decreased in patients with SLE and is negatively correlated with the severity of disease (Qian et al., 2017). The B cells of mice bearing a targeted null mutation in *Tnfaip3* are resistant to Fas-mediated cell death and are increased in germinal centers. As a result, the mice develop autoantibodies and glomerular immunoglobulin deposits (Tavares et al., 2010). Mice bearing the same mutation targeted to their DC develop splenomegaly, anti-dsDNA antibodies and nephritis. Their DC captured apoptotic cells more efficiently, resisted the inhibitory effects of apoptotic cells, and induced self-reactive effector lymphocytes, resulting in the accumulation of plasma cells and conversion of CD4 T cells to IFN-γ-producing effector cells (Kool et al., 2011).

Other variants in this region are also associated with RA (Raychaudhuri et al., 2008), celiac disease (Dubois et al., 2010), MS (IMSGC et al., 2013), psoriasis (Tsoi et al., 2012), and inflammatory bowel disease (Jostins et al., 2012).

The Hom et al. (2008) study was followed up with a large-scale replication study of an independent sample of 1963 cases and 4329 healthy controls from the United States and Sweden, using a custom Illumina Infinium II SNP array targeted at genomic regions that had shown nominal (or better) evidence of association in the original GWAS. The three datasets were combined by converting *P* values to *z* scores, which were weighted by each sample size, combined and converted back to a *P* value for each locus (Gateva et al., 2009). They confirmed at a genome-wide level of significance *HLA*, *STAT4*, *IRF5*, *ITGAM*, *BLK*, and *PHRF1* and identified six new loci: 1p13 (gene of interest *PTPN22*), 5q33 (*TNIP1*), 6q21 (*PRDM1*), 7p15 (*JAZF1*), 6p21 (*UHRF1BP1*), and 1q32 (*IL10*).

### **TNF Alpha-Induced Protein 3 Interacting Protein 1**

The most strongly SLE-associated variant at 5q33, rs7708392G > C, lies within an intron of *TNIP1*, which encodes TNFAIP3 Interacting Protein 1 (TNIP1). TNIP1 inhibits NF-κB activation by facilitating TNFAIP3-mediated deubiquitylation of NF-kappa-B Essential Modulator (NEMO), an inhibitor of IKK-γ. TNIP1 is decreased in expression in PBMC of patients with SLE, and it negatively correlates with the severity of disease (Qian et al., 2017).

Another variant at 5q33, rs2233278 G > C, is associated with psoriasis (Tsoi et al., 2012).

### **PRDM1**

The most strongly SLE-associated variant at 6q21, rs6568431 A > C, lies 31 kb 3' of *PRDM1*, which encodes B-Lymphocyte-Induced Maturation Protein 1 (BLIMP1), a transcriptional repressor of *CITA*, *GCSAM*, *LMO2*,

*MK167*, *MYC*, and *PCNA* (TRRUST2; Han et al., 2017). It inhibits proliferation by regulating p53 and plays a critical role in the differentiation of many lymphocyte subsets, including plasma cells, Th2 CD4 T cells, and effector (IL-10-secreting) Treg cells, as well as inhibition of differentiation of Th1, Th17, and Tfh cells (Fu et al., 2017). *PRDM1* mRNA expression in SLE patients is more than two times greater than that in healthy controls, as it is in MRL/lpr SLE-prone mice (Luo et al., 2013). *Prdm1* expression in mice is increased by the expression of the SLE-associated gene *Irf5* and decreased by *Irf5* targeted deletion (Panchanathan et al., 2012). Suppression of *Prdm1* expression by siRNA in MRL-Fas(lpr) lupus-prone mice reduced anti-dsDNA antibody levels, improved histological signs of glomerulonephritis, and decreased proteinuria (Luo et al., 2015).

Other variants in this region are associated with Crohn's disease (Franke et al., 2010) and ulcerative colitis (Anderson et al., 2011).

## IL10

The most strongly SLE-associated variant at 1q32, rs3024505 G > A, lies about 1 kb 3' of *IL10*, which encodes IL-10, an immunoregulatory cytokine produced by leukocytes, especially monocytes. IL-10 suppresses cell-mediated immunity while supporting B-cell survival, proliferation, and antibody production. The spontaneous in vitro production of IL-10 by monocytes and B cells of patients with SLE is over 30-fold higher than in healthy controls (Llorente et al., 1993) and can correlate with the disease severity as measured by SLE disease activity index, ds-DNA titers, and C3 levels (Houssiau et al., 1995). In vitro the addition of IL-10 PBMC from SLE patients resulted in the production of IgM, IgG, and IgA, which was inhibited by the addition of anti-IL-10 mAb (Llorente et al., 1995).

The SLE-associated rs3024505 C > T variant is also associated with T1D (Gateva et al., 2009), inflammatory bowel disease (Jostins et al., 2012), ulcerative colitis (Anderson et al., 2011), and Crohn's disease (Franke et al., 2010). While the rs3024505T allele is associated with SLE, ulcerative colitis and Crohn's disease, T1D is associated with the C allele.

Cunningham Graham et al. (2011) performed a replication study of loci that had, in Gateva et al. (2009), previously shown moderate evidence of association with SLE ( $P < 5 \times 10^{-3}$ ) without reaching genome-wide significance ( $P < 5 \times 10^{-8}$ ). They genotyped 23 independent variants in 905 British patients with SLE and 5551 controls that included British 1958 Birth Cohort samples and data from the WTCCC2 project. Eight of the variants tested had not been previously genotyped in the WTCCC2 samples, so they were imputed from the existing data. Of these 23 markers, 12 showed evidence of association ( $P < .05$ ). A meta-analysis was then performed incorporating data from Gateva et al. (2009) using Fisher's combined probability test. Five novel loci were identified that showed significant association at a genome-wide level: 1q25 (gene of interest *NCF2*), 2q24 (*IFIH1*), 7p12 (*IKZF1*), 16q24 (*IRF8*), and 19p13 (*TYK2*).

## NCF2

The most strongly SLE-associated variant at 1q25, rs10911363 G > T, lies within an intron of, and is an eQTL for, *NCF2* (GTEx Portal on 07/01/18), which encodes Neutrophil Cytosolic Factor 2 (NCG2), the 67 kDa cytosolic subunit of neutrophil Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase complex responsible for the superoxide burst of phagosomes in monocytes, macrophages, and neutrophils. Fine mapping attributed this association to linkage disequilibrium with a nonsynonymous coding variant in exon 12, rs17849502 G > A/G > C/G > T, which results in a substitution of a His to a Gln at position 389 in the PB1 domain of the NCF2 protein. This substitution reduces the binding efficiency of NCF2 to the ZF domain of the guanine nucleotide exchange factor VAV1. As this interaction plays a critical role in a positive feedback cycle for the Rac GTPase (another component of the NADPH oxidase complex), the substitution results in a twofold decrease in reactive oxygen species production induced following the activation of the Vav-dependent Fc $\gamma$  receptor-elicited NADPH oxidase (Jacob et al., 2012). The NZM 2328 mouse strain spontaneously develops anti-dsDNA IgG autoantibodies by 5 months of age and glomerulonephritis associated with IgG and C3 deposits and proteinuria by 12 months. The presence of one or two deletional mutant alleles of *Ncf2* in NZM 2328 mice significantly accelerated the onset of glomerulonephritis and proteinuria by several months (Jacob et al., 2017).

The rs17849502 G > A/G > C/G > T variant is also associated with risk of childhood-onset SLE (Jacob et al., 2012).

## IRF8

The SLE-associated variant at 16q24, rs2280381 C > T, lies approximately 62 kb 3' of *IRF8* (GTEx Portal on 09/01/18) and encodes the transcription factor IRF8, which controls the expression of a wide range of

immunologically related genes, including the SLE susceptibility gene *NCF2* (TRRUST2; Han et al., 2017). IRF8-deficient NZB. *Ifi8*<sup>-/-</sup> mice produced no Coomb's antibody or antichromatin IgG, reduced glomerular immunoglobulin and complement deposition, and had milder glomerulonephritis than wild-type mice (Baccala et al., 2013).

Variants in the same region are associated with MS (IMSGC et al., 2013), Sjögren's syndrome (Lessard et al., 2013), RA (Eyre et al., 2012), inflammatory bowel disease (Jostins et al., 2012), and primary biliary cirrhosis (Liu et al., 2012).

### UBE2L3

Wang et al. (2012) performed a targeted association study of the 22q1 region, which had been identified repeatedly in SLE linkage and association studies without reaching a genome-wide level of significance. They genotyped 57 variants in the region and a panel of ancestral markers in 8922 independent SLE cases and 8077 independent controls across five ethnic populations. The most strongly SLE-associated variant, rs7444 T > C (combined  $P = 2.21 \times 10^{-14}$ ), lies within the 3' untranslated region (UTR) and is an eQTL for (GTEx Portal on 21/01/18), *UBE2L3*, which encodes Ubiquitin Conjugating Enzyme E2 L3 (*UBE2L3*). *UBE2L3* contributes to the ubiquitination and degradation of p53, c-FOS, and the NF- $\kappa$ B precursor p105. Homozygosity for the risk-associated, (minor) allele, rs7444C (OR 1.23), is associated with twofold increased expression of transcripts and protein in EBV-transformed B-cell lines. Another SLE-associated marker in the region, rs140490 G > A/G > T, lies within an intron of, and is also an eQTL for (GTEx Portal on 21/01/18), *UBE2L3*. The risk-associated rs140490T allele was associated with increased NF- $\kappa$ B translocation in primary human cells from healthy individuals and correlated with basal NF- $\kappa$ B activation in unstimulated B cells and monocytes (Lewis et al., 2015).

Variants in the 22q1 region are associated with psoriasis (Tsoi et al., 2012), RA (Okada et al., 2014), juvenile idiopathic arthritis (Hinks et al., 2013), and celiac disease (Trynka et al., 2011).

The largest GWAS of SLE in subjects of European ancestry published to date studied 7219 cases and 15,991 healthy controls (Bentham et al., 2015). It applied a staged strategy, involving (1) a large GWAS (4036 cases and 6959 controls); (2) a meta-analysis combining the discovery study with the data from the Hom et al. (2008) study (1165 cases, 2107 controls); (3) a replication study (2018 cases, 6925 controls) and postreplication meta-analysis. The initial GWAS identified 25 regions associated with SLE at a genome-wide level. Twenty-one had been previously published: *PTPN22*, *FCGR2A*, *TNFSF4*, *NCF2*, *IFIH1*, *STAT4*, *BANK1*, *TNIP1*, MHC class III, *PRDM1*, *TNFAIP3*, *IRF5*, *BLK*, *WDFY4*, *IRF7*, *CD44*, *ITGAM*, *IRF8*, *TYK2*, *UBE2L3*, and *IRAK1*. Two had been identified previously but not at a genome-wide level of significance: 5q33 (*MIR146A*) and 15q24 (*CSK*), and three were novel: 2p14 (*SPRED2*), 2q34 (*IKZF2*), and 5q31 (*TCF7*).

The replication study and postreplication meta-analysis confirmed nine additional loci that had previously been associated at a whole genome level of significance: *IL10*, *IFIH1*, *PXK*, *UHRF1BP1*, *JAZF1*, *ARID5B*, *ETS1*, *SLC154A*, *IKZF3*, and confirmed *LYST*, which had been previously identified, but not at a whole genome level of significance. Seven newly identified loci were 3q25 (*IL12A*), 11q13 (*DHCR7*), 12q24 (*SH2B3*), 14q24 (*RAD51B*), 16p13 (*SOCS1*), 17p13 (*PLD2*), and Xp21 (*CXorf21*).

### IL12A

The most significant SLE-associated variant at 3q25, rs564799 C > T, lies 15 kb 3' of, and is an eQTL for (GTEx Portal on 01/21/18), *IL12A*, which encodes IL 12A, the p35 (35 kDa) subunit of the proinflammatory heterodimeric cytokine IL-12. The binding partner, p40, is encoded by *IL12B* and is also a component of IL-23. IL-12 is produced by professional APC and acts on T cells and NK cells via STAT4 translocation; it is essential for the secretion of IFN- $\gamma$ , which is required for the differentiation of CD8 T cells into effector cytotoxic cells. *IL12A* transcripts are reduced in the PBMC of untreated patients with SLE and fall further on treatment with corticosteroids; in contrast, *IL12B* transcripts are normal before treatment but drop significantly on treatment. Both transcripts are expressed at higher levels in patients with more severe disease (Huang et al., 2007).

Variants in 3q25 are also associated with celiac disease (Dubois et al., 2010), Sjögren's syndrome (Lessard et al., 2013), systemic sclerosis (Mayes et al., 2014), primary biliary cirrhosis (Liu et al., 2012), and MS (IMSGC et al., 2013).

## CONCLUDING COMMENTS

Over the last two decades, GWASs have been extraordinarily successful in identifying candidate autoimmune disease susceptibility genes. For most autoimmune diseases, 50–100 loci have been identified. Testing these

candidates requires a combination of in vitro and in vivo models. As a generalization, the existing models, supplemented by the molecular manipulation of gene expression, serve this purpose well, so long as the limitations of each model are taken into consideration.

Even at this relatively early stage of functional validation, clear patterns of gene activity associated with each disease are emerging. For example, in the case of MS, which has a relatively low heritability by virtue of the major effects of its environmental risk factors (EBV, sun exposure, and smoking), several candidate genes have been implicated in responding to these factors. For example, the high levels of expression of DQB1 and DRB1 associated with the HLA-DR15 haplotype facilitates EBV attachment and cellular entry. This, rather than antigenic restriction, may be enough to explain the observed HLA association with the disease. This hypothesis is consistent with the clinical observation that MS is not characterized by a single, or even a primary, autoantigen. Both T- and B-cell responses are oligoclonal and vary in specificity between patients. Genetic associations with sun exposure are also observed in MS. The regulatory regions of risk genes *HLA-DRB1* and *IRF8* contain VDREs, CYP27B1 is involved in Vitamin D metabolism, and the phosphorylation of STAT3 and STAT4 in response to IL-12 receptor binding is inhibited by Vitamin D.

In contrast to MS, in T1D the primary autoantigen has been identified: proinsulin within the beta cells of the islets of Langerhans in the pancreas. Insulin is probably targeted because the promoter of its gene contains a VNTR that results in reduced thymic expression and increased pancreatic expression of insulin in people carrying the T1D-associated VNTR allele. This probably results in a mismatch in central and peripheral signaling intensities and consequently impaired central tolerance. The association with HLA-DR3 and HLA-DR4, and particularly CD3/4 heterozygotes, may therefore reflect the antigen restriction structures with highest affinity to insulin peptides. Other T1D candidate genes relate specifically to allelic vulnerabilities within beta cells. For example, variants in *CLEC16A* affect the regulation of mitophagy (selective degradation of mitochondria by autophagy), causing, in those bearing risk alleles, a reduction in cellular ATP, which is required for insulin secretion. Similar defects are seen in other classes of diabetes. Similarly, variants in *PTPN2* may be associated with differences in beta-cell sensitivity to cytokine-induced programmed cell death and impaired glucose tolerance under a high-fat diet. One of the effects of variation at *BACH2* is to modulate the expression of *PTPN2*, potentially explaining its association with T1D.

Other patterns of candidate gene function suggest, not so much "pathways" but, networked interactions associated with emerging complex phenomena. The fundamental cell biological activities of programmed cell death and receptor-mediated activation are affected by candidate genes in most autoimmune diseases: *BACH2*, a derepressor of the antiapoptotic factors *BCL2* and *BCL-XL*, maintains the longevity of Tregs; variants in *BACH2* are associated with T1D, MS, and many other autoimmune diseases. In SLE the expression of *TNFAIP3* is decreased; it functions as a negative regulator of NF- $\kappa$ B signaling, and its deficiency results in NF- $\kappa$ B driven proliferation instead of death-receptor-mediated programmed cell death. Again, variants associated with *TNFAIP3* are associated with many autoimmune diseases: RA, celiac disease, inflammatory bowel disease, psoriasis, and MS. Another SLE candidate gene, *TNIP1*, encodes a protein that interacts with *TNFAIP3*. *TNIP1* is decreased in expression in PBMC of patients with SLE, and its expression negatively correlates with the severity of disease.

Many candidate autoimmune disease susceptibility genes are implicated in the regulation or stimulation of leukocyte activation and proliferation. Confusingly, the direction of activity is inconsistent between genes. For example, while T1D-associated alleles at variants near *SH2B3* are associated with its reduced expression and increased T-cell proliferation, the T1D-associated alleles at variants in *PTPN22* are associated with the gain of function and decreased T-cell proliferation. One possible explanation for paradoxes of this sort is the existence of two activation thresholds for CD4 T cells: One for initiating effector function and the other for Treg differentiation. Candidate genes related to leukocyte activation in MS include *IL2RA*, *CD5*, *CD6*, *CD40*, *STAT3*, and *STAT4*, and most of these have been implicated in multiple other autoimmune diseases.

A third class of candidate autoimmune susceptibility genes encompasses the genes associated with specific immune mechanisms. Perhaps the best recognized in this category are those involved in the phagocytosis and lysis of immune complexes in SLE. For example, *ITGAM*, *FCGR2A*, *FCGR3A*, *FCGR3B*, *C1Q*, *C2*, and *C4B* are all involved in immune complex clearance, while *NCF2* contributes to the oxidative burst in phagosomes. These genes are relatively restricted in their disease associations to SLE and SLE-like disease, such as childhood-onset lupus. In contrast, *IL2RA* and *BACH2*, which contribute to the differentiation and maintenance of Treg, are tagged by variants that are associated with T1D, celiac disease, autoimmune thyroid disease, MS, RA, juvenile idiopathic arthritis, alopecia areata, primary sclerosing cholangitis, inflammatory bowel disease, and Crohn's disease. Another example of a specific immune mechanism associated with autoimmune disease commonly targeted by candidate susceptibility genes is that of immune deviation. *IL-12B* is a component of IL-23 and IL-12, both of

which signal via STAT3 and STAT4, and all of which are required for the differentiation of destructive Th1 and Th17 immune responses. As a generalization, increased STAT3/4 phosphorylation mediates more severe autoimmune disease. Variants in these genes are associated with SLE, Sjögren's syndrome, RA, systemic sclerosis, juvenile idiopathic arthritis, primary biliary cirrhosis, celiac disease, Crohn's disease, ulcerative colitis, and psoriasis.

These collections of interacting genes, which are both shared between diseases and create disease-specific patterns, elaborate and enhance our previous understanding of disease mechanisms. As we complete the identification of causative mutations and the identification of tissue-specific eQTL, this mapping from location to function and drugable target will only improve.

## Acknowledgments

This work was supported by MS Research Australia, Lions Clubs of Australia, National Health and Medical Research Council of Australia. AGB is grateful for the kind hospitality of the staff and students at the Peter Doherty Institute for Infection and Immunity for hosting him on sabbatical while he worked on this manuscript.

## References

- Acheson, E.D., Bachrach, C.A., Wright, F.M., 1960. Some comments on the relationship of the distribution of multiple sclerosis to latitude, solar radiation, and other variables. *Acta Psychiatr. Scand. Suppl.* 35, 132–147.
- Aderka, D., Wysenbeek, A., Engelmann, H., et al., 1993. Correlation between serum levels of soluble tumor necrosis factor receptor and disease activity in systemic lupus erythematosus. *Arthritis Rheum.* 36, 1111–1120.
- Agnello, V., De Bracco, M.M., Kunkel, H.G., 1972. Hereditary C2 deficiency with some manifestations of systemic lupus erythematosus. *J. Immunol.* 108, 837–840.
- Ahmed, A.R., Yunis, J.J., Marcus-Bagley, D., et al., 1993. Major histocompatibility complex susceptibility genes for dermatitis herpetiformis compared with those for gluten-sensitive enteropathy. *J. Exp. Med.* 178, 2067–2075.
- Alarcón-Segovia, D., Alarcón-Riquelme, M.E., Cardiel, M.H., et al., 2005. Grupo Latinoamericano de Estudio del Lupus Eritematoso (GLADEL). Familial aggregation of systemic lupus erythematosus, rheumatoid arthritis, and other autoimmune diseases in 1,177 lupus patients from the GLADEL cohort. *Arthritis Rheum.* 52, 1138–1147.
- Alcina, A., Abad-Grau Mdel, M., Fedetz, M., et al., 2012. Multiple sclerosis risk variant HLA-DRB1\*1501 associates with high expression of DRB1 gene in different human populations. *PLoS One* 7, e29819.
- American College of Rheumatology Ad Hoc Committee on Systemic Lupus Erythematosus Guidelines, 1999. Guidelines for referral and management of systemic lupus erythematosus in adults. *Arthritis Rheum.* 42, 1785–1796.
- Amiel, J.L., 1967. Study of the leukocyte phenotypes in Hodgkin's disease. In: Curtoni, E.S., Mattiuz, P.L., Tosi, R.M. (Eds.), *Histocompatibility Testing*. Munksgaard, Copenhagen, pp. 79–81.
- Amos, C.I., Chen, W.V., Lee, A., et al., 2006. High-density SNP analysis of 642 Caucasian families with rheumatoid arthritis identifies two new linkage regions on 11p12 and 2q33. *Genes Immun.* 7, 277–286.
- Anderson, C.A., Boucher, G., Lees, C.W., et al., 2011. Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. *Nat. Genet.* 43, 246–252.
- Araki, M., Chung, D., Liu, S., et al., 2009. Genetic evidence that the differential expression of the ligand-independent isoform of CTLA-4 is the molecular basis of the Idd5.1 type 1 diabetes region in nonobese diabetic mice. *J. Immunol.* 183, 5146–5157.
- Arellano, J., Vallejo, M., Jimenez, J., et al., 1984. HLA-B27 and ankylosing spondylitis in the Mexican Mestizo population. *Tissue Antigens* 23, 112–116.
- Aringer, M., Houssiau, F., Gordon, C., et al., 2009. Adverse events and efficacy of TNF-alpha blockade with infliximab in patients with systemic lupus erythematosus: long-term follow-up of 13 patients. *Rheumatology (Oxford)* 48, 1451–1454.
- Armstrong, D.L., Zidovetzki, R., Alarcón-Riquelme, M.E., et al., 2014. GWAS identifies novel SLE susceptibility genes and explains the association of the HLA region. *Genes Immun.* 15, 347–354.
- Ascherio, A., Munger, K.L., 2007. Environmental risk factors for multiple sclerosis. Part I: The role of infection. *Ann. Neurol.* 61, 288–299.
- Australia and New Zealand Multiple Sclerosis Genetics Consortium (ANZgene), 2009. Genome-wide association study identifies new multiple sclerosis susceptibility loci on chromosomes 12 and 20. *Nat. Genet.* 41, 824–828.
- Axtell, R.C., Webb, M.S., Barnum, S.R., Raman, C., 2004. Cutting edge: critical role for CD5 in experimental autoimmune encephalomyelitis: inhibition of engagement reverses disease in mice. *J. Immunol.* 173, 2928–2932.
- Axtell, R.C., Xu, L., Barnum, S.R., Raman, C., 2006. CD5-CK2 binding/activation-deficient mice are resistant to experimental autoimmune encephalomyelitis: protection is associated with diminished populations of IL-17-expressing T cells in the central nervous system. *J. Immunol.* 177, 8542–8549.
- Baccala, R., Gonzalez-Quintal, R., Blasius, A.L., et al., 2013. Essential requirement for IRF8 and SLC15A4 implicates plasmacytoid dendritic cells in the pathogenesis of lupus. *Proc. Natl. Acad. Sci. U.S.A.* 110, 2940–2945.
- Baechler, E.C., Batliwalla, F.M., Karypis, G., et al., 2003. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *Proc. Natl. Acad. Sci. U.S.A.* 100, 2610–2615.
- Bailey, R., Cooper, J.D., Zeitels, L., et al., 2007. Association of the vitamin D metabolism gene CYP27B1 with type 1 diabetes. *Diabetes* 56, 2616–2621.
- Bain, S.C., Prins, J.B., Hearne, C.M., et al., 1992. Insulin gene region-encoded susceptibility to type 1 diabetes is not restricted to HLA-DR4-positive individuals. *Nat. Genet.* 2, 212–215.

- Ban, M., Stewart, G.J., Bennetts, B.H., et al., 2002. A genome screen for linkage in Australian sibling-pairs with multiple sclerosis. *Genes Immun.* 3, 464–469.
- Bankovich, A.J., Shiow, L.R., Cyster, J.G., 2010. CD69 suppresses sphingosine 1-phosphate receptor-1 (S1P1) function through interaction with membrane Helix 4. *J. Biol. Chem.* 285, 22328–22337.
- Baranzini, S.E., Wang, J., Gibson, R.A., et al., 2009. Genome-wide association analysis of susceptibility and clinical phenotype in multiple sclerosis. *Hum. Mol. Genet.* 18, 767–778.
- Barcellos, L.F., Sawcer, S., Ramsay, P.P., et al., 2006. Heterogeneity at the HLA-DRB1 locus and risk for multiple sclerosis. *Hum. Mol. Genet.* 15, 2813–2824.
- Barnes, B.J., Moore, P.A., Pitha, P.M., 2001. Virus-specific activation of a novel interferon regulatory factor, IRF-5, results in the induction of distinct interferon alpha genes. *J. Biol. Chem.* 276, 23382–23390.
- Barratt, B.J., Payne, F., Lowe, C.E., et al., 2004. Remapping the insulin gene/IDDM2 locus in type 1 diabetes. *Diabetes* 53, 1884–1889.
- Barrett, J.C., Hansoul, S., Nicolae, D.L., et al., 2008. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat. Genet.* 40, 955–962.
- Barrett, J.C., Clayton, D.G., Concannon, P., et al., 2009. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. *Nat. Genet.* 41, 703–707.
- Baxter, A.G., 1997. Immunogenetics and the cause of autoimmune disease. *Autoimmunity* 25, 177–189.
- Baxter, A.G., 2007. The origin and application of experimental autoimmune encephalomyelitis. *Nat. Rev. Immunol.* 7, 904–912.
- Begovich, A.B., Carlton, V.E., Honigberg, L.A., et al., 2004. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. *Am. J. Hum. Genet.* 75, 330–337.
- Bell, G.I., Horita, S., Karam, J.H., 1984. A polymorphic locus near the human insulin gene is associated with insulin-dependent diabetes mellitus. *Diabetes* 33, 176–183.
- Bennett, S.T., Lucassen, A.M., Gough, S.C., et al., 1995. Susceptibility to human type 1 diabetes at IDDM2 is determined by tandem repeat variation at the insulin gene minisatellite locus. *Nat. Genet.* 9, 284–292.
- Bentham, J., Morris, D.L., Graham, D.S.C., et al., 2015. Genetic association analyses implicate aberrant regulation of innate and adaptive immunity genes in the pathogenesis of systemic lupus erythematosus. *Nat. Genet.* 47, 1457–1464.
- Bergamaschi, L., Leone, M.A., Fasano, M.E., et al., 2010. HLA-class I markers and multiple sclerosis susceptibility in the Italian population. *Genes Immun.* 11, 173–180.
- Bertrams, J., 1984. The HLA association of insulin-dependent (type I) diabetes mellitus. *Behring Inst. Mitt.* 75, 89–99.
- Betz, R.C., Petukhova, L., Ripke, S., et al., 2015. Genome-wide meta-analysis in alopecia areata resolves HLA associations and reveals two new susceptibility loci. *Nat. Commun.* 6, 5966.
- Blanco-Kelly, F., Matesanz, F., Alcina, A., et al., 2010. CD40: novel association with Crohn's disease and replication in multiple sclerosis susceptibility. *PLoS One* 5, e11520.
- Block, S.R., Winfield, J.B., Lockshin, M.D., et al., 1975. Studies of twins with systemic lupus erythematosus. A review of the literature and presentation of 12 additional sets. *Am. J. Med.* 59, 533–552.
- Blomhoff, A., Lie, B.A., Myhre, A.G., et al., 2004. Polymorphisms in the cytotoxic T lymphocyte antigen-4 gene region confer susceptibility to Addison's disease. *J. Clin. Endocrinol. Metab.* 89, 3474–3476.
- Bonifacio, E., Bingley, P.J., Shattock, M., et al., 1990. Quantification of islet-cell antibodies and prediction of insulin-dependent diabetes. *Lancet* 335, 147–149.
- Bossingham, D., 2003. Systemic lupus erythematosus in the far north of Queensland. *Lupus* 12, 327–331.
- Bottini, N., Musumeci, L., Alonso, A., et al., 2004. A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. *Nat. Genet.* 36, 337–338.
- Bowness, P., Davies, K.A., Norsworthy, P.J., et al., 1994. Hereditary C1q deficiency and systemic lupus erythematosus. *QJM* 87, 455–464.
- Bradfield, J.P., Qu, H.Q., Wang, K., et al., 2011. A genome-wide meta-analysis of six type 1 diabetes cohorts identifies multiple associated loci. *PLoS Genet.* 7 (9), e1002293.
- Brewerton, D.A., Caffrey, M., Hart, F.D., et al., 1973. Ankylosing spondylitis and HL-A 27. *Lancet* 1, 904–907.
- Bright, J.J., Sriram, S., 1998. TGF-beta inhibits IL-12-induced activation of Jak-STAT pathway in T lymphocytes. *J. Immunol.* 161, 1772–1777.
- Broadley, S., Sawcer, S., D'Alfonso, S., et al., 2001. A genome screen for multiple sclerosis in Italian families. *Genes Immun.* 2, 205–210.
- Cardon, L.R., Bell, J.I., 2001. Association study designs for complex diseases. *Nat. Rev. Genet.* 2, 91–99.
- Carton, H., Vlietinck, R., Debruyne, J., et al., 1997. Risks of multiple sclerosis in relatives of patients in Flanders, Belgium. *J. Neurol. Neurosurg. Psychiatry* 62, 329–333.
- Cayrol, R., Wosik, K., Berard, J.L., et al., 2008. Activated leukocyte cell adhesion molecule promotes leukocyte trafficking into the central nervous system. *Nat. Immunol.* 9, 137–145.
- Cheng, Y., Chikwava, K., Wu, C., et al., 2016. LNK/SH2B3 regulates IL-7 receptor signaling in normal and malignant B-progenitors. *J. Clin. Invest.* 126, 1267–1281.
- Christiansen, F.T., Zhang, W.J., Griffiths, M., et al., 1991. Major histocompatibility complex (MHC) complement deficiency, ancestral haplotypes and systemic lupus erythematosus (SLE): C4 deficiency explains some but not all of the influence of the MHC. *J. Rheumatol.* 18, 1350–1358.
- Chung, S.A., Taylor, K.E., Graham, R.R., et al., 2011. Differential genetic associations for systemic lupus erythematosus based on anti-dsDNA autoantibody production. *PLoS Genet.* 7, e1001323.
- Compston, A., 1991. Limiting and repairing the damage in multiple sclerosis. *J. Neurol. Neurosurg. Psychiatry* 54, 945–948.
- Concannon, P., Chen, W.M., Julier, C., et al., 2009. Genome-wide scan for linkage to type 1 diabetes in 2,496 multiplex families from the Type 1 Diabetes Genetics Consortium. *Diabetes* 58, 1018–1022.
- Concannon, P., Gogolin-Ewens, K.J., Hinds, D.A., et al., 1998. A second-generation screen of the human genome for susceptibility to insulin-dependent diabetes mellitus. *Nat. Genet.* 19, 292–296.

- Concannon, P., Erlich, H.E., Julier, C., et al., 2005. Type 1 diabetes: evidence for susceptibility loci from four genome-wide linkage scans in 1435 multiplex families. *Diabetes* 54, 2995–3001.
- Cooper, J.D., Smyth, D.J., Smiles, A.M., et al., 2008. Meta-analysis of genome-wide association study data identifies additional type 1 diabetes risk loci. *Nat. Genet.* 40, 1399–1401.
- Cooper, J.D., Simmonds, M.J., Walker, N.M., et al., 2012. Seven newly identified loci for autoimmune thyroid disease. *Hum. Mol. Genet.* 21, 5202–5208.
- Coraddu, F., Sawcer, S., D'Alfonso, S., et al., 2001. A genome screen for multiple sclerosis in Sardinian multiplex families. *Eur. J. Hum. Genet.* 9, 621–626.
- Cornall, R.J., Prins, J.B., Todd, J.A., et al., 1991. Type 1 diabetes in mice is linked to the interleukin-1 receptor and Lsh/Ity/Bcg genes on chromosome 1. *Nature* 353, 262–265.
- Cree, B.A., Rioux, J.D., McCauley, J.L., et al., 2010. A major histocompatibility class I locus contributes to multiple sclerosis susceptibility independently from HLA-DRB1\*15:01. *PLoS One* 5, e11296.
- Cross, A.H., Girard, T.J., Giacopello, K.S., et al., 1995. Long-term inhibition of murine experimental autoimmune encephalomyelitis using CTLA-4-Fc supports a key role for CD28 costimulation. *J. Clin. Invest.* 95, 2783–2789.
- Cucca, F., Lampis, R., Congia, M., et al., 2001. A correlation between the relative predisposition of MHC class II alleles to type 1 diabetes and the structure of their proteins. *Hum. Mol. Genet.* 10, 2025–2037.
- Cunningham Graham, D.S., Graham, R.R., Manku, H., et al., 2008. Polymorphism at the TNF superfamily gene TNFSF4 confers susceptibility to systemic lupus erythematosus. *Nat. Genet.* 40, 83–89.
- Cunningham Graham, D.S., Morris, D.L., Bhangale, T.R., Criswell, L.A., Syvänen, A.C., Rönnblom, L., et al., 2011. Association of NCF2, IKZF1, IRF8, IFIH1, and TYK2 with systemic lupus erythematosus. *PLoS Genet.* 7 (10), e1002341.
- Dausset, J., 1958. Iso-leuco-anticorps. *Acta Haematol.* 20, 156–166.
- Davies, K.A., Erlendsson, K., Beynon, H.L., et al., 1993. Splenic uptake of immune complexes in man is complement-dependent. *J. Immunol.* 151, 3866–3873.
- Davies, J.L., Kawaguchi, Y., Bennett, S.T., et al., 1994. A genome-wide search for human type 1 diabetes susceptibility genes. *Nature* 371, 130–136.
- Davies, K.A., Peters, A.M., Beynon, H.L., et al., 1992. Immune complex processing in patients with systemic lupus erythematosus. In vivo imaging and clearance studies. *J. Clin. Invest.* 90, 2075–2083.
- De Jager, P.L., Simon, K.C., Munger, K.L., et al., 2008. Integrating risk factors: HLA-DRB1\*1501 and Epstein-Barr virus in multiple sclerosis. *Neurology* 70, 1113–1118.
- De Jager, P.L., Baecher-Allan, C., Maier, L.M., et al., 2009a. The role of the CD58 locus in multiple sclerosis. *Proc. Natl. Acad. Sci. U.S.A.* 106, 5264–6269.
- De Jager, P.L., Jia, X., Wang, J., et al., 2009b. Meta-analysis of genome scans and replication identify CD6, IRF8 and TNFRSF1A as new multiple sclerosis susceptibility loci. *Nat. Genet.* 41, 776–782.
- Deapen, D., Escalante, A., Weinrib, L., et al., 1992. A revised estimate of twin concordance in systemic lupus erythematosus. *Arthritis Rheum.* 35, 311–318.
- Delépine, M., Pociot, F., Habita, C., et al., 1997. Evidence of a non-MHC susceptibility locus in type I diabetes linked to HLA on chromosome 6. *Am. J. Hum. Genet.* 60, 174–187.
- Dendrou, C.A., Plagnol, V., Fung, E., et al., 2009. Cell-specific protein phenotypes for the autoimmune locus IL2RA using a genotype-selectable human bioresource. *Nat. Genet.* 41, 1011–1015.
- Denny, P., Lord, C.J., Hill, N.J., et al., 1997. Mapping of the IDDM locus Idd3 to a 0.35-cM interval containing the interleukin-2 gene. *Diabetes* 46, 695–700.
- Devendra, D., Eisenbarth, G.S., 2003. Immunologic endocrine disorders. *J. Allergy Clin. Immunol.* 111, S624–S636.
- Dietrich, W.F., Katz, H., Lincoln, S.E., et al., 1992. A genetic map of the mouse suitable for typing intraspecific crosses. *Genetics* 131, 423–447.
- Dietrich, W.F., Miller, J., Steen, R., et al., 1996. A comprehensive genetic map of the mouse genome. *Nature* 34, 149–152.
- Dijstelbloem, H.M., Bijl, M., Fijnheer, R., et al., 2000. Fc gamma receptor polymorphisms in systemic lupus erythematosus: association with disease and in vivo clearance of immune complexes. *Arthritis Rheum.* 43, 2793–2800.
- Donner, H., Braun, J., Seidl, C., et al., 1997. Codon 17 polymorphism of the cytotoxic T lymphocyte antigen 4 gene in Hashimoto's thyroiditis and Addison's disease. *J. Clin. Endocrinol. Metab.* 82, 4130–4132.
- Dubois, P.C., Trynka, G., Franke, L., et al., 2010. Multiple common variants for celiac disease influencing immune gene expression. *Nat. Genet.* 42, 295–302.
- Duits, A.J., Bootsma, H., Derkx, R.H., et al., 1995. Skewed distribution of IgG Fc receptor IIa (CD32) polymorphism is associated with renal disease in systemic lupus erythematosus patients. *Arthritis Rheum.* 38, 1832–1836.
- Ebers, G.C., Bulman, D.E., Sadovnick, A.D., et al., 1986. A population-based study of multiple sclerosis in twins. *N. Engl. J. Med.* 315, 1638–1642.
- Ebers, G.C., Kukay, K., Bulman, D.E., et al., 1996. A full genome search in multiple sclerosis. *Nat. Genet.* 13, 472–476.
- Ebringer, A., 1992. Ankylosing spondylitis is caused by *Klebsiella*. Evidence from immunogenetic, microbiologic, and serologic studies. *Rheum. Dis. Clin. North Am.* 18, 105–121.
- Eichhorst, H., 1896. Veber infantile und hereditare multiple sclerosis. *Arch. Pathol. Anat.* 146, 173–192.
- Ettahar, A., Ferrigno, O., Zhang, M.Z., et al., 2013. Identification of PHRF1 as a tumor suppressor that promotes the TGF-β cytostatic program through selective release of TGIF-driven PML inactivation. *Cell Rep.* 4, 530–541.
- Eyre, S., Bowes, J., Diogo, D., et al., 2012. High-density genetic mapping identifies new susceptibility loci for rheumatoid arthritis. *Nat. Genet.* 44, 1336–1340.
- Fanciulli, M., Norsworthy, P.J., Petretto, E., et al., 2007. FCGR3B copy number variation is associated with susceptibility to systemic, but not organ-specific, autoimmunity. *Nat. Genet.* 39, 721–723.

- Field, L.L., Tobias, R., Thomson, G., Plon, S., 1996. Susceptibility to insulin-dependent diabetes mellitus maps to a locus (IDDM11) on human chromosome 14q24.3-q31. *Genomics* 33, 1–8.
- Fielder, A.H., Walport, M.J., Batchelor, J.R., et al., 1983. Family study of the major histocompatibility complex in patients with systemic lupus erythematosus: importance of null alleles of C4A and C4B in determining disease susceptibility. *Br. Med. J. (Clin. Res. Ed.)* 286, 425–428.
- Fisher, M., Pusey, C.D., Vaughan, R.W., et al., 1997. Susceptibility to Goodpasture's disease is strongly associated with HLA-DRB1 genes. *Kidney Int.* 51, 222–229.
- Fogdell, A., Hillert, J., Sachs, C., et al., 1995. The multiple sclerosis- and narcolepsy-associated HLA class II haplotype includes the DRB5\*0101 allele. *Tissue Antigens* 46, 333–336.
- Fogdell-Hahn, A., Ligers, A., Gronning, M., et al., 2000. Multiple sclerosis: a modifying influence of HLA class I genes in an HLA class II associated autoimmune disease. *Tissue Antigens* 55, 140–148.
- Francis, D.A., Compston, D.A., Batchelor, J.R., et al., 1987a. A reassessment of the risk of multiple sclerosis developing in patients with optic neuritis after extended follow-up. *J. Neurol. Neurosurg. Psychiatry* 50, 758–765.
- Francis, D.A., Batchelor, J.R., McDonald, W.I., et al., 1987b. Multiple sclerosis in north-east Scotland. An association with HLA-DQw1. *Brain* 110, 181–196.
- Franke, A., McGovern, D.P., Barrett, J.C., et al., 2010. Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. *Nat. Genet.* 42, 1118–1125.
- Freedman, B.I., Spray, B.J., Heise, E.R., et al., 1993. A race-controlled human leukocyte antigen frequency analysis in lupus nephritis. The South-Eastern Organ Procurement Foundation. *Am. J. Kidney Dis.* 21, 378–382.
- Frisullo, G., Angelucci, F., Caggiula, M., et al., 2006. pSTAT1, pSTAT3, and T-bet expression in peripheral blood mononuclear cells from relapsing-remitting multiple sclerosis patients correlates with disease activity. *J. Neurosci. Res.* 84, 1027–1036.
- Frisullo, G., Nociti, V., Iorio, R., et al., 2008. The persistency of high levels of pSTAT3 expression in circulating CD4+ T cells from CIS patients favors the early conversion to clinically defined multiple sclerosis. *J. Neuroimmunol.* 205, 126–134.
- Fu, Q., Zhao, J., Qian, X., et al., 2011. Association of a functional IRF7 variant with systemic lupus erythematosus. *Arthritis Rheum.* 63, 749–754.
- Fu, S.H., Yeh, L.T., Chu, C.C., Yen, B.L., Sytwu, H.K., 2017. New insights into Blimp-1 in T lymphocytes: a divergent regulator of cell destiny and effector function. *J. Biomed. Sci.* 24, 49.
- Gaffney, P.M., Kearns, G.M., Shark, K.B., et al., 1998. A genome-wide search for susceptibility genes in human systemic lupus erythematosus sib-pair families. *Proc. Natl. Acad. Sci. U.S.A.* 95, 14875–14879.
- Gaffney, P.M., Ortmann, W.A., Selby, S.A., et al., 2000. Genome screening in human systemic lupus erythematosus: results from a second Minnesota cohort and combined analyses of 187 sib-pair families. *Am. J. Hum. Genet.* 66, 547–556.
- Garavito, G., Yunis, E.J., Egea, E., et al., 2004. HLA-DRB1 alleles and HLA-DRB1 shared epitopes are markers for juvenile rheumatoid arthritis subgroups in Colombian mestizos. *Hum. Immunol.* 65, 359–365.
- Gateva, V., Sandling, J.K., Hom, G., et al., 2009. A large-scale replication study identifies TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL10 as risk loci for systemic lupus erythematosus. *Nat. Genet.* 41, 1228–1233.
- Ge, Y., Paisie, T.K., Newman, J.R.B., McIntyre, L.M., Concannon, P., 2017. UBASH3A mediates risk for type 1 diabetes through inhibition of T-cell receptor-induced NF-κB signaling. *Diabetes* 66, 2033–2043.
- Genetic Analysis of Psoriasis Consortium & the Wellcome Trust Case Control Consortium 2, Strange, A., Capon, F., et al., 2010. A genome-wide association study identifies new psoriasis susceptibility loci and an interaction between HLA-C and ERAP1. *Nat. Genet.* 42, 985–990.
- Gerold, K.D., Zheng, P., Rainbow, D.B., et al., 2011. The soluble CTLA-4 splice variant protects from type 1 diabetes and potentiates regulatory T-cell function. *Diabetes* 60, 1955–1963.
- Gonzalez-Escribano, M.F., Rodriguez, R., Valenzuela, A., et al., 1999. CTLA4 polymorphisms in Spanish patients with rheumatoid arthritis. *Tissue Antigens* 53, 296–300.
- Gorer, P.A., 1937. The genetic and antigenic basis of tumour transplantation. *J. Pathol. Bacteriol.* 44, 691–697.
- Graham, R.R., Kozyrev, S.V., Baechler, E.C., et al., 2006. A common haplotype of interferon regulatory factor 5 (IRF5) regulates splicing and expression and is associated with increased risk of systemic lupus erythematosus. *Nat. Genet.* 38, 550–555.
- Graham, R.R., Cotsapas, C., Davies, L., et al., 2008. Genetic variants near TNFAIP3 on 6q23 are associated with systemic lupus erythematosus. *Nat. Genet.* 40, 1059–1061.
- Grant, S.F., Qu, H.Q., Bradfield, J.P., et al., 2009. Follow-up analysis of genome-wide association data identifies novel loci for type 1 diabetes. *Diabetes* 58, 290–295.
- Gregersen, P.K., Amos, C.I., Lee, A.T., et al., 2009. REL, encoding a member of the NF-κB family of transcription factors, is a newly defined risk locus for rheumatoid arthritis. *Nat. Genet.* 41, 820–823.
- Gregory, S.G., Schmidt, S., Seth, P., et al., 2007. Interleukin 7 receptor alpha chain (IL7R) shows allelic and functional association with multiple sclerosis. *Nat. Genet.* 39, 1083–1091.
- Gregory, A.P., Dendrou, C.A., Attfield, K.E., et al., 2012. TNF receptor 1 genetic risk mirrors outcome of anti-TNF therapy in multiple sclerosis. *Nature* 488, 508–511.
- Guggenbuhl, P., Jean, S., Jego, P., et al., 1998. Primary Sjögren's syndrome: role of the HLA-DRB1\*0301-\*1501 heterozygotes. *J. Rheumatol.* 25, 900–905.
- Gyapay, G., Morissette, J., Vignal, A., et al., 1994. The 1993–94 Genethon human genetic linkage map. *Nat. Genet.* 7, 246–339.
- Haines, J.L., Ter-Minassian, M., Bazyk, A., et al., 1996. A complete genomic screen for multiple sclerosis underscores a role for the major histocompatibility complex. The Multiple Sclerosis Genetics Group. *Nat. Genet.* 13, 469–471.
- Haines, J.L., Terwedow, H.A., Burgess, K., et al., 1998. Linkage of the MHC to familial multiple sclerosis suggests genetic heterogeneity. The Multiple Sclerosis Genetics Group. *Hum. Mol. Genet.* 7, 1229–1234.
- Hajeer, A.H., Hutchinson, I.V., 2001. Influence of TNFalpha gene polymorphisms on TNFalpha production and disease. *Hum. Immunol.* 62, 1191–1199.

- Hakonarson, H., Grant, S.F., Bradfield, J.P., et al., 2007. A genome-wide association study identifies KIAA0350 as a type 1 diabetes gene. *Nature* 448, 591–594.
- Hammarström, L., Smith, E., Möller, E., et al., 1975. Myasthenia gravis: studies on HL-A antigens and lymphocyte subpopulations in patients with myasthenia gravis. *Clin. Exp. Immunol.* 21, 202–215.
- Han, H., Cho, J.W., Lee, S., et al., 2017. TRRUST v2: an expanded reference database of human and mouse transcriptional regulatory interactions. *Nucleic Acids Res.* 46, D380–D386. Available from: <https://doi.org/10.1093/nar/gkx1013>.
- Harbo, H.F., Celius, E.G., Vartdal, F., et al., 1999. CTLA4 promoter and exon 1 dimorphisms in multiple sclerosis. *Tissue Antigens* 53, 106–110.
- Hashimoto, L., Habita, C., Beressi, J., et al., 1994. Genetic mapping of a susceptibility locus for insulin-dependent diabetes mellitus on chromosome 11q. *Nature* 371, 161–164.
- Hatta, Y., Tsuchiya, N., Ohashi, J., et al., 1999. Association of Fc gamma receptor IIIB, but not of Fc gamma receptor IIA and IIIA polymorphisms with systemic lupus erythematosus in Japanese. *Genes Immun.* 1, 53–60.
- Hawkes, C.H., Macgregor, A.J., 2009. Twin studies and the heritability of MS: a conclusion. *Mult. Scler.* 15, 661–667.
- Healy, B.C., Liguori, M., Tran, D., et al., 2010. HLA B\*44: protective effects in MS susceptibility and MRI outcome measures. *Neurology* 75, 634–640.
- Hinks, A., Cobb, J., Marion, M.C., et al., 2013. Dense genotyping of immune-related disease regions identifies 14 new susceptibility loci for juvenile idiopathic arthritis. *Nat. Genet.* 45, 664–669.
- Hirsch, R.L., Panitch, H.S., Johnson, K.P., 1985. Lymphocytes from multiple sclerosis patients produce elevated levels of gamma interferon in vitro. *J. Clin. Immunol.* 5, 386–389.
- Hisanaga-Oishi, Y., Nishiwaki-Ueda, Y., Nojima, K., Ueda, H., 2014. Analysis of the expression of candidate genes for type 1 diabetes susceptibility in T cells. *Endocr. J.* 61, 577–588.
- Hom, G., Graham, R.R., Modrek, B., et al., 2008. Association of systemic lupus erythematosus with C8orf13-BLK and ITGAM-ITGAX. *N. Engl. J. Med.* 358, 900–909.
- Hoppenbrouwers, I.A., Aulchenko, Y.S., Ebers, G.C., et al., 2008. EVI5 is a risk gene for multiple sclerosis. *Genes Immun.* 9, 334–337.
- Hoppenbrouwers, I.A., Aulchenko, Y.S., Janssens, A.C., et al., 2009. Replication of CD58 and CLEC16A as genome-wide significant risk genes for multiple sclerosis. *J. Hum. Genet.* 54, 676–680.
- Horiuchi, M., Itoh, A., Pleasure, D., et al., 2011. Cooperative contributions of interferon regulatory factor 1 (IRF1) and IRF8 to interferon- $\gamma$ -mediated cytotoxic effects on oligodendroglial progenitor cells. *J. Neuroinflammation* 8, 8.
- Houssiau, F.A., Lefebvre, C., Vanden Berghe, M., Lambert, M., Devogelaer, J.P., Renauld, J.C., 1995. Serum interleukin 10 titers in systemic lupus erythematosus reflect disease activity. *Lupus* 4, 393–395.
- Howard, P.F., Hochberg, M.C., Bias, W.B., et al., 1986. Relationship between C4 null genes, HLA-D region antigens, and genetic susceptibility to systemic lupus erythematosus in Caucasian and black Americans. *Am. J. Med.* 81, 187–193.
- Huang, X., Hua, J., Shen, N., Chen, S., 2007. Dysregulated expression of interleukin-23 and interleukin-12 subunits in systemic lupus erythematosus patients. *Mod. Rheumatol.* 17, 220–223.
- Hyttinen, V., Kaprio, J., Kinnunen, L., et al., 2003. Genetic liability of type 1 diabetes and the onset age among 22,650 young Finnish twin pairs: a nationwide follow-up study. *Diabetes* 52, 1052–1055.
- International Consortium for Systemic Lupus Erythematosus Genetics, Harley, J.B., Alarcón-Riquelme, M.E., et al., 2008. Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in ITGAM, PXK, KIAA1542 and other loci. *Nat. Genet.* 40, 204–210.
- International Genetics of Ankylosing Spondylitis Consortium (IGAS), Cortes, A., Hadler, J., et al., 2013. Identification of multiple risk variants for ankylosing spondylitis through high-density genotyping of immune-related loci. *Nat. Genet.* 45, 730–738.
- International Multiple Sclerosis Genetics Consortium (IMSGC), Beecham, A.H., Patsopoulos, N.A., et al., 2013. Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. *Nat. Genet.* 45, 1353–1360.
- International Multiple Sclerosis Genetics Consortium (IMSGC), Hafler, D.A., Compston, A., et al., 2007. Risk alleles for multiple sclerosis identified by a genomewide study. *N. Engl. J. Med.* 357, 851–862.
- International Multiple Sclerosis Genetics Consortium (IMSGC), Wellcome Trust Case Control Consortium 2, Sawcer, S., et al., 2011. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature* 476, 214–219.
- International Multiple Sclerosis Genetics Consortium (IMSGC), 2009. The expanding genetic overlap between multiple sclerosis and type I diabetes. *Genes Immun.* 10, 11–14.
- International Multiple Sclerosis Genetics Consortium (IMSGC), 2010. IL12A, MPHOSPH9/CDK2AP1 and RGS1 are novel multiple sclerosis susceptibility loci. *Genes Immun.* 11, 397–405.
- International Multiple Sclerosis Genetics Consortium (IMSGC), 2011. Genome-wide association study of severity in multiple sclerosis. *Genes Immun.* 12, 615–625.
- Jacob, C.O., McDevitt, H.O., 1988. Tumour necrosis factor-alpha in murine autoimmune 'lupus' nephritis. *Nature* 331, 356–358.
- Jacob, C.O., Fronek, Z., Lewis, G.D., et al., 1990. Heritable major histocompatibility complex class II-associated differences in production of tumor necrosis factor alpha: relevance to genetic predisposition to systemic lupus erythematosus. *Proc. Natl. Acad. Sci. U.S.A.* 87, 1233–1237.
- Jacob, C.O., Eisenstein, M., Dinwiddie, M.C., et al., 2012. Lupus-associated causal mutation in neutrophil cytosolic factor 2 (NCF2) brings unique insights to the structure and function of NADPH oxidase. *Proc. Natl. Acad. Sci. U.S.A.* 109, E59–E67.
- Jacob, C.O., Yu, N., Yoo, D.G., et al., 2017. Haploinsufficiency of NADPH oxidase subunit neutrophil cytosolic factor 2 is sufficient to accelerate full-blown lupus in NZM 2328 mice. *Arthritis Rheum.* 69, 1647–1660.
- Jersild, C., Fog, T., 1972. Histocompatibility (HL-A) antigens associated with multiple sclerosis. *Acta Neurol. Scand. Suppl.* 51, 377.
- Jersild, C., Svegaard, A., Fog, T., 1972. HL-A antigens and multiple sclerosis. *Lancet* 1, 1240–1241.
- Jin, Y., Birlea, S.A., Fain, P.R., et al., 2010. Variant of TYR and autoimmunity susceptibility loci in generalized vitiligo. *N. Engl. J. Med.* 362, 1686–1697.

- Jin, Y., Birlea, S.A., Fain, P.R., et al., 2012. Genome-wide association analyses identify 13 new susceptibility loci for generalized vitiligo. *Nat. Genet.* 44, 676–680.
- Johnston, C., Millward, B.A., Hoskins, P., et al., 1989. Islet-cell antibodies as predictors of the later development of type 1 (insulin-dependent) diabetes. A study in identical twins. *Diabetologia* 32, 382–386.
- Jordan, M.A., Silveira, P.A., Shepherd, D.P., et al., 2000. Linkage analysis of systemic lupus erythematosus induced in diabetes-prone nonobese diabetic mice by *Mycobacterium bovis*. *J. Immunol.* 165, 1673–1684.
- Jostins, L., Ripke, S., Weersma, R.K., et al., 2012. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* 491, 119–124.
- Julier, C., Hyer, R.N., Daviews, J., et al., 1991. Insulin-IGF2 region on chromosome 11p encodes a gene implicated in HLA-DR4-dependent diabetes susceptibility. *Nature* 354, 155–159.
- Kachru, R.B., Sequeira, W., Mittal, K.K., et al., 1984. A significant increase of HLA-DR3 and DR2 in systemic lupus erythematosus among blacks. *J. Rheumatol.* 11, 471–474.
- Kallenberg, C.G., Van der Voort-Beelen, J.M., D'Amaro, J., et al., 1981. Increased frequency of B8/DR3 in scleroderma and association of the haplotype with impaired cellular immune response. *Clin. Exp. Immunol.* 43, 478–485.
- Kavvoura, F.K., Ioannidis, J.P., 2005. CTLA-4 gene polymorphisms and susceptibility to type 1 diabetes mellitus: a HuGE review and meta-analysis. *Am. J. Epidemiol.* 162, 3–16.
- Kelly, M.A., Rayner, M.L., Mijovic, C.H., et al., 2003. Molecular aspects of type 1 diabetes. *Mol. Pathol.* 56, 1–10.
- Kennedy, G.C., German, M.S., Rutter, W.J., 1995. The minisatellite in the diabetes susceptibility locus IDDM2 regulates insulin transcription. *Nat. Genet.* 9, 293–298.
- Kent, S.C., Chen, Y., Bregoli, L., et al., 2005. Expanded T cells from pancreatic lymph nodes of type 1 diabetic subjects recognize an insulin epitope. *Nature* 435, 224–228.
- Khalil, I., d'Auriol, L., Gobet, M., et al., 1990. A combination of HLA-DQ beta Asp57-negative and HLA DQ alpha Arg52 confers susceptibility to insulin-dependent diabetes mellitus. *J. Clin. Invest.* 85, 1315–1319.
- Khan, M.A., Braun, W.E., Kushner, I., et al., 1977. HLA B27 in ankylosing spondylitis: differences in frequency and relative risk in American Blacks and Caucasians. *J. Rheumatol. Suppl.* 3, 39–43.
- Kim, E.H., Gasper, D.J., Lee, S.H., Plisch, E.H., Svaren, J., Suresh, M., 2014. Bach2 regulates homeostasis of Foxp3+ regulatory T cells and protects against fatal lung disease in mice. *J. Immunol.* 192, 985–995.
- Koene, H.R., Kleijer, M., Swaak, A.J., et al., 1998. The Fc gammaRIIIA-158F allele is a risk factor for systemic lupus erythematosus. *Arthritis Rheum.* 41, 1813–1818.
- Kofler, D.M., Severson, C.A., Mousissian, N., et al., 2011. The CD6 multiple sclerosis susceptibility allele is associated with alterations in CD4+ T cell proliferation. *J. Immunol.* 187, 3286–3291.
- Kong, A., Cox, N.J., 1997. Allele-sharing models: LOD scores and accurate linkage tests. *Am. J. Hum. Genet.* 61, 1179–1188.
- Kool, M., van Loo, G., Waelput, W., et al., 2011. The ubiquitin-editing protein A20 prevents dendritic cell activation, recognition of apoptotic cells, and systemic autoimmunity. *Immunity* 35, 82–96.
- Kotsa, K., Watson, P.F., Weetman, A.P., 1997. A CTLA-4 gene polymorphism is associated with both Graves disease and autoimmune hypothyroidism. *Clin. Endocrinol. (Oxf.)* 46, 551–554.
- Kouki, T., Sawai, Y., Gardine, C.A., et al., 2000. CTLA-4 gene polymorphism at position 49 in exon 1 reduces the inhibitory function of CTLA-4 and contributes to the pathogenesis of Graves' disease. *J. Immunol.* 165, 6606–6611.
- Kozyrev, S.V., Abelson, A.K., Wojcik, J., et al., 2008. Functional variants in the B-cell gene BANK1 are associated with systemic lupus erythematosus. *Nat. Genet.* 40, 211–216.
- Kruglyak, L., 1999. Prospects for whole-genome linkage disequilibrium mapping of common disease genes. *Nat. Genet.* 22, 139–144.
- Kumar, D., Gemayel, N.S., Deapen, D., et al., 1993. North-American twins with IDDM. Genetic, etiological, and clinical significance of disease concordance according to age, zygosity, and the interval after diagnosis in first twin. *Diabetes* 42, 1351–1363.
- Kuokkanen, S., Gschwend, M., Rioux, J.D., et al., 1997. Genomewide scan of multiple sclerosis in Finnish multiplex families. *Am. J. Hum. Genet.* 61, 1379–1387.
- Kyogoku, C., Langefeld, C.D., Ortmann, W.A., et al., 2004. Genetic association of the R620W polymorphism of protein tyrosine phosphatase PTPN22 with human SLE. *Am. J. Hum. Genet.* 75, 504–507.
- Lander, E., Kruglyak, L., 1995. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat. Genet.* 11, 241–247.
- Lang, H.L., Jacobsen, H., Ikemizu, S., et al., 2002. A functional and structural basis for TCR cross-reactivity in multiple sclerosis. *Nat. Immunol.* 3, 940–943.
- Langrish, C.L., Chen, Y., Blumenschein, W.M., et al., 2005. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J. Exp. Med.* 201, 233–240.
- Lavrikova, E.Y., Nikitin, A.G., Kuraeva, T.L., et al., 2011. The carriage of the type 1 diabetes-associated R262W variant of human LNK correlates with increased proliferation of peripheral blood monocytes in diabetic patients. *Pediatr. Diabetes* 12, 127–132.
- Lawrence, J.S., Martins, C.L., Drake, G.L., 1987. A family survey of lupus erythematosus. *J. Rheumatol.* 14, 913–921.
- Lee, P.W., Smith, A.J., Yang, Y., Selhorst, A.J., Liu, Y., Racke, M.K., et al., 2017. IL-23R-activated STAT3/STAT4 is essential for Th1/Th17-mediated CNS autoimmunity. *JCI Insight* 2, 91663. Available from: <https://doi.org/10.1172/jci.insight.91663>.
- Leikfoss, I.S., Keshari, P.K., Gustavsen, M.W., et al., 2015. Multiple sclerosis risk allele in CLEC16A acts as an expression quantitative trait locus for CLEC16A and SOCS1 in CD4+ T cells. *PLoS One* 10, e0132957.
- Lessard, C.J., Li, H., Adrianto, I., et al., 2013. Variants at multiple loci implicated in both innate and adaptive immune responses are associated with Sjögren's syndrome. *Nat. Genet.* 45, 1284–1292.
- Lewis, M.J., Vyse, S., Shields, A.M., et al., 2015. UBE2L3 polymorphism amplifies NF-κB activation and promotes plasma cell development, linking linear ubiquitination to multiple autoimmune diseases. *Am. J. Hum. Genet.* 96, 221–234.

- Li, Q., Spriggs, M.K., Kovats, S., et al., 1997. Epstein-Barr virus uses HLA class II as a cofactor for infection of B lymphocytes. *J. Virol.* 71, 4657–4662.
- Li, Y., He, X., Schembri-King, J., Jakes, S., Hayashi, J., 2000. Cloning and characterization of human Lnk, an adaptor protein with pleckstrin homology and Src homology 2 domains that can inhibit T cell activation. *J. Immunol.* 164, 5199–5206.
- Li, Y.J., Li, X.Y., Guo, X.R., et al., 2012. Absence of SH2B3 mutation in nonobese diabetic mice. *Genet. Mol. Res.* 11, 1266–1271.
- Ligers, A., Xu, C., Saarinen, S., et al., 1999. The CTLA-4 gene is associated with multiple sclerosis. *J. Neuroimmunol.* 97, 182–190.
- Lindqvist, A.K., Steinsson, K., Johannesson, B., et al., 2000. A susceptibility locus for human systemic lupus erythematosus (hSLE1) on chromosome 2q. *J. Autoimmun.* 14, 169–178.
- Lipsker, D.M., Schreckenberg-Gilliot, C., Uring-Lambert, B., et al., 2000. Lupus erythematosus associated with genetically determined deficiency of the second component of the complement. *Arch. Dermatol.* 136, 1508–1514.
- Liu, X., Lee, Y.S., Yu, C.R., Egwuagu, C.E., 2008. Loss of STAT3 in CD4+ T cells prevents development of experimental autoimmune diseases. *J. Immunol.* 180, 6070–6076.
- Liu, J.Z., Almarri, M.A., Gaffney, D.J., et al., 2012. Dense fine-mapping study identifies new susceptibility loci for primary biliary cirrhosis. *Nat. Genet.* 44, 1137–1141.
- Liu, J.Z., Hov, J.R., Folseraas, T., et al., 2013. Dense genotyping of immune-related disease regions identifies nine new risk loci for primary sclerosing cholangitis. *Nat. Genet.* 45, 670–675.
- Llorente, L., Richaud-Patin, Y., Wijdenes, J., et al., 1993. Spontaneous production of interleukin-10 by B lymphocytes and monocytes in systemic lupus erythematosus. *Eur. Cytokine Netw.* 4, 421–427.
- Llorente, L., Zou, W., Levy, Y., et al., 1995. Role of interleukin 10 in the B lymphocyte hyperactivity and autoantibody production of human systemic lupus erythematosus. *J. Exp. Med.* 181, 839–844.
- Long, S.A., Cerosaletti, K., Wan, J.Y., Ho, J.C., Tatum, M., Wei, S., et al., 2011. An autoimmune-associated variant in PTPN2 reveals an impairment of IL-2R signaling in CD4(+) T cells. *Genes Immun.* 12, 116–125.
- Long, A.E., Gillespie, K.M., Rokni, S., et al., 2012. Rising incidence of type 1 diabetes is associated with altered immunophenotype at diagnosis. *Diabetes* 61, 683–686.
- Lowe, C.E., Cooper, J.D., Brusko, T., et al., 2007. Large-scale genetic fine mapping and genotype-phenotype associations implicate polymorphism in the IL2RA region in type 1 diabetes. *Nat. Genet.* 39, 1074–1082.
- Luo, D.F., Bui, M.M., Muir, A., et al., 1995. Affected-sib-pair mapping of a novel susceptibility gene to insulin-dependent diabetes mellitus (IDDM8) on chromosome 6q25-q27. *Am. J. Hum. Genet.* 57, 911–919.
- Luo, D.F., Buzzetti, R., Rotter, J.I., et al., 1996. Confirmation of three susceptibility genes to insulin-dependent diabetes mellitus: IDDM4, IDDM5 and IDDM8. *Hum. Mol. Genet.* 5, 693–698.
- Luo, J., Niu, X., Liu, H., Zhang, M., Chen, M., Deng, S., 2013. Up-regulation of transcription factor Blimp1 in systemic lupus erythematosus. *Mol. Immunol.* 56, 574–582.
- Luo, J., Niu, X., Zhang, M., Chen, M., Deng, S., 2015. Inhibition of B lymphocyte-induced maturation protein-1 reduces the production of autoantibody and alleviates symptoms of systemic lupus erythematosus. *Autoimmunity* 48, 80–86.
- Maier, L.M., Lowe, C.E., Cooper, J., et al., 2009a. IL2RA genetic heterogeneity in multiple sclerosis and type 1 diabetes susceptibility and soluble interleukin-2 receptor production. *PLoS Genet.* 5, e1000322.
- Maier, L.M., Anderson, D.E., Severson, C.A., et al., 2009b. Soluble IL-2RA levels in multiple sclerosis subjects and the effect of soluble IL-2RA on immune responses. *J. Immunol.* 182, 1541–1547.
- Malek, T.R., Bayer, A.L., 2004. Tolerance, not immunity, crucially depends on IL-2. *Nat. Rev. Immunol.* 4, 665–674.
- Manzotti, C.N., Tipping, H., Perry, L.C., et al., 2002. Inhibition of human T cell proliferation by CTLA-4 utilizes CD80 and requires CD25+ regulatory T cells. *Eur. J. Immunol.* 32, 2888–2896.
- Marron, M.P., Raffel, L.J., Garchon, H.J., et al., 1997. Insulin-dependent diabetes mellitus (IDDM) is associated with CTLA4 polymorphisms in multiple ethnic groups. *Hum. Mol. Genet.* 6, 1275–1282.
- Marroquí, L., Santin, I., Dos Santos, R.S., et al., 2014. BACH2, a candidate risk gene for type 1 diabetes, regulates apoptosis in pancreatic β-cells via JNK1 modulation and crosstalk with the candidate gene PTPN2. *Diabetes* 63, 2516–2527.
- Martin, E.R., Monks, S.A., Warren, L.L., Kaplan, N.L., 2000. A test for linkage and association in general pedigrees: the pedigree disequilibrium test. *Am. J. Hum. Genet.* 67, 146–154.
- Masterman, T., Ligers, A., Olsson, T., et al., 2000. HLA-DR15 is associated with lower age at onset in multiple sclerosis. *Ann. Neurol.* 48, 211–219.
- Mayes, M.D., Bossini-Castillo, L., Gorlova, O., et al., 2014. Immunochip analysis identifies multiple susceptibility loci for systemic sclerosis. *Am. J. Hum. Genet.* 94, 47–61.
- McKinney, C., Merriman, T.R., 2012. Meta-analysis confirms a role for deletion in FCGR3B in autoimmune phenotypes. *Hum. Mol. Genet.* 21, 2370–2376.
- Mein, C.A., Esposito, L., Dunn, M.G., et al., 1998. A search for type 1 diabetes susceptibility genes in families from the United Kingdom. *Nat. Genet.* 19, 297–300.
- Mells, G.F., Floyd, J.A., Morley, K.I., et al., 2011. Genome-wide association study identifies 12 new susceptibility loci for primary biliary cirrhosis. *Nat. Genet.* 43, 329–332.
- Merriman, T., Twells, R., Merriman, M., et al., 1997. Evidence by allelic association-dependent methods for a type 1 diabetes polygene (IDDM6) on chromosome 18q21. *Hum. Mol. Genet.* 6, 1003–1010.
- Misu, T., Onodera, H., Fujihara, K., et al., 2001. Chemokine receptor expression on T cells in blood and cerebrospinal fluid at relapse and remission of multiple sclerosis: imbalance of Th1/Th2-associated chemokine signaling. *J. Neuroimmunol.* 114, 207–212.
- Montomoli, C., Prokopenko, I., Caria, A., et al., 2002. Multiple sclerosis recurrence risk for siblings in an isolated population of Central Sardinia, Italy. *Genet. Epidemiol.* 22, 265–271.
- Moore, F., Colli, M.L., Cnops, M., et al., 2009. PTPN2, a candidate gene for type 1 diabetes, modulates interferon-gamma-induced pancreatic beta-cell apoptosis. *Diabetes* 58, 1283–1291.

- Moore, K.W., de Waal Malefyt, R., Coffman, R.L., et al., 2001. Interleukin-10 and the interleukin-10 receptor. *Annu. Rev. Immunol.* 19, 683–765.
- Morahan, G., Huang, D., Tait, B.D., et al., 1996. Markers on distal chromosome 2q linked to insulin-dependent diabetes mellitus. *Science* 272, 1811–1813.
- Mori, T., Iwasaki, Y., Seki, Y., et al., 2014. Lnk/Sh2b3 controls the production and function of dendritic cells and regulates the induction of IFN- $\gamma$ -producing T cells. *J. Immunol.* 193, 1728–1736.
- Moriguchi, K., Miyamoto, K., Tanaka, N., Yoshie, O., Kusunoki, S., 2013. The importance of CCR4 and CCR6 in experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* 257, 53–58.
- Moriguchi, K., Miyamoto, K., Tanaka, N., et al., 2016. C-C chemokine receptor type 4 antagonist Compound 22 ameliorates experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* 291, 54–58.
- Moser, K.L., Neas, B.R., Salmon, J.E., et al., 1998. Genome scan of human systemic lupus erythematosus: evidence for linkage on chromosome 1q in African-American pedigrees. *Proc. Natl. Acad. Sci. U.S.A.* 95, 14869–14874.
- Munger, K.L., Levin, L.I., Hollis, B.W., et al., 2006. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. *JAMA* 296, 2832–2838.
- Muthian, G., Raikwar, H.P., Rajasingh, J., Bright, J.J., 2006. 1,25 Dihydroxyvitamin-D3 modulates JAK-STAT pathway in IL-12/IFNgamma axis leading to Th1 response in experimental allergic encephalomyelitis. *J. Neurosci. Res.* 83, 1299–1309.
- Myhr, K.M., Riise, T., Barrett-Connor, E., et al., 1998. Altered antibody pattern to Epstein-Barr virus but not to other herpesviruses in multiple sclerosis: a population based case-control study from western Norway. *J. Neurol. Neurosurg. Psychiatry* 64, 539–542.
- Naito, S., Namerow, N., Mickey, M.R., et al., 1972. Multiple sclerosis: association with HL-A3. *Tissue Antigens* 2, 1–4.
- Nakai, H., Morito, T., Tanimoto, K., et al., 1977. Reduced Fc-receptor bearing cells in peripheral bloods of patients with systemic lupus erythematosus and in rheumatoid synovial fluids. *J. Rheumatol.* 4, 405–413.
- Nath, S.K., Han, S., Kim-Howard, X., et al., 2008. A nonsynonymous functional variant in integrin-alpha(M) (encoded by ITGAM) is associated with systemic lupus erythematosus. *Nat. Genet.* 40, 152–154.
- Naves, M., Hajeer, A.H., The, L.S., et al., 1998. Complement C4B null allele status confers risk for systemic lupus erythematosus in a Spanish population. *Eur. J. Immunogenet.* 25, 317–320.
- Nerup, J., Platz, P., Ortved-Andersen, O., et al., 1974. HL-A antigens and diabetes mellitus. *Lancet* 2, 864–866.
- Ng, M.C., Shriner, D., Chen, B.H., et al., 2014. Meta-analysis of genome-wide association studies in African Americans provides insights into the genetic architecture of type 2 diabetes. *PLoS Genet.* 10, e1004517.
- Nielsen, T.R., Rostgaard, K., Askling, J., et al., 2009. Effects of infectious mononucleosis and HLA-DRB1\*15 in multiple sclerosis. *Mult. Scler.* 15, 431–436.
- Nieves, J.F., Cosman, J., Herbert, V., et al., 1994. High prevalence of vitamin D deficiency and reduced bone mass in multiple sclerosis. *Neurology* 44, 1687–1692.
- Nisticò, L., Buzzetti, R., Pritchard, L.E., et al., 1996. The CTLA-4 gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes. *Hum. Mol. Genet.* 5, 1075–1080.
- Noble, J.A., Valdes, A.M., Cook, M., et al., 1996. The role of HLA class II genes in insulin-dependent diabetes mellitus: molecular analysis of 180 Caucasian, multiplex families. *Am. J. Hum. Genet.* 59, 1134–1148.
- Nordmark, G., Kristjansdottir, G., Theander, E., et al., 2009. Additive effects of the major risk alleles of IRF5 and STAT4 in primary Sjögren's syndrome. *Genes Immun.* 10, 68–76.
- Oaks, M.K., Hallett, K.M., Penwell, R.T., et al., 2000. A native soluble form of CTLA-4. *Cell Immunol.* 201, 144–153.
- Okada, Y., Wu, D., Trynka, G., et al., 2014. Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature* 506, 376–381.
- Oliveira, M.I., Gonçalves, C.M., Pinto, M., et al., 2012. CD6 attenuates early and late signaling events, setting thresholds for T-cell activation. *Eur. J. Immunol.* 42, 195–205.
- Onengut-Gumuscu, S., Ewens, K.G., Spielman, R.S., et al., 2004. A functional polymorphism (1858C/T) in the PTPN22 gene is linked and associated with type I diabetes in multiplex families. *Genes Immun.* 5, 678–680.
- Onengut-Gumuscu, S., Chen, W.-M., Burren, O., et al., 2015. Fine mapping of type 1 diabetes susceptibility loci and evidence for colocalization of causal variants with lymphoid gene enhancers. *Nat. Genet.* 47, 381–386.
- Onkamo, P., Väänänen, S., Karvonen, M., et al., 1999. Worldwide increase in incidence of type I diabetes—the analysis of the data on published incidence trends. *Diabetologia* 42, 1395–1403. Erratum in: *Diabetologia* 2000; 43, 685.
- Owerbach, D., Gabbay, K.H., 1995. The HOXD8 locus (2q31) is linked to type I diabetes. Interaction with chromosomes 6 and 11 disease susceptibility genes. *Diabetes* 44, 132–136.
- Panchanathan, R., Liu, H., Liu, H., et al., 2012. Distinct regulation of murine lupus susceptibility genes by the IRF5/Blimp-1 axis. *J. Immunol.* 188, 270–278.
- Panitch, H.S., Hirsch, R.L., Haley, A.S., Johnson, K.P., 1987. Exacerbations of multiple sclerosis in patients treated with gamma interferon. *Lancet* 1, 893–895.
- Parkes, M., Cortes, A., van Heel, D.A., Brown, M.A., 2013. Genetic insights into common pathways and complex relationships among immune-mediated diseases. *Nat. Rev. Genet.* 14, 661–673.
- Paterson, A.D., Rahman, P., Petronis, A., 1999. IDDM9 and a locus for rheumatoid arthritis on chromosome 3q appear to be distinct. *Hum. Immunol.* 60, 883–885.
- Payne, R., Rolfs, M.R., 1958. Fetomaternal leukocyte incompatibility. *J. Clin. Invest.* 37, 1756–1762.
- Petukhova, L., Duvic, M., Hordinsky, M., et al., 2010. Genome-wide association study in alopecia areata implicates both innate and adaptive immunity. *Nature* 466, 113–117.
- Pociot, F., McDermott, M.F., 2002. Genetics of type 1 diabetes mellitus (review). *Genes Immun.* 3, 235–249.
- Price, P., Witt, C., Allcock, R., et al., 1999. The genetic basis for the association of the 8.1 ancestral haplotype (A1, B8, DR3) with multiple immunopathological diseases. *Immunol. Rev.* 167, 257–274.
- Provost, T.T., Arnett, F.C., Reichlin, M., 1983. Homozygous C2 deficiency, lupus erythematosus, and anti-Ro (SSA) antibodies. *Arthritis Rheum.* 26, 1279–1282.

- Qian, T., Chen, F., Shi, X., et al., 2017. Upregulation of the C/EBP  $\beta$  LAP isoform could be due to decreased TNFAIP3/TNIP1 expression in the peripheral blood mononuclear cells of patients with systemic lupus erythematosus. *Mod. Rheumatol.* 27, 657–663.
- Ramagopalan, S.V., Maugeri, N.J., Handunnetthi, L., et al., 2009. Expression of the multiple sclerosis-associated MHC class II allele HLA-DRB1\*1501 is regulated by vitamin D. *PLoS Genet.* 5, e1000369.
- Ramagopalan, S.V., Heger, A., Berlanga, A.J., et al., 2010. A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution. *Genome Res.* 20, 1352–1360.
- Raveney, B.J., Oki, S., Hohjoh, H., et al., 2015. Eomesodermin-expressing T-helper cells are essential for chronic neuroinflammation. *Nat. Commun.* 6, 8437.
- Raychaudhuri, S., Remmers, E.F., Lee, A.T., et al., 2008. Common variants at CD40 and other loci confer risk of rheumatoid arthritis. *Nat. Genet.* 40, 1216–1223.
- Remmers, E.F., Plenge, R.M., Lee, A.T., et al., 2007. STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus. *N. Engl. J. Med.* 357, 977–986.
- Rieck, M., Arechiga, A., Onengut-Gumuscu, S., et al., 2007. Genetic variation in PTPN22 corresponds to altered function of T and B lymphocytes. *J. Immunol.* 179, 4704–4710.
- Roberts, K.G., Morin, R.D., Zhang, J., et al., 2012. Genetic alterations activating kinase and cytokine receptor signaling in high-risk acute lymphoblastic leukemia. *Cancer Cell* 22, 153–166.
- Robertson, N.P., Clayton, D., Fraser, M., et al., 1996. Clinical concordance in sibling pairs with multiple sclerosis. *Neurology* 47, 347–352.
- Roychoudhuri, R., Hirahara, K., Mousavi, K., et al., 2013. BACH2 represses effector programs to stabilize T(reg)-mediated immune homeostasis. *Nature* 498, 506–510.
- Rubio, J.P., Stankovich, J., Field, J., et al., 2008. Replication of KIAA0350, IL2RA, RPL5 and CD58 as multiple sclerosis susceptibility genes in Australians. *Genes Immun.* 9, 624–630.
- Rudwaleit, M., Tikly, M., Khamashita, M., et al., 1996. Interethnic differences in the association of tumor necrosis factor promoter polymorphisms with systemic lupus erythematosus. *J. Rheumatol.* 23, 1725–1728.
- Sadovnick, A.D., Armstrong, H., Rice, G.P., et al., 1993. A population-based study of multiple sclerosis in twins: update. *Ann. Neurol.* 33, 281–285.
- Sadovnick, A.D., Baird, P.A., 1988. The familial nature of multiple sclerosis: age-corrected empiric recurrence risks for children and siblings of patients. *Neurology* 38, 990–991.
- Salloum, R., Franek, B.S., Kariuki, S.N., et al., 2010. Genetic variation at the IRF7/PHRF1 locus is associated with autoantibody profile and serum interferon-alpha activity in lupus patients. *Arthritis Rheum.* 62, 553–561.
- Salmon, J.E., Millard, S., Schachter, L.A., et al., 1996. Fc gamma RIIA alleles are heritable risk factors for lupus nephritis in African Americans. *J. Clin. Invest.* 97, 1348–1354.
- Salmon, J.E., Ng, S., Yoo, D.H., et al., 1999. Altered distribution of Fcgamma receptor IIIA alleles in a cohort of Korean patients with lupus nephritis. *Arthritis Rheum.* 42, 818–819.
- Santin, I., Moore, F., Colli, M.L., et al., 2011. PTPN2, a candidate gene for type 1 diabetes, modulates pancreatic  $\beta$ -cell apoptosis via regulation of the BH3-only protein Bim. *Diabetes* 60, 3279–3288.
- Sawcer, S., Jones, H.B., Feakes, R., et al., 1996. A genome screen in multiple sclerosis reveals susceptibility loci on chromosome 6p21 and 17q22. *Nat. Genet.* 13, 464–468.
- Schifferli, J.A., Ng, Y.C., Peters, D.K., 1986. The role of complement and its receptor in the elimination of immune complexes. *N. Engl. J. Med.* 315, 488–495.
- Schlosstein, L., Terasaki, P.I., Bluestone, R., et al., 1973. High association of an HL-A antigen, W27, with ankylosing spondylitis. *N. Engl. J. Med.* 288, 704–708.
- Segal, B.M., Constantinescu, C.S., Raychaudhuri, A., et al., 2008. Repeated subcutaneous injections of IL12/23 p40 neutralising antibody, ustekinumab, in patients with relapsing-remitting multiple sclerosis: a phase II, double-blind, placebo-controlled, randomised, dose-ranging study. *Lancet Neurol.* 7, 796–804.
- Seidi, O.A., Semra, Y.K., Sharief, M.K., 2002. Expression of CD5 on B lymphocytes correlates with disease activity in patients with multiple sclerosis. *J. Neuroimmunol.* 133, 205–210.
- Shai, R., Quismorio Jr, F.P., Li, L., et al., 1999. Genome-wide screen for systemic lupus erythematosus susceptibility genes in multiplex families. *Hum. Mol. Genet.* 8, 639–644.
- Sigurdsson, S., Nordmark, G., Göring, H.H., et al., 2005. Polymorphisms in the tyrosine kinase 2 and interferon regulatory factor 5 genes are associated with systemic lupus erythematosus. *Am. J. Hum. Genet.* 76, 528–537.
- Simpfendorfer, K.R., Olsson, L.M., Manjarrez Orduño, N., et al., 2012. The autoimmunity-associated BLK haplotype exhibits cis-regulatory effects on mRNA and protein expression that are prominently observed in B cells early in development. *Hum. Mol. Genet.* 21, 3918–3925.
- Smyth, D.J., Cooper, J.D., Collins, J.E., et al., 2004. Replication of an association between the lymphoid tyrosine phosphatase locus LYP/PTPN22 with type 1 diabetes, and evidence for its role as a general autoimmunity locus. *Diabetes* 53, 3020–3023.
- Smyth, D.J., Cooper, J.D., Bailey, R., et al., 2006. A genome-wide association study of nonsynonymous SNPs identifies a type 1 diabetes locus in the interferon-induced helicase (IFIH1) region. *Nat. Genet.* 38, 617–619.
- So, A.K., Fielder, A.H., Warner, C.A., et al., 1990. DNA polymorphism of major histocompatibility complex class II and class III genes in systemic lupus erythematosus. *Tissue Antigens* 35, 144–147.
- Soleimanpour, S.A., Gupta, A., Bakay, M., et al., 2014. The diabetes susceptibility gene Clec16a regulates mitophagy. *Cell* 157, 1577–1590.
- Soleimanpour, S.A., Ferrari, A.M., Raum, J.C., et al., 2015. Diabetes susceptibility genes Pdx1 and Clec16a function in a pathway regulating mitophagy in  $\beta$ -cells. *Diabetes* 64, 3475–3484.
- Stahl, E.A., Raychaudhuri, S., Remmers, E.F., et al., 2010. Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. *Nat. Genet.* 42, 508–514.
- Steck, A.K., Rewers, M.J., 2011. Genetics of type 1 diabetes. *Clin. Chem.* 57, 176–185.
- Steck, A.K., Liu, S.Y., McFann, K., et al., 2006. Association of the PTPN22/LYP gene with type 1 diabetes. *Pediatr. Diabetes* 7, 274–278.

- Steck, A.K., Baschal, E.E., Jasinski, J.M., et al., 2009. rs2476601 T allele (R620W) defines high-risk PTPN22 type I diabetes-associated haplotypes with preliminary evidence for an additional protective haplotype. *Genes Immun.* 10, S21–S26.
- Stoll, T., Seifert, B., Isenberg, D.A., 1996. SLICC/ACR damage index is valid, and renal and pulmonary organ scores are predictors of severe outcome in patients with systemic lupus erythematosus. *Br. J. Rheumatol.* 35, 248–254.
- Stretell, M.D., Thomson, L.J., Donaldson, P.T., et al., 1997. HLA-C genes and susceptibility to type 1 autoimmune hepatitis. *Hepatology* 26, 1023–1026.
- Sullivan, K.E., Petri, M.A., Schmeckpeper, B.J., et al., 1994. Prevalence of a mutation causing C2 deficiency in systemic lupus erythematosus. *J. Rheumatol.* 21, 1128–1133.
- Sumaya, C.V., Myers, L.W., Ellison, G.W., et al., 1985. Increased prevalence and titer of Epstein-Barr virus antibodies in patients with multiple sclerosis. *Ann. Neurol.* 17, 371–377.
- Takaoka, A., Yanai, H., Kondo, S., et al., 2005. Integral role of IRF-5 in the gene induction programme activated by Toll-like receptors. *Nature* 434, 243–249.
- Tan, F.K., Arnett, F.C., 1998. The genetics of lupus. *Curr. Opin. Rheumatol.* 10, 399–408.
- Tavares, R.M., Turer, E.E., Liu, C.L., et al., 2010. The ubiquitin modifying enzyme A20 restricts B cell survival and prevents autoimmunity. *Immunity* 33, 181–191.
- Taylor, K.E., Remmers, E.F., Lee, A.T., et al., 2008. Specificity of the STAT4 genetic association for severe disease manifestations of systemic lupus erythematosus. *PLoS Genet.* 4, e1000084.
- Texido, G., Su, I.H., Mecklenbräuker, I., et al., 2000. The B-cell-specific Src-family kinase Blk is dispensable for B-cell development and activation. *Mol. Cell. Biol.* 20, 1227–1233.
- Thakker, P., Leach, M.W., Kuang, W., et al., 2007. IL-23 is critical in the induction but not in the effector phase of experimental autoimmune encephalomyelitis. *J. Immunol.* 178, 2589–2598.
- The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group, 1999. TNF neutralization in MS: results of a randomized, placebo-controlled multicenter study. *Neurology* 53, 457–465.
- Thorsby, E., 1997. Invited anniversary review: HLA associated diseases. *Hum. Immunol.* 53, 1–11.
- Thorsby, E., 2009. A short history of HLA. *Tissue Antigens* 74, 101–116.
- Tillil, H., Köbberling, J., 1987. Age-corrected empirical genetic risk estimates for first-degree relatives of IDDM patients. *Diabetes* 36, 93–99.
- Todd, J.A., 1992. Genetic analysis of susceptibility to type 1 diabetes. *Springer Semin. Immunopathol.* 14, 33–58.
- Todd, J.A., 1995. Genetic analysis of type 1 diabetes using whole genome approaches. *Proc. Natl. Acad. Sci. U.S.A.* 92, 8560–8565.
- Todd, J.A., Bell, J.I., McDevitt, H.O., 1987. HLA-DQ beta gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. *Nature* 329, 599–604.
- Todd, J.A., Walker, N.M., Cooper, J.D., et al., 2007. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. *Nat. Genet.* 39, 857–864.
- Trynka, G., Hunt, K.A., Bockett, N.A., et al., 2011. Dense genotyping identifies and localizes multiple common and rare variant association signals in celiac disease. *Nat. Genet.* 43, 1193–1201.
- Tsang, S., Sun, Z., Luke, B., et al., 2005. A comprehensive SNPbased genetic analysis of inbred mouse strains. *Mamm. Genome.* 16, 476–480.
- Tsao, B.P., Cantor, R.M., Kalunian, K.C., et al., 1997. Evidence for linkage of a candidate chromosome 1 region to human systemic lupus erythematosus. *J. Clin. Invest.* 99, 725–731.
- Tsoi, L.C., Spain, S.L., Knight, J., et al., 2012. Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity. *Nat. Genet.* 44, 1341–1348.
- Tsuchiya, N., Komata, T., Matsushita, M., et al., 2000. New single nucleotide polymorphisms in the coding region of human TNFR2: association with systemic lupus erythematosus. *Genes Immun.* 1, 501–503.
- Ueda, H., Howson, J.M., Esposito, L., et al., 2003. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* 423, 506–511.
- Vafiadis, P., Bennett, S.T., Todd, J.A., et al., 1997. Insulin expression in human thymus is modulated by INS VNTR alleles at the IDDM2 locus. *Nat. Genet.* 15, 289–292.
- Valdes, A.M., Noble, J.A., Genin, E., et al., 2001. Modeling of HLA class II susceptibility to type 1 diabetes reveals an effect associated with DPB1. *Genet. Epidemiol.* 21, 212–223.
- Valdes, A.M., Erlich, H.A., Noble, J.A., 2005. Human leukocyte antigen class I B and C loci contribute to type 1 diabetes (T1D) susceptibility and age at T1D onset. *Hum. Immunol.* 66, 301–313.
- van Rood, J.J., Eernisse, J.G., van Leeuwen, A., 1958. Leucocyte antibodies in sera from pregnant women. *Nature* 181, 1735–1736.
- Vandenborre, K., Van Gool, S.W., Kasran, A., et al., 1999. Interaction of CTLA-4 (CD152) with CD80 or CD86 inhibits human T-cell activation. *Immunology* 98, 413–421.
- Vang, T., Congia, M., Macis, M.D., et al., 2005. Autoimmune-associated lymphoid tyrosine phosphatase is a gain-of-function variant. *Nat. Genet.* 37, 1317–1319.
- Vazquez, B.N., Laguna, T., Notario, L., et al., 2012. Evidence for an intronic cis-regulatory element within CD69 gene. *Genes Immun.* 13, 356–362.
- Velazquez, L., Cheng, A.M., Fleming, H.E., et al., 2002. Cytokine signaling and hematopoietic homeostasis are disrupted in Lnk-deficient mice. *J. Exp. Med.* 195, 1599–1611.
- Vella, A., Cooper, J.D., Lowe, C.E., et al., 2005. Localization of a type 1 diabetes locus in the IL2RA/CD25 region by use of tag single-nucleotide polymorphisms. *Am. J. Hum. Genet.* 76, 773–779.
- Vijayakrishnan, L., Slavik, J.M., Illés, Z., et al., 2004. An autoimmune disease-associated CTLA-4 splice variant lacking the B7 binding domain signals negatively in T cells. *Immunity* 20, 563–575.
- Villar, L.M., Espiño, M., Roldán, E., et al., 2011. Increased peripheral blood CD5+ B cells predict earlier conversion to MS in high-risk clinically isolated syndromes. *Mult. Scler.* 17, 690–694.
- Vyse, T.J., Kotzin, B.L., 1998. Genetic susceptibility to systemic lupus erythematosus. *Annu. Rev. Immunol.* 16, 261–292.

- Walport, M.J., 1993. The Roche rheumatology prize lecture. Complement deficiency and disease. *Br. J. Rheumatol.* 32, 269–273.
- Walunas, T.L., Lenschow, D.J., Bakker, C.Y., et al., 1994. CTLA-4 can function as a negative regulator of T cell activation. *Immunity* 1, 405–413.
- Wang, W.Y., Barratt, B.J., Clayton, D.G., et al., 2005. Genome-wide association studies: theoretical and practical concerns. *Nat. Rev. Genet.* 6, 109–118.
- Wang, H., Jin, Y., Reddy, M.V., Podolsky, R., et al., 2010. Genetically dependent ERBB3 expression modulates antigen presenting cell function and type 1 diabetes risk. *PLoS One* 5, e11789.
- Wang, S., Adrianto, I., Wiley, G.B., et al., 2012. A functional haplotype of UBE2L3 confers risk for systemic lupus erythematosus. *Genes Immun.* 13, 380–387.
- Wapelhorst, B., Bell, G.I., Risch, N., et al., 1995. Linkage and association studies in insulin-dependent diabetes with a new dinucleotide repeat polymorphism at the GAD65 locus. *Autoimmunity* 21, 127–130.
- Weber, F., Fontaine, B., Cournu-Rebeix, I., et al., 2008. IL2RA and IL7RA genes confer susceptibility for multiple sclerosis in two independent European populations. *Genes Immun.* 9, 259–263.
- Weiss, K.M., Terwilliger, J.D., 2000. How many diseases does it take to map a gene with SNPs? *Nat. Genet.* 26, 151–157.
- Wellcome Trust Case Control Consortium, Australo-Anglo-American Spondylitis Consortium (TASC), Burton, P.R., et al., 2007. Association scan of 14,500 nonsynonymous SNPs in four common diseases identifies variants involved in autoimmunity. *Nat. Genet.* 39, 1329–1337.
- Wiede, F., Shields, B.J., Chew, S.H., et al., 2011. T cell protein tyrosine phosphatase attenuates T cell signaling to maintain tolerance in mice. *J. Clin. Invest.* 121, 4758–4774.
- Wilson, A.G., Gordon, C., di Giovine, F.S., et al., 1994. A genetic association between systemic lupus erythematosus and tumor necrosis factor alpha. *Eur. J. Immunol.* 24, 191–195.
- Windhagen, A., Newcombe, J., Dangond, F., et al., 1995. Expression of costimulatory molecules B7-1 (CD80), B7-2 (CD86), and interleukin 12 cytokine in multiple sclerosis lesions. *J. Exp. Med.* 182, 1985–1996.
- Wu, J., Edberg, J.C., Redecha, P.B., et al., 1997. A novel polymorphism of Fc gamma RIIIa (CD16) alters receptor function and predisposes to autoimmune disease. *J. Clin. Invest.* 100, 1059–1070.
- Xi, Y., Liu, S., Bettaieb, A., et al., 2015. Pancreatic T cell protein–tyrosine phosphatase deficiency affects beta cell function in mice. *Diabetologia* 58, 122–131.
- Yamada, H., Watanabe, A., Mimori, A., et al., 1990. Lack of gene deletion for complement C4A deficiency in Japanese patients with systemic lupus erythematosus. *J. Rheumatol.* 17, 1054–1057.
- Yanagawa, T., Hidaka, Y., Guimaraes, V., et al., 1995. CTLA-4 gene polymorphism associated with Graves' disease in a Caucasian population. *J. Clin. Endocrinol. Metab.* 80, 41–45.
- Yu, D.T., Choo, S.Y., Schaack, T., 1989. Molecular mimicry in HLA-B27-related arthritis. *Ann. Intern. Med.* 111, 581–591.
- Zheng, W., She, J.X., 2005. Genetic association between a lymphoid tyrosine phosphatase (PTPN22) and type 1 diabetes. *Diabetes* 54, 906–908.
- Ziegler, A.G., Pflueger, M., Winkler, C., et al., 2011. Accelerated progression from islet autoimmunity to diabetes is causing the escalating incidence of type 1 diabetes in young children. *J. Autoimmun.* 37, 3–7.
- Zimmerman, A.W., Joosten, B., Torensma, R., Parnes, J.R., van Leeuwen, F.N., Figdor, C.G., 2006. Long-term engagement of CD6 and ALCAM is essential for T-cell proliferation induced by dendritic cells. *Blood* 107, 3212–3220.

# Sexual Dimorphism in the Immune System

Pamela A. McCombe and Judith M. Greer

The University of Queensland, UQ Centre for Clinical Research, Royal Brisbane & Women's Hospital,  
Brisbane, QLD, Australia

## OUTLINE

Introduction	419	Role of the Sex Chromosomes in Immunity	423
Overview of Sexual Dimorphism	419	X Chromosome	423
		Y Chromosome	423
Sexual Dimorphism in the Immune System	420	Environmental Effects on Sex Differences in Immunity	424
Effects of Hormones on the Immune System	421	Consequences for Autoimmunity of Sexual Dimorphism in the Immune System	424
Estrogens	421		
Progesterone	422		
Androgens	422	References	425

## INTRODUCTION

Sexual dimorphism is the term that refers to differences between males and females of the same species and is most obvious as differences in external appearances. However, there can also be sexual dimorphism of internal organs and biological functions, including the immune system. Sexual dimorphism in the immune system is important in medicine because it can lead to sex differences in the responses to infection and vaccination and sex differences in the development of autoimmune disease (McCombe et al., 2009). This chapter focuses on the effects of sexual dimorphism on immunocompetence, and on some of the possible mechanisms underlying these effects, including the effects of the sex hormones, sex chromosomes, and sexually dimorphic cultural and environmental effects on immunity. The possible consequences for autoimmunity of sexual dimorphism in the immune system are also considered.

## OVERVIEW OF SEXUAL DIMORPHISM

The sex chromosomes evolved many millions of years ago (Livernois et al., 2012). In humans, most males have X and Y chromosomes and most females have two X chromosomes, although other combinations of sex chromosomes are found, with such as one X chromosome (Turner's syndrome) or XXY (Klinefelter's syndrome). Sexual dimorphism was noted by Darwin (1871) who thought it was due to sexual selection, as, for example, larger stronger males might have a reproductive advantage. Other possibilities for the evolution of sexual dimorphism include intersexual competition for food (Hedrick and Temeles, 1989) and other ecological factors (Kruger et al., 2014). More recently, it has been suggested that males and females have evolved to be dimorphic

in the “Pace of Life” because of differing requirements (Immonen et al., 2018). Overall, in humans, males are larger than females, with greater strength, but shorter life expectancy. In medicine, there are sex differences in the prevalence of many diseases, and there can be sex differences in response to medications.

Sexual dimorphism is related to the sex chromosome complement of an individual (Wijchers and Festenstein, 2011). Many genes show sex-specific differences in expression (Mank, 2009; Ellegren and Parsch, 2007; Mank, 2017). There are also reports of sex differences in DNA methylation (Ho et al., 2018). We will now discuss the sex differences in the immune system.

## SEXUAL DIMORPHISM IN THE IMMUNE SYSTEM

Immunocompetence is a word used to describe the overall level of function of the immune system and is a complex genetic trait (Flori et al., 2011). It has been recognized that females have increased immunoreactivity (immunocompetence) compared to males and suggested that this leads to increased autoimmune disease in females (Zandman-Goddard et al., 2007). In the past, it has been suggested that the expression of male sexual traits is inversely related to immunocompetence (the immunocompetence handicap hypothesis) (Folstad and Karter, 1992). However, further studies have not confirmed this (Roberts et al., 2004) and others have suggested that the differences in overall immunocompetence between males and females are related to the pressures of selection on the basis of fitness. It is thought that fitness in females is maximized by lengthening the lifespan through greater investment in immune defenses whereas in males require fitness early in life to maximize sexual mating and so do not need to invest in immune defenses to prolong the lifespan (Nunn et al., 2009). There are also differences between males and females in immunosenescence, which is the decline in immune function in later life, with greater decline in males (Yan et al., 2010; Das et al., 2008).

Overall immunocompetence is the sum of the elements of the immune system. There is sexual dimorphism of many of the individual components of the immune system. In terms of cell counts, there are numerical differences between the males and females. In males the total lymphocyte count is similar to that in females (Bouman et al., 2004; Giltay et al., 2000), but the percentage of T cells within the lymphocyte population is lower (Bouman et al., 2004). The ratio of CD4<sup>+</sup>:CD8<sup>+</sup> cells is greater in females than in males (Wikby et al., 2008; Das et al., 2008). There are estrogen receptors (ERs) on regulatory T cells (Tregs); this could be important in autoimmunity (Aristimuno et al., 2012).

There are no reports of differences in B-cell counts between females and males; however, there are long-recognized differences in levels of IgM but not in other immunoglobulin isotypes, with females showing increased levels for all ages  $>6$  years (Butterworth et al., 1967; Lichtman et al., 1967). The reproductive phase of females also influences the immune system, as postmenopausal women, compared to fertile women, have fewer total lymphocytes [as a consequence of decreased numbers of B and T helper cells (Th cells)] (Giglio et al., 1994; Yang et al., 2000).

In laboratory animals, there are also sex differences in the structure of primary immune organs: in male rats the thymus is heavier, has a greater yield of cells, and contains a higher percentage of double negative CD4<sup>-</sup>CD8<sup>-</sup> cells (Leposavic et al., 1996) and catecholamine-containing cells (Pilipovic et al., 2008) than thymi of female rats. However, in humans, in autopsy studies, no difference was found between the weight of adult thymi from females or males, after correction for body weight (Narongchai and Narongchai, 2008). Possibly the differences are more clear-cut in inbred laboratory animals.

There are differences in the function of the immune system of males and females. Generally, females produce more vigorous humoral and cellular immune responses than males (Ansar et al., 1985; Weinstein et al., 1984), shown in mice in a variety of test systems such as an augmented responses to a variety of antigens (Terres et al., 1968), the ability to reject allografts more rapidly than males (Kongshavn and Bliss, 1970), and in mice and humans by better in vitro responses to mitogens (Santoli et al., 1976; Weinstein et al., 1984) and relative resistance to the induction of immune tolerance (Bebo et al., 1999). Following trauma-hemorrhage, immune functions are severely depressed in young male mice, ovariectomized and aged females but are maintained in proestrus females (Choudhry et al., 2007; Kahlke et al., 2000). The survival rate in proestrus females following trauma-hemorrhage and the induction of subsequent sepsis are significantly higher than in age-matched males and ovariectomized females.

Furthermore, females are more successful than males in resisting a variety of bacterial, viral, and parasitic infestations and this enhanced immune capability might explain, in part at least, why the life expectancy of females exceeds that of males. For example, female mice have a greater ability than male to combat various

infections, including Leishmaniasis and amebic infection with liver abscess (Lotter et al., 2006), which is thought to be due to the sex difference in Th1 and Th2 responses (Mock and Nacy, 1988). Moreover, in Wistar rats infected with *Trypanosoma cruzi*, there is less parasitemia in females than males (dos Santos et al., 2005). Of relevance to autoimmunity, there are gender differences in resistance to inflammation of the heart after Coxsackie virus infection, which are inferior in males and related to poorer Treg responses in males than females (Frisancho-Kiss et al., 2007). In immunized mice, females show greater production of the Th1 cytokine interferon (IFN)- $\gamma$  than males (Huygen and Palfiet, 1984). These differences in Th1 and Th2 response are mediated in part through IL-13 (Sinha et al., 2008).

In humans, the antibody response to a variety of vaccines and test antigens, including flagellin protein (Rowley and Mackay, 1969), influenza, hepatitis B, rubella, tetanus (Cook, 2008), and a variety of other viral vaccines (Klein et al., 2010), is more vigorous in females than in males. In women, the innate immune response is also stronger.

Another, less quantifiable, functional difference between females and males is seen in the immune response to injury, according to reports on greater degrees of immunosuppression in men than in women after physical trauma (Choudhry et al., 2005) and after abdominal surgery (Wichmann et al., 2003) as measured by B-lymphocyte, T-lymphocyte, Th cell counts, and natural killer (NK) cell counts and circulating levels of IL-6.

There are many examples of sex differences in biochemical pathways involved in immunity. Sexual dimorphism is also expressed at a cellular level, since stimulation of invariant natural killer T cells with  $\alpha$ -galactosylceramide leads to higher concentrations of IFN- $\gamma$  in the serum of female mice (Gourdy et al., 2005) and the concentration of serotonin and histamine in white blood cells and mast cells is greater in females than in male rats (Csaba et al., 2003).

## EFFECTS OF HORMONES ON THE IMMUNE SYSTEM

Sexual dimorphism in the immune system could be due to the effects of sex and reproductive hormones. These hormones have cognate receptors that are widely expressed on many cell types, including cells of the immune system. There is evidence that sexual dimorphism in immune functions in C57Bl/6 mice is dependent on puberty, which suggests that the effects of the sex hormones are important (Lamason et al., 2006). There are numerous effects of the sex hormones in human immunity (Bouman et al., 2005). The effects of the various sex hormones on immune function are summarized below.

### Estrogens

There are three estrogenic hormones, estrogen (E1), estradiol (E2), and estriol (E3), with E3 being produced only in pregnancy. As well as being present in high levels in females, estrogens are also present in males, in whom they have widespread effects (Sharpe, 1998). Estrogens act on intracellular ERs. The best known ERs (ER $\alpha$  and ER $\beta$ ) belong to the class known as ligand-regulated nuclear transcription factors (Matthews and Gustafsson, 2003). There is also a membrane-bound G-protein-coupled ER (Prossnitz et al., 2007), that is prominent on vascular tissue and has rapid "nongenomic" effects (Simoncini et al., 2004). ERs are widespread and have important effects on the growth of cells. ER $\alpha$  and ER $\beta$  are expressed on antigen-presenting cells (Nalbandian and Kovats, 2005), and at higher levels in CD4 $^+$  T cells and B cells than in CD8 $^+$  T cells; however, whereas B cells express higher levels of ER $\beta$  than ER $\alpha$ , CD4 $^+$  T cells show higher levels of ER $\alpha$  than ER $\beta$  (Pernis, 2007).

The results of many experiments indicate that estrogen can affect immune function: for example, T lymphocytes from patients with systemic lupus erythematosus (SLE), but not from healthy controls, can be activated via ER $\alpha$  and ER $\beta$  to increase expression of the T-cell activation markers CD154 and calcineurin (Rider et al., 2006). Furthermore, different types of estrogens can have differential effects (Ding and Zhu, 2008). Estrogen induces thymic atrophy by preventing the proliferation of early thymic progenitor cells (Zoller and Kersh, 2006), and this is due in part to the effects of estrogen on the membrane-bound ER (Wang et al., 2008). Estrogen has effects on dendritic cells (DCs) (Ding and Zhu, 2008), modulates the effects of lipopolysaccharide on monocytes (Pioli et al., 2007), and inhibits monocyte adhesion to endothelium (Nilsson, 2007). Such results, however, may not relate to the role of estrogens at physiological levels. Much of what has been observed *in vitro* is likely to be what occurs in high estrogen states, such as pregnancy, rather than in nonpregnant women.

Estrogen has numerous effects on the function of CD4<sup>+</sup> T cells (Pernis, 2007). Low doses of estrogen, as would occur in nonpregnant women, enhance Th1 responses whereas high levels, as would occur in pregnancy, enhance Th2 responses. Estrogen also influences chemokine production by activated splenocytes (Lengi et al., 2007) and influences T-cell trafficking. E2 can convert CD25<sup>-</sup> cells to CD25<sup>+</sup> Tregs (Tai et al., 2008). Thus enhancement of Th2 responses and induction of Treg cells by high doses of estrogens could ameliorate Th1-mediated autoimmune diseases. Estrogen enhances antibody production in response to antigen immunization (Da Silva, 1999a). This effect differs between E3 and E2 (Ding and Zhu, 2008), with E3 strongly stimulating antibody response to bacteria (Da Silva, 1999b).

There is some evidence that estrogens are more directly involved in autoimmunity. Thus in patients with myasthenia gravis, there is an alteration of expression of ER in the thymus (Nancy and Berrih-Aknin, 2005), a site wherein the disease may be generated (Hill et al., 2008). In SLE, estrogen promotes the survival of B cells with affinity for DNA (as does prolactin) (Grimaldi, 2006). Genetic studies among Japanese show that there are associations of polymorphisms in the ER in rheumatoid arthritis (Takagi et al., 2000), although not in autoimmune diabetes (Ban et al., 2000). In females with multiple sclerosis (MS) who carry HLA-DR2, carriage of an ER $\alpha$ 4 polymorphism conveys an additional risk of developing disease (Mattila et al., 2001).

## Progesterone

Progesterone is a steroid hormone that is present in the blood during the menstrual cycle and in high levels in pregnancy. Adult males have levels similar to those in women during the follicular phase of the menstrual cycle. In women, progesterone is produced in the ovary as well as the placenta during pregnancy. In males progesterone is produced by the adrenal gland and testes. The action of progesterone is mediated through cytosolic progesterone receptors, of which there are three isoforms, belonging to the nuclear hormone receptor superfamily of transcription factors. The different isoforms are widely distributed and have specific tissue expression patterns that can be modified following exposure to hormones. Ligand binding induces changes in gene expression through direct binding to promoter elements or through protein–protein interactions with other transcription factors (Tait et al., 2008). In some cells, including T cells (Dosiou et al., 2008), progesterone may also act via membrane G-coupled protein receptors that rapidly transduce hormone-induced signals across the cell membrane (BoonyaratanaKornkit et al., 2008). Since these additional phenomena do not depend on the genome, these actions are called nongenomic or extranuclear effects (Wehling and Losel, 2006).

Progesterone has immunomodulatory effects (Tait et al., 2008) which are generally antiinflammatory, including inhibition of glucocorticoid-mediated thymocyte apoptosis (McMurray et al., 2000), and reduction of nitric oxide production (Miller et al., 1996) and expression of Toll-like receptors (TLRs) (Jones et al., 2008) by macrophages. Progesterone promotes Th2 differentiation in vitro (Piccinni et al., 1995) and also influences antibody production, especially the production of antibodies that are asymmetrically glycosylated in such a way that they fail to trigger immune effector mechanisms. Such antibodies are thought to be self-protective (Canellada et al., 2002). High doses of progesterone enhance differentiation of human and mouse DCs in vitro and expression of the costimulatory molecules CD80, CD86, and CD40, as well as major histocompatibility complex (MHC) class II (Ivanova et al., 2005; Liang et al., 2006).

## Androgens

Androgens, the best known of which is testosterone, stimulate or control the development and maintenance of masculine characteristics; however, they have other functions, including maintenance of bone mass in both women and men. Androgens are also the precursors of all estrogens. In males, androgens, primarily testosterone, are present in high levels, but these decline with increasing age (Mitchell et al., 1995). In contrast, in females, lower levels of androgens (particularly the adrenal-derived androgens dehydroepiandrosterone (DHEA) and DHEA-S) persist throughout life (Davison et al., 2005). The best known androgen receptors are nuclear receptors, but nongenomic signaling also occurs (Lutz et al., 2003).

The potent and rapidly occurring immunosuppressive effects of testosterone in mice occur at least in part by induction of thymic atrophy, and cessation of testosterone production by castration of males results in thymic hypertrophy; these effects occur via interaction of sex hormones with receptors on thymic stromal cells (Hince et al., 2008). Testosterone also impairs thymocyte proliferation in response to the mitogen Con A (Yao and Shang, 2005). In men treated with medical castration, there is a reduction in levels of CD4<sup>+</sup> CD25<sup>+</sup> Tregs and in IFN- $\gamma$  production following mitogen-induced activation of CD8<sup>+</sup> T cells (Page et al., 2006).

## ROLE OF THE SEX CHROMOSOMES IN IMMUNITY

The differences between males and females are due to differences in sex chromosomes. People with a Y chromosome show masculinization, due largely to the effects of the testis forming gene, *sry*, on the Y chromosome, and the effects of high levels of testosterone. However, the other genes on the sex chromosomes play a role in sexual dimorphism, largely through epigenetic effects, and the *sry* gene also has other effects than in testis formation (Wijchers et al., 2010). We will now examine the effects of sex chromosomes in immunity.

### X Chromosome

The increased prevalence of autoimmunity in females has generated considerable interest in the X chromosome in immunity, particularly since there was shown to be a direct influence of the overall complement of X chromosomes to the female bias toward autoimmune disease (Smith-Bouvier et al., 2008). This could be related to autoimmune susceptibility genes on the X chromosome.

There are genes on the X chromosome of potential relevance for autoimmunity, including those encoding the IL-2R $\gamma$  chain (also known as the common  $\gamma$  chain because it is shared by receptors for IL-2, IL-7, IL-15, and IL-21) (Alves et al., 2007), and *Foxp3*, which is important in the development and function of Tregs (Zheng and Rudensky, 2007). Mutations of these genes lead to X-linked severe combined immune deficiency (Leonard et al., 1994) and IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome (Bennett et al., 2001), respectively. Other molecules encoded by genes on the X chromosome that might be important for autoimmunity are CD154 (CD40 ligand), which is overexpressed on CD4 $^{+}$  T cells in some autoimmune diseases (Toubi and Shoenfeld, 2004), tissue inhibitors of metalloproteinases 1–4, which are important in inflammation (Anderson and Brown, 2002), the X-linked inhibitor of apoptosis that regulates T-cell function (Zehntner et al., 2007), and the pattern recognition receptors TLR7 and TLR8, both of which are important in recognition of single-stranded RNA and which have also been associated with asthma and other inflammatory diseases (Moller-Larsen et al., 2008; Fish, 2008).

In women with Turners syndrome, who have X monosomy in all cells, there is an increased incidence of autoimmune disease overall, and especially thyroid autoimmunity (Wilson et al., 1996). Interestingly, this thyroid disease is associated with an X isochromosome, which is a condition when one arm of a chromosome is deleted and replaced with an exact copy of that arm from the sister chromosome. This means that both of these identical arms would have the same epigenetic modifications—depending on whether the arm came originally from mother or father (Elsheikh et al., 2001).

Males who have SLE in addition to Klinefelter's syndrome (wherein they have at least two X chromosomes and at least one Y chromosome) share many characteristics of the female with SLE (Lahita, 1999), including increased oxidation of androgens, suggesting that there is some role for the two X chromosomes in this process, although exactly what this role is may be is not yet known.

### Y Chromosome

The Y chromosome contains few genes. Most of the DNA is male specific and the remainder is autosomal. The Y chromosome encodes at least 27 proteins, some of which are confined to testis and some of which are more widely expressed (Skaletsky et al., 2003). The most important Y chromosome gene is *Sry*, which is the gene responsible for the formation of testes and masculine features.

There is some effect of Y chromosome genes on development of experimental autoimmune encephalomyelitis (EAE), the animal model of multiple sclerosis, since female offspring of backcross strains carrying the susceptible SJL/J Y chromosome behaved more like their male siblings than like the female mice from three other birth crosses carrying the resistant C57BL/6 Y chromosome, both in lesion severity (reduced) and clinical sensitivity to CNS damage (increased) (Teuscher et al., 2006). With increased age, male SJL/J mice show increased susceptibility to EAE, and this has been shown to be due to a polymorphism on the Y chromosome (Spach et al., 2009). Y chromosomes candidate genes potentially responsible for these effects include *Hya*, *Yaa*, and *Sry* (Teuscher et al., 2006).

Interestingly, *Yaa*, which predisposes to autoimmunity in mice, is actually a translocation of a cluster of X chromosome genes, including the gene encoding TLR7 (Subramanian et al., 2006). It has been demonstrated that even relatively modest increases in the expression of TLR7 can result in dramatic lymphocyte activation and autoimmunity in mice (Deane et al., 2007). It has been suggested that a similar effect in humans could dictate

susceptibility to SLE. However, a study of 190 male and female SLE patients and controls found no lupus-related difference in copy number of the *TLR7* gene, suggesting that human lupus is not commonly due to a *Yaa*-like mutation (Kelley et al., 2007).

## ENVIRONMENTAL EFFECTS ON SEX DIFFERENCES IN IMMUNITY

As with many issues in biology, there can be an interaction between genes and the environment. The sex differences noted above are what is observed in humans living in natural conditions, and it is possible that there is plasticity in the immune responses in response to the environment. It is theorized that the sex differences are a result of the pressures of the environment (availability of food, availability of sexual partners) and that the males and females differ in traits that limit their fitness, so the response to the environment will differ. It is possible that alteration of the environment could alter the sexual dimorphism in the immune system. In experiments with *Drosophila*, increased availability of food led to increased female immune function and increased availability of mates led to decreased male immune function (McKean and Nunney, 2005). It is possible that gender differences in human immune function could be affected by environmental and social factors. It is now known that the gut microbiota plays an important role in the shaping of the immune system, and in autoimmunity. There is evidence that there is sexual dimorphism in the interaction of the gut, nutrition, and the immune system.

## CONSEQUENCES FOR AUTOIMMUNITY OF SEXUAL DIMORPHISM IN THE IMMUNE SYSTEM

There is a marked female preponderance in many autoimmune diseases (McCombe et al., 2009; Ngo et al., 2014) and it is likely that this is due, at least in part, to the stronger female immune system. When autoimmune disease occurs in males, this could mean that these individuals have immune systems that are particularly vulnerable to disease. This theory is known as the Carter effect, which is applicable to polygenic disorders (Carter, 1969), and suggests that if a subject has an innate biological resistance to a particular disease, then there will be a greater burden of genetic risk factors required to overcome that resistance for disease to occur. Thus for a male to develop a true autoimmune disorder would require that there needs to be a much heavier load of other contributing elements, be they genetic or environmental: males who do present with diseases such as autoimmune thyroid disorder, primary biliary cirrhosis, or lupus may warrant particular scrutiny, as nonsex-related causal elements might be more readily identifiable.

There may also be other gender-specific differences that indirectly affect immune reactivity and contribute to skewing of susceptibility to autoimmune disease in females (McCombe et al., 2009; Greer and McCombe, 2011; Pan and Chang, 2012; Borchers and Gershwin, 2012; Pollard, 2012; Nussinovitch and Shoenfeld, 2012; Quintero et al., 2012). These differences could include sexual dimorphism in the vulnerability of the target organ to

**TABLE 24.1** Immune Functions that Show Sexual Dimorphism

Immune functions that show sexual dimorphism	Difference between males and females
Percentage of lymphocytes in total leukocyte population	Lower in males (Bouman et al., 2004)
Immunosenescence	Greater in males
IgM levels	Greater in females (Butterworth et al., 1967; Lichtman et al., 1967)
Allograft rejection	Greater in females (Kongshavn and Bliss, 1970)
In vitro response to mitogens	Greater in females (Santoli et al., 1976; Weinstein et al., 1984)
Resistance to the induction of immune tolerance	Greater in females (Bebo et al., 1999)
Ability to combat infection	Greater in females (Lotter et al., 2006; dos Santos et al., 2005)
Response to vaccination	Greater in women (Klein et al., 2010)
CD4 + :CD8 + ratio	Greater in women (Wikby et al., 2008; Das et al., 2008)
Th1 responses	Greater in females (Huygen and Palfriet, 1984)

autoimmune attack; epigenetic effects of the sex hormones or gender-specific environmental or lifestyle exposures on genetic risk factors; differences in genomic imprinting in males and females; and effects related to female reproduction. The future challenge is to determine which of these differences between males and females is most important in the development of autoimmunity, since understanding the influences of sexual dimorphism in autoimmune diseases is likely to provide opportunities for better therapies (Table 24.1).

## References

- Alves, N.L., Arosa, F.A., Van lier, R.A., 2007. Common gamma chain cytokines: dissidence in the details. *Immunol. Lett.* 108, 113–120.
- Anderson, C.L., Brown, C.J., 2002. Variability of X chromosome inactivation: effect on levels of TIMP1 RNA and role of DNA methylation. *Hum. Genet.* 110, 271–278.
- Ansar, A.S., Penhale, W.J., Talal, N., 1985. Sex hormones, immune responses, and autoimmune diseases. Mechanisms of sex hormone action. *Am. J. Pathol.* 121, 531–551.
- Aristimuno, C., Teijeiro, R., Valor, L., Alonso, B., Tejera-Alhambra, M., De andres, C., et al., 2012. Sex-hormone receptors pattern on regulatory T-cells: clinical implications for multiple sclerosis. *Clin. Exp. Med.* 12, 247–255.
- Ban, Y., Taniyama, M., Tozaki, T., Tomita, M., 2000. Estrogen receptor alpha dinucleotide repeat polymorphism in Japanese patients with autoimmune thyroid diseases. *BMC. Med. Genet.* 1, 1.
- Bebo Jr., B.F., Adlard, K., Schuster, J.C., Unsicker, L., Vandenbergk, A.A., Offner, H., 1999. Gender differences in protection from EAE induced by oral tolerance with a peptide analogue of MBP-Ac1-11. *J. Neurosci. Res.* 55, 432–440.
- Bennett, C.L., Christie, J., Ramsdell, F., Brunkow, M.E., Ferguson, P.J., Whitesell, L., et al., 2001. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat. Genet.* 27, 20–21.
- Boonyaratelanakornkit, V., Bi, Y., Rudd, M., Edwards, D.P., 2008. The role and mechanism of progesterone receptor activation of extra-nuclear signaling pathways in regulating gene transcription and cell cycle progression. *Steroids* 73, 922–928.
- Borchers, A.T., Gershwin, M.E., 2012. Sociological differences between women and men: implications for autoimmunity. *Autoimmun. Rev.* 11, A413–A421.
- Bouman, A., Schipper, M., Heineman, M.J., Faas, M.M., 2004. Gender difference in the non-specific and specific immune response in humans. *Am. J. Reprod. Immunol.* 52, 19–26.
- Bouman, A., Heineman, M.J., Faas, M.M., 2005. Sex hormones and the immune response in humans. *Hum. Reprod. Update* 11, 411–423.
- Butterworth, M., McClellan, B., Allansmith, M., 1967. Influence of sex in immunoglobulin levels. *Nature* 214, 1224–1225.
- Canellada, A., Blois, S., Gentile, T., Margni idehu, R.A., 2002. In vitro modulation of protective antibody responses by estrogen, progesterone and interleukin-6. *Am. J. Reprod. Immunol.* 48, 334–343.
- Carter, C.O., 1969. Genetics of common disorders. *Br. Med. Bull.* 25, 52–57.
- Choudhry, M.A., Schwacha, M.G., Hubbard, W.J., Kerby, J.D., Rue, L.W., Bland, K.I., et al., 2005. Gender differences in acute response to trauma-hemorrhage. *Shock* 24 (Suppl. 1), 101–106.
- Choudhry, M.A., Bland, K.I., Chaudry, I.H., 2007. Trauma and immune response—effect of gender differences. *Injury* 38, 1382–1391.
- Cook, I.F., 2008. Sexual dimorphism of humoral immunity with human vaccines. *Vaccine* 26, 3551–3555.
- Csaba, G., Kovacs, P., Pallinger, E., 2003. Gender differences in the histamine and serotonin content of blood, peritoneal and thymic cells: a comparison with mast cells. *Cell Biol. Int.* 27, 387–389.
- Darwin, C., 1871. *The Sessent of Man and Selection in Relation to Sex*. John Murray.
- Das, B.R., Bhanushali, A.A., Khadapkar, R., Jeswani, K.D., Bhavasar, M., Dasgupta, A., 2008. Reference ranges for lymphocyte subsets in adults from western India: influence of sex, age and method of enumeration. *Indian J. Med. Sci.* 62, 397–406.
- Da Silva, J.A., 1999a. Sex hormones and glucocorticoids: interactions with the immune system. *Ann. N.Y. Acad. Sci.* 876, 102–117.
- Da Silva, J.A.P., 1999b. Sex hormones and glucocorticoids: interactions with the immune system. *Ann. N.Y. Acad. Sci.* 876, 102–118.
- Davison, S.L., Bell, R., Donath, S., Montalto, J.G., Davis, S.R., 2005. Androgen levels in adult females: changes with age, menopause, and oophorectomy. *J. Clin. Endocrinol. Metab.* 90, 3847–3853.
- Deane, J.A., Pisitkun, P., Barrett, R.S., Feigenbaum, L., Town, T., Ward, Jerrold M., et al., 2007. Control of toll-like receptor 7 expression is essential to restrict autoimmunity and dendritic cell proliferation. *Immunity* 27, 801–810.
- Ding, J., Zhu, B.T., 2008. Unique effect of the pregnancy hormone estriol on antigen-induced production of specific antibodies in female BALB/c mice. *Steroids* 73, 289–298.
- Dosiou, C., Hamilton, A.E., Pang, Y., Overgaard, M.T., Tulac, S., Dong, J., et al., 2008. Expression of membrane progesterone receptors on human T lymphocytes and Jurkat cells and activation of G-proteins by progesterone. *J. Endocrinol.* 196, 67–77.
- dos Santos, C.D., Toldo, M.P., DO Prado Junior, J.C., 2005. Trypanosoma cruzi: the effects of dehydroepiandrosterone (DHEA) treatment during experimental infection. *Acta Trop.* 95, 109–115.
- Ellegren, H., Parsch, J., 2007. The evolution of sex-biased genes and sex-biased gene expression. *Nat. Rev. Genet.* 8, 689–698.
- Elsheikh, M., Wass, J.A., Conway, G.S., 2001. Autoimmune thyroid syndrome in women with Turner's syndrome—the association with karyotype. *Clin. Endocrinol. (Oxf.)* 55, 223–226.
- Fish, E.N., 2008. The X-files in immunity: sex-based differences predispose immune responses. *Nat. Rev. Immunol.* 8, 737–744.
- Flori, L., Gao, Y., Laloe, D., Lemonnier, G., Leplat, J.J., Teillaud, A., et al., 2011. Immunity traits in pigs: substantial genetic variation and limited covariation. *PLoS One* 6, e22717.
- Folstad, I., Karter, A.J., 1992. Parasites, bright males, and the immunocompetence handicap. *Am. Nat.* 139, 603–622.
- Frisancho-Kiss, S., Davis, S.E., Nyland, J.F., Frisancho, J.A., Cihakova, D., Barrett, M.A., et al., 2007. Cutting edge: cross-regulation by TLR4 and T cell Ig mucin-3 determines sex differences in inflammatory heart disease. *J. Immunol.* 178, 6710–6714.
- Giglio, T., Imro, M.A., Filaci, G., Scudeletti, M., Puppo, F., De, C.L., et al., 1994. Immune cell circulating subsets are affected by gonadal function. *Life Sci.* 54, 1305–1312.

- Giltay, E.J., Fonk, J.C., Von blomberg, B.M., Drexhage, H.A., Schalkwijk, C., Gooren, L.J., 2000. In vivo effects of sex steroids on lymphocyte responsiveness and immunoglobulin levels in humans. *J. Clin. Endocrinol. Metab.* 85, 1648–1657.
- Gourdy, P., Araujo, L.M., Zhu, R., Garmy-Susini, B., Diem, S., Laurell, H., et al., 2005. Relevance of sexual dimorphism to regulatory T cells: estradiol promotes IFN-gamma production by invariant natural killer T cells. *Blood* 105, 2415–2420.
- Greer, J.M., McCombe, P.A., 2011. Role of gender in multiple sclerosis: clinical effects and potential molecular mechanisms. *J. Neuroimmunol.* 234, 7–18.
- Grimaldi, C.M., 2006. Sex and systemic lupus erythematosus: the role of the sex hormones estrogen and prolactin on the regulation of auto-reactive B cells. *Curr. Opin. Rheumatol.* 18, 456–461.
- Hedrick, A.V., Temeles, E.J., 1989. The evolution of sexual dimorphism in animals: hypotheses and tests. *Trends Ecol. Evol.* 4, 136–138.
- Hill, M.E., Shiono, H., Newsom-Davis, J., Willcox, N., 2008. The myasthenia gravis thymus: a rare source of human autoantibody-secreting plasma cells for testing potential therapeutics. *J. Neuroimmunol.* 201–202, 50–56.
- Hince, M., Sakkal, S., Vlahos, K., Dudakov, J., Boyd, R., Chidgey, A., 2008. The role of sex steroids and gonadectomy in the control of thymic involution. *Cell Immunol.* 252, 122–138.
- Ho, B., Greenlaw, K., Al tuwaijri, A., Moussette, S., Martinez, F., Giorgio, E., et al., 2018. X chromosome dosage and presence of SRY shape sex-specific differences in DNA methylation at an autosomal region in human cells. *Biol. Sex Differ.* 9, 10.
- Huygen, K., Palfriet, K., 1984. Strain variation in interferon gamma production of BCG-sensitized mice challenged with PPD II. Importance of one major autosomal locus and additional sexual influences. *Cell Immunol.* 85, 75–81.
- Immonen, E., Hamalainen, A., Schuett, W., Tarka, M., 2018. Evolution of sex-specific pace-of-life syndromes: genetic architecture and physiological mechanisms. *Behav. Ecol. Sociobiol.* 72, 60.
- Ivanova, E., Kyurkchiev, D., Altankova, I., Dimitrov, J., Binakova, E., Kyurkchiev, S., 2005. CD83 monocyte-derived dendritic cells are present in human decidua and progesterone induces their differentiation in vitro. *Am. J. Reprod. Immunol.* 53, 199–205.
- Jones, L.A., Anthony, J.P., Henriquez, F.L., Lyons, R.E., Nickdel, M.B., Carter, K.C., et al., 2008. Toll-like receptor-4-mediated macrophage activation is differentially regulated by progesterone via the glucocorticoid and progesterone receptors. *Immunology* 125, 59–69.
- Kahlke, V., Angele, M.K., Schwacha, M.G., Ayala, A., Cioffi, W.G., Bland, K.I., et al., 2000. Reversal of sexual dimorphism in splenic T lymphocyte responses after trauma-hemorrhage with aging. *Am. J. Physiol. Cell Physiol.* 278, C509–C516.
- Kelley, J., Johnson, M.R., Alarcón, G.S., Kimberly, R.P., Edberg, J.C., 2007. Variation in the relative copy number of the *TLR7* gene in patients with systemic lupus erythematosus and healthy control subjects. *Arthritis Rheum.* 56, 3375–3378.
- Klein, S.L., Jedlicka, A., Pekosz, A., 2010. The Xs and Y of immune responses to viral vaccines. *Lancet Infect. Dis.* 10, 338–349.
- Kongshavn, P.A., Bliss, J.Q., 1970. Sex differences in survival of H-2 incompatible skin grafts in mice treated with antithymocyte serum. *Nature* 226, 451.
- Kruger, O., Wolf, J.B., Jonker, R.M., Hoffman, J.I., Trillmich, F., 2014. Disentangling the contribution of sexual selection and ecology to the evolution of size dimorphism in pinnipeds. *Evolution* 68, 1485–1496.
- Lahita, R.G., 1999. Emerging concepts for sexual predilection in the disease systemic lupus erythematosus. *Ann. N.Y. Acad. Sci.* 876, 64–70.
- Lamason, R., Zhao, P., Rawat, R., Davis, A., Hall, J.C., Chae, J.J., et al., 2006. Sexual dimorphism in immune response genes as a function of puberty. *BMC Immunol.* 7, 2.
- Lengi, A.J., Phillips, R.A., Karpuzoglu, E., Ahmed, S.A., 2007. Estrogen selectively regulates chemokines in murine splenocytes. *J. Leukoc. Biol.* 81, 1065–1074.
- Leonard, W.J., Noguchi, M., Russell, S.M., McBride, O.W., 1994. The molecular basis of X-linked severe combined immunodeficiency: the role of the interleukin-2 receptor gamma chain as a common gamma chain, gamma c. *Immunol. Rev.* 138, 61–86.
- Leposavic, G., Karapetrovic, B., Obradovic, S., Vidiic, D.B., Kosec, D., 1996. Differential effects of gonadectomy on the thymocyte phenotypic profile in male and female rats. *Pharmacol. Biochem. Behav.* 54, 269–276.
- Liang, J., Sun, L., Wang, Q., Hou, Y., 2006. Progesterone regulates mouse dendritic cells differentiation and maturation. *Int. Immunopharmacol.* 6, 830–838.
- Lichtman, M.A., Vaughan, J.H., Hames, C.G., 1967. The distribution of serum immunoglobulins, anti-gamma-G globulins ("rheumatoid factors") and antinuclear antibodies in White and Negro subjects in Evans County, Georgia. *Arthritis Rheum.* 10, 204–215.
- Livernois, A.M., Graves, J.A., Waters, P.D., 2012. The origin and evolution of vertebrate sex chromosomes and dosage compensation. *Heredity* 108, 50–58.
- Lotter, H., Jacobs, T., Gaworski, I., Tannich, E., 2006. Sexual dimorphism in the control of amebic liver abscess in a mouse model of disease. *Infect. Immun.* 74, 118–124.
- Lutz, L.B., Jamnongjit, M., Yang, W.H., Jahani, D., Gill, A., Hammes, S.R., 2003. Selective modulation of genomic and nongenomic androgen responses by androgen receptor ligands. *Mol. Endocrinol.* 17, 1106–1116.
- Mank, J.E., 2009. Sex chromosomes and the evolution of sexual dimorphism: lessons from the genome. *Am. Nat.* 173, 141–150.
- Mank, J.E., 2017. The transcriptional architecture of phenotypic dimorphism. *Nat. Ecol. Evol.* 1, 6.
- Matthews, J., Gustafsson, J.A., 2003. Estrogen signaling: a subtle balance between ER alpha and ER beta. *Mol. Interv.* 3, 281–292.
- Mattila, K.M., Luomala, M., Lehtimaki, T., Laippala, P., Koivula, T., Elovaara, I., 2001. Interaction between ESR1 and HLA-DR2 may contribute to the development of MS in women. *Neurology* 56, 1246–1247.
- McCombe, P.A., Greer, J.M., Mackay, I.R., 2009. Sexual dimorphism in autoimmune disease. *Curr. Mol. Med.* 9, 1058–1079.
- McKean, K.A., Nunney, L., 2005. Bateman's principle and immunity: phenotypically plastic reproductive strategies predict changes in immunological sex differences. *Evolution* 59, 1510–1517.
- McMurray, R.W., Wilson, J.G., Bigler, L., Xiang, L., Lagoo, A., 2000. Progesterone inhibits glucocorticoid-induced murine thymocyte apoptosis. *Int. J. Immunopharmacol.* 22, 955–965.
- Miller, L., Alley, E.W., Murphy, W.J., Russell, S.W., Hunt, J.S., 1996. Progesterone inhibits inducible nitric oxide synthase gene expression and nitric oxide production in murine macrophages. *J. Leukoc. Biol.* 59, 442–450.
- Mitchell, R., Hollis, S., Rothwell, C., Robertson, W.R., 1995. Age related changes in the pituitary-testicular axis in normal men: lower serum testosterone results from decreased bioactive LH drive. *Clin. Endocrinol. (Oxf.)* 42, 501–507.

- Mock, B.A., Nacy, C.A., 1988. Hormonal modulation of sex differences in resistance to *Leishmania major* systemic infections. *Infect. Immun.* 56, 3316–3319.
- Moller-Larsen, S., Nyegaard, M., Haagerup, A., Vestbo, J., Kruse, T.A., Borglum, A.D., 2008. Association analysis identifies TLR7 and TLR8 as novel risk genes in asthma and related disorders. *Thorax* 63, 1064–1069.
- Nalbandian, G., Kovats, S., 2005. Understanding sex biases in immunity: effects of estrogen on the differentiation and function of antigen-presenting cells. *Immunol. Res.* 31, 91–106.
- Nancy, P., Berrih-Akinin, S., 2005. Differential estrogen receptor expression in autoimmune myasthenia gravis. *Endocrinology* 146, 2345–2353.
- Narongchai, P., Narongchai, S., 2008. Study of the normal internal organ weights in Thai population. *J. Med. Assoc. Thai.* 91, 747–753.
- Ngo, S.T., Steyn, F.J., McCombe, P.A., 2014. Gender differences in autoimmune disease. *Front. Neuroendocrinol.* 35, 347–369.
- Nilsson, B.O., 2007. Modulation of the inflammatory response by estrogens with focus on the endothelium and its interactions with leukocytes. *Inflamm. Res.* 56, 269–273.
- Nunn, C.L., Lindenfors, P., Pursall, E.R., Rolff, J., 2009. On sexual dimorphism in immune function. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 364, 61–69.
- Nussinovitch, U., Shoenfeld, Y., 2012. The role of gender and organ specific autoimmunity. *Autoimmun. Rev.* 11, A377–A385.
- Page, S.T., Plymate, S.R., Bremner, W.J., Matsumoto, A.M., Hess, D.L., Lin, D.W., et al., 2006. Effect of medical castration on CD4 + CD25 + T cells, CD8 + T cell IFN-gamma expression, and NK cells: a physiological role for testosterone and/or its metabolites. *Am. J. Physiol. Endocrinol. Metab.* 290, E856–E863.
- Pan, Z., Chang, C., 2012. Gender and the regulation of longevity: implications for autoimmunity. *Autoimmun. Rev.* 11, A393–A403.
- Pernis, A.B., 2007. Estrogen and CD4 + T cells. *Curr. Opin. Rheumatol.* 19, 414–420.
- Piccinni, M.P., Giudizi, M.G., Biagiotti, R., Beloni, L., Giannarini, L., Sampognaro, S., et al., 1995. Progesterone favors the development of human T helper cells producing Th2-type cytokines and promotes both IL-4 production and membrane CD30 expression in established Th1 cell clones. *J. Immunol.* 155, 128–133.
- Pilipovic, I., Vidic-Dankovic, B., Perisic, M., Radojevic, K., Colic, M., Todorovic, V., et al., 2008. Sexual dimorphism in the catecholamine-containing thymus microenvironment: a role for gonadal hormones. *J. Neuroimmunol.* 195, 7–20.
- Pioli, P.A., Jensen, A.L., Weaver, L.K., Amiel, E., Shen, Z., Shen, L., et al., 2007. Estradiol attenuates lipopolysaccharide-induced CXC chemokine ligand 8 production by human peripheral blood monocytes. *J. Immunol.* 179, 6284–6290.
- Pollard, K.M., 2012. Gender differences in autoimmunity associated with exposure to environmental factors. *J. Autoimmun.* 38, J177–J186.
- Prossnitz, E.R., Arterburn, J.B., Sklar, L.A., 2007. GPR30: a G protein-coupled receptor for estrogen. *Mol. Cell Endocrinol.* 265-266, 138–142.
- Quintero, O.L., Amador-Patarroyo, M.J., Montoya-Ortiz, G., Rojas-Villarraga, A., Anaya, J.M., 2012. Autoimmune disease and gender: plausible mechanisms for the female predominance of autoimmunity. *J. Autoimmun.* 38, J109–J119.
- Rider, V., Li, X., Peterson, G., Dawson, J., Kimler, B.F., Abdou, N.I., 2006. Differential expression of estrogen receptors in women with systemic lupus erythematosus. *J. Rheumatol.* 33, 1093–1101.
- Roberts, M.L., Buchanan, K.L., Evans, M.R., 2004. Testing the immunocompetence handicap hypothesis: a review of the evidence. *Anim. Behav.* 68, 227–239.
- Rowley, M.J., Mackay, I.R., 1969. Measurement of antibody-producing capacity in man. I. The normal response to flagellin from *Salmonella adelaide*. *Clin. Exp. Immunol.* 5, 407–418.
- Santoli, D., Trinchieri, G., Zmijewski, C.M., Koprowski, H., 1976. HLA-related control of spontaneous and antibody-dependent cell-mediated cytotoxic activity in humans. *J. Immunol.* 117, 765–770.
- Sharpe, R.M., 1998. The roles of oestrogen in the male. *Trends Endocrinol. Metab.* 9, 371–377.
- Simoncini, T., Mannella, P., Fornari, L., Caruso, A., Varone, G., Genazzani, A.R., 2004. Genomic and non-genomic effects of estrogens on endothelial cells. *Steroids* 69, 537–542.
- Sinha, S., Kaler, L.J., Proctor, T.M., Teuscher, C., Vandebark, A.A., Offner, H., 2008. IL-13-mediated gender difference in susceptibility to autoimmune encephalomyelitis. *J. Immunol.* 180, 2679–2685.
- Skaletsky, H., Kuroda-Kawaguchi, T., Minx, P.J., Cordum, H.S., Hillier, L., Brown, L.G., et al., 2003. The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. *Nature* 423, 825–837.
- Smith-Bouvier, D.L., Divekar, A.A., Sasidhar, M., Du, S., Tiwari-Woodruff, S.K., King, J.K., et al., 2008. A role for sex chromosome complement in the female bias in autoimmune disease. *J. Exp. Med.* 205, 1099–1108.
- Spach, K.M., Blake, M., Bunn, J.Y., McElvany, B., Noubade, R., Blankenhorn, E.P., et al., 2009. Cutting edge: the Y chromosome controls the age-dependent experimental allergic encephalomyelitis sexual dimorphism in SJL/J mice. *J. Immunol.* 182, 1789–1793.
- Subramanian, S., Tus, K., Li, Q.Z., Wang, A., Tian, X.H., Zhou, J., et al., 2006. A Tlr7 translocation accelerates systemic autoimmunity in murine lupus. *Proc. Natl. Acad. Sci. U.S.A.* 103, 9970–9975.
- Tai, P., Wang, J., Jin, H., Song, X., Yan, J., Kang, Y., et al., 2008. Induction of regulatory T cells by physiological level estrogen. *J. Cell Physiol.* 214, 456–464.
- Tait, A.S., Butts, C.L., Sternberg, E.M., 2008. The role of glucocorticoids and progestins in inflammatory, autoimmune, and infectious disease. *J. Leukoc. Biol.* 84, 924–931.
- Takagi, H., Ishiguro, N., Iwata, H., Kanamono, T., 2000. Genetic association between rheumatoid arthritis and estrogen receptor microsatellite polymorphism. *J. Rheumatol.* 27, 1638–1642.
- Terres, G., Morrison, S.L., Habicht, G.S., 1968. A quantitative difference in the immune response between male and female mice. *Proc. Soc. Exp. Biol. Med.* 127, 664–667.
- Teuscher, C., Noubade, R., Spach, K., McElvany, B., Bunn, J.Y., Fillmore, P.D., et al., 2006. Evidence that the Y chromosome influences autoimmune disease in male and female mice. *Proc. Natl. Acad. Sci. U.S.A.* 103, 8024–8029.
- Toubi, E., Shoenfeld, Y., 2004. The role of CD40-CD154 interactions in autoimmunity and the benefit of disrupting this pathway. *Autoimmunity* 37, 457–464.
- Wang, C., Dehghani, B., Magrisso, I.J., Rick, E.A., Bonhomme, E., Cody, D.B., et al., 2008. GPR30 contributes to estrogen-induced thymic atrophy. *Mol. Endocrinol.* 22, 636–648.

- Wehling, M., Losel, R., 2006. Non-genomic steroid hormone effects: membrane or intracellular receptors? *J. Steroid Biochem. Mol. Biol.* 102, 180–183.
- Weinstein, Y., Ran, S., Segal, S., 1984. Sex-associated differences in the regulation of immune responses controlled by the MHC of the mouse. *J. Immunol.* 132, 656–661.
- Wichmann, M.W., Muller, C., Meyer, G., Adam, M., Angele, M.K., Eisenmenger, S.J., et al., 2003. Different immune responses to abdominal surgery in men and women. *Langenbecks Arch. Surg.* 387, 397–401.
- Wijchers, P.J., Festenstein, R.J., 2011. Epigenetic regulation of autosomal gene expression by sex chromosomes. *Trends Genet.: TIG* 27, 132–140.
- Wijchers, P.J., Yandim, C., Panousopoulou, E., Ahmad, M., Harker, N., Saveliev, A., et al., 2010. Sexual dimorphism in mammalian autosomal gene regulation is determined not only by Sry but by sex chromosome complement as well. *Dev. Cell.* 19, 477–484.
- Wikby, A., Mansson, I.A., Johansson, B., Strindhall, J., Nilsson, S.E., 2008. The immune risk profile is associated with age and gender: findings from three Swedish population studies of individuals 20–100 years of age. *Biogerontology* 9, 299–308.
- Wilson, R., Chu, C.E., Donaldson, M.D., Thomson, J.A., Mckillop, J.H., Connor, J.M., 1996. An increased incidence of thyroid antibodies in patients with Turner's syndrome and their first degree relatives. *Autoimmunity* 25, 47–52.
- Yan, J., Greer, J.M., Hull, R., O'sullivan, J.D., Henderson, R.D., Read, S.J., et al., 2010. The effect of ageing on human lymphocyte subsets: comparison of males and females. *Immun. Age.* 7, 4.
- Yang, J.H., Chen, C.D., Wu, M.Y., Chao, K.H., Yang, Y.S., Ho, H.N., 2000. Hormone replacement therapy reverses the decrease in natural killer cytotoxicity but does not reverse the decreases in the T-cell subpopulation or interferon-gamma production in postmenopausal women. *Fertil. Steril.* 74, 261–267.
- Yao, G., Shang, X.J., 2005. A comparison of modulation of proliferation of thymocyte by testosterone, dehydroisoandrosterone and androstenedione in vitro. *Arch. Androl.* 51, 257–265.
- Zandman-Goddard, G., Peeva, E., Shoenfeld, Y., 2007. Gender and autoimmunity. *Autoimmun. Rev.* 6, 366–372.
- Zehntner, S.P., Bourbonniere, L., Moore, C.S., Morris, S.J., Methot, D., St jean, M., et al., 2007. X-linked inhibitor of apoptosis regulates T cell effector function. *J. Immunol.* 179, 7553–7560.
- Zheng, Y., Rudensky, A.Y., 2007. Foxp3 in control of the regulatory T cell lineage. *Nat. Immunol.* 8, 457–462.
- Zoller, A.L., Kersh, G.J., 2006. Estrogen induces thymic atrophy by eliminating early thymic progenitors and inhibiting proliferation of beta-selected thymocytes. *J. Immunol.* 176, 7371–7378.

# Epigenetics of Autoimmune Diseases

Moncef Zouali<sup>1,2</sup>

<sup>1</sup>Inserm-S 1132, Paris, France <sup>2</sup>University Paris Diderot, Sorbonne Paris Cité, Paris, France

## OUTLINE

<b>Epigenetic Modifications</b>			
DNA Methylation	430	Histone Modifications in Multiple Sclerosis	441
Histone Posttranslational Modifications	430	Generation of Neo-Epitopes	442
Noncoding RNAs	431	MicroRNAs in Multiple Sclerosis	443
Cross Talk Between Epigenetic Regulations	434		
Epigenetic Stability	434	<b>Systemic Lupus Erythematosus</b>	443
	435	DNA Methylation in Systemic Lupus Erythematosus	443
<b>Rheumatoid Arthritis</b>	435	Histone Modifications in Systemic Lupus Erythematosus	445
Acetylation Marks in Rheumatoid Arthritis Immune Cells	435	MicroRNA in Systemic Lupus Erythematosus	445
Histone Modifications in Rheumatoid Arthritis Synovial Fibroblasts	435		
DNA Methylation in Rheumatoid Arthritis Immune Cells	436	<b>Sjögren's Syndrome</b>	446
Genomic DNA Hypomethylation and the Activated Phenotype of Rheumatoid Arthritis Synovial Fibroblasts	436	DNA Methylation in Sjögren's Syndrome	446
Aberrant SUMOylation	437	miRNAs in Sjögren's Syndrome	447
miRNA and the Destructive Potential of Rheumatoid Arthritis Synovial Fibroblasts	437		
<b>Autoimmune Thyroid Diseases</b>	438	<b>Systemic Sclerosis</b>	447
DNA Methylation in Autoimmune Thyroid Diseases	438	DNA Methylation in Scleroderma	447
Histone Tail Modifications in Autoimmune Thyroid Diseases	438	Histone Modifications in Scleroderma	447
Noncoding RNAs in Autoimmune Thyroid Diseases	438	MicroRNAs in Scleroderma	448
<b>Type-1 Diabetes</b>	439		
DNA Methylation Profiling in Type-1 Diabetes Chromatin Remodeling and Histone Acetylation in Type-1 Diabetes	439	<b>Antineutrophil Cytoplasmic Autoantibodies–Associated Vasculitis</b>	448
Histone Deacetylase Inhibitors in Type-1 Diabetes Preclinical Studies	440		
<b>Multiple Sclerosis</b>	441	<b>Epigenetics of Immune Tolerance to Self</b>	449
DNA Methylation and Multiple Sclerosis	441	Dysruption of B Cell Tolerance to Self	449
		Epigenetic Regulators of Tolerant T Cells	449
		DNA Methylation in Treg Development and Function	450
		Impacts of Histone Acetylation on Development and Function of Regulatory T Cells	450
		Epigenetic Modulation of Regulatory T-Cell Stability	451
		<b>Genetic and Epigenetic Interactions in Autoimmune Diseases</b>	451
		<b>Epigenetics Changes Associated With Environment Triggers in Autoimmunity</b>	452
		Exposures to Ultraviolet Light	452

Drugs	452	Potential Disease Biomarkers	454
Toxic Chemicals	453	Epigenetic Therapy	454
Microbiome Epigenetics	453	Conclusions and Future Prospects	457
Dietary Components and Nutri-Epigenomics	453		
Translational Applications of Epigenetics	454	References	458

Alterations in genome architecture can lead to human diseases. In autoimmune diseases, however, although strong genetic bases have been found in genome-wide association studies, no unique genetic mechanism underlying immune tolerance breakdown was identified, and the significant genetic associations described are present only in a relatively small proportion of patients. As seen in other complex disorders, linkage studies and genome-wide profiling arrays have contributed to the identification of multiple genes that may exert a combinatorial effect in predisposing individuals to develop the disease (Kochi, 2016). The largely incomplete concordance rates of autoimmune diseases in monozygotic twins strongly support other complementary mechanisms involved in gene regulation, ultimately causing overt autoimmunity (Zouali, 2009). It is, therefore, becoming increasingly evident that gene expression based on DNA sequence alterations or mutations is not sufficient to explain the variety of manifestations observed in disease states (Feil and Fraga, 2012), and that epigenetic deregulation contributes to the severity of these diseases.

## EPIGENETIC MODIFICATIONS

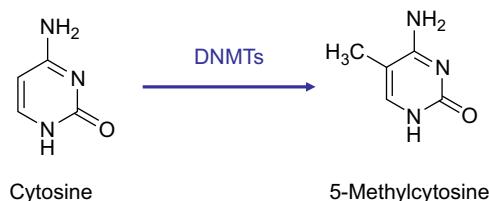
Epigenetics can be defined by the heritable changes that occur in gene expression caused by mechanisms other than alterations in the underlying DNA sequence. These modifications are based on a set of molecular processes that can activate, reduce, or completely disable the activity of particular genes or entire genomic regions: methylation of cytosine residues in the DNA, remodeling of chromatin structure through covalent modification of histone proteins, and recruitment of chromatin-associated small RNA molecules that provide the sequence specificity to target transcriptional silencing. The combination of these different modifications, commonly referred to as the “epigenetic code,” adds a layer of complexity to the information present in the genetic code.

Several observations indicate that environmental changes can produce modifications in gene expression, suggesting that epigenetics can have a potential role in environmental/genetic interactions. First, when the diet *agouti* pregnant rodents were supplemented with foods rich in methyl donors, the offsprings exhibited coat color changes, compared to mothers fed with a standard diet (Wolff et al., 1998). This observation was explained by an altered DNA methylation process that silenced the intracisternal A particle retroviral insertional element, ultimately limiting the appearance of *agouti* alleles. A second remarkable example comes from Dutch individuals who were exposed to famine during intrauterine life and childhood during World War II. Compared to nonexposed subjects, they had a well-conserved hypomethylation status of a region regulating the insulin-like growth factor 2 (IGF2) (Heijmans et al., 2008), providing an example of epigenetic imprinting in human.

### DNA Methylation

In mammals, approximately 60% of the cytosine-phosphate-guanine (CpG) dinucleotides are methylated, and DNA methyltransferases (DNMTs) control DNA methylation of CpG residues and maintenance of methylation during cell differentiation (Bird, 2011). As a regulator of transcription, DNA methylation generally occurs on cytosines in cytosine-guanosine (CG) dinucleotides located within the promoter, most often between 1 and 0.8 kb upstream of the transcription start site (Fig. 25.1).

DNA methylation results from the addition of a methyl group from *S*-adenosylmethionine (SAM) to the C5 position of a cytosine nucleotide, generally located within CpG dinucleotides. DNA sequences that are rich in CpG are called CpG islands. They map essentially to the promoter regions of many genes. Methylation generates a more condensed configuration of the DNA, which hinders the accessibility of transcription factors and attracts methyl-CpG-binding domain (MBD) proteins, thereby leading to repression of gene transcription. By contrast, a low methylation at the promoter region results in an increased transcriptional activity (Jones, 2012).

**FIGURE 25.1** DNA methylation.

Methylation of DNA at 5-position of cytosine by DNMTs that control DNA methylation of CpG residues and maintenance of methylation during cell differentiation. *DNMTs*, DNA methyltransferases; *CpG*, cytosine-phosphate-guanine.

Altered CpG island methylation may change chromatin structure, modulating promoter-transcription factor interactions within the transcription machinery. Although DNA methylation was initially thought to be a stable modification with only passive removal of methyl groups possible, accumulating evidence suggests that DNA demethylation occurs actively and appears to be key in a variety of cellular responses to environmental stimuli, including hypoxia, hormonal signaling, and viral latency and reactivation. Furthermore, aberrant hypo- and hypermethylation may occur in specific genes within the same cell, as is the case in some neoplastic cells.

The three DNMTs that catalyze DNA methylation, DNMT1, DNMT3A, and DNMT3B, have unique sequences, expression patterns, and regulatory mechanisms (Jurkowska et al., 2011). DNMT1 sustains a methylation pattern during cell division and DNA replication and is, therefore, considered a maintenance methyltransferase. The two others, DNMT3A and DNMT3B, catalyze de novo methylation, essentially during embryonic development.

DNA methylation is dynamically controlled and can be reversed through either passive demethylation, a process wherein methyl groups are lost during cell division and DNA replication, or active demethylation mediated by enzyme catalysis. In this process, methylcytosines are modified sequentially by hydroxylation, deamination, oxidation, and DNA repair (Bhutani et al., 2011). Active demethylation requires the following enzymes: the ten-eleven translocation (TET), the activation-induced cytidine deaminase/apolipoprotein B messenger RNA (mRNA) editing enzyme component 1 (AID/APOBEC), and the base excision repair (BER) (Bhutani et al., 2011). Oxidation of 5-methylcytosine (5-mC) gives rise to 5-hydroxymethylcytosine (5-hmC), which is often considered an indicator for active demethylation.

Methylated CpGs recruit transcriptional repressors, such as MBD proteins and histone deacetylases (HDACs), which introduce positive charges in the histone tail, allowing tight binding to negatively charged nucleic acids and leading to the formation of closely compact nucleosomes that prevent transcription. Importantly, these proteins play essential roles in orchestrating the interaction between DNA methylation, histone modifications, and chromatin structure. Eleven MBD members have been described: the methyl-CpG-binding protein (MeCP2), MBD1–6, SETDB1, SETDB2, BAZ2A, and BAZ2B (Baymaz et al., 2014; Hendrich and Tweedie, 2003). Of these proteins, only MeCP2 and MBD1–4 can bind to 5-mC and, therefore, are considered the core MBD proteins. However, even though MBDs are generally considered transcriptional repressors, there is evidence to indicate that these proteins also can behave as transcriptional activators (Chahrour et al., 2008). In physiological conditions, DNA methylation is required for several cellular processes, including genomic imprinting, embryonic development, cell differentiation, X chromosome inactivation, repression of repetitive elements, and maintenance of cellular identity (Martin-Subero, 2011).

## Histone Posttranslational Modifications

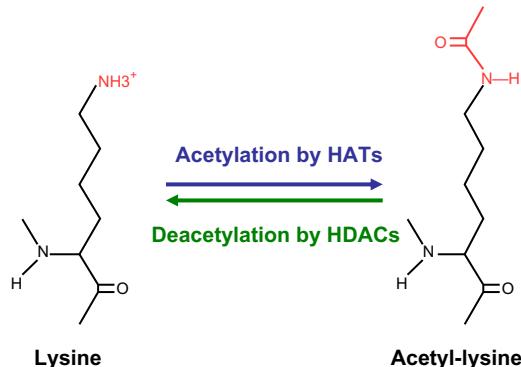
Histone proteins can undergo a number of posttranslational modifications (PTMs) that represent major epigenetic regulatory mechanisms. A variety of histone PTMs that affect chromatin structure have been described, including acetylation, methylation, phosphorylation, ubiquitination, sumoylation, and ADP ribosylation. They can be dynamically added or removed by a plethora of specific enzymes that can work to add or remove functional groups, which are, in turn, recognized by nuclear factors. Together with the diverse nucleosome composition, these modifications can cause a change in the net charge of nucleosomes, alter DNA–histone interactions, and affect chromatin structure. In addition, individual histone PTMs, or specific combinations of them that form the “histone code,” can provide signals to other proteins able to influence chromatin structure, thereby regulating gene expression by the formation of transcriptionally active or repressed states (Li et al., 2007; Berger, 2007).

Histones are highly conserved proteins that reside within nuclei of eukaryotic cells and form the nucleosome cores. They can be categorized into two main groups: (1) core histones (H2A, H2B, H3, and H4), and (2) linker histones (H1 and H5). Two of each of the core histones assemble to form an octameric nucleosome core particle by wrapping approximately 147 base pairs of DNA around the protein spool in a 1.7 left-handed super-helical turn (Luger et al., 1997), thus providing DNA condensation and organization in the nucleus and modulating DNA accessibility to the transcription machinery.

The interaction between histones and the surrounding DNA impact gene expression. Whereas a closed chromatin configuration is associated with transcriptional repression, an open chromatin conformation favors transcriptional activation. These DNA–histone interactions are affected by histone modifications that target specific amino acid residues in the side chains of histones and alter chromatin structure and accessibility. Mechanistically, specific amino acids of histone tails are the targets for several PTM, including acetylation, phosphorylation, poly-ADP ribosylation, ubiquitination, and methylation. Likewise, each histone subtype can be modified by different chemical alterations at defined amino acids, leading to transcriptional modulation and, therefore, cell cycle regulation, development, and differentiation. Acetylation of histones in the nucleosome increases their net negative charge, thereby interrupting their interaction with DNA and leading to an open chromatin conformation with negatively charged DNA—a structure permissive for recruitment of a transcriptional machinery that can initiate gene transcription and expression.

### ***Histone Acetylation and Deacetylation***

These processes are among the most important gene expression regulatory mechanisms (Strahl and Allis, 2000). They involve the conserved ε-amino group of lysine residues at the amino-terminus of nucleosomal histones (Fig. 25.2). Histone acetylation can present as mono-, di-, or tri-acetylation and, usually, occurs at lysine residues on H3 or H4. Acetylation abrogates the positive charges on the histone side chains, which reduces the interaction between the negatively charged DNA and histones, and results in an open chromatin conformation encompassing gene promoters that are more accessible to transcription factors, thereby favoring gene transcription. During histone acetylation, an acetyl group from acetyl coenzyme A is transferred to the NH<sub>3</sub><sup>+</sup> groups on lysine. This transfer is mediated by histone acetyltransferases (HATs) that include P300/CBP, PCAF, and MYST and leads to increased gene expression through transcriptional activation. For example, acetylation of lysine 27 of H3 (H3K27ac) is considered an epigenetic mark associated with transcriptional activation. Histone deacetylation, on the other hand, is catalyzed by several HDACs. According to size, subcellular expression, number of enzymatic domains, and structure, HDACs are divided into four classes. Class I HDACs (HDACs 1, 2, 3, and 8) are detected in the nucleus and ubiquitously expressed in different tissues and cell lines. Class II HDACs (HDACs 4, 5, 6, 7, 9, and 10) shuttle between the nucleus and cytoplasm and are expressed in a tissue-specific manner. For example, human HDAC4 is more abundant in skeletal muscle, brain, heart, and ovary, but undetectable in liver, lung, spleen, and placenta. HDAC5 is expressed in mouse skeletal muscle, liver, and brain, but not in spleen (Brandl et al., 2009). Class III HDACs are structurally unrelated to class I and class II HDACs, but use a unique enzymatic mechanism of action that requires the cofactor NAD<sup>+</sup> for their activity. Finally, HDAC1 is the only



**FIGURE 25.2** Acetylation and deacetylation of lysine residues in histone molecules.

Histone acetylation is catalyzed by HATs. In general, histone acetylation is linked to transcriptional activation and associated with euchromatin. HDACs introduce positive charges in the histone tail, which bind tightly to negatively charged nucleic acids and lead to the formation of closely compact nucleosomes that prevent transcription. *HATs*, Histone acetyltransferases; *HDACs*, histone deacetylases.

member of class IV HDACs, and its classification is still under debate. As a result, HATs decrease the overall positive charge of histones, thereby decreasing their affinity for negatively charged DNA and providing a platform for the binding of transcription factors to the chromatin template. Thus the transfer of acetyl groups to lysine by HATs promotes gene expression. By contrast, removal of acetyl groups by HDACs is generally associated with gene repression (Thiagalingam et al., 2003).

### **Histone Methylation**

Another form of histone PTM is histone methylation, the effects of which depend on the position of the modified lysine residue within the histone tail and on the number of methyl groups added to such residues. Depending on the number of methyl groups that are added and the location of the amino acid being methylated, this process, which occurs frequently, can either increase or decrease gene transcription. For example, whereas trimethylation of histone H3 at lysine 4 (H3K4me3) is an active mark for transcription, the triple methylation of residues 9 or 27 (H3K27me3) is repressive (Kouzarides, 2007). The methylation status of histones is under the control of histone methyltransferases (HMTs) and histone demethylases (HDMs) (Teperino et al., 2010). Three classes of HMTs have been described: (1) SET domain lysine methyltransferase, (2) non-SET domain lysine methyltransferase, and (3) arginine methyltransferase (Smith and Denu, 2009). On the other hand, HDMs have been categorized based on their demethylase domains: (1) LSD1 domain and (2) JmjC domain (Teperino et al., 2010).

Importantly, the interactions between histone modifications and readers are dynamic and exhibit a level of promixuity (Margueron et al., 2009). Each HMT or HDM can target different lysines or arginines, and each lysine or arginine can be methylated or demethylated by more than one HMT or HDM. A single domain from a reader can recognize several histone modifications (i.e., EED can read H3K9me3, H3K27me3, and H1K26me3), and several readers can recognize one histone modification (i.e., H3K4me3 has eight readers). Thus H3K27 can be methylated by EZH2, which can also methylate H1K26. H3K27 can be demethylated by UTX or JMJD3, which can demethylate H3K4.

### **Arginine Methylation**

Arginine can also be methylated/demethylated by specific enzymes (Chen et al., 1999). Methylation of arginine residue 3 on histone H4 (H4R3) and arginine 17 on histone H3 (H3R17) has been shown to induce gene activation. Remarkably, the occupancy of coactivator-associated arginine methyltransferase-1 (CARM1), known to enhance transcriptional activation by nuclear receptors, together with histone H3R17 methylation and citrullination, is regulated at the promoters of inflammatory genes in monocytes, suggesting a role for histone arginine modifications in inflammatory diseases (Miao et al., 2006). Therefore similar to histone lysine methylation, interest in histone arginine methylation is growing. Based on the current knowledge, the histone modification status of H3R17 in cells can exist in at least three modes: (1) arginine, (2) methylated arginine, and (3) citrullinated arginine. The biological meaning of the three modes—or the “H3R17 code”—is slowly emerging but needs further investigation. It seems that H3R17 methylation is associated with gene activation and that H3 arginine citrullination might be associated with the repression of nuclear receptor and NF- $\kappa$ B-regulated genes. This could suggest additional connections between arginine epigenetic markers and human diseases.

### **Ubiquitination**

Ubiquitin is a 76 amino acid protein involved in specific protein labeling. Ubiquitinated proteins are committed to proteosomal degradation, and, thus, ubiquitination controls the stability and intracellular localization of numerous proteins. Ultimately, it influences the status of histone methylation or acetylation and modulates gene expression, as in the case of the NF- $\kappa$ B pathway.

### **Writers, Readers, and Erasers of Histone Modifications**

Among the tail histone modifications described above, enzymes that add groups on histones, that is, HATs and HMTs, are called “writers” of the histone code, and those that remove groups, that is, HDACs and HDMs, are referred to as “erasers.” In addition to writers and erasers, histone modifications also can be regulated by protein effectors to control gene transcription, which are called “readers” (Musselman et al., 2012). Even though these histone readers exhibit structural differences, they have in common one or more conserved domains capable of recognizing specific histone modifications (Yun et al., 2011). Protein modular domains include bromodomains that bind to acetyl-lysines, plant homeodomain that binds methyl-lysines, and “Royal Family” of reader

modules—including Tudor, chromo, PWWP, and MBT domains—that recognize methylated lysine or methylated arginine residues (Margueron et al., 2009).

It is important to note that as a result of these PTMs histone tail modifications can be, directly or indirectly, involved in the regulation of gene expression but also in DNA repair, replication, and recombination (Bannister and Kouzarides, 2011). Thus, histone acetyltransferases mediate histone tail acetylation, resulting in an open chromatin structure that facilitates transcription (Berger, 2007). Methylation on histone tail residues also is a well characterized, but more complex, epigenetic mark that can shape chromatin conformation. Because several methylation sites are located on each histone tail, methylation of core histones H3 and H4 can be associated with either chromatin condensation or relaxation.

## Noncoding RNAs

Noncoding RNAs (ncRNAs) do not code for proteins, but they can impact gene expression through several mechanisms. On the basis of size, ncRNAs can be categorized into three groups: (1) small ncRNAs with less than 50 nt include microRNA (miRNA) and PIWI-interacting RNA; (2) medium-sized ncRNAs (<200 nt) include small nucleolar RNA and promoter-associated small RNAs; and (3) long ncRNAs (lncRNAs), the length of which exceeds 200 nt (Esteller, 2011). miRNAs and lncRNAs are the most studied in autoimmune disease pathogenesis.

Only recently has it been discovered that miRNA can regulate gene expression via mRNA degradation and translational repression (Bartel, 2004). miRNAs are small RNA molecules, the size of which ranges between 18 and 25 nucleotides that bind to the 3' untranslated region of target mRNAs and mediate their posttranscriptional regulation (Sato et al., 2011). Depending on the degree of sequence complementarity, miRNAs, which target about 60% of all genes, can lead to either degradation or translational inhibition. Their synthesis is a step-wise process, which starts with the transcription of primary miRNAs from noncoding regions of the genome (Krol et al., 2010). These longer transcripts are then processed by RNase III enzymes, that is, Drosha and Dicer, into mature double-stranded miRNAs. Subsequently, each strand of the complementary miRNA is incorporated in the RNA-induced silencing complex to promote mRNA degradation. With such a tightly regulated process, miRNAs can repress gene transcription through either mRNA degradation or repression of mRNA translation.

This group of posttranscriptional regulators is involved in several biological processes, including development, differentiation, proliferation, and apoptosis (Filipowicz et al., 2008). miRNAs are genome-encoded and transcribed by RNA polymerase II, similar to ordinary protein-coding RNAs, and it is estimated that miRNA target ~30% of the human transcriptome (Lewis et al., 2005). The role of miRNA in immunity is being explored. For example, miR-146 was identified as a key player in innate immunity (Taganov et al., 2006), and miR-181a was found to modulate adaptive immunity, particularly T-cell sensitivity and selection (Li et al., 2007). Therefore miRNAs are being investigated in autoimmune and chronic inflammatory conditions. A growing body of evidence suggests impaired expression of ncRNAs, and particularly of miRNAs, in autoimmune diseases (Liu et al., 2017).

lncRNAs are thought to make up the largest portion of the noncoding transcriptome (Mercer et al., 2009). This group of heterogeneous transcripts are over 200 nt long. They are involved in RNA silencing, but also in chromatin remodeling, transcriptional activation, posttranscriptional regulation, and DNA methylation (Schmitz et al., 2016).

## Cross Talk Between Epigenetic Regulations

It is noteworthy that epigenetic mechanisms act in concert. Two or more epigenetic mechanisms, including RNA-mediated silencing, histone modifications, and DNA methylation, can act in concert to control gene transcription, and, hence, genome-encoded functions. Thus the regulation of specific DNA methylation patterns that control gene transcription is dependent on local chromatin conformations, and histone methylation marks affect de novo DNA methylation by guiding DNMTs to specific regions of the genome (Brenner and Fuks, 2007).

DNA methylation, for its part, is mediated and maintained by DNMTs, and MBD proteins, which associate with methylcytosine, and recruit silencing complexes that participate in transcriptional repression. These latter complexes contain HDACs and HMTs, which enable a cross talk between DNA methylation, histone modifications, and nucleosome remodeling (Ehrlich and Lacey, 2013). In parallel, miRNAs frequently interact with other epigenetic mechanisms, including DNA methylation, histone modification, and other epigenetic modifiers, thereby shaping the overall gene expression profile.

## Epigenetic Stability

Maintenance of epigenetic marks during cellular division is important to maintain cell lineage commitment in progeny cells and to shape a memory of transcriptional status. Several mechanisms are required for the transmission of epigenetic information through multiple cell divisions. These mechanisms also mediate the inheritance of epimutations (alterations to epigenetic marks that can be transmitted from parents to offspring via their germ-line), which can lead to changes in chromatin structure and transcription levels of genes in disease conditions, such as cancer, imprinting disorders, and, possibly, autoimmune diseases.

Thus even though DNA methylation shows dynamic changes during developmental stages, DNA methylation marks have been initially suggested to be relatively stable over time in adult individuals (Dolinoy et al., 2007). However, human and experimental studies revealed that DNA methylation can exhibit various temporal behaviors, ranging from a nearly absolute stability of the DNA sequence to the rapid variations typical of mRNA levels (Hoyo et al., 2009). Consistently, a recent study showed that DNA methylation markers in human-blood DNA exhibit different degrees of short-term variability (Byun et al., 2012). Whether such variations can be extended to other cell types remains to be determined.

## RHEUMATOID ARTHRITIS

Like several other chronic immune-mediated diseases, the physiopathology of rheumatoid arthritis (RA) involves excessive production of inflammatory mediators, and large quantities of cytokines and chemokines are detectable in the synovial fluid. Another hallmark of RA is the hyperplasia of the synovium, with increased cell density and infiltration of inflammatory cells. The aggressive and invasive behavior of RA synovial fibroblasts (RASFs) and their increased resistance to apoptosis account for the fact that they also are referred to as cells with a “tumor-like phenotype” (Fassbender et al., 1992). Together with cell–cell contacts, the inflammatory mediators can activate stromal fibroblast–like synoviocytes (FLS), leading to the production of enzymes that degrade cartilage and bone. While there is no genetic background for these alterations, several studies suggest that epigenetic modifications could contribute to the characteristic changes of RASFs.

### Acetylation Marks in Rheumatoid Arthritis Immune Cells

Hyperacetylation and hypoacetylation are postulated to tightly regulate the production of inflammatory cytokines at multiple levels, including regulation of transcription factor access to gene promoters, posttranscriptional mRNA processing, and protein secretion. Given the complexity of histone modifications that stabilize an open or repressive chromatin state, a number of investigators focused on the expression of histone-modifying enzymes, including HDACs, particularly because they could have promising therapeutic applications. The analysis of human RA monocytes revealed that HDAC inhibitors (HDACis) are potent antiinflammatory agents (Leoni et al., 2002). More recently, RA PBMCs were found to exhibit increased HDAC activity compared to PBMCs from healthy individuals, and the increase was unaltered after 12 weeks of etanercept therapy (Gillespie et al., 2012).

### Histone Modifications in Rheumatoid Arthritis Synovial Fibroblasts

In initial studies of synovial fibroblasts, there were indications that decreased expression of HDACs in synovial tissue could contribute to RA pathogenesis, and nuclear extracts of RA synovial tissue samples showed lower HDAC activity than those of osteoarthritis (OA) tissue samples (Huber et al., 2007). In further investigations, histone hyperacetylation at the Interleukin (IL) 6 promoter was connected to increased IL-6 expression (Wada et al., 2014). In a more detailed study, significantly higher marks were found for the active H3K4me3 mark in the promoters of MMP-1, MMP-3, MMP-9, and MMP-13 and lower marks of the repressive H3K27me3 in the promoters for MMP-1 and MMP-9 of RA patients, as compared to OA (Araki et al., 2016). These epigenetic modifications could account for the heightened basal MMP levels in RASFs and their aggressive behavior.

In murine models of RA, nonselective HDACis have been used with positive results, including reduced proinflammatory cytokine expression, joint destruction, and disease severity (Lin et al., 2007). In ex vivo experiments, TSA treatment of synovial macrophages reduced the level of IL-6 production and the reduction correlated with enhanced mRNA degradation in RA macrophages and RASFs (Grabiec et al., 2010). Consistently, TSA was a potent inhibitor of TNF- $\alpha$  and IL-6 production in both RA and healthy PBMCs (Gillespie et al., 2012). By contrast,

another HDACi (MI192) inhibited TNF- $\alpha$  production at high concentrations and dose-dependently inhibited IL-6 in RA PBMCs, but not in healthy PBMCs. In parallel, HDACi were found to reduce RA FLS IL-6 production induced by cytokines and Toll-like receptor (TLR) ligands (Grabiec et al., 2012), providing a mechanistic evidence by which HDACi might suppress inflammatory cytokine production and supporting the view that HDACi may represent a novel therapeutic approach for RA.

Importantly, even though blockade of HDACs and bromodomain proteins—reader proteins of histone acetylation—generally leads to antiinflammatory effects (Klein et al., 2016), not all HDACs inhibitors exhibit antiinflammatory actions on synovial fibroblasts. However, one member of the HDCA, namely, HDAC3, represents a promising target because its inhibition was reportedly associated with a suppression of proinflammatory factors in RASFs (Angiolilli et al., 2017). Further characterization of HDACis is needed to better establish their role in the management of RA, and changes in RA acetylation patterns have to be firmly determined before molecular therapeutic targeting becomes feasible.

## DNA Methylation in Rheumatoid Arthritis Immune Cells

Defective DNA methylation also was described in other lineages, such as lymphocytes (Schwab and Illges, 2001), potentially leading to the emergence of autoreactive T- and/or B-cell clones in RA. The *CD21* and *IL-6* promoters were also demethylated in RA PBMC and synovial fluid cells (Nile et al., 2008). In further studies, DNA of monocytes from RA patients was found to be globally hypomethylated in T cells (de Andres et al., 2015). While in the latter study no change in global DNA methylation between healthy and RA B cells could be detected, genome-wide DNA methylation profiling, changes between healthy and RA B cells were described, even in treatment-naïve RA patients (Glossop et al., 2014; Glossop et al., 2016).

In epigenetic studies of PBMCs, the potential confounding effects of differences in the cellular composition cannot be excluded. As a result, the identification of cell-specific epigenetic changes requires that the data must be corrected for differences in the amount of the various cell types present in PBMCs. In one such study, the authors described various DMRs that mapped essentially to the major histocompatibility complex (MHC) region associated with RA genetic risk (Liu et al., 2013). Investigation of the function of regulatory T cells (Tregs) in RA patients disclosed a specific region within the *CTLA-4* promoter that was hypermethylated in Tregs from RA patients, as compared to healthy controls. This DNA hypermethylation could prevent the binding of a transcription factor (NF-AT2), thereby leading to a lower *CTLA-4* expression (Cribbs et al., 2014). In addition to showing that changes in methylation have the potential to subvert cellular function, these epigenetic marks could have therapeutic applications.

## Genomic DNA Hypomethylation and the Activated Phenotype of Rheumatoid Arthritis Synovial Fibroblasts

Converging observations support the hypothesis that progressive loss of methylation marks may give rise to the activated phenotype of RASFs. For example, methylation of CpG islands in the promoter region of the death receptor 3 of RASFs results in a higher resistance to apoptosis (Takami et al., 2006). In further experiments, hypomethylated nuclei were reportedly present in the synovial tissue of RA patients, and RASFs retained their demethylation profile ex vivo (Karouzakis et al., 2009). Chronic treatment of normal synovial fibroblasts with the DNMT inhibitor 5-AZA (5-aza-2'-deoxycytidine) changed the cellular profile into a RASF-like phenotype. Overall, the data support the hypothesis that genomic hypomethylation could account for the activated phenotype of RASFs, in particular with respect to their destructive potential. This hypomethylation could also explain the increased expression of multiple receptors, adhesion molecules, and matrix-degrading enzymes, which play a role in RA. It remains unclear whether the epigenetic modifications of RASFs can contribute to RA chronicity and can be responsible, at least in part, for the fact that current therapies are not effective in all patients.

To determine whether the differentially methylated CpGs in RASFs also are differentially methylated in peripheral blood subsets from RA patients, 371 genome-wide DNA methylation profiles were recently measured in  $CD14^+$  monocytes,  $CD19^+$  B cells,  $CD4^+$  memory T cells, and  $CD4^+$  naïve T cells (Rhead et al., 2017). Of 5532 hypermethylated RASF candidate CpGs, 1056 were hypermethylated in naïve T cells from RA. In addition, 1 significantly hypermethylated CpG in memory T cells, 6 hypomethylated, and 12 hypermethylated CpG were observed in naïve T cells, indicating that DNA methylation signatures of peripheral cells could represent useful risk biomarkers for RA.

## Aberrant SUMOylation

In addition to DNA and histone modifications, posttranslational processes can have direct or indirect effects on epigenetic events. Thus ubiquitin and a related family of proteins, the small ubiquitin-like modifiers (SUMOs), have been shown to impact the potential of RASFs to react to Fas-induced apoptosis. In RA, SUMO is overexpressed in synovial tissue and synovial fibroblasts (Meinecke et al., 2007). However, despite the availability of chemical modulators of ubiquitination and SUMOylation, a better understanding of the molecular mechanisms that underlie these modifications must be achieved before clinical applications can be considered.

## miRNA and the Destructive Potential of Rheumatoid Arthritis Synovial Fibroblasts

Accumulating evidence implicates an important role of miRNAs in the regulation of immune responses and the development of autoimmunity, and recent observations suggest that altered expression and function of miRNAs may also be involved in RA pathogenesis. In one study, treatment of RASFs with TNF- $\alpha$ , IL-1 $\beta$ , lipopolysaccharide, or poly(I-C) led to an upregulation of miR-155 and miR-146, and these two miRNAs were constitutively more highly expressed in RASFs than in synovial fibroblasts of OA patients (Stanczyk et al., 2008). Peripheral blood monocytes of RA patients also displayed higher levels of miR-155. Furthermore, enforced expression of miR-155 repressed the levels of matrix metalloproteinase-3 (MMP-3) and reduced the induction of MMP-3 and MMP-1 by TLR ligands and cytokines (Stanczyk et al., 2008). The repressive effect of miR-155 on MMPs may suggest that miR-155 plays a role in the modulation of the destructive behavior of RASFs (Stanczyk et al., 2008). This conclusion is supported by another study showing an enhanced expression of miR-146 in RA synovial tissue (Nakasa et al., 2008). In that study, the expression levels of miR-146 in RASFs were increased upon stimulation with TNF- $\alpha$  and IL-1 $\beta$ . Consistently, the investigation of 33 RA patients revealed that synovial fluid and peripheral blood CD4 $^+$  T cells exhibit upregulation of miR-146a and downregulation of miR-363 and miR-498 (Li et al., 2010). Moreover, the levels of miR-146a expression were positively correlated with those of TNF- $\alpha$ , and miR-146a overexpression was found to suppress T-cell apoptosis, suggesting a role for miR-146a in RA pathogenesis (Li et al., 2010).

Further evidence for the involvement of miR-146a in inflammation and cytokine production comes from the observation that the expression of miR-146a was associated with that of IL-17 in the PBMC and synovium of RA patients, and that the increased expression of both molecules correlated with disease activity (Niimoto et al., 2010). A parallel survey further demonstrated that a polymorphism in the 3'-UTR of IL-1 receptor-associated kinase (IRAK1), a target gene of miR-146a, is associated with RA susceptibility (Chatzikyriakidou et al., 2010). Since the overexpression of miR-146a was found to significantly deregulate the function of Th1 cells, and because miR-146a treatment ex vivo could induce protein expression of key proinflammatory cytokines (Guo et al., 2010), the results support the hypothesis that miR-146a may be directly involved in the pathogenesis of RA and, possibly, other inflammatory diseases. Thus in reports from different research groups, miR-146a and miR-155 have been consistently found to be upregulated in RASFs, PBMCs, synovial fluid, PBMC-derived CD4 $^+$  T cells, and Th17 cells from patients with RA when compared with healthy controls or OA patients (Stanczyk et al., 2008; Li et al., 2010; Pauley et al., 2008).

In more recent investigations, miRNA-155 was described to be a regulator of chemokine and proinflammatory chemokine receptor expression in RA monocytes (Elmesmari et al., 2016). The fact that its levels were in correlation with tender and swollen joint counts could indicate that it is an active player in promoting inflammation in the RA synovium. In addition to playing a role in innate immunity, miRNA-155 seems to be required for adaptive immune responses that lead to autoimmune arthritis. In one study, miR-155 was found to target a transcription factor, PU.1, implicated in early B-cell commitment and upregulated in memory B cells at an early stage of RA (Elmesmari et al., 2016). Because blockade of endogenous miRNA-155 in B cells of RA patients restored PU.1 levels and decreased antibody production (Elmesmari et al., 2016), this miRNA could represent a promising therapeutic target.

In line with the observation that the expression of both miRNA-146a and miRNA-155 is characteristically abnormal in peripheral naïve and Treg subsets from RA patients (Simmonds et al., 2014), injection of miRNA-146a into CIA mice could inhibit osteoclastogenesis, prevent joint destruction, but, only partially, ameliorated inflammation (Nakasa et al., 2011). miRNA-124/miRNA-124 is another regulator of osteoclastogenesis that acts through downregulation of NFATc1. When injected into the ankles of rats, it ameliorated adjuvant-induced arthritis (Nakamachi et al., 2016). Also overexpressed in synovial fibroblasts, T cells, monocytes, synovial fluid, and serum of RA patients are miRNA-223 (Ogando et al., 2016). It targets aryl hydrocarbon receptor nuclear translocator and acts as an antiinflammatory factor by reducing the production of inflammatory cytokines (Ogando et al., 2016).

## AUTOIMMUNE THYROID DISEASES

Both Graves' disease (GD) and Hashimoto's thyroiditis (HT) are organ-specific autoimmune diseases characterized by lymphocytic infiltration of the thyroid gland, with evidence of humoral and cellular immune system hyperactivation and female preponderance (Weetman, 2001). Whereas in GD the autoimmune process results in the production of thyroid-stimulating antibodies that lead to hyperthyroidism, in HT the immune response is destructive and leads essentially to hypothyroidism (Brix and Hegedus, 2012). In patients with autoimmune thyroid diseases (AITD), there is a significant increased risk for the occurrence of other autoimmune diseases (Fallahi et al., 2016). The investigation of genetic risk factors has revealed that some genes are unique for GD or HT, while others are common to both diseases and, sometimes, shared by other autoimmune diseases (Tomer, 2014).

### DNA Methylation in Autoimmune Thyroid Diseases

Epigenetic profiling of blood samples, lymphocytes, and thyrocytes from patients revealed impaired DNA methylation in AITD. In one study of peripheral blood mononuclear cells (PBMCs) from three GD patients and three age- and gender-matched controls, 82 hypermethylated and 103 hypomethylated genes were reported (Cai et al., 2015). Some of these genes had been previously associated with GD or other autoimmune diseases, including the immunoregulatory factor *ADRB2*, *ICAM1* that codes for a cell surface glycoprotein, and *B3GNT2* that plays a role in the regulation of lymphocyte activity. In addition, the transcription of the genes coding for both DNMT1 and the MBD protein MECP2 was significantly decreased in GD patients compared with normal controls (Cai et al., 2015). In another study of CD4<sup>+</sup> and CD8<sup>+</sup> T cells from 38 GD patients and 31 matched controls, 365 and 3322 differentially methylated CpG sites were described in CD4<sup>+</sup> and CD8<sup>+</sup> T cells, respectively (Limbach et al., 2016). Some of the hypermethylated CpG sites are present on genes involved in T-cell signaling, including *CD247*, *LCK*, *ZAP70*, *CD3D*, *CD3E*, *CD3G*, *CTLA4*, and *CD8A* (Limbach et al., 2016). Interestingly, the first intron of the thyroid-stimulating hormone receptor (*TSHR*) gene, known to contain several GD-associated polymorphisms, was hypermethylated (Limbach et al., 2016). A third study showed abnormal DNA methylation of the *ICAM1* gene promoter in the thyrocytes of 35 AITD patients, as compared to 35 sex- and age-matched controls (Liu et al., 2017).

### Histone Tail Modifications in Autoimmune Thyroid Diseases

One study of PBMCs from GD patients showed reduced global histone H4 acetylation levels and increased levels of HDACs, as compared to healthy controls (Yan et al., 2015). In an analysis of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, also from GD patients, there was a decreased expression of H3K4me3 (histone 3 lysine 4 trimethylation) and H3K27ac (histone 3 lysine 27 acetylation), two epigenetic marks usually present on the vicinity of active promoters (Limbach et al., 2016). Together with the DNA methylation studies, it appears that the gene promoter methylation observed in cells from GD patients is associated with changes in chromatin structure that allow silencing of gene expression.

### Noncoding RNAs in Autoimmune Thyroid Diseases

Several studies probed miRNAs expression in AITD. For example, the expression of miR-154\*, miR-376b, and miR-431\* was found to be suppressed in PBMC from initial GD patients, as compared to healthy controls (Qin et al., 2015). In studies attempting to decipher the biological significance of miRNA deregulation, it has been suggested that increased miR-155 and decreased miR-146a could promote ocular inflammation and proliferation in Graves' ophthalmopathy (Li et al., 2014). Similarly, a decreased expression of miR-125a-3p was suggested to upregulate IL-23 receptor expression in patients with HT (Peng et al., 2015). In other investigations, there was a differential expression of 23 miRNAs in thyroid tissue of GD patients, resulting in the upregulation of 1271 mRNAs and in downregulated expression of 777 mRNAs (Qin et al., 2015). Further evidence for a possible involvement of miRNAs in AITD pathogenesis also comes from studies linking miRNA gene polymorphisms to increased risk of thyroid disease (Cai et al., 2017). However, given the variability in miRNA expression, additional studies are required to discriminate between miRNAs that are deregulated in a given disease.

## TYPE-1 DIABETES

Whereas type-2 diabetes is associated with a relative lack of insulin, most commonly due to the failure of the beta cells to compensate for insulin resistance caused by obesity, type-1 diabetes (T1D) is associated with absolute insulin deficiency due to selective destruction of beta cells. Affecting more than 30 million people worldwide, T1D is a complex autoimmune disease caused by a combination of genetic and nongenetic factors, leading to immune destruction of insulin-secreting islet cells.

It is thought that the destructive process causing T1D is immune-mediated and involves a destructive autoimmune reaction. The fact that predisposing susceptibility loci map at immune response genes and that other autoimmune diseases share common genetic risk factors supports the view that the disease has an immunological basis. In T1D, the associated genes code for proteins that exhibit four broad functions, namely, decreased T-cell signaling, antigen presentation and T-cell repertoire formation, increased type-1 interferon (IFN) responses, and cytokine signaling (Li et al., 2015).

Several lines of evidence suggest that T1D could be subject to nongenetically determined components (Salvetti et al., 2000). First, MZ twins exhibit a high discordance rate, particularly when they are aged over 15 years at diagnosis. Thus even though MZ twins are genetically identical, only approximately 50% of the twins of T1D-affected cotwins will develop the disease (Leslie and Delli Castelli, 2004; Redondo et al., 2001; Hyttinen et al., 2003). Second, over recent years, the risk of developing T1D has increased at a rate that cannot be accounted for solely by genetic changes. Third, the risk for an offspring of a father with T1D is higher than that of an offspring of a mother with T1D, that is, 6% versus 1%, respectively. Fourth, it is the age at diabetes onset, and not the haplotype, that accounts for diabetes risk, suggesting that epigenetic/environmental factors can accelerate T1D progression. Factors that could contribute to the etiology of this complex autoimmune disease include viral infections, dietary factors, or vitamin D deficiency (Knip et al., 2005).

### DNA Methylation Profiling in Type-1 Diabetes

To determine whether epigenetic variation can underlie some of the nongenetic components of T1D etiology, an epigenome-wide association study was devised (Rakyan et al., 2011). To rule out genetic differences and to establish the temporal origins of T1D-associated epigenetic variation, the study combined T1D–discordant MZ twins with longitudinally sampled pre-T1D singletons. Genome-wide DNA methylation profiling led to the identification of 132 different CpG sites at which the direction of the intra-MZ pair DNA methylation difference significantly correlated with the diabetic state, that is, T1D-associated methylation variable positions (T1D–MVPs). Since these T1D–MVPs were found in MZ twins, they cannot be due to genetic differences. Additional experiments revealed that some of these T1D–MVPs are found in individuals before T1D diagnosis, suggesting that they arise very early in the process that leads to overt T1D and that they are not simply due to postdisease associated factors, that is, medication or long-term metabolic changes. However, the origin of these T1D–MVPs remains unclear. First, given that stochastic epigenetic variation in humans is more common than previously appreciated, as demonstrated by genome-scale analysis of DNA methylation profiles in 114 MZ and 80 dizygotic (DZ) twins (Kaminsky et al., 2009), these T1D–MVPs could be of early life stochastic origin. Second, since disease-relevant environmental factors can operate in early life to influence disease-risk (Knip et al., 2010), the T1D–MVPs could have been induced environmentally in MZ twins who were exposed to similar, but not identical, environments. The analysis of individuals before they present with autoantibodies should establish whether T1D–MVPs are valuable disease biomarkers, capable of augmenting the predictive power of autoantibodies and genetic variants.

In a second genome-wide DNA methylation quantitative trait locus, the analysis of human pancreatic islets using 574,553 SNPs with genome-wide DNA methylation data of 468,787 CpG sites from 89 donors, 383 CpG sites showed significant associations after correction for multiple testing, including known diabetes loci (Olsson et al., 2014). Importantly, functional analysis revealed that the identified candidate genes, that is, GPX7, GSTT1, and SNX19, could affect chief biological processes that include proliferation and apoptosis in pancreatic beta cells. Thus genome-wide genetic and epigenetic variations have the potential to interact in a manner that impacts gene expression, islet function, and potential T1D risk.

DNA methylation of promoter regions is generally inherited and exhibits high concordance rates in identical twins, indicating that twin pairs discordant for a particular disease represent an optimal opportunity to probe epigenetic differences that might be involved in that disease. In an epigenome-wide association study of T cells,

B cells and monocytes from 52 monozygotic twin pairs discordant for T1D, a striking concordance between twins of each pair was observed (Paul et al., 2016), consistent with a strong shared genetic/nongenetic effect on CpG methylation in DNA promoter regions. While this observation could suggest that epigenetic changes can contribute to disease pathogenesis, it is also possible that the epigenetic changes could result from other abnormalities that arise in T1D. It will be important to focus on prediabetic subjects and interrogate distal enhancer regions.

### Chromatin Remodeling and Histone Acetylation in Type-1 Diabetes

HDAC inhibition is known to modify innate and adaptive immune responses. In monocytes isolated from patients with T1D or T2D, histone H3 was hyperacetylated in the promoters of TNF- $\alpha$  and the inflammatory-associated enzyme cyclooxygenase (COX)-2 (Miao et al., 2004), suggesting a potential importance of HATs and HDACs in the expression of proinflammatory genes.

A more recent study was based on the view that environmental factors can trigger epigenetic changes and that variations in histone PTMs at the promoter/enhancer regions of T1D susceptible genes may be associated with T1D (Miao et al., 2012). Likewise, histone PTM variations at known T1D susceptibility genes were evaluated in blood cells from T1D patients versus healthy nondiabetic controls, and key histone PTMs were profiled. Marked variations in H3K9 acetylation (H3K9Ac) levels were observed at the upstream regions of *HLA-DRB1* and *HLA-DQB1* in T1D monocytes relative to controls (Miao et al., 2012). Additional experiments demonstrated increased expression of *HLA-DRB1* and *HLA-DQB1* in response to IFN- $\gamma$  and TNF- $\alpha$  treatment that was accompanied by changes in H3K9Ac at the same promoter regions. These results suggest that the H3K9Ac status of *HLA-DRB1* and *HLA-DQB1*, two loci highly associated with T1D, may be relevant to their regulation and transcriptional response toward external stimuli. Thus the promoter/enhancer architecture and chromatin status of key susceptible loci could be important determinants in their functional association with T1D susceptibility. In summary, the findings point to inappropriate chromatin remodeling and histone acetylation as important pathogenetic factors in diabetes.

### Histone Deacetylase Inhibitors in Type-1 Diabetes Preclinical Studies

In ex vivo experiments, increased histone acetylation can be induced by high-glucose concentrations and HDACis in monocytes from diabetics (Miao et al., 2004). Similarly, production of the inflammatory cytokines IL-1 $\beta$  and TNF- $\alpha$  was induced by high-glucose concentrations through the activation of NF- $\kappa$ B (Shanmugam et al., 2003), suggesting that hyperacetylation is a consequence of hyperglycemia or other metabolic aberrancies of diabetes, rather than a cause. Such studies suggest that acetylation favors insulin expression and that HDAC activity decreases insulin expression. In support of this view, TSA and sodium butyrate (NaB) increase histone H4 acetylation and enhance insulin expression at low glucose levels (3 mmol/L), supporting a repressive role of HDACs on pre/proinsulin transcription (Mosley and Ozcan, 2003). Of note, TSA and NaB do not potentiate acetylation of H4 after exposure to high concentrations (30 mmol/L) of glucose (Mosley and Ozcan, 2003). A stimulatory effect of sodium valproate (VPA) on insulin release has also been reported in human islets incubated in low glucose concentrations (2.8 mmol/L). In contrast, accumulated insulin release from rat islets incubated in 11 mmol/L glucose was unaffected by SAHA and the HDACi ITF2357, but was slightly inhibited by TSA (Lundh et al., 2010).

Overall, converging observations indicate that there is evidence for genetic association between diabetes and HDACs, and that HDACs are involved in several biological pathways relevant for the etiology and pathogenesis of not only T1D, but also T2D. The observations that HDACi can promote  $\beta$ -cell development, proliferation, differentiation, and function; prevent  $\beta$ -cell inflammatory damage; improve insulin resistance; and positively affect late diabetic microvascular complications provide a strong rationale for continuing preclinical studies, with the aim of testing the clinical utility of HDACi in diabetes. On the basis of the preclinical evidence, inhibition of various HDACs represents a promising novel therapeutic principle to correct the insulin-resistant state. However, further studies are needed to clarify the differential importance of various HDAC subtypes and, thereby, different HDACi, and to optimize the concentrations of HDACi to be used. The use of more specific HDACi, along with careful titration studies, should provide even more encouraging preclinical results. Lastly, the enigma of how HDAC inhibition, an apparently nonspecific treatment, can exert therapeutic benefits in so many diverse disorders needs to be unraveled.

## MULTIPLE SCLEROSIS

In this chronic, inflammatory and demyelinating neurological disease of the central nervous system, the precise mode of inheritance is not established. Even though the prevalence of multiple sclerosis (MS) in Europe and the United States approximates 0.1%, the disease frequently affects young adults at the age range of 20–45 and leads to considerable economic burden. Approximately 15%–20% of the patients have one or more affected relatives, and first-, second-, and third-degree relatives are more likely to develop the disease than the general population (Sadovnick et al., 1988). If genetic information were the sole determinant for MS susceptibility, one would expect that MZ twins, who are genetically identical, should display the same risk for developing the disease. By contrast, in MS the concordance rate for MZs is only 20%–30% (Hansen et al., 2005; Willer et al., 2003), implying a multi-factorial etiology, with interactions among genetic, environmental and stochastic factors (Dyment et al., 1997). The disease is almost two times more prevalent in women rather in men, implicating the involvement of epigenetic mechanisms in MS development, a disparity that could be due to an epigenetic deregulation of the X chromosome in females.

Environmental risk factors for the development of MS include smoking, which has been documented to impact DNA methylation, histone acetylation, and miRNA expression (Allione et al., 2015). Low serum levels of vitamin D and Epstein–Barr virus infection also have been proposed to act on the epigenome of susceptible patients. Thus vitamin D could alter gene expression by recruiting histone acetyltransferases or HDACs (Fetahu et al., 2014).

### DNA Methylation and Multiple Sclerosis

DNA isolated from the white matter of MS brains contained only about one-third of the amount of methylcytosine found in DNA from normal subjects (Mastronardi et al., 2007). This decreased methylation of cytosines in CpG islands was not the result of decreased DNMT activity, but rather the result of a twofold higher DNMT activity (Mastronardi et al., 2007). Importantly, it was specific for MS because DNAs from the thymus of the same MS patients and from patients with Alzheimer's, Parkinson's, and Huntington's diseases were normally methylated, and the DNMT activity was unaffected (Mastronardi et al., 2007). An important target of this hypomethylation in MS brains is the activation of the PAD2 locus. Its promoter has a 74% GC content, and sequence analysis revealed that this region is hypomethylated in MS brains compared to controls (Mastronardi et al., 2007). Together with the findings of increased DNA demethylase, the data suggest that specific genes are reactivated in MS brains, due to epigenetic modulation. It is, for example, possible that the hypomethylated PAD2 promoter underlies overexpression of PAD2 in the normal-appearing white matter (NAWM), which could be responsible for citrullination of MBP (Baranzini et al., 2010). In addition to cellular DNA, relapsing-remitting MS (RRMS) patients exhibit unique disease- and state-specific methylation abnormalities of cell-free plasma DNA, an observation that could be useful for developing new MS biomarkers (Liggett et al., 2010).

Genome-wide DNA methylation profiling of CD4<sup>+</sup> T cells from monozygotic twin pairs discordant for MS showed differences in DNA methylation patterns (Baranzini et al., 2010). Consistently, the analysis of approximately 485,000 CpG sites throughout the whole genome of peripheral blood CD4<sup>+</sup> T cells from patients with MS and healthy controls disclosed 74 significantly different methylated CpG sites in MS, as compared to controls (Graves et al., 2014). Among these CpG sites, 30 mapped to genes that were previously associated with MS, including IL-32 and the T-cell antigen receptor. Another genome-wide methylation analysis of NAWM from MS patients showed 319 significantly hypermethylated and 220 significantly hypomethylated regions (Huynh and Casaccia, 2013). Hypermethylation and hypomethylation targeted genes known to be involved in oligodendrocyte survival and immune responses, respectively.

### Histone Modifications in Multiple Sclerosis

HATs and HDACs fine-tune cellular acetylation, targeting not only histones but also a variety of proteins with key roles in cell metabolism, signaling, and death. Several observations indicate that, within neural cells, aberrant regulation of acetylation homeostasis may represent a common pathogenetic mechanism underlying neurodegeneration in neurological diseases (Sweet et al., 2012). In an animal model of MS, experimental autoimmune encephalomyelitis (EAE) in SJL mice, the HDACi sodium phenylbutyrate and its metabolite sodium phenylacetate (SPA) almost completely abrogated the development of adoptive transfer of EAE (Dasgupta et al., 2003).

Similarly, SPA suppressed neurological impairment in MBP-primed T-cell recipient mice. Both SPA pretreatment of donor EAE mice *in vivo* or MBP-primed T cells *ex vivo* were able to reduce adoptive EAE symptoms and neuropathology in recipient mice (Dasgupta et al., 2003). In C57BL/6 mice immunized with the myelin oligodendrocyte glycoprotein peptide MOG35–55, injection of TSA reduced neurological impairment (Camelo et al., 2005). In that study, increased histone acetylation levels in the spinal cord of TSA-treated EAE mice correlated with reduced levels of caspase 3 and 9. In addition, TSA treatment reduced transcripts for IL-2 receptor, IL-8 receptor, IL-12, and the costimulatory molecule CD28 in the spleen of EAE mice, and T-cell proliferation to MOG35–55 as well as to nonspecific T-cell activators (Camelo et al., 2005). The findings indicate that TSA treatment during EAE severely affects the development of the autoimmune response.

Other studies demonstrated that immunological functions of dendritic cells (DCs), Th1, Th17, and Tregs, as well as glial cells, are affected profoundly by HDACi. For example, exposure of *ex vivo*–generated human DCs to the HDACis valproic acid or entinostat (MS-275) impaired cell differentiation and immunogenicity (Nencioni et al., 2007). Likewise, DC production of proinflammatory cytokines (IL-6, IL-12, TNF- $\alpha$ ) was reduced by the two HDACi. Consistently, two other HDACis (SAHA and ITF2357) reportedly suppressed IL-6, IL-12, and TNF- $\alpha$  expression by mouse bone marrow–derived DCs challenged with different activators (Reddy et al., 2008). The two HDACi also impaired costimulatory molecule expression by DCs as well as their T-cell stimulatory capacity. In addition to the HDACi-dependent DC suppression, which might be relevant to MS therapy during different disease phases, different HDACi are able to impair Th1 and Th17 cell actions. Thus the HDACis TSA and SAHA could reduce the amount of IL-12 and IL-23 (two key cytokines necessary for DC-dependent polarization of T cells toward Th1 and Th17 cells) produced by human DCs *ex vivo* or mouse DCs *in vivo* (Bosisio et al., 2008).

Further underscoring the therapeutic relevance of HDACi to MS therapy, several lines of evidence indicate that HDACs negatively regulate Treg generation and function. Whereas Treg transfer affords protection from EAE in rodents (Kohm et al., 2002), reduced numbers and functional deficiency of Treg have been reported in MS patients (Zhang et al., 2004). In further studies, TSA was found to assist proliferation of Treg as well as their suppressive function on effector T cells *in vivo* (Tao et al., 2007). These effects are due to inhibition of HDAC9-dependent Foxp3 transcription factor deacetylation. Additional evidence indicates that the functional enhancement also is prompted in freshly isolated and expanded human Tregs by several HDACi, and the promotion of Treg functions by HDACi seems to be due to increased expression of the negative immune regulator CTLA-4, also called CD152 (Akimova et al., 2010).

Inhibitors of HDACs might exert protection from the autoimmune response to the nervous system via both immunosuppressive effects and the promotion of neuronal survival. The preclinical evidence suggests that pharmacological inhibition of HDACs is a promising therapeutic strategy for the treatment of neurological disorders, including MS. However, based on preclinical data, the drugs also might exert detrimental effects that may contribute to MS pathogenesis. In light of the emerging role of drug-induced remyelination in protection from EAE, for example, the observation that HDACi can impair remyelination in cuprizone-treated mice (Taveggia et al., 2010) may reduce the therapeutic potential of these compounds in MS. It is also worth noting that the various HDACi used in preclinical studies are pan-HDACis with off-target effects (Kazantsev and Thompson, 2008). It is, therefore, hoped that isoform-selective HDACi will be available shortly.

Mitochondrial changes, including decreased expression of electron transport chain subunit, have been described in MS, but their relevance to MS pathogenesis remains the focus of the investigation. In studies of histone H3 methylation in the gray matter from postmortem MS and control cortical samples, reduced betaine homocysteine methyltransferase levels in cortical neurons from MS patients were in correlation with altered methylation of H3K4me3 (Singhal et al., 2015), which could account for the downregulation of oxidative phosphorylation genes and, consequently, respiratory defects in the MS cortex. It remains to be seen whether dietary betaine supplementation could support mitochondrial respiration in the clinic.

## Generation of Neo-Epitopes

It is possible that environmental factors can break tolerance through PTMs and molecular mimicry to induce self-antigen modifications that, subsequently, can trigger a range of immune responses. In MS, episodes of neuroinflammation lead to myelin loss and multifocal loci of demyelination, called “plaques,” that are often characterized by the appearance of new myelin at the lesion edge. Pathological studies of postmortem tissue obtained from MS brains revealed that the composition of myelin in the NAWM is different from that of non-MS brains (Moscarello et al., 1994). The coexistence of multiple cell populations in the NAWM and the observation

that HDAC is necessary for the definition of an epigenetic signature of oligodendrocytes in developing rodents (He et al., 2007) led to the proposal that, as a result of a deregulated “epigenetic identity,” disease progression may result from the generation of neo-epitopes.

Further evidence that epigenetic deregulation could lead to the emergence of immunogenic forms of MBP comes from studies of PAD2, a peptidylarginine deiminase that catalyzes the conversion of the guanidino group of arginine to citrulline in MBP. PAD2 levels are elevated in MS and in a transgenic animal model of the disease (Mastronardi et al., 2007). While in normal brains only a very small proportion of MBP is citrullinated, in chronic MS, citrullinated MBP comprises approximately 40% of the total MBP (Moscarello et al., 1994). The observation that there was over 90% citrullination of MBP in a rare acute Marburg’s form of MS suggested a relationship between the amount of citrullinated MBP and MS severity. The conversion of the majority of arginines into citrullines resulted in a visible apparent mass change of MBP, and in a net reduction of its positive charge, thereby affecting the interactions of MBP with other myelin components, and leading to an aberrant localization of the citrullinated form of MBP in human brain white matter biopsies (Wood et al., 1996). Altogether, citrullination of MBP caused by epigenetic deregulation of PAD2 could lead to the release of modified and/or highly immunogenic forms of MBP, which may affect disease progression (Mastronardi et al., 2007; Tranquill et al., 2000; Musse and Harauz, 2007). In one scenario, citrullination could increase the generation of immunodominant peptides, due to increased autocleavage of the protein. Alternatively, citrullination could disrupt the physicochemical properties of MBP and thereby affect its localization within the myelin membrane. Finally, the importance of sex hormones in MS suggests that sex steroids could epigenetically modulate PAD2 gene expression by affecting chromatin components and transcriptional complexes (Kaminsky et al., 2006).

## MicroRNAs in Multiple Sclerosis

A number of research groups reported different expression profiles of miRNAs in whole blood, peripheral blood-derived lymphocytes, or serum from MS patients, but discordant profiles were found. These discrepancies could be due to differences in sample types, cell populations, numbers of miRNAs surveyed, or the techniques used.

Since epigenetic modifications, including miRNA expression profiles, are unique to each cell subset, evaluation of miRNA expression in purified cell populations, instead of heterogeneous cells contained in PBMCs, would be optimal to pinpoint aberrant miRNA profiles in MS patients.

In a study of CD4<sup>+</sup> T cells isolated from peripheral blood, significantly different expression patterns of 85 miRNAs were found in samples from MS patient, as compared to control subjects (Guerau-de-Arellano et al., 2011). Also of interest is the profiling of 368 miRNA in the plasma samples from MS patients (Gandhi et al., 2013). It revealed specific patterns of 29 miRNAs in RRMS patients and 35 miRNAs in secondary progressive MS (SPMS) patients. Remarkably, in RRMS patients, miR-92a-1 expression correlated with disability score and disease duration.

## SYSTEMIC LUPUS ERYTHEMATOSUS

In lupus, there is strong evidence that the presence of one risk allele can influence other risk alleles across different loci. With the identification of a number of gene–gene epistatic interactions (Hughes et al., 2012), it is becoming clear that epigenetic factors play a key role in systemic lupus erythematosus (SLE) pathogenesis. Likewise, interactions between environmental and genetic factors have been proposed to explain why certain individuals develop the disease while others do not, and to account for the discordance rates for the disease in MZ twins (Zouali, 2005; Gourley and Miller, 2007; Zouali, 2011).

## DNA Methylation in Systemic Lupus Erythematosus

This epigenetic modification has emerged as an important contributing factor in lupus. Patients exhibit global T-cell hypomethylation (Richardson et al., 1990) and the changes correlate with increased disease activity (Deng et al., 2001). T-cell DNA hypomethylation has been implicated in the development of drug-induced lupus by hydralazine and procainamide, and in the pathogenesis of lupus-prone MRL-lpr mice (Yung et al., 1997).

Interruption of the ERK signaling pathway in T cells and subsequent inhibition of DNMT1 induces anti-dsDNA antibody production and a lupus-like gene expression profile in mice (Sawalha et al., 2008).

The *CD40LG* gene, located on the X chromosome, is hypomethylated in T cells from healthy men, while healthy women have one methylated and one hypomethylated allele (Lu et al., 2007). Treatment of CD4<sup>+</sup> T cells with the DNA methylation inhibitor 5-azacytidine demethylated *CD40LG* and doubled its expression in normal healthy women, but not men (Lu et al., 2007; Zhou et al., 2009). These findings suggest that hypomethylation of the normally inactivated and silenced X chromosome might be related to the higher prevalence of lupus in women. Indeed, men with Klinefelter's syndrome (47, XXY) have a similar risk to develop lupus as normal women (46, XX) and approximately a 14 times higher risk to develop lupus compared to normal men (46, XY). This gene–dose effect may be achieved by hypomethylation of the X chromosome, making more than one X chromosome available for transcription (Scofield et al., 2008).

Other abnormally methylated genes include the *ITGAL* (CD11a), which is important for cell–cell adhesion (Lu et al., 2002), *TNFSF7* (CD70); which is required for T-cell proliferation, clonal expansion and promotion of effector T-cell formation (Oelke et al., 2004), and *CD40L*; and which stimulates IgG overproduction (Lu et al., 2007). Other factors include the gene encoding perforin 1 (PRF1) (Kaplan et al., 2004), which contributes to autoreactive killing of macrophages and release of apoptotic material, CD3  $\zeta$  chain, CTLA4, IL-2, IL-4, IL-10, and IFN- $\gamma$  (Kaplan et al., 2004; Richardson et al., 1992; Lu et al., 2005; Mishra et al., 2001; Lee et al., 2002; Thomas et al., 2005; Januchowski and Jagodzinski, 2005; Schmidl et al., 2009). In resting SLE B cells, the E1B promoter of the *CD5* gene was reportedly hypomethylated (Lu et al., 2002). These data show that DNA methylation and chromatin structure can influence the expression of SLE-related genes. In mice, demethylating drugs can trigger overexpression of these same molecules and induce both autoreactive T cells and a lupus-like syndrome (Yung et al., 1997).

The observation that several susceptibility genes (*STAT4* or *MECP2*) bear connections with DNA methylation and the existence of polymorphisms in genes that can influence DNA methylation prompted further investigation of epigenetic changes in lupus. Initial candidate gene studies revealed several pathways in which aberrant gene expression due to DNA demethylation was linked with SLE development (Huck and Zouali, 1996). Abnormal hypomethylation of CD4<sup>+</sup> T-cell DNA could contribute to the pathogenesis of lupus-like diseases (Ballestar et al., 2006), and this phenomenon was associated with reduced expression and activity of DNMT1 in patient CD4<sup>+</sup> T cells (Deng et al., 2001; Dubroff and Reid, 1980; Balada et al., 2008; Lei et al., 2009). In a genome-wide DNA methylation study, 236 hypomethylated and 105 hypermethylated CG sites were identified among 27,578 CG sites located within the promoter regions of 14,495 genes in lupus CD4<sup>+</sup> T cells, as compared to normal controls (Jeffries et al., 2011). Among the hypermethylated genes identified, several genes are overrepresented in gene ontologies of metabolic pathways and responses to micronutrients, suggesting a link between environmental factors and epigenetic modifications. They also suggest that DNA methylation changes in specific loci correlate with lupus disease activity.

Studies of T cells from lupus patients also showed reduced expression of DNMT1 and DNMT3a, as compared to normal subjects, but other investigations did not reveal such differences (Balada et al., 2008). These disparities could be due to the inclusion of distinct ethnic groups of patients or different disease activities. It is also possible that an mRNA expression was not a reflection of protein activity of the DNMTs. Mechanisms that could contribute to reduced DNMT1 expression include increased expression of protein phosphatase 2A, known to suppress ERK signaling and DNMT1 activity (Sunahori et al., 2013). Altered activation of protein kinase C delta also can reduce activation of extracellular signal-regulated kinases (ERKs) and impair DNMT1 activity, which could result in reduced DNA methylation and overexpression of costimulatory molecules (CD11A, CD70, and CD40L), proinflammatory cytokines (IL-17A) and IFN-regulated genes (Gorelik et al., 2015).

Because lupus PBMCs express high levels of IL-10 mRNA and protein, and since the serum levels of the potent B-cell stimulator IL-10 correlate with disease activity and severity, it has been hypothesized that the Th2 cytokines IL-10 and IL-13 may be upregulated in SLE due to an aberrant DNA methylation of their promoter regions in CD4<sup>+</sup> T cells, thereby contributing to the activation of the humoral immune arm and triggering lupus disease activity. In one study, methylation of C/G pairs within the IL-10 and IL-13 promoters was found to be significantly reduced in T cells from SLE CD4<sup>+</sup> T cells, as compared with healthy control samples, and the methylation status was inversely correlated with the levels of IL-10 and IL-13 transcripts and proteins, as well as with disease severity (Zhao et al., 2010). Furthermore, treatment of healthy CD4<sup>+</sup> T cells with the methylation inhibitor 5-azacytidine triggered IL-10 and IL-13 expression to levels similar to those observed in SLE CD4<sup>+</sup> T cells, suggesting an important role of DNA methylation in regulating the expression of Th2 cytokines in SLE. These findings may provide a basis for the design of a new therapy aimed at reversing the epigenetic alterations of gene expression in SLE patients.

## Histone Modifications in Systemic Lupus Erythematosus

In addition to altered DNA methylation, abnormal histone marks are thought to contribute to the pathophysiology of SLE. Global H3 and H4 hypoacetylation characterizes CD4<sup>+</sup> T cells from SLE patients (Hu et al., 2008). In one study, significant clusters of aberrantly expressed genes were found in SLE, including those coding for chemokines, which are strongly associated with altered H4 acetylation (Zhang et al., 2010). Other investigators reported that patient T cells exhibit permissive modifications in histone proteins at the IL-17 locus (increased H3K18ac and reduced levels of H3K27me3), which could contribute to the uncontrolled expression of proinflammatory IL-17A (Hedrich et al., 2012). In parallel, the same research group found that the *IL-2* gene undergoes epigenetic silencing in T cells from lupus patients, with histone modifications, impaired histone acetylation and increased methylation being repressive (Hedrich et al., 2012). It is possible that these histone modifications contribute to shape the effector phenotype of T cells from lupus patients.

Overexpression of the CD70 receptor on lupus CD4<sup>+</sup> T cell was proposed to be due to the hypomethylation of the promoter of its encoding gene, *TNFSF7*. Yet studies of lupus CD4<sup>+</sup> T cells also indicated that, in addition to DNMT inhibitors, HDAC inhibitors, such as TSA, could impact CD70 overexpression. Consistently, permissive histone modifications (H3K4 hypoacetylation and H3K4 dimethylation) are frequently increased in lupus patients and are in correlation with disease activity. Thus both deregulated histone marks and DNA hypomethylation within the *TNFSF7* promoter likely contribute to lupus pathogenesis by the promotion of CD70 expression (Yang et al., 2015).

## MicroRNA in Systemic Lupus Erythematosus

miRNAs play an important role at different stages of cell activity, and in lupus, several observations suggest that miRNA expression could represent an indicator of disease progression. In a study of 23 SLE patients and 10 healthy controls, seven miRNAs (miR-196a, miR-17-5p, miR-409-3p, miR-141, miR-383, miR-112, and miR-184) were downregulated and nine miRNAs (miR-189, miR-61, miR-78, miR-21, miR-142-3p, miR-342, miR299-3p, miR-198, and miR-298) were upregulated in patients, as compared with healthy controls (Dai et al., 2007). In further studies using pilot expression profiling, underexpression of miR-146a was identified in PBMCs from SLE patients (Tang et al., 2009), and miR-146a was found to negatively regulate type-I IFN induction by TLR-7 signaling. The strong association between miR-146a levels and clinical disease activity suggested that it may serve as a new disease biomarker and that miRNA could serve as a therapeutic target for SLE treatment (Tang et al., 2009). In another study, miR-125a was found to be reduced, and the expression of its predicted target gene, KLF13 (encoding a transcription factor) that regulates the expression of the chemokine (C-C motif) ligand 5 (CCL5) in T lymphocytes was increased (Zhao et al., 2010). In addition, miR-125a negatively regulated RANTES expression by targeting KLF13 in activated T cells. In another study, screening of approximately 1000 human miRNAs indicated altered expression that was related to disease development and activity (Amarilyo and La Cava, 2012). Methylation profiling also revealed that *IL-10* and *IL-1R2* genes exhibit decreased methylation levels in SLE, as compared to healthy controls, and these decreases correlated with greater disease activity (Lin et al., 2012). It is intriguing that *IL-6* gene hypomethylation was proposed to play a role in lupus kidney damage. Consistently, hypomethylated *IL-6* and *IL-6* overexpression were found to correlate with the presence of autoantibodies and immune complexes.

In SLE, as well as in the antiphospholipid syndrome (APS), there is an increased tissue factor (TF) expression in monocytes and endothelial cells (ECs). The fact that, in several web databases and algorithm target predictions, miR-19b and miR-20a were reported to target TF expression led to a study that tested whether miRNA levels may influence TF levels in patients. In APS, the levels of these two miRNAs were decreased in comparison with monocytes from healthy controls. In monocytes from SLE, miR-20a levels were also lower than those from healthy subjects (Teruel et al., 2011). In addition, the reduced expression of miR-19b and miR-20a was inversely correlated with TF cell surface expression.

In line with these findings, one investigation disclosed that among the 11 miRNA that showed increased or decreased expression in SLE CD4<sup>+</sup> T cells, miR-126 was significantly overexpressed, and its upregulation was inversely correlated with DNMT1 protein levels (Zhao et al., 2011). miR-126 reportedly could directly inhibit DNMT1 translation by interacting with its 3'-UTR, leading to a significant reduction in DNMT1 protein levels. This observation suggests that overexpression of miR-126 can cause demethylation and upregulation of genes encoding LFA-1 (CD11a) and CD70, two receptors that have been linked to disease activity. Importantly, knocking down miR-126 in SLE CD4<sup>+</sup> T cells reduced their autoactivity and their stimulatory effect on IgG production

in cocultured B cells (Zhao et al., 2011). This link between miRNA126 expression and reduced MAPK activity, which could subsequently result in reduced DNMT1 expression in lupus T cells (Zhao et al., 2011), illustrates how epigenetic alterations can influence the expression of ncRNA.

Recent investigations suggest that, in SLE, miRNAs also can regulate DNA methylation by targeting the DNA methylation machinery. In one study, miR-21 and miR-148a were found to be upregulated in CD4<sup>+</sup> T cells from both patients with lupus and in MRL-*lpr* mice (Pan et al., 2010). Whereas miR-21 indirectly downregulated DNMT1 by targeting its upstream regulator (Ras guanyl-releasing protein 1), miR-148a directly downregulated DNMT1 by targeting the protein-coding region of its transcript, leading to a derepression of autoimmune-associated methylation-sensitive genes in CD4<sup>+</sup> T cells, such as CD70 and LFA-1 (CD11a). An alleviation of hypomethylation could also be induced in CD4<sup>+</sup> T cells from lupus patients by transfection with miR-21 and miR-148a inhibitors (Pan et al., 2010).

## SJÖGREN'S SYNDROME

In this disease, studies have focused on analyzing DNA methylation alterations in mechanotransduction, a mechanism that allows the conversion of mechanical stimuli into chemical activity. By translating mechanical forces and deformations into biochemical signals, mechanotransduction enables cells to sense their physical three-dimensional environment. In turn, these signals can adjust cellular and extracellular structures. Experimental evidence suggests that a physical continuum directly connects the extracellular matrix (ECM) to the cellular nucleus (Herrmann et al., 2007). An example of a mechanotransduction-mediated mechanism is the production of lactotransferrin by glandular cells: high levels of lactotransferrin mRNA have been detected in a cell fraction enriched in epithelial cells from salivary glands of patients with Sjögren's syndrome (pSS), together with an altered distribution of  $\alpha 6\beta 4$  integrin and an acinar cell shape (Perez et al., 2009). Increased transcription of the lactotransferrin gene suggests a role of mechanotransduction-signaling pathways in pSS etiopathogenesis.

### DNA Methylation in Sjögren's Syndrome

The role of epigenetic processes in the development of glandular damage was investigated through hemidesmosome (HD) organization–mediated mechanisms. HDs are protein complexes that mediate epithelial cell adhesion to the ECM and are composed of an  $\alpha 6\beta 4$  integrin dimer that binds to laminin, plectin, and other proteins (bullous pemphigoid antigens BP180 and BP230). Suggestive of epigenetic control, reduced levels of BP230 mRNA were found in epithelial cells of patients with pSS in comparison with controls, and, in contrast, an accumulation of BP230 on the basal surface of acini was documented (Gonzalez et al., 2011). The reduced levels of BP230 mRNA could be related to an increased methylation index of CpG islands. In addition, differential methylation changes of the *BP230* gene promoter may explain the up- and downregulations detected in patients with pSS. It is of related interest that CD70 expression was elevated, and the changes correlated with a decrease in TNFSF7 promoter methylation in pSS CD4<sup>+</sup> T cells compared to controls (Yin et al., 2010). As for SLE, demethylation of the CD70 promoter regulatory elements could contribute to CD70 overexpression in pSS CD4<sup>+</sup> T cells and, thereby, to autoreactivity.

More recently, genome-wide DNA methylation profiles were analyzed in CD4<sup>+</sup> T cells and CD19<sup>+</sup> B cells from female pSS patients (Miceli-Richard et al., 2016). Remarkably, methylation alterations were mainly detected in B cells, with an enrichment of genes with differentially methylated positions (DMPs) in at-risk loci, including Interferon regulated genes. In addition, DFPs were overrepresented in B cells from patients with active disease.

In another genome-wide DNA methylation study of human labial salivary glands (LSGs) from 28 female pSS patients, 7820 DMPs associated with disease status were reported, including 5699 hypomethylated and 2121 hypermethylated DMPs (Cole et al., 2016). The investigators identified 57 genes, the promoters of which were enriched for DMPs and implicated pathways previously thought to be involved in disease-related processes. However, since the basal DNA methylation level in lymphocytes is lower than in epithelial cells, it remains possible that lymphocyte infiltration could account, at least partly, for the aberrant methylation described in LSG tissues.

## miRNAs in Sjögren's Syndrome

Studies of miRNAs in pSS pathogenesis have centered on analyzing miRNAs from salivary exosomes. Thus miRNA profiling of salivary glands of healthy controls and patients with pSS who were classified according to a high or a low focus score revealed patterns that could distinguish control subjects and the two pSS groups (Alevizos et al., 2011). In particular, the expression of two miRNAs (768-3p and 574) was inversely correlated with the focus score, and downregulation of the mir-17–92 cluster was observed in half of the pSS patients with a high focus score (a “focus” refers to a cluster in a LSG biopsy of at least 50 lymphocytes, based on survey of at least 4 lobules). Here, it is remarkable that previous studies had associated downregulation of the mir-17–92 cluster with an accumulation in pro-B cells and a marked reduction of pre-B cells, which have been associated with lymphoproliferative and autoimmune diseases (Mendell, 2008; Xiao et al., 2008). However, a large number of studies are required to validate the usefulness of miRNAs in salivary glands as diagnostic markers of pSS.

## SYSTEMIC SCLEROSIS

Systemic sclerosis (scleroderma, SSc) is a multisystem disorder of unknown etiology. The disease is characterized by vascular injury, immune activation, and skin fibrosis. Tissue fibrosis of internal organs often leads to organ dysfunction, causing morbidity and mortality. Because of the complex nature of the disease, there is no efficacious treatment for SSc. Its pathogenesis is believed to involve four cell types, namely, microvascular ECs, lymphocytes, macrophages, and fibroblasts, and to require three self-amplifying events, namely, microvascular alterations, immune activation, and fibrosis in multiple organs. The disease has a genetic component. For example, having a family history of SSc represents a high-risk factor for developing the disease (Arnett et al., 2001); but environmental factors, such as silica, organic solvents, welding fumes, viruses, and drugs, are thought to be involved in triggering SSc. It is possible that these factors are able to affect gene expression through epigenetic mechanisms that could contribute to the onset, disease severity, and response to medication of scleroderma.

## DNA Methylation in Scleroderma

DNA methylation studies revealed that global hypomethylation is common among various cell types, and a number of genes are differentially methylated. Early studies showed a significant reduction in DNMT1, MBD3, and MBD4, possibly leading to a reduction of DNA methylation in CD4<sup>+</sup> T cells (Lei et al., 2009). Similarly, overexpression of CD40L in CD4<sup>+</sup> T cells of female SSc patients was suggested to be due to DNA hypomethylation of CD40L promoter and enhancer (Lian et al., 2012). In parallel, DNA methylation was proposed to account for perturbation of X chromosome inactivation (Selmi et al., 2012), supporting the view that epigenetic mechanisms could indeed contribute to the gender bias in SSc.

On the other hand, studies of fibroblasts indicated upregulation of a number of epigenetic mediators in SSc, as compared to normal fibroblasts (Wang et al., 2006; O'Reilly et al., 2016). In a genome-wide level DNA methylation analysis covering over 485,000 CpG sites in the entire genome, a total of 2710 and 1021 differentially methylated CpG sites were identified in diffuse SSc and limited SSc, respectively, with a large proportion of them being hypomethylated (Altorok et al., 2015). Remarkably, this unbiased approach led to the identification of genes that code for collagen, ECM, transcription factors, and those that are involved in the TGF- $\beta$  pathway and the Wnt pathway. In addition, there were significant differences in DNA methylation patterns between diffuse and limited SSc patients, suggesting that these two different disease subsets are epigenetically distinct.

## Histone Modifications in Scleroderma

As regards histone modifications, aberrant expression of histone writers and erasers, together with histone codes in different cell types were reported. In one study, hypermethylation of CpG islands and deacetylation in the FLI-1 promoter region in SSc fibroblasts and skin biopsy specimens were found to be associated with an increased production of type I collagen (Wang et al., 2006). Treating the cells with both the DNMT inhibitor 5-aza-2'-deoxycytidine and the HDACi TSA resulted in decreased collagen levels. Remarkably, there was a direct influence of DNMT3A on the degree of histone modification; and a reduced DNMT3A expression resulted in an enhanced histone acetylation, further underlining the repressive nature of DNMT3A on acetylation of core histones. The beneficial therapeutic use of HDACi has been shown in an animal model of SSc. Knockdown of

HDAC7 in skin fibroblasts and treatment of bleomycin-induced skin fibrosis in mice with TSA remarkably reduced the accumulation of ECM proteins and fibrosis (Huber et al., 2007).

More recently, studies of SSc B cells showed global H4 hyperacetylation and H3K9 hypomethylation, together with expression changes in JHDM2A, HDAC2, HDAC7, and SUV39H2 (Wang et al., 2013). Whereas global H4 acetylation correlated with disease activity, HDAC2 expression negatively correlated with skin thickness, which may suggest that these histone modifications could be pathogenic. Examining the impact of histone modifications on SSc fibrosis, other investigators reported an increase in HDAC4 and HDAC5 expression in SSc ECs and a reduction in HDAC6 expression (Tsou et al., 2016). In addition, levels of EZH2 and H3K27me3 were upregulated in SSc ECs, as compared to normal ECs (Tsou et al., 2016). It is possible that by repressing proangiogenic genes or activating antiangiogenic genes, EZH2 inhibits angiogenesis in SSc ECs.

## MicroRNAs in Scleroderma

A number of studies were devoted to probing ncRNAs in different cell types and tissues from SSc patients. In the investigation of vascular dysfunction, miR-193b was found to be significantly downregulated in SSc fibroblasts and skin (Iwamoto et al., 2016). Interestingly, manipulation of this miRNA in SSc dermal fibroblasts altered urokinase-type plasminogen activator (uPA) expression, which may suggest a role of this pathway in SSc pathogenesis. Not only uPA induced vascular smooth muscle cell proliferation, but it also inhibited cell apoptosis. Since downregulation of miR-193b led to increased uPA production, this pathway could contribute to the proliferative vasculopathy seen in SSc.

The TGF- $\beta$  pathway is thought to be a key regulator of fibrosis, and a number of ncRNA studies focused on activation of this pathway and its downstream signaling effectors. In one study, miR-150, which targets integrin  $\beta$ 3 and activates the latent form of TGF- $\beta$ , was downregulated in SSc skin and dermal fibroblasts (Honda et al., 2013). In addition, miR-21 can be regarded as an enhancer that amplifies the effect of TGF- $\beta$  in SSc fibrosis (Zhu et al., 2013). Specifically, the induction of miR-21 by TGF- $\beta$  could downregulate Smad7, which inhibits profibrotic signals of TGF- $\beta$ .

miR-29 exerts a classical antifibrotic potential, and several studies support the conclusion that it plays an antifibrotic role in SSc (Iwamoto et al., 2016; Maurer et al., 2010). Its overexpression, which can be modulated by TGF- $\beta$ , decreased type I and type III collagen expression in SSc dermal fibroblasts. In addition to miR-29a, TGF- $\beta$  also can negatively modulate miR-196a and let-7a expression in fibroblasts and modulates the expression of their target, type I collagen.

Beside TGF- $\beta$ , miR-155 reportedly enhanced Wnt/b-catenin and Akt signaling pathways in SSc fibroblasts by directly targeting casein kinase 1a (CK1a) and src homology 2-containing inositol phosphatase-1 (SHIP1) (Yan et al., 2016). Consistently, mice deficient in miR-155 showed reduced skin fibrosis and inhibition of the Wnt/b-catenin and Akt pathways.

In more recent studies, miRNA profiles were determined in serum and their relevance to disease was assessed. For example, a statistical model was built from 26 circulating miRNAs derived from 120 SSc individuals to distinguish healthy controls, SSc, and SLE patients (Steen et al., 2015). A combination of the miR-17–92 cluster could discriminate between the control and diseased groups, indicating that analyzing a cluster of circulating miRNA could represent a promising strategy for disease classification and prediction.

## ANTINEUTROPHIL CYTOPLASMIC AUTOANTIBODIES—ASSOCIATED VASCULITIS

In this pathology of blood vessel walls, inflammation can lead to heterogeneous clinical manifestations. The activation of neutrophils represents a hallmark of antineutrophil cytoplasmic autoantibodies (ANCA)—associated vasculitis. It results in an increased adherence and transmigration to the vascular endothelium, where neutrophils produce reactive oxygen species and release granule constituents, including proteolytic enzymes (Kallenberg et al., 2006). To account for this activation, it has been proposed that the major neutrophil granule protein ANCA autoantigens—proteinase 3 (PR3) and myeloperoxidase (MPO)—are aberrantly expressed in mature neutrophils of ANCA patients (Ohlsson et al., 2005). In contrast, silencing mechanisms would maintain normal expression of PR3 and MPO in mature neutrophils of healthy controls. Support to this view comes from observations showing that aberrant expression of proteolytic enzymes in ANCA disease patients could result from active gene transcription, suggesting a failure in normal gene silencing (Ciavatta et al., 2010). Specifically, at the PR3 and MPO loci, levels of H3K27me3, a mark of transcriptionally silent chromatin, were reduced in

neutrophils of ANCA patients relative to those of healthy controls. This reduced H3K27me3 signature was associated with altered histone modification mechanisms, that is, an overexpression of the H3K27me3 demethylase jumonji C domain-containing protein 3 (JMJD3) in patients. The decreased histone methylation could result from a defective recruitment of the H3K27 methyltransferase enhancer of zeste homolog 2 (EZH2). A potential additional silencing mechanism may be DNA methylation because a CpG island in the *MPO* gene was found to be unmethylated in patients, and there was a significant increase in methylated DNA at this CpG island in healthy controls (Ciavatta et al., 2010). Overall, the evidence suggests that the transcriptional regulation of *PR3* and *MPO* genes may involve an epigenetic process in normal neutrophils that is perturbed in neutrophils of ANCA-associated patients. A decreased histone methylation and an increased histone demethylation would give rise to a permissive chromatin structure at the *MPO* and *PR3* genes.

## EPIGENETICS OF IMMUNE TOLERANCE TO SELF

### Dysruption of B Cell Tolerance to Self

In the B cell compartment, immune tolerance operates through several mechanisms (Zouali, 2007). Their epigenetic regulation has been discussed (Zouali, 2013). In the B-cell compartment, self-tolerance operates by several processes, including deletion, anergy and receptor editing, a powerful mechanism that allows autoreactive B cells to revise their receptors and to escape apoptotic death (Radic and Zouali, 1996). Several studies found that receptor editing is impaired in SLE and RA and that this abnormality could account for the ineffective silencing of B cells that have acquired autoreactive receptors (Bensimon et al., 1994; Suzuki et al., 1996; Samuels et al., 2005; Yurasov et al., 2005; Faber et al., 2006; Zouali, 2008). Further insight into the molecular basis of inefficient editing as a potential cause of autoimmunity comes from studies based on different approaches. One line of evidence comes from studies on drug-induced lupus. This autoimmune disease can be induced by more than 40 medications, such as the antihypertensive drug hydralazine (Zouali, 2011). Interestingly, aromatic amines and hydralazine derivatives can be found in a wide variety of compounds used in agriculture and industry, and commercial applications. Hydralazine itself also occurs in tobacco and tobacco smoke, and a lupus-like syndrome has been reported in individuals who have been in contact with these agents (Reidenberg et al., 1993). To test the hypothesis that drugs that alter DNA methylation and trigger a lupus-like syndrome can disrupt the mechanisms that maintain B cell tolerance, the effects of hydralazine on receptor editing were investigated in mice harboring human transgenic immunoglobulins. The studies revealed that by disrupting the ERK signaling pathway, hydralazine alters DNA methylation, reduces receptor editing in B lymphocytes, and contributes to the generation of pathogenic autoreactivity (Mazari et al., 2007). The data also support the view that epigenetic alterations contribute to exacerbated activation or deregulation of the mechanisms that maintain tolerance to self-antigens. Future studies will determine whether other drugs and xenobiotics that trigger autoimmunity in humans also can act through similar mechanisms.

For T cells, central tolerance takes place in the thymus through negative selection, where developing thymocytes are deleted if they react too strongly with self-antigens presented by the MHC. However, since not all peripheral self-antigens are adequately presented in the thymus, central tolerance is incomplete, and self-reactive T cells escape negative selection, and migrate to secondary lymphoid organs. In the periphery, autoreactive lymphocytes must be inactivated by deletion or by suppression by Tregs, and/or by cell-intrinsic programs that lead to a state of functional unresponsiveness, called anergy. Understanding the regulatory mechanisms that maintain or break T-cell tolerance to self-antigens is required to prevent autoimmunity.

### Epigenetic Regulators of Tolerant T Cells

Studies of histone acetylation in vitro and ex vivo suggested that immune tolerance is under epigenetic influences. A variety of HDACis, such as butyrate or trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA), induce anergy in Th1 cell cultures (Edens et al., 2006), as characterized by an inhibition of proliferation accompanied by a reduction of IL-2 production. The effect was attributed to chromatin remodeling at the IL-2 promoter, as well as to acetylation of transcription factors, such as NF- $\kappa$ B (Matsuoka et al., 2007).

To identify genes and pathways critical for maintenance of unresponsiveness, an in vivo mouse model was used to perform a microarray analysis of naïve (N), memory (M), and tolerant (T) T cells (Schietinger et al., 2012). The study revealed that T cells harbor a tolerance-specific gene signature markedly distinct from that of N and M cells, and 164 genes were identified as “tolerance-specific” gene sets representing uniquely overexpressed genes,

including negative regulators of cell signaling and proliferation, transcription factors, and phosphatases. Genes modulating cell cycle, cell division, nucleosome assembly, mitosis, and DNA replication were also highly over-represented and upregulated in T cells. Thus T cells “remember” the tolerance program established during the initial encounter(s) with self-antigen in the periphery, raising the question of how such memory is encoded. The fact that transcripts of numerous genes regulating chromatin modification were enriched in T cells (e.g., *Jmjd3*, *Dnmt1*, *Hat1*, *Hdac2*, *Hdac3*) supports the view that epigenetic factors are involved. Consistently, genome-wide miRNA profiling revealed that N, M, and T cells had distinct miRNA expression patterns (Schietinger et al., 2012). Thus these insights into the regulatory mechanisms that maintain or break self-tolerance may lead to new epigenetic strategies for the treatment of autoimmunity.

## DNA Methylation in Treg Development and Function

*Foxp3* is a member of forkhead/winged-helix family of transcription factors that act as a “master” regulator for the development and suppressive function of Tregs. Its constitutive expression is necessary for the suppressive function of Tregs, and mutation or deficiency of *Foxp3* leads to the development of autoimmune diseases. Genetic defects in *Foxp3* cause the *scurfy* phenotype in mice and IPEX syndrome (immune dysfunction, polyendocrinopathy, enteropathy, X-linked syndrome) in humans (Khattari et al., 2003). In the thymus, a subset of CD4<sup>+</sup>CD25<sup>+</sup> T cells develops into Foxp3<sup>+</sup>CD4<sup>+</sup> T cells, known as natural Tregs (nTregs). However, in peripheral lymphoid organs, TGF-β can induce naïve CD4<sup>+</sup>CD25<sup>-</sup> T cells to develop into Foxp3<sup>+</sup>CD4<sup>+</sup> T cells, known as adaptive or induced Tregs (iTregs). Ex vivo culture of peripheral naïve CD4<sup>+</sup>CD25<sup>-</sup> T cells in the presence of TGF-β induces expression of *Foxp3* and provides a method to generate Foxp3<sup>+</sup> Tregs ex vivo. Importantly, nTregs and iTregs exhibit different functional characteristics (Tran et al., 2007), and nTregs are more stable than iTregs, a feature that may be related to the epigenetic regulation of *Foxp3* (Lal et al., 2009; Polansky et al., 2008).

Early studies showed that the differentiation of T helper cells is under epigenetic regulation through CpG methylation (Lee et al., 2001; Wilson et al., 2009). As regards Tregs, different regulatory *cis*-elements are present in the *Foxp3* locus, upstream of the transcriptional start site (−600 to −1 bp) at the proximal promoter where the methylation status of the CpG residues plays an essential role in *Foxp3* expression. Thus 10%–45% of the CpG sites in the *Foxp3* proximal promoter (−250 to +1) are methylated in naïve CD4<sup>+</sup>CD25<sup>-</sup> T cells, whereas all are demethylated in nTregs (Kim and Leonard, 2007), and TGF-β induces demethylation of CpG at this site in CD4<sup>+</sup>CD25<sup>-</sup> T cells. That methylation of the proximal promoter is an important regulator of *Foxp3* expression also is supported by the observation that this region is ≈70% methylated in human CD4<sup>+</sup>CD25<sup>lo</sup> cells compared with ≈5% in CD4<sup>+</sup>CD25<sup>hi</sup> T cells (Janson et al., 2008). Further support comes from the fact that CpG residues in an intronic region (+4201 to +4500) are completely methylated in naïve CD4<sup>+</sup>CD25<sup>-</sup> T cells and fully demethylated in mouse (Polansky et al., 2008; Kim and Leonard, 2007) and human nTregs (Baron et al., 2007). Also, an upstream enhancer (−5786 to −5558 bp) is methylated in naïve CD4<sup>+</sup>CD25<sup>-</sup> T cells, activated CD4<sup>+</sup> T cells, and iTFoxp3<sup>+</sup> Tregs but is demethylated in nTregs (Lal et al., 2009). Together, these reports demonstrate that *Foxp3* is regulated by complex mechanisms where many extracellular signals control transacting factors as well as chromatin remodeling through covalent modification of CpG DNA. Understanding these signals and their cumulative intracellular effects on *Foxp3* *cis*-elements at different T-cell developmental stages will be a key for therapeutic manipulation of T-cell responses.

## Impacts of Histone Acetylation on Development and Function of Regulatory T Cells

In addition to DNA methylation, Treg development and function are under influences of histone acetylation. For example, following TCR stimulation of murine T cells, class II HDACs are mainly expressed in Tregs (Tao et al., 2007). On the other hand, HDACi enhances *Foxp3* expression in both CD4<sup>+</sup>CD25<sup>-</sup> and CD4<sup>+</sup>CD25<sup>+</sup> T cells, suggesting that deacetylation directly regulates *Foxp3* expression and enhances Treg suppressive functions (Tao et al., 2007; Tao et al., 2007; Lucas et al., 2009). Another class II HDAC, HDAC9, interacts with *Foxp3* and down-regulates its acetylation. Treatment with the class I and II HDACi TSA enhances *Foxp3* acetylation and Treg function (Li et al., 2007). In mice, administration of pan-HDACi increased *Foxp3* gene expression, as well as the production and suppressive function of Tregs in vitro. In addition, HDAC9 proved particularly important in regulating *Foxp3*-dependent suppression, as demonstrated by studies in HDAC9-knockout mice (Tao et al., 2007). At the molecular level, optimal functions of Tregs required acetylation of several lysines in the forkhead domain of *Foxp3* (Wang et al., 2009).

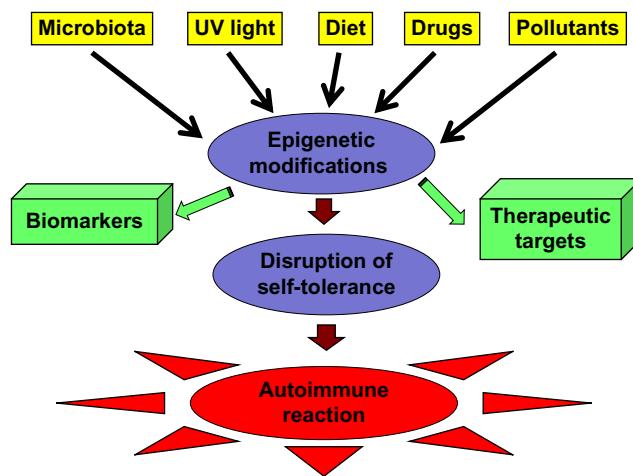
## Epigenetic Modulation of Regulatory T-Cell Stability

Several observations indicate that  $\text{Foxp}3^+ \text{CD4}^+$  Tregs encompass a heterogeneous population in mice (Komatsu et al., 2009) and humans (Miyara et al., 2009) and that their phenotype and functions are not as stable as initially thought (Zhou et al., 2009; Lee et al., 2009). For example, a fraction of  $\text{Foxp}3^+ \text{CD4}^+$  nTregs adoptively transferred to lymphopenic mice converted into  $\text{Foxp}3^-$  T cells (Komatsu et al., 2009). Under inflammatory conditions,  $\text{Foxp}3^+$  Tregs lose  $\text{Foxp}3$  expression and suppressive functions in an IL-6-dependent manner (Pasare and Medzhitov, 2003). The fact that different subsets of Tregs exhibit different levels of CpG DNA methylation at the  $\text{Foxp}3$  locus suggests that Treg instability may be under epigenetic controls (Miyara et al., 2009). Thus the increased methylation of CpG at the  $\text{Foxp}3$  locus was linked with a lower  $\text{Foxp}3$  expression, decreased Treg stability, and reduced suppressive Treg function (Lal et al., 2009; Miyara et al., 2009). In addition, nTregs possess demethylated CpG at the  $\text{Foxp}3$  locus and show stable  $\text{Foxp}3$  expression, whereas TGF- $\beta$ -induced Tregs show methylated CpG and do not maintain constitutive  $\text{Foxp}3$  expression after restimulation in the absence of TGF- $\beta$ . It is of further interest that HDACi can modulate Th cellular phenotypes. In one study, the HDACi TSA could induce a profound negative effect on the emergence of IL-17-producing cells from Tregs (Koenen et al., 2008). Whereas sorted Tregs could be polarized from the Treg phenotype to Th17 cells in a defined cytokine milieu, this could be prevented by the presence of TSA (Koenen et al., 2008). Finally, while it is known that various cross talks between histone methylation and acetylation exist, their impact on T-cell polarization is being addressed. Investigators identified a highly conserved CpG island within a FoxP3 enhancer region that is methylated in peripheral CD4 $^+$  T cells, but not in nTreg (Lal et al., 2009). This region was histone H3 acetylated and bound by Sp1 and TGF-inducible early gene-1 (TIEG1). Removing this methylation in non-Tregs resulted in an increased and stable FoxP3 expression. This effect is similar to other reported modulations of FoxP3 expression via methylation (Polansky et al., 2008).

## GENETIC AND EPIGENETIC INTERACTIONS IN AUTOIMMUNE DISEASES

It is plausible that genetic and nongenetic effects interact to induce complex diseases. This scenario is likely to true for autoimmune diseases. For example, exposure of females who are slow acetylators—a genetically determined trait—and bear the HLA-DR4 genotype to hydralazine, an antihypertensive, demethylating drug, can trigger SLE (Russell et al., 1987). Similarly, subjects who carry the HLA-DR4 risk allele and smoke are at increased risk of developing RA (Meng et al., 2017). Specifically, DNA methylation of the CpG promoter site cg21325723 can mediate gene-environment interactions between the RA-associated single-nucleotide polymorphism rs6933349 and smoking. Remarkably, this interaction imparts the risk of developing RA-associated anti-trullinated peptide autoantibodies and has been observed in both Caucasian and Asian subjects (Meng et al., 2017). Thus interactions resulting from altered gene expression linked to epigenetic changes and nongenetic factors have the potential to trigger an autoimmune process that leads to autoimmune disease (Fig. 25.3).

Direct comparison of identical twins represents a unique approach to test “environmental epigenetics” because DNA sequence differences, including single-nucleotide polymorphisms, which would be abundant in singleton-based studies, cannot interfere. Not surprisingly, twin studies demonstrated the existence of genome-wide epigenetic differences that potentially could explain phenotypic differences (Kaminsky et al., 2009; Fraga et al., 2005). They demonstrated how epigenetic differences between MZ twins become more pronounced with age, supporting the notion that “epigenetic drift” plays a role in the divergence of MZ phenotypes (Fraga et al., 2005). More recently, a collection of identical twins discordant for SLE and two other related systemic autoimmune diseases (RA and dermatomyositis) was used to perform a high-throughput analysis of DNA methylation changes (Javierre et al., 2010). Only SLE samples exhibited significant changes in the DNA methylation status at both the global and sequence-specific levels in comparison with their healthy, discordant twins and compared with unrelated matched normal controls. Comparison of SLE twins with their corresponding healthy twins showed a decrease in global 5-methylcytosine content and a change in DNA methylation status of the CpG-rich region of the ribosomal DNA repeat, which contains the transcribed 18S and 28S genes. The study also yielded a list of epigenetically deregulated DNA sequences in SLE. Importantly, gene ontology analysis revealed an enrichment in categories associated with immune function, and individual analysis confirmed the relevance of DNA methylation changes in genes to SLE pathogenesis, supporting the notion that epigenetic alterations may be critical in the clinical manifestations (Zouali, 2011; Javierre et al., 2010). The results reinforce the notion that, for a particular genetic background, environmental factors can modulate SLE onset. However, it remains unclear whether



**FIGURE 25.3** A current trend of linking epigenetics to autoimmune diseases.

In genetically susceptible individuals (single nucleotide polymorphisms, copy number variation, etc.), the complex interplay between the environment, the epigenome, and the genome can lead to a variety of epigenetic modifications. The list of environmental factors covers a range of not only deleterious factors (toxic materials, radiation, drugs, and pollution) but also lifestyle (diet, smoking, and stress). The resulting epigenetic alterations can affect normal gene expression and lead to loss of immune tolerance to self in both the B- and T-cell compartments. This tolerance break could account for the emergence and/or progression of autoimmune diseases. The abnormal epigenetic marks identified in this group of disorders could allow the discovery of biomarkers for disease diagnosis and monitoring, and, possibly, the design of novel therapeutic strategies.

environmental factors caused the epigenetic changes observed, or whether environmental factors cause disease by some unknown mechanisms, possibly associated with inflammatory and immune responses that give rise to epigenetic changes.

## EPIGENETICS CHANGES ASSOCIATED WITH ENVIRONMENT TRIGGERS IN AUTOIMMUNITY

It is possible that a close interplay between environmental triggers, epigenetic, and genetic factors is responsible for the loss of immunological tolerance to self, leading to autoimmune disease. There is evidence to suggest that the environment can subvert epigenetic regulatory pathways, causing overexpression of normally silenced genes. Environmental factors that could play a role include exposure to ultraviolet (UV) light, toxic chemicals, dietary components, drugs, and infection.

### Exposures to Ultraviolet Light

In SLE, up to 73% of the patients develop photosensitivity, and UV light has long been associated with disease exacerbation (Wysenbeek et al., 1989). UV irradiation is known to lead to specific demethylation events during subsequent rounds of replication (Wysenbeek et al., 1989; Kastan et al., 1982). It reduces DNMT1 mRNA expression and impairs DNA methylation in T cells from SLE patients (Zhu et al., 2013). Yet, it remains unclear how UV-B affects disease by altering DNA methylation.

### Drugs

Over 40 drugs are able to induce a lupus-like syndrome, including procainamide, hydralazine, propylthiouracil, sulfonamides, D-penicillamine, isoniazid, phenytoin, carbamazepine, methyldopa, quinidine, captopril, acebutolol, and chlorpromazine (Rubin, 2005; Tsay and Zouali, 2008). The mechanisms of drug-induced lupus remain unknown, and several possibilities have been put forward. Two drugs, in particular, are high-risk inducers: procainamide, an antiarrhythmic drug, and hydralazine, an antihypertensive medication. Hydralazine-induced antihistone antibodies are characterized by anti-H3 and anti-H2 IgM antibodies, and procainamide-induced antihistone antibodies by anti-H2a/H2b complex antibodies. Both drugs inhibit DNMT1. Procainamide

acts as a competitive inhibitor, and hydralazine as an indirect inhibitor through its action on the ERK that controls DNMTS transcription. In an experimental model, hydralazine was documented to be able to subvert B cell tolerance to self and to trigger autoantibody production (Mazari et al., 2007).

## Toxic Chemicals

Experimental studies and clinical observations showed that autoimmune disease may be triggered by chronic exposure to various chemicals, including xenobiotic organic and inorganic compounds, silica dust, organic solvents, and petroleum (Pollard et al., 2010). Notorious among them is hydrazine and its derivatives present in a variety of compounds and used in agriculture and industry, including herbicides, photographic supplies, textiles, synthesis of plastics, anticorrosives, rubber products, preservatives, dyes, and pharmaceuticals. Hydrazine is present in mushrooms, penicillium, tobacco, and tobacco smoke; and smokers exhibit a significant increased risk to develop SLE and RA (Klareskog et al., 2007).

In experimental animals, tetramethylpentadecane (TMPD), commonly known as pristane, can trigger autoimmune phenomena. In the test tube, TMPD, which can be found in processed food for human consumption, induces chromatin changes, and, potentially, changes in gene expression (Garrett and Cuchens, 1991). Also of interest is the common industrial solvent trichloroethylene (TCE), known to be able to exacerbate autoimmunity (Cai et al., 2008). This compound was found to alter DNA methylation patterns (Palbykin et al., 2011). Exposure to heavy metals, which have been postulated to be able to trigger autoimmune diseases, can modify gene expression by a variety of epigenetic mechanisms (Martinez-Zamudio and Ha, 2011).

## Microbiome Epigenetics

Recent evidence indicates that the gut microbiome is perturbed in several autoimmune diseases, including SLE, RA, inflammatory bowel disease (IBD), and T1D. Since environmental signals can impact gene expression through epigenetic mechanisms, the human microbiome, as an environmental component inside the human body, could influence host gene expression by epigenetic modifications. Several studies showed that the changes of the composition and/or the function of the human microbiome could have effects on the status of the human epigenome. Translocation of microbial epigenetic enzymes or ncRNAs of the microbiome into host cells could influence the expression of host genes. On the other hand, microbial metabolites can impact the activity of epigenetic enzymes. For example, short-chain fatty acids (SCFAs) can act as HDACis, leading to suppression of inflammation in PBMCs (Vinolo et al., 2011), macrophages (Chang et al., 2014), T cells (Arpaia et al., 2013), and DCs (Singh et al., 2010).

Importantly, the interplay between epigenetics and microbiome is bidirectional. That is, human epigenetic changes can, in turn, result in dysbacteriosis. For example, miRNAs, which are detectable in host feces in the form of extracellular vesicles, can enter bacteria and impact bacterial gene expression. Neonatal mice do not develop a conventional gut microbiota when their Dicer, the cleaving enzyme required for miRNA formation, was inactivated (Liu et al., 2016).

There are examples of the relationship between dysbacteriosis and epigenetic changes in some autoimmune diseases. Thus an inverse correlation between miR-30C and miR-130A levels and autophagy-related proteins was reported in ileal biopsy samples of patients with Crohn's disease, which may suggest that microbiome regulation of miRNA expression and autophagy could be important for IBD pathogenesis (Nguyen et al., 2014). In an experimental model of T1D, pathological changes and gut dysbacteriosis in virus-induced LEW1.WR1 rats could be reversed by HDACis (Hara et al., 2014). However, convincing evidence for the association between microbiome and epigenetics in autoimmune diseases is still missing. It is possible that future work based on the analysis of multiomics of the microbiome and the epigenome could lead to the identification of biomarkers and novel therapeutic targets for autoimmune diseases.

## Dietary Components and Nutri-Epigenomics

A significant increase in the incidence of autoimmune diseases, such as diabetes and MS, in the industrialized countries has led to the hypothesis that diet is a potential environmental risk factor for such disorders. In parallel, studies of the role of dietary influences on gene expression have led to the emergence of a new discipline termed

nutri-epigenomics. It is, for example, conceivable that DNA methylation events and dietary practices, such as micronutrient intake, can influence disease induction and/or progression.

Importantly, epigenetic marks, such as DNA and histone modifications, are dependent on diet-derived substrates and/or intermediary metabolism products. For example, levels of SAM, which plays a pivotal role as a methyl donor in a myriad of biological and biochemical events, including DNA methylation, are dependent on dietary micronutrients, including vitamins B2, B6 and B12, folate, methionine, zinc, and choline (Oaks and Perl, 2014). Since methyl groups from SAM are needed for DNA methylation, diets with insufficient sources of methyl groups can impair SAM synthesis and lead to global DNA hypomethylation (Selhub, 2002).

In lupus patients, there is a reduced global DNA methylation, which could result from alterations of the SAM cycle and/or expression of the gene encoding DNMTs. Patients who have low DNMT1 levels due to environmental exposures, that is, UV light, smoking or infections, could be sensitive to a diet poor in methyl donors. Levels of S-adenosylhomocysteine (SAH), an inhibitor of transmethylation reactions, are often increased, and transmethylation micronutrient levels are decreased in patients with active lupus (Wu et al., 2012). Experimentally, dietary micronutrients that affect DNA methylation could exacerbate or ameliorate disease in a transgenic mouse lupus model (Strickland et al., 2013). It is, therefore, possible that nutritional intakes able to restore a balanced SAM could be useful in the management of disorders exhibiting reduced global DNA hypomethylation.

## TRANSLATIONAL APPLICATIONS OF EPIGENETICS

As in other disorders, epigenetic marks are thought to represent potential therapeutic targets and/or diagnostic markers for disease progression and/or response to treatment.

### Potential Disease Biomarkers

In several autoimmune diseases, differentially methylated regions in the genome were proposed to represent putative disease markers. In RA, for example, peripheral naïve T cells were found to share hypermethylation sites with FLS (Rhead et al., 2017). It will be of interest to confirm that the epigenetic signatures identified can represent valuable biomarkers for the risk of RA or for disease status.

miRNAs are detectable and stable in a variety of body fluids (blood, plasma, synovial fluid, etc.) and are relatively easy to quantify using accessible experimental approaches. In lupus nephritis (LN), miR-26a and miR-30b have been proposed to play an important role. Their expression is downregulated in renal tissues and urine of LN patients (Costa-Reis et al., 2015). In addition, the expression of miR-26a is increased in urinary exosomes, which are cell-derived vesicles that play a key role in intercellular signaling (Ichii et al., 2014). In parallel, miR-29c expression was reported to be predictive of lupus kidney damage (Sole et al., 2015), suggesting that miR-26a and miR-29c could serve as biomarkers of early LN progression. The fact that the expression levels of miRNAs in the serum are generally stable suggests that they could be used as disease biomarkers. However, despite their abnormal expression in SLE, whether miRNAs could serve as biomarkers in stratifying lupus disease remains uncertain.

Caution should be exercised for data interpretation (Churov et al., 2015). First, miRNA expression is under the influence of sex, lifestyle, and environmental factors (Vrijens et al., 2015; Khalifa et al., 2016). Second, data analysis requires using appropriate reference samples and needs to be standardized. Third, the specificity of the changes observed needs to be expressed relative to other inflammatory disorders. Recent observations made during a clinical trial illustrate some of these limitations in using miRNAs as disease biomarkers. In a discovery cohort of RA patients treated with either adalimumab (anti-TNF antibody) or etanercept (a recombinant protein encompassing the TNF receptor and the constant end of the IgG1 antibody), the predicted miRNAs that were targeted by the treatment could not be validated (Cuppen et al., 2016). These disappointing results call for validation of miRNA candidates in independent cohorts, and standardization of tests that would make them suitable for implementation into clinical practice.

### Epigenetic Therapy

It is clear that epigenetic regulation plays a role in disease pathogenesis. However, it remains unresolved whether the epigenetic abnormalities are a consequence of altered disease pathways or the true trigger of the

pathogenesis. Within this limitation, targeting epigenetic pathways for therapeutic purposes could be an attractive approach for treating autoimmune diseases.

The use of drugs to correct epigenetic defects, referred to as epigenetic therapy, is a new and rapidly developing area of pharmacology. Compared to genetic defects, which are permanent, epigenetic defects are more easily reversible with pharmacological intervention. Therefore epigenetic therapy could give rise to agents capable of controlling and, possibly, preventing various diseases (Egger et al., 2004). This offers the possibility of using epigenetic drugs to reverse patterns of epigenetic alterations and to relieve particular conditions, and several drugs initially approved for cancer treatment could, hopefully, possess beneficial properties for multiple autoimmune diseases.

### **Targeting DNA Methylation**

For this epigenetic mark, only an all-or-nothing targeting is possible. The prototypic inhibitor 5-azacytidine was developed as a cytotoxic agent and, subsequently, has been discovered to possess a potent DNMT inhibitor activity. Since the drug is converted into nucleotide triphosphates and is incorporated in place of cytosines into replicating DNA, it is more active in the S-phase of cells. Because it binds both RNA and DNA, it both interrupts mRNA translation and inhibits methylation by trapping DNMTs. In animal models, DNMT inhibitors, such as 5-aza-2'-deoxycytidine, reversed the aberrant hypermethylation of DKK1, SFRP1, and FLI1 in fibroblasts from SSc patients, and normalized Wnt signaling and type I collagen expression (Dees et al., 2014). In mice with EAE, a model of the human demyelinating disease MS, 5-aza-2'-deoxycytidine treatment increased the immunosuppressive function of  $\text{Foxp3}^+$  Tregs, prevented EAE development and suppressed CNS inflammation (Vojinovic et al., 2011; Chan et al., 2014).

However, DNA methylation inhibitors exhibit double-edged effects on the immune system. For example, 5-azacytidine could promote Treg function via demethylation of FOXP3 but, at the same time, promoted increased IgG production by B cells (Lal et al., 2009; Wang et al., 2014), making its potential effects difficult to predict in autoimmune patients whose cells already often exhibit global hypomethylation.

The disadvantages of azanucleosides (instability in aqueous solutions and toxicity) might be overcome by the use of other analogs, such as zebularine, procainamide (used to treat cardiac arrhythmia), 5-fluoro-2'-deoxycytidine, and hydralazine (used to treat hypertension). Several natural products derived from tea and sponges, such as epigallocatechin-3-gallate, also show DNMT inhibitory activity (Fang et al., 2003). Clinical trials that target DNMT1 are underway in tumors (<http://clinicaltrials.gov>).

In human autoimmune diseases, inhibiting DNA methylation would not be the optimal therapeutic option because the changes identified to date are hypomethylation, but not hypermethylation. Furthermore, DNA demethylating agents such as hydralazine have been shown to subvert B lymphocyte tolerance and to contribute to the generation of pathogenic autoreactivity (Mazari et al., 2007). Likewise, in autoimmune diseases exhibiting a global DNA hypo methylation (SLE and RA), drugs should be designed to specifically increase the DNA methylation without triggering undesirable hypermethylation-related tumorigenesis.

It is of related interest that in clinical settings, the epigenetic effects of some drugs may account for their effectiveness. For example, methotrexate can induce depletion of SAM, a substrate of DNMTs during DNA methylation, and thereby reduces DNMT1 activity (Nihal et al., 2014). In RA patients, treatment with methotrexate was found to reduce DNA hydroxymethylation (de Andres et al., 2015). Similarly, cyclophosphamide treatment can perturb the activity of DNMT1 and increase DNA methylation (Zhang et al., 2011).

### **Histone Deacetylase Inhibitors**

HDAC and HAT enzymes play an important role in regulating gene transcription through chromatin remodeling, which comprises histone protein–DNA complexes that tightly package to form chromosomes. HATs promote the transcriptionally active “decondensed” chromatin state by catalyzing the addition of acetyl groups onto the N-terminal tails of histone lysine residues, causing spatial distortion and allowing transcription factor and RNA polymerase complex recruitment. On the other hand, HDACs catalyze the removal of acetyl groups from lysine tails and restore the transcriptionally inactive “condensed” state.

The development of HDACis is relatively advanced. They show promising antiinflammatory properties, as demonstrated in an increasing number of animal and cellular models of inflammatory diseases (Halili et al., 2009). As indicated by their name, the molecular function of HDACs was thought to be restricted to histone deacetylation, but recent advances in phylogenetic analysis suggested that HDACs regulate the activity of a wide range of nonhistone proteins; and 3600 acetylation sites (of which only 61 were on histones) were found on 1750 proteins, including cytoplasmic proteins. Thus the impact of acetylation in terms of posttranslational regulation

is comparable to that of phosphorylation. A growing number of HDACi are being developed for the treatment of an expanding range of conditions, from cancer to neurodegenerative and other inflammatory diseases.

To date, HDACi, such as SAHA and TSA, have proved to be useful for relieving lupus disease in mice (Ballestar et al., 2006). The effects of TSA on human T cells are predominantly immunosuppressive and reminiscent of the signaling aberrations that have been described in patients with SLE (Hasler and Zouali, 2001; Zouali and Sarmay, 2004). Since global histone acetylation is reduced in T cells from SLE patients, increased histone acetylation was suggested to be beneficial in SLE. HDACi, such as suberoylanilide hydroxamic acid (SAHA) and TSA, have been reported to induce clinical improvement of lupus disease in animal models (Mishra et al., 2003; Reilly et al., 2008). However, valproic acid, used to treat bipolar mood disorder and seizures, also inhibits HDAC. In epilepsy patients, it was found to sometimes result in lupus-like symptoms (Mau and Yung, 2014). Such adverse reactions may limit the potential of epigenetic treatments.

In juvenile idiopathic arthritis, HDAC inhibition provides beneficial effects (Vojinovic et al., 2011). In studies of experimental diabetes, acetylation is recognized to regulate NF- $\kappa$ B, the master transcription factor in inflammation, the activation of which is critical in IL-1 $\beta$ -induced  $\beta$ -cell death. As a result, HDAC inhibition exerts protective effects on  $\beta$  cells exposed to toxicity-mediating cytokines.

In SSc, the HDACi TSA has demonstrated inhibitory effects on fibroblast activation by restoring the expression of FLI1, a negative regulator of collagen expression (Wang et al., 2006). The inhibition of HDAC7 by TSA down-regulated the expression of the fibrosis-related genes COL1A1 and COL3A1 (Hemmatazad et al., 2009). In RA synoviocytes, TSA impaired the stability of IL-6 mRNA and disrupted inflammatory cytokine production (Grabiec et al., 2012). In T cells from SLE patients, TSA reversed the aberrant increase of CD40L and IL-10 expression and the skewed IFN- $\gamma$  expression (Mishra et al., 2001).

In a clinical trial of 17 patients with systemic juvenile idiopathic arthritis, the orally active pan-HDACi givinostat, also called ITF2357, gave rise to six adverse events in three patients, while two-thirds of the patients achieved significant therapeutic benefit after 12 weeks, particularly in terms of the arthritic component of the disease (Vojinovic et al., 2011). This therapeutic benefit could be due, in part, to decreased production of IL-6 by givinostat. Currently, selective HDACi are being developed to increase therapeutic efficacy and limit toxicity (Lee et al., 2015).

Studies of chemically induced experimental colitis that mimics human ulcerative colitis also show a similar trend. For example, inhibition of HDAC in trinitrobenzene- and oxazolone-induced colitis resulted in amelioration (Glauben et al., 2006; Glauben et al., 2008). HDACi of different classes (the hydroxamic acids SAHA and ITF2357 as well as the SCFA valproic acid) suppressed the inflammatory parameters in acute dextran sulfate sodium-induced colitis, and ameliorated weight loss, bleeding, and colon shortening (Glauben et al., 2008). These macroscopic data were paralleled by a reduction of proinflammatory cytokines at the site of inflammation in colon cultures and by a reduced histological inflammation score (Glauben et al., 2006).

However, since each HDAC can target several genes, it can potentially alter the expression of other genes and lead to unacceptable toxicity (Kroesen et al., 2014). As a result, HDACi could behave as a double-edged sword in epigenetic therapy. A similar reasoning applies to the use of DNMT inhibitors in the clinic. Therefore investigations should be performed to identify safer therapeutic targets.

### **MicroRNAs-Targeting Therapeutics**

miRNAs have been demonstrated to represent potential diagnostic biomarkers for autoimmune diseases. Therefore the modulation of miRNA expression using miRNA mimics or inhibitors could represent an attractive therapeutic avenue for autoimmune diseases. Antisense oligonucleotides that target miRNA (anti-miRs) are highly complementary to the target miRNA. Upon delivery to cells in culture, anti-miRs specifically suppress the function of the target miRNAs and can even induce degradation of the targeted miRNAs.

In studies of collagen-induced arthritis, injection of recombinant lentiviral vectors silencing miR-223 to mice led to amelioration of the score and the incidence of arthritis, and bone erosion in the joints (Li et al., 2012). In a clinical trial of five SLE patients who had high IFN scores, manipulation of miR-146a levels reduced expression of selected genes, suggesting that this approach could provide a therapeutic benefit to SLE patients (Tang et al., 2009).

Inhibition of miRNA expression can be achieved using antisense oligonucleotides, small-molecule inhibitors, and miRNA sponges. This latter vector-based strategy is based on mRNA sequences containing multiple artificial miRNA-binding sites that can act as a decoy or a sponge. Transfection of a vector encoding a sponge into cells in culture selectively depletes endogenous miRNAs, allowing translation of the target mRNAs (Ebert et al., 2007). In studies of MS, in vivo silencing of miR-326 by miRNA sponges reduced Th17 cell numbers and disease severity,

suggesting that miR-326 plays a role in Th17 polarization during the pathogenesis of this disease (Du et al., 2009). Thus the antisense oligonucleotide approach could open up new avenues for treating autoimmune diseases.

### ***Epigenetic Generation of Regulatory T Cells***

A variety of methods can be used to generate Tregs expressing Foxp3 protein, which is generally considered a specific Treg marker. Since the stability of Foxp3 expression in Tregs is important for their therapeutic use, conversion of antigen-specific Tregs into effector T cells can have detrimental effects and limit their clinical applicability (Roncarolo and Battaglia, 2007). In addition to Foxp3 activity, the development and function of stable Tregs also are governed by epigenetic mechanisms, including DNA methylation, histone modifications, nucleosome positioning, chromatin remodeling, and miRNA expression (Arvey et al., 2015; Huehn and Beyer, 2015).

Therefore epigenetic regulation may be an effective therapeutic strategy to generate stable, suppressive Tregs. In vivo injection of HDAC9, a member of the class II HDACi, increases Treg numbers and function in mice (Tao et al., 2007) and in rhesus monkeys (Johnson et al., 2008). Remarkably, pan-HDACi, but not class I-specific HDACi, increased the function of Foxp3<sup>+</sup> Tregs and prevented and reduced established colitis in mice. These pan-HDACi-mediated effects were associated with increased numbers of Foxp3<sup>+</sup> Tregs within the lamina propria.

As regards DNMT inhibitors, they induce strong Foxp3 expression, but with cell toxicity as well as induction of Th1 and Th2 cytokines, which limits their use (Lal et al., 2009). Similarly, DNA methyltransferase inhibitors were found to strongly induce Foxp3 expression but are associated with high toxicity and induction of Th1 and Th2 cytokines, which also hampers their clinical use.

In humans, an epigenetic approach was followed to generate enhanced Foxp3<sup>+</sup> suppressive Tregs that could be used in cell therapy (Lal et al., 2009). Human Foxp3<sup>+</sup>CD4<sup>+</sup> T cells are very heterogeneous, based on CD25, CD62L, CD45RA, HLA-DR, and ICOS expression, but they can be divided into three different subsets of Foxp3<sup>+</sup>CD4<sup>+</sup> T cells in the peripheral blood (Miyara et al., 2009). CD45RA<sup>+</sup>Foxp3<sup>lo</sup> resting Tregs and CD45RA<sup>-</sup>Foxp3<sup>hi</sup> activated Tregs show completely demethylated CpG at the proximal promoter (-256 to -16 bp) and more than 85% demethylation of the intronic region promoter (+3824 to +3937 bp), and are suppressive (Miyara et al., 2009). However, CD45RA<sup>-</sup>Foxp3<sup>lo</sup> Tregs show less than 50% demethylation at the intronic region, secrete cytokines such as IL-2 and IFN- $\gamma$  and do not show suppressive functions (Miyara et al., 2009). These findings suggest that epigenetic mechanisms may help designing better approaches to generate suppressive human Tregs. Stabilization of the activity of Foxp3 and Treg function by epigenetic mechanisms could open up development of novel therapeutic strategies for autoimmune diseases.

---

## CONCLUSIONS AND FUTURE PROSPECTS

---

As summarized above, autoimmune disease concordance rates in monozygotic twins range from 13% to 61%, suggesting that environmental factors play a direct or indirect role in the pathogenesis of autoimmune diseases. They include drugs, such as the methyl donor hydralazine, vaccination and antibiotics, iodine levels, cigarette smoking, increasing wealth, temperate, decreased infection rates, increased hygiene, UV irradiation, wheat consumption, and gut microbiota. Some of these factors have the potential to induce epigenetic changes that have been reported in cells from patients with autoimmune diseases. It will be important to further define the role of environmental factors and to decipher how they influence gene expression in autoimmune diseases. There is a need for additional investigations to confirm the observed alterations and, hopefully, relate them to abnormal pathways that could be unique to a given disease. In addition, it would be essential to pinpoint common pathways in several autoimmune diseases that could account for the occurrence of different autoimmune phenotypes in family members.

The above hypothesis-driven studies are being complemented by resource-generating activities. Thus the International Human Epigenome Consortium makes available comprehensive sets of reference epigenomes relevant to health and disease (Bujold et al., 2016). It will be important to compare epigenome maps of model organisms relevant to human health and disease and to encompass a variety of human cell and tissue types from a variety of disease states, including autoimmune diseases. This could lead to the discovery and validation of epigenetic markers for diagnostic use and for the development of novel and more efficacious treatments.

A better understanding of the paths involved in disease pathogenesis should open up avenues for potential diagnostic and prognostic tools, and, possibly, for epigenetic interventions based on miRNA silencing and/or

chromatin remodeling agents. Progress in understanding epigenetic deregulation in autoimmune diseases invites investigations aiming at identifying biomarkers for early diagnosis and to develop novel therapeutic approaches. In experimental animals, several therapeutic agents have been found to modify the epigenome. However, since the epigenetic modifications reported in autoimmune diseases are complex, treatment strategies that target the epigenome are limited by off-target effects, and may result in undesirable effects or may cause severe adverse events. Despite encouraging preclinical observations, epigenetic therapy may have limitations. Because of lack of specificity, DNMT and HDACi may activate oncogenes, potentially resulting in accelerated tumor progression. In addition, once corrected, epigenetic states may revert to the original state because of the reversible nature of DNA methylation patterns. Finally, the findings that crosstalk mechanisms between DNA methylation, histone modification, and miRNAs regulate the expression of epigenome would suggest that epigenetic targeting for therapeutic purposes and using epigenetic modifications as biomarkers will not be of use in the short term in autoimmune diseases.

## References

- Akimova, T., Ge, G., Golovina, T., Mikheeva, T., Wang, L., Riley, J.L., et al., 2010. Histone/protein deacetylase inhibitors increase suppressive functions of human FOXP3<sup>+</sup> Tregs. *Clin. Immunol.* 136 (3), 348–363.
- Alevizos, I., Alexander, S., Turner, R.J., Illei, G.G., 2011. MicroRNA expression profiles as biomarkers of minor salivary gland inflammation and dysfunction in Sjogren's syndrome. *Arthritis Rheum.* 63 (2), 535–544.
- Allione, A., Marcon, F., Fiorito, G., Guarnera, S., Siniscalchi, E., Zijno, A., et al., 2015. Novel epigenetic changes unveiled by monozygotic twins discordant for smoking habits. *PLoS One* 10 (6), e0128265.
- Altork, N., Tsou, P.S., Coit, P., Khanna, D., Sawalha, A.H., 2015. Genome-wide DNA methylation analysis in dermal fibroblasts from patients with diffuse and limited systemic sclerosis reveals common and subset-specific DNA methylation aberrancies. *Ann. Rheum. Dis.* 74 (8), 1612–1620.
- Amarilyo, G., La Cava, A., 2012. miRNA in systemic lupus erythematosus. *Clin. Immunol.* 144 (1), 26–31.
- de Andres, M.C., Perez-Pampin, E., Calaza, M., Santaclara, F.J., Ortega, I., Gomez-Reino, J.J., et al., 2015. Assessment of global DNA methylation in peripheral blood cell subpopulations of early rheumatoid arthritis before and after methotrexate. *Arthritis Res. Ther.* 17, 233.
- Angiolilli, C., Kabala, P.A., Grabiec, A.M., Van Baarsen, I.M., Ferguson, B.S., Garcia, S., et al., 2017. Histone deacetylase 3 regulates the inflammatory gene expression programme of rheumatoid arthritis fibroblast-like synoviocytes. *Ann. Rheum. Dis.* 76 (1), 277–285.
- Araki, Y., Tsuzuki Wada, T., Aizaki, Y., Sato, K., Yokota, K., Fujimoto, K., et al., 2016. Histone methylation and STAT-3 differentially regulate interleukin-6-induced matrix metalloproteinase gene activation in rheumatoid arthritis synovial fibroblasts. *Arthritis Rheumatol.* 68 (5), 1111–1123.
- Arnett, F.C., Cho, M., Chatterjee, S., Aguilar, M.B., Reveille, J.D., Mayes, M.D., 2001. Familial occurrence frequencies and relative risks for systemic sclerosis (scleroderma) in three United States cohorts. *Arthritis Rheum.* 44 (6), 1359–1362.
- Arpaia, N., Campbell, C., Fan, X., Dikiy, S., van der Veeken, J., deRoos, P., et al., 2013. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 504 (7480), 451–455.
- Arvey, A., van der Veeken, J., Plitas, G., Rich, S.S., Concannon, P., Rudensky, A.Y., 2015. Genetic and epigenetic variation in the lineage specification of regulatory T cells. *Elife* 4, e07571.
- Balada, E., Ordi-Ros, J., Serrano-Acedo, S., Martinez-Lostao, L., Rosa-Leyva, M., Vilardell-Tarres, M., 2008. Transcript levels of DNA methyltransferases DNMT1, DNMT3A and DNMT3B in CD4<sup>+</sup> T cells from patients with systemic lupus erythematosus. *Immunology* 124 (3), 339–347.
- Ballestar, E., Esteller, M., Richardson, B.C., 2006. The epigenetic face of systemic lupus erythematosus. *J. Immunol.* 176 (12), 7143–7147.
- Bannister, A.J., Kouzarides, T., 2011. Regulation of chromatin by histone modifications. *Cell Res.* 21 (3), 381–395.
- Baranzini, S.E., Mudge, J., van Velkinburgh, J.C., Khankhanian, P., Khrebtukova, I., Miller, N.A., et al., 2010. Genome, epigenome and RNA sequences of monozygotic twins discordant for multiple sclerosis. *Nature* 464 (7293), 1351–1356.
- Baron, U., Floess, S., Wieczorek, G., Baumann, K., Grutzkau, A., Dong, J., et al., 2007. DNA demethylation in the human FOXP3 locus discriminates regulatory T cells from activated FOXP3(+) conventional T cells. *Eur. J. Immunol.* 37 (9), 2378–2389.
- Bartel, D.P., 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116 (2), 281–297.
- Baymaz, H.I., Fournier, A., Laget, S., Ji, Z., Jansen, P.W., Smits, A.H., et al., 2014. MBD5 and MBD6 interact with the human PR-DUB complex through their methyl-CpG-binding domain. *Proteomics* 14 (19), 2179–2189.
- Bensimon, C., Chastagner, P., Zouali, M., 1994. Human lupus anti-DNA autoantibodies undergo essentially primary V kappa gene rearrangements. *EMBO J.* 13 (13), 2951–2962.
- Berger, S.L., 2007. The complex language of chromatin regulation during transcription. *Nature* 447 (7143), 407–412.
- Bhutani, N., Burns, D.M., Blau, H.M., 2011. DNA demethylation dynamics. *Cell* 146 (6), 866–872.
- Bird, A., 2011. Putting the DNA back into DNA methylation. *Nat. Genet.* 43 (11), 1050–1051.
- Bosisio, D., Vulcano, M., Del Prete, A., Sironi, M., Salvi, V., Salogni, L., et al., 2008. Blocking TH17-polarizing cytokines by histone deacetylase inhibitors in vitro and in vivo. *J. Leukoc. Biol.* 84 (6), 1540–1548.
- Brandl, A., Heinzel, T., Kramer, O.H., 2009. Histone deacetylases: salesmen and customers in the post-translational modification market. *Biol. Cell* 101 (4), 193–205.
- Brenner, C., Fuks, F., 2007. A methylation rendezvous: reader meets writer. *Dev. Cell* 12 (6), 843–844.
- Brix, T.H., Hegedus, L., 2012. Twin studies as a model for exploring the aetiology of autoimmune thyroid disease. *Clin. Endocrinol. (Oxf.)* 76 (4), 457–464.

- Bujold, D., Morais, D.A.L., Gauthier, C., Cote, C., Caron, M., Kwan, T., et al., 2016. The International Human Epigenome Consortium data portal. *Cell Syst.* 3 (5), 496–499.e2.
- Byun, H.M., Nordio, F., Coull, B.A., Tarantini, L., Hou, L., Bonzini, M., et al., 2012. Temporal stability of epigenetic markers: sequence characteristics and predictors of short-term DNA methylation variations. *PLoS One* 7 (6), e39220.
- Cai, P., Konig, R., Boor, P.J., Kondraganti, S., Kaphalia, B.S., Khan, M.F., et al., 2008. Chronic exposure to trichloroethylene causes early onset of SLE-like disease in female MRL +/+ mice. *Toxicol. Appl. Pharmacol.* 228 (1), 68–75.
- Cai, T., Li, J., An, X., Yan, N., Li, D., Jiang, Y., et al., 2017. Polymorphisms in MIR499A and MIR125A gene are associated with autoimmune thyroid diseases. *Mol. Cell Endocrinol.* 440, 106–115.
- Cai, T.T., Muhalil, F.S., Song, R.H., Qin, Q., Wang, X., Shi, L.F., et al., 2015. Genome-wide DNA methylation analysis in Graves' disease. *Genomics* 105 (4), 204–210.
- Camelo, S., Iglesias, A.H., Hwang, D., Due, B., Ryu, H., Smith, K., et al., 2005. Transcriptional therapy with the histone deacetylase inhibitor trichostatin A ameliorates experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* 164 (1–2), 10–21.
- Chahrour, M., Jung, S.Y., Shaw, C., Zhou, X., Wong, S.T., Qin, J., et al., 2008. MeCP2, a key contributor to neurological disease, activates and represses transcription. *Science* 320 (5880), 1224–1229.
- Chan, M.W., Chang, C.B., Tung, C.H., Sun, J., Suen, J.L., Wu, S.F., 2014. Low-dose 5-aza-2'-deoxycytidine pretreatment inhibits experimental autoimmune encephalomyelitis by induction of regulatory T cells. *Mol. Med.* 20, 248–256.
- Chang, P.V., Hao, L., Offermanns, S., Medzhitov, R., 2014. The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. *Proc. Natl. Acad. Sci. U.S.A.* 111 (6), 2247–2252.
- Chatzikyriakidou, A., Voulgari, P.V., Georgiou, I., Drosos, A.A., 2010. A polymorphism in the 3'-UTR of interleukin-1 receptor-associated kinase (IRAK1), a target gene of miR-146a, is associated with rheumatoid arthritis susceptibility. *Joint Bone Spine* 77 (5), 411–413.
- Chen, D., Ma, H., Hong, H., Koh, S.S., Huang, S.M., Schurter, B.T., et al., 1999. Regulation of transcription by a protein methyltransferase. *Science* 284 (5423), 2174–2177.
- Churov, A.V., Oleinik, E.K., Knip, M., 2015. MicroRNAs in rheumatoid arthritis: altered expression and diagnostic potential. *Autoimmun. Rev.* 14 (11), 1029–1037.
- Ciavatta, D.J., Yang, J., Preston, G.A., Badhwar, A.K., Xiao, H., Hewins, P., et al., 2010. Epigenetic basis for aberrant upregulation of autoantigen genes in humans with ANCA vasculitis. *J. Clin. Invest.* 120 (9), 3209–3219.
- Cole, M.B., Quach, H., Quach, D., Baker, A., Taylor, K.E., Barcellos, L.F., et al., 2016. Epigenetic signatures of salivary gland inflammation in Sjogren's syndrome. *Arthritis Rheumatol.* 68 (12), 2936–2944.
- Costa-Reis, P., Russo, P.A., Zhang, Z., Colonna, L., Maurer, K., Gallucci, S., et al., 2015. The Role of microRNAs and human epidermal growth factor receptor 2 in proliferative lupus nephritis. *Arthritis Rheumatol.* 67 (9), 2415–2426.
- Cribbs, A.P., Kennedy, A., Penn, H., Read, J.E., Amjadi, P., Green, P., et al., 2014. Treg cell function in rheumatoid arthritis is compromised by cta-4 promoter methylation resulting in a failure to activate the indoleamine 2,3-dioxygenase pathway. *Arthritis Rheumatol.* 66 (9), 2344–2354.
- Cuppen, B.V., Rossato, M., Fritsch-Stork, R.D., Concepcion, A.N., Schenk, Y., Bijlsma, J.W., et al., 2016. Can baseline serum microRNAs predict response to TNF-alpha inhibitors in rheumatoid arthritis? *Arthritis Res. Ther.* 18, 189.
- Dai, Y., Huang, Y.S., Tang, M., Lv, T.Y., Hu, C.X., Tan, Y.H., et al., 2007. Microarray analysis of microRNA expression in peripheral blood cells of systemic lupus erythematosus patients. *Lupus* 16 (12), 939–946.
- Dasgupta, S., Zhou, Y., Jana, M., Banik, N.L., Pahan, K., 2003. Sodium phenylacetate inhibits adoptive transfer of experimental allergic encephalomyelitis in SJL/J mice at multiple steps. *J. Immunol.* 170 (7), 3874–3882.
- Dees, C., Schlottmann, I., Funke, R., Distler, A., Palumbo-Zerr, K., Zerr, P., et al., 2014. The Wnt antagonists DKK1 and SFRP1 are downregulated by promoter hypermethylation in systemic sclerosis. *Ann. Rheum. Dis.* 73 (6), 1232–1239.
- Deng, C., Kaplan, M.J., Yang, J., Ray, D., Zhang, Z., McCune, W.J., et al., 2001. Decreased Ras-mitogen-activated protein kinase signaling may cause DNA hypomethylation in T lymphocytes from lupus patients. *Arthritis Rheum.* 44 (2), 397–407.
- Dolinoy, D.C., Weidman, J.R., Jirtle, R.L., 2007. Epigenetic gene regulation: linking early developmental environment to adult disease. *Reprod. Toxicol.* 23 (3), 297–307.
- Du, C., Liu, C., Kang, J., Zhao, G., Ye, Z., Huang, S., et al., 2009. MicroRNA miR-326 regulates TH-17 differentiation and is associated with the pathogenesis of multiple sclerosis. *Nat. Immunol.* 10 (12), 1252–1259.
- Dubroff, L.M., Reid Jr., R.J., 1980. Hydralazine-pyrimidine interactions may explain hydralazine-induced lupus erythematosus. *Science* 208 (4442), 404–406.
- Dyment, D.A., Sadovnick, A.D., Ebers, G.C., 1997. Genetics of multiple sclerosis. *Hum. Mol. Genet.* 6 (10), 1693–1698.
- Ebert, M.S., Neilson, J.R., Sharp, P.A., 2007. MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. *Nat. Methods* 4 (9), 721–726.
- Edens, R.E., Dagtas, S., Gilbert, K.M., 2006. Histone deacetylase inhibitors induce antigen specific anergy in lymphocytes: a comparative study. *Int. Immunopharmacol.* 6 (11), 1673–1681.
- Egger, G., Liang, G., Aparicio, A., Jones, P.A., 2004. Epigenetics in human disease and prospects for epigenetic therapy. *Nature* 429 (6990), 457–463.
- Ehrlich, M., Lacey, M., 2013. DNA methylation and differentiation: silencing, upregulation and modulation of gene expression. *Epigenomics* 5 (5), 553–568.
- Elmesmari, A., Fraser, A.R., Wood, C., Gilchrist, D., Vaughan, D., Stewart, L., et al., 2016. MicroRNA-155 regulates monocyte chemokine and chemokine receptor expression in Rheumatoid Arthritis. *Rheumatology (Oxford)* 55 (11), 2056–2065.
- Esteller, M., 2011. Non-coding RNAs in human disease. *Nat. Rev. Genet.* 12 (12), 861–874.
- Faber, C., Morbach, H., Singh, S.K., Girschick, H.J., 2006. Differential expression patterns of recombination-activating genes in individual mature B cells in juvenile idiopathic arthritis. *Ann. Rheum. Dis.* 65 (10), 1351–1356.
- Fallahi, P., Ferrari, S.M., Ruffilli, I., Elia, G., Biricotti, M., Vita, R., et al., 2016. The association of other autoimmune diseases in patients with autoimmune thyroiditis: review of the literature and report of a large series of patients. *Autoimmun. Rev.* 15 (12), 1125–1128.

- Fang, M.Z., Wang, Y., Ai, N., Hou, Z., Sun, Y., Lu, H., et al., 2003. Tea polyphenol (−)-epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines. *Cancer Res.* 63 (22), 7563–7570.
- Fassbender, H.G., Seibel, M., Hebert, T., 1992. Pathways of destruction in metacarpal and metatarsal joints of patients with rheumatoid arthritis. *Scand. J. Rheumatol.* 21 (1), 10–16.
- Feil, R., Fraga, M.F., 2012. Epigenetics and the environment: emerging patterns and implications. *Nat. Rev. Genet.* 13 (2), 97–109.
- Fetahu, I.S., Hobaus, J., Kallay, E., 2014. Vitamin D and the epigenome. *Front. Physiol.* 5, 164.
- Filipowicz, W., Bhattacharyya, S.N., Sonenberg, N., 2008. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat. Rev. Genet.* 9 (2), 102–114.
- Fraga, M.F., Ballestar, E., Paz, M.F., Ropero, S., Setien, F., Ballestar, M.L., et al., 2005. Epigenetic differences arise during the lifetime of monozygotic twins. *Proc. Natl. Acad. Sci. U.S.A.* 102 (30), 10604–10609.
- Gandhi, R., Healy, B., Gholipour, T., Egorova, S., Musallam, A., Hussain, M.S., et al., 2013. Circulating microRNAs as biomarkers for disease staging in multiple sclerosis. *Ann Neurol.* 73 (6), 729–740.
- Garrett, L.R., Cuchens, M.A., 1991. Pristane induced effects on chromatin of rat lymphoid cells. *J. Cell Biochem.* 45 (3), 311–316.
- Gillespie, J., Savic, S., Wong, C., Hempshall, A., Inman, M., Emery, P., et al., 2012. Histone deacetylases are dysregulated in rheumatoid arthritis and a novel histone deacetylase 3-selective inhibitor reduces interleukin-6 production by peripheral blood mononuclear cells from rheumatoid arthritis patients. *Arthritis Rheum.* 64 (2), 418–422.
- Glauben, R., Batra, A., Fedke, I., Zeitz, M., Lehr, H.A., Leoni, F., et al., 2006. Histone hyperacetylation is associated with amelioration of experimental colitis in mice. *J. Immunol.* 176 (8), 5015–5022.
- Glauben, R., Batra, A., Stroh, T., Erben, U., Fedke, I., Lehr, H.A., et al., 2008. Histone deacetylases: novel targets for prevention of colitis-associated cancer in mice. *Gut* 57 (5), 613–622.
- Glossop, J.R., Emes, R.D., Nixon, N.B., Haworth, K.E., Packham, J.C., Dawes, P.T., et al., 2014. Genome-wide DNA methylation profiling in rheumatoid arthritis identifies disease-associated methylation changes that are distinct to individual T- and B-lymphocyte populations. *Epigenetics* 9 (9), 1228–1237.
- Glossop, J.R., Emes, R.D., Nixon, N.B., Packham, J.C., Fryer, A.A., Matthey, D.L., et al., 2016. Genome-wide profiling in treatment-naïve early rheumatoid arthritis reveals DNA methylome changes in T and B lymphocytes. *Epigenomics* 8 (2), 209–224.
- Gonzalez, S., Aguilera, S., Allende, C., Urzua, U., Quest, A.F., Herrera, L., et al., 2011. Alterations in type I hemidesmosome components suggestive of epigenetic control in the salivary glands of patients with Sjogren's syndrome. *Arthritis Rheum.* 63 (4), 1106–1115.
- Gorelik, G., Sawalha, A.H., Patel, D., Johnson, K., Richardson, B., 2015. T cell PKC $\delta$  kinase inactivation induces lupus-like autoimmunity in mice. *Clin. Immunol.* 158 (2), 193–203.
- Gourley, M., Miller, F.W., 2007. Mechanisms of disease: environmental factors in the pathogenesis of rheumatic disease. *Nat. Clin. Pract. Rheumatol.* 3 (3), 172–180.
- Grabiec, A.M., Krausz, S., de Jager, W., Burakowski, T., Groot, D., Sanders, M.E., et al., 2010. Histone deacetylase inhibitors suppress inflammatory activation of rheumatoid arthritis patient synovial macrophages and tissue. *J. Immunol.* 184 (5), 2718–2728.
- Grabiec, A.M., Korchynskyi, O., Tak, P.P., Reedquist, K.A., 2012. Histone deacetylase inhibitors suppress rheumatoid arthritis fibroblast-like synoviocyte and macrophage IL-6 production by accelerating mRNA decay. *Ann. Rheum. Dis.* 71 (3), 424–431.
- Graves, M.C., Benton, M., Lea, R.A., Boyle, M., Tajouri, L., Macartney-Coxson, D., et al., 2014. Methylation differences at the HLA-DRB1 locus in CD4+ T-cells are associated with multiple sclerosis. *Mult. Scler.* 20 (8), 1033–1041.
- Guerau-de-Arellano, M., Smith, K.M., Godlewski, J., Liu, Y., Winger, R., Lawler, S.E., et al., 2011. Micro-RNA dysregulation in multiple sclerosis favours pro-inflammatory T-cell-mediated autoimmunity. *Brain* 134 (Pt 12), 3578–3589.
- Guo, M., Mao, X., Ji, Q., Lang, M., Li, S., Peng, Y., et al., 2010. miR-146a in PBMCs modulates Th1 function in patients with acute coronary syndrome. *Immunol. Cell Biol.* 88 (5), 555–564.
- Halili, M.A., Andrews, M.R., Sweet, M.J., Fairlie, D.P., 2009. Histone deacetylase inhibitors in inflammatory disease. *Curr. Top. Med. Chem.* 9 (3), 309–319.
- Hansen, T., Skytthe, A., Stenager, E., Petersen, H.C., Kyvik, K.O., Bronnum-Hansen, H., 2005. Risk for multiple sclerosis in dizygotic and monozygotic twins. *Mult. Scler.* 11 (5), 500–503.
- Hara, N., Alkanani, A.K., Dinarello, C.A., Zipris, D., 2014. Histone deacetylase inhibitor suppresses virus-induced proinflammatory responses and type 1 diabetes. *J. Mol. Med. (Berl.)* 92 (1), 93–102.
- Hasler, P., Zouali, M., 2001. B cell receptor signaling and autoimmunity. *FASEB J.* 15 (12), 2085–2098.
- He, Y., Dupree, J., Wang, J., Sandoval, J., Li, J., Liu, H., et al., 2007. The transcription factor Yin Yang 1 is essential for oligodendrocyte progenitor differentiation. *Neuron* 55 (2), 217–230.
- Hedrich, C.M., Rauen, T., Kis-Toth, K., Kyttaris, V.C., Tsokos, G.C., 2012. cAMP-responsive element modulator alpha (CREMalpha) suppresses IL-17F protein expression in T lymphocytes from patients with systemic lupus erythematosus (SLE). *J. Biol. Chem.* 287 (7), 4715–4725.
- Heijmans, B.T., Tobi, E.W., Stein, A.D., Putter, H., Blauw, G.J., Susser, E.S., et al., 2008. Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc. Natl. Acad. Sci. U.S.A.* 105 (44), 17046–17049.
- Hemmatazad, H., Rodrigues, H.M., Maurer, B., Brentano, F., Pilecky, M., Distler, J.H., et al., 2009. Histone deacetylase 7, a potential target for the antifibrotic treatment of systemic sclerosis. *Arthritis Rheum.* 60 (5), 1519–1529.
- Hendrich, B., Tweedie, S., 2003. The methyl-CpG binding domain and the evolving role of DNA methylation in animals. *Trends Genet.* 19 (5), 269–277.
- Herrmann, H., Bar, H., Kreplak, L., Strelkov, S.V., Aebi, U., 2007. Intermediate filaments: from cell architecture to nanomechanics. *Nat. Rev. Mol. Cell Biol.* 8 (7), 562–573.
- Honda, N., Jinnin, M., Kira-Etoh, T., Makino, K., Kajihara, I., Makino, T., et al., 2013. miR-150 down-regulation contributes to the constitutive type I collagen overexpression in scleroderma dermal fibroblasts via the induction of integrin beta3. *Am. J. Pathol.* 182 (1), 206–216.
- Hoyo, C., Murphy, S.K., Jirtle, R.L., 2009. Imprint regulatory elements as epigenetic biosensors of exposure in epidemiological studies. *J. Epidemiol. Community Health* 63 (9), 683–684.

- Hu, N., Qiu, X., Luo, Y., Yuan, J., Li, Y., Lei, W., et al., 2008. Abnormal histone modification patterns in lupus CD4 + T cells. *J. Rheumatol.* 35 (5), 804–810.
- Huber, L.C., Brock, M., Hemmatazad, H., Giger, O.T., Moritz, F., Trenkmann, M., et al., 2007. Histone deacetylase/acetylase activity in total synovial tissue derived from rheumatoid arthritis and osteoarthritis patients. *Arthritis Rheum.* 56 (4), 1087–1093.
- Huber, L.C., Distler, J.H., Moritz, F., Hemmatazad, H., Hauser, T., Michel, B.A., et al., 2007. Trichostatin A prevents the accumulation of extracellular matrix in a mouse model of bleomycin-induced skin fibrosis. *Arthritis Rheum.* 56 (8), 2755–2764.
- Huck, S., Zouali, M., 1996. DNA methylation: a potential pathway to abnormal autoreactive lupus B cells. *Clin. Immunol. Immunopathol.* 80 (1), 1–8.
- Huehn, J., Beyer, M., 2015. Epigenetic and transcriptional control of Foxp3 + regulatory T cells. *Semin. Immunol.* 27 (1), 10–18.
- Hughes, T., Adler, A., Kelly, J.A., Kaufman, K.M., Williams, A.H., Langefeld, C.D., et al., 2012. Evidence for gene-gene epistatic interactions among susceptibility loci for systemic lupus erythematosus. *Arthritis Rheum.* 64 (2), 485–492.
- Huynh, J.L., Casaccia, P., 2013. Epigenetic mechanisms in multiple sclerosis: implications for pathogenesis and treatment. *Lancet Neurol.* 12 (2), 195–206.
- Hyttinen, V., Kaprio, J., Kinnunen, L., Koskenvuo, M., Tuomilehto, J., 2003. Genetic liability of type 1 diabetes and the onset age among 22,650 young Finnish twin pairs: a nationwide follow-up study. *Diabetes* 52 (4), 1052–1055.
- Ichii, O., Otsuka-Kanazawa, S., Horino, T., Kimura, J., Nakamura, T., Matsumoto, M., et al., 2014. Decreased miR-26a expression correlates with the progression of podocyte injury in autoimmune glomerulonephritis. *PLoS One* 9 (10), e110383.
- Iwamoto, N., Vettori, S., Maurer, B., Brock, M., Pachera, E., Jungel, A., et al., 2016. Downregulation of miR-193b in systemic sclerosis regulates the proliferative vasculopathy by urokinase-type plasminogen activator expression. *Ann. Rheum. Dis.* 75 (1), 303–310.
- Janson, P.C., Winerdal, M.E., Marits, P., Thorn, M., Ohlsson, R., Winqvist, O., 2008. FOXP3 promoter demethylation reveals the committed Treg population in humans. *PLoS One* 3 (2), e1612.
- Januchowski, R., Jagodzinski, P.P., 2005. Effect of 5-azacytidine and procainamide on CD3-zeta chain expression in Jurkat T cells. *Biomed. Pharmacother.* 59 (3), 122–126.
- Javierre, B.M., Fernandez, A.F., Richter, J., Al-Shahrour, F., Martin-Subero, J.I., Rodriguez-Ubreva, J., et al., 2010. Changes in the pattern of DNA methylation associate with twin discordance in systemic lupus erythematosus. *Genome Res.* 20 (2), 170–179.
- Jeffries, M.A., Dozmorov, M., Tang, Y., Merrill, J.T., Wren, J.D., Sawalha, A.H., 2011. Genome-wide DNA methylation patterns in CD4 + T cells from patients with systemic lupus erythematosus. *Epigenetics* 6 (5), 593–601.
- Johnson, J., Pahuja, A., Graham, M., Hering, B., Hancock, W.W., Bansal-Pakala, P., 2008. Effects of histone deacetylase inhibitor SAHA on effector and FOXP3 + regulatory T cells in rhesus macaques. *Transplant. Proc.* 40 (2), 459–461.
- Jones, P.A., 2012. Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat. Rev. Genet.* 13 (7), 484–492.
- Jurkowska, R.Z., Jurkowski, T.P., Jeltsch, A., 2011. Structure and function of mammalian DNA methyltransferases. *ChemBioChem* 12 (2), 206–222.
- Kallenberg, C.G., Heeringa, P., Stegeman, C.A., 2006. Mechanisms of disease: pathogenesis and treatment of ANCA-associated vasculitides. *Nat. Clin. Pract. Rheumatol.* 2 (12), 661–670.
- Kaminsky, Z., Wang, S.C., Petronis, A., 2006. Complex disease, gender and epigenetics. *Ann. Med.* 38 (8), 530–544.
- Kaminsky, Z.A., Tang, T., Wang, S.C., Ptak, C., Oh, G.H., Wong, A.H., et al., 2009. DNA methylation profiles in monozygotic and dizygotic twins. *Nat. Genet.* 41 (2), 240–245.
- Kaplan, M.J., Lu, Q., Wu, A., Attwood, J., Richardson, B., 2004. Demethylation of promoter regulatory elements contributes to perforin overexpression in CD4 + lupus T cells. *J. Immunol.* 172 (6), 3652–3661.
- Karouzakis, E., Gay, R.E., Michel, B.A., Gay, S., Neidhart, M., 2009. DNA hypomethylation in rheumatoid arthritis synovial fibroblasts. *Arthritis Rheum.* 60 (12), 3613–3622.
- Kastan, M.B., Gowans, B.J., Lieberman, M.W., 1982. Methylation of deoxycytidine incorporated by excision-repair synthesis of DNA. *Cell.* 30 (2), 509–516.
- Kazantsev, A.G., Thompson, L.M., 2008. Therapeutic application of histone deacetylase inhibitors for central nervous system disorders. *Nat. Rev. Drug Discov.* 7 (10), 854–868.
- Khalifa, O., Pers, Y.M., Ferreira, R., Senechal, A., Jorgensen, C., Apparailly, F., et al., 2016. X-linked miRNAs associated with gender differences in rheumatoid arthritis. *Int. J. Mol. Sci.* 17 (11), 1852.
- Khattri, R., Cox, T., Yasayko, S.A., Ramsdell, F., 2003. An essential role for Scurfin in CD4 + CD25 + T regulatory cells. *Nat. Immunol.* 4 (4), 337–342.
- Kim, H.P., Leonard, W.J., 2007. CREB/ATF-dependent T cell receptor-induced FoxP3 gene expression: a role for DNA methylation. *J. Exp. Med.* 204 (7), 1543–1551.
- Klareskog, L., Padyukov, L., Alfredsson, L., 2007. Smoking as a trigger for inflammatory rheumatic diseases. *Curr. Opin. Rheumatol.* 19 (1), 49–54.
- Klein, K., Kabala, P.A., Grabiec, A.M., Gay, R.E., Kolling, C., Lin, L.L., et al., 2016. The bromodomain protein inhibitor I-BET151 suppresses expression of inflammatory genes and matrix degrading enzymes in rheumatoid arthritis synovial fibroblasts. *Ann. Rheum. Dis.* 75 (2), 422–429.
- Knip, M., Veijola, R., Virtanen, S.M., Hyoty, H., Vaarala, O., Akerblom, H.K., 2005. Environmental triggers and determinants of type 1 diabetes. *Diabetes* 54 (Suppl. 2), S125–S136.
- Knip, M., Virtanen, S.M., Seppa, K., Ilonen, J., Savilahti, E., Vaarala, O., et al., 2010. Dietary intervention in infancy and later signs of beta-cell autoimmunity. *N. Engl. J. Med.* 363 (20), 1900–1908.
- Kochi, Y., 2016. Genetics of autoimmune diseases: perspectives from genome-wide association studies. *Int. Immunopharmacol.* 28 (4), 155–161.
- Koenen, H.J., Smeets, R.L., Vink, P.M., van Rijssen, E., Boots, A.M., Joosten, I., 2008. Human CD25highFoxp3pos regulatory T cells differentiate into IL-17-producing cells. *Blood* 112 (6), 2340–2352.
- Kohm, A.P., Carpentier, P.A., Anger, H.A., Miller, S.D., 2002. Cutting edge: CD4 + CD25 + regulatory T cells suppress antigen-specific auto-reactive immune responses and central nervous system inflammation during active experimental autoimmune encephalomyelitis. *J. Immunol.* 169 (9), 4712–4716.

- Komatsu, N., Mariotti-Ferrandiz, M.E., Wang, Y., Malissen, B., Waldmann, H., Hori, S., 2009. Heterogeneity of natural Foxp3<sup>+</sup> T cells: a committed regulatory T-cell lineage and an uncommitted minor population retaining plasticity. *Proc. Natl. Acad. Sci. U.S.A.* 106 (6), 1903–1908.
- Kouzarides, T., 2007. Chromatin modifications and their function. *Cell* 128 (4), 693–705.
- Kroesen, M., Gielen, P., Brok, I.C., Armandari, I., Hoogerbrugge, P.M., Adema, G.J., 2014. HDAC inhibitors and immunotherapy; a double edged sword? *Oncotarget* 5 (16), 6558–6572.
- Krol, J., Loedige, I., Filipowicz, W., 2010. The widespread regulation of microRNA biogenesis, function and decay. *Nat. Rev. Genet.* 11 (9), 597–610.
- Lal, G., Zhang, N., van der Touw, W., Ding, Y., Ju, W., Bottinger, E.P., et al., 2009. Epigenetic regulation of Foxp3 expression in regulatory T cells by DNA methylation. *J. Immunol.* 182 (1), 259–273.
- Lee, D.U., Agarwal, S., Rao, A., 2002. Th2 lineage commitment and efficient IL-4 production involves extended demethylation of the IL-4 gene. *Immunity* 16 (5), 649–660.
- Lee, J., Hong, E.C., Jeong, H., Hwang, J.W., Kim, H., Bae, E.K., et al., 2015. A novel histone deacetylase 6-selective inhibitor suppresses synovial inflammation and joint destruction in a collagen antibody-induced arthritis mouse model. *Int. J. Rheum. Dis.* 18 (5), 514–523.
- Lee, P.P., Fitzpatrick, D.R., Beard, C., Jessup, H.K., Lehar, S., Makar, K.W., et al., 2001. A critical role for Dnmt1 and DNA methylation in T cell development, function, and survival. *Immunity* 15 (5), 763–774.
- Lee, Y.K., Mukasa, R., Hatton, R.D., Weaver, C.T., 2009. Developmental plasticity of Th17 and Treg cells. *Curr. Opin. Immunol.* 21 (3), 274–280.
- Lei, W., Luo, Y., Yan, K., Zhao, S., Li, Y., Qiu, X., et al., 2009. Abnormal DNA methylation in CD4<sup>+</sup> T cells from patients with systemic lupus erythematosus, systemic sclerosis, and dermatomyositis. *Scand. J. Rheumatol.* 38 (5), 369–374.
- Leoni, F., Zaliani, A., Bertolini, G., Porro, G., Pagani, P., Pozzi, P., et al., 2002. The antitumor histone deacetylase inhibitor suberoylanilide hydroxamic acid exhibits antiinflammatory properties via suppression of cytokines. *Proc. Natl. Acad. Sci. U.S.A.* 99 (5), 2995–3000.
- Leslie, R.D., Delli Castelli, M., 2004. Age-dependent influences on the origins of autoimmune diabetes: evidence and implications. *Diabetes* 53 (12), 3033–3040.
- Lewis, B.P., Burge, C.B., Bartel, D.P., 2005. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 120 (1), 15–20.
- Li, B., Carey, M., Workman, J.L., 2007. The role of chromatin during transcription. *Cell* 128 (4), 707–719.
- Li, B., Samanta, A., Song, X., Iacono, K.T., Bembas, K., Tao, R., et al., 2007. FOXP3 interactions with histone acetyltransferase and class II histone deacetylases are required for repression. *Proc. Natl. Acad. Sci. U.S.A.* 104 (11), 4571–4576.
- Li, J., Wan, Y., Guo, Q., Zou, L., Zhang, J., Fang, Y., et al., 2010. Altered microRNA expression profile with miR-146a upregulation in CD4<sup>+</sup> T cells from patients with rheumatoid arthritis. *Arthritis Res. Ther.* 12 (3), R81.
- Li, K., Du, Y., Jiang, B.L., He, J.F., 2014. Increased microRNA-155 and decreased microRNA-146a may promote ocular inflammation and proliferation in Graves' ophthalmopathy. *Med. Sci. Monit.* 20, 639–643.
- Li, Q.J., Chau, J., Ebert, P.J., Sylvester, G., Min, H., Liu, G., et al., 2007. miR-181a is an intrinsic modulator of T cell sensitivity and selection. *Cell* 129 (1), 147–161.
- Li, Y.R., Li, J., Zhao, S.D., Bradfield, J.P., Mentch, F.D., Maggadottir, S.M., et al., 2015. Meta-analysis of shared genetic architecture across ten pediatric autoimmune diseases. *Nat. Med.* 21 (9), 1018–1027.
- Li, Y.T., Chen, S.Y., Wang, C.R., Liu, M.F., Lin, C.C., Jou, I.M., et al., 2012. Brief report: amelioration of collagen-induced arthritis in mice by lentivirus-mediated silencing of microRNA-223. *Arthritis Rheum.* 64 (10), 3240–3245.
- Lian, X., Xiao, R., Hu, X., Kanekura, T., Jiang, H., Li, Y., et al., 2012. DNA demethylation of CD40L in CD4<sup>+</sup> T cells from women with systemic sclerosis: a possible explanation for female susceptibility. *Arthritis Rheum.* 64 (7), 2338–2345.
- Liggett, T., Melnikov, A., Tilwalli, S., Yi, Q., Chen, H., Replogle, C., et al., 2010. Methylation patterns of cell-free plasma DNA in relapsing-remitting multiple sclerosis. *J. Neurol. Sci.* 290 (1–2), 16–21.
- Limbach, M., Saare, M., Tserel, L., Kisand, K., Eglit, T., Sauer, S., et al., 2016. Epigenetic profiling in CD4<sup>+</sup> and CD8<sup>+</sup> T cells from Graves' disease patients reveals changes in genes associated with T cell receptor signaling. *J. Autoimmun.* 67, 46–56.
- Lin, H.S., Hu, C.Y., Chan, H.Y., Liew, Y.Y., Huang, H.P., Lepescheux, L., et al., 2007. Anti-rheumatic activities of histone deacetylase (HDAC) inhibitors in vivo in collagen-induced arthritis in rodents. *Br. J. Pharmacol.* 150 (7), 862–872.
- Lin, S.Y., Hsieh, S.C., Lin, Y.C., Lee, C.N., Tsai, M.H., Lai, L.C., et al., 2012. A whole genome methylation analysis of systemic lupus erythematosus: hypomethylation of the IL10 and IL1R2 promoters is associated with disease activity. *Genes Immun.* 13 (3), 214–220.
- Liu, S., da Cunha, A.P., Rezende, R.M., Cialic, R., Wei, Z., Bry, L., et al., 2016. The host shapes the gut microbiota via fecal microRNA. *Cell Host Microbe* 19 (1), 32–43.
- Liu, T., Sun, J., Wang, Z., Yang, W., Zhang, H., Fan, C., et al., 2017. Changes in the DNA methylation and hydroxymethylation status of the intercellular adhesion molecule 1 gene promoter in thyrocytes from autoimmune thyroiditis patients. *Thyroid* 27 (6), 838–845.
- Liu, Y., Aryee, M.J., Padyukov, L., Fallin, M.D., Hesselberg, E., Runarsson, A., et al., 2013. Epigenome-wide association data implicate DNA methylation as an intermediary of genetic risk in rheumatoid arthritis. *Nat. Biotechnol.* 31 (2), 142–147.
- Lu, Q., Kaplan, M., Ray, D., Zacharek, S., Gutsch, D., Richardson, B., 2002. Demethylation of ITGAL (CD11a) regulatory sequences in systemic lupus erythematosus. *Arthritis Rheum.* 46 (5), 1282–1291.
- Lu, Q., Wu, A., Richardson, B.C., 2005. Demethylation of the same promoter sequence increases CD70 expression in lupus T cells and T cells treated with lupus-inducing drugs. *J. Immunol.* 174 (10), 6212–6219.
- Lu, Q., Wu, A., Tesmer, L., Ray, D., Yousif, N., Richardson, B., 2007. Demethylation of CD40LG on the inactive X in T cells from women with lupus. *J. Immunol.* 179 (9), 6352–6358.
- Lucas, J.L., Mirshahpanah, P., Haas-Stapleton, E., Asadullah, K., Zollner, T.M., Numerof, R.P., 2009. Induction of Foxp3<sup>+</sup> regulatory T cells with histone deacetylase inhibitors. *Cell Immunol.* 257 (1–2), 97–104.
- Luger, K., Mader, A.W., Richmond, R.K., Sargent, D.F., Richmond, T.J., 1997. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature*. 389 (6648), 251–260.

- Lundh, M., Christensen, D.P., Rasmussen, D.N., Mascagni, P., Dinarello, C.A., Billestrup, N., et al., 2010. Lysine deacetylases are produced in pancreatic beta cells and are differentially regulated by proinflammatory cytokines. *Diabetologia* 53 (12), 2569–2578.
- Margueron, R., Justin, N., Ohno, K., Sharpe, M.L., Son, J., Drury 3rd, W.J., et al., 2009. Role of the polycomb protein EED in the propagation of repressive histone marks. *Nature* 461 (7265), 762–767.
- Martin-Sabero, J.I., 2011. How epigenomics brings phenotype into being. *Pediatr. Endocrinol. Rev.* 9 (Suppl. 1), 506–510.
- Martinez-Zamudio, R., Ha, H.C., 2011. Environmental epigenetics in metal exposure. *Epigenetics* 6 (7), 820–827.
- Mastronardi, F.G., Noor, A., Wood, D.D., Paton, T., Moscarello, M.A., 2007. Peptidyl argininedeiminase 2 CpG island in multiple sclerosis white matter is hypomethylated. *J. Neurosci. Res.* 85 (9), 2006–2016.
- Matsuoka, H., Fujimura, T., Mori, H., Aramori, I., Mutoh, S., 2007. Mechanism of HDAC inhibitor FR235222-mediated IL-2 transcriptional repression in Jurkat cells. *Int. Immunopharmacol.* 7 (11), 1422–1432.
- Mau, T., Yung, R., 2014. Potential of epigenetic therapies in non-cancerous conditions. *Front. Genet.* 5, 438.
- Maurer, B., Stanczyk, J., Jungel, A., Akhmetshina, A., Trenkmann, M., Brock, M., et al., 2010. MicroRNA-29, a key regulator of collagen expression in systemic sclerosis. *Arthritis Rheum.* 62 (6), 1733–1743.
- Mazari, L., Ouarzane, M., Zouali, M., 2007. Subversion of B lymphocyte tolerance by hydralazine, a potential mechanism for drug-induced lupus. *Proc. Natl. Acad. Sci. U.S.A.* 104 (15), 6317–6322.
- Meinecke, I., Cinski, A., Baier, A., Peters, M.A., Dankbar, B., Wille, A., et al., 2007. Modification of nuclear PML protein by SUMO-1 regulates Fas-induced apoptosis in rheumatoid arthritis synovial fibroblasts. *Proc. Natl. Acad. Sci. U.S.A.* 104 (12), 5073–5078.
- Mendell, J.T., 2008. miRiad roles for the miR-17-92 cluster in development and disease. *Cell* 133 (2), 217–222.
- Meng, W., Zhu, Z., Jiang, X., Too, C.L., Uebe, S., Jagodic, M., et al., 2017. DNA methylation mediates genotype and smoking interaction in the development of anti-citrullinated peptide antibody-positive rheumatoid arthritis. *Arthritis Res. Ther.* 19 (1), 71.
- Mercer, T.R., Dinger, M.E., Mattick, J.S., 2009. Long non-coding RNAs: insights into functions. *Nat. Rev. Genet.* 10 (3), 155–159.
- Miao, F., Gonzalo, I.G., Lanting, L., Natarajan, R., 2004. In vivo chromatin remodeling events leading to inflammatory gene transcription under diabetic conditions. *J. Biol. Chem.* 279 (17), 18091–18097.
- Miao, F., Li, S., Chavez, V., Lanting, L., Natarajan, R., 2006. Coactivator-associated arginine methyltransferase-1 enhances nuclear factor-kappaB-mediated gene transcription through methylation of histone H3 at arginine 17. *Mol. Endocrinol.* 20 (7), 1562–1573.
- Miao, F., Chen, Z., Zhang, L., Liu, Z., Wu, X., Yuan, Y.C., et al., 2012. Profiles of epigenetic histone post-translational modifications at type 1 diabetes susceptible genes. *J. Biol. Chem.* 287 (20), 16335–16345.
- Miceli-Richard, C., Wang-Renault, S.F., Boudaoud, S., Busato, F., Lallemand, C., Bethune, K., et al., 2016. Overlap between differentially methylated DNA regions in blood B lymphocytes and genetic at-risk loci in primary Sjögren's syndrome. *Ann. Rheum. Dis.* 75 (5), 933–940.
- Mishra, N., Brown, D.R., Olorenshaw, I.M., Kammer, G.M., 2001. Trichostatin A reverses skewed expression of CD154, interleukin-10, and interferon-gamma gene and protein expression in lupus T cells. *Proc. Natl. Acad. Sci. U.S.A.* 98 (5), 2628–2633.
- Mishra, N., Reilly, C.M., Brown, D.R., Ruiz, P., Gilkeson, G.S., 2003. Histone deacetylase inhibitors modulate renal disease in the MRL-lpr/lpr mouse. *J. Clin. Invest.* 111 (4), 539–552.
- Miyara, M., Yoshioka, Y., Kitoh, A., Shima, T., Wing, K., Niwa, A., et al., 2009. Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor. *Immunity* 30 (6), 899–911.
- Moscarello, M.A., Wood, D.D., Ackerley, C., Boulias, C., 1994. Myelin in multiple sclerosis is developmentally immature. *J. Clin. Invest.* 94 (1), 146–154.
- Mosley, A.L., Ozcan, S., 2003. Glucose regulates insulin gene transcription by hyperacetylation of histone h4. *J. Biol. Chem.* 278 (22), 19660–19666.
- Musse, A.A., Haraz, G., 2007. Molecular “negativity” may underlie multiple sclerosis: role of the myelin basic protein family in the pathogenesis of MS. *Int. Rev. Neurobiol.* 79, 149–172.
- Musselman, C.A., Lalonde, M.E., Cote, J., Kutateladze, T.G., 2012. Perceiving the epigenetic landscape through histone readers. *Nat. Struct. Mol. Biol.* 19 (12), 1218–1227.
- Nakamachi, Y., Ohnuma, K., Uto, K., Noguchi, Y., Saegusa, J., Kawano, S., 2016. MicroRNA-124 inhibits the progression of adjuvant-induced arthritis in rats. *Ann. Rheum. Dis.* 75 (3), 601–608.
- Nakasa, T., Miyaki, S., Okubo, A., Hashimoto, M., Nishida, K., Ochi, M., et al., 2008. Expression of microRNA-146 in rheumatoid arthritis synovial tissue. *Arthritis Rheum.* 58 (5), 1284–1292.
- Nakasa, T., Shibuya, H., Nagata, Y., Niimoto, T., Ochi, M., 2011. The inhibitory effect of microRNA-146a expression on bone destruction in collagen-induced arthritis. *Arthritis Rheum.* 63 (6), 1582–1590.
- Nencioni, A., Beck, J., Werth, D., Grunebach, F., Patrone, F., Ballesterro, A., et al., 2007. Histone deacetylase inhibitors affect dendritic cell differentiation and immunogenicity. *Clin. Cancer Res.* 13 (13), 3933–3941.
- Nguyen, H.T., Dalmasso, G., Muller, S., Carriere, J., Seibold, F., Darfeuille-Michaud, A., 2014. Crohn's disease-associated adherent invasive Escherichia coli modulate levels of microRNAs in intestinal epithelial cells to reduce autophagy. *Gastroenterology* 146 (2), 508–519.
- Nihal, M., Wu, J., Wood, G.S., 2014. Methotrexate inhibits the viability of human melanoma cell lines and enhances Fas/Fas-ligand expression, apoptosis and response to interferon-alpha: rationale for its use in combination therapy. *Arch. Biochem. Biophys.* 563, 101–107.
- Niimoto, T., Nakasa, T., Ishikawa, M., Okuhara, A., Izumi, B., Deie, M., et al., 2010. MicroRNA-146a expresses in interleukin-17 producing T cells in rheumatoid arthritis patients. *BMC Musculoskelet Disord.* 11, 209.
- Nile, C.J., Read, R.C., Akil, M., Duff, G.W., Wilson, A.G., 2008. Methylation status of a single CpG site in the IL6 promoter is related to IL6 messenger RNA levels and rheumatoid arthritis. *Arthritis Rheum.* 58 (9), 2686–2693.
- O'Reilly, S., Ciechomska, M., Fullard, N., Przyborski, S., van Laar, J.M., 2016. IL-13 mediates collagen deposition via STAT6 and microRNA-135b: a role for epigenetics. *Sci. Rep.* 6, 25066.
- Oaks, Z., Perl, A., 2014. Metabolic control of the epigenome in systemic Lupus erythematosus. *Autoimmunity* 47 (4), 256–264.
- Oelke, K., Lu, Q., Richardson, D., Wu, A., Deng, C., Hanash, S., et al., 2004. Overexpression of CD70 and overstimulation of IgG synthesis by lupus T cells and T cells treated with DNA methylation inhibitors. *Arthritis Rheum.* 50 (6), 1850–1860.

- Ogando, J., Tardaguila, M., Diaz-Alderete, A., Usategui, A., Miranda-Ramos, V., Martinez-Herrera, D.J., et al., 2016. Notch-regulated miR-223 targets the aryl hydrocarbon receptor pathway and increases cytokine production in macrophages from rheumatoid arthritis patients. *Sci. Rep.* 6, 20223.
- Ohlsson, S., Hellmark, T., Pieters, K., Sturfelt, G., Wieslander, J., Segelmark, M., 2005. Increased monocyte transcription of the proteinase 3 gene in small vessel vasculitis. *Clin. Exp. Immunol.* 141 (1), 174–182.
- Olsson, A.H., Volkov, P., Bacos, K., Dayeh, T., Hall, E., Nilsson, E.A., et al., 2014. Genome-wide associations between genetic and epigenetic variation influence mRNA expression and insulin secretion in human pancreatic islets. *PLoS Genet.* 10 (11), e1004735.
- Palbykin, B., Borg, J., Caldwell, P.T., Rowles, J., Papoutsis, A.J., Romagnolo, D.F., et al., 2011. Trichloroethylene induces methylation of the Serca2 promoter in H9c2 cells and embryonic heart. *Cardiovasc. Toxicol.* 11 (3), 204–214.
- Pan, W., Zhu, S., Yuan, M., Cui, H., Wang, L., Luo, X., et al., 2010. MicroRNA-21 and microRNA-148a contribute to DNA hypomethylation in lupus CD4+ T cells by directly and indirectly targeting DNA methyltransferase 1. *J. Immunol.* 184 (12), 6773–6781.
- Pasare, C., Medzhitov, R., 2003. Toll pathway-dependent blockade of CD4+CD25+ T cell-mediated suppression by dendritic cells. *Science* 299 (5609), 1033–1036.
- Paul, D.S., Teschendorff, A.E., Dang, M.A., Lowe, R., Hawa, M.I., Ecker, S., et al., 2016. Increased DNA methylation variability in type 1 diabetes across three immune effector cell types. *Nat. Commun.* 7, 13555.
- Pauley, K.M., Satoh, M., Chan, A.L., Bubb, M.R., Reeves, W.H., Chan, E.K., 2008. Upregulated miR-146a expression in peripheral blood mononuclear cells from rheumatoid arthritis patients. *Arthritis Res. Ther.* 10 (4), R101.
- Peng, H., Liu, Y., Tian, J., Ma, J., Tang, X., Yang, J., et al., 2015. Decreased expression of microRNA-125a-3p upregulates interleukin-23 receptor in patients with Hashimoto's thyroiditis. *Immunol. Res.* 62 (2), 129–136.
- Perez, P., Anaya, J.M., Aguilera, S., Urzua, U., Munroe, D., Molina, C., et al., 2009. Gene expression and chromosomal location for susceptibility to Sjogren's syndrome. *J. Autoimmun.* 33 (2), 99–108.
- Polansky, J.K., Kretschmer, K., Freyer, J., Floess, S., Garbe, A., Baron, U., et al., 2008. DNA methylation controls Foxp3 gene expression. *Eur. J. Immunol.* 38 (6), 1654–1663.
- Pollard, K.M., Hultman, P., Kono, D.H., 2010. Toxicology of autoimmune diseases. *Chem. Res. Toxicol.* 23 (3), 455–466.
- Qin, Q., Wang, X., Yan, N., Song, R.H., Cai, T.T., Zhang, W., et al., 2015. Aberrant expression of miRNA and mRNAs in lesioned tissues of Graves' disease. *Cell Physiol. Biochem.* 35 (5), 1934–1942.
- Radic, M.Z., Zouali, M., 1996. Receptor editing, immune diversification and self-tolerance. *Immunity* 5, 505–511.
- Rakyan, V.K., Beyan, H., Down, T.A., Hawa, M.I., Maslau, S., Aden, D., et al., 2011. Identification of type 1 diabetes-associated DNA methylation variable positions that precede disease diagnosis. *PLoS Genet.* 7 (9), e1002300.
- Reddy, P., Sun, Y., Toubai, T., Duran-Struuck, R., Clouthier, S.G., Weisiger, E., et al., 2008. Histone deacetylase inhibition modulates indoleamine 2,3-dioxygenase-dependent DC functions and regulates experimental graft-versus-host disease in mice. *J. Clin. Invest.* 118 (7), 2562–2573.
- Redondo, M.J., Yu, L., Hawa, M., Mackenzie, T., Pyke, D.A., Eisenbarth, G.S., et al., 2001. Heterogeneity of type I diabetes: analysis of monozygotic twins in Great Britain and the United States. *Diabetologia* 44 (3), 354–362.
- Reidenberg, M.M., Drayer, D.E., Lorenzo, B., Strom, B.L., West, S.L., Snyder, E.S., et al., 1993. Acetylation phenotypes and environmental chemical exposure of people with idiopathic systemic lupus erythematosus. *Arthritis Rheum.* 36 (7), 971–973.
- Reilly, C.M., Thomas, M., Gogal Jr., R., Olgun, S., Santo, A., Sodhi, R., et al., 2008. The histone deacetylase inhibitor trichostatin A upregulates regulatory T cells and modulates autoimmunity in NZB/W F1 mice. *J. Autoimmun.* 31 (2), 123–130.
- Rhead, B., Holingue, C., Cole, M., Shao, X., Quach, H.L., Quach, D., et al., 2017. Rheumatoid arthritis naive T cells share hypermethylation sites with synoviocytes. *Arthritis Rheumatol.* 69 (3), 550–559.
- Richardson, B., Scheinbart, L., Strahler, J., Gross, L., Hanash, S., Johnson, M., 1990. Evidence for impaired T cell DNA methylation in systemic lupus erythematosus and rheumatoid arthritis. *Arthritis Rheum.* 33 (11), 1665–1673.
- Richardson, B.C., Strahler, J.R., Piviroto, T.S., Quddus, J., Bayliss, G.E., Gross, L.A., et al., 1992. Phenotypic and functional similarities between 5-azacytidine-treated T cells and a T cell subset in patients with active systemic lupus erythematosus. *Arthritis Rheum.* 35 (6), 647–662.
- Roncarolo, M.G., Battaglia, M., 2007. Regulatory T-cell immunotherapy for tolerance to self antigens and alloantigens in humans. *Nat. Rev. Immunol.* 7 (8), 585–598.
- Rubin, R.L., 2005. Drug-induced lupus. *Toxicology* 209 (2), 135–147.
- Russell, G.I., Bing, R.F., Jones, J.A., Thurston, H., Swales, J.D., 1987. Hydralazine sensitivity: clinical features, autoantibody changes and HLA-DR phenotype. *Q. J. Med.* 65 (246), 845–852.
- Sadovnick, A.D., Baird, P.A., Ward, R.H., 1988. Multiple sclerosis: updated risks for relatives. *Am. J. Med. Genet.* 29 (3), 533–541.
- Salvetti, M., Ristori, G., Bompuzzi, R., Pozzilli, P., Leslie, R.D., 2000. Twins: mirrors of the immune system. *Immunol. Today* 21 (7), 342–347.
- Samuels, J., Ng, Y.S., Coupillaud, C., Paget, D., Meffre, E., 2005. Impaired early B cell tolerance in patients with rheumatoid arthritis. *J. Exp. Med.* 201 (10), 1659–1667.
- Sato, F., Tsuchiya, S., Meltzer, S.J., Shimizu, K., 2011. MicroRNAs and epigenetics. *FEBS J.* 278 (10), 1598–1609.
- Sawalha, A.H., Jeffries, M., Webb, R., Lu, Q., Gorelik, G., Ray, D., et al., 2008. Defective T-cell ERK signaling induces interferon-regulated gene expression and overexpression of methylation-sensitive genes similar to lupus patients. *Genes Immun.* 9 (4), 368–378.
- Schietinger, A., Delrow, J.J., Basom, R.S., Blattman, J.N., Greenberg, P.D., 2012. Rescued tolerant CD8T cells are preprogrammed to reestablish the tolerant state. *Science* 335 (6069), 723–727.
- Schmidl, C., Klug, M., Boeld, T.J., Andreesen, R., Hoffmann, P., Edinger, M., et al., 2009. Lineage-specific DNA methylation in T cells correlates with histone methylation and enhancer activity. *Genome Res.* 19 (7), 1165–1174.
- Schmitz, S.U., Grote, P., Herrmann, B.G., 2016. Mechanisms of long noncoding RNA function in development and disease. *Cell Mol. Life Sci.* 73 (13), 2491–2509.
- Schwab, J., Illges, H., 2001. Silencing of CD21 expression in synovial lymphocytes is independent of methylation of the CD21 promoter CpG island. *Rheumatol. Int.* 20 (4), 133–137.
- Scofield, R.H., Bruner, G.R., Namjou, B., Kimberly, R.P., Ramsey-Goldman, R., Petri, M., et al., 2008. Klinefelter's syndrome (47, XXY) in male systemic lupus erythematosus patients: support for the notion of a gene-dose effect from the X chromosome. *Arthritis Rheum.* 58 (8), 2511–2517.

- Selhub, J., 2002. Folate, vitamin B12 and vitamin B6 and one carbon metabolism. *J. Nutr. Health Aging.* 6 (1), 39–42.
- Selmi, C., Feghali-Bostwick, C.A., Lleo, A., Lombardi, S.A., De Santis, M., Cavaciocchi, F., et al., 2012. X chromosome gene methylation in peripheral lymphocytes from monozygotic twins discordant for scleroderma. *Clin. Exp. Immunol.* 169 (3), 253–262.
- Shanmugam, N., Reddy, M.A., Guha, M., Natarajan, R., 2003. High glucose-induced expression of proinflammatory cytokine and chemokine genes in monocytic cells. *Diabetes* 52 (5), 1256–1264.
- Simmonds, M.J., Kavvoura, F.K., Brand, O.J., Newby, P.R., Jackson, L.E., Hargreaves, C.E., et al., 2014. Skewed X chromosome inactivation and female preponderance in autoimmune thyroid disease: an association study and meta-analysis. *J. Clin. Endocrinol. Metab.* 99 (1), E127–E131.
- Singh, N., Thangaraju, M., Prasad, P.D., Martin, P.M., Lambert, N.A., Boettger, T., et al., 2010. Blockade of dendritic cell development by bacterial fermentation products butyrate and propionate through a transporter (Slc5a8)-dependent inhibition of histone deacetylases. *J. Biol. Chem.* 285 (36), 27601–27608.
- Singhal, N.K., Li, S., Arning, E., Alkhayer, K., Clements, R., Sarcy, Z., et al., 2015. Changes in methionine metabolism and histone H3 trimethylation are linked to mitochondrial defects in multiple sclerosis. *J. Neurosci.* 35 (45), 15170–15186.
- Smith, B.C., Denu, J.M., 2009. Chemical mechanisms of histone lysine and arginine modifications. *Biochim. Biophys. Acta* 1789 (1), 45–57.
- Sole, C., Cortes-Hernandez, J., Felip, M.L., Vidal, M., Ordi-Ros, J., 2015. miR-29c in urinary exosomes as predictor of early renal fibrosis in lupus nephritis. *Nephrol. Dial. Transpl.* 30 (9), 1488–1496.
- Stanczyk, J., Pedrioli, D.M., Brentano, F., Sanchez-Pernaute, O., Kolling, C., Gay, R.E., et al., 2008. Altered expression of MicroRNA in synovial fibroblasts and synovial tissue in rheumatoid arthritis. *Arthritis Rheum.* 58 (4), 1001–1009.
- Steen, S.O., Iversen, L.V., Carlsen, A.L., Burton, M., Nielsen, C.T., Jacobsen, S., et al., 2015. The circulating cell-free microRNA profile in systemic sclerosis is distinct from both healthy controls and systemic lupus erythematosus. *J. Rheumatol.* 42 (2), 214–221.
- Strahl, B.D., Allis, C.D., 2000. The language of covalent histone modifications. *Nature* 403 (6765), 41–45.
- Strickland, F.M., Hewagama, A., Wu, A., Sawalha, A.H., Delaney, C., Hoeltzel, M.F., et al., 2013. Diet influences expression of autoimmune-associated genes and disease severity by epigenetic mechanisms in a transgenic mouse model of lupus. *Arthritis Rheum.* 65 (7), 1872–1881.
- Sunahori, K., Nagpal, K., Hedrich, C.M., Mizui, M., Fitzgerald, L.M., Tsokos, G.C., 2013. The catalytic subunit of protein phosphatase 2A (PP2Ac) promotes DNA hypomethylation by suppressing the phosphorylated mitogen-activated protein kinase/extracellular signal-regulated kinase (ERK) kinase (MEK)/phosphorylated ERK/DNMT1 protein pathway in T-cells from controls and systemic lupus erythematosus patients. *J. Biol. Chem.* 288 (30), 21936–21944.
- Suzuki, N., Harada, T., Mihara, S., Sakane, T., 1996. Characterization of a germline  $\text{V}\kappa$  gene encoding cationic anti-DNA antibody and role of receptor editing for development of the autoantibody in patients with systemic lupus erythematosus. *J. Clin. Invest.* 98, 1843–1850.
- Sweet, M.J., Shakespear, M.R., Kamal, N.A., Fairlie, D.P., 2012. HDAC inhibitors: modulating leukocyte differentiation, survival, proliferation and inflammation. *Immunol. Cell Biol.* 90 (1), 14–22.
- Taganov, K.D., Boldin, M.P., Chang, K.J., Baltimore, D., 2006. NF- $\kappa$ B-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc. Natl. Acad. Sci. U.S.A.* 103 (33), 12481–12486.
- Takami, N., Osawa, K., Miura, Y., Komai, K., Taniguchi, M., Shiraishi, M., et al., 2006. Hypermethylated promoter region of DR3, the death receptor 3 gene, in rheumatoid arthritis synovial cells. *Arthritis Rheum.* 54 (3), 779–787.
- Tang, Y., Luo, X., Cui, H., Ni, X., Yuan, M., Guo, Y., et al., 2009. MicroRNA-146A contributes to abnormal activation of the type I interferon pathway in human lupus by targeting the key signaling proteins. *Arthritis Rheum.* 60 (4), 1065–1075.
- Tao, R., de Zoeten, E.F., Ozkaynak, E., Wang, L., Li, B., Greene, M.I., et al., 2007. Histone deacetylase inhibitors and transplantation. *Curr Opin Immunol.* 19 (5), 589–595.
- Tao, R., de Zoeten, E.F., Ozkaynak, E., Chen, C., Wang, L., Porrett, P.M., et al., 2007. Deacetylase inhibition promotes the generation and function of regulatory T cells. *Nat. Med.* 13 (11), 1299–1307.
- Taveggia, C., Feltri, M.L., Wrabetz, L., 2010. Signals to promote myelin formation and repair. *Nat. Rev. Neurol.* 6 (5), 276–287.
- Teperino, R., Schoonjans, K., Auwerx, J., 2010. Histone methyl transferases and demethylases; can they link metabolism and transcription? *Cell Metab.* 12 (4), 321–327.
- Teruel, R., Perez-Sanchez, C., Corral, J., Herranz, M.T., Perez-Andreu, V., Saiz, E., et al., 2011. Identification of miRNAs as potential modulators of tissue factor expression in patients with systemic lupus erythematosus and antiphospholipid syndrome. *J. Thromb. Haemost.* 9 (10), 1985–1992.
- Thiagalingam, S., Cheng, K.H., Lee, H.J., Mineva, N., Thiagalingam, A., Ponte, J.F., 2003. Histone deacetylases: unique players in shaping the epigenetic histone code. *Ann. N. Y. Acad. Sci.* 983, 84–100.
- Thomas, R.M., Gao, L., Wells, A.D., 2005. Signals from CD28 induce stable epigenetic modification of the IL-2 promoter. *J. Immunol.* 174 (8), 4639–4646.
- Tomer, Y., 2014. Mechanisms of autoimmune thyroid diseases: from genetics to epigenetics. *Annu. Rev. Pathol.* 9, 147–156.
- Tran, D.Q., Ramsey, H., Shevach, E.M., 2007. Induction of FOXP3 expression in naive human CD4+FOXP3 T cells by T-cell receptor stimulation is transforming growth factor-beta dependent but does not confer a regulatory phenotype. *Blood* 110 (8), 2983–2990.
- Tranquill, L.R., Cao, L., Ling, N.C., Kalbacher, H., Martin, R.M., Whitaker, J.N., 2000. Enhanced T cell responsiveness to citrulline-containing myelin basic protein in multiple sclerosis patients. *Mult. Scler.* 6 (4), 220–225.
- Tsay, G.J., Zouali, M., 2008. Toxicogenomics – a novel opportunity to probe lupus susceptibility and pathogenesis. *Int. Immunopharmacol.* 8 (10), 1330–1337.
- Tsou, P.S., Wren, J.D., Amin, M.A., Schiopu, E., Fox, D.A., Khanna, D., et al., 2016. Histone deacetylase 5 is overexpressed in scleroderma endothelial cells and impairs angiogenesis via repression of proangiogenic factors. *Arthritis Rheumatol.* 68 (12), 2975–2985.
- Vinolo, M.A., Rodrigues, H.G., Hatanaka, E., Sato, F.T., Sampaio, S.C., Curi, R., 2011. Suppressive effect of short-chain fatty acids on production of proinflammatory mediators by neutrophils. *J. Nutr. Biochem.* 22 (9), 849–855.
- Vojinovic, J., Damjanov, N., D'Urzo, C., Furlan, A., Susic, G., Pasic, S., et al., 2011. Safety and efficacy of an oral histone deacetylase inhibitor in systemic-onset juvenile idiopathic arthritis. *Arthritis Rheum.* 63 (5), 1452–1458.

- Vrijens, K., Bollati, V., Nawrot, T.S., 2015. MicroRNAs as potential signatures of environmental exposure or effect: a systematic review. *Environ. Health Perspect.* 123 (5), 399–411.
- Wada, T.T., Araki, Y., Sato, K., Aizaki, Y., Yokota, K., Kim, Y.T., et al., 2014. Aberrant histone acetylation contributes to elevated interleukin-6 production in rheumatoid arthritis synovial fibroblasts. *Biochem. Biophys. Res. Commun.* 444 (4), 682–686.
- Wang, L., Tao, R., Hancock, W.W., 2009. Using histone deacetylase inhibitors to enhance Foxp3(+) regulatory T-cell function and induce allograft tolerance. *Immunol. Cell Biol.* 87 (3), 195–202.
- Wang, Y., Fan, P.S., Kahaleh, B., 2006. Association between enhanced type I collagen expression and epigenetic repression of the FLI1 gene in scleroderma fibroblasts. *Arthritis Rheum.* 54 (7), 2271–2279.
- Wang, Y., Yang, Y., Luo, Y., Yin, Y., Wang, Q., Li, Y., et al., 2013. Aberrant histone modification in peripheral blood B cells from patients with systemic sclerosis. *Clin. Immunol.* 149 (1), 46–54.
- Wang, Y., Shu, Y., Xiao, Y., Wang, Q., Kanekura, T., Li, Y., et al., 2014. Hypomethylation and overexpression of ITGAL (CD11a) in CD4(+) T cells in systemic sclerosis. *Clin. Epigenetics.* 6 (1), 25.
- Weetman, A.P., 2001. Determinants of autoimmune thyroid disease. *Nat. Immunol.* 2 (9), 769–770.
- Willer, C.J., Dymert, D.A., Risch, N.J., Sadovnick, A.D., Ebers, G.C., 2003. Twin concordance and sibling recurrence rates in multiple sclerosis. *Proc. Natl. Acad. Sci. U.S.A.* 100 (22), 12877–12882.
- Wilson, C.B., Rowell, E., Sekimata, M., 2009. Epigenetic control of T-helper-cell differentiation. *Nat. Rev. Immunol.* 9 (2), 91–105.
- Wolff, G.L., Kodell, R.L., Moore, S.R., Cooney, C.A., 1998. Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. *FASEB J.* 12 (11), 949–957.
- Wood, D.D., Bilbao, J.M., O'Connors, P., Moscarello, M.A., 1996. Acute multiple sclerosis (Marburg type) is associated with developmentally immature myelin basic protein. *Ann. Neurol.* 40 (1), 18–24.
- Wu, T., Xie, C., Han, J., Ye, Y., Weiel, J., Li, Q., et al., 2012. Metabolic disturbances associated with systemic lupus erythematosus. *PLoS One* 7 (6), e37210.
- Wysenbeek, A.J., Block, D.A., Fries, J.F., 1989. Prevalence and expression of photosensitivity in systemic lupus erythematosus. *Ann. Rheum. Dis.* 48 (6), 461–463.
- Xiao, C., Srinivasan, L., Calado, D.P., Patterson, H.C., Zhang, B., Wang, J., et al., 2008. Lymphoproliferative disease and autoimmunity in mice with increased miR-17-92 expression in lymphocytes. *Nat. Immunol.* 9 (4), 405–414.
- Yan, N., Zhou, J.Z., Zhang, J.A., Cai, T., Zhang, W., Wang, Y., et al., 2015. Histone hypoacetylation and increased histone deacetylases in peripheral blood mononuclear cells from patients with Graves' disease. *Mol. Cell Endocrinol.* 414, 143–147.
- Yan, Q., Chen, J., Li, W., Bao, C., Fu, Q., 2016. Targeting miR-155 to treat experimental scleroderma. *Sci. Rep.* 6, 20314.
- Yang, Y., Tang, Q., Zhao, M., Liang, G., Wu, H., Li, D., et al., 2015. The effect of mycophenolic acid on epigenetic modifications in lupus CD4 + T cells. *Clin. Immunol.* 158 (1), 67–76.
- Yin, H., Zhao, M., Wu, X., Gao, F., Luo, Y., Ma, L., et al., 2010. Hypomethylation and overexpression of CD70 (TNFSF7) in CD4 + T cells of patients with primary Sjögren's syndrome. *J. Dermatol. Sci.* 59 (3), 198–203.
- Yun, M., Wu, J., Workman, J.L., Li, B., 2011. Readers of histone modifications. *Cell Res.* 21 (4), 564–578.
- Yung, R., Chang, S., Hemati, N., Johnson, K., Richardson, B., 1997. Mechanisms of drug-induced lupus. IV. Comparison of procainamide and hydralazine with analogs in vitro and in vivo. *Arthritis Rheum.* 40 (8), 1436–1443.
- Yurasov, S., Wardemann, H., Hammersen, J., Tsuji, M., Meffre, E., Pascual, V., et al., 2005. Defective B cell tolerance checkpoints in systemic lupus erythematosus. *J. Exp. Med.* 201 (5), 703–711.
- Zhang, J., Yuan, B., Zhang, F., Xiong, L., Wu, J., Pradhan, S., et al., 2011. Cyclophosphamide perturbs cytosine methylation in Jurkat-T cells through LSD1-mediated stabilization of DNMT1 protein. *Chem. Res. Toxicol.* 24 (11), 2040–2043.
- Zhang, X., Koldzic, D.N., Izikson, L., Reddy, J., Nazareno, R.F., Sakaguchi, S., et al., 2004. IL-10 is involved in the suppression of experimental autoimmune encephalomyelitis by CD25 + CD4 + regulatory T cells. *Int. Immunol.* 16 (2), 249–256.
- Zhang, Z., Maurer, K., Perin, J.C., Song, L., Sullivan, K.E., 2010. Cytokine-induced monocyte characteristics in SLE. *J. Biomed. Biotechnol.* 2010, 507475.
- Zhao, M., Tang, J., Gao, F., Wu, X., Liang, Y., Yin, H., et al., 2010. Hypomethylation of IL10 and IL13 promoters in CD4 + T cells of patients with systemic lupus erythematosus. *J. Biomed. Biotechnol.* 2010, 931018.
- Zhao, S., Wang, Y., Liang, Y., Zhao, M., Long, H., Ding, S., et al., 2011. MicroRNA-126 regulates DNA methylation in CD4 + T cells and contributes to systemic lupus erythematosus by targeting DNA methyltransferase 1. *Arthritis Rheum.* 63 (5), 1376–1386.
- Zhao, X., Tang, Y., Qu, B., Cui, H., Wang, S., Wang, L., et al., 2010. MicroRNA-125a contributes to elevated inflammatory chemokine RANTES levels via targeting KLF13 in systemic lupus erythematosus. *Arthritis Rheum.* 62 (11), 3425–3435.
- Zhou, X., Bailey-Bucktrout, S., Jeker, L.T., Bluestone, J.A., 2009. Plasticity of CD4(+) FoxP3(+) T cells. *Curr. Opin. Immunol.* 21 (3), 281–285.
- Zhou, Y., Yuan, J., Pan, Y., Fei, Y., Qiu, X., Hu, N., et al., 2009. T cell CD40LG gene expression and the production of IgG by autologous B cells in systemic lupus erythematosus. *Clin. Immunol.* 132 (3), 362–370.
- Zhu, H., Luo, H., Li, Y., Zhou, Y., Jiang, Y., Chai, J., et al., 2013. MicroRNA-21 in scleroderma fibrosis and its function in TGF-beta-regulated fibrosis-related genes expression. *J. Clin. Immunol.* 33 (6), 1100–1109.
- Zhu, X., Li, F., Yang, B., Liang, J., Qin, H., Xu, J., 2013. Effects of ultraviolet B exposure on DNA methylation in patients with systemic lupus erythematosus. *Exp. Ther. Med.* 5 (4), 1219–1225.
- Zouali, M., 2005. Taming lupus. *Sci. Am.* 292 (3), 58–65.
- Zouali, M., 2007. Immunological tolerance: mechanisms. *Encyclopedia of Life Sciences.* John Wiley & Sons, Ltd, pp. 1–9.
- Zouali, M., 2008. Receptor editing and receptor revision in rheumatic autoimmune diseases. *Trends Immunol.* 29 (3), 103–109.
- Zouali, M., 2009. (an imprint of John Wiley & Sons Ltd) *The Epigenetics of Autoimmune Diseases.* Wiley-Blackwell, p. 480.
- Zouali, M., 2011. Epigenetics in lupus. *Ann. N. Y. Acad. Sci.* 1217, 154–165.
- Zouali, M., 2013. The epigenetic landscape of B lymphocyte tolerance to self. *FEBS Lett.* 587, 2067–2073.
- Zouali, M., Sarmay, G., 2004. B lymphocyte signaling pathways in systemic autoimmunity: implications for pathogenesis and treatment. *Arthritis Rheum.* 50 (9), 2730–2741.

# Genetic Predisposition to Autoimmune Diseases Conferred by the Major Histocompatibility Complex: Utility of Animal Models

Veena Taneja

Department of Immunology and Division of Rheumatology, Mayo Clinic, Rochester, MN, United States

## O U T L I N E

<b>Major Histocompatibility Complex</b>	468	<b><i>Porphyromonas gingivalis</i> and Rheumatoid Arthritis</b>	475
<b>Major Histocompatibility Complex and Autoimmunity</b>	468	<b>Genetic Factors, Gut Microbiome in Autoimmune Diseases</b>	475
<b>The Mystery of Human Leukocyte Antigen-B27 and Spondyloarthropathies</b>	469	<b>Human Leukocyte Antigen, Microbiome, and Rheumatoid Arthritis</b>	476
<i>Human Leukocyte Antigen-B27 Transgenic Mice</i>	469	<i>Vitamin D in Autoimmune Diseases</i>	477
<i>Human Leukocyte Antigen-B27 and Autophagy</i>	470	<i>Posttranslational Modifications in Autoimmunity</i>	477
<i>Human Leukocyte Antigen-B27 and AIDS</i>	470	<i>Deimination</i>	478
<i>Human Leukocyte Antigen-B27 and Peptide Binding</i>	470	<i>Deamidation</i>	478
<i>Human Leukocyte Antigen-B27 and Natura Killer Cells</i>	471	<i>Humanized Animal Models of Autoimmunity</i>	479
<i>Human Leukocyte Antigen-B27 and Evolution</i>	471	<i>Collagen-Induced Arthritis</i>	479
<b>Human Leukocyte Antigen Class II Association With Autoimmune Diseases</b>	471	<i>Nonrheumatoid Arthritis-Associated Human Leukocyte Antigen Alleles Can Predispose to Autoimmunity</i>	480
<b>Predisposition</b>	472	<b>Human Leukocyte Antigen-DR Transgenic Mice With Experimental Autoimmune Encephalomyelitis as an Animal Model of Multiple Sclerosis</b>	481
<b>Onset</b>	473		
<b>Environmental Factors</b>	473		
<b>Infectious Agent</b>	473		
<b>Smoking and Autoimmunity</b>	474		

Role of DQ Molecule in Predisposition to Multiple Sclerosis	481	Human Leukocyte Antigen Class II Molecules Regulate Autoimmunity by Antigen-Specific T Regulatory Cells	484
Animal Model of Celiac Disease	482	Concluding Remarks	485
Animal Model for Type 1 Diabetes	483	References	485
Human Leukocyte Antigen Class II Molecule Regulate Infection Through Modulation of Cytokine Networks	483		

## MAJOR HISTOCOMPATIBILITY COMPLEX

Evolution of pathogens including viruses, bacteria, and parasites provides the biggest threat to human survival. The human immune system has evolved to combat the invading pathogens via innate immunity which is the first line of defense. However, specific response to various pathogens and memory to clear a second infection by the same pathogen required an adaptive immunity. The main feature of the adaptive immunity is its diverse nature of immune response. The major players for adaptive immunity are the genes encoded within the major histocompatibility complex (MHC). While everybody is exposed to infections at some point in their life, the response generated to infectious pathogens varies among individuals. The MHC plays a very important role in generating the immune response to clear invading pathogens. Human MHC is divided into three main regions, human leukocyte antigen (HLA) class I, II, and III. HLA class I and class II genes are most polymorphic genes in human genome. The high polymorphism in the MHC region can be attributed to strong selection pressure as the human population moved to different parts of the world and encountered new infections leading to diversity in MHC genes through mutation, gene duplication, or gene conversion. A population with diverse HLA class I and II alleles leads to herd resistance to infection and have a survival advantage.

The MHC is the most gene-dense region in human chromosome. Human MHC molecules are called HLA that are homologous to the H-2 of mice. The HLA complex is located on the short arm of chromosome 6 in humans and chromosome 17 in mice and is 3500 kb long. HLA class I molecules contain one heavy chain of 44 kDa and one non-MHC encoded nonpolymorphic  $\beta 2$  microglobulin of 12 kDa. The heavy chain consists of 3 extracellular domains of 90 residues each. The polymorphic residues in class I molecules are located on  $\alpha 1$  and  $\alpha 2$  domains of the heavy chain. There are three class I genes designated HLA-A, HLA-B, HLA-C that are expressed on all nucleated cells. Class I molecules can bind 8–10 amino acids long peptides that have been processed endogenously.

HLA Class II molecules are present as heterodimers on the cell surface consisting of an alpha chain (32–34 kDa) and a  $\beta$  chain (29–32 kDa) each with two extracellular domains of about 90 amino acid long ( $\alpha 1$ ,  $\alpha 2$  and  $\beta 1$ ,  $\beta 2$ ). The beta chains of three major histocompatibility complex class II genes, HLA-DR, DQ, and DP are highly polymorphic, while alpha chains are generally nonpolymorphic. The class II molecules are expressed on antigen-presenting cells such as B lymphocytes, macrophages, dendritic cells, endothelial, and other organ-specific antigen presenting cells (APCs). In general, class II molecules can accommodate peptides up to 10–25 residues. In humans, DR, DQ, and DP are in linkage disequilibrium and are inherited en bloc as a haplotype. MHC genes are expressed codominantly in each individual. Crystal structures of the DR and DQ genes have shown that MHC molecule has a single peptide-binding cleft, which can accommodate a variety of peptides, which depends on the charge, stability, and binding affinity.

## MAJOR HISTOCOMPATIBILITY COMPLEX AND AUTOIMMUNITY

During evolution, HLA alleles offering an advantage during reproductive years have been selected and occur in populations with much higher frequency compared to other alleles. Abundance of HLA-DR2/DQ6, DR4/DQ8, and DR3/DQ2 haplotypes in various populations suggesting the importance of these haplotypes in clearing infections and survival of the species. It has been hypothesized that HLA class II molecules may be involved in autoimmunity due, in part, to their selection of T cells capable of producing cytokines such as IFN $\gamma$ , IL-4, and IL-17 (Mangalam et al., 2013). These cytokines are required for clearing pathogens, extracellular and intracellular,

as well as parasitic infections. While Th1 (IFN $\gamma$ ) can provide protection from intracellular pathogens, Th17 (IL-17) producing CD4 T cells are required for extracellular pathogens (Mangalam et al., 2013). Polymorphism in HLA class II has been associated with immune responses to vaccinations (Poland et al., 2008). HLA-DRB1\*0401 was found to be increased in individuals with seroprotective response to influenza vaccine (Moss et al., 2013). Involvement of class II molecules and CD4 T cells in clearing influenza has been shown (Miller et al., 2015). Although these haplotypes offered a survival advantage in terms of fighting infection, they can also generate an autoreactive response, thus predisposing to a number of autoimmune diseases. Even though there are more than 450 DRB1 alleles and 75 DQB1 alleles with the possibility of generating thousands of haplotypes, only few alleles of these 3 haplotypes are associated with most of the autoimmune diseases. DRB1 has the maximum allelic diversity suggesting a rapidly evolving region due to positive or negative selective pressures. Thus nature leads to the selection of alleles offering advantage over other alleles. Studies have suggested that HLA heterozygosity has an advantage over homozygosity in the generation of immune response. HLA diversity evolves via mutations, gene conversion, and genetic recombination. The most conserved haplotype, B8-DR3-DQ2, has been associated with autoimmunity suggesting this haplotype provides an advantage over other haplotypes and is supported by a high gene frequency of HLA-DR3 in centenarians. Most of the autoimmune diseases show association with HLA class II alleles except the HLA-B27 associated spondyloarthropathies.

## THE MYSTERY OF HUMAN LEUKOCYTE ANTIGEN-B27 AND SPONDYLOARTHROPATHIES

Ankylosing spondylitis (AS) is a joint disease that affects the spine, the sacroiliac joints, and shoulders. In 1973 Brewerton in England showed that the MHC gene, HLA-B27, is linked to susceptibility to ankylosis spondylitis (Brewerton et al., 1973). While B27 was found in 5% of the general population, 95% of the AS patients were HLA-B27 positive. This was the first MHC gene found to be associated with an autoimmune type disease. Eventually, several other closely related diseases such as psoriatic arthritis, arthritis associated with inflammatory bowel disease, and reactive arthritis were also linked to HLA-B27, although at a much lower frequency (Allen et al., 1999b). Several enterobacteria were found to be implicated in the onset of the disease (Kvien et al., 1994). These diseases are now referred to as spondyloarthropathies. In order to understand the role of HLA-B27 in these diseases, Taurog et al. developed a transgenic rat expressing human HLA-B27 gene from an AS patient (Hammer et al., 1990). Transgenic rats developed spontaneous symptoms characteristic of spondyloarthropathy (SPA), but the disease only developed in high gene copy number rats.

### Human Leukocyte Antigen-B27 Transgenic Mice

Mice, expressing intact HLA-B27 on the cell surface, developed normally and showed no disease symptoms. Assuming the disease may require the human  $\beta$ 2m gene, we initiated studies to replace mouse  $\beta$ 2m with human  $\beta$ 2m. During this process, we noticed that mice lacking  $\beta$ 2m altogether showed some symptoms of spondyloarthropathies (Khare et al., 1995). We found that these mice expressed about 10% of  $\beta$ 2m free B27 heavy chains on their cell surface. Free heavy chains of class I molecules are rarely seen on the cell surface. This suggested that B27 may be a unique class I molecule capable of reaching the cell surface as free heavy chains. The frequency of symptoms increased after the mice were brought outside the pathogen-free barrier facility, suggesting that environmental factors may play a role. Studies with B27+ CD4– and CD8– mice indicated that CD4 T cells were critical for the disease process. Computer simulation studies indicated that the pocket of a  $\beta$ 2m free class I heavy chain may resemble a class II molecule with open ends that can load long exogenous peptides. Studies in Strominger's laboratory confirmed that some human B27 molecules were bound with 14–16 amino acid peptides rather than 8–9 amino acid peptides normally found in most class I molecules (Madden et al., 1991). Transporter-associated proteins (TAPs) interact with a unique class I confirmation, whereas calnexin associates with multiple class I forms. Spontaneous inflammatory disease in HLA-B27 transgenic mice does not require TAP (Khare et al., 2001) and is independent of class II molecules, suggesting a direct role for B27 heavy chains and not B27-derived peptides (Khare et al., 1998).

On the basis of our studies with the HLA-B27 transgenic mice, we proposed the following hypothesis. HLA-B27 is a unique class I molecule that can be expressed on the cell surface as free heavy chains. They can load

14–16 amino acids long exogenous bacterial peptides and activate autoreactive CD4 T cells. Activated CD4 T cells migrate to a target tissue and destroy cartilage causing various symptoms related to spondyloarthropathies.

### Human Leukocyte Antigen-B27 and Autophagy

In B27 transgenic rats, free heavy chains of B27 were suggested to accumulate in endoplasmic reticulum causing stress and leading to unfolded protein response (Colbert et al., 2010). However, it was difficult to prove the unfolded protein response (UPR) in patients with AS. The patients with AS were shown to have abnormal autophagy in the gut, which would suggest that misfolded protein is not cleared. Endoplasmic reticulum-associated degradation protein deficiency is linked to autophagy. In addition, altered endoplasmic reticulum aminopeptidase 1 (ERAP1) and ERAP2 have been shown to lead to an aberrant trimming of peptides causing abnormal peptide presentation by B27 and triggering an inflammatory response by stimulating IL-17 production. Thus the aberrant presentation of peptides due to variants may lead to the activation of inflammatory pathways.

### Human Leukocyte Antigen-B27 and AIDS

A major breakthrough came in the HLA-B27 field when it was identified as one of a very few HLA class I genes that protected HIV-infected individuals from progressing to full-blown disease (McMichael and Klenerman, 2002). The HLA-B27 molecules were able to bind and present multiple viral epitopes to generate a robust T-cell response to clear infection (Streeck et al., 2007). The CD4 T-cell level remained high and there was an efficient B cell response also. Further proof came when it was shown that B27+ individuals were also protected against hepatitis C infections and endemic malaria (Mathieu et al., 2008).

Andrew McMichael and his group at the University of Cambridge became interested in the role of HLA-B27 in clearing viral infection. They initiated in-depth studies to understand how the B27 molecule is processed and loaded with peptides in human cells. When they tried to generate B27 tetramers for their studies, they noticed that B27 heavy chains were reaching the cell surface in the absence of  $\beta 2m$  and peptide. Further studies showed that these free chains were actually dimers of heavy chains (Allen et al., 1999a). This confirmed the findings on the HLA-B27 transgenic mice. The McMichael group expanded their studies to analyze the expression of HLA-B27 molecule in patients with spondyloarthropathies.

### Human Leukocyte Antigen-B27 and Peptide Binding

There are several critical residues in an HLA-B27 molecule that may influence protection versus susceptibility against viral infection as well as autoimmunity. One such residue is at position 97 in the floor of the peptide-binding pocket (McMichael and Jones, 2010). While most HLA class I molecules have arginine at this position, HLA-B27 has asparagine. Arginine is known to be a hindrance for peptide binding in the groove. HLA-B27 molecules in the thymus bind to multiple peptides, but there is a major difference in the affinity of the binding. While B27 bind very few peptides with high affinity, they bind many peptides with low affinity. Thus fewer self-reactive T cells are deleted in the thymus, resulting in the positive selection of autoreactive T cells. On the other hand, they may select virus-specific T cells to broader fine specificity that could reduce virus options to escape by mutations, thus enhancing viral clearance.

Another key residue on the HLA-B27 molecule is at position 67 where B27 molecules have free cysteine (Bird et al., 2003). This residue enables the formation of dimers of the free heavy chains. The homodimer formation is dependent upon disulfide bonding through Cys67. Such bonding would require unwinding of the  $\alpha 1$  helix, enabling binding of longer peptides in a class II-like groove.

The HLA-B27 molecules have several other unique characteristics. The HLA-B27 molecules continue to be associated with calnexin after making a complex with  $\beta 2$  microglobulin unlike other HLA molecules. However, they can be expressed on the cell surface in the absence of TAP and tapasin, which are involved in the loading of peptides into the class I molecule. We have previously shown that B27 mice in the context of the TAP (knockout) still get spontaneous disease, suggesting that TAP may not be involved.

Thus HLA-B27 can be expressed on the cell surface in many different forms: as an intact molecule loaded with an endogenous peptide, or as  $\beta 2m$  free heavy chains, homodimers or multimers either loaded with peptide or as empty molecules. CD4 T cells have been found to recognize HLA-B27 either as homodimers or heterodimers

when they are devoid of peptide (Boyle and Hill Gaston, 2003). HLA-B27 reactive CD4+ T cells have been identified in HLA-B27+ AS patients.

### Human Leukocyte Antigen-B27 and Natura Killer Cells

We had shown a potential role of natural killer cells on HLA-B27 associated arthritis (Marietta et al., 2000). Introduction into T cells of NKB1, an allelic form of KIR3DL1, produces severe arthritis in HLA-B27 transgenic mice. Thus the interaction of free MHC heavy chains with MHC receptors could play a role in the development of arthritis in our HLA-B27 transgenic mice. Killer cell immunoglobulin-like receptors (KIR family) are expressed on Natural Killer (NK) cells, T cells, and NK T cells. KIR is polymorphic and demonstrates allele-specific recognition with a cognate KIR for HLA-B27 being the 3 domain, KIR3DL1. Our work in the mice was confirmed by Kollnberger et al. (2002) who showed that HLA-B27 heavy chain homodimers and receptors for HLA-B27 homodimers are expressed on populations of peripheral blood, B and T lymphocytes, and synovial monocytes from patients with spondyloarthritis. Thus the interaction of HLA-B27 heavy chain with immunoreceptors on cells of the myelomonocytic cell lineage or lymphocytes might also be involved in the pathogenesis of spondyloarthritis.

### Human Leukocyte Antigen-B27 and Evolution

If HLA-B27 was a bad gene causing all these diseases, it should have been eliminated during evolutionary time. On the contrary, HLA-B27 is one of the oldest MHC class I genes and has survived thousands of years. It is found on every continent, every geographical area, and in all ethnic and racial groups. It has one of the highest gene frequencies and is very polymorphic with over 50 subtypes. These facts suggest that HLA-B27 gene was positively selected during evolution and had to be a good gene.

The main function of MHC molecules is to clear infection. During thousands of years of evolution, HLA-B27 emerged as a class I molecule capable of presenting multiple epitopes of infectious agents to activate T cells to clear infection as well as to generate cytotoxic T cells and help B cells to generate antibodies to clear infection. This could have happened by many different mechanisms. Key mutations in the peptide-binding pocket could enable more promiscuous binding of multiple peptides for presentation to T cells. Mutations could have also enabled the B27 molecule to be expressed on the cell surface as free heavy chains, capable of loading exogenous peptides and activating CD4 T cells. Thus B27+ individuals would have survived many infectious episodes and bottlenecks, spread all over the world, and reproduced.

Unfortunately, HLA-B27 molecules can also generate adverse effects. The free chains of HLA-B27 are also expressed in the thymus where they can bind many self-peptides with low affinity and activate autoreactive CD4 T cells. These autoreactive CD4 T cells in the periphery can be activated by various mechanisms including molecular mimicry to cause autoimmunity.

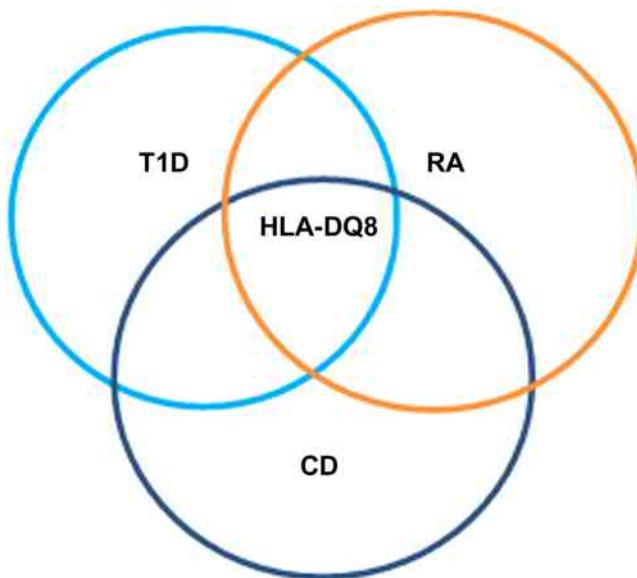
In conclusion, the many forms of HLA-B27 can activate many different T-cell populations. An intact B27 molecule would activate CD8 T cells. A free heavy chain or a homodimer loaded with an exogenous peptide will activate a CD4 T cell. B27 homodimers in complex with KIR molecules can activate NK cells. Finally, the  $\beta 2m$  free heavy chains may be able to directly interact and activate antigen-presenting cells such as B cells. Thus the HLA-B27 molecule can generate a robust immune response against infectious agents to clear infections and maintain the health of the individual. On the other hand, the aberrant activation of an autoreactive CD4 T cell could result in autoimmune diseases.

## HUMAN LEUKOCYTE ANTIGEN CLASS II ASSOCIATION WITH AUTOIMMUNE DISEASES

The first studies showing an association between MHCII and autoimmunity came from the studies from Noel Rose who suggested a connection between MHCII and autoimmune experiential thyroiditis induced in the mouse and spontaneously model in the chicken (Vladutiu and Rose, 1971; Bacon et al., 1974). This was followed by a collagen-induced model showing that MHCII predicted arthritis susceptibility (Wooley et al., 1981). Similar to experimental models, susceptibility to the majority of autoimmune diseases is associated with the presence of certain HLA-DR and -DQ alleles (Table 26.1). Despite the presence of a high degree of polymorphism, only a few HLA class II alleles and their linked haplotypes are associated with autoimmune diseases. Type 1 diabetes,

**TABLE 26.1** Association of Various Autoimmune Diseases With Human Leukocyte Antigen Class II Alleles and Haplotypes

Disease	Alleles	Haplotype
Rheumatoid arthritis	DRB1*04, DQB1*0302	DRB1*04\ DQB1*0302 DRB1*04\ DQB1*0301 DRB1*10\ DQB1*0501
Insulin-dependent Type I diabetes	DRB1*03, DRB1*04 DQB1*0302	DRB1*04\ DQB1*0302 DRB1*0301\ DQB1*02
Multiple sclerosis	DRB1*15 DRB1*0301	DRB1*1501\ DQB1*0602 DRB1*0301\ DQB1*0201
Celiac disease	DRB1*0301, DRB1*0701 DQB1*0302	DRB1*0301\ DQB1*02 DRB1*0701\ DQB1*02
Graves' disease	DRB1*0301, DQB1*02	DRB1*0301, DQB1*02
Autoimmune thyroid disease	DRB1*0301	DRB1*0301, DQB1*02
Myasthenia gravis	DRB1*03	DRB1*0301, DQB1*02

**FIGURE 26.1** Many autoimmune diseases share the predisposing HLA allele. HLA-DQ8 has been associated with predisposition to T1D, RA, and CD. *HLA*, Human leukocyte antigen; *T1D*, type 1 diabetes; *RA*, rheumatoid arthritis; *CD*, celiac disease.

rheumatoid arthritis (RA), and celiac disease (CD) are all associated with the presence of HLA-DQ8 suggesting it to be an autoimmune predisposing allele (Fig. 26.1). Although HLA class II genes are the strongest genetic factors associated with the development of autoimmune diseases, the exact role of these molecules in disease etiology is still being deciphered.

## PREDISPOSITION

In the thymus, T cells are positively selected based on their ability to bind to self-MHC molecules expressed in the thymus; T cells binding to self-peptide-MHC molecules with moderate affinity are selected, while T cells binding self-peptide-MHC complex with strong affinity or weak affinity are deleted (von Boehmer and Kisielow, 1990; Huang et al., 2004). Although effective, there is some leakiness as some T cells binding with weak affinity to self-peptide-MHC complex escape the negative selection and circulate into the periphery. In most individuals, these autoreactive T cells are kept in check through peripheral tolerance.

However, in some individuals, these autoreactive T cells can get activated through unknown mechanism and lead to the onset of autoimmune disease. Such a mechanism might explain the selection of a T-cell repertoire that is protective or susceptible to autoreactivity. The elution of peptides from HLA class II molecules suggests that some of these peptides may indeed be derived from HLA molecules themselves (Chicz et al., 1994). Thus HLA molecules not only function in the thymus by presenting other peptides but can also serve as a donor of self-peptides. This intricate relationship between MHC, self-peptides, and the T-cell receptor (TCR) could determine the specificity of T cells in the periphery. Thus the peripheral pool of T cells can recognize nonself-antigen to clear infection, recognize self-antigens to cause autoimmunity, or can become tolerant/anergic. However, studies to resolve these issues in humans have been hampered by the lack of knowledge of "culprit" autoantigens as well as the difficulty in obtaining samples from affected organs. The other confounding problem has been the linkage disequilibrium of HLA class II alleles—DR and —DQ making it difficult to interpret the association of a disease with a haplotype or specific allele. Despite this risk of autoimmunity in certain individuals, nature persisted with these HLA class II alleles due to the survival advantage associated with these genes. Thus autoimmunity is a price that we have to pay in order to control widespread infections and maintain the survival of human population. Based on the observations in animal models, it was hypothesized that autoimmune-associated HLA alleles positively select T cells programmed to produce inflammatory cytokines when activated, thereby influencing autoreactive response (Mangalam et al., 2013; Mangalam et al., 2008). This was recently confirmed in mice expressing arthritis-susceptible and -resistant genes, where susceptible mice generated Th17 response and also harbor memory T cells reactive to autoantigens (Luckey et al., 2014).

## ONSET

Among the individuals carrying disease predisposing HLA allele/haplotype, only a small percentage develops autoimmune disease. This indicates that the precipitation of disease requires a second hit in addition to genetic predisposition. Interestingly, besides genetic factors, most of the autoimmune diseases also show association with environmental factors. A number of environmental factors, both infectious and noninfectious, have been shown to play an important role in the onset of disease in genetically predisposed individuals (Klareskog et al., 2006). One mechanism could be molecular mimicry between infected agents and self-molecules activating autoreactive T cells. Till now extraintestinal infections were considered to be the culprits, but recent observations demonstrating an association between gut microbes and autoimmunity suggest that opportunistic commensals can also lead to autoreactive response. Another mechanism by which pathogens can cause autoreactivity is when posttranslational modification of proteins occurs to clear infections, it may inadvertently lead to activation of self-reactive T cells.

## ENVIRONMENTAL FACTORS

Genome-wide association studies (GWAS) have shown that the presence of a genetic factor, linked to an autoimmune disease, does not necessarily lead to the development of autoimmunity. Oftentimes, autoimmune disease in patients can be linked to environmental factors that they have been exposed to (Goris and Liston, 2012). Some examples of these environmental factors include infections, smoking, food, drugs, and bacteria (Symmons et al., 1997; Campbell, 2014; Vassallo et al., 2014; Luckey et al., 2013; Lunemann et al., 2007). Further complicating this idea is the fact that environmental and genetic contribution can interact in an additive or even synergistic manner, resulting in higher disease rates (Marietta et al., 2015). This complex association is highlighted in RA patients, where there exists an association between smoking and HLA class II alleles, resulting in higher disease rates of RA patients who smoke as compared to their nonsmoking counterparts (Klareskog et al., 2006).

## INFECTIOUS AGENT

Among all the infectious agents associated with autoimmune diseases, such as RA, multiple sclerosis (MS), and systemic lupus erythematosus, the strongest association had been reported with the presence of viruses, especially Epstein–Barr virus (EBV) (Pender, 2003). The EBV is a ubiquitous virus found in populations all over

the world. After the primary infection, virus resides in memory B cells and secretes latent protein such as EBV nuclear antigen (EBNA). After an unknown period of latency, the virus reactivates and goes into lytic phase infecting more cells. It has been hypothesized that EBV may cause autoimmunity by the activation of autoreactive T cells either through molecular mimicry or bystander activation and release of proinflammatory cytokines. There is strong evidence that EBV plays a central role in the etiology of autoimmune diseases including RA and MS (Alspaugh et al., 1981; Lunemann et al., 2008).

A pathophysiological link between EBV and RA was first described when 67% of the patients with RA were shown to produce antibodies to EBNA (Alspaugh et al., 1981; Baboonian et al., 1989). Also, EBV-encoded protein gp110, a major replicative phase glycoprotein required for infection, shares sequence similarities with the QKRAA amino acid motif (the “shared epitope”) of HLA-DRB1\*0401, suggesting that molecular mimicry may be one-way EBV could be involved in the pathogenesis of RA. In addition, the presence of HLA-DR4 is associated with 10-fold high synovial EBV DNA loads compared to controls and low T-cell response in RA patients. A clonal expansion of CD8+ EBV specific T cells that are suggested to be dysfunctional has also been observed in RA patients. Patients with RA have higher numbers of circulating EBV-reactive B cells compared to controls (Tosato et al., 1984). Thus a combination of impaired T-cell response and presence of antibodies could lead to an immune complex disease.

The mechanism linking MS and EBV is not well understood but could involve a pathological role of antibodies to EBV antigens and activation of CNS myelin-specific T cells by cross-recognition of EBV specific T cells. Patients with MS have increased antibody reactivity against several EBNA domains, of which antibodies against EBNA-1 in HLA DRB1\*1501 positive individuals were associated with a 24-fold increase in risk for MS (Sundstrom et al., 2009). Further, MS patients show a selective increase of CD4+ T-cell response to the EBNA1 which also cross-reacted with myelin antigens (Lunemann et al., 2008). These reports indicate that EBNA1-specific antibodies and/or myelin cross-reactive CD4+ T cells in the presence of a susceptible HLA class II allele (HLA-DRB1\*1501) could potentially contribute to the development of MS. These reports suggest that molecular mimicry may be one way that EBV-derived protein could cause pathogenesis in autoimmune diseases.

## SMOKING AND AUTOIMMUNITY

Several retrospective and prospective studies have shown the association between cigarette smoking and susceptibility to a number of autoimmune diseases such as RA, MS, Type I diabetes (T1D), thyroiditis, primary biliary cirrhosis, and Crohn’s disease (Wingerchuk, 2012). This has led to the emergence of smoking as a risk factor linked to the onset and the clinical development of these autoimmune diseases in genetically predisposed individuals. Although these studies show an association, there is no experimental proof of a direct link between smoking and autoimmune diseases, RA and MS. Numerous studies have provided evidence suggesting an association of smoking with the development of RA. Smoking has been associated with extraarticular features of RA-like nodules and lung disease, which is the third major reason for mortality in arthritis patients (Harel-Meir et al., 2007). An interaction between smoking and DRB1 alleles has been suggested to confer an increased risk of anti-citrullinated protein antibodies (ACPA)-positive RA. An increase in the presence of citrulline-modified proteins observed in the lungs of smokers may be due to an increase in the peptidyl arginine deiminase (PAD) enzyme (Makrygiannakis et al., 2008). Although the mechanism of interaction between the RA-susceptible class II alleles and environmental factors such as smoking has not been elucidated, it is thought that RA onset may occur later in life (median age for RA onset is around 60 years), but the process of autoreactivity starts earlier. This is supported by the fact that autoantibodies in shared epitope positive individuals precede clinical disease, suggesting a role for smoking in triggering antibody production. Cigarette smoke exposure in humanized mice showed an exacerbation of disease in mice expressing DQ8 that was associated with Th17 response in lungs and enhanced antibody production to citrullinated proteins (Vassallo et al., 2014). On the other hand, DRB1\*0401 mice showed a suppression of arthritis after an exposure to cigarette smoke. Most human data shows an association with DRB1\*0401 and smoking; this study suggested that host haplotype interaction with cigarette smoke may determine the overall immune response. This was further supported by recent observations where enhanced immune response to citrullinated Vimentin was shown in mice expressing RA-susceptible, \*0401, as well as RA-resistant, \*0402, alleles confirming previous observations that an overall Th response may determine the outcome (Bidkar et al., 2016; Ummarino, 2016). A recent study using multinational QUEST-RA database showed no significant differences in the clinical profile of RA patients that are smokers and nonsmokers except for an increase in the presence of

nodules in the smoker group (Naranjo et al., 2010). A study with a 3-year follow-up of patients with current smokers showed fewer swollen joints and no difference in radiologic damage suggesting smoking may be contributing to disease by the production of autoantibodies (Harrison et al., 2001). Cigarette smoking is the most characterized environmental factor associated with the pathogenesis of RA and suggests that the onset of RA may begin at a site other than joints.

Cigarette smoke can interact with other environmental factors such as EBV to increase the risk of MS, as smokers were twice as likely to have MS with high titers of anti-EBNA antibody compared to nonsmokers (Simon et al., 2010). Smoking can increase the risk of MS through a number of pathways such as (1) modulation of systemic immune response; (2) increasing the blood–brain barrier permeability; and (3) direct injury to CNS by neurotoxic chemicals present in smoke.

## PORPHYROMONAS GINGIVALIS AND RHEUMATOID ARTHRITIS

*Porphyromonas gingivalis*, a Gram-negative facultative anaerobe, is the major cause of an inflammatory condition of oral cavity called periodontitis (McGraw et al., 1999). Smoking has been shown to be the leading susceptibility factor for periodontitis (Klareskog et al., 2006; Lundberg et al., 2010). Further, an association between the presence of HLA-DR4 and periodontitis has been described indicating similarities with RA. *P. gingivalis* is present in more than 80% of the RA patients. Recent epidemiology studies have shown an association between periodontal disease and RA (Farquharson et al., 2012). Antibodies to *P. gingivalis* are increased in RA patients and correlate with ACPAs. The potential role of *P. gingivalis* in RA pathogenesis has been suggested to be due to the presence of bacterial PAD enzyme, even though there is no similarity with human PAD (Farquharson et al., 2012). These enzymes can deiminate an arginine residue to citrulline in antigens, thus changing their binding affinity to HLA molecules. Further, more than 40% of the RA patients are positive for antibodies to an immunodominant peptide, citrullinated alpha-enolase peptide 1, that bears sequence similarity and cross-reactivity with enolase from *P. gingivalis* (Lundberg et al., 2010). These studies suggest that molecular mimicry and immune response to a bacterial epitope may result in the production of antibodies. Recent studies have shown that bacterial PAD enzyme can deiminate host fibrinogen peptides (Wegner et al., 2010), which may lead to the generation of new epitopes, so triggering of an immune response in a genetically predisposed individual. The presence of antibodies to *P. gingivalis* in patients was associated with smoking and DRB1, suggesting that it could be a trigger for RA (Kharlamova et al., 2016).

Besides oral microbiota, recent studies have shown that intestinal microbiota is perturbed in patients with RA and could also contribute to pathogenesis (Marietta et al., 2016; Scher et al., 2013; Zhang et al., 2015).

## GENETIC FACTORS, GUT MICROBIOME IN AUTOIMMUNE DISEASES

Recently, the role and importance of the microbiome have been realized by researchers, as the microbes in the gut are involved in the homeostasis of immune response and any changes to the microbiota can disrupt this immune response. The human intestine is colonized by a large number of microorganisms, including around  $10^{14}$  types of bacteria that are involved in various functions. The colonization of the gut begins at birth and stabilizes by year 3 but changes throughout the life based on various factors, forming a unique intestinal microbiota for each individual. The gut microbiota forms a protective barrier between the environment and the intestine and helps maintain a healthy gut. A mutualistic relation exists between the host and gut microbiota (Backhed et al., 2005). The composition of an individual's gut microbiota is influenced by many factors including diet, geographical location, genetics, age, and gender (Marietta et al., 2015; Gomez et al., 2012, 2015). Most of the autoimmune diseases are sex-biased with a higher prevalence in women. The major factors in sex-bias have been suggested to be the expression of X-linked immune genes such as FoxP3, CD40L, TLR7, and hormones. Since genetic factors and hormones impact gut microbiota, it is possible that the gut is involved in the pathogenesis of autoimmune diseases. Although sex-biased microbiota has been shown in mice (Gomez et al., 2012), human data has been inconsistent (Schnorr et al., 2014; Mueller et al., 2006; Arumugam et al., 2011) probably due to lifestyle differences.

Recent studies have shown alterations in the gut microbiota, also called dysbiosis, in patients with various autoimmune diseases including diabetes, MS, lupus, and RA among others. T1D, an autoimmune disease, has been associated with reduced diversity and a decrease in Bacteroidetes:Firmicutes ratio, 2 of the main phyla, in human gut (Brown et al., 2011). Similarly, RA and MS have also been associated with dysbiosis

(Zhang et al., 2015; Chen et al., 2016; Jangi et al., 2016). Since these autoimmune diseases have strong genetic predisposition, the question arose if the gut microbiota is under the control of host genetic factors. Analysis of gut microbiota in monozygotic and dizygotic twins showed greater similarity between MZ twins compared to DZ twins (Benson et al., 2010). Many host genetic factors have been shown to influence gut microbial composition including HLA genes, Mediterranean fever, resistin-like molecule  $\beta$ , and obese genes (Marietta et al., 2015). Certain genes are involved in limiting bacterial interaction with the epithelium lining of the intestine. Mucin gene (MUC-2) is involved in protection via mucus layer, and MyD88 (myeloid differentiation primary response gene 88) pathway regulates the production of antimicrobial peptide Reg III $\gamma$  (Bergstrom et al., 2010; Vaishnava et al., 2011; Asquith et al., 2010). Yet other genes can control microbial composition. Receptors such as Toll-like receptors (TLRs) and nucleotide-binding oligomerization-domain protein-like receptors (NLRs) act as sensors for microbe-associated molecular patterns. Recent studies have shown a role of these receptors in controlling microbial composition and their association with arthritis. TLRs can bind structurally conserved molecular patterns of microbes and activate innate immune system. Nonobese diabetic (NOD)-like receptor family pyrin domain containing 6 (NLRP6) is expressed in cytosol and can bind procaspase 1 leading to the activation of inflammasomes. Mice deficient in these receptors have altered intestinal microbiota suggesting that microbial interaction with various host proteins may control colonization (Elinav et al., 2011; Oh et al., 2014). Moreover, inflammasomes have been associated with various autoimmune diseases such as MS, RA, and diabetes (Guo et al., 2015). A role of gut microbiome was further supported by mouse models. Humanized mice expressing RA-associated gene showed a loss of age-driven changes in the gut microbiota, while mice expressing RA-resistant HLA gene showed a dynamic change in the gut microbiota suggesting immunogenetic control of the microbiota (Gomez et al., 2012). A correlation between segmented filamentous bacteria and protection from diabetes and susceptibility to arthritis reaffirms a role of gut microbiome in the pathogenesis of and protection from autoimmune diseases (Wu et al., 2010).

## HUMAN LEUKOCYTE ANTIGEN, MICROBIOME, AND RHEUMATOID ARTHRITIS

The mechanism by which HLA genes control intestinal microbial composition is unknown. However, one can speculate that the role of HLA molecules in immune response may determine the colonization of the species in the intestine. Another mechanism by which HLA class II alleles influence microbial colonization could be by the HLA-mediated presentation of bacterial polysaccharides (PSA) by dendritic cells. Indeed the presentation of PSA of *Bacteroides fragilis* generates immunoregulatory response (Surana and Kasper, 2012) that may influence colonization in the gut. Autoimmune predisposing HLA class II alleles may lead to the colonization of opportunistic bacteria in the gut, which under certain inflammatory conditions can lead to break in tolerance. In addition to class II alleles, a role of HLA class I has also been suggested in bacterial colonization as bacterial adhesion protein called curli has been shown to bind immunoglobulin-like domain of the heavy chain and  $\beta 2m$  as well as fibronectin and lead to the production of cytokines (Johansson et al., 2001).

Recent studies have shown perturbed gut and oral microbiome in patients with RA (Zhang et al., 2015; Chen et al., 2016). Patients showed dysbiosis with reduced microbial diversity. RA was characterized by an abundance of rare taxa of phylum Actinobacteria, *Collinsella aerofaciens* and *Eggerthella lenta*, with a decrease in the presence of *Faecalibacterium prausnitzii* (Chen et al., 2016). Fecal microbiota analysis of naive humanized mice expressing RA-susceptible DRB1\*0401 showed the difference from DRB1\*0402 expressing mice (Gomez et al., 2012). Interestingly, naïve DRB1\*0401 mice lost sex-dependent microbial differences while \*0402 mice showed significant gut microbial composition between male and female mice. Moreover, naïve \*0401 mice showed an increase in gut permeability as they aged. MHC molecules have been suggested to regulate diseases by determining cytokine profile by the selection of T cells (Mangalam et al., 2013). HLA class II provides the strongest risk factor for autoimmune diseases. One can envisage that by controlling the immune system, HLA genes can control the colonization of microbes in the gut. It's possible that autoimmune-associated HLA class II molecules generate an environment in the gut that is favorable for microbes that, if present in abundance, can cause inflammation. Intestinal microbial composition is influenced by other factors associated with autoimmune diseases such as smoking and infections. However, since not all individuals carrying the autoimmune-susceptible HLA alleles develop disease, it would appear that another insult to immune system via gut may lead to dysbiosis and inflammation that breaks tolerance. In patients who do not carry the autoimmune-prone HLA genes, other intestinal bacteria or bacterial products may be involved. Interestingly, *E. lenta*, observed with abundance in RA, is involved in ornithine metabolic pathway resulting in the production of citrulline (Chen et al., 2016). While citrulline generated may not be directly involved in the citrullination process, it is transported out of the intestine and

converted into arginine that is again utilized for protein synthesis. Also, microbes can lead to the differentiation of T cells into T follicular cells resulting in the formation of germination centers in the gut and the production of antibodies (Taneja, 2017). Thus an abundance of a taxa or species can generate an abundance of certain byproducts, and based on the specific species, it can cause a significant increase of the byproducts. As many microbes may be able to perform a similar function, metabolites produced as byproducts of pathways microbes are involved in or bacterial products may provide targets for treatment.

## VITAMIN D IN AUTOIMMUNE DISEASES

Epidemiological studies have suggested that vitamin D deficiency is associated with a number of autoimmune diseases. Patients with autoimmune diseases such as RA, MS, T1D as well as lupus have decreased serum levels of vitamin D3. However, clinical trials for supplementation with vitamin D in various autoimmune conditions including, RA, MS, T1D, and CD have elucidated minor improvements in some studies, while others show no effect (Dankers et al., 2016). In MS, there are both a strong north-to-south gradient and high altitude areas with low sunlight (decreased levels of vitamin D) that are associated with an increased disease incidence (Hogancamp et al., 1997; Kurtzke, 2005). African-American patients are three times more likely to develop lupus as compared to Caucasian populations carrying a similar genotype. Together, these studies point toward an important role of vitamin D in predisposition to autoimmune diseases. Cells involved in immune response express vitamin D receptors. Since vitamin D shows a strong immune-modulatory effect, it's hypothesized that vitamin D suppresses autoimmune disease by inhibiting disease promoting proinflammatory response and maintaining immune tolerance (Wen and Baker, 2011). Induction of regulatory T cells and suppressor macrophages by vitamin D had been suggested to play an important role in maintaining homeostasis of the immune system.

Interaction between environmental and genetic factors in etiology of autoimmune diseases can be explained by a "Causal Pie Model" (van der Mei et al., 2011), where MHC represents the major fixed slice, while other slices represent one or another individual components (genetic or environmental) and these can interact to cause disease. To develop an autoimmune disease such as MS or RA, a person must complete the pie, though different people or groups of people might have different slices in their pie. In RA patients an inverse relationship has been described between disease activity and levels of vitamin D metabolites (Patel et al., 2007). Further, an association between a polymorphism of the vitamin D receptor and onset of RA has been observed (Gomez-Vaquero et al., 2007). Vitamin D metabolites have been shown to inhibit the production of IL-17A and stimulate IL-4 production in early RA patients (Colin et al., 2010). Thus a deficiency of vitamin D may lead to proinflammatory conditions. Patients carrying susceptible genotypes may require one or more factors, such as smoking, infection, or deficiency in vitamin D, to precipitate RA.

Similarly, in a subset of MS patients carrying the HLA-DRB1\*1501 allele, immune reactivity to EBV and smoking might complete the pie, leading to clinical disease. In other individuals, vitamin D and smoking or EBV and vitamin D might complete the pie and cause the precipitation of disease (Ascherio and Munger, 2007). EBV may cause MS through molecular mimicry as T/B cells against the EBV antigen have been shown to cross-react with myelin antigen, leading to a break in tolerance. Smoking can also increase immune reactivity to viruses such as EBV, and T cells specific to EBV antigen can further activate myelin-specific T cells through molecular mimicry (Lunemann et al., 2008). These interactions might lead to MS-related immune dysregulation that involves the activation of T cells, the modulation of dendritic cell (DC) function, and blood–brain barrier permeability.

## POSTTRANSLATIONAL MODIFICATIONS IN AUTOIMMUNITY

Posttranslational modifications of proteins occur *in vivo* frequently. In most cases, this process enables the clearance of infection and malignant cells. For example, the modification of viral antigen could enable its binding to MHC molecules for the activation of T cells. Similarly, malignant self-tissue could be modified to make them more antigenic. This process also occurs occasionally in normal proteins (Gyorgy et al., 2006; Greer and Shi, 2012). Unfortunately, the posttranslational modification of self-antigen could activate autoreactive cells, for example, posttranslational modification of synovial or joint-related antigen could trigger an immune response that would activate autoreactive T cells in the genetically predisposed individuals. There are many types of posttranslational modifications (PTMs). Modifications that change an amino acid include phosphorylation, methylation, and glycosylation while enzymatic conversions include deamidation and deimination.

## Deimination

The enzymatic process by which citrulline, a noncoded amino acid, is inserted in proteins is called deimination. PAD is the enzyme required for catalytic deimination of peptidyl arginine to citrulline, a process called citrullination, first described by [Fearon \(1939\)](#). Citrullination of proteins occurs under many conditions; however, an aberrant B cell response to citrullinated proteins is specific to RA. The presence of ACPA has been shown to be specific to RA patients.

Rheumatoid arthritis is associated with the presence of DRB1\*0401. GWAS using genotyping for single nucleotide polymorphisms of RA patients have confirmed the association of DRB1 with RA ([Harel-Meir et al., 2007](#)). Studies in some populations have shown an association of RA with the PADI4 gene encoding for PAD isotype 4 ([Suzuki et al., 2003](#)). The peptide-binding region of \*0401 has a positive charge; thus antigens with positive-charged arginine cannot bind. Citrullination of arginine changes the charge to neutral, enabling the antigen to bind DRB1\*0401 molecules for the generation of an immune response. This is supported by studies suggesting that ACPAs in RA sera strongly associate with the presence of DRB1 shared epitope alleles. In RA patients, the presence of ACPAs is used for clinical diagnosis in association with rheumatoid factor (RF). Sera from RA patients have been shown to carry antibodies to citrullinated synovial proteins that include CII, fibrinogen, vimentin, and fibronectin. Citrullination of these proteins can potentially alter their antigenicity and function; and in genetically predisposed individuals, this might lead to an autoreactive immune response. Studies using DR4.1E transgenic mice have shown that citrullinated fibrinogen can induce arthritis although no significant difference was observed in T-cell response to citrullinated and native fibrinogen. In DR4+ RA patients, T cells reactive to citrullinated vimentin-derived peptides rather than native peptides have been described, suggesting a crucial role of citrullinated vimentin in RA pathogenesis. Thus posttranslational conversion of peptidyl arginine to peptidyl citrulline by PAD enzymes appears to be an essential feature of many autoantigens in RA. A recent study utilizing humanized mice showed that RA-resistant \*0402 can also present citrullinated Vimentin and generate response suggesting that this phenomenon is not unique for \*0401 ([Bidkar et al., 2016](#)). However, immune response generated to citrullinated vimentin was different by RA-susceptible and resistant strains. In addition to HLA-DR molecules, it has been shown that DQ8 can also present citrullinated peptides ([Vassallo et al., 2014](#)) suggesting both \*0401 and DQ8 as haplotype may have an additive effect on the presentation of citrullinated proteins leading to abnormal immune response.

Estrogen can increase the production of the PAD enzymes required for citrullination of proteins ([Senshu et al., 1989](#)). A primary risk factor for the production of ACPAs in RA is the presence of the DRB1 shared epitope. Immunization with native CII-derived peptides that are nonresponders in native form in DRB1\*0401 mice followed by challenge in vitro with citrullinated peptide generated a higher response in female mice compared to males ([Behrens et al., 2010](#)). A higher antigen presentation has been shown by splenic cells and epithelial cells during proestrus when estrogen is high. One can speculate that the increased expression of PAD enzyme during estrous cycle could cause an increase in the citrullination of peptides, not only in the uterus, but other tissues including synovial tissue. This could result in the citrullination of multiple synovial specific autoantigens and their presentation by DRB1\*0401 molecule leading to the activation of autoreactive CD4 T cells and autoimmunity. This could be one of the reasons for a higher incidence of autoimmune diseases in females.

## Deamidation

Around 90% of proteins undergo some form of PTMs, which increases the structural and functional diversity of proteome. PTMs such as deamidation have been shown to be associated with autoimmunity. Deamidation is a chemical reaction in which an amide functional group is removed from a protein leading to the degradation of the protein because it damages the amide-containing side chains of the amino acids. Asparagine (Asn) and, to a lesser extent, glutamine (Gln) are prone to spontaneous deamidation changing them to aspartic/isoaspartic acid and glutamic acid, respectively. The rate at which Asn and Gln residues are deamidated is dependent on the neighboring residues in the peptide. The presence of glycine and serine as neighboring residues to Asn and Gln has a destabilizing effect, making them a target for deamidation.

In addition to spontaneous deamidation, enzymatic deamidation also occurs and requires transglutaminase (TG2). Transglutaminase is a calcium-dependent enzyme that is involved in the deamidation of glutamine side chains. Enzymatic deamidation of glutamine converts it to glutamic acid. Tissue transglutaminase (tTg) is widely distributed and is ubiquitously present in the cytosol and the extracellular space within many connective tissues and is involved in many functions. Deamidation by tTg is dependent on the presence of either a proline residue

or a hydrophobic amino acid such as C-terminal Phe of the target glutamine. In CD patients, an immune response to a wheat-derived protein, gliadin, can generate an autoimmune response. CD is a good example of how modified proteins induce an immune response even though the initiating antigen may not involve a self-protein. T cells of patients with CD recognize peptides of gliadin that are deamidated (Glu to Gln).

CD is associated with the presence of HLA-DQ2 in the majority of patients, and the remaining patients are generally positive for DQ8. Deamidation of gliadin peptides changes the charge leading to a better binding of peptides to DQ2 and DQ8 and the generation of an immune response. Studies in CD have shown that tissue transglutaminase enzymes can deamidate peptides of gliadin that are presented by DQ8 or DQ2 leading to gluten sensitivity.

## HUMANIZED ANIMAL MODELS OF AUTOIMMUNITY

The advent of transgenic mice lacking endogenous class II molecules ( $A\beta^0$ ) but expressing human HLA-DR and HLA-DQ genes has significantly advanced the understanding of the role of individual HLA class II molecules (Mangalam et al., 2008; Taneja and David, 2010). The introduction of HLA class II transgenes in  $A\beta^0$  mice led to the expression of functional HLA class II molecules and reconstituted the CD4 T-cell compartment, thus resulting in CD4 restricted immune response to various peptides. The HLA transgene in these mice is self and so they are tolerant to it. Experimental data from various laboratories have shown that the HLA class II molecules in these mice function in a way similar to that in humans. The first evidence came from in vivo and in vitro studies done with super antigens in HLA transgenic mice. Bacterial super antigens (SAg) have a lower affinity for mouse

MHC class II than for human MHC class II molecules. Because of this biological characteristic, SAg-induced toxicity is much lower in mouse models than it is in humans. When immunized with lower concentrations of SEB, HLA-DR3 transgenic mice respond vigorously, activate multiple T cells, and secrete higher levels of proinflammatory cytokines than wild-type mice (DaSilva et al., 2002). The response to super antigens in transgenic mice can result in toxic shock and is dependent on the polymorphism of HLA class II alleles.

The second evidence comes from the peptide presentation by HLA class II molecules in transgenic mice. The HLA transgenic mice respond to similar epitopes as observed in humans. In a comparison study, DR3.  $A\beta^0$  mice recognized only one epitope comprising aa 1–20 for heat shock protein 65 of *Mycobacterium tuberculosis* as observed with human DR3-restricted T cells (Geluk et al., 1998). The response was specific in DR3 mice as DQ8 mice did not respond to this peptide. These studies suggest that processing and presentation of the antigens in the context of class II molecules is similar in transgenic mice and humans.

The third evidence that HLA transgenes in mice function similar to humans comes from studies with experimental autoimmune myasthenia gravis in DR3 transgenic mice (Infante et al., 2003). The wild-type mice show a highly conserved TCR-BV gene usage and CDR3 sequences in response to acetylcholine receptor (AChR), an autoantigen for myasthenia gravis patients. However, DR3-restricted murine hybridomas generated from DR3 mice immunized with AChR expressed a diverse set of TCR  $\beta$  chains, similar to that observed in humans. The TCR-BV sequences from human MG patients were homologous to DR3-restricted murine clones, suggesting that human and mice can recognize similar epitopes and use similar CDR3 sequences for the recognition of the same peptide/MHC complex. Thus transgenic mice can provide an important insight into peptide presentation by different class II alleles and the resulting pathogenesis.

## COLLAGEN-INDUCED ARTHRITIS

Collagen-induced arthritis has been used as a model for inflammatory arthritis where the immunization of susceptible strains of mice with type II collagen leads to the development of arthritis with features similar to those of human inflammatory arthritis. The first model of autoimmunity to determine the role of human class II molecules in arthritis was established by using DQA1\*0301, DQB1\*0302 (DQ8) transgenic mice. DQ8 occurs in linkage disequilibrium with DR4 and has been shown to be associated with RA in certain ethnic groups (Taneja et al., 1992). Collagen-induced arthritis, an animal model for RA, was studied in vivo in  $A\beta^0$ .DQ8 mice. The immunization of  $A\beta^0$ .DQ8 mice with heterologous type II collagen led to a pathogenic autoimmune CD4 mediated response leading to severe arthritis and antibodies to self-type II collagen (Nabozny et al., 1996). Both CD4 T cells and B cells are required for the development of arthritis in transgenic mice (Taneja et al., 2005, 2007b). This

is the first model where arthritic mice produced RF, one of the major features in patients with RA (Taneja et al., 2002). The scenario in the development of arthritis in transgenic mice can be compared to that of RA in human. Both require the presentation of an arthritogenic epitope by HLA class II molecules to CD4 T cells, leading to the proliferation of autoreactive cells and the production of RF by B cells, subsequently leading to joint pathology. Further, studies using CD4-deficient and CD8-deficient mice suggested that the disease was mediated by CD4+ T cells while CD8+ T cells may be the regulatory cells as CD8-deficient mice transgenic for DQ8 develop high amounts of autoantibodies including RF and antinuclear antibodies (ANA) (Taneja et al., 2002). A similar phenomenon can be envisaged in RA where the production of autoantibodies such as RF and ANAs could be related to the functional status of CD8 T cells. On the other hand, mice expressing DQA1\*0103, DQB1\*0601 (DQ6) were resistant to develop CIA. The double transgenic mice expressing both DQ6 and DQ8 developed moderate CIA when compared with the severe arthritis observed in DQ8 transgenic mice (Bradley et al., 1998) much like RA patients bearing both susceptible and nonsusceptible HLA haplotypes. These observations contributed to the concept that polymorphism in DQ may be a major contributing factor in human RA.

Predisposition to RA has been associated with the expression of some subtypes of HLA-DR4 in most human studies ever since the first association of DW4 and RA shown by Stastny (1978). However, most of the studies in different ethnic groups have observed only two alleles, DRB1\*0401 and \*0404, which occur with an increased frequency in RA patients. Thus while DRB1\*0401, 0404 are associated with predisposition, DRB1\*0402 is associated with resistance or protection against RA. To determine the role of DR4 molecules in RA, DRB1\*0401 mice were generated in complete MHC knockout mice (AE – / –). DRB1\*0401.AE – / – mice develop arthritis that mimics human arthritis in sex-bias and autoantibodies production (Taneja et al., 2007a). AE – / – mice have the deletion of an 80 kb region of MHC class II such that none of the classical murine class II molecules are expressed. Human T cell is unique from mice in the expression of MHC class II molecules on their cell surface. Interestingly, similar to humans, T cells from AE – / – DR transgenic mice express HLA-DR molecules on their cell surface and can present peptide antigen. Thus the presentation of an antigen in the context of HLA by an activated T cell might also contribute toward severity. Interestingly, antigen presentation by B cells was shown to generate a sex-bias response, and treatment with B cell depleting antibody shows sex-biased response in humanized mice (Behrens et al., 2017).

To simulate human haplotypes, double transgenic mice expressing autoimmune-associated DQ and DR alleles were generated on the AE – / – background. DR4\|DQ8.AE – / – mice developed arthritis similar to DQ8 but also showed gender differences. Gender differences in CIA were not observed in DQ8 mice suggesting the modulation of DQ8-restricted disease by DR4 molecules. To understand the modulation by DR polymorphism, DRB1\*0402 mice were studied (Taneja et al., 2008) for their influence on CIA. DRB1\*0402 mice are not susceptible to arthritis, similar to humans. Mice expressing both susceptible and resistant DR4 subtypes, 0401/0402, developed arthritis with lower incidence than \*0401 mice (Taneja et al., 2003). Further, data in DR\*0401/DQ8 mice suggested that antigen-presenting cells present DR-restricted peptides differentially suggesting a role of hormones in influencing APCs (Behrens et al., 2010; Luckey et al., 2012). Based on the data in transgenic mice, it can be speculated that hormone-influenced PAD enzymes may lead to citrullination of antigens in females, thus enhancing the presentation of certain antigens. The experimental and human data led us to hypothesize that DQ polymorphism may be responsible for susceptibility, while DR may be involved in the modulation of disease. Thus both DQ and DR alleles as a haplotype influence the development of disease (Taneja et al., 1998). From the studies on DQ and DR transgenic mice, it can be extrapolated that gene complementation or interaction between DQ and DR molecules mediates susceptibility to RA in the human. Depending on the haplotypes carried by an individual, they could be susceptible to severe or mild disease. A homozygous haplotype for predisposing DQ and permissive DR will lead to severe disease. Also, heterozygous RA-susceptible haplotypes will result in very severe disease since there will be two predisposing DQ molecules. However, one predisposing and one protective haplotype should show less severity and low incidence.

## NONRHEUMATOID ARTHRITIS-ASSOCIATED HUMAN LEUKOCYTE ANTIGEN ALLELES CAN PREDISPOSE TO AUTOIMMUNITY

HLA-DQ6 alleles, DQB1\*0601 and \*0604, are not associated with susceptibility to develop arthritis. DQB1\*0601 occurs in linkage with DQA1\*0103 and DQB1\*0604 with DQA1\*0102. To understand if transheterodimers of two nonsusceptible HLA alleles may have a role in rendering susceptibility to develop arthritis, we generated mice expressing DQB1\*0601/ DQA1\*0103 (DQ6.1) and DQB1\*0604/DQA1\*0103 (DQ6.4). Immunization of transgenic

mice leads to the development of severe CIA in DQ6.4 but not DQ6.1 mice (Behrens et al., 2011). Further, data showed that DQ6.4 molecules could present CII-derived peptides similar to those presented by the CIA-susceptible DQ8 allele. These studies suggested that transheterodimer molecules between two DQB1 and DQA1 alleles may result in the presentation of unique antigens and susceptibility to develop arthritis. Molecular modeling of the CII peptides showed that DQB1\*0604/DQA1\*0103 shares the p4 pocket with the arthritis-susceptible DQB1\*0302 allele and further a critical role of p4 and p9 pockets is suggested with susceptibility to arthritis. In some conditions, DQA1\*0103 can form heterodimer with CD74 and be expressed on the cell surface and present antigen leading to the development of arthritis (David et al., 2016). This provides an explanation for the presence of nonsusceptible alleles in some RA patients and a mechanism by which they can predispose to develop arthritis.

### HUMAN LEUKOCYTE ANTIGEN-DR TRANSGENIC MICE WITH EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS AS AN ANIMAL MODEL OF MULTIPLE SCLEROSIS

Multiple sclerosis (MS), a chronic inflammatory disease of the central nervous system, shows strong linkage with the presence of HLA-DR2 haplotypes (DRB1\*1501, DRB5\*0101, DQA1\*0102, and DQB1\*0602) (DRB1\*1501, DRB5\*0101, DQA1\*0102, and DQB1\*0602) (Oksenberg et al., 1993). Besides DR2, MS has also been associated with HLA-DR3 and -DR4 haplotypes (Weinshenker et al., 1998; Coraddu et al., 2001). HLA DR3/DQ2 haplotypes have been shown to confer an increased risk for relapsing-remitting MS in individuals (Weinshenker et al., 1998). The lack of a complete association to a particular HLA allele suggests that MS is a heterogeneous disease at the molecular level. MS is hypothesized to be mediated by autoreactive T cells against a variety of myelin antigens including myelin basic protein (MBP), proteolipid protein (PLP), and myelin oligodendrocytic glycoprotein (MOG).

Experimental autoimmune encephalomyelitis (EAE) has been used as an animal model of MS where the immunization of susceptible strains of mice with autoantigens, such as PLP, MBP, or MOG, can lead to the development of inflammation and demyelination in CNS, similar to MS pathology observed in patients (Mangalam et al., 2004; Rich et al., 2004). Induction of EAE with recombinant myelin oligodendrocyte glycoprotein (rMOG) in DR2 mice (DRB1\*1501, DRB1\*1502, and DRB1\*1503) showed that all three strains developed EAE; however, the disease incidence and severity was higher in DRB1\*1501 transgenic mice. Almost all the T-cell epitopes identified in HLA transgenic mice using overlapping epitopes of MBP, PLP, and MOG are similar to those identified among T cells isolated from peripheral blood mononuclear cells of MS patients (Khare et al., 2003; Kawamura et al., 2000). The administration of PLP91-110 peptide-induced EAE in DR3 transgenic mice (Mangalam et al., 2009). Recently we have also observed that PLP178-197 peptide can induce EAE in HLA-DR4 (DRB1\*0401) transgenic mice, while HLA-DR4 (DRB1\*0402) transgenic mice were resistant to disease. The disease in transgenic mice was characterized by the paralysis of limbs with mild demyelination, while in humans demyelination is the major feature of MS. CD4+ T cells have been shown to infiltrate the CNS and are responsible for inflammation and demyelination in MS. It is speculated that class II molecules on T cells may present myelin antigen in CNS and exacerbate the disease. To replicate the human disease, DR3.AEo mice were utilized for EAE with PLP peptide (Mangalam et al., 2006). The DR3.AEo transgenic mice with EAE showed severe inflammation and demyelination in the meningeal, stratum, and brain-stem regions, a hallmark for human MS. HLA class II expression was detected in the CNS, especially on microglial cells. These experiments suggest that humanized HLA class II transgenic mice simulate human MS and are a good model to study the role of class II molecules in its pathogenesis.

### ROLE OF DQ MOLECULE IN PREDISPOSITION TO MULTIPLE SCLEROSIS

To simulate the human haplotype, we generated double transgenic mice expressing HLA-DR2 and DQ8 (DR2/DQ8). When immunized with rMOG, 90% of the mice-developed disease accompanied with severe inflammatory and demyelinating lesions in CNS as compared to DR2 single transgenic mice (Khare et al., 2005). Similarly, the expression of DQ8 on a disease susceptible DR3 background (DR3/DQ8 double transgenic mice) led to the development of severe EAE upon immunization with PLP91-110 peptide (Mangalam et al., 2009). The disease in DR3/DQ8 double transgenic mice was characterized by earlier disease onset, higher clinical score and increased inflammation, and demyelination in CNS compared to single DR3 transgenic mice. Since HLA-DQ8

mice were resistant to EAE, our data suggested that DQ8 allele synergizes with disease susceptible HLA-DR allele(s) for a more severe disease phenotype suggesting its pathogenic role in disease etiology. We further determined that the increased susceptibility in DQ8/DR3 mice was due to the increased production of the proinflammatory cytokines IL-17 and GM-CSF. Higher IL-17 levels might lead to the activation and recruitment of more inflammatory cells inside the CNS, while GM-CSF has been shown to increase antigen presentation, both in the periphery and CNS.

In contrast, HLA-DQB1\*0601, observed frequently in Japanese and Asian populations, is known to be protective in MS ([Amirzargar et al., 1998](#); [Marrosu et al., 2001](#)). DQB1\*0601 transgenic mice were resistant to EAE on immunization with MOG, MBP, or PLP antigens. The expression of HLA-DQB\*0601 allele in disease-susceptible HLA-DR2 or -DR3 transgenic mice led to a decrease in disease incidence and severity suggesting a protective role of the allele in disease pathogenesis. Immunization of DR2/DQB1\*0601 mice with rMOG led to lower disease incidence compared to DR2 single transgenic mice. Similar findings were also observed in DR3/DQ6 mice immunized with whole myelin or human PLP91-110 ([Das et al., 2000](#)). We have further characterized that the protective effect of DQ6 molecules is due to high levels of IFN $\gamma$  produced by DQ6 restricted T cells, which suppressed the proliferation of encephalitogenic DR3-restricted T cells by inducing apoptosis. Our study suggests that DQ6 modifies the PLP91-110 specific T-cell response in DR3 through antiinflammatory effects of IFN $\gamma$ .

From the EAE data generated using whole myelin (CNS extract), rMOG and PLP91-110, we have observed that the presence of (1) HLA-DR molecules is required for the development of disease; (2) HLA-DQ6 or DQ8 molecules alone are not sufficient for disease induction; (3) HLA-DQ8 on disease susceptible HLA-DR2 or HLA-DR3 transgenic mice increased the severity and incidence of disease; (4) DQ6 (DQB1\*0601) molecules suppress disease incidence and severity on disease susceptible HLA-DR2 or HLA-DR3 transgenic mice; (5) DQ6 or DQ8 on a disease-resistant background had no effect on disease induction as none of the HLA-DR2/DQ6, DR2/DQ8, or DR4/DQ8 double transgenic mice developed EAE on immunization with PLP91-110 peptide. These data would suggest that while the presence of certain DR gene(s) predisposes an individual to MS, polymorphism in DQ gene(s) might play a modulating role. This study also points out that MS is a heterogeneous disease and HLA association is specific for various autoantigens involved.

## ANIMAL MODEL OF CELIAC DISEASE

We have used transgenic mice that express HLA-DQ8 to evaluate the response to gliadin (done in collaboration with Dr. Joseph Murray and Dr. Eric Marietta; [Marietta et al., 2011](#)). Immunization of DQ8 mice with gluten led to a strong T-cell response and IgG antibodies against gluten, but no enteropathy was observed. In order to introduce an autoimmune component to our mouse model of CD, we backcrossed DQ8 transgenic mice to a NOD genetic background that was deficient in endogenous murine MHCII. This resulted in transgenic NOD mice that expressed human DQ8. With parenteral sensitization to gluten, these mice developed blistering upon the ears, reminiscent of dermatitis herpetiformis (DH), the skin manifestation that is found in 3%–5% of the patients with CD ([Marietta et al., 2004](#)). This model had all the elements of DH, including IgA deposits at the dermal–epidermal junction, infiltration of neutrophils into the dermis of the lesional tissue and, most importantly, the resolution of the disease with a gluten-free diet and/or dapsone treatment. However, unlike the majority of DH patients, these mice did not develop enteropathy with gluten sensitization.

Using DQ8 mice, it was shown that T-cell hybridomas generated to native peptides mounted a heteroclitic response against the deamidated peptide, suggesting a role of the class II molecule in amplifying the T-cell response against dietary gluten. Thus an aberrant innate immune response to gliadin could activate tTg that would result in deamidated forms of gliadin-derived peptides. This could result in an enhanced T-cell response to gliadin, causing damage and enteropathy. This heteroclitic response would also explain why most adult celiac patients have a strong T-cell response against the deamidated forms of the specific gliadin-derived peptide.

In transgenic mice that express both HLA-DQ2 and DR3 ([de Kauwe et al., 2009](#); [Du Pre et al., 2011](#)), sensitization to gliadin resulted in a strong T-cell response to gliadin, but no overt enteropathy. One group went further and generated a DQ2/DR3 mouse that expressed a gliadin-specific TCR that had been identified in the gliadin-sensitization studies ([de Kauwe et al., 2009](#)). These mice responded to deamidated gliadin peptide and generated the Th1 response present in CD patients but did not develop enteropathy, suggesting that a second insult to mucosa may be required for the development of enteropathy in humans. The absence of pathology in the presence of antigen-specific CD4 T cells may suggest that other systemic factors may be involved in disease

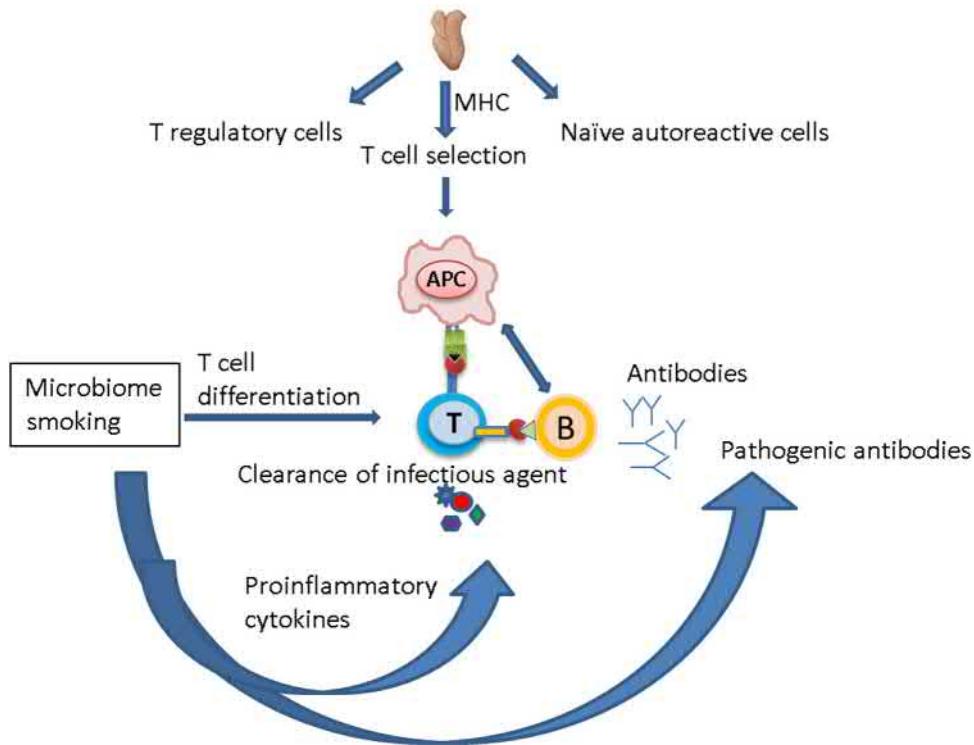
pathogenesis. The patients with CD have upregulated the expression of IL-15 in lamina propria and intestinal epithelium (Abadie and Jabri, 2014). Mice overexpressing IL-15 in the presence of antigen-reactive T cells did not lead to enteropathy suggesting other pathways are involved in breaking tolerance (Korneychuk et al., 2015).

## ANIMAL MODEL FOR TYPE 1 DIABETES

Type 1 diabetes show familial clustering and is linked with the presence of HLA-DQ8/DR4 and HLA-DQ2/DR3 haplotypes. Within the HLA-DQ8/DR4 haplotype, HLA-DQ8 is believed to be the predisposing class II molecule. Most knowledge about autoimmune diabetes comes from a special strain of NOD mice that develop spontaneous diabetes. Elegant genetic studies performed in these mice have mapped the development of disease to the presence of MHC class II molecule H2-A<sup>g7</sup>. Interestingly, the H2-A<sup>g7</sup> molecule shows strong structural and functional homology with human HLA-DQ8 molecules, thus strengthening the major pathogenic role of DQ8 in human T1D. Although HLA-DQ8 transgenic mice do not develop spontaneous diabetes, they lost tolerance to self-GAD65 antigen, leading to activation of autoreactive T cells in the periphery and pancreas. These transgenic mice show insulitis but do not progress to diabetes. As with DQ8 transgenic mice, the presence of a T1D predisposing MHC allele in certain individuals could result in the escape of autoreactive T cells against pancreatic antigens. However, the onset of disease might require a second hit in the pancreas. This second hit can come in the form of virus/bacterial infection, and/or the overproduction of a cytokine, the overexpression of an accessory molecule, or other genetically determined variations completing the causal pie and precipitation of disease in these individuals. To simulate such an insult in the pancreas, we generated HLA-DQ8 (disease susceptible) and HLA-DQ6 (disease resistant) transgenic mice expressing the costimulatory molecule CD80 (B7.1) in islet  $\beta$  cells under the rat insulin promoter (RIP) (Wen et al., 2000). HLA-DQ8/RIP/B7.1 mice developed spontaneous diabetes, while no disease was seen in DQ6/RIP/B7.1 transgenic mice. These studies further confirmed that while the HLA class II allele is the major predisposing gene for the development of most, if not all, autoimmune diseases, other insults or signals are required for precipitation of the disease. Further studies using double transgenic mice expressing DQ8 with HLA-DR3 or HLA-DR4 indicated that the HLA-DR molecule can modulate the diabetogenic effect of DQ8 (Rajagopalan et al., 2003), which in turn suggests that interaction between various MHC class II molecules in *cis* as well as *trans* plays an important role in predisposition, onset as well progression of the disease.

## HUMAN LEUKOCYTE ANTIGEN CLASS II MOLECULE REGULATE INFECTION THROUGH MODULATION OF CYTOKINE NETWORKS

The MHC has evolved over millions of years to fight infection. Mediators such as cytokines and chemokines produced following antigen presentation play an important role in the clearance of pathogens. The association of a particular HLA class II haplotype with disease seems to depend on geographical locations as well as the ethnic composition of the populations. Evolutionarily DQ is a stable molecule with less polymorphism than observed in DRB1 locus. The DQ8 molecule has not undergone many changes during evolution and occurs with only three known subtypes. This could be because DQ8 has the advantage of being able to bind and present multiple peptides. On the other hand, DR has many subtypes. For example, DRB1\*04 has at least 50 subtypes; however, not all the subtypes are associated with susceptibility to autoimmune diseases. We speculate that DR4 polymorphism occurred to counteract the predisposition to autoimmunity imposed by the DQ8 molecule. Thus multiple subtypes of DR4 lead to many possible DR4\DQ8 haplotypes. Only those with an advantage of presenting microbial antigens, clearing infections, and with the capacity to counter the autoimmune responses may eventually be selected. Therefore it's possible that HLA molecules were selected based on their ability to induce subsets of T cells for the production of a particular cytokine that is responsible for controlling one set of microorganisms. Interferon gamma (IFN $\gamma$ ) is one of the signature cytokines of Th1 type of T cells and controls intracellular pathogens. At the same time, IL-17, a signature cytokine of Th17 T cells, controls extracellular pathogens. Thus one set of class II genes might have evolved to produce IL-17 for controlling the spread of extracellular pathogen, while others produce Th1/Th2 to control other pathogens. Using our transgenic mice, we have observed that while transgenic mice expressing HLA-DQ8 induce more TH17 type of T cells, HLA-DQ6 transgenic mice induce more IFN $\gamma$ -producing CD4 T cells. This might result in a cytokine milieu that can regulate disease onset and progression (Mangalam et al., 2013; Luckey et al., 2014). A scenario

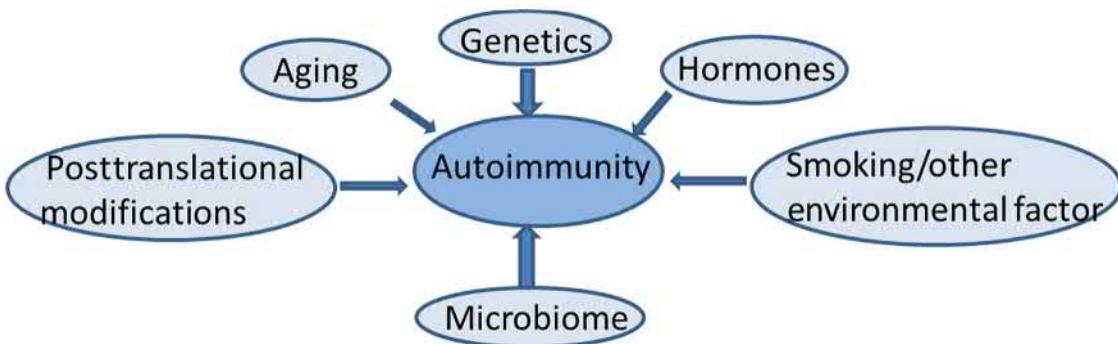


**FIGURE 26.2** HLA molecules select T-cell repertoire in the thymus by presenting peptides and also serving as donors of self-peptides. T cells are selected on the basis of their interaction with self-MHC molecules expressed in thymus. Thus T cells reactive with self-peptide binding with high affinity to MHC are negatively selected in the thymus while those with weak interactions may escape negative selection. Such a mechanism may explain the selection of an autoreactive T-cell repertoire. In the periphery, positively selected T cells can recognize nonself-antigens, produce cytokines and antibodies to clear infections. The autoreactive T cells that have escaped can recognize viral peptide that mimics self-antigen, or cryptic epitopes that are exposed during infection or posttranslational modification causing the activation of autoreactive T cells. These T cells can migrate to various organs, and depending on the host genotype and its interaction with the nongenetic factors, they can cause inflammation locally. The response can be exacerbated in the presence of self-peptides, that when presented by antigen-presenting cells in the tissue leading to the production of proinflammatory cytokines and autoantibodies ensuing an autoimmune response. Environmental factors such as microbiome can lead to differentiation of T cells into T follicular cells that with the help of B cells produce antibodies. Smoking can augment the modification of proteins and contribute to proinflammatory cytokine production, thus enhancing disease severity. *HLA*, Human leukocyte antigen; *MHC*, major histocompatibility complex.

where interaction between HLA molecules and environmental factors leads to the onset of an autoimmune response and disease can be envisaged (Fig. 26.2).

## HUMAN LEUKOCYTE ANTIGEN CLASS II MOLECULES REGULATE AUTOIMMUNITY BY ANTIGEN-SPECIFIC T REGULATORY CELLS

As suggested above, cytokines play a major role in driving an overall proinflammatory or antiinflammatory response based on the HLA polymorphism. Now there is evidence that HLA-mediated protection from autoimmune diseases may be associated with T regulatory cells specific to an antigen involved in the disease. The evidence for this comes from two elegant studies. Vimentin is considered an autoantigen in RA pathogenesis as ACPA+ antibodies have antivimentin antibodies. Using DRB1\*0401 tetramers coupled with citrullinated Vim-59-71 peptide, Scally et al. (2013) showed that while healthy individuals also carried CD4+ T cells specific to the peptide, the ratio of T regulatory to effector memory cells was higher in healthy controls as compared to RA patients. This data suggested that the disproportionate expansion of antigen-specific Treg and effector cells may be associated with RA. In another elegant study, using HLA-DR15 and DR1 transgenic mice, authors investigated a role of antigen-specific T cells in the pathogenesis of autoimmune Goodpasture disease (Ooi et al., 2017).



**FIGURE 26.3** Autoimmunity is multifactorial that involves both genetic and environmental factors. Among genetic factors, HLA class II alleles provide the strongest risk. Among environmental factors, microbiome and smoking are the prime risk factors. Host genotype determines an individual's core gut microbiome, and certain species may contract or expand depending on the exposure to various environmental factors, thus influencing the immune system locally in the gut as well as adaptive immune system. A role of hormones and age-associated changes in immune system may contribute to altered immune response in autoimmunity. Responses to posttranslational modified proteins, generated for efficient presentation, augment proinflammatory response and enhance autoreactivity. *HLA*, Human leukocyte antigen.

The presence of CD4+ T cells to an epitope derived from the  $\alpha 3$  chain of type IV collagen 135–145 is linked to Goodpasture disease. Using DR15 antigen-specific tetramers, authors showed that in DR15 mice, these cells exhibit a proinflammatory profile and cause kidney disease. On the other hand, DR1-antigen-specific tetramers + CD4 T cells express tolerogenic CD4+ FoxP3+ Treg cells. These studies suggest that the autoimmune-associated HLA molecules may predispose or protect based on what T cells are expanded and the cytokines produced by those T cells. The question what determines the expansion of pathogenic T cells may lie in the role of environmental factors as discussed above.

## CONCLUDING REMARKS

From various *in vivo* and *in vitro* studies, it has become clear that MHC and non-MHC genetic components are common elements for various autoimmune diseases, and in corresponding animal models (Fig. 26.3). Thus polymorphism in MHC is an advantage for human survival, but autoimmunity is the price that the population must pay for combating infection effectively and for survival. In evolutionary terms, only those HLA genes that can generate strong immune responses to infections are selected. The constant mutations observed might be leading to the generation of new subtypes of an allele to circumvent autoimmunity. Haplotypes and not a single class II allele function in the selection of the T-cell repertoire and susceptibility to disease.

## References

- Abadie, V., Jabri, B., 2014. IL-15: a central regulator of celiac disease immunopathology. *Immunol. Rev.* 260 (1), 221–234.
- Allen, R.L., O'Callaghan, C.A., McMichael, A.J., Bowness, P., 1999a. Cutting edge: HLA-B27 can form a novel beta 2-microglobulin-free heavy chain homodimer structure. *J. Immunol.* 162 (9), 5045–5048.
- Allen, R.L., Bowness, P., McMichael, A., 1999b. The role of HLA-B27 in spondyloarthritis. *Immunogenetics* 50 (3-4), 220–227.
- Alspaugh, M.A., Henle, G., Lennette, E.T., Henle, W., 1981. Elevated levels of antibodies to Epstein-Barr virus antigens in sera and synovial fluids of patients with rheumatoid arthritis. *J. Clin. Invest.* 67 (4), 1134–1140.
- Amirzargar, A., Mytilineos, J., Yousefipour, A., Farjadian, S., Scherer, S., Opelz, G., et al., 1998. HLA class II (DRB1, DQA1 and DQB1) associated genetic susceptibility in Iranian multiple sclerosis (MS) patients. *Eur. J. Immunogenet.* 25 (4), 297–301.
- Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D.R., et al., 2011. Enterotypes of the human gut microbiome. *Nature* 473 (7346), 174–180.
- Ascherio, A., Munger, K.L., 2007. Environmental risk factors for multiple sclerosis. Part I: The role of infection. *Ann. Neurol.* 61 (4), 288–299.
- Asquith, M.J., Boulard, O., Powrie, F., Maloy, K.J., 2010. Pathogenic and protective roles of MyD88 in leukocytes and epithelial cells in mouse models of inflammatory bowel disease. *Gastroenterology* 139 (2), 519–529. 29 e1-2.
- Baboonian, C., Halliday, D., Venables, P.J., Pawlowski, T., Millman, G., Maini, R.N., 1989. Antibodies in rheumatoid arthritis react specifically with the glycine alanine repeat sequence of Epstein-Barr nuclear antigen-1. *Rheumatol. Int.* 9 (3-5), 161–166.
- Backhed, F., Ley, R.E., Sonnenburg, J.L., Peterson, D.A., Gordon, J.I., 2005. Host-bacterial mutualism in the human intestine. *Science* 307 (5717), 1915–1920.

- Bacon, L.D., Kite Jr., J.H., Rose, N.R., 1974. Relation between the major histocompatibility (B) locus and autoimmune thyroiditis in obese chickens. *Science (New York, N Y)*. 186 (4160), 274–275.
- Behrens, M., Trejo, T., Luthra, H., Griffiths, M., David, C.S., Taneja, V., 2010. Mechanism by which HLA-DR4 regulates sex-bias of arthritis in humanized mice. *J. Autoimmun.* 35 (1), 1–9.
- Behrens, M., Papadopoulos, G.K., Moustakas, A., Smart, M., Luthra, H., David, C.S., et al., 2011. Trans heterodimer between two non-arthritis-associated HLA alleles can predispose to arthritis in humanized mice. *Arthritis Rheum.* 63 (6), 1552–1561.
- Behrens, M., Luckey, D., Luthra, H., David, C., Taneja, V., 2017. B cells influence sex specificity of arthritis via myeloid suppressors and chemokines in humanized mice. *Clin. Immunol.* 178, 10–19.
- Benson, A.K., Kelly, S.A., Legge, R., Ma, F., Low, S.J., Kim, J., et al., 2010. Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. *Proc. Natl. Acad. Sci. U.S.A.* 107 (44), 18933–18938.
- Bergstrom, K.S., Kissoon-Singh, V., Gibson, D.L., Ma, C., Montero, M., Sham, H.P., et al., 2010. Muc2 protects against lethal infectious colitis by disassociating pathogenic and commensal bacteria from the colonic mucosa. *PLoS Pathog.* 6 (5), e1000902.
- Bidkar, M., Vassallo, R., Luckey, D., Smart, M., Mouapi, K., Taneja, V., 2016. Cigarette smoke induces immune responses to vimentin in both, arthritis-susceptible and -resistant humanized mice. *PLoS One* 11 (9), e0162341.
- Bird, L.A., Peh, C.A., Kollnberger, S., Elliott, T., McMichael, A.J., Bowness, P., 2003. Lymphoblastoid cells express HLA-B27 homodimers both intracellularly and at the cell surface following endosomal recycling. *Eur. J. Immunol.* 33 (3), 748–759.
- von Boehmer, H., Kisielow, P., 1990. Self-nonself discrimination by T cells. *Science* 248 (4961), 1369–1373.
- Boyle, L.H., Hill Gaston, J.S., 2003. Breaking the rules: the unconventional recognition of HLA-B27 by CD4+ T lymphocytes as an insight into the pathogenesis of the spondyloarthropathies. *Rheumatology (Oxford)* 42 (3), 404–412.
- Bradley, D.S., Das, P., Griffiths, M.M., Luthra, H.S., David, C.S., 1998. HLA-DQ6/8 double transgenic mice develop auricular chondritis following type II collagen immunization: a model for human relapsing polychondritis. *J. Immunol.* 161 (9), 5046–5053.
- Brewerton, D.A., Hart, F.D., Nicholls, A., Caffrey, M., James, D.C., Sturrock, R.D., 1973. Ankylosing spondylitis and HL-A 27. *Lancet* 1 (7809), 904–907.
- Brown, C.T., Davis-Richardson, A.G., Giongo, A., Gano, K.A., Crabb, D.B., Mukherjee, N., et al., 2011. Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. *PLoS One* 6 (10), e25792.
- Campbell, A.W., 2014. Autoimmunity and the gut. *Autoimmune Dis.* 2014 (152428).
- Chen, J., Wright, K., Davis, J.M., Jeraldo, P., Marietta, E.V., Murray, J., et al., 2016. An expansion of rare lineage intestinal microbes characterizes rheumatoid arthritis. *Genome Med.* 8 (1), 43.
- Chicz, R.M., Lane, W.S., Robinson, R.A., Trucco, M., Strominger, J.L., Gorga, J.C., 1994. Self-peptides bound to the type I diabetes associated class II MHC molecules HLA-DQ1 and HLA-DQ8. *Int. Immunol.* 6 (11), 1639–1649.
- Colbert, R.A., DeLay, M.L., Klenk, E.I., Layh-Schmitt, G., 2010. From HLA-B27 to spondyloarthritis: a journey through the ER. *Immunol. Rev.* 233, 181–202.
- Colin, E.M., Asmawidjaja, P.S., van Hamburg, J.P., Mus, A.M., van Driel, M., Hazes, J.M., et al., 2010. 1,25-dihydroxyvitamin D3 modulates Th17 polarization and interleukin-22 expression by memory T cells from patients with early rheumatoid arthritis. *Arthritis Rheum.* 62 (1), 132–142.
- Coraddu, F., Sawcer, S., D'Alfonso, S., Lai, M., Hensiek, A., Solla, E., et al., 2001. A genome screen for multiple sclerosis in Sardinian multiplex families. *Eur. J. Hum. Genet.* 9 (8), 621–626.
- DaSilva, L., Welcher, B.C., Ulrich, R.G., Aman, M.J., David, C.S., Bavari, S., 2002. Humanlike immune response of human leukocyte antigen-DR3 transgenic mice to staphylococcal enterotoxins: a novel model for superantigen vaccines. *J. Infect. Dis.* 185 (12), 1754–1760.
- Dankers, W., Colin, E.M., van Hamburg, J.P., Lubberts, E., 2016. Vitamin D in autoimmunity: molecular mechanisms and therapeutic potential. *Front. Immunol.* 7 (697).
- Das, P., Drescher, K.M., Geluk, A., Bradley, D.S., Rodriguez, M., David, C.S., 2000. Complementation between specific HLA-DR and HLA-DQ genes in transgenic mice determines susceptibility to experimental autoimmune encephalomyelitis. *Hum. Immunol.* 61 (3), 279–289.
- David, L., Gokhale, A., Jois, S., Johnson, A., Behrens, M., Luthra, H., et al., 2016. CD74/DQA1 dimers predispose to the development of arthritis in humanized mice. *Immunology* 147 (2), 204–211.
- Du Pre, M.F., Kozijn, A.E., van Berkel, L.A., ter Borg, M.N., Lindenbergh-Kortleve, D., Jensen, L.T., et al., 2011. Tolerance to ingested deamidated gliadin in mice is maintained by splenic, type 1 regulatory T cells. *Gastroenterology* 141 (2), 610–620. 20 e1–2.
- Elinav, E., Strowig, T., Kau, A.L., Henao-Mejia, J., Thaiss, C.A., Booth, C.J., et al., 2011. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. *Cell* 145 (5), 745–757.
- Farquharson, D., Butcher, J.P., Culshaw, S., 2012. Periodontitis, porphyromonas, and the pathogenesis of rheumatoid arthritis. *Mucosal Immunol.* 5 (2), 112–120.
- Fearon, W.R., 1939. The carbamido diacetyl reaction: a test for citrulline. *Biochem. J.* 33 (6), 902–907.
- Geluk, A., Taneja, V., van Meijgaarden, K.E., Zanelli, E., Abou-Zeid, C., Thole, J.E., et al., 1998. Identification of HLA class II-restricted determinants of *Mycobacterium tuberculosis*-derived proteins by using HLA-transgenic, class II-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* 95 (18), 10797–10802.
- Gomez, A., Luckey, D., Yeoman, C.J., Marietta, E.V., Berg Miller, M.E., Murray, J.A., et al., 2012. Loss of sex and age driven differences in the gut microbiome characterize arthritis-susceptible 0401 mice but not arthritis-resistant 0402 mice. *PLoS One* 7 (4), e36095.
- Gomez, A., Luckey, D., Taneja, V., 2015. The gut microbiome in autoimmunity: sex matters. *Clin. Immunol.* 159 (2), 154–162.
- Gomez-Vaquero, C., Fiter, J., Enjuanes, A., Nogues, X., Diez-Perez, A., Nolla, J.M., 2007. Influence of the Bsml polymorphism of the vitamin D receptor gene on rheumatoid arthritis clinical activity. *J. Rheumatol.* 34 (9), 1823–1826.
- Goris, A., Liston, A., 2012. The immunogenetic architecture of autoimmune disease. *Cold Spring Harb. Perspect. Biol.* 4 (3), pii: a007260.
- Greer, E.L., Shi, Y., 2012. Histone methylation: a dynamic mark in health, disease and inheritance. *Nat. Rev. Genet.* 13 (5), 343–357.
- Guo, H., Callaway, J.B., Ting, J.P., 2015. Inflammasomes: mechanism of action, role in disease, and therapeutics. *Nat. Med.* 21 (7), 677–687.
- Gyorgy, B., Toth, E., Tarcsa, E., Falus, A., Buzas, E.I., 2006. Citrullination: a posttranslational modification in health and disease. *Int. J. Biochem. Cell Biol.* 38 (10), 1662–1677.

- Hammer, R.E., Maika, S.D., Richardson, J.A., Tang, J.P., Taurog, J.D., 1990. Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human beta 2m: an animal model of HLA-B27-associated human disorders. *Cell* 63 (5), 1099–1112.
- Harel-Meir, M., Sherer, Y., Shoenfeld, Y., 2007. Tobacco smoking and autoimmune rheumatic diseases. *Nat. Clin. Pract. Rheumatol.* 3 (12), 707–715.
- Harrison, B.J., Silman, A.J., Wiles, N.J., Scott, D.G., Symmons, D.P., 2001. The association of cigarette smoking with disease outcome in patients with early inflammatory polyarthritis. *Arthritis Rheum.* 44 (2), 323–330.
- Hogancamp, W.E., Rodriguez, M., Weinshenker, B.G., 1997. The epidemiology of multiple sclerosis. *Mayo Clin. Proc.* 72 (9), 871–878.
- Huang, Y.H., Li, D., Winoto, A., Robey, E.A., 2004. Distinct transcriptional programs in thymocytes responding to T cell receptor, Notch, and positive selection signals. *Proc. Natl. Acad. Sci. U.S.A.* 101 (14), 4936–4941.
- Infante, A.J., Baillargeon, J., Kraig, E., Lott, L., Jackson, C., Hammerling, G.J., et al., 2003. Evidence of a diverse T cell receptor repertoire for acetylcholine receptor, the autoantigen of myasthenia gravis. *J. Autoimmun.* 21 (2), 167–174.
- Jangi, S., Gandhi, R., Cox, L.M., Li, N., von Glehn, F., Yan, R., et al., 2016. Alterations of the human gut microbiome in multiple sclerosis. *Nat. Commun.* 7, 12015.
- Johansson, C., Nilsson, T., Olsen, A., Wick, M.J., 2001. The influence of curli, a MHC-I-binding bacterial surface structure, on macrophage-T cell interactions. *FEMS Immunol. Med. Microbiol.* 30 (1), 21–29.
- de Kauwe, A.L., Chen, Z., Anderson, R.P., Keech, C.L., Price, J.D., Wijburg, O., et al., 2009. Resistance to celiac disease in humanized HLA-DR3-DQ2-transgenic mice expressing specific anti-gliadin CD4+ T cells. *J. Immunol.* 182 (12), 7440–7450.
- Kawamura, K., Yamamura, T., Yokoyama, K., Chui, D.H., Fukui, Y., Sasazuki, T., et al., 2000. Hla-DR2-restricted responses to proteolipid protein 95–116 peptide cause autoimmune encephalitis in transgenic mice. *J. Clin. Invest.* 105 (7), 977–984.
- Khare, S.D., Luthra, H.S., David, C.S., 1995. Spontaneous inflammatory arthritis in HLA-B27 transgenic mice lacking beta 2-microglobulin: a model of human spondyloarthropathies. *J. Exp. Med.* 182 (4), 1153–1158.
- Khare, S.D., Bull, M.J., Hanson, J., Luthra, H.S., David, C.S., 1998. Spontaneous inflammatory disease in HLA-B27 transgenic mice is independent of MHC class II molecules: a direct role for B27 heavy chains and not B27-derived peptides. *J. Immunol.* 160 (1), 101–106.
- Khare, S.D., Lee, S., Bull, M.J., Hanson, J., Luthra, H.S., David, C.S., 2001. Spontaneous inflammatory disease in HLA-B27 transgenic mice does not require transporter of antigenic peptides. *Clin. Immunol.* 98 (3), 364–369.
- Khare, M., Rodriguez, M., David, C.S., 2003. HLA class II transgenic mice authenticate restriction of myelin oligodendrocyte glycoprotein-specific immune response implicated in multiple sclerosis pathogenesis. *Int. Immunol.* 15 (4), 535–546.
- Khare, M., Mangalam, A., Rodriguez, M., David, C.S., 2005. HLA DR and DQ interaction in myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis in HLA class II transgenic mice. *J. Neuroimmunol.* 169 (1-2), 1–12.
- Kharlamova, N., Jiang, X., Sherina, N., Potempa, B., Israelsson, L., Quirke, A.M., et al., 2016. Antibodies to *Porphyromonas gingivalis* indicate interaction between oral infection, smoking, and risk genes in rheumatoid arthritis etiology. *Arthritis Rheumatol.* 68 (3), 604–613.
- Klareskog, L., Padyukov, L., Lorentzen, J., Alfredsson, L., 2006. Mechanisms of disease: genetic susceptibility and environmental triggers in the development of rheumatoid arthritis. *Nat. Clin. Pract. Rheumatol.* 2 (8), 425–433.
- Kollnberger, S., Bird, L., Sun, M.Y., Retiere, C., Braud, V.M., McMichael, A., et al., 2002. Cell-surface expression and immune receptor recognition of HLA-B27 homodimers. *Arthritis Rheum.* 46 (11), 2972–2982.
- Korneychuk, N., Meresse, B., Cerf-Bensussan, N., 2015. Lessons from rodent models in celiac disease. *Mucosal Immunol.* 8 (1), 18–28.
- Kurtzke, J.F., 2005. Epidemiology and etiology of multiple sclerosis. *Phys. Med. Rehabil. Clin. N. Am.* 16 (2), 327–349.
- Kvien, T.K., Glennas, A., Melby, K., Granfors, K., Andrup, O., Karstensen, B., et al., 1994. Reactive arthritis: incidence, triggering agents and clinical presentation. *J. Rheumatol.* 21 (1), 115–122.
- Luckey, D., Medina, K., Taneja, V., 2012. B cells as effectors and regulators of sex-biased arthritis. *Autoimmunity.* 45, 364–376.
- Luckey, D., Gomez, A., Murray, J., White, B., Taneja, V., 2013. Bugs & us: the role of the gut in autoimmunity. *Indian J. Med. Res.* 138 (5), 732–743.
- Luckey, D., Behrens, M., Smart, M., Luthra, H., David, C.S., Taneja, V., 2014. DRB1\*0402 may influence arthritis by promoting naive CD4+ T-cell differentiation into regulatory T cells. *Eur. J. Immunol.* 44 (11), 3429–3438.
- Lundberg, K., Wegner, N., Yucel-Lindberg, T., Venables, P.J., 2010. Periodontitis in RA—the citrullinated enolase connection. *Nat. Rev. Rheumatol.* 6 (12), 727–730.
- Lunemann, J.D., Kamradt, T., Martin, R., Munz, C., 2007. Epstein-Barr virus: environmental trigger of multiple sclerosis? *J. Virol.* 81 (13), 6777–6784.
- Lunemann, J.D., Jelcic, I., Roberts, S., Lutterotti, A., Tackenberg, B., Martin, R., et al., 2008. EBNA1-specific T cells from patients with multiple sclerosis cross react with myelin antigens and co-produce IFN-gamma and IL-2. *J. Exp. Med.* 205 (8), 1763–1773.
- Madden, D.R., Gorga, J.C., Strominger, J.L., Wiley, D.C., 1991. The structure of HLA-B27 reveals nonamer self-peptides bound in an extended conformation. *Nature* 353 (6342), 321–325.
- Makrygiannakis, D., Hermansson, M., Ulfgren, A.K., Nicholas, A.P., Zendman, A.J., Eklund, A., et al., 2008. Smoking increases peptidylarginine deiminase 2 enzyme expression in human lungs and increases citrullination in BAL cells. *Ann. Rheum. Dis.* 67 (10), 1488–1492.
- Mangalam, A.K., Khare, M., Krco, C., Rodriguez, M., David, C., 2004. Identification of T cell epitopes on human proteolipid protein and induction of experimental autoimmune encephalomyelitis in HLA class II-transgenic mice. *Eur. J. Immunol.* 34 (1), 280–290.
- Mangalam, A., Rodriguez, M., David, C., 2006. Role of MHC class II expressing CD4+ T cells in proteolipid protein(91-110)-induced EAE in HLA-DR3 transgenic mice. *Eur. J. Immunol.* 36 (12), 3356–3370.
- Mangalam, A.K., Rajagopalan, G., Taneja, V., David, C.S., 2008. HLA class II transgenic mice mimic human inflammatory diseases. *Adv. Immunol.* 97, 65–147.
- Mangalam, A., Luckey, D., Basal, E., Jackson, M., Smart, M., Rodriguez, M., et al., 2009. HLA-DQ8 (DQB1\*0302)-restricted Th17 cells exacerbate experimental autoimmune encephalomyelitis in HLA-DR3-transgenic mice. *J. Immunol.* 182 (8), 5131–5139.
- Mangalam, A.K., Taneja, V., David, C.S., 2013. HLA class II molecules influence susceptibility versus protection in inflammatory diseases by determining the cytokine profile. *J. Immunol.* 190 (2), 513–518.

- Marietta, E., Trejo, T., Luthra, H., David, C., 2000. The role of natural killer (NK) cells on HLA-B27 associated arthritis. *Arthritis Rheum.* 43 (9), S263.
- Marietta, E., Black, K., Camilleri, M., Krause, P., Rogers 3rd, R.S., David, C., et al., 2004. A new model for dermatitis herpetiformis that uses HLA-DQ8 transgenic NOD mice. *J. Clin. Invest.* 114 (8), 1090–1097.
- Marietta, E.V., David, C.S., Murray, J.A., 2011. Important lessons derived from animal models of celiac disease. *Int. Rev. Immunol.* 30 (4), 197–206.
- Marietta, E., Rishi, A., Taneja, V., 2015. Immunogenetic control of the intestinal microbiota. *Immunology* 145 (3), 313–322.
- Marietta, E.V., Murray, J.A., Luckey, D.H., Jeraldo, P.R., Lamba, A., Patel, R., et al., 2016. Suppression of inflammatory arthritis by human gut-derived *Prevotella histicola* in humanized mice. *Arthritis Rheumatol.* 68 (12), 2878–2888.
- Marrosu, M.G., Murru, R., Murru, M.R., Costa, G., Zavattari, P., Whalen, M., et al., 2001. Dissection of the HLA association with multiple sclerosis in the founder isolated population of Sardinia. *Hum. Mol. Genet.* 10 (25), 2907–2916.
- Mathieu, A., Cauli, A., Fiorillo, M.T., Sorrentino, R., 2008. HLA-B27 and ankylosing spondylitis geographic distribution as the result of a genetic selection induced by malaria endemic? A review supporting the hypothesis. *Autoimmun. Rev.* 7 (5), 398–403.
- McGraw, W.T., Potempa, J., Farley, D., Travis, J., 1999. Purification, characterization, and sequence analysis of a potential virulence factor from *Porphyromonas gingivalis*, peptidylarginine deiminase. *Infect. Immun.* 67 (7), 3248–3256.
- McMichael, A., Klenerman, P., 2002. HIV/AIDS. HLA leaves its footprints on HIV. *Science* 296 (5572), 1410–1411.
- McMichael, A.J., Jones, E.Y., 2010. Genetics. First-class control of HIV-1. *Science* 330 (6010), 1488–1490.
- van der Mei, I.A., Simpson Jr, S., Stankovich, J., Taylor, B.V., 2011. Individual and joint action of environmental factors and risk of MS. *Neurol. Clin.* 29 (2), 233–255.
- Miller, M.A., Ganeshan, A.P., Luckashenak, N., Mendonca, M., Eisenlohr, L.C., 2015. Endogenous antigen processing drives the primary CD4+ T cell response to influenza. *Nat. Med.* 21 (10), 1216–1222.
- Moss, A.J., Gaughran, F.P., Karasu, A., Gilbert, A.S., Mann, A.J., Gelder, C.M., et al., 2013. Correlation between human leukocyte antigen class II alleles and HAI titers detected post-influenza vaccination. *PLoS One* 8 (8), e71376.
- Mueller, S., Saunier, K., Hanisch, C., Norin, E., Alm, L., Midtvedt, T., et al., 2006. Differences in fecal microbiota in different European study populations in relation to age, gender, and country: a cross-sectional study. *Appl. Environ. Microbiol.* 72 (2), 1027–1033.
- Nabozny, G.H., Baisch, J.M., Cheng, S., Cosgrove, D., Griffiths, M.M., Luthra, H.S., et al., 1996. HLA-DQ8 transgenic mice are highly susceptible to collagen-induced arthritis: a novel model for human polyarthritis. *J. Exp. Med.* 183 (1), 27–37.
- Naranjo, A., Toloza, S., Guimaraes da Silveira, I., Lazovskis, J., Hetland, M.L., Hamoud, H., et al., 2010. Smokers and non smokers with rheumatoid arthritis have similar clinical status: data from the multinational QUEST-RA database. *Clin. Exp. Rheumatol.* 28 (6), 820–827.
- Oh, J.Z., Ravindran, R., Chassaing, B., Carvalho, F.A., Maddur, M.S., Bower, M., et al., 2014. TLR5-mediated sensing of gut microbiota is necessary for antibody responses to seasonal influenza vaccination. *Immunity* 41 (3), 478–492.
- Oksenberg, J.R., Begovich, A.B., Erlich, H.A., Steinman, L., 1993. Genetic factors in multiple sclerosis. *JAMA* 270 (19), 2362–2369.
- Ooi, J.D., Petersen, J., Tan, Y.H., Huynh, M., Willett, Z.J., Ramarathnam, S.H., et al., 2017. Dominant protection from HLA-linked autoimmunity by antigen-specific regulatory T cells. *Nature* 545 (7653), 243–247.
- Patel, S., Farragher, T., Berry, J., Bunn, D., Silman, A., Symmons, D., 2007. Association between serum vitamin D metabolite levels and disease activity in patients with early inflammatory polyarthritis. *Arthritis Rheum.* 56 (7), 2143–2149.
- Pender, M.P., 2003. Infection of autoreactive B lymphocytes with EBV, causing chronic autoimmune diseases. *Trends Immunol.* 24 (11), 584–588.
- Poland, G.A., Ovsyannikova, I.G., Jacobson, R.M., 2008. Immunogenetics of seasonal influenza vaccine response. *Vaccine* 26 (Suppl 4), D35–D40.
- Rajagopalan, G., Kudva, Y.C., Chen, L., Wen, L., David, C.S., 2003. Autoimmune diabetes in HLA-DR3/DQ8 transgenic mice expressing the co-stimulatory molecule B7-1 in the {beta} cells of islets of Langerhans. *Int. Immunopharmacol.* 15 (9), 1035–1044.
- Rich, C., Link, J.M., Zamora, A., Jacobsen, H., Meza-Romero, R., Offner, H., et al., 2004. Myelin oligodendrocyte glycoprotein-35-55 peptide induces severe chronic experimental autoimmune encephalomyelitis in HLA-DR2-transgenic mice. *Eur. J. Immunol.* 34 (5), 1251–1261.
- Scally, S.W., Petersen, J., Law, S.C., Dudek, N.L., Nel, H.J., Loh, K.L., et al., 2013. A molecular basis for the association of the HLA-DRB1 locus, citrullination, and rheumatoid arthritis. *J. Exp. Med.* 210 (12), 2569–2582.
- Scher, J.U., Sczesnak, A., Longman, R.S., Segata, N., Ubeda, C., Bielski, C., et al., 2013. Expansion of intestinal *Prevotella copri* correlates with enhanced susceptibility to arthritis. *Elife* 2, e01202.
- Schnorr, S.L., Candela, M., Rampelli, S., Centanni, M., Consolandi, C., Basaglia, G., et al., 2014. Gut microbiome of the Hadza hunter-gatherers. *Nat. Commun.* 5, 3654.
- Senshu, T., Akiyama, K., Nagata, S., Watanabe, K., Hikichi, K., 1989. Peptidylarginine deiminase in rat pituitary: sex difference, estrous cycle-related changes, and estrogen dependence. *Endocrinology* 124 (6), 2666–2670.
- Simon, K.C., van der Mei, I.A., Munger, K.L., Ponsonby, A., Dickinson, J., Dwyer, T., et al., 2010. Combined effects of smoking, anti-EBNA antibodies, and HLA-DRB1\*1501 on multiple sclerosis risk. *Neurology* 74 (17), 1365–1371.
- Stastny, P., 1978. Association of the B-cell alloantigen DRw4 with rheumatoid arthritis. *New Engl. J. Med.* 298 (16), 869–871.
- Streeck, H., Lichterfeld, M., Alter, G., Meier, A., Teigen, N., Yassine-Diab, B., et al., 2007. Recognition of a defined region within p24 gag by CD8+ T cells during primary human immunodeficiency virus type 1 infection in individuals expressing protective HLA class I alleles. *J. Virol.* 81 (14), 7725–7731.
- Sundstrom, P., Nyström, M., Ruuth, K., Lundgren, E., 2009. Antibodies to specific EBNA-1 domains and HLA DRB1\*1501 interact as risk factors for multiple sclerosis. *J. Neuroimmunol.* 215 (1-2), 102–107.
- Surana, N.K., Kasper, D.L., 2012. The yin yang of bacterial polysaccharides: lessons learned from *B. fragilis* PSA. *Immunol. Rev.* 245 (1), 13–26.
- Suzuki, A., Yamada, R., Chang, X., Tokuhiro, S., Sawada, T., Suzuki, M., et al., 2003. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. *Nat. Genet.* 34 (4), 395–402.

- Symmons, D.P., Bankhead, C.R., Harrison, B.J., Brennan, P., Barrett, E.M., Scott, D.G., et al., 1997. Blood transfusion, smoking, and obesity as risk factors for the development of rheumatoid arthritis: results from a primary care-based incident case-control study in Norfolk, England. *Arthritis Rheum.* 40 (11), 1955–1961.
- Taneja, V., 2017. Microbiome in 2016: T follicular helper cells and the gut microbiome in arthritis. *Nat. Rev. Rheumatol.* 13 (2), 72–74.
- Taneja, V., David, C.S., 2010. Role of HLA class II genes in susceptibility/resistance to inflammatory arthritis: studies with humanized mice. *Immunol. Rev.* 233 (1), 62–78.
- Taneja, V., Mehra, N.K., Chandershekaran, A.N., Ahuja, R.K., Singh, Y.N., Malaviya, A.N., 1992. HLA-DR4-DQw8, but not DR4-DQw7 haplotypes occur in Indian patients with rheumatoid arthritis. *Rheumatol. Int.* 11 (6), 251–255.
- Taneja, V., Griffiths, M.M., Luthra, H., David, C.S., 1998. Modulation of HLA-DQ-restricted collagen-induced arthritis by HLA-DRB1 polymorphism. *Int. Immunol.* 10 (10), 1449–1457.
- Taneja, V., Taneja, N., Paisansinsup, T., Behrens, M., Griffiths, M., Luthra, H., et al., 2002. CD4 and CD8 T cells in susceptibility/protection to collagen-induced arthritis in HLA-DQ8-transgenic mice: implications for rheumatoid arthritis. *J. Immunol.* 168 (11), 5867–5875.
- Taneja, V., Taneja, N., Behrens, M., Pan, S., Trejo, T., Griffiths, M., et al., 2003. HLA-DRB1\*0402 (DW10) transgene protects collagen-induced arthritis-susceptible H2Aq and DRB1\*0401 (DW4) transgenic mice from arthritis. *J. Immunol.* 171 (8), 4431–4438.
- Taneja, V., Taneja, N., Behrens, M., Griffiths, M.M., Luthra, H.S., David, C.S., 2005. Requirement for CD28 may not be absolute for collagen-induced arthritis: study with HLA-DQ8 transgenic mice. *J. Immunol.* 174 (2), 1118–1125.
- Taneja, V., Behrens, M., Mangalam, A., Griffiths, M.M., Luthra, H.S., David, C.S., 2007a. New humanized HLA-DR4-transgenic mice that mimic the sex bias of rheumatoid arthritis. *Arthritis Rheum.* 56 (1), 69–78.
- Taneja, V., Krco, C.J., Behrens, M.D., Luthra, H.S., Griffiths, M.M., David, C.S., 2007b. B cells are important as antigen presenting cells for induction of MHC-restricted arthritis in transgenic mice. *Mol Immunol.* 44 (11), 2988–2996.
- Tosato, G., Steinberg, A.D., Yarchoan, R., Heilman, C.A., Pike, S.E., De Seau, V., et al., 1984. Abnormally elevated frequency of Epstein-Barr virus-infected B cells in the blood of patients with rheumatoid arthritis. *J. Clin. Invest.* 73 (6), 1789–1795.
- Ummarino, D., 2016. Rheumatoid arthritis: smoking influences autoimmunity to vimentin. *Nat. Rev. Rheumatol.* 12 (11), 624.
- Vaishnavi, S., Yamamoto, M., Severson, K.M., Ruhn, K.A., Yu, X.F., Koren, O., et al., 2011. The antibacterial lectin RegIII gamma promotes the spatial segregation of microbiota and host in the intestine. *Science* 334 (6053), 255–258.
- Vassallo, R., Luckey, D., Behrens, M., Madden, B., Luthra, H., David, C., et al., 2014. Cellular and humoral immunity in arthritis are profoundly influenced by the interaction between cigarette smoke effects and host HLA-DR and DQ genes. *Clin. Immunol.* 152 (1-2), 25–35.
- Vladutiu, A.O., Rose, N.R., 1971. Autoimmune murine thyroiditis relation to histocompatibility (H-2) type. *Science* 174 (4014), 1137–1139.
- Wegner, N., Wait, R., Sroka, A., Eick, S., Nguyen, K.A., Lundberg, K., et al., 2010. Peptidylarginine deiminase from *Porphyromonas gingivalis* citrullinates human fibrinogen and alpha-enolase: implications for autoimmunity in rheumatoid arthritis. *Arthritis Rheum.* 62 (9), 2662–2672.
- Weinshenker, B.G., Santrach, P., Bissonet, A.S., McDonnell, S.K., Schaid, D., Moore, S.B., et al., 1998. Major histocompatibility complex class II alleles and the course and outcome of MS: a population-based study. *Neurology* 51 (3), 742–747.
- Wen, H., Baker, J.F., 2011. Vitamin D, immunoregulation, and rheumatoid arthritis. *J. Clin. Rheumatol.* 17 (2), 102–107.
- Wen, L., Wong, F.S., Tang, J., Chen, N.Y., Altieri, M., David, C., et al., 2000. In vivo evidence for the contribution of human histocompatibility leukocyte antigen (HLA)-DQ molecules to the development of diabetes. *J. Exp. Med.* 191 (1), 97–104.
- Wingerchuk, D.M., 2012. Smoking: effects on multiple sclerosis susceptibility and disease progression. *Ther. Adv. Neurol. Disord.* 5 (1), 13–22.
- Wooley, P.H., Luthra, H.S., Stuart, J.M., David, C.S., 1981. Type II collagen-induced arthritis in mice. I. Major histocompatibility complex (I region) linkage and antibody correlates. *J. Exp. Med.* 154 (3), 688–700.
- Wu, H.J., Ivanov, I.I., Darce, J., Hattori, K., Shima, T., Umesaki, Y., et al., 2010. Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. *Immunity* 32 (6), 815–827.
- Zhang, X., Zhang, D., Jia, H., Feng, Q., Wang, D., Liang, D., et al., 2015. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. *Nat. Med.* 21 (8), 895–905.

# Animal Models of Organ-Specific Autoimmune Disease

Ken Coppieters<sup>1</sup>, Matthias von Herrath<sup>2</sup> and Dirk Homann<sup>3</sup>

<sup>1</sup>Global Research, Research Project Management, Måløv, Denmark <sup>2</sup>Type 1 Diabetes Research Center, Novo Nordisk, Seattle, WA, United States <sup>3</sup>Icahn School of Medicine at Mount Sinai, New York, NY, United States

## O U T L I N E

<b>What Can Animal Models Teach Us About Organ-Specific Autoimmunity?</b>	<b>493</b>	<i>Pernicious Anemia</i>	501
<i>Animal Models in Basic Science: Understanding the Complexity of Organ-Specific Autoimmunity</i>	494	<i>Ulcerative Colitis and Crohn's Disease</i>	502
<i>Animal Models in Drug Development: Picking the Winners</i>	495	<i>Autoimmune Hepatitis</i>	502
<i>Summary of Advantages and Disadvantages of Animal Models</i>	497	<i>Primary Biliary Cirrhosis</i>	503
<b>A Survey of Animal Models for Organ-Specific Autoimmune Diseases</b>	<b>498</b>	<i>Vitiligo</i>	503
<i>Hashimoto's Thyroiditis and Graves' Disease</i>	499	<i>Alopecia Areata</i>	503
<i>Type 1 Diabetes</i>	500	<i>Dermatitis Herpetiformis</i>	504
<i>Addison's Disease</i>	501	<i>Multiple Sclerosis</i>	504
<i>Celiac Disease</i>	501	<i>Narcolepsy</i>	505
		<i>Immune Thrombocytopenic Purpura</i>	505
		<i>Giant Cell Arteritis</i>	505
		<b>Conclusions</b>	506
		<b>References</b>	506

The problem of science will consist precisely in this, to seek the unitary character of physiological and pathological phenomena in the midst of the infinite variety of their particular manifestations. *Bernard (1865)*.

All models are wrong but some are useful. *Box (1979)*.

## WHAT CAN ANIMAL MODELS TEACH US ABOUT ORGAN-SPECIFIC AUTOIMMUNITY?

Throughout recorded history, animals have claimed a principal place in our imagination and inspired a broad range of practices that substantially shaped the course of our collective pursuit to organize and control the natural world. Based on the notion that humans and other animal species apparently share certain anatomic and physiological characteristics, the heuristic use of animals to specifically address questions about human health and disease emerged at least 2500 years ago and the potential, limitations, and ethical implications of "animal

experimentation" have been discussed ever since (Ericsson et al., 2013; Franco, 2013). The related concept of "animal model" can therefore draw on a rich history of practical and discursive exploration but its actual formalization emerged only in the late 20th century and includes the proposed definition of "a living organism in which normative biology or behavior can be studied, or in which a spontaneous or induced pathological process can be investigated, and in which the phenomenon in one or more respects resembles the same phenomenon in humans or other species of animal" (CMBR, 1985; Hau, 2003; Held, 1980; Wessler, 1976). The contemporary concretization of these ideas further integrates the ethical core principles that build on Russell and Burch's original "3Rs" (replacement, reduction, and refinement) as a guide for animal studies and that continue to evolve to meet the concerns and challenges arising from an ongoing effort to balance the importance of empiric and value judgments (Tannenbaum, 2017). Since "the definitions of key concepts used in any scientific endeavor express the fundamental aims and priorities of that endeavor" (Tannenbaum and Bennett, 2015), it is within this larger context that animal models for organ-specific autoimmune diseases have to be considered (Cohen and Miller, 1994; Morel, 2004; Taneja and David, 2001; Wekerle et al., 2012; Williams, 2010; Yu et al., 2015). We submit that beyond the practical aspects of harnessing animal models for the study of specific autoimmune disorders, a historically and epistemologically informed perspective ultimately will precipitate the development of more productive research practices by allowing for more accurate diagnoses of current shortcomings and better prescriptions for needed course corrections. In what follows, we will first sketch out examples of scientific questions that are typically answered with the aid of animal models before embarking on a broad survey of their use and utility for the study of major organ-specific autoimmune diseases.

## Animal Models in Basic Science: Understanding the Complexity of Organ-Specific Autoimmunity

Arguably the most important and certainly most obvious path toward an improved and actionable understanding of organ-specific autoimmune diseases is the combination of clinical observation and intervention as well as experimentation with human blood and/or tissue samples. In as much as certain aspects of pathogenesis and pathology can be reproduced in animal models, such experimental strategies may complement and expand the scope of interrogation beyond the restrictions of human tissue access, availability, and ethical considerations; relevant observations and insights may then in turn inform and guide further human research endeavors. These "dialogical dynamics" of human and animal model research, including their possible pitfalls, are readily illustrated by the history of type 1 diabetes (T1D) research over the past half-century. The conception of T1D as an autoimmune disease in the mid-1970s emerged in the wake of seminal discoveries made primarily through investigations of the human disease (inflammatory infiltrates affecting the pancreatic islets, islet cell antibodies, HLA associations) and provided the impetus for the subsequent development of suitable animal models (Gale, 2001). However, the relative ease and success with which the animal models supported, refined, and enriched the autoimmune hypothesis over the ensuing three decades also detracted from the further pursuit of the logically, practically, and ethically more challenging interrogation of the human pancreas. Only more recent assessments have emphasized the inherent limitations of *in vivo* T1D models, their potential to promote a biased or even distorted understanding of the disease process, and the importance to realign preclinical investigations according to relevant pathogenetic parameters of the human disease (Roep, 2007; von Herrath and Nepom, 2009). As a consequence, interest in the detailed histopathology of the pre/diabetic human pancreas has been rekindled (Morgan et al., 2014; Richardson et al., 2014), and its pursuit greatly facilitated by the creation of initiatives such as the Network for Pancreatic Organ Donors with Diabetes (nPOD), a T1D tissue repository that collects, processes, and distributes pancreatic tissues to accredited T1D research teams (Campbell-Thompson et al., 2012; Kaddis et al., 2015). The direct study of the diseased organ can now provide an important referent and corrective for future studies of accessible human tissues such as peripheral blood, and for the more effective and adjusted use of T1D animal models. Disease-specific and technological constraints further define the potential scope of experimental exploration; here the fact that biopsy of the human pancreas is difficult, risky, raises ethical concerns, and, given the regional and lobular distribution of the pathological processes as revealed in recent nPOD studies, may not necessarily capture representative biological material (Atkinson, 2014; Gianani et al., 2010; Krogvold et al., 2014).

Even when human tissue samples are readily accessible, interpretation of experimental results can be difficult due to the lack of tissue or organism context. Let's take as an example here intestinal diseases such as Crohn's disease (CD), where biopsies are relatively easily obtained. Even under the best possible culturing conditions, how can an isolated slice of intestinal epithelium faithfully reproduce the milieu of the T cells it harbors? Stress responses, disrupted interactions with microbiota, altered gravitational, and osmotic conditions are only a few of

the confounding factors. That is not to say that one cannot obtain valuable data from these *ex vivo* systems. But some type of validation within the context of a living organism is typically required to corroborate the physiological validity of such findings. This applies particularly to results obtained using human-derived cell lines, which represent often aberrantly functioning clones from a single cell, from a single organ, and from a single individual, thus missing even the slightest organismal context.

Animal models have helped us to understand the complexity of autoimmune disease processes by partially replicating these conditions within an organismal context. This is an important notion, as it acknowledges that no animal model should ever be studied in isolation. Chemically destroying the intestinal barrier, for example, will reproduce certain mechanisms associated with cellular stress, altered microbial sensing, and tissue repair as observed in inflammatory bowel diseases (IBDs) (Chassaing et al., 2014). It will not, however, provide information the primary role of regulatory T cells (Tregs) in maintaining immune homeostasis in the gut, for which a T-cell transfer model would be more suitable (Eri et al., 2012). In other words, our understanding of human disease has historically been built on the combined datasets emerging from work on cell lines, primary cells, tissues and organ cultures, and all available animal models. Neither a single human explant study nor a particular animal model should be relied upon to generate a complete picture because each has its specific limitations.

In the immunogenetic arena, animal models have provided particularly useful insights. Genome-wide association study (GWAS) technology has revealed a tremendous degree of detail on the array of genes associated with human autoimmune diseases. Gene-function relationships are extremely difficult to study in humans and genetically modified animals have historically filled that void. The role of the IL-10 pathway in IBD (Kuhn et al., 1993), interferon signaling in multiple sclerosis (MS) (Chu et al., 2000), and insulin autoreactivity in T1D (Nakayama et al., 2005) are examples where transgenic animals have elucidated what are now believed to be the key aspects of the immunopathology. Moreover, broad concepts that apply to multiple forms of autoimmunity have been discovered or characterized in mice, with AIRE-mediated thymic T-cell selection (Anderson et al., 2002) and Foxp3-regulated natural Treg development and function (Hori et al., 2003) being prime examples. Overall, the comparison between mouse models and humans deficient in respective genes greatly contributes to our understanding of autoimmunity, even if complete phenotypic homology between knockout mice and the respective human genetic deficiency is often lacking.

Finally, despite intensive research over the last few years, there are some crucial elements in organ-specific autoimmunity that we are yet to fully comprehend. One major problem is that for many organ-specific autoimmune diseases, we still do not know the targets of the initial autoimmune response, although in others such as in pemphigus vulgaris (Lin et al., 1997), myasthenia gravis (Fambrough et al., 1973), and autoimmune gastritis (Karlsson et al., 1988), the driving autoantigen has been defined. This is a research question which needs to originate from or at least be confirmed by human studies, not animal models. The same applies to studies on inciting environmental factors, where epidemiological studies have much more relevance and power. Ideally, animal models should then be designed based on that knowledge. We believe that human polygenic autoimmune diseases are likely triggered by a wide variety of environmental factors in individual patients, making it unlikely that we will ever be able to define a single or a small set of environmental risk factors. We should therefore rather focus on common downstream disease pathways, for which animal models are excellent tools.

## Animal Models in Drug Development: Picking the Winners

During the course of commercial drug development, animal models are primarily used during the early discovery phase to support drug targets for further progression into the pipeline. The important decision to select drug targets for progression toward further analog design and screening is rarely ever based on the outcome of a single animal model. Typically, the criteria for progression involve genetic association, target protein, or mRNA association in human disease tissue, *in vitro* and *ex vivo* human cell or tissue experiments demonstrating beneficial responses, and, in addition to all of this, successful treatment with a research lead molecule in multiple animal models.

There are some general preferences in the drug industry when it comes to the use of animal models. Firstly, experimental disease should be reliably induced or occur spontaneously within a predictable time period and should exhibit a reproducible disease phenotype. A popular animal model for rheumatoid arthritis (RA) for instance is the collagen-induced arthritis (CIA) model in DBA/1 mice (Brand et al., 2007), because it fulfills these criteria and can thus be used in a relatively high-throughput *in vivo* screening approach. A variant of the model, induced with autologous collagen, may well mimic the remitting–relapsing course of RA better, but it takes much longer to induce, shows more variability, and has therefore never really enjoyed widespread use (Malfait et al., 2001).

Secondly, models with an established track record of translatability are favored. Returning to the CIA example, we have long known that standard-of-care biological therapies such as anti-TNF agents reproducibly alter the model's disease course (Williams et al., 1992). So, even though the acute nature of the model may not faithfully reproduce human disease, many drugs that are efficacious in humans can serve as positive controls for experimental compounds. On the contrary, many have argued that the nonobese diabetic (NOD) model for diabetes has proven unreliable in predicting clinical translatability, although we would argue that perhaps the model has historically not been used correctly. One example in the T1D arena is CTLA-4-targeted therapy, which is able to prevent diabetes in the NOD mouse when given early but is incapable of reversing clinical disease (Lenschow et al., 1995). In a subsequent clinical trial, 2-year continued administration in recently diagnosed patients was only effective in the initial months of treatment, which to some extent serves to confirm what was predicted by the animal data (Orban et al., 2011). Thus the choice of drug and trial design does not always seem to be rationally based upon animal research and is often more governed by the availability of certain drugs.

Thirdly, a robust understanding of the model's disease mechanisms is required to enable proper usage. Sticking with the CIA example, one could argue that after decades of use, there is substantial information available on every imaginable gene, cell, or protein that drives the disease process (Luross and Williams, 2001). We know that the eventual effector mechanisms are multifaceted and to some extent redundant. Asking the original question of whether TNF plays a primordial role in this model makes sense and the result of those experiments supported the medical hypothesis that spurred the development of anti-TNF biologicals for RA. Likewise, it would be suitable to study agents affecting the IL-6 pathway (Alonzi et al., 1998), currently a major class of drug target in RA. It would, however, not make sense in our opinion to treat animals that transgenically overexpress TNF with a TNF inhibitor because that result would not be very informative based on the fact that the mechanism is known to be primarily driven by TNF. That would be stacking the deck in favor of the therapy under observation.

When all these criteria are fulfilled and the experimental approach is planned, we cannot stress enough the need for properly powered study designs and sufficient numbers of experimental repetitions. Not a single animal model we know of doesn't show at least some degree of heterogeneity, be it in disease penetrance, progression rate, or severity. Even relatively subtle environmental changes can cause alterations in for instance the colony's microbiome, leading to variations in disease development. Nevertheless, rather than trying to control all possible variables in order to achieve reproducible drug effects, one should expect a sufficiently potent drug to confer mildly variable levels of protection regardless of environment, animal vendor, injection volume, etc. We have evaluated preclinical intervention strategies developed by others that are reproducible only if all of the aforementioned parameters are precisely controlled. When the ultimate aim is to take robust treatment strategies to an outbred human population, with vastly differing environmental conditions and notoriously heterogeneous disease courses, treatment efficacy contingent on tightly controlled conditions constitutes a considerable limitation (Harris, 2017).

An example of a theoretically attractive niche where animal models still have to deliver on predictive potential is the area of antigen-specific therapies (Coppieters et al., 2012b). This treatment approach holds significant promise since it specifically targets only immune cell subsets responsible for disease pathogenesis rather than inducing generalized immunosuppression with its associated complications. From a translational angle, it is still unclear whether tolerizing the "driver" T-cell clones would be sufficient to reverse an ongoing autoimmune process and has therapeutic value. Some reports suggest a striking oligoclonality, which in turn indicates that deletional immunotherapy using one or a few autoantigenic determinants might be feasible (Hafler et al., 1988; Kent et al., 2005). Animal models also suggest that T-cell receptor (TCR) avidity plays a pivotal role, as it was shown that low-avidity clonotypes can act protectively (Han et al., 2005). Numerous other reports, however, suggest that low-affinity clones might in fact be the driving force. For example, in the slow-onset variant of the RIP-LCMV model (mice susceptible to T1D after lymphocytic choriomeningitis virus infection due to transgenic expression of LCMV proteins under control of the rat insulin promoter), low-affinity T cells are responsible for disease induction (von Herrath et al., 1994). In human T1D, similar conclusions were drawn from studies using a preproinsulin-specific human CD8 T-cell clone that is capable of inducing beta cell death (Skowera et al., 2008). Remarkably, the TCR of this clone was of extraordinarily low affinity, redefining the understanding of what constitutes a functionally relevant TCR–pMHC interaction (Bulek et al., 2012).

Within that framework of knowledge, animal models have been used to evaluate antigen-specific therapies that have now completed early clinical trials, including proinsulin plasmid (Roep et al., 2013), peptide therapies (Alhadj Ali et al., 2017), and oral insulin (Bonifacio et al., 2015; Greenbaum, 2017) for T1D. The jury is still out on the former two approaches with only safety data being available, while the book can probably be closed on the latter. In fact, we recently attempted to elicit antigen-specific tolerance using oral insulin of various sources, formulations, and doses but failed to generate support for robust efficacy (Pham et al., 2016).

Finally, one interface between discovery research and clinical development that may benefit from animal model research is the field of biomarker discovery. Well-characterized animal models are suitable tools to pose questions that often cannot be asked in humans, such as whether peripheral blood cell populations reflect the events at the target organ, change in response to therapy, or correlate with therapeutic benefit. One could then take such mode-of-action-related biomarkers into Phase 1 trials to assess whether they can be reliably measured and then correlate levels to efficacy in Phase 2 proof-of-concept trials. Again, rather than offering ultimate proof, animal models should be seen as part of the hypothesis generation, which ultimately would be tested in the clinic.

### Summary of Advantages and Disadvantages of Animal Models

As illustrated in [Box 27.1](#) and as outlined earlier, there are several areas of investigation, where we have learned much from animal models of organ-specific autoimmunity. The first is the investigation of immune responses at locations that are inaccessible in humans. Moreover, in these diseases, much of what we know has often been derived from peripheral blood samples but it is unclear how this correlates to immune status at the target organ. We, for instance, were able to pioneer the *in vivo* visualization of immune responses at cellular resolution in the mouse pancreas during the development of autoimmune diabetes ([Coppieters et al., 2012a](#)); such information can not be gathered in the human target organ with any of the technologies currently available.

It is difficult to establish the proof of concept in humans. Many techniques that are frequently used to unequivocally identify cellular subsets that are important in the etiology or progression of autoimmunity are impossible to implement in a clinical setting. Examples include adoptive transfer experiments, knockout technologies, bone marrow chimeras, and many more. Accordingly, the continued and refined use of animal models remains at present indispensable to provide initial preclinical support for novel drugs and to define suitable dose ranges and regimens for immunotherapies.

How far reaching should the conclusions that we draw from observations in a given animal model be? The prevailing approach in scientific communication is still that a discovery in one of the animal models thought to faithfully represent the human disease is sufficient for acceptance for publication. If the same result is found in a subsequent submission, it is typically considered “confirmatory;” if a different result is discovered, the “secondary” model is frequently labeled as not-as-good or even flawed, for which various reasons are cited. This, we believe, can be treacherous and may hamper the translation of research performed in animal models, because our pathogenetic insight into human diseases is often still rather limited due to ethical constraints. For example, only about 10% of patients with T1D exhibit the same clinical features as the NOD diabetic mouse, which is characterized by a polyglandular autoimmune syndrome affecting thyroid, salivary glands, and testes ([Atkinson and Leiter, 1999](#); [Roep et al., 2004](#)). However, there are also striking similarities between NOD and human diabetes, for example, the occurrence of autoantibodies that precedes the development of clinical disease in NOD mice and humans ([Pietropaolo and Eisenbarth, 2001](#)).

#### BOX 27.1

### WHAT CAN ANIMAL MODELS TEACH US THAT WE CANNOT LEARN OTHERWISE?

- *In vivo* immune kinetics at *sites that are difficult to access in humans.*
- *Proof of concept* using techniques that cannot be used in humans, such as adoptive transfers, genetic knockouts, bone marrow chimeras, etc.
- *Preclinical drug validation*, large-scale assessment of *dose range, toxicity, and immunization sites* in drug and vaccine development.
- A single model is unlikely to cover all aspects of human pathology; concepts should therefore ideally be confirmed in multiple models.
- Genetic knockout models do not mimic the subtle and complex genetic imbalances that are thought to underlie human diseases.
- Environmental factors are usually not known and thus cannot be mimicked.

Reasons to be *cautious with animal data:*

We can now be certain that one single genetic defect or polymorphism will not be the cause for most human autoimmune disorders. We have learned from the multitude of GWAS that the far more common scenario is a substantial degree of polygenic complexity. This is likely to involve many protective and predisposing genes that act in concert, leading to disease manifestation in a fraction of their bearers. In addition to major histocompatibility complex (MHC) class II molecules that are found in association with several organ-specific autoimmune diseases such as MS and T1D, many less frequent polymorphisms in immune-related genes contribute to the expression of autoimmune disease (Bluestone et al., 2010). Immunogenetic studies in genetically identical mice can therefore not be expected to fully mimic the diverse array of polymorphisms that lead to disease in genetically distinct human subjects. Thus, seeking direct relevance to the human disorder becomes very important, and the assumption that there is a necessity to identify genetic defects in animal models in order to obtain the best pathways to cure human disease may not be correct. Indeed, in today's medicine, there is little evidence that the optimal treatment for a given disease is always the elimination of its cause. However, immunogenetics in animal models might still help the adoption of novel predictive strategies and subclassifications for autoimmune diseases, which may in the future allow for more individualized treatments.

Finally, most of these genetic associations are weak enough to allow for the possibility that environmental factors in addition to genetic determinants precipitate penetrance of disease. These factors, however, are poorly defined in most organ-specific autoimmune diseases. In the NOD mouse, for example, there appears to be a role for the gastrointestinal microbiome (Wen et al., 2008), although under normal specific pathogen-free conditions, diabetes develops without the need for an external stimulus. In human T1D, the strongest evidence for a potential trigger points toward enteroviral infection (Yeung et al., 2011). While viral infection can also accelerate disease in the NOD model under certain conditions, it is normally not required for onset. It is thus obvious that the NOD model does not faithfully integrate the environmental factors that seem to characterize the human disease. Similar discordances are present in models for MS, where neuronal antigens are often injected in adjuvant to induce demyelinating disease. Such nonphysiological conditions should always be kept in mind when interpreting results from animal models.

## A SURVEY OF ANIMAL MODELS FOR ORGAN-SPECIFIC AUTOIMMUNE DISEASES

Parsing animal models for organ-specific autoimmune diseases is a taxonomic exercise that readily evokes the "ambiguities, redundancies, and deficiencies" so brilliantly illustrated by Borges in his "*Celestial Emporium of Benevolent Knowledge*", an apocryphal classification of the animals that distinguishes, amongst others, "*those that belong to the emperor*", "*those that are included in this classification*", "*those that have just broken the flower vase*", and "*those that, at a distance, resemble flies*" Borges (1942). So, while the basic distinction between "spontaneous" and "induced" autoimmune disease models appears sensible (Hau, 2003), the oft-used addition of "genetically engineered animals" as a third category generates a logical conundrum since genetic manipulation may of course be employed to refine existing or create new models for both spontaneous or induced autoimmune disease. Additional subdivisions such as those made according to inducing agents can be equally confounding [proteins/peptides and adjuvant, pathogens, specific immune effectors (antibodies or T cells), chemicals, etc.], some "proof-of-principle" demonstrations may exude a whiff of circular logic [e.g., combining tissue-specific expression of "neo-autoantigens" with TCR-transgenic (TCRtg) T cells recognizing that very antigen], and certain interventions may stretch the limits of what can be considered autoimmunity (e.g., toxin-induced destruction of selected target tissues); all of this poses a challenge to constructive classification, yet it also constitutes a celebration of the manifold and at times wondrous combinatorial possibilities afforded by experimental autoimmunology. Since the topic of our survey therefore proves somewhat resistant to a convincing organization of its constituents, namely, the relevant animal models available for study of organ-specific autoimmune diseases, we have aimed to avoid an otherwise arbitrary selection of models for further discussion by grounding our choices in a ranking of human autoimmune diseases according to their overall prevalence (Hayter and Cook, 2012). Building and expanding on just a few earlier reports, Hayter and Cook identified 81 human autoimmune diseases that together affect an estimated ~4.5% of the population; 23 autoimmune diseases can be considered as "non-rare" (i.e., present at a frequency of >1/10,000) and of these, 17 are classified as organ specific (Table 27.1). In terms of prevalence, these 17 organ-specific diseases represent ~75% of all autoimmune disorders, and we provide here a brief overview of the corresponding animal models developed to emulate and study these conditions a clear majority of which (~80%) involves endocrine or digestive systems. For a more detailed discussion of the various diseases and their in vivo models, the reader is referred to respective review articles cited later as well as the disease-focused chapters in this book.

**TABLE 27.1** The Most Common Organ-Specific Autoimmune Diseases in Humans

Organ system	Organ-specific autoimmune disorder	Prevalence	
		(per 10 <sup>5</sup> )	Ratio F:M
Endocrine system	Hashimoto's thyroiditis (HT)	791.7	19:1
Digestive system	Celiac disease	750.0	1.3:1
Endocrine system	Graves' disease (GD)	629.0	7.3:1
Endocrine system	Type 1 diabetes (T1D)	480.0	0.8:1
Integumentary system	Vitiligo	400.2	1.1:1
Digestive system	Pernicious anemia/autoimmune gastritis	150.9	2.0:1
Integumentary system	Alopecia areata (AA)	150.0	1.0:1
Hematopoietic system	Immune thrombocytopenic purpura (ITP)	72.0	2.3:1
Nervous system	Multiple sclerosis (MS)	58.3	1.8:1
Nervous system	Narcolepsy	30.6	0.6:1
Cardiovascular system	Giant cell arteritis (GCA)/temporal arteritis	30.0	5.7:1
Digestive system	Ulcerative colitis (UC)	30.0	1.9:1
Digestive system	Crohn's disease (CD)	25.0	0.7:1
Digestive system	Autoimmune hepatitis (AIH) types 1 and 2	19.9	3.6:1
Digestive system	Primary biliary cirrhosis (PBC)	14.6	8.1:1
Endocrine system	Addison's disease (AD)	14.0	1.7:1
Integumentary system	dermatitis herpetiformis (DH)	11.2	0.6:1

Modified from Hayter, S.M., Cook, M.C., 2012. Updated assessment of the prevalence, spectrum and case definition of autoimmune disease. *Autoimmun. Rev.* 11, 754–765.

## Hashimoto's Thyroiditis and Graves' Disease

Thyroid-specific autoimmunity covers the spectrum from hypo- [Hashimoto's thyroiditis (HT)] to hyperthyroidism [Graves' disease (GD)] and preferentially targets thyroglobulin, thyroid peroxidase, and the thyroid-stimulating hormone receptor (TSHR). Antibodies and T cells specific for these autoantigens are detected not only in HT and GD patients but also healthy individuals reinforcing the concept that autoimmunity in fact represents a continuum stretching from the physiological to the pathological (Coppieters et al., 2013). Of historical interest is a series of studies conducted by Rose and Witebsky in the mid-1950s that explored the consequences of immunizing rabbits with leporine thyroid extracts in adjuvant and that noted the generation of autoantibodies and mononuclear thyroid infiltrations (Rose and Witebsky, 1956; Witebsky et al., 1957); this observation was complemented by the parallel demonstration of Roitt et al. (1956) that sera from HT patients contain thyroglobulin-specific autoantibodies. The work of Rose and Witebsky not only paved the way for the development of “experimental autoimmune thyroiditis (EAT)” to model the most common of organ-specific autoimmune diseases but in fact changed the course of autoimmune research *at large* by finally, if reluctantly, rejecting a persistent misreading of P. Ehrlich's half-century old “horror autotoxicus” (Silverstein, 2009). EAT is readily induced in genetically susceptible mice by immunization with thyroglobulin (and more recently also with thyroid peroxidase), following neonatal thymectomy in certain mouse and rat strains, after transfer of specific T cells but not antibodies, and through various other modulations of T-cell immunity. In addition, spontaneous thyroiditis occurs in several species including obese strain chickens, biobreeding (BB) rats, and NOD mice lacking the chemokine receptor CCR7 or expressing the H-2A<sup>k</sup> allele (the latter mice are diabetes-resistant, spontaneous disease is exacerbated by dietary iodine and reproduces hallmarks of HT such as high titers of TG antibody and cellular infiltration of the thyroid). Altogether, these models have contributed to the identification of genetic, cellular, molecular, and environmental pathogenesis determinants, have established HT as a paradigm for other T-cell-mediated organ-specific autoimmune diseases, and have provided fundamental insights into peripheral tolerance mechanisms by

precipitating the second wave of regulatory T-cell research (Kong et al., 2009; Ludgate, 2008; Nagayama and Abiru, 2011; Stassi and De Maria, 2002). For historical, conceptual, and practical reasons, the strategies to model HT therefore occupy a role unlike any other in the field of autoimmunity research. In contrast, a truly satisfactory GD model has not yet been established. An autoimmune condition apparently unique to humans, GD is caused by agonistic TSHR-specific antibodies that mimic the action of TSH and drive uncontrolled thyroid hormone production. No spontaneous GD model exists, and traditional protein/adjuvant approaches to TSHR immunization have failed to promote hyperthyroidism. Over the past 20 years, several GD models have been developed that are all based on immunization against and, *in vivo* expression of, the TSHR; they can be broadly divided into injection of TSHR-expressing cells and genetic immunization with TSHR-DNA plasmids or recombinant adenoviral vectors. Overall, the A-subunit of TSHR is more efficient in inducing disease than the holoreceptor, and more recent methodological refinements employing repetitive adenoviral vaccinations or the *in vivo* electroporation of TSHR expression plasmids have succeeded in reproducing some aspects of Graves' orbitopathy (McLachlan et al., 2005; Moshkelgoshia et al., 2015; Nagayama et al., 2015; Wiesweg et al., 2013).

## Type 1 Diabetes

In a notably broad array of animal models, hyperglycemia or T1D-like disease develops either spontaneously or in the wake of chemical, surgical, infectious, and/or genetic manipulations (Chatenoud, 2008; King and Austin, 2017; King, 2012; Van Belle et al., 2009). Streptozotocin (STZ) and alloxan are toxic glucose analogs (Lenzen, 2008) routinely used to model end-stage disease in rodents for the purpose of testing drugs (e.g., new insulin formulations) and interventions (e.g., islet transplantation) that specifically aim to lower blood glucose. An important and perhaps underused alternative to chemical T1D induction is the Akita mouse that exhibits hypoinsulinemia and pronounced hyperglycemia as a consequence of an insulin 2 gene mutation, overload with misfolded proteins and resultant ER stress. Hyperglycemia may also be induced in pigs and nonhuman primates through tailored STZ treatment or pancreatectomy but all of the preceding approaches obviously are of limited value for the elucidation of genuine autoimmune processes. Certain viral infections (coxsackie B virus, encephalomyocarditis virus, and Kilham rat virus) may promote direct and/or immune-mediated beta cell destruction, and transgenic expression of model antigens in beta cells [ovalbumin, influenza hemagglutinin, or LCMV glycoprotein (LCMV-GP)] renders them susceptible to attack by TCR<sup>T</sup> T cells specific for the same antigens. Of these models, the LCMV-GP and related mouse strains are particularly useful since T1D can also be induced following LCMV infection and efficient beta cell destruction by the endogenous virus-specific T-cell response (Ohashi et al., 1991; Oldstone et al., 1991). Collectively, the transgenic models offer a substantial degree of experimental freedom that greatly facilitates the visualization, quantification, characterization, and manipulation of antigen-specific, diabetogenic T-cell immunity and, in particular, allows for a thorough interrogation of pathogenic mechanisms and potential therapeutic interventions. However, the models do not feature the gradually accumulating, diversified, and endogenous beta cell antigen-specific and mostly low-affinity T-cell populations present in T1D patients; they do not capture aspects of genetic susceptibility other than the engineered components; and, in the absence of TCR<sup>T</sup> T cells, they do not develop spontaneous or even peptide/protein immunization-induced disease. The latter observations support the general conclusion about the not inconsiderable challenges to break peripheral tolerance and bolster the importance of spontaneous T1D models. The four major models in this category are the NOD mouse and three rat strains (BB, Komeda diabetes-prone, and LEW.1AR1-iddm rats) (King and Austin, 2017; Lenzen, 2017; Whalen et al., 2001); other spontaneous T1D models such as canine autoimmune diabetes may offer distinct advantages in terms of pathophysiological similarities with the human disease but are otherwise not widely employed (O'Kell et al., 2017). The NOD mouse was discovered in Japan in 1974, introduced to the public in 1980, and by 1987 had become a fully established T1D model (Tochino, 1987). Since then, some 11,000 articles have been published (corresponding to an average of one publication per day over the past 30 years) making the NOD mouse the most widely studied autoimmune disease model. In these mice, a slow and seemingly stochastic progression from initial insulitis to beta cell destruction culminates in frank hyperglycemia by 30 weeks in ~80% of females and ~30% of males; T1D development is affected by various husbandry practices, "cleanliness" of the facility, viral infections, and intestinal microbiota (King and Sarvetnick, 2011; Paun et al., 2017; von Herrath et al., 2011) and therefore overall disease incidence may vary substantially in different mouse colonies. Immunopathogenetically, a complex interplay of T and B cells, dendritic cells, macrophages, and NK cells initiates and perpetuates spontaneous T1D in NOD mice, and various approaches can accelerate disease onset for practical purposes (cyclophosphamide injection, diabetogenic T-cell transfer, islet transplantation to

overtly diabetic recipients). Because of its remarkable breadth and depth, the cumulative delineation of genetic, pathologic, and immunologic factors in NOD diabetes as well as the development of multiple effective prophylactic and therapeutic interventions (Chaparro and Dilorenzo, 2010; Shoda et al., 2005) constitutes a fundamental and fascinating contribution to T1D research and beyond. The seeming failure to translate these insights into treatment modalities for human T1D, however, has caused an early backlash and of late an abundance of discussions that attempt to reassess the potential and limitations of NOD research from a translational perspective (Driver et al., 2011, 2012; Graham and Schuurman, 2015; Pearson et al., 2016; Reed and Herold, 2015; Roep et al., 2004; von Herrath and Nepom, 2009). The arguments are overall nuanced; the utility of NOD (and other preclinical T1D) models has to be ascertained in an appropriate, detailed, and highly context-specific manner; and among the promising recommendations is an increased investment in humanized mouse models (Serreze et al., 2016). Lastly, it should be noted that the profound immune dysregulation in NOD mice (Anderson and Bluestone, 2005) is not restricted to promotion of beta cell destruction and can affect multiple other organ systems such that wild-type, congenic, transgenic, and/or immunodeficient NOD mice may be harnessed for the study of other major organ-specific autoimmune diseases [HT, Addison's disease (AD), celiac disease, autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), dermatitis herpetiformis (DH), and MS] as well as autoimmune cholangitis, sialadenitis, orchitis, neuritis, and other conditions.

### Addison's Disease

Throughout the second half of the 20th century, various attempts to model AD employed the immunization of guinea pigs, rabbits, rats, or mice with adrenal extracts and adjuvant. Collectively, "experimental autoimmune adrenalitis" emulates the aspects of the human disease (lymphocytic infiltration of adrenal glands, autoantibodies) and emphasizes the dominance of cell-mediated autoimmune processes (adoptive transfer of cells but not serum from immunized rodents causes adrenalitis in recipients) but remains of limited value due to the different histopathological presentation of adrenal lesions, their transient nature, and, with only two exceptions, a lack of adrenocortical insufficiency; similar considerations apply to the "spontaneous" lymphocytic infiltrations observed in NOD mice in the absence of clinical disease (Betterle et al., 2008; Bratland and Husebye, 2011). In contrast, both cats and dogs can develop spontaneous symptomatic AD (accompanied by adrenal infiltrations but usually not autoantibodies) and the identification of orthologous genetic susceptibility loci in canine and human AD suggests a common underlying autoimmune etiology (Mitchell and Pearce, 2012). In an interesting twist to animal experimentation, however, human AD studies appear to have been useful for research in dogs but not vice versa (Mitchell and Pearce, 2012), and a genetically well-defined, robust AD model remains to be developed.

### Celiac Disease

Defined as an autoimmune enteropathy induced by dietary gluten in genetically predisposed individuals, "spontaneous" celiac disease is also observed in Irish Setters, Rhesus macaques, and horses (Marietta and Murray, 2012). However, since the utility of these animals as research models is restricted by unclear or nonexistent disease associations with MHC-II genes (the main risk factor in humans) as well as practical and ethical considerations, considerable effort has been invested in the development of suitable murine models (Costes et al., 2015; Korneychuk et al., 2015; Verdu et al., 2015). Collectively, however, studies conducted with wild-type, immunodeficient, and especially humanized mice expressing HLA-DQ2.5 or -DQ8 haplotypes and challenged with gluten containing diet or gliadin have been hampered by the robust tolerance to food antigens in rodents. Accordingly, experimental systems have gained in complexity and emphasized the cooperation of multiple potentially pathogenic pathways such as specific CD4<sup>+</sup> T-cell immunity, the cytokine IL-15, intestinal microbiota and barrier function, and activation of transglutaminase 2 (a target of celiac disease-specific IgA autoantibodies) to break tolerance. Thus, despite some proof-of-principle demonstrations, the successful modeling of celiac disease in mice remains an outstanding challenge (Korneychuk et al., 2015).

### Pernicious Anemia

Pernicious anemia, the end-stage of autoimmune gastritis, presents an unusual case in the spectrum of autoimmune disorders: it is one of the most common and previously fatal autoimmune diseases, yet a highly effective

treatment (vitamin B12 replacement) based in part on pioneering canine studies (Whipple et al., 1920) became available before a detailed understanding of the pathogenic mechanisms could be developed. From the 1980s onward, murine models of experimental autoimmune gastritis contributed to the generation of pertinent insights by exploring spontaneous and lymphopenia-induced disease courses, immunization with gastric H<sup>+</sup>/K<sup>+</sup> ATPase, the use of infectious agents, and transgenic approaches (TCRtg T cells or GM-CSF expression by parietal cells). Notably, and in apparent contrast to many other autoimmune diseases, these models are considered to be particularly reliable and robust since they share key features with the human disease including H<sup>+</sup>/K<sup>+</sup> ATPase as the major autoantigen targeted by both antibodies and T cells (Field et al., 2005; Toh et al., 2012). Perhaps as an unintended consequence of this unusually favorable constellation of therapeutic efficacy in the human disease, access to excellent in vivo models and an overall compelling grasp of major pathogenetic mechanisms, the current literature on pernicious anemia animal models is limited to but a few review articles and book chapters (van Driel et al., 2014). Nevertheless, we emphasize that the precise nature of disease initiating events remains little understood and that vitamin B12 therapy does not tackle the underlying cause of the disease which is associated with an increased risk for gastric cancer; further research and an exploration of additional treatment strategies are therefore warranted.

### Ulcerative Colitis and Crohn's Disease

An impressive spectrum of IBD models established and/or refined over the past two decades has elucidated fundamental principles of human IBD pathogenesis including the unique tissue-specific constraints that drive intestinal inflammation through a complex interplay of microbiota, disrupted epithelial barriers, and dysregulated immune responses (in particular polarized T helper and innate immunity) (Jamwal and Kumar, 2017). In a recent authoritative review, Kiesler et al. (2015) have organized IBD models into five major groups comprising dextran sulfate sodium colitis, TNBS (trinitrobenzene sulfonic acid) colitis, oxazolone colitis, adoptive cell transfer-induced colitis, and IL-10-deficient mice. Informed by the notion that no single model captures the complexities of human IBD, relevant insights can nevertheless be generated as composite constructs that draw on the strength of individual models to illuminate particular aspects of specific diseases. Thus, instead of emphasizing the lack or shortcomings of mouse models for ulcerative colitis (UC) or CD, this model- rather than disease-centered conception advocates for a deliberately distributed approach to disease study that builds on the specific contribution of multiple models to progressively reconstruct human IBD entities (Kiesler et al., 2015). Obviously, the particular extent to which individual models prove useful in this context can vary considerably. For example, oxazolone colitis is considered a useful "UC model" on the basis of morphological and immunopathogenetic aspects shared with the human disease; at the same time, IL-10-deficient mice may highlight other aspects of UC development since polymorphisms at the IL-10 locus confer increased disease risk in humans. IL-10 polymorphisms or IL-10R mutations are also associated with an enhanced CD risk or a familial form of early onset CD, respectively, demonstrating the utility of IL-10-deficient mice in CD research. Adoptive transfer colitis and especially TNBS colitis models further reproduce important features of CD but an integrated "CD model" has not yet been established. Lastly, we note that the experimental modeling of IBD pathogenesis goes well beyond the major (and minor) mouse models mentioned here and ranges from invertebrates (nematodes, insects, fish) to other rodents (rats, guinea pigs) and related species (rabbits) as well as pigs, ruminants, dogs, and nonhuman primates (Jiminez et al., 2015).

### Autoimmune Hepatitis

Attempts to establish animal models of AIH, beyond a few scattered earlier reports, slowly gained traction from the 1960s onward with an almost exclusive focus on immunization with liver antigens in adjuvant (Lohse and Meyer zum Büschenthal, 1994). Since the early 1990s, more than two dozen murine AIH models were introduced and include protocols for immunization with surrogate antigens; transgenic antigen expression, use of TCRtg T cells, or a combination thereof; certain systemic or conditional immunodeficiencies; dendritic cell immunization; viral infection; and challenge with AIH type-2 antigens (CYP2D6: 2D6 isoform of the large cytochrome P450 enzyme family; FTCD: formiminotransferase cyclodeaminase). These models have been reviewed in great detail (Christen and Hintermann, 2015, 2016; Czaja, 2010; Hardtke-Wolenski et al., 2012; Yuksel et al., 2014) and collectively support the sobering conclusion that truly satisfactory and reliable mouse models reflecting the clinical and histopathological features of human AIH have not yet been developed. In the majority of models, an

acute breakdown of tolerance to liver antigens is readily achieved but cannot be maintained due to the inherent tolerogenic capacity of the liver and the relative efficacy of immunoregulatory mechanisms. Immunization with adenoviral vectors expressing CYP2D6 or FTCD shows promise based on its capacity to promote the development of chronic hepatitis and fibrosis, and these models can conceivably be improved through a concurrent inhibition of peripheral tolerance pathways. However, these experimental strategies are restricted to the modeling of type-2 AIH and mouse models specific for the ~sixfold more frequent type-1 AIH have not yet been established (though the targeting of PD-1, Tim-3, and/or IL-4R pathways is being considered).

## Primary Biliary Cirrhosis

Apart from reports on an age-associated development of PBC-like liver lesions in C57BL/6 mice some 25 years ago, PBC appeared somewhat resistant to the study in suitable small animal models. However, over the past 15 years, about a dozen murine PBC models have been reported that feature both spontaneous and induced development of disease (Concepcion and Medina, 2015; Katsumi et al., 2015; Leung et al., 2012; Pollheimer and Fickert, 2015; Wang et al., 2014). The former models employ congenic mice including spontaneously occurring mutations (scurfy, MRL/lpr) and genetic modification such as IL-2Ra deficiency or T-cell-specific suppression of TGF $\beta$  signaling while the latter approach includes xenobiotic immunizations and bacterial infections. Several recent and comprehensive reviews offer a consensus assessment that the complex nature of PBC pathogenesis and pathology cannot be captured in a single animal model but may now be studied in complementary models that adequately recapitulate specific aspects of the disease (Concepcion and Medina, 2015; Katsumi et al., 2015; Leung et al., 2012; Pollheimer and Fickert, 2015; Wang et al., 2014).

## Vitiligo

Spontaneous vitiligo develops in horses carrying the dominant Gray allele (Arabians, Andalusians, and Lipizzaners), Sinclair miniature swine, certain dog breeds, water buffalo, and Smyth line chicken (Erf, 2010; Essien and Harris, 2014). Among these, the Smyth line chicken constitutes the most important vitiligo model since it recapitulates many aspects of the human condition and permits an integrated evaluation of genetic determinants, melanocyte defects, and autoimmune responses throughout all stages of the disease (Erf, 2010). In the absence of a spontaneous mouse model (the “vitiligo mouse” [mi<sup>vit</sup>/mi<sup>vit</sup>] does not reflect mechanisms operative in the human disease since disease develops independent of a functional immune system, and the underlying point mutation in the *Mitf* gene does not correspond to any mutation in human vitiligo), the induction of vitiligo in mice has been achieved through chemical sensitization, immunization with melanocyte antigens, and, perhaps most prominently, TCRtg CD4 $^{+}$  and CD8 $^{+}$  T-cell populations recognizing melanocyte or model antigens (Essien and Harris, 2014). As with other autoimmune disorders, the complexities of the human disease cannot be reproduced by a single animal model and necessitate a composite approach that takes into account specific strengths and weaknesses of individual models.

## Alopecia Areata

Similar to humans, adult onset alopecia areata (AA) can be observed in dogs, horses, cattle, and nonhuman primates but these animals typically do not serve as experimental disease models (Sundberg et al., 2015). The first reported animal model for AA was the “Dundee experimental balding rat,” and although it is no longer in use, it was instrumental in demonstrating that AA is a T-cell-mediated autoimmune disorder. Currently, the most prominent models are inbred C3H/HeJ mice that develop spontaneous or induced AA-like hair loss, and the xenotransplantation of healthy human scalp onto SCID mice followed by intracutaneous injection of IL-2-activated human PBMC; the latter model is noteworthy for the fact that it effectively phenocopies a major autoimmune disease using previously healthy primary human tissue (Gilhar et al., 2016). Among other insights, the C3H/HeJ model confirmed a central role for IFN $\gamma$  in AA pathogenesis and provided the proof-of-principle that Janus kinase inhibitors are suitable agents for AA management (Gilhar et al., 2016). Of potentially farther reaching importance is the notion that the considerable progress in preclinical AA research may establish AA as a “model disease” that offers both conceptual and practical tools for the study of less accessible organ-specific autoimmune diseases that share certain pathogenic determinants such as T1D and MS.

## Dermatitis Herpetiformis

DH is an autoantibody-mediated autoimmune blistering disease caused by gluten consumption and may be considered a skin manifestation of celiac disease. Accordingly, the challenges pertaining to the establishment of a robust celiac disease model also apply to DH. To date, the first and only DH model that captures some of the genetic and clinical features of the human disease is a transgenic NOD mouse expressing HLA-DQ8 instead of murine MHC-II, and similar treatment responses in humans and HLA-DQ8 transgenic NOD mice further underscore their experimental utility for exploration of pathogenic mechanisms and novel therapeutic modalities (Marietta and Murray, 2012; Marietta et al., 2012; Pollmann and Eming, 2017). While it is not unreasonable to predict a refinement and diversification of DH models in the future, we also note that no new models have been reported since the original introduction of the HLA-DQ8 NOD mouse a dozen years ago.

## Multiple Sclerosis

With no other animal species developing a spontaneous demyelinating disease of sufficient similarity, MS appears to be an autoimmune disorder unique to humans (Ransohoff, 2012). This, however, has not hampered the creation of multiple animal models that are typically grouped into the three main categories of experimental autoimmune encephalomyelitis (EAE), virus-induced chronic demyelinating disease, and toxin-induced demyelination. Collectively, these models have established and illuminated various pathogenetic, clinical, and therapeutic correlates of the decidedly heterogeneous human disease, and numerous review articles have discussed in exquisite detail their roles in past, present, and potentially future investigations, their principal prospects and apparent limitations, and their translational relevance or lack thereof (Baker and Amor, 2015; Denic et al., 2011; Kipp et al., 2017; Mix et al., 2010; Procaccini et al., 2015; Stimmer et al., 2018). Perhaps more so than anything else, historical contingency has turned EAE into one of the most prominent autoimmune disease models with some 10,000 articles on the topic currently featured in the PubMed database. The earliest evidence that encephalomyelitis could be triggered by challenging the immune system with “self-proteins” came from side effects associated with the use of Pasteur’s rabies vaccine at the end of the 19th century (Mackay, 2010). The vaccine, made from the dried spinal cord of rabies-infected rabbits, in some cases led to ascending paralysis. This postvaccination encephalomyelitis was shown in the late 1920s to also occur in rabbits immunized with extracts of normal human spinal cord or sheep brain; paralysis in challenged monkeys was shown to correlate with defined histological lesions and demyelination in the 1930s; and more formal demonstrations that EAE could be provoked in animals via a short course of injections of normal brain combined with Freund’s adjuvant were provided in the 1940s. In time, EAE research converged from nonhuman primates and larger rodents toward mice and to some extent relinquished neuropathological and -behavioral complexity in favor of greater experimental flexibility and control, economic feasibility, and important ethical considerations that limit the use of higher species in animal research. Altogether, EAE research has proved fruitful for the identification of immunological and genetic determinants of disease pathogenesis as well as the study of histopathological features but decidedly less so for an evaluation of new treatment modalities. Among the other classes of MS animal models, infection with Theiler’s murine encephalomyelitis virus constitutes a well-defined experimental system that has contributed important discoveries about the initiation and perpetuation of autoimmune processes, demyelination, and even behavioral alterations. Similarly, the mouse hepatitis virus system provides a controlled framework for a comprehensive analysis of specific immune responses at the interface of pathogen control and targeted immunopathology. Lastly, toxin-induced models, the most popular of which are feeding of the copper chelator cuprizone and the microinjection of lysophosphatidyl choline, are particularly useful for the study of remyelination and therefore have important translational potential (although they do not address the autoimmune nature of MS) (Baker and Amor, 2015; Denic et al., 2011; Kipp et al., 2017; Mix et al., 2010; Procaccini et al., 2015; Ransohoff, 2012; Stimmer et al., 2018). Beyond the applied specifics of EAE research in particular and MS models in general, two aspects are worth emphasizing: (1) basic experimental strategies originally established for EAE studies have come to serve, arguably for better and worse, as a general template for the development of many other organ-specific “experimental autoimmune” models and (2) the long history and extraordinarily rich record of EAE experimentation offer an abundance of detailed and diversified insights that can and should inform important discussions about the utility, constraints, pitfalls, and future directions of animal studies in autoimmune disease research. It is certainly not a coincidence that the apparent limitations of animal research have been most readily identified and critically assessed in the very fields that benefitted from a profusion of published *in vivo* studies (i.e., MS and T1D research); the resultant recommendations, namely, a careful selection of particular animal

models to address specific and only partial aspects of the human disease, may appear as a more modest goal but in fact emphasize the fundamentally dialogical nature of animal experimentation, that is, the necessity to better define human pathophysiology so as to better tailor the corresponding animal studies.

## Narcolepsy

Narcolepsy type 1 (NT1) arises from the presumed autoimmune-mediated destruction of hypocretin/orexin-producing neurons in the hypothalamus (NT2, despite many clinical similarities, is a poorly defined heterogeneous disorder of unknown origin). However, the evidence supporting an autoimmune pathogenesis for NT1 is at best indirect (autoreactive antibodies and T cells detected only in some cases in blood but not hypothalamus) and circumstantial (association with certain HLA haplotypes, likely role of environmental triggers) (Kornum et al., 2017). Narcolepsy has been observed in several animal species (cat, horse, sheep, and cattle), and familial canine forms of the disease, established in the 1970s as a model system, were instrumental for the identification of orexin 2 receptor mutations as an underlying gene defect in 1999. At the same time, a narcolepsy phenotype was described in hypocretin-deficient mice, and since then, multiple genetically engineered strains have been generated including a model for inducible ablation of hypocretin neurons (Chen et al., 2009; Sakurai, 2015; Sinton, 2010; Toth and Bhargava, 2013). Among the attempts to create a mouse model that better mimics the potential autoimmune pathogenesis of NT1, a most recent approach demonstrated that mice expressing a “neo-self-antigen” specifically in hypocretin neurons developed NT1-like symptoms after transfer of CD8<sup>+</sup> but not CD4<sup>+</sup> effector T cells specific for that antigen (Bernard-Valnet et al., 2016). However, despite the experimental utility of the NT1 models generated to date, their contributions to an elucidation of autoimmune processes operative in the human disease have been limited (Kornum et al., 2017).

## Immune Thrombocytopenic Purpura

Primary chronic immune thrombocytopenic purpura (ITP), resulting from the autoantibody-mediated targeting and destruction of platelets, is not limited to humans and has been reported for various domesticated animals such as dogs, cats, pigs, and horses; similarly, a remarkably broad range of animals (mice, rats, guinea pigs, rabbits, marmosets, swine, sheep, cattle, and horses) has served as recipients of heterologous anti-platelet serum in the so-called passive ITP model (Semple, 2010). However, out of practical, economic, and ethical considerations, ITP research has moved over the past 20 years toward mice as the primary model (Neschadim and Branch, 2015). In addition to the popular passive transfer model nowadays mostly employing monoclonal anti-platelet antibodies, “secondary ITP” models encompass various experimental scenarios wherein ITP emerges as a consequence of an underlying disease (e.g., “spontaneous” ITP in the [NZWxBXSB]F1 lupus mouse), drug therapy, infection or immunization, and “platelet-induced” ITP models make use of platelet GP-deficient mice and/or adoptive transfer of sensitized T cells. The development of a convincing humanized ITP mouse model, however, has apparently not yet been achieved (Neschadim and Branch, 2015; Semple, 2010). Overall, the available ITP models have served quite well in reproducing critical features of the human disease and therefore will continue to inform investigations into pathogenic determinants and novel treatment options.

## Giant Cell Arteritis

The estimated prevalence of giant cell arteritis (GCA), also known as temporal arteritis, seems to be on par with that of UC or CD; yet in contrast to the considerable investment into the development of animal models for IBD, *in vivo* studies of GCA appear to be limited to a single experimental strategy introduced some 20 years ago. Originally conceived by Weyand et al. (Brack et al., 1997a,b), temporal artery-SCID mouse chimeras (NOD-SCID mice subcutaneously engrafted with temporal artery specimens obtained from GCA patients) have been used in about a dozen studies investigating aspects of disease pathogenesis and/or therapeutic modalities (Weyand and Goronzy, 2013). An explanation for the lack of other GCA models certainly has to consider disease-specific constraints and challenges in developing suitable rodent models but contributing factors are probably the advanced age of the patient population and the overall excellent response to high dose corticosteroid treatment (Frohman et al., 2016).

## CONCLUSIONS

Building largely on the established concepts and hypotheses but facilitated and accelerated by remarkable technological advances, many new, refined, and improved animal models for organ-specific autoimmune diseases have been introduced in the early 21st century. Altogether, they have considerably contributed to our understanding of fundamental immunological processes in health and autoimmune disease yet the degree to which they have precipitated therapeutic progress is less clear. The reasons for this shortcoming are undoubtedly manifold, but we contend that it is above all a relative “lack of diversity” that has compromised the reproducibility, robustness, and relevance of preclinical animal studies. (1) Reproducibility: several recent publications reexamining successful strategies for T1D prevention and/or reversion in the NOD model have failed to replicate previously published results (Gill et al., 2016; Grant et al., 2013; Pham et al., 2016) and thus are symptoms for the more pervasive “reproducibility crisis” afflicting animal studies in diabetes research (Ackermann et al., 2018; van der Meulen et al., 2018) and beyond (Drucker, 2016). Confronting these challenges require nothing short of a recalibration of our research culture (Drucker, 2016; Flier, 2017) that in particular will have to incentivize independent verification of potentially significant observations by implementation of applicable experimental protocols under deliberately varied conditions (other research teams, facilities, locations, etc.). (2) Robustness: specific inbred and genetically defined mouse strains evaluated under strictly controlled environmental conditions not only offer unique research opportunities but also impose considerable constraints that limit any generalization of experimental findings to outbred populations; the deliberate diversification and extension of investigations to other strains as well as “wild” (outbred) and “dirty” (subjected to natural environmental exposures including pathogens) mice may lend much needed robustness to consequential conclusions (Abolins et al., 2017; Masopust et al., 2017; Sellers et al., 2012; Tao and Reese, 2017). (3) Relevance: while it is generally agreed upon that no single autoimmune disease model captures all relevant aspects of the human disorder, principal limitations observed in particular for murine models need to be considered more carefully (Davis, 2008; Mestas and Hughes, 2004; Zschaler et al., 2014); the translational potential of animal studies therefore increases with the degree to which experimental findings are supported by a mosaic of carefully chosen, complementary, and diversified model systems. Lastly, we need to consider the possibility that animal models may at times hamper rather than promote progress. For example, in their excellent 2007 review of mouse models for psoriasis, Gudjonsson et al. (2007) issue the lament that “lack of a suitable animal model has greatly hindered research into the pathogenesis of psoriasis”); Guttman-Yassky and Krueger (2007) on the other hand argue in a contemporaneous article that animal models may sometimes “hinder the overall translational enterprise” that, in the case of psoriasis, has brought about “rapid advances in pathogenic understanding and development of new therapeutics”. The notion that animal models can distort our perspective onto the human disease is also echoed by most recent histopathological findings in human T1D that challenge some of the central tenets about T1D pathogenesis as established in rodent models (Battaglia and Atkinson, 2015). The potential dangers of narrowing our investigative scope along the dictates of the “murine-industrial complex” are further elaborated and vividly illustrated in a journalistic essay aptly entitled “The Mouse Trap” (Engber, 2011). The specific utility, value, and importance of animal research arise only in a larger context of biomedical knowledge formation that draws on extraordinarily wide-ranging ideas, disciplines, tools, and practices and that integrates the specific contributions of animal models in an essentially cumulative and dialectical (or perhaps better dialogical) fashion (Carbone, 2012). The study of T1D and MS animal models in particular has generated an impressive wealth of data, information, and knowledge that also has illuminated the very limitations of this type of research endeavor (Wekerle et al., 2012). Moving forward from this point can neither mean “business as usual” nor a radical break with the past but perhaps a strategic “retreat from the seductions of model organisms to something more diverse—a throwback, perhaps, to the slower, more comparative style of the 19th century, when theories were constructed from the differences among the many, rather than the similarities of the few” (Engber, 2011).

## References

- Abolins, S., King, E.C., Lazarou, L., Weldon, L., Hughes, L., Drescher, P., et al., 2017. The comparative immunology of wild and laboratory mice, *Mus musculus domesticus*. *Nat. Commun.* 8, 14811.
- Ackermann, A.M., Moss, N.G., Kaestner, K.H., 2018. GABA and artesunate do not induce pancreatic alpha-to-beta cell transdifferentiation in vivo. *Cell Metab.* 28, 787–792. e783.
- Alhadj Ali, M., Liu, Y.F., Arif, S., Tatovic, D., Shariff, H., Gibson, V.B., et al., 2017. Metabolic and immune effects of immunotherapy with pro-insulin peptide in human new-onset type 1 diabetes. *Sci. Transl. Med.* 9.

- Alonzi, T., Fattori, E., Lazzaro, D., Costa, P., Probert, L., Kollias, G., et al., 1998. Interleukin 6 is required for the development of collagen-induced arthritis. *J. Exp. Med.* 187, 461–468.
- Anderson, M.S., Bluestone, J.A., 2005. The NOD mouse: a model of immune dysregulation. *Annu. Rev. Immunol.* 23, 447–485.
- Anderson, M.S., Venanzio, E.S., Klein, L., Chen, Z., Berzins, S.P., Turley, S.J., et al., 2002. Projection of an immunological self shadow within the thymus by the aire protein. *Science* 298, 1395–1401.
- Atkinson, M.A., 2014. Pancreatic biopsies in type 1 diabetes: revisiting the myth of Pandora's box. *Diabetologia* 57, 656–659.
- Atkinson, M.A., Leiter, E.H., 1999. The NOD mouse model of type 1 diabetes: as good as it gets? *Nat. Med.* 5, 601–604.
- Baker, D., Amor, S., 2015. Mouse models of multiple sclerosis: lost in translation? *Curr. Pharm. Des.* 21, 2440–2452.
- Battaglia, M., Atkinson, M.A., 2015. The streetlight effect in type 1 diabetes. *Diabetes* 64, 1081–1090.
- Bernard, C., 1865. *Introduction à l'étude de la médecine expérimentale*. J.-B. Baillière & Fils, Paris.
- Bernard-Valnet, R., Yshii, L., Queriault, C., Nguyen, X.H., Arthaud, S., Rodrigues, M., et al., 2016. CD8 T cell-mediated killing of orexinergic neurons induces a narcolepsy-like phenotype in mice. *Proc. Natl. Acad. Sci. U.S.A.* 113, 10956–10961.
- Betterle, C., Zanchetta, R., Presotto, F., 2008. Addison's disease. In: Weetman, A.P. (Ed.), *Autoimmune Diseases in Endocrinology*. Humana Press, New Jersey, pp. 303–330.
- Bluestone, J.A., Herold, K., Eisenbarth, G., 2010. Genetics, pathogenesis and clinical interventions in type 1 diabetes. *Nature* 464, 1293–1300.
- Bonifacio, E., Ziegler, A.G., Klingensmith, G., Schober, E., Bingley, P.J., Rottenkolber, M., et al., 2015. Effects of high-dose oral insulin on immune responses in children at high risk for type 1 diabetes: the Pre-POINT randomized clinical trial. *JAMA* 313, 1541–1549.
- Borges, J.L., 1942. El idioma analítico de John Wilkins. In: La Nación, Argentina. <[https://en.wikipedia.org/wiki/Celestial\\_Emporium\\_of\\_Benevolent\\_Knowledge](https://en.wikipedia.org/wiki/Celestial_Emporium_of_Benevolent_Knowledge)>.
- Box, G.E.P., 1979. Robustness in the strategy of scientific model building. In: Launer, R.L., Wilkinson, G.N. (Eds.), *Robustness in Statistics*. Academic Press, New York, pp. 201–236.
- Brack, A., Geisler, A., Martinez-Taboada, V.M., Younge, B.R., Goronzy, J.J., Weyand, C.M., 1997a. Giant cell vasculitis is a T cell-dependent disease. *Mol. Med.* 3, 530–543.
- Brack, A., Rittner, H.L., Younge, B.R., Kaltschmidt, C., Weyand, C.M., Goronzy, J.J., 1997b. Glucocorticoid-mediated repression of cytokine gene transcription in human arteritis-SCID chimeras. *J. Clin. Invest.* 99, 2842–2850.
- Brand, D.D., Latham, K.A., Rosloniec, E.F., 2007. Collagen-induced arthritis. *Nat. Protoc.* 2, 1269–1275.
- Bratland, E., Husebye, E.S., 2011. Cellular immunity and immunopathology in autoimmune Addison's disease. *Mol. Cell Endocrinol.* 336, 180–190.
- Bulek, A.M., Cole, D.K., Skowera, A., Dolton, G., Gras, S., Madura, F., et al., 2012. Structural basis for the killing of human beta cells by CD8 (+) T cells in type 1 diabetes. *Nat. Immunol.* 13, 283–289.
- Campbell-Thompson, M., Wasserfall, C., Kaddis, J., Albanese-O'Neill, A., Staeva, T., Nierras, C., et al., 2012. Network for pancreatic organ donors with diabetes (nPOD): developing a tissue biobank for type 1 diabetes. *Diabetes Metab. Res. Rev.* 28, 608–617.
- Carbone, L., 2012. The utility of basic animal research. In: Hastings Cent Rep Suppl, pp. S12–S15.
- Chaparro, R.J., Dilorenzo, T.P., 2010. An update on the use of NOD mice to study autoimmune (Type 1) diabetes. *Exp. Rev. Clin. Immunol.* 6, 939–955.
- Chassaing, B., Aitken, J.D., Malleshappa, M., Vijay-Kumar, M., 2014. Dextran sulfate sodium (DSS)-induced colitis in mice. *Curr. Protoc. Immunol.* 104, Unit15.25.
- Chatenoud, L., 2008. Animal models of type 1 diabetes mellitus. In: Weetman, A.P. (Ed.), *Contemporary Endocrinology: Autoimmune Diseases in Endocrinology*. Humana Press, Totowa, NJ, pp. 217–241.
- Chen, L., Brown, R.E., McKenna, J.T., McCarley, R.W., 2009. Animal models of narcolepsy. *CNS Neurol. Disorders Drug Targets* 8, 296–308.
- Christen, U., Hintermann, E., 2015. An update on animal models of autoimmune hepatitis: are we there yet? *Curr. Pharm. Des.* 21, 2391–2400.
- Christen, U., Hintermann, E., 2016. Immunopathogenic mechanisms of autoimmune hepatitis: how much do we know from animal models? *Int. J. Mol. Sci.* 17, 2007.
- Chu, C.Q., Wittmer, S., Dalton, D.K., 2000. Failure to suppress the expansion of the activated CD4 T cell population in interferon gamma-deficient mice leads to exacerbation of experimental autoimmune encephalomyelitis. *J. Exp. Med.* 192, 123–128.
- CMBR, 1985. What is a model? In: Morowitz, H.J. (Ed.), *Models for Biomedical Research: A New Perspective*. National Academy Press, Washington, DC, pp. 12–23.
- Cohen, I.R., Miller, A., 1994. *Autoimmune Disease Models: A Guidebook*. Academic Press, San Diego, CA.
- Concepcion, A.R., Medina, J.F., 2015. Mouse models of primary biliary cirrhosis. *Curr. Pharm. Des.* 21, 2401–2413.
- Coppiepers, K., Amirian, N., von Herrath, M., 2012a. Intravital imaging of CTLs killing islet cells in diabetic mice. *J. Clin. Invest.* 122, 119–131.
- Coppiepers, K.T., Sehested Hansen, B., von Herrath, M.G., 2012b. Clinical potential of antigen-specific therapies in type 1 diabetes. *Rev. Diabetic Stud.* 9, 328–337.
- Coppiepers, K.T., von Herrath, M.G., Homann, D., 2013. Autoimmunity and autoimmune diseases. In: Paul, W. (Ed.), *Fundamental Immunology*. Lippincott Williams & Wilkins, Philadelphia, PA.
- Costes, L.M., Meresse, B., Cerf-Bensussan, N., Samsom, J.N., 2015. The role of animal models in unravelling therapeutic targets in coeliac disease. *Best Pract. Res. Clin. Gastroenterol.* 29, 437–450.
- Czaja, A.J., 2010. Animal models of autoimmune hepatitis. *Expert Rev. Gastroenterol. Hepatol.* 4, 429–443.
- Davis, M.M., 2008. A prescription for human immunology. *Immunity* 29, 835–838.
- Denic, A., Johnson, A.J., Bieber, A.J., Warrington, A.E., Rodriguez, M., Pirko, I., 2011. The relevance of animal models in multiple sclerosis research. *Pathophysiology* 18, 21–29.
- Driver, J.P., Serreze, D.V., Chen, Y.G., 2011. Mouse models for the study of autoimmune type 1 diabetes: a NOD to similarities and differences to human disease. *Semin. Immunopathol.* 33, 67–87.
- Driver, J.P., Chen, Y.G., Mathews, C.E., 2012. Comparative genetics: synergizing human and NOD mouse studies for identifying genetic causation of type 1 diabetes. *Rev. Diabetic Stud.* 9, 169–187.
- Drucker, D.J., 2016. Never waste a good crisis: Confronting reproducibility in translational research. *Cell Metab.* 24, 348–360.
- Engber, D., 2011. The mouse trap. In: Slate. <[http://www.slate.com/articles/health\\_and\\_science/the\\_mouse\\_trap/2011/11/the\\_mouse\\_trap.html](http://www.slate.com/articles/health_and_science/the_mouse_trap/2011/11/the_mouse_trap.html)>.

- Erf, G.F., 2010. Animal models. In: Picardo, M., Taieb, A. (Eds.), *Vitiligo*. Springer, Heidelberg, pp. 205–218.
- Eri, R., McGuckin, M.A., Wadley, R., 2012. T cell transfer model of colitis: a great tool to assess the contribution of T cells in chronic intestinal inflammation. *Methods Mol. Biol.* 844, 261–275.
- Ericsson, A.C., Crim, M.J., Franklin, C.L., 2013. A brief history of animal modeling. *Mol. Med.* 110, 201–205.
- Essien, K.I., Harris, J.E., 2014. Animal models of vitiligo: matching the model to the question. *Dermatol. Sin.* 32, 240–247.
- Fambrough, D.M., Drachman, D.B., Satyamurti, S., 1973. Neuromuscular junction in myasthenia gravis: decreased acetylcholine receptors. *Science* 182, 293–295.
- Field, J., Biondo, M.A., Murphy, K., Alderuccio, F., Toh, B.H., 2005. Experimental autoimmune gastritis: mouse models of human organ-specific autoimmune disease. *Int. Rev. Immunol.* 24, 93–110.
- Flier, J.S., 2017. Irreproducibility of published bioscience research: diagnosis, pathogenesis and therapy. *Mol. Metab.* 6, 2–9.
- Franco, N.H., 2013. Animal experiments in biomedical research: a historical perspective. *Animals (Basel)* 3, 238–273.
- Frohman, L., Wong, A.B., Matheos, K., Leon-Alvarado, L.G., Danesh-Meyer, H.V., 2016. New developments in giant cell arteritis. *Surv. Ophthalmol.* 61, 400–421.
- Gale, E.A., 2001. The discovery of type 1 diabetes. *Diabetes* 50, 217–226.
- Gianani, R., Campbell-Thompson, M., Sarkar, S.A., Wasserfall, C., Pugliese, A., Solis, J.M., et al., 2010. Dimorphic histopathology of long-standing childhood-onset diabetes. *Diabetologia* 53, 690–698.
- Gilhar, A., Schrum, A.G., Etzioni, A., Waldmann, H., Paus, R., 2016. Alopecia areata: animal models illuminate autoimmune pathogenesis and novel immunotherapeutic strategies. *Autoimmun. Rev.* 15, 726–735.
- Gill, R.G., Pagni, P.P., Kupfer, T., Wasserfall, C.H., Deng, S., Posgai, A., et al., 2016. A preclinical consortium approach for assessing the efficacy of combined anti-CD3 plus IL-1 blockade in reversing new-onset autoimmune diabetes in NOD mice. *Diabetes* 65, 1310–1316.
- Graham, M.L., Schuurman, H.J., 2015. Validity of animal models of type 1 diabetes, and strategies to enhance their utility in translational research. *Eur. J. Pharmacol.* 759, 221–230.
- Grant, C.W., Moran-Paul, C.M., Duclos, S.K., Guberski, D.L., Arreaza-Rubin, G., Spain, L.M., 2013. Testing agents for prevention or reversal of type 1 diabetes in rodents. *PLoS One* 8, e72989.
- Greenbaum, C.J., 2017. Type 1 Diabetes TrialNetOral Insulin Trial. ADA 77th Scientific Sessions.
- Gudjonsson, J.E., Johnston, A., Dyson, M., Valdimarsson, H., Elder, J.T., 2007. Mouse models of psoriasis. *J. Invest. Dermatol.* 127, 1292–1308.
- Guttman-Yassky, E., Krueger, J.G., 2007. Psoriasis: evolution of pathogenic concepts and new therapies through phases of translational research. *Br. J. Dermatol.* 157, 1103–1115.
- Hafler, D.A., Duby, A.D., Lee, S.J., Benjamin, D., Seidman, J.G., Weiner, H.L., 1988. Oligoclonal T lymphocytes in the cerebrospinal fluid of patients with multiple sclerosis. *J. Exp. Med.* 167, 1313–1322.
- Han, B., Serra, P., Amrani, A., Yamanouchi, J., Maree, A.F., Edelstein-Keshet, L., et al., 2005. Prevention of diabetes by manipulation of anti-IGRP autoimmunity: high efficiency of a low-affinity peptide. *Nat. Med.* 11, 645–652.
- Hardtke-Wolenski, M., Taubert, R., Jaeckel, E., 2012. Animal models for autoimmune liver disease—what is relevant for immune-mediated liver disease. *Dig. Dis.* 30 (Suppl. 1), 2–10.
- Harris, R.F., 2017. *Rigor Mortis: How Sloppy Science Creates Worthless Cures, Crushes Hope, and Wastes Billions*. Basic Books, an imprint of Perseus Books, LLC, a subsidiary of Hachette Book Group, Inc., New York.
- Hau, J., 2003. Animal models. In: Hau, J., Van Hoosier Jr, G.L. (Eds.), *Handbook of Laboratory Animal Science*, pp. 1–9.
- Hayter, S.M., Cook, M.C., 2012. Updated assessment of the prevalence, spectrum and case definition of autoimmune disease. *Autoimmun. Rev.* 11, 754–765.
- Held, J.R., 1980. Muhlbock Memorial Lecture: considerations in the provision and characterization of animal models. In: Spiegel, A., Erichson, S., Solleveld, H.A. (Eds.), *Animal Quality and Models in Biomedical Research*, 7th ICLAS Symposium Utrecht 1979, Gustav Fisher Verlag.
- Hori, S., Nomura, T., Sakaguchi, S., 2003. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 299, 1057–1061.
- Jamwal, S., Kumar, P., 2017. Animal models of inflammatory bowel disease. In: Conn, M.P. (Ed.), *Animal Models for the Study of Human Disease*. Academic Press, San Diego, CA, pp. 467–480.
- Jiminez, J.A., Uwiera, T.C., Douglas Inglis, G., Uwiera, R.R., 2015. Animal models to study acute and chronic intestinal inflammation in mammals. *Gut Pathog.* 7, 29.
- Kaddis, J.S., Pugliese, A., Atkinson, M.A., 2015. A run on the biobank: what have we learned about type 1 diabetes from the nPOD tissue repository? *Curr. Opin. Endocrinol. Diabetes Obes.* 22, 290–295.
- Karlsson, F.A., Burman, P., Loof, L., Mardh, S., 1988. Major parietal cell antigen in autoimmune gastritis with pernicious anemia is the acid-producing H<sup>+</sup>,K<sup>+</sup>-adenosine triphosphatase of the stomach. *J. Clin. Invest.* 81, 475–479.
- Katsumi, T., Tomita, K., Leung, P.S., Yang, G.X., Gershwin, M.E., Ueno, Y., 2015. Animal models of primary biliary cirrhosis. *Clin. Rev. Allergy Immunol.* 48, 142–153.
- Kent, S.C., Chen, Y., Bregoli, L., Clemmings, S.M., Kenyon, N.S., Ricordi, C., et al., 2005. Expanded T cells from pancreatic lymph nodes of type 1 diabetic subjects recognize an insulin epitope. *Nature* 435, 224–228.
- Kiesler, P., Fuss, I.J., Strober, W., 2015. Experimental models of inflammatory bowel diseases. *Cell Mol. Gastroenterol. Hepatol.* 1, 154–170.
- King, A.J., 2012. The use of animal models in diabetes research. *Br. J. Pharmacol.* 166, 877–894.
- King, A., Austin, A., 2017. Animal models of type 1 and type 2 diabetes mellitus. In: Conn, M.P. (Ed.), *Animal Models for the Study of Human Disease*. Academic Press, San Diego, CA, pp. 245–265.
- King, C., Sarvetnick, N., 2011. The incidence of type-1 diabetes in NOD mice is modulated by restricted flora not germ-free conditions. *PLoS One* 6, e17049.
- Kipp, M., Nyamoya, S., Hochstrasser, T., Amor, S., 2017. Multiple sclerosis animal models: a clinical and histopathological perspective. *Brain Pathol.* 27, 123–137.
- Kong, Y.C., Morris, G.P., Brown, N.K., Yan, Y., Flynn, J.C., David, C.S., 2009. Autoimmune thyroiditis: a model uniquely suited to probe regulatory T cell function. *J. Autoimmun.* 33, 239–246.
- Korneychuk, N., Meresse, B., Cerf-Bensussan, N., 2015. Lessons from rodent models in celiac disease. *Mucosal Immunol.* 8, 18–28.
- Kornum, B.R., Knudsen, S., Ollila, H.M., Pizza, F., Jenum, P.J., Dauvilliers, Y., et al., 2017. Narcolepsy. *Nat. Rev. Dis. Primers* 3, 16100.

- Krogvold, L., Edwin, B., Buanes, T., Ludvigsson, J., Korsgren, O., Hyoty, H., et al., 2014. Pancreatic biopsy by minimal tail resection in live adult patients at the onset of type 1 diabetes: experiences from the DiViD study. *Diabetologia* 57, 841–843.
- Kuhn, R., Lohler, J., Rennick, D., Rajewsky, K., Muller, W., 1993. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 75, 263–274.
- Lenschow, D.J., Ho, S.C., Sattar, H., Rhee, L., Gray, G., Nabavi, N., et al., 1995. Differential effects of anti-B7-1 and anti-B7-2 monoclonal antibody treatment on the development of diabetes in the nonobese diabetic mouse. *J. Exp. Med.* 181, 1145–1155.
- Lenzen, S., 2008. The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia* 51, 216–226.
- Lenzen, S., 2017. Animal models of human type 1 diabetes for evaluating combination therapies and successful translation to the patient with type 1 diabetes. *Diabetes Metab. Res. Rev.* 33.
- Leung, P.S.C., Yang, G.X., Dhirapong, A., Tsuneyama, K., Ridgway, W.M., Gershwin, M.E., 2012. Animal models of primary biliary cirrhosis: materials and methods. In: Perl, A. (Ed.), *Autoimmunity Methods in Molecular Biology (Methods and Protocols)*. Humana Press, Totowa, NJ, pp. 291–316.
- Lin, M.S., Swartz, S.J., Lopez, A., Ding, X., Fernandez-Vina, M.A., Stastny, P., et al., 1997. Development and characterization of desmoglein-3 specific T cells from patients with pemphigus vulgaris. *J. Clin. Invest.* 99, 31–40.
- Lohse, A.W., Meyer zum Büschenfelde, K.-H., 1994. Experimental hepatitis. In: Cohen, I.R., Miller, A. (Eds.), *Autoimmune Disease Models*. Academic Press, Inc, San Diego, CA, pp. 191–199.
- Ludgate, M., 2008. Animal models of autoimmune thyroid disease. In: Weetman, A.P. (Ed.), *Contemporary Endocrinology: Autoimmune Diseases in Endocrinology*. Humana Press, Totowa, NJ, pp. 79–93.
- Luross, J.A., Williams, N.A., 2001. The genetic and immunopathological processes underlying collagen-induced arthritis. *Immunology* 103, 407–416.
- Mackay, I.R., 2010. Travels and travails of autoimmunity: a historical journey from discovery to rediscovery. *Autoimmun. Rev.* 9, A251–258.
- Malfait, A.M., Williams, R.O., Malik, A.S., Maini, R.N., Feldmann, M., 2001. Chronic relapsing homologous collagen-induced arthritis in DBA/1 mice as a model for testing disease-modifying and remission-inducing therapies. *Arthritis Rheum.* 44, 1215–1224.
- Marietta, E.V., Murray, J.A., 2012. Animal models to study gluten sensitivity. *Semin. Immunopathol.* 34, 497–511.
- Marietta, E.V., Rashtak, S., Pittelkow, M.R., 2012. Experiences with animal models of dermatitis herpetiformis: a review. *Autoimmunity* 45, 81–90.
- Masopust, D., Sivula, C.P., Jameson, S.C., 2017. Of mice, dirty mice, and men: using mice to understand human immunology. *J. Immunol.* 199, 383–388.
- McLachlan, S.M., Nagayama, Y., Rapoport, B., 2005. Insight into Graves' hyperthyroidism from animal models. *Endocr. Rev.* 26, 800–832.
- Mestas, J., Hughes, C.C., 2004. Of mice and not men: differences between mouse and human immunology. *J. Immunol.* 172, 2731–2738.
- Mitchell, A.L., Pearce, S.H., 2012. Autoimmune Addison disease: pathophysiology and genetic complexity. *Nat. Rev. Endocrinol.* 8, 306–316.
- Mix, E., Meyer-Rienecker, H., Hartung, H.P., Zettl, U.K., 2010. Animal models of multiple sclerosis—potentials and limitations. *Prog. Neurobiol.* 92, 386–404.
- Morel, L., 2004. Mouse models of human autoimmune diseases: essential tools that require the proper controls. *PLoS Biol.* 2, E241.
- Morgan, N.G., Leete, P., Foulis, A.K., Richardson, S.J., 2014. Islet inflammation in human type 1 diabetes mellitus. *IUBMB Life* 66, 723–734.
- Moshkelgosha, S., So, P.W., Diaz-Cano, S., Banga, J.P., 2015. Preclinical models of Graves' disease and associated secondary complications. *Curr. Pharm. Des.* 21, 2414–2421.
- Nagayama, Y., Abiru, N., 2011. Animal models of autoimmune thyroid disease. In: Eisenbarth, G.S. (Ed.), *Immunoendocrinology: Scientific and Clinical Aspects*. Humana Press, pp. 415–426.
- Nagayama, Y., Nakahara, M., Abiru, N., 2015. Animal models of Graves' disease and Graves' orbitopathy. *Curr. Opin. Endocrinol. Diabetes Obes.* 22, 381–386.
- Nakayama, M., Abiru, N., Moriyama, H., Babaya, N., Liu, E., Miao, D., et al., 2005. Prime role for an insulin epitope in the development of type 1 diabetes in NOD mice. *Nature* 435, 220–223.
- Neschadim, A., Branch, D.R., 2015. Mouse models of autoimmune diseases: immune thrombocytopenia. *Curr. Pharm. Des.* 21, 2487–2497.
- Ohashi, P.S., Oehen, S., Buerki, K., Pircher, H., Ohashi, C.T., Odermatt, B., et al., 1991. Ablation of “tolerance” and induction of diabetes by virus infection in viral antigen transgenic mice. *Cell* 65, 305–317.
- O'Kell, A.L., Wasserfall, C., Catchpole, B., Davison, L.J., Hess, R.S., Kushner, J.A., et al., 2017. Comparative pathogenesis of autoimmune diabetes in humans, NOD mice, and canines: has a valuable animal model of type 1 diabetes been overlooked? *Diabetes* 66, 1443–1452.
- Oldstone, M.B., Nerenberg, M., Southern, P., Price, J., Lewicki, H., 1991. Virus infection triggers insulin-dependent diabetes mellitus in a transgenic model: role of anti-self (virus) immune response. *Cell* 65, 319–331.
- Orban, T., Bundy, B., Becker, D.J., DiMeglio, L.A., Gitelman, S.E., Goland, R., et al., 2011. Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial. *Lancet* 378, 412–419.
- Paun, A., Yau, C., Danska, J.S., 2017. The influence of the microbiome on type 1 diabetes. *J. Immunol.* 198, 590–595.
- Pearson, J.A., Wong, F.S., Wen, L., 2016. The importance of the non obese diabetic (NOD) mouse model in autoimmune diabetes. *J. Autoimmun.* 66, 76–88.
- Pham, M.N., Gibson, C., Ryden, A.K., Perdue, N., Boursalian, T.E., Pagni, P.P., et al., 2016. Oral insulin (human, murine, or porcine) does not prevent diabetes in the non-obese diabetic mouse. *Clin. Immunol.* 164, 28–33.
- Pietropaolo, M., Eisenbarth, G.S., 2001. Autoantibodies in human diabetes. *Curr. Dir. Autoimmun.* 4, 252–282.
- Pollheimer, M.J., Fickert, P., 2015. Animal models in primary biliary cirrhosis and primary sclerosing cholangitis. *Clin. Rev. Allergy Immunol.* 48, 207–217.
- Pollmann, R., Eming, R., 2017. Research techniques made simple: mouse models of autoimmune blistering diseases. *J. Invest. Dermatol.* 137, e1–e6.
- Procaccini, C., De Rosa, V., Pucino, V., Formisano, L., Matarese, G., 2015. Animal models of multiple sclerosis. *Eur. J. Pharmacol.* 759, 182–191.
- Ransohoff, R.M., 2012. Animal models of multiple sclerosis: the good, the bad and the bottom line. *Nat. Neurosci.* 15, 1074–1077.
- Reed, J.C., Herold, K.C., 2015. Thinking bedside at the bench: the NOD mouse model of T1DM. *Nat. Rev. Endocrinol.* 11, 308–314.
- Richardson, S.J., Morgan, N.G., Foulis, A.K., 2014. Pancreatic pathology in type 1 diabetes mellitus. *Endocr. Pathol.* 25, 80–92.
- Roep, B.O., 2007. Are insights gained from NOD mice sufficient to guide clinical translation? Another inconvenient truth. *Ann. N. Y. Acad. Sci.* 1103, 1–10.

- Roep, B.O., Atkinson, M., von Herrath, M., 2004. Satisfaction (not) guaranteed: re-evaluating the use of animal models of type 1 diabetes. *Nat. Rev. Immunol.* 4, 989–997.
- Roep, B.O., Solvason, N., Gottlieb, P.A., Abreu, J.R.F., Harrison, L.C., Eisenbarth, G.S., et al., 2013. Plasmid-encoded proinsulin preserves C-peptide while specifically reducing proinsulin-specific CD8(+) T cells in type 1 diabetes. *Sci. Transl. Med.* 5, 191ra182.
- Roitt, I.M., Doniach, D., Campbell, P.N., Hudson, R.V., 1956. Auto-antibodies in Hashimoto's disease (lymphadenoid goitre). *Lancet* 271, 820–821.
- Rose, N.R., Witebsky, E., 1956. Studies on organ specificity. V. Changes in the thyroid glands of rabbits following active immunization with rabbit thyroid extracts. *J. Immunol.* 76, 417–427.
- Sakurai, T., 2015. Animal models of narcolepsy. In: Sakurai, T., Pandi-Perumal, S.R., Monti, J.M. (Eds.), *Orexin and Sleep*. Springer, pp. 203–212.
- Sellers, R.S., Clifford, C.B., Treuting, P.M., Brayton, C., 2012. Immunological variation between inbred laboratory mouse strains: points to consider in phenotyping genetically immunomodified mice. *Vet. Pathol.* 49, 32–43.
- Semple, J.W., 2010. Animal models of immune thrombocytopenia (ITP). *Ann. Hematol.* 89 (Suppl. 1), 37–44.
- Serreze, D.V., Niens, M., Kulik, J., DiLorenzo, T.P., 2016. Bridging mice to men: using HLA transgenic mice to enhance the future prediction and prevention of autoimmune type 1 diabetes in humans. *Methods Mol. Biol.* 1438, 137–151.
- Shoda, L.K., Young, D.L., Ramanujan, S., Whiting, C.C., Atkinson, M.A., Bluestone, J.A., et al., 2005. A comprehensive review of interventions in the NOD mouse and implications for translation. *Immunity* 23, 115–126.
- Silverstein, A.M., 2009. *A History of Immunology*, second ed. Academic Press, New York.
- Sinton, C.M., 2010. Animal models of narcolepsy: development, findings and perspectives. In: Goswami, M., Thorpy, M.J., Pandi-Perumal, S.R. (Eds.), *Narcolepsy: A Clinical Guide*. Humana Press, pp. 23–37.
- Skowera, A., Ellis, R.J., Varela-Calvino, R., Arif, S., Huang, G.C., Van-Krinks, C., et al., 2008. CTLs are targeted to kill beta cells in patients with type 1 diabetes through recognition of a glucose-regulated preproinsulin epitope. *J. Clin. Invest.* 118, 3390–3402.
- Stassi, G., De Maria, R., 2002. Autoimmune thyroid disease: new models of cell death in autoimmunity. *Nat. Rev. Immunol.* 2, 195–204.
- Stimmer, L., Fovet, C.M., Serguera, C., 2018. Experimental models of autoimmune demyelinating diseases in nonhuman primates. *Vet. Pathol.* 55 (1), 27–41. Available from: <http://dx.doi.org/10.1177/0300985817712794>.
- Sundberg, J.P., McElwee, K., Brehm, M.A., Su, L., King Jr., L.E., 2015. Animal models for alopecia areata: what and where? *J. Investig. Dermatol. Symp. Proc.* 17, 23–26.
- Taneja, V., David, C.S., 2001. Lessons from animal models for human autoimmune diseases. *Nat. Immunol.* 2, 781–784.
- Tannenbaum, J., 2017. Ethics in biomedical animal research: the key role of the investigator. *Animal Models for the Study of Human Disease*. Academic Press, San Diego, CA, pp. 3–46.
- Tannenbaum, J., Bennett, B.T., 2015. Russell and Burch's 3Rs then and now: the need for clarity in definition and purpose. *J. Am. Assoc. Lab. Anim. Sci.* 54, 120–132.
- Tao, L., Reese, T.A., 2017. Making mouse models that reflect human immune responses. *Trends Immunol.* 38, 181–193.
- Tochino, Y., 1987. The NOD mouse as a model of type I diabetes. *Crit. Rev. Immunol.* 8, 49–81.
- Toh, B.H., Chan, J., Kyaw, T., Alderuccio, F., 2012. Cutting edge issues in autoimmune gastritis. *Clin. Rev. Allergy Immunol.* 42, 269–278.
- Toth, L.A., Bhargava, P., 2013. Animal models of sleep disorders. *Comp. Med.* 63, 91–104.
- Van Belle, T.L., Taylor, P., von Herrath, M.G., 2009. Mouse models for type 1 diabetes. *Drug Discov. Today Dis. Models* 6, 41–45.
- van der Meulen, T., Lee, S., Noordeloos, E., Donaldson, C.J., Adams, M.W., Noguchi, G.M., et al., 2018. Artemether does not turn alpha cells into beta cells. *Cell Metab.* 27, 218–225. e214.
- van Driel, I.R., Tu, E., Gleeson, P.A., 2014. Autoimmune gastritis and pernicious anemia. In: Rose, N.R., Mackay, C.R. (Eds.), *The Autoimmune Diseases*. Academic Press, pp. 619–631.
- Verdu, E.F., Galipeau, H.J., Jabri, B., 2015. Novel players in coeliac disease pathogenesis: role of the gut microbiota. *Nat. Rev. Gastroenterol. Hepatol.* 12, 497–506.
- von Herrath, M., Nepom, G.T., 2009. Animal models of human type 1 diabetes. *Nat. Immunol.* 10, 129–132.
- von Herrath, M.G., Dockter, J., Oldstone, M.B., 1994. How virus induces a rapid or slow onset insulin-dependent diabetes mellitus in a transgenic model. *Immunity* 1, 231–242.
- von Herrath, M., Filippi, C., Coppieters, K., 2011. How viral infections enhance or prevent type 1 diabetes—from mouse to man. *J. Med. Virol.* 83, 1672.
- Wang, J., Yang, G.X., Tsuneyama, K., Gershwin, M.E., Ridgway, W.M., Leung, P.S., 2014. Animal models of primary biliary cirrhosis. *Semin. Liver Dis.* 34, 285–296.
- Wekerle, H., Flugel, A., Fugger, L., Schett, G., Serreze, D., 2012. Autoimmunity's next top models. *Nat. Med.* 18, 66–70.
- Wen, L., Ley, R.E., Volchkov, P.Y., Stranges, P.B., Avanesyan, L., Stonebraker, A.C., et al., 2008. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. *Nature* 455, 1109–1113.
- Wessler, S., 1976. Introduction: what is a model? *Animal Models of Thrombosis and Hemorrhagic Diseases*. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Washington, DC, pp. xi–xvi.
- Weyand, C.M., Goronzy, J.J., 2013. Immune mechanisms in medium and large-vessel vasculitis. *Nat. Rev. Rheumatol.* 9, 731–740.
- Whalen, B.J., Mordes, J.P., Rossini, A.A., 2001. The BB rat as a model of human insulin-dependent diabetes mellitus. *Curr. Protoc. Immunol.* Chapter 15, Unit 15.13.
- Whipple, G.H., Hooper, C.W., Robscheidt, F.S., 1920. Blood regeneration following anemia. *Am. J. Physiol.* 53, 236–262.
- Wiesweg, B., Johnson, K.T., Eckstein, A.K., Berchner-Pfannschmidt, U., 2013. Current insights into animal models of Graves' disease and orbitopathy. *Horm. Metab. Res.* 45, 549–555.
- Williams, R., 2010. Autoimmune disease: animal models. *Encyclopedia of Life Sciences (ELS)*. John Wiley & Sons, Ltd, Chichester.
- Williams, R.O., Feldmann, M., Maini, R.N., 1992. Anti-tumor necrosis factor ameliorates joint disease in murine collagen-induced arthritis. *Proc. Natl. Acad. Sci. U.S.A.* 89, 9784–9788.

- Witebsky, E., Rose, N.R., Terplan, K., Paine, J.R., Egan, R.W., 1957. Chronic thyroiditis and autoimmunization. *J. Am. Med. Assoc.* 164, 1439–1447.
- Yeung, W.C., Rawlinson, W.D., Craig, M.E., 2011. Enterovirus infection and type 1 diabetes mellitus: systematic review and meta-analysis of observational molecular studies. *BMJ* 342, d35.
- Yu, X., Huang, Q., Petersen, F., 2015. History and milestones of mouse models of autoimmune diseases. *Curr. Pharm. Des.* 21, 2308–2319.
- Yuksel, M., Laukens, D., Heindryckx, F., Van Vlierberghe, H., Geerts, A., Wong, F.S., et al., 2014. Hepatitis mouse models: from acute-to-chronic autoimmune hepatitis. *Int. J. Exp. Pathol.* 95, 309–320.
- Zschaler, J., Schlorke, D., Arnhold, J., 2014. Differences in innate immune response between man and mouse. *Crit. Rev. Immunol.* 34, 433–454.

# Autoimmunity in Primary Immunodeficiency Disorders

Maleewan Kitcharoensakkul and Megan A. Cooper

Department of Pediatrics, Divisions of Rheumatology and Allergy, Immunology, and Pulmonary Medicine,  
Washington University School of Medicine, St. Louis, MO, United States

## OUTLINE

<b>Introduction</b>	513	<i>Monogenic Defects Affecting T-Cell Selection and Homeostasis</i>	522
<b>Immunodeficiencies Affecting Cellular and Humoral Immunity</b>	515	<i>Disorders of Regulatory T Cells</i>	524
Severe Combined Immunodeficiency	515	<b>Gain-of-Function Disorders of Cytokine Signaling</b>	525
Combined Immune Deficiencies	516		
Combined Immunodeficiencies With Syndromic Features	518	<b>Innate Immune Defects</b>	526
<b>Predominantly Antibody Deficiencies</b>	520	<b>Conclusion</b>	527
<b>Disorders of Immune Dysregulation: T-Cell Tolerance</b>	522	<b>References</b>	527

## INTRODUCTION

There are more than 300 distinct primary immunodeficiencies, the majority being defined by single-gene defects (Picard et al., 2015). With an increased identification of primary immunodeficiencies through genomic sequencing, it is clear that many primary immunodeficiencies are characterized not only by susceptibility to infection but also by dysregulation of the immune response manifesting as autoimmunity. With improved prevention of infectious complications, even well-characterized “classic” primary immunodeficiencies such as T and B-cell deficiencies are recognized to have immune dysregulation. The clinical findings together with delineation of the genetic basis for many of these “experiments of nature” have led to an expanded understanding of various pathways involved in immune homeostasis and the maintenance of self-tolerance. In this chapter the major primary immunodeficiency disorders associated with immune dysregulation and autoimmunity are presented (Table 28.1), paying particular attention to the mechanisms by which defects in different arms of the immune system cause autoimmunity. Immunodeficiencies presented here are classified based on the primary immunologic defect in each disorder, for the most part using classifications proposed by the Primary Immunodeficiency Expert Committee of the International Union of Immunological Societies (Picard et al., 2015 and [www.iuisonline.org](http://www.iuisonline.org)), with a focus on primary immunodeficiencies with infectious susceptibility and/or

**TABLE 28.1** Autoimmune Clinical Manifestations by Organ System of Select Primary Immunodeficiency Syndromes<sup>a</sup>

Gene	Disease name	AI cytopenias	Skin disease	Enteropathy	Lung	Endocrinopathy	Arthritis	Other
<b>SCID</b>								
RAG1, RAG2	Omenn	++	++++	+++	-	+	-	++
PNP	SCID	+++	-	-	-	-	-	-
<b>CIDS</b>								
CD40L	XL HIGM	+++	++	++	-	++	++	++
ICOS	ICOS	+	++	+++	+	-	++	++
PIK3CD, PIK3R1	APDS	++	+	++	+++	++	++	++
22q11.2	DiGeorge	++	++	+	-	++	+	+
WAS	WAS	+++	++++	+	-	-	++	++
STIM1	STIM1 def	++++	-	+	-	-	-	-
STK4	MST1 def	+++	-	-	-	-	-	-
<b>PREDOMINANTLY ANTIBODY DEFICIENCIES</b>								
BTK	XLA	++	+	++	-	-	++	-
Multiple/unknown	CVID	++	++	++	++	++	++	++
<b>DISORDERS OF IMMUNE DYSREGULATION</b>								
AIRE	APECED	-	-	-	++	++++	-	+++
FAS, FASL, CASP10	ALPS	+++	-	-	-	-	-	++
FOXP3	IPEX	++	+++	+++	-	+++	+	-
STAT5B	STAT5B LOF	++	++	+	+++	++	+	-
LRBA	LATAIE	++	+	++	++	+	++	+
CTLA4	CHAI	++	-	+++	++	++	++	+
<b>GOF DISORDERS OF CYTOKINE SIGNALING</b>								
STAT1	STAT1 GOF	++	++	+	-	++	-	+
STAT3	STAT3 GOF	+++	++	++	++	++	++	++
<b>INNATE IMMUNE DEFECTS</b>								
Multiple	CGD	+	-	+++	-	-	+	+

<sup>a</sup>Primary immunodeficiency syndromes are classified based on their major immunologic defect, however there is significant overlap in the mechanisms of each, and most syndromes are not strictly limited to one type of cellular defect.

AI, Autoimmune; HIGM, hyper-IgM; WAS, Wiskott–Aldrich syndrome; XLA, x-linked agammaglobulinemia; CVID, common variable immunodeficiency; APECED, autoimmune polyendocrinopathy, candidiasis, ectodermal dysplasia; ALPS, autoimmune lymphoproliferative syndrome; IPEX, immunodysregulation, polyendocrinopathy, enteropathy, x-linked; LRBA, lipopolysaccharide-responsive beige-like anchor; LATAIE, LRBA deficiency with autoantibodies; CHAI, CTLA4 haploinsufficiency with autoimmune infiltration; LOF, loss-of-function; GOF, gain-of-function; CGD, chronic granulomatous disease; LAD, leukocyte adhesion deficiency; SCID, severe combined immunodeficiency; PNP, purine nucleoside phosphorylase; RAG, recombinase-activating genes; AIRE, autoimmune regulator; CIDS, combined immune deficiencies; CD40L, CD40 ligand; STK4, serine-threonine protein kinase; ICOS, inducible T-cell costimulator; APDS, activated phosphoinositide-3-kinase δ syndrome; STIM, stromal interaction molecule; BTK, Bruton's tyrosine kinase; FOXP3, forkhead box P3 protein; FASL, FAS ligand; STAT, signal transducer and activator of transcription. Other includes vasculitis, uveitis, hepatitis, systemic lupus erythematosus, and others. +++, a key clinical feature seen in most patients; ++, a major feature (>30%); ++, observed in <30% of the patients; +, <5%; -, not a feature of disease.

dysregulated T lymphocytes. In addition to the primary immunodeficiencies presented here, there are a variety of other monogenic disorders of the immune response whose sole and/or major clinical manifestations are autoimmune or autoinflammatory, including primary autoinflammatory disorders such as the periodic fever syndromes, early-onset inflammatory bowel disease (e.g., IL-10 pathway defects), early complement disorders associated primarily with autoimmunity risk and not infection (i.e., C1q, C1r/C1s, C4, or C2), and interferonopathies such as TREX1-associated disease, which are reviewed in detail in this text and elsewhere.

## IMMUNODEFICIENCIES AFFECTING CELLULAR AND HUMORAL IMMUNITY

This group of immunodeficiencies includes diseases with significant T-cell dysfunction, due to either defects in T-cell differentiation or function. There has been an increasing appreciation for the association of T-cell developmental defects producing a combination of immune deficiency and dysregulation. In addition to intrinsic defects in T-cell function, impaired T-cell cross talk and interactions with other components of the immune system, particularly B cell or humoral immunity, lead to more global immune defects contributing to autoimmunity and immune dysregulation.

### Severe Combined Immunodeficiency

Severe combined immunodeficiency (SCID) is a group of genetic defects characterized by severe defects in T-cell numbers and immunity. Patients with typical SCID have severe deficiency of naïve T cells with varied effects on B cells and natural killer (NK) cells depending on the genetic defect. The resulting immune deficiency is of such severity that without hematopoietic cell transplantation (HCT) early in life, patients have a high likelihood of developing failure to thrive and recurrent opportunistic infections that result in death within the first 2 years of life. In the United States, the initiation of newborn screening for SCID has dramatically changed the way that this disease is detected and treated, and infants are typically identified within the first 2 weeks of life and treated with HCT within the first few months of life ([Dorsey et al., 2017](#)).

Patients with SCID not detected in the newborn period typically present with severe and recurrent respiratory and gastrointestinal (GI) infection before 6 months of age. Despite the characteristic lack of T-cell immunity, autoimmunity and immune dysregulation can also be a clinical features of SCID. The immune dysregulation in SCID patients typically occurs due to hypomorphic mutations of genetic defects causing SCID associated with residual, but dysregulated, T-cell function such as Omenn syndrome (OS) ([Somech et al., 2009; Cassani et al., 2010](#)). Autoimmunity and immune dysregulation can also be seen with complete loss-of-function (LOF) of certain SCID-associated genes as described in adenosine deaminase (ADA), purine nucleoside phosphorylase (PNP), and zeta chain-associated protein kinase 70 (ZAP70) genes.

OS is a description for patients with different genetic forms of SCID presenting with opportunistic infections and generalized erythroderma, alopecia, failure to thrive, autoimmunity, particularly hepatitis and enteritis resembling graft-versus-host disease (GVHD). Additional findings include hepatosplenomegaly, lymphadenopathy, increased serum IgE, lymphocytosis, and eosinophilia suggesting an involvement of a Th2 polarized immune response. The age of onset is usually in the first weeks of life. OS is classically caused by hypomorphic mutations in the recombinase-activating genes 1 and 2 (*RAG1* and *RAG2*) ([Marrella et al., 2011](#)) associated with classic T-/B-/NK + SCID. *RAG1* and *RAG2* proteins are the key enzymes in the V(D)J recombination process, important for the assembly and diversity of T- and B-cell antigen receptors. The phenotypes of patients with RAG deficiencies depend principally on the residual quantity of V(D)J activity ([Niehues et al., 2010](#)). Patients with OS have residual V(D)J recombination and, unlike classical SCID, have significant numbers of circulating T cells due to clonal expansions of T cells with restricted T-cell receptor (TCR) repertoire. The circulating cells are highly activated based on CD45RO and HLA-DR expression, and they are primarily autoreactive and prove to be incapable of providing appropriate T-cell responses to microbial pathogens. The genetic defects associated with OS also include mutations in other SCID-associated genes encoding other proteins including Artemis, DNA ligase IV, common-gamma chain, IL-7 receptor alpha chain, ADA, ZAP70, and the ribonuclease mitochondrial RNA processing complex ([Villa et al., 2008](#)). The association of *RAG1/2* and other SCID genotypes with an altered clinical phenotype sets the stage for investigations that have uncovered further expansion of the clinical phenotypes linked to hypomorphic or leaky SCID. The range of findings appears to be linked to the level of protein function with less severe phenotypes in the aspect of infectious susceptibility associated with mutations that result in residual protein activity. Syndromic disorders with T-cell defects such as DiGeorge syndrome (DGS) and coloboma of the eye, heart defects, atresia of the choanae, retardation of growth and/or development, genital and/or urinary abnormalities, and ear abnormalities and deafness (CHARGE) syndrome may also be associated with Omenn-like manifestations ([Pirovano et al., 2003; Gennery et al., 2008](#)).

Autoimmunity has evolved as a major common feature of patients with OS including autoimmune cytopenias, granulomas, alopecia, vitiligo, myasthenia gravis, vasculitis, and psoriasis ([Notarangelo et al., 2016](#)). It has also been shown that patients with hypomorphic *RAG* mutations can present with delayed-onset combined

immunodeficiency with granulomas and autoimmunity similar to clinical features of common variable immunodeficiency (CVID) (Avila et al., 2010; Abolhassani et al., 2014). These patients may produce a wide range of autoantibodies including neutralizing antibodies specific for interferon-alpha and interferon- $\omega$  (Walter et al., 2015).

The combination of autoimmunity and immune deficiency associated with defective T-cell development may be explained by abnormalities in the thymic stroma found in experimental models of "leaky" SCID (Rucci et al., 2011). The predominant finding in these models is a reduction in the pool of medullary thymic epithelial cells (mTECs) and reduction in the level of self-antigen expression in the thymic medulla, which appears to diminish the effectiveness of negative selection of self-reactive T cells during thymopoiesis. There also appears to be a contribution of defective B-cell tolerance associated with RAG gene mutations (Walter et al., 2010; Lee et al., 2016b). In addition, findings in these models also identify a marked reduction in thymic-derived regulatory T cells (Tregs), an observation that is also likely to be contributing to the development of autoimmunity (Cassani et al., 2010). In summary, impaired negative thymic selection, B-cell tolerance, Tregs development, and/or homeostatic proliferation may largely account for the development of autoimmunity in patients with OS.

ZAP70 is a tyrosine kinase that is involved in TCR signaling. The complete lack of this enzyme results in combined immunodeficiency characterized by the selective absence of CD8 $^{+}$  T cells with recurrent infections in the first few months of life. However, there have been case reports of patients with hypomorphic ZAP70 defects, potentially both LOF and gain-of-function (GOF), associated with early-onset autoimmunity including inflammatory bowel disease, eczematous skin lesions, immune thrombocytopenia purpura (ITP), bullous pemphigoid, and autoimmune hypothyroidism (Chan et al., 2016; Liu et al., 2017). ZAP70 deficiency is associated with reduced numbers of autoimmune regulator-positive (AIRE $^{+}$ ) mTECs, thymic dendritic cells, and thymic-derived natural Tregs, which could explain the predisposition to autoimmunity in these patients (Poliani et al., 2013).

ADA and PNP are the key enzymes in the purine salvage pathway. The deficiency of either of these enzymes results in an accumulation of substrates that are toxic to lymphocytes. Both ADA and PNP are expressed ubiquitously in all cells. Hence, the lack of either of these enzymes leads to systemic manifestations in addition to immune dysfunction. Hereditary deficiency of ADA is the second most common cause of SCID in North America. This type of autosomal-recessive SCID is characterized by progressive depletion to nearly the absence of T, B, and NK cells. Patients frequently have nonimmune defects including skeletal alteration, neurological abnormalities, hepatic, and renal diseases. It has been shown that alterations in the adenosine metabolism affect Treg functions (Sauer et al., 2012). Autoimmune manifestations have been observed in milder forms of this disease including autoimmune hemolytic anemia (AIHA), immune thrombocytopenia (ITP), neutropenia (Grunebaum et al., 2013), type I diabetes (Notarangelo et al., 1992), and autoimmune thyroiditis causing acquired hypothyroidism (Nagpala et al., 2007). PNP deficiency is less common than ADA. The deficiency of PNP leads to progressive decline of T-cell number and function but tends to spare B and NK cells (Grunebaum et al., 2013). Infectious and neurological manifestations including developmental delay, hypotonia, and spasticity are predominant symptoms. Approximately one-third of the patients develop autoimmune manifestations including hemolytic anemia and immune thrombocytopenia (Markert, 1991), and these can be presenting symptoms (Rich et al., 1979). The basis of autoimmunity in patients with this enzymatic defect is not known but must be linked in some fashion to the toxic metabolite's impact on T-cell (and possibly B-cell) function.

*SCID with maternal engraftment.* A subset of SCID patients may have an engraftment by transplacentally acquired maternal T and B cells (Okano et al., 2017). If these maternal cells are functional, patients may have a delayed clinical presentation of SCID (Al-Muhsen, 2010). Immune dysregulation from the oligoclonal expansion of these maternal cells can lead to cutaneous GVHD of skin presenting as diffuse scaly erythematous morbilliform rashes and even erythroderma similar to OS (Muller et al., 2001). Liver involvement and cytopenia have also been described in these patients (Palmer et al., 2007).

## Combined Immune Deficiencies

Combined immune deficiencies (CIDs) include primary immunodeficiencies with T-cell defects that are generally less severe than those seen in SCID, sometimes presenting later in childhood, and not universally requiring HCT.

*Immunodeficiency with hyper-IgM (HIGM) syndrome* is a heterogeneous group of disorders caused by defects in genes that critically involved in immunoglobulin class switch recombination or somatic hypermutation

(Jesus et al., 2008). X-linked forms of HIGM are caused by defects in the CD40 ligand (CD40L) and autosomal-recessive defects in CD40. The serological hallmark of these disorders is a normal to increased serum level of IgM in the face of decreased levels of IgG, IgA, and IgE. Other monogenic primary immunodeficiencies manifesting with a HIGM phenotype include missense variants in the gene encoding nuclear factor- $\kappa$ B (NF- $\kappa$ B) essential modulator (NEMO) which leads to defects in multiple immune cell types (see the “Combined Immunodeficiencies with syndromic features” section) (Qamar and Fuleihan, 2014), and disease caused by defects in enzymes involved in generation or repair of DNA breaks during immunoglobulin isotype switching including activation-induced cytidine deaminase (AID) and uracil-DNA glycosylase (UNG) (presented in the “Predominantly antibody deficiencies” section), as well as postmeiotic segregation 2 (Qamar and Fuleihan, 2014).

X-linked CD40L deficiency is the most common cause of HIGM syndrome (70%) and manifests clinically before the age of 2 years with recurrent pyogenic infections as well as with an increased risk of infections with certain viruses, fungi, and parasites, most notably *Pneumocystis jiroveci* and *Cryptosporidium* species (Notarangelo et al., 2006; Jesus et al., 2008). CD40L is expressed by T lymphocytes, and much of the infectious susceptibility in this disorder is thought to be due to impaired T-cell function. In addition, a substantial number of patients develop autoimmune disorders (Jesus et al., 2008). In particular, autoimmune neutropenia occurs in up to 50% of the patients. AIHA and ITP have been estimated to occur in 2%–10% of the patients (Hirbod-Mobarakah et al., 2014). Other autoimmune manifestations in CD40L-deficient patients include seronegative arthritis, hypoparathyroidism, hypothyroidism, nephritis, autoimmune retinopathy, inflammatory bowel disease, and cutaneous sarcoid-like granulomas (Jesus et al., 2008; Levy et al., 1997). A less common form of HIGM associated with an autosomal-recessive *CD40* deficiency presents clinically similar to *CD40L* deficiency.

A review of the function of CD40L, a member of the TNF superfamily, provides a context for understanding these seemingly unrelated clinical phenotypes (Hirbod-Mobarakah et al., 2014). It is expressed transiently on activated T-helper cells and interacts with its counter-receptor, CD40, on B cells, dendritic cells, and macrophages. The engagement on B cells is a critical proximal step in the isotype switch of naïve B cells following their encounter with T-dependent antigens. This cognate interaction initiates the activation of a family of TNF receptor-associated factors, which leads to the activation of nuclear translocation of NF- $\kappa$ B and the expression of the genes *AID* and *UNG* triggering isotype class switch. In the absence of CD40L, patients have low serum IgA and IgG levels, normal-to-elevated levels of IgM, and show impaired IgG antibody responses to T-dependent antigens together with a marked reduction of circulating isotype-switched memory B cells. CD40L/CD40 interaction promotes germinal center of lymph nodes, and lymph nodes of affected patients show primary lymphoid follicles that are devoid of germinal centers. The engagement of CD40 molecules expressed is required for the upregulation of CD80/CD86 on B cells, macrophages, and dendritic cells. The interaction of these costimulatory molecules with CD28 on the collaborating T cells leads to full T-cell activation and promotes the survival of these antigen-presenting cells. Thus it is not surprising that X-linked CD40L-deficient patients experience opportunistic infections and develop malignancies in tissues infected by the prototypical organisms. Defects in peripheral B-cell tolerance contribute to immune dysregulation of CD40L-deficient patients (Herve et al., 2007). Their mature naïve B cells express a high proportion of autoreactive antibodies. It has also been shown that the lack of CD40L results in a defect of Tregs both quantitatively and qualitatively (Herve et al., 2007; Tang et al., 2014). Finally, serum levels of B cell-activating factor of the tumor necrosis factor family (BAFF), a molecule that promotes the B-cell survival, are increased in X-linked CD40L deficiency, offering another factor that might contribute to the expression of B-cell autoimmunity (Herve et al., 2007).

*Major histocompatibility complex (MHC) class I deficiency* is associated with a defect in the surface expression of class I MHC proteins (often referred to as the bare lymphocyte syndrome) that present with low numbers of CD8<sup>+</sup> T cells and recurrent infections. The underlying defect in this disorder is associated with mutations in genes for specific proteins, including transporter-associated with antigen processing (TAP) 1, TAP2, and TAPBP, required for the transport of antigenic peptides to the endoplasmic reticulum necessary to assemble the MHC protein peptide complex that is normally expressed on the cell surface. MHC class I deficiency is a very rare disorder clinically characterized by recurrent respiratory tract infections, bronchiectasis, and necrotizing granulomatous skin lesions typically involving extremities and the midface that required a differentiation from granulomatosis with polyangiitis (Zimmer and Ollert, 2015; Villa-Forte et al., 2008). Nonerosive polyarthritides and leukocytoclastic vasculitis have also been reported in TAP-deficient patients (Gadola et al., 2000).

*Serine-threonine protein kinase (STK4 or MST1) deficiency* is an autosomal-recessive CID that was first described in 2012 in two consanguineous kindreds, presenting with recurrent bacterial and viral infections and autoimmunity (Nehme et al., 2012). The disease is caused by homozygous mutations in the STK4 gene, coding for protein MST1. The STK4-deficiency patients have T- and B-cell lymphopenia and reduced in vitro T-cell proliferation.

Dermatitis, autoimmune neutropenia, AIHA, and hypergammaglobulinemia associated with increased titers of autoantibodies have been described as clinical features of this novel primary immunodeficiency disease (Abdollahpour et al., 2012).

*Inducible T-cell costimulatory (ICOS)* deficiency is associated with defects in activated T cells and clinically resembles CVID-like disease (Grimbacher et al., 2003). ICOS is a costimulatory molecule upregulated on activated T cells and plays a role in T-cell help for late B-cell differentiation, class-switching, and memory B-cell generation. ICOS also has a distinct role in the generation and maintenance of germinal centers in lymphatic tissue, and ICOS deficiency leads to severe reduction of memory B cells and impaired switched antibody responses (Warnatz et al., 2006). ICOS deficiency in patients typically manifests with recurrent bacterial infections of respiratory tracts (onset from childhood to adults) and GI involvement including giardiasis, inflammatory bowel disease salmonellosis, and nodular lymphoid hyperplasia (Warnatz et al., 2006). These patients were reported to have total B-cell lymphopenia, severe reduction of switched memory B cells, hypogammaglobulinemia, and impaired antibody responses to vaccines. Importantly, the majority of 15 patients in multicenter studies developed autoimmunity (73%, 11/15) including enteropathy (9), psoriasis (4), arthritis (3), and interstitial pneumonitis (1) (Schepp et al., 2017). Patient also can develop thrombocytopenia from splenomegaly. The precise mechanism of autoimmunity is unclear. However, it has been shown that plasmacytoid dendritic cells prime IL-10-producing Tregs via ICOS-L.

*Activated phosphoinositide-3-kinase δ syndrome (APDS)* is a combined T- and B-cell immunodeficiency resulting from heterozygous GOF mutations in *PIK3CD* encoding the p110 $\delta$  catalytic subunit or *PIK3R1* encoding the p85 $\alpha$  regulatory subunit of class IA phosphoinositide-3-kinase (class IA PI3K) (Angulo et al., 2013; Lucas et al., 2014, 2016). Class IA PI3Ks are heterodimeric proteins that function as signaling complexes, with the p110 $\delta$  subunit restricted to immune cells. p110 $\delta$  is downstream of multiple receptors in the immune system, including T and B-cell receptors, as well as a variety of cytokine receptors. The signaling cascade downstream of class IA PI3K leads to the activation of AKT and mTOR (mammalian target of rapamycin).

The clinical phenotype of APDS is heterogeneous and characterized by recurrent bacterial infections, severe/persistent herpes virus infections, lymphoproliferation, and autoimmunity. Immunologic findings described in APDS include progressive T- and B-cell lymphopenia, decreased numbers of naïve T cells, expansion of terminally differentiated senescent effector CD8 $^{+}$  T cells, relatively increased numbers of transitional B cells, and hypogammaglobulinemia (Elgizouli et al., 2016). Autoimmune manifestations found in 34% of the p110 $\delta$  GOF (also known as APDS1) patients in an international cohort included cytopenias, glomerulonephritis, exocrine pancreatic insufficiency, thyroiditis, seronegative arthritis, sclerosing cholangitis, systemic lupus erythematosus, and enteropathy with lymphoid nodular hyperplasia (Coulter et al., 2017). For patients with APDS2 from GOF of p85 $\alpha$ , 17% had autoimmune complications including cytopenias, insulin-dependent diabetes, chronic arthritis, autoimmune hepatitis, and eczema (Elkaim et al., 2016). Given the pathology of APDS is in part caused by increased mTOR signaling, the treatment of patients with mTOR inhibitors has been described with some success (Lucas et al., 2014). An open-label clinical trial of a selective PI3Kδ inhibitor in six patients with APDS resulted in improvement of lymphoproliferation in all patients, suggesting this might be an effective targeted therapy for this disease.

## Combined Immunodeficiencies With Syndromic Features

*DGS* results from defective third and fourth pharyngeal pouch development during embryogenesis, which in the majority of cases is associated with a deletion at chromosome 22q11.2. Heterozygous LOF mutations in *TBX1* can cause a similar clinical phenotype. As a result of the developmental abnormality, patients with DGS typically have a combination of conotruncal cardiac anomalies, hypocalcemia due to hypoparathyroidism, dysmorphic facial features (e.g., low set ears, shortened philtrum, high arched, or cleft palate), and T-cell immunodeficiency from thymic hypoplasia. In addition, patients with DGS have an increased incidence of autoimmunity including ITP, AIHA, autoimmune neutropenia, and organ-specific autoimmunity particularly juvenile idiopathic arthritis (JIA) and autoimmune thyroid diseases (Zembla et al., 2010; Gennery, 2012; Sullivan et al., 1997). Based on a cohort study of 130 pediatric patients, 8.5% of the partial DGS had autoimmune diseases with autoimmune cytopenias and hypothyroidism were the most common manifestations (Tison et al., 2011). From an immunologic perspective, a small minority of DGS patients (1%–2%) have thymic aplasia that results in marked T-cell lymphopenia with increased susceptibility to opportunistic infections, and among this patient group, chromosomal microdeletions are only found in about 50% of the patients. This group is referred to as complete DGS, and

among these patients is a subgroup that carries a diagnosis of complete atypical DGS with a clinical phenotype that is virtually indistinguishable from OS (Markert et al., 2004). Due to the severity of the infectious complications associated with complete (including atypical) DGS, the thymic transplantation is indicated to reconstitute T-cell function. However, due to the autoreactive T cells present in the complete atypical DGS presentation, aggressive immunosuppression is required to control the autoreactive cells prior to initiating the thymus transplantation. Alternatively, fully matched donor T-cell transplant has also been suggested as therapeutic option and in this case, reconstitution is presumably derived from long-lived donor T cells since there is no thymus present for new T-cell development (Gennery et al., 2010). Long-term follow-up studies of complete DGS (including atypical complete DGS) patients post successful thymic transplantation have demonstrated an increased incidence of autoimmune thyroid disease as well as autoimmune cytopenias (Markert et al., 2007). It has been shown that the decreased Tregs and homeostatic dysregulation of CD4<sup>+</sup> T cells may contribute to autoimmunity in DGS (Sullivan et al., 2002; Ferrando-Martinez et al., 2014). However, the mechanism is likely more complex beyond Tregs and could be similar to the pathophysiology of immune dysregulation in OS.

*Wiskott–Aldrich syndrome* (WAS) is an X-linked primary immunodeficiency that variously presents with thrombocytopenia involving small platelets, eczema, recurrent infections, and high incidence of autoimmunity and malignancies (Notarangelo et al., 2008; Thrasher and Burns, 2010). Affected patients are particularly susceptible to infections with encapsulated bacteria and herpes viruses. This disorder is caused by mutations within the gene encoding the WAS protein (WASP), a critical factor in actin polymerization and skeletal remodeling initiated by cellular activation. In particular, the above-described clinical phenotype is caused by mutations that lead to abrogation of WASP expression. By contrast, missense mutations in exon 1–3 of WASP gene resulting in some WASP expression generally lead to a milder clinical phenotype characterized by X-linked thrombocytopenia (XLT) (Notarangelo et al., 2002).

As WASP is ubiquitously expressed in all nonerythroid hematopoietic cells, it is not surprising that affected individuals present with a number of immunological abnormalities. These include progressive T-cell lymphopenia, impaired in vitro T-cell proliferation and IL-2 production following TCR cross-linking, low serum IgM levels often associated with elevated serum IgA and IgE levels, impaired IgG antibody responses to both T-dependent and T-independent antigens, impaired migratory responses of monocytes, dendritic cells, T and B cells and neutrophils to chemokines, and impaired NK-cell cytolytic activity.

As is true for many immunodeficiency disorders, the clinical phenotype of WAS does not show consistent fidelity with the genotype. This was demonstrated in a report of monozygotic twins, associated with WAS including autoimmune disease in one twin and a milder XLT phenotype in the other (Buchbinder et al., 2011). Interestingly, CpG methylation, diminished WASP gene transcripts, diminished WASP expression, and more impaired cellular function were detected in the more symptomatic twin. These findings are consistent with the previously reported effects of epigenetic changes on the clinical expression of genetically based autoimmune and primary immunodeficiency disorders.

Autoimmune disease affects as many as 70% of the WAS patients with clinical presentation in rank order including AIHA, ITP, vasculitis, renal disease, Henoch–Schönlein-like purpura, arthritis, and inflammatory bowel disease (Notarangelo et al., 2008; Thrasher and Burns, 2010; Walter et al., 2016; Sullivan et al., 1994). Autoimmunity most commonly occurs in patients with WAS but occasionally is also present in patients with XLT. Early explanations for the pathogenesis of autoimmunity referred to possible contributions of recurrent exposure to microbes and chronic inflammation as well as reduced IL-2 production and impaired clearance of apoptotic cells/bodies and debris due to the defective migration of phagocytic cells. Further studies have shown impaired functions of Tregs (Marangoni et al., 2007) and regulatory B cells (Bregs) (Bouma et al., 2014), dysregulated plasmacytoid dendritic cells, and diminished NK-cell cytotoxicity in WASP-deficient mice and/or humans that may collectively contribute to immune dysregulation in WAS (Crestani et al., 2015). WASP-dependent intrinsic B defects in the setting of normal T-cell function may also account to the pathology of systemic autoimmunity in WAS (Becker-Herman et al., 2011). This may contribute to the findings that posthematopoietic stem cell-transplantation autoimmunity is particularly problematic in patients who remain mixed chimeras for B cells suggesting that the WASP-negative B cells may be mediating autoimmunity in this situation.

*NEMO syndrome* is caused by defects in NF-κB activation due to hypomorphic mutations in the *IKBKG* gene encoding NEMO on the X-chromosome. Males with NEMO syndrome have ectodermal dysplasia associated with immunodeficiency and susceptibility to recurrent pyogenic, mycobacterial, and serious viral infections (Orange et al., 2004), sometimes with a HIGM-like phenotype (Qamar and Fuleihan, 2014). HIGM and hyper-IgA were reported in 15% and 27% of the patients in hypomorphic NEMO mutation database (Hanson et al., 2008). These patients also develop a range of inflammatory and autoimmune conditions (25%) including inflammatory colitis,

AIHA, and chronic arthritis. This association suggests that in addition to defective host defense, there is also some degree of immune dysregulation in this disorder.

*Schimke immune-osseous dysplasia (SIOD)* is an autosomal-recessive disease characterized by spondyloepiphyseal dysplasia, focal segmental glomerulosclerosis, renal failure, and T-cell deficiency caused by biallelic mutations in SMARCLA1 (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin, subfamily-a-like-1) gene. Based on the report of 41 patients with SIOD, 9 had autoimmune manifestations including ITP, AIHA, enteropathy, and pericarditis (Zieg et al., 2011).

*Immunodeficiency associated with  $\text{Ca}^{2+}$  release-activated  $\text{Ca}^{2+}$  (CRAC) channelopathy* due to LOF mutations in genes encoding ORAI1 and stromal interaction molecule (STIM1) proteins have been identified as etiologies of severe T-cell immunodeficiencies (Picard et al., 2009; McCarl et al., 2009). ORAI1 is a pore-forming subunit of the CRAC channel that is activated by STIM1 that is localized in endoplasmic reticulum where it senses the concentration of stored  $\text{Ca}^{2+}$  (Feske et al., 2010). As store-operated calcium influx via CRAC channels is a requirement for lymphocyte activation, ORAI1 and STIM1-deficient patients were found to have severe defects in T-cell function leading to recurrent and severe infection similar to SCID patients. Their clinical phenotypes are similar and characterized by SCID-like disease, autoimmunity, muscular hypotonia, and ectodermal dysplasia (Lacruz and Feske, 2015). Interestingly, these patients have normal number of lymphocyte subsets including T, B, and NK cells; however, they have a loss of naïve and increase in memory CD4 $^{+}$  and CD8 $^{+}$  T cells with impaired lymphocyte proliferative responses to mitogen and skin delayed-type hypersensitivity reactions.

Autoimmune manifestations of LOF mutations of STIM1 include lymphoproliferation, AIHA, neutropenia, thrombocytopenia, and colitis (Lacruz and Feske, 2015). These autoimmunities are less common in ORAI1 due to unclear etiology. One presumed mechanism of autoimmunity in STIM1-deficient patients is a reduction in Treg cells based on observations in one patient as well as a murine model with T cell-specific deletion of STIM1 and STIM2 demonstrating reduced numbers and diminished function of Treg cells (Picard et al., 2009).

## PREDOMINANTLY ANTIBODY DEFICIENCIES

*X-linked agammaglobulinemia (XLA)* was the first primary antibody deficiency identified and is caused by mutations in Bruton's tyrosine kinase (BTK) gene necessary for B-cell differentiation and signaling (Tsukada et al., 1993). In BTK-deficient patients, the B lymphocyte lineage is arrested at the pro- to pre-B-cell stage of differentiation resulting in a marked deficiency of circulating B cells and panhypogammaglobulinemia but intact T-cell function. Affected individuals usually present clinically by 5–6 months of age with recurrent sinopulmonary infections involving encapsulated bacteria following the loss of maternally transferred IgG antibodies. Up to 15% of the XLA patients develop autoimmune disease including chronic arthritis, inflammatory bowel disease, alopecia, AIHA, glomerulonephritis, and type 1 diabetes (Winkelstein et al., 2006; Howard et al., 2006; Barmettler et al., 2017).

The mechanism of autoimmunity in XLA remains uncertain. BTK has been shown to play a crucial role in setting the threshold of B-cell activation and negative selection of autoreactive T cells in mice (Kil et al., 2012), and the selective inhibition of BTK prevents murine lupus and antibody-mediated glomerulonephritis in mice (Rankin et al., 2013). The absence of B cells and immunoglobulins in these patients suggests that immunoglobulin-independent, BTK-dependent processes may be involved in the pathogenesis of autoimmune disorders. Bregs, described in mice and humans, appear to contribute to suppression of the development and expression of inflammatory reactions, particularly those mediated by T cells suggesting an important role in immune homeostasis and prevention of autoimmune and inflammatory diseases (Klinker and Lundy, 2012). The absence of Bregs in XLA could also contribute to the risk of developing autoimmunity.

*HIGM syndrome due to B-cell defects.* Deficiency of AID and UNG are autosomal recessive forms of HIGM that present with an infectious phenotype consisting of recurrent sinopulmonary infections with encapsulated bacteria but without opportunistic infections (Jesus et al., 2008). AID-deficient patients typically present with enlarged tonsils and lymph nodes presumably related to expansion of the B cells attempting to undergo class switch as evidenced by massive germinal center expansion. Autoimmune manifestations are found in 25% of the AID-deficient patients (Jesus et al., 2008) including diabetes mellitus (DM), polyarthritis, hepatitis, AIHA, ITP, Crohn's disease, and chronic uveitis (Quartier et al., 2004). Patients with UNG deficiency clinically resemble those with AID deficiency.

*CVID* is a group of heterogeneous disorders with a common feature of hypogammaglobulinemia and failure to mount adequate antibody response to pathogens and vaccination (Bonilla et al., 2016). Patients with CVID

have low serum IgG and frequently low IgA and/or IgM due to a failure of terminal B-cell differentiation, due to either intrinsic B-cell defects and/or impairment of helper T-cell function. The onset of the varied clinical manifestations of CVID can occur at any age. Based on the worldwide study in 2212 patients with CVID, one-third of the patients had an onset of symptoms before the age of 10 years (Gathmann et al., 2014). While there is no sex preference in adults, in pediatric patients, the male-to-female ratio was 2:1 (Gathmann et al., 2014). Clinical phenotypes of CVID include recurrent pneumonia (32%), autoimmunity (29%), splenomegaly (26%), bronchiectasis (23%), granuloma (9%), enteropathy (9%), and others (<5%). The onset of autoimmune manifestations was before the diagnosis of CVID in 50% of the patients. They also found that autoimmune manifestations were independently associated with the presence of splenomegaly and granulomas. Due to the nature of data collection, the study did not specify the type of autoimmunity in their cohort. In a cohort study of 473 CVID patients in the United States, hematologic and organ-specific autoimmune diseases were found in 29% of the patients. These manifestations include ITP (14%), AIHA (7%), Evans syndrome (4%), inflammatory bowel disease (4%), rheumatoid arthritis (3%), and other less common conditions (<2%) including alopecia, DM, pernicious anemia, uveitis, multiple sclerosis, neutropenia, primary biliary cirrhosis, systemic lupus erythematosus, autoimmune thyroid disease, vasculitis, psoriasis, and vitiligo (Chapel et al., 2012).

The pathophysiology of autoimmunity in CVID is unclear. The immunologic phenomena found to be associated with autoimmunity in CVID includes the reduction of switched memory B cells and expansion of CD21 low B cells, reduction of naïve and Tregs, dysregulated cytokine responses with increased levels of a proliferation-inducing ligand (APRIL) and BAFF, upregulated interferon signature expression, and increased circulating inflammatory innate lymphoid cells (Maglione, 2016). The heterogeneity of these studies is in part due to complex genetic origins of CVID that are not entirely understood.

Monogenic causes of CVID have been found in only 2%–10% of the patients, but these patients have provided an insight into mechanisms of immune dysregulation leading to antibody deficiency (Bogaert et al., 2016; Bonilla et al., 2016). Genetic discovery has led to a better appreciation of the heterogeneity of this complex disease. For example, some patients, previously classified as “CVID” or “CVID-like,” have monogenic disorders of T-cell function such as defects in ICOS or PI3K-pathway genes (see the “Combined immune deficiencies” section) or T-cell tolerance due to altered Treg function, including disease associated with CTLA4 and lipopolysaccharide-responsive beige-like anchor (LRBA) (see the “Disorders of regulatory T cells” section). Here, we highlight the current knowledge and clinical phenotypes of two genetic pathways associated with a primary antibody deficiency and CVID-like disease and autoimmunity, transmembrane activator and calcium modular and cyclophilin ligand interactor (TACI), and Ikaros.

TACI is encoded by TNFRSF13B gene. It is one of the receptors of BAFF and APRIL. TACI is a regulator in immune responses. Biallelic and monoallelic LOF variants in TACI have been associated with some degrees of antibody deficiency with variable penetrance and clinical outcomes (Bogaert et al., 2016). In certain cohorts, 7%–10% of the CVID patients carry allelic mutation in this gene. However, the family members and approximately 2% of the healthy control subjects who lacked immune abnormalities shared the same heterozygous mutations (Martinez-Gallo et al., 2013). This finding may suggest that other factors, potentially genetic or epigenetic modifiers and/or environmental triggers, interact to promote the CVID clinical phenotype. The incidence of autoimmunity is high in CVID patients with heterozygous mutation of TACI, and they have increased levels of circulating T follicular helper cells and antinuclear antibodies. Interestingly, patients with homozygous TACI deficiency have less severe disease compared to patients with heterozygous TACI deficiency, and they do not have increased predisposition for autoimmunity (Maglione, 2016).

Ikaros deficiency is caused by heterozygous germline-dominant LOF mutations of IKZF1 gene encoding Ikaros, a transcription factor that plays a critical role in hematopoiesis and B-cell differentiation. The disease is characterized by progressive loss of B cells with a lack of plasma cells and decreased serum immunoglobulins with a CVID-like phenotype (Kuehn et al., 2016; Hoshino et al., 2017). In the largest cohort of 29 patients, most had recurrent infections, one patient had ITP, two had B-cell acute lymphocytic leukemia, and some were asymptomatic despite mildly low IgG (Kuehn et al., 2016). Of nine patients described in a subsequent cohort, three presented with recurrent sinopulmonary infection and three had no significant infections but had autoimmune manifestations including one with SLE and Evans syndrome (Hoshino et al., 2017). Other manifestations described in this cohort were ITP and IgA vasculitis.

Selective IgA deficiency (sIgAD) is the most common primary immune deficiency affecting humans. The diagnosis of sIgAD is defined by a lack of serum IgA of <7 mg/dL with normal serum IgM and IgG levels in an individual older than 4 years, and exclusion of other causes of immunodeficiency (Bonilla et al., 2015). The prevalence of sIgAD varies across studied ethnic populations with a high of 1:143 on the Arabian Peninsula to a

low of 1:18:500 in Japan. In the United States the range is 1:223–1:1000 in community studies. There is considerable variability in clinical presentation with the majority of patients reported as being asymptomatic. The most common clinical manifestation occurring in the roughly 10%–15% symptomatic patients is recurrent sinopulmonary infections caused by encapsulated bacteria (Geha et al., 2007). IgA-deficient patients are also considered to have an increased frequency of allergic disorders, and this is particularly true in younger patients (4–32 years of age) where a prevalence of 84% was reported in one study.

The prevalence of autoimmune disorders in sIgAD patients varies from 7% to 36% with more than 40% of the patients having abnormal serum antibodies to cells or tissues (Jacob et al., 2008; Edwards et al., 2004; Abolhassani et al., 2015). Autoimmune manifestations described in these patients include ITP, JIA, thyroiditis, vitiligo, inflammatory bowel diseases, myasthenia gravis, Henoch–Schönlein purpura, type 1 DM, and SLE. The role of positive serum autoantibodies in sIgAD patients is unclear since it is commonly found in the absence of clinical manifestations of autoimmune diseases. First-degree relatives of IgA-deficient patients display an increased frequency of autoimmune disorders. In addition, patients are at risk for the development of GI complications including gluten-sensitive celiac disease, lactose intolerance, nodular lymphoid hyperplasia, giardiasis, malabsorption, chronic active hepatitis, and ulcerative colitis.

The pathogenesis of sIgAD remains obscure in that patients have immature B cells that express surface IgA as well as IgM and IgD but fail to differentiate into IgA secreting plasma cells (Conley and Cooper, 1981). While some investigators have provided evidence of an intrinsic B-cell defect, others have found reduced number of Tregs and decreased or impaired helper T-cell activation in sIgAD patients (Yazdani et al., 2017; Soheili et al., 2013). Similarly, the basis of the association of this PID and autoimmunity has yet to be elucidated, although several hypotheses have been proposed, including altered mucosal immunity.

No single genetic defect has been reported in sIgAD patients, although a small number of TACI deficient patients have been found with sIgAD (Castigli et al., 2005). Indeed, the same TACI mutation has been found in IgA-deficient patients and patients with CVID. Some patients with sIgAD progress to developing full-blown CVID, adding support to the contention that both disorders fall along a continuum of the same overarching PID (Aghamohammadi et al., 2008). Further support for this argument is the observation that both IgA deficiency and CVID are associated with the same common extended MHC haplotype, HLA A1, B8, DR3, and DQ2 (Mohammadi et al., 2010).

## DISORDERS OF IMMUNE DYSREGULATION: T-CELL TOLERANCE

T-cell tolerance is essential for maintenance of a pool of effectors capable of recognizing pathogens and tumor cells but not host. There are multiple mechanisms by which the immune system maintains T-cell tolerance, and monogenic disorders disrupting these pathways highlight the importance of the pathways discussed here.

### Monogenic Defects Affecting T-Cell Selection and Homeostasis

*Autoimmune polyendocrinopathy, candidiasis, ectodermal dysplasia (APECED)*, also referred to as autoimmune polyendocrinopathy syndrome type 1, is a human disorder caused by a deficiency in the AIRE protein. AIRE is a transcriptional regulator that is critical for ectopic expression of tissue-specific antigens by mTECs in the thymus and T cell-negative selection (Proekt et al., 2017). As classically defined, APECED presents with a triad of findings including hypoparathyroidism, adrenal insufficiency, and chronic mucocutaneous candidiasis (CMC) with ectodermal dysplasia. As advances in genetic sequencing have allowed for the identification of additional patients based on a genetic diagnosis, it has become clear that there are many additional autoimmune features, some of which may be as prominent as those traditionally described. A recent cohort of 35 patients, primarily from the United States, demonstrated additional major features including urticarial, autoimmune hepatitis, gastritis, intestinal dysfunction, pneumonitis, and Sjögren-like disease (Ferre et al., 2016). Additional less common autoimmune features described in APECED patients include gonadal failure, hypothyroidism, type 1 diabetes, celiac disease, B12 deficiency, and others.

APECED was initially described as an autosomal-recessive disorder caused by LOF in the AIRE protein. However, more recently autosomal-dominant AIRE-associated disease has been described associated with dominant-negative variants in the protein (Oftedal et al., 2015). There is a higher incidence of APECED in certain populations including Iranian Jews (1:9000), Sardinians (1:14,000), and Finns (1:25,000) with lower prevalence in

Slovenia (1:43,000), Norway (1:80,000), and Poland (1:129,000) (Kisand and Peterson, 2011). In these settings, there tend to be predominantly specific AIRE mutations. Importantly, the same mutation in different patients can present with varied clinical phenotypes, pointing out that additional factors impacting the clinical presentation.

AIRE-induced expression of tissue-specific antigens by mTECs is critical in the negative selection of antigen-specific autoreactive T cells within the thymic medulla. AIRE also appears to play a role in Treg-cell development and studies in APECED patients have identified a decrease in forkhead box P3 protein (FOXP3) expression together with diminished in vitro suppressive capacity of circulating Treg cells (Laakso et al., 2010). It is clear that in APECED patients, many of the autoimmune disorders are accompanied by the production of autoantibodies that in some cases may be linked to disease and in others simply serve as biomarkers of disease. The explanation for CMC in this disorder was linked to the development of high affinity, neutralizing autoantibodies to IL-17A and IL-22 (Puel et al., 2010). This finding was noted in all APECED patients evaluated but was not detected in any other conditions tested. This finding fits well with prior descriptions of autoantibodies to another cytokine ( $\alpha$ -interferon) found in the majority of these patients. The finding of the neutralizing anticytokine antibody establishes a new paradigm with an autoantibody inhibiting the activity of a critical cytokine resulting in the development of a specific host defense defect. This type of autoimmune process (i.e., anticytokine autoantibody) has also been linked to susceptibility to nontuberculous mycobacterial infections associated with high titer, neutralizing autoantibodies to gamma interferon.

*Autoimmune lymphoproliferative syndrome (ALPS)* is a lymphoproliferative disorder characterized by an increased percentage of peripheral blood alpha–beta TCR double-negative T cells and abnormal lymphocyte apoptosis (Oliveira et al., 2010). The underlying defect in the majority of ALPS patients is an abnormality in the extrinsic apoptotic pathway linked to defective signaling via the FAS receptor (Lenardo et al., 2010). Clinically, ALPS patients have a high incidence of autoimmune cytopenias and a substantially increased risk for the development of non-Hodgkin and Hodgkin lymphoma. The typical course of disease in ALPS patients begins during childhood with the development of nonmalignant lymphadenopathy and splenomegaly. In many of the patients, the lymphoid accumulation is followed by the development of autoimmunity that is almost exclusively immune-mediated cytopenias, most commonly AIHA and ITP. Some ALPS patients also develop neutropenia and dermatologic findings that can include an urticarial rash. Rare autoimmune findings reported in ALPS include glomerulonephritis, Guillain–Barré syndrome, autoimmune hepatitis, and polyneuropathy. A very significant problem in the most common genetic form of ALPS associated with defects in the FAS gene is an increased risk for the development of Hodgkin and non-Hodgkin lymphoma.

The homotrimeric FAS receptor is a member of the TNF receptor superfamily and plays a critical role in lymphocyte homeostasis as well as in immune-mediated cytotoxicity following receptor engagement by FAS ligand (FASL). Among patients diagnosed with ALPS, 65%–70% have a heterozygous defect in the *FAS* gene that acts as a dominant negative mutation in most patients (i.e., autosomal dominant). These patients are now categorized as ALPS-FAS with the majority having a heterozygous germline mutation. However, a significant percentage of ALPS-FAS patients have a heterozygous somatic mutation affecting FAS (categorized as ALPS-sFAS) that can be detected by performing sequencing of circulating alpha–beta double-negative T cells. There are additional, rare, and genetic causes of ALPS including mutations in the genes encoding FASL (categorized as ALPS-FASLG) and caspase-10 (categorized as ALPS-CASP). In addition, there remain ~20% of the patients with an ALPS clinical phenotype who do not have a defined genetic defect (categorized as ALPS-U). In all ALPS cases with genetically defined mutations, there is a defect in the death inducing signal mediated by FAS. This can be analyzed in vitro, and the degree of the apoptotic defect varies based on the site of the FAS mutation with those affecting exons coding for the intracellular part of the protein generally conferring more severe disease with higher disease penetrance. Additional biomarkers have been identified that are linked with both germline and somatic FAS mutations including elevation in soluble FASL, IL-10, and vitamin B12. Combining an elevation in one of these biomarkers with increased alpha–beta double-negative T cells yields a greater than 95% likelihood of a FAS mutation (either germline or somatic).

The defect in the FAS-dependent extrinsic apoptotic pathway presumably interferes with peripheral tolerance and the normal control of specific autoreactive T cells (and possibly autoreactive B cells). Clearly, based on varied penetrance observed in extended family pedigrees, the FAS gene defect requires additional (currently unidentified) factors for the development of clinical disease. This situation is analogous to the murine model of autoimmunity associated with a FAS defect, the *lpr* mouse, where strain variations can significantly impact the degree of autoimmunity. The range of autoimmunity in ALPS is quite limited and suggests that the FAS-mediated

pathway of tolerance has a more restricted role in controlling self-reactivity, and the increased risk of lymphoma in ALPS patients suggests that FAS also serves as a tumor suppressor pathway.

## Disorders of Regulatory T Cells

*Immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome* is a severe disorder of immune function due to loss of Treg differentiation. Patients with IPEX syndrome usually present during infancy with severe enteropathy, insulin-dependent type I DM, and dermatitis (Ochs et al., 2007; Verbsky and Chatila, 2013). The GI disease produces intractable diarrhea and malabsorption causing failure to thrive. The histopathology of the enteropathy includes villous atrophy and lymphocytic infiltration of the mucosa.

The type I DM in IPEX syndrome can be present as early as birth and is often difficult to control with typical insulin regimens. Dermatitis is a generalized eczematous-like rash. Additional autoimmune features include cytopenias, thyroid disease, renal disease, and hepatitis. Immunologic laboratory findings include eosinophilia and hypergammaglobulinemia including elevation of serum IgE, as well as the presence of a variety of autoantibodies (Tsuda et al., 2010). Early recognition of this syndrome is crucial as mortality is extremely high without therapy, which initially consists of aggressive immunosuppression to control the autoreactivity and hematopoietic stem-cell transplantation to provide curative immune reconstitution and a source of Tregs (Rao et al., 2007).

The underlying genetic basis for IPEX is an X-linked recessive defect in the gene encoding FOXP3, a member of the forkhead/winged helix family of transcription factors located at Xp11.23. There have been a wide range of mutations found including single base substitutions, deletions, and splicing mutations. Defective FOXP3 expression leads to a defect in Treg production, and this is reflected by the virtual absence of circulating CD4<sup>+</sup>/CD25<sup>+</sup>/FOXP3<sup>+</sup> T cells in these patients. Additional patients with hypomorphic variants of FOXP3 deficiency have been described, and these patients may express normal, or even increased, levels of protein that is dysfunctional. Such patients frequently present later in life with milder symptoms but may also present during the neonatal period. Therefore the presence of FOXP3<sup>+</sup> T cells in the periphery does not exclude IPEX syndrome, and patients suspected of having this disorder should have genetic testing.

The actual mechanism(s) mediating the immunosuppressive activity of Tregs in humans is not fully understood, although it appears to involve both inhibition by cell–cell contact and the secretion of immunomodulatory cytokines including transforming growth factor-β and IL-10. The consistently targeted organs in the IPEX syndrome suggest that Treg function is particularly important in developing and maintaining self-tolerance in the GI tract, (selected) endocrine organs, blood cells, and skin. However, owing to the severity of this syndrome, it is unclear if autoimmunity to other target organs could arise over time in this patient population.

*IPEX-like syndromes* caused by genetic defects in other genes important for Treg differentiation highlight the importance of these cells for maintenance of T-cell tolerance (Verbsky and Chatila, 2013). The first involves an autosomal-recessive defect in the gene encoding the CD25 protein, a component of the high-affinity IL-2 receptor heterotrimer (IL-2 receptor alpha chain). In this disorder, patients not only have an autoimmune phenotype that resembles IPEX but also develop a SCID-like picture with early onset of opportunistic infections (Caudy et al., 2007). Findings in the murine model of CD25 deficiency suggest that Treg development is normal but the survival, maintenance, and competitive fitness of these cells are defective due to the absence of IL-2 signaling associated with the defective IL-2 receptor.

Another overlap syndrome is an autosomal-recessive disorder due to LOF mutations in the gene encoding signal transducer and activator of transcription (STAT) 5B (STAT5B), which is a transcription factor necessary for IL-2 signaling. In this disorder, patients also have an IPEX-like clinical picture together with a marked immune deficiency, growth hormone–resistant dwarfism, and other clinical features found in IPEX syndrome including eczema and endocrinopathy with less prominent enteropathy (Bernasconi et al., 2006; Hwa et al., 2007; Kanai et al., 2012). Patients with STAT5B deficiency have prominent lung disease with lymphoid interstitial pneumonitis, and the mechanism of this is uncertain. The growth defect in STAT5B deficiency is due to impaired growth hormone signaling, which also uses this transcription factor. Studies in these very rare patients have demonstrated decreased numbers of circulating Tregs that expressed diminished levels of FOXP3 and were functionally ineffective when tested in vitro.

*LRBA protein deficiency with autoantibodies (LATAIE)* is caused by LOF autosomal-recessive LOF mutations in the LRBA gene and manifests clinically with hypogammaglobulinemia, autoimmune cytopenias, splenomegaly, and lymphadenopathy. LATAIE disease shows LRBA-deficient patients frequently present with organ-specific autoimmunity including severe inflammatory bowel disease, thyroiditis, and arthritis (Kostel Bal et al., 2017;

Levy et al., 2016; Lopez-Herrera et al., 2012; Alangari et al., 2012; Alkhairy et al., 2016). The immune dysregulation in LRBA deficiency is thought to be due to increased turnover of CTLA4 protein and impaired trafficking and cell-surface expression CTLA4 (Lo et al., 2015). CTLA4 is expressed by Tregs and activated T cells and is a key inhibitory checkpoint protein that inhibits T-cell activation. Immunologic abnormalities in this disease include defects in class-switched B cells and increased T-cell apoptosis similar to ALPS (Alkhairy et al., 2016). Symptoms of immune dysregulation in these patients are responsive to abatacept therapy (soluble CTLA4/IgG1 fusion protein), offering proof of concept for the ability to translate molecular targets into personalized therapies for patients with primary immunodeficiency (Lo et al., 2015). Chloroquine increased the ability of CTLA4 to traffic to the cell surface of T cell in vitro, and partially restore protein expression on Tregs and activated T cells, suggesting this is another potential therapeutic option (Lo et al., 2015).

*CTLA4 haploinsufficiency with autoimmune infiltration (CHAI)* is an autosomal-dominant immune dysregulation syndrome due to heterozygous LOF mutations in the gene encoding CTLA4 (Schubert et al., 2014; Kuehn et al., 2014). CHAI is a monogenic disease with incomplete penetrance. In mice, deficiency of CTLA4 results in a lethal autoimmune phenotype. In humans, CHAI patients have some clinical features resembling ALPS, but with other autoimmune manifestations, similar to LATAIE patients. The majority of CHAI patients present with symptoms of immune dysregulation including diarrhea/enteropathy (78%), hypogammaglobulinemia (76%), granulomatous lymphocytic interstitial lung disease (66%), respiratory infections (57%), organ infiltration (50%), and splenomegaly (50%) (Schubert et al., 2014). Other autoimmune manifestations include ITP, AIHA, psoriasis and other skin disease, autoimmune thyroiditis, and autoimmune arthritis. Lab findings in CHAI are normal-to-low IgG and/or IgM, normal IgA, decreased or normal antibody response to polysaccharide vaccines, increased double-negative T cells, and decreased FOXP3/CD25 expression on Tregs with decreased Treg suppression activity. As both CHAI and LATAIE are diseases caused by CTLA4 checkpoint deficiency, their manifestations are quite similar. However, CHAI is present in older children or young adults, while LATAIE frequently has an onset in preschool children. Abatacept and sirolimus, a rapamycin inhibitor, have been used for the treatment of autoimmunity and systemic inflammation in CHAI patients with some improvement of their symptoms (Lee et al., 2016a).

## GAIN-OF-FUNCTION DISORDERS OF CYTOKINE SIGNALING

The development, homeostasis, and function of immune cells are critically dependent on cytokine signaling. Defective cytokine signaling has been well recognized to lead to impaired immunity, such as SCID associated with common-gamma chain deficiency. More recently, GOF variants in cytokine signaling have been discovered as a cause of primary immunodeficiency and autoimmunity. Most cytokine signals are mediated through the janus kinase (JAK)/STAT pathway (O'Shea et al., 2013). Following binding of cytokines to their receptors, phosphorylation of JAKs leads to the recruitment and activation of STAT molecules which then form homo- or heterodimers and translocate to the nucleus to induce transcriptional changes. Genetic variants causing a GOF in these pathways have been discovered in genes encoding STAT and JAK proteins.

*STAT1 GOF* due to heterozygous mutations in the *STAT1* gene was originally described in association with susceptibility to mycobacterial infection due to defective Th17-mediated immunity (Liu et al., 2011; van de Veerdonk et al., 2011). Functional assays demonstrated hyperphosphorylation of STAT1 with cytokine activation of primary immune cells. Identification of additional patients and careful analysis of the clinical phenotype have revealed a wide spectrum of disease, including prominent autoimmunity. A recent analysis of 274 patients from 167 kindreds demonstrates a wide variety of other associated clinical symptoms including recurrent infections caused by bacteria, mycobacteria, and viruses, autoimmunity (37%), increased risk of aneurysms (6%), and tumors (6%), and asthma/eczema (20%) (Toubiana et al., 2016). Autoimmune thyroid disease was seen in 22% of the patients, and other autoimmune features include endocrinopathies (4%), skin disease (10%), GI disease (4%), autoimmune hepatitis (2%), and autoimmune cytopenias (4%).

T cells from STAT1 GOF patients have increased expression of PD-L1 on their naïve T cells which may inhibit Th17 lineage commitment (Romberg et al., 2013). In addition, STAT1 GOF increases B-cell apoptosis, which may contribute to progressive loss of B cells and susceptibility to infection. Autoimmunity is unlikely to be associated with a defect in Th17 differentiation, since other patients with monogenic defects in IL-17A/F do not have autoimmunity. In addition, it has been reported that STAT1 GOF does not alter Tregs, although disease similar to IPEX has been reported in patients (Uzel et al., 2013), thus the mechanism of autoimmunity with STAT1 GOF is uncertain.

Patients with STAT1 GOF are treated with antimicrobial prophylaxis and specific therapy for their autoimmune manifestations. An international cohort of 15 patients who received HCT was recently reported (Leiding et al., 2017). Retrospective analysis of these patients treated with a variety of conditioning regimens demonstrated 40% survival, with poor event-free survival and frequent graft failure, suggesting that HCT has significant risk in this population. Targeted therapy with the JAK inhibitor ruxolitinib has been reported to be well tolerated and leads to the improvement of clinical symptoms including autoimmunity in a small number of patients (Weinacht et al., 2017; Higgins et al., 2015), suggesting that this targeted approach may be more successful.

STAT3 GOF syndrome is caused by heterozygous genetic variants in the gene encoding STAT3 (Vogel et al., 2015; Milner et al., 2015; Flanagan et al., 2014; Haapaniemi et al., 2015). Patients with STAT3 GOF present at a young age, typically less than 4 years, with poly-autoimmunity. Majority of the patients exhibit lymphoproliferation with hepatosplenomegaly and/or lymphadenopathy, autoimmune cytopenias, and many patients have been described to have GI tract disease including enteropathy and celiac-like disease. Additional autoimmune manifestations that have been described include skin disease (atopic dermatitis and scleroderma), autoimmune hepatitis, neonatal-onset type I DM, lymphocytic interstitial lung disease, and arthritis. Most patients have hypogammaglobulinemia, and some demonstrated susceptibility to a variety of infections, including viral upper respiratory tract infections, sepsis, candidiasis, herpes zoster, osteomyelitis, mycobacterial disease, and others, although the contribution of immunosuppressive therapy versus intrinsic risk of infection with STAT3 GOF is unclear. Other features of STAT3 GOF include postnatal short stature and an increased risk of malignancy with reports of associated Hodgkin lymphoma and large granular lymphocytic leukemia (Milner et al., 2015; Haapaniemi et al., 2015).

STAT3 is an important transcriptional regulator of the balance between Tregs and Th17 cells, with inhibitory effects on peripheral Treg differentiation and stimulation of Th17 immunity. Patients with STAT3 GOF have a decreased percentage of Tregs, and there is evidence that these cells from patients have decreased suppressive capacity, likely due to observed decreased level of the IL-2 high-affinity CD25 receptor on Tregs. Defects in T cell IL-17 production have been reported; however, other patients have normal production of IL-17 by CD4<sup>+</sup> T cells after stimulation (Haapaniemi et al., 2015; Milner et al., 2015). These studies have been limited to peripheral blood samples, and it is unknown whether tissue-resident immune cells (T cells and innate lymphoid cells) have an altered IL-17 phenotype. Additional immunologic abnormalities assessed in small numbers of patients include defects in B-cell maturation and dendritic cell subsets (Haapaniemi et al., 2015). Interestingly, patients with STAT3 GOF do not exhibit constitutive phosphorylation of STAT3 in primary immune cells, and the majority of GOF defects in STAT3 do not cause hyperphosphorylation of the protein but result in increased transcriptional activity of the activated protein and in some cases delayed dephosphorylation of STAT3. Targeting the initial activation of STAT3 using therapy with an anti-IL-6R biologic therapy or JAK inhibitors has been reported to lead to improvement of autoimmunity in some patients. HCT of patients with STAT3 GOF has also been reported with varying success.

JAK1 GOF was recently described in a single family who presented with liver cysts, atopic dermatitis, eosinophilia with infiltration of the GI tract, hepatosplenomegaly, thyroiditis, and failure to thrive (Del Bel et al., 2017). JAK1 is downstream of multiple cytokine receptors, including those utilizing the common gamma chain (e.g., IL-2, -7, -9, -15, and -21) the gp130 receptor family (including IL-6 family members), type I interferons, and IL-10. Immune cells from affected patients had increased phosphorylation of STAT1 and STAT3 after cytokine activation. The immunologic defects in this disease are unknown, and the discovery of additional patients will be important to uncover the immunologic and clinical phenotype of this new disorder. Treatment with a JAK inhibitor was reported to be effective with clinical resolution of dermatitis and hepatosplenomegaly (Del Bel et al., 2017).

## INNATE IMMUNE DEFECTS

While the majority of primary immunodeficiencies associated with autoimmunity are those leading to disruption of adaptive T and B lymphocytes, there is also an increased risk of autoimmune disease with some defects of innate immunity. Complement deficiencies, predominantly those without a high risk of infection, including C1q, C1r, C1s, C2, and C4, predispose to autoimmunity, particularly systemic lupus erythematosus, and are discussed in a separate chapter in this text.

Chronic granulomatous disease (CGD) includes a group of genetic defects affecting the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system in neutrophils (and other cells) that results in recurrent bacterial and fungal infections. NADPH oxidase is the enzyme complex that produces superoxide and other reactive

oxygen species upon neutrophil ingestion of microbes required for killing a variety of bacteria and fungi. This disorder is also characterized by dysregulated inflammation resulting in an inflammatory process that typically affects the GI and genitourinary tracts often producing strictures (Rosenzweig, 2008; Kang et al., 2011). In addition, these patients can develop inflammatory bowel disease that resembles Crohn's disease. Up to 80% of the CGD patients have some inflammatory GI manifestations, with up to 30% having IBD-like illness, and in some instances, this is the initial clinical presentation for CGD (Uzzan et al., 2016). The histology of the GI lesions in CGD differs from Crohn's disease with the development of well-defined granulomata that are surrounded by dense lymphocytic infiltrates. CGD patients may also develop chorioretinal lesions that appear as asymptomatic "punch-out" retinal scars localized along retinal vessels.

The mechanism underlying the increased inflammatory responses is unknown and has been observed more commonly in CGD patients with defects in the membrane bound components of NADPH oxidase (gp91phox, p22phox) that are generally associated with a greater degree of compromised NADPH oxidase function. This suggests that the oxidase pathway is not only involved in host protection but also in controlling inflammation. However, the actual explanation for the inflammatory responses is more complicated since X-linked carriers of CGD, who show mosaicism in their neutrophils, are at increased risk for discoid lupus, oral ulcers, and other evidence of immune dysregulation, whereas the majority of carriers do not have an increased risk for infections.

## CONCLUSION

Although at one time a perplexing paradox, coexistent autoimmunity and primary immunodeficiency now stand as two sides of the coin of immunologic dysregulation. In most of the disorders affecting cellular and humoral immunity, including combined immunodeficiencies, autoimmunity occurs primarily as a consequence of the impairment of one of the more critical checkpoints in the development/maintenance of immune cells leading to defects in tolerance. Immunodeficiencies manifest primarily by antibody deficiencies, including CVID and HIGM syndrome, highlight the importance of proper differentiation of B cells in the pathogenesis of autoimmunity. The prominent autoimmunity in monogenic disorders of immune dysregulation, including the key checkpoints in T-cell tolerance such as APECED, ALPS, and disorders of Tregs, reveals the importance of proper T-cell selection and control of aberrant T-cell activation. GOF disorders in cytokine signaling lead to autoimmunity through skewing of immune responses that are typically tightly regulated by the cytokine milieu and signaling patterns. Finally, defects in neutrophil oxidase (CGD) associated with autoimmunity highlight the potential for chronic infection and uncontrolled inflammation to lead to autoimmune complications. Importantly, all of the immunodeficiency disorders highlighted here can be quite heterogeneous with regard to their clinical phenotype and whether or not they are complicated by autoimmunity. This suggests that additional genetic, epigenetic, and environmental factors yet to be elucidated likely contribute to the clinical phenotype of these primary immunodeficiencies.

In addition to instructing our understanding of how autoimmunity develops, patients with primary immunodeficiency and autoimmunity present a clinical dilemma for clinicians attempting to balance therapies that will alleviate autoimmune symptoms while not rendering affected individuals more susceptible to infection. With the identification of molecular defects underlying many primary immunodeficiencies, it is now possible to provide targeted therapies, as highlighted above for patients with APDS, LRBA, CTLA4, STAT1/3 GOF, and JAK1 GOF-associated disease. Over the past decade, there has also been increasing use of early transplantation (HCT) in patients with primary immunodeficiency and immune dysregulation, offering the potential for a definitive "cure" for both immunodeficiency and autoimmunity in these patients, with the caveat that this procedure has significant risks and varying success in different disorders. Thus a better understanding of the molecular and cellular mechanisms of autoimmunity in primary immunodeficiencies has the potential to lead to enhanced therapeutic options for these patients.

## References

- Abdollahpour, H., Appaswamy, G., Kotlarz, D., Diestelhorst, J., Beier, R., Schaffer, A.A., et al., 2012. The phenotype of human STK4 deficiency. *Blood* 119, 3450–3457.
- Abolhassani, H., Wang, N., Aghamohammadi, A., Rezaei, N., Lee, Y.N., Frugoni, F., et al., 2014. A hypomorphic recombination-activating gene 1 (RAG1) mutation resulting in a phenotype resembling common variable immunodeficiency. *J. Allergy Clin. Immunol.* 134, 1375–1380.

- Abolhassani, H., Gharib, B., Shahinpour, S., Masoom, S.N., Havaei, A., Mirminachi, B., et al., 2015. Autoimmunity in patients with selective IgA deficiency. *J. Investig. Allergol. Clin. Immunol.* 25, 112–119.
- Aghamohammadi, A., Mohammadi, J., Parvaneh, N., Rezaei, N., Moin, M., Espanol, T., et al., 2008. Progression of selective IgA deficiency to common variable immunodeficiency. *Int. Arch. Allergy Immunol.* 147, 87–92.
- Al-Muhsen, S.Z., 2010. Delayed presentation of severe combined immunodeficiency due to prolonged maternal T cell engraftment. *Ann. Saudi Med.* 30, 239–242.
- Alangari, A., Alsultan, A., Adly, N., Massaad, M.J., Kiani, I.S., Aljebreen, A., et al., 2012. LPS-responsive beige-like anchor (LRBA) gene mutation in a family with inflammatory bowel disease and combined immunodeficiency. *J. Allergy Clin. Immunol.* 130, 481–488.e2.
- Alkhairy, O.K., Abolhassani, H., Rezaei, N., Fang, M., Andersen, K.K., Chavoshzadeh, Z., et al., 2016. Spectrum of phenotypes associated with mutations in LRBA. *J. Clin. Immunol.* 36, 33–45.
- Angulo, I., Vadas, O., Garcon, F., Banham-Hall, E., Plagnol, V., Leahy, T.R., et al., 2013. Phosphoinositide 3-kinase delta gene mutation predisposes to respiratory infection and airway damage. *Science* 342, 866–871.
- Avila, E.M., Uzel, G., Hsu, A., Milner, J.D., Turner, M.L., Pittaluga, S., et al., 2010. Highly variable clinical phenotypes of hypomorphic RAG1 mutations. *Pediatrics* 126, e1248–e1252.
- Barmettler, S., Otani, I.M., Minhas, J., Abraham, R.S., Chang, Y., Dorsey, M.J., et al., 2017. Gastrointestinal manifestations in X-linked agammaglobulinemia. *J. Clin. Immunol.* 37, 287–294.
- Becker-Herman, S., Meyer-Bahlburg, A., Schwartz, M.A., Jackson, S.W., Hudkins, K.L., Liu, C., et al., 2011. WASp-deficient B cells play a critical, cell-intrinsic role in triggering autoimmunity. *J. Exp. Med.* 208, 2033–2042.
- Bernasconi, A., Marino, R., Ribas, A., Rossi, J., Ciaccio, M., Oleastro, M., et al., 2006. Characterization of immunodeficiency in a patient with growth hormone insensitivity secondary to a novel STAT5b gene mutation. *Pediatrics* 118, e1584–e1592.
- Bogaert, D.J., Dullaers, M., Lambrecht, B.N., Vermaelen, K.Y., De Baere, E., Haerynck, F., 2016. Genes associated with common variable immunodeficiency: one diagnosis to rule them all? *J. Med. Genet.* 53, 575–590.
- Bonilla, F.A., Khan, D.A., Ballas, Z.K., Chinen, J., Frank, M.M., Hsu, J.T., et al., 2015. Practice parameter for the diagnosis and management of primary immunodeficiency. *J. Allergy Clin. Immunol.* 136, 1186–1205.e1–78.
- Bonilla, F.A., Barlan, I., Chapel, H., Costa-Carvalho, B.T., Cunningham-Rundles, C., de la Morena, M.T., et al., 2016. International consensus document (ICON): common variable immunodeficiency disorders. *J. Allergy Clin. Immunol. Pract.* 4, 38–59.
- Bouma, G., Carter, N.A., Recher, M., Malinova, D., Adriani, M., Notarangelo, L.D., et al., 2014. Exacerbated experimental arthritis in Wiskott-Aldrich syndrome protein deficiency: modulatory role of regulatory B cells. *Eur. J. Immunol.* 44, 2692–2702.
- Buchbinder, D., Nadeau, K., Nugent, D., 2011. Monozygotic twin pair showing discordant phenotype for X-linked thrombocytopenia and Wiskott-Aldrich syndrome: a role for epigenetics? *J. Clin. Immunol.* 31, 773–777.
- Cassani, B., Poliani, P.L., Moratto, D., Sobacchi, C., Marrella, V., Imperatori, L., et al., 2010. Defect of regulatory T cells in patients with Omenn syndrome. *J. Allergy Clin. Immunol.* 125, 209–216.
- Castigli, E., Wilson, S.A., Garibyan, L., Rachid, R., Bonilla, F., Schneider, L., et al., 2005. TACI is mutant in common variable immunodeficiency and IgA deficiency. *Nat. Genet.* 37, 829–834.
- Caudy, A.A., Reddy, S.T., Chatila, T., Atkinson, J.P., Verbsky, J.W., 2007. CD25 deficiency causes an immune dysregulation, polyendocrinopathy, enteropathy, X-linked-like syndrome, and defective IL-10 expression from CD4 lymphocytes. *J. Allergy Clin. Immunol.* 119, 482–487.
- Chan, A.Y., Punwani, D., Kadlecik, T.A., Cowan, M.J., Olson, J.L., Mathes, E.F., et al., 2016. A novel human autoimmune syndrome caused by combined hypomorphic and activating mutations in ZAP-70. *J. Exp. Med.* 213, 155–165.
- Chapel, H., Lucas, M., Patel, S., Lee, M., Cunningham-Rundles, C., Resnick, E., et al., 2012. Confirmation and improvement of criteria for clinical phenotyping in common variable immunodeficiency disorders in replicate cohorts. *J. Allergy Clin. Immunol.* 130, 1197–1198.e9.
- Conley, M.E., Cooper, M.D., 1981. Immature IgA B cells in IgA-deficient patients. *N. Engl. J. Med.* 305, 495–497.
- Coulter, T.I., Chandra, A., Bacon, C.M., Babar, J., Curtis, J., Screamton, N., et al., 2017. Clinical spectrum and features of activated phosphoinositide 3-kinase delta syndrome: a large patient cohort study. *J. Allergy Clin. Immunol.* 139, 597–606.e4.
- Crestani, E., Volpi, S., Candotti, F., Giliani, S., Notarangelo, L.D., Chu, J., et al., 2015. Broad spectrum of autoantibodies in patients with Wiskott-Aldrich syndrome and X-linked thrombocytopenia. *J. Allergy Clin. Immunol.* 136, 1401–1404.e1–3.
- Del Bel, K.L., Ragotte, R.J., Saferali, A., Lee, S., Vercauteren, S.M., Mostafavi, S.A., et al., 2017. JAK1 gain-of-function causes an autosomal dominant immune dysregulatory and hypereosinophilic syndrome. *J. Allergy Clin. Immunol.* 139, 2016–2020.e5.
- Dorsey, M.J., Dvorak, C.C., Cowan, M.J., Puck, J.M., 2017. Treatment of infants identified as having severe combined immunodeficiency by means of newborn screening. *J. Allergy Clin. Immunol.* 139, 733–742.
- Edwards, E., Razvi, S., Cunningham-Rundles, C., 2004. IgA deficiency: clinical correlates and responses to pneumococcal vaccine. *Clin. Immunol.* 111, 93–97.
- Elgizouli, M., Lowe, D.M., Speckmann, C., Schubert, D., Hulsdunker, J., Eskandarian, Z., et al., 2016. Activating PI3Kdelta mutations in a cohort of 669 patients with primary immunodeficiency. *Clin. Exp. Immunol.* 183, 221–229.
- Elkaim, E., Neven, B., Bruneau, J., Mitsui-Sekinaka, K., Stanislas, A., Heurtier, L., et al., 2016. Clinical and immunologic phenotype associated with activated phosphoinositide 3-kinase delta syndrome 2: a cohort study. *J. Allergy Clin. Immunol.* 138, 210–218.e9.
- Ferrando-Martinez, S., Lorente, R., Gurbido, D., De Jose, M.I., Leal, M., Munoz-Fernandez, M.A., et al., 2014. Low thymic output, peripheral homeostasis deregulation, and hastened regulatory T cells differentiation in children with 22q11.2 deletion syndrome. *J. Pediatr.* 164, 882–889.
- Ferre, E.M., Rose, S.R., Rosenzweig, S.D., Burbelo, P.D., Romito, K.R., Niemela, J.E., et al., 2016. Redefined clinical features and diagnostic criteria in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *JCI Insight*. 1.
- Feske, S., Picard, C., Fischer, A., 2010. Immunodeficiency due to mutations in ORAI1 and STIM1. *Clin. Immunol.* 135, 169–182.
- Flanagan, S.E., Haapaniemi, E., Russell, M.A., Caswell, R., Lango Allen, H., De Franco, E., et al., 2014. Activating germline mutations in STAT3 cause early-onset multi-organ autoimmune disease. *Nat. Genet.* 46, 812–814.
- Gadola, S.D., Moins-Tisserenc, H.T., Trowsdale, J., Gross, W.L., Cerundolo, V., 2000. TAP deficiency syndrome. *Clin. Exp. Immunol.* 121, 173–178.

- Gathmann, B., Mahlaoui, N., Ceredih, Gerard, L., Oksenhendler, E., Warnatz, K., et al., 2014. Clinical picture and treatment of 2212 patients with common variable immunodeficiency. *J. Allergy Clin. Immunol.* 134, 116–126.
- Geha, R.S., Notarangelo, L.D., Casanova, J.L., Chapel, H., Conley, M.E., Fischer, A., et al., 2007. Primary immunodeficiency diseases: an update from the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee. *J. Allergy Clin. Immunol.* 120, 776–794.
- Gennery, A.R., 2012. Immunological aspects of 22q11.2 deletion syndrome. *Cell. Mol. Life Sci.* 69, 17–27.
- Gennery, A.R., Slatter, M.A., Rice, J., Hoefsloot, L.H., Barge, D., McLean-Tooke, A., et al., 2008. Mutations in CHD7 in patients with CHARGE syndrome cause T-B+ natural killer cell + severe combined immune deficiency and may cause Omenn-like syndrome. *Clin. Exp. Immunol.* 153, 75–80.
- Gennery, A.R., Slatter, M.A., Grandin, L., Taupin, P., Cant, A.J., Veys, P., et al., 2010. Transplantation of hematopoietic stem cells and long-term survival for primary immunodeficiencies in Europe: entering a new century, do we do better? *J. Allergy Clin. Immunol.* 126, 602–610.e1–11.
- Grimbacher, B., Hutloff, A., Schlesier, M., Glocker, E., Warnatz, K., Drager, R., et al., 2003. Homozygous loss of ICOS is associated with adult-onset common variable immunodeficiency. *Nat. Immunol.* 4, 261–268.
- Grunebaum, E., Cohen, A., Roifman, C.M., 2013. Recent advances in understanding and managing adenosine deaminase and purine nucleoside phosphorylase deficiencies. *Curr. Opin. Allergy Clin. Immunol.* 13, 630–638.
- Haapaniemi, E.M., Kaustio, M., Rajala, H.L., van Adrichem, A.J., Kainulainen, L., Glumoff, V., et al., 2015. Autoimmunity, hypogammaglobulinemia, lymphoproliferation, and mycobacterial disease in patients with activating mutations in STAT3. *Blood* 125, 639–648.
- Hanson, E.P., Monaco-Shawver, L., Solt, L.A., Madge, L.A., Banerjee, P.P., May, M.J., et al., 2008. Hypomorphic nuclear factor-kappaB essential modulator mutation database and reconstitution system identifies phenotypic and immunologic diversity. *J. Allergy Clin. Immunol.* 122, 1169–1177.e16.
- Herve, M., Isnardi, I., Ng, Y.S., Bussel, J.B., Ochs, H.D., Cunningham-Rundles, C., et al., 2007. CD40 ligand and MHC class II expression are essential for human peripheral B cell tolerance. *J. Exp. Med.* 204, 1583–1593.
- Higgins, E., Al Shehri, T., McAleer, M.A., Conlon, N., Feighery, C., Lilic, D., et al., 2015. Use of ruxolitinib to successfully treat chronic mucocutaneous candidiasis caused by gain-of-function signal transducer and activator of transcription 1 (STAT1) mutation. *J. Allergy Clin. Immunol.* 135, 551–553.
- Hirbod-Mobarakeh, A., Aghamohammadi, A., Rezaei, N., 2014. Immunoglobulin class switch recombination deficiency type 1 or CD40 ligand deficiency: from bedside to bench and back again. *Expert Rev. Clin. Immunol.* 10, 91–105.
- Hoshino, A., Okada, S., Yoshida, K., Nishida, N., Okuno, Y., Ueno, H., et al., 2017. Abnormal hematopoiesis and autoimmunity in human subjects with germline IKZF1 mutations. *J. Allergy Clin. Immunol.* 140, 223–231.
- Howard, V., Greene, J.M., Pahwa, S., Winkelstein, J.A., Boyle, J.M., Kocak, M., et al., 2006. The health status and quality of life of adults with X-linked agammaglobulinemia. *Clin. Immunol.* 118, 201–208.
- Hwa, V., Camacho-Hubner, C., Little, B.M., David, A., Metherell, L.A., El-Khatib, N., et al., 2007. Growth hormone insensitivity and severe short stature in siblings: a novel mutation at the exon 13-intron 13 junction of the STAT5b gene. *Horm Res.* 68, 218–224.
- Jacob, C.M., Pastorino, A.C., Fahl, K., Carneiro-Sampaio, M., Monteiro, R.C., 2008. Autoimmunity in IgA deficiency: revisiting the role of IgA as a silent housekeeper. *J. Clin. Immunol.* 28 (Suppl 1), S56–S61.
- Jesus, A.A., Duarte, A.J., Oliveira, J.B., 2008. Autoimmunity in hyper-IgM syndrome. *J. Clin. Immunol.* 28 (Suppl 1), S62–S66.
- Kanai, T., Jenks, J., Nadeau, K.C., 2012. The STAT5b pathway defect and autoimmunity. *Front. Immunol.* 3, 234.
- Kang, E.M., Marciano, B.E., DeRavin, S., Zarembra, K.A., Holland, S.M., Malech, H.L., 2011. Chronic granulomatous disease: overview and hematopoietic stem cell transplantation. *J. Allergy Clin. Immunol.* 127, 1319–1326. quiz 27–28.
- Kil, L.P., de Bruijn, M.J., van Nimwegen, M., Corneth, O.B., van Hamburg, J.P., Dingjan, G.M., et al., 2012. Btk levels set the threshold for B-cell activation and negative selection of autoreactive B cells in mice. *Blood* 119, 3744–3756.
- Kisand, K., Peterson, P., 2011. Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy: known and novel aspects of the syndrome. *Ann. N.Y. Acad. Sci.* 1246, 77–91.
- Klinker, M.W., Lundy, S.K., 2012. Multiple mechanisms of immune suppression by B lymphocytes. *Mol. Med.* 18, 123–137.
- Kostel Bal, S., Haskologlu, S., Serwas, N.K., Islamoglu, C., Aytekin, C., Kendirli, T., et al., 2017. Multiple presentations of LRBA deficiency: a single-center experience. *J. Clin. Immunol.* 37, 790–800.
- Kuehn, H.S., Ouyang, W., Lo, B., Deenick, E.K., Niemela, J.E., Avery, D.T., et al., 2014. Immune dysregulation in human subjects with heterozygous germline mutations in CTLA4. *Science* 345, 1623–1627.
- Kuehn, H.S., Boisson, B., Cunningham-Rundles, C., Reichenbach, J., Stray-Pedersen, A., Gelfand, E.W., et al., 2016. Loss of B cells in patients with heterozygous mutations in IKAROS. *N. Engl. J. Med.* 374, 1032–1043.
- Laakso, S.M., Laurinolli, T.T., Rossi, L.H., Lehtoviita, A., Sairanen, H., Perheentupa, J., et al., 2010. Regulatory T cell defect in APECED patients is associated with loss of naive FOXP3(+) precursors and impaired activated population. *J. Autoimmun.* 35, 351–357.
- Lacruz, R.S., Feske, S., 2015. Diseases caused by mutations in ORAI1 and STIM1. *Ann. N.Y. Acad. Sci.* 1356, 45–79.
- Lee, S., Moon, J.S., Lee, C.R., Kim, H.E., Baek, S.M., Hwang, S., et al., 2016a. Abatacept alleviates severe autoimmune symptoms in a patient carrying a de novo variant in CTLA-4. *J. Allergy Clin. Immunol.* 137, 327–330.
- Lee, Y.N., Frugoni, F., Dobbs, K., Tirosh, I., Du, L., Ververs, F.A., et al., 2016b. Characterization of T and B cell repertoire diversity in patients with RAG deficiency. *Sci. Immunol.* 1, eaah6109.
- Leiding, J.W., Okada, S., Hagin, D., Abinun, M., Shcherbina, A., Balashov, D.N., et al., 2017. Hematopoietic stem cell transplantation in patients with gain-of-function signal transducer and activator of transcription 1 mutations. *J. Allergy Clin. Immunol.* 141, 704–717.e5.
- Lenardo, M.J., Oliveira, J.B., Zheng, L., Rao, V.K., 2010. ALPS-ten lessons from an international workshop on a genetic disease of apoptosis. *Immunity* 32, 291–295.
- Levy, J., Espanol-Boren, T., Thomas, C., Fischer, A., Tovo, P., Bordigoni, P., et al., 1997. Clinical spectrum of X-linked hyper-IgM syndrome. *J. Pediatr.* 131, 47–54.

- Levy, E., Stolzenberg, M.C., Bruneau, J., Breton, S., Neven, B., Sauvion, S., et al., 2016. LRBA deficiency with autoimmunity and early onset chronic erosive polyarthritis. *Clin. Immunol.* 168, 88–93.
- Liu, L., Okada, S., Kong, X.F., Kreins, A.Y., Cypowij, S., Abhyankar, A., et al., 2011. Gain-of-function human STAT1 mutations impair IL-17 immunity and underlie chronic mucocutaneous candidiasis. *J. Exp. Med.* 208, 1635–1648.
- Liu, Q., Wang, Y.P., Liu, Q., Zhao, Q., Chen, X.M., Xue, X.H., et al., 2017. Novel compound heterozygous mutations in ZAP70 in a Chinese patient with leaky severe combined immunodeficiency disorder. *Immunogenetics.* 69, 199–209.
- Lo, B., Zhang, K., Lu, W., Zheng, L., Zhang, Q., Kanelloupolou, C., et al., 2015. Autoimmune disease. Patients with LRBA deficiency show CTLA4 loss and immune dysregulation responsive to abatacept therapy. *Science* 349, 436–440.
- Lopez-Herrera, G., Tampella, G., Pan-Hammarstrom, Q., Herholz, P., Trujillo-Vargas, C.M., Phadwal, K., et al., 2012. Deleterious mutations in LRBA are associated with a syndrome of immune deficiency and autoimmunity. *Am. J. Hum. Genet.* 90, 986–1001.
- Lucas, C.L., Kuehn, H.S., Zhao, F., Niemela, J.E., Deenick, E.K., Palendira, U., et al., 2014. Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110delta result in T cell senescence and human immunodeficiency. *Nat. Immunol.* 15, 88–97.
- Lucas, C.L., Chandra, A., Nejentsev, S., Condliffe, A.M., Okkenhaug, K., 2016. PI3Kdelta and primary immunodeficiencies. *Nat. Rev. Immunol.* 16, 702–714.
- Maglione, P.J., 2016. Autoimmune and lymphoproliferative complications of common variable immunodeficiency. *Curr. Allergy Asthma Rep.* 16, 19.
- Marangoni, F., Trifari, S., Scaramuzza, S., Panaroni, C., Martino, S., Notarangelo, L.D., et al., 2007. WASP regulates suppressor activity of human and murine CD4(+)CD25(+)FOXP3(+) natural regulatory T cells. *J. Exp. Med.* 204, 369–380.
- Markert, M.L., 1991. Purine nucleoside phosphorylase deficiency. *Immunodefic. Rev.* 3, 45–81.
- Markert, M.L., Alexieff, M.J., Li, J., Sarzotti, M., Ozaki, D.A., Devlin, B.H., et al., 2004. Complete DiGeorge syndrome: development of rash, lymphadenopathy, and oligoclonal T cells in 5 cases. *J. Allergy Clin. Immunol.* 113, 734–741.
- Markert, M.L., Devlin, B.H., Alexieff, M.J., Li, J., McCarthy, E.A., Gupton, S.E., et al., 2007. Review of 54 patients with complete DiGeorge anomaly enrolled in protocols for thymus transplantation: outcome of 44 consecutive transplants. *Blood* 109, 4539–4547.
- Marrella, V., Maina, V., Villa, A., 2011. Omenn syndrome does not live by V(D)J recombination alone. *Curr. Opin. Allergy Clin. Immunol.* 11, 525–531.
- Martinez-Gallo, M., Radigan, L., Almejun, M.B., Martinez-Pomar, N., Matamoros, N., Cunningham-Rundles, C., 2013. TACI mutations and impaired B-cell function in subjects with CVID and healthy heterozygotes. *J. Allergy Clin. Immunol.* 131, 468–476.
- McCarl, C.A., Picard, C., Khalil, S., Kawasaki, T., Rother, J., Papulos, A., et al., 2009. ORAI1 deficiency and lack of store-operated Ca<sup>2+</sup> entry cause immunodeficiency, myopathy, and ectodermal dysplasia. *J. Allergy Clin. Immunol.* 124, 1311–1318.e7.
- Milner, J.D., Vogel, T.P., Forbes, L., Ma, C.A., Stray-Pedersen, A., Niemela, J.E., et al., 2015. Early-onset lymphoproliferation and autoimmunity caused by germline STAT3 gain-of-function mutations. *Blood* 125, 591–599.
- Mohammadi, J., Ramanujam, R., Jarefors, S., Rezaei, N., Aghamohammadi, A., Gregersen, P.K., et al., 2010. IgA deficiency and the MHC: assessment of relative risk and microheterogeneity within the HLA A1 B8, DR3 (8.1) haplotype. *J. Clin. Immunol.* 30, 138–143.
- Muller, S.M., Ege, M., Potthast, A., Schulz, A.S., Schwarz, K., Friedrich, W., 2001. Transplacentally acquired maternal T lymphocytes in severe combined immunodeficiency: a study of 121 patients. *Blood* 98, 1847–1851.
- Nagpala, P., Newfield, R., Bastian, J., Gottschalk, M., 2007. Autoimmune thyroiditis and acquired hypothyroidism in an infant with severe combined immunodeficiency due to adenosine deaminase deficiency. *Thyroid.* 17, 585–587.
- Nehme, N.T., Schmid, J.P., Debeurme, F., Andre-Schmutz, I., Lim, A., Nitschke, P., et al., 2012. MST1 mutations in autosomal recessive primary immunodeficiency characterized by defective naive T-cell survival. *Blood* 119, 3458–3468.
- Niehues, T., Perez-Becker, R., Schuetz, C., 2010. More than just SCID—the phenotypic range of combined immunodeficiencies associated with mutations in the recombinase activating genes (RAG) 1 and 2. *Clin. Immunol.* 135, 183–192.
- Notarangelo, L.D., Stoppoloni, G., Toraldo, R., Mazzolari, E., Coletta, A., Airo, P., et al., 1992. Insulin-dependent diabetes mellitus and severe atopic dermatitis in a child with adenosine deaminase deficiency. *Eur. J. Pediatr.* 151, 811–814.
- Notarangelo, L.D., Mazza, C., Giliani, S., D'Aria, C., Gandellini, F., Ravelli, C., et al., 2002. Missense mutations of the WASP gene cause intermittent X-linked thrombocytopenia. *Blood* 99, 2268–2269.
- Notarangelo, L.D., Lanzi, G., Peron, S., Durandy, A., 2006. Defects of class-switch recombination. *J. Allergy Clin. Immunol.* 117, 855–864.
- Notarangelo, L.D., Miao, C.H., Ochs, H.D., 2008. Wiskott-Aldrich syndrome. *Curr. Opin. Hematol.* 15, 30–36.
- Notarangelo, L.D., Kim, M.S., Walter, J.E., Lee, Y.N., 2016. Human RAG mutations: biochemistry and clinical implications. *Nat. Rev. Immunol.* 16, 234–246.
- O'Shea, J.J., Holland, S.M., Staudt, L.M., 2013. JAKs and STATs in immunity, immunodeficiency, and cancer. *N. Engl. J. Med.* 368, 161–170.
- Ochs, H.D., Gambineri, E., Torgerson, T.R., 2007. IPEX, FOXP3 and regulatory T-cells: a model for autoimmunity. *Immunol. Res.* 38, 112–121.
- Oftedal, B.E., Hellesen, A., Erichsen, M.M., Bratland, E., Vardi, A., Perheentupa, J., et al., 2015. Dominant mutations in the autoimmune regulator AIRE are associated with common organ-specific autoimmune diseases. *Immunity* 42, 1185–1196.
- Okano, T., Nishikawa, T., Watanabe, E., Watanabe, T., Takashima, T., Yeh, T.W., et al., 2017. Maternal T and B cell engraftment in two cases of X-linked severe combined immunodeficiency with IgG1 gammopathy. *Clin. Immunol.* 183, 112–120.
- Oliveira, J.B., Bleesing, J.J., Dianzani, U., Fleisher, T.A., Jaffe, E.S., Lenardo, M.J., et al., 2010. Revised diagnostic criteria and classification for the autoimmune lymphoproliferative syndrome (ALPS): report from the 2009 NIH International Workshop. *Blood* 116, e35–e40.
- Orange, J.S., Jain, A., Ballas, Z.K., Schneider, L.C., Geha, R.S., Bonilla, F.A., 2004. The presentation and natural history of immunodeficiency caused by nuclear factor kappaB essential modulator mutation. *J. Allergy Clin. Immunol.* 113, 725–733.
- Palmer, K., Green, T.D., Roberts, J.L., Sajaroff, E., Cooney, M., Parrott, R., et al., 2007. Unusual clinical and immunologic manifestations of transplacentally acquired maternal T cells in severe combined immunodeficiency. *J. Allergy Clin. Immunol.* 120, 423–428.
- Picard, C., Al-Herz, W., Bousfiha, A., Casanova, J.L., Chatila, T., Conley, M.E., et al., 2015. Primary immunodeficiency diseases: an update on the classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency 2015. *J. Clin. Immunol.* 35, 696–726.

- Picard, C., McCarl, C.A., Papolos, A., Khalil, S., Luthy, K., Hivroz, C., et al., 2009. STIM1 mutation associated with a syndrome of immunodeficiency and autoimmunity. *N. Engl. J. Med.* 360, 1971–1980.
- Pirovano, S., Mazzolari, E., Pasic, S., Albertini, A., Notarangelo, L.D., Imberti, L., 2003. Impaired thymic output and restricted T-cell repertoire in two infants with immunodeficiency and early-onset generalized dermatitis. *Immunol Lett.* 86, 93–97.
- Poliani, P.L., Fontana, E., Roifman, C.M., Notarangelo, L.D., 2013. Zeta chain-associated protein of 70 kDa (ZAP70) deficiency in human subjects is associated with abnormalities of thymic stromal cells: Implications for T-cell tolerance. *J. Allergy Clin. Immunol.* 131, 597–600.e1-2.
- Proekt, I., Miller, C.N., Lionakis, M.S., Anderson, M.S., 2017. Insights into immune tolerance from AIRE deficiency. *Curr Opin Immunol.* 49, 71–78.
- Puel, A., Doffinger, R., Natividad, A., Chrabieh, M., Barcenas-Morales, G., Picard, C., et al., 2010. Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. *J. Exp. Med.* 207, 291–297.
- Qamar, N., Fuleihan, R.L., 2014. The hyper IgM syndromes. *Clin. Rev. Allergy Immunol.* 46, 120–130.
- Quartier, P., Bustamante, J., Sanal, O., Plebani, A., Debre, M., Deville, A., et al., 2004. Clinical, immunologic and genetic analysis of 29 patients with autosomal recessive hyper-IgM syndrome due to activation-induced cytidine deaminase deficiency. *Clin. Immunol.* 110, 22–29.
- Rankin, A.L., Seth, N., Keegan, S., Andreyeva, T., Cook, T.A., Edmonds, J., et al., 2013. Selective inhibition of BTK prevents murine lupus and antibody-mediated glomerulonephritis. *J. Immunol.* 191, 4540–4550.
- Rao, A., Kamani, N., Filipovich, A., Lee, S.M., Davies, S.M., Dalal, J., et al., 2007. Successful bone marrow transplantation for IPEX syndrome after reduced-intensity conditioning. *Blood* 109, 383–385.
- Rich, K.C., Arnold, W.J., Palella, T., Fox, I.H., 1979. Cellular immune deficiency with autoimmune hemolytic anemia in purine nucleoside phosphorylase deficiency. *Am. J. Med.* 67, 172–176.
- Romberg, N., Morbach, H., Lawrence, M.G., Kim, S., Kang, I., Holland, S.M., et al., 2013. Gain-of-function STAT1 mutations are associated with PD-L1 overexpression and a defect in B-cell survival. *J. Allergy Clin. Immunol.* 131, 1691–1693.
- Rosenzweig, S.D., 2008. Inflammatory manifestations in chronic granulomatous disease (CGD). *J. Clin. Immunol.* 28 (Suppl. 1), S67–S72.
- Rucci, F., Poliani, P.L., Caraffi, S., Paganini, T., Fontana, E., Giliani, S., et al., 2011. Abnormalities of thymic stroma may contribute to immune dysregulation in murine models of leaky severe combined immunodeficiency. *Front. Immunol.* 2, 15.
- Sauer, A.V., Brigida, I., Carriglio, N., Hernandez, R.J., Scaramuzza, S., Clavenna, D., et al., 2012. Alterations in the adenosine metabolism and CD39/CD73 adenosinergic machinery cause loss of Treg cell function and autoimmunity in ADA-deficient SCID. *Blood* 119, 1428–1439.
- Schepp, J., Chou, J., Skrabl-Baumgartner, A., Arkwright, P.D., Engelhardt, K.R., Hambleton, S., et al., 2017. 14 years after discovery: clinical follow-up on 15 patients with inducible co-stimulator deficiency. *Front. Immunol.* 8, 964.
- Schubert, D., Bode, C., Kenefek, R., Hou, T.Z., Wing, J.B., Kennedy, A., et al., 2014. Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. *Nat. Med.* 20, 1410–1416.
- Soheili, H., Abolhassani, H., Arandi, N., Khazaei, H.A., Shahinpour, S., Hirbod-Mobarakeh, A., et al., 2013. Evaluation of natural regulatory T cells in subjects with selective IgA deficiency: from senior idea to novel opportunities. *Int. Arch. Allergy Immunol.* 160, 208–214.
- Somech, R., Simon, A.J., Lev, A., Dalal, I., Spire, Z., Goldstein, I., et al., 2009. Reduced central tolerance in Omenn syndrome leads to immature self-reactive oligoclonal T cells. *J. Allergy Clin. Immunol.* 124, 793–800.
- Sullivan, K.E., McDonald-McGinn, D.M., Driscoll, D.A., Zmijewski, C.M., Ellabban, A.S., Reed, L., et al., 1997. Juvenile rheumatoid arthritis-like polyarthritis in chromosome 22q11.2 deletion syndrome (DiGeorge anomalous/velocardiofacial syndrome/conotruncal anomaly face syndrome). *Arthritis Rheum.* 40, 430–436.
- Sullivan, K.E., Mullen, C.A., Blaese, R.M., Winkelstein, J.A., 1994. A multiinstitutional survey of the Wiskott-Aldrich syndrome. *J. Pediatr.* 125, 876–885.
- Sullivan, K.E., McDonald-McGinn, D., Zackai, E.H., 2002. CD4(+) CD25(+) T-cell production in healthy humans and in patients with thymic hypoplasia. *Clin. Diagn. Lab. Immunol.* 9, 1129–1131.
- Tang, W.J., An, Y.F., Dai, R.X., Wang, Q.H., Jiang, L.P., Tang, X.M., et al., 2014. Clinical, molecular, and T cell subset analyses in a small cohort of Chinese patients with hyper-IgM syndrome type 1. *Hum. Immunol.* 75, 633–640.
- Thrasher, A.J., Burns, S.O., 2010. WASP: a key immunological multitasker. *Nat. Rev. Immunol.* 10, 182–192.
- Tison, B.E., Nicholas, S.K., Abramson, S.L., Hanson, I.C., Paul, M.E., Seeborg, F.O., et al., 2011. Autoimmunity in a cohort of 130 pediatric patients with partial DiGeorge syndrome. *J. Allergy Clin. Immunol.* 128, 1115–1117.e1-3.
- Toubiana, J., Okada, S., Hiller, J., Oleastro, M., Lagos Gomez, M., Aldave Becerra, J.C., et al., 2016. Heterozygous STAT1 gain-of-function mutations underlie an unexpectedly broad clinical phenotype. *Blood* 127, 3154–3164.
- Tsuda, M., Torgerson, T.R., Selmi, C., Gambineri, E., Carneiro-Sampaio, M., Mannurita, S.C., et al., 2010. The spectrum of autoantibodies in IPEX syndrome is broad and includes anti-mitochondrial autoantibodies. *J. Autoimmun.* 35, 265–268.
- Tsukada, S., Saffran, D.C., Rawlings, D.J., Parolini, O., Allen, R.C., Klisak, I., et al., 1993. Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. *Cell* 72, 279–290.
- Uzel, G., Sampaio, E.P., Lawrence, M.G., Hsu, A.P., Hackett, M., Dorsey, M.J., et al., 2013. Dominant gain-of-function STAT1 mutations in FOXP3 wild-type immune dysregulation-polyendocrinopathy-enteropathy-X-linked-like syndrome. *J. Allergy Clin. Immunol.* 131, 1611–1623.
- Uzzan, M., Ko, H.M., Mehandru, S., Cunningham-Rundles, C., 2016. Gastrointestinal disorders associated with common variable immune deficiency (CVID) and chronic granulomatous disease (CGD). *Curr. Gastroenterol. Rep.* 18, 17.
- van de Veerdonk, F.L., Plantinga, T.S., Hoischen, A., Smeekens, S.P., Joosten, L.A., Gilissen, C., et al., 2011. STAT1 mutations in autosomal dominant chronic mucocutaneous candidiasis. *N. Engl. J. Med.* 365, 54–61.
- Verbsky, J.W., Chatila, T.A., 2013. Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) and IPEX-related disorders: an evolving web of heritable autoimmune diseases. *Curr. Opin. Pediatr.* 25, 708–714.
- Villa, A., Notarangelo, L.D., Roifman, C.M., 2008. Omenn syndrome: inflammation in leaky severe combined immunodeficiency. *J. Allergy Clin. Immunol.* 122, 1082–1086.
- Villa-Forte, A., de la Salle, H., Fricker, D., Hentges, F., Zimmer, J., 2008. HLA class I deficiency syndrome mimicking Wegener's granulomatosis. *Arthritis Rheum.* 58, 2579–2582.

- Vogel, T.P., Milner, J.D., Cooper, M.A., 2015. The Ying and Yang of STAT3 in human disease. *J. Clin. Immunol.* 35, 615–623.
- Walter, J.E., Farmer, J.R., Foldvari, Z., Torgerson, T.R., Cooper, M.A., 2016. Mechanism-based strategies for the management of autoimmunity and immune dysregulation in primary immunodeficiencies. *J. Allergy Clin. Immunol. Pract.* 4, 1089–1100.
- Walter, J.E., Rucci, F., Patrizi, L., Recher, M., Regenass, S., Paganini, T., et al., 2010. Expansion of immunoglobulin-secreting cells and defects in B cell tolerance in Rag-dependent immunodeficiency. *J. Exp. Med.* 207, 1541–1554.
- Walter, J.E., Rosen, L.B., Csomas, K., Rosenberg, J.M., Mathew, D., Keszei, M., et al., 2015. Broad-spectrum antibodies against self-antigens and cytokines in RAG deficiency. *J. Clin. Invest.* 125, 4135–4148.
- Warnatz, K., Bossaller, L., Salzer, U., Skrabl-Baumgartner, A., Schwinger, W., van der Burg, M., et al., 2006. Human ICOS deficiency abrogates the germinal center reaction and provides a monogenic model for common variable immunodeficiency. *Blood* 107, 3045–3052.
- Weinacht, K.G., Charbonnier, L.M., Alroqi, F., Plant, A., Qiao, Q., Wu, H., et al., 2017. Ruxolitinib reverses dysregulated T helper cell responses and controls autoimmunity caused by a novel signal transducer and activator of transcription 1 (STAT1) gain-of-function mutation. *J. Allergy Clin. Immunol.* 139, 1629–1640.e2.
- Winkelstein, J.A., Marino, M.C., Lederman, H.M., Jones, S.M., Sullivan, K., Burks, A.W., et al., 2006. X-linked agammaglobulinemia: report on a United States registry of 201 patients. *Medicine (Baltimore)* 85, 193–202.
- Yazdani, R., Azizi, G., Abolhassani, H., Aghamohammadi, A., 2017. Selective IgA deficiency: epidemiology, pathogenesis, clinical phenotype, diagnosis, prognosis and management. *Scand. J. Immunol.* 85, 3–12.
- Zemble, R., Luning Prak, E., McDonald, K., McDonald-McGinn, D., Zackai, E., Sullivan, K., 2010. Secondary immunologic consequences in chromosome 22q11.2 deletion syndrome (DiGeorge syndrome/velocardiofacial syndrome). *Clin. Immunol.* 136, 409–418.
- Zieg, J., Krepelova, A., Baradaran-Heravi, A., Levchenko, E., Guillen-Navarro, E., Balascakova, M., et al., 2011. Rituximab resistant Evans syndrome and autoimmunity in Schimke immuno-osseous dysplasia. *Pediatr. Rheumatol. Online J.* 9, 27.
- Zimmer, J., Ollert, M., 2015. HLA class I deficiency as an additional cause of bronchiectasis. *Respirology* 20, 1145.

# Animal Models: Systemic Autoimmune Diseases

Masayuki Mizui<sup>1</sup> and George C. Tsokos<sup>2</sup>

<sup>1</sup>Department of Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan <sup>2</sup>Division of Rheumatology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, United States

## OUTLINE

<b>Introduction</b>	533	Complement and Complement Receptor Proteins	542
<b>Spontaneous Models of Systemic Autoimmunity</b>	<b>534</b>	Clearance of Dead Cells	543
Overview of Spontaneous Autoimmune Disease	534	Innate Immune Cell Signaling	543
Models and Autoimmune-Susceptibility Loci	534	<b>Induced Models of Systemic Autoimmunity</b>	544
New Zealand Mixed Mice	534	Pristane-Induced Lupus Model	544
MRL/lpr and gld Mice	534	Graft-Versus-Host Reaction—Induced	
BXSB Mice	535	Autoimmunity	544
Palmerston North Mice	535	Collagen-Induced Arthritis Model	545
Ank/ank Mice	535	Proteoglycan-Induced Arthritis Model	545
K/BxN Mice	535	Collagen Antibody—Induced Arthritis Model	545
SKG Mice	536	<b>Conclusion</b>	545
<b>Genetically Manipulated Models of Systemic Autoimmunity</b>	<b>536</b>	<b>References</b>	546
Lymphocyte Activation Molecules	536	<b>Further Reading</b>	551
Ubiquitination-Related Enzymes	540		
Cytokines and Their Receptors	541		

## INTRODUCTION

Animal models have greatly facilitated the study of systemic autoimmune diseases, notably systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), and helped to develop rational new treatments. In addition, autoimmunity-prone mice have served as important tools in the study of genes involved in the expression of autoimmunity and related disease. Genes that facilitate or inhibit disease have been identified and these have in turn facilitated the study on immunogenetics and immunopathogenesis of human systemic autoimmune diseases. The etiology of both SLE (Tsokos, 2011) and RA (McInnes and Schett, 2011) is heterogeneous and complicated, but animal models bring a consistent understanding of disease pathogenesis. Mouse models of systemic autoimmune disease can be grouped into three types: spontaneous, gene manipulation-derived, and induced.

## SPONTANEOUS MODELS OF SYSTEMIC AUTOIMMUNITY

### Overview of Spontaneous Autoimmune Disease Models and Autoimmune-Susceptibility Loci

Commonly studied spontaneous models of lupus include the MRL-Fas<sup>lpr/lpr</sup> (MRL/*lpr*) [New Zealand Black (NZB) x New Zealand White (NZW)] F1, and BXSB mice. These murine models develop SLE spontaneously and their study has generated significant information on the role of hormones (Roubinian et al., 1978; Fernandes and Talal, 1986; Dhaher et al., 2000; Svenson et al., 2008) and the contribution of aberrant immune regulation (Handwerger et al., 1994) in the expression of the disease. They have been used to identify loci that contribute to the genetic pool required for the development of disease (Drake et al., 1995; Reilly and Gilkeson, 2002; Kono and Theofilopoulos, 2006). Forward genetic screening by gene complementation studies has shed light on the epistatic interactions of genes (Wakeland et al., 1997; Morel and Wakeland, 1998). Candidate genes have been identified including those of the CD2/SLAM family in the Sle1b locus (Wandstrat et al., 2004), the interferon (IFN)-inducible genes (Rozzo et al., 2001) for the NZB-derived Nba2 locus, and the complement receptor gene Cr2 for the NZW-derived Sle1c locus (Boackle et al., 2001). Numerous genes susceptible to lupus were identified and well characterized (Kono and Theofilopoulos, 2011). Identification of disease candidate genes by quantitative trait loci (QTLs) mapping strategy has been prompted to proceed analysis of mice deficient of targeted genes (reverse genetics). For example, it has been shown that the introduction of an IFN- $\alpha/\beta$  receptor null gene into NZB mouse results in decreased production of antierythrocytic antibodies (Santiago-Raber et al., 2003). Because IFN- $\alpha$  has been found to be increased in patients with SLE and to promote dendritic cell maturation (Blanco et al., 2001), a case has been made for the construction of biologics to limit the action of type I IFNs in systemic autoimmunity. Moreover, identification of contributing genes and loci in animal models has guided the search for orthologs in humans with systemic autoimmune diseases (Tsao et al., 1997, 2002; Gaffney et al., 1998, 2000; Harley et al., 1998; Moser et al., 1999).

### New Zealand Mixed Mice

The NZB (H-2<sup>d</sup>) mouse develops anemia due to an antierythrocyte antibody. Hybrids with NZW (H-2<sup>z</sup>) (NZBxNZW) F1 mice, develop systemic autoimmune disease with a high titer of anti-DNA antibodies and severe glomerulonephritis (GN) that becomes apparent at 5–6 months of age. The decreased average lifespan of these mice is 8 months for females and 13 months for males (Vyse et al., 1998; Ibnoou-Zekri et al., 1999). Among New Zealand Mixed (NZM) mice generated by (NZBxNZW) F1 and NZW backcross and sib mating, the NZM2328 and NZM2410 strains were found to develop lupus-like disease. Importantly, three lupus susceptibility loci, *Sle1–3*, were identified by the lupus linkage analysis of NZM2410 mice (Morel et al., 1994). From the findings in mice, variations in many genes in these loci have been directly associated with human SLE. *Sle1b* corresponds to polymorphisms in four signaling lymphocytic activation molecule family (SLAMF) member genes (Wandstrat et al., 2004), including Ly108, which was directly implicated in the regulation of B-cell tolerance (Kumar et al., 2006). Variants of SLAMF3 (Ly9) and SLAMF4 (CD244) have been associated with human SLE and RA (Cunningham Graham et al., 2008; Suzuki et al., 2008).

### MRL/lpr and gld Mice

The MRL mouse strain was derived from several inbred lines that included LG/J (75%), AKR/J (12.6%), C3H/Dehi (12.1%), and C57BL/6 (0.3%). MRL/MpJ-Fas<sup>lpr</sup>/J (MRL/*lpr*) mice, bearing two doses of mutation in the Fas gene, develop accelerated autoimmune disease characterized by severe lymphadenopathy due to the accumulation of CD3<sup>+</sup>CD4<sup>-</sup>CD8<sup>-</sup>B220<sup>+</sup> (double-negative) T cells. Disease onset with severe dermatitis and/or lymphadenopathy is seen from 10 to 12 weeks and death occurs at around 25 weeks. The disease is severe in female and the average lifespan for female MRL/*lpr* mice is 17 weeks. Mice display high concentrations of immunoglobulins including elevated levels of autoantibodies such as antinuclear antibodies (ANA), anti-ssDNA, anti-dsDNA, anti-Sm, and rheumatoid factors (Andrews et al., 1978). B and T cells from these mice have a defect in apoptosis due to the lack of functional Fas receptor (Reap et al., 1995). A mutation of Fas ligand (FasL) gene leads to generalized lymphoproliferative disease (gld) similar to that caused by the *lpr* mutation (Takahashi et al., 1994). In humans, defective Fas signaling can lead to the development of autoimmune lymphoproliferative syndrome which shares many manifestations with SLE (see Chapter 22: Autoimmune Diseases: The Role for Vaccines). Further study of the intense lymphoproliferation has enabled

the identification of genes whose products are central to the expression of systemic disease. For example, while deletion of the cyclin-dependent kinase inhibitor p21 does not lead to autoimmunity (Lawson et al., 2002, 2004), transfer of the *p21* null gene into the MRL *lpr/lpr* mouse results in a reduced autoimmune disease by allowing T-cell death and decreasing the accumulation of G0/G1 arrested T cells (Lawson et al., 2004). Expression of the *lpr* mutation in nonautoimmune strains such as C3H/HeJ and C57BL/6 leads to the development of lymphoproliferation and autoantibody production but limited GN in female mice. C3H/HeJ<sup>Fas/gld</sup>/J (gld) mice, the point mutation of cognate receptor for Fas, FasL in C3H/HeJ background, also develop autoimmunity with lymphocyte expansion similar to that of MRL/lpr mice. However, they have a longer lifespan than MRL/lpr mice due to reduced renal pathology (Roths et al., 1984).

## BXSB Mice

Male only C57BL/6J × SB/Le sa bg/sa bg (BXSB) mice develop severe GN and autoantibodies. The genetic locus responsible for the expression of the disease is located in the Y chromosome and is known as *Yaa* (Y chromosome–accelerated autoimmunity) (Izui et al., 1995). *Yaa* was first identified from a cross between a C57BL/6 female and an SB/Le male that produced the BXSB hybrid line. BXSB mice develop SLE at much higher incidence and with earlier onset in males compared to females, whereas mice from the reciprocal cross (SB/LE female and B6 male) do not show the same acceleration of disease in males. The disease is heavily dependent on the presence of the H-2b allele, because its replacement with the H-2d allele results in prolonged survival. Of interest, the (BXSBxNZW) F1 mouse develops thrombocytopenia and coronary artery disease accompanied by the presence of antiphospholipid antibodies (Kono and Theofilopoulos, 2006). Toll-like receptor 7 (TLR7), a single-stranded RNA-binding innate immune receptor, was identified as a gene responsible for the Y chromosome–linked autoimmune accelerator, as duplication of the *Tlr7* gene in *Yaa*<sup>+</sup> mice was shown to be associated with the induction of autoreactive B cells (Pisitkun et al., 2006). Moreover, deletion of the endogenous copy of *Tlr7* from X chromosome abrogates *Yaa*-induced monocytosis, lymphoid activation, splenomegaly, and GN, with decreased mortality (Deane et al., 2007; Santiago-Raber et al., 2008). Consistently, a human allele of the *Tlr7* gene was recently reported to be associated with increased risk for SLE development in males (Shen et al., 2010).

## Palmerston North Mice

Palmerston North (PN) mice develop spontaneous autoimmunity manifested by the production of autoantibodies against ssDNA, dsDNA, caldiolipin, and other phospholipids (Handwerger et al., 1999). PN mice exhibit vasculitis and GN from the age of 6 month and die at 11–12 months. Male mice also develop autoimmune diseases but they occur later and less severe than those in female mice (Walker et al., 1978; Luzina et al., 1999).

## Ank/ank Mice

Mice homozygous for the *ank* mutation. *Ank/ank* mice develop early-onset joint ankylosis in the spine and peripheral joints resembling human spondyloarthritis (Krug et al., 1989). The *Ank* gene encodes a transmembrane protein expressed in joints that controls pyrophosphate levels and may be irrelevant to autoimmunity.

## K/BxN Mice

K/BxN T-cell receptor mice, the F1 generation mice from the crossing of a T cell receptor (TCR) transgenic KRN mice on C57BL/6 background with the autoimmune-prone nonobese diabetic strain (Monach et al., 2004; Kouskoff et al., 1996), develop spontaneously an autoimmune disease with most (although not all) of the clinical, histological, and immunological features of RA in humans. The murine disease is critically dependent on both T and B cells and is joint specific, but it is initiated and perpetuated by T- and B-cell autoreactivity to a ubiquitously expressed antigen, glucose-6-phosphate isomerase (GPI). Transfer of serum (or purified anti-GPI immunoglobulins) from arthritic K/BxN mice into healthy animals regularly provokes arthritis within a few days, even when recipients are devoid of lymphocytes (Ji et al., 2001; Christensen et al., 2016). Complement components, Fc receptors, and mast cells are important for the expression of the disease. The relevance of the K/BxN model to human RA is supported by one report showing that serum from almost two-thirds of patients with RA contains anti-GPI antibodies, which are absent from serum from normal individuals or of patients with Lyme arthritis or

Sjogren's syndrome (Matsumoto et al., 2003). However, not all investigators agree. The K/BxN model has been particularly useful in illustrating the role of immune-inflammatory components in the development of arthritis and notably the characterization of the role of mast cells (Lee et al., 2002); yet, this model offers little evidence that autoantibodies are involved in the pathogenesis of RA.

### SKG Mice

SKG mice develop spontaneously T cell-mediated arthritis due to a mutation of the gene encoding a Src homology 2 (SH2) domain of  $\zeta$ -associated protein of 70 kDa (ZAP-70), a key signal transduction molecule in T cells. The disturbance in the thymic T-cell selection from the absence of ZAP-70 results in the generation of arthritogenic T cells. Besides synovitis, SKG mice develop extraarticular lesions, including pneumonitis and vasculitis. Serologically, they develop high levels of rheumatoid factor (Marshall et al.) and autoantibodies specific for type II collagen (CII) (Sakaguchi et al., 2003). A molecule termed synoviolin/Hrd1 was found to play a key role in the development of synovitis. This represents an E3 ubiquitin ligase, which, by promoting the growth of synoviocytes, facilitates the development of arthritis. Mice lacking synoviolin are resistant to arthritis (Amano et al., 2003).

Mice that develop spontaneous autoimmunity have helped our understanding of hormonal and immunoregulatory influences in autoimmunity, but obvious restraints limit the direct transfer of this information to human disease. Human disease develops in individuals with a permissive genetic background in conjunction with environmental and stressful factors, possibly acting over a long period. However, these murine models allow us to analyze disease mechanisms and are very useful to test potential therapies. Moreover, analysis of gene-manipulated mice in these genetic backgrounds enables investigators to identify a number of molecules involved in the development of disease pathogenesis (Table 29.1). A number of cytokine/cytokine receptor signals are reported to be important for the pathogenesis of SLE, including IFN- $\gamma$ /IFN- $\gamma$ R (Balomenos et al., 1998; Haas et al., 1997), IL-6 (Cash et al., 2010), IFN $\alpha$ R (Santiago-Raber et al., 2003), IL-21/IL-21R (Bubier et al., 2009; Herber et al., 2007), and IL-23R (Kytaris et al., 2010; Zhang et al., 2009). Intracellular signaling molecules such as phosphoinositide 3-kinase gamma (Barber et al., 2005), mammalian target of rapamycin (Lui et al., 2008), spleen tyrosine kinase (Syk) (Deng et al., 2010; Bahjat et al., 2008), and calmodulin-dependent kinase IV (Ichinose et al., 2011) were also identified to be involved in the development of lupus. These molecules could represent therapeutic targets for the treatment of human SLE.

## GENETICALLY MANIPULATED MODELS OF SYSTEMIC AUTOIMMUNITY

Loss of tolerance is a fundamental immunological abnormality in SLE. Numerous studies of mice with genetic manipulation such as gene deletions and transgenes that develop autoantibodies and other features of SLE on nonautoimmune genetic backgrounds have provided great insights into mechanisms that govern tolerance and autoimmunity and suggested novel rational treatments (Fig. 29.1 and Table 29.1).

### Lymphocyte Activation Molecules

Human SLE T cells are known to express less TCR- $\zeta$  chain (Liossis et al., 1998) and to use alternative signaling through the FcR- $\gamma$  chain (Enyedy et al., 2001). Mice that lack TCR- $\zeta$  chain develop autoimmune manifestation and display disturbed positive and negative thymic selection (Yamazaki et al., 1997), and the phenotype can be rescued successfully by introducing the FcR- $\gamma$  chain (Shores and Love, 1997). The pathophysiology for the murine and human phenotypes may well differ and, although in mice lack of the  $\zeta$  chain may lead to autoimmunity by altering early thymic events that limit the export of autoimmune T cells, in humans the rewiring of TCR with the newly upregulated FcR- $\gamma$  chain will lead to increased TCR-mediated signaling processes (Tsokos et al., 2003).

The B7-CD28/CTLA-4 costimulatory pathway is pivotal for T-cell activation. Signaling through this pathway is complex due to the presence of at least two B7 family members, CD80 (B7-1) and CD86 (B7-2), and two counter receptors CD28 and CD152 (CTLA-4). CTLA-4-deficient mice rapidly develop lymphoproliferative disease with multiorgan lymphocyte proliferation and tissue destruction, with particularly severe myocarditis and pancreatitis, and die by 3–4 weeks of age (Waterhouse et al., 1995; Tivol et al., 1995). CTLA-4-Ig limits effectively murine

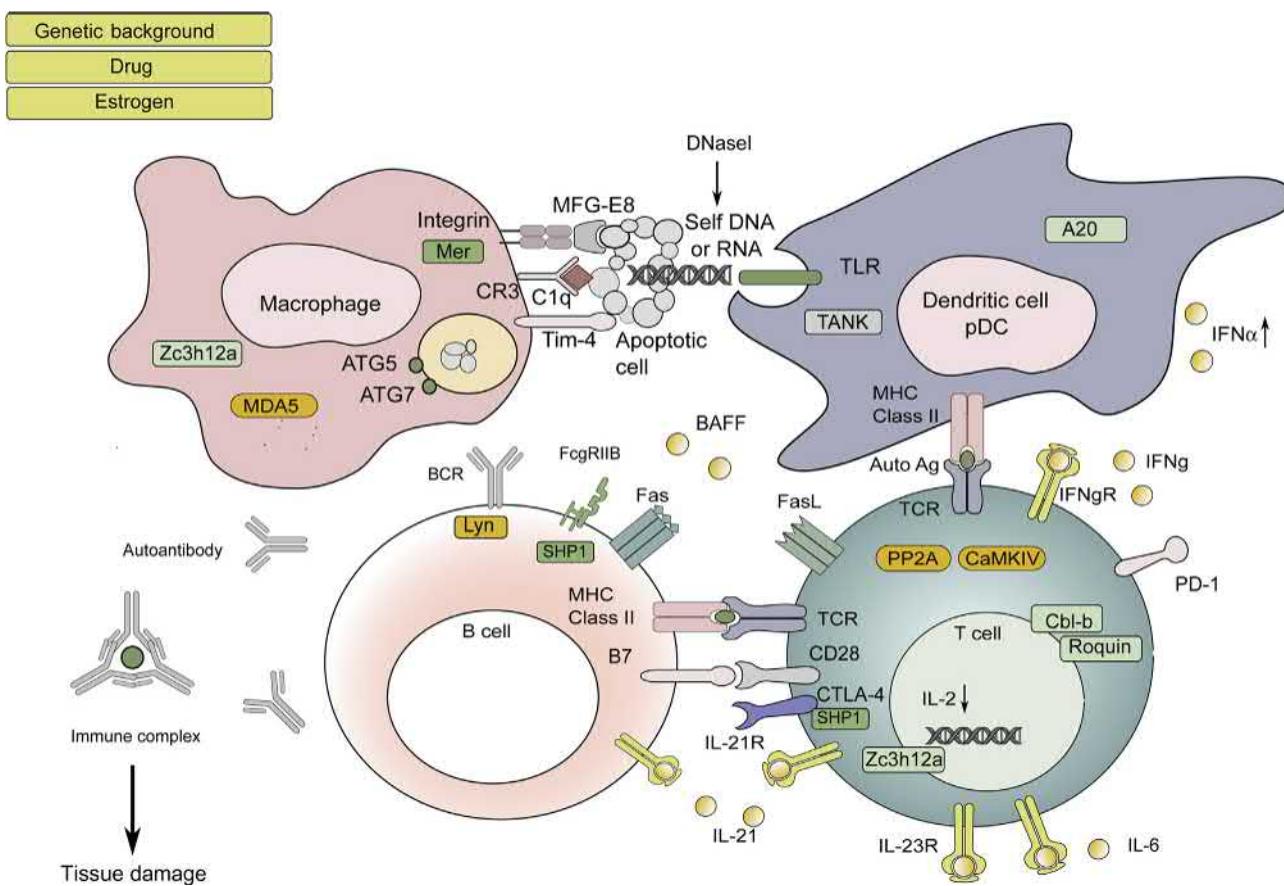
**TABLE 29.1** Representatives of Mice Models of Systemic Autoimmune Disease

Strain/name induction method	Target gene	Autoantibody production	Arthritis	Glomerulonephritis	Approximate life span (weeks)	Reference(s)
NZB		+				Ibnou-Zekri et al. (1999)
NZW		+ / -				Ibnou-Zekri et al. (1999)
(NZBxNZW)F1		++		++	60–70	Ibnou-Zekri et al. (1999)
NZM2328		++		++		Waters et al. (2001)
NZM2410		++		++		Morel et al. (1994)
MRL/MpJ-Fas <sup>lpr</sup> /J	Fas mutation	+++	+	+++	17	Andrews et al. (1978)
C3H/HeJ-Fasl <sup>gld</sup> /J	Fasl mutation	++		++	48	Roths et al. (1984)
BXSB (male)		+		+++	18	Izui et al. (1995)
Palmerston North		++		++	44–18	Walker et al. (1978)
						Luzina et al. (1999)
K/BxN			+			Monach et al. (2004)
SKG	Zap70 mutation		+			Marshall et al. (2003)
						Sakaguchi et al. (2003)
Ank/ank	Ank mutation		+			Krug et al. (1989)
<b>GENE MANIPULATION–DERIVED MODELS</b>						
<b>LYMPHOCYTE ACTIVATION</b>						
	PD-1 KO	+	+	+ (B6)		Nishimura et al. (2001)
	SHP1 KO	+			3	Kozlowski et al. (1993)
						Tsui et al. (1993)
						Bignon and Smininovilch (1994)
	SHP1 KO	+			12 (median)	Helgason et al. (2000)
	Mgat5 KO	+		+ (-30%)		Demetriou et al. (2001)
	Lyn KO	+		+	58% survive at 25 weeks	Hibbs et al. (1995)
	FcyRIIb KO	+		++(B6)	36	Bolland and Ravetch (2000)
	PEP-R619W Knock-in	+		+		Dai et al. (2013)
<b>UBIQUITINATION-PROTEIN LIGASES</b>						
	CD11cCre-A20 <sup>nymx</sup>	+		+		Kool et al. (2011)
	Cbl-b KO	+		+		Bachmaier et al. (2000)
	Roquin KO	+		+		Vinuesa et al. (2005)

(Continued)

TABLE 29.1 (Continued)

Strain/name induction method	Target gene	Autoantibody production	Arthritis	Glomerulonephritis	Approximate life span (weeks)	Reference(s)
<b>CYTOKINES AND THEIR RECEPTORS</b>						
	IL-2/IL-2R KO	+			12	Sharfe et al. (1997)
	BAFF TG	+		+		Mackay et al. (1999)
	TNF $\alpha$ TG		+			Keffer et al. (1991)
<b>COMPLEMENT</b>						
	B6/129 C1q KO	+		+(25%)		Botto et al. (1998)
	B6/129 C4 KO	+		+		Chen et al. (2000)
<b>MOLECULES INVOLVED IN DEAD CELL CLEARANCE</b>						
	DNase1 KO	+		+		Napirei et al. (2000)
	B6/129 MGF-E8 KO	+		+		Hanayama et al. (2002)
	Tim-4 KO	+		+		Miyanishi et al. (2007)
	Mer <sup>KD</sup>	+		+		Cohen et al. (2002)
	LysM-Cre Atg5 <sup>flox/flox</sup>	+		+		Martinez et al. (2016)
<b>INNATE IMMUNE CELL SIGNALING MOLECULES</b>						
	Ifih1 <sup>5k</sup> Knock-in	+		+		Funabiki et al. (2014)
	TANK KO	+		+	50% survive at 56 weeks	Kawagoe et al. (2009)
	Zc3h12a KO	+			15	Matsushita et al. (2009)
<b>INDUCED MODELS</b>						
Pristane		+	+	+		Satoh and Reeves (1994)
GVHD		+		+		Morris et al. (1990)
Collagen type II			+			Stuart et al. (1983)
Proteoglycan			+			Watson et al. (1987)
Collagen antibody			+			Glant et al. (2003)
						Holmdahl et al. (1986)



**FIGURE 29.1** Pathogenesis of systemic autoimmunity and molecules involved in the development of autoimmune disease, compiled from studies on mice and humans with SLE. Similar to organ-specific autoimmunity, systemic autoimmunity is a function of a deleterious combination of genetic and environmental factors that lead in concert to the loss of self-tolerance. T cells display numerous aberrations in the form of impaired cytokine productions, defective immunoregulatory function, and increased ability to provide help to B cells to produce autoantibodies. In systemic autoimmunity, autoantigens are frequently directed against nuclear antigens, with which they form immune complexes. Defective clearance of such immune complexes is the result of defective expression of Fc and complement receptors, and increased levels of nuclear materials may propagate the autoimmune responses. A number of cytokines including IFN $\alpha$ , IL-6, IL-21, IL-17, BAFF, and their receptors play critical roles for the development of lupus. *IFN*, Interferon; *IL*, interleukin; *MHC*, major histocompatibility complex; *SLE*, systemic lupus erythematosus.

lupus (Finck et al., 1994) and the use of CTLA-4-Ig biologics (Abatacept) has helped patients with RA (Kremer et al., 2003) and psoriatic arthritis (Abrams et al., 2000). Abatacept is now one of the biological agents for the treatment of RA. Phase II/III trial is undergoing for psoriatic arthritis, although clinical trials for SLE have failed to achieve their primary outcome (Pimentel-Quiroz et al., 2016).

Programmed death-1 (PD-1) is a member of the CD28 family of receptors and its intracytoplasmic domain defines an immunoreceptor tyrosine-based inhibition motif (ITIM); engagement of PD-1 with its ligands PD-L1 and PD-L2 delivers a negative signal. Mice lacking the PD-1 gene develop either autoantibody-mediated cardiomyopathy in BALB/c (Okazaki et al., 2003) or GN on a C57BL/6 background (Nishimura et al., 2001). PD-1 polymorphism has been identified among SLE patients (Prokunina et al., 2002) and/or RA patients (Lin et al., 2004). PD-L1-deficient mice show accelerated autoimmune inflammation in MRL/MpJ and MRL/lpr mice (Lucas et al., 2008).

Src homology 2-containing phosphatase 1 (SHP1) is one of the best-characterized protein tyrosine phosphatases (PTPase). SHP inhibits cell activation through its recruitment by negative regulatory molecules containing ITIM. The homozygous loss of SHP1 (*Ptpn6*<sup>me-v/me-v</sup>) in mice leads to the “moth-eaten” phenotype characterized by spotty hair loss and abnormalities in the immune system that lead to systemic autoimmunity and skin inflammation (Kozlowski et al., 1993; Tsui et al., 1993; Bignon and Siminovitch, 1994). T lymphocytes from these mice are hyperresponsive to TCR stimulation (Pani et al., 1996). Since neutrophils from SHP1-deficient

moth-eaten mice demonstrate increased oxidant production, surface expression of CD18, and adhesion to protein-coated plastic, the autoimmune phenotype in these mice may not directly reflect lymphocyte aberrations (Kruger et al., 2000). Moth-eaten *Rag1*<sup>-/-</sup> mice lack B and T cells but still develop severe systemic inflammatory disease (Yu et al., 1996). Furthermore, only B cell–specific deletion of SHP1 leads to systemic autoimmune disease (Pao et al., 2007).

Mice with beta1,6 N-acetylglucosaminyltransferase V (Mgat5) deficiency display decreased N-glycosylation of T-cell membrane proteins, which prevents galectin binding and thereby disrupts the galectin-glycoprotein lattice leading to increased clustering of the TCR (Demetriou et al., 2001). Increased TCR clustering in Mgat5-deficient mice results in autoimmunity, which implicate a phenotype compatible with human SLE, involving lowered T-cell activation thresholds and increased TCR signaling. In the case of T cells of human SLE, increased association of the TCR- $\zeta$  chain with lipid rafts, as well as membrane clustering, is claimed to lead to decreased tolerance and abnormal signaling (Nambiar et al., 2002; Krishnan et al., 2004). Thus defects in the expression and function of glycosylation processes may predispose to autoimmunity and, along the same lines, glycosylation of the transcription factor Elf-1, which enables the 80-kDa form to transform to the DNA-binding 98-kDa form, is defective in human SLE T cells (Juang et al., 2002).

Other lines of evidence suggest T cells from lupus-prone mice that are transgenic for a specific receptor have decreased antigen-initiated T-cell stimulation thresholds (Vratsanos et al., 2001; Bouzahzah et al., 2003). A similar concept of an “overexcitable” antigen-initiated proximal lymphocyte signaling phenotype has been proposed for human SLE (Tsokos et al., 2003). For example, protein tyrosine phosphatase nonreceptor 22 (PTPN22) is involved in negative regulation of T-cell signaling through the encoded lymphocyte-specific PTPase LYP (see Chapter 5: Immunological Tolerance—T Cells). Single nucleotide polymorphism (SNP) of PTPN22 at position 1858 was reported to be associated with autoimmune diseases such as type I diabetes, SLE, and RA. The mice orthologous proline, glutamic acid, serine and threonine (PEST) domain phosphatase (PEP) mutant (R619W) protein knock-in mice exhibit systemic autoimmunity with the elevation of autoantibody due to hyperactivation of T cells (Dai et al., 2013). Furthermore, only B cell–specific insertion of mutant protein is enough for the induction of autoimmune diseases.

A state of B-lymphocyte hyperactivity resembling SLE is seen in mice lacking the Src-family kinase Lyn. Lyn is an essential inhibitory component on B-cell receptor (BCR) signaling. Negative regulation of the BCR is a complex quantitative trait in which Lyn, the coreceptor CD22, and the tyrosine phosphatase SHP1 are each limiting elements (Cornall et al., 1998). When immunoreceptor tyrosine-based motif (ITIM) is phosphorylated by Lyn, ITIM recruits and activates SHP1 and SHIP-1 (SH2 domain-containing inositol-5-phosphatase). Lyn-deficient mice display decreased numbers of mature peripheral B cells, greatly elevated serum IgM and IgA (Bignon and Siminovitch), and production of autoantibodies that cause autoimmune GN reminiscent of SLE (Chan et al., 1997). Paradoxically, sustained activation of Lyn in vivo using a targeted gain-of-function mutation (*Lyn*<sup>up/up</sup> mice) led to the development of autoantibodies and lethal autoimmune GN. Interestingly, B cells from *Lyn*<sup>up/up</sup> mice show a heightened calcium flux in response to BCR stimulation (Hibbs et al., 2002). These data in humans and mice suggest that mechanisms that lead to sustained BCR signaling may override control mechanisms and lead to autoimmunity. SHIP-1 deficiency in C57BL6 background exhibits severe autoimmune disease characterized by a greater number of activated B cells, plasmacytosis, and intense GN (Maxwell et al., 2011).

Antigen presented in the context of immune complexes engages not only the BCR but also the Fc $\gamma$ RIIb, which exert an immunosuppressive effect through the phosphorylation of ITIM. Introduction of the Fc $\gamma$ RIIb null phenotype into the C57BL/6 background caused production of autoantibodies and GN (Bolland and Ravetch, 2000) and Fc $\gamma$ RIIb deficiency exacerbates autoimmunity in B6/lpr mice (Yajima et al., 2003). Yet the same null phenotype on the BALB/c background did not result in autoimmunity. In humans, polymorphisms of the Fc receptors have been associated with systemic autoimmunity and particularly with SLE and granulomatous polyangiitis (Kimberly et al., 1995).

## Ubiquitination-Related Enzymes

The ubiquitination system is an essential regulator for cellular processes such as differentiation, cell cycle, and DNA repair. Disruption of ubiquitination system could induce aberrant immune responses. The ubiquitin editing enzyme A20 [tumor necrosis factor  $\alpha$  (TNF $\alpha$ )-induced protein, Tnfp3], a cytoplasmic zinc-finger protein, restricts and terminates inflammatory responses through modulation of the ubiquitination status

of a central component in NF $\kappa$ B, IRF3, and apoptosis signaling components (Vereecke et al., 2011). In humans, polymorphisms in genomic A20 locus are associated with several autoimmune diseases including SLE, RA, and psoriasis. A20-deficient mice die prematurely from a severe wasting disease with multiorgan inflammation (Lee et al., 2000). Mice with dendritic cell-specific deletion of A20 develop lupus-like features with elevated anti-dsDNA, antiphospholipid syndrome, GN, and lymphosplenomegaly (Kool et al., 2011).

The Cbl-b (casitas B-lineage lymphoma-b) and Cbl adaptor proteins are E3 ubiquitin ligases that inhibit receptor and nonreceptor tyrosine kinases by promoting ubiquitination (Bachmaier et al., 2000). Loss of Cbl-b rescues reduced calcium mobilization of anergic T cells, which was attributed to Cbl-b-mediated regulation of PLC $\gamma$ -1 phosphorylation. Loss of Cbl-b in mice results in impaired induction of T-cell tolerance both in vitro and in vivo and shows exacerbated autoimmunity (Jeon et al., 2004). Moreover, B cell-specific deficiency of Cbl/Cbl-b in mice leads to impaired BCR downmodulation and anergy to self-antigen, and development of spontaneous lupus-like disease with anti-dsDNA, ANA, massive leukocytic infiltrates in multiple organs, and immune complex GN (Kitaura et al., 2007).

Roquin (Rc3h1), a RING-type ubiquitin ligase family member, was identified as a negative regulator of follicular helper T (Tfh) cell development. This molecule is identified by a novel forward genetic strategy: male C57BL/6 mice were treated with ethylnitrosourea (ENU), a mutagenic agent, and bred the variant genome sequences to homozygosity and progeny were screened for autoimmunity. M199R mutation within the roquin (ROQ) domain of Roquin was generated by ENU mutagenesis that resulted in ANA, anti-dsDNA, GN, necrotizing hepatitis, anemia, and immune thrombocytopenia (Vinuesa et al., 2005). This mouse displays increased germinal center formation and expansion of memory/effector CD4 $^{+}$  T cells, particularly Tfh cells. Tfh cells have been established as a T-helper cell subset specialized for providing help to B cells in germinal centers (GCs) (King et al., 2008). Overpresentation of Tfh cells is associated with the development of systemic autoimmunity (Linterman et al., 2009) and the expansion of circulating Tfh-like cells was also detected in human SLE and associated with severe disease (Simpson et al., 2010).

## Cytokines and Their Receptors

Mice that are deficient in IL-2 and IL-2a have disrupted immunological homeostasis that eventually leads to fatal autoimmune manifestations (Nelson, 2002). Specifically, these mice develop autoimmune hemolytic anemia and colitis with lymphoproliferation, expansion of effector/memory phenotype T cells, polyclonal hypergammaglobulinemia, and autoantibodies. IL-2RB – / – mice likewise develop anemia, splenomegaly, and lymphadenopathy, but not colitis. In humans, IL-2 deficiency is clinically manifested as severe combined immunodeficiency, whereas a patient lacking IL-2a was declared immunocompromised, and several organs were infiltrated with inflammatory cells (Sharfe et al., 1997). The autoimmune manifestations depend on the presence of both T and B cells and environmental antigens since, if the mice are kept under pathogen-free conditions, they do not develop autoimmunity. Activation-induced cell death (AICD) is central for the elimination of activated autoreactive cells, and this depends on IL-2 signaling. Defective AICD obviously plays a role in the development of autoimmunity. Humans with SLE have defective AICD that appears to be multifunctional: defective TNF $\alpha$  (Kovacs et al., 1996) and IL-2 production (Tsokos et al., 1996). The downregulation of IL-2 production in SLE patients was found to be mediated by Ser/Thr protein phosphatase 2A (PP2A). PP2A dephosphorylates transcription factor SP-1, which results in strong binding of cyclic-AMP responsive element modulator to the IL-2 promoter (Juang et al., 2011). T cells from patients with SLE have increased PP2A (Katsiari et al., 2005). Overexpression of PP2A in T cells in B6 mice does not lead to autoimmunity but only to granulocytosis and increased levels of IL-17 and, when challenged with an antiglomerular basement membrane antibody, they developed exuberant GN (Crispin et al., 2012). On the other hand, mice with PP2A-deficient regulatory T cells (Treg) develop severe lymphoproliferative autoimmune disease due to functional impairment of Treg (Apostolidis et al., 2016). IL-2 plays a central role in the development and maintenance of Treg cells, which have been proven to be of pathogenic relevance in the development of autoimmune disease (La cava, 2008). Low-dose IL-2 treatment in patients of chronic graft-versus-host disease (GVHD) (Koreth et al., 2011) and of hepatitis C virus-induced vasculitis (Saadoun et al., 2011) was reported to be effective with an accompanying increase of Treg cells. *Vaccinia virus*-mediated in vivo IL-2 introduction ameliorates disease progression in MRL/lpr mice (Gutierrez-Ramos et al., 1990). These results indicate that correction of IL-2 production could have therapeutic potential by restoring the function of T cells.

The TNF/TNF receptor (TNFR) system acts on the homeostasis of the immune system in different ways. Among them, TNFSF13B (BAFF, BlyS) and TNFRSF13B (TACI) are implicated in the development of autoimmune disease. BAFF is critical for B-cell survival and BAFF-transgenic or TACI-knockout mice show lupus-like disease. Moreover, serum BAFF is elevated in both BWF1 and MRL/*lpr* mice and blocking BAFF function with a soluble TACI-IgGFc protein can inhibit proteinuria and prolong survival (Gross et al., 2000). Surprisingly, T cell-deficient BAFF-transgenic mice can develop lupus-like disease indistinguishable from that of BAFF-transgenic mice and the development of the disease is dependent on myeloid differentiation factor 88 (MyD88) (Groom et al., 2007; Mackay et al., 1999). Clinical trials (phase II/III) using the chimeric molecule TACI-Ig (Atacicept) in SLE show effectiveness for inhibition of new flare (Isenberg et al., 2015) and the clinical use of anti-BAFF/BlyS (Belimumab) for active SLE patients was recently approved (Navarra et al., 2011).

The successful clinical introduction of treatment with anti-TNF $\alpha$  confirmed the biological relevance of TNF $\alpha$  function in chronic inflammatory diseases, particularly RA and Crohn's disease. The introduction of a modified human TNF-globin hybrid transgene in mice was the first demonstration in animal models that TNF has arthritogenic properties. These mice (Tg197) spontaneously develop (with 100% penetrance and a predictable time of onset) a chronic, erosive, inflammatory polyarthritis with histological lesions resembling human RA (Kontoyiannis et al., 1999; Keffer et al., 1991). Adenylate-uridylate-rich (AU-rich) elements (Holmdahl et al.) are important for TNF $\alpha$  mRNA destabilization and translational repression in hematopoietic and stromal cells. Development of two specific pathologies in mutant mice—chronic inflammatory arthritis and colitis akin to Crohn's disease—suggests a defective function in analogous human pathologies. These mice have proven to be quite informative in dissecting the pleiotropic effects of TNF $\alpha$  on immune responses and on the expression of various forms of autoimmune pathology.

## Complement and Complement Receptor Proteins

Activation of the classical pathway typically starts by interaction of C1q with immune complexes and the action of complement is the main effector mechanism of antibody-mediated immunity. The complement system also has an important role in clearing immune complexes from the circulation. It can also bind apoptotic cells and helps to eliminate these cells from tissue (Taylor et al., 2000). If the complement system fails in this function, waste material can accumulate and evoke an autoimmune response. Deficiencies of the classical pathway are associated with an increased risk to develop SLE or allied diseases in human (Manderson et al., 2004). More than 90% of the individuals with C1q and C1r/C1s deficiency develop an SLE-like disorder, and 10%–20% of the individuals with C2 deficiency develop SLE (Truedsson et al., 2007). These phenotypes in humans do not parallel in full those in mice. Deficiency of C1q in C57BL/6 mice does not lead to the development of autoimmunity. By contrast, C1q-deficient MRL/MpJ mice display accelerated disease onset and increased levels of ANA and of GN, particularly in females which developed severe crescentic GN. Moreover, C1q-deficient mice on a B6x129 genetic background were shown to develop higher levels of autoantibodies compared to strain-matched controls and to develop GN by 8–10 months of age (Manderson et al., 2004; Botto et al., 1998). Thus the expression of autoimmunity in C1q-deficient mice is strongly influenced by additional background genes (Botto and Walport, 2002; Botto et al., 1998).

The B6/*lpr* mouse develops minor autoimmune features. Deficiency in this strain of the complement receptor 1 and complement receptor 2 (CR1/CR2, CD35/CD21), encoded by the Cr2 gene, permits the development of intense autoimmune features (Boackle and Holers, 2003), indicating that complement receptors are important in the elimination of B cells that display reactivity with self-antigens. This explanation assumes that self-antigens initiate a strong B-cell signal, which leads to B-cell death and the absence of the CR2-mediated enhancement of the signal permits their survival (Tsokos et al., 1990; Dempsey et al., 1996). CR1 and CR2 have been proposed to play a role in the development of SLE. Patients with SLE have around 50% lower levels of these receptors on their B cells (Levy et al., 1992; Marquart et al., 1995). MRL/*lpr* mice exhibit lower levels of these receptors on B cells prior to the onset of overt disease, suggesting that the reduction of CR1/CR2 expression may be pathogenic (Takahashi et al., 1997). Also, evidence for the involvement of Cr2 in systemic autoimmunity comes from the NZM2410 mouse. The congenic interval corresponding to *Sle1c*, one of the SLE susceptibility loci, *Sle1*, contains the Cr2 gene. NZM2410/NZW Cr2 exhibits a single nucleotide polymorphism that introduces a novel glycosylation site, resulting in higher molecular weight proteins. This polymorphism, located in the C3d binding domain, reduces ligand binding and receptor-mediated cell signaling. Molecular modeling based on the CR2 structure in complex with C3d has revealed that this glycosylation interferes with receptor dimerization (Boackle et al., 2001).

Since disruption of the C1q, C4, and CR1/CR2 leads to reduced selection against autoreactive B cells and to impaired humoral responses, C1 and C4 could act through CR1/CR2 to enhance humoral immunity and suppress autoimmunity, but each complement component appears to act independently. High titers of spontaneous ANA and SLE-like autoimmunity develop in all C4<sup>-/-</sup> mice and most male mice but not in Cr2<sup>-/-</sup> mice. The fact that the clearance of circulating immune complexes is impaired in preautoimmune C4<sup>-/-</sup>, but not Cr2<sup>-/-</sup> mice, favors the role of nuclear antigen–ANA immune complexes in the development of autoimmune disease (Chen et al., 2000).

## Clearance of Dead Cells

Effective degradation of nucleotides from dead cells and digestion of cellular components by macrophages allow noninflammatory clearance and a recycle of dead cells (Nagata et al., 2010). DNaseI is the major nuclease present in the blood, urine, and secretions. DNaseI deficiency in nonautoimmune background mice was reported to increase the incidence of SLE manifestations, including positive ANA, anti-DNA, and immune complex GN (Napirei et al., 2000). Reduced DNaseI activity is observed in the sera of lupus patients, which may contribute to SLE susceptibility (Tsukumo and Yasutomo, 2004). Intriguingly, an identical heterozygous nonsense mutation in DNASE1 was detected in two SLE patients (Yasutomo et al., 2001).

Deficiency in the clearance of apoptotic cells is proposed to be one of the causes of SLE. Unengulfed apoptotic cells are present in the germinal centers of the lymph nodes of some SLE patients and macrophages from these patients often show a reduced ability to engulf apoptotic cells (Gaapl et al., 2006). Milk fat globule-EGF factor 8 (MFG-E8) protein functions as a bridging protein between phosphatidylserine (PS) on apoptotic cells to avb3 or avb5 integrins on phagocytic cells (Hanayama et al., 2002). MFG-E8 is primarily expressed on CD68-positive tangible body macrophages within germinal centers. MFG-E8-deficient female mice on a B6x129 gene background develop SLE-like autoimmune disease with anti-dsDNA, ANA, and GN by 40 weeks of age (Hanayama et al., 2004).

T-cell immunoglobulin and mucin domain-containing 4 (Tim-4) is another PS receptor. Peritoneal resident macrophages highly express Tim-4 that mediates phagocytosis of apoptotic cells. Tim-4-deficient mice develop lupus-like disease with the elevation of autoantibody titers; however, GN or other organ damages were not reported (Miyanishi et al., 2007; Rodriguez-Manzanet et al., 2010).

TAM family members (Tyro3, Axl, and Mer), which are tyrosine kinase receptors expressed on antigen-presenting cells, promote clearance of apoptotic cells and mice expressing a kinase-dead mutant of Mer (MerKD) develop SLE-like autoimmunity (Scott et al., 2001; Cohen et al., 2002). TAM receptors negatively regulate the innate immune reaction and TAM-deficient dendritic cells overproduce IL-6, IFN, and TNF $\alpha$  that might be responsible for the induction of autoimmunity (Lemke and Rothlin, 2008).

Genome-wide association studies in human revealed that autophagy-related genes are predisposed SLE disease susceptibility, including Atg5 and Atg7. Therefore autophagy and autophagy-relevant proteins have been implicated to autoimmunity (see Chapter 18: Effector Mechanisms in Autoimmunity). Microtubule-associated protein light chain 3 (LC3) is a key autophagy-related protein that is recruited to the double-membrane autophagosome. Recently, LC3 was found to be recruited to the single-membrane phagosome, called LC3-associated phagocytosis (LAP), distinct from endocytosis or autophagy. LAP vesicles contain engulfed particles including dying cells, indicating an important role for the clearance of dead cells. A number of autophagy-related genes such as Atg5, Atg7, Beclin-1, Rubicon, and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-2 (Nox2) are involved in LAP processes. Mice with macrophage-deficient of these autophagy-related genes were recently reported to exhibit lupus-like autoimmune diseases. Importantly, autoimmunity was observed in mice lacking proteins required for both LAP and autophagy (Atg5, Atg7, Beclin-1) or LAP alone (Nox2, Rubicon) but not in animals lacking proteins required for autophagy but dispensable for LAP (FIP200, ULK1) (Martinez et al., 2016). Previous report showed that Nox2-deficient and -heterozygous MRL/lpr mice had exacerbated lupus, indicating a protective role of Nox2 in autoimmune disease (Campbell et al., 2012). Taken together, this noncanonical autophagy process LAP might control immune regulation for preventing autoimmune reaction.

## Innate Immune Cell Signaling

Recent studies demonstrate that DNA and RNA in apoptotic material can activate B cells and dendritic cells through TLR9, TLR7, and TLR8 (Leadbetter et al., 2002; Boule et al., 2004; Vollmer et al., 2005). As described

above, overexpression of TLR7 is responsible for the development of autoimmune diseases in BXSB mice. These results indicate that TLR signaling is linked to the development of autoimmune disease and aberrant activation of innate immunity may contribute to systemic autoimmune diseases including RA and SLE (Marshak-Rothstein, 2006).

While TLRs recognize pathogen-associated molecular patterns (PAMPs) at the cell surface or in endosomes, RIG-I-like receptors such as RIG-I and MDA5 are recognized by intracellular viral RNAs. Constitutive active MDA5-carrying knock-in (*Ifih1<sup>gs/+</sup>*) mice develop severe autoimmune diseases with high autoantibody titer and GN (Funabiki et al., 2014).

TANK (also known as I-TRAF) is a TNF receptor–associated factor (TRAF)–binding protein and binds to TRAF1, 2, 3, 5, and 6, all of which are crucial for TLR signaling. TANK is a negative regulator of proinflammatory cytokine production induced by TLR signaling and TANK-deficient mice spontaneously develop lupus-like autoimmune diseases with fatal GN, ANA, and anti-dsDNA. Autoantibody production in TANK-deficient mice is abrogated by antibiotic treatment or the absence of IL-6 or MyD88, indicating that TANK controls TLR signaling by intestinal commensal microbiota (Kawagoe et al., 2009).

Zc3h12a (regnase-1) is an RNase activated by TLR signaling that promotes the degradation of mRNA. Zc3h12a-deficient mice have early mortality associated with severe hemolytic anemia, lymphoproliferation, and ANA with an increased number of activated B cells, T cells, and plasma cells due to excessive cytokine transcription notably IL-6 and IL-12 (Matsushita et al., 2009). Zc3h12a deficiency also induces hyperactivation of CD4<sup>+</sup> T cells due to impairment of degradation of IL-2, Ox40, and c-Rel. Consequently, mice with regnase-1-deficient CD4<sup>+</sup> T cells develop severe autoimmunity with the elevation of antinuclear antibody (Uehata et al., 2013).

## INDUCED MODELS OF SYSTEMIC AUTOIMMUNITY

Information has been acquired over many years from the study of models based on induction of autoimmunity, including immunoregulatory events that lead to the expression of clinical disease and proximal events associated with triggering of autoimmune disease.

### Pristane-Induced Lupus Model

The isoprenoid alkane pristane (2, 6, 10, and 14 tetramethylpentadecane) induces autoantibodies characteristic of SLE, including anti-Sm, anti-dsDNA, and antiribosomal P in BALB/c and SJL/J mice and CD1d deficiency exacerbates lupus nephritis induced by pristane (Satoh and Reeves, 1994). Pristane induces type I IFN production from Ly6C<sup>hi</sup> monocyte through TLR7 and myeloid differentiation factor 88 (MyD88) pathway and triggers autoantibody production (Lee et al., 2008). Also, IL-12 and IFN $\gamma$ , but not IL-4, are involved in the development of pristane-induced lupus, indicating that T-helper type 1 responses are dominant. For unexplained reasons, the *lpr* and *gld* mutations protect mice from the production of antibodies routinely induced by pristane. In addition to nephritis, hemorrhagic pulmonary capillaritis and arthritis have also been observed in pristane-treated mice (Chowdhary et al., 2007; Wooley et al., 1989). The arthritis symptoms in this model include synovial hyperplasia, perostitis, and progressive marginal erosions. Pristane-induced arthritis is TNF $\alpha$  mediated, as treatment with neutralizing anti-TNF $\alpha$  antibody ameliorates the arthritis symptoms (Beech and Thompson, 1997).

### Graft-Versus-Host Reaction–Induced Autoimmunity

Graft-versus-host-induced models of systemic autoimmunity involve the injection of parent cells into F1 offspring and clarified early events in the induction of autoimmunity. When lymphocytes from DBA mice are transferred into (B6 X DBA) F1 mice, donor CD4<sup>+</sup> cells are stimulated by recipient MHC class II cells, which presumably present a chromatin-associated nuclear antigen and produce initially IL-2 and later on IL-4 and IL-10, while the generation of CD8<sup>+</sup> CTL cells is silenced. These responses result in chronic GVHD with autoantibody and immune complex–mediated GN. When donor B6/H-2bm12 lymphocytes, whose MHC class II locus confers a three amino acid substitution in H-2<sup>b</sup>, are transferred into B6 hosts, there also is a development of chronic GVHD with autoantibodies and GN. This model works equally with opposite donor and recipient strains (Morris et al., 1990).

## Collagen-Induced Arthritis Model

Injection of allogeneic CII or certain peptides of this protein in complete or incomplete Freund's adjuvant into susceptible strains of rats or mice results in collagen-induced arthritis (CIA) resembling RA. Disease susceptibility is strongly linked to MHC class II molecules. H-2<sup>d</sup> mice are susceptible to CIA when immunized with bovine, chicken, and human CII, whereas H-2<sup>r</sup> mice are susceptible with bovine or porcine CII but not with chicken or human CII (Stuart et al., 1983; Watson et al., 1987). C57BL/6 (H-2<sup>b</sup>) mice are also susceptible but the incidence and severity are not severe. CD4<sup>+</sup> cells and various cytokines including IL-1 and TNF $\alpha$ , as well as antibody to CII, have been shown to participate in the expression of CIA (Rosloniec et al., 2010).

## Proteoglycan-Induced Arthritis Model

Immunization of BALB/c mice with partially deglycosylated human aggrecan induces chronic progressive polyarthritis and spondylitis (Glant et al., 2003). This proteoglycan (aggrecan)-induced arthritis (PGIA) resembles RA, as judged by observation, laboratory tests, radiography, and histopathology of the peripheral joints. The occurrence of PGIA depends on the development of cross-reactive T- and B-cell responses between the immunizing human and self (mouse) cartilage aggrecan. CIA and PGIA are two most commonly used RA models for QTL mapping for the identification of RA susceptibility loci.

## Collagen Antibody-Induced Arthritis Model

Collagen antibody-induced arthritis (CAIA) is induced by injection of specific monoclonal CII antibodies (Holmdahl et al., 1986). The model was developed based on the findings that serum from arthritic mice or RA patients could transfer arthritis to naïve mice (Wooley et al., 1984; Stuart and Dixon, 1983). CAIA resembles CIA but is more acute and has a rapid onset, a few days after injection. Normally, the disease heals after a month and mice remain healthy. CAIA is inducible independently of MHC and T- and B-cell interaction (Nandakumar et al., 2004).

## CONCLUSION

Animal models of systemic human autoimmune disease have served us well for understanding autoimmunity. Human systemic autoimmune diseases are highly heterogeneous both at the clinical and pathogenic level to the point that we do not serve the field properly by lumping them along antedated criteria-counting approaches. We have presented a critical review of the animal models which have been used to understand disease processes and perform preclinical trials of putative new drugs and biologics.

1. No animal model represents any systemic autoimmune disease.
2. Spontaneous models of disease can be used to obtain early insight on the consequences of lost tolerance in terms of organ damage.
3. Spontaneous models may serve preclinical testing of new rationally developed new drugs and biologics although their predictive clinical value has been rather disappointing.
4. Animals which develop systemic autoimmune disease after modulation of a specific molecule known to be important in the control of maintenance of tolerance or of the immune response may only serve to address specific mechanistic questions.
5. In the study of these animals, the concepts of autoimmunity and autoimmunity-associated organ damage should be carefully considered separately.
6. Construction of animals in which modulated molecules previously identified important in the aberrant function of SLE or RA immune cells may provide valuable information on the development of putative-specific new drugs.
7. These novel animals constructed with intelligence generated from the study of human cells should provide definitive information on the relative contribution of each aberrantly expressed molecule in the expression of human systemic autoimmune disease. The B6. CD2. PP2Ac mouse is such an example (Crispin et al., 2012).

## References

- Abrams, J.R., Kelley, S.L., Hayes, E., Kikuchi, T., Brown, M.J., Kang, S., et al., 2000. Blockade of T lymphocyte costimulation with cytotoxic T lymphocyte-associated antigen 4-immunoglobulin (CTLA4Ig) reverses the cellular pathology of psoriatic plaques, including the activation of keratinocytes, dendritic cells, and endothelial cells. *J. Exp. Med.* 192, 681–694.
- Amano, T., Yamasaki, S., Yagishita, N., Tsuchimochi, K., Shin, H., Kawahara, K., et al., 2003. Synoviolin/Hrd1, an E3 ubiquitin ligase, as a novel pathogenic factor for arthropathy. *Genes Dev.* 17, 2436–2449.
- Andrews, B.S., Eisenberg, R.A., Theofilopoulos, A.N., Izui, S., Wilson, C.B., McConahey, P.J., et al., 1978. Spontaneous murine lupus-like syndromes. Clinical and immunopathological manifestations in several strains. *J. Exp. Med.* 148, 1198–1215.
- Apostolidis, S.A., Rodriguez-Rodriguez, N., Suarez-Fueyo, A., Dioufa, N., Ozcan, E., Crispin, J.C., et al., 2016. Phosphatase PP2A is requisite for the function of regulatory T cells. *Nat. Immunol.* 17, 556–564.
- Bachmaier, K., Krawczyk, C., Kozieradzki, I., Kong, Y.Y., Sasaki, T., Oliveira-Dos-Santos, A., et al., 2000. Negative regulation of lymphocyte activation and autoimmunity by the molecular adaptor Cbl-b. *Nature* 403, 211–216.
- Bahjat, F.R., Pine, P.R., Reitsma, A., Cassafer, G., Baluom, M., Grillo, S., et al., 2008. An orally bioavailable spleen tyrosine kinase inhibitor delays disease progression and prolongs survival in murine lupus. *Arthritis Rheum.* 58, 1433–1444.
- Balomenos, D., Rumold, R., Theofilopoulos, A.N., 1998. Interferon-gamma is required for lupus-like disease and lymphoaccumulation in MRL-lpr mice. *J. Clin. Invest.* 101, 364–371.
- Barber, D.F., Bartolome, A., Hernandez, C., Flores, J.M., Redondo, C., Fernandez-Arias, C., et al., 2005. PI3Kgamma inhibition blocks glomerulonephritis and extends lifespan in a mouse model of systemic lupus. *Nat. Med.* 11, 933–935.
- Beech, J.T., Thompson, S.J., 1997. Anti-tumour necrosis factor therapy ameliorates joint disease in a chronic model of inflammatory arthritis. *Br. J. Rheumatol.* 36, 1129.
- Bignon, J.S., Siminovitch, K.A., 1994. Identification of PTP1C mutation as the genetic defect in motheaten and viable motheaten mice: a step toward defining the roles of protein tyrosine phosphatases in the regulation of hemopoietic cell differentiation and function. *Clin. Immunol. Immunopathol.* 73, 168–179.
- Blanco, P., Palucka, A.K., Gill, M., Pascual, V., Banchereau, J., 2001. Induction of dendritic cell differentiation by IFN-alpha in systemic lupus erythematosus. *Science* 294, 1540–1543.
- Boackle, S.A., Holers, V.M., 2003. Role of complement in the development of autoimmunity. *Curr. Dir. Autoimmun.* 6, 154–168.
- Boackle, S.A., Holers, V.M., Chen, X., Szakonyi, G., Karp, D.R., Wakeland, E.K., et al., 2001. Cr2, a candidate gene in the murine Sle1c lupus susceptibility locus, encodes a dysfunctional protein. *Immunity* 15, 775–785.
- Bolland, S., Ravetch, J.V., 2000. Spontaneous autoimmune disease in Fc(gamma)RIIB-deficient mice results from strain-specific epistasis. *Immunity* 13, 277–285.
- Botto, M., Walport, M.J., 2002. C1q, autoimmunity and apoptosis. *Immunobiology* 205, 395–406.
- Botto, M., Dell'agnola, C., Bygrave, A.E., Thompson, E.M., Cook, H.T., Petry, F., et al., 1998. Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. *Nat. Genet.* 19, 56–59.
- Boule, M.W., Broughton, C., Mackay, F., Akira, S., Marshak-Rothstein, A., Rifkin, I.R., 2004. Toll-like receptor 9-dependent and -independent dendritic cell activation by chromatin-immunoglobulin G complexes. *J. Exp. Med.* 199, 1631–1640.
- Bouzahzah, F., Jung, S., Craft, J., 2003. CD4+ T cells from lupus-prone mice avoid antigen-specific tolerance induction in vivo. *J. Immunol.* 170, 741–748.
- Bubier, J.A., Sproule, T.J., Foreman, O., Spolski, R., Shaffer, D.J., Morse, H.C., et al., 2009. A critical role for IL-21 receptor signaling in the pathogenesis of systemic lupus erythematosus in BXSB-Yaa mice. *Proc. Natl. Acad. Sci. U.S.A.* 106, 1518–1523.
- Campbell, A.M., Kashgarian, M., Shlomchik, M.J., 2012. NADPH oxidase inhibits the pathogenesis of systemic lupus erythematosus. *Sci. Transl. Med.* 4, 157ra141.
- Cash, H., Relle, M., Menke, J., Brochhausen, C., Jones, S.A., Topley, N., et al., 2010. Interleukin 6 (IL-6) deficiency delays lupus nephritis in MRL-Faslpr mice: the IL-6 pathway as a new therapeutic target in treatment of autoimmune kidney disease in systemic lupus erythematosus. *J. Rheumatol.* 37, 60–70.
- Cunningham Graham, D.S., Vyse, T.J., Fortin, P.R., Montpetit, A., Cai, Y.C., Lim, S., et al., 2008. Association of LY9 in UK and Canadian SLE families. *Genes Immun.* 9, 93–102.
- Chan, V.W., Meng, F., Soriano, P., Defranco, A.L., Lowell, C.A., 1997. Characterization of the B lymphocyte populations in Lyn-deficient mice and the role of Lyn in signal initiation and down-regulation. *Immunity* 7, 69–81.
- Chen, Z., Koralov, S.B., Kelsoe, G., 2000. Complement C4 inhibits systemic autoimmunity through a mechanism independent of complement receptors CR1 and CR2. *J. Exp. Med.* 192, 1339–1352.
- Chowdhary, V.R., Grande, J.P., Luthra, H.S., David, C.S., 2007. Characterization of haemorrhagic pulmonary capillaritis: another manifestation of pristane-induced lupus. *Rheumatology (Oxford)* 46, 1405–1410.
- Christensen, A.D., Haase, C., Cook, A.D., Hamilton, J.A., 2016. K/BxN serum-transfer arthritis as a model for human inflammatory arthritis. *Front. Immunol.* 7, 213.
- Cohen, P.L., Caricchio, R., Abraham, V., Camenisch, T.D., Jennette, J.C., Roubey, R.A., et al., 2002. Delayed apoptotic cell clearance and lupus-like autoimmunity in mice lacking the c-mer membrane tyrosine kinase. *J. Exp. Med.* 196, 135–140.
- Cornall, R.J., Cyster, J.G., Hibbs, M.L., Dunn, A.R., Otipoby, K.L., Clark, E.A., et al., 1998. Polygenic autoimmune traits: Lyn, CD22, and SHP-1 are limiting elements of a biochemical pathway regulating BCR signaling and selection. *Immunity* 8, 497–508.
- Crispin, J.C., Apostolidis, S.A., Rosetti, F., Keszei, M., Wang, N., Terhorst, C., et al., 2012. Cutting edge: protein phosphatase 2A confers susceptibility to autoimmune disease through an IL-17-dependent mechanism. *J. Immunol.* 188, 3567–3571.
- Dai, X., James, R.G., Habib, T., Singh, S., Jackson, S., Khim, S., et al., 2013. A disease-associated PTPN22 variant promotes systemic autoimmunity in murine models. *J. Clin. Invest.* 123, 2024–2036.
- Deane, J.A., Pisitkun, P., Barrett, R.S., Feigenbaum, L., Town, T., Ward, J.M., et al., 2007. Control of toll-like receptor 7 expression is essential to restrict autoimmunity and dendritic cell proliferation. *Immunity* 27, 801–810.

- Demetriou, M., Granovsky, M., Quaggin, S., Dennis, J.W., 2001. Negative regulation of T-cell activation and autoimmunity by Mga5 N-glycosylation. *Nature* 409, 733–739.
- Dempsey, P.W., Allison, M.E., Akkaraju, S., Goodnow, C.C., Fearon, D.T., 1996. C3d of complement as a molecular adjuvant: bridging innate and acquired immunity. *Science* 271, 348–350.
- Deng, G.M., Liu, L., Bahjat, F.R., Pine, P.R., Tsokos, G.C., 2010. Suppression of skin and kidney disease by inhibition of spleen tyrosine kinase in lupus-prone mice. *Arthritis Rheum.* 62, 2086–2092.
- Dhaher, Y.Y., Greenstein, B., DE Fougerolles nunn, E., Khamashta, M., Hughes, G.R., 2000. Strain differences in binding properties of estrogen receptors in immature and adult BALB/c and MRL/MP-lpr/lpr mice, a model of systemic lupus erythematosus. *Int. J. Immunopharmacol.* 22, 247–254.
- Drake, C.G., Rozzo, S.J., Vyse, T.J., Palmer, E., Kotzin, B.L., 1995. Genetic contributions to lupus-like disease in (NZB × NZW)F1 mice. *Immunol. Rev.* 144, 51–74.
- Enyedy, E.J., Nambiar, M.P., Liassis, S.N., Dennis, G., Kammer, G.M., Tsokos, G.C., 2001. Fc epsilon receptor type I gamma chain replaces the deficient T cell receptor zeta chain in T cells of patients with systemic lupus erythematosus. *Arthritis Rheum.* 44, 1114–1121.
- Fernandes, G., Talal, N., 1986. SLE: hormones and diet. *Clin. Exp. Rheumatol.* 4, 183–185.
- Finck, B.K., Linsley, P.S., Wofsy, D., 1994. Treatment of murine lupus with CTLA4Ig. *Science* 265, 1225–1227.
- Funabiki, M., Kato, H., Miyachi, Y., Toki, H., Motegi, H., Inoue, M., et al., 2014. Autoimmune disorders associated with gain of function of the intracellular sensor MDA5. *Immunity* 40, 199–212.
- Gaffney, P.M., Kearns, G.M., Shark, K.B., Ortmann, W.A., Selby, S.A., Malmgren, M.L., et al., 1998. A genome-wide search for susceptibility genes in human systemic lupus erythematosus sib-pair families. *Proc. Natl. Acad. Sci. U.S.A.* 95, 14875–14879.
- Gaffney, P.M., Ortmann, W.A., Selby, S.A., Shark, K.B., Ockenden, T.C., Rohlf, K.E., et al., 2000. Genome screening in human systemic lupus erythematosus: results from a second Minnesota cohort and combined analyses of 187 sib-pair families. *Am. J. Hum. Genet.* 66, 547–556.
- Gaip, U.S., Kuhn, A., Sheriff, A., Munoz, L.E., Franz, S., Voll, R.E., et al., 2006. Clearance of apoptotic cells in human SLE. *Curr. Dir. Autoimmun.* 9, 173–187.
- Glant, T.T., Finnegan, A., Mikecz, K., 2003. Proteoglycan-induced arthritis: immune regulation, cellular mechanisms, and genetics. *Crit. Rev. Immunol.* 23, 199–250.
- Groom, J.R., Fletcher, C.A., Walters, S.N., Grey, S.T., Watt, S.V., Sweet, M.J., et al., 2007. BAFF and MyD88 signals promote a lupuslike disease independent of T cells. *J. Exp. Med.* 204, 1959–1971.
- Gross, J.A., Johnston, J., Mudri, S., Enselman, R., Dillon, S.R., Madden, K., et al., 2000. TACI and BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease. *Nature* 404, 995–999.
- Gutierrez-Ramos, J.C., Andreu, J.L., Revilla, Y., Vinuela, E., Martinez, C., 1990. Recovery from autoimmunity of MRL/lpr mice after infection with an interleukin-2/vaccinia recombinant virus. *Nature* 346, 271–274.
- Haas, C., Ryffel, B., Le hir, M., 1997. IFN-gamma is essential for the development of autoimmune glomerulonephritis in MRL/Ipr mice. *J. Immunol.* 158, 5484–5491.
- Hanayama, R., Tanaka, M., Miwa, K., Shinohara, A., Iwamatsu, A., Nagata, S., 2002. Identification of a factor that links apoptotic cells to phagocytes. *Nature* 417, 182–187.
- Hanayama, R., Tanaka, M., Miyasaka, K., Aozasa, K., Koike, M., Uchiyama, Y., et al., 2004. Autoimmune disease and impaired uptake of apoptotic cells in MFG-E8-deficient mice. *Science* 304, 1147–1150.
- Handwerger, B.S., Rus, V., Da silva, L., Via, C.S., 1994. The role of cytokines in the immunopathogenesis of lupus. *Springer Semin. Immunopathol.* 16, 153–180.
- Handwerger, B.S., Storror, C.E., Wasson, C.S., Movafagh, F., Reichlin, M., 1999. Further characterization of the autoantibody response of Palmerston North mice. *J. Clin. Immunol.* 19, 45–57.
- Harley, J.B., Moser, K.L., Gaffney, P.M., Behrens, T.W., 1998. The genetics of human systemic lupus erythematosus. *Curr. Opin. Immunol.* 10, 690–696.
- Herber, D., Brown, T.P., Liang, S., Young, D.A., Collins, M., Dunussi-Joannopoulos, K., 2007. IL-21 has a pathogenic role in a lupus-prone mouse model and its blockade with IL-21R. Fc reduces disease progression. *J. Immunol.* 178, 3822–3830.
- Helgason, C.D., Kalberer, C.P., Damen, J.E., Chappel, S.M., Pineault, N., Krystal, G., et al., 2000. A dual role for Src homology 2 domain-containing inositol-5-phosphatase (SHIP) in immunity: aberrant development and enhanced function of b lymphocytes in ship -/- mice. *J. Exp. Med.* 191, 781–794.
- Hibbs, M.L., Tarlinton, D.M., Armes, J., Grail, D., Hodgson, G., Maglitto, R., et al., 1995. Multiple defects in the immune system of Lyn-deficient mice, culminating in autoimmune disease. *Cell* 83, 301–311.
- Hibbs, M.L., Harder, K.W., Armes, J., Kountouri, N., Quilici, C., Casagranda, F., et al., 2002. Sustained activation of Lyn tyrosine kinase in vivo leads to autoimmunity. *J. Exp. Med.* 196, 1593–1604.
- Holmdahl, R., Rubin, K., Klareskog, L., Larsson, E., Wigzell, H., 1986. Characterization of the antibody response in mice with type II collagen-induced arthritis, using monoclonal anti-type II collagen antibodies. *Arthritis Rheum.* 29, 400–410.
- Ibnou-Zekri, N., Vyse, T.J., Rozzo, S.J., Iwamoto, M., Kobayakawa, T., Kotzin, B.L., et al., 1999. MHC-linked control of murine SLE. *Curr. Top. Microbiol. Immunol.* 246, 275–280. discussion 281.
- Ichinose, K., Rauen, T., Juang, Y.T., Kis-Toth, K., Mizui, M., Koga, T., et al., 2011. Cutting edge: calcium/calmodulin-dependent protein kinase type IV is essential for mesangial cell proliferation and lupus nephritis. *J. Immunol.* 187, 5500–5504.
- Isenberg, D., Gordon, C., Licu, D., Copt, S., Rossi, C.P., Wofsy, D., 2015. Efficacy and safety of atacicept for prevention of flares in patients with moderate-to-severe systemic lupus erythematosus (SLE): 52-week data (APRIL-SLE randomised trial). *Ann. Rheum. Dis.* 74, 2006–2015.
- Izui, S., Iwamoto, M., Fossati, L., Merino, R., Takahashi, S., Ibnou-Zekri, N., 1995. The Yaa gene model of systemic lupus erythematosus. *Immunol. Rev.* 144, 137–156.
- Jeon, M.S., Atfield, A., Venuprasad, K., Krawczyk, C., Sarao, R., Elly, C., et al., 2004. Essential role of the E3 ubiquitin ligase Cbl-b in T cell anergy induction. *Immunity* 21, 167–177.

- Ji, H., Gauguier, D., Ohmura, K., Gonzalez, A., Duchatelle, V., Danoy, P., et al., 2001. Genetic influences on the end-stage effector phase of arthritis. *J. Exp. Med.* 194, 321–330.
- Juang, Y.T., Solomou, E.E., Rellahan, B., Tsokos, G.C., 2002. Phosphorylation and O-linked glycosylation of Elf-1 leads to its translocation to the nucleus and binding to the promoter of the TCR zeta-chain. *J. Immunol.* 168, 2865–2871.
- Juang, Y.T., Rauen, T., Wang, Y., Ichinose, K., Benedyk, K., Tenbrock, K., et al., 2011. Transcriptional activation of the cAMP-responsive modulator promoter in human T cells is regulated by protein phosphatase 2A-mediated dephosphorylation of SP-1 and reflects disease activity in patients with systemic lupus erythematosus. *J. Biol. Chem.* 286, 1795–1801.
- Katsiari, C.G., Kyttaris, V.C., Juang, Y.T., Tsokos, G.C., 2005. Protein phosphatase 2A is a negative regulator of IL-2 production in patients with systemic lupus erythematosus. *J. Clin. Invest.* 115, 3193–3204.
- Kawagoe, T., Takeuchi, O., Takabatake, Y., Kato, H., Isaka, Y., Tsujimura, T., et al., 2009. TANK is a negative regulator of Toll-like receptor signaling and is critical for the prevention of autoimmune nephritis. *Nat. Immunol.* 10, 965–972.
- Keffer, J., Probert, L., Cazlaris, H., Georgopoulos, S., Kaslaris, E., Kioussis, D., et al., 1991. Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. *EMBO J.* 10, 4025–4031.
- Kimberly, R.P., Salmon, J.E., Edberg, J.C., 1995. Receptors for immunoglobulin G. Molecular diversity and implications for disease. *Arthritis Rheum.* 38, 306–314.
- King, C., Tangye, S.G., Mackay, C.R., 2008. T follicular helper (TFH) cells in normal and dysregulated immune responses. *Annu. Rev. Immunol.* 26, 741–766.
- Kitaura, Y., Jang, I.K., Wang, Y., Han, Y.C., Inazu, T., Cadera, E.J., et al., 2007. Control of the B cell-intrinsic tolerance programs by ubiquitin ligases Cbl and Cbl-b. *Immunity* 26, 567–578.
- Kono, D.H., Theofilopoulos, A.N., 2006. Genetics of SLE in mice. *Springer Semin. Immunopathol.* 28, 83–96.
- Kono, D.H., Theofilopoulos, A.N., 2011. Genetics of lupus in mice. In: Lahita, R.G. (Ed.), *Systemic Lupus Erythematosus*, fifth ed. Academic Press, San Diego, CA, pp. 63–105.
- Kontoyiannis, D., Pasparakis, M., Pizarro, T.T., Cominelli, F., Kollias, G., 1999. Impaired on/off regulation of TNF biosynthesis in mice lacking TNF AU-rich elements: implications for joint and gut-associated immunopathologies. *Immunity* 10, 387–398.
- Kool, M., Van loo, G., Waelput, W., De prijck, S., Muskens, F., Sze, M., et al., 2011. The ubiquitin-editing protein A20 prevents dendritic cell activation, recognition of apoptotic cells, and systemic autoimmunity. *Immunity* 35, 82–96.
- Koreth, J., Matsuoka, K., Kim, H.T., Mcdonough, S.M., Bindra, B., Alyea, E.P., et al., 2011. Interleukin-2 and regulatory T cells in graft-versus-host disease. *N. Engl. J. Med.* 365, 2055–2066.
- Kouskoff, V., Korganow, A.S., Duchatelle, V., Degott, C., Benoist, C., Mathis, D., 1996. Organ-specific disease provoked by systemic autoimmunity. *Cell* 87, 811–822.
- Kovacs, B., Vassilopoulos, D., Vogelgesang, S.A., Tsokos, G.C., 1996. Defective CD3-mediated cell death in activated T cells from patients with systemic lupus erythematosus: role of decreased intracellular TNF-alpha. *Clin. Immunol. Immunopathol.* 81, 293–302.
- Kozlowski, M., Mlinaric-Rascan, I., Feng, G.S., Shen, R., Pawson, T., Siminovitch, K.A., 1993. Expression and catalytic activity of the tyrosine phosphatase PTP1C is severely impaired in motheaten and viable motheaten mice. *J. Exp. Med.* 178, 2157–2163.
- Kremer, J.M., Westhovens, R., Leon, M., Di giorgio, E., Alten, R., Steinfeld, S., et al., 2003. Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4Ig. *N. Engl. J. Med.* 349, 1907–1915.
- Krishnan, S., Nambiar, M.P., Warke, V.G., Fisher, C.U., Mitchell, J., Delaney, N., et al., 2004. Alterations in lipid raft composition and dynamics contribute to abnormal T cell responses in systemic lupus erythematosus. *J. Immunol.* 172, 7821–7831.
- Krug, H.E., Mahowald, M.L., Clark, C., 1989. Progressive ankylosis (ank/ank) in mice: an animal model of spondyloarthropathy. III. Proliferative spleen cell response to T cell mitogens. *Clin. Exp. Immunol.* 78, 97–101.
- Kruger, J., Butler, J.R., Cherapanov, V., Dong, Q., Ginzberg, H., Govindarajan, A., et al., 2000. Deficiency of Src homology 2-containing phosphatase 1 results in abnormalities in murine neutrophil function: studies in motheaten mice. *J. Immunol.* 165, 5847–5859.
- Kumar, K.R., Li, L., Yan, M., Bhaskarabhatla, M., Mobley, A.B., Nguyen, C., et al., 2006. Regulation of B cell tolerance by the lupus susceptibility gene Ly108. *Science* 312, 1665–1669.
- Kyttaris, V.C., Zhang, Z., Kuchroo, V.K., Oukka, M., Tsokos, G.C., 2010. Cutting edge: IL-23 receptor deficiency prevents the development of lupus nephritis in C57BL/6-lpr/lpr mice. *J. Immunol.* 184, 4605–4609.
- La cava, A., 2008. T-regulatory cells in systemic lupus erythematosus. *Lupus* 17, 421–425.
- Lawson, B.R., Kono, D.H., Theofilopoulos, A.N., 2002. Deletion of p21 (WAF-1/Cip1) does not induce systemic autoimmunity in female BXSB mice. *J. Immunol.* 168, 5928–5932.
- Lawson, B.R., Baccala, R., Song, J., Croft, M., Kono, D.H., Theofilopoulos, A.N., 2004. Deficiency of the cyclin kinase inhibitor p21(WAF-1/CIP-1) promotes apoptosis of activated/memory T cells and inhibits spontaneous systemic autoimmunity. *J. Exp. Med.* 199, 547–557.
- Leadbetter, E.A., Rifkin, I.R., Hohlbaum, A.M., Beaudette, B.C., Shlomchik, M.J., Marshak-Rothstein, A., 2002. Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. *Nature* 416, 603–607.
- Lee, D.M., Friend, D.S., Gurish, M.F., Benoist, C., Mathis, D., Brenner, M.B., 2002. Mast cells: a cellular link between autoantibodies and inflammatory arthritis. *Science* 297, 1689–1692.
- Lee, E.G., Boone, D.L., Chai, S., Libby, S.L., Chien, M., Lodolce, J.P., et al., 2000. Failure to regulate TNF-induced NF-kappaB and cell death responses in A20-deficient mice. *Science* 289, 2350–2354.
- Lee, P.Y., Kumagai, Y., Li, Y., Takeuchi, O., Yoshida, H., Weinstein, J., et al., 2008. TLR7-dependent and Fc gamma R-independent production of type I interferon in experimental mouse lupus. *J. Exp. Med.* 205, 2995–3006.
- Lemke, G., Rothlin, C.V., 2008. Immunobiology of the TAM receptors. *Nat. Rev. Immunol.* 8, 327–336.
- Levy, E., Ambrus, J., Kahl, L., Molina, H., Tung, K., Holers, V.M., 1992. T lymphocyte expression of complement receptor 2 (CR2/CD21): a role in adhesive cell-cell interactions and dysregulation in a patient with systemic lupus erythematosus (SLE). *Clin. Exp. Immunol.* 90, 235–244.
- Lin, S.C., Yen, J.H., Tsai, J.J., Tsai, W.C., Ou, T.T., Liu, H.W., et al., 2004. Association of a programmed death 1 gene polymorphism with the development of rheumatoid arthritis, but not systemic lupus erythematosus. *Arthritis Rheum.* 50, 770–775.

- Linterman, M.A., Rigby, R.J., Wong, R.K., Yu, D., Brink, R., Cannons, J.L., et al., 2009. Follicular helper T cells are required for systemic autoimmunity. *J. Exp. Med.* 206, 561–576.
- Liossis, S.N., Ding, X.Z., Dennis, G.J., Tsokos, G.C., 1998. Altered pattern of TCR/CD3-mediated protein-tyrosyl phosphorylation in T cells from patients with systemic lupus erythematosus. Deficient expression of the T cell receptor zeta chain. *J. Clin. Invest.* 101, 1448–1457.
- Lucas, J.A., Menke, J., Rabacal, W.A., Schoen, F.J., Sharpe, A.H., Kelley, V.R., 2008. Programmed death ligand 1 regulates a critical checkpoint for autoimmune myocarditis and pneumonitis in MRL mice. *J. Immunol.* 181, 2513–2521.
- Lui, S.L., Tsang, R., Chan, K.W., Zhang, F., Tam, S., Yung, S., et al., 2008. Rapamycin attenuates the severity of established nephritis in lupus-prone NZB/W F1 mice. *Nephrol. Dial. Transplant* 23, 2768–2776.
- Luzina, I.G., Knitzer, R.H., Atamas, S.P., Gause, W.C., Papadimitriou, J.C., Sztein, M.B., et al., 1999. Vasculitis in the Palmerston North mouse model of lupus: phenotype and cytokine production profile of infiltrating cells. *Arthritis Rheum.* 42, 561–568.
- Mackay, F., Woodcock, S.A., Lawton, P., Ambrose, C., Baetscher, M., Schneider, P., et al., 1999. Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. *J. Exp. Med.* 190, 1697–1710.
- Manderson, A.P., Botto, M., Walport, M.J., 2004. The role of complement in the development of systemic lupus erythematosus. *Annu. Rev. Immunol.* 22, 431–456.
- Marquart, H.V., Svendsen, A., Rasmussen, J.M., Nielsen, C.H., Junker, P., Svehag, S.E., et al., 1995. Complement receptor expression and activation of the complement cascade on B lymphocytes from patients with systemic lupus erythematosus (SLE). *Clin. Exp. Immunol.* 101, 60–65.
- Marshak-Rothstein, A., 2006. Toll-like receptors in systemic autoimmune disease. *Nat. Rev. Immunol.* 6, 823–835.
- Martinez, J., Cunha, L.D., Park, S., Yang, M., Lu, Q., Orchard, R., et al., 2016. Noncanonical autophagy inhibits the autoinflammatory, lupus-like response to dying cells. *Nature* 533, 115–119.
- Matsumoto, I., Lee, D.M., Goldbach-Mansky, R., Sumida, T., Hitchon, C.A., Schur, P.H., et al., 2003. Low prevalence of antibodies to glucose-6-phosphate isomerase in patients with rheumatoid arthritis and a spectrum of other chronic autoimmune disorders. *Arthritis Rheum.* 48, 944–954.
- Matsushita, K., Takeuchi, O., Standley, D.M., Kumagai, Y., Kawagoe, T., Miyake, T., et al., 2009. Zc3h12a is an RNase essential for controlling immune responses by regulating mRNA decay. *Nature* 458, 1185–1190.
- Maxwell, M.J., Duan, M., Armes, J.E., Anderson, G.P., Tarlinton, D.M., Hibbs, M.L., 2011. Genetic segregation of inflammatory lung disease and autoimmune disease severity in SHIP-1<sup>-/-</sup> mice. *J. Immunol.* 186, 7164–7175.
- McInnes, I.B., Schett, G., 2011. The pathogenesis of rheumatoid arthritis. *N. Engl. J. Med.* 365, 2205–2219.
- Miyanishi, M., Tada, K., Koike, M., Uchiyama, Y., Kitamura, T., Nagata, S., 2007. Identification of Tim4 as a phosphatidylserine receptor. *Nature* 450, 435–439.
- Monach, P.A., Benoist, C., Mathis, D., 2004. The role of antibodies in mouse models of rheumatoid arthritis, and relevance to human disease. *Adv. Immunol.* 82, 217–248.
- Morel, L., Wakeland, E.K., 1998. Susceptibility to lupus nephritis in the NZB/W model system. *Curr. Opin. Immunol.* 10, 718–725.
- Morel, L., Rudofsky, U.H., Longmate, J.A., Schiffenbauer, J., Wakeland, E.K., 1994. Polygenic control of susceptibility to murine systemic lupus erythematosus. *Immunity* 1, 219–229.
- Morris, S.C., Cohen, P.L., Eisenberg, R.A., 1990. Experimental induction of systemic lupus erythematosus by recognition of foreign Ia. *Clin. Immunol. Immunopathol.* 57, 263–273.
- Moser, K.L., Gray-McGuire, C., Kelly, J., Asundi, N., Yu, H., Bruner, G.R., et al., 1999. Confirmation of genetic linkage between human systemic lupus erythematosus and chromosome 1q41. *Arthritis Rheum.* 42, 1902–1907.
- Nagata, S., Hanayama, R., Kawane, K., 2010. Autoimmunity and the clearance of dead cells. *Cell* 140, 619–630.
- Nambiar, M.P., Enyedy, E.J., Fisher, C.U., Krishnan, S., Warke, V.G., Gilliland, W.R., et al., 2002. Abnormal expression of various molecular forms and distribution of T cell receptor zeta chain in patients with systemic lupus erythematosus. *Arthritis Rheum.* 46, 163–174.
- Nandakumar, K.S., Backlund, J., Vestberg, M., Holmdahl, R., 2004. Collagen type II (CII)-specific antibodies induce arthritis in the absence of T or B cells but the arthritis progression is enhanced by CII-reactive T cells. *Arthritis Res. Ther.* 6, R544–R550.
- Napirei, M., Karsunky, H., Zevnik, B., Stephan, H., Mannherz, H.G., Moroy, T., 2000. Features of systemic lupus erythematosus in Dnase1-deficient mice. *Nat. Genet.* 25, 177–181.
- Navarra, S.V., Guzman, R.M., Gallacher, A.E., Hall, S., Levy, R.A., Jimenez, R.E., et al., 2011. Efficacy and safety of belimumab in patients with active systemic lupus erythematosus: a randomised, placebo-controlled, phase 3 trial. *Lancet* 377, 721–731.
- Nelson, B.H., 2002. Interleukin-2 signaling and the maintenance of self-tolerance. *Curr. Dir. Autoimmun.* 5, 92–112.
- Nishimura, H., Okazaki, T., Tanaka, Y., Nakatani, K., Hara, M., Matsumori, A., et al., 2001. Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. *Science* 291, 319–322.
- Okazaki, T., Tanaka, Y., Nishio, R., Mitsuiye, T., Mizoguchi, A., Wang, J., et al., 2003. Autoantibodies against cardiac troponin I are responsible for dilated cardiomyopathy in PD-1-deficient mice. *Nat. Med.* 9, 1477–1483.
- Pani, G., Fischer, K.D., Mlinaric-Rascan, I., Siminovitch, K.A., 1996. Signaling capacity of the T cell antigen receptor is negatively regulated by the PTP1C tyrosine phosphatase. *J. Exp. Med.* 184, 839–852.
- Pao, L.I., Lam, K.P., Henderson, J.M., Kutok, J.L., Alimzhanov, M., Nitschke, L., et al., 2007. B cell-specific deletion of protein-tyrosine phosphatase Shp1 promotes B-1a cell development and causes systemic autoimmunity. *Immunity* 27, 35–48.
- Pimentel-Quiroz, V.R., Ugarte-Gil, M.F., Alarcon, G.S., 2016. Abatacept for the treatment of systemic lupus erythematosus. *Expert Opin. Investig. Drugs* 25, 493–499.
- Pisitkun, P., Deane, J.A., Difilippantonio, M.J., Tarasenko, T., Satterthwaite, A.B., Bolland, S., 2006. Autoreactive B cell responses to RNA-related antigens due to TLR7 gene duplication. *Science* 312, 1669–1672.
- Prokunina, L., Castillejo-Lopez, C., Oberg, F., Gunnarsson, I., Berg, L., Magnusson, V., et al., 2002. A regulatory polymorphism in PDCD1 is associated with susceptibility to systemic lupus erythematosus in humans. *Nat. Genet.* 32, 666–669.
- Reap, E.A., Leslie, D., Abrahams, M., Eisenberg, R.A., Cohen, P.L., 1995. Apoptosis abnormalities of splenic lymphocytes in autoimmune lpr and gld mice. *J. Immunol.* 154, 936–943.

- Reilly, C.M., Gilkeson, G.S., 2002. Use of genetic knockouts to modulate disease expression in a murine model of lupus, MRL/lpr mice. *Immunol Res* 25, 143–153.
- Rodriguez-Manzanet, R., Sanjuan, M.A., Wu, H.Y., Quintana, F.J., Xiao, S., Anderson, A.C., et al., 2010. T and B cell hyperactivity and autoimmunity associated with niche-specific defects in apoptotic body clearance in TIM-4-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* 107, 8706–8711.
- Rosloniec, E.F., Cremer, M., Kang, A.H., Myers, L.K., Brand, D.D., 2010. Collagen-induced arthritis. *Curr. Protoc. Immunol.* 15 (5), 1–25 (Chapter 15, Unit).
- Roths, J.B., Murphy, E.D., Eicher, E.M., 1984. A new mutation, gld, that produces lymphoproliferation and autoimmunity in C3H/HeJ mice. *J. Exp. Med.* 159, 1–20.
- Roubinian, J.R., Talal, N., Greenspan, J.S., Goodman, J.R., Siiteri, P.K., 1978. Effect of castration and sex hormone treatment on survival, anti-nucleic acid antibodies, and glomerulonephritis in NZB/NZW F1 mice. *J. Exp. Med.* 147, 1568–1583.
- Rozzo, S.J., Allard, J.D., Choubey, D., Vyse, T.J., Izui, S., Peltz, G., et al., 2001. Evidence for an interferon-inducible gene, Ifi202, in the susceptibility to systemic lupus. *Immunity* 15, 435–443.
- Saadoun, D., Rosenzwajg, M., Joly, F., Six, A., Carrat, F., Thibault, V., et al., 2011. Regulatory T-cell responses to low-dose interleukin-2 in HCV-induced vasculitis. *N. Engl. J. Med.* 365, 2067–2077.
- Sakaguchi, N., Takahashi, T., Hata, H., Nomura, T., Tagami, T., Yamazaki, S., et al., 2003. Altered thymic T-cell selection due to a mutation of the ZAP-70 gene causes autoimmune arthritis in mice. *Nature* 426, 454–460.
- Santiago-Raber, M.L., Baccala, R., Haraldsson, K.M., Choubey, D., Stewart, T.A., Kono, D.H., et al., 2003. Type-I interferon receptor deficiency reduces lupus-like disease in NZB mice. *J. Exp. Med.* 197, 777–788.
- Santiago-Raber, M.L., Kikuchi, S., Borel, P., Uematsu, S., Akira, S., Kotzin, B.L., et al., 2008. Evidence for genes in addition to Tlr7 in the Yaa translocation linked with acceleration of systemic lupus erythematosus. *J. Immunol.* 181, 1556–1562.
- Satoh, M., Reeves, W.H., 1994. Induction of lupus-associated autoantibodies in BALB/c mice by intraperitoneal injection of pristane. *J. Exp. Med.* 180, 2341–2346.
- Scott, R.S., McMahon, E.J., Pop, S.M., Reap, E.A., Caricchio, R., Cohen, P.L., et al., 2001. Phagocytosis and clearance of apoptotic cells is mediated by MER. *Nature* 411, 207–211.
- Sharfe, N., Dadi, H.K., Shahar, M., Roifman, C.M., 1997. Human immune disorder arising from mutation of the alpha chain of the interleukin-2 receptor. *Proc. Natl. Acad. Sci. U.S.A.* 94, 3168–3171.
- Shen, N., Fu, Q., Deng, Y., Qian, X., Zhao, J., Kaufman, K.M., et al., 2010. Sex-specific association of X-linked Toll-like receptor 7 (TLR7) with male systemic lupus erythematosus. *Proc. Natl. Acad. Sci. U.S.A.* 107, 15838–15843.
- Shores, E.W., Love, P.E., 1997. TCR zeta chain in T cell development and selection. *Curr. Opin. Immunol.* 9, 380–389.
- Simpson, N., Gatenby, P.A., Wilson, A., Malik, S., Fulcher, D.A., Tangye, S.G., et al., 2010. Expansion of circulating T cells resembling follicular helper T cells is a fixed phenotype that identifies a subset of severe systemic lupus erythematosus. *Arthritis Rheum.* 62, 234–244.
- Stuart, J.M., Dixon, F.J., 1983. Serum transfer of collagen-induced arthritis in mice. *J. Exp. Med.* 158, 378–392.
- Stuart, J.M., Tomoda, K., Yoo, T.J., Townes, A.S., Kang, A.H., 1983. Serum transfer of collagen-induced arthritis. II. Identification and localization of autoantibody to type II collagen in donor and recipient rats. *Arthritis Rheum.* 26, 1237–1244.
- Suzuki, A., Yamada, R., Kochi, Y., SAWADA, T., Okada, Y., Matsuda, K., et al., 2008. Functional SNPs in CD244 increase the risk of rheumatoid arthritis in a Japanese population. *Nat. Genet.* 40, 1224–1229.
- Svenson, J.L., Eudaly, J., Ruiz, P., Korach, K.S., Gilkeson, G.S., 2008. Impact of estrogen receptor deficiency on disease expression in the NZM2410 lupus prone mouse. *Clin. Immunol.* 128, 259–268.
- Takahashi, K., Kozono, Y., Waldschmidt, T.J., Berthiaume, D., Quigg, R.J., Baron, A., et al., 1997. Mouse complement receptors type 1 (CR1; CD35) and type 2 (CR2; CD21): expression on normal B cell subpopulations and decreased levels during the development of autoimmunity in MRL/lpr mice. *J. Immunol.* 159, 1557–1569.
- Takahashi, T., Tanaka, M., Brannan, C.I., Jenkins, N.A., Copeland, N.G., Suda, T., et al., 1994. Generalized lymphoproliferative disease in mice, caused by a point mutation in the Fas ligand. *Cell* 76, 969–976.
- Taylor, P.R., Carugati, A., Fadok, V.A., Cook, H.T., Andrews, M., Carroll, M.C., et al., 2000. A hierarchical role for classical pathway complement proteins in the clearance of apoptotic cells in vivo. *J. Exp. Med.* 192, 359–366.
- Tivol, E.A., Borriello, F., Schweitzer, A.N., Lynch, W.P., Bluestone, J.A., Sharpe, A.H., 1995. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity* 3, 541–547.
- Truedsson, L., Bengtsson, A.A., Sturfelt, G., 2007. Complement deficiencies and systemic lupus erythematosus. *Autoimmunity* 40, 560–566.
- Tsao, B.P., Cantor, R.M., Kalunian, K.C., Chen, C.J., Badsha, H., Singh, R., et al., 1997. Evidence for linkage of a candidate chromosome 1 region to human systemic lupus erythematosus. *J. Clin. Invest.* 99, 725–731.
- Tsao, B.P., Cantor, R.M., Grossman, J.M., Kim, S.K., Strong, N., Lau, C.S., et al., 2002. Linkage and interaction of loci on 1q23 and 16q12 may contribute to susceptibility to systemic lupus erythematosus. *Arthritis Rheum.* 46, 2928–2936.
- Tsokos, G.C., 2011. Systemic lupus erythematosus. *N. Engl. J. Med.* 365, 2110–2121.
- Tsokos, G.C., Lambris, J.D., Finkelman, F.D., Anastassiou, E.D., June, C.H., 1990. Monovalent ligands of complement receptor 2 inhibit whereas polyvalent ligands enhance anti-Ig-induced human B cell intracytoplasmic free calcium concentration. *J. Immunol.* 144, 1640–1645.
- Tsokos, G.C., Kovacs, B., Sfikakis, P.P., Theocharis, S., Vogelgesang, S., Via, C.S., 1996. Defective antigen-presenting cell function in patients with systemic lupus erythematosus. *Arthritis Rheum.* 39, 600–609.
- Tsokos, G.C., Nambiar, M.P., Tenbrock, K., Juang, Y.T., 2003. Rewiring the T-cell: signaling defects and novel prospects for the treatment of SLE. *Trends Immunol.* 24, 259–263.
- Tsui, H.W., Siminovitch, K.A., De souza, L., Tsui, F.W., 1993. Motheaten and viable motheaten mice have mutations in the hematopoietic cell phosphatase gene. *Nat. Genet.* 4, 124–129.
- Tsukumo, S., Yasutomo, K., 2004. DNaseI in pathogenesis of systemic lupus erythematosus. *Clin. Immunol.* 113, 14–18.
- Uehata, T., Iwasaki, H., Vandenberg, A., Matsushita, K., Hernandez-Cuellar, E., Kuniyoshi, K., et al., 2013. Malt1-induced cleavage of regnase-1 in CD4(+) helper T cells regulates immune activation. *Cell* 153, 1036–1049.

- Vereecke, L., Beyaert, R., Van loo, G., 2011. Genetic relationships between A20/TNFAIP3, chronic inflammation and autoimmune disease. *Biochem. Soc. Trans.* 39, 1086–1091.
- Vinuesa, C.G., Cook, M.C., Angelucci, C., Athanasopoulos, V., Rui, L., Hill, K.M., et al., 2005. A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. *Nature* 435, 452–458.
- Vollmer, J., Tluk, S., Schmitz, C., Hamm, S., Jurk, M., Forsbach, A., et al., 2005. Immune stimulation mediated by autoantigen binding sites within small nuclear RNAs involves Toll-like receptors 7 and 8. *J. Exp. Med.* 202, 1575–1585.
- Vratsanos, G.S., Jung, S., Park, Y.M., Craft, J., 2001. CD4(+) T cells from lupus-prone mice are hyperresponsive to T cell receptor engagement with low and high affinity peptide antigens: a model to explain spontaneous T cell activation in lupus. *J. Exp. Med.* 193, 329–337.
- Vyse, T.J., Rozzo, S.J., Drake, C.G., Appel, V.B., Lemeur, M., Izui, S., et al., 1998. Contributions of Ea(z) and Eb(z) MHC genes to lupus susceptibility in New Zealand mice. *J. Immunol.* 160, 2757–2766.
- Wakeland, E.K., Morel, L., Mohan, C., Yui, M., 1997. Genetic dissection of lupus nephritis in murine models of SLE. *J. Clin. Immunol.* 17, 272–281.
- Walker, S.E., Gray, R.H., Fulton, M., Wigley, R.D., Schnitzer, B., 1978. Palmerston North mice, a new animal model of systemic lupus erythematosus. *J. Lab. Clin. Med.* 92, 932–945.
- Wandstrat, A.E., Nguyen, C., Limaye, N., Chan, A.Y., Subramanian, S., Tian, X.H., et al., 2004. Association of extensive polymorphisms in the SLAM/CD2 gene cluster with murine lupus. *Immunity* 21, 769–780.
- Waters, S.T., Fu, S.M., Gaskin, F., Deshmukh, U.S., Sung, S.S., Kannapell, C.C., et al., 2001. NZM2328: a new mouse model of systemic lupus erythematosus with unique genetic susceptibility loci. *Clin Immunol.* 100, 372–383.
- Waterhouse, P., Penninger, J.M., Timms, E., Wakeham, A., Shahinian, A., Lee, K.P., et al., 1995. Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. *Science* 270, 985–988.
- Watson, W.C., Brown, P.S., Pitcock, J.A., Townes, A.S., 1987. Passive transfer studies with type II collagen antibody in B10. D2/old and new line and C57Bl/6 normal and beige (Chediak-Higashi) strains: evidence of important roles for C5 and multiple inflammatory cell types in the development of erosive arthritis. *Arthritis Rheum.* 30, 460–465.
- Wooley, P.H., Luthra, H.S., Singh, S.K., Huse, A.R., Stuart, J.M., David, C.S., 1984. Passive transfer of arthritis to mice by injection of human anti-type II collagen antibody. *Mayo Clin. Proc.* 59, 737–743.
- Wooley, P.H., Seibold, J.R., Whalen, J.D., Chapdelaine, J.M., 1989. Pristane-induced arthritis. The immunologic and genetic features of an experimental murine model of autoimmune disease. *Arthritis Rheum.* 32, 1022–1030.
- Yajima, K., Nakamura, A., Sugahara, A., Takai, T., 2003. Fc $\gamma$ RIIB deficiency with Fas mutation is sufficient for the development of systemic autoimmune disease. *Eur. J. Immunol.* 33, 1020–1029.
- Yamazaki, T., Arase, H., Ono, S., Ohno, H., Watanabe, H., Saito, T., 1997. A shift from negative to positive selection of autoreactive T cells by the reduced level of TCR signal in TCR-transgenic CD3 zeta-deficient mice. *J. Immunol.* 158, 1634–1640.
- Yasutomo, K., Horiuchi, T., Kagami, S., Tsukamoto, H., Hashimura, C., Urushihara, M., et al., 2001. Mutation of DNASE1 in people with systemic lupus erythematosus. *Nat. Genet.* 28, 313–314.
- Yu, C.C., Tsui, H.W., Ngan, B.Y., Shulman, M.J., Wu, G.E., Tsui, F.W., 1996. B and T cells are not required for the viable motheaten phenotype. *J. Exp. Med.* 183, 371–380.
- Zhang, Z., Kyttaris, V.C., Tsokos, G.C., 2009. The role of IL-23/IL-17 axis in lupus nephritis. *J. Immunol.* 183, 3160–3169.

## Further Reading

- Furie, R., Khamashta, M., Merrill, J.T., Werth, V.P., Kalunian, K., Brohawn, P., et al., 2017. Anifrolumab, an anti-interferon-alpha receptor monoclonal antibody, in moderate-to-severe systemic lupus erythematosus. *Arthritis Rheum.* 69, 376–386.
- Khamashta, M., Merrill, J.T., Werth, V.P., Furie, R., Kalunian, K., Illei, G.G., et al., 2016. Sifalimumab, an anti-interferon-alpha monoclonal antibody, in moderate to severe systemic lupus erythematosus: a randomised, double-blind, placebo-controlled study. *Ann. Rheum. Dis.* 75, 1909–1916.
- Marshall, D., Dangerfield, J.P., Bhatia, V.K., Larbi, K.Y., Nourshargh, S., Haskard, D.O., 2003. MRL/lpr lupus-prone mice show exaggerated ICAM-1-dependent leucocyte adhesion and transendothelial migration in response to TNF-alpha. *Rheumatology (Oxford)* 42, 929–934.

# Systemic Lupus Erythematosus

Jagtar Singh Nijjar and Kenneth G C Smith

Department of Medicine and Cambridge Institute of Therapeutic Immunology and Infectious Disease, University of Cambridge, Cambridge, United Kingdom

## OUTLINE

<b>Introduction</b>	<b>555</b>	<b>Disease Features</b>	<b>560</b>
<b>Epidemiology</b>	<b>555</b>	<i>Cutaneous and Mucosal Disease</i>	562
<b>Pathogenesis of Disease</b>	<b>556</b>	<b>Therapeutics in Systemic Lupus Erythematosus</b>	<b>565</b>
<i>Genetic and Associated Studies</i>	556	<i>Measurement of Disease Activity</i>	565
<i>Transcriptional Analysis in Systemic Lupus Erythematosus</i>	556	<i>Disease Modifying Drugs</i>	566
<i>Autoantibodies in Systemic Lupus Erythematosus</i>	557	<b>Future Perspectives</b>	<b>569</b>
<i>Cellular Players in Systemic Lupus Erythematosus</i>	557	<b>References</b>	<b>569</b>
<i>Animal Models of Systemic Lupus Erythematosus</i>	560		

## INTRODUCTION

Systemic lupus erythematosus (SLE) is systemic autoimmune disease causing significant morbidity and mortality worldwide, particularly in the women of child-bearing age. It is typically associated with antinuclear antibodies (ANA), in particular antidouble-stranded DNA (anti-dsDNA) antibodies, which form immune complexes and can cause multiorgan damage by activating various cell types in genetically susceptible individuals.

Due to the chronic and heterogenous nature of the condition, clinical trials of novel therapeutics or treatment strategies are challenging, with some newer drugs failing to meet their primary endpoint in trial. SLE manifestations are protean, with the treatment of lupus nephritis (LN) being a priority area for study given its association with end-stage renal failure (ESRF). SLE remains a disease with significant unmet need, but there is hope that the study of larger, well-characterized cohorts along with further genetic and experimental medicine studies, will lead to new treatments and improved patient outcomes.

## EPIDEMIOLOGY

Studies of the incidence and prevalence of SLE have been hampered by the lack of high-quality census data, population mobility, and also the diagnostic definition of SLE. As well as census data, countries such as the United Kingdom have disease registries that have also helped to clarify the incidence and prevalence of the disease. Incidence rates vary worldwide from just over 2 per 100,000 in Denmark (Hermansen et al., 2016) to 4 per 100,000 in the United Kingdom (Rees et al., 2016), 7 per 100,000 in the United States (Furst et al., 2013), and 8 per

100,000 in Taiwan (Chiu and Lai, 2010). Differences in incidence are due, at least in part, to genetics, but study design also has an impact on the data available. Recent systematic reviews of the incidence and prevalence of SLE worldwide (Rees et al., 2017) and the effect of ethnicity and genetic ancestry (Lewis and Jawad, 2017) further discuss these complicated issues.

A large recent study from the United Kingdom examined a primary care research database and showed an incidence of 4.9 per 100,000 person-years. The incidence was 5.8 times higher in females at all ages, with a peak onset of 40–49 years for females and 60–69 for males. Although the study was carried out in the United Kingdom, data linkage allowed the assessment of ethnicity, with Afro-Caribbeans having the highest incidence (31.46/100,000 person-years) compared to Caucasians (6.73/100,000 person-years) (Rees et al., 2016). This and other studies have demonstrated an increase in prevalence from the historical figures of 40 per 100,000 to almost 100 per 100,000: it is likely this is largely due to a true increase in disease prevalence, but better patient survival and increased case identification may also contribute.

Studies in the United States have confirmed the higher incidence and prevalence in African American patients with 186 per 100,000 females being affected by the disease compared to 87 per 100,000 Caucasian females (Somers et al., 2014). Furthermore, African American had an earlier disease onset and disease severity was increased, with 40% of the patients having renal disease and 15% ESRF compared to 19% and 5%, respectively, in Caucasian patients.

Hispanic and South Asian populations are also affected to higher degree compared to Caucasians and are more likely to progress to renal replacement therapy (Rees et al., 2016; Feldman et al., 2013). The same study, using the US Medicaid database, showed that Native American populations had a prevalence and incidence similar to Hispanic and Asian populations but lower than African Americans. The incidence of SLE in this study (23.2 per 100,000 person-years) was also higher than previous studies from the United States but may reflect the high-risk and poorer population covered by Medicaid.

A large study from Taiwan showed an incidence rate of 8.1 per 100,000 person-years (Chiu and Lai, 2010) which is higher than the general incidence rate from UK studies but lower than those from the United States. Earlier studies from Africa may have underestimated the rate of SLE (Tiffin et al., 2014), but the studies of African, South Asian and Caribbean migrants to the United Kingdom show higher rates of SLE compared to Caucasians (Johnson et al., 1995).

## PATHOGENESIS OF DISEASE

### Genetic and Associated Studies

SLE is a classical polygenic disease, with multiple genetic variants with small effect sizes contributing to disease risk, although monogenic causes are now increasingly recognized as the causing cases of SLE, particularly in younger patients. This section considers the contributions made by genetic studies in the understanding of disease pathways and ethnic differences and goes on to describe transcriptomic studies, including the interferon signature, in SLE.

There is an increased risk of SLE and other autoimmune diseases such as rheumatoid arthritis (RA) in the siblings of patients with SLE (Alarcón-Segovia et al., 2005). A high sibling recurrence ratio ( $\lambda_s$ ) of 29 was observed in SLE compared to, for example, 5 in RA, and a polygenic additive model was thought to be most suitable for SLE (Alarcón-Segovia et al., 2005). Early twin studies suggest a monozygous concordance rate of up to 69% but were likely subject to overreporting bias, as subsequent twin-registry studies demonstrated 25% of monozygotic twins and 2% of dizygotic twins were disease concordant (Deapen et al., 1992). A large study of all national health insurance records in Taiwan suggested a heritability of over 40% for SLE by comparing relative risk of SLE in twins, first-degree relatives, and spouses without genetic similarity (Kuo et al., 2015).

Deficiencies in early complement proteins from the classical pathway, such as C1q and C4, have been linked to pediatric SLE (as well as predisposing to infection). However, these mutations are rare, and the mutation does not have complete penetrance, indicating that a combination of genetic and/or environmental factors is required. Mutations in TREX1, a DNA exonuclease, have also been associated with lupus, with the accumulation of intracellular single-stranded DNA and subsequent Toll-like receptor (TLR)-driven secretion of interferon as a postulated mechanism (Lee-Kirsch et al., 2007; Stetson et al., 2008). In addition, genes in pathways implicated in type I

interferon production, nucleic acid sensing, clearance of self-antigen, apoptosis, and tolerance are causes of monogenic lupus and have been recently reviewed (Lo, 2016).

Early linkage disequilibrium studies showed that various regions of the genome associated with risk of SLE and other autoimmune diseases, with the MHC and STAT4 being implicated early (Remmers et al., 2007). The first genome-wide association studies (GWAS) included predominantly European cases, and controls confirmed the findings of early linkage studies and added substantially to them. The regions and genes associated with SLE included HLA-DRB1, IRF5, STAT4, FCGR2A, and PTPN22 [International Consortium for Systemic Lupus Erythematosus Genetics (SLEGEN) et al., 2008]. Association of variants at loci containing BLK, ITGAM-ITGAX, and BANK1 genes implicated B cells in the pathogenesis of SLE (Hom et al., 2008; Kozyrev et al., 2008). Currently, 62 loci have strong evidence of genetic association with SLE, with recent estimates suggesting these explain around 27% of the heritability of SLE (Morris et al., 2016).

Studies in Han Chinese populations and populations of mixed Asian ancestry replicated findings from original GWAS and further identified loci containing ETS1, WDFY1, IKZF1, and others which may be specific to this ethnic group (Han et al., 2009; Yang et al., 2010). Ethnic differences in SLE incidence and severity may therefore be driven by genetic differences, and balancing selection may provide an evolutionary explanation for some of these. Genetic variants that reduce the expression or function of Fc $\gamma$ RIIb, an inhibitory Fc receptor, predispose to SLE in mice and humans (Niederer et al., 2010). Interestingly, they also protect against malaria, consistent with the increased frequency of the main SLE-associated FCGR2B variant in African and Asian populations (Willcocks et al., 2010).

## Transcriptional Analysis in Systemic Lupus Erythematosus

Transcriptomic studies of whole blood or cell subsets from patients with SLE have given insight into mechanisms of disease or prognostic factors. Comparison of the transcriptomes of peripheral blood mononuclear cells (PBMCs) from patients with pediatric SLE, juvenile arthritis, and healthy controls found that genes that were differentially regulated in SLE were overrepresented in the interferon (IFN) pathways, and that this “interferon signature” correlated with disease activity (Bennett et al., 2003). A subsequent study confirmed the existence of an interferon signature, and divided patients with SLE into “interferon-high” and “interferon-low” groups, with the “interferon-high” group having more severe and active disease (Baechler et al., 2003). These studies thus emphasized a role for type I interferons in SLE and suggested they could be therapeutic targets (see Other agents and Treatment Options).

Transcriptional studies of purified cell subsets can overcome the “noise” caused by the variable cellular contents of PBMC between individuals. One such study found signature in purified CD8 T cells that correlated with time to flare in SLE, anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV), and inflammatory bowel disease, and was associated with CD8 T-cell exhaustion (McKinney et al., 2015, 2010). This could be of practical value if predicting a poor course could allow preemptive intensive therapy, and demonstrates the benefits of studying cell subsets, something now taken to a higher level with single-cell transcriptomic analysis.

## Autoantibodies in Systemic Lupus Erythematosus

Over 100 autoantibodies (Sherer et al., 2004) have been described in patients with SLE and include ANA, described further below, as well as antibodies targeting blood cells (e.g., antiplatelet), endothelial cells, and antigens in the nervous system to name a few. Development of autoantibodies predates disease onset (Arbuckle et al., 2003), and some may drive disease features such as fetal transfer of anti-Ro antibodies resulting in congenital heart block.

ANA are a diverse group of autoantibodies that are categorized according to their antigenic targets: DNA and DNA-binding proteins and histones; RNA and RNA-associated proteins; as well as autoantibodies associated with phospholipids and cell membrane proteins. Certain ANA are strongly associated with particular autoimmune rheumatic diseases: antitopoisomerase I with progressive systemic sclerosis, anticentromere with the limited cutaneous form of systemic sclerosis, and anti-Jo1 with myositis, although many more autoantibodies have been discovered in the latter disease.

Almost every patient with SLE is positive for ANA, which may also include specific antibodies associated with organ involvement (see the following table). Anti-dsDNA and anti-Sm are the most specific for SLE, anti-C1q is associated with aggressive LN, and anti-Ro, when present during pregnancy, can lead to congenital heart block and neonatal lupus.

ANA	Clinical association
Anti-dsDNA, anti-Sm, anti-C1q	Lupus nephritis
Anti-Ro	Neonatal lupus, congenital heart block, subacute cutaneous lupus
Anti-Ro and anti-La	Secondary Sjögren's syndrome and sicca symptoms
Anti-RNP	
Anti-P	Neuropsychiatric manifestations
Anti-β2-glycoprotein 1	Antiphospholipid syndrome

ANA, Antinuclear antibodies; anti-dsDNA, antidualle-stranded DNA; RNP, ribonucleoprotein.

Anti-dsDNA antibodies are the most widely assayed autoantibody in SLE and are more specific for SLE than ANA. DNA can be released from dead and dying cells by three main methods: apoptosis (an active process resulting in apoptotic bodies and microparticles containing DNA), necrosis, and via the development of “neutrophil extracellular traps”—so-called NETosis (Brinkmann et al., 2004).

Free DNA and dsDNA antibodies combine to form antigen–antibody complexes, which are deposited in sites such as the skin and kidney to nonspecifically activate the immune system and cause tissue damage via antibody dependent cellular cytotoxicity and complement activation. DNA-containing immune-complexes can also activate immune cells by signaling through surface and endosomal TLRs.

ANA can be measured with a variety of assays; however due to the wide variety of targets, not every autoantibody is detected during screening and specific autoantibody testing may be needed if clinical suspicion of a particular manifestation is high. ANA was originally measured using the “gold standard” indirect immunofluorescence assay (IIFA) first described in the 1950s (Coons and Kaplan, 1950). This assay involves serial dilutions of sera and a determination of the staining pattern on Hep-2 cells. The reproducibility and interpretation of this time-consuming assay can be variable, and so higher throughput standardized assays such as enzyme-linked immunosorbent assays (ELISA) or chemiluminescence assays have been employed to improve consistency between laboratories worldwide. Using the IIFA method, with increasing age up to 13% of the healthy population can develop a positive ANA (Mariz et al., 2011). In individuals with autoimmune diseases, however, the ANA titer is higher with a nuclear homogenous, nuclear coarse speckled, nuclear centromeric, or nuclear fine-speckled pattern occurring. Healthy individuals could also exhibit a nuclear fine-speckled pattern, but at a lower titer, whilst a nuclear dense fine-speckled pattern only occurred in healthy individuals (Mariz et al., 2011).

The IIFA method using HEp-2 or Hep-2000 cells has been recognized as the gold standard for ANA screening (Agmon-Levin et al., 2014). Thereafter anti-dsDNA antibodies should be measured in a consistent manner using an ELISA, Farr method or *Crithidia luciliae* immunofluorescence test. ELISA-based methods allow standardization and quantitative monitoring during disease course and treatment. The *C. luciliae* assay or Farr assay offer the best specificity at the time of diagnosis, although these are rarely done now as both assays are time consuming and the latter requires the use of radioactive material.

Antibodies to extractable nuclear antigens, such as those that bind RNA and RNA-binding proteins, can also be present in SLE, but are also seen in other autoimmune diseases. Anti-Ro and Anti-La antibodies are associated with Sjögren's syndrome and anti-Ro is associated with congenital heart block and neonatal lupus. Anti-RNP antibodies bind to complexes of RNA with small nuclear ribonucleoproteins, but are not specific for SLE as they are also present in mixed connective tissue disease.

ANA testing remains a hallmark in the diagnosis and management of SLE, but their frequent occurrence in the healthy population precludes their use as a screening test. Certain ANA are associated with organ-specific manifestations of disease, but whether these define genetically distinct disease subsets [as has been shown in RA (Padyukov et al., 2011) and ANCA associated vasculitis (Lyons et al., 2012)] or can be used to stratify management remains to be determined.

## Cellular Players in Systemic Lupus Erythematosus

The adaptive immune system is clearly implicated in the pathogenesis of SLE given the central role of autoreactive B and T cells (Moulton et al., 2017). However, dysregulation of the innate immune system is also

critical. A complete review of the cellular pathogenesis of SLE is outside the scope of this chapter, which will concentrate only on well-described cell types, while accepting that many others (including, e.g., innate lymphoid cells, natural killer (NK) and stromal cells, and tissue resident lymphocytes) are also likely to be involved in pathogenesis.

The break of tolerance to self in SLE occurs many years before clinical presentation. Studies using serum repositories from armed forces personnel in the United States have shown that ANA are usually present up to a decade before clinical manifestations, and that anti-dsDNA antibodies are present on average 2.2 years before diagnosis (Arbuckle et al., 2003). However, a retrospective review of medical records also show that clinical features are present before diagnosis, and symptoms such as arthritis and seizures can precede diagnosis by almost 2 years (Heinlen et al., 2007). Nevertheless, as with other autoimmune diseases such as RA and type 1 diabetes, autoantibodies are present before the onset of clinical disease.

### **Adaptive Immunity**

#### **B CELLS**

SLE is characterized by autoantibody production, and SLE-associated genetic variants occur in genes, such as BLK, BANK1, and ITGAM, together implicating B cells in disease pathogenesis. B-cell tolerance is maintained at a series of central and peripheral “checkpoints,” at which autoreactive B cells are deleted or functionally silenced through a process termed anergy (Goodnow, 2007). Defects in central tolerance, anergy, and peripheral (germinal center) tolerance have all been implicated in predisposing to SLE (Malkiel et al., 2016; Yurasov et al., 2005), and B-cell survival factors such as the B-lymphocyte stimulator (BlyS) are elevated in patients with SLE (Petri et al., 2008) which might contribute to persistence of autoreactive B cells.

B cells contribute to disease by producing autoantibodies but may also play a role in antigen presentation to autoreactive T cells. In addition, populations of CD27-negative memory B cells are expanded in patients with SLE (but not in RA or chronic hepatitis C), although their role in pathogenesis is not clear (Wei et al., 2007). “Regulatory” B cells, which can suppress Th1 responses via IL-10, appear impaired in SLE (Blair et al., 2010). These findings suggest that, in addition to producing autoantibodies, B cells may also predispose to SLE by contributing to immune dysregulation more generally.

#### **T CELLS**

T cells are important in the pathogenesis of SLE. Their roles are diverse and include B-cell help via CD4 T helper cells, in particular T follicular helper (Tfh) cells, cellular cytotoxicity via CD8 T cells and roles for T regulatory cells and Th17 cells. Only a few key features will be highlighted here.

CD4 T cells can drive B cell differentiation more efficiently than IL21, B cell activating factors or TLR ligands. Upon activation, CD4 T cells from patients with SLE persistently express CD40 ligand resulting in more B cell activation and differentiation. Recently, a subset of CD4 T cells, the Tfh cell, which is found in the follicular zone of lymphoid organs, has been shown to drive B-cell maturation and class-switched antibody production by secreting IL-21. Tfh cells, and others that secrete IL-21, have been associated with the formation of extrafollicular germinal centers in organs inflamed by SLE, such as kidney.

Tfh cells express the chemokine receptor CXCR5 and PD1 and are found at increased frequency in the lymph nodes of patients with SLE, as are Tfh-like cells in the blood (Choi et al., 2015). In SLE patients CD4 helper T cells have a defect in IL-2 production, which may reduce the effectiveness of regulatory T cells in controlling inflammation (Lieberman and Tsokos, 2010). Consistent with this, low-dose IL-2 therapy increased regulatory T-cell numbers and improved disease activity in a proof of concept study in humans (He et al., 2016). CD8 T cells have been indirectly implicated in SLE, as their exhaustion is associated with an improved prognosis (McKinney et al., 2015). An increase in the number of Th17 cells, and in IL-17 levels, has been observed in patients with SLE. Consistent with these cells playing a role, ustekinumab, an IL-12/23 blocker which interrupts signaling upstream of IL-17, has shown some efficacy in the treatment of SLE (van Vollenhoven et al., 2017a).

### **Innate Immunity**

#### **DENDRITIC CELLS**

Dendritic cells are “professional” antigen presenting cells and also help prime lymphocytes and other immune cells. Two major subtypes exist: myeloid and plasmacytoid dendritic cells (mDC and pDC), with both thought to have roles in the pathogenesis and perpetuation of disease in SLE.

In particular, pDCs have an important role in the production of type I interferons. Immune complexes can activate pDCs via TLRs, and this leads to the production of IFN $\alpha$  and other proinflammatory cytokines (Blanco et al., 2008). pDCs have been shown to accumulate in the end organs where damage occurs, such as the kidney, and play a role in orchestrating tissue damage (Tucci et al., 2008). IFN $\alpha$  activity is elevated prior to the diagnosis of SLE, and both type I and II IFNs appear to play poorly defined roles in the initiation and perpetuation of disease (Munroe et al., 2016). Type I IFNs are implicated in the wider pathogenesis of SLE, activating neutrophils, promoting mDC differentiation and increasing B-cell responsiveness. Type I IFN may also be important for the malaise and intractable fatigue prominent in SLE. Type I IFN has been targeted clinical by agents such as sifalimumab (Khamashtha et al., 2016) and rontalizumab (Kalunian et al., 2016) with promising but inconclusive results, and further trials are underway, many using variants on the IFN transcriptomic signature described above as a biomarker.

### **Neutrophils and Extracellular Traps**

Neutrophils are first-line cells in host protection against bacteria and are activated by complement and cytokines to phagocytose pathogens. Neutrophils can undergo a particular type of cell death called NETosis, where their cellular contents including DNA form networks of fibers (Brinkmann et al., 2004) that in health trap bacteria but in autoimmunity might lead to prolonged presentation of self-antigen (Kessenbrock et al., 2009). Neutrophils also carry a toxic payload of lytic enzymes and which could exacerbate tissue damage.

### **Animal Models of Systemic Lupus Erythematosus**

Although animal models do not fully recapitulate human disease, they are useful in exploring mechanisms of disease in a controlled system. Mouse models, in particular the New Zealand Black (NZB)/New Zealand White (NZW) and Murphy Roths Large (MRL)/*lpr*, have been invaluable to our early understanding of SLE, as they share similar histopathological features and syntenic genetic susceptibility loci to human disease.

The first generation of a cross between NZB and NZW, known as NZB/NZW F1, develop a lupus-like illness (Andrews et al., 1978). Female mice are more often affected and they develop lymphadenopathy and splenomegaly as well as ANA. In addition, these mice develop a glomerulonephritis that is immune complex-mediated and so have been used as a model of chronic SLE. Further congenic crosses resulted in the New Zealand mixed (NZM) strains each of which has slight differences in pathology (Rudofsky et al., 1993). The NZM2410 strain showed particularly severe pathology. Three congenic lines were generated through backcrossing onto a C57BL/6 background: B6.Sle1 (Mohan et al., 1998), B6.Sle2 (Mohan et al., 1997; Morel et al., 2000), and B6.Sle3 (Nguyen et al., 2002). These contained genetic loci that are syntenic to those implicated in human SLE, including the major histocompatibility complex (MHC) region, Fc receptor, and signalling lymphocyte activation molecule (SLAM) family loci to name but a few. Each had subtle immune abnormalities, but when intercrossed developed SLE, demonstrating how genetic variants combine to cause polygenic disease.

The MRL strain was developed in Japan and developed chronic autoimmune and vasculitis features, and a substrain derived from it—the MRL/*lpr*—developed SLE-like disease more rapidly, along with glomerulonephritis, prominent lymphadenopathy, and abnormal T-cell accumulation. The *lpr* variant was found to be due to disruption of the *Fas* gene resulting in reduced T-cell apoptosis (Adachi et al., 1993). Disease in the BXSB mouse is accelerated in the BXSB-Yaa strain, with more aggressive disease in males, as *Yaa* is a translocation of the X chromosome region containing TLR7 on to the Y chromosome (Murphy and Roths, 1979; Maibaum et al., 2000), effectively doubling the dose of this and neighboring genes in males (Santiago-Raber et al., 2008).

## **DISEASE FEATURES**

Precise disease delineation in SLE is difficult because the variety of clinical manifestations and disease subsets can be poorly defined or indeed cryptic. The Systemic Lupus International Collaborating Clinics (SLICC) 2012 criteria are the latest published for the classification of SLE (Petri et al., 2012). These improved on the American College of Rheumatology (ACR) 1982 and 1997 criteria (Tan et al., 1982; Hochberg, 1997) by, for example, allowing a diagnosis of SLE with biopsy proven LN with either positive ANA or dsDNA antibodies. Furthermore,

they were found to be more sensitive than the 1997 ACR criteria and were shown to be useful in clinical practice rather than solely in the realm of clinical trials ([Inês et al., 2015](#)). Although at least four criteria are needed for

**SLICC Classification Criteria for Systemic Lupus Erythematosus 2012**

≥ 4 Criteria are required (at least one from clinical and one immunologic) or biopsy proven lupus nephritis with either ANA positive or anti-dsDNA antibodies

Clinical	Immunologic
Acute cutaneous lupus	ANA
Chronic cutaneous lupus	Anti-dsDNA
Oral or nasal ulcers	Anti-Sm
Nonscarring alopecia	Antiphospholipid antibodies
Arthritis	Low complement
Serositis	Direct Coombs' test positive (but not in the presence of hemolytic anemia)
Renal	
Neurologic	
Hemolytic anemia	
Leucopenia	
Thrombocytopenia	

ANA, Antinuclear antibodies.

the diagnosis of SLE, in the early stages of disease these may not be present. If SLE is suspected but insufficient features are present for a formal diagnosis, a forme fruste diagnosis of “incomplete lupus” can be used ([Ugarte-Gil and Alarcón, 2016](#)). Drug-induced SLE can be caused by hydralazine, sulfasalazine, and TNF $\alpha$  inhibitors, etc., and needs careful assessment as both immunologic and clinical features can evolve even once the medication has been stopped.

Constitutional symptoms such as fever, weight loss, and fatigue may precede clinical diagnosis and can be associated with a wide differential diagnosis, which can delay diagnosis and treatment initiation. Furthermore, these symptoms can be some of the most troubling and intractable for patients and have a bearing on the psychosocial wellbeing of the patient and their family and carers.

---

**Disease features with prevalence in parentheses ([Font et al., 2004](#))**

---

Cutaneous and mucosal (59%)

- Malar rash (54%)
- Discoid lupus (8%)
- Livedo reticularis (5%)

Musculoskeletal (83%)

- Arthralgia
- Jaccoud’s deforming arthropathy of SLE

Cardiac (13%)

- Pericarditis
- Accelerated atherosclerosis

(Continued)

(Continued)

Disease features with prevalence in parentheses (Font et al., 2004)

- Valvular heart disease including Libman–Sacks

Vascular (32%)

- Raynaud's phenomenon
- Antiphospholipid syndrome

Pulmonary (3%)

- Pleural effusion
- Interstitial fibrosis
- Interstitial pneumonitis

Renal (35%)

- Glomerulonephritis

Neurologic (18%)

- Seizures
- Psychosis
- Stroke and transient ischemic attacks

Hematologic (75%)

- Hemolytic anemia
- Thrombocytopenia
- Leucopenia

Constitutional symptoms (42%)

- Fever
- Lymphadenopathy
- Hair loss

Gastrointestinal

- Oral ulcers
- Regional enteritis
- Vasculitis

SLE, Systemic lupus erythematosus.

## Cutaneous and Mucosal Disease

Acute malar rash is a classical feature of lupus and may be photosensitive. Other forms of SLE-associated rash include bullous lupus, epidermal necrolysis, and a maculopapular lupus rash. Subacute lupus rashes may require input from dermatologists as they can be psoriaform and occasionally be associated with depigmentation and telangiectasia.

Chronic forms include, but are not limited to, a discoid rash, lupus panniculitis, and chilblain lupus. Mucosal surfaces can be involved with oral ulcers of the tongue, palate, and buccal area. Nasal ulceration is also a feature although infectious causes should be ruled out. Both hair loss and fragile hair can occur but reversible causes

such as iron deficiency should be excluded. SLE can be diagnosed on the basis of three forms of mucocutaneous disease and a positive ANA, and therefore a careful examination of the skin and mucus membranes must always be carried out.

### **Musculoskeletal Manifestations**

Synovitis of at least two joints or tenderness of at least two joints with early morning stiffness is part of the SLICC SLE arthritis criteria. Jaccoud's arthropathy of SLE occurs in 5%–10% of patients, and while it is not erosive it can have some clinical features in keeping with RA, such as ulnar deviation and swan-neck deformities. These deformities are due to ligamentous laxity, although in some patients an overlap syndrome can be present where they also have rheumatoid factor and antibodies against citrullinated peptides. Synovitis and radiographic erosion can be present in these circumstances, necessitating consideration as a more inflammatory arthritis in these cases.

Although not an autoimmune manifestation of disease, avascular necrosis can be a complication of steroid therapy. Commonly affected sites are the hip and tibial plateau, and the investigation of choice is MRI to detect early change when radiographs may be normal. Management of avascular necrosis in younger patients is challenging, and efforts are made to delay joint replacement surgery with conservative management where possible.

Myositis is an uncommon manifestation of SLE, but myalgia is more widespread and may also be related to medication such as statins that are used to manage comorbidities. Elevated creatine kinase levels raise the possibility of myositis, and muscle tissue biopsy can help to rule out other conditions. Long-term steroid use causes steroid-related myopathy that can be disabling and affect quality of life.

### **Cardiac**

Pericarditis and pericardial effusion are the commonest cardiac manifestations of SLE. Myocardial and endocardial inflammation, such as Libman–Sacks endocarditis, can also occur. Comorbidities are common, in the form of ischemic heart disease and infective endocarditis in patients who are on high-dose and long-term corticosteroids. SLE patients are at risk for accelerated cardiovascular disease and have a higher rate of arterial and venous thromboembolic events compared to the healthy population (Mok et al., 2005).

### **Vascular**

Secondary Raynaud's phenomenon occurs in 20% of patients with SLE and may need management with nifedipine or iloprost. In the case of digital ulceration, sildenafil and bosentan may be indicated. Antiphospholipid antibody positivity can be determined by measuring lupus anticoagulant, medium-to-high titer of IgA, IgG, or IgM antiphospholipid antibody or positive IgA, IgG, or IgM anti- $\beta_2$  glycoprotein antibody. Catastrophic antiphospholipid syndrome can result in devastating thrombosis and can be associated with recurrent miscarriage.

### **Pulmonary**

Exudative pleural effusions can occur during disease flare and may need drainage. Pleuritis can be challenging to manage and the possibility of pulmonary embolism should be considered in the differential diagnosis. D-Dimers may be nonspecifically raised in inflammation and therefore may not be helpful.

### **Renal**

The International Society for Nephrology/Renal Pathology Society (ISN/RPS) 2003 classification for LN (Weening et al., 2004) superseded the World Health Organization 1995 classification. LN is a presenting feature in up to 35% of patients with SLE, and although overall SLE survival is 92% at 10 years, this is reduced to 88% at 10 years in the presence of LN. Survival is poorer for African Americans, Hispanics, and Asians, and despite treatment, 10%–20% of patients with LN will progress to ESRF (Group KDIGO/KW, 2012; Bertasias et al., 2012; Hahn et al., 2012).

Indications for biopsy include an unexplained fall in renal function, proteinuria ( $\geq 0.5 \text{ g}/24 \text{ h}$  or 3+ on urine dipstick) and active urinary sediment or hematuria, although there is no international consensus (Wilhelms et al., 2016). Biopsies are then classified according to the ISN/RPS criteria and treatment initiated according to diagnostic class. Treatment should not be delayed if renal biopsy is not readily available, and in patients with refractory disease, repeat biopsy should be considered.

**International Society of Nephrology/Renal Pathology Society 2003 classification of LN**

Class	Description with subtypes	Histological changes
Class I	Minimal mesangial LN	Normal on LM. Mesangial immune deposits by EM or IF
Class II	Mesangial proliferative LN	Mesangial hypercellularity or mesangial matrix expansion with mesangial immune deposits. Isolated subepithelial or subendothelial deposits by EM or IF
Class III	Focal LN (<50% of glomeruli) III (A): active lesions III (A/C): active and chronic lesions III (C): chronic lesions	Active or inactive focal, segmental, or global endocapillary or extracapillary glomerulonephritis, with or without mesangial alterations. Focal subendothelial immune deposits by EM or IF
Class IV	Diffuse LN ( $\geq 50\%$ glomeruli) Diffuse segmental (IV-S) or global (IV-G) LN IV (A): active lesions IV (A/C): active and chronic lesions IV (C): chronic lesions	Active or inactive diffuse, segmental, or global endocapillary or extracapillary glomerulonephritis, with or without mesangial alterations. Diffuse subendothelial immune deposits by EM or IF
Class V	Membranous LN (can occur in combination with class III or IV)	Global or segmental subepithelial immune deposits (EM or IF) or morphological sequelae by LM with or without mesangial alterations
Class VI	Advanced sclerosing LN ( $\geq 90\%$ globally sclerosed glomeruli without residual activity)	Globally sclerosed glomeruli

EM, Electron microscopy; IF, immunofluorescence; LM, light microscopy; LN, lupus nephritis.

In general, classes I and II disease do not require immunosuppression, although some guidelines suggest that proteinuria in the context of class II disease should be treated with immunosuppressants. Patients with class III or IV disease require aggressive immunosuppression, and regimes using glucocorticoids, cyclophosphamide, and other immunosuppressants, including that biologics are discussed in the treatment section. Class V disease in the presence of class III or IV disease should also be treated with aggressive immunosuppression, although there is inconsistency in guidance for the treatment of class V disease alone. Class VI disease is not treated with immunosuppression, in general, although it may be required in the presence of extrarenal manifestations, and preparation should be made for renal replacement therapy.

Patients with active LN should be monitored regularly and have their body weight, blood pressure, serum creatinine, proteinuria, complement levels, and anti-dsDNA antibody levels measured. In addition, management of comorbidities should include blood pressure control ( $< 130/80$  mmHg), treatment of hyperlipidemia with statins, and management of proteinuria with renin–angiotensin–aldosterone system inhibition.

The ISN/RPS classification does not take account of tubulointerstitial lesions, although they have been shown to have prognostic value independent of glomerular lesions. Vascular lesions such as immune complex deposition, lupus vasculopathy, thrombotic microangiopathy (TMA), and overt vasculitis are not taken account of in the ISN/RPS criteria. TMA can occur in thrombotic thrombocytopenia, and some guidelines advise to treat it with plasma exchange. Anticoagulation may be needed in the context of significant proteinuria ( $> 3$  g/24 h) or in antiphospholipid antibody–associated nephropathy.

### **Neuropsychiatric**

Neuropsychiatric lupus is a difficult syndrome to diagnose and manage and the prevalence of the condition is unclear. Neurological manifestations of SLE can range from seizures through cranial nerve palsies and peripheral neuropathies to acute confusional states and psychosis. A standardized nomenclature exists for neuropsychiatric SLE (Kozora et al., 2004), but a major unmet need is the lack of specific biomarkers to differentiate neuropsychiatric lupus from its differentials.

## Hematologic

Hemolytic anemia, leucopenia, and thrombocytopenia all occur as features of SLE and can be difficult to manage in the context of treatments such as cyclophosphamide or other disease modifying drugs, many of which can cause bone marrow suppression.

# THERAPEUTICS IN SYSTEMIC LUPUS ERYTHEMATOSUS

## Measurement of Disease Activity

Measuring disease activity in a multisystemic disorder such as SLE is particularly challenging but is necessary to measure treatment responses both in routine clinical care and in the context of research trials and longitudinal studies. In addition to the time taken to fill out these measures by patients and clinical staff, there is often a significant administrative burden of calculating scores, especially for more extensive measures such as BILAG (British Isles Lupus Assessment Group) 2004 (Isenberg et al., 2005).

The BILAG score assesses nine systems and requires a degree of training in order to administer it efficiently. The test itself can take up to 20 minutes depending on the complexity of disease, but the administrative time taken to record the data can be up to double that. The SLEDAI-2K (Gladman et al., 2002) instrument assesses nine systems but uses a simple point-based additive scale, and so is faster to administer. Both the BILAG and SLEDAI-2K can be used to quantify severity of disease flares and so have clinical utility and can help direct therapeutic escalation decisions. Alongside the score based methods, ESR and C-reactive protein (CRP) should be measured and if both are elevated, the possibility of concomitant infection should be considered. Rising antibodies to ds-DNA and falling levels of C3 and C4 may herald an imminent lupus flare, especially if there is a change from baseline (Fernando and Isenberg, 2005).

Prediction of SLE flares to allow preemptive treatment has proven difficult. In a large post hoc analysis of the belimumab trials, rising dsDNA antibody levels, proteinuria, CRP, and BlyS levels predicted flare (Petri et al., 2013), but studies from the Hopkins lupus cohort showed that decreased dsDNA antibody levels and decreased complement levels were most common around the time of flare (Ho et al., 2001a,b; Petri et al., 2009) and earlier studies from the BILAG group showed similar results (Symmons et al., 1988). Others have found no association with dsDNA antibody or complement levels but did find an association with baseline ESR, anemia, lymphopenia, and ANA titer (Mirzayan et al., 2000). Predicting flare in SLE based on clinical or simple antibody parameters thus leads to conflicting conclusions, and a biomarker predicting flares remains a significant unmet need for patients.

Cumulative damage can be captured using the SDI (SLICC/American College of Rheumatology Damage Index) (Gladman et al., 1996). This tool assesses 12 organ systems and is generally only used in “steady states” of disease. At diagnosis each patient has a score of 0 and subsequent accumulation of damage is a predictor of mortality (Rahman et al., 2001). As with other measures, the length of time to complete the assessment is increased in complex patients with multiorgan involvement.

Disease subsets in SLE are likely to exist, although there is no consensus on classification. Common “phenotypes” include clinically active but serologically quiescent disease (Gladman et al., 2003); serologically active but clinically quiescent disease (Steiman et al., 2010); and serologically quiescent, clinically quiescent disease (Steiman et al., 2012). These phenotypes may overlap during the course of disease in a patient and show that we need validated biomarkers to help characterize disease pathology and prognosis in a particular patient, at a particular time.

“Treating to target” refers to treating until a predetermined disease activity state has been achieved, rather than for a fixed treatment “course,” and has been an effective strategy in diseases such as hypertension, diabetes, and RA. Consensus statements from expert groups have established the principles of treating to target in SLE (van Vollenhoven et al., 2014) and have stated that the aim for each patient should be to treat to remission. In 2016 a consensus definition was established for a lupus low disease activity state (LLDAS) (Franklyn et al., 2016). Essentially this state required five components: a SLEDAI-2K  $\leq 4$ , a SLEDAI physician global assessment of  $\leq 1$  (scale 0–3), no new lupus disease activity compared to the last assessment, a prednisolone dose  $\leq 7.5$  mg daily, and well-tolerated biologic or immunosuppressive agents. Those who achieved more than 50% of their time in LLDAS had reduced organ damage compared to those who did not. However, LLDAS is not equal to remission and therefore international frameworks have been developed to help define and validate an accepted definition of remission in SLE (van Vollenhoven et al., 2017b).

### **Management of Comorbidities**

In addition to managing the direct manifestations of SLE, reduced disease activity and also treatment side effects can lead to significant comorbidity. Premature atherosclerosis results in increased cardiovascular disease. In addition, the metabolic syndrome is more prevalent in patients with SLE compared to matched controls. Osteoporosis can be induced by glucocorticoids, and therefore monitoring of bone mineral density and of vitamin D levels is required in patients who are at high risk. Immunosuppression carries risks that include infection and malignancy, and some drugs can cause infertility (e.g., cyclophosphamide) or are teratogenic (e.g., mycophenolate).

### **Disease Modifying Drugs**

#### ***Hydroxychloroquine***

Antimalarial medication such as hydroxychloroquine (HCQ) and chloroquine are anchor drugs in the treatment of SLE during the acute and quiescent phase. HCQ is used in both discoid lupus and SLE, and has been associated with renal remission in patients who are treated with mycophenolate mofetil (MMF) for membranous LN ([Kasitanon et al., 2006](#)). Furthermore, a randomized trial of withdrawal of HCQ by [Canadian Hydroxychloroquine Study Group \(1991\)](#) showed that patients who are taking HCQ and have stable SLE are at a 2.5 times lower risk of having a disease flare.

HCQ has also been shown to be protective against thrombosis in a cohort study of SLE patients from Spain and also demonstrated a survival benefit in those taking the drug ([Ruiz-Irastorza et al., 2006](#)). A further study using a multiethnic cohort from the United States demonstrated a survival benefit but also took account of a propensity score, which aims to control for the fact that patients taking HCQ often had milder disease and higher socioeconomic status ([Alarcón et al., 2007](#)).

Optimizing drug levels of HCQ does not appear to confer added benefit, although this area requires further investigation ([Costedoat-Chalumeau et al., 2013](#)). Systematic reviews of the literature have also shown that toxicity is infrequent and the drug is relatively safe in pregnancy-controlling disease activity without seemingly having an effect on the new-born ([Ruiz-Irastorza et al., 2010](#)). Recent guidelines regarding retinal toxicity suggest a maximum daily HCQ dose of  $\leq 5.0$  mg/kg real weight with the risk of toxicity up to 5 years being  $<1\%$  and up to 10 years being  $<2\%$ . Risk factors for toxicity include higher dose, longer duration of use, preexisting renal or retinal disease, and the use of tamoxifen ([Marmor et al., 2016](#)). These guidelines recommend baseline fundoscopy to rule out preexisting maculopathy and then to commence annual screening after 5 years of drug treatment; sooner if major risk factors are present.

#### ***Azathioprine***

Azathioprine has commonly been used as a steroid-sparing agent, especially in the context of lupus with renal involvement. The BILAG multicenter trial comparing cyclosporin and azathioprine showed that there was no difference between the two agents with regard to either steroid reduction from baseline or disease flares during the course of the trial ([Griffiths et al., 2010](#)). Azathioprine has been used as a maintenance therapy following high-dose steroid and cyclophosphamide induction therapy for LN. However, the ALMS trial showed that mycophenolate was superior to azathioprine in preventing disease relapses and in maintaining a renal response ([Dooley et al., 2011](#)).

Although mycophenolate was shown to be superior to azathioprine, it is contraindicated during pregnancy whereas azathioprine has been shown to be relatively safe. Switching mycophenolate to azathioprine in pregnant patients, with quiescent LN, rarely led to renal flares ([Fischer-Betz et al., 2013](#)). Azathioprine is also well tolerated and leucocytopenias are an uncommon reason for cessation of therapy. Adequate dosage of azathioprine is a contentious issue, with not all services measuring thiopurine methyltransferase levels. Measurement of azathioprine metabolites may help to determine whether patients fail because of true disease resistance or inadequate dosage and therefore undertreatment ([Croyle et al., 2015](#)). Therefore in particular circumstances, such as during pregnancy or in mycophenolate intolerance, there is still a role for azathioprine in the treatment of SLE.

#### ***Mycophenolate***

MMF has been used for over a decade in the treatment of LN and was originally used in patients who were resistant to other therapies ([Karim et al., 2002](#)). Subsequent trials have demonstrated that MMF can, at least in some circumstances, be of comparable efficacy to intravenous cyclophosphamide when used as induction therapy

in LN (Appel et al., 2009; Ginzler et al., 2005). Furthermore, a Cochrane review of treatment for LN analyzed 50 clinical trials in LN and concluded that mycophenolate was as effective as cyclophosphamide and was also associated with less alopecia and ovarian failure (Henderson et al., 2012).

MMF is also more effective as a maintenance therapy for LN compared to azathioprine, although it is contraindicated in pregnancy (Dooley et al., 2011). In addition to treating renal manifestations, it has also been shown to be effective at controlling extrarenal disease in SLE (Ginzler et al., 2010). Enteric-coated mycophenolate sodium has a better gastrointestinal tolerability profile, with a recent trial showing that it is more effective than azathioprine at treating SLE and preventing relapses as measured by the SLEDAI-2K and BILAG scoring system (Ordi-Ros et al., 2017).

### **Calcineurin Inhibitors**

Although cyclosporin A and tacrolimus have been used in solid organ transplant for many years, their role in SLE treatment has been less clear. Calcineurin inhibitor-related side effects such as hypertension, gingival hyperplasia, and hirsutism occur less with tacrolimus than with cyclosporin. A trial of tacrolimus versus MMF as induction therapy in LN showed no difference between the two agents (Mok et al., 2016). These treatments can be useful when mycophenolate, azathioprine, or cyclophosphamide cause significant leucopenia or other toxicity.

Drug monitoring of calcineurin inhibitors often makes their use in clinical practice more complicated than mycophenolate, although newer agents such as voclosporin have a higher therapeutic window. Pilot trials of voclosporin and mycophenolate as a combination therapy in LN have suggested greater efficacy (Dooley et al., 2016) but also more serious adverse events, and larger trials of voclosporin are underway in lupus. Topical tacrolimus can also be used to treat discoid lupus and cutaneous manifestations of lupus (Wang et al., 2015).

### **Intravenous Steroids and Cyclophosphamide**

Cyclophosphamide has been a mainstay of treatment in acute LN and has been shown to be superior to steroids alone (Gourley et al., 1996) or azathioprine monotherapy in establishing remission (Steinberg and Steinberg, 1991). The Euro-Lupus trial demonstrated that low-dose intermittent cyclophosphamide was as effective as high dose at achieving remission in patients with active LN (Houssiau et al., 2002). Cyclophosphamide is, however, associated with ovarian failure in females, and this is a significant problem in a disease with a female:male ratio of 9:1. Therefore in certain cases cyclophosphamide has been superseded by mycophenolate as induction therapy.

## **Biologic Agents and Small Molecule Inhibitors in Systemic Lupus Erythematosus**

### **RITUXIMAB**

B cells and the antibodies they produce play a central role in the pathogenesis of SLE, and it is therefore not surprising that B-cell depletion has been considered as a therapeutic option. Rituximab is a chimeric monoclonal antibody against CD20 and which depletes B cells but not plasma cells, and both case reports and series suggested a beneficial role in renal and extrarenal SLE (Smith et al., 2006).

Formal trials of the use of rituximab in SLE have not been successful. The EXPLORER trial of rituximab in moderate-to-severely active SLE failed to reach the primary endpoint, which was the ability of rituximab or placebo to achieve a complete or partial clinical response at week 52 (Merrill et al., 2010). Many believe this was due to defects in trial design rather than true lack of efficacy, perhaps including trial size, background immunosuppression, and high steroid use, along with difficulty of measuring outcomes in a heterogeneous disease like SLE. Subsequent analyses showed that rituximab was superior to placebo in reducing the risk of severe flares (defined as BILAG A) and also the total number of flares (Merrill et al., 2011). However, this was a post hoc analysis and therefore could not be used to support regulatory authorization for the use of rituximab in SLE. The LUNAR trial, which assessed the role of rituximab in active proliferative LN, also failed to achieve the primary endpoint of renal response at 52 weeks (Rovin et al., 2012). However, clinical experience, together with long-term follow-up data and post hoc analyses, has meant that, despite negative phase three clinical trials, rituximab is often used “off-label” in renal and extrarenal SLE.

Based on regimens from the transplant community, rituximab is being trialed as a treatment for LN but without tapered oral steroids (RITUXILUP), after a cohort study found that the majority of LN patients achieved a complete or partial response by 37 weeks, suggesting that oral steroids can be avoided (Condon et al., 2013). These and subsequent trials could lead to a step-change in the treatment of SLE without steroids, but longer term

follow-up data will be crucial, especially in the case of a B-cell depleting therapy that can result in secondary hypogammaglobulinemia.

### BELIMUMAB

Belimumab inhibits the BLyS, which is a key factor on B-cell survival and has been shown to be upregulated in patients with SLE. BLyS inhibition has the effect of reducing CD20-positive B cells, short-lived plasmablasts, and anti-dsDNA autoantibodies. The BLISS-52 trial showed that belimumab was superior to placebo with higher SLE Responder Index improvement at week 52 (Navarra et al., 2011) and no significant safety signals. The SLE responder index is defined as a  $\geq 4$  point reduction in SELENA-SLEDAI score, no deterioration from baseline in a physician global assessment and no new BILAG A scores and no more than one new BILAG B score. Belimumab was therefore licensed for the treatment of antibody-positive SLE active despite standard of care therapy. Subsequent analysis of the BLISS trials showed that the effect of belimumab was greatest in those with highest disease activity, and it therefore provides another licensed treatment option in patients who are nonresponsive or cannot tolerate other therapies (van Vollenhoven et al., 2012). The role of belimumab in the treatment of LN is being examined in on-going clinical trials, but questions around treatment length still need to be answered, with some patients experiencing severe flares on discontinuation of medication.

### ATACICEPT

Atacicept is a fusion protein of the Transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) receptor with human IgG. TACI binds both BLyS and APRIL (a proliferation-inducing ligand), and therefore it was thought that inhibiting both would have a more potent effect in patients with active SLE. The first trial of atacicept, APRIL-SLE, was stopped early because of two deaths in the higher dose treatment arm due to lung infections possibly related to drug (Isenberg et al., 2015). There was no difference in the primary endpoint between placebo and 75 mg atacicept, although post hoc analysis suggested a benefit of 150 mg atacicept versus placebo. The ADDRESS II trial reexamined efficacy and safety in patients with active SLE who had up-to-date vaccination status for *Streptococcus pneumoniae* and influenza prior to enrolment. The trial failed to reach the primary endpoint but a subsequent prespecified subpopulation analysis showed benefit in those with high disease activity (Merrill et al., 2018). Upper respiratory tract infections and diarrhea were more common in the atacicept-treated groups. Further evaluation of this drug is required.

### **Other Agents and Treatment Options**

Ustekinumab, a monoclonal antibody against the p40 subunit of IL-12 and IL-23, is effective in the treatment of psoriasis and psoriatic arthritis. In a phase II trial, ustekinumab was associated with a higher Systemic Lupus Erythematosus Responder Index (SRI) response compared to placebo at 24 weeks in patients with active SLE (van Vollenhoven et al., 2017a). Ustekinumab targets T cell-derived cytokines, thus opening a new therapeutic pathway. Although TNF $\alpha$  blockade is commonly used in diseases such as RA, it is not commonly used in SLE. A small study from the Smolen group showed that it may be effective at controlling LN, but over half of the infliximab-treated patients experienced serious adverse events (Aringer et al., 2009). Janus kinase (JAK) inhibitors are also licensed for RA and offer an oral small molecule treatment approach. The evaluation of JAK inhibitors such as tofacitinib is underway in lupus and may be beneficial given their effect on interferon signaling. Epratuzumab, a monoclonal antibody targeting CD22 on B cells, that results in altered signaling without significant reduction in B-cell numbers, failed to show benefit over standard of care in SLE (Clowse et al., 2017).

GWAS and transcriptomic studies have implicated type I interferons and associated pathways in the pathogenesis of SLE, although therapeutic targeting has given mixed results. Sifalimumab, a human monoclonal antibody that binds to IFN- $\alpha$ , met the primary endpoint in patients with moderate-to-severely active SLE (Khamashta et al., 2016). Rontalizumab, another human monoclonal antibody that targets all subtypes of IFN- $\alpha$ , failed to meet primary endpoints in a trial of moderate-to-severe lupus (Kalunian et al., 2016). Anifrolumab, a type I IFN receptor antagonist, reduced disease activity compared to placebo in patients with moderate-to-severely active SLE (Furie et al., 2017). A greater effect size was seen in patients with a high interferon signature at baseline, although the treatment was associated with more herpes zoster infections.

Bortezomib, a proteasome inhibitor, has been shown to be clinically effective in refractory SLE in a small case series, with plasma cell depletion and a reduction in anti-dsDNA antibodies, although controlled trials are awaited (Alexander et al., 2015). Other measures such as plasma exchange (Kronbichler et al., 2016) may be used in refractory SLE and reduction of antibodies by targeting FcRN is under evaluation. Autologous hematopoietic stem-cell transplantation (HSCT) can result in 50% disease-free survival, at 1 year, in patients with refractory disease but is associated with significant morbidity and mortality (12%) (Jayne et al., 2004). Mesenchymal stem-cell

transplant, in severe and treatment refractory patients, resulted in 27% of patients in complete remission after 5 years with fewer adverse events compared to HSCT (Wang et al., 2018).

## FUTURE PERSPECTIVES

SLE is a clinically and pathologically diverse disease with significant unmet needs in terms of biomarkers for diagnosis and prognosis and effective treatment options with acceptable tolerability. The major challenge in achieving each of these goals might lie in disease stratification; the identification of disease subsets that are driven by distinct pathological processes and therefore may respond to specific targeted therapeutics. The genetics and “omics” revolution might assist in the identification of such disease subgroups, whilst also giving insight into new therapeutic targets. In addition to new targets, current clinical trial design where a therapy needs to be effective in “all types” of SLE will need to evolve if we are to realize the benefit of these approaches. In the future, adequate power to detect differences in patient subpopulations will be a critical issue in order to overcome past issues of clinical trials in SLE.

SLE is still a relatively rare disease, and this, together with the likely existence of unrecognized disease subgroups, makes the conduct of clinical trials difficult. Novel methods of recruitment, such as via social media, need to be considered. Screening for disease is possible in certain populations, such as African American females, and screening for renal LN in the same manner as for hypertension in the barbershop trial (Victor et al., 2018) might address a significant clinical problem in a novel manner.

Despite research and development into biologics for SLE, only one agent, belimumab, has a license for use in this disease. Nonetheless many other novel agents are currently under development. Patients with SLE and those caring for them, now more than ever, have the ability to take part in clinical trials of these newer agents, such as those targeting type I IFN and IL-23. Increased patient participation in translational research, patient stratification, and target identification through genetics/genomics and modern clinical trial design should result in better outcomes for this chronic disease.

## References

- Adachi, M., Watanabe-Fukunaga, R., Nagata, S., 1993. Aberrant transcription caused by the insertion of an early transposable element in an intron of the Fas antigen gene of lpr mice. *Proc. Natl. Acad. Sci. U.S.A.* 90 (5), 1756–1760.
- Agmon-Levin, N., Damoiseaux, J., Kallenberg, C., Sack, U., Witte, T., Herold, M., et al., 2014. International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. *Ann. Rheum. Dis.* 73, 17–23.
- Alarcón, G.S., McGwin, G., Bertoli, A.M., Fessler, B.J., Calvo-Alén, J., Bastian, H.M., et al., 2007. Effect of hydroxychloroquine on the survival of patients with systemic lupus erythematosus: data from LUMINA, a multiethnic US cohort (LUMINA L). *Ann. Rheum. Dis.* 66 (9), 1168–1172.
- Alarcón-Segovia, D., Alarcón-Riquelme, M.E., Cardiel, M.H., Caeiro, F., Massardo, L., Villa, A.R., et al., 2005. Familial aggregation of systemic lupus erythematosus, rheumatoid arthritis, and other autoimmune diseases in 1,177 lupus patients from the GLADEL cohort. *Arthritis Rheum.* 52 (4), 1138–1147.
- Alexander, T., Sarfert, R., Klotsche, J., Kühl, A.A., Rubbert-Roth, A., Lorenz, H.-M., et al., 2015. The proteasome inhibitor bortezomib depletes plasma cells and ameliorates clinical manifestations of refractory systemic lupus erythematosus. *Ann. Rheum. Dis.* 74 (7), 1474–1478.
- Andrews, B.S., Eisenberg, R.A., Theofilopoulos, A.N., Izui, S., Wilson, C.B., McConahey, P.J., et al., 1978. Spontaneous murine lupus-like syndromes. Clinical and immunopathological manifestations in several strains. *J. Exp. Med.* 148 (5), 1198–1215.
- Appel, G.B., Contreras, G., Dooley, M.A., Ginzler, E.M., Isenberg, D., Jayne, D., et al., 2009. Mycophenolate mofetil versus cyclophosphamide for induction treatment of lupus nephritis. *J. Am. Soc. Nephrol.* 20 (5), 1103–1112.
- Arbuckle, M.R., McClain, M.T., Rubertone, M.V., Scofield, R.H., Dennis, G.J., James, J.A., et al., 2003. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N. Engl. J. Med.* 349 (16), 1526–1533.
- Aringer, M., Houssiau, F., Gordon, C., Graninger, W.B., Voll, R.E., Rath, E., et al., 2009. Adverse events and efficacy of TNF-alpha blockade with infliximab in patients with systemic lupus erythematosus: long-term follow-up of 13 patients. *Rheumatology (Oxford)* 48 (11), 1451–1454.
- Baehler, E.C., Batliwalla, F.M., Karypis, G., Gaffney, P.M., Ortmann, W.A., Espe, K.J., et al., 2003. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *Proc. Natl. Acad. Sci. U.S.A.* 100 (5), 2610–2615.
- Bennett, L., Palucka, A.K., Arce, E., Cantrell, V., Borvak, J., Banchereau, J., et al., 2003. Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. *J. Exp. Med.* 197 (6), 711–723.
- Bertsias, G.K., Tektonidou, M., Amoura, Z., Aringer, M., Bajema, I., Berden, J.H.M., et al., 2012. Joint European League Against Rheumatism and European Renal Association-European Dialysis and Transplant Association (EULAR/ERA-EDTA) recommendations for the management of adult and paediatric lupus nephritis. *BMJ* 71, 1771–1782.
- Blair, P.A., NoreNa, L.Y., Flores-Borja, F., Rawlings, D.J., Isenberg, D.A., Ehrenstein, M.R., et al., 2010. CD19(+)CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic lupus erythematosus patients. *Immunity* 32 (1), 129–140.

- Blanco, P., Palucka, A.K., Pascual, V., Banchereau, J., 2008. Dendritic cells and cytokines in human inflammatory and autoimmune diseases. *Cytokine Growth Factor Rev.* 19 (1), 41–52.
- Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D.S., et al., 2004. Neutrophil extracellular traps kill bacteria. *Science* 303 (5663), 1532–1535.
- Canadian Hydroxychloroquine Study Group, 1991. A randomized study of the effect of withdrawing hydroxychloroquine sulfate in systemic lupus erythematosus. *N. Engl. J. Med.* 324 (3), 150–154.
- Chiu, Y.-M., Lai, C.-H., 2010. Nationwide population-based epidemiologic study of systemic lupus erythematosus in Taiwan. *Lupus* 19 (10), 1250–1255.
- Choi, J.-Y., Ho, J.H.-E., Pasoto, S.G., Bunin, V., Kim, S.T., Carrasco, S., et al., 2015. Circulating follicular helper-like T cells in systemic lupus erythematosus: association with disease activity. *Arthritis Rheumatol.* 67 (4), 988–999.
- Clowse, M.E.B., Wallace, D.J., Furie, R.A., Petri, M.A., Pike, M.C., Leszczyński, P., et al., 2017. Efficacy and safety of epratuzumab in moderately to severely active systemic lupus erythematosus: results from two phase III randomized, double-blind, placebo-controlled trials. *Arthritis Rheumatol.* 69 (2), 362–375.
- Condon, M.B., Ashby, D., Pepper, R.J., Cook, H.T., Levy, J.B., Griffith, M., et al., 2013. Prospective observational single-centre cohort study to evaluate the effectiveness of treating lupus nephritis with rituximab and mycophenolate mofetil but no oral steroids. *Ann. Rheum. Dis.* 72 (8), 1280–1286.
- Coons, A.H., Kaplan, M.H., 1950. Localization of antigen in tissue cells; improvements in a method for the detection of antigen by means of fluorescent antibody. *J. Exp. Med.* 91 (1), 1–13.
- Costedoat-Chalumeau, N., Galicier, L., Aumaître, O., Francès, C., Le Guern, V., Lioté, F., et al., 2013. Hydroxychloroquine in systemic lupus erythematosus: results of a French multicentre controlled trial (PLUS Study). *Ann. Rheum. Dis.* 72 (11), 1786–1792.
- Croyle, L., Hoi, A., Morand, E.F., 2015. Characteristics of azathioprine use and cessation in a longitudinal lupus cohort. *Lupus Sci. Med.* 2 (1), e000105.
- Deapen, D., Escalante, A., Weinrib, L., Horwitz, D., Bachman, B., Roy-Burman, P., et al., 1992. A revised estimate of twin concordance in systemic lupus erythematosus. *Arthritis Rheum.* 35 (3), 311–318.
- Dooley, M.A., Jayne, D., Ginzler, E.M., Isenberg, D., Olsen, N.J., Wofsy, D., et al., 2011. Mycophenolate versus azathioprine as maintenance therapy for lupus nephritis. *N. Engl. J. Med.* 365 (20), 1886–1895.
- Dooley, M.A., et al., 2016. Speed of remission with the use of voclosporin, MMF and low dose steroids: results of a global lupus nephritis study – ACR Meeting abstracts. 2016. *Arthritis Rheumatol.* 68 (Suppl. 10).
- Feldman, C.H., Hiraki, L.T., Liu, J., Fischer, M.A., Solomon, D.H., Alarcón, G.S., et al., 2013. Epidemiology and sociodemographics of systemic lupus erythematosus and lupus nephritis among US adults with Medicaid coverage, 2000–2004. *Arthritis Rheum.* 65 (3), 753–763.
- Fernando, M.M.A., Isenberg, D.A., 2005. How to monitor SLE in routine clinical practice. *Ann. Rheum. Dis.* 64 (4), 524–527.
- Fischer-Betz, R., Specker, C., Brinks, R., Aringer, M., Schneider, M., 2013. Low risk of renal flares and negative outcomes in women with lupus nephritis conceiving after switching from mycophenolate mofetil to azathioprine. *Rheumatology (Oxford)* 52 (6), 1070–1076.
- Font, J., Cervera, R., Ramos-Casals, M., García-Carrasco, M., Sents, J., Herrero, C., et al., 2004. Clusters of clinical and immunologic features in systemic lupus erythematosus: analysis of 600 patients from a single center. *Semin. Arthritis Rheum.* 33 (4), 217–230.
- Franklyn, K., Lau, C.S., Navarra, S.V., Louthrenoo, W., Lateef, A., Hamijoyo, L., et al., 2016. Definition and initial validation of a Lupus Low Disease Activity State (LLDAS). *Ann. Rheum. Dis.* 75 (9), 1615–1621.
- Furie, R., Khamashta, M., Merrill, J.T., Werth, V.P., Kalunian, K., Brohawn, P., et al., 2017. Anifrolumab, an anti-interferon- $\alpha$  receptor monoclonal antibody, in moderate-to-severe systemic lupus erythematosus. *Arthritis Rheumatol.* 69 (2), 376–386.
- Furst, D.E., Clarke, A.E., Fernandes, A.W., Bancroft, T., Greth, W., Iorga, S.R., et al., 2013. Incidence and prevalence of adult systemic lupus erythematosus in a large US managed-care population. *Lupus* 22 (1), 99–105.
- Ginzler, E.M., Dooley, M.A., Aranow, C., Kim, M.Y., Buyon, J., Merrill, J.T., et al., 2005. Mycophenolate mofetil or intravenous cyclophosphamide for lupus nephritis. *N. Engl. J. Med.* 353 (21), 2219–2228.
- Ginzler, E.M., Wofsy, D., Isenberg, D., Gordon, C., Lisk, L., Dooley, M.A., et al., 2010. Nonrenal disease activity following mycophenolate mofetil or intravenous cyclophosphamide as induction treatment for lupus nephritis: findings in a multicenter, prospective, randomized, open-label, parallel-group clinical trial. *Arthritis Rheum.* 62 (1), 211–221.
- Gladman, D., Ginzler, E., Goldsmith, C., Fortin, P., Liang, M., Urowitz, M., et al., 1996. The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for systemic lupus erythematosus. *Arthritis Rheum.* 39, 363–369.
- Gladman, D.D., Ibañez, D., Urowitz, M.B., 2002. Systemic lupus erythematosus disease activity index 2000. *J. Rheumatol.* 29 (2), 288–291.
- Gladman, D.D., Hirani, N., Ibañez, D., Urowitz, M.B., 2003. Clinically active serologically quiescent systemic lupus erythematosus. *J. Rheumatol.* 30 (9), 1960–1962.
- Goodnow, C.C., 2007. Multistep pathogenesis of autoimmune disease. *Cell* 130 (1), 25–35.
- Gourley, M.F., Austin, H.A., Scott, D., Yarboro, C.H., Vaughan, E.M., Muir, J., et al., 1996. Methylprednisolone and cyclophosphamide, alone or in combination, in patients with lupus nephritis. A randomized, controlled trial. *Ann. Intern. Med.* 125 (7), 549–557.
- Griffiths, B., Emery, P., Ryan, V., Isenberg, D., Akil, M., Thompson, R., et al., 2010. The BILAG multi-centre open randomized controlled trial comparing ciclosporin vs azathioprine in patients with severe SLE. *Rheumatology (Oxford)* 49 (4), 723–732.
- Group KDIGO, 2012. KDIGO clinical practice guideline for glomerulonephritis. *Kidney Int.* 2 (2), 139–274.
- Hahn, B.H., McMahon, M.A., Wilkinson, A., Wallace, W.D., Daikh, D.I., FitzGerald, J.D., et al., 2012. American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis. *Arthritis Care Res.* 55 (9), 797–808.
- Han, J.-W., Zheng, H.-F., Cui, Y., Sun, L.-D., Ye, D.-Q., Hu, Z., et al., 2009. Genome-wide association study in a Chinese Han population identifies nine new susceptibility loci for systemic lupus erythematosus. *Nat. Genet.* 41 (11), 1234–1237.
- He, J., Zhang, X., Wei, Y., Sun, X., Chen, Y., Deng, J., et al., 2016. Low-dose interleukin-2 treatment selectively modulates CD4(+) T cell subsets in patients with systemic lupus erythematosus. *Nat. Med.* 22 (9), 991–993.

- Heinlen, L.D., McClain, M.T., Merrill, J., Akbarali, Y.W., Edgerton, C.C., Harley, J.B., et al., 2007. Clinical criteria for systemic lupus erythematosus precede diagnosis, and associated autoantibodies are present before clinical symptoms. *Arthritis Rheum.* 56 (7), 2344–2351.
- Henderson, L., Masson, P., Craig, J.C., Flanc, R.S., Roberts, M.A., Strippoli, G.F.M., et al., 2012. Treatment for lupus nephritis. *Cochrane Database Syst. Rev.* 12, CD002922.
- Hermansen, M.-L.F., Lindhardsen, J., Torp-Pedersen, C., Faurschou, M., Jacobsen, S., 2016. Incidence of systemic lupus erythematosus and lupus nephritis in denmark: a nationwide cohort study. *J. Rheumatol.* 43 (7), 1335–1339.
- Ho, A., Magder, L.S., Barr, S.G., Petri, M., 2001a. Decreases in anti-double-stranded DNA levels are associated with concurrent flares in patients with systemic lupus erythematosus. *Arthritis Rheum.* 44 (10), 2342–2349.
- Ho, A., Barr, S.G., Magder, L.S., Petri, M., 2001b. A decrease in complement is associated with increased renal and hematologic activity in patients with systemic lupus erythematosus. *Arthritis Rheum.* 44 (10), 2350–2357.
- Hochberg, M.C., 1997. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* 40 (9), 1725.
- Hom, G., Graham, R.R., Modrek, B., Taylor, K.E., Ortmann, W., Garnier, S., et al., 2008. Association of systemic lupus erythematosus with C8orf13-BLK and ITGAM-ITGAX. *N. Engl. J. Med.* 358 (9), 900–909.
- Houssiau, F.A., Vasconcelos, C., D'Cruz, D., Sebastiani, G.D., Garrido Ed Ede, R., Danieli, M.G., et al., 2002. Immunosuppressive therapy in lupus nephritis: the Euro-Lupus Nephritis Trial, a randomized trial of low-dose versus high-dose intravenous cyclophosphamide. *Arthritis Rheum.* 46 (8), 2121–2131.
- Inês, L., Silva, C., Galindo, M., López-Longo, F.J., Terroso, G., Romão, V.C., et al., 2015. Classification of systemic lupus erythematosus: Systemic Lupus International Collaborating Clinics versus American College of Rheumatology Criteria. A comparative study of 2,055 patients from a real-life, International Systemic Lupus Erythematosus Cohort. *Arthritis Care Res.* 67 (8), 1180–1185.
- International Consortium for Systemic Lupus Erythematosus Genetics (SLEGEN), Harley, J.B., Alarcón-Riquelme, M.E., Criswell, L.A., Jacob, C.O., Kimberly, R.P., et al., 2008. Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in ITGAM, PXK, KIAA1542 and other loci. *Nat. Genet.* 40 (2), 204–210.
- Isenberg, D.A., Rahman, A., Allen, E., Farewell, V., Akil, M., Bruce, I.N., et al., 2005. BILAG 2004. Development and initial validation of an updated version of the British Isles Lupus Assessment Group's disease activity index for patients with systemic lupus erythematosus. *Rheumatology* 44 (7), 902–906.
- Isenberg, D., Gordon, C., Licu, D., Copt, S., Rossi, C.P., Wofsy, D., 2015. Efficacy and safety of atacicept for prevention of flares in patients with moderate-to-severe systemic lupus erythematosus (SLE): 52-week data (APRIL-SLE randomised trial). *Ann. Rheum. Dis.* 74 (11), 2006–2015.
- Jayne, D., Passweg, J., Marmont, A., Farge, D., Zhao, X., Arnold, R., et al., 2004. Autologous stem cell transplantation for systemic lupus erythematosus. *Lupus* 13 (3), 168–176.
- Johnson, A.E., Gordon, C., Palmer, R.G., Bacon, P.A., 1995. The prevalence and incidence of systemic lupus erythematosus in Birmingham, England. Relationship to ethnicity and country of birth. *Arthritis Rheum.* 38 (4), 551–558.
- Kalunian, K.C., Merrill, J.T., Maciucă, R., McBride, J.M., Townsend, M.J., Wei, X., et al., 2016. A phase II study of the efficacy and safety of rontalizumab (rhuMAb interferon- $\alpha$ ) in patients with systemic lupus erythematosus (ROSE). *Ann. Rheum. Dis.* 75 (1), 196–202.
- Karim, M.Y., Alba, P., Cuadrado, M.-J., Abbs, I.C., D'Cruz, D.P., Khamashta, M.A., et al., 2002. Mycophenolate mofetil for systemic lupus erythematosus refractory to other immunosuppressive agents. *Rheumatology* 41 (8), 876–882.
- Kasitanon, N., Fine, D.M., Haas, M., Magder, L.S., Petri, M., 2006. Hydroxychloroquine use predicts complete renal remission within 12 months among patients treated with mycophenolate mofetil therapy for membranous lupus nephritis. *Lupus* 15 (6), 366–370.
- Kessenbrock, K., Krumbholz, M., Schönermarck, U., Back, W., Gross, W.L., Werb, Z., et al., 2009. Netting neutrophils in autoimmune small-vessel vasculitis. *Nat. Med.* 15 (6), 623–625.
- Khamashta, M., Merrill, J.T., Werth, V.P., Furie, R., Kalunian, K., Illei, G.G., et al., 2016. Sifalimumab, an anti-interferon- $\alpha$  monoclonal antibody, in moderate to severe systemic lupus erythematosus: a randomised, double-blind, placebo-controlled study. *Ann. Rheum. Dis.* 75 (11), 1909–1916.
- Kozora, E., Ellison, M.C., West, S., 2004. Reliability and validity of the proposed American College of Rheumatology neuropsychological battery for systemic lupus erythematosus. *Arthritis Rheum.* 51 (5), 810–818.
- Kozyrev, S.V., Abelson, A.-K., Wojcik, J., Zaghlool, A., Linga Reddy, M.V.P., Sánchez, E., et al., 2008. Functional variants in the B-cell gene BANK1 are associated with systemic lupus erythematosus. *Nat. Genet.* 40 (2), 211–216.
- Kronbichler, A., Brezina, B., Quintana, L.F., Jayne, D.R.W., 2016. Efficacy of plasma exchange and immunoabsorption in systemic lupus erythematosus and antiphospholipid syndrome: a systematic review. *Autoimmun. Rev.* 15 (1), 38–49.
- Kuo, C.-F., Grainge, M.J., Valdes, A.M., See, L.-C., Luo, S.-F., Yu, K.-H., et al., 2015. Familial aggregation of systemic lupus erythematosus and coaggregation of autoimmune diseases in affected families. *JAMA Intern. Med.* 175 (9), 1518–1526.
- Lee-Kirsch, M.A., Gong, M., Chowdhury, D., Senenko, L., Engel, K., Lee, Y.-A., et al., 2007. Mutations in the gene encoding the 3'-5' DNA exonuclease TREX1 are associated with systemic lupus erythematosus. *Nat. Genet.* 39 (9), 1065–1067.
- Lewis, M.J., Jawad, A.S., 2017. The effect of ethnicity and genetic ancestry on the epidemiology, clinical features and outcome of systemic lupus erythematosus. *Rheumatology (Oxford)* 56 (suppl. 1), i67–i77.
- Lieberman, L.A., Tsokos, G.C., 2010. The IL-2 defect in systemic lupus erythematosus disease has an expansive effect on host immunity. *J. Biomed. Biotechnol.* 2010, 740619.
- Lo, M.S., 2016. Monogenic lupus. *Curr. Rheumatol. Rep.* 18 (12), 71.
- Lyons, P.A., Rayner, T.F., Trivedi, S., Holle, J.U., Watts, R.A., Jayne, D.R.W., et al., 2012. Genetically distinct subsets within ANCA-associated vasculitis. *N. Engl. J. Med.* 367 (3), 214–223.
- Maibaum, M.A., Haywood, M.E., Walport, M.J., Morley, B.J., 2000. Lupus susceptibility loci map within regions of BXSB derived from the SB/Le parental strain. *Immunogenetics* 51 (4–5), 370–372.
- Malkiel, S., Jeganathan, V., Wolfson, S., Manjarrez Orduño, N., Marasco, E., Aranow, C., et al., 2016. Checkpoints for autoreactive B cells in the peripheral blood of lupus patients assessed by flow cytometry. *Arthritis Rheumatol.* 68 (9), 2210–2220.

- Mariz, H.A., Sato, E.I., Barbosa, S.H., Rodrigues, S.H., Dellavance, A., Andrade, L.E.C., 2011. Pattern on the antinuclear antibody-HEp-2 test is a critical parameter for discriminating antinuclear antibody-positive healthy individuals and patients with autoimmune rheumatic diseases. *Arthritis Rheum.* 63 (1), 191–200.
- Marmor, M.F., Kellner, U., Lai, T.Y.Y., Melles, R.B., Mieler, W.F., American Academy of Ophthalmology, 2016. Recommendations on screening for chloroquine and hydroxychloroquine retinopathy (2016 revision). *Ophthalmology* 138–1394.
- McKinney, E.F., Lyons, P.A., Carr, E.J., Hollis, J.L., Jayne, D.R.W., Willcocks, L.C., et al., 2010. A CD8+ T cell transcription signature predicts prognosis in autoimmune disease. *Nat. Med.* 16 (5), 586–591.
- McKinney, E.F., Lee, J.C., Jayne, D.R.W., Lyons, P.A., Smith, K.G.C., 2015. T-cell exhaustion, co-stimulation and clinical outcome in autoimmunity and infection. *Nature* 523 (7562), 612–616.
- Merrill, J.T., Neuwelt, C.M., Wallace, D.J., Shanahan, J.C., Latinis, K.M., Oates, J.C., et al., 2010. Efficacy and safety of rituximab in moderately-to-severely active systemic lupus erythematosus: the randomized, double-blind, phase II/III systemic lupus erythematosus evaluation of rituximab trial. *Arthritis Rheum.* 62 (1), 222–233.
- Merrill, J., Buyon, J., Furie, R., Latinis, K., Gordon, C., Hsieh, H.-J., et al., 2011. Assessment of flares in lupus patients enrolled in a phase II/III study of rituximab (EXPLORER). *Lupus* 20 (7), 709–716.
- Merrill, J.T., Wallace, D.J., Wax, S., Kao, A., Fraser, P.A., Chang, P., et al., 2018. Efficacy and safety of atacicept in patients with systemic lupus erythematosus: results of a twenty-four-week, multicenter, randomized, double-blind, placebo-controlled, parallel-arm, phase IIb study. *Arthritis Rheumatol.* 70 (2), 266–276.
- Mirzayan, M.J., Schmidt, R.E., Witte, T., 2000. Prognostic parameters for flare in systemic lupus erythematosus. *Rheumatology* 39 (12), 1316–1319.
- Mohan, C., Morel, L., Yang, P., Wakeland, E.K., 1997. Genetic dissection of systemic lupus erythematosus pathogenesis: Sle2 on murine chromosome 4 leads to B cell hyperactivity. *J. Immunol.* 159 (1), 454–465.
- Mohan, C., Alas, E., Morel, L., Yang, P., Wakeland, E.K., 1998. Genetic dissection of SLE pathogenesis. Sle1 on murine chromosome 1 leads to a selective loss of tolerance to H2A/H2B/DNA subnucleosomes. *J. Clin. Invest.* 101 (6), 1362–1372.
- Mok, C.C., Tang, S.S.K., To, C.H., Petri, M., 2005. Incidence and risk factors of thromboembolism in systemic lupus erythematosus: a comparison of three ethnic groups. *Arthritis Rheum.* 52 (9), 2774–2782.
- Mok, C.C., Ying, K.Y., Yim, C.W., Siu, Y.P., Tong, K.H., To, C.H., et al., 2016. Tacrolimus versus mycophenolate mofetil for induction therapy of lupus nephritis: a randomised controlled trial and long-term follow-up. *Ann. Rheum. Dis.* 75 (1), 30–36.
- Morel, L., Croker, B.P., Blenman, K.R., Mohan, C., Huang, G., Gilkeson, G., et al., 2000. Genetic reconstitution of systemic lupus erythematosus immunopathology with polycongenic murine strains. *Proc. Natl. Acad. Sci. U.S.A.* 97 (12), 6670–6675.
- Morris, D.L., Sheng, Y., Zhang, Y., Wang, Y.-F., Zhu, Z., Tombleson, P., et al., 2016. Genome-wide association meta-analysis in Chinese and European individuals identifies ten new loci associated with systemic lupus erythematosus. *Nat. Genet.* 48 (8), 940–946.
- Moulton, V.R., Suarez-Fueyo, A., Meidan, E., Li, H., Mizui, M., Tsokos, G.C., 2017. Pathogenesis of human systemic lupus erythematosus: a cellular perspective. *Trends Mol. Med.* 23 (7), 615–635.
- Munroe, M.E., Lu, R., Zhao, Y.D., Fife, D.A., Robertson, J.M., Guthridge, J.M., et al., 2016. Altered type II interferon precedes autoantibody accrual and elevated type I interferon activity prior to systemic lupus erythematosus classification. *Ann. Rheum. Dis.* 75 (11), 2014–2021.
- Murphy, E.D., Roths, J.B., 1979. A Y chromosome associated factor in strain BXSB producing accelerated autoimmunity and lymphoproliferation. *Arthritis Rheum.* 22 (11), 1188–1194.
- Navarra, S.V., Guzmán, R.M., Gallacher, A.E., Hall, S., Levy, R.A., Jimenez, R.E., et al., 2011. Efficacy and safety of belimumab in patients with active systemic lupus erythematosus: a randomised, placebo-controlled, phase 3 trial. *Lancet* 377 (9767), 721–731.
- Nguyen, C., Limaye, N., Wakeland, E.K., 2002. Susceptibility genes in the pathogenesis of murine lupus. *Arthritis. Res.* 4 (Suppl. 3), S255–S263.
- Niederer, H.A., Willcocks, L.C., Rayner, T.F., Yang, W., Lau, Y.L., Williams, T.N., et al., 2010. Copy number, linkage disequilibrium and disease association in the FCGR locus. *Hum. Mol. Genet.* 19 (16), 3282–3294.
- Ordi-Ros, J., Sáez-Comet, L., Pérez-Conesa, M., Vidal, X., Mitjavila, F., Castro Salomó, A., et al., 2017. Enteric-coated mycophenolate sodium versus azathioprine in patients with active systemic lupus erythematosus: a randomised clinical trial. *Ann. Rheum. Dis.* 76 (9), 1575–1582.
- Padyukov, L., Seielstad, M., Ong, R.T.H., Ding, B., Rönnelid, J., Seddighzadeh, M., et al., 2011. A genome-wide association study suggests contrasting associations in ACPA-positive versus ACPA-negative rheumatoid arthritis. *Ann. Rheum. Dis.* 70 (2), 259–265.
- Petri, M., Stohl, W., Chatham, W., McCune, W.J., Chevrier, M., Ryel, J., et al., 2008. Association of plasma B lymphocyte stimulator levels and disease activity in systemic lupus erythematosus. *Arthritis Rheum.* 58 (8), 2453–2459.
- Petri, M., Singh, S., Tesfayone, H., Malik, A., 2009. Prevalence of flare and influence of demographic and serologic factors on flare risk in systemic lupus erythematosus: a prospective study. *J. Rheumatol.* 36 (11), 2476–2480.
- Petri, M., Orbai, A.-M., Alarcón, G.S., Gordon, C., Merrill, J.T., Fortin, P.R., et al., 2012. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum.* 64 (8), 2677–2686.
- Petri, M.A., van Vollenhoven, R.F., Buyon, J., Levy, R.A., Navarra, S.V., Cervera, R., et al., 2013. Baseline predictors of systemic lupus erythematosus flares: data from the combined placebo groups in the phase III belimumab trials. *Arthritis Rheum.* 65 (8), 2143–2153.
- Rahman, P., Gladman, D.D., Urowitz, M.B., Hallett, D., Tam, L.-S., 2001. Early damage as measured by the SLICC/ACR damage index is a predictor of mortality in systemic lupus erythematosus. *Lupus* 10 (2), 93–96.
- Rees, F., Doherty, M., Grainge, M., Davenport, G., Lanyon, P., Zhang, W., 2016. The incidence and prevalence of systemic lupus erythematosus in the UK, 1999–2012. *Ann. Rheum. Dis.* 75 (1), 136–141.
- Rees, F., Doherty, M., Grainge, M.J., Lanyon, P., Zhang, W., 2017. The worldwide incidence and prevalence of systemic lupus erythematosus: a systematic review of epidemiological studies. *Rheumatology (Oxford)* 56 (11), 1945–1961.
- Remmers, E.F., Plenge, R.M., Lee, A.T., Graham, R.R., Hom, G., Behrens, T.W., et al., 2007. STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus. *N. Engl. J. Med.* 357 (10), 977–986.
- Rovin, B.H., Furie, R., Latinis, K., Looney, R.J., Fervenza, F.C., Sánchez-Guerrero, J., et al., 2012. Efficacy and safety of rituximab in patients with active proliferative lupus nephritis: the lupus nephritis assessment with rituximab study. *Arthritis Rheum.* 64 (4), 1215–1226.

- Rudofsky, U.H., Evans, B.D., Balaban, S.L., Mottironi, V.D., Gabrielsen, A.E., 1993. Differences in expression of lupus nephritis in New Zealand mixed H-2<sup>z</sup> homozygous inbred strains of mice derived from New Zealand black and New Zealand white mice. Origins and initial characterization. *Lab. Invest.* 68 (4), 419–426.
- Ruiz-Irastorza, G., Egurbide, M.-V., Pijoan, J.-I., Garmendia, M., Villar, I., Martinez-Berriotxoa, A., et al., 2006. Effect of antimalarials on thrombosis and survival in patients with systemic lupus erythematosus. *Lupus* 15 (9), 577–583.
- Ruiz-Irastorza, G., Ramos-Casals, M., Brito-Zeron, P., Khamashta, M.A., 2010. Clinical efficacy and side effects of antimalarials in systemic lupus erythematosus: a systematic review. *Ann. Rheum. Dis.* 69 (1), 20–28.
- Santiago-Raber, M.-L., Kikuchi, S., Borel, P., Uematsu, S., Akira, S., Kotzin, B.L., et al., 2008. Evidence for genes in addition to Tlr7 in the Yaa translocation linked with acceleration of systemic lupus erythematosus. *J. Immunol.* 181 (2), 1556–1562.
- Sherer, Y., Gorstein, A., Fritzler, M.J., Shoenfeld, Y., 2004. Autoantibody explosion in systemic lupus erythematosus: more than 100 different antibodies found in SLE patients. *Semin. Arthritis Rheum.* 34 (2), 501–537.
- Smith, K.G.C., Jones, R.B., Burns, S.M., Jayne, D.R.W., 2006. Long-term comparison of rituximab treatment for refractory systemic lupus erythematosus and vasculitis: remission, relapse, and re-treatment. *Arthritis Rheum.* 54 (9), 2970–2982.
- Somers, E.C., Marder, W., Cagnoli, P., Lewis, E.E., DeGuire, P., Gordon, C., et al., 2014. Population-based incidence and prevalence of systemic lupus erythematosus: the Michigan Lupus Epidemiology and Surveillance program. *Arthritis Rheumatol.* 66 (2), 369–378.
- Steiman, A.J., Gladman, D.D., Ibañez, D., Urowitz, M.B., 2010. Prolonged serologically active clinically quiescent systemic lupus erythematosus: frequency and outcome. *J. Rheumatol.* 37 (9), 1822–1827.
- Steiman, A.J., Gladman, D.D., Ibañez, D., Urowitz, M.B., 2012. Outcomes in patients with systemic lupus erythematosus with and without a prolonged serologically active clinically quiescent period. *Arthritis Care Res.* 64 (4), 511–518.
- Steinberg, A.D., Steinberg, S.C., 1991. Long-term preservation of renal function in patients with lupus nephritis receiving treatment that includes cyclophosphamide versus those treated with prednisone only. *Arthritis Rheum.* 34 (8), 945–950.
- Stetson, D.B., Ko, J.S., Heidmann, T., Medzhitov, R., 2008. Trex1 prevents cell-intrinsic initiation of autoimmunity. *Cell* 134 (4), 587–598.
- Symmons, D.P., Cockcroft, J.S., Bacon, P.A., Bresnihan, B., Isenberg, D.A., Maddison, P., et al., 1988. Development and assessment of a computerized index of clinical disease activity in systemic lupus erythematosus. Members of the British Isles Lupus Assessment Group (BILAG). *Q. J. Med.* 69 (259), 927–937.
- Tan, E.M., Cohen, A.S., Fries, J.F., Masi, A.T., McShane, D.J., Rothfield, N.F., et al., 1982. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* 25 (11), 1271–1277.
- Tiffin, N., Hodgkinson, B., Okpechi, I., 2014. Lupus in Africa: can we dispel the myths and face the challenges? *Lupus* 23 (1), 102–111.
- Tucci, M., Quatraro, C., Lombardi, L., Pellegrino, C., Dammacco, F., Silvestris, F., 2008. Glomerular accumulation of plasmacytoid dendritic cells in active lupus nephritis: role of interleukin-18. *Arthritis Rheum.* 58 (1), 251–262.
- Ugarte-Gil, M.F., Alarcón, G.S., 2016. Incomplete systemic lupus erythematosus: early diagnosis or overdiagnosis? *Arthritis Care Res.* 68 (3), 285–287.
- van Vollenhoven, R.F., Petri, M.A., Cervera, R., Roth, D.A., Ji, B.N., Kleoudis, C.S., et al., 2012. Belimumab in the treatment of systemic lupus erythematosus: high disease activity predictors of response. *Ann. Rheum. Dis.* 71 (8), 1343–1349.
- van Vollenhoven, R.F., Mosca, M., Bertsias, G., Isenberg, D., Kuhn, A., Lerstrøm, K., et al., 2014. Treat-to-target in systemic lupus erythematosus: recommendations from an international task force. *Ann. Rheum. Dis.* 73, 958–967.
- van Vollenhoven, R., Hahn, B.H., Tsokos, G.C., Wagner, C., Lipsky, P., Hsu, B., et al., 2017a. Efficacy and safety of ustekinumab, an interleukin 12/23 inhibitor, in patients with active systemic lupus erythematosus: results of a phase 2, randomized placebo-controlled study. *Arthritis Rheumatol.* 69 (Suppl. 10).
- van Vollenhoven, R., Voskuyl, A., Bertsias, G., Aranow, C., Aringer, M., Arnaud, L., et al., 2017b. A framework for remission in SLE: consensus findings from a large international task force on definitions of remission in SLE (DORIS). *Ann. Rheum. Dis.* 76, 554–561.
- Victor, R.G., Lynch, K., Li, N., Blyler, C., Muhammad, E., Handler, J., et al., 2018. A cluster-randomized trial of blood-pressure reduction in black barbershops. *N. Engl. J. Med.* 378 (14), 1291–1301.
- Wang, X., Zhang, L., Luo, J., Wu, Z., Mei, Y., Wang, Y., et al., 2015. Tacrolimus 0.03% ointment in labial discoid lupus erythematosus: a randomized, controlled clinical trial. *J. Clin. Pharmacol.* 55 (11), 1221–1228.
- Wang, D., Zhang, H., Liang, J., Wang, H., Hua, B., Feng, X., et al., 2018. A long-term follow-up study of allogeneic mesenchymal stem/stromal cell transplantation in patients with drug-resistant systemic lupus erythematosus. *Stem Cell Rep.* 10 (3), 933–941.
- Weening, J.J., D'Agati, V.D., Schwartz, M.M., Seshan, S.V., Alpers, C.E., Appel, G.B., et al., 2004. The classification of glomerulonephritis in systemic lupus erythematosus revisited. *J. Am. Soc. Nephrol.* 15 (2), 241–250.
- Wei, C., Anolik, J., Cappione, A., Zheng, B., Pugh-Bernard, A., Brooks, J., et al., 2007. A new population of cells lacking expression of CD27 represents a notable component of the B cell memory compartment in systemic lupus erythematosus. *J. Immunol.* 178 (10), 6624–6633.
- Wilhelmus, S., Bajema, I.M., Bertsias, G.K., Boumpas, D.T., Gordon, C., Lightstone, L., et al., 2016. Lupus nephritis management guidelines compared. *Nephrol. Dial. Transplant.* 31 (6), 904–913.
- Willcocks, L.C., Carr, E.J., Niederer, H.A., Rayner, T.F., Williams, T.N., Yang, W., et al., 2010. A defunctioning polymorphism in FCGR2B is associated with protection against malaria but susceptibility to systemic lupus erythematosus. *Proc. Natl. Acad. Sci. U.S.A.* 107 (17), 7881–7885.
- Yang, W., Shen, N., Ye, D.-Q., Liu, Q., Zhang, Y., Qian, X.-X., et al., 2010. Genome-wide association study in Asian populations identifies variants in ETS1 and WDFY4 associated with systemic lupus erythematosus. *PLoS Genet.* 6 (2), e1000841.
- Yurasov, S., Wardemann, H., Hammersen, J., Tsuiji, M., Meffre, E., Pascual, V., et al., 2005. Defective B cell tolerance checkpoints in systemic lupus erythematosus. *J. Exp. Med.* 201 (5), 703–711.

# Systemic Sclerosis (Scleroderma)

*Shervin Assassi<sup>1</sup> and John Varga<sup>2</sup>*

<sup>1</sup>The University of Texas Health Science Center at Houston, Houston, United States

<sup>2</sup>Division of Rheumatology, Northwestern University, Chicago, United States

## OUTLINE

<b>Definition and Classification</b>	<b>575</b>	<b>Organ Involvement</b>	<b>588</b>
Epidemiology	577	Raynaud's Phenomenon	588
Pathogenesis	580	Skin Features	588
Genetics	580	Pulmonary Features	589
Preclinical Disease Models	583	<b>Gastrointestinal Involvement</b>	590
Microvascular Disease in Systemic Sclerosis	583	Upper Gastrointestinal Tract Involvement	590
Inflammation and Autoimmunity	584	Lower Gastrointestinal Tract and Anorectal	590
Cellular Immunity	584	Involvement	591
Humoral Autoimmunity	585	<b>Renal Involvement: Scleroderma</b>	592
Fibrosis	585	Renal Crisis	592
Pathology	585	Cardiac Involvement	592
Skin	586	<b>Musculoskeletal Complications</b>	593
Lungs	586	Less Recognized Disease Manifestations	593
Gastrointestinal Tract	586	Cancer	594
Kidneys	586	<b>Biomarkers and Autoantibodies in</b>	594
Heart	586	<b>Systemic Sclerosis</b>	594
Pathology in Other Organs	586	Screening and Follow-Up Evaluation	595
Clinical Features	587	Natural History and Prognosis	598
Overview	587	References	599
Initial Clinical Presentation	587		

## DEFINITION AND CLASSIFICATION

Systemic sclerosis (SSc), historically called scleroderma, is an autoimmune connective tissue disease with complex and poorly understood pathogenesis. SSc has protean clinical manifestations, follows a chronic and commonly progressive course, and is associated with substantially diminished quality of life, disability, and excess mortality. In addition to skin involvement, the hallmark of the disease, virtually every organ can be affected (Fig. 31.1). A recently adopted set of classification criteria are highly sensitive and specific for SSc (Table 31.1). According to these criteria, skin thickening of the fingers of both hands extending proximal to the metacarpophalangeal joint is



**FIGURE 31.1** Clinical features of systemic sclerosis. (A) Facial features with oral furrowing and telangiectasia; (B) palmar telangiectasia; (C) calcinosis on the digital tip; (D) calcinosis on the elbow; (E and F) digital ulcer leading to gangrene. *Clinical pictures courtesy of Dr. Maureen D. Mayes*

**TABLE 31.1** Classification Criteria for Diagnosis of Systemic Sclerosis

Item	Subitem	Weight/score
Bilateral skin thickening—fingers extending proximal to MCP joints		9
Skin thickening of fingers only	Puffy fingers	2
	Sclerodactyly (skin thickened distal to MCP joints)	4
Fingertip lesions	Digital tip ulcer or pitting scar	2
		3
Mucocutaneous telangiectasia		2
Abnormal nailfold capillary pattern		2
Lung involvement	PAH	2
	Interstitial lung disease	2
Raynaud's phenomenon		3
SSc-specific autoantibodies	ACA	3
	Scl-70	
	RNA polymerase III	

ACA, Anticentromere; MCP, metacarpophalangeal joint; PAH, pulmonary arterial hypertension.

sufficient for the diagnosis of SSc. If proximal skin thickening is absent, a constellation of symptoms presented in Table 31.1 is used, and more than nine points is required for the classification as SSc (van den Hoogen et al., 2013).

SSc shows marked patient-to-patient variability in its patterns of skin involvement, complications, autoantibody profiles, disease progression, response to treatment, and survival (Allanore et al., 2015). In its earliest stages, SSc is marked by prominent immune and inflammatory features, but over the time as the disease advances, irreversible structural alterations in multiple vascular beds and visceral organ dysfunction and failure due to fibrosis and atrophy emerge.

Indurated skin (*scleroderma*) is the distinguishing hallmark of SSc. However, similar indurative skin changes can also be seen in localized forms of scleroderma. The localized sclerodermas represent a family of fibroinflammatory conditions that affect the skin and underlying tissue (hypodermis and occasionally underlying muscle and bone) but spare internal organs. In this chapter, we focus on the systemic form of scleroderma or SSc. In addition to localized scleroderma, skin induration can also occur in a diverse group of metabolic, inherited, and autoimmune disorders that should not be confused with SSc (Table 31.2).

SSc is customarily divided into two partially overlapping subsets that are defined by the pattern of skin involvement, along with clinical and serological features (Table 31.3). The natural history of these two SSc subsets is quite distinct. Patients with diffuse cutaneous SSc (dcSSc) typically have extensive, and often rapidly progressive, skin induration ascending from the distal fingers (sclerodactyly) to proximal limbs (above elbows and/or knees) and the trunk. Interstitial lung disease (ILD) and acute scleroderma renal crisis occur in these patients and characteristically develop relatively early in the disease course. In contrast, limited cutaneous SSc (lcSSc) tends to be indolent and is characterized by Raynaud's phenomenon preceding other disease manifestations, sometimes by years. In lcSSc, the skin induration shows little progression and remains confined to the fingers, distal limbs, and face. Many patients with lcSSc display a characteristic constellation of clinical findings (calcinosis cutis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and mucocutaneous telangiectasia) that lead to the designation *CREST syndrome*. However, CREST features can also be present in patients with dcSSc. In contrast to dcSSc, visceral organ involvement in lcSSc tends to follow an indolent course, with digital ischemic ulcers and pulmonary arterial hypertension (PAH) as late complications. Some individuals may present with Raynaud's phenomenon accompanied by characteristic clinical, capillaroscopic, and serological features of SSc in the absence of detectable skin induration (*sine scleroderma*).

## EPIDEMIOLOGY

The reported incidence and prevalence of SSc vary significantly in different geographic regions. This might reflect true variability in disease frequency based on geographic and ethnic backgrounds but might also reflect differences in the case definition and ascertainment methods used. A large-scale survey in the Detroit area from 1988 to 1991 indicated an incidence rate of 21 new cases/year and a prevalence rate of 276 cases per million adults. A diagnosis was verified based on the 1980 American Rheumatism Association (ARA) classification criteria (Leroy et al., 1980) or presence of sclerodactyly in addition to two other CREST features (Mayes et al., 2003). A Canadian study using physician billing and hospitalization databases reported a prevalence of 443 per million in the province of Quebec in 2003 (Bernatsky et al., 2009). Epidemiological data from Europe reveal a north-south gradient for SSc, with Southern European countries having a higher frequency. An Italian retrospective study (Lo Monaco et al., 2011) from outpatient clinics from 1999 through 2007 reported annual incidence and prevalence rates of 43 and 341 cases per million, respectively (Leroy and Medsger, 2001). These rates decrease to an annual incidence and prevalence rates of 32 and 254 per million cases, respectively, when the more restrictive 1980 ARA classification criteria were applied. A study from Iceland reported an annual incidence of 3.8 per million during the period 1975–90 and prevalence of 71 per million (Geirsson et al., 1994). Epidemiological studies from Northeast England (Allcock et al., 2004) and Norway show similar prevalence data (88 and 99 cases per million, respectively). The most recent epidemiological study from Europe reported a prevalence of 228 cases per million in the Alsace region of France (Meyer et al., 2016). SSc seems to be less frequent in Asia. For instance, a recent study based on the Taiwan National Health Registry data indicated that the annual incidence rate and prevalence of SSc were 10.9 and 56.3 cases per million (Kuo et al., 2011). Of note, the 2013 classification can diagnose milder cases, potentially leading to higher SSc incidence and prevalence estimates in future epidemiological studies (van den Hoogen et al., 2013).

SSc has a female preponderance with most estimates of female-to-male ratios ranging from 3:1 to 9:1 [reviewed in Chifflot et al. (2008); Coral-Alvarado et al. (2009)]. In a large European SSc database (EUSTAR) 86% of the patients were female (6.2:1 ratio) (Meier et al., 2012). The female predominance in SSc is similar to other autoimmune rheumatic diseases such as systemic lupus erythematosus and rheumatoid arthritis. It is unclear why SSc occurs more frequently in females. Hormonal influences and pregnancy-related events have been postulated. A case-sibling study investigating the influence of birth order and gravity/parity in 974 sibships showed that the risk of SSc increased with increasing birth order (OR 1.25 for birth order 2–5; OR 2.22 for birth order 6–9; and OR 3.53 for birth order 10–15). Furthermore, gravidity/parity in 168 sibships showed that a history of one or more pregnancy was associated with SSc (Cockrill et al., 2010). These results are congruent with the notion that

**TABLE 31.2** Conditions Associated With Skin Induration (scleroderma)

SSc

- Limited cutaneous SSc  
 Diffuse cutaneous SSc  
 Localized scleroderma  
 Guttate (plaque) morphea, diffuse morphea, bullous morphea  
 Linear scleroderma, coup de sabre, hemifacial atrophy (Parry-Romberg)  
 Pansclerotic morphea  
 Overlap syndromes  
 Mixed connective tissue disease  
 SSc/polymyositis  
 Scleromyxedema (papular mucinosis)  
 Paraneoplastic syndromes  
 Diabetic scleredema and scleredema of Buschke  
 Chronic graft-versus-host disease  
 Diffuse fasciitis with eosinophilia (Shulman's disease, eosinophilic fasciitis)  
 Stiff skin syndrome  
 Pachydermoperiostosis (primary hypertrophic osteoarthropathy)  
 Chemically induced and drug-associated scleroderma-like conditions  
 Vinyl chloride–induced disease  
 Eosinophilia-myalgia syndrome (associated with L-tryptophan contaminant exposure)  
 Nephrogenic systemic fibrosis (associated with gadolinium exposure)

SSc, Systemic sclerosis.

**TABLE 31.3** Prominent Features of Limited Versus Diffuse Cutaneous Systemic Sclerosis (SSc)

Feature	Limited cutaneous SSc	Diffuse cutaneous SSc
Skin involvement	Indolent; limited to fingers, distal to elbows, face	Rapid onset. Diffuse: fingers, extremities, face, trunk; progression
Raynaud's phenomenon	Antedates skin involvement, sometimes by years; may be associated with critical ischemia in the digits	Onset coincident with skin involvement; critical ischemia less common
Calcinosis cutis	Common, may be prominent	Less common, mild
Musculoskeletal	Arthralgia, small joints of hands	Carpal tunnel syndrome, joint contractures; tendon friction rubs
Interstitial lung disease	Slowly progressive, generally mild	Frequent, early onset and progression, can be severe
Pulmonary arterial hypertension	Frequent, late, may occur as an isolated complication	Often occurs in association with interstitial lung disease
Scleroderma renal crisis	Uncommon	Occurs in 15%; generally early (<4 years from disease onset)
Calcinosis cutis	Common and often prominent	Less common, mild
Characteristic autoantibodies	Anticentromere	Antitopoisomerase I (Scl-70), anti-RNA polymerase III

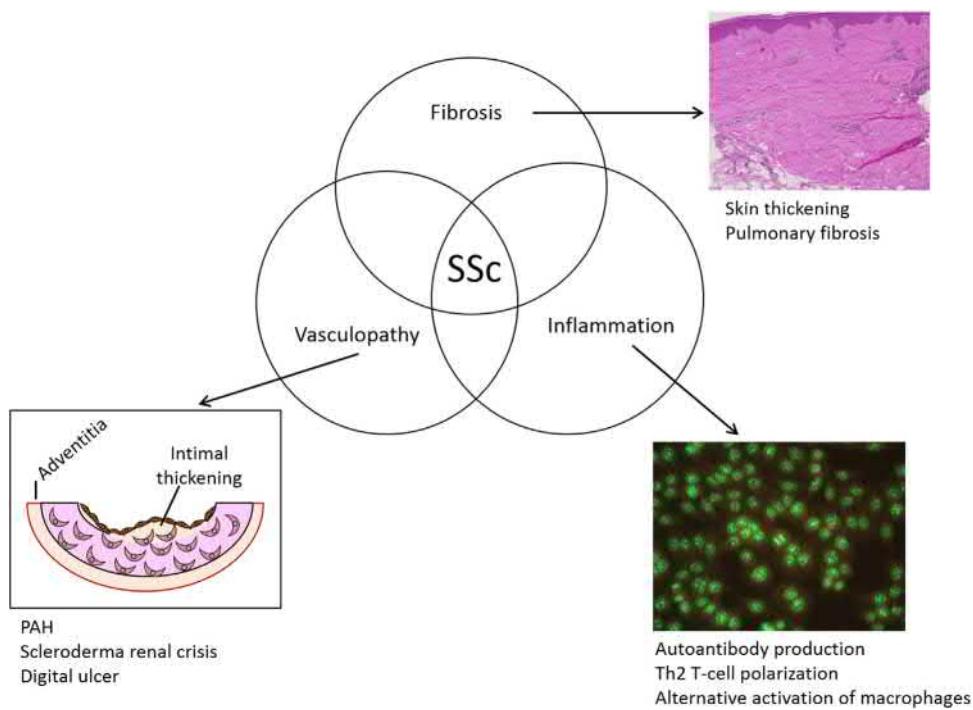
microchimerism might contribute to the female preponderance of SSc. Microchimerism occurs when cells are transferred between mother and fetus during pregnancy, resulting in the persistence of genetically distinct cell populations in the host (Bianchi et al., 1996). Male microchimerism was more frequent in peripheral blood cell mononuclear cells of parous women with SSc than of matched controls (Lambert et al., 2002), although this study had a small sample size (57 SSc and 57 controls). An alternative explanation for female predominance in SSc implicates preferential X-chromosome inactivation toward one parental source, leading to a deviation from the theoretical 1:1 inactivation ratio. The skewed pattern of X-chromosome inactivation in SSc has been confirmed in three independent studies with sample sizes ranging from 68 to 217 (Kanaan et al., 2016; Broen et al., 2010; Ozbalkan et al., 2005). It is worth noting that single-nucleotide polymorphisms (SNPs) of the genes for Interleukin-1 receptor-associated kinase-1 (IRAK1) and methyl-CpG-binding protein 2 (MECP2), both located on X-chromosome, have been linked to SSc. IRAK1 has an important immune function by modulating IL-1-mediated nuclear factor- $\kappa$ B (NF- $\kappa$ B) activation, whereas MECP2 encodes a nuclear protein that binds specifically to methylated DNA and plays a central role in the transcriptional regulation. A nonsynonymous IRAK1 variant (rs1059702) was most strongly associated with dcSSc and SSc-ILD (Dieude et al., 2011). A follow-up study found that the IRAK1 variant rs1059702 and MECP2 rs17435 were specifically associated with dcSSc in female patients. These two SNPs showed moderate linkage disequilibrium ( $r^2 = 0.6$ ). However, conditional logistic regression analysis indicated that the association of IRAK1 rs1059702 with dcSSc was explained by the effect of MECP2 rs17435. The IRAK1 variant rs1059702 was associated with the presence of ILD (Carmona et al., 2013). Of note, in both the studies, the odds ratios for association with the disease were less than 1.5, indicating that these significant associations have a low effect size.

The age of onset in SSc varies based on sex and ethnicity. African American patients have an earlier age of onset. In a large US study, the mean age at diagnosis did not differ based on sex. The mean age at SSc diagnosis was 48.1 for Whites and 41.0 for Blacks (Steen et al., 1997). In the EUSTAR database (primarily White patients) the mean age at disease onset (earlier than the age at diagnosis) was 45.9 years (Meier et al., 2012).

SSc is associated with significant excess mortality. A metaanalysis of nine studies conducted between 1960 and 2010 found a pooled standardized mortality ratio (compared to the age and gender-matched general population) of 3.53 (Elhai et al., 2011), which was higher than the standardized mortality ratio (SMR) in other autoimmune rheumatic diseases (Toledano et al., 2012). Of note, the SMR of SSc had not changed significantly over time, although studies from single-center cohorts or national registries indicate improvement in SSc-related mortality over time (Ferri et al., 2002; Steen and Medsger, 2007). A study from Spanish Scleroderma Registry indicated that death from SSc-related causes decreased from 72% in the time period of 1990–99 to 48% within 2000–09 (Rubio-Rivas et al., 2017). The cause of SSc-related deaths has also changed over time. In a large single-center study of patients seen between 1972 and 1997, the frequency of death due scleroderma renal crisis decreased from 42% to 6%, while the proportion of deaths due to SSc-ILD increased from 6% to 33%. The frequency of deaths due to PAH also increased during this period (Steen and Medsger, 2007). Confirming these findings, a recent analysis of the EUSTAR database with 5860 SSc patients indicated that 55% of the deaths were attributable directly to SSc, while 41% were due to non-SSc causes. Nineteen percent were due SSc-related ILD, 14% due to PAH, 14% due to SSc-related myocardial involvement, 4% due to renal causes, and 3% due to gastrointestinal (GI) complications. Among the non-SSc-related death, 13% were due to infections (predominantly pneumonia), 13% due to malignancies, and 12% due to cardiovascular causes (Tyndall et al., 2010).

Environmental factors are thought to play an etiologic role in the SSc. Occupational silica exposure is the best established of the potential environmental triggers of SSc. A metaanalysis of 16 original studies indicated that silica exposure is a risk factor for SSc, especially in male patients (McCormic et al., 2010). However, most of the SSc patients (male or female) have not had occupational silica exposure. Environmental toxins have also been linked to scleroderma-like conditions. For instance, the toxic oil syndrome was linked to the consumption of contaminated rapeseed oil used for cooking in Spain, while the epidemic of eosinophilia-myalgia syndrome in the United States was traced to the ingestion of contaminated L-tryptophan dietary supplements. Although both of these novel toxic-epidemic syndromes were characterized by scleroderma-like chronic skin changes and variable visceral organ involvement, they were associated with clinical, pathologic, and laboratory features distinguishing them from SSc.

Viral exposures have been also investigated as a potential trigger of SSc. Cytomegalovirus and Epstein–Barr virus have been both implicated. However, epidemiological studies of the SSc link with viruses have been hampered by the fact that they are highly prevalent in the general population. Although case reports and series describing SSc in women with silicone breast implants had raised concern regarding a possible causal role of



**FIGURE 31.2** The pathophysiologic triad of vasculopathy, autoimmunity/inflammation, and fibrosis underlies protean clinical manifestations of systemic sclerosis.

silicone exposure in SSc, a metaanalysis did not show any relation between silicone breast implants and SSc or any other rheumatic disease (Janowsky et al., 2000).

## PATHOGENESIS

The complex pathogenesis of SSc reflects a distinct triad of pathomechanistic processes: inflammation/autoimmunity (van den Hoogen et al., 2013), microangiopathy (Allanore et al., 2015), and fibrosis in multiple organs (Leroy et al., 1980) (Fig. 31.2). The relative severity and contribution of these three interrelated processes to the overall clinical picture and disease activity varies among patients and during disease evolution over time (Gabrielli et al., 2009). Autoimmunity/Inflammation and altered vascular reactivity occur early in the disease course and, therefore, lead to subsequent vascular loss, fibrosis, and atrophy in multiple organs.

## GENETICS

SSc is a polygenic disease associated with several genetic susceptibility loci, with each having low-to-moderate effect size. The combination of environmental and stochastic factors converging upon individuals of a particular genetic background seems to ultimately lead to the development of the disease.

In a study of 703 SSc patients from three US cohorts, 11 multiplex families were identified, resulting in familial accumulation rate of 1.5%–1.7%. Compared to the estimated prevalence in the general population, familial relative risk in the first-degree relatives was 13 and the relative risk in siblings was 15, which represent the strongest risk factors for the disease established to date. However, the absolute risk for each family member remains <1% (Arnett et al., 2001). A follow-up study indicated that affected first-degree relatives with SSc had a significantly higher concordance for SSc-related antibodies and human leukocyte antigen (HLA) class II haplotypes than expected by chance, supporting the role of genetics in autoantibody expression (Assassi et al., 2007).

To date, only a single twin study has been carried out in SSc. This study based on 42 pairs of twins (24 monozygotic twins) showed low concordance for SSc (4.7%), while stronger concordance was observed for the presence of antinuclear antibodies (ANA), which was detected in 40% in dizygotic twins versus 90% in monozygotic

twins. These results suggest that genetic predisposition alone is not sufficient for the development of SSc, but it might establish a susceptible host with autoantibody expression (Feghali-Bostwick et al., 2003).

The advent of genome-wide association studies (GWAS) and large-scale international collaborations have dramatically improved our understanding of SSc genetics. While the strongest disease associations have been observed in the HLA region, over 20 susceptibility loci outside the HLA region have been also robustly linked to SSc. Unbiased genetic association studies have provided new insights into SSc pathogenesis and can potentially identify novel therapeutic targets. The majority of SSc risk genes appears to be involved in immune regulation and is also susceptibility loci for systemic lupus erythematosus and other autoimmune diseases (Martin et al., 2012). This finding is also supported by a study examining the aggregation of autoimmune diseases in 4612 first-degree relatives of 1071 SSc patients. The most striking increase for familial autoimmunity compared to the general population was observed in systemic lupus erythematosus ( $OR = 17$ ,  $P = .004$ ), indicating that common genetic risk factors (and potentially early environment factors) predispose to SSc and systemic lupus erythematosus (Arora-Singh et al., 2010).

Large-scale candidate gene studies, four GWAS (Allanore et al., 2011; Radstake et al., 2010; Zhou et al., 2009; Terao et al., 2017) and two Immunochip studies (Mayes et al., 2014; Zochling et al., 2014), have led to the identification of robustly validated SSc susceptibility loci. The strongest associations in the genome-wide studies were observed on chromosome 6 in the HLA class II region. This finding provides important support for the notion that SSc is fundamentally an autoimmune disease. A large study of HLA class II genes (*DRB1*, *DQB1*, *DQA1*, *DPB1*) was carried out in 1300 SSc cases (961 Caucasians) and 1000 controls (Arnett et al., 2010). SSc was associated with the *DRB1\*11:04*, *DQA1\*05:01*, and *DQB1\*03:01* haplotypes and the *DQB1* allele. However, many other alleles or haplotypes were associated with autoantibody subtypes of disease. For example, HLA-*DPB1\*13:01* had an OR of 14 in antitopoisomerase I (ATA)-positive SSc patients in comparison to unaffected controls. The notion that the HLA class II associations represent a pathogenic contribution mainly through regulating autoantibody expression was supported by a GWAS follow-up study. In this study, different patterns of genetic associations were found for ATA and anticentromere (ACA)-positive SSc patients within HLA region, while the genetic associations outside the HLA region tended to be in the concordant direction for the antibody subgroups, although the strength of association varied (Gorlova et al., 2011).

**Table 31.4** shows a selected list of non-HLA susceptibility loci which were associated with SSc in at least one large-scale discovery/replication cohort study. Most of the non-HLA susceptibility genes are involved directly in innate and adaptive immunity.

Several genes directly involved in type I interferon (IFN) signaling have been associated with SSc. Specifically, four IFN regulatory factors (IRFs) are risk loci for SSc (*IRF4*, *IRF5*, *IRF7*, and *IRF8*). Several *IFR5* SNPs have been shown to be associated with SSc (Radstake et al., 2010; Dieude et al., 2009a). In a follow-up study, the minor allele of one of these (rs4728142) was associated with longer survival and higher forced vital capacity (FVC). This gene variant was also associated with lower *IRF5* transcript levels in peripheral blood monocytes from both SSc patients and controls (Sharif et al., 2012). The genetic ablation of *IRF5* in mice ameliorated experimental scleroderma induced by subcutaneous bleomycin injections, with reduced fibroblast activation, Th2 skewing of the immune response, and attenuation of vascular pathology in the skin and the lungs (Saigusa et al., 2015). Similarly, *IRF4* (Lopez-Isac et al., 2016b), *IRF7* (Carmona et al., 2012), and *IRF8* (Gorlova et al., 2011; Arismendi et al., 2015) have been associated with SSc. These findings support an important role of type I IFN signaling in the pathogenesis of SSc.

Genes involved in T- and B-cell signaling have also identified as SSc risk loci. *CD247* encodes the zeta subunit of the T-cell receptor and is associated with SSc (Radstake et al., 2010). Similarly, *TNFSF4*, encoding the OX40 ligand involved in the interaction between T cells, antigen presentation, and activation of T and B cells was also identified as a risk locus in several independent studies (Arismendi et al., 2015; Coustet et al., 2012). In mouse models of scleroderma, pharmacological OX40L blockade prevented inflammation-driven skin, lung, and vascular fibrosis and induced the regression of established dermal fibrosis (Elhai et al., 2016). Similarly, *BLK* proto-oncogene, which transduces the downstream B-cell receptor signal, has been linked to SSc (Gourh et al., 2010; Ito et al., 2010). Moreover, two nonsynonymous and functional SNPs of *BANK1* (rs10516487 and rs3733197), which encodes the B cell–specific scaffold protein with ankyrin repeats, were linked to SSc in two independent studies (Dieude et al., 2009b; Rueda et al., 2010). The above studies potentially implicate adaptive immunity in SSc pathogenesis.

Recently identified SSc susceptibility loci have also “converged” on NF- $\kappa$ B and IL12, two signaling pathways involved in innate immunity. *TNFAIP3* encodes the A20 ubiquitin-editing enzyme, a zinc finger protein that is required for negative feedback control for NF- $\kappa$ B and Toll-like receptors (TLR) signaling. *TNFAIP3* was linked to

**TABLE 31.4** Selected Genetic Loci Associated With SSc Susceptibility

Gene	Chr	Type of study	Function
TNFSF4 ( <i>OX40L</i> )	<u>1q25</u>	Candidate gene (American population)	Adaptive immunity
PTPN22	<u>1p13.2</u>	Candidate gene (with replication in an independent population)	Adaptive immunity
CD247	<u>1q24.2</u>	Whole-genome	Adaptive immunity
STAT4	<u>2q32</u>	Candidate gene and whole-genome	Adaptive immunity-IL12 pathway
BANK1	<u>4q24</u>	Candidate gene	Adaptive immunity
HLA class II	<u>6</u>	Candidate gene and whole-genome	Adaptive and innate immunity
TNFAIP3	<u>6q23</u>	Candidate gene (European Caucasian population)	NFkB signaling
PRDM1	<u>6q21</u>	Whole genome in multiethnic metaanalysis	Adaptive immunity
IRF5	<u>7q32</u>	Candidate gene and whole-genome	Innate immunity—Interferon pathway
<i>C8orf13/BLK</i>	<u>8p23-p22</u>	Candidate gene (with replication in an independent population)	Adaptive immunity
IRF8	<u>16q24.1</u>	Candidate gene	Innate immunity—Interferon pathway
IRF7	<u>11p15.5</u>	Candidate gene	Innate immunity—Interferon pathway
TLR2	<u>4q32</u>	Candidate gene	Innate immunity
CCR6	<u>6q27</u>	Candidate gene	Adaptive immunity
BANK1	<u>4q24</u>	Candidate gene	Adaptive immunity
BLK	<u>8p23-p22</u>	Candidate gene	Adaptive immunity
GRB10	<u>7p12.2</u>	Secondary analyses of GWAS	Insulin-like growth factor
CAV1	<u>7q31.1</u>	Candidate gene	Fibrosis
SOX5	<u>12p12.1</u>	Secondary analyses of GWAS	Apoptosis
CSK	<u>15q24.1</u>	Secondary analyses of GWAS	Fibrosis—Src kinase
DNASE1L3	<u>3p14.3</u>	Immunochip	Apoptosis—DNA fragmentation
ATG5	<u>6q21</u>	Immunochip	Autophagy
GSDMA		Whole-genome in multiethnic metaanalysis	Gene expression regulation
IL12RB2	<u>1p31.3</u>	Secondary analyses of GWAS	IL12 pathway
IL12RB1	<u>19p13.11</u>	Immunochip	IL12 pathway
IL21	<u>4q27</u>	Candidate gene	Adaptive immunity
IRAK1/MECP2	<u>Xq28</u>	Candidate gene	NFkB signaling/DNA methylation
TYK2	<u>19p13.2</u>	Secondary analysis of Immunochip	IL12 pathway
IRF4	<u>6p25.3</u>	Secondary analysis of GWAS	Interferon pathway
NFKB	<u>4q24</u>	Candidate gene	NFkB signaling

GWAS, Genome-wide association studies.

SSc susceptibility in two independent studies (Mayes et al., 2014; Koumakis et al., 2012a). Interestingly, decreased A20 expression was associated with enhanced transforming growth factor  $\beta$  (TGF- $\beta$ ) signaling and augmented fibrotic responses in normal dermal fibroblasts, suggesting an important role for A20 in fibrosis (Bhattacharya et al., 2016). An SNP in TNIP1 (ABIN), which can downregulate NF- $\kappa$ B signaling in part by interacting with TNFAIP3, was also identified as SSc risk locus in two independent studies (Allanore et al. 2011; Bossini-Castillo et al., 2013). However, both TNFAIP3 and TNIP1 are also strongly implicated in autoimmune diseases, including rheumatoid arthritis, lupus, psoriasis, and inflammatory bowel disease. Remarkably, NF- $\kappa$ B itself was identified as a risk locus for SSc (Arismendi et al., 2015). Five genes in the IL12 pathway have been also linked to SSc susceptibility. An *IL12RB2* intronic SNP was associated with SSc in a GWAS follow-up study (Bossini-Castillo et al.,

2012). Similarly, an SNP in the intergenic region of *SHIP1-IL12A* was linked to SSc with a relatively high OR (2.57) in the North American/European Caucasian Immunochip study (Mayes et al., 2014). Furthermore, an SNP in the RB1 subunit of IL12 was also linked to SSc risk in an Immunochip follow-up study (Lopez-Isac et al., 2014). Coexpression of IL12R $\beta$ 1 and IL12R $\beta$ 2 is required to form the high-affinity IL12 receptor, which induces activation of STAT4, itself linked to SSc susceptibility in four independent and follow-up GWAS (Allanore et al., 2011; Radstake et al., 2010). The tyrosine kinase TYK2 mediating the IL12 signaling was also associated with SSc susceptibility (Lopez-Isac et al., 2016a). While these studies implicate IL12 in SSc, the functional role of IL12 pathway variants and their pathogenic mechanisms remain to be investigated in mechanistic studies.

The majority of SSc susceptibility loci identified to date are located in intronic (noncoding) gene regions and have relatively modest effect size (typically <1.5). However, it is possible that these loci are in linkage disequilibrium with SNPs in gene coding regions. In addition, these loci might influence the transcription of noncoding regulatory RNAs such as microRNAs, circular RNAs, or long noncoding RNAs. Large-scale whole-exome or whole-genome sequencing studies in SSc are currently underway. It is anticipated that ongoing genetic efforts will reveal novel, rare mutations linked to risk of SSc, or specific disease endophenotypes, which might have substantially higher effect sizes. A further considerable challenge remains the functional characterization of the contribution of identified disease-linked genetic variants to the cellular and molecular alterations that underlie disease manifestations.

## PRECLINICAL DISEASE MODELS

Currently, no single animal model of SSc phenocopies the full spectrum of the human disease. Some existing models recapitulate selected features and therefore have substantial utility for understanding the pathobiology or preclinical evaluation of promising therapeutic interventions (Beyer et al., 2010).

Tight-skin mice (*Tsk1/+*) develop spontaneous skin fibrosis due to a fibrillin-1 mutation, which causes aberrant activation of TGF- $\beta$ . These mice do not develop lung fibrosis or characteristic vascular features. In humans, mutations in fibrillin-1 cause Marfan's disease and a familial scleroderma-like condition termed stiff skin syndrome; however, fibrillin-1 variants have not been linked to SSc. Skin and lung fibrosis accompanied by vasculopathy, inflammation, and autoimmunity can be induced in mice by subcutaneous injection of bleomycin, angiotensin, or HOCl, by transplantation of HLA-mismatched bone marrow or spleen cells, or by immunization with topoisomerase-1 (Marangoni et al., 2016). Targeted genetic modifications in mice are increasingly used for investigating individual molecules, pathways, and cell types in disease models. For example, mice constitutively overexpressing  $\beta$ -catenin, Fra-2, PDGFR-alpha, uPAR, IRF5, or adiponectin show either resistance, or hypersensitivity, to experimental scleroderma, and help shed light on specific aspects of SSc pathogenesis (Marangoni et al., 2017).

## MICROVASCULAR DISEASE IN SYSTEMIC SCLEROSIS

Vascular injury is a common early event in SSc that plays a primary role in pathogenesis. Widespread damage in multiple vascular beds ensues and underlies protean clinical manifestations of SSc.

The clinical sequelae of SSc microangiopathy include Raynaud's phenomenon and ischemic digital ulcers, watermelon stomach, mucocutaneous telangiectasia, scleroderma renal crisis, PAH, and myocardial involvement (Matucci-Cerinic et al., 2013).

Raynaud's phenomenon is an initially reversible vascular abnormality characterized by altered blood-flow response to cold challenge in small digital arteries. Raynaud's phenomenon is associated with autonomic and peripheral nervous system alterations, with impaired production of calcitonin gene-related peptide from sensory afferent nerves and heightened  $\alpha_2$ -adrenergic receptor sensitivity on vascular smooth muscle cells. Primary or idiopathic Raynaud's disease is common, generally nonprogressive and benign. In contrast, secondary Raynaud's phenomenon associated with SSc tends to be progressive and complicated by irreversible structural changes, culminating in digital tip ulcers, necrosis, and amputation.

The triggers for SSc-associated vascular endothelial cell injury are not fully known, but likely include viruses, circulating cytotoxic factors, chemokines, thrombogenic microparticles, and activation of the alternate complement pathways. Recent studies have also described functional autoantibodies targeting endothelial cells and phospholipids. Endothelial damage leads to dysregulated production of vasodilatory (nitric oxide and

prostacyclin) and vasoconstricting (endothelin-1) substances and upregulation of intercellular adhesion molecule 1 (ICAM-1). Injured microvessels show enhanced permeability and transendothelial leukocyte diapedesis, activation of coagulation cascades, elevated thrombin production, impaired fibrinolysis, and spontaneous platelet aggregation. Activated platelets release serotonin, platelet-derived growth factor (PDGF), and the potent vasoconstrictor thromboxane, contributing to further vascular damage.

In the vascular media, myointimal cells proliferate, the basement membrane is thickened and reduplicated, and adventitial fibrosis surrounding the blood vessels develops. The vasculopathic process affects capillaries, arterioles, and less commonly large vessels in many organs. In late-stage SSc, progressive luminal occlusion due to intimal and medial hypertrophy, combined with adventitial fibrosis, culminates in loss of small blood vessels (vascular rarefaction) and tissue ischemia. Revascularization or vasculogenesis, a process distinct from angiogenesis that normally restores circulation in ischemic tissue, is paradoxically impaired in SSc despite elevated levels of angiogenic factors. This is possibly related to reduced number and function of bone marrow–derived circulating endothelial progenitor cells. Capillary loss, fibro-obliterative vasculopathy of small and medium-sized arteries, coupled with impaired ability to repair and replace damaged vessels, underlie progressive SSc vascular disease.

## INFLAMMATION AND AUTOIMMUNITY

### Cellular Immunity

Inflammation and autoimmunity appear to play key roles in SSc. This is supported by the presence of disease-specific autoantibodies; the familial clustering of SSc with other autoimmune diseases ([Arora-Singh et al., 2010](#)); T and B cells with oligoclonal antigen receptors, in target organs; prominent type I IFN gene signatures ([Tan et al., 2006](#)); the elevated circulating levels of cytokines and chemokines such as interleukin-6 (IL-6), IL-4, IL-17, IL-33, CCL2, and CXCL4; and fibrosis resolution and vascular regeneration reported in some SSc patients treated with immunoablative hematopoietic stem-cell therapies. Genetic studies further reveal the strong associations of SSc with HLA, as well as numerous non-HLA-linked genes implicated in adaptive and innate immune responses ([Martin et al., 2012](#)).

In the early-stage (edematous) phase of the disease, mononuclear cell infiltrates comprising activated T and B cells, monocytes/macrophages, and dendritic cells are prominent in lesional skin and lungs even prior to vascular damage or fibrosis. Regulatory T cells, in contrast, are reduced in number. Dendritic cells can be found in close proximity to activated fibroblasts and myofibroblasts. By expressing TLR and secreting IFN, IL-10, thymic stromal lymphopoietin (TSLP), and CXCL4, they shape the adaptive immune response and contribute to loss of immune tolerance. Tissue-infiltrating T cells display restricted T-cell receptor signatures indicative of recognition of as-yet-unknown antigen, supporting the premise that an abnormal antigen might act as an initial trigger for the autoimmune response in SSc.

Peripheral blood T cells in SSc express chemokine receptors and  $\alpha_1$  integrin, which facilitates binding to endothelium and to fibroblasts, while endothelial cells express ICAM-1 and other vascular adhesion molecules that facilitate leukocyte diapedesis. T cells show a  $T_{H}2$ -polarized immune response and enhanced production of IL-4, IL-13, IL-33, and TSLP. These Th2 cytokines generally induce fibroblast activation, whereas the  $T_{H}1$  cytokine IFN- $\gamma$  is antifibrotic. Recent evidence also implicates altered Th17 and regulatory T-cell function in SSc. Type 2 innate lymphoid cells are a recently discovered lymphoid cell population that are also elevated in SSc skin biopsies. Alternately activated macrophages, which promote angiogenesis and tissue remodeling, are activated and increased in lesional skin and bind to fibroblasts.

Circulating B cells express CD19 and costimulatory molecules CD80 and CD86, suggesting chronic activation. Serum levels of a proliferation-inducing ligand (APRIL) and B cell–activating factor are elevated in SSc and associate with the extent of skin and lung involvement. Since B cells secrete IL-6, TGF- $\beta$ , and other profibrotic cytokines implicated in SSc, B-cell hyperactivity might directly contribute to the inflammatory and fibrotic processes, as well as the generation of disease-specific autoantibodies. Tissue transcriptome studies identified a distinct subset of SSc skin biopsies that show elevated expression of inflammation-related genes ([Martyanov and Whitfield, 2016](#)). Evidence of ongoing TLR signaling is prominent in both circulating immunocompetent cells as well as tissue-resident stromal cells within target organs and may reflect impaired ability to restrict innate immunity.

## Humoral Autoimmunity

Circulating ANA occur in the vast majority of patients with SSc and appear to predate clinical manifestations of the disease. Certain SSc-specific autoantibodies show robust associations with distinct disease endophenotypes and clinical/laboratory manifestations. Owing to their high degree of specificity, mutual exclusivity, association with particular disease patterns, and rates of progression, SSc-associated autoantibodies have gained substantial clinical utility as diagnostic and prognostic markers. Some SSc-specific antibodies appear to be associated with cancer (Joseph et al., 2014a). Antibodies directed against matrix metalloproteinases, fibrillin-1, and the surface receptors for angiotensin II, endothelin-1, muscarinic 3, and PDGF, have also been reported in patients with SSc, although their disease specificity, clinical utility, and potential pathogenic significance, if any, remain established.

A variety of mechanisms have been postulated to account for the emergence of these disease-specific autoantibodies in SSc. Proteolytic cleavage, altered expression or subcellular localization of normal proteins, or somatic mutations in the case of certain cancer-associated forms of SSc, might give rise to immune recognition of cryptic neoepitopes, breaking immune tolerance.

## Fibrosis

Tissue fibrosis is characterized by replacement of normal architecture with rigid avascular and acellular connective tissue (Ho et al., 2014). Fibrosis in SSc is caused by fibroblasts and myofibroblasts activated as a consequence of inflammation, autoimmunity, and microvascular damage. Fibroblasts are tissue-resident multifunctional stromal cells responsible for the functional and structural integrity of connective tissue. Resident fibroblasts are normally in a quiescent state. Upon their activation by extracellular cues, fibroblasts proliferate; migrate; induce a canonical secretome of collagens and other matrix molecules, growth factors, chemokines, and cytokines; and transdifferentiate into contractile myofibroblasts. Under normal conditions, these responses are spatially and temporally limited to completing physiologic repair and regeneration. When they become sustained and amplified, pathologic fibrosis results (Bhattacharyya et al., 2011). Paracrine fibrotic mediators including TGF- $\beta$ , IL-6, IL-13, Wnt ligands, lysophosphatidic acid, connective tissue growth factor, PDGF, hypoxia, reactive oxygen radicals (ROS), thrombin, and biomechanical forces have all been implicated in sustained fibroblast activation underlying SSc fibrosis. Damage-associated endogenous ligands for the innate pattern recognition receptors TLR4 (HMGB1, EDA-fibronectin, and tenascin-C) and TLR9 (mitochondrial DNA) further contribute to aberrant fibroblast activation underlying nonresolving fibrosis in SSc.

In addition to tissue-resident fibroblasts, endothelial cells, vascular mural pericytes, and other mesenchymal progenitor cells and preadipocytes are potential sources of activated myofibroblasts in fibrosis (El Agha et al., 2017). Although myofibroblasts are transiently found in normal wound healing, their persistence in lesional tissue, possibly due to mechanotransduction-mediated resistance to apoptosis, contributes to fibrosis (Nanchahal and Hinz, 2016).

Explanted fibroblasts from SSc skin biopsies display an abnormally activated phenotype ex vivo, with variably increased rates of extracellular matrix (ECM) production, spontaneous ROS generation, prominent alpha smooth muscle actin stress fibers, and a secretome characteristic of cellular aging. Persistence of the “scleroderma phenotype” during serial ex vivo passage is likely to reflect altered microRNA expressions, or acquired and stable cell type–specific epigenetic modifications such as DNA methylation or chromatin remodeling via histone acetylation. The heterogeneity of activated fibroblast populations within lesional skin and other tissues is just beginning to be investigated with the application of advanced technologies such as single-cell RNA sequencing.

## PATHOLOGY

The distinguishing pathological triad of SSc irrespective of the organ system affected is a capillary loss, and obliterative microangiopathy, combined with fibrosis. In early-stage disease, mononuclear inflammatory cell infiltrates can be detected around blood vessels in multiple organs. Obliterative microangiopathy is the prominent late finding in the lungs, kidneys, heart, and GI tract. Excessive buildup of a fibrotic extracellular matrix composed of collagens, proteoglycans, and other structural matrix macromolecules progressively disrupts normal architecture, resulting in impaired function and failure of affected organs. The process is most evident in the skin, lungs, cardiovascular and GI systems, tendon sheaths, perifascicular tissue surrounding skeletal muscle, and thyroid glands, while the brain, spinal cord, and liver are largely spared.

## Skin

The lesional skin is rigid and thickened, with broad bundles of homogenized collagen within the dermis oriented parallel to the epidermis. Loss of periadnexal and intradermal white adipose tissue and its replacement with a collagenous matrix can be striking. Perivascular mononuclear cell infiltrates may be seen early, while in established skin fibrosis inflammation is largely absent.

## Lungs

In SSc autopsy studies, evidence of interstitial lung involvement is nearly universal. The most common histopathological pattern is nonspecific interstitial pneumonia (NSIP), characterized by interstitial fibrosis and mild chronic inflammation. The pattern of usual interstitial pneumonia (UIP), with spatial/temporal heterogeneity of inflammation, fibrosis, and fibrotic foci that is characteristic of idiopathic pulmonary fibrosis, is less common in SSc-associated lung disease ([Herzog et al., 2014](#)). Fibrosis of the alveolar septae results in obliteration of the airspaces and loss of pulmonary blood vessels, impairing gas exchange. Intimal thickening of the pulmonary arteries underlies SSc-associated PAH, a frequent late complication, and is often associated with pulmonary emboli and myocardial fibrosis.

## Gastrointestinal Tract

Pathologic changes can be prominent at any level in the GI tract from the mouth to the rectum. In the lower esophagus, atrophy and fibrosis of the muscularis propria and characteristic vascular lesions are prominent, while the upper third of the esophagus is largely spared. Chronic gastroesophageal reflux can lead to esophageal inflammation, mucosal ulceration, and stricture formation. Barrett's metaplasia with an attendant risk of adenocarcinoma can occur. Collagenous replacement of the GI wall results in impaired smooth muscle contractility and diminished peristaltic activity, with bacterial overgrowth and small-bowel pseudo-obstruction as late sequelae.

## Kidneys

In the kidneys, vascular lesions affecting the interlobular and arcuate arteries predominate. Biopsies in acute scleroderma renal crisis show acute fibrinoid necrosis and intimal proliferation (onion-skin pattern) of the afferent arterioles, sometimes associated with ischemic glomerular collapse and sclerosis. These changes are strongly reminiscent of thrombotic microangiopathies (TMAs) such as thrombotic thrombocytopenic purpura (TTP) and atypical hemolytic-uremic syndrome, resulting in occasional misclassification. Additional findings include thrombosis, thrombocytopenia due to platelet consumption, and microangiopathic hemolysis. Presence of extensive vascular thrombosis, glomerular collapse, and peritubular capillary deposits is predictive of irreversible renal failure. As in other forms of TMAs, evidence of intravascular complement activation can be prominent, suggesting a potential pathogenic role of the complement system in scleroderma renal crisis ([Ghossein et al., 2016](#)).

## Heart

While cardiac involvement in SSc is often subclinical, prominent pathological involvement of the myocardium and pericardium is frequent. The characteristic arteriolar lesions are concentric intimal hypertrophy and luminal narrowing, accompanied by patchy contraction band necrosis, loss of cardiac myocytes, and myocardial fibrosis that are thought to be due to microvascular involvement and ischemia-reperfusion injury. Fibrosis of the conduction system, especially at the sinoatrial node, can be found. Pericardial involvement with chronic inflammatory infiltrates, fibrinous exudates, and pericardial effusions is common.

## Pathology in Other Organs

Skeletal muscle inflammation and, in the later stages, atrophy and fibrosis are common findings, which are similar to those in polymyositis ([Paik et al., 2015](#)). Inflammatory synovitis is seen in some patients with early SSc; with disease progression, the synovium becomes fibrotic, and in contrast to rheumatoid arthritis, pannus formation or bone resorption are uncommon. Fibrosis and atrophy of the thyroid and the minor salivary glands may

be seen. Placentas from SSc pregnancies show decidual vasculopathy, which is associated with poor perinatal outcomes and fetal death.

## CLINICAL FEATURES

### Overview

SSc can affect virtually any organ (Table 31.5). Although the widely employed dichotomous subgrouping of disease into dcSSc and lcSSc is clinically useful (Table 31.2), different endophenotypes with unique constellations of symptoms occur within each subgroup. The SSc-related autoantibodies are associated with certain disease manifestations and are the best predictive biomarkers identified thus far (Steen, 2005). SSc can coexist with other autoimmune diseases, most commonly rheumatoid arthritis, Sjögren's syndrome, polymyositis, systemic lupus erythematosus, and primary biliary cirrhosis.

### Initial Clinical Presentation

Characteristic initial presentation is quite different in patients with the diffuse and limited cutaneous forms of the disease. In dcSSc, the interval between Raynaud's phenomenon and the onset of other disease manifestations is typically short (weeks to months), and in some cases, Raynaud's phenomenon may even be preceded by other disease manifestations. Puffy fingers and soft tissue swelling accompanied by intense pruritus are signs of the early inflammatory "edematous" phase. The disease typically starts in fingers and face. Diffuse hyperpigmentation of the skin, carpal tunnel syndrome, arthralgias, fatigue, and decreased joint mobility are common. During the ensuing weeks to months, the inflammatory edematous phase evolves into the "fibrotic" phase, with skin thickening associated with loss of hair. Progressive flexion contractures of the fingers can follow. Inflammatory joint pain and associated morning stiffness in the wrists, elbows, shoulders, hip girdles, knees, and ankles can be present. While advancing skin involvement is the most visible manifestation of early dcSSc, important and clinically silent internal organ involvement can occur during this stage. Gastrointestinal reflux disease (GERD) can be an early symptom. The initial 4–5 years from disease onset generally delineates the period of most rapidly evolving pulmonary and renal damage. If significant organ failure does not emerge during this phase of dcSSc, the systemic process may stabilize, although certain organ manifestations such as ILD and GI involvement can progress even after the initial period.

Compared to dcSSc, the course of lcSSc tends to be more indolent. The interval between onset of Raynaud's phenomenon and other disease manifestations such as distal skin thickening, GERD, and calcinosis can be several years or even decades. Scleroderma renal crisis, significant ILD, and tendon friction rub are rare in lcSSc, while PAH, and overlap with keratoconjunctivitis sicca, polyarthritis, cutaneous vasculitis, and biliary cirrhosis can develop in the later stages of this disease subtype.

**TABLE 31.5** Clinically Significant Organ Involvement IN Systemic Sclerosis (SSc)

Feature	Limited cutaneous SSc (%)	Diffuse cutaneous SSc (%)
Skin involvement	90 <sup>a</sup>	100
Raynaud's phenomenon	99	98
Digital ischemic ulcers	50	25
Esophageal involvement	90	80
Interstitial lung disease	35	65
Pulmonary arterial hypertension	15	15
Myopathy	11	23
Clinical cardiac involvement	9	12
Scleroderma renal crisis	0–2	15

<sup>a</sup>Approximately 10% of the patients limited cutaneous SSc patients have SSc sine scleroderma.

## ORGAN INVOLVEMENT

### Raynaud's Phenomenon

Raynaud's phenomenon is the most frequent extracutaneous manifestation of SSc. It is characterized by episodes of reversible vasoconstriction in the fingers and toes, sometimes also affecting the tip of the nose and earlobes. Episodes ("attacks") can be triggered by cold temperature, as well as emotional stress and vibration, and characteristically start with digital pallor due to decrease in blood supply, followed by cyanosis of variable duration. Hyperemia ensues spontaneously or with rewarming of the digit and is typically the most painful phase of Raynaud's phenomenon. The progression of the three color phases reflects the underlying vasoconstriction, ischemia, and reperfusion. If Raynaud's phenomenon occurs without any underlying disease, it is classified as primary. Primary Raynaud's phenomenon (Raynaud's disease) is relatively common in the general population. A recent metaanalysis of 33 studies assessing 33,733 participants showed that the pooled prevalence of primary Raynaud's phenomenon is 4.85% ([Garner et al., 2015](#)). Secondary Raynaud's phenomenon occurs in SSc as well as in other connective tissue diseases, hematological conditions associated with hyperviscosity, and occupational disorders, and can complicate treatment with beta-blockers and anticancer drugs such as cisplatin and bleomycin. Distinguishing primary from secondary Raynaud's phenomenon is important, as secondary Raynaud's phenomenon can be an early symptom of SSc or other underlying conditions. Primary Raynaud's phenomenon is supported by the following: earlier age of onset; absence of an underlying cause; absence of digital tissue necrosis or ischemic ulceration; and a negative ANA. Secondary Raynaud's phenomenon tends to occur at an older age (>30 years), is more severe (episodes more frequent, prolonged, and painful), and is associated with ischemic digital ulcers and loss of digits.

Nailfold capillaroscopy using videocapillaroscopy, low-power stereoscopic microscope, or ophthalmoscope permits visualization of nailfold cutaneous capillaries. Primary Raynaud's phenomenon is associated with evenly spaced parallel vascular loops at the nailfolds, whereas in secondary Raynaud's phenomenon, widened capillaries, reduction of capillary density (drop-outs), irregular new capillary formations can be observed. Thus nailfold capillaroscopy can be a helpful bedside tool for differentiating primary from secondary Raynaud's phenomenon, moreover, nailfold capillaroscopic findings (enlarged capillaries and/or capillary loss with or without pericapillary hemorrhages) are included as criteria in the revised SSc classification ([van den Hoogen et al., 2013](#)).

### Skin Features

Bilateral symmetrical skin thickening is the hallmark of SSc that distinguishes it from other connective tissue diseases. Skin involvement starts in the fingers and characteristically advances from distal to proximal extremities in an ascending fashion. Some patients notice diffuse hyperpigmentation without sun exposure early in the course of the disease. In dark-skinned individuals, vitiligo-like hypopigmentation may occur. Because the pigment in the perifollicular areas is spared, the skin may have a "salt-and-pepper" appearance that is most prominent on the scalp, upper back, and chest. Dermal sclerosis obliterating hair follicles, sweat glands, and eccrine and sebaceous glands causes hair loss, decreased sweating, and dry and itchy skin on the extremities. Pruritus is a prevalent manifestation and is associated with decreased perceived health ([El-Baalbaki et al., 2010](#)). In a recent observational study, pruritus was present in 43% of the patients and was associated with more extensive skin and GI involvement ([Razykov et al., 2013](#)). Skin thickening can lead to fixed flexion contracture and shortening of tendons and muscle atrophy in the underlying tissue. Thick ridges at the neck due to firm adherence of skin to the underlying platysma muscle can limit neck extension.

In established SSc, the face assumes a characteristic "mauskopf" appearance with taut and shiny skin, and loss of wrinkles. In advanced disease, the patients display an expressionless Parkinsonian facies due to reduced mobility of the eyelids, cheeks, and mouth. Thinning of the lips with accentuation of the central incisor teeth and prominent perioral radial furrowing (rhytides) complete the picture. Reduced oral aperture (microstomia) can interfere with oral hygiene and dental care. This can be especially problematic because SSc patients are at risk for accelerated dental decay and gum disease due to xerostomia. The nose assumes a pinched, beak-like appearance.

In late-stage disease, the skin is thin and atrophic, and loss of subcutaneous fat tissue leads to a "bound-down," tethered skin. Dilated skin capillaries 2–20 mm in diameter (telangiectasia) that are reminiscent of hereditary hemorrhagic telangiectasia occur, most frequently on the face, hands, lips, and buccal mucosa. Presence of telangiectasia has been included in the revised SSc classification criteria ([van den Hoogen et al., 2013](#)). Moreover,

the number of telangiectasia correlates with the severity of microvascular disease and PAH. The breakdown of atrophic skin can lead to recurrent ulcerations at the extensor surfaces of the proximal interphalangeal joints and bony prominences such as elbows and malleoli. These ulcers can be painful, tend to heal slowly, and can become secondarily infected requiring treatment with antibiotics. Healing of ischemic fingertip ulcerations leaves characteristic fixed digital “pits.” Loss of soft tissue at the fingertips and associated resorption of the terminal phalanges (acro-osteolysis) due to ischemia can also occur.

Dystrophic calcifications in the skin, subcutaneous, and soft tissues (calcinosis cutis) in the presence of normal serum calcium and phosphate levels occur in up to 25% of the patients. In a multicenter observational study of 1290 SSc patients, ATA, anti-RNA polymerase III (RNAP), and U1-RNP autoantibodies were significantly less common in patients with calcinosis, while ACA, anti-PM/Scl, and anticardiolipin antibodies were more frequent. In the multivariable model, digital ulcers, female gender, telangiectasia, cardiac disease, GI involvement, ACA positivity, and osteoporosis were independent clinical variables associated with calcinosis (Valenzuela et al., 2016). Calcific deposits represent dystrophic calcifications composed of calcium hydroxyapatite crystals that vary in size from tiny punctate lesions to large conglomerate masses and can be readily visualized on plain radiographs or dual-energy CT. It is thought that tissue damage by inflammation, hypoxia, or local trauma trigger calcium deposition, but the exact underlying mechanism remains elusive. The distal finger pads, palms, extensor surfaces of the forearms, and the olecranon and prepatellar bursae are common locations for calcinosis. The lesions can cause pain and nerve compression, ulcerate through the overlying skin with drainage of chalky white material and secondary infections. Paraspinal sheet calcifications may lead to neurologic complications.

## Pulmonary Features

The two principal forms of lung involvement in SSc, ILD, and PAH, are common and the leading causes of disease-related mortality (Steen and Medsger, 2007; Tyndall et al., 2010). Survival is particularly poor in SSc patients with concurrent presence of these two processes. Less frequent pulmonary complications of SSc include recurrent aspiration complicating chronic GERD, pulmonary hemorrhage due to endobronchial telangiectasia, obliterative bronchiolitis, pleural reactions, restrictive physiology due to chest wall fibrosis, spontaneous pneumothorax, and drug-induced lung toxicity.

### **Interstitial Lung Disease**

While evidence of ILD can be found in up to 65% of the SSc patients by high-resolution computed tomography (HRCT), clinically significant ILD develops in 16%–43%; the frequency varies depending on the method used for its detection (Herzog et al., 2014). Black race, diffuse cutaneous involvement, severe GERD, presence of ATA, or absence of ACA has been shown to be risk factors for SSc-associated ILD. Low FVC or single-breath diffusing capacity of the lung for carbon monoxide ( $D_{LCO}$ ) at initial presentation can indicate the presence of clinically significantly ILD, which need to be confirmed by HRCT imaging. Esophageal dilatation with chronic GERD in SSc causes microaspiration, which can contribute to the development and progression of ILD. In an observational study of 270 SSc patients, increased esophageal diameter on HRCT was associated with more severe radiographic ILD, lower lung volumes, and lower  $D_{LCO}$  (Richardson et al., 2016).

Pulmonary involvement in SSc can remain asymptomatic until it is quite advanced. The most common presenting respiratory symptoms—exertional dyspnea, fatigue, and reduced exercise tolerance—are nonspecific, subtle, and slowly progressive. Chronic dry cough can be a prominent accompanying symptom. In a recent randomized controlled trial (Scleroderma Lung Study II), 61% of the patients with SSc-ILD reported frequent cough at baseline, which was associated with greater dyspnea, more extensive lung involvement on HRCT, lower  $D_{LCO}$ , and more frequent GERD symptoms (Tashkin et al., 2017). Physical examination may reveal fine “Velcro” crackles at the lung bases. Pulmonary function testing (PFT) is only moderately sensitive for detecting early pulmonary involvement. It helps to identify a restrictive respiratory pattern and exclude obstructive disease (e.g., chronic obstructive pulmonary disease). The characteristic constellation is low FVC, normal FEV1/FVC ratio, and decreased  $D_{LCO}$ . A disproportionately decreased  $D_{LCO}$  compared to the change in FVC might point to pulmonary vascular disease (i.e., PAH) in SSc. Oxygen desaturation with exercise is common.

Chest radiography can be used to rule out infection and other causes of pulmonary involvement, but compared to HRCT, it is relatively insensitive for detection of early ILD. HRCT may demonstrate bilateral lower lobe subpleural reticular linear opacities and ground-glass opacifications, even in asymptomatic patients with

normal PFTs. Mediastinal lymphadenopathy, pulmonary nodules, and traction bronchiectasis can also be found on HRCT. The typical radiographic finding is the NSIP pattern. However, a minority of patients show the UIP pattern on HRCT with prominent honeycombing.

### Pulmonary Arterial Hypertension

PAH resulting from the vascular remodeling of small ( $<500\text{ }\mu\text{m}$ ) pulmonary arteries develops in 8%–12% of the patients with SSc and occurs as an isolated abnormality or in association with ILD. PAH must be confirmed by right-heart catheterization and is defined based on the following hemodynamic parameters: mean pulmonary artery pressure  $\geq 25\text{ mmHg}$  at rest with a pulmonary capillary wedge pressure  $\leq 15\text{ mmHg}$  and pulmonary vascular resistance  $>3$  Wood units. The natural history of SSc-associated PAH is highly variable but can show rapid deterioration once right-heart failure develops. The observational Pulmonary Hypertension Assessment and Recognition of Outcomes in Scleroderma Registry of 131 SSc patients with PAH showed 1-, 2-, and 3-year cumulative survival rates of 93%, 88%, and 75%, respectively. On the multivariable analysis, age  $>60$  years, male sex, functional class IV,  $D_{\text{LCO}} < 39\%$  were significant predictors of mortality (Chung et al., 2014a). Mutations in TGF- $\beta$  receptor genes, including BMPR2, which are risk factors for both idiopathic and familial forms of PAH, are not risk loci for SSc-PAH (Koumakis et al., 2012b).

Often asymptomatic in early stages, SSc-PAH may present with symptoms of exertional dyspnea and reduced exercise tolerance. With progression, angina, near-syncope, and symptoms and signs of right-sided heart failure appear. Tachycardia, a loud pulmonic component of the  $S_2$  heart sound, pulmonic/tricuspid regurgitation murmur, palpable right ventricular heave, elevated jugular venous pressure, and dependent edema can be found on physical examination. Doppler echocardiography provides a widely used noninvasive screening method for estimating the pulmonary arterial pressure. While it can be used for screening of PAH, cardiac catheterization is virtually always required to make the diagnosis, because the specificity of Doppler echocardiogram for PAH is poor. Right-heart catheterization can also assess the degree of right-heart dysfunction and rule out other causes of increased right ventricular systolic pressure, including left-heart impairment due to diastolic dysfunction. Distinguishing PAH from pulmonary hypertension secondary to pulmonary fibrosis and hypoxia in SSc can be difficult. Serum levels of N-terminal probrain natriuretic peptide (NT proBNP) correlate with the presence and severity of PAH, as well as survival. While NT proBNP measurements can be useful in screening for PAH and in monitoring the response to treatment, its elevation is not specific for PAH and can be seen in both right and/or left-heart disease.

Despite more favorable hemodynamics, the prognosis of SSc-associated PAH is worse than idiopathic PAH, and treatment responses are poorer. This difference is thought to reflect frequent concurrence of ILD, GI, and cardiac disease in SSc. Moreover, patients with SSc-PAH have higher mortality than those with PAH due to other connective tissue diseases. In the Registry to Evaluate Early and Long-Term PAH Management database, SSc-PAH patients had a 3-year survival rate of 61% versus 81% in those with PAH associated with other connective tissue diseases. In multivariable analysis, male sex, age  $>60$  years, systolic BP  $\leq 110\text{ mmHg}$ , 6-minute walk distance  $<165$ , and elevated right arterial pressure and pulmonary vascular resistance were independent predictors of mortality in SSc-PAH (Chung et al., 2014b).

## GASTROINTESTINAL INVOLVEMENT

GI involvement occurs in up to 90% of the SSc patients and is equally prevalent in dcSSc and lcSSc (Table 31.6). The pathologic findings of smooth muscle atrophy accompanied by fibrosis and variable inflammatory infiltrates are similar throughout the length of the GI tract. GI involvement contributes to a reduced quality of life, malnutrition, fatigue, and increased mortality (Tyndall et al., 2010; Assassi et al., 2011).

### Upper Gastrointestinal Tract Involvement

Decreased oral aperture interferes with regular dental hygiene. Teeth are loosened due to the loss of periodontal ligament attaching teeth to the alveolar bone. In addition, dental and oral problems are often aggravated by xerostomia, shortened frenulum, and resorption of the mandibular condyles. Most of the patients with SSc develop symptomatic GERD with prominent heartburn, regurgitation, and dysphagia, and GERD can be a presenting symptom of the disease. GERD is caused by a combination of reduced lower esophageal sphincter

**TABLE 31.6** SSc-Related Autoantibodies and Their Demographic and Clinical Correlates

Antibody	Demographic and clinical associations
Antitopoisomerase I (Scl-70)	Diffuse disease, ILD, poorer survival
Anticentromere	Limited disease, calcinosis, better survival
Anti-RNA polymerase III	Diffuse disease, PAH, scleroderma renal crisis, GAVE, cancer
Anti-Pm-Scl	Limited disease, polymyositis and dermatomyositis, better survival
Anti-U3-RNP (antifibrillarin)	African American race, PAH, poorer survival

GAVE, gastric antral vascular ectasia; ILD, interstitial lung disease; PAH, pulmonary arterial hypertension.

pressure resulting in reflux, impaired esophageal clearance of refluxed gastric contents due to diminished motility, and delayed gastric emptying. Calcium channel antagonists and phosphodiesterase inhibitors that are commonly used in SSc to treat Raynaud's phenomenon can further worsen GERD. Esophageal manometry shows abnormal motility in most of the patients, even in the absence of GERD symptoms. Extraesophageal manifestations of GERD include hoarseness, chronic cough, and recurrent microaspiration, which can result in pulmonary infections and worsening ILD. A dilated patulous esophagus with intraluminal air is often seen on HRCT. While endoscopy is not always necessary to diagnose upper GI tract involvement in patients with SSc, this diagnostic modality is occasionally indicated to rule out opportunistic infections with *Candida*, herpes virus, and cytomegalovirus. Since SSc patients with Barrett's metaplasia, a complication of chronic reflux, are at risk for developing adenocarcinoma, such patients should undergo regular surveillance endoscopy with biopsy. SSc patients can also develop esophageal strictures which can cause severe dysphagia and weight loss. Esophageal strictures can be treated with endoscopic dilation.

Gastroparesis is common in SSc and is manifested by early satiety, abdominal distention, and aggravated reflux symptoms. Barium contrast studies are neither sensitive nor specific for evaluation of gastric involvement in this setting. Gastric antral vascular ectasia (GAVE) in the antrum can be detected. These subepithelial lesions, reflecting the diffuse small-vessel vasculopathy of SSc, are described as "watermelon stomach" due to their characteristic endoscopic appearance (Watson et al., 1996). GAVE lesions can intermittently bleed, leading to chronic anemia, or less commonly severe acute anemia. Asymptomatic GAVE lesions appear to be more common in SSc than initially thought. In a recent randomized controlled trial (Scleroderma: Cyclophosphamide or Transplant), 22% of the 103 SSc patients with early-stage diffuse cutaneous disease who underwent upper GI endoscopy were found to have evidence of GAVE (Hung et al., 2013). GAVE lesion with active bleed can be treated with endoscopic cauterization.

### Lower Gastrointestinal Tract and Anorectal Involvement

Weight loss and malnutrition due to impaired intestinal motility, malabsorption, and chronic diarrhea secondary to bacterial overgrowth are significant complications of SSc involvement of the lower GI tract, and can lead to fat and protein malabsorption, vitamin B<sub>12</sub>, and vitamin D deficiency. The disturbed intestinal motor function can also lead to intestinal pseudo-obstruction. The symptoms can be indistinguishable from those of delayed gastric emptying. The recurrent episodes of acute abdominal pain, nausea, and vomiting, and radiographic studies showing acute intestinal obstruction are presenting symptoms and signs. A major diagnostic challenge in the acute setting is differentiating pseudo-obstruction, which typically responds to supportive care and intravenous nutritional supplementation, from mechanical obstruction requiring surgical intervention. SSc patients are also at risk for bacterial overgrowth, characteristically presenting as severe diarrhea alternating with episodes of constipation.

Fecal incontinence is a commonly underrecognized GI manifestation of SSc and can be the source of considerable patient distress and embarrassment. An occasional radiologic finding is pneumatisis cystoides intestinalis, caused by air trapping in the bowel wall that may rarely rupture and cause benign pneumoperitoneum. In the setting of SSc, pneumatisis cystoides is usually benign and responds to conservative management (Kaneko et al., 2016). Primary liver involvement is rare in SSc. However, biliary cirrhosis is seen in 2% of the patients and is generally associated with ACA positivity (Assassi et al., 2009a).

## RENAL INVOLVEMENT: SCLERODERMA RENAL CRISIS

Scleroderma renal crisis is a major complication of SSc and was a leading cause of death in the preangiotensin-converting enzyme (ACE) inhibitor era. Patients present with accelerated hypertension accompanied by progressive failure. This complication develops in 5%–15% of the patients, and almost invariably occurs within 4 years of disease onset (Penn et al., 2007). Rarely, scleroderma renal crisis can be the presenting manifestation of SSc. Prior to the advent of ACE inhibitor therapy, short-term survival in scleroderma renal crisis was <10%. The pathogenesis involves obliterative vasculopathy and luminal narrowing of the renal arcuate and interlobular arteries, with consequent intravascular (microangiopathic) hemolysis. Progressive reduction in renal blood flow, aggravated by vasospasm, leads to augmented juxtaglomerular renin secretion and activation of angiotensin II, with further renal vasoconstriction resulting in a vicious cycle that culminates in accelerated hypertension. Risk factors for scleroderma renal crisis include early-stage disease, diffuse cutaneous involvement, and the presence of tendon friction rub (Avouac et al., 2016; Hudson et al., 2014). Up to 50% of the patients with scleroderma renal crisis have RNAP3 antibodies, whereas this complication is exceedingly rare in SSc patients with ACA antibodies. Palpable tendon friction rubs, pericardial effusion, new unexplained anemia, and thrombocytopenia may be the clinical signs of impending scleroderma renal crisis. Daily home monitoring of blood pressure and immediate notification of treating physician in case of hypertension are the recommended strategies in SSc patients at risk for scleroderma renal crisis. Treatment with prednisone has been linked to scleroderma renal crisis and should be avoided in high-risk patients. If prednisone is absolutely required, it should be prescribed in doses below <10 mg and given for short periods of time (Steen and Medsger, 1998). Patients with scleroderma renal crisis characteristically present with accelerated hypertension (generally >150/90 mmHg) and progressive oliguric renal insufficiency. However, approximately 10% of the patients with renal crisis do not have elevated blood pressure. Normotensive renal crisis is associated with poorer outcome, which might be related to delayed diagnosis.

Symptoms of elevated blood pressure such as headache, blurred vision, encephalopathy, congestive heart failure, and pulmonary edema may be present. Urinalysis typically shows mild proteinuria, granular casts, and microscopic hematuria; moderate thrombocytopenia and microangiopathic hemolysis with fragmented red blood cells can be seen. If scleroderma renal crisis is untreated, rapidly progressive oliguric renal failure generally ensues. Scleroderma renal crisis is occasionally misdiagnosed as TTP or other forms of TMAs. Prompt differentiation from TTP is important because these two conditions are treated differently, with early ACE-inhibitors effective in the former while plasma exchange used in the latter. When the differential between scleroderma renal crisis and TTP remains unsettled, cautious renal biopsy and determination of serum vWF-cleaving protease (ADAMTS13) activity can aid in establishing the correct diagnosis. Baseline oliguria or serum creatinine >3.0 mg/dL predicts poor outcome of renal crisis (permanent hemodialysis or early mortality). In addition, biopsy findings of extensive renal vascular thrombosis and glomerular ischemic collapse portend poor outcomes.

Rarely, rapidly progressive crescentic glomerulonephritis occurs in the setting of SSc and may be associated with myeloperoxidase-specific antineutrophil cytoplasmic antibodies. Asymptomatic renal function impairment occurs in up to half of SSc patients. This is associated with other vascular manifestations of SSc and rarely progresses to renal failure.

## CARDIAC INVOLVEMENT

Although it is often clinically silent and asymptomatic, cardiac involvement is detected in 10%–50% of the SSc patients screened with sensitive diagnostic tools. Clinically apparent cardiac involvement occurs more frequently in patients with dcSSc and inflammatory myositis. It can be primary, or secondary to SSc-associated PAH, ILD, or renal involvement, and is a marker of poor outcomes. All three anatomic components of heart, endocardium, myocardium, and pericardium may be affected separately or together. Pericardial manifestations include pericarditis, mild-to-moderate pericardial effusions, constrictive pericarditis, and, rarely, cardiac tamponade. Conduction system fibrosis may be manifested by heart block. Electrocardiogram (EKG) abnormalities are common in SSc patients and are associated with more severe heart and lung involvement. In particular, complete right bundle branch block in setting of SSc is highly predictive of mortality (Draeger et al., 2013).

Widespread microvascular involvement, recurrent vasospasm, and ischemia-reperfusion injury contribute to patchy myocardial fibrosis, which can lead to systolic or diastolic left ventricular dysfunction that may be

asymptomatic or progress to overt heart failure. Diastolic dysfunction, in particular, is common in SSc. In an observational study of 153 patients, left ventricular diastolic dysfunction was present in 23%, while left ventricular systolic dysfunction occurred in 5%, particularly in patients with longer disease duration (Hinchcliff et al., 2012). Acute or subacute myocarditis leading to left ventricular dysfunction may occur and are best identified using cardiac magnetic resonance imaging (cMRI), while endomyocardial biopsy is used less commonly. The typical cMRI finding is delayed enhancement which is thought to correspond to fibrotic bands found on histology. While conventional echocardiography has low sensitivity for detecting preclinical heart involvement in SSc, newer diagnostic modalities such as tissue Doppler echocardiography, cMRI, and nuclear imaging (single-photon emission CT) reveal a high prevalence of myocardial function or perfusion abnormalities. The serum levels of NT proBNP have utility as markers of primary cardiac involvement; however, this biomarker can also be elevated in other forms of SSc cardiac involvement and pulmonary hypertension.

## MUSCULOSKELETAL COMPLICATIONS

Musculoskeletal complications are very common in SSc. Carpal tunnel syndrome, generalized arthralgia, and stiffness are prominent in early disease and may be the presenting symptoms. The mobility of both small and large joints is progressively impaired, and fixed contractures develop at the proximal interphalangeal joints and wrists. Large joint contractures can be seen in dcSSc and, when they affect the knees or ankles, can lead to fixed contractures that interfere with walking. Tendon friction rubs in dcSSc are characterized by coarse leathery crepitation heard or palpated upon passive joint movement. Tendon rubs are due to extensive fibrosis and adhesion of the tendon sheaths and fascial planes at the affected joint and are associated with increased risk for renal crisis and progression of skin disease (Avouac et al., 2016). Synovitis can occur and is an independent predictor of skin progression (Avouac et al., 2016). Occasional SSc patients develop a seronegative erosive polyarthritis in the hands, and up to 10% have an overlap disease with seropositive rheumatoid arthritis. In SSc, detection of synovitis on physical examination can be hampered by the presence of puffy hands, and the swollen joint count has a poor interobserver reliability in this setting. Musculoskeletal ultrasound is therefore increasingly utilized for the diagnosis of SSc-related synovitis (Gordon et al., 2017). Muscle weakness is common in SSc, and multifactorial deconditioning, disuse atrophy, malnutrition, muscle inflammation, and fibrosis may all contribute. In patients with dcSSc, a chronic noninflammatory myopathy, characterized by atrophy and fibrosis with only mildly elevated muscle enzymes, may be commonly seen. Active inflammatory myositis with proximal muscle weakness and elevated muscle enzyme levels may also occur. Bone resorption is prominent in the terminal phalanges and can lead to loss of the distal tufts (acro-osteolysis). Resorption of the mandibular condyles can cause bite difficulties. Osteolysis may also affect the ribs and distal clavicles.

## LESS RECOGNIZED DISEASE MANIFESTATIONS

Dry eyes and dry mouth (sicca complex) are common complications of SSc. Biopsy of the minor salivary glands typically shows fibrosis rather than focal lymphocytic infiltration characteristic of primary Sjögren's syndrome. Hypothyroidism resulting from Hashimoto disease is common, particularly in lcSSc, and may be underrecognized. The central nervous system is generally not affected in patients with SSc, but unilateral or bilateral sensory trigeminal neuropathy has been recognized. Increased prevalence of peripheral neuropathy manifesting as loss of vibration sensation and/or inability to sense fine filaments has been reported (Frech et al., 2013). Erectile dysfunction is a frequent complication and may be an unrecognized initial manifestation of the disease. Inability to attain or maintain penile erection is caused by vascular insufficiency and fibrosis of corporal smooth muscle. Phosphodiesterase inhibitors have been safely used for the treatment of SSc-associated erectile dysfunction. Sexual performance is also adversely affected in women with SSc. While fertility does not appear to be significantly impaired in SSc, the rate of pregnancy complications is increased. In a prospective study of 99 pregnant patients with SSc, a higher frequency of preterm deliveries and intrauterine growth restrictions was reported. While disease remained stable in most of the SSc patients during pregnancy, four cases of disease progression within 1-year postpartum occurred, all in patients who were positive for ATA (Taraborelli et al., 2012).

## Cancer

Multiple epidemiologic studies indicate an increased cancer risk in patients with SSc. In particular, substantially increased risk of lung cancer (relative risk 4.3) and hematological malignancies (relative risk: 2.2) was confirmed in the metaanalysis (Bonifazi et al., 2013). Occasionally, cancer occurs in close temporal association with the onset of SSc, particularly in patients with RNAP antibodies. A close temporal relationship between disease onset and cancer diagnosis has also been recently described in SSc patients with rare anti-RNPC-3 antibodies with speckled immunofluorescence (Shah et al., 2017; Xu et al., 2016). In these contexts, SSc may represent a paraneoplastic syndrome that is triggered by the antitumor immune response.

## BIMARKERS AND AUTOANTIBODIES IN SYSTEMIC SCLEROSIS

SSc is associated with highly specific autoantibodies, which to date are the best biomarkers predictive of specific organ manifestations. SSc-specific autoantibodies tend to be mutually exclusive and highly specific (Arora-Singh et al., 2010; Steen 2005). However, multiple antibodies can be detected occurring in combination when determined by novel multiplex assays (Mehra et al., 2013). The relative frequency of the SSc-associated autoantibodies varies according to geographic regions and patient ethnicity.

The importance of SSc-autoantibodies in the clinical setting is underscored by the fact that presence of ACA, ATA, or anti-RNA polymerase III (RNAP) antibodies is included in the 2013 American College of Rheumatology/European League Against Rheumatism Classification Criteria (Table 31.1) (van den Hoogen et al., 2013).

Approximately 96% of the SSc patients have ANA. While ANA-negative SSc patients experience less vasculopathic complications (e.g., PAH, telangiectasia, ischemic digital ulcers, and pits) compared to ANA-positive SSc patients, they are more likely to have GI manifestations (Salazar et al., 2014).

Table 31.6 shows the frequency and prominent clinical associations of SSc-antibodies. ATA (also called anti-Scl-70) antibody is more common among Black and Asian patients, and is associated with diffuse cutaneous involvement and increased risk for progressive ILD (Steen, 2005; Nihtyanova et al., 2014; Assassi et al., 2010a; Wang et al., 2013; Steen et al., 2012). By contrast, ACA, more common in whites, is associated with limited cutaneous involvement, while clinically significant ILD is rare and survival rates are better. ACA is also associated with calcinosis and cooccurrence of biliary cirrhosis (Nihtyanova et al., 2014; Steen et al., 2012; Ioannidis et al., 2005; Assassi et al., 2009b). The frequency of anti-RNAP3 antibodies in SSc varies widely based on ethnicity and geographic region. With a prevalence of 15%–17%, it is more common in the United States, United Kingdom, and Australia (Nihtyanova et al., 2014; Nikpour et al., 2011; Nandiwada et al., 2016) than in the continental Europe (around 6%) (Sobanski et al., 2014), and appears to be rare in Asia (1%–4%) (Wang et al., 2013). RNAP is associated with diffuse cutaneous involvement and scleroderma renal crisis (odds ratios ranging from 5 to 17.5) (Nihtyanova et al., 2014; Nikpour et al., 2011; Nguyen et al., 2011) and GAVE (Ghrenassia et al., 2014). Clinically advanced ILD is rare in SSc patients with RNAP, while PAH was common in a large single-center study (Nihtyanova et al., 2014).

A higher prevalence of contemporaneous onset of cancer and SSc in patients with RNAP compared with the other two SSc-specific autoantibodies has been reported in five independent cohorts (Nikpour et al., 2011; Shah et al., 2010; Moinzadeh et al., 2014; Airo' et al., 2011; Lazzaroni et al., 2017). Levels of nucleolar RNA polymerase III were increased in tumor samples from SSc patients with RNAP but not in SSc patients with other SSc-specific autoantibodies (Shah et al., 2010). Moreover, alterations of the gene encoding polymerase III polypeptide A were found in the tumors from six out of eight SSc patients with RNAP, but none of eight RNAP-negative SSc patients, supporting a potential link between cancer-associated somatic mutations and an anticancer immune response driving disease in this serological subset (Joseph et al., 2014b).

Two less common SSc-related antibodies (anti-PM-Scl and anti-U3-RNP; also called antifibrillarin) with a nucleolar immunofluorescence pattern are increasingly recognized. PM-Scl antibody is associated with SSc overlap with other connective tissue diseases (particularly polymyositis-dermatomyositis), limited cutaneous involvement, calcinosis, and better survival (D'Aoust et al., 2014; Koschik et al., 2012). Anti-U3-RNP antibodies are found in about 5% of the White patients but are considerably more frequent among Black (18%–27%) and Native North American patients (Mejia Otero et al., 2017; Aggarwal et al., 2009). These antibodies are associated with PAH and increased mortality (Nihtyanova et al., 2014; Mejia Otero et al., 2017; Aggarwal et al., 2009).

The specificity and clinical utility of other SSc biomarkers remain less well-established. Elevation in the levels of C-reactive protein (CRP) in SSc was associated with more severe skin and lung involvement in two independent cohorts. More importantly, higher CRP was associated with faster ILD progression and shorter survival (Muangchan et al., 2012; Liu et al., 2013a). In a recent randomized trial of the anti-IL-6 agent tocilizumab, high CRP at baseline was used as a strategy to enrich the study population for patients with progressive disease (Khanna et al., 2016). There are contradicting results on the utility of serum proteins as markers of SSc-associated ILD. In a recent Japanese study, the elevated baseline serum levels of Krebs von den Lungen-6 (KL-6) in SSc were predictive of end-stage ILD (Kuwana et al., 2016). Other studies have identified the chemokine CXCL4, and serum amyloid A, as potential biomarkers for SSc-associated ILD (Lakota et al., 2015; van et al., 2013; Volkmann et al., 2016).

SSc is associated with distinct transcriptome profiles in both blood cells and tissue biopsies (Tan et al., 2006; Whitfield et al., 2003). A type 1 IFN gene expression profile in peripheral blood cells in SSc is similar to that noted in patients with SLE and correlates with the severity of skin, lung, and muscle involvement (Assassi et al., 2010b; Liu et al., 2013b). Furthermore, it has been suggested that SSc patients can be categorized on the basis of their skin gene expression profile, including a subgroup with a prominent inflammatory gene signature (Assassi et al., 2015; Milano et al., 2008). Emerging evidence suggests that SSc patients in this gene expression subgroup might have a better response to immunosuppression (Hinchcliff et al., 2013). Sufficiently powered longitudinal studies will be needed to determine whether tissue-specific transcriptome signatures robustly define specific SSc subsets and predict therapeutic responses and whether they contribute to clinical decision-making and selection of focused and effective treatments in a precision medicine approach (Varga and Hinchcliff, 2014).

## SCREENING AND FOLLOW-UP EVALUATION

Once the diagnosis of SSc is established, patients undergo initial screening for the presence of internal organ involvement. Screening includes Doppler echocardiography, chest HRCT, and PFT to detect evidence of cardiopulmonary involvement. In patients with SSc, HRCT is a sensitive method to detect early signs of ILD, even in patients with normal lung function, and is generally obtained at the initial visit. Bronchoalveolar lavage and lung biopsy are generally not required to establish the diagnosis of SSc-ILD but might be indicated in some cases of SSc to rule out infection or cancer in the setting of atypical imaging or clinical symptomatology. Basic metabolic panel and urinalysis can aid in identifying renal involvement, while muscle enzymes screen for myopathy or inflammatory myositis. Determination of ANA and SSc-related autoantibody profiles provides valuable prognostic information. The extent of skin involvement is typically assessed using the modified Rodnan Skin Score, a validated clinical outcome measure to assess skin thickness (distinct from skin tethering) in 17 distinct body surface areas assigned a score on a scale 0–3 (0 = normal skin; 1 = mild thickness; 2 = moderate thickness; 3 = severe thickness with inability to pinch the skin into a fold) (Furst et al., 2007; Furst et al., 1998). The modified Rodnan Skin Score is the sum of scores in the 17 assessed body areas.

Repeated assessment of the skin score during follow-up visits (optimally performed by the same examiner) can establish the temporal trajectory of skin involvement. PFT and echocardiographic assessments are generally performed on a regular (yearly) basis to monitor for development or progression of cardiopulmonary involvement. The FVC% predicted is a validated outcome measure for SSc-ILD and can be followed longitudinally to assess the course of ILD in this setting (Furst et al., 2007). The  $D_{LCO}$  is also decreased in ILD, but a decrease that is disproportionate (compared to  $D_{LCO}$ ) might indicate pulmonary vascular involvement. While the extent of radiologic lung fibrosis on HRCT has prognostic value for disease course and mortality (Goh et al., 2008), HRCT imaging is not repeated routinely due to concerns regarding radiation exposure. Repeat HRCT may be indicated in SSc if an unexplained decline in lung volumes on PFT, worsening dyspnea, or other respiratory symptoms occurs.

In light of the poor prognosis of untreated PAH, and better therapeutic response in early-stage disease, all SSc patients should be screened for PAH at initial evaluation, followed by serial reevaluation performed annually. Doppler echocardiography is an informative and widely used tool for PAH screening. Estimated pulmonary artery systolic pressure >40 mmHg at rest or tricuspid regurgitation jet velocities >3 m/s on echocardiography suggest the presence of PAH. However, as these findings are not specific, suspected PAH needs to be verified by hemodynamic measurements determined by right-heart catheterization. Right-heart catheterization is also necessary for excluding alternate causes of pulmonary hypertension such as diastolic dysfunction.

A multicenter cross-sectional study of 466 at-risk patients (disease duration >3 years and  $D_{LCO} < 60\%$ ) aimed at optimizing PAH screening strategies. In 19% of the patients, PAH was confirmed by right-heart catheterization. A two-step approach was proposed for determining which SSc patients should undergo right-heart catheterization. In step 1, a prediction score based on the presence of ACA, telangiectasia, right axis deviation on EKG, the  $FVC\% / D_{LCO}\%$  ratio, and elevated levels of serum urate and NT proBNP was developed for a referral to echocardiography. In step 2, a prediction score based on the score from step 1 and the presence of two echocardiographic variables (right atrium area and tricuspid regurgitation velocity) is used for proceeding to right-heart catheterization. This two-step algorithm had a sensitivity of 96% and specificity of 48% for detecting PAH in SSc patients. Interestingly, dyspnea and physical findings related to the right heart and WHO functional class were insufficiently predictive of PAH (Coghlan et al., 2014).

## MANAGEMENT OF SYSTEMIC SCLEROSIS

### General Principles

Although no therapy to date has been shown to significantly alter the natural history of SSc, selected interventions can be highly effective in alleviating specific symptoms, slowing progressive accrual of organ damage, and mitigating disability. In light of the marked patient-to-patient heterogeneity in SSc manifestations and natural history, a thoughtful “precision medicine” approach that is tailored specifically to each individual’s unique needs is recommended.

Our approach to the patient with SSc is guided by certain general principles: accurate and timely diagnosis and risk stratification based on clinical, laboratory, and serological evaluation at baseline; prompt recognition and staging of organ-based complications and assessment of their likelihood of progression; regular monitoring for new complications; and adjusting therapy. To reduce the risk of irreversible organ damage, we advocate a proactive approach with regular screening and initiation of appropriate intervention at the earliest possible opportunity. In light of the complex multisystemic nature of the SSc, a coordinated multispecialty team approach that integrates all appropriate specialists should be pursued. Treatment regimens typically include combinations of therapies that impact different aspects of the disease.

### Disease-Modifying Immunomodulatory Therapy

Immunosuppressive agents that are effective in other rheumatic and autoimmune diseases have generally shown only modest or no benefit in SSc. As an example, glucocorticoids reduce stiffness and aching in early-stage dcSSc, but fail to influence disease progression, and their use is associated with an increased risk of scleroderma renal crisis.

Both oral and intravenously administered cyclophosphamides were shown to slow progression of SSc-associated ILD, with stabilization and modest improvement of respiratory symptoms, pulmonary

function, HRCT findings, and skin induration. These benefits must be carefully balanced against drug-induced bone marrow suppression, opportunistic infections, hemorrhagic cystitis and bladder cancer, premature ovarian failure, and late secondary malignancies.

Mycophenolate mofetil was shown to ameliorate skin induration and ILD and is generally well tolerated in SSc (Tashkin et al., 2016). Tocilizumab, a monoclonal antibody directed against the IL-6 receptor, also showed modest benefit in randomized SSc clinical trials (Khanna et al., 2016). In addition, both open-label studies and small trials support the use of rituximab, a monoclonal antibody directed against the mature B-cell marker CD20. Randomized clinical trials in SSc evaluating the efficacy of abatacept, a fusion protein that inhibits T-cell costimulation, are currently underway. Extracorporeal photochemotherapy (photopheresis) has been shown to have modest clinical efficacy in ameliorating skin involvement in SSc, although the underlying mechanism of action remains largely unknown. Ablation of the immune system with high-dose chemotherapy followed by autologous hematopoietic stem-cell reconstitution (bone marrow transplantation) was associated with durable remission and improved long-term survival in several randomized clinical trials (van Laar et al., 2014). This form of immunomodulatory therapy carries morbidity and even potential treatment-related mortality, as well as significant cost, and is therefore still reserved for carefully selected patients. While cyclosporine, azathioprine, plaquenil, thalidomide, and rapamycin are often used in the treatment of SSc, their efficacy in this setting is currently inadequately supported by the literature.

### Therapy Targeting Fibrosis

Because tissue fibrosis underlies progressive multorgan damage in SSc, drugs that interfere with the fibrotic process or speed its resolution represent rational therapeutic options (Distler et al., 2017). Recent studies show modest benefit of two novel drugs, pirfenidone and the multikinase inhibitor nintedanib, in patients with idiopathic pulmonary fibrosis. Whether these

*(cont'd)*

antifibrotic drugs have comparable efficacy and tolerability in SSc-associated ILD and other manifestations of the disease, is currently under investigation.

### Vascular Therapy

The goal of vascular therapy is to reduce the frequency, duration, and severity of vasospastic episodes, enhance ischemic ulcer healing, prevent critical ischemic episodes, and slow the progression of generalized obliterative vasculopathy. Dihydropyridine calcium channel blockers (such as amlodipine and diltiazem) are effective in ameliorating Raynaud's, but their use can be limited by palpitations, dependent edema, and worsening gastroesophageal reflux. While widely used for treating Raynaud's, ACE inhibitors do not appear to reduce the frequency or severity of episodes. In contrast, angiotensin II receptor blockers such as losartan are effective and well tolerated in this setting. Patients unresponsive to these therapies may require the addition of 5-phosphodiesterase inhibitors (e.g., sildenafil), topical nitroglycerine, and in severe cases, intermittent IV infusions of prostaglandins such as treprostinil. Low-dose aspirin and dipyridamole inhibit platelet aggregation and may have a role as adjunctive agents. Patients with non-healing ischemic digital ulcerations may respond to the endothelin-1 receptor antagonists, digital sympathectomy, and intradigital injections of botulinum (Botox) or nerve block. Empirical long-term therapy with statins and antioxidants may retard microvascular damage and obliteration, but their efficacy in SSc has not been demonstrated. While there is limited evidence-based information regarding the treatment of cardiac complications of SSc, nondihydropyridine calcium channel blockers are often used for atrial arrhythmias, and nonselective alpha/beta-blockers such as carvediol for improving myocardial perfusion and left ventricular systolic function.

### Treatment of Gastrointestinal Complications

Common SSc-associated oral problems include reduced saliva production, and decreased oral aperture, gum recession, periodontal disease, and teeth loss. Gastroesophageal reflux may occur in the absence of symptoms. Patients should be instructed to elevate the head of the bed, eat frequent small meals, and avoid known reflux exacerbants such as alcohol, caffeine, or bedtime meals. Proton pump inhibitors are commonly used, sometimes at relatively high doses. Prokinetic agents such as metoclopramide, erythromycin (a motilin agonist), domperidone, and prucalopride may be helpful but are frequently associated with side effects. Surgical antireflux procedures such as Nissen fundoplication can result in secondary achalasia

and generally should be avoided in patients with SSc. Recurrent episodic bleeding from GAVE may be controlled with endoscopic ablation using laser or argon plasma photocoagulation, although recurrences are common. When GI dysmotility is advanced, enteral feeding and decompression via percutaneous gastrostomy or jejunostomy might be required. Bacterial overgrowth secondary to dysmotility of the small bowel causes bloating and diarrhea and may lead to malabsorption and severe malnutrition. In such cases, repeated short courses of rotating broad-spectrum antibiotics such as metronidazole, erythromycin, and rifaximin can be effective by eradicating bacterial overgrowth. Fecal incontinence may respond to antidiarrheal medication, biofeedback therapy, sphincter augmentation, and sacral neuromodulation.

### Treatment of Interstitial Lung Disease

While ILD is a very frequent SSc complication and is a leading cause of death, its course is unpredictable. Some patients with SSc-ILD show little progression of lung disease over time. It is therefore important to identify patients at high risk for ILD deterioration in the absence of treatment. Treatment with cyclophosphamide, given IV or orally for 6–12 months and in combination with low-dose prednisone, or mycophenolate mofetil for up to 2 years, have been shown to slow declining lung function and improve respiratory symptoms (Tashkin et al., 2016). The tolerability and efficacy of antifibrotic agents recently approved for idiopathic pulmonary fibrosis, alone or in combination with immunomodulatory therapies, are currently under investigation for SSc-associated ILD. Carefully screened patients who show continued progression of ILD despite maximal medical therapy might be candidates for lung transplantation. Recurrence of ILD in the lung allograft in SSc patients has not been reported.

### Management of Renal Crisis

The outcome of scleroderma renal crisis has improved considerably since the introduction of ACE inhibitors. Outcomes are largely determined by the extent of renal damage at initiation of aggressive antihypertensive therapy, and prompt recognition of impending or early scleroderma renal crisis is therefore paramount (Woodworth et al., 2016). SSc patients deemed at high risk for renal crisis (early-stage disease, progressive skin involvement, palpable tendon friction rubs, and positive anti-RNA polymerase III antibodies) should monitor their blood pressure daily and promptly report significant alterations. Glucocorticoids should be used sparingly if at all. Patients

(cont'd)

presenting with scleroderma renal crisis should be hospitalized and started on short-acting ACE inhibitors such as captopril. In cases of persistent hypertension, addition of calcium channel blockers, angiotensin II receptor blockers, endothelin-1 receptor blockers, prostacyclins, direct renin inhibitors, and complement pathway inhibitors such as eculizumab could be considered, although so far the evidence for their efficacy comes mostly from case reports. While up to two-thirds of patients with scleroderma renal crisis necessitate dialysis, substantial renal recovery can occur for up to 2 years, and 50% ultimately discontinue dialysis. Outcomes for SSc patients undergoing kidney transplantation for renal crisis are comparable to other indications, and recurrence is rare.

### Treatment of Pulmonary Arterial Hypertension

Despite considerable recent advances in management, PAH still carries a poor prognosis in SSc and accounts

for 30% of the deaths. Treatment is generally started with an oral endothelin-1 receptor antagonist such as bosentan or a phosphodiesterase 5 inhibitor such as sildenafil alone or in combination. Recently, the soluble guanylate cyclase stimulator riociguat, which acts via nitric oxide, and the selective prostacyclin receptor agonist selexipag, were shown to improve PAH symptoms and survival. The prostacyclin analogs epoprostenol or treprostинil can be given by continuous IV or SC infusion, or via intermittent nebulized inhalations. Studies demonstrate that early combination therapy in SSc-PAH with different classes of drugs acting additively or synergistically, is associated with improved outcomes. Lung transplantation remains an option for SSc patients who fail medical therapy for PAH, with survival rates (64% at 2 years) comparable to those of patients transplanted for idiopathic ILD or PAH.

## NATURAL HISTORY AND PROGNOSIS

The natural history of SSc is highly variable and not well predictable. Patient with dcSSc characteristically develop organ complications early and tend to follow a more rapidly progressive course than those with lcSSc. Inflammatory symptoms, such as edema, aching, and pruritus, dominate early-stage disease but generally subside and skin induration reaches a plateau after 2–4 years. However, it is during the early disease stage that life-threatening visceral organ involvement develops. For instance, scleroderma renal crisis almost invariably occurs within the first 4 years of disease. In late-stage disease (>6 years), the skin is usually thin and atrophic, except on the fingers (sclerodactyly). Relapse, progression, or recurrence of skin thickening after the plateau stage has been reached is uncommon. Patients with lcSSc tend to follow a trajectory that is markedly different from dcSSc. In these patients, Raynaud's phenomenon characteristically precedes other disease manifestations by years or even decades. Skin involvement is limited and shows little progression, while visceral organ complications such as PAH and biliary cirrhosis generally develop late and tend to follow an indolent course.

The risk of premature death is substantially increased in all forms of SSc, and age- and gender-adjusted mortality rates are eightfold higher compared to the general population. More than 50% of the SSc patients will die from their disease. In dcSSc, 5- and 10-year survival rates are 70% and 55%, respectively, whereas in lcSSc, 5- and 10-year survival rates are 90% and 75%, respectively. Predictors of poor prognosis include male gender, African American race, older age at onset of disease, extensive skin thickening with truncal involvement, palpable tendon friction rubs, and progressive visceral organ involvement. Laboratory predictors at baseline evaluation include an elevated ESR, anemia, proteinuria, and ATA antibodies. In one study, SSc patients with extensive skin involvement, vital capacity <55% predicted, significant GI involvement (pseudo-obstruction or malabsorption), clinical cardiac involvement, or scleroderma renal crisis had a 9-year survival <40%. Nevertheless, survival rates are improving. For instance, overall 10-year survival rates have increased from <60% in the 1970s to >66%–78% in the 1990s. This positive trend, which is likely reflecting both earlier diagnosis and detection of complications, and better management, is expected to continue.

## References

- Aggarwal, R., Lucas, M., Fertig, N., Oddis, C.V., Medsger Jr., T.A., 2009. Anti-U3 RNP autoantibodies in systemic sclerosis. *Arthritis Rheum.* 60 (4), 1112–1118. Available from: <https://doi.org/10.1002/art.24409>.
- Airo', P., Ceribelli, A., Cavazzana, I., Taraborelli, M., Zingarelli, S., Franceschini, F., 2011. Malignancies in Italian patients with systemic sclerosis positive for anti-RNA polymerase III antibodies. *J. Rheumatol.* 38 (7), 1329–1334. Available from: <https://doi.org/10.3899/jrheum.101144> [pii];10.3899/jrheum.101144.
- Allanore, Y., Saad, M., Dieude, P., Avouac, J., Distler, J.H., Amouyel, P., et al., 2011. Genome-wide scan identifies TNIP1, PSORS1C1, and RHOB as novel risk loci for systemic sclerosis. *PLoS Genet.* 7 (7), e1002091. Available from: <https://doi.org/10.1371/journal.pgen.1002091>. PGENETICS-D-10-00215[pii].
- Allanore, Y., Simms, R., Distler, O., Trojanowska, M., Pope, J., Denton, C.P., et al., 2015. Systemic sclerosis. *Nat. Rev. Dis. Primers* 1. Available from: <https://doi.org/10.1038/nrdp.2015.2>. PubMed PMID: 27189141. 15002. Epub 2015/01/01.
- Allcock, R.J., Forrest, I., Corris, P.A., Crook, P.R., Griffiths, I.D., 2004. A study of the prevalence of systemic sclerosis in northeast England. *Rheumatology (Oxford)* 43 (5), 596–602. Available from: <https://doi.org/10.1093/rheumatology/keh124>. PubMed PMID: 14872101.
- Arismendi, M., Giraud, M., Ruzehaji, N., Dieude, P., Koumakis, E., Ruiz, B., et al., 2015. Identification of NF-kappaB and PLCL2 as new susceptibility genes and highlights on a potential role of IRF8 through interferon signature modulation in systemic sclerosis. *Arthritis Res. Ther.* 17, 71. Available from: <https://doi.org/10.1186/s13075-015-0572-y>. PubMed PMID: 25880423; PMCID: PMC4422604. Epub 2015/04/17.
- Arnett, F.C., Cho, M., Chatterjee, S., Aguilar, M.B., Reveille, J.D., Mayes, M.D., 2001. Familial occurrence frequencies and relative risks for systemic sclerosis (scleroderma) in three United States cohorts. *Arthritis Rheum.* 44 (6), 1359–1362.
- Arnett, F.C., Gourh, P., Shete, S., Ahn, C.W., Honey, R., Agarwal, S.K., et al., 2010. Major Histocompatibility Complex (MHC) class II alleles, haplotypes, and epitopes which confer susceptibility or protection in the fibrosing autoimmune disease systemic sclerosis: analyses in 1300 Caucasian, African-American and Hispanic cases and 1000 controls. *Ann. Rheum. Dis.* 69 (5), 822–827.
- Arora-Singh, R.K., Assassi, S., del Junco, D.J., Arnett, F.C., Perry, M., Irfan, U., et al., 2010. Autoimmune diseases and autoantibodies in the first degree relatives of patients with systemic sclerosis. *J. Autoimmun.* 35 (1), 52–57.
- Assassi, S., Arnett, F.C., Reveille, J.D., Gourh, P., Mayes, M.D., 2007. Clinical, immunologic, and genetic features of familial systemic sclerosis. *Arthritis Rheum.* 56 (6), 2031–2037.
- Assassi, S., Fritzler, M.J., Arnett, F.C., Norman, G.L., Shah, K.R., Gourh, P., et al., 2009a. Primary biliary cirrhosis (PBC), PBC autoantibodies, and hepatic parameter abnormalities in a large population of systemic sclerosis patients. *J. Rheumatol.* 36 (10), 2250–2256.
- Assassi, S., Del, J.D., Sutter, K., McNearney, T.A., Reveille, J.D., Karnavas, A., et al., 2009b. Clinical and genetic factors predictive of mortality in early systemic sclerosis. *Arthritis Rheum.* 61 (10), 1403–1411.
- Assassi, S., Sharif, R., Lasky, R.E., McNearney, T.A., Estrada, Y.M.R., Draeger, H.T., et al., 2010a. Predictors of interstitial lung disease in early systemic sclerosis: a prospective longitudinal study of the GENIOS cohort. *Arthritis Res. Ther.* 12 (5), R166.
- Assassi, S., Mayes, M.D., Arnett, F.C., Gourh, P., Agarwal, S.K., McNearney, T.A., et al., 2010b. Systemic sclerosis and lupus: points in an interferon-mediated continuum. *Arthritis Rheum.* 62 (2), 589–598.
- Assassi, S., Leyva, A.L., Mayes, M.D., Sharif, R., Nair, D.K., Fischbach, M., et al., 2011. Predictors of fatigue severity in early systemic sclerosis: a prospective longitudinal study of the GENIOS cohort. *PLoS One* 6 (10), e26061. Available from: <https://doi.org/10.1371/journal.pone.0026061>. PONE-D-11-06905[pii].
- Assassi, S., Swindell, W.R., Wu, M., Tan, F.D., Khanna, D., Furst, D.E., et al., 2015. Dissecting the heterogeneity of skin gene expression patterns in systemic sclerosis. *Arthritis Rheumatol.* 67 (11), 3016–3026. Available from: <https://doi.org/10.1002/art.39289>. PubMed PMID: 26238292; PMCID: PMC5394431.
- Avouac, J., Walker, U.A., Hachulla, E., Riemarken, G., Cuomo, G., Carreira, P.E., et al., 2016. Joint and tendon involvement predict disease progression in systemic sclerosis: a EUSTAR prospective study. *Ann. Rheum. Dis.* 75 (1), 103–109. Available from: <https://doi.org/10.1136/annrheumdis-2014-205295>. PubMed PMID: 25165035. Epub 2014/08/29.
- Bernatsky, S., Joseph, L., Pineau, C.A., Belisle, P., Hudson, M., Clarke, A.E., 2009. Scleroderma prevalence: demographic variations in a population-based sample. *Arthritis Rheum.* 61 (3), 400–404.
- Beyer, C., Schett, G., Distler, O., Distler, J.H., 2010. Animal models of systemic sclerosis: prospects and limitations. *Arthritis Rheum.* 62 (10), 2831–2844. Available from: <https://doi.org/10.1002/art.27647>. PubMed PMID: 20617524. Epub 2010/07/10.
- Bhattacharyya, S., Wei, J., Varga, J., 2011. Understanding fibrosis in systemic sclerosis: shifting paradigms, emerging opportunities. *Nat. Rev. Rheumatol.* 8 (1), 42–54. Available from: <https://doi.org/10.1038/nrrheum.2011.149>. PubMed PMID: 22025123; PMCID: PMC3954787. Epub 2011/10/26.
- Bhattacharyya, S., Wang, W., Graham, L.V., Varga, J., 2016. A20 suppresses canonical Smad-dependent fibroblast activation: novel function for an endogenous inflammatory modulator. *Arthritis Res. Ther.* 18 (1), 216. Available from: <https://doi.org/10.1186/s13075-016-1118-7>. PubMed PMID: 27716397; PMCID: PMC5048449. Epub 2016/10/08.
- Bianchi, D.W., Zickwolf, G.K., Weil, G.J., Sylvester, S., DeMaria, M.A., 1996. Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. *Proc. Natl. Acad. Sci. U.S.A.* 93 (2), 705–708. PubMed PMID: 8570620; PMCID: PMC40117.
- Bonifazi, M., Tramacere, I., Pomponio, G., Gabrielli, B., Avvedimento, E.V., La Vecchia, C., et al., 2013. Systemic sclerosis (scleroderma) and cancer risk: systematic review and meta-analysis of observational studies. *Rheumatology (Oxford)* 52 (1), 143–154. Available from: <https://doi.org/10.1093/rheumatology/kes303>. PubMed PMID: 23175568. Epub 2012/11/24.
- Bossini-Castillo, L., Martin, J.E., Broen, J., Gorlova, O., Simeon, C.P., Beretta, L., et al., 2012. A GWAS follow-up study reveals the association of the IL12RB2 gene with systemic sclerosis in Caucasian populations. *Hum. Mol. Genet.* 21 (4), 926–933. Available from: <https://doi.org/10.1093/hmg/ddr522> [pii];10.1093/hmg/ddr522.
- Bossini-Castillo, L., Martin, J.E., Broen, J., Simeon, C.P., Beretta, L., Gorlova, O.Y., et al., 2013. Confirmation of TNIP1 but not RHOB and PSORS1C1 as systemic sclerosis risk factors in a large independent replication study. *Ann. Rheum. Dis.* 72 (4), 602–607. Available from: <https://doi.org/10.1136/annrheumdis-2012-201888>. PubMed PMID: 22896740; PMCID: PMC3887516. Epub 2012/08/17.

- Broen, J.C., Wolvers-Tettero, I.L., Geurts-van Bon, L., Vonk, M.C., Coenen, M.J., Lafyatis, R., et al., 2010. Skewed X chromosomal inactivation impacts T regulatory cell function in systemic sclerosis. *Ann. Rheum. Dis.* 69 (12), 2213–2216. Available from: <https://doi.org/10.1136/ard.2010.129999>. PubMed PMID: 20699236. Epub 2010/08/12.
- Carmona, F.D., Gutala, R., Simeon, C.P., Carreira, P., Ortego-Centeno, N., Vicente-Rabaneda, E., et al., 2012. Novel identification of the IRF7 region as an anticentromere autoantibody propensity locus in systemic sclerosis. *Ann. Rheum. Dis.* 71 (1), 114–119. Available from: <https://doi.org/10.1136/annrheumdis-2011-200275> [pii];10.1136/annrheumdis-2011-200275.
- Carmona, F.D., Cenit, M.C., Diaz-Gallo, L.M., Broen, J.C., Simeon, C.P., Carreira, P.E., et al., 2013. New insight on the Xq28 association with systemic sclerosis. *Ann. Rheum. Dis.* Available from: <https://doi.org/10.1136/annrheumdis-2012-202742> [pii];10.1136/annrheumdis-2012-202742.
- Chifflot, H., Fautrel, B., Sordet, C., Chatelus, E., Sibilia, J., 2008. Incidence and prevalence of systemic sclerosis: a systematic literature review. *Semin. Arthritis Rheum.* 37 (4), 223–235. Available from: <https://doi.org/10.1016/j.semarthrit.2007.05.003>. PubMed PMID: 17692364.
- Chung, L., Domsic, R.T., Lingala, B., Alkassab, F., Bolster, M., Csuka, M.E., et al., 2014a. Survival and predictors of mortality in systemic sclerosis-associated pulmonary arterial hypertension: outcomes from the pulmonary hypertension assessment and recognition of outcomes in scleroderma registry. *Arthritis Care Res. (Hoboken)* 66 (3), 489–495. Available from: <https://doi.org/10.1002/acr.22121>. PubMed PMID: 23983198. Epub 2013/08/29.
- Chung, L., Farber, H.W., Benza, R., Miller, D.P., Parsons, L., Hassoun, P.M., et al., 2014b. Unique predictors of mortality in patients with pulmonary arterial hypertension associated with systemic sclerosis in the REVEAL registry. *Chest* 146 (6), 1494–1504. Available from: <https://doi.org/10.1378/chest.13-3014>. PubMed PMID: 24992469; PMCID: PMC4251613. Epub 2014/07/06.
- Cockrill, T., del Junco, D.J., Arnett, F.C., Assassi, S., Tan, F.K., McNearney, T., et al., 2010. Separate influences of birth order and gravidity/parturition on the development of systemic sclerosis. *Arthritis Care Res. (Hoboken)* 62 (3), 418–424. Available from: <https://doi.org/10.1002/acr.20096>. PubMed PMID: 20391489; PMCID: PMC2876718.
- Coghlan, J.G., Denton, C.P., Grunig, E., Bonderman, D., Distler, O., Khanna, D., et al., 2014. Evidence-based detection of pulmonary arterial hypertension in systemic sclerosis: the DETECT study. *Ann. Rheum. Dis.* 73 (7), 1340–1349. Available from: <https://doi.org/10.1136/annrheumdis-2013-203301> [pii];10.1136/annrheumdis-2013-203301.
- Coral-Alvarado, P., Pardo, A.L., Castano-Rodriguez, N., Rojas-Villarraga, A., Anaya, J.M., 2009. Systemic sclerosis: a world wide global analysis. *Clin. Rheumatol.* 28 (7), 757–765. Available from: <https://doi.org/10.1007/s10067-009-1144-9>. PubMed PMID: 19277816.
- Coustet, B., Bouaziz, M., Dieude, P., Guedj, M., Bossini-Castillo, L., Agarwal, S., et al., 2012. Independent replication and metaanalysis of association studies establish TNFSF4 as a susceptibility gene preferentially associated with the subset of anticentromere-positive patients with systemic sclerosis. *J. Rheumatol.* 39 (5), 997–1003. Available from: <https://doi.org/10.3899/jrheum.111270> [pii];10.3899/jrheum.111270.
- D'Aoust, J., Hudson, M., Tatibouet, S., Wick, J., Mahler, M., Baron, M., et al., 2014. Clinical and serologic correlates of anti-PM/Scl antibodies in systemic sclerosis: a multicenter study of 763 patients. *Arthritis Rheumatol.* 66 (6), 1608–1615. Available from: <https://doi.org/10.1002/art.38428>.
- Dieude, P., Guedj, M., Wipff, J., Avouac, J., Fajardy, I., Diot, E., et al., 2009a. Association between the IRF5 rs2004640 functional polymorphism and systemic sclerosis: a new perspective for pulmonary fibrosis. *Arthritis Rheum.* 60 (1), 225–233.
- Dieude, P., Wipff, J., Guedj, M., Ruiz, B., Melchers, I., Hachulla, E., et al., 2009b. BANK1 is a genetic risk factor for diffuse cutaneous systemic sclerosis and has additive effects with IRF5 and STAT4. *Arthritis Rheum.* 60 (11), 3447–3454. Available from: <https://doi.org/10.1002/art.24885>.
- Dieude, P., Bouaziz, M., Guedj, M., Riemekasten, G., Airo, P., Muller, M., et al., 2011. Evidence of the contribution of the X chromosome to systemic sclerosis susceptibility: association with the functional IRAK1 196Phe/532Ser haplotype. *Arthritis Rheum.* 63 (12), 3979–3987. Available from: <https://doi.org/10.1002/art.30640>.
- Distler, J.H., Feghali-Bostwick, C., Soare, A., Asano, Y., Distler, O., Abraham, D.J., 2017. Review: frontiers of antifibrotic therapy in systemic sclerosis. *Arthritis Rheumatol.* 69 (2), 257–267. Available from: <https://doi.org/10.1002/art.39865>. PubMed PMID: 27636741. Epub 2016/09/17.
- Draeger, H.T., Assassi, S., Sharif, R., Gonzalez, E.B., Harper, B.E., Arnett, F.C., et al., 2013. Right bundle branch block: a predictor of mortality in early systemic sclerosis. *PLoS One* 8 (10), e78808. Available from: <https://doi.org/10.1371/journal.pone.0078808>. PONE-D-13-20243[pii].
- El Agha, E., Kramann, R., Schneider, R.K., Li, X., Seeger, W., Humphreys, B.D., et al., 2017. Mesenchymal stem cells in fibrotic disease. *Cell Stem Cell* 21 (2), 166–177. Available from: <https://doi.org/10.1016/j.stem.2017.07.011>. PubMed PMID: 28777943. Epub 2017/08/05.
- El-Baalbaki, G., Razikov, I., Hudson, M., Bassel, M., Baron, M., Thombs, B.D., et al., 2010. Association of pruritus with quality of life and disability in systemic sclerosis. *Arthritis Care Res. (Hoboken)* 62 (10), 1489–1495. Available from: <https://doi.org/10.1002/acr.20257>. PubMed PMID: 20506531. Epub 2010/05/28.
- Elhai, M., Meune, C., Avouac, J., Kahan, A., Allanore, Y., 2011. Trends in mortality in patients with systemic sclerosis over 40 years: a systematic review and meta-analysis of cohort studies. *Rheumatology (Oxford)* 51 (6), 1017–1026. Available from: <https://doi.org/10.1093/rheumatology/ker269> [pii];10.1093/rheumatology/ker269.
- Elhai, M., Avouac, J., Hoffmann-Vold, A.M., Ruzejhaji, N., Amiar, O., Ruiz, B., et al., 2016. OX40L blockade protects against inflammation-driven fibrosis. *Proc. Natl. Acad. Sci. U.S.A.* 113 (27), E3901–E3910. Available from: <https://doi.org/10.1073/pnas.1523512113>. PubMed PMID: 27298374; PMCID: PMC4941508. Epub 2016/06/15.
- Feghali-Bostwick, C., Medsger Jr., T.A., Wright, T.M., 2003. Analysis of systemic sclerosis in twins reveals low concordance for disease and high concordance for the presence of antinuclear antibodies. *Arthritis Rheum.* 48 (7), 1956–1963.
- Ferri, C., Valentini, G., Cozzi, F., Sebastiani, M., Michelassi, C., La, M.G., et al., 2002. Systemic sclerosis: demographic, clinical, and serologic features and survival in 1,012 Italian patients. *Medicine (Baltimore)* 81 (2), 139–153.
- Frech, T.M., Smith, G., Reilly, M., Chamberlain, J., Murtaugh, M.A., Penrod, J., et al., 2013. Peripheral neuropathy: a complication of systemic sclerosis. *Clin. Rheumatol.* 32 (6), 885–888. Available from: <https://doi.org/10.1007/s10067-013-2206-6>. PubMed PMID: 23404236. Epub 2013/02/14.

- Furst, D.E., Clements, P.J., Steen, V.D., Medsger Jr, T.A., Masi, A.T., D'Angelo, W.A., et al., 1998. The modified Rodnan skin score is an accurate reflection of skin biopsy thickness in systemic sclerosis. *J. Rheumatol.* 25 (1), 84–88.
- Furst, D., Khanna, D., Matucci-Cerinic, M., Clements, P., Steen, V., Pope, J., et al., 2007. Systemic sclerosis—continuing progress in developing clinical measures of response. *J. Rheumatol.* 34 (5), 1194–1200.
- Gabrielli, A., Avvedimento, E.V., Krieg, T., 2009. Scleroderma. *N Engl J Med* 360 (19), 1989–2003. Available from: <https://doi.org/10.1056/NEJMra0806188>. PubMed PMID: 19420368. Epub 2009/05/08.
- Garner, R., Kumari, R., Lanyon, P., Doherty, M., Zhang, W., 2015. Prevalence, risk factors and associations of primary Raynaud's phenomenon: systematic review and meta-analysis of observational studies. *BMJ Open* 5 (3), e006389. Available from: <https://doi.org/10.1136/bmjopen-2014-006389>. PubMed PMID: 25776043; PMCID: PMC4368987. Epub 2015/03/18.
- Geirsson, A.J., Steinsson, K., Guthmundsson, S., Sigurthsson, V., 1994. Systemic sclerosis in Iceland. A nationwide epidemiological study. *Ann. Rheum. Dis.* 53 (8), 502–505. PubMed PMID: 7944633; PMCID: PMC1005388.
- Ghossein, C., Varga, J., Fenves, A.Z., 2016. Recent developments in the classification, evaluation, pathophysiology, and management of scleroderma renal crisis. *Curr. Rheumatol. Rep.* 18 (1), 5. Available from: <https://doi.org/10.1007/s11926-015-0551-y>. PubMed PMID: 26711696. Epub 2015/12/30.
- Ghrenassia, E., Avouac, J., Khanna, D., Derk, C.T., Distler, O., Suliman, Y.A., et al., 2014. Prevalence, correlates and outcomes of gastric antral vascular ectasia in systemic sclerosis: a EUSTAR case-control study. *J. Rheumatol.* 41 (1), 99–105. Available from: <https://doi.org/10.3899/jrheum.130386> [pii];10.3899/jrheum.130386.
- Goh, N.S., Desai, S.R., Veeraraghavan, S., Hansell, D.M., Copley, S.J., Maher, T.M., et al., 2008. Interstitial lung disease in systemic sclerosis: a simple staging system. *Am. J. Respir. Crit. Care Med.* 177 (11), 1248–1254.
- Gordon, J.K., Girish, G., Berrocal, V.J., Zhang, M., Hatzis, C., Assassi, S., et al., 2017. Reliability and validity of the tender and swollen joint counts and the modified Rodnan skin score in early diffuse cutaneous systemic sclerosis: analysis from the prospective registry of early systemic sclerosis cohort. *J. Rheumatol.* 44 (6), 791–794. Available from: <https://doi.org/10.3899/jrheum.160654>. PubMed PMID: 28298560; PMCID: PMC5457319. Epub 2017/03/17.
- Gorlova, O., Martin, J.E., Rueda, B., Koeleman, B.P., Ying, J., Teruel, M., et al., 2011. Identification of novel genetic markers associated with clinical phenotypes of systemic sclerosis through a genome-wide association strategy. *PLoS Genet.* 7 (7), e1002178. Available from: <https://doi.org/10.1371/journal.pgen.1002178>. PGENETICS-D-10-00536[pii].
- Gourh, P., Agarwal, S.K., Martin, E., Divecha, D., Rueda, B., Bunting, H., et al., 2010. Association of the C8orf13-BLK region with systemic sclerosis in North-American and European populations. *J. Autoimmun.* 34 (2), 155–162.
- Herzog, E.L., Mathur, A., Tager, A.M., Feghali-Bostwick, C., Schneider, F., Varga, J., 2014. Review: interstitial lung disease associated with systemic sclerosis and idiopathic pulmonary fibrosis: how similar and distinct? *Arthritis Rheumatol.* 66 (8), 1967–1978. Available from: <https://doi.org/10.1002/art.38702>. PubMed PMID: 24838199; PMCID: PMC4340472. Epub 2014/05/20.
- Hinchcliff, M., Desai, C.S., Varga, J., Shah, S.J., 2012. Prevalence, prognosis, and factors associated with left ventricular diastolic dysfunction in systemic sclerosis. *Clin. Exp. Rheumatol.* 30 (2Suppl 71), S30–S37. Epub 2012/02/18. PubMed PMID: 22338601; PMCID: PMC3507505.
- Hinchcliff, M., Huang, C.C., Wood, T.A., Matthew, M.J., Martyanov, V., Bhattacharyya, S., et al., 2013. Molecular signatures in skin associated with clinical improvement during mycophenolate treatment in systemic sclerosis. *J. Invest. Dermatol.* Available from: <https://doi.org/10.1038/jid2013130> [pii];10.1038/jid.2013.130.
- Ho, Y.Y., Lagares, D., Tager, A.M., Kapoor, M., 2014. Fibrosis—a lethal component of systemic sclerosis. *Nat. Rev. Rheumatol.* 10 (7), 390–402. Available from: <https://doi.org/10.1038/nrrheum.2014.53>. PubMed PMID: 24752182. Epub 2014/04/23.
- Hudson, M., Baron, M., Tatibouet, S., Furst, D.E., Khanna, D., 2014. International Scleroderma Renal Crisis Study I. Exposure to ACE inhibitors prior to the onset of scleroderma renal crisis—results from the International Scleroderma Renal Crisis Survey. *Semin. Arthritis Rheum.* 43 (5), 666–672. Available from: <https://doi.org/10.1016/j.semarthrit.2013.09.008>. PubMed PMID: 24176729. Epub 2013/11/02.
- Hung, E.W., Mayes, M.D., Sharif, R., Assassi, S., Machicao, V.I., Hosing St, C., et al., 2013. Gastric antral vascular ectasia and its clinical correlates in patients with early diffuse systemic sclerosis in the SCOT trial. *J. Rheumatol.* 40 (4), 455–460. Available from: <https://doi.org/10.3899/jrheum.121087> [pii];10.3899/jrheum.121087.
- Ioannidis, J.P., Vlachoyiannopoulos, P.G., Haidich, A.B., Medsger Jr, T.A., Lucas, M., Michet, C.J., et al., 2005. Mortality in systemic sclerosis: an international meta-analysis of individual patient data. *Am. J. Med.* 118 (1), 2–10.
- Ito, I., Kawaguchi, Y., Kawasaki, A., Hasegawa, M., Ohashi, J., Kawamoto, M., et al., 2010. Association of the FAM167A-BLK region with systemic sclerosis. *Arthritis Rheum.* 62 (3), 890–895. Available from: <https://doi.org/10.1002/art.27303>. PubMed PMID: 20131239. Epub 2010/02/05.
- Janowsky, E.C., Kupper, L.L., Hulka, B.S., 2000. Meta-analyses of the relation between silicone breast implants and the risk of connective-tissue diseases. *N. Engl. J. Med.* 342 (11), 781–790. Available from: <https://doi.org/10.1056/NEJM200003163421105>. PubMed PMID: 10717013. Epub 2000/03/16.
- Joseph, C.G., Darrah, E., Shah, A.A., Skora, A.D., Casciola-Rosen, L.A., Wigley, F.M., et al., 2014a. Association of the autoimmune disease scleroderma with an immunologic response to cancer. *Science* 343 (6167), 152–157. Available from: <https://doi.org/10.1126/science.1246886>. PubMed PMID: 24310608; PMCID: PMC4038033. Epub 2013/12/07.
- Joseph, C.G., Darrah, E., Shah, A.A., Skora, A.D., Casciola-Rosen, L.A., Wigley, F.M., et al., 2014b. Association of the autoimmune disease scleroderma with an immunologic response to cancer. *Science* 343 (6167), 152–157. Available from: <https://doi.org/10.1126/science.1246886> [pii];10.1126/science.1246886.
- Kanaan, S.B., Onat, O.E., Balandraud, N., Martin, G.V., Nelson, J.L., Azzouz, D.F., et al., 2016. Evaluation of X chromosome inactivation with respect to HLA genetic susceptibility in rheumatoid arthritis and systemic sclerosis. *PLoS One* 11 (6), e0158550. Available from: <https://doi.org/10.1371/journal.pone.0158550>. PubMed PMID: 27355582; PMCID: PMC4927113. Epub 2016/06/30.
- Kaneko, M., Sasaki, S., Teruya, S., Ozaki, K., Ishimaru, K., Terai, E., et al., 2016. Pneumatosis cystoides intestinalis in patients with systemic sclerosis: a case report and review of 39 Japanese cases. *Case Rep. Gastrointest. Med.* 2016, . Available from: <https://doi.org/10.1155/2016/2474515>. PubMed PMID: 27651961; PMCID: PMC5019915. 2474515. Epub 2016/09/22.

- Khanna, D., Denton, C.P., Jahreis, A., van Laar, J.M., Frech, T.M., Anderson, M.E., et al., 2016. Safety and efficacy of subcutaneous tofacitinib in adults with systemic sclerosis (faSScinate): a phase 2, randomised, controlled trial. *Lancet* 387 (10038), 2630–2640. Available from: [https://doi.org/10.1016/S0140-6736\(16\)00232-4](https://doi.org/10.1016/S0140-6736(16)00232-4). PubMed PMID: 27156934.
- Koschik, R.W., Fertig, N., Lucas, M.R., Domsic, R.T., Medsger Jr., T.A., 2012. Anti-PM-Scl antibody in patients with systemic sclerosis. *Clin. Exp. Rheumatol.* 30 (2Suppl 71), S12–S16. doi: 3813 [pii].
- Koumakis, E., Giraud, M., Dieude, P., Cohignac, V., Cuomo, G., Airo, P., et al., 2012a. Brief report: candidate gene study in systemic sclerosis identifies a rare and functional variant of the TNFAIP3 locus as a risk factor for polyautoimmunity. *Arthritis Rheum.* 64 (8), 2746–2752. Available from: <https://doi.org/10.1002/art.34490>. PubMed PMID: 22488580. Epub 2012/04/11.
- Koumakis, E., Wipff, J., Dieude, P., Ruiz, B., Bouaziz, M., Revillod, L., et al., 2012b. TGFbeta receptor gene variants in systemic sclerosis-related pulmonary arterial hypertension: results from a multicentre EUSTAR study of European Caucasian patients. *Ann Rheum Dis* 71 (11), 1900–1903. Available from: <https://doi.org/10.1136/annrheumdis-2012-201755>. PubMed PMID: 22896741. Epub 2012/08/17.
- Kuo, C.F., See, L.C., Yu, K.H., Chou, I.J., Tseng, W.Y., Chang, H.C., et al., 2011. Epidemiology and mortality of systemic sclerosis: a nationwide population study in Taiwan. *Scand. J. Rheumatol.* 40 (5), 373–378. Available from: <https://doi.org/10.3109/03009742.2011.553736>. PubMed PMID: 21388247.
- Kuwana, M., Shirai, Y., Takeuchi, T., 2016. Elevated serum krebs von den Lungen-6 in early disease predicts subsequent deterioration of pulmonary function in patients with systemic sclerosis and interstitial lung disease. *J. Rheumatol.* 43 (10), 1825–1831. Available from: <https://doi.org/10.3899/jrheum.160339>. PubMed PMID: 27481907. Epub 2016/08/03.
- Lakota, K., Carns, M., Podlusky, S., Mrak-Polsak, K., Hinchliff, M., Lee, J., et al., 2015. Serum amyloid A is a marker for pulmonary involvement in systemic sclerosis. *PLoS One* 10 (1), e0110820. Available from: <https://doi.org/10.1371/journal.pone.0110820>. PubMed PMID: 25629975; PMCID: PMC4321755. Epub 2015/01/30.
- Lambert, N.C., Lo, Y.M., Erickson, T.D., Tylee, T.S., Guthrie, K.A., Furst, D.E., et al., 2002. Male microchimerism in healthy women and women with scleroderma: cells or circulating DNA? A quantitative answer. *Blood* 100 (8), 2845–2851. Available from: <https://doi.org/10.1182/blood-2002-01-0295>. PubMed PMID: 12351394.
- Lazzaroni, M.G., Cavazzana, I., Colombo, E., Dobrota, R., Hernandez, J., Hesselstrand, R., et al., 2017. Malignancies in patients with anti-RNA polymerase III antibodies and systemic sclerosis: analysis of the EULAR scleroderma trials and research cohort and possible recommendations for screening. *J. Rheumatol.* 44 (5), 639–647. Available from: <https://doi.org/10.3899/jrheum.160817>. PubMed PMID: 28089973. Epub 2017/01/17.
- Leroy, E.C., Medsger Jr., T.A., 2001. Criteria for the classification of early systemic sclerosis. *J. Rheumatol.* 28 (7), 1573–1576.
- Leroy, C., Altman, R.D., Kirsner, J.B., Myers, J.A., McShane, D., Masi, D., et al., 1980. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. *Arthritis Rheum.* 23 (5), 581–590.
- Liu, X., Mayes, M.D., Phd, C.P., Draeger, H.T., Gonzalez, E.B., Harper, B.E., et al., 2013a. C-reactive protein predicts the long-term progression of interstitial lung disease and survival in patients with early systemic sclerosis. *Arthritis Care Res (Hoboken)*. Available from: <https://doi.org/10.1002/acr.21968>.
- Liu, X., Mayes, M.D., Tan, F.K., Wu, M., Reveille, J.D., Harper, B.E., et al., 2013b. Correlation of interferon-inducible chemokine plasma levels with disease severity in systemic sclerosis. *Arthritis Rheum.* 65 (1), 226–235. Available from: <https://doi.org/10.1002/art.37742>.
- Lo Monaco, A., Bruschi, M., La Corte, R., Volpinari, S., Trotta, F., 2011. Epidemiology of systemic sclerosis in a district of northern Italy. *Clin. Exp. Rheumatol.* 29 (2Suppl 65), S10–S14. PubMed PMID: 21586212.
- Lopez-Isac, E., Bossini-Castillo, L., Guerra, S.G., Denton, C., Fonseca, C., Assassi, S., et al., 2014. Identification of IL12RB1 as a novel systemic sclerosis susceptibility locus. *Arthritis Rheumatol.* 66 (12), 3521–3523. Available from: <https://doi.org/10.1002/art.38870>.
- Lopez-Isac, E., Campillo-Davo, D., Bossini-Castillo, L., Guerra, S.G., Assassi, S., Simeon, C.P., et al., 2016a. Influence of TYK2 in systemic sclerosis susceptibility: a new locus in the IL-12 pathway. *Ann. Rheum. Dis.* 75 (8), 1521–1526. Available from: <https://doi.org/10.1136/annrheumdis-2015-208154> [pii];10.1136/annrheumdis-2015-208154.
- Lopez-Isac, E., Martin, J.E., Assassi, S., Simeon, C.P., Carreira, P., Ortego-Centeno, N., et al., 2016b. Brief report: IRF4 newly identified as a common susceptibility locus for systemic sclerosis and rheumatoid arthritis in a cross-disease meta-analysis of genome-wide association studies. *Arthritis Rheumatol.* 68 (9), 2338–2344. Available from: <https://doi.org/10.1002/art.39730>. PubMed PMID: 27111665; PMCID: PMC5530728. Epub 2016/04/26.
- Marangoni, R.G., Varga, J., Tourtellotte, W.G., 2016. Animal models of scleroderma: recent progress. *Curr. Opin. Rheumatol.* 28 (6), 561–570. Available from: <https://doi.org/10.1097/BOR.0000000000000331>. PubMed PMID: 27533324. Epub 2016/08/18.
- Marangoni, R.G., Masui, Y., Fang, F., Korman, B., Lord, G., Lee, J., et al., 2017. Adiponectin is an endogenous anti-fibrotic mediator and therapeutic target. *Sci. Rep.* 7 (1), 4397. Available from: <https://doi.org/10.1038/s41598-017-04162-1>. PubMed PMID: 28667272; PMCID: PMC5493638. Epub 2017/07/02.
- Martin, J.E., Bossini-Castillo, L., Martin, J., 2012. Unraveling the genetic component of systemic sclerosis. *Hum. Genet.* 131 (7), 1023–1037. Available from: <https://doi.org/10.1007/s00439-011-1137-z>.
- Martyanov, V., Whitfield, M.L., 2016. Molecular stratification and precision medicine in systemic sclerosis from genomic and proteomic data. *Curr. Opin. Rheumatol.* 28 (1), 83–88. Available from: <https://doi.org/10.1097/BOR.0000000000000237>. PubMed PMID: 26555452; PMCID: PMC4722537. Epub 2015/11/12.
- Matucci-Cerinic, M., Kahaleh, B., Wigley, F.M., 2013. Review: evidence that systemic sclerosis is a vascular disease. *Arthritis Rheum.* 65 (8), 1953–1962. Available from: <https://doi.org/10.1002/art.37988>. PubMed PMID: 23666787. Epub 2013/05/15.
- Mayes, M.D., Lacey Jr., J.V., Beebe-Dimmer, J., Gillespie, B.W., Cooper, B., Laing, T.J., et al., 2003. Prevalence, incidence, survival, and disease characteristics of systemic sclerosis in a large US population. *Arthritis Rheum.* 48 (8), 2246–2255.
- Mayes, M.D., Bossini-Castillo, L., Gorlova, O., Martin, J.E., Zhou, X., Chen, W.V., et al., 2014. Immunochip analysis identifies multiple susceptibility loci for systemic sclerosis. *Am. J. Hum. Genet.* 94 (1), 47–61. Available from: <https://doi.org/10.1016/j.ajhg.2013.12.002>. S0002-9297 (13)00571-5 [pii];10.1016/j.ajhg.2013.12.002.

- McCormic, Z.D., Khuder, S.S., Aryal, B.K., Ames, A.L., Khuder, S.A., 2010. Occupational silica exposure as a risk factor for scleroderma: a meta-analysis. *Int. Arch. Occup. Environ. Health* 83 (7), 763–769. Available from: <https://doi.org/10.1007/s00420-009-0505-7>. PubMed PMID: 20047060. Epub 2010/01/05.
- Mehra, S., Walker, J., Patterson, K., Fritzler, M.J., 2013. Autoantibodies in systemic sclerosis. *Autoimmun. Rev.* 12 (3), 340–354. Available from: <https://doi.org/10.1016/j.autrev.2012.05.011>. PubMed PMID: 22743034. Epub 2012/06/30.
- Meier, F.M., Frommer, K.W., Dinser, R., Walker, U.A., Czirjak, L., Denton, C.P., et al., 2012. Update on the profile of the EUSTAR cohort: an analysis of the EULAR Scleroderma Trials and Research group database. *Ann. Rheum. Dis.* 71 (8), 1355–1360. Available from: <https://doi.org/10.1136/annrheumdis-2011-200742>. PubMed PMID: 22615460. Epub 2012/05/23.
- Mejia Otero, C., Assassi, S., Hudson, M., Mayes, M.D., Estrada, Y.M.R., Pedroza, C., et al., 2017. Antifibrillarin antibodies are associated with native North American ethnicity and poorer survival in systemic sclerosis. *J. Rheumatol.* 44 (6), 799–805. Available from: <https://doi.org/10.3899/jrheum.160574>. PubMed PMID: 28365584; PMCID: PMC5457664. Epub 2017/04/04.
- Meyer, A., Chifflet, H., Chatelus, E., Kleinmann, J.F., Ronde-Ousteau, C., Klein, D., et al., 2016. Brief report: spatial heterogeneity of systemic sclerosis in France: high prevalence in the Northeast Region. *Arthritis Rheumatol.* 68 (7), 1731–1737. Available from: <https://doi.org/10.1002/art.39613>. PubMed PMID: 26816302.
- Milano, A., Pendergrass, S.A., Sargent, J.L., George, L.K., McCalmont, T.H., Connolly, M.K., et al., 2008. Molecular subsets in the gene expression signatures of scleroderma skin. *PLoS One* 3 (7), e2696.
- Moinzadeh, P., Fonseca, C., Hellmich, M., Shah, A.A., Chighizola, C., Denton, C.P., et al., 2014. Association of anti-RNA polymerase III autoantibodies and cancer in scleroderma. *Arthritis Res. Ther.* 16 (1), R53. Available from: <https://doi.org/10.1186/ar4486> [pii];10.1186/ar4486.
- Muangchan, C., Harding, S., Khimdas, S., Bonner, A., Group, C.S.R., Baron, M., et al., 2012. Association of C-reactive protein with high disease activity in systemic sclerosis: results from the Canadian Scleroderma Research Group. *Arthritis Care Res. (Hoboken)* 64 (9), 1405–1414. Available from: <https://doi.org/10.1002/acr.21716>.
- Nanchahal, J., Hinz, B., 2016. Strategies to overcome the hurdles to treat fibrosis, a major unmet clinical need. *Proc. Natl. Acad. Sci. U.S.A.* 113 (27), 7291–7293. Available from: <https://doi.org/10.1073/pnas.1607896113>. PubMed PMID: 27342865; PMCID: PMC4941454. Epub 2016/06/28.
- Nandiwada, S.L., Peterson, L.K., Mayes, M.D., Jaskowski, T.D., Malmberg, E., Assassi, S., et al., 2016. Ethnic differences in autoantibody diversity and hierarchy: more clues from a US cohort of patients with systemic sclerosis. *J. Rheumatol.* 43 (10), 1816–1824. Available from: <https://doi.org/10.3899/jrheum.160106>. PubMed PMID: 27481902. Epub 2016/08/03.
- Nguyen, B., Mayes, M.D., Arnett, F.C., Del, J.D., Reveille, J.D., Gonzalez, E.B., et al., 2011. HLA-DRB1\*0407 and \*1304 are risk factors for scleroderma renal crisis. *Arthritis Rheum.* 63 (2), 530–534. Available from: <https://doi.org/10.1002/art.30111>.
- Nihtyanova, S.I., Schreiber, B.E., Ong, V.H., Rosenberg, D., Moinzadeh, P., Coghlan, J.G., et al., 2014. Prediction of pulmonary complications and long-term survival in systemic sclerosis. *Arthritis Rheumatol.* 66 (6), 1625–1635. Available from: <https://doi.org/10.1002/art.38390>.
- Nikpour, M., Hissaria, P., Byron, J., Sahhar, J., Micallef, M., Paspariatis, W., et al., 2011. Prevalence, correlates and clinical usefulness of antibodies to RNA polymerase III in systemic sclerosis: a cross-sectional analysis of data from an Australian cohort. *Arthritis Res. Ther.* 13 (6), R211. Available from: <https://doi.org/10.1186/ar3544> [pii];10.1186/ar3544.
- Ozbalkan, Z., Bagislar, S., Kiraz, S., Akyerli, C.B., Ozer, H.T., Yavuz, S., et al., 2005. Skewed X chromosome inactivation in blood cells of women with scleroderma. *Arthritis Rheum.* 52 (5), 1564–1570. Available from: <https://doi.org/10.1002/art.21026>. PubMed PMID: 15880831. Epub 2005/05/10.
- Paik, J.J., Wigley, F.M., Lloyd, T.E., Corse, A.M., Casciola-Rosen, L., Shah, A.A., et al., 2015. Spectrum of muscle histopathologic findings in forty-two scleroderma patients with weakness. *Arthritis Care Res. (Hoboken)* 67 (10), 1416–1425. Available from: <https://doi.org/10.1002/acr.22620>. PubMed PMID: 25989455; PMCID: PMC4580502. Epub 2015/05/20.
- Penn, H., Howie, A.J., Kingdon, E.J., Bunn, C.C., Stratton, R.J., Black, C.M., et al., 2007. Scleroderma renal crisis: patient characteristics and long-term outcomes. *QJM* 100 (8), 485–494.
- Radstake, T.R., Gorlova, O., Rueda, B., Martin, J.E., Alizadeh, B.Z., Palomino-Morales, R., et al., 2010. Genome-wide association study of systemic sclerosis identifies CD247 as a new susceptibility locus. *Nat. Genet.* 42 (5), 426–429.
- Razykov, I., Levis, B., Hudson, M., Baron, M., Thombs, B.D., Canadian Scleroderma Research G, 2013. Prevalence and clinical correlates of pruritus in patients with systemic sclerosis: an updated analysis of 959 patients. *Rheumatology (Oxford)* 52 (11), 2056–2061. Available from: <https://doi.org/10.1093/rheumatology/ket275>. PubMed PMID: 23946437. Epub 2013/08/16.
- Richardson, C., Agrawal, R., Lee, J., Almagor, O., Nelson, R., Varga, J., et al., 2016. Esophageal dilatation and interstitial lung disease in systemic sclerosis: a cross-sectional study. *Semin. Arthritis Rheum.* 46 (1), 109–114. Available from: <https://doi.org/10.1016/j.semarthrit.2016.02.004>. PubMed PMID: 27033049; PMCID: PMC5500283. Epub 2016/04/02.
- Rubio-Rivas, M., Simeon-Aznar, C.P., Velasco, C., Mari-Alfonso, B., Espinosa, G., Corbella, X., et al., 2017. Changes in the pattern of death of 987 patients with systemic sclerosis from 1990 to 2009 from the nationwide Spanish Scleroderma Registry (RESCLE). *Clin. Exp. Rheumatol.* Epub 2017/02/24. PubMed PMID: 28229826.
- Rueda, B., Gourh, P., Broen, J., Agarwal, S.K., Simeon, C., Ortego-Centeno, N., et al., 2010. BANK1 functional variants are associated with susceptibility to diffuse systemic sclerosis in Caucasians. *Ann. Rheum. Dis.* 69 (4), 700–705. Available from: <https://doi.org/10.1136/ard.2009.118174> [pii];10.1136/ard.2009.118174.
- Saigusa, R., Asano, Y., Taniguchi, T., Yamashita, T., Ichimura, Y., Takahashi, T., et al., 2015. Multifaceted contribution of the TLR4-activated IRF5 transcription factor in systemic sclerosis. *Proc. Natl. Acad. Sci. U.S.A.* 112 (49), 15136–15141. Available from: <https://doi.org/10.1073/pnas.1520997112> [pii];10.1073/pnas.1520997112.
- Salazar, G.A., Assassi, S., Wigley, F., Hummers, L., Varga, J., Hinckle, M., et al., 2014. Antinuclear antibody-negative systemic sclerosis. *Semin. Arthritis Rheum.* Available from: <https://doi.org/10.1016/j.semarthrit.2014.11.006> S0049-0172(14)00293-5 [pii];10.1016/j.semarthrit.2014.11.006.
- Shah, A.A., Rosen, A., Hummers, L., Wigley, F., Casciola-Rosen, L., 2010. Close temporal relationship between onset of cancer and scleroderma in patients with RNA polymerase I/III antibodies. *Arthritis Rheum.* 62 (9), 2787–2795. Available from: <https://doi.org/10.1002/art.27549>.

- Shah, A.A., Xu, G., Rosen, A., Hummers, L.K., Wigley, F.M., Elledge, S.J., et al., 2017. Brief report: anti-RNPC-3 antibodies as a marker of cancer-associated scleroderma. *Arthritis Rheumatol.* 69 (6), 1306–1312. Available from: <https://doi.org/10.1002/art.40065>. PubMed PMID: 28217959; PMCID: PMC5449218. Epub 2017/02/22.
- Sharif, R., Mayes, M.D., Tan, F.K., Gorlova, O.Y., Hummers, L.K., Shah, A.A., et al., 2012. IRF5 polymorphism predicts prognosis in patients with systemic sclerosis. *Ann. Rheum. Dis.* Available from: <https://doi.org/10.1136/annrheumdis-2011-200901> [pii];10.1136/annrheumdis-2011-200901.
- Sobanski, V., Dauchet, L., Lefevre, G., Lambert, M., Morell-Dubois, S., Sy, T., et al., 2014. Prevalence of anti-RNA polymerase III antibodies in systemic sclerosis: new data from a French cohort and a systematic review and meta-analysis. *Arthritis Rheumatol.* 66 (2), 407–417. Available from: <https://doi.org/10.1002/art.38219>. PubMed PMID: 24504813. Epub 2014/02/08.
- Steen, V.D., 2005. Autoantibodies in systemic sclerosis. *Semin. Arthritis Rheum.* 35 (1), 35–42.**
- Steen, V.D., Medsger Jr., T.A., 1998. Case-control study of corticosteroids and other drugs that either precipitate or protect from the development of scleroderma renal crisis. *Arthritis Rheum.* 41 (9), 1613–1619. Available from: [https://doi.org/10.1002/1529-0131\(199809\)41:9<1613::AID-ART11>3.0.CO;2-O](https://doi.org/10.1002/1529-0131(199809)41:9<1613::AID-ART11>3.0.CO;2-O).
- Steen, V.D., Medsger, T.A., 2007. Changes in causes of death in systemic sclerosis, 1972–2002. *Ann. Rheum. Dis.* 66 (7), 940–944.
- Steen, V.D., Oddis, C.V., Conte, C.G., Janoski, J., Casterline, G.Z., Medsger Jr., T.A., 1997. Incidence of systemic sclerosis in Allegheny County, Pennsylvania. A twenty-year study of hospital-diagnosed cases, 1963–1982. *Arthritis Rheum.* 40 (3), 441–445.
- Steen, V., Domsic, R.T., Lucas, M., Fertig, N., Medsger, T.A., 2012. A clinical and serologic comparison of African-American and Caucasian patients with systemic sclerosis. *Arthritis Rheum.* Available from: <https://doi.org/10.1002/art.34482>.
- Tan, F.K., Zhou, X., Mayes, M.D., Gourh, P., Guo, X., Marcum, C., et al., 2006. Signatures of differentially regulated interferon gene expression and vasculotrophism in the peripheral blood cells of systemic sclerosis patients. *Rheumatology (Oxford)* 45 (6), 694–702.**
- Taraborelli, M., Ramoni, V., Brucato, A., Airo, P., Bajocchi, G., Bellisai, F., et al., 2012. Brief report: successful pregnancies but a higher risk of preterm births in patients with systemic sclerosis: an Italian multicenter study. *Arthritis Rheum.* 64 (6), 1970–1977. Available from: <https://doi.org/10.1002/art.34350>. PubMed PMID: 22213060. Epub 2012/01/04.
- Tashkin, D.P., Roth, M.D., Clements, P.J., Furst, D.E., Khanna, D., Kleerup, E.C., et al., 2016. Mycophenolate mofetil versus oral cyclophosphamide in scleroderma-related interstitial lung disease (SLS II): a randomised controlled, double-blind, parallel group trial. *Lancet Respir. Med.* 4 (9), 708–719. Available from: [https://doi.org/10.1016/S2213-2600\(16\)30152-7](https://doi.org/10.1016/S2213-2600(16)30152-7) [pii];10.1016/S2213-2600(16)30152-7.
- Tashkin, D.P., Volkmann, E.R., Tseng, C.H., Roth, M.D., Khanna, D., Furst, D.E., et al., 2017. Improved cough and cough-specific quality of life in patients treated for scleroderma-related interstitial lung disease: results of scleroderma lung study II. *Chest* 151 (4), 813–820. Available from: <https://doi.org/10.1016/j.chest.2016.11.052>. PubMed PMID: 28012804; PMCID: PMC5472514. Epub 2016/12/26.
- Terao, C., Kawaguchi, T., Dieude, P., Varga, J., Kuwana, M., Hudson, M., et al., 2017. Transthoracic meta-analysis identifies GSDMA and PRDM1 as susceptibility genes to systemic sclerosis. *Ann. Rheum. Dis.* 76 (6), 1150–1158. Available from: <https://doi.org/10.1136/annrheumdis-2016-210645>. PubMed PMID: 28314753. Epub 2017/03/21.
- Toledano, E., Candelas, G., Rosales, Z., Martinez Prada, C., Leon, L., Abasolo, L., et al., 2012. A meta-analysis of mortality in rheumatic diseases. *Reumatol. Clin.* 8 (6), 334–341. Available from: <https://doi.org/10.1016/j.reuma.2012.05.006>. PubMed PMID: 22789463. Epub 2012/07/14.
- Tyndall, A.J., Bannert, B., Vonk, M., Airo, P., Cozzi, F., Carreira, P.E., et al., 2010. Causes and risk factors for death in systemic sclerosis: a study from the EULAR Scleroderma Trials and Research (EUSTAR) database. *Ann. Rheum. Dis.* 69 (10), 1809–1815. Available from: <https://doi.org/10.1136/ard.2009.114264> [pii];10.1136/ard.2009.114264.
- Valenzuela, A., Baron, M., Canadian Scleroderma Research G, Herrick, A.L., Proudman, S., Stevens, W., et al., 2016. Calcinosis is associated with digital ulcers and osteoporosis in patients with systemic sclerosis: A Scleroderma Clinical Trials Consortium study. *Semin. Arthritis Rheum.* 46 (3), 344–349. Available from: <https://doi.org/10.1016/j.semarthrit.2016.05.008>. PubMed PMID: 27371996; PMCID: PMC5500288. Epub 2016/07/04.
- van, B.L., Affandi, A.J., Broen, J., Christmann, R.B., Marijnissen, R.J., Stawski, L., et al., 2013. Proteome-wide analysis and CXCL4 as a biomarker in systemic sclerosis. *N. Engl. J. Med.* Available from: <https://doi.org/10.1056/NEJMoa1114576>.
- van den Hoogen, F., Khanna, D., Fransen, J., Johnson, S.R., Baron, M., Tyndall, A., et al., 2013. 2013 classification criteria for systemic sclerosis: an American college of rheumatology/European league against rheumatism collaborative initiative. *Ann. Rheum. Dis.* 72 (11), 1747–1755. Available from: <https://doi.org/10.1136/annrheumdis-2013-204424> [pii];10.1136/annrheumdis-2013-204424.
- van Laar, J.M., Farge, D., Sont, J.K., Naraghi, K., Marjanovic, Z., Larghero, J., et al., 2014. Autologous hematopoietic stem cell transplantation vs intravenous pulse cyclophosphamide in diffuse cutaneous systemic sclerosis: a randomized clinical trial. *JAMA* 311 (24), 2490–2498. Available from: <https://doi.org/10.1001/jama.2014.6368>. 1883019 [pii];10.1001/jama.2014.6368.
- Varga, J., Hinchliff, M., 2014. Connective tissue diseases: systemic sclerosis: beyond limited and diffuse subsets? *Nat. Rev. Rheumatol.* 10 (4), 200–202. Available from: <https://doi.org/10.1038/nrrheum.2014.22>. PubMed PMID: 24535544; PMCID: PMC5438483. Epub 2014/02/19.
- Volkmann, E.R., Tashkin, D.P., Roth, M.D., Clements, P.J., Khanna, D., Furst, D.E., et al., 2016. Changes in plasma CXCL4 levels are associated with improvements in lung function in patients receiving immunosuppressive therapy for systemic sclerosis-related interstitial lung disease. *Arthritis Res. Ther.* 18 (1), 305. Available from: <https://doi.org/10.1186/s13075-016-1203-y>. PubMed PMID: 28038680; PMCID: PMC5203703. Epub 2017/01/01.
- Wang, J., Assassi, S., Guo, G., Tu, W., Wu, W., Yang, L., et al., 2013. Clinical and serological features of systemic sclerosis in a Chinese cohort. *Clin. Rheumatol.* 32 (5), 617–621. Available from: <https://doi.org/10.1007/s10067-012-2145-7>.
- Watson, M., Hally, R.J., McCue, P.A., Varga, J., Jimenez, S.A., 1996. Gastric antral vascular ectasia (watermelon stomach) in patients with systemic sclerosis. *Arthritis Rheum.* 39 (2), 341–346.
- Whitfield, M.L., Finlay, D.R., Murray, J.I., Troyanskaya, O.G., Chi, J.T., Pergamenshchikov, A., et al., 2003. Systemic and cell type-specific gene expression patterns in scleroderma skin. *Proc. Natl. Acad. Sci. U.S.A.* 100 (21), 12319–12324.
- Woodworth, T.G., Suliman, Y.A., Furst, D.E., Clements, P., 2016. Scleroderma renal crisis and renal involvement in systemic sclerosis. *Nat. Rev. Nephrol.* 12 (11), 678–691. Available from: <https://doi.org/10.1038/nrneph.2016.124>. PubMed PMID: 27641135. Epub 2016/09/20.

- Xu, G.J., Shah, A.A., Li, M.Z., Xu, Q., Rosen, A., Casciola-Rosen, L., et al., 2016. Systematic autoantigen analysis identifies a distinct subtype of scleroderma with coincident cancer. *Proc. Natl. Acad. Sci. U.S.A.* 113 (47), E7526–E7534. Available from: <https://doi.org/10.1073/pnas.1615990113>. PubMed PMID: 27821747; PMCID: PMC5127349. Epub 2016/11/09.
- Zhou, X., Lee, J.E., Arnett, F.C., Xiong, M., Park, M.Y., Yoo, Y.K., et al., 2009. HLA-DPB1 and DPB2 are genetic loci for systemic sclerosis: a genome-wide association study in Koreans with replication in North Americans. *Arthritis Rheum.* 60 (12), 3807–3814. Available from: <https://doi.org/10.1002/art.24982>.
- Zochling, J., Newell, F., Charlesworth, J.C., Leo, P., Stankovich, J., Cortes, A., et al., 2014. An Immunochip-based interrogation of scleroderma susceptibility variants identifies a novel association at DNASE1L3. *Arthritis Res. Ther.* 16 (5), 438. Available from: <https://doi.org/10.1186/s13075-014-0438-8>. PubMed PMID: 25332064; PMCID: PMC4230517. Epub 2014/10/22.

## Antiphospholipid Syndrome

Ora Shovman<sup>1,2</sup> and Yehuda Shoenfeld<sup>1,3,4</sup>

<sup>1</sup>The Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Tel-Hashomer, Affiliated to Tel Aviv University, Tel Aviv, Israel <sup>2</sup>Department of Medicine ‘B’, Sheba Medical Center, Tel-Hashomer, Israel <sup>3</sup>Past Incumbent of the Laura Schwarz-Kipp Chair for Research of Autoimmune Diseases, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel <sup>4</sup>Laboratory of the Mosaics of Autoimmunity, Saint Petersburg University, Saint Petersburg, Israel

### O U T L I N E

General Introduction and Historical Aspects	607	Diagnostic Procedures	621
Epidemiology	608	The Mechanisms of Antiphospholipid Antibodies-Mediated Disease Expressions:	
Clinical Features and Disease Associations	609	Clinical Trials and Animal Models	621
Obstetric Antiphospholipid Syndrome	610	Thrombotic Manifestations	623
Thrombotic Antiphospholipid Syndrome	611	Obstetric Manifestations	624
Noncriteria Antiphospholipid Syndrome	611	The Complement System in Antiphospholipid Syndrome	625
Manifestations	614		
Catastrophic Antiphospholipid Syndrome			
The Antiphospholipid Antibodies	615	Mortality in the Antiphospholipid Syndrome	626
Criteria-Relevant Antiphospholipid Antibodies	615	Treatment of Antiphospholipid Syndrome	626
Noncriteria Antiphospholipid Antibodies	616	Conclusions and Future Aspects	627
Seronegative Antiphospholipid Syndrome	619	References	628
Risk Assessment in Antiphospholipid Syndrome	620	Further Reading	634
Genetics	620		
Classification Criteria Versus Diagnostic Criteria	621		

### GENERAL INTRODUCTION AND HISTORICAL ASPECTS

In 1983, a discrete syndrome was described by Graham Hughes in which lupus patients with antiphospholipid antibodies (aPL) were prone to arterial/venous thrombosis, recurrent abortions, neurological manifestations, and occasional thrombocytopenia (Hughes, 1983). In the following years, the Hughes' syndrome was delineated as a systemic disease that can affect both children and adults and can present as a primary disorder or as secondary to other autoimmune disease (Shoenfeld et al., 2009). The definition of this classical

**TABLE 32.1** Summary of the Sydney Consensus Statement on Investigational Classification Criteria for APS<sup>a</sup>

Antiphospholipid antibody syndrome is present if at least one of the clinical criteria and one of the laboratory criteria that follow are met

#### Clinical criteria

1. Vascular thromboses: One or more documented episodes of arterial, venous, or small vessel thrombosis—other than superficial venous thrombosis—in any tissue or organ. Thrombosis must be confirmed by objective validated criteria. For histopathologic confirmation, thrombosis should be present without significant evidence of inflammation in the vessel wall
2. Pregnancy morbidity:
  - a. One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus, or
  - b. One or more premature births of a morphologically normal neonate before the 34th week of gestation because of (1) eclampsia or severe preeclampsia defined according to standard definitions or (2) recognized features of placental insufficiency, or
  - c. Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded

#### Laboratory criteria

1. LA present in plasma, on two or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Haemostasis (Scientific Subcommittee on LAs/phospholipid-dependent antibodies)
2. aCL antibody of IgG and/or IgM isotype in serum or plasma, present in medium or high level (i.e., 40 GPL or MPL, or the 99th percentile), on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA
3. Anti-b2-glycoprotein-I antibody of IgG and/or IgM isotype in serum or plasma (in level, the 99th percentile), present on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA, according to recommended procedures

<sup>a</sup>Miyakis et al. (2006).

aCL, anticardiolipin; LA, Lupus anticoagulant.

autoimmune syndrome has greatly advanced from the original reports and classification criteria for antiphospholipid syndrome (APS) formulated in Sapporo, Japan, in 1998 to the current ones published in 2006 (Miyakis et al., 2006; Cervera and Ra, 2008) (Table 32.1). For the determination of APS, at least one classical clinical criterion [i.e., vascular thrombosis or pregnancy morbidity (PM)] and one serological criterion [i.e., the persistent presence of anticardiolipin (aCL) and/or anti-b2-glycoprotein-I antibody (anti-B2GPI) of IgG or IgM isotype at medium-to-high titers or lupus anticoagulant (LAC)] have to be met. Several new aPL specificities were found, including antibodies directed against domains of B2GPI or coagulation cascade proteins such as prothrombin itself or complexes of prothrombin with phospholipids (i.e., phosphatidylserine/prothrombin complex) (Bertolaccini et al., 2014).

## EPIDEMIOLOGY

aPL may be detected in up to 1%–5% of the general population, but only the minority of aPL-positive individuals develop APS. aPL appearing in healthy subjects are usually detected transiently at low levels and are frequently clinically insignificant (Shoenfeld et al., 2008a; Biggioggero and Meroni, 2010). They are normally found in older individuals and in association with infections, vaccinations, malignancies, and exposure to certain drugs. The prevalence of aPL in systemic lupus erythematosus (SLE) patients ranges between 30% and 40% (Biggioggero and Meroni, 2010).

The Antiphospholipid Syndrome Alliance For Clinical Trials and International Networking conducted a literature review and analyzed the frequency of aPL in patients with PM, stroke (ST), myocardial infarction (MI), and deep venous thrombosis (DVT) (Andreoli et al., 2013a). According to this review, aPL were positive in about 6% of the patients with PM, 13.5% of the patients with ST, 11% of the patients with MI, and 9.5% of the patients with DVT (Andreoli et al., 2013a).

It has been estimated that the incidence of APS is approximately 5 new cases per 100,000 individuals per year and the prevalence is 40–50 cases per 100,000 individuals (Cervera, 2017).

Secondary APS is estimated to appear in 10%–15% of SLE patients and less in other autoimmune diseases. In contrast with aPL-positive healthy individuals, patients with APS either primary or secondary typically present with persistent (more than 12 weeks), high-level aPL seropositivity and significant related morbidity and mortality (Cervera et al., 2009). Catastrophic APS (CAPS) is rare and occurs in less than 1% of all APS cases (Asherson et al., 2003).

## CLINICAL FEATURES AND DISEASE ASSOCIATIONS

APS is usually diagnosed following obstetric or thrombotic morbidity. Nonetheless, the clinical spectrum of APS is considered nowadays to be wider and includes systemic and organ-specific symptoms induced by both thrombotic and immune-mediated mechanisms (Shoenfeld, 2007; Marai et al., 2004). In addition, a wide spectrum of clinical presentations may arise due to occlusions in one or several vessels.

The clinical manifestations of 1000 patients with APS that were in the follow up for 10 years within the Euro-Phospholipid project are summarized in **Table 32.2**.

**TABLE 32.2** The Most Common APS Manifestations, According to the “Euro-Phospholipid Project” (at the Beginning of the Study)

Manifestations	%
Peripheral thrombosis	
Deep vein thrombosis	38.9
Superficial thrombophlebitis in legs	11.7
Arterial thrombosis in legs	4.3
Venous thrombosis in arms	3.4
Arterial thrombosis in arms	2.7
Subclavian vein thrombosis	1.8
Jugular vein thrombosis	0.9
Neurologic manifestations	
Migraine	20.2
Stroke	19.8
Transient ischemic attack	11.1
Epilepsy	7.0
Multiinfarct dementia	2.5
Chorea	1.3
Acute encephalopathy	1.1
Pulmonary manifestations	
Pulmonary embolism	14.1
Pulmonary hypertension	2.2
Pulmonary microthrombosis	1.5
Cardiac manifestations	
Valve thickening/dysfunction	11.6
Myocardial infarction	5.5
Angina	2.7
Myocardiopathy	2.9
Vegetations	2.7
Coronary by-pass rethrombosis	1.1
Intraabdominal manifestations	
Renal manifestations (glomerular thrombosis, renal infarction, renal artery thrombosis, renal vein thrombosis)	2.7

*(Continued)*

**TABLE 32.2** (Continued)

Manifestations	%
Gastrointestinal manifestations (esophageal or mesenteric ischemia)	1.5
Splenic infarction	1.1
Cutaneous manifestations	
<i>Livedo reticularis</i>	24.1
Ulcers	5.5
Pseudovasculitic lesions	3.9
Digital gangrene	3.3
Cutaneous necrosis	2.1
Osteo-articular manifestations	
Arthralgia	38.7
Arthritis	27.1
Avascular necrosis of bone	2.4
Ophthalmologic manifestations	
Amaurosis fugax	5.4
Retinal artery thrombosis	1.5
E.N.T. manifestations	
Nasal septum perforation	0.8
Hematological manifestations	
Thrombocytopenia (< 100,000 per $\mu\text{L}$ )	29.6
Hemolytic anemia	9.7
Obstetric manifestations (pregnant female = 590)	
Preeclampsia	9.5
Eclampsia	4.4
<i>Abruptio placentae</i>	2.0
Fetal manifestations (pregnancies = 1580)	
Early fetal losses (< 10 weeks)	35.4
Late fetal losses ( $\geq 10$ weeks)	16.9
Live births	47.7
Prematures	10.6

Adopted from Cervera, R., Piette, J.C., Font, J., Khamashta, M.A., Shoenfeld, Y., Camps, M.T., et al., 2002. Antiphospholipid syndrome: clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients. *Arthritis Rheum.* 46, 1019–1027.

## Obstetric Antiphospholipid Syndrome

Obstetric complications are a hallmark of APS including fetal and maternal complications and are now recognized as a distinct entity from vascular APS.

Early recurrent miscarriages (less than 10 weeks of gestation) occur in about 1% of the general obstetric population, and out of them 15% are associated with APS.

Fetal complications in obstetric APS (OAPS) patients include prematurity, intrauterine growth restriction due to placental insufficiency, and early and late pregnancy loss. According to the Euro-Phospholipid Project study, during a 10-year period, the prevalence of these events in APS pregnancies was 48%, 26%, 16.5%, and 5%,

respectively (Cervera et al., 2015). Fetal death is considered to be the most specific feature of OAPS, and strong association between fetal loss and the presence of aPL was found (Abou-Nassar et al., 2011; Silver et al., 2013). The most common maternal manifestation of APS is preeclampsia, followed by eclampsia and abruptio placentae.

The assessment of risk for PM in aPL-positive patients is difficult in general because similar clinical manifestations may be associated with different patterns and combinations of aPL. According to the classification criteria, only women with PM and medium-to-high titer aPL should be diagnosed with OAPS. However, patients with low titer aPL can experience poor pregnancy outcomes similar to high titer patients, and therefore current classification criteria do not incorporate all the OAPS cases (Ofer-Shiber and Molad, 2015).

The presence of an associated systemic autoimmune disease, in particular SLE, a history of previous thrombotic events, and complement reduction, has been identified as being predictive of a poor pregnancy outcome (Fredi et al., 2015). In addition, there is evidence that LA and triple positivity may serve as markers of worse outcome (Alijotas-Reig et al., 2015; Yelnik et al., 2016). Angiogenic biomarkers measured as early as the 12th week of pregnancy, in combination with clinical criteria, may be useful to identify patients with aPL at risk of severe adverse pregnancy outcomes (Kim et al., 2016). The possible association between aPL and sterility remains unknown.

No thrombosis or SLE was found in babies born to mothers with APS included in European aPL Forum despite the transplacental transfer of maternal aPL autoantibodies (Mekinian et al., 2013).

One study has reported high prolactin levels in 12% of APS patients, and this hyperprolactinemia was associated with reproductive failure, including early and late pregnancy loss, and intrauterine growth retardation (Praprotnik et al., 2010).

## Thrombotic Antiphospholipid Syndrome

Venous and/or arterial thrombosis are distinctive characteristics of APS (Taraborelli et al., 2012), and vessels in any site and of any size may be involved (Saponjski et al., 2011). Such thrombotic events are the main causes of morbidity and mortality in APS, and they tend to recur particularly in untreated patients (Taraborelli et al., 2012).

The confirmation of thrombosis by an objective method is a requirement of the Sapporo criteria, nowadays performed by a diversity of invasive and noninvasive methods such as angiography, ultrasound, computed tomography (i.e., 64 multislice computed tomographic angiography), and others (Saponjski et al., 2011). Histopathology may also be utilized to confirm the diagnosis of APS-associated thrombosis once there is no evidence of inflammation in the vessel wall (Taraborelli et al., 2012).

DVT, pulmonary embolism (PE), and STs are the most commonly reported. In line with these data, thrombosis was the most common manifestation of APS in the 1000 patients involved in the Euro-Phospholipid project (Cervera et al., 2002). Venous thrombosis in particular DVT of the lower limbs was a presenting symptom in 39% of the patients. Thrombotic events appeared in 166 (16.6%) patients during the first 5-year period of observation and in 118 patients (15.3%) during the second 5-year period. Among these thrombotic events that developed during the 10-year period, the most common were STs (5.3% of the total cohort), transient ischemic attack (TIA) (4.7%), DVT (4.3%), and PE (3.5%). These events were observed in similar proportions during both periods of the study (Cervera et al., 2009, 2015).

Furthermore, thrombotic APS manifestations occurring in atypical sites may cause additional clinical syndromes, including acute coronary syndromes; hepatic arterial or venous (Budd–Chiari syndrome) thrombosis; portal, mesenteric, or splenic ischemia; and pancreatic and adrenal insufficiency secondary to acute vascular infarction.

## Noncriteria Antiphospholipid Syndrome Manifestations

The first definitive classification criteria for APS were formulated during the 8th International Congress on aPL held in Sapporo and were published in 1999 (Wilson et al., 1999). A subsequent revision was later completed during the 11th International Congress on aPL held in Sydney (Miyakis et al., 2006). Initially, these criteria were designed for scientific clinical studies and included major manifestations of APS. Subsequently, they have been adapted for the diagnosis of APS in routine clinical practice.

According to the APS Clinical Features Task Force of the 14th International Congress on aPL, additional common non-criteria manifestations including thrombocytopenia, heart valve disease, renal microangiopathy

(aPL nephropathy), chorea, and longitudinal myelitis may be added to the APS classification criteria (Abreu et al., 2015). However, these features are not yet included in the International Consensus Criteria (Sapporo criteria).

### **Neurological Antiphospholipid Syndrome**

Neurological manifestations were detailed in the original description of APS and have since remained a main cause for morbidity and mortality among patients affected by this syndrome (Hughes, 1983). In APS, ST is the most common and severe complication and is the only manifestation included in APS criteria (Appenzeller et al., 2012). APS-related STs often occur in young patients and have a tendency to recur without an adequate therapy (Andreoli et al., 2013a).

In the Euro-Phospholipid Project Group, it has been demonstrated that the prevalence of ST and TIAs in 1000 APS patients at the beginning of the study was 19.8% and 11.1%, respectively (Cervera et al., 2002). During the 10 years follow-up, STs and TIA occurred in 5.3% and 4.7% of the total cohort, correspondingly (Cervera et al., 2015). In addition to local thrombosis mediated by aPL, valvular heart disease may become a source of emboli and related cerebrovascular events (Andreoli et al., 2013a).

Yet, in the last 30 years, aPL positivity has been correlated with a wide variety of non-ST neurological expressions including headache, cognitive dysfunction, psychosis, depression, dementia, migraine, convulsions, chorea, transverse myelitis, Guillain–Barré syndrome, and a multiple sclerosis-like illness (Chapman et al., 2003; Katzav et al., 2003; Shoenfeld et al., 2004; Rodrigues et al., 2010).

Some syndromes, such as migraine or cognitive dysfunction, are frequently described in APS whereas other neurological manifestations are rare. In addition, cognitive dysfunction is strongly associated with aPL, more than any other neurological non-ST APS presentation (Coín et al., 2015). One study reported that epilepsy was strongly associated with STs and TIA, presence of SLE, valvulopathy, and livedo reticularis (Shoenfeld et al., 2004). A different rare severe manifestation of APS is transverse myelitis, which requires early aggressive immunosuppressive treatment (Sherer et al., 2002).

In addition, APS should be considered in all the unexplained cases of retinal arterial and venous thromboses, as well as in the cases of unusual ocular inflammations, particularly in young individuals (Arnson et al., 2010).

### **Hematologic Antiphospholipid Syndrome**

Thrombocytopenia occurs in 20%–40% of APS patients (Cervera et al., 2002, 2011b) and is associated with systemic involvement (Krause et al., 2005a). According to the Euro-Phospholipid Project Group, thrombocytopenia appeared in 8.7% of the APS patients during the 10 years follow-up (Cervera et al., 2015). It is usually moderate, with platelet counts greater than 50K, rarely associated with major bleeding episodes, and does not require therapeutic intervention.

aPL-positive patients who develop thrombocytopenia without fulfilling the clinical criteria for APS have a potential risk of developing thrombosis. Smoking, LAC, and higher titers of aPL were found to be risk factors for the development of thrombocytopenia (Demetrio Pablo et al., 2017). Hyperferritinemia was found to be linked to thrombocytopenia, LAC, and aCL in SLE patients and it may serve as an early marker for secondary APS in this cohort (Zandman-Goddard et al., 2013).

Autoimmune hemolytic anemia is less frequent and was previously reported to occur in 6%–10% of APS patients (Taraborelli et al., 2012; Rottem et al., 2006). According to Euro-Phospholipid Project, it has appeared in 4% of the patients within during 10 years follow-up (Cervera et al., 2015). A significant association between the presence of aPL and a positive Coombs test as well as the cooccurrence of both autoimmune thrombocytopenia and hemolytic anemia (named Evans syndrome) has been described (Rottem et al., 2006; Cervera et al., 2011b). Lymphopenia and neutropenia are well-recognized features of SLE and therefore are found more commonly in patients with APS associated with SLE.

### **Dermatologic Antiphospholipid Syndrome**

There are several different dermatologic features of APS. Although these features are nonspecific and not included in the classification criteria, they are common (49% of APS patients) and may be the first clinical presentations in 30%–40% of the cases (Francès et al., 2005). The most frequent is livedo reticularis, a red or bluish alteration of the skin with a net-like pattern attributed to blood stasis in capillaries and venules (Toubi et al., 2005). The pathophysiology of livedo reticularis is not well characterized, but the relationship with arterial thrombosis such as Sneddon's syndrome suggests a possible role for an interaction of aPL with the endothelial cell or other cellular element in blood vessels (Taraborelli et al., 2012). Livedo reticularis may be a prognostic marker of a

more severe disease, which may be possibly used as a criterion for APS (Toubi and Shoenfeld, 2007). It is associated with arterial and venous thrombosis and PM, irrespective of the presence of aPL (Francès et al., 1999, 2005; Toubi et al., 2005).

Cutaneous necrosis is observed in 5.5% of the patients with APS and the most commonly involved sites are the upper and lower extremities, helices of ears, cheeks, trunk, and forehead. These lesions may appear in the postphlebitic state (following a thrombosis) or caused by a circumscribed skin necrosis. A rare association has been found between cryoglobulinemia and APS in patients with cutaneous necrosis (Shachaf and Yair, 2016).

APS should be in the differential for pyoderma gangrenosum-like lesions unresponsive to the usual treatment. Subungual splinter hemorrhages may be seen along with thrombotic events elsewhere (Francès et al., 2005).

Cutaneous digital gangrene, with preceding ischemic symptoms, has also been observed in up to 7.5% of the patients with APS. Many nonspecific skin lesions of which some resemble vasculitis (pseudovasculitis) including red macules, purpura, cyanotic lesions on the hands and feet, ecchymoses, and painful skin nodules as well as primary anetoderma have also been reported.

### **Cardiac Antiphospholipid Syndrome**

The heart is one of the organs targeted in APS. Cardiac features include asymptomatic valve lesions, cardiac vegetations causing recurrent STs, accelerated atherosclerosis, MI, intracardiac thrombus, pulmonary hypertension, cardiomyopathy, and diastolic dysfunction (Soltész et al., 2007).

Valve abnormalities, vegetations, and/or thickening, termed Libman–Sacks endocarditis, are the most common manifestations described in 30%–50% of the patients (Ziporen et al., 1996; Blank et al., 2004; Cervera et al., 2011a). It should be noted that the valve damage is more frequent in patients with secondary APS and is highly associated with the presence of aPL (Nesher et al., 1997). aPL represent an independent risk factor for valvular heart disease in SLE patients, along with the disease itself (Watad et al., 2017). In addition, aCL IgG/IgM positivity was connected to valvular abnormalities in primary and secondary APS patients, and higher levels of those antibodies were correlated with an increased risk for these manifestations (Djokovic et al., 2014). Valve abnormalities can lead to an increased risk of embolism and may rarely (4%–6%) require replacement. The mitral valve is most commonly affected followed by the aortic valve.

The presence of cardiac valve pathology may be a risk factor for several types of CNS involvement in primary APS including epilepsy, migraine, STs, and TIA (Krause et al., 2005b).

Pulmonary hypertension is the second common cardiac manifestation in APS and occurred in about 11% of APS patients in one study (Pardos-Gea et al., 2015). It has been previously demonstrated that pulmonary hypertension occurs mostly due to venous thromboembolic disease with PE.

Myocardial ischemia may result from coronary thromboembolism, accelerated atherosclerosis of the coronary arteries, or microvascular injury. MI is diagnosed in 5.5% of the patients with APS (Cervera et al., 2002) and is also significantly associated with the presence of aPL (Cervera et al., 2011a). Similarly, clinically silent myocardial ischemia have been found in young patients with primary APS, and it have also been associated with elevated levels of the IgG class of both aCL and anti-B2GPI antibodies (Padjas et al., 2016). Accelerated atherosclerosis has been found in APS patients more frequently than in the general population (Shoenfeld et al., 2005) and may be driven by different immunopathological factors associated with disease itself.

Ventricular dysfunction in APS is rare and may result from valvular disorders, myocardial ischemia, or probably from the direct effect of the aPL on the myocardium. Primary APS seems to be related to diastolic dysfunction while SLE and APS appear to be associated with systolic dysfunction. Moreover, the right ventricle seems to be more involved than left ventricle, especially in primary APS (Tektonidou et al., 2001). Owing to the high incidence of cardiac involvement, echocardiographic follow-up is recommended for all APS patients (Cervera et al., 2011a). Cardiovascular magnetic resonance may be able to identify a high prevalence of occult myocardial scarring and endomyocardial fibrosis in APS and may have a great value in the diagnosis and follow-up of both clinically overt and silent cardiac diseases in APS patients (Mavrogeni et al., 2015).

### **Pulmonary Antiphospholipid Syndrome**

PE and infarction constitute the most frequent pulmonary manifestation of APS. In one study that included 1000 APS patients, PE was the presenting manifestation in 14% of them and additional 3.5% developed this condition during 10 years of follow-up (Cervera et al., 2002, 2015). Leaving aside this classical thrombotic manifestation, other pulmonary expressions including pulmonary hypertension, acute respiratory distress syndrome

(ARDS), intraalveolar hemorrhage, and fibrosing alveolitis have been described in the clinical spectrum of APS features (Stojanovich et al., 2012a). Notably, the presence of aPL correlated with distinct pulmonary types of involvement, such as the link between aCL and pulmonary arterial thrombosis, adult respiratory distress syndrome, and fibrosing alveolitis (Stojanovich et al., 2012a). In patients, with CAPS, small vessel thrombosis and systemic inflammatory response may lead to ARDS (Erkan et al., 2010).

Diffuse alveolar hemorrhage may occasionally present as the initial manifestation of APS and cause cough, dyspnea, and fever with or without hemoptysis and, in some cases, progress to acute respiratory failure (Cartin-Ceba et al., 2014). The aPL positivity may help in the identification of SLE patients who are at risk for developing pulmonary hypertension and pulmonary arterial hypertension (Zuily et al., 2017).

### **Renal Antiphospholipid Syndrome**

APS-mediated thrombosis can affect different parts of the kidney depending on the type and size of the vessels. These thrombotic events may result in various manifestations that reflect the site and size of the involved vessel. Large vessel involvement is usually in the form of thrombosis and/or stenosis that present as marked hypertension, renal dysfunction, and pain. In the case of occlusive lesions, both *in situ* thrombosis and embolization from heart lesions can take place (Taraborelli et al., 2012).

Renal involvement in APS also includes small vessel vaso-occlusive nephropathy defined as “APS nephropathy” or “aPL-associated nephropathy.” The major clinical presentations of this condition are hypertension, hematuria, proteinuria (ranging from mild to nephrotic level), and renal insufficiency (Tektonidou, 2014). Investigation in such cases should include a renal biopsy (Chaturvedi et al., 2011; Tektonidou, 2014).

In a histological view, aPL-associated nephropathy is characterized by acute lesions with thrombotic microangiopathy, and less commonly, chronic lesions with fibrous, intimal hyperplasia, organizing thrombi with or without recanalization, fibrous occlusions of arteries or arterioles, and focal cortical atrophy. Similar clinical and histological characteristics have been found within all the different groups of patients with positive aPL (primary APS, SLE-related APS, CAPS, and SLE/non-APS with positive aPL) (Tektonidou, 2014).

In SLE patients undergoing kidney biopsy, aPL-associated nephropathy, isolated or concomitant with SLE nephritis, should be considered. Moreover, it has been demonstrated that the inclusion of renal vascular lesions in the histological classification system of lupus nephritis improves renal outcome predictions. Thus thrombotic microangiopathy was associated with the poorest renal outcome among the other renal vascular lesions (Wu et al., 2013). aPL screening should be performed when aPL-associated nephropathy lesions are found in kidney biopsy, after the exclusion of other reasons for similar histological lesions such as malignant hypertension, thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, scleroderma, or human immunodeficiency (Tektonidou, 2014).

### **Catastrophic Antiphospholipid Syndrome**

The term CAPS was first used in 1992 to define an accelerated form of APS resulting in multiorgan failure (Asherson, 1992). CAPS is characterized by widespread intravascular thrombosis within a short period of time resulting in multiorgan failure (Carmi et al., 2017). CAPS usually involves multiple small vessels, but sometimes large vessels may be concomitantly occluded as well (Erkan et al., 2010).

The international “CAPS Registry” was created in 2000 by the European Forum on Antiphospholipid Antibodies and included a large cohort of 500 patients with CAPS (Rodríguez-Pintó et al., 2016). In this cohort, 60% had primary APS and 40% had APS associated with another autoimmune disease, mainly SLE (75%). In 65% of CAPS episodes, the most common precipitating factor was infections (49%), especially in young patients. In this cohort, several organs and systems were involved, including kidneys (73%), lungs (60%), brain (56%), heart (50%), and skin (47%). Among laboratory features, thrombocytopenia was the most common (67%). Schistocytes, the markers of thrombotic microangiopathic hemolytic anemia, were present in 22%. LAC, aCL IgG, and anti-B2GPI IgG antibodies were the most frequently observed aPL (83%, 81%, and 78% respectively). Interestingly, in a series of 14 patients with CAPS, high levels of ferritin have been reported in 71% of the cases (Agmon-Levin et al., 2013). In the aforementioned CAPS Registry, the 12-year mortality rate in the general cohort was 37%, while a higher mortality of 48% was found in SLE patients secondary to severe cardiac and brain involvement (Rodríguez-Pintó et al., 2016).

## THE ANTIPHOSPHOLIPID ANTIBODIES

### Criteria-Relevant Antiphospholipid Antibodies

aPL is a group of over 20 different autoantibodies directed against a variety of antigens including negatively charged phospholipids, phospholipid-binding proteins, and factors related to hemostasis (de Groot et al., 2012). However, the revised laboratory criteria for the classification of APS include only LAC, aCL, and anti-B2GPI antibodies (Miyakis et al., 2006).

In recent years, the role of these three aPL in the pathogenesis of APS and their relation to APS manifestations have been challenged.

#### **Lupus Anticoagulant**

LAC defines a heterogeneous group of immunoglobulins that inhibits phospholipid-dependent coagulation reactions in vitro and is detected by prolongation of functional coagulation assay.

LAC is associated with severe clinical manifestations and considered the most important acquired risk factor for thrombosis and fetal loss. Thus a high incidence of LAC was found in patients with ST, DVT, and both early and late PM (Andreoli et al., 2013a). These findings have been further demonstrated by a recent metaanalysis showing that LAC is associated with a higher risk for thrombotic events in comparison with aCL and anti-B2GPI antibodies (Reynaud et al., 2014). Finally, LAC positivity has been shown to be the strongest predictor of poor pregnancy outcomes after 12 weeks of pregnancy (Lockshin et al., 2012; Yelnik et al., 2016). A “weak” positive LAC result (a measurement that results in value just above the mean + 2SD) should be considered positive when making clinical decisions, according to the 14th International Congress on Antiphospholipid Antibodies Task Force (Bertolaccini et al., 2014).

#### **Anticardiolipin**

The thrombotic risk of aCL antibodies, particularly high titer IgG aCL, has been previously demonstrated in patients with primary APS and APS associated with SLE (Long et al., 1991; Danowski et al., 2009). However, the importance of aCL in OAPS has been recently challenged by the several studies, which observed that isolated aCL positivity had no significant influence on adverse pregnancy outcomes in aPL-positive women with and without SLE (Lockshin et al., 2012; Yelnik et al., 2016). aCL antibodies were associated with an increased risk of venous thromboembolism and arterial thrombosis in patients without SLE, but this trend was less statistically significant than a similar association between LAC and thrombotic events (Reynaud et al., 2014).

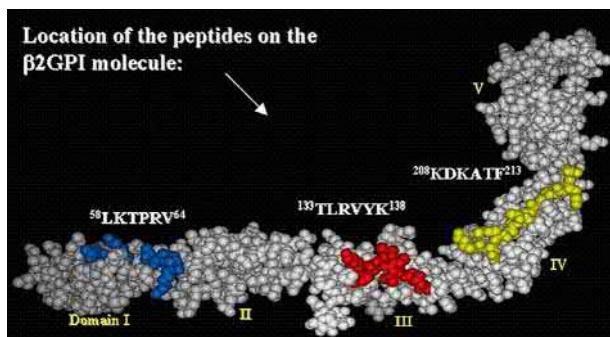
The effects of aCL antibodies' isotypes and their ability to predict different clinical manifestations of APS remain controversial issues. The IgG aCL isotype has been shown to be an independent risk factor for thrombosis, especially venous, in several previous and recent studies (Danowski et al., 2009; Domingues et al., 2016). Although IgM aCL is included in the Sydney APS Classification Criteria, the clinical importance of isolated IgM aCL is under debate. One study hasn't shown an increased incidence of venous or arterial thrombosis in patients with IgM aCL positivity (Danowski et al., 2009), and a different study has shown an association between IgM aCL and venous thrombosis (Samarkos et al., 2006).

It has been lately shown that the risk for jugular venous thrombosis and cerebral venous sinus thrombosis was correlated with the titer of aCL IgM, and no correlation with aCL IgG was found (Stojanovich et al., 2012b).

#### **Anti-b2-Glycoprotein-I Antibody**

Originally, it was assumed that aPL bind to phospholipids and were thus named “aPL antibodies.” However, in the 1990s it has been shown that aPL recognize phospholipids indirectly via phospholipid-binding plasma proteins (Galli et al., 1990). B2GPI is the most important phospholipid-binding protein, present either in a circulating form or bound to cells (de Groot et al., 2012). The B2GPI molecule has five homologous domains (I–V) (Fig. 32.1).

B2GPI participates in the innate immune responses [i.e., lipopolysaccharide (LPS) scavenging and clearance of unwanted anionic microparticles] and has antithrombotic properties. It has been proposed previously that B2GPI is the most important antigenic target (Meroni et al., 2011). However, the predominant role of anti-B2GPI in the pathogenesis and clinical manifestations of APS has been recently challenged. According to a critical analysis of published data regarding the pathogenic role of anti-B2GPI, this antibody may not play a central role in the pathogenesis and clinical manifestations of APS (Lackner and Müller-Calleja, 2016). Thus no association has been



**FIGURE 32.1** The plasma protein B2GPI is a phospholipid-binding protein. It is constructed of five domains (I–V) that can present in a circular nonactive form or in an active, open J-shaped conformation, as presented in the figure. Each of the 5 homologous domains of B2GPI includes approximately 60 amino acids, and certain peptides in each domain have been identified as autoantigens (3 of which are detailed in this figure). Anti-B2GPI antibodies can form a complex with B2GPI and activate intracellular signaling only in its open conformation while cryptic epitopes are exposed. *With acknowledgment to Prof. Miri Blank (The Zabludovitz Center for Autoimmune Diseases, Israel).* B2GPI, B(2)-glycoprotein I.

found between anti-B2GPI antibodies, both IgG and IgM, and adverse pregnancy outcomes in women with aPL and/or SLE (Lockshin et al., 2012; Yelnik et al., 2016).

Large systematic literature review and metaanalysis have showed that anti-B2GPI antibodies were associated with an increased risk of arterial thrombosis (OR 3.12, 95% CI 1.51–6.44) but only a nonsignificant trend was identified for venous thromboembolism (Reynaud et al., 2014).

In addition, in one recent study, anti-B2GPI antibodies were the most common antibodies in patients with thrombotic APS and had the highest sensitivity and negative predictive value with regard to APS diagnosis during the first visit (Ahluwalia et al., 2016). Higher titers of anti-B2GPI, both IgM and IgG, were observed in patients with SLE and secondary APS compared to negative aPL SLE patients and positive aPL SLE patients without APS clinical features. In this study, anti-B2GPI positivity was accompanied by almost a sixfold increased risk of secondary APS in SLE patients (Dima et al., 2015).

### Noncriteria Antiphospholipid Antibodies

Only three aPL are regarded as APS-relevant criteria, despite the fact that more than 30 different antibodies have been described in APS patients (the so-called autoantibody explosion in APS) (Shoenfeld et al., 2008b).

#### ***Antiphospholipid Antibodies of the IgA Isotype***

These are present in up to 40% of SLE patients and are especially common in Afro-Caribbean individuals. aCL antibodies of the IgA isotype were found to be thrombogenic in mice, and anti-B2GPI IgA was documented in a subgroup of seronegative women with recurrent pregnancy losses. IgA class aPL were also an independent risk factor for cardiovascular mortality and thrombotic events in hemodialysis patients (Serrano et al., 2012) and SLE patients (Swiss et al., 2010). Interestingly, unlike IgG directed at domains IV (DIV) and V (DV) of B2GPI, IgA antibodies that bind to these domains may contribute to thrombosis atherosclerosis (Iverson et al., 2006) and early pregnancy loss (Staub et al., 2006).

Several studies failed to show the usefulness of IgA aCL and IgA anti-B2GPI testing, owing to several reasons, including the low prevalence of these antibodies, the fact that they are found along with other aPL in most cases, and, mainly, the failure to enhance the diagnostic accuracy of routine testing (Meijide et al., 2013). Recent studies suggest that isolated IgA anti-B2GPI may identify additional patients with clinical features of APS and hence recommended testing for these antibodies when other aPL are negative and APS is suspected (Murthy et al., 2013). Based on the published evidence, IgA aPL testing may contribute to the risk assessment of thrombosis or/and PM in selected cases, mainly in SLE patients (Andreoli et al., 2013b). The above-discussed clinical utility of IgA aCL and IgA anti-B2GPI has been a subject of debate in the 14th International Congress on Antiphospholipid Antibodies Task Force (Bertolaccini et al., 2014).

### **Low Level Antiphospholipid Antibodies**

The value and clinical significance of low positive aPL values have been the topic of several publications. According to the 2006 classification criteria update, medium-to-high titers of the IgG and/or IgM isotypes of aCL and/or anti-B2GPI antibodies and/or a positive test for LAC should be present on two consecutive occasions, at least 12 weeks apart. The threshold between low and medium aCL or anti-B2GPI antibody titer is 40 GPL or MPL units, or the 99th percentile of the values obtained from the reference subjects for both aCL and anti-B2GPI antibodies, respectively. It has been previously reported that high levels of aPL are correlated with a worse outcome (Simchen et al., 2011). However, even lower levels should be taken into consideration by the treating physician.

Thus a recent study in this field has demonstrated that persistent low titers of aCL and/or anti-B2GPI antibodies were present in the patients with OAPS (Gardiner et al., 2013). In another study, the overall risk for vascular and obstetrical manifestations of APS was found to be similar both in patients with low titer of aCL/anti-B2GPI IgG/IgM antibody and in patients with moderate-to-high titers. This indicates that low titer aCL/B2GPI IgG/IgM tested positive twice 12 weeks apart may be sufficient for diagnosing APS in routine clinical practice (Ofer-Shiber and Molad, 2015).

Moreover, technical aspects may play a role while evaluating aPL levels. Given the variability of aPL assays, it is difficult to find a standard numerical cutoff value distinguishing between low- and medium-high titers of antibodies. The definition of medium-positive antibody titers depends on the performance characteristics of the particular assay, the statistical method, and the reference population. The most appropriate approach was summarized in the 14th International Congress on Antiphospholipid Antibodies Task Force, and according to it, the significance of a low positive aPL result depends on the whole risk profile of the patient with respect to clinical manifestations (Bertolaccini et al., 2014).

### **Autoantibodies to Domain 1 of b2-Glycoprotein-I Antibody**

The role of anti-B2GPI antibodies directed at different domains of B2GPI has become a focus of attention. Antibodies directed against domain I of B2GPI (anti-B2GPI-DI) have been previously found to be associated with an increased risk of thrombosis and pregnancy complications (de Laat et al., 2005, 2009) and represent one of the noncriteria aPL. Recent promising data support this association between anti-B2GPI-DI and APS clinical manifestation (Pengo et al., 2015a). Additional studies have highlighted the strong association between IgG anti-B2GPI-DI antibodies and thrombosis. Thus assessment of IgG anti-B2GPI-DI in 35 patients with primary APS and 51 patients with secondary APS indicated that anti-B2GPI-DI antibodies, but not non-DI anti-B2GPI antibodies, were significantly correlated with thrombotic events (OR 3.27; 95% CI 1.59–6.71) (Zhang et al., 2016). In this report, IgG anti-B2GPI-DI antibodies were detected in 48.6% of the patients with primary APS and in 45.1% of the patients with secondary APS, and their levels were significantly increased in APS patients in comparison with controls. IgG anti-B2GPI-DI antibodies were found in 81.4% of APS patients with positive anti-B2GPI antibodies, supporting the idea that DI is the major epitope in B2GPI.

Recently, the domain profile of anti-B2GPI antibodies has been evaluated in a large cohort of patients. In this work, no association has been found between anti-B2GPI-DI or anti-B2GPI-DIV/DV antibodies and APS clinical criteria. However, a high ratio ( $>1.5$ ) of anti-B2GPI-DI to anti-B2GPI-DIV/DV may predict systemic autoimmunity and, consequently, may be a useful biomarker for APS (Andreoli et al., 2015). An additional study evaluating aCL and/or aB2GPI-positive patients suggests that the addition of anti-B2GPI-DI positivity increases the likelihood of APS by three to five times. Positivity for IgG, IgA, or IgM anti-B2GPI-DI antibodies increased the strength of association between aCL and/or anti-B2GPI antibodies and thrombotic manifestations in APS (Pericleous et al., 2016). Recent studies have found that patients with multiple aPL have a higher prevalence and higher titers of anti-B2GPI-DI antibodies (Pengo et al., 2015a). Significantly higher levels of IgG aB2GP1-DI were found in patients with triple aPL positivity, compared with patients with double and single aPL positivity (Zhang et al., 2016).

Specific autoantibodies directed at other domains of B2GPI were less extensively studied. Nonetheless, the presence of an antibody directed at a peptide derived from domain III of B2GPI was found to be a significant predictor of recurrent spontaneous abortions (Shoenfeld et al., 2003).

### **Antiphosphatidylethanolamine Antibodies**

Antiphosphatidylethanolamine (aPE) antibodies were found to be associated with fetal loss and venous thrombosis (Pierangeli et al., 2011). In a different cohort, it has been reported that aPE antibodies were found in 19% of

the patients with unexplained recurrent early or mid-to-late pregnancy losses, suggesting an association between these antibodies and pregnancy losses (Sugi et al., 2004). The pathogenic role of aPE in pregnancy complications was assessed in a mice model, and it was found that passive immunization with aPE antibodies significantly induced thrombosis and hemorrhage in the placenta (Velayuthaprabhu et al., 2011).

In contrast, several studies failed to find an association between aPE and PM and thrombosis in SLE patients (Bertolaccini et al., 2012). The role of aPE in patients with seronegative APS remains a field of broad and current interest that requires additional investigation. The diagnostic value of aPE is still not established and aPE assays are not yet standardized. Similarly, there are no definitive therapeutic recommendations for patients with thrombotic or obstetric events and isolated aPE.

### **Antiphosphatidylserine Antibodies**

Antiphosphatidylserine (aPS) positivity was documented in women with recurrent pregnancy losses who were negative for aCL (Sater et al., 2012). In addition, aPS can inhibit trophoblast development and invasion and impede syncytiotrophoblast formation further supporting their pathogenic role (Blank and Shoenfeld, 2004).

The diagnostic value of aPS antibodies in APS has been recently assessed in a prospective observational study consisting of 212 patients with thrombosis and recurrent pregnancy failure. In this study, aPS antibodies were detected in 47.6% of the patients with thrombosis and in 52% of the patients with pregnancy failure. This prevalence of aPS antibodies in these two groups was similar to the prevalence of LAC (57% and 53%), anti-B2GPI antibodies (45.7% and 56%), and aCL (52% and 56%). In this study, 75% of the patients with confirmed primary APS had aPS antibodies. Thus aPS may be used as a diagnostic tool in clinical cases when other aPL are absent (Khogeer et al., 2015).

### **Antiprothrombin Antibodies**

Antiprothrombin antibodies (aPT) similarly to anti-B2GPI are phospholipid-binding proteins. The coexistence of IgG aPT and LACs was found to be an essential risk factor for venous thromboembolism in patients with SLE (Nojima et al., 2001) and to play a role in the pathogenesis of thrombosis in APS (de Groot et al., 1998; Von Landenberg et al., 2003; Haj-Yahia et al., 2003). Besides, aPT was linked to thrombosis and disease progression in other conditions such as acute ischemic STs in young women (Cojocaru et al., 2008), fetal death in women with previous uneventful pregnancies and seronegative aPL (Marozio et al., 2011), and more advanced primary biliary cirrhosis (Agmon-Levin et al., 2010). A recent systematic review of 38 studies on aPT has shown that the presence of aPT is linked to a higher risk of thrombosis (OR 1.82, 95% CI 1.44–2.75) (Sciascia et al., 2014). aPT may be detected by directly coating prothrombin on irradiated ELISA plates (aPT) (Bertolaccini et al., 2013).

### **Antiphosphatidylserine/Prothrombin Antibodies**

Antibodies directed at the complex phosphatidylserine/prothrombin (aPS/PT) were reported to be markers of APS with a high degree of concordance with LAC activity (Hoxha et al., 2012; Vlagea et al., 2013; Fabris et al., 2014). In a recent study that included 62 patients with inconclusive LAC analysis, the prevalence of aPS/PT antibodies was found to be 48% and allowed to discover a significant number of previously unrecognized aPL-positive cases with negative aCL and aB2GPI. Therefore, testing for aPS/PT antibodies may enhance the diagnostic performance for APS (Fabris et al., 2014).

In a cohort of 295 individuals that included primary and secondary APS and APS-related diseases, aPS/PT correlated with particular expressions of APS, namely venous thrombosis (OR 7.44; 95% CI 3.97–13.92) and obstetric abnormalities (OR 2.37; 95% CI 1.04–5.43), but not with arterial thrombosis (Vlagea et al., 2013). According to the systematic review of the literature, aPS/PT antibodies were found to be a strong risk factor for arterial (more significant) or venous thrombosis (less significant) (Sciascia et al., 2014). In line with this, a recent study has demonstrated that aPS/PT antibodies were found in 63% of 108 APS patients and were significantly associated with thrombosis (OR 3.4), especially arterial thrombosis (OR 4.8), independently of aCL and anti-B2GPI (Zhu et al., 2017).

Controversial data have been published regarding the connection between aPS/PT and PM. In one earlier study that included 208 women with history of pregnancy complications relevant to APS, the prevalence of aPS/PT was 13.0%. Moreover, 6.5% of these patients had isolated aPS/PT positivity (Žigon et al., 2015). In contrast, according to another study, no association has been found between aPS/PT positivity and history of PM (Zhu et al., 2017). The presence of aPS/PT antibodies may be a risk factor for more severe APS. Thus

aPS/PT antibodies, especially in high titers, were correlated with severe thrombosis, severe pregnancy complications inducing prematurity, and vascular microangiopathy (Hoxha et al., 2017). In addition, the inclusion of aPS/PT as a second-level assay to confirm APS classification was recommended in one study (Vlagea et al., 2013).

A recent report presented at the 14th International Congress on aPL antibodies concluded that testing for aPS/PT can contribute to a better identification of patients with APS and to the assessment of the risk of thrombosis (Bertolaccini et al., 2014).

### **Antiannexin A5 Antibodies**

Annexin A5 is one of the most important proteins in the annexin A group. It has an anticoagulant activity that prevents the binding and activation of clotting factors by the phospholipid shell that covers cells. aPL disturb this shield and expose phospholipids, thereby accelerating blood coagulation reactions. A significantly high correlation has been reported between annexin A5 resistance measured by flow cytometric assay (FCA) and the diagnosis of APS (Rodríguez-García et al., 2015). In a different recent report, positive FCA results were correlated with the presence of aCL and anti-B2GPI and were associated with APS features among patients with SLE (Avriel et al., 2016). A possible mechanism of annexin A5 resistance is linked to neutralization of annexin A5 by antiannexin A5 antibodies, which exposes the phospholipids on cell membranes for the accessibility of phospholipid-dependent coagulation enzymes.

In one recent study conducted on primary and secondary APS patients and disease and healthy controls, the levels of both IgG and IgM antiannexin A5 antibodies were significantly increased in patients with APS, compared with controls. Significant correlations were found between IgG antiannexin A5 antibodies and arterial thrombotic events (OR 2.60; 95% CI 1.44–4.71) and between IgG antiannexin A5 antibodies and venous thrombotic events (OR 2.80; 95% CI 1.55–5.06). In contrast, obstetric complications and IgG or IgM antiannexin A5 antibodies were not correlated. It has been suggested that antiannexin A5 antibodies could serve as a diagnostic biomarker for patients with APS (Zhang et al., 2017).

### **Metaanalysis: Prevalence of Different Noncriteria Antiphospholipid Antibodies**

Several additional investigations have addressed themselves to identify the prevalence of different noncriteria aPL in patients with APS. A recent metaanalysis of 16 such retrospective studies was conducted, incorporating 1404 APS patients, 1839 disease control patients, and 797 healthy patients. This analysis was based on studies published before June 2014 and reviewed the following noncriteria antibodies: IgA aCL, IgA anti-B2GPI, aPS, aPE, antiphosphatidylinositol, anti-B2GPI-DI, aPT, antiphosphatidylcholine, antivimentin/cardiolipin complex, and antiphosphatidic acid. Studies that evaluated the resistance to annexin A5 in APS patients were also included.

It has been shown that the prevalence of all noncriteria aPL was significantly increased in APS patients compared with controls. The most common laboratory characteristics were IgA anti-B2GPI antibodies (56.3%), resistance to annexin A5 (53.4%), and IgG anti-B2GPI-DI antibodies (44.0%) (Rodríguez-García et al., 2015).

---

## **SERONEGATIVE ANTIPHOSPHOLIPID SYNDROME**

---

The seronegative APS definition has been suggested for patients with clinical manifestations indicative of APS but with persistently negative results utilizing assays to detect criteria aPL (Rodríguez-García et al., 2012). Apparently these patients exhibit similar frequencies of thrombotic events and obstetric morbidity. Transient or false-negative aPL tests may explain some of these cases, as even today anti-B2GPI is routinely tested in only a small number of laboratories, and standardization of other criteria aPL is yet to be accomplished. In addition, noncriterion aPL are currently tested in only a few research laboratories (Cervera et al., 2012).

Therefore, new antigenic targets and different methodological approaches have been investigated in order to discover aPL in seronegative APS. One study analyzed the sera from 24 seronegative APS patients utilizing several methods: thin layer chromatography (TLC immunostaining) for all aPL, ELISA for antivimentin/cardiolipin, antiannexin A5 and aPT antibodies, and dot blot that was also used for antiannexin A5 and aPT antibodies (Conti et al., 2014).

This study has found that 54.2% of the cohort were positive for aCL antibodies according to the TLC immunostaining assay. In addition, 45.8% of them had IgG antibodies against vimentin/cardiolipin, 12.5% against prothrombin, and 4.2% against annexin A5. At least one aPL/cofactor antibody was detected using these assays in 79.2% of the patients. By combining TLC immunostaining for aCL and ELISA for antivimentin/cardiolipin

antibodies, aPL/cofactors were detected in two-thirds of the seronegative APS patients. Furthermore, recent evidence suggests that anti-B2GPI-DI, IgA aCL, or IgA anti-B2GPI may be used to identify seronegative APS patients (Meroni et al., 2014).

## RISK ASSESSMENT IN ANTIOPHOSPHOLIPID SYNDROME

APS manifestations may vary greatly between different patients. Some patients with aPL remain healthy, while others develop PM and/or thrombosis.

The value of different aPL profiles in the stratification of risks for thrombosis or obstetric events is under current investigation. Among aPL, LAC, triple positivity, and isolated persistently positive aCL at medium-high titers have been shown to be associated with a high risk for thrombosis (reviewed in Khamashta et al., 2016). In line with this, LAC and triple positivity were also correlated with worse pregnancy outcome (Alijotas-Reig et al., 2015).

While the presence of aPL is a risk factor for APS, additional risk factors have been incorporated in the global APS score (GAPSS) that was developed in 2013 (Sciascia et al., 2013). The GAPPS combines independent risk factors for thrombosis and pregnancy loss, including aPL profiles (criteria aPL and noncriteria aPL), and conventional cardiovascular risk factors and autoimmune antibody profiles, including anti-nuclear antibodies (ANA), extractable nuclear antigens antibodies (ENA), and anti-double stranded DNA antibodies (dsDNA), among others.

It has been demonstrated that arterial hypertension, hyperlipidemia, LAC, and IgG/IgM isotypes of aCL, anti-B2GPI, and aPS/PT were independent risk factors for thrombosis and/or PM.

Each risk factor was assigned a number of points within the GAPSS, as follows: IgG/IgM aCL (5 points), IgG/IgM anti-B2GPI (4 points), LAC (4 points), IgG/IgM aPS/PT (3 points), hyperlipidemia (3 points), and arterial hypertension (1 point).

The GAPSS model was initially developed in patients with SLE, and higher GAPSS scores were associated with thrombosis and/or pregnancy loss (Sciascia et al., 2013). Later, the GAPSS scoring system was evaluated in a cohort 62 patients with primary APS (Sciascia et al., 2015). Higher values of GAPSS were found in APS patients who had thrombosis alone, in comparison with patients who had previous pregnancy loss alone. In addition, patients with GAPSS values higher or equal to 11 had a higher risk of recurrent thrombotic events. Furthermore, a different study has shown that GAPSS correlated with a history of APS manifestations, particularly with thrombosis, suggesting that it may be used as a quantitative marker for APS (Oku et al., 2015).

Recently, GAPSS above 16 was reported as a predictor of thrombosis in a study that included APS and SLE patients (Zuily et al., 2015).

## GENETICS

The role of various genetic factors in the pathogenesis of APS was evaluated in several studies, including animal model studies, family studies, and population studies.

The current data suggest the existence of a genetic predisposition to APS, both as a primary condition or in association with SLE. This genetic predisposition is partly associated with the HLA system, and several family studies have shown that specific haplotypes, especially those containing DR4 and DRw53, may be correlated with aPL production or APS itself (reviewed in Sebastiani et al., 2016).

According to different population studies, the HLA loci relevant to the pathogenesis of primary APS appeared to be DRB1\*04, DR7, DRw53, DQB1\*0301/4, DQB1\*0604/5/6/7/9, DQA1\*0102, and DQA1\*0301/2.

With regard to aPL in SLE patients, the majority of the reports indicate that these antibodies are associated with DR4, DR7, the closely linked antigen DRw53, and DQB1\*0302 (Sebastiani et al., 2016). Several other genes unrelated to the HLA system may also increase the susceptibility to APS, including STAT 4 (Yin et al., 2009), 12q24.12 (Ochoa et al., 2013), and PTPN22 (Bottini et al., 2006). In addition, in a recent metaanalysis including 1507 APS patients, a strong association was found between valine/leucine (247) polymorphism of B2GPI and APS, thrombosis, and anti-B2GPI positivity (Lee et al., 2012).

In addition to genetic factors, epigenetic processes such as DNA methylation, histone modification, noncoding RNA, and nucleosome remodeling may be the topics of future research and provide additional insights regarding APS (Zhang and Zhang, 2015).

## CLASSIFICATION CRITERIA VERSUS DIAGNOSTIC CRITERIA

To date, there are no diagnostic criteria available for APS and therefore physicians should consider this diagnosis in the presence of minor features, even when major manifestations are absent. As mentioned earlier, there are minor clinical features and several noncriteria antibodies that may be potentially included in the disease's classification criteria.

Recently, it has been suggested to classify APS as definite, probable/possible, or uncertain. According to this suggestion, if the patient satisfies the 2006 Sydney APS Classification Criteria and has triple positivity, the diagnosis is considered definite. These patients may not require repeat testing after 12 weeks and have a high risk for recurrence of thrombosis and pregnancy loss. The diagnosis of APS is considered as probable in patients with double positivity (mostly LAC negative but with aCL IgG or IgM >99th percentile and anti-B2GPI IgG or IgM of the same isotype >99th percentile) and proven venous/arterial thrombosis and/or pregnancy loss. The diagnosis is "uncertain" if only one of the tests turns positive. Low titer single test positivity may be significant in obstetric cases only ([Pengo et al., 2015b](#)).

## DIAGNOSTIC PROCEDURES

Great efforts have been made to standardize the tests evaluating the presence of aPL. Several committees published guidelines regarding different coagulation and immunological tests. In 2009, the subcommittee on LAC/aPL antibodies of the International Society of Thrombosis and Haemostasis (ISTH) initiated the new guidelines for the detection of LAC ([Pengo et al., 2009](#)). Generally, two tests are used for the detection of LAC: dilute Russell venom time (dRVVT) and a sensitive activated thromboplastin time (aPTT).

The test for the detection of LAC consists of three steps: screening for prolongation in a phospholipid-sensitive test, mixing with normal plasma to discriminate between the presence of an inhibitor and coagulation factor deficiencies, and confirmation through result shortening in a phospholipid-rich test screening, mixing, and confirmatory studies. It has been found that the mixing test is essential to avoid false-positives in patients with prolonged dRVVT ([Devreese and de Laat, 2015](#)). In addition, the Scientific and Standardization Committee of the ISTH initiated new recommendations regarding aCL and anti-B2GPI ELISAs. In particular, the aCL ELISA cutoff should be calculated by the 99th percentile based on a population of healthy volunteers, a value that is quite different from the usually adopted cutoff of 40 GPL ([Devreese et al., 2014](#)).

Novel assay techniques have been proposed for aPL testing, such as chemiluminescence-based methods, fluorescence enzyme immunoassays, or line immunoassays (LIAs). A comparison of the LIA and ELISA techniques in 56 APS patients, 24 aPL carriers, and 73 aPL-positive patients with infectious diseases revealed a good inter-method agreement for the detection of IgG/IgM anti-B2GPI and aCL in APS patients. Unlike ELISA, according to LIA, the prevalence of aCL and anti-B2GPI IgG in aPL carriers and in aPL-positive patients with infectious diseases was significantly reduced in comparison with APS patients. This indicates that LIA discriminates patients with APS from aPL-positive asymptomatic carriers and infectious patients ([Roggenbuck et al., 2016](#)).

Confirmation of aPL positivity in order to avoid the detection of transient antibodies is a mandatory part of the classification criteria for APS. However, it may delay the diagnosis of APS and influence the treatment decisions. It has been demonstrated that initial aPL profiles may predict aPL persistence after 12 weeks. For example, repeated testing of 161 aPL-positive individuals who initially had one or more aPL showed that in 98% of subjects with triple positivity at initial testing, aPL profile was confirmed after 12 weeks. The aPL profile confirmation rates in double-positive and single-positive groups were 84% and 40%, respectively. These results have raised the possibility that triple positivity at initial screening may help in the early identification of APS patients ([Pengo et al., 2013](#)).

## THE MECHANISMS OF ANTIPHOSPHOLIPID ANTIBODIES-MEDIATED DISEASE EXPRESSIONS: CLINICAL TRIALS AND ANIMAL MODELS

The pathogenic effects of these antibodies are exerted through binding to receptor on target cells, including monocytes, endothelial cells, and trophoblasts, leading to the recruitment of cell surface receptors and subsequent perturbation of intracellular signaling pathways ([Blank et al., 1991; Giannakopoulos and Krilis, 2013](#)).

In the last three decades, much effort has been devoted to clarify the pathogenic potential of aPL (Bakimer et al., 1992). Both vascular thrombosis and pregnancy loss are the classical clinical expressions of the APS, therefore aPL pathogenicity was studied focusing on these clinical aspects. Notably, aPL-related vascular thrombosis can occur both on the arterial and the venous side, making this acquired procoagulant condition very peculiar in human disease. The first evidence of the aPL-related pathogenic potential was obtained through animals that carried aPL as part of their disease (i.e., lupus-like-prone mice), or alternatively naive mice that were infused with antibodies or stimulated to produce their own aPL (Ziporen et al., 1997; Katzav et al., 2010). In a long series of studies, different polyclonal and monoclonal aPL with various specificities were utilized inducing pregnancy losses and enhancing the coagulation process. Probably in no other autoimmune disorders has there been so much evidence produced to show the pathogenicity *in vivo* of these autoantibodies. This body of evidence was pivotal in proving autoantibody-mediated damage and at the same time prompted further *in vitro* studies aiming to define the cellular pathogenic effects of aPL. Originally both pregnancy loss and vascular occlusions were ascribed to the thrombogenic effect of aPL. Nowadays, it is clear that placental thrombosis can be responsible for pregnancy failures, but at the same time it is accepted that aPL can exert a direct nonthrombotic effect on trophoblast cells (Di Simone et al., 2000) and other cells (Del Papa et al., 1995). Over the years, the concept of aPL itself has also been redefined. In fact, starting from the hypothesis that these autoantibodies were directed to phospholipids, we now know that in most cases they are directed to phospholipid-binding proteins (Giannopoulos et al., 2011). Even if these antibodies are detected with different assays, the main target of the so-called aPL was identified as B2GPI, a phospholipid-binding protein. Almost all pathogenic effects of aPL have been shown using highly purified anti-B2GPI antibodies (Meroni et al., 2011). However, at that time, antibodies that bind to phospholipids in a cofactor-independent manner were generally considered irrelevant for the pathogenesis of APS. In recent years, there is direct evidence that cofactor-independent aPL presented in the blood of APS patients may contribute to the pathogenesis of the APS.

Thus two human monoclonal cofactor-independent aPL that had been obtained from patients with APS stimulated proinflammatory and procoagulant responses in monocytes and endothelial cells (Müller-Calleja et al., 2015). Endosomal NADPH-oxidase 2 (NOX2) activation was mediated by cofactor-independent aPL with subsequent induction of cytokines, inflammasome components, and tissue factor (TF). All effects induced by the monoclonal aPL were reproduced with IgG fractions of APS patients suggesting that effects of the monoclonal aPL are relevant for the APS. In a different study, two monoclonal human aPL-induced thrombus formation *in vivo* mouse model (Manukyan et al., 2016). Rapid induction of thrombus formation within 3 hours after infusion of the aPL was observed, and this thrombogenic effect of aPL was not reintroduced in NOX2-deficient mice. It has been found that thrombus formation depends on TF induction in monocytes. These accumulating data suggest that cofactor-independent aPL play an important role in pathogenesis of APS (Lackner and Müller-Calleja, 2016).

Several noncriteria autoantibodies such as aPS/PT, aPT, and anti-B2GPI-DI have been proposed to be relevant to the pathogenesis of APS. The prothrombotic property of aPS-PT antibodies has been demonstrated *in vitro* (Oku et al., 2013) and *in vivo* (Yamada et al., 2017). Both purified IgG fractions obtained from the sera of aPS/PT positive patients with APS and murine monoclonal aPS/PT antibodies induced TF expression and shortening of coagulation time in cells in the presence of prothrombin in procoagulant cells via p38 mitogen-activated protein kinase (MAPK) phosphorylation pathway.

The pathogenicity of aPS-PT antibodies *in vivo* has also been demonstrated in rat model of thrombosis induced by intravenous injection of aPS-PT antibodies (Yamada et al., 2017).

A direct demonstration of the pathogenic effect of anti-B2GPI-DI antibodies has been recently observed in animal model, in which infusion of human monoclonal anti-B2GPI-DI antibodies resulted in fetal losses in pregnant mice and blood clots in rat mesenteric microcirculation following priming with LPS. A variant of this antibody, lacking the CH2 domain, is effective in preventing blood clot formation and fetal loss induced by aPL (Agostinis et al., 2014). In a different work, intraperitoneal injection of two polyclonal IgG fractions (anti-B2GPI-DI rich and anti-B2GPI-DI poor IgG) isolated from APS patients *in vivo* mouse model demonstrated that anti-B2GPI-DI-rich IgG induced significantly larger thrombi compared with anti-B2GPI-DI poor IgG. Similarly, anti-B2GPI-DI-rich IgG significantly increased the procoagulant activity of the carotid artery endothelium and peritoneal macrophages isolated from the experimental animals (Pericleous et al., 2015).

## Thrombotic Manifestations

Many factors are involved in the pathogenesis of thrombotic manifestations. Several mechanisms have been proposed regarding the development of thrombosis, including the activation of endothelial cells, monocytes, platelets, coagulation, and complement pathways, along with the inhibition of fibrinolytic and anticoagulation pathways (Merashli et al., 2015). Consequently, it has been recently indicated that vasculopathy, related mainly to severe intimal hyperplasia, may also play a role in arterial vascular occlusion secondary to stenotic lesions and in PM. In this regard, the vascular endothelium of proliferating intrarenal vessels from patients with APS nephropathy demonstrated signs of mTOR activation, which is the mammalian target of rapamycin (Canaud et al., 2014). It has been shown that in cultured vascular endothelial cells, IgG antibodies from patients with APS stimulated mTOR through the phosphatidylinositol 3-kinase–AKT pathway. Treatment with sirolimus (rapamycin) led to the inhibition of mTOR in APS patients who underwent renal transplantation, which in turn inhibited the recurrence of vascular lesions and decreased vascular proliferation on biopsy, compared with APS patients who were not treated with sirolimus. Therefore, it was concluded that the activation of mTOR stimulates intimal hyperplasia, leading to the formation of the chronic vascular lesions seen in APS. It has also been found that mTOR is able to induce a prothrombogenic phenotype leading to thrombosis (Canaud and Terzi, 2014).

## **Antiphospholipid Antibodies and the Coagulation Cascade**

Several mechanisms have been described to explain the thrombophilic properties of aPL, generally related to their interaction with the coagulation and fibrinolysis systems or with cells involved in thrombus formation. These include B2GPI–anti-B2GPI complexes that were found to be localized to atherosclerotic plaques, mainly to oxidized low-density lipoprotein, to induce autoimmune thrombogenesis (Matsuura et al., 2003), and to amplify thrombus size (Arad et al., 2011).

Activated protein C (APC) is a natural anticoagulant that interacts with factors Va and VIIIa impairing their procoagulant activity. In the presence of B2GPI–anti-B2GPI complexes, APC cannot exert its action possibly because it is unable to bind Va/VIIIa or, alternatively, the formed APC/Va/VIIIa complex is sterically impaired in its binding to a phospholipid surface (Vlachoyiannopoulos and Routsias, 2010). aPL antibodies can also impair the anticoagulant function of antithrombin. This can occur because some of the targets such as thrombin or activated factor IX, when bound by aPL, are no longer available for antithrombin action (Chen et al., 2010). On the other hand, aPL can interfere with the fibrinolytic cascade.

Fibrin degradation, which allows thrombus remodeling and dissolution, is mediated by the active enzyme plasmin which is derived from the conversion of plasminogen. This conversion is mediated by tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator, and both of them are controlled by specific inhibitors -plasminogen activator inhibitors PAI-1 and PAI-2. In the presence of aPL, PAI-1 activity was reported as enhanced resulting in reduction of tPA and plasminogen activation. Apparently B2GPI protects tPA from the action of PAI-1, thereby promoting fibrinolysis; if B2GPI is bound by its specific antibodies, it cannot exert its protective action with the consequent prevalent action of the inhibitory effect of PAI-1 (Vlachoyiannopoulos and Routsias, 2010). Another impairment of fibrinolytic activity seems to be due to a possible direct binding of aPL to plasmin, followed by its inactivation (Yang et al., 2004). The data reported so far represent only some of the plausible mechanisms by which aPL interfere with the coagulation process, and recently another possible explanation has been formulated (Lambrianides et al., 2011). Many of the proteins involved in coagulation/fibrinolysis processes belong to the family of serine protease (SP) enzymes, such as APC, thrombin, plasmin, tPA, and others. These proteins share a high homology region at their catalytic domain. Most aPL recognize a conformational epitope that is shared by B2GPI molecule and the catalytic domain of SP. Therefore the presence of aPL can result in binding of SP molecules and impairment of their enzymatic activity, resulting in enhanced risk of thrombosis.

## **Antiphospholipid Antibodies Cellular Interactions**

One of the most studied pathogenic effects of aPL deals with the interaction of antibodies with the cells involved in the coagulation process. This basically occurs through the recognition of B2GPI that has adhered to cell membranes. It is known that anti-B2GPI antibodies can upregulate adhesion molecules such as endothelial leukocyte adhesion molecule-1, vascular cell adhesion molecule-1, intracellular adhesion molecule-1, and TF, conditioning their expression on the cell surface. This process was described in vitro on monolayers of human umbilical vein endothelial cells (HUVECs) (Meroni et al., 1996) and its consequences were shown in vivo on CD1 mice

infused with aPL: in fact in this model, leukocytes were seen to adhere to vascular endothelium favoring clotting (Pierangeli et al., 1995). In the presence of aPL, HUVECs also significantly increase the production of some proinflammatory cytokines such as IL-6 and IL-1B. Therefore the consequence of the aPL effect on endothelium is the shift toward a proadhesive/procoagulant as well as proinflammatory phenotype.

These profound modifications are basically due to the presence of B2GPI on the endothelial surface. A number of possible receptors for B2GPI have been described on the endothelial cell surface. Heparin sulfate, annexin 2 receptor, Toll-like receptors (TLR) 2 and 4, and apolipoprotein E receptor 2 were all shown to be involved in the thrombogenic mechanisms related to aPL using *in vitro* and *in vivo* models (Poulton et al., 2012). It was also observed that animals lacking one of these receptors are only partially resistant to the pathogenic potential of aPL, suggesting that they play a similar, probably redundant, role (Meroni et al., 2011). The binding of anti-B2GPI to its antigen on the endothelial surface should produce intracellular signaling, able to upregulate the cellular expression of adhesion molecules and TF. The signal can start from the receptors that are sensitive to the clustering of B2GPI that follows their specific antibody binding. Among receptors, those having a cytoplasmic tail, TLR 2 and 4 are most likely to be the favorite candidate. Several intracellular pathways have been described as activated, including nuclear factor  $\kappa$ B, p38 MAPK, myeloid differentiation primary response protein (MyD88), and tumor necrosis factor receptor-associated factor 6. The above-reported mechanisms are well defined in endothelial cells, but they are also at least partially described in monocytes, platelets, and in other cells serving as a possible target of aPL-mediated damage (Meroni et al., 2011; Poulton et al., 2012). The thrombophilic effect of aPL was also investigated at platelet level. In subjects with aPL, platelet activation was proven by the increase of thromboxane B2 and the decrease in vascular prostacyclin. Receptors of B2GPI at the platelet surface seem to be the apolipoprotein E receptor 2 and the platelet glycoprotein Ib alpha chain (Cognasse et al., 2005; Urbanus et al., 2008). In *vitro*, the pathogenic effect of affinity purified anti-B2GPI antibodies was shown as an increased aggregation and intracellular signaling activation of platelets in the presence of low doses of thrombin stimulation. In *vivo*, the B2GP1–anti-B2GP1 complex binds to platelets and activates thrombus-associated platelets. This enhanced platelet activation leads to increased activation of the endothelium and fibrin generation (Proulle et al., 2014). In this respect, a recent study (Vlachoyiannopoulos and Routsias, 2010) has underlined the importance of platelet factor 4, a protein derived from platelet alpha granules and belonging to the chemokine family. Platelet factor 4 is secreted by platelets but can also bind to the platelet surface as well as anionic molecules and B2GPI, both in solid phase and in solution. Platelet factor 4 is able to gather two B2GPI molecules, so favoring an efficient antibody binding. Complexes containing platelet factor 4, dimerized B2GPI, and anti-B2GPI antibodies can induce an activated procoagulant phenotype in platelets. Notably, platelet factor 4 is expressed in different cells such as endothelial cells, monocytes, T cells, and dendritic cells suggesting that its capacity to dimerize B2GPI could favor immune system sensitization and the production of pathogenic anti-B2GPI antibodies.

Neutrophil extracellular traps (NETs) represent an important activator of the coagulation cascade and an important component of arterial and venous thrombi. In one study, freshly isolated APS neutrophils demonstrated enhanced NET release in a spontaneous manner. Circulating aPL both purified IgG fractions and anti-B2GPI monoclonals can promote NET release from neutrophils. A positive correlation was found between circulating levels of NETs and IgG anti-B2GPI positivity, LAC positivity, and triple positivity in APS patients (Yalavarthi et al., 2015). A decreased degradation of NETs was found in a subgroup of patients with both primary and secondary APS and was associated with antibodies against NETs in patients with secondary APS (Leffler et al., 2014).

Cell-released vesicles and exosomes are important systems of intercellular communication. aPL may promote pathogenic effects on vascular cells (endothelial cells, platelets, monocytes) through the release of extracellular vesicles, which include exosomes and microparticles. In this regard, an increased number of monocyte and endothelial microparticles was found in APS patients, in comparison with healthy controls (Vikerfors et al., 2012). Notably, endothelial cells exposed to polyclonal IgG from APS patients produced significantly more endothelial microparticles than those exposed to polyclonal IgG from healthy subjects (Pericleous et al., 2013).

## Obstetric Manifestations

The pathogenesis of pregnancy failure in APS patients seems to be multifactorial, and it is well known that aPL are associated with reproductive failure (reviewed in Blank and Shoenfeld, 2010).

The mechanisms responsible for aPL-mediated obstetric manifestations include intraplacental thrombosis, inflammation, interference with annexin A5 function, inhibition of syncytium-trophoblast differentiation, defective placentation/placental apoptosis, and complement activation (reviewed in Arachchillage et al., 2017).

Although many different obstetric expressions of APS have been described, the most typical are midpregnancy losses, mainly related to defective placentation and intrauterine growth restriction. Therefore, the first pathogenic mechanism to be investigated was the presence of intraplacental thrombosis (Inbar et al., 1993; Levy et al., 1998). Indeed the above-described thrombophilic properties of aPL favor an increased thrombosis occurrence, particularly during pregnancy, that can work as a “second hit” since pregnancy per se is characterized by an increased thrombosis risk, even in the general population. A potent anticoagulant acting mainly, but not exclusively, on the trophoblast surface is annexin A5 that produces the so-called protective shield, by binding negatively charged phospholipids such as phosphatidylserine. The presence of annexin A5 at the intervillous surface was found to be significantly reduced in patients with APS, thus confirming that B2GPI–anti-B2GPI complexes are able to displace in vivo annexin A5 from the cell surface as shown in vitro (Hunt et al., 2011). Furthermore, it is recognized that aPL can also directly interact with trophoblast during syncytium formation (Di Simone et al., 2000) when it expresses phosphatidylserine at its outer surface. As shown by in vitro experiments, B2GPI can bind to negatively charged phospholipids such as phosphatidylserine on the cell surface and become a target of circulating anti-B2GPI antibodies.

Direct placental damage induced by aPL may be caused by several mechanisms including inhibition of trophoblast differentiation and syncytialization, induction of trophoblast apoptosis, impairment of trophoblast invasiveness and trophoblast expression of adhesion molecules, and also inhibition of the angiogenic factors production by trophoblasts (Tong et al., 2015). An additional mechanism for preeclampsia is related to the internalization of aPL by trophoblasts with the subsequent acceleration of cell death and release of debris that can activate maternal endothelial cells (Viall et al., 2013).

Inflammation has been described as one of the main mechanisms of aPL-induced PM, and it has recently gained additional support from an in vitro study, which showed that aPL can induce trophoblasts to produce interleukin-1 $\beta$  by inflammasome activation (Müller-Calleja et al., 2015).

A novel mechanism of trophoblast inflammation may be related to endothelial microparticles production upon exposure to aPL. MicroRNA released via exosomes may induce the trophoblast to secrete the proinflammatory cytokines IL-8 through activation of TLR 8 (Gysler et al., 2016).

Current research focuses on the identification of aPL targets on cell membranes and on the intracellular signaling pathways.

In particular, a recent study suggests that apolipoprotein E receptor 2 may be the key molecule mediating trophoblast dysfunction in a mouse model (Ulrich et al., 2016). In a different study, it was shown that TLR 4 mediated the inhibition of trophoblast invasion in vitro by purified aPL IgG from patients with OAPS, but this effect was not observed when aPL IgG from non-OAPS was used (Poulton et al., 2015).

## The Complement System in Antiphospholipid Syndrome

Complement is apparently involved in APS as antibodies cannot exert their pathogenic effect in animals that lack complement factors or receptors. This effect has been observed in both pregnancy loss (Holers et al., 2002) and in a thrombosis model (Fischetti et al., 2005). If the binding of aPL to their target cells (endothelial cells, trophoblasts) can activate complement, several activation molecules can be released. A recently described damage mechanism focused on C5a as a possible activator of neutrophils via its receptor on the cell surface (Girardi, 2010); according to this model, the consequence of C5a binding to neutrophils is TF production that can exert procoagulant and proinflammatory actions, and these can cause both thrombosis and fetal loss. Direct evidence that activation of the complement system is an important mechanism for aPL-mediated thrombosis was demonstrated in an APS mouse model of induced thrombosis (Romay-Penabad et al., 2014). In this study, the thrombosis was induced in a femoral vein pinch manner, and it was found that mice treated with IgG from APS patients with high levels of aPL developed larger thrombi and higher soluble TF activity than controls. Furthermore, the coadministration of rEV576 (coversin), a recombinant protein inhibitor of C5 activation, resulted in significantly smaller thrombi and reduced TF activity (Romay-Penabad et al., 2014). These data suggest that complement inhibition may significantly reduce aPL-mediated venous thrombosis and TF production. Hypocomplementemia was frequently observed in a cohort of 36 patients with primary APS, reflecting complement activation and consumption, and was also correlated with LAC activity but not with particular

clinical manifestations (Oku et al., 2009). In contrast, one large study that included 2399 aPL-positive SLE patients found that in aCL-positive patients, the presence of hypocomplementemia (both low C3 and C4) was strongly associated with DVT (Durcan et al., 2016).

The assumption that the complement system is involved in pregnancy morbidities in APS patients is supported by histopathological evaluation of the placentae in women with aPL (Viall and Chamley, 2015). While lower complement levels were observed in patients with OAPS, no correlation has been found between hypocomplementemia and obstetric complications in these patients (Reggia et al., 2012).

The reason of complement activation in APS has not been fully clarified yet. One recent study that included primary APS patients detected the presence of antibodies directed against C1q which is the initiator of the classical complement pathway. These antibodies bind to C1q on anionic phospholipids and accelerate complement activation. Thus autoantibodies against C1q were detected in 36% of APS patients and their titers were significantly higher compared to normal healthy controls. Neither the prevalence nor the titers of anti-C1q antibodies were correlated with a specific manifestation of APS (arterial thrombosis, venous thrombosis, PM) and no correlation was found between titers of anti-C1q and titers of aPL. In addition, patients with refractory APS tended to have higher serum titers of anti-C1q antibodies than patients with nonrefractory APS (Oku et al., 2016). It has been suggested that the presence of aPL alone is not enough to cause thrombus formation. Thus the activation of the complement pathway due to the existence of anti-C1q antibodies may play an additional role in the pathogenesis of APS.

## MORTALITY IN THE ANTIIPHOSPHOLIPID SYNDROME

The mortality rate in patients with APS is still relatively high despite the current treatment. One of the most prominent studies that investigated mortality in APS patients was performed within the Euro-Phospholipid project (Cervera et al., 2015). In this study, the mortality rate observed during a 10-year period (1999–2009) was 9.3% (5.3% in the first 5-year period and 4% in the second 5-year period), and the mean age of death was 59 years. No differences were detected in the mortality rate or the causes of death between patients with primary APS and patients with APS associated with SLE. Similarly, there were no differences in the causes of death between patients receiving different treatments (immunosuppressive or anticoagulant agents). The causes of death were severe thrombotic events including MI, STs, and PE (36.5%), followed by infections (26.9%) and hemorrhages (10.7%). In addition, among the nine patients who developed CAPS, five patients (55.6%) died. Despite the similar mortality rates in both the first and second 5-year periods of the study, there were some differences in the causes of death: fatal thrombotic events were more frequent in the first period, while malignancies were more frequent during the second period. According to this study, no clinical or immunological parameter with prognostic significance for mortality was identified.

## TREATMENT OF ANTIIPHOSPHOLIPID SYNDROME

In contrast to the large body of evidence on the pathogenesis, mechanisms, and diagnosis of APS, there is still a gap in our knowledge on appropriate therapy for patients affected by this disease. The management of patients with APS is currently directed to antithrombotic medications. Balancing an individual's risk of thrombosis against the benefits and risks of antithrombotic therapies is crucial for optimizing management and preventing morbidity in patients with APS or aPL-positive-asymptomatic ones.

Primary thromboprophylaxis in aPL carriers is mainly based on controlling any additional vascular risk factors that should be treated according to the cardiovascular disease prevention guidelines for the general population. All aPL carriers should receive thromboprophylaxis with usual doses of low molecular weight heparin (LMWH) in high-risk situations including surgery, prolonged immobilization, and puerperium period.

Estrogen-containing oral contraceptives are not recommended while progestin-only contraception is considered to be safe. Low-dose aspirin (LDA, 75–100 mg/day) is recommended in subjects with persistent positivity of multiple and/or high titer aPL (Ruiz-Iraistorza et al., 2010). In accordance with a recent metaanalysis, a lower rate of first thrombotic events was found in aPL-positive patients receiving aspirin in comparison with nontreated patients (7.8% vs 15.2%,  $P < .0001$ ). According to the subgroup analysis, aspirin had a significant protective effect in asymptomatic aPL-positive individuals (OR 0.50, 95% CI 0.25–0.99), in SLE patients (OR 0.55, 95% CI 0.31–0.98), and in OAPS patients (OR 0.25, 95% CI 0.10–0.62) (Arnaud et al., 2014, 2015).

No difference in the frequency of thrombosis was observed in aPL-positive patients treated with low-intensity anticoagulation target INR (1.5 and LDA) in comparison with those treated with LDA only ([Cuadrado et al., 2014](#)).

Definite APS patients with a first venous thrombosis event should receive oral anticoagulant therapy to a target of INR 2.0–3.0. Patients with definite APS and arterial thrombosis and/or recurrent events should be treated with oral anticoagulant therapy target with an INR of over 3.0, or receive anticoagulation therapy combined with antiaggregant agents, with an INR target between 2.0 and 3.0. In addition, indefinite antithrombotic therapy is recommended in patients with definite APS and thrombosis ([Sciascia et al., 2017](#)).

It has been recently demonstrated that persistent negative aPL profile is not an indication to stop oral anticoagulant therapy in APS patients. Thus in one study, thrombosis recurrence appeared in 46% of 24 primary APS patients with persistent negative aPL profile ([Medina et al., 2017](#)).

Oral anticoagulation therapies have been developed during the last years including direct anti-Xa inhibitors, including rivaroxaban, apixaban, and edoxaban, and a direct thrombin inhibitor named dabigatran etexilate. According to the rivaroxaban in antiphospholipid syndrome trial, rivaroxaban might be an effective alternative in patients with APS and previous venous thromboembolism ([Cohen et al., 2016](#)). The use of new oral anticoagulants in APS patients with arterial events and/or high-risk aPL profile requires further investigation. With regard to pregnancy management in women with APS, after pregnancy confirmation, the patient should discontinue oral anticoagulants because of teratogenicity and switch to LDA in combination with LMWH.

LMWH is commonly prescribed at prophylactic doses in women without previous thrombosis, or therapeutic doses in women with previous thrombotic episodes ([Erkan et al., 2014; Levy et al., 2015](#)).

Several clinical studies have examined the role of immunosuppressive therapy in APS patients and aPL-positive subjects. It has been previously shown that hydroxychloroquine (HCQ) has a beneficial effect on primary arterial and venous thromboses prevention in aPL-positive individuals and in SLE patients with or without aPL ([Tektonidou et al., 2009](#)). The use of HCQ as an additional treatment in refractory APS cases is also recommended ([Negrini et al., 2017](#)). Furthermore, an addition of HCQ to the current therapy may be considered in cases of OAPS when a standard treatment with aspirin and a heparin agent has failed, or in selected cases including women with previous thrombosis and/or ischemic placenta-mediated complications ([Sciascia et al., 2016](#)).

Rituximab, an anti-CD20 chimeric monoclonal antibody, may be considered in difficult-to-treat APS patients, possibly in those with hematologic and microthrombotic/microangiopathic manifestations ([Erkan et al., 2014](#)).

In cases of CAPS, an aggressive therapy is highly recommended using anticoagulation, glucocorticoids, and plasma exchange and/or intravenous immunoglobulins ([Puente et al., 2009](#)). This combination was retrospectively assessed and found to be advantageous ([Espinosa et al., 2011](#)), noting that APS has been revealed as a complex syndrome with multiple pathophysiological mechanisms previously unknown. In this context, new therapeutic approaches have been defended and empirically tested, with potentially promising results. For patients with refractory CAPS, rituximab has been reported to be safe and effective ([Sukara et al., 2015](#)). In addition, several reports have described the successful use of eculizumab, a humanized monoclonal antibody against complement protein C5, in CAPS and severe cases of APS, including APS and thrombotic microangiopathy (reviewed in [Sciascia et al., 2017](#)).

Peptide therapy and mTOR inhibition are new potential targets in APS that require additional investigation ([Andrade and Tektonidou, 2016](#)).

## CONCLUSIONS AND FUTURE ASPECTS

APS is a systemic autoimmune disease, mediated by autoantibodies directed at phospholipids and phospholipid-binding proteins. Since its definition some 40 years ago, our understanding of the mechanisms underlining this disease has greatly improved. Nonetheless, much is yet to be accomplished regarding the definition of subgroups of APS patients, the accurate diagnosis and interpretation of criteria and noncriteria aPL, and the appropriate treatments for APS- and aPL-affected patients. These goals may be achieved by conducting well-designed, large-scale, multicenter clinical trials to explore and address the unmet needs of better, safer, and targeted management of APS.

## References

- Abou-Nassar, K., Carrier, M., Ramsay, T., Rodger, M.A., 2011. The association between antiphospholipid antibodies and placenta mediated complications: a systematic review and meta-analysis. *Thromb. Res.* 128, 77–85.
- Abreu, M.M., Danowski, A., Wahl, D.G., Amigo, M.C., Tektonidou, M., Pacheco, M.S., et al., 2015. The relevance of “non-criteria” clinical manifestations of antiphospholipid syndrome: 14th International Congress on Antiphospholipid Antibodies Technical Task Force Report on Antiphospholipid Syndrome Clinical Features. *Autoimmun. Rev.* 14, 401–414.
- Agmon-Levin, N., Shapira, Y., Selmi, C., Barzilai, O., Ram, M., Szyper-Kravitz, M., et al., 2010. A comprehensive evaluation of serum autoantibodies in primary biliary cirrhosis. *J. Autoimmun.* 34, 55–58.
- Agmon-Levin, N., Rosário, C., Katz, B.S., Zandman-Goddard, G., Meroni, P., Cervera, R., et al., 2013. Ferritin in the antiphospholipid syndrome and its catastrophic variant (cAPS). *Lupus* 22, 1327–1335.
- Agostinis, C., Durigutto, P., Sblattero, D., Borghi, M.O., Grossi, C., Guida, F., et al., 2014. A non-complement-fixing antibody to  $\beta$ 2 glycoprotein I as a novel therapy for antiphospholipid syndrome. *Blood* 123, 3478–3487.
- Ahluwalia, J., Sreedharanunni, S., Kumar, N., Masih, J., Bose, S.K., Varma, N., et al., 2016. Thrombotic primary antiphospholipid syndrome: the profile of antibody positivity in patients from North India. *Int. J. Rheum. Dis.* 19, 903–912.
- Alijotas-Reig, J., Ferrer-Oliveras, R., Ruffatti, A., Tincani, A., Lefkou, E., Bertero, M.T., et al., 2015. The European Registry on Obstetric Antiphospholipid Syndrome (EUROAPS): a survey of 247 consecutive cases. *Autoimmun. Rev.* 14, 387–395.
- Andrade, D., Tektonidou, M., 2016. Emerging therapies in antiphospholipid syndrome. *Curr. Rheumatol. Rep.* 18, 22.
- Andreoli, L., Chighizola, C.B., Banzato, A., Pons-Estel, G.J., Ramire de Jesus, G., Erkan, D., 2013a. Estimated frequency of antiphospholipid antibodies in patients with pregnancy morbidity, stroke, myocardial infarction, and deep vein thrombosis: a critical review of the literature. *Arthritis Care Res. (Hoboken)* 65, 1869–1873.
- Andreoli, L., Fredi, M., Nalli, C., Piantoni, S., Reggia, R., Dall'Ara, F., et al., 2013b. Clinical significance of IgA anti-cardiolipin and IgA anti- $\beta$ 2 glycoprotein I antibodies. *Curr. Rheumatol. Rep.* 15, 343.
- Andreoli, L., Chighizola, C.B., Nalli, C., Gerosa, M., Borghi, M.O., Pagnolato, F., et al., 2015. Clinical characterization of antiphospholipid syndrome by detection of IgG antibodies against  $\beta$ 2-glycoprotein i domain 1 and domain 4/5: ratio of anti-domain 1 to anti-domain 4/5 as a useful new biomarker for antiphospholipid syndrome. *Arthritis Rheumatol.* 67, 2196–2204.
- Appenzeller, S., Lapa, A.T., Guirau, C.R., De Carvalho, J.F., Shoenfeld, Y., 2012. Cognitive impairment in antiphospholipid syndrome: evidence from animal models. *Clin. Rheumatol.* 31, 403–406.
- Arachchilage, D.R.J., Laffan, M., 2017. Pathogenesis and management of antiphospholipid syndrome. *Br. J. Haematol.* 178 (2), 181–195.
- Arad, A., Proulle, V., Furie, R.A., Furie, B.C., Furie, B., 2011.  $\beta$ (2)-Glycoprotein-1 autoantibodies from patients with antiphospholipid syndrome are sufficient to potentiate arterial thrombus formation in a mouse model. *Blood* 117, 3453–3459.
- Arnaud, L., Mathian, A., Ruffatti, A., Erkan, D., Tektonidou, M., Cervera, R., et al., 2014. Efficacy of aspirin for the primary prevention of thrombosis in patients with antiphospholipid antibodies: an international and collaborative meta-analysis. *Autoimmun. Rev.* 13, 281–291.
- Arnaud, L., Mathian, A., Devilliers, H., Ruffatti, A., Tektonidou, M., Forastiero, R., et al., 2015. Patient-level analysis of five international cohorts further confirms the efficacy of aspirin for the primary prevention of thrombosis in patients with antiphospholipid antibodies. *Autoimmun. Rev.* 14 (3), 192–200.
- Arnson, Y., Shoenfeld, Y., Alon, E., Amital, H., 2010. The antiphospholipid syndrome as a neurological disease. *Semin. Arthritis Rheum.* 40, 97–108.
- Asherson, R.A., 1992. The catastrophic antiphospholipid syndrome. *J. Rheumatol.* 19, 508–512.
- Asherson, R.A., Cervera, R., de Groot, P.G., et al., 2003. Catastrophic antiphospholipid syndrome: international consensus statement on classification criteria and treatment guidelines. *Lupus* 12, 530–534.
- Avriel, A., Fleischer, S., Friger, M., Shovman, O., Neuman, G., Shoenfeld, Y., et al., 2016. Prediction of Antiphospholipid syndrome using annexin A5 competition assay in patients with SLE. *Clin. Rheumatol.* 35, 2933–2938.
- Bakimer, R., Fishman, P., Blank, M., Sredni, B., Djaldetti, M., Shoenfeld, Y., 1992. Induction of primary antiphospholipid syndrome in mice by immunization with a human monoclonal anticardiolipin antibody (H-3). *J. Clin. Invest.* 89, 1558–1563.
- Bertolaccini, M.L., Murru, V., Sciascia, S., Sanna, G., Khamashta, M.A., 2012. The clinical value of testing for antibodies to phosphatidylethanolamine (aPE) in patients with systemic lupus erythematosus (SLE). *Thromb. Res.* 130, 914–918.
- Bertolaccini, M.L., Sciascia, S., Murru, V., Garcia-Fernandez, C., Sanna, G., Khamashta, M.A., 2013. Prevalence of antibodies to prothrombin in solid phase (aPT) and to phosphatidylserine-prothrombin complex (aPS/PT) in patients with and without lupus anticoagulant. *Thromb. Haemost.* 109, 207–213.
- Bertolaccini, M.L., Amengual, O., Andreoli, L., Atsumi, T., Chighizola, C.B., Forastiero, R., et al., 2014. 14th International Congress on Antiphospholipid Antibodies Task Force. Report on antiphospholipid syndrome laboratory diagnostics and trends. *Autoimmun. Rev.* 13, 917–930.
- Biggiogero, M., Meroni, P.L., 2010. The geoepidemiology of the antiphospholipid antibody syndrome. *Autoimmun. Rev.* 9, A299–A304.
- Blank, M., Shoenfeld, Y., 2004. Antiphosphatidylserine antibodies and reproductive failure. *Lupus* 13, 661–665.
- Blank, M., Shoenfeld, 2010. Antiphospholipid antibody-mediated reproductive failure in antiphospholipid syndrome. *Clin. Rev. Allergy Immunol.* 38, 141–147.
- Blank, M., Shani, A., Goldberg, I., Kopolovic, J., Amigo, M.C., Magrini, L., et al., 2004. Libman-Sacks endocarditis associated with antiphospholipid syndrome and infection. *Thromb. Res.* 114, 589–592.
- Blank, M., Cohen, J., Toder, V., Shoenfeld, Y., 1991. Induction of antiphospholipid syndrome in naive mice with mouse lupus monoclonal and human polyclonal anti-cardiolipin antibodies. *Proc. Natl. Acad. Sci. U.S.A.* 88, 3069–3073.
- Bottini, N., Vang, T., Cucca, F., Mustelin, T., 2006. Role of PTPN22 in type 1 diabetes and other autoimmune diseases. *Semin. Immunol.* 18, 207–213.
- Canaud, G., Terzi, F., 2014. Inhibition of the mTORC pathway in the antiphospholipid syndrome. *N. Engl. J. Med.* 371, 1554–1555.
- Canaud, G., Bienaimé, F., Tabarin, F., Bataillon, G., Seilhean, D., Noël, L.H., et al., 2014. Inhibition of the mTORC pathway in the antiphospholipid syndrome. *N. Engl. J. Med.* 371, 303–312.

- Carmi, O., Berla, M., Shoenfeld, Y., Levy, Y., 2017. Diagnosis and management of catastrophic antiphospholipid syndrome. *Expert Rev. Hematol.* 10, 365–374.
- Cartin-Ceba, R., Peikert, T., Ashrani, A., Keogh, K., Wylam, M.E., Ytterberg, S., et al., 2014. Primary antiphospholipid syndrome-associated diffuse alveolar hemorrhage. *Arthritis Care Res. (Hoboken)* 66, 301–310.
- Cervera, R., 2017. Antiphospholipid syndrome. *Thromb. Res.* 151 (Suppl. 1), S43–S47.
- Cervera, R., Ra, A., 2008. Antiphospholipid syndrome. *Diagnostic Criteria in Autoimmune Diseases*. Humana Press.
- Cervera, R., Piette, J.C., Font, J., Khamashta, M.A., Shoenfeld, Y., Camps, M.T., et al., 2002. Antiphospholipid syndrome: clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients. *Arthritis Rheum.* 46, 1019–1027.
- Cervera, R., Khamashta, M.A., Shoenfeld, Y., Camps, M.T., Jacobsen, S., Kiss, E., et al., 2009. Morbidity and mortality in the antiphospholipid syndrome during a 5-year period: a multicentre prospective study of 1000 patients. *Ann. Rheum. Dis.* 68, 1428–1432.
- Cervera, R., Tektonidou, M.G., Espinosa, G., Cabral, A.R., Gonzalez, E.B., Erkan, D., et al., 2011a. Task force on catastrophic antiphospholipid syndrome (APS) and non-criteria APS manifestations (I): catastrophic APS, APS nephropathy and heart valve lesions. *Lupus* 20, 165–173.
- Cervera, R., Tektonidou, M.G., Espinosa, G., Cabral, A.R., Gonzalez, E.B., Erkan, D., et al., 2011b. Task force on catastrophic antiphospholipid syndrome (APS) and non-criteria APS manifestations (II): thrombocytopenia and skin manifestations. *Lupus* 20, 174–181.
- Cervera, R., Conti, F., Doria, A., Iaccarino, L., Valesini, G., 2012. Does seronegative antiphospholipid syndrome really exist? *Autoimmun. Rev.* 11, 581–584.
- Cervera, R., Serrano, R., Pons-Estel, G.J., Ceberio-Hualde, L., Shoenfeld, Y., de Ramón, E., et al., 2015. Morbidity and mortality in the antiphospholipid syndrome during a 10-year period: a multicentre prospective study of 1000 patients. *Ann. Rheum. Dis.* 74, 1011–1018.
- Chapman, J., Rand, J.H., Brey, R.L., Levine, S.R., Blatt, J., Khamashta, M.A., et al., 2003. Non-stroke neurological syndromes associated with antiphospholipid antibodies: evaluation of clinical and experimental studies. *Lupus* 12, 514–517.
- Chaturvedi, S., Branda, L., Geary, D., Licht, C., 2011. Primary antiphospholipid syndrome presenting as renal vein thrombosis and membranous nephropathy. *Pediatr. Nephrol.* 26, 979–985.
- Chen, P.P., Wu, M., Hahn, B.H., 2010. Some antiphospholipid antibodies bind to various serine proteases in hemostasis and tip the balance toward hypercoagulant states. *Lupus* 19, 365–369.
- Cognasse, F., Hamzeh, H., Chavarin, P., Acquart, S., Genin, C., Garraud, O., 2005. Evidence of Toll-like receptor molecules on human platelets. *Immunol. Cell Biol.* 83, 196–198.
- Cohen, H., Hunt, B.J., Efthymiou, M., Arachchilage, D.R., Mackie, I.J., Clawson, S., et al., 2016. Rivaroxaban versus warfarin to treat patients with thrombotic antiphospholipid syndrome, with or without systemic lupus erythematosus (RAPS): a randomised, controlled, open-label, phase 2/3, non-inferiority trial. *Lancet Haematol.* 3, e426–e436.
- Coin, M.A., Vilar-López, R., Peralta-Ramírez, I., Hidalgo-Ruzzante, N., Callejas-Rubio, J.L., Ortego-Centeno, N., et al., 2015. The role of anti-phospholipid autoantibodies in the cognitive deficits of patients with systemic lupus erythematosus. *Lupus* 24, 875–879.
- Cojocaru, I.M., Cojocaru, M., Tanasescu, R., Burcin, C., Mitu, A.C., Iliescu, I., et al., 2008. Detecting anti-prothrombin antibodies in young women with acute ischemic stroke. *Rom. J. Intern. Med.* 46, 337–341.
- Conti, F., Capozzi, A., Truglia, S., Lococo, E., Longo, A., Misasi, R., et al., 2014. The mosaic of “seronegative” antiphospholipid syndrome. *J. Immunol. Res.* 2014, 389601.
- Cuadrado, M.J., Bertolaccini, M.L., Seed, P.T., Tektonidou, M.G., Aguirre, A., Mico, L., et al., 2014. Low-dose aspirin vs low-dose aspirin plus low-intensity warfarin in thromboprophylaxis: a prospective, multicentre, randomized, open, controlled trial in patients positive for anti-phospholipid antibodies (ALIWAPAS). *Rheumatology (Oxford)* 53 (2), 275–284.
- Danowski, A., de Azevedo, M.N., de Souza Papi, J.A., Petri, M., 2009. Determinants of risk for venous and arterial thrombosis in primary anti-phospholipid syndrome and in antiphospholipid syndrome with systemic lupus erythematosus. *J. Rheumatol.* 36, 1195–1199.
- de Groot, P.G., Horbach, D.A., Simmelink, M.J., Van Oort, E., Derkzen, R.H., 1998. Anti-prothrombin antibodies and their relation with thrombosis and lupus anticoagulant. *Lupus* 7 (Suppl. 2), S32–S36.
- de Groot, P.G., Meijers, J.C., Urbanus, R.T., 2012. Recent developments in our understanding of the antiphospholipid syndrome. *Int. J. Lab. Hematol.* 34, 223–231.
- de Laat, B., Derkzen, R.H., Urbanus, R.T., de Groot, P.G., 2005. IgG antibodies that recognize epitope Gly40-Arg43 in domain I of beta 2-glycoprotein I cause LAC, and their presence correlates strongly with thrombosis. *Blood* 105, 1540–1545.
- de Laat, B., Pengo, V., Pabinger, I., Musial, J., Voskuyl, A.E., Bultink, I.E., et al., 2009. The association between circulating antibodies against domain I of beta2-glycoprotein I and thrombosis: an international multicenter study. *J. Thromb. Haemost.* 7, 1767–1773.
- Del Papa, N., Guidali, L., Spatola, L., Bonara, P., Borghi, M.O., Tincani, A., et al., 1995. Relationship between anti-phospholipid and anti-endothelial cell antibodies III: beta 2 glycoprotein I mediates the antibody binding to endothelial membranes and induces the expression of adhesion molecules. *Clin. Exp. Rheumatol.* 13, 179–185.
- Demetrio Pablo, R., Muñoz, P., López-Hoyos, M., Calvo, V., Riancho, L., Martínez-Taboada, V.M., 2017. Thrombocytopenia as a thrombotic risk factor in patients with antiphospholipid antibodies without disease criteria. *Med. Clin. (Barc.)* 148, 394–400.
- Devreese, K.M., de Laat, B., 2015. Mixing studies in lupus anticoagulant testing are required at least in some type of samples. *J. Thromb. Haemost.* 13, 1475–1478.
- Devreese, K.M., Pierangeli, S.S., de Laat, B., Tripodi, A., Atsumi, T., Ortell, T.L., 2014. Testing for antiphospholipid antibodies with solid phase assays: guidance from the SSC of the ISTH. *J. Thromb. Haemost.* 12, 792–795.
- Di Simone, N., Meroni, P.L., De Papa, N., Raschi, E., Caliandro, D., De Carolis, C.S., et al., 2000. Antiphospholipid antibodies affect trophoblast gonadotropin secretion and invasiveness by binding directly and through adhered beta2-glycoprotein I. *Arthritis Rheum.* 43, 140–150.
- Dima, A., Caraiola, S., Jurcut, C., Balanescu, E., Balanescu, P., Ramba, D., et al., 2015. Extended antiphospholipid antibodies screening in systemic lupus erythematosus patients. *Rom. J. Intern. Med.* 53, 321–328.
- Djokovic, A., Stojanovich, L., Kontic, M., Stanisavljevic, N., Radovanovic, S., Marisavljevic, D., et al., 2014. Association between cardiac manifestations and antiphospholipid antibody type and level in a cohort of Serbian patients with primary and secondary antiphospholipid syndrome. *Isr. Med. Assoc. J.* 16, 162–167.

- Domingues, V., Magder, L.S., Petri, M., 2016. Assessment of the independent associations of IgG, IgM and IgA isotypes of anticardiolipin with thrombosis in SLE. *Lupus Sci. Med.* 3, e000107.
- Durcan, L., Fu, W., Petri, M., 2016. OP0183 Hypocomplementemia associates with thrombosis in SLE patients with antiphospholipid antibodies. *Ann. Rheum. Dis.* 75 (Suppl. 2), 126.
- Erkan, D., Espinosa, G., Cervera, R., 2010. Catastrophic antiphospholipid syndrome: updated diagnostic algorithms. *Autoimmun. Rev.* 10, 74–79.
- Erkan, D., Aguiar, C.L., Andrade, D., Cohen, H., Cuadrado, M.J., Danowski, A., et al., 2014. 14th International Congress on Antiphospholipid Antibodies: task force report on antiphospholipid syndrome treatment trends. *Autoimmun. Rev.* 13, 685–696.
- Espinosa, G., Berman, H., Cerveraser, R., 2011. Management of refractory cases of catastrophic antiphospholipid syndrome. *Autoimmun. Rev.* 10, 664–668.
- Fabris, M., Giacomello, R., Poz, A., Pantarotto, L., Tanzi, N., Curcio, F., et al., 2014. The introduction of anti-phosphatidylserine/prothrombin autoantibodies in the laboratory diagnostic process of anti-phospholipid antibody syndrome: 6 months of observation. *Auto Immun. Highlights.* 5, 63–67.
- Fischetti, F., Durigutto, P., Pellis, V., Debeus, A., Macor, P., Bulla, R., et al., 2005. Thrombus formation induced by antibodies to beta2-glycoprotein I is complement dependent and requires a priming factor. *Blood* 106, 2340–2346.
- Frances, C., Papo, T., Wechsler, B., Laporte, J.L., Bioussé, V., Piette, J.C., 1999. Sneddon syndrome with or without antiphospholipid antibodies. A comparative study in 46 patients. *Medicine (Baltimore)* 78, 209–219.
- Frances, C., Niang, S., Laffitte, E., Pelletier, F., Costedoat, N., Piette, J.C., 2005. Dermatologic manifestations of the antiphospholipid syndrome: two hundred consecutive cases. *Arthritis Rheum.* 52, 1785–1793.
- Fredi, M., Aggogeri, E., Bettiga, E., Andreoli, L., Lazzaroni, M.G., Le Guern, V., et al., 2015. A multicenter evaluation of obstetric and maternal outcome in prospectively followed pregnant patients with confirmed positivity for antiphospholipid antibodies (aPL). *Arthritis Rheumatol.* 67 (Suppl. 10), [http://acrabstracts.org/abstract/a-multicenter-evaluation-of-obstetric-and-maternal-outcome-in-prospectively-followed-pregnant-patients-with-confirmed-positivity-for-antiphospholipid-antibodies-apl/\[abstract\]](http://acrabstracts.org/abstract/a-multicenter-evaluation-of-obstetric-and-maternal-outcome-in-prospectively-followed-pregnant-patients-with-confirmed-positivity-for-antiphospholipid-antibodies-apl/[abstract]) (accessed 06.06.17.).
- Galli, M., Comfurius, P., Maassen, C., Hemker, H.C., de Baets, M.H., van Breda-Vriesman, P.J., et al., 1990. Anticardiolipin antibodies (ACA) directed not to cardiolipin but to a plasma protein cofactor. *Lancet* 335 (8705), 1544–1547.
- Gardiner, C., Hills, J., Machin, S.J., Cohen, H., 2013. Diagnosis of antiphospholipid syndrome in routine clinical practice. *Lupus* 22, 18–25.
- Giannakopoulos, B., Krilis, S.A., 2013. The pathogenesis of the antiphospholipid syndrome. *N. Engl. J. Med.* 368, 1033–1044.
- Giannakopoulos, B., Mirarabshahi, P., Krilis, S.A., 2011. New insights into the biology and pathobiology of beta2-glycoprotein I. *Curr. Rheumatol. Rep.* 13, 90–95.
- Girardi, G., 2010. Role of tissue factor in the maternal immunological attack of the embryo in the antiphospholipid syndrome. *Clin. Rev. Allergy Immunol.* 39, 160–165.
- Gysler, S.M., Mulla, M.J., Guerra, M., Brosens, J.J., Salmon, J.E., Chamley, L.W., et al., 2016. Antiphospholipid antibody-induced miR-146a-3p drives trophoblast interleukin-8 secretion through activation of Toll-like receptor 8. *Mol. Hum. Reprod.* 22, 465–474.
- Haj-Yahia, S., Sherer, Y., Blank, M., Kaetsu, H., Smolinsky, A., Shoenfeld, Y., 2003. Anti-prothrombin antibodies cause thrombosis in a novel qualitative ex-vivo animal model. *Lupus* 12, 364–369.
- Holers, V.M., Girardi, G., Mo, L., Guthridge, J.M., Molina, H., Pierangeli, S.S., et al., 2002. Complement C3 activation is required for antiphospholipid antibody-induced fetal loss. *J. Exp. Med.* 195, 211–220.
- Hoxha, A., Ruffatti, A., Tonello, M., Bontadi, A., Salvani, E., Banzato, A., et al., 2012. Antiphosphatidylserine/prothrombin antibodies in primary antiphospholipid syndrome. *Lupus* 21, 787–789.
- Hoxha, A., Mattia, E., Tonello, M., Grava, C., Pengo, V., Ruffatti, A., 2017. Antiphosphatidylserine/prothrombin antibodies as biomarkers to identify severe primary antiphospholipid syndrome. *Clin. Chem. Lab. Med.* 55, 890–898.
- Hughes, G.R., 1983. Thrombosis, abortion, cerebral disease, and the lupus anticoagulant. *Clin. Res. (Ed.)*, Br. Med. J. 287, 1088–1089.
- Hunt, B.J., Wu, X.X., de Laat, B., Arslan, A.A., Stuart-Smith, S., Rand, J.H., 2011. Resistance to annexin A5 anticoagulant activity in women with histories for obstetric antiphospholipid syndrome. *Am. J. Obstet. Gynecol.* 205 (485), e17–e23.
- Inbar, O., Blank, M., Faden, D., Tincani, A., Lorber, M., Shoenfeld, Y., 1993. Prevention of fetal loss in experimental antiphospholipid syndrome by low-molecular-weight heparin. *Am. J. Obstet. Gynecol.* 169, 423–426.
- Iverson, G.M., Von Muhlen, C.A., Staub, H.L., Lassen, A.J., Binder, W., Norman, G.L., 2006. Patients with atherosclerotic syndrome, negative in anti-cardiolipin assays, make IgA autoantibodies that preferentially target domain 4 of beta2-GPI. *J. Autoimmun.* 27, 266–271.
- Katzav, A., Chapman, J., Shoenfeld, Y., 2003. CNS dysfunction in the antiphospholipid syndrome. *Lupus* 12, 903–907.
- Katzav, A., Shoenfeld, Y., Chapman, J., 2010. The pathogenesis of neural injury in animal models of the antiphospholipid syndrome. *Clin. Rev. Allergy Immunol.* 38, 196–200.
- Khamashita, M., Taraborelli, M., Sciascia, S., Tincani, A., 2016. Antiphospholipid syndrome. *Best Pract. Res. Clin. Rheumatol.* 30, 133–148.
- Khogeer, H., Alfattani, A., Al Kaff, M., Al Shehri, T., Khojah, O., Owaideh, T., 2015. Antiphosphatidylserine antibodies as diagnostic indicators of antiphospholipid syndrome. *Lupus* 24, 186–190.
- Kim, M.Y., Buyon, J.P., Guerra, M.M., Rana, S., Zhang, D., Laskin, C.A., et al., 2016. Angiogenic factor imbalance early in pregnancy predicts adverse outcomes in patients with lupus and antiphospholipid antibodies: results of the PROMISSE study. *Am. J. Obstet. Gynecol.* 214, 108.
- Krause, I., Blank, M., Fraser, A., Lorber, M., Stojanovich, L., Rovensky, J., et al., 2005a. The association of thrombocytopenia with systemic manifestations in the antiphospholipid syndrome. *Immunobiology* 210, 749–754.
- Krause, I., Lev, S., Fraser, A., Blank, M., Lorber, M., Stojanovich, L., et al., 2005b. Close association between valvular heart disease and central nervous system manifestations in the antiphospholipid syndrome. *Ann. Rheum. Dis.* 64, 1490–1493.
- Lackner, K.J., Müller-Calleja, N., 2016. Pathogenesis of the antiphospholipid syndrome revisited: time to challenge the dogma. *J. Thromb. Haemost.* 14, 1117–1120.
- Lambrianides, A., Turner-Stokes, T., Pericleous, C., Ehsanullah, J., Papadimitraki, E., Poulton, K., et al., 2011. Interactions of human monoclonal and polyclonal antiphospholipid antibodies with serine proteases involved in hemostasis. *Arthritis Rheum.* 63, 3512–3521.

- Lee, Y.H., Choi, S.J., Ji, J.D., Song, G.G., 2012. Association between the valine/leucine 247 polymorphism of  $\beta$ 2-glycoprotein I and susceptibility to anti-phospholipid syndrome: a meta-analysis. *Lupus* 21, 865–871.
- Leffler, J., Stojanovich, L., Shoenfeld, Y., Bogdanovic, G., Hesselstrand, R., Blom, A.M., 2014. Degradation of neutrophil extracellular traps is decreased in patients with antiphospholipid syndrome. *Clin. Exp. Rheumatol.* 32, 66–70.
- Levy, R.A., Avvad, E., Oliveira, J., Porto, L.C., 1998. Placental pathology in antiphospholipid syndrome. *Lupus* 7 (Suppl. 2), S81–S85.
- Levy, R.A., Dos Santos, F.C., de Jesús, G.R., de Jesús, N.R., 2015. Antiphospholipid antibodies and antiphospholipid syndrome during pregnancy: diagnostic concepts. *Front. Immunol.* 6, 205.
- Lockshin, M.D., Kim, M., Laskin, C.A., Guerra, M., Branch, D.W., Merrill, J., et al., 2012. Prediction of adverse pregnancy outcome by the presence of lupus anticoagulant, but not anticardiolipin antibody, in patients with antiphospholipid antibodies. *Arthritis Rheum.* 64, 2311–2318.
- Long, A.A., Ginsberg, J.S., Brill-Edwards, P., Johnston, M., Turner, C., et al., 1991. The relationship of antiphospholipid antibodies to thromboembolic disease in systemic lupus erythematosus: a cross-sectional study. *Thromb. Haemost.* 66, 520–524.
- Manukyan, D., Müller-Calleja, N., Jäckel, S., Luchmann, K., Mönnikes, R., Kiouptsi, K., et al., 2016. Cofactor-independent human antiphospholipid antibodies induce venous thrombosis in mice. *J. Thromb. Haemost.* 14, 1011–1020.
- Mari, I., Zandman-Goddard, G., Shoenfeld, Y., 2004. The systemic nature of the antiphospholipid syndrome. *Scand. J. Rheumatol.* 33, 365–372.
- Marozio, L., Curti, A., Botta, G., Canuto, E.M., Salton, L., Tavella, A.M., et al., 2011. Anti-prothrombin antibodies are associated with adverse pregnancy outcome. *Am. J. Reprod. Immunol.* 66, 404–409.
- Matsuura, E., Kobayashi, K., Koike, T., Shoenfeld, Y., Khamashta, M.A., Hughes, G.R., 2003. Oxidized low-density lipoprotein as a risk factor of thrombosis in antiphospholipid syndrome. *Lupus* 12, 550–554.
- Mavrogeni, S.I., Sfikakis, P.P., Kitas, G.D., Kolovou, G., Tektonidou, M.G., 2015. Cardiac involvement in antiphospholipid syndrome: The diagnostic role of noninvasive cardiac imaging. *Semin. Arthritis Rheum.* 45, 611–616.
- Medina, G., Briones-García, E., Cruz-Domínguez, M.P., Flórez-Durante, O.I., Jara, L.J., 2017. Antiphospholipid antibodies disappearance in primary antiphospholipid syndrome: thrombosis recurrence. *Autoimmun. Rev.* 16 (4), 352–354.
- Meijide, H., Sciascia, S., Sanna, G., Khamashta, M.A., Bertolaccini, M.L., 2013. The clinical relevance of IgA anticardiolipin and IgA anti- $\beta$ 2 glycoprotein I antiphospholipid antibodies: a systematic review. *Autoimmun. Rev.* 12, 421–425.
- Mekinian, A., Lachassinne, E., Nicaise-Roland, P., Carbillon, L., Motta, M., Vicaut, E., et al., 2013. European registry of babies born to mothers with antiphospholipid syndrome. *Ann. Rheum. Dis.* 72, 217–222.
- Merashli, M., Noureldine, M.H., Uthman, I., Khamashta, M., 2015. Antiphospholipid syndrome: an update. *Eur. J. Clin. Invest.* 45, 653–662.
- Meroni, P.L., Papa, N.D., Beltrami, B., Tincani, A., Balestrieri, G., Krilis, S.A., 1996. Modulation of endothelial cell function by antiphospholipid antibodies. *Lupus* 5, 448–450.
- Meroni, P.L., Borghi, M.O., Raschi, E., Tedesco, F., 2011. Pathogenesis of antiphospholipid syndrome: understanding the antibodies. *Nat. Rev. Rheumatol.* 7, 330–339.
- Meroni, P.L., Chighizola, C.B., Rovelli, F., Gerosa, M., 2014. Antiphospholipid syndrome in 2014: more clinical manifestations, novel pathogenic players and emerging biomarkers. *Arthritis Res. Ther.* 16, 209.
- Miyakis, S., Lockshin, M.D., Atsumi, T., Branch, D.W., Brey, R.L., Cervera, R., et al., 2006. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J. Thromb. Haemost.* 4, 295–306.
- Müller-Calleja, N., Köhler, A., Siebald, B., Canisius, A., Orning, C., Radsak, M., et al., 2015. Cofactor-independent antiphospholipid antibodies activate the NLRP3-inflammasome via endosomal NADPH-oxidase: implications for the antiphospholipid syndrome. *Thromb. Haemost.* 113, 1071–1083.
- Murthy, V., Willis, R., Romay-Penabad, Z., Ruiz-Limón, P., Martínez-Martínez, L.A., Jatwani, S., et al., 2013. Value of isolated IgA anti- $\beta$ 2-glycoprotein I positivity in the diagnosis of the antiphospholipid syndrome. *Arthritis Rheum.* 65, 3186–3193.
- Negrini, S., Pappalardo, F., Murdaca, G., Indiveri, F., Puppo, F., 2017. The antiphospholipid syndrome: from pathophysiology to treatment. *Clin. Exp. Med.* 17, 257–267.
- Nesher, G., Ilany, J., Rosenmann, D., Abraham, A.S., 1997. Valvular dysfunction in antiphospholipid syndrome: prevalence, clinical features, and treatment. *Semin. Arthritis Rheum.* 27, 27–35.
- Nojima, J., Kuratsune, H., Suehisa, E., Futsukaichi, Y., Yamanishi, H., Machii, T., et al., 2001. Anti-prothrombin antibodies combined with lupus anti-coagulant activity is an essential risk factor for venous thromboembolism in patients with systemic lupus erythematosus. *Br. J. Haematol.* 114, 647–654.
- Ochoa, E., Iriondo, M., Bielsa, A., Ruiz-Irastorza, G., Estonba, A., Zubiaga, A.M., 2013. Thrombotic antiphospholipid syndrome shows strong haplotypic association with SH2B3-ATXN2 locus. *PLoS One* 8 (7), e67897.
- Ofer-Shiber, S., Molad, Y., 2015. Frequency of vascular and pregnancy morbidity in patients with low vs. moderate-to-high titers of antiphospholipid antibodies. *Blood Coagul. Fibrinolysis.* 26, 261–266.
- Oku, K., Atsumi, T., Bohgaki, M., Amengual, O., Kataoka, H., Horita, T., et al., 2009. Complement activation in patients with primary antiphospholipid syndrome. *Ann. Rheum. Dis.* 68, 1030–1035.
- Oku, K., Amengual, O., Zigon, P., Horita, T., Yasuda, S., Atsumi, T., 2013. Essential role of the p38 mitogen-activated protein kinase pathway in tissue factor gene expression mediated by the phosphatidylserine-dependent antiprothrombin antibody. *Rheumatology (Oxford)* 52, 1775–1784.
- Oku, K., Amengual, O., Bohgaki, T., Horita, T., Yasuda, S., Atsumi, T., 2015. An independent validation of the Global Anti-Phospholipid Syndrome Score in a Japanese cohort of patients with autoimmune diseases. *Lupus* 24, 774–775.
- Oku, K., Amengual, O., Hisada, R., Ohmura, K., Nakagawa, I., Watanabe, T., et al., 2016. Autoantibodies against a complement component 1 q subcomponent contribute to complement activation and recurrent thrombosis/pregnancy morbidity in anti-phospholipid syndrome. *Rheumatology (Oxford)* 55, 1403–1411.
- Padjas, A., Plazak, W., Celińska-Lowenhoff, M., Mazurek, A., Perricone, C., Podolec, P., et al., 2016. Myocardial ischaemia, coronary atherosclerosis and pulmonary pressure elevation in antiphospholipid syndrome patients. *Adv. Clin. Exp. Med.* 25, 1199–1205.

- Pardos-Gea, J., Avegliano, G., Evangelista, A., Vilardell, M., Ordi-Ros, J., 2015. Cardiac manifestations other than valvulopathy in antiphospholipid syndrome: long-time echocardiography follow-up study. *Int. J. Rheum. Dis.* 18, 76–83.
- Pengo, V., Tripodi, A., Reber, G., Rand, J.H., Ortel, T.L., Galli, M., et al. Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis 2009. Update of the guidelines for lupus anticoagulant detection. Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis. *J. Thromb. Haemost.* 7, 1737–1740.
- Pengo, V., Ruffatti, A., Del Ross, T., Tonello, M., Cuffaro, S., Hoxha, A., et al., 2013. Confirmation of initial antiphospholipid antibody positivity depends on the antiphospholipid antibody profile. *J. Thromb. Haemost.* 11, 1527–1531.
- Pengo, V., Ruffatti, A., Tonello, M., Cuffaro, S., Banzato, A., Bison, E., et al., 2015a. Antiphospholipid syndrome: antibodies to Domain 1 of  $\beta$ 2-glycoprotein I correctly classify patients at risk. *J. Thromb. Haemost.* 13, 782–787.
- Pengo, V., Denas, G., Padayattil, S.J., Zoppellaro, G., Bison, E., Banzato, A., et al., 2015b. Diagnosis and therapy of antiphospholipid syndrome. *Pol. Arch. Med. Wewn.* 125 (9), 672–677.
- Pericleous, C., Clarke, L.A., Brogan, P.A., Latchman, D.S., Isenberg, D.A., Ioannou, Y., et al., 2013. Endothelial microparticle release is stimulated in vitro by purified IgG from patients with the antiphospholipid syndrome. *Thromb. Haemost.* 109, 72–78.
- Pericleous, C., Ruiz-Limón, P., Romay-Penabad, Z., Marín, A.C., Garza-García, A., Murfitt, L., et al., 2015. Proof-of-concept study demonstrating the pathogenicity of affinity-purified IgG antibodies directed to domain I of  $\beta$ 2-glycoprotein I in a mouse model of anti-phospholipid antibody-induced thrombosis. *Rheumatology (Oxford)* 54, 722–727.
- Pericleous, C., Ferreira, I., Borghi, O., Pagnolato, F., McDonnell, T., Garza-García, A., et al., 2016. Measuring IgA anti- $\beta$ 2-glycoprotein I and IgG/IgA anti-domain I antibodies adds value to current serological assays for the antiphospholipid syndrome. *PLoS One* 11, e0156407.
- Pierangeli, S.S., Liu, X.W., Barker, J.H., Anderson, G., Harris, E.N., 1995. Induction of thrombosis in a mouse model by IgG, IgM and IgA immunoglobulins from patients with the antiphospholipid syndrome. *Thromb. Haemost.* 74, 1361–1367.
- Pierangeli, S.S., de Groot, P.G., Dlott, J., Favaloro, E., Harris, E.N., Lakos, G., et al., 2011. Criteria" aPL tests: report of a task force and pre-conference workshop at the 13th International Congress on Antiphospholipid Antibodies, Galveston, Texas, April 2010. *Lupus* 20, 182–190.
- Poulton, K., Rahman, A., Giles, I., 2012. Examining how antiphospholipid antibodies activate intracellular signaling pathways: a systematic review. *Semin. Arthritis Rheum.* 41, 720–736.
- Poulton, K., Ripoll, V.M., Pericleous, C., Meroni, P.L., Gerosa, M., Ioannou, Y., et al., 2015. Purified IgG from patients with obstetric but not IgG from non-obstetric antiphospholipid syndrome inhibit trophoblast invasion. *Am. J. Reprod. Immunol.* 73, 390–401.
- Praprotnik, S., Agmon-Levin, N., Porat-Katz, B.S., Blank, M., Meroni, P.L., Cervera, R., et al., 2010. Prolactin's role in the pathogenesis of the antiphospholipid syndrome. *Lupus* 19, 1515–1519.
- Proulle, V., Furie, R.A., Merrill-Skoloff, G., Furie, B.C., Furie, B., 2014. Platelets are required for enhanced activation of the endothelium and fibrinogen in a mouse thrombosis model of APS. *Blood* 124, 611–622.
- Puente, D., Pombo, G., Forastiero, R., 2009. Current management of antiphospholipid syndrome-related thrombosis. *Expert. Rev. Cardiovasc. Ther.* 7, 1551–1558.
- Reggia, R., Ziglioli, T., Andreoli, L., Bellisai, F., Iuliano, A., Gerosa, M., et al., 2012. Primary antiphospholipid syndrome: any role for serum complement levels in predicting pregnancy complications? *Rheumatology (Oxford)* 51, 2186–2190.
- Reynaud, Q., Lega, J.C., Mismetti, P., Chapelle, C., Wahl, D., Cathébras, P., et al., 2014. Risk of venous and arterial thrombosis according to type of antiphospholipid antibodies in adults without systemic lupus erythematosus: a systematic review and meta-analysis. *Autoimmun. Rev.* 13, 595–608.
- Rodrigues, C.E., Carvalho, J.F., Shoenfeld, Y., 2010. Neurological manifestations of antiphospholipid syndrome. *Eur. J. Clin. Invest.* 40, 350–359.
- Rodríguez-García, J.L., Bertolaccini, M.L., Cuadrado, M.J., Sanna, G., Ateka-Barrutia, O., Khamashta, M.A., 2012. Clinical manifestations of antiphospholipid syndrome (APS) with and without antiphospholipid antibodies (the so-called "seronegative APS"). *Ann. Rheum. Dis.* 71, 242–244.
- Rodríguez-García, V., Ioannou, Y., Fernández-Nebro, A., Isenberg, D.A., Giles, I.P., 2015. Examining the prevalence of non-criteria anti-phospholipid antibodies in patients with anti-phospholipid syndrome: a systematic review. *Rheumatology (Oxford)* 54 (11), 2042–2050.
- Rodríguez-Pintó, I., Moitinho, M., Santacreu, I., Shoenfeld, Y., Erkan, D., Espinosa, G., et al., 2016. Catastrophic antiphospholipid syndrome (CAPS): descriptive analysis of 500 patients from the International CAPS Registry. *Autoimmun. Rev.* 15, 1120–1124.
- Roggewick, D., Borghi, M.O., Somma, V., Büttner, T., Schierack, P., Hanack, K., et al., 2016. Antiphospholipid antibodies detected by line immunoassay differentiate among patients with antiphospholipid syndrome, with infections and asymptomatic carriers. *Arthritis Res. Ther.* 18, 111.
- Romay-Penabad, Z., Carrera Marin, A.L., Willis, R., Weston-Davies, W., Machin, S., Cohen, H., et al., 2014. Complement C5-inhibitor rEV576 (coversin) ameliorates in-vivo effects of antiphospholipid antibodies. *Lupus* 23, 1324–1326.
- Rottem, M., Krause, I., Fraser, A., Stojanovich, L., Rovensky, J., Shoenfeld, Y., 2006. Autoimmune hemolytic anaemia in the antiphospholipid syndrome. *Lupus* 15, 473–477.
- Ruiz-Irastorza, G., Crowther, M., Branch, W., Khamashta, M.A., 2010. Antiphospholipid syndrome. *Lancet* 376 (9751), 1498–1509.
- Samarkos, M., Davies, K.A., Gordon, C., Loizou, S., 2006. Clinical significance of IgA anticardiolipin and anti-beta2-GP1 antibodies in patients with systemic lupus erythematosus and primary antiphospholipid syndrome. *Clin. Rheumatol.* 25, 199–204.
- Saponjski, J., Stojanovich, L., Djokovic, A., Petkovic, M., Mrda, D., 2011. Systemic vascular diseases in the antiphospholipid syndrome. What is the best diagnostic choice? *Autoimmun. Rev.* 10, 235–237.
- Sater, M.S., Finan, R.R., Abu-Hijleh, F.M., Abu-Hijleh, T.M., Almawi, W.Y., 2012. Anti-phosphatidylserine, anti-cardiolipin, anti-beta2 glycoprotein I and anti-prothrombin antibodies in recurrent miscarriage at 8–12 gestational weeks. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 163, 170–174.
- Sciascia, S., Sanna, G., Murru, V., Roccatello, D., Khamashta, M.A., Bertolaccini, M.L., 2013. GAPSS: the Global Anti-Phospholipid Syndrome Score. *Rheumatology (Oxford)* 52, 1397–1403.

- Sciascia, S., Sanna, G., Murru, V., Roccatello, D., Khamashta, M.A., Bertolaccini, M.L., 2014. Anti-prothrombin (aPT) and anti-phosphatidylserine/prothrombin (aPS/PT) antibodies and the risk of thrombosis in the antiphospholipid syndrome. A systematic review. *Thromb. Haemost.* 111, 354–364.
- Sciascia, S., Sanna, G., Murru, V., Roccatello, D., Khamashta, M.A., Bertolaccini, M.L., 2015. The global anti-phospholipid syndrome score in primary APS. *Rheumatology (Oxford)* 54, 134–138.
- Sciascia, S., Branch, D.W., Levy, R.A., Middeldorp, S., Pavord, S., Roccatello, D., et al., 2016. The efficacy of hydroxychloroquine in altering pregnancy outcome in women with antiphospholipid antibodies. *Evid. Clin. Judgment* 115 (2), 285–290.
- Sciascia, S., Radin, M., Bazzan, M., Roccatello, D., 2017. Novel diagnostic and therapeutic frontiers in thrombotic anti-phospholipid syndrome. *Intern. Emerg. Med.* 12 (1), 1–7.
- Sebastiani, G.D., Iuliano, A., Cantarini, L., Galeazzi, M., 2016. Genetic aspects of the antiphospholipid syndrome: an update. *Autoimmun. Rev.* 15, 433–439.
- Serrano, A., Garcia, F., Serrano, M., Ramirez, E., Alfaro, F.J., Lora, D., et al., 2012. IgA antibodies against beta2 glycoprotein I in hemodialysis patients are an independent risk factor for mortality. *Kidney Int.* 81, 1239–1244.
- Shachaf, S., Yair, M., 2016. The correlation between antiphospholipid syndrome and cryoglobulinemia: case series of 4 patients and review of the literature. *Rev. Bras. Reumatol. Engl. Ed.* 56, 2–7.
- Sherer, Y., Hassin, Y., Shoenfeld, Y., Levy, Y., Livneh, A., Ohry, A., et al., 2002. Transverse myelitis in patients with antiphospholipid antibodies – the importance of early diagnosis and treatment. *Clin. Rheumatol.* 21, 207–210.
- Shoenfeld, Y., 2007. APS—more systemic disease than SLE. *Clin. Rev. Allergy Immunol.* 32, 129–130.
- Shoenfeld, Y., Krause, I., Kvapil, F., Sulkes, J., Lev, S., Von Landenberg, P., et al., 2003. Prevalence and clinical correlations of antibodies against six beta2-glycoprotein-I-related peptides in the antiphospholipid syndrome. *J. Clin. Immunol.* 23, 377–383.
- Shoenfeld, Y., Lev, S., Blatt, I., Blank, M., Font, J., Von Landenberg, P., et al., 2004. Features associated with epilepsy in the antiphospholipid syndrome. *J. Rheumatol.* 31, 1344–1348.
- Shoenfeld, Y., Gerli, R., Doria, A., Matsuura, E., Cerinic, M.M., Ronda, N., et al., 2005. Accelerated atherosclerosis in autoimmune rheumatic diseases. *Circulation* 112, 3337–3347.
- Shoenfeld, Y., Meroni, P.L., Cervera, R., 2008a. Antiphospholipid syndrome dilemmas still to be solved: 2008 status. *Ann. Rheum. Dis.* 67, 438–442.
- Shoenfeld, Y., Twig, G., Katz, U., Sherer, Y., 2008b. Autoantibody explosion in antiphospholipid syndrome. *J. Autoimmun.* 30, 74–83.
- Shoenfeld, Y., Meroni, P.L., Toubi, E., 2009. Antiphospholipid syndrome and systemic lupus erythematosus: are they separate entities or just clinical presentations on the same scale? *Curr. Opin. Rheumatol.* 21, 495–500.
- Silver, R.M., Parker, C.B., Reddy, U.M., Goldenberg, R., Coustan, D., Dudley, D.J., et al., 2013. Antiphospholipid antibodies in stillbirth. *Obstet. Gynecol.* 122, 641–657.
- Simchen, M.J., Dulitzki, M., Rofe, G., Shani, H., Langevitz, P., Schiff, E., et al., 2011. High positive antibody levels and adverse pregnancy outcome in women with antiphospholipid syndrome. *Acta Obstet. Gynecol. Scand.* 90, 1428–1433.
- Soltész, P., Szekanecz, Z., Kiss, E., Shoenfeld, Y., 2007. Cardiac manifestations in antiphospholipid syndrome. *Autoimmun. Rev.* 6, 379–386.
- Staub, H.L., Von Muhlen, C.A., Norman, G.L., 2006. Beta2-glycoprotein I IgA antibodies and ischaemic stroke. *Rheumatology (Oxford)* 45, 645–646.
- Stojanovich, L., Kontic, M., Djokovic, A., Ilijevski, N., Stanisavljevic, N., Marisavljevic, D., 2012a. Pulmonary events in antiphospholipid syndrome: influence of antiphospholipid antibody type and levels. *Scand. J. Rheumatol.* 41, 223–226.
- Stojanovich, L., Markovic, O., Marisavljevic, D., Elezovic, I., Ilijevski, N., Stanisavljevic, N., 2012b. Influence of antiphospholipid antibody levels and type on thrombotic manifestations: results from the Serbian National Cohort Study. *Lupus* 21, 338–345.
- Sugi, T., Matsabayashi, H., Inomo, A., Dan, L., Makino, T., 2004. Antiphosphatidylethanolamine antibodies in recurrent early pregnancy loss and mid-to-late pregnancy loss. *J. Obstet. Gynaecol. Res.* 30, 326–332.
- Sukara, G., Baresic, M., Sentic, M., Brčić, L., Anic, B., 2015. Catastrophic antiphospholipid syndrome associated with systemic lupus erythematosus treated with rituximab: case report and a review of the literature. *Acta Reumatol. Port.* 40, 169–175.
- Sweiss, N.J., Bo, R., Kapadia, R., Manst, D., Mahmood, F., Adhikari, T., et al., 2010. IgA anti-beta2-glycoprotein I autoantibodies are associated with an increased risk of thromboembolic events in patients with systemic lupus erythematosus. *PLoS One* 5, e12280.
- Taraborelli, M., Andreoli, L., Tincani, A., 2012. Much more than thrombosis and pregnancy loss: the antiphospholipid syndrome as a 'systemic disease'. *Best Pract. Res. Clin. Rheumatol.* 26, 79–90.
- Tektonidou, M.G., 2014. Identification and treatment of APS renal involvement. *Lupus* 23, 1276–1278.
- Tektonidou, M.G., Ioannidis, J.P., Moyssakis, I., Boki, K.A., Vassiliou, V., Vlachoyiannopoulos, P.G., et al., 2001. Right ventricular diastolic dysfunction in patients with anticardiolipin antibodies and antiphospholipid syndrome. *Ann. Rheum. Dis.* 60, 43–48.
- Tektonidou, M.G., Laskari, K., Panagiotakos, D.B., Moutsopoulos, H.M., 2009. Risk factors for thrombosis and primary thrombosis prevention in patients with systemic lupus erythematosus with or without antiphospholipid antibodies. *Arthritis Rheum.* 61 (1), 29–36.
- Tong, M., Viall, C.A., Chamley, L.W., 2015. Antiphospholipid antibodies and the placenta: a systematic review of their in vitro effects and modulation by treatment. *Hum Reprod Update* 21, 97–118.
- Toubi, E., Shoenfeld, Y., 2007. Livedo reticularis as a criterion for antiphospholipid syndrome. *Clin. Rev. Allergy Immunol.* 32, 138–144.
- Toubi, E., Krause, I., Fraser, A., Lev, S., Stojanovich, L., Rovensky, J., et al., 2005. Livedo reticularis is a marker for predicting multisystem thrombosis in antiphospholipid syndrome. *Clin. Exp. Rheumatol.* 23, 499–504.
- Ulrich, V., Gelber, S.E., Vukelic, M., Sacharidou, A., Herz, J., Urbanus, R.T., et al., 2016. ApoE receptor 2 mediation of trophoblast dysfunction and pregnancy complications induced by antiphospholipid antibodies in mice. *Arthritis Rheumatol.* 68, 730–739.
- Urbanus, R.T., Pennings, M.T., Derkx, R.H., de Groot, P.G., 2008. Platelet activation by dimeric beta2-glycoprotein I requires signaling via both glycoprotein Ib alpha and apolipoprotein E receptor 20. *J. Thromb. Haemost.* 6, 1405–1412.
- Velayuthaprabhu, S., Matsabayashi, H., Sugi, T., Nakamura, M., Ohnishi, Y., Ogura, T., et al., 2011. A unique preliminary study on placental apoptosis in mice with passive immunization of anti-phosphatidylethanolamine antibodies and anti-factor XII antibodies. *Am. J. Reprod. Immunol.* 66, 373–384.

- Viall, C.A., Chamley, L.W., 2015. Histopathology in the placentae of women with antiphospholipid antibodies: a systematic review of the literature. *Autoimmun. Rev.* 14, 446–471.
- Viall, C.A., Chen, Q., Liu, B., Hickey, A., Snowise, S., Salmon, J.E., et al., 2013. Antiphospholipid antibodies internalised by human syncytiotrophoblast cause aberrant cell death and the release of necrotic trophoblast debris. *J. Autoimmun.* 47, 45–57.
- Vikerfors, A., Mobarrez, F., Bremme, K., Holmström, M., Ågren, A., Eelde, A., et al., 2012. Studies of microparticles in patients with the anti-phospholipid syndrome (APS). *Lupus* 21, 802–805.
- Vlachoyiannopoulos, P.G., Routsias, J.G., 2010. A novel mechanism of thrombosis in antiphospholipid antibody syndrome. *J. Autoimmun.* 35, 248–255.
- Vlagea, A., Gil, A., Cuesta, M.V., Arribas, F., Diez, J., Lavilla, P., et al., 2013. Antiphosphatidylserine/prothrombin antibodies (aPS/PT) as potential markers of antiphospholipid syndrome. *Clin. Appl. Thromb. Hemost.* 19, 289–296.
- Von Landenberg, P., Matthias, T., Zaech, J., Schultz, M., Lorber, M., Blank, M., et al., 2003. Antiprothrombin antibodies are associated with pregnancy loss in patients with the antiphospholipid syndrome. *Am. J. Reprod. Immunol.* 49, 51–56.
- Watad, A., Tiosano, S., Grysman, N., Comaneshter, D., Cohen, A.D., Shoenfeld, Y., et al., 2017. The association between systemic lupus erythematosus and valvular heart disease: an extensive data analysis. *Eur. J. Clin. Invest.* 47, 366–371.
- Wilson, W.A., Gharavi, A.E., Koike, T., Lockshin, M.D., Branch, D.W., Piette, J.C., et al., 1999. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. *Arthritis Rheum.* 42, 1309–1311.
- Wu, L.H., Yu, F., Tan, Y., Qu, Z., Chen, M.H., Wang, S.X., et al., 2013. Inclusion of renal vascular lesions in the 2003 ISN/RPS system for classifying lupus nephritis improves renal outcome predictions. *Kidney Int.* 83, 715–723.
- Yalavarthi, S., Gould, T.J., Rao, A.N., Mazza, L.F., Morris, A.E., Núñez-Álvarez, C., et al., 2015. Antiphospholipid antibodies promote the release of neutrophil extracellular traps: a new mechanism of thrombosis in the antiphospholipid syndrome. *Arthritis Rheumatol.* 67, 2990–3003.
- Yamada, M., Kawakami, T., Takashima, K., Nishioka, Y., Nishibata, Y., Masuda, S., et al., 2017. Establishment of a rat model of thrombosis induced by intravenous injection of anti-phosphatidylserine-prothrombin complex antibody. *Rheumatology (Oxford)* 56, 1013–1018.
- Yang, C.D., Hwang, K.K., Yan, W., Gallagher, K., Fitzgerald, J., Grossman, J.M., et al., 2004. Identification of anti-plasmin antibodies in the antiphospholipid syndrome that inhibit degradation of fibrin. *J. Immunol.* 172, 5765–5773.
- Yelnik, C.M., Laskin, C.A., Porter, T.F., Branch, D.W., Buyon, J.P., Guerra, M.M., et al., 2016. Lupus anticoagulant is the main predictor of adverse pregnancy outcomes in aPL-positive patients: validation of PROMISSE study results. *Lupus Sci. Med.* 3, e000131.
- Yin, H., Borghi, M.O., Delgado-Vega, A.M., Tincani, A., Meroni, P.L., Alarcón-Riquelme, M.E., 2009. Association of STAT4 and BLK, but not BANK1 or IRF5, with primary antiphospholipid syndrome. *Arthritis Rheum.* 60, 2468–2471.
- Zandman-Goddard, G., Orbach, H., Agmon-Levin, N., Boaz, M., Amital, H., Szekanecz, Z., et al., 2013. Hyperferritinemia is associated with serologic antiphospholipid syndrome in SLE patients. *Clin. Rev. Allergy Immunol.* 44, 23–30.
- Zhang, S., Wu, Z., Chen, S., Li, J., Wen, X., Li, L., et al., 2016. Evaluation of the diagnostic potential of antibodies to beta2-glycoprotein 1 domain 1 in Chinese patients with antiphospholipid syndrome. *Sci. Rep.* 6, 23839.
- Zhang, S., Wu, Z., Li, J., Wen, X., Li, L., Zhang, W., et al., 2017. Evaluation of the clinical relevance of anti-annexin-A5 antibodies in Chinese patients with antiphospholipid syndrome. *Clin. Rheumatol.* 36, 407–412.
- Zhang, Z., Zhang, R., 2015. Epigenetics in autoimmune diseases: pathogenesis and prospects for therapy. *Autoimmun. Rev.* 14, 854–863.
- Zhu, L., Li, C., Liu, N., Yang, X., Jia, R.L., Mu, R., et al., 2017. Diagnostic value of antibodies to phosphatidylserine/prothrombin complex for antiphospholipid syndrome in Chinese patients. *Clin. Rheumatol.* 36 (2), 401–406.
- Ziporen, L., Goldberg, I., Arad, M., Hojnik, M., Ordi-Ros, J., Afek, A., et al., 1996. Libman-Sacks endocarditis in the antiphospholipid syndrome: immunopathologic findings in deformed heart valves. *Lupus* 5, 196–205.
- Ziporen, L., Shoenfeld, Y., Levy, Y., Korczyn, A.D., 1997. Neurological dysfunction and hyperactive behavior associated with antiphospholipid antibodies. A mouse model. *J. Clin. Invest.* 100, 613–619.
- Zuily, S., de Laat, B., Mohamed, S., Kelchtermans, H., Shums, Z., Albesa, R., et al., 2015. Validity of the global anti-phospholipid syndrome score to predict thrombosis: a prospective multicentre cohort study. *Rheumatology (Oxford)* 54, 2071–2075.
- Zuily, S., Domingues, V., Suty-Selton, C., Eschwège, V., Bertoletti, L., Chaouat, A., et al., 2017. Antiphospholipid antibodies can identify lupus patients at risk of pulmonary hypertension: a systematic review and meta-analysis. *Autoimmun. Rev.* 16, 576–586.
- Žigon, P., Perdan Pirkmajer, K., Tomšić, M., Kveder, T., Božič, B., Sodin Šemrl, S., et al., 2015. Anti-phosphatidylserine/prothrombin antibodies are associated with adverse pregnancy outcomes. *J. Immunol. Res.* 2015, 975704.

## Further Reading

- Agmon-Levin, N., Blank, M., Zandman-Goddard, G., Orbach, H., Meroni, P.L., Tincani, A., et al., 2011. Vitamin D: an instrumental factor in the anti-phospholipid syndrome by inhibition of tissue factor expression. *Ann. Rheum. Dis.* 70, 145–150.
- Cimaz, R., Meroni, P.L., Shoenfeld, Y., 2006. Epilepsy as part of systemic lupus erythematosus and systemic antiphospholipid syndrome (Hughes syndrome). *Lupus* 15, 191–197.
- de Groot, P.G., Meijers, J.C., 2011. beta(2)-Glycoprotein I: evolution, structure and function. *J. Thromb. Haemost.* 9, 1275–1284.
- Di Prima, F.A., Valenti, O., Hyseni, E., Giorgio, E., Faraci, M., Renda, E., et al., 2011. Antiphospholipid syndrome during pregnancy: the state of the art. *J. Prenat. Med.* 5, 41–53.
- Erkan, D., Vega, J., Ramón, G., Kozora, E., Lockshin, M.D., 2013. A pilot open-label phase II trial of rituximab for non-criteria manifestations of antiphospholipid syndrome. *Arthritis Rheum.* 65 (2), 464–471.

## 33

# Sjogren's Syndrome

*Julian L. Ambrus, Jr.*

Division of Allergy, Immunology and Rheumatology, SUNY at Buffalo School of Medicine, Buffalo, NY, United States

## OUTLINE

General Introduction	635	Lymphoma and Other Hematological Manifestations	641
Historical Aspects	636	Pathological Features	641
Epidemiology	636	Autoimmune Features	643
Clinical Features and Disease Associations	637	Genetics	643
Eyes	637	Animal Models Including Relevance	644
Oral Cavity	637	Diagnostic Procedures	646
Lungs	637	Treatment	647
Kidney	638	Perspectives	649
Gastrointestinal Tract	638	References	650
Nervous System	639		
Genitourinary	639		
Vascular System	640		
Musculoskeletal and Constitutional Symptoms	640		

## GENERAL INTRODUCTION

Sjogren's syndrome (SS) is one of the most common autoimmune diseases. It may exist as either a primary syndrome or as a secondary syndrome in association with other autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus (SLE), systemic sclerosis, and primary biliary cirrhosis (Brito-Zerón et al., 2016; Delaleu et al., 2005; Fox, 2005; Mavragani and Moutsopoulos, 2014). As with all autoimmune diseases, there is a great deal of clinical variability such that some patients may only have dry eyes and/or dry mouth, while others may have systemic manifestations including lung disease, kidney disease, and lymphoma. The 2016 American College of Rheumatology (ACR)—European League Against Rheumatism (EULAR) criteria for SS include symptoms of oral and/or ocular dryness or one extraglandular manifestation along with object indicators including a minor salivary gland biopsy showing lymphocytic infiltration, anti-Ro antibodies, positive ocular staining score, reduced Schirmer's test, and/or reduced unstimulated salivary flow (Shiboski et al., 2017). These criteria have undergone and will continue to undergo revision as more is learned about the disease and its protean manifestations. Because of its often-subtle presentation, SS may not be identified in patients until they have had the disease for several years (Akpek et al., 2015; Beckman et al., 2017).

Treatment of reversible manifestations, however, requires early identification. Animal models and genetic studies have provided novel insights that may lead to early identification of the disease and improved forms of therapy (Burbelo et al., 2014; Delaleu et al., 2011). I will highlight in this chapter the current understanding of SS and introduce some of the areas where current and future research is directed.

## HISTORICAL ASPECTS

Dr. Johann von Mikulicz-Rdeck, a surgeon from Cernowitz, Austria, is credited with identifying the first patient with SS in 1892 based on round-cell infiltrate and acinar atrophy of the parotid and lacrimal glands (Fox, 2005; Parke and Buchanan, 1998). The syndrome is named after an ophthalmologist from Jonkoping, Sweden, Dr. Henrik Sjogren, who noted a patient with low secretions from the salivary and lacrimal glands in 1930. He subsequently published series of patients with "keratoconjunctivitis sicca" in 1933 and 1951 that brought the syndrome to worldwide medical attention (Parke and Buchanan, 1998; Sjogren, 1951). He importantly distinguished the primary disease from other causes of keratoconjunctivitis sicca, such as vitamin A deficiency and tuberculosis. In the 1950s and 1960s SS was felt to be a relatively rare disorder and the appreciation of its association with other autoimmune diseases was just starting (Bloch et al., 1965). In the 1970s immunological abnormalities associated with SS, especially hypergammaglobulinemia, monoclonal gammopathy, and various lymphoid abnormalities, were just being elucidated. The association between SS and lymphomas was noted. Minor salivary gland biopsies were used for diagnosis. Immunological therapies, such as the use of corticosteroids, were attempted. The association of SS with HLA-B8 and DR3 was made. Animal models for SS were first examined (Anderson and Talal, 1972; Keyes et al., 1977; Strand and Talal, 1979). In the 1980s came the association of SS with anti-Sjogren's syndrome A (SSA) and anti-Sjogren's syndrome B (SSB) antibodies (Eisenberg, 1985). SS was noted to have a strong correlation with chronic fatigue. Cholinergic agents were used to increase salivary gland flow (Fox, 1987). Immunological studies focused on the hypothesis that SS involves abnormal B cells and T cells of the adaptive immune system producing autoreactive antibodies and direct cytotoxicity (Moutsopoulos, 1988). Genetic studies identified particular human leukocyte antigen (HLA) associations with SS (Harley et al., 1986). The 21st century has seen a blossoming of research in SS that has led to improved understanding of clinical manifestations, greater appreciation of disease pathophysiology including the involvement of the innate immune system, role of epithelial cells and other cell types, determination of contributory genetic factors, development of improved animal models, and therapeutic trials with encouraging outcomes. These will be discussed in the remainder of this chapter.

## EPIDEMIOLOGY

The true prevalence of SS is very hard to determine because of the frequent changes in diagnostic criteria, variation in the method of diagnosis based on the training of the clinician making the diagnosis (i.e., ophthalmologist vs oral surgeon vs rheumatologist vs internist), and novel diagnostic markers that are potentially more sensitive. While SS was once considered a rare disease, it is now considered the second most common autoimmune disease, after rheumatoid arthritis. Its prevalence is estimated at 1% (0.1%–4.8%) with an incidence of 7 per 100,000 in the United States. It is felt that roughly 4 million Americans have SS with 90% of them being women and 50% of them having SS in association with another autoimmune disease (Helmick et al., 2008; Reksten and Jonsson, 2014; Thomas et al., 1998). The incidence of SS is felt to be lower in China and higher in Japan (Qin et al., 2015). While SS is felt to be a disease predominantly of women, with a female-to-male ratio of 9:1, the incidence of SS is likely higher in males than is currently estimated, because males tend to make a different pattern of autoantibodies than females and are often missed with the current diagnostic criteria (Beckman et al., 2017; Gondran et al., 2008). The reason for the difference in the estimated prevalence of SS in various countries is likely due to many factors, but certainly different genetic backgrounds and environmental influences could explain some of these variations. SS, depending upon how it is defined, may in fact turn out to be more common than rheumatoid arthritis.

## CLINICAL FEATURES AND DISEASE ASSOCIATIONS

As with all autoimmune diseases, the expression of SS is due to combinations of various genetic and environmental factors that result in wide variability in phenotype in different patients. The clinical manifestations that define the disease are dry eyes and dry mouth. There are patients with predominantly dry eyes without dry mouth, patient with predominantly dry mouth with minimal dry eyes, and patients with both. Some of these patients will go on to have extraglandular manifestations, but some will not. It is also possible that some patients have bouts of autoimmune salivary and/or lacrimal gland injury with recovery before permanent damage to the salivary and/or lacrimal glands is noted. It is estimated that the average SS patient has had disease for 3 years before they are diagnosed (Akpek et al., 2015).

### Eyes

Involvement of the eyes is one of the defining features of SS. Dry eye disease is one of the most common diseases seen by ophthalmologists and can be due to environmental toxins, drugs, various infections, genetic factors accelerated by aging, various diseases including sarcoidosis, radiation therapy, malignancies, and SS (Abelson et al., 2009; Akpek et al., 2015; Basak et al., 2012; Gayton, 2009; Li et al., 2015a). It has been estimated in the United States that as many as 25% of the dry eye patients have SS. In China, one study estimated that only 1.9% of the dry eye patients had SS (Akpek et al., 2015; Li et al., 2015b). Both primary and secondary SS are included in these numbers.

Patients note dryness, a feeling of gravel in their eyes, and often associated pain in their eyes. These symptoms are often responsible for life alterations (Akpek et al., 2015). Patients can have problems with both aqueous and lipid secretions as well as meibomian gland dysfunction. Complications in the eye can include corneal ulceration and perforation, conjunctivitis, uveitis, scleritis and episcleritis, optic neuritis, and orbital inflammation all of which can be infectious and/or “autoimmune” (Akpek et al., 2015).

### Oral Cavity

Involvement of the salivary glands is the second defining feature of SS. There are three major salivary glands, the submandibular gland, the parotid gland, and the sublingual gland. The submandibular gland, which secretes both serous and mucous secretions, tends to be involved first and is responsible for the majority of the symptoms in the oral cavity. The parotid gland produces predominantly serous secretions and tends to be involved second. The sublingual glands, which also secrete serous secretions, may or may not be involved but cannot compensate for the loss of submandibular gland function (Chen et al., 2016; Dugonjic et al., 2014; Pijpe et al., 2007). Similarly, there are numerous minor salivary glands that cannot compensate for loss of submandibular and parotid function. As with dry eyes, dry mouth can occur for many reasons including mouth breathing, genetic factors accelerated by aging, environmental toxins, drugs, radiation, infections, chronic diseases such as sarcoidosis, malignancies, and SS. It is unclear what percentage of patients with dry mouth has SS. This has never been carefully studied.

Patients with SS experience dry mouth; burning sensation in their mouth; loss of sense of taste and smell; inability to eat, chew, and swallow food; speaking difficulty; and weight loss. Complications of SS in the oral cavity include dental caries, gingivitis, dry and cracked lips, depapillation of the tongue, oral ulcers, and infections especially with fungi (Napenas and Rouleau, 2014).

### Lungs

Lung involvement occurs in 9%–75% of the patients with SS, depending upon what criteria are utilized to define pulmonary disease and whether primary and/or secondary causes of lung disease are being considered (Parke, 2008; Quismorio, 1996). The most common lung finding in SS patients, which occurs in roughly 1% of the patients, is lymphocytic interstitial pneumonia (LIP) (Parambil et al., 2006). LIP that is indistinguishable from what is seen in SS can be seen in SLE, systemic sclerosis, polymyositis, and other autoimmune diseases. In one

study of patients with LIP, 25% had SS (Koss et al., 1987). Patients with LIP are often asymptomatic but may have chronic cough and dyspnea on exertion (Swigris et al., 2002). In SS, cough can also be secondary to dryness in the upper airway and trachea.

Other less frequent airway manifestations in SS include other forms of interstitial pneumonitis (usual interstitial pneumonia (UIP), nodular interstitial pneumonia (NIP), and bronchiolitis obliterans with organizing pneumonia (BOOP)), lymphocytic bronchitis, pleuritis with or without pleural effusions, pulmonary vasculitis usually in the setting of systemic vasculitis, pseudo-lymphoma, and lymphoma (Parke, 2008). Especially in SS with secondary antiphospholipid antibody syndrome, one can see transient and/or permanent pulmonary hypertension as well as thrombosis and pulmonary embolism (Ostrowski and Robinson, 2008; Paran et al., 2007). In secondary SS, it may be hard to distinguish whether the lung disease is secondary to the primary disorder (i.e., rheumatoid arthritis and systemic sclerosis) or SS. Furthermore, patients with SS are more likely than normal individuals to get various infections in both the upper and lower airways because of the deficiency in protective mucous secretions.

## Kidney

Kidney involvement occurs in approximately 5% of the patients with primary SS (Evans et al., 2015; Francois and Mariette, 2016; Ren et al., 2008). The majority of SS patients have lymphocyte predominant tubulointerstitial nephritis (TIN), although some patients have glomerular disease. Variability is seen in studies done in different locations. In one study of 130 patients with SS, 80% had TIN and 20% had glomerular disease while another study of 33 patients with SS found 35% with TIN, 52% with glomerular disease, and 12% with both (Kaufman et al., 2008; Ren et al., 2008).

TIN frequently leads to distal renal tubular acidosis that results in hypokalemia, nephrolithiasis, and nephrocalcinosis. In some patients, antibodies to carbonic anhydrase II are demonstrated. One may also see diabetes insipidus and hypomagnesemia. Patients may notice polydipsia, polyuria, pain from kidney stones, muscle cramps, and rarely paralysis (Both et al., 2015; Evans et al., 2015; Francois and Mariette, 2016; Pertovaara et al., 2011).

Glomerular disease is generally immune complex-mediated and may occur in the setting of systemic, often cryoglobulinemic, vasculitis. It is generally asymptomatic until renal failure occurs, at which point patients note oliguria, fatigue, and problems with hypertension (Evans et al., 2015; Francois and Mariette, 2016; Ren et al., 2008).

## Gastrointestinal Tract

Almost all patients with SS will have some issues with the gastrointestinal tract, as the mouth is a part of the gastrointestinal tract (discussed previously) (Ebert, 2012). Beyond the mouth, however, various problems can be seen. In the esophagus, dysphagia may be seen because of the lack of saliva. Dysmotility with associated dysphagia and gastroesophageal reflux disease may occur because of muscarinic receptor 3 antibodies, acetylcholine receptor antibodies, other forms of nerve injury, and/or secondary metabolic muscle problems (Bengtsson et al., 2011; Ebert, 2012; Park et al., 2013; Pierce et al., 2016).

In the stomach, chronic inflammation and glandular atrophy may be noted. Secondary *Helicobacter pylori* infections may be seen. This may lead to lymphoma. Some patients have antiparietal cell antibodies, but not all of these develop pernicious anemia (Fox et al., 1984).

With regards to small and large bowel disease, abdominal bloating, constipation, diarrhea, nausea, and malabsorption of various nutrients including vitamin D and iron may be seen. Celiac disease has been noted in up to 15% of the patients with SS as well as other food hypersensitivities (Barton and Murray, 2008; Kim-Lee et al., 2015; Pittman and Holub, 1965). Many of these patients were initially labeled as having irritable bowel syndrome. Several patients with SS have been described to have inflammatory bowel disease (Gainey et al., 1985). In patients with vasculitis, the small and large bowel are frequently involved (Scofield, 2011; Tsokos et al., 1987). The neurological and muscular problems affecting the esophagus and stomach are likely to involve the small and large bowel in a similar manner. Furthermore, lymphoma in patients with SS is frequently seen in the small and large bowel in the presence or absence of lymphoma in the salivary glands (Thieblemont et al., 1995).

Involvement of the pancreas in SS can occur for many reasons. There can be autoimmune attack of the pancreatic cells, injury to the biliary tract from sclerosing cholangitis or biliary cirrhosis, inspissation of biliary secretions

because of poor glandular function, ischemic injury because of vasculitis or vasculopathy (commonly associated with antiphospholipid antibodies), and toxic injury because of medications (Ebert, 2012; Lin et al., 2010). Disorders in pancreatic exocrine function have been seen in 18%–37% of the patients with primary SS. However, acute or chronic pancreatitis is identified in 0%–7% of the patients with SS. Rarely neoplasms of the pancreas have been seen, probably not more commonly than in the general population. Patients notice abdominal pain and sometimes diarrhea associated with eating.

The liver is generally not a target organ in primary SS. However, secondary SS can be seen in association with various autoimmune liver diseases including primary sclerosing cholangitis, primary biliary cirrhosis, and autoimmune hepatitis (Floreani et al., 2014; Sun et al., 2015). Furthermore, hepatitis C infection is associated not only with keratoconjunctivitis sicca but also many of the autoantibodies described in patients with primary SS (Buskila, 2009; Ramos-Casals et al., 2001).

## Nervous System

Neurological involvement in SS can involve both the peripheral and central nervous system (CNS) and may be the presenting symptoms in several patients. In two large studies, peripheral nervous system (PNS) was seen in 16% and 10.4% of primary SS patients, respectively, while CNS involvement occurred in 3.6% and 2.7%, respectively. One study estimated CNS involvement to be as high as 8% of the patients with SS (Carvajal Alegria et al., 2016).

PNS manifestations included pure sensory neuropathy, sensorimotor neuropathy, cranial nerve involvement, mononeuritis multiplex, and polyradiculoneuropathy (Mellgren et al., 2007). CNS manifestations included meningitis, encephalitis, transverse myelitis, seizure, stroke, multiple sclerosis-like disease, vasculitis, and various cognitive and psychiatric disorders (Massara et al., 2010). Some degree of autonomic dysfunction was noted in 37.5% of the patients with SS and most strongly associated with the presence of Raynaud's and fatigue (Koh et al., 2017). It was noted that SS patients with one form of neurological disease are likely to develop other neurologic manifestations as their disease progresses.

The mechanisms by which the nervous system is injured in SS are poorly understood but may involve several overlapping pathophysiological processes. There may be autoantibodies affecting nerve function, such as aquaporin 4 antibodies that have been associated with the multiple sclerosis-like disease involving the optic nerve, cerebrum, brainstem, and spinal cord (Dellavance et al., 2012). Cell-mediated injury may occur. The presence of predominantly CD4<sup>+</sup> T lymphocytes has been noted in some peripheral nerve biopsies in patients with primary SS (Mellgren et al., 2007). Immune complex–mediated vasculitis is frequently associated with mononeuritis multiplex and seen more commonly in SS than in most other autoimmune diseases (Cacoub et al., 2015; Jamilloux et al., 2014). Certainly, other forms of nerve ischemia can occur with the vasculopathy of antiphospholipid antibody syndrome seen in association with SS (Acheson and Sanders, 1994). Cytokines produced in the course of the disease can have variable influences on neurological function. Tumor necrosis factor (TNF) at high levels, for example, can induce depression and fatigue (Hermann et al., 2005; Patejdl et al., 2016). Metabolic dysfunction occurring as part of the disease initiating process or as a result of the inflammation seen in SS may certainly contribute to various forms of neurological dysfunction, including autonomic dysfunction and fatigue (Morris et al., 2015; Pagano et al., 2013; Suresh et al., 2014). Not to be forgotten are neural toxicities that can occur because of medications and/or infections (that may occur secondarily to particular medications or to the disease itself) in patients with SS. As with other autoimmune diseases that are treated with immunosuppressive medication, it is not always clear what is a primary disease manifestation and what manifestations occur as secondary processes.

Patients with neurological manifestations of SS are as likely to be seen first by a neurologist as they are to be seen by an ophthalmologist or rheumatologist. It is important that the care of these patients involves a multidisciplinary approach.

## Genitourinary

The involvement of the genitourinary tract is one of the most disabling manifestations of SS for women. Vaginal xerosis has been identified in 53% of SS patients and is often associated with dyspareunia and sexual dysfunction (Priori et al., 2015; van Nimwegen et al., 2015). These problems contribute to the social isolation that is exacerbated by fatigue and depression. The cause for the vaginal xerosis is unknown but is felt to be secondary

to similar processes that cause the loss of secretory function in the salivary and lacrimal glands. No studies have evaluated the effects of SS on male sexual function.

A disorder of bladder inflammation, interstitial cystitis, has been reported in several patients with SS (Darrieutort-Laffite et al., 2015). This disorder can result from many different causes, which were not investigated in these studies (Patnaik et al., 2017). The true incidence of interstitial cystitis in SS is unclear. It is also unclear whether it is actually a manifestation of SS.

Patients with SS also have a higher risk of spontaneous abortion than normal controls (Ballester et al., 2017; De Carolis et al., 2014; Upala et al., 2016). The contribution of secondary antiphospholipid antibodies to this problem has not been adequately studied. Furthermore, because many patients with SS have anti-Ro antibodies, their children may be subject to neonatal lupus, which consists of skin rash and/or heart block (Ballester et al., 2017; Cimaz et al., 2003; Luo et al., 2015).

## Vascular System

The vascular system is commonly involved in patients with SS. The most common manifestation is Raynaud's, which occurs in 30%–50% of the patients (GarciaCarrasco et al., 2002; Kraus et al., 1992; Vivino et al., 2016). Besides causing transient color changes noticed in the hands and feet, Raynaud's can cause reversible CNS vasospasm, causing migraine headaches and transient ischemic attacks, reversible pulmonary hypoxemia, Prinzmetal's angina, and abdominal angina (Gupta et al., 2014; Herrick, 2016; Wigley, 2002). Raynaud's may or may not be associated with the presence of antiphospholipid antibodies (Cervera et al., 2002). The true incidence of antiphospholipid antibodies in SS is not known, but it is probably lower than in SLE, as the incidence of thrombosis is significantly lower in SS than in SLE (Ungprasert et al., 2015). Nonetheless, the presence of antiphospholipid antibodies in SS, as in other autoimmune diseases, is associated with an increased incidence of stroke (Pasoto et al., 2012).

Vasculitis was noted in 10.5% of 152 patients followed over a 25-year period. In another study, 6% of the 1115 patients with SS developed vasculitis (Abrol et al., 2014; Baldini et al., 2014; Retamozo et al., 2016). The vasculitis frequently involved the skin and bowel but can involve other organs such as peripheral nerves, kidney, and muscle. Patients will notice palpable purpura on their skin and crampy abdominal pain, with or without bloody diarrhea. Vasculitis is commonly associated with hypergammaglobulinemia (often predominantly IgM), high titers of autoantibodies and rheumatoid factor (RF), hypocomplementemia, and cryoglobulinemia (Scofield, 2011; Tsokos et al., 1987). While some vasculitis in SS is limited to the skin, as in most other autoimmune diseases, a systemic vasculitis with organ involvement similar to polyarteritis nodosa is more commonly seen in SS than in other autoimmune diseases (Scofield, 2011).

Atherosclerotic cardiovascular disease is increased in patients with SS as well as in patients with SLE (Bartoloni et al., 2015; Birt et al., 2017; Valim et al., 2016). How much this is due to SS and how much to medications, especially corticosteroids used to treat SS, are unclear. Evaluation of potential atherosclerotic disease should be a part of the care of all patients with SS.

## Musculoskeletal and Constitutional Symptoms

Musculoskeletal complaints occur in most patients with SS. The most common complaints are arthralgias without frank arthritis although synovitis can occur in 15%–35% of the patients (Fauchais et al., 2010; Malladi et al., 2012). The most typical joint involvement is the knees and the small joints of the hands and wrists. The arthritis tends to be nondeforming and not associated with erosions. It is important to remember, however, that inflammation in the gastrointestinal tract from either food hypersensitivities or inflammatory bowel disease can lead to an arthritis involving predominantly the hands, wrists, and knees (Holden et al., 2003). Furthermore, SS is frequently seen secondary to rheumatoid arthritis (Anaya et al., 2016).

One of the most debilitating complaints of patients with SS is fatigue. Fatigue is a complex symptom that can occur for many reasons, including sleep disorders, metabolic disorders, cytokines released as part of chronic inflammation, and psychiatric disorders such as anxiety and depression (Norheim et al., 2011). Any or all of these may be relevant to particular patients with SS. It is estimated that 30% of the patients with SS have fatigue (Segal et al., 2009; Vivino et al., 2016). Several studies have identified sleep disorders in patients with SS, although none indicated the true incidence of sleep disorders in this population. It was observed that patients with SS and

obstructive sleep apnea demonstrated improvement in their fatigue when appropriately treated with continuous positive airway pressure (CPAP) (Hackett et al., 2016; Priori et al., 2016; Usmani et al., 2012).

The role of metabolic disorders in various autoimmune diseases has become a recent topic of investigation. Metabolic disorders have been observed in patients with SLE and systemic sclerosis (Fernandez and Perl, 2009; Marra et al., 2015). In SS, decreased antioxidant molecules have been observed in the sera and saliva of patients, evidence of oxidative stress has been observed in the salivary glands and mutations in a mitochondrial carrier protein SLC24A40 have been found in a number of SS patients with fatigue (Morris et al., 2015; Norheim et al., 2014; Ryo et al., 2006). Preliminary studies have observed frequent disorders of aerobic and anaerobic metabolism in patients with SS and fatigue. Treatment of these disorders resulted in symptomatic improvement of fatigue (Ambrus et al., 2015; Kim-Lee et al., 2015).

Various cytokines, especially TNF, are associated with symptoms of fatigue and depression (Norheim et al., 2011). These symptoms are improved with the use of various medications blocking TNF. This effect has been observed in rheumatoid arthritis but has not been systematically studied in SS.

The presence of anxiety and depression in patients with SS has been observed in several studies. The true incidence in SS is unclear, but it is more common than what was observed in normal controls (Epstein et al., 2014; Kocer et al., 2016). Furthermore, the cause for the anxiety and depression is likely to be multifactorial and could involve distress over life changes that occur because of the disease, metabolic disorders, and cytokine effects. It is noted that attention to anxiety or depression in patients with SS is an important part of their care.

## Lymphoma and Other Hematological Manifestations

The incidence of lymphoma is increased in many autoimmune diseases, but most notably in SS. The incidence of lymphoma in patients with primary SS has been estimated to be between 5% and 10% (Voulgarelis et al., 2012; Zintzaras et al., 2005). The malignancies of patients with SS are generally non-Hodgkin's B-cell lymphomas that may be various histological subtypes including follicular lymphoma, large B-cell lymphoma (LBCL), and marginal zone lymphoma (Smedby et al., 2008). Interestingly, the tumors may start not only in the salivary glands but also in other mucosal lymphoid tissues, such as the Peyer's patches, suggesting that there is systemic dysregulations of the B cells. Little is known regarding the evolution of the chronic inflammation of SS into lymphoma, but one hypothesis is that the rapid proliferation of autoreactive cells leads to accumulation of mutations that ultimately allow the malignant state to prevail. Another hypothesis is that there are intrinsic abnormalities in the B cells of SS patients that allow malignancy to develop after further environmental insults (Mackay and Rose, 2001). Malignancy generally requires the dysregulation of at least three genes, one putting the cells into the cell cycle, one inducing proliferation, and one inhibiting apoptosis (BernalMizrachi et al., 2006). It has been observed clinically that SS patients with hypergammaglobulinemia, monoclonal gammopathy, and low C4 are most likely to go on to develop lymphoma. Some of these patients may develop multiple myeloma instead (Giannouli and Voulgarelis, 2014). Studies evaluating LBCL have shown that LBCL from patients with autoimmune diseases is more aggressive and harder to treat than LBCL from patients with no predisposing factors. The LBCL from patients with autoimmune diseases is more commonly the activated B-cell type (ABC) than the germinal center type and is associated with NF- $\kappa$ B activation and a poorer prognosis (Smedby et al., 2008; Song et al., 2009).

Other hematological manifestations of SS include hemolytic anemia, autoimmune thrombocytopenia, and leukopenia (Brito-Zeron et al., 2009; Manganelli et al., 2006). Both hemolytic anemia and thrombocytopenia can be related to the presence of antiphospholipid antibodies (de Groot et al., 2012). Alternatively, they can occur as part of a primary disorder such as SLE. Patients with SS can also have hematological manifestations related to drug reactions, gastrointestinal blood loss, and malabsorption of nutrients, renal failure, and secondary infections.

## PATHOLOGICAL FEATURES

Several issues complicate understanding the histopathology of SS. First, as with all autoimmune disease, SS is a heterogeneous disease determined by different genetic and environmental factors. Different immunological events may contribute to injury of the salivary and lacrimal glands in different patients. Second, SS is a disease that is often not diagnosed until it has been present for several years. Analysis of patients with SS frequently

misses the immunological events involved with the initiation of the disease. In addition, some of the observed changes in patients with SS may be related to attempts of the immune system to correct the problems at hand, rather than being causative for the disease. And third, SS inevitably transitions through various stages, and not all patients are at the same stage of the disease when they are being evaluated. In this section, I will discuss the observations that have been made in patients with SS. In a later section, I will discuss how animal models have been used to put these observations into perspective and to provide new insights into disease pathogenesis, especially initiating factors and disease progression.

When SS was first described, the diagnosis involved the presence of clinical symptoms of dry eyes and/or dry mouth along with the presence of a mononuclear cell infiltrate in the glands that presumably was responsible for these symptoms and ultimately led to the total destruction of the glands (Parke and Buchanan, 1998). Among the current criteria for the diagnosis of SS is the demonstration of  $>1$  mononuclear cell/ $4\text{ mm}^2$  surface area of a minor salivary gland biopsy, called the focus score (Shiboski et al., 2017). Much work has gone into the characterization of these cells and the products that they produce. Lymphocytic foci in the interlobular and intralobular ducts are initially predominantly CD4 $^+$  T cells. These cells have been shown to produce interferon- $\gamma$ , interleukin (IL)-17, IL-21, and IL-22 (Ciccia et al., 2012; Furuzawa-Carballeda et al., 2014; Katsifis et al., 2007). Patients with long-standing SS sometimes have CD4 $^-$ , CD8 $^-$  "double negative" T cells in their salivary glands. These cells were first described in the peripheral blood of patients with SLE (Alunno et al., 2014; Crispin et al., 2008). B cells are commonly found in these lesions and have the phenotype of transitional type 2 B cells (marginal zone B-cell precursors) and marginal zone B cells (Ambrus et al., 2016; Bohnhorst et al., 2002; Daridon et al., 2006; Jonsson et al., 2007; Mariette, 2008; Pers and Youinou, 2014). These B cells have been shown to produce lymphotoxin, which has been implicated in direct injury to the salivary glands (Pillai et al., 2005; Shen et al., 2016, 2010). Plasma cells are identified and are felt to be the distinguishing feature between SS and other forms of salivary gland inflammation (Llamas-Gutierrez et al., 2014). Production of anti-Ro antibodies has been described by plasma cells in the salivary glands of patients with SS (Maier-Moore et al., 2014). Approximately 25% of SS patients have ectopic germinal center-like structures in their salivary glands. The presence of these germinal centers correlates with a higher likelihood of developing lymphoma (Delaleu et al., 2016).

Much work has focused on determining the signals that attract lymphocytes to the salivary and lacrimal glands. Current hypotheses emphasize a role for injury to salivary gland epithelial cells (SGEC) by a viral or other environmental trigger causing them to release CCL17, CCL19, CCL22, CXCL10, CXCL12, and CXCL13 that attract various populations of lymphocytes and dendritic cells to the glands (Amft and Bowman, 2001; Barone et al., 2008). B-cell activating factor (BAFF) produced by SGEC contributes to the abnormal activation of B cells (Mariette, 2008; Pers and Youinou, 2014). Salivary gland epithelial cells also produce IL-7 and have reduced levels of thymic stromal lymphopoietin that influence the activity and survival of T cells (Bikker et al., 2012; Hillen et al., 2016). Local plasmacytoid dendritic cells produce type 1 interferons, which are responsible for the "interferon signature" seen in patients with SS (Bave et al., 2005; Gottenberg et al., 2006). The stimulus for production of type 1 interferons, the timing of type 1 interferon production in the course of the disease, and the roles of type 1 interferon in the pathophysiology of the disease at different stages of the disease are poorly understood (Maria et al., 2015; Mitsias et al., 2002; Nezos et al., 2015; Shen et al., 2013).

Additional studies have emphasized abnormalities in glandular structure and function that may be observed in the presence or absence of cellular infiltrate. These changes may be induced by infections or environmental toxins and/or by cytokines and other mediators released by local cells in response to these toxins. Normal luminal localization of aquaporin 5 is lost in the glands of patients with SS (Gresz et al., 2015). This causes some of the fluid that should be part of the secretions to leak into the interstitium. Furthermore, increased production of cholinesterase and muscarinic receptor antibodies prevents normal production of salivary and lacrimal gland secretions (Dawson et al., 2006; Sumida et al., 2012). Normal signaling through the muscarinic receptors involves import of calcium, release of intracellular calcium, and activation of protein kinase C. Abnormalities in calcium loading via stromal interaction molecules 1 and 2 and calcium signaling have been observed in the cells of the patients with SS that can be mimicked by treating normal salivary glands with lymphotoxin (Cheng et al., 2012; Sneyd et al., 2014; Teos et al., 2015). Finally, the remodeling of the basement membrane of the glands that occurs every 200 days in normal individuals is defective in patients with SS (Konttinen et al., 2006). Extensive acinar cell atrophy and loss are seen in conjunction with ductal cell hypertrophy. Increased production of matrix metalloproteinase 9, which can degrade collagen type IV, and trypsin-2 in patients with SS have been implicated in these changes (Molina et al., 2006).

## AUTOIMMUNE FEATURES

SS is an autoimmune disease because organ damage is demonstrated in the presence of autoantibodies, in the absence of other explanations for the organ damage. Infections that are known to cause disease similar to SS are excluded from the diagnosis, including hepatitis B and C, HTLV-1 mumps, and CMV. Other causes of glandular dysfunction, such as anticholinergic drugs, radiation therapy, aging, and other systemic inflammatory diseases, such as sarcoidosis, must also be excluded before a diagnosis can be made (Cornec et al., 2015). What is unclear, however, is the mechanism(s) by which the actual organ injury occurs.

As with other autoimmune diseases, such as type 1 diabetes (T1D) and rheumatoid arthritis, evaluation of patients with very early disease often reveals damaged tissue with some associated macrophages, but not lymphocytes (McNelis and Olefsky, 2014; Tapinos et al., 1999). Furthermore, as discussed below, several animal models for SS have demonstrated loss of salivary and lacrimal gland function before any lymphocytes are seen in the glandular tissue. This suggests that lymphocyte-directed killing of the target organ is not a major factor in the early course of the disease, at least in some patients. Whether it is a feature in later stages of the disease is unclear as well.

Autoantibodies, such as anti-Ro, which are used to define SS as a disease entity, may have many functions as well. Studies in various autoimmune diseases have suggested that some autoantibodies, especially IgM autoantibodies, may be utilized by the immune system to recognize damaged tissues and bring in cells that can participate in tissue repair (Gronwall and Silverman, 2014; Nguyen et al., 2015). If the tissue is repaired and inflammation is stopped, the individual can return to a normal state. Perhaps what defines the development of this as well as other autoimmune diseases is the inability to turn the immune system off once it has initiated a response—normal low-affinity autoreactivity is converted to a high-affinity autoimmune response that is (1) capable of injuring self—for example, IgG autoantibodies can induce antibody-mediated cytotoxicity while IgM autoantibodies cannot and (2) memory B and T cells are formed which are not as susceptible to growth inhibitory and apoptotic signals as primary B and T lymphocytes (Kurosaki et al., 2015; Sallusto et al., 2004). Studies have demonstrated autoantibodies in some patients with SS and SLE years before clinical disease was evident (Arbuckle et al., 2003; Theander et al., 2015). Did this merely demonstrate that they were responding to an environmental challenge that they were able to confine without leading to overt disease? Overt disease occurred later when more potent environmental challenges occurred that could not be contained. And/Or, did it demonstrate that they had a genetic predisposition to have difficulty turning off their immune systems once the wrong kind of environmental trigger occurred. All autoantibodies can be seen in normal individuals, but not in the titers, isotypes, and affinities seen in patients with autoimmune diseases.

Once more is learned about the pathophysiology of SS, the environmental triggers, the predisposing genes, the sequence of events leading to organ malfunction, and the sequence of events leading to the progression from a localized disease of the salivary and lacrimal glands to a systemic disease involving multiple other organs and ultimately lymphoma, a greater understanding will be available of what it means that SS is an “autoimmune” disease.

## GENETICS

SS, like all other autoimmune diseases, has a strong genetic component. All centers studying patients with SS have patients whose family members have SS as well as other related autoimmune diseases (Reksten et al., 2016). However, in contrast to SLE and T1D, twin studies have never been done in this disorder to document a definitive genetic contribution. Attempts to understand the genetic components of SS have largely been done by genome-wide association studies (GWAS). These studies are hampered by the fact that large numbers of patients and controls are needed to get statistically significant data and the patients being studied have variable ethnic backgrounds and distinct clinical phenotypes. Even in patients with the same clinical phenotype, the genetic factors contributing to the development of disease may be variable. These factors are likely responsible for differences in the results of the GWAS studies with SS patients in the United States and China (Lessard et al., 2013; Li et al., 2013). Furthermore, genetic association studies done in various other locations with smaller numbers of patients identify potential genetic associations not appreciated in the larger GWAS studies (Bolstad and Jonsson, 2002; Miceli-Richard and Criswell, 2014).

The first genetic studies done on patients with SS at the National Institutes of Health in Bethesda, Maryland (United States) evaluated HLA genes because of their importance in antigen presentation. These studies revealed a strong association of SS with HLA-Dw3, HLA-B8, HLA-DRw3, HLA-DR3, and HLA-Dw52. Later studies revealed a strong association of North American SS patients with HLA-DRB1\*0301-DRB3\*0101-DQA1\*0501-DQB1\*0201. Japanese SS patients were associated with HLA DRB1\*0405-DRB4\*0101-DQA1\*0301-DQB1\*0401 and Chinese SS patients with DRB1\*0803-DQA1\*0103-DQB1\*0601 (Kang et al., 1993). In Finland, the association of SS was with HLA-DRB1\*0301-DQA1\*0501-DQB1\*0201 (Kerttula et al., 1996). Some variability has been seen in all different SS patient populations, depending upon their ethnic background and location.

Besides the HLA genes, GWAS studies have identified statistically significant polymorphisms involved with various cellular functions (Reksten et al., 2016). These include IRF5 (interferon regulatory factor 5), a transcription factor induced by type 1 interferon that induces production of IL-6, IL-12, type 1 interferon, and tumor necrosis factor alpha (TNF- $\alpha$ ). IRF5 also induces the production of BLIMP-1, which is important for the differentiation and function of plasma cells and B1 cells (Reksten et al., 2016; Salloum and Niewold, 2011). Genetic associations with SS were also found with several gene polymorphisms relevant to IL-12 function, the IL-12 receptor subunit B, signal transducer and activation of transcription 4, and interferon regulatory factor 8 (Korman et al., 2008; Low and Witte, 2011; Reksten et al., 2016). IL-12 is important for various aspects of innate and cell-mediated immunity. With regards to B-cell function, polymorphisms increased in patients with SS were found in the B lymphocyte tyrosine kinase, Ras guanyl-releasing protein 3, diacylglycerol kinase theta, and cytoplasmic polyadenylation element binding protein 4 all of which are involved with intracellular signaling in B cells (Reksten et al., 2016). In addition, polymorphisms were found in CXCR5, the chemokine receptor for CXCL13 that is involved with bringing B and T cells into germinal centers (Kramer et al., 2013). In other studies, polymorphisms were seen in the genes for lymphotoxin- $\alpha$  and its receptor tumor necrosis factor receptor 1, tumor necrosis factor super family 4, and the TNF- $\alpha$ -induced protein 3 (Bolstad et al., 2012; Fletes-Rayas et al., 2016; Nocturne et al., 2016; Nordmark et al., 2011). These proteins may all be involved with induction of cellular cytotoxicity and inflammation.

The significance of these genetic polymorphisms in the pathophysiology of SS is poorly understood, but they do suggest areas of cellular function that deserve further attention. They also emphasize the importance of studying genetic of SS in patients with the same form and stage of disease. This is currently a problem in the study of all autoimmune diseases.

## ANIMAL MODELS INCLUDING RELEVANCE

The use of animal models in the study of SS has several caveats. On the plus side, spontaneous animal models of SS allow study of the time course of various physiological changes in the disease, they allow introducing genetic changes to see how they influence the disease process and they allow the investigation of particular therapeutic strategies. Animals can be autopsied to make complete assessment of the status of each organ at any particular stage of disease. The initiation of disease can be investigated, which can almost never be appreciated in patients with SS. On the negative side, since SS is a heterogeneous disease, each animal model likely represents a form of disease that may be present in some but not all patients. Second, depending upon how the animal model is established, the cure of the disease may involve reversing the mechanism by which the disease was induced rather than doing something that would be relevant to treating the human disease. And, finally animals may have different ways of dealing with particular immunological challenges than people, so all observations made in animals may not be directly referable to human disease. Any observations made in animal models of SS need to be reassessed in SS patients, when possible (Delaleu et al., 2011; Lee et al., 2012).

The first murine models used to study SS were in fact animal models already being utilized to study SLE (Keyes et al., 1977). The (NZB × NZW) F1 mouse develops many of the features of SLE and has been used to identify genes involved in the pathogenesis of the disease (Wakeland et al., 1999). These mice do not develop sicca symptoms but can be induced to develop them with agonists for Toll-like receptor 3 which induced production of type 1 interferons (Deshmukh et al., 2008). The MRL/lpr mouse has also been used to study SLE, although its disease is largely due to a mutation in fas, which make them a model for the human lymphoproliferative syndrome (Turbyville and Rao, 2010). These mice develop lymphocytic infiltration of salivary glands and in some cases anti-Ro antibodies, but they do not develop either keratoconjunctivitis sicca or loss of salivation (Jabs and Prendergast, 1988).

The first animal model that developed salivary and lacrimal gland dysfunction more consistent with SS was the NOD mouse (Gao et al., 2006). These mice develop both T1D and SS, although these two autoimmune diseases developed by different mechanisms. >20 chromosomal regions contribute to the development of T1D. Deleting Toll 2 from the NOD genome eliminates the T1D but does not influence the development of SS (Kim et al., 2011). Blocking intercellular adhesion molecule-1 (ICAM-1) early in the course of the disease prevents the salivary gland inflammation without affecting the T1D (Roescher et al., 2011). Two genetic loci of the NOD strain, *Idd3* (also known as *Aec1*—autoimmune exocrinopathy 1) and *Idd5* (*Aec2*) were noted to be critical for the development of SS. A new strain of mice expressing these genetic loci on a C57Bl/6 background was developed, the C57BL/6.NOD-Aec1Aec2 mouse. This mouse does not develop T1D but demonstrates loss of salivary gland function before lymphocytic infiltration of the salivary glands, activated B and T lymphocytes, autoantibodies, and inflammatory cytokines (Nguyen et al., 2006; Peck et al., 2011). Another derivative of the NOD mouse is the NOD.B10Sn-H2<sup>b</sup>/J (NOD.B10) mouse that was generated by replacing the MHC locus of the NOD mouse with the MHC of the C57BL/6 strain. These mice also develop SS without developing T1D. They develop lymphocytic infiltration of the salivary and lacrimal glands as well as pulmonary disease, renal disease, and autoantibodies (Coursey et al., 2016).

Various animal models for SS have been developed based on mediators felt to be important in the development of human disease. One of the hypotheses for the development of SS is that viral infection of the salivary and lacrimal glands leads to the initial tissue injury that sets off the disease process in a person with the appropriate genetic background (Igoe and Scofield, 2013). Murine cytomegalovirus was used to infect C57BL/6 lpr-deficient mice (C57BL/6.lpr/lpr). The mice developed sialadenitis that persisted after the infection was cleared. Chronic inflammation with T cells and high titers of anti-Ro antibodies were noted (Ohyama et al., 2006).

In patients with SS, increased expression of BAFF was noted in epithelial cells and was felt to be responsible for the abnormal activation of B cells and survival of autoreactive B cells (Kalled, 2005). BAFF transgenic mice develop a disease very similar to human SS except they develop a proliferative glomerulonephritis and fail to develop lymphoma (Mackay et al., 1999). BAFF transgenic mice lacking the TNF- $\alpha$  gene do develop lymphoma (Sutherland et al., 2006). BAFF transgenic mice lacking marginal zone B cells fail to develop features of SS, although they still develop glomerulonephritis (Fletcher et al., 2006). Marginal zone B cells produce lymphotoxin, which was felt to be critical for the development of SS (Pillai et al., 2005; Shen et al., 2016).

Similarly, patients with SS, as well as MRL/lpr, (NZB × NZW) F1 and C57BL/6.NOD-Aec1Aec2 produce IL-14 at high levels, a cytokine felt to be important for the development and survival of memory B cells (Shen et al., 2006). One hypothesis is that the development of autoimmune disease occurs when normal low-affinity autoreactivity is converted to a high-affinity memory response. IL-14 could drive this abnormal memory B-cell function. IL-14 transgenic mice were demonstrated to develop features of SS seen in the patients with SS in the same relative time frame: hypergammaglobulinemia, autoantibodies, loss of salivary gland function before infiltration of the salivary glands with lymphocytes, lymphocytic infiltration of the submandibular and lacrimal glands before the parotid glands, lymphocytic interstitial lung disease, mild renal disease, and in old age LBCLs (Shen et al., 2009). Features of SS could be eliminated from IL-14 transgenic mice by deletion of either marginal zone B cells or lymphotoxin (Shen et al., 2010, 2016). Deletion of the type 1 interferon receptor gene from IL-14 transgenic mice prevented late manifestations of the disease (parotid gland, lung and kidney disease as well as lymphoma) but not the early manifestations of the disease (loss of salivary gland function and lymphocytic infiltration of the submandibular and lacrimal glands) (Shen et al., 2013). Autoantibodies to the salivary and lacrimal gland antigens, salivary gland protein 1, carbonic anhydrase 6, and parotid secretory protein were demonstrated to occur in the mice before antibodies to Ro (Shen et al., 2012). These observations have been confirmed in other animal models for SS as well as in patients (Everett et al., 2017; Shen et al., 2014; Suresh et al., 2015).

Anti-Ro antibodies are commonly seen in SS, and in fact part of the diagnostic criteria for SS (Shiboski et al., 2017). BALB/c mice were immunized with Ro peptides to generate these autoantibodies. Decreased salivary gland flow and infiltration of the salivary glands with lymphocytes were noted. Neither lacrimal gland disease nor disease of the lungs, kidneys, or lymph nodes were noted (Scofield et al., 2005). Similarly, antimuscarinic receptor 3 antibodies are seen in a subgroup of patients with SS (Zuo et al., 2016). T cells from C57Bl/6-M3R  $-/-$  mice immunized with M3R peptides transferred into C57BL/6-Rag-1  $-/-$  mice caused decrease in salivary flow and infiltration of the salivary glands with lymphocytes (Sumida et al., 2012).

Other proteins that are aberrantly expressed in SS have also been investigated in animal models of SS. Bone morphogenic protein 6 (BMP6) was noted on microarray studies to be selectively increased in the salivary glands

of patients with SS compared to normal controls. Increased expression of BMP6 in the salivary gland cells of C57BL/6 mice led to loss of salivary gland function in the absence of infiltrating lymphocytes (Lai et al., 2016). Protein kinase C-delta expression is reduced in lymphocytes of patients with SS (Varin et al., 2012). Deletion of protein kinase C-delta gene from C57BL/6 mice led to lymphocytic infiltration of multiple organs including the salivary glands with resulting hyposalivation (Miyamoto et al., 2002). Phosphatidylinositol-3-kinase (PI3K) is critical for downstream signaling of the T-cell receptor as well as other receptor molecules on T cells (Garcon et al., 2008). Deletion of PI3K from T cells of C57BL/6 mice led to lacrimal gland selective loss of secretory function (Oak et al., 2006). Another molecule important for signaling through the T-cell receptor, inhibitor of differentiation 3, was also deleted from C57BL/6 mice (Yokota, 2001). These mice showed loss of both lacrimal gland and salivary gland function without infiltration of the glands with lymphocytes or production of either anti-Ro or anti-La antibodies (Pan et al., 1999).

Overall, these animal models for SS raise several important issues that were largely missed in studies looking exclusively at patients with SS. First, loss of salivary and lacrimal gland function may occur before or even without identifiable lymphocytic infiltration of the glands. This does not mean, however, that lymphocytes do not participate in the pathophysiology of the disorder. Second, the innate immune system is important in the pathophysiology of the disease and this is not simply a disorder of the adaptive immune system in which self-tolerance has been lost. Third, salivary and lacrimal gland injury may occur via multiple different mechanism that may not be the same in all patients. In some cases, marginal zone B cells and the lymphotoxin they produce are important in the pathophysiology of the disorder in its early stages. SS progresses through many stages of disease that may occur over a course of many years in patients. The significance of particular cytokines, such as type 1 interferons and IL-17, may be variable at different stages of the disease. In chronic viral infections, type 1 interferon has very different effects at the onset of the infection compared to the state in which a chronic infection has been established (Muller et al., 1994; Taleb et al., 2017). Finally, events necessary for the progression of SS through its various stages including the development of lymphoma will have to be studied further in animal models before headway will be made in understanding what patients with SS undergo. Animal models will help to delineate parameters that will allow SS to be divided into its various subtypes.

## DIAGNOSTIC PROCEDURES

The ACR/EULAR 2016 criteria for the diagnosis of SS require a positive response to at least one of the following questions (Shiboski et al., 2017):

1. Have you had daily, persistent, troublesome dry eyes for >3 months?
2. Do you have a recurrent sensation of sand or gravel in the eyes?
3. Do you use tear substitutes >3 times a day?
4. Have you had a daily feeling of dry mouth for >3 months?
5. Do you frequently drink liquids to aid in swallowing dry food?

Exclusion of the following entities:

1. History of head and neck radiation treatment
2. Active hepatitis C infection (with confirmation by polymerase chain reaction)
3. AIDS
4. Sarcoidosis
5. Amyloidosis
6. Graft-versus-host disease
7. IgG4-related disease

And a score of >4 on the following weighted tests:

1. Labial salivary gland with focal lymphocytic sialadenitis and focus score >1 foci/4 mm<sup>2</sup> (weight = 3)
2. Anti-Ro positive (weight = 3)
3. Ocular staining score >5 in at least one eye (weight = 1)
4. Schirmer's test <5 mm/5 minutes in at least one eye (weight = 1)
5. Unstimulated whole saliva flow rate <0.1 mL/min (weight = 1)

The questions for the diagnosis of SS are straightforward and usually not a challenge for the patients. Some may feel, however, the mild dry eye is normal however. The exclusion criteria require a thorough history and physical as well as potentially laboratory and radiographic studies for further analysis. An oral surgeon or other trained physician can do the lip biopsy. Rheumatologists in specialized SS clinics often do them. However, the key is to have the labial biopsy read by a pathologist who has experience with them. There are many mistakes that can be made in interpreting them (Caporali et al., 2008). Similarly an experienced lab should do the identification of anti-Ro antibodies in sera. There is no national standard for this test, so variability may be seen in the test results among different labs. Furthermore, the titers of anti-Ro antibodies in sera may vary over time in individual patients. An experienced ophthalmologist should do the ocular staining score and Schirmer's test. Variability in the results of these studies depends on not only the experience of the person doing the study but also day-to-day fluctuation in individual patients (Valim et al., 2015). The collection of saliva requires appropriate cooperation of the patient and may show day-to-day variability but is generally not technically challenging.

These diagnostic criteria will certainly undergo further modification in the next several years.

Other studies used to establish abnormalities in the salivary and lacrimal glands, although not part of the diagnostic criteria, can also be helpful. Some investigators have utilized ultrasound including ultrasound-guided biopsies, but this is not routinely done at most centers (Astori et al., 2016; Delli et al., 2015). Technetium 99m Tc-sodium pertechnetate scintigraphy can be used to evaluate saliva formation as well as resting and stimulated function. The utility of this study is dependent upon the experience and expertise of the center doing the study. Magnetic resonance imaging (MRI) including MR sialography, computed tomography, and 18F-FDG PET/CT are utilized to evaluate swelling in the salivary glands, especially when a diagnosis of lymphoma is being considered (Cohen et al., 2013).

The investigation of the extraglandular manifestations is done according to the organ system being studied. All patients should have a complete blood count to look for hematological abnormalities, basic metabolic profile to look for liver or kidney abnormalities, urinalysis to look for kidney abnormalities, especially renal tubular acidosis, quantitative IgG, IgA, and IgM levels to look for hypergammaglobulinemia, and serum protein electrophoresis to look for monoclonal gammopathy. If breathing abnormalities are suggested, a high-resolution chest CT may be indicated to look for interstitial lung disease. Gastrointestinal problems may indicate a workup for celiac sprue, food hypersensitivities, inflammatory bowel disease, and/or motility disorders. Neurological abnormalities may indicate nerve conduction studies, MRI studies of the brain and spinal cord as well as additional serological studies. The presence of Raynaud's should trigger a workup for antiphospholipid antibodies. The presence of profound fatigue may indicate the need for a sleep study as well as a workup for metabolic abnormalities. If vasculitis is likely based on skin findings, appropriate angiograms and/or biopsies may be necessary before therapy is initiated.

The complete workup for each SS patient is individualized based on the initial history and physical examination. It is important to remember the types of organ involvement that can occur with SS so each patient gets the appropriate workup and therapy as their disease evolves.

## TREATMENT

Rheumatologists and internists have ignored SS historically for several reasons. First, they believed that it was a very rare disease. We now know that this is not true. Second, they thought that it was a benign disease, since no one dies from dry eyes and dry mouth. We now know that dry eye, dry mouth, fatigue, and dyspareunia can be very debilitating and socially isolating for patients. And, we understand that of all the autoimmune diseases, SS has the highest incidence of systemic vasculitis and lymphoma, both of which can be fatal (Singh et al., 2016). And finally, studies done in the 1980s and 1990s suggested that there were really no therapies for SS. We now know that various symptomatic therapies can be beneficial for the day-to-day lives of SS patients. More importantly, as we learn more about SS, various therapies are emerging that show promise to control the underlying disease. Beyond the specific treatment of SS itself, however, each patient is managed according to their organ involvement. For example, if a SS patient has LIP, they are treated with rituximab, cyclophosphamide, or a similar immunosuppressive therapy that has been shown to work in LIP in patients not only with SS but also with SLE, scleroderma, polymyositis, and other autoimmune diseases.

Recent treatment recommendations have been published by the Sjogren's Foundation for the management of patients with SS ([Vivino et al., 2016](#)):

1. Management of oral problems is recommended that a topical fluoride be used. Attempts should be made to increase salivary flow and these include masticatory stimulation, sugar free lozenges or gums, mannitol, xylitol, and either Pilocarpine or Cevimeline. Chlorhexidine mouthwash is used to reduce dental caries. Nonfluoride remineralizing agents are encouraged.
2. Management of eye problems includes artificial tears, gels, and ointments. For more severe disease, topical steroids, topical cyclosporine, punctal plugs, and secretagogues (Pilocarpine and Cevimeline) are recommended. For the next level of disease severity, contact lenses, topical autologous serum, and permanent punctal occlusion are recommended. For the most severe patients, eyelid surgery and systemic antiinflammatory therapy are recommended.
3. Regarding musculoskeletal pain, hydroxychloroquine is recommended as a first-line agent with the addition of methotrexate if hydroxychloroquine alone is not adequate. Short and long term (>1 month), corticosteroids are recommended in the worst cases. The use of other disease-modifying agents, such as leflunomide, sulfasalazine, azathioprine, and cyclosporine, are only weakly recommended.
4. Regarding fatigue, exercise and dehydroepiandrosterone received strong recommendations. A strong recommendation against the use of TNF- $\alpha$  inhibitors is included.
5. The only biological agent to be given a positive recommendation is rituximab, which is recommended moderately for cryoglobulinemia, vasculitis, severe parotid swelling, inflammatory arthritis, pulmonary disease, and peripheral neuropathy. Only weak recommendation is given for the use of rituximab for keratoconjunctivitis sicca and xerostomia.

A new treatment for eye disease, lifitegrast ophthalmic solution 5%, which works by blocking the interaction between lymphocyte function-associated antigen (LFA-1) and ICAM-1, was not considered in this publication because it is new and has no long-term studies. It has been shown in three trials to improve inferior corneal staining score and eye dryness score ([Keating, 2017](#)).

The use of hydroxychloroquine for musculoskeletal disease is accepted. The use of hydroxychloroquine to help symptoms of dry eyes is debated. Both positive and negative studies have reported its use in this indication ([Gottenberg et al., 2014](#); [Yavuz et al., 2011](#)). Interestingly, in SLE patients with antiphospholipid antibodies, the use of hydroxychloroquine has been associated with a lower incidence of thrombosis ([Belizna, 2015](#)).

With regards to the use of biological agents in SS, rituximab is the only agent that has received a positive recommendation. Interestingly, several trials using rituximab in SS have been done. The trials that utilized patients with early SS showed more positive results than the trials done in patients with more advanced disease ([Carubbi et al., 2013](#); [Devauchelle-Pensec et al., 2014](#); [Pijpe et al., 2005](#); [Souza et al., 2016](#)). The issue of patient selection and the timing of drug administration plays an important role in all the ongoing trials with other biological agents, which to date have not seen significant positive results to warrant their use: Belimumab, a monoclonal antibody that blocks BAFF ([Ginzler et al., 2014](#)); Abatacept, a soluble CTLA-4-Ig that interferes with T-cell activation ([Meiners et al., 2013](#)); and Baminercept, a soluble lymphotoxin beta receptor IgG fusion protein that blocks the actions of lymphotoxin beta ([St Clair et al., 2015](#)). Additional biological agents are in the early phases of the study. These include a monoclonal antibody that blocks CD 40, to inhibit B-cell activation, a monoclonal antibody to inducible T cell costimulator-ligand (ICOS-L) to block the formation of T follicular helper cells and production of high-affinity autoantibodies, a PI3K inhibitor, to block B- and T-cell activation, and a monoclonal antibody to the IL-6 receptor, to block the formation of plasma cells ([Vivino et al., 2016](#)).

Management of extraglandular manifestations of SS often involves therapies that have been studied in other related disease entities, but not necessarily specifically in SS. As mentioned previously, the LIP can be managed with rituximab, but other immunosuppressive agents, including cyclophosphamide and mycophenolate mofetil (MMF), have been used successfully in patients with SS and other autoimmune disease ([Parambil et al., 2006](#); [Wells and Denton, 2014](#)).

Renal disease is managed according to the nature of the problem ([Francois and Mariette, 2016](#); [Ren et al., 2008](#)). TIN has generally been treated with corticosteroids alone, although MMF has been proposed as a "steroid-sparing" agent in patients who require long-term therapy. With renal tubular acidosis, the acidification is corrected with sodium bicarbonate and secondary hypokalemia is corrected with potassium. Glomerular disease is treated according to the nature of the abnormality. If an immune complex glomerulonephritis occurs because of cryoglobulinemia, rituximab can be utilized, as can other immunosuppressive drugs including cyclophosphamide and MMF.

Treatment of the gastrointestinal manifestations in SS depends upon the nature of the gastrointestinal problem. If the patient has celiac sprue or another food hypersensitivity, an elimination diet of the offending food is warranted (Aziz et al., 2016; Farrell and Kelly, 2002; Kim-Lee et al., 2015). In some cases, antiinflammatory agents targeting the gastrointestinal tract are indicated. Similarly, with inflammatory bowel disease, various immunosuppressive agents can be utilized, including budesonide, mesalamine, and TNF- $\alpha$  inhibitors, depending upon the nature and severity of the disease (Abraham et al., 2017). Other biological agents, such as an inhibitor of the integrin  $\alpha 4\beta 7$ , are used in special cases (Perez et al., 2017). Motility disorders can be treated with various agents stimulating motility, including erythromycin, loperamide, and metoclopramide. However, an endocrine and/or metabolic workup may be indicated to look for an underlying treatable cause (Gidwaney et al., 2016; Sanger and Pasricha, 2017). Vascular disease of the gastrointestinal tract may require specific therapy, as does vascular disease anywhere (see later).

Treatment of nervous system problems depends upon the nature of the underlying problem. Rituximab is shown to improve peripheral neuropathy related to either vascular disease or autoantibody-mediated injury (Mekinian et al., 2012; Tracy and Dyck, 2010). Corticosteroids and other immunosuppressive agents have also been used in this setting. Other neurological problems are treated according to the pathophysiology of the disorder (Carvajal Alegria et al., 2016; Jayaramaiah et al., 2014; Massara et al., 2010; Zhong et al., 2017).

Raynaud's is treated with the appropriate vasodilators (Bakst et al., 2008; Hughes and Herrick, 2016). Calcium channel blockers, such as nifedipine and verapamil; alpha-blockers, such as Doxazosin; nitrates, such as isosorbide mononitrate; and phosphodiesterase inhibitors, such as Cilostazol, can all be utilized. If Raynaud's occurs in the CNS, something called reversible CNS vasospasm, the calcium channel blockers nimodipine and nicardipine are most effective (Gupta et al., 2014; Rao and Muthuchellappan, 2016; Singhal et al., 2011). Raynaud's uncontrollable with oral agents, which rarely occurs in SS, can be treated with intravenous prostaglandin E1 (Hauptman et al., 1991). If antiphospholipid antibodies are present, patients should be on an antiplatelet drug, preferably aspirin. If thrombosis has occurred, systemic anticoagulation is indicated (Scoble et al., 2011).

The vasculitis of SS can be restricted to the skin. This can be managed with hydroxychloroquine and in rare cases rituximab (Chen and Carlson, 2008). If patients with SS have vasculitis involving major organs or larger blood vessels, however, rituximab or an immunosuppressive agent, such as cyclophosphamide, is utilized (Scofield, 2011; Tsokos et al., 1987).

Treatment of fatigue in SS is dependent upon the cause of the fatigue. If sleep apnea is present, this should be treated with CPAP, bilevel positive airway pressure (BiPAP), or a dental appliance (Usmani et al., 2012). If a metabolic disorder is present, this should be treated according to the underlying problem. Patients with mitochondrial problems may respond to a combination of CoQ10, creatine, carnitine, folic acid, and alpha lipoic acid (Abdullah et al., 2012; Finsterer and Bindu, 2015; Nicolson, 2014; Tarnopolsky, 2008). Patients with glycogen storage diseases eliminate complex carbohydrates from their diets and substitute them with simple sugars (Adler and Shieh, 2015; Weinstein and Wolfsdorf, 2002). Patients with myoadenylate deaminase deficiency use a protein-restricted diet along with ribose (Goebel and Bardosi, 1987; Teijeira et al., 2009). If SS patients have significant anxiety and/or depression, counseling is indicated and in some cases appropriate pharmacological intervention as well (Kotsis et al., 2014; Milin et al., 2016).

The lymphomas associated with SS are treated according to their histology and genetic make-up. The most common lymphoma seen in SS is diffuse LBCLs of the ABC type (Blenk et al., 2007; Theander et al., 2006). This is a difficult lymphoma to treat. Recent therapies have utilized a combination of rituximab with cyclophosphamide, doxorubicin, vincristine, and prednisone (Fu et al., 2008; Zhou et al., 2017). New forms of therapy are being developed as more is learned about the characteristics of these tumors.

Overall, the treatment of patients with SS must be individualized to the particular manifestations that are present. Attention to each of the existing abnormalities is necessary to provide the SS patient with needed symptomatic relief. Appreciation of the genetic variability contributing to SS in different patients may also demand different approaches to similar disease manifestations in individual patients. Improved therapies are being developed as more is learned about the pathophysiology of SS as well as its variability.

## PERSPECTIVES

SS is an interesting disorder to look at from a historical perspective. It has gone from being considered a rare disorder to being recognized as one of the most common autoimmune diseases. It has gone from being considered an inconsequential and untreatable disorder to one that causes significant patient morbidity and mortality.

Various treatment options exist and are being developed. The research in SS has blossomed in the last decade and will hopefully continue at a similar pace.

The research in SS using both patients and animal models has focused attention on several paradigm shifts in the field of autoimmunity, which have been observed in other autoimmune diseases as well. For a disease that was once considered due to a lack of self-tolerance in the adaptive immune system, we now recognize the importance of the innate immune system in the initiation and perpetuation of the disease (Kramer, 2014; Low and Witte, 2011; Shikhagaie et al., 2017). We recognize that autoreactivity is often part of the normal immune system and not equivalent to autoimmune disease (Tiller et al., 2007). We recognize that some autoantibodies, especially IgM autoantibodies, may be part of the immune system's attempt to regulate itself as well as to repair tissue injury. All autoantibodies are not necessarily pathological (Bayry et al., 2004; Carselli et al., 2005; Gronwall and Silverman, 2014; Panda and Ding, 2015). We have begun to appreciate the complexity of SS as it evolves through various stages (Delaleu et al., 2011; Haldorsen et al., 2008; Jonsson et al., 2006; Roescher et al., 2012; Shen et al., 2013; Xuan et al., 2013). Particular mediators and cytokines may play various pathophysiological roles in different stages of the disease. We appreciate the phenotypic and genetic variability of the disease. We understand the overlapping nature of the genes predisposing to SS and other autoimmune diseases (Bolstad and Jonsson, 2002; Lessard et al., 2013; Reksten et al., 2016; Teruel and Alarcon-Riquelme, 2016). Many patients will have clinical features that overlap what is expected in the textbook definitions of particular autoimmune diseases including SS, SLE, celiac sprue, inflammatory bowel disease, and many others. Improved care of patients with SS requires more in-depth understanding of the genetic and environmental factors involved, the dysfunction that has occurred in various tissues, the dysregulation that has occurred in the immune system, and the way that these changes evolve over time. For example, understanding that all autoimmune diseases involve the inability to turn off the immune system once activated provides insights into the causes of flares in these diseases. One must look for and eliminate the agent that activated the immune system, be it an infection, drug reaction, or other environmental trigger, before using immunosuppression to quiet down the over reactive cells.

## References

- Abdullah, M., Vishwanath, S., Elbalkhi, A., Ambrus Jr., J.L., 2012. Mitochondrial myopathy presenting as fibromyalgia: a case report. *J. Med. Case Rep.* 6, 55.
- Abelson, M.B., Ousler, G.W., Maffei, C., 2009. Dry eye in 2008. *Curr. Opin. Ophthalmol.* 20, 282–286.
- Abraham, B.P., Ahmed, T., Ali, T., 2017. Inflammatory bowel disease: pathophysiology and current therapeutic approaches. *Handb. Exp. Pharmacol.* 239, 115–146.
- Abrol, E., Gonzalez-Pulido, C., Praena-Fernandez, J.M., Isenberg, D.A., 2014. A retrospective study of long-term outcomes in 152 patients with primary Sjogren's syndrome: 25-year experience. *Clin. Med.* 14, 157–164.
- Acheson, J.F., Sanders, M.D., 1994. Coagulation abnormalities in ischaemic optic neuropathy. *Eye* 8, 89–92.
- Adler, M., Shieh, P.B., 2015. Metabolic myopathies. *Semin. Neurol.* 35, 385–397.
- Akpek, E.K., Mathews, P., Hahn, S., Hessen, M., Kim, J., Grader-Beck, T., et al., 2015. Ocular and systemic morbidity in a longitudinal cohort of Sjogren's syndrome. *Ophthalmology* 122, 56–61.
- Alunno, A., Carubbi, F., Bistoni, O., Caterbi, S., Bartoloni, E., Bigerna, B., et al., 2014. CD4(-)CD8(-) T-cells in primary Sjogren's syndrome: association with the extent of glandular involvement. *J. Autoimmun.* 51, 38–43.
- Ambrus, J.L., Vishwanath, S., Shen, L., Suresh, L., 2015. Sjogren's syndrome and metabolic disorders. *Scand. J. Immunol.* 81, 370.
- Ambrus, J.L., Suresh, L., Peck, A., 2016. Multiple roles for B-lymphocytes in Sjogren's syndrome. *J. Clin. Med.* 5, pii: E87.
- Amft, N., Bowman, S.J., 2001. Chemokines and cell trafficking in Sjogren's syndrome. *Scand. J. Immunol.* 54, 62–69.
- Anaya, J.M., Rojas-Villarraga, A., Mantilla, R.D., Arcos-Burgos, M., Sarmiento-Monroy, J.C., 2016. Polyautoimmunity in Sjogren syndrome. *Rheum. Dis. Clin. N. Am.* 42, 457–+.
- Anderson, L.G., Talal, N., 1972. The spectrum of benign to malignant lymphoproliferation in Sjogren's syndrome. *Clin. Exp. Immunol.* 10, 199–221.
- Arbuckle, M.R., McClain, M.T., Rubertone, M.V., Scofield, R.H., Dennis, G.J., James, J.A., et al., 2003. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N. Engl. J. Med.* 349, 1526–1533.
- Astorri, E., Sutcliffe, N., Richards, P.S., Suchak, K., Pitzalis, C., Bombardieri, M., et al., 2016. Ultrasound of the salivary glands is a strong predictor of labial gland biopsy histopathology in patients with sicca symptoms. *J. Oral Pathol. Med.* 45, 450–454.
- Aziz, I., Dwivedi, K., Sanders, D.S., 2016. From coeliac disease to noncoeliac gluten sensitivity; should everyone be gluten free? *Curr. Opin. Gastroenterol.* 32, 120–127.
- Bakst, R., Merola, J.F., Franks Jr., A.G., Sanchez, M., 2008. Raynaud's phenomenon: pathogenesis and management. *J. Am. Acad. Dermatol.* 59, 633–653.
- Baldini, C., Pepe, P., Quartuccio, L., Priori, R., Bartoloni, E., Alunno, A., et al., 2014. Primary Sjogren's syndrome as a multi-organ disease: impact of the serological profile on the clinical presentation of the disease in a large cohort of Italian patients. *Rheumatology* 53, 839–844.
- Ballester, C., Grobst, V., Roblot, P., Pourrat, O., Pierre, F., Laurichesse-Delmas, H., et al., 2017. Pregnancy and primary Sjogren's syndrome: management and outcomes in a multicentre retrospective study of 54 pregnancies. *Scand. J. Rheumatol.* 46, 56–63.

- Barone, F., Bombardieri, M., Rosado, M.M., Morgan, P.R., Challacombe, S.J., De Vita, S., et al., 2008. CXCL13, CCL21, and CXCL12 expression in salivary glands of patients with Sjogren's syndrome and MALT lymphoma: association with reactive and malignant areas of lymphoid organization. *J. Immunol.* 180, 5130–5140.
- Bartoloni, E., Baldini, C., Schillaci, G., Quartuccio, L., Priori, R., Carubbi, F., et al., 2015. Cardiovascular disease risk burden in primary Sjogren's syndrome: results of a population-based multicentre cohort study. *J. Intern. Med.* 278, 185–192.
- Barton, S.H., Murray, J.A., 2008. Celiac disease and autoimmunity in the gut and elsewhere. *Gastroenterol. Clin. N. Am.* 37, 411–+.
- Basak, S.K., Pal, P.P., Basak, S., Bandyopadhyay, A., Choudhury, S., Sar, S., 2012. Prevalence of dry eye diseases in hospital-based population in West Bengal, Eastern India. *J. Indian Med. Assoc.* 110, 789–794.
- Bave, U., Nordmark, G., Lovgren, T., Ronnelid, J., Cajander, S., Eloranta, M.L., et al., 2005. Activation of the type I interferon system in primary Sjogren's syndrome – a possible etiopathogenic mechanism. *Arthritis Rheum.* 52, 1185–1195.
- Bayry, J., LacroixDesmazes, S., DonkovaPetrini, V., Carboneil, C., Misra, N., Lepelletier, Y., et al., 2004. Natural antibodies sustain differentiation and maturation of human dendritic cells. *Proc. Natl. Acad. Sci. U.S.A.* 101, 14210–14215.
- Beckman, K.A., Luchs, J., Milner, M.S., Ambrus Jr., J.L., 2017. The potential role for early biomarker testing as part of a modern, multidisciplinary approach to Sjogren's syndrome diagnosis. *Adv. Ther.* 34, 799–812.
- Belizna, C., 2015. Hydroxychloroquine as an anti-thrombotic in antiphospholipid syndrome. *Autoimmun. Rev.* 14, 358–362.
- Bengtsson, M., Hammar, O., Mandl, T., Ohlsson, B., 2011. Evaluation of gastrointestinal symptoms in different patient groups using the visual analogue scale for irritable bowel syndrome (VAS-IBS). *BMC Gastroenterol.* 11, 122.
- BernalMizrachi, L., Lovly, C.M., Ratner, L., 2006. The role of NF-kappa B-1 and NF-kappa B-2-mediated resistance to apoptosis in lymphomas. *Proc. Natl. Acad. Sci. U.S.A.* 103, 9220–9225.
- Bikker, A., Moret, F.M., Kruize, A.A., Bijlsma, J.W.J., Lafeber, F., van Roon, J.A.G., 2012. IL-7 drives Th1 and Th17 cytokine production in patients with primary SS despite an increase in CD4 T cells lacking the IL-7R alpha. *Rheumatology* 51, 996–1005.
- Birt, J.A., Tan, Y., Mozaffarian, N., 2017. Sjogren's syndrome: managed care data from a large United States population highlight real-world health care burden and lack of treatment options. *Clin. Exp. Rheumatol.* 35, 98–107.
- Blenk, S., Engelmann, J., Weniger, M., Schultz, J., Dittrich, M., Rosenwald, A., et al., 2007. Germinal center B cell-like (GCB) and activated B cell-like (ABC) type of diffuse large B cell lymphoma (DLBCL): analysis of molecular predictors, signatures, cell cycle state and patient survival. *Cancer Inform.* 3, 399–420.
- Bloch, K.J., Buchanan, W.W., Wohl, M.J., Bunim, J.J., 1965. Sjogren's syndrome. A clinical, pathological, and serological study of sixty-two cases. *Medicine (Baltimore)* 44, 187–231.
- Bohnhorst, J.O., Bjorgan, M.B., Thoen, J.E., Jonsson, R., Natvig, J.B., Thompson, K.M., 2002. Abnormal B cell differentiation in primary Sjogren's syndrome results in a depressed percentage of circulating memory B cells and elevated levels of soluble CD27 that correlate with serum IgG concentration. *Clin. Immunol.* 103, 79–88.
- Bolstad, A.I., Jonsson, R., 2002. Genetic aspects of Sjogren's syndrome. *Arthritis Res.* 4, 353–359.
- Bolstad, A.I., Le Hellard, S., Kristjansdottir, G., Vasaitis, L., Kvarnstrom, M., Sjowall, C., et al., 2012. Association between genetic variants in the tumour necrosis factor/lymphotoxin alpha/lymphotoxin beta locus and primary Sjogren's syndrome in Scandinavian samples. *Ann. Rheum. Dis.* 71, 981–988.
- Both, T., Hoorn, E.J., Zietse, R., van Laar, J.A., Dalm, V.A., Brkic, Z., et al., 2015. Prevalence of distal renal tubular acidosis in primary Sjogren's syndrome. *Rheumatology* 54, 933–939.
- Brito-Zeron, P., Soria, N., Munoz, S., Bove, A., Akasbi, M., Belenguer, R., et al., 2009. Prevalence and clinical relevance of autoimmune neutropenia in patients with primary Sjogren's syndrome. *Semin. Arthritis Rheum.* 38, 389–395.
- Brito-Zeron, P., Baldini, C., Bootsma, H., Bowman, S.J., Jonsson, R., Mariette, X., et al., 2016. Sjogren syndrome. *Nat. Rev. Dis. Primers* 2.
- Burbelo, P.D., Arnbatipudi, K., Alevizos, I., 2014. Genome-wide association studies in Sjogren's syndrome: what do the genes tell us about disease pathogenesis? *Autoimmun. Rev.* 13, 756–761.
- Buskila, D., 2009. Hepatitis C-associated rheumatic disorders. *Rheum. Dis. Clin. N. Am.* 35, 111–+.
- Cacoub, P., Comarmond, C., Domont, F., Savey, L., Saadoun, D., 2015. Cryoglobulinemia vasculitis. *Am. J. Med.* 128, 950–955.
- Caporali, R., Bonacci, E., Epis, O., Bobbio-Pallavicini, F., Morbini, P., Montecucco, C., 2008. Safety and usefulness of minor salivary gland biopsy: retrospective analysis of 502 procedures performed at a single center. *Arthritis Rheum.-Arthritis Care Res.* 59, 714–720.
- Carsetti, R., Rosado, M.M., Donnanno, S., Guazzi, V., Soresina, A., Meini, M., et al., 2005. The loss of IgM memory B cells correlates with clinical disease in common variable immunodeficiency. *J. Allerg. Clin. Immunol.* 115, 412–417.
- Carubbi, F., Cipriani, P., Marrelli, A., Di Benedetto, P., Ruscitti, P., Berardicurti, O., et al., 2013. Efficacy and safety of rituximab treatment in early primary Sjogren's syndrome: a prospective, multi-center, follow-up study. *Arthritis Res. Ther.* 15, R172.
- Carvajal Alegria, G., Guellec, D., Mariette, X., Gottenberg, J.E., Dernis, E., Dubost, J.J., et al., 2016. Epidemiology of neurological manifestations in Sjogren's syndrome: data from the French ASSESS Cohort. *RMD Open* 2, e000179.
- Cervera, R., Piette, J.C., Font, J., Khamashta, M.A., Shoenfeld, Y., Camps, M.T., et al., 2002. Antiphospholipid syndrome: clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients. *Arthritis Rheum.* 46, 1019–1027.
- Chen, K.R., Carlson, J.A., 2008. Clinical approach to cutaneous vasculitis. *Am. J. Clin. Dermatol.* 9, 71–92.
- Chen, S., Wang, Y., Zhang, G., Chen, S., 2016. Combination of salivary gland ultrasonography and virtual touch quantification for diagnosis of Sjogren's syndrome: a preliminary study. *Biomed. Res. Int.* 2016, 2793898.
- Cheng, K.T., Alevizos, I., Liu, X., Swaim, W.D., Yin, H., Feske, S., et al., 2012. STIM1 and STIM2 protein deficiency in T lymphocytes underlies development of the exocrine gland autoimmune disease, Sjogren's syndrome. *Proc. Natl. Acad. Sci. U.S.A.* 109, 14544–14549.
- Ciccia, F., Guggino, G., Rizzo, A., Ferrante, A., Raimondo, S., Giardina, A., et al., 2012. Potential involvement of IL-22 and IL-22-producing cells in the inflamed salivary glands of patients with Sjogren's syndrome. *Ann. Rheum. Dis.* 71, 295–301.
- Cimaz, R., Spence, D.L., Hornberger, L., Silverman, E.D., 2003. Incidence and spectrum of neonatal lupus erythematosus: a prospective study of infants born to mothers with anti-Ro autoantibodies. *J. Pediatr.* 142, 678–683.
- Cohen, C., Mekinian, A., Uzunhan, Y., Fauchais, A.L., Dhote, R., Pop, G., et al., 2013. 18F-fluorodeoxyglucose positron emission tomography/computer tomography as an objective tool for assessing disease activity in Sjogren's syndrome. *Autoimmun. Rev.* 12, 1109–1114.

- Cornec, D., Saraux, A., Jousse-Joulin, S., Pers, J.O., Boisrame-Gastrin, S., Renaudineau, Y., et al., 2015. The differential diagnosis of dry eyes, dry mouth, and parotidomegaly: a comprehensive review. *Clin. Rev. Allergy Immunol.* 49, 278–287.
- Coursey, T.G., Bian, F., Zaheer, M., Pflugfelder, S.C., Volpe, E.A., de Paiva, C.S., 2016. Age-related spontaneous lacrimal keratoconjunctivitis is accompanied by dysfunctional T regulatory cells. *Mucosal Immunol.* 10, 743–756.
- Crispin, J.C., Oukka, M., Bayliss, G., Cohen, R.A., Van Beek, C.A., Stillman, I.E., et al., 2008. Expanded double negative T cells in patients with systemic lupus erythematosus produce IL-17 and infiltrate the kidneys. *J. Immunol.* 181, 8761–8766.
- Daridon, C., Pers, J.O., Devauchelle, V., MartinsCarvalho, C., Hutin, P., Pennec, Y.L., et al., 2006. Identification of transitional type IIB cells in the salivary glands of patients with Sjogren's syndrome. *Arthritis Rheum.* 54, 2280–2288.
- Darrieutort-Laffite, C., Andre, V., Hayem, G., Saraux, A., Le Guern, V., Le Jeunne, C., et al., 2015. Sjogren's syndrome complicated by interstitial cystitis: a case series and literature review. *Joint Bone Spine* 82, 245–250.
- Dawson, L.J., Stanbury, J., Venn, N., Hasdimir, B., Rogers, S.N., Smith, P.M., 2006. Antimuscarinic antibodies in primary Sjogren's syndrome reversibly inhibit the mechanism of fluid secretion by human submandibular salivary acinar cells. *Arthritis Rheum.* 54, 1165–1173.
- De Carolis, S., Salvi, S., Botta, A., Garofalo, S., Garufi, C., Ferrazzani, S., et al., 2014. The impact of primary Sjogren's syndrome on pregnancy outcome: Our series and review of the literature. *Autoimmun. Rev.* 13, 103–107.
- de Groot, P.G., Meijers, J.C.M., Urbanus, R.T., 2012. Recent developments in our understanding of the antiphospholipid syndrome. *Int. J. Lab. Hematol.* 34, 223–231.
- Delaleu, N., Jonsson, R., Koller, M.M., 2005. Sjogren's syndrome. *Eur. J. Oral Sci.* 113, 101–113.
- Delaleu, N., Nguyen, C.Q., Peck, A.B., Jonsson, R., 2011. Sjogren's syndrome: studying the disease in mice. *Arthritis Res. Ther.* 13, 217.
- Delaleu, N., Mydel, P., Brun, J.G., Jonsson, M.V., Alimonti, A., Jonsson, R., 2016. Sjogren's syndrome patients with ectopic germinal centers present with a distinct salivary proteome. *Rheumatology* 55, 1127–1137.
- Dellavance, A., Alvarenga, R.R., Rodrigues, S.H., Kok, F., de Souza, A.W.S., Andrade, L.E.C., 2012. Anti-aquaporin-4 antibodies in the context of assorted immune-mediated diseases. *Eur. J. Neurol.* 19, 248–252.
- Delli, K., Dijkstra, P.U., Stel, A.J., Bootsma, H., Vissink, A., Spijkervet, F.K.L., 2015. Diagnostic properties of ultrasound of major salivary glands in Sjogren's syndrome: a meta-analysis. *Oral Dis.* 21, 792–800.
- Deshmukh, U.S., Ohyama, Y., Bagavant, H., Guo, X., Gaskin, F., Fu, S.M., 2008. Inflammatory stimuli accelerate Sjogren's syndrome-like disease in (NZB × NZW)F-1 mice. *Arthritis Rheum.* 58, 1318–1323.
- Devauchelle-Pensec, V., Mariette, X., Jousse-Joulin, S., Berthelot, J.M., Perdriger, A., Puechal, X., et al., 2014. Treatment of primary Sjogren syndrome with rituximab A randomized trial. *Ann. Intern. Med.* 160, 233–242.
- Dugonjic, S., Stefanovic, D., Ethurovic, B., Spasic-Jokic, V., Ajdinovic, B., 2014. Evaluation of diagnostic parameters from parotid and submandibular dynamic salivary glands scintigraphy and unstimulated sialometry in Sjogren's syndrome. *Hell J. Nucl. Med.* 17, 116–122.
- Ebert, E.C., 2012. Gastrointestinal and hepatic manifestations of Sjogren syndrome. *J. Clin. Gastroenterol.* 46, 25–30.
- Eisenberg, R.A., 1985. Association between the Ro and La antigenic determinants: immunodiffusion analysis of human spleen extract. *J. Immunol.* 135, 1707–1713.
- Epstein, L.C., Masse, G., Harmatz, J.S., Scott, T.M., Papas, A.S., Greenblatt, D.J., 2014. Characterization of cognitive dysfunction in Sjogren's syndrome patients. *Clin. Rheumatol.* 33, 511–521.
- Evans, R., Zdebik, A., Ciurtin, C., Walsh, S.B., 2015. Renal involvement in primary Sjogren's syndrome. *Rheumatology (Oxford, England)* 54, 1541–1548.
- Everett, S., Vishwanath, S., Cavero, V., Shen, L., Suresh, L., Malyavantham, K., et al., 2017. Analysis of novel Sjogren's syndrome autoantibodies in patients with dry eyes. *BMC Ophthalmol.* 17, 20.
- Farrell, R.J., Kelly, C.P., 2002. Current concepts – celiac sprue. *N. Engl. J. Med.* 346, 180–188.
- Fauchais, A.L., Ouattara, B., Gondran, G., Lalloue, F., Petit, D., Ly, K., et al., 2010. Articular manifestations in primary Sjogren's syndrome: clinical significance and prognosis of 188 patients. *Rheumatology* 49, 1164–1172.
- Fernandez, D., Perl, A., 2009. Metabolic control of T cell activation and death in SLE. *Autoimmun. Rev.* 8, 184–189.
- Finsterer, J., Bindu, P.S., 2015. Therapeutic strategies for mitochondrial disorders. *Pediat. Neurol.* 52, 302–313.
- Fletcher, C.A., Sutherland, A.P., Groom, J.R., Batten, M.L., Ng, L.G., Gommerman, J., et al., 2006. Development of nephritis but not sialadenitis in autoimmune-prone BAFF transgenic mice lacking marginal zone B cells. *Eur. J. Immunol.* 36, 2504–2514.
- Fletes-Rayas, A.L., Palafox-Sanchez, C.A., Munoz-Valle, J.F., Orozco-Barocio, G., Navarro-Hernandez, R.E., Oregon-Romero, E., 2016. TNFR1-383 A > C polymorphism association with clinical manifestations in primary Sjogren's syndrome patients. *Genet Mol. Res.* 15.
- Floreani, A., Franceschet, I., Cazzagon, N., 2014. Primary biliary cirrhosis: overlaps with other autoimmune disorders. *Semin. Liver Dis.* 34, 352–360.
- Fox, P.C., 1987. Systemic therapy of salivary gland hypofunction. *J. Dental Res.* 66 (Spec No), 689–692.
- Fox, R.I., 2005. Sjogren's syndrome. *Lancet* 366, 321–331.
- Fox, R.I., Howell, F.V., Bone, R.C., Michelson, P., 1984. Primary Sjogren syndrome: clinical and immunopathologic features. *Semin. Arthritis Rheum.* 14, 77–105.
- Francois, H., Mariette, X., 2016. Renal involvement in primary Sjogren syndrome. *Nat. Rev. Nephrol.* 12, 82–93.
- Fu, K., Weisenburger, D.D., Choi, W.W., Perry, K.D., Smith, L.M., Shi, X., et al., 2008. Addition of rituximab to standard chemotherapy improves the survival of both the germinal center B-cell-like and non-germinal center B-cell-like subtypes of diffuse large B-cell lymphoma. *J. Clin. Oncol.* 26, 4587–4594.
- Furuzawa-Carballeda, J., Sanchez-Guerrero, J., Betanzos, J.L., Enriquez, A.B., Avila-Casado, C., Llorente, L., et al., 2014. Differential cytokine expression and regulatory cells in patients with primary and secondary Sjogren's syndrome. *Scand. J. Immunol.* 80, 432–440.
- Gainey, R., Rooney, P.J., Alspaugh, M., 1985. Sjogren's syndrome and Crohn's disease. *Clin. Exp. Rheumatol.* 3, 67–69.
- Gao, J.H., Killadar, S., Cornelius, J.G., Nguyen, C., Cha, S.H., Peck, A.B., 2006. Sjogren's syndrome in the NOD mouse model is an interleukin-4 time-dependent, antibody isotype-specific autoimmune disease. *J. Autoimmun.* 26, 90–103.
- GarciaCarrasco, M., Siso, A., RamosCasals, M., Rosas, J., delaRed, G., Gil, V., et al., 2002. Raynaud's phenomenon in primary Sjogren's syndrome. Prevalence and clinical characteristics in a series of 320 patients. *J. Rheumatol.* 29, 726–730.

- Garcon, F., Patton, D.T., Emery, J.L., Hirsch, E., Rottapel, R., Sasaki, T., et al., 2008. CD28 provides T-cell costimulation and enhances PI3K activity at the immune synapse independently of its capacity to interact with the p85/p110 heterodimer. *Blood* 111, 1464–1471.
- Gayton, J.L., 2009. Etiology, prevalence, and treatment of dry eye disease. *Clin. Ophthalmol.* 3, 405–412.
- Giannouli, S., Voulgarelis, M., 2014. Predicting progression to lymphoma in Sjogren's syndrome patients. *Expert Rev. Clin. Immunol.* 10, 501–512.
- Gidwaney, N.G., Bajpai, M., Chokhavatia, S.S., 2016. Gastrointestinal dysmotility in the elderly. *J. Clin. Gastroenterol.* 50, 819–827.
- Ginzler, E.M., Wallace, D.J., Merrill, J.T., Furie, R.A., Stohl, W., Chatham, W.W., et al., 2014. Disease control and safety of belimumab plus standard therapy over 7 years in patients with systemic lupus erythematosus. *J. Rheumatol.* 41, 300–309.
- Goebel, H.H., Bardosi, A., 1987. Myoadenylate deaminase deficiency. *Klin. Wochenschr.* 65, 1023–1033.
- Gondran, G., Fauchais, A.L., Lambert, M., Ly, K., Launay, D., Queyrel, V., et al., 2008. Primary Sjogren's syndrome in men. *Scand. J. Rheumatol.* 37, 300–305.
- Gottenberg, J.E., Cagnard, N., Lucchesi, C., Letourneur, F., Mistou, S., Lazure, T., et al., 2006. Activation of IFN pathways and plasmacytoid dendritic cell recruitment in target organs of primary Sjogren's syndrome. *Proc. Natl. Acad. Sci. U.S.A.* 103, 2770–2775.
- Gottenberg, J.E., Ravaud, P., Puechal, X., Le Guern, V., Sibilia, J., Goeb, V., et al., 2014. Effects of hydroxychloroquine on symptomatic improvement in primary Sjogren syndrome the JOQUER randomized clinical trial. *JAMA* 312, 249–258.
- Gresz, V., Horvath, A., Gera, I., Nielsen, S., Zelles, T., 2015. Immunolocalization of AQP5 in resting and stimulated normal labial glands and in Sjogren's syndrome. *Oral Dis.* 21, E114–E120.
- Gronwall, C., Silverman, G.J., 2014. Natural IgM: beneficial autoantibodies for the control of inflammatory and autoimmune disease. *J. Clin. Immunol.* 34, S12–S21.
- Gupta, S., Zivadinov, R., Ramasamy, D., Ambrus, J.L., 2014. Reversible cerebral vasoconstriction syndrome (RCVS) in antiphospholipid antibody syndrome (APLA): the role of centrally acting vasodilators. Case series and review of literature. *Clin. Rheumatol.* 33, 1829–1833.
- Hackett, K.L., Gotts, Z.M., Ellis, J., Deary, V., Rapley, T., Ng, W.F., et al., 2016. An investigation into the prevalence of sleep disturbances in primary Sjogren's syndrome: a systematic review of the literature. *Rheumatology* 56, 570–580.
- Haldorsen, K., Moen, K., Jacobsen, H., Jonsson, R., Brun, J.G., 2008. Exocrine function in primary Sjogren syndrome: natural course and prognostic factors. *Ann. Rheum. Dis.* 67, 949–954.
- Harley, J.B., Reichlin, M., Arnett, F.C., Alexander, E.L., Bias, W.B., Provost, T.T., 1986. Gene interaction at HLA-DQ enhances autoantibody production in primary Sjogren's syndrome. *Science* 232, 1145–1147.
- Hauptman, H.W., Ruddy, S., Roberts, W.N., 1991. Reversal of the vasospastic component of lupus vasculopathy by infusion of prostaglandin E1. *J. Rheumatol.* 18, 1747–1752.
- Helmick, C.G., Felson, D.T., Lawrence, R.C., Gabriel, S., Hirsch, R., Kwoh, C.K., et al., 2008. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part I. *Arthritis Rheum.* 58, 15–25.
- Hermann, G.E., Holmes, G.M., Rogers, R.C., 2005. TNF( $\alpha$ ) modulation of visceral and spinal sensory processing. *Curr. Pharm. Des.* 11, 1391–1409.
- Herrick, A.L., 2016. Recent advances in the pathogenesis and management of Raynaud's phenomenon and digital ulcers. *Curr. Opin. Rheumatol.* 28, 577–585.
- Hillen, M.R., Moret, F.M., Giovannone, B., Kruize, A.A., Radstake, T., van Roon, J.A.G., 2016. Size matters: decreased glandular levels of anti-inflammatory short thymic stromal lymphopoietin in primary Sjogren's syndrome. *Clin. Exp. Rheumatol.* 34, 959–960.
- Holden, W., Orchard, T., Wordsworth, P., 2003. Enteropathic arthritis. *Rheum. Dis. Clin. North Am.* 29, 513–530. viii.
- Hughes, M., Herrick, A.L., 2016. Raynaud's phenomenon. *Best Pract. Res. Clin. Rheumatol.* 30, 112–132.
- Igoe, A., Scofield, R.H., 2013. Autoimmunity and infection in Sjogren's syndrome. *Curr. Opin. Rheumatol.* 25, 480–487.
- Jabs, D.A., Prendergast, R.A., 1988. Murine models of Sjogren's syndrome. Immunohistologic analysis of different strains. *Invest. Ophthalmol. Vis. Sci.* 29, 1437–1443.
- Jamilloux, Y., Magy, L., Hurtevent, J.F., Gondran, G., de Seze, J., Launay, D., et al., 2014. Immunological profiles determine neurological involvement in Sjogren's syndrome. *Eur. J. Intern. Med.* 25, 177–181.
- Jayarangaiah, A., Sehgal, R., Epperla, N., 2014. Sjogren's syndrome and Neuromyelitis Optica spectrum disorders (NMOSD) – a case report and review of literature. *BMC Neurol.* 14, 200.
- Jonsson, M.V., Delaleu, N., Brokstad, K.A., Berggreen, E., Skarstein, K., 2006. Impaired salivary gland function in NOD mice – association with changes in cytokine profile but not with histopathologic changes in the salivary gland. *Arthritis Rheum.* 54, 2300–2305.
- Jonsson, R., Nginamau, E., Szyszko, E., Brokstad, K.A., 2007. Role of B cells in Sjogren's syndrome – from benign lymphoproliferation to overt malignancy. *Front. Biosci.* 12, 2159–2170.
- Kalled, S.L., 2005. The role of BAFF in immune function and implications for autoimmunity. *Immunol. Rev.* 204, 43–54.
- Kang, H.I., Fei, H.M., Saito, I., Sawada, S., Chen, S.L., Yi, D., et al., 1993. Comparison of HLA class II genes in Caucasoid, Chinese, and Japanese patients with primary Sjogren's syndrome. *J. Immunol.* 150, 3615–3623.
- Katsifis, G.E., Moutsopoulos, N.M., Wahl, S.M., 2007. T lymphocytes in Sjogren's syndrome: contributors to and regulators of pathophysiology. *Clin. Rev. Allergy Immunol.* 32, 252–264.
- Kaufman, I., Schwartz, D., Caspi, D., Paran, D., 2008. Sjogren's syndrome – not just Sicca: renal involvement in Sjogren's syndrome. *Scand. J. Rheumatol.* 37, 213–218.
- Keating, G.M., 2017. Lifitegrast ophthalmic solution 5%: a review in dry eye disease. *Drugs* 77, 201–208.
- Kerttula, T.O., Collin, P., Polvi, A., Korpela, M., Partanen, J., Maki, M., 1996. Distinct immunologic features of Finnish Sjogren's syndrome patients with HLA alleles DRB1\*0301, DQA1\*0501, and DQB1\*0201. Alterations in circulating T cell receptor gamma/delta subsets. *Arthritis Rheum.* 39, 1733–1739.
- Keyes, G.G., Vickers, R.A., Kersey, J.H., 1977. Immunopathology of Sjogren-like disease in NZB/HZW mice. *J. Oral Pathol.* 6, 288–295.
- Kim, D.H., Lee, J.C., Kim, S., Oh, S.H., Lee, M.K., Kim, K.W., et al., 2011. Inhibition of autoimmune diabetes by TLR2 tolerance. *J. Immunol.* 187, 5211–5220.

- Kim-Lee, C., Suresh, L., Ambrus, J.L., 2015. Gastrointestinal disease in Sjogren's syndrome: related to food hypersensitivities. *Springerplus* 4, 766.
- Kocer, B., Tezcan, M.E., Batur, H.Z., Haznedaroglu, S., Goker, B., Irkec, C., et al., 2016. Cognition, depression, fatigue, and quality of life in primary Sjogren's syndrome: correlations. *Brain Behav.* 6, e00586.
- Koh, J.H., Kwok, S.K., Lee, J., Park, S.H., 2017. Autonomic dysfunction in primary Sjogren's syndrome: a prospective cohort analysis of 154 Korean patients. *Kor. J. Intern. Med.* 32, 165–+.
- Konttinen, Y.T., Porola, P., Konttinen, L., Laine, M., Poduval, P., 2006. Immunohistopathology of Sjogren's syndrome. *Autoimmun. Rev.* 6, 16–20.
- Korman, B.D., Alba, M.I., Le, J.M., Alevizos, I., Smith, J.A., Nikolov, N.P., et al., 2008. Variant form of STAT4 is associated with primary Sjogren's syndrome. *Genes Immun.* 9, 267–270.
- Koss, M.N., Hochholzer, L., Langloss, J.M., Wehunt, W.D., Lazarus, A.A., 1987. Lymphoid interstitial pneumonia: clinicopathological and immunopathological findings in 18 cases. *Pathology* 19, 178–185.
- Kotsis, K., Voulgari, P.V., Tsifetaki, N., Drosos, A.A., Carvalho, A.F., Hyphantis, T., 2014. Illness perceptions and psychological distress associated with physical health-related quality of life in primary Sjogren's syndrome compared to systemic lupus erythematosus and rheumatoid arthritis. *Rheumatol. Int.* 34, 1671–1681.
- Kramer, J.M., 2014. Early events in Sjogren's Syndrome pathogenesis: the importance of innate immunity in disease initiation. *Cytokine* 67, 92–101.
- Kramer, J.M., Klimatcheva, E., Rothstein, T.L., 2013. CXCL13 is elevated in Sjogren's syndrome in mice and humans and is implicated in disease pathogenesis. *J. Leukoc. Biol.* 94, 1079–1089.
- Kraus, A., Caballero-Uribe, C., Jakez, J., Villa, A.R., Alarcon-Segovia, D., 1992. Raynaud's phenomenon in primary Sjogren's syndrome. Association with other extraglandular manifestations. *J. Rheumatol.* 19, 1572–1574.
- Kurosaki, T., Kometani, K., Ise, W., 2015. Memory B cells. *Nat. Rev. Immunol.* 15, 149–159.
- Lai, Z.N., Yin, H.G., Cabrera-Perez, J., Guimaro, M.C., Afione, S., Michael, D.G., et al., 2016. Aquaporin gene therapy corrects Sjogren's syndrome phenotype in mice. *Proc. Natl. Acad. Sci. U.S.A.* 113, 5694–5699.
- Lee, B.H., Gauna, A.E., Pauley, K.M., Park, Y.J., Cha, S., 2012. Animal models in autoimmune diseases: lessons learned from mouse models for Sjogren's syndrome. *Clin. Rev. Allergy Immunol.* 42, 35–44.
- Lessard, C.J., Li, H., Adrianto, I., Ice, J.A., Rasmussen, A., Grundahl, K.M., et al., 2013. Variants at multiple loci implicated in both innate and adaptive immune responses are associated with Sjogren's syndrome. *Nat. Genet.* 45, 1284–1292.
- Li, Y., Zhang, K., Chen, H., Sun, F., Xu, J., Wu, Z., et al., 2013. A genome-wide association study in Han Chinese identifies a susceptibility locus for primary Sjogren's syndrome at 7q11.23. *Nat. Genet.* 45, 1361–1365.
- Li, J., Zheng, K., Deng, Z., Zheng, J., Ma, H., Sun, L., et al., 2015a. Prevalence and risk factors of dry eye disease among a hospital-based population in southeast China. *Eye Contact Lens* 41, 44–50.
- Li, X.M., Xu, B., Ma, Y., Li, X.P., Cheng, Q., Wang, X.M., et al., 2015b. Clinical and laboratory profiles of primary Sjogren's syndrome in a Chinese population: a retrospective analysis of 315 patients. *Int. J. Rheum. Dis.* 18, 439–446.
- Lin, D.F., Yan, S.M., Zhao, Y., Zhang, W., Li, M.T., Zeng, X.F., et al., 2010. Clinical and prognostic characteristics of 573 cases of primary Sjogren's syndrome. *Chin. Med. J.* 123, 3252–3257.
- Llamas-Gutierrez, F.J., Reyes, E., Martinez, B., Hernandez-Molina, G., 2014. Histopathological environment besides the focus score in Sjogren's syndrome. *Int. J. Rheum. Dis.* 17, 898–903.
- Low, H.Z., Witte, T., 2011. Aspects of innate immunity in Sjogren's syndrome. *Arthritis Res. Ther.* 13, 218.
- Luo, Y., Zhang, L., Fei, Y., Li, Y., Hao, D., Liu, Y., et al., 2015. Pregnancy outcome of 126 anti-SSA/Ro-positive patients during the past 24 years—a retrospective cohort study. *Clin. Rheumatol.* 34, 1721–1728.
- Mackay, I.R., Rose, N.R., 2001. Autoimmunity and lymphoma: tribulations of B cells. *Nat. Immunol.* 2, 793–795.
- Mackay, F., Woodcock, S.A., Lawton, P., Ambrose, C., Baetscher, M., Schneider, P., et al., 1999. Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. *J. Exp. Med.* 190, 1697–1710.
- Maier-Moore, J.S., Koelsch, K.A., Smith, K., Lessard, C.J., Radfar, L., Lewis, D., et al., 2014. Antibody-secreting cell specificity in labial salivary glands reflects the clinical presentation and serology in patients with Sjogren's syndrome. *Arthritis Rheum.* 66, 3445–3456.
- Malladi, A., Sack, K., Shibuski, S., Shibuski, C., Baer, A., Banushree, R., et al., 2012. Primary Sjogren's syndrome as a systemic disease: a study of participants enrolled in an international Sjogren's syndrome registry. *Arthritis Care Res.* 64, in press.
- Manganelli, P., Fietta, P., Quaini, F., 2006. Hematologic manifestations of primary Sjogren's syndrome. *Clin. Exp. Rheumatol.* 24, 438–448.
- Maria, N.I., van Helden-Meeuwesen, C.G., Steenwijk, E.C., Ijpma, A.S., Brkic, Z., van Daele, P.L., et al., 2015. Systemic interferon type I and type II signatures present in distinct subsets of primary Sjogren's syndrome: en route towards more selective targeting. *Arthritis Rheum.* 67.
- Mariette, X., 2008. Therapeutic potential for B-cell modulation in Sjogren's syndrome. *Rheum. Dis. Clin. N. Am.* 34, 1025–+.
- Marra, A.M., Arcopinto, M., Bossone, E., Ehlik, N., Cittadini, A., Grunig, E., 2015. Pulmonary arterial hypertension-related myopathy: an overview of current data and future perspectives. *Nutr. Metab. Cardiovasc. Dis.* 25, 131–139.
- Massara, A., Bonazza, S., Castellino, G., Caniatti, L., Trotta, F., Borrelli, M., et al., 2010. Central nervous system involvement in Sjogren's syndrome: unusual, but not unremarkable-clinical, serological characteristics and outcomes in a large cohort of Italian patients. *Rheumatology* 49, 1540–1549.
- Mavragani, C.P., Moutsopoulos, H.M., 2014. Sjogren's syndrome. *Annu. Rev. Pathol.: Mech. Dis.* 9, 273–285.
- McNelis, J.C., Olefsky, J.M., 2014. Macrophages, immunity, and metabolic disease. *Immunity* 41, 36–48.
- Meiners, P., Vissink, A., Kroese, F., Spijkervet, F., Haacke, E., Abdulahad, W., et al., 2013. Abatacept treatment reduces disease activity in early primary Sjogren's syndrome (phase ii open label ASAP study). *Ann. Rheum. Dis.* 72, 89.
- Mekinian, A., Ravaud, P., Hatron, P.Y., Larroche, C., Leone, J., Gombert, B., et al., 2012. Efficacy of rituximab in primary Sjogren's syndrome with peripheral nervous system involvement: results from the AIR registry. *Ann. Rheum. Dis.* 71, 84–87.
- Mellgren, S.I., Goransson, L.G., Omdal, R., 2007. Primary Sjogren's syndrome associated neuropathy. *Can. J. Neurol. Sci.* 34, 280–287.

- Miceli-Richard, C., Criswell, L.A., 2014. Genetic, genomic and epigenetic studies as tools for elucidating disease pathogenesis in primary Sjogren's syndrome. *Expert Rev. Clin. Immunol.* 10, 437–444.
- Milin, M., Cornea, D., Chastaing, M., Griner, V., Berrouiguet, S., Nowak, E., et al., 2016. Sicca symptoms are associated with similar fatigue, anxiety, depression, and quality-of-life impairments in patients with and without primary Sjogren's syndrome. *Joint Bone Spine* 83, 681–685.
- Mitsias, D.I., Tzioufas, A.G., Veiropoulou, C., Zintzaras, E., Tassios, I.K., Kogopoulou, O., et al., 2002. The Th1/Th2 cytokine balance changes with the progress of the immunopathological lesion of Sjogren's syndrome. *Clin. Exp. Immunol.* 128, 562–568.
- Miyamoto, A., Nakayama, K., Imaki, H., Hirose, S., Jiang, Y., Abe, M., et al., 2002. Increased proliferation of B cells and auto-immunity in mice lacking protein kinase C delta. *Nature* 416, 865–869.
- Molina, C., Allende, C., Aguilera, S., Kwon, Y.J., Leyton, L., Martinez, B., et al., 2006. Basal lamina disorganisation of the acini and ducts of labial salivary glands from patients with Sjogren's syndrome: association with mononuclear cell infiltration. *Ann. Rheum. Dis.* 65, 178–183.
- Morris, G., Berk, M., Walder, K., Maes, M., 2015. Central pathways causing fatigue in neuro-inflammatory and autoimmune illnesses. *BMC Med.* 13, 28.
- Moutsopoulos, H.M., 1988. Immunopathogenesis of Sjogren's syndrome. *Isr. J. Med. Sci.* 24, 737–739.
- Muller, U., Steinhoff, U., Reis, L., Hemmi, S., Pavlovic, J., Zinkernagel, R.M., et al., 1994. Functional role of type I and type II interferons in antiviral defense. *Science* 264, 1918–1921.
- Napenas, J.J., Rouleau, T.S., 2014. Oral complications of Sjogren's syndrome. *Oral Maxillofac. Surg. Clin. N. Am.* 26, 55–+.
- Nezos, A., Gravani, F., Tassidou, A., Kapsogeorgou, E.K., Voulgarelis, M., Koutsilieris, M., et al., 2015. Type I and II interferon signatures in Sjogren's syndrome pathogenesis: contributions in distinct clinical phenotypes and Sjogren's related lymphomagenesis. *J. Autoimmun.* 63, 47–58.
- Nguyen, C., Singson, E., Kim, J.Y., Cornelius, J.G., Attia, R., Doyle, M.E., et al., 2006. Sjogren's syndrome-like disease of C57BL/6.NOD-Aec1Aec2 mice: gender differences in keratoconjunctivitis sicca defined by a cross-over in the chromosome 3 Aec1 locus. *Scand. J. Immunol.* 64, 295–307.
- Nguyen, T.T.T., Elsner, R.A., Baumgarth, N., 2015. Natural IgM prevents autoimmunity by enforcing B cell central tolerance induction. *J. Immunol.* 194, 1489–1502.
- Nicolson, G.L., 2014. Mitochondrial dysfunction and chronic disease: treatment with natural supplements. *Altern. Therap. Health Med.* 20 (Suppl 1), 18–25.
- Nocturne, G., Tarn, J., Boudaoud, S., Locke, J., Miceli-Richard, C., Hachulla, E., et al., 2016. Germline variation of TNFAIP3 in primary Sjogren's syndrome-associated lymphoma. *Ann. Rheum. Dis.* 75, 780–783.
- Nordmark, G., Kristjansdottir, G., Theander, E., Appel, S., Eriksson, P., Vasaitis, L., et al., 2011. Association of EBF1, FAM167A(C8orf13)-BLK and TNFSF4 gene variants with primary Sjogren's syndrome. *Genes Immun.* 12, 100–109.
- Norheim, K.B., Jonsson, G., Omdal, R., 2011. Biological mechanisms of chronic fatigue. *Rheumatology* 50, 1009–1018.
- Norheim, K.B., Le Hellard, S., Nordmark, G., Harboe, E., Goransson, L., Brun, J.G., et al., 2014. A possible genetic association with chronic fatigue in primary Sjogren's syndrome: a candidate gene study. *Rheumatol. Int.* 34, 191–197.
- Oak, J.S., Deane, J.A., Kharas, M.G., Luo, J., Lane, T.E., Cantley, L.C., et al., 2006. Sjogren's syndrome-like disease in mice with T cells lacking class 1A phosphoinositide-3-kinase. *Proc. Natl. Acad. Sci. U.S.A.* 103, 16882–16887.
- Ohyama, Y., Carroll, V.A., Deshmukh, U., Gaskin, F., Brown, M.G., Fu, S.M., 2006. Severe focal sialadenitis and dacryoadenitis in NZM2328 mice induced by MCMV: a novel model for human Sjogren's syndrome. *J. Immunol.* 177, 7391–7397.
- Ostrowski, R.A., Robinson, J.A., 2008. Antiphospholipid antibody syndrome and autoimmune diseases. *Hematol.-Oncol. Clin. N. Am.* 22, 53–+.
- Pagano, G., Castello, G., Pallardo, F.V., 2013. Sjogren's syndrome-associated oxidative stress and mitochondrial dysfunction: prospects for chemoprevention trials. *Free Radic. Res.* 47, 71–73.
- Pan, L., Sato, S., Frederick, J.P., Sun, X.H., Zhuang, Y., 1999. Impaired immune responses and B-cell proliferation in mice lacking the Id3 gene. *Mol. Cell Biol.* 19, 5969–5980.
- Panda, S., Ding, J.L., 2015. Natural antibodies bridge innate and adaptive immunity. *J. Immunol.* 194, 13–20.
- Parambil, J.G., Myers, J.L., Lindell, R.M., Matteson, E.L., Ryu, J.H., 2006. Interstitial lung disease in primary Sjogren syndrome. *Chest* 130, 1489–1495.
- Paran, D., Fireman, E., Levartovsky, D., Elkayam, O., Kaufman, I., Litinsky, I., et al., 2007. Pulmonary dysfunction in systemic lupus erythematosus and anti-phospholipid syndrome patients. *Scand. J. Rheumatol.* 36, 285–290.
- Park, K., Park, S., Jackson, M.W., 2013. The inhibitory effects of antimuscarinic autoantibodies in the sera of primary Sjogren syndrome patients on the gastrointestinal motility. *Mol. Immunol.* 56, 583–587.
- Parke, A.L., 2008. Pulmonary manifestations of primary Sjogren's syndrome. *Rheum. Dis. Clin. N. Am.* 34, 907–+.
- Parke, A.L., Buchanan, W.W., 1998. Sjogren's syndrome: history, clinical and pathological features. *Inflammopharmacology* 6, 271–287.
- Pasoto, S.G., Chakkour, H.P., Natalino, R.R., Viana, V.S., Bueno, C., Lianza, A.C., et al., 2012. Lupus anticoagulant: a marker for stroke and venous thrombosis in primary Sjogren's syndrome. *Clin. Rheumatol.* 31, 1331–1338.
- Patejdl, R., Penner, I.K., Noack, T.K., Zettl, U.K., 2016. Multiple sclerosis and fatigue: a review on the contribution of inflammation and immune-mediated neurodegeneration. *Autoimmun. Rev.* 15, 210–220.
- Patnaik, S.S., Lagana, A.S., Vitale, S.G., Buttice, S., Noventa, M., Gizzo, S., et al., 2017. Etiology, pathophysiology and biomarkers of interstitial cystitis/painful bladder syndrome. *Arch. Gynecol. Obstet.* 295, 1341–1359.
- Peck, A.B., Saylor, B.T., Nguyen, L., Sharma, A., She, J.X., Nguyen, C.Q., et al., 2011. Gene expression profiling of early-phase Sjogren's syndrome in C57BL/6.NOD-Aec1Aec2 mice identifies focal adhesion maturation associated with infiltrating leukocytes. *Invest. Ophthal. Vis. Sci.* 52, 5647–5655.
- Perez, M.J., Martin, R.V., Trillo, V.M., Gande, R.G., 2017. Inflammatory bowel disease: new therapeutic options in the post anti-TNFalpha era. *Curr. Drug Metab.* 18, 666–679.

- Pers, J.O., Youinou, P., 2014. Are the B cells cast with the leading part in the Sjogren's syndrome scenario? *Oral Dis.* 20, 529–537.
- Pertovaara, M., Bootorabi, F., Kuuslahti, M., Pasternack, A., Parkkila, S., 2011. Novel carbonic anhydrase autoantibodies and renal manifestations in patients with primary Sjogren's syndrome. *Rheumatology* 50, 1453–1457.
- Pierce, J.L., Tanner, K., Merrill, R.M., Miller, K.L., Kendall, K.A., Roy, N., 2016. Swallowing disorders in Sjogren's syndrome: prevalence, risk factors, and effects on quality of life. *Dysphagia* 31, 49–59.
- Pijpe, J., vanImhoff, G.W., Spijkervet, F.K.L., Roodenburg, J.L.N., Wolbink, G.J., Mansour, K., et al., 2005. Rituximab treatment in patients with primary Sjogren's syndrome. *Arthritis Rheum.* 52, 2740–2750.
- Pijpe, J., Kalk, W.W., Bootsma, H., Spijkervet, F.K., Kallenberg, C.G., Vissink, A., 2007. Progression of salivary gland dysfunction in patients with Sjogren's syndrome. *Ann. Rheum. Dis.* 66, 107–112.
- Pillai, S., Cariappa, A., Moran, S.T., 2005. Marginal zone B cells. *Annu. Rev. Immunol.* 23, 161–196.
- Pittman, F.E., Holub, D.A., 1965. Sjogren's syndrome and adult celiac disease. *Gastroenterology* 48, 869–876.
- Priori, R., Minniti, A., Derme, M., Antonazzo, B., Brancatisano, F., Ghirini, S., et al., 2015. Quality of sexual life in women with primary Sjogren syndrome. *J. Rheumatol.* 42, 1427–1431.
- Priori, R., Minniti, A., Antonazzo, B., Fusconi, M., Valesini, G., Curcio, G., 2016. Sleep quality in patients with primary Sjogren's syndrome. *Clin. Exp. Rheumatol.* 34, 373–379.
- Qin, B., Wang, J., Yang, Z., Yang, M., Ma, N., Huang, F., et al., 2015. Epidemiology of primary Sjogren's syndrome: a systematic review and meta-analysis. *Ann. Rheum. Dis.* 74, 1983–1989.
- Quismorio Jr., F.P., 1996. Pulmonary involvement in primary Sjogren's syndrome. *Curr. Opin. Pulm. Med.* 2, 424–428.
- RamosCasals, M., GarciaCarrasco, M., Cervera, R., Rosas, J., Trejo, O., delaRed, G., et al., 2001. Hepatitis C virus infection mimicking primary Sjogren syndrome – a clinical and immunologic description of 35 cases. *Medicine* 80, 1–8.
- Rao, G.S.U., Muthuchellappan, R., 2016. Cerebral vasospasm: current understanding. *Curr. Opin. Anesthesiol.* 29, 544–551.
- Reksten, T.R., Jonsson, M.V., 2014. Sjogren's syndrome an update on epidemiology and current insights on pathophysiology. *Oral Maxillofac. Surg. Clin. N. Am.* 26, 1–+.
- Reksten, T.R., Lessard, C.J., Sivils, K.L., 2016. Genetics in Sjogren syndrome. *Rheum. Dis. Clin. N. Am.* 42, 435.
- Ren, H., Wang, W.M., Chen, X.N., Zhang, W., Pan, X.X., Wang, X.L., et al., 2008. Renal involvement and followup of 130 patients with primary Sjogren's syndrome. *J. Rheumatol.* 35, 278–284.
- Retamozo, S., Gheitasi, H., Quartuccio, L., Kostov, B., Corazza, L., Bove, A., et al., 2016. Cryoglobulinaemic vasculitis at diagnosis predicts mortality in primary Sjogren syndrome: analysis of 515 patients. *Rheumatology* 55, 1443–1451.
- Roescher, N., Vosters, J.L., Yin, H.E., Illei, G.G., Tak, P.P., Chiorini, J.A., 2011. Effect of soluble ICAM-1 on a Sjogren's syndrome-like phenotype in NOD mice is disease stage dependent. *PLoS One* 6, e19962.
- Roescher, N., Lodde, B.M., Vosters, J.L., Tak, P.P., Catalan, M.A., Illei, G.G., et al., 2012. Temporal changes in salivary glands of non-obese diabetic mice as a model for Sjogren's syndrome. *Oral Dis.* 18, 96–106.
- Ryo, K., Yamada, H., Nakagawa, Y., Tai, Y., Obara, K., Inoue, H., et al., 2006. Possible involvement of oxidative stress in salivary gland of patients with Sjogren's syndrome. *Pathobiology* 73, 252–260.
- Salloum, R., Niewold, T.B., 2011. Interferon regulatory factors in human lupus pathogenesis. *Transl. Res.* 157, 326–331.
- Sallusto, F., Geginat, J., Lanzavecchia, A., 2004. Central memory and effector memory T cell subsets: function, generation, and maintenance. *Annu. Rev. Immunol.* 22, 745–763.
- Sanger, G.J., Pasricha, P.J., 2017. Investigational drug therapies for the treatment of gastroparesis. *Expert Opin. Investig. Drugs* 26, 331–342.
- Scoble, T., Wijetilleka, S., Khamashta, M.A., 2011. Management of refractory anti-phospholipid syndrome. *Autoimmun. Rev.* 10, 669–673.
- Scofield, R.H., 2011. Vasculitis in Sjogren's syndrome. *Curr. Rheumatol. Rep.* 13, 482–488.
- Scofield, R.H., Asfa, S., Obeso, D., Jonsson, R., Kurien, B.T., 2005. Immunization with short peptides from the 60-kDa Ro antigen recapitulates the serological and pathological findings as well as the salivary gland dysfunction of Sjogren's syndrome. *J. Immunol.* 175, 8409–8414.
- Segal, B., Bowman, S.J., Fox, P.C., Vivino, F.B., Murukutla, N., Brodscholl, J., et al., 2009. Primary Sjogren's Syndrome: health experiences and predictors of health quality among patients in the United States. *Health Qual. Life Outcomes* 7, 46.
- Shen, L., Zhang, C., Wang, T., Brooks, S., Ford, R.J., Lin-Lee, Y.C., et al., 2006. Development of autoimmunity in IL-14 alpha-transgenic mice. *J. Immunol.* 177, 5676–5686.
- Shen, L., Suresh, L., Li, H., Zhang, C.J., Kumar, V., Panekewycz, O., et al., 2009. IL-14 alpha, the nexus for primary Sjogren's disease in mice and humans. *Clin. Immunol.* 130, 304–312.
- Shen, L., Suresh, L., Wu, J., Xuan, J.X., Li, H., Zhang, C.J., et al., 2010. A role for lymphotoxin in primary Sjogren's disease. *J. Immunol.* 185, 6355–6363.
- Shen, L., Suresh, L., Lindemann, M., Xuan, J., Kowal, P., Malyavantham, K., et al., 2012. Novel autoantibodies in Sjogren's syndrome. *Clin. Immunol.* 145, 251–255.
- Shen, L., Suresh, L., Malyavantham, K., Kowal, P., Xuan, J.X., Lindemann, M.J., et al., 2013. Different stages of primary Sjogren's syndrome involving lymphotoxin and type 1 IFN. *J. Immunol.* 191, 608–613.
- Shen, L., Kapsogeorgou, E.K., Yu, M.X., Suresh, L., Malyavantham, K., Tzioufas, A.G., et al., 2014. Evaluation of salivary gland protein 1 antibodies in patients with primary and secondary Sjogren's syndrome. *Clin. Immunol.* 155, 42–46.
- Shen, L., Gao, C., Suresh, L., Xian, Z., Song, N., Chaves, L.D., et al., 2016. Central role for marginal zone B cells in an animal model of Sjogren's syndrome. *Clin. Immunol.* 168, 30–36.
- Shiboski, C.H., Shiboski, S.C., Seror, R., Criswell, L.A., Labetoulle, M., Lietman, T.M., et al., 2017. 2016 American College of Rheumatology/European League Against Rheumatism classification criteria for primary Sjogren's syndrome a consensus and data-driven methodology involving three international patient cohorts. *Arthritis Rheumatol.* 69, 35–45.
- Shikhagaie, M.M., Germar, K., Bal, S.M., Ros, X.R., Spits, H., 2017. Innate lymphoid cells in autoimmunity: emerging regulators in rheumatic diseases. *Nat. Rev. Rheumatol.* 13, 164–173.
- Singh, A.G., Singh, S., Matteson, E.L., 2016. Rate, risk factors and causes of mortality in patients with Sjogren's syndrome: a systematic review and meta-analysis of cohort studies. *Rheumatology* 55, 450–460.

- Singhal, A.B., Hajj-Ali, R.A., Topcuoglu, M.A., Fok, J., Bena, J., Yang, D.S., et al., 2011. Reversible cerebral vasoconstriction syndromes analysis of 139 cases. *Arch. Neurol.* 68, 1005–1012.
- Sjogren, H., 1951. Some problems concerning keratoconjunctivitis sicca and the sicca-syndrome. *Acta Ophthalmol. (Copenh)* 29, 33–47.
- Smedby, K.E., Vajdic, C.M., Falster, M., Engels, E.A., Martinez-Maza, O., Turner, J., et al., 2008. Autoimmune disorders and risk of non-Hodgkin lymphoma subtypes: a pooled analysis within the InterLymph consortium. *Blood* 111, 4029–4038.
- Sneyd, J., Crampin, E., Yule, D., 2014. Multiscale modelling of saliva secretion. *Math. Biosci.* 257, 69–79.
- Song, M.K., Chung, J.S., Shin, D.H., Seol, Y.M., Shin, H.J., Choi, Y.J., et al., 2009. Prognostic significance of the Bcl-2 negative germinal centre in patients with diffuse large B cell lymphoma treated with R-CHOP. *Leuk. Lymphoma* 50, 54–61.
- Souza, F.B.D., Porfirio, G.J.M., Andriolo, B.N.G., de Albuquerque, J.V., Trevisani, V.F.M., 2016. Rituximab effectiveness and safety for treating primary Sjögren's syndrome (pSS): systematic review and meta-analysis. *PLoS One* 11, e0150749.
- St Clair, E.W., Baer, A.N., Noaish, G., Parke, A., Coca, A., Utset, T., et al., 2015. The clinical efficacy and safety of baminercept, a lymphotoxin-beta receptor fusion protein, in primary Sjögren's syndrome: results from a randomized, double-blind, placebo-controlled phase II trial. *Arthritis Rheum.* 67.
- Strand, V., Talal, N., 1979. Advances in the diagnosis and concept of Sjögren's syndrome (autoimmune exocrinopathy). *Bull. Rheum. Dis.* 30, 1046–1052.
- Sumida, T., Iizuka, M., Asashima, H., Tsuboi, H., Matsumoto, I., 2012. Pathogenic role of anti-M3 muscarinic acetylcholine receptor immune response in Sjögren's syndrome. *Presse. Med.* 41, e461–466.
- Sun, Y., Zhang, W., Li, B., Zou, Z., Selmi, C., Gershwin, M.E., 2015. The coexistence of Sjögren's syndrome and primary biliary cirrhosis: a comprehensive review. *Clin. Rev. Allergy Immunol.* 48, 301–315.
- Suresh, L., Ambrus, J., Shen, L., Vishwanath, S., 2014. Metabolic disorders causing fatigue in Sjögren's syndrome. *Arthritis Rheumatol.* 66, S1113.
- Suresh, L., Malyavantham, K., Shen, L., Ambrus, J.L., 2015. Investigation of novel autoantibodies in Sjögren's syndrome utilizing Sera from the Sjögren's international collaborative clinical alliance cohort. *BMC Ophthalmol.* 15, 38.
- Sutherland, A.P., Mackay, F., Mackay, C.R., 2006. Targeting BAFF: immunomodulation for autoimmune diseases and lymphomas. *Pharmacol. Ther.* 112, 774–786.
- Swigris, J.J., Berry, G.J., Raffin, T.A., Kuschner, W.G., 2002. Lymphoid interstitial pneumonia: a narrative review. *Chest* 122, 2150–2164.
- Taleb, K., Auffray, C., Villefroy, P., Pereira, A., Hosmalin, A., Gaudry, M., et al., 2017. Chronic type I IFN is sufficient to promote immunosuppression through accumulation of myeloid-derived suppressor cells. *J. Immunol.* 198, 1156–1163.
- Tapinos, N.I., Polihronis, M., Tzioufas, A.G., Moutsopoulos, H.M., 1999. Sjögren's syndrome. Autoimmune epithelitis. *Adv. Exp. Med. Biol.* 455, 127–134.
- Tarnopolsky, M.A., 2008. The mitochondrial cocktail: rationale for combined nutraceutical therapy in mitochondrial cytopathies. *Adv. Drug Deliv. Rev.* 60, 1561–1567.
- Teijeira, S., Millan, B.S., Fernandez, J.M., Rivas, E., Vieitez, I., Miranda, S., et al., 2009. Myoadenylate deaminase deficiency: clinicopathological and molecular study of a series of 27 Spanish cases. *Clin. Neuropathol.* 28, 136–142.
- Teos, L.Y., Zhang, Y., Cotrim, A.P., Swaim, W., Won, J.H., Ambrus, J., et al., 2015. IP3R deficit underlies loss of salivary fluid secretion in Sjögren's Syndrome. *Sci. Rep.* 5, 13953.
- Teruel, M., Alarcon-Riquelme, M.E., 2016. Genetics of systemic lupus erythematosus and Sjögren's syndrome: an update. *Curr. Opin. Rheumatol.* 28, 506–514.
- Theander, E., Henriksson, G., Ljungberg, O., Mandl, T., Manthorpe, R., Jacobsson, L.T.H., 2006. Lymphoma and other malignancies in primary Sjögren's syndrome: a cohort study on cancer incidence and lymphoma predictors. *Ann. Rheum. Dis.* 65, 796–803.
- Theander, E., Jonsson, R., Sjostrom, B., Brokstad, K., Olsson, P., Henriksson, G., 2015. Prediction of Sjögren's syndrome years before diagnosis and identification of patients with early onset and severe disease course by autoantibody profiling. *Arthritis Rheum.* 67, 2427–2436.
- Thieblemont, C., Berger, F., Coiffier, B., 1995. Mucosa-associated lymphoid tissue lymphomas. *Curr. Opin. Oncol.* 7, 415–420.
- Thomas, E., Hay, E.M., Hajeer, A., Silman, A.J., 1998. Sjögren's syndrome: a community-based study of prevalence and impact. *Br. J. Rheumatol.* 37, 1069–1076.
- Tiller, T., Tsuji, M., Yurasov, S., Velinzon, K., Nussenzweig, M.C., Wardemann, H., 2007. Autoreactivity in human IgG(+) memory B cells. *Immunity* 26, 205–213.
- Tracy, J.A., Dyck, P.J.B., 2010. Investigations and treatment of chronic inflammatory demyelinating polyradiculoneuropathy and other inflammatory demyelinating polyneuropathies. *Curr. Opin. Neurol.* 23, 242–248.
- Tsokos, M., Lazarou, S.A., Moutsopoulos, H.M., 1987. Vasculitis in primary Sjögren's syndrome. Histologic classification and clinical presentation. *Am. J. Clin. Pathol.* 88, 26–31.
- Turbyville, J.C., Rao, V.K., 2010. The autoimmune lymphoproliferative syndrome: a rare disorder providing clues about normal tolerance. *Autoimmun. Rev.* 9, 488–493.
- Ungprasert, P., Srivali, N., Kittanamongkolchai, W., 2015. Risk of venous thromboembolism in patients with Sjögren's syndrome: a systematic review and meta-analysis. *Clin. Exp. Rheumatol.* 33, 746–750.
- Upala, S., Yong, W.C., Sanguankeo, A., 2016. Association between primary Sjögren's syndrome and pregnancy complications: a systematic review and meta-analysis. *Clin. Rheumatol.* 35, 1949–1955.
- Usmani, Z.A., Hlavac, M., Rischmueller, M., Heraganahally, S.S., Hilditch, C.J., Lester, S., et al., 2012. Sleep disordered breathing in patients with primary Sjögren's syndrome: a group controlled study. *Sleep Med.* 13, 1066–1070.
- Valim, V., Trevisani, V.F.M., de Sousa, J.M., Vilela, V., Belfort, R., 2015. Current approach to dry eye disease. *Clin. Rev. Allergy Immunol.* 49, 288–297.
- Valim, V., Gerdts, E., Jonsson, R., Ferreira, G.A., Brokstad, K.A., Brun, J.G., et al., 2016. Atherosclerosis in Sjögren's syndrome: evidence, possible mechanisms and knowledge gaps. *Clin. Exp. Rheumatol.* 34, 133–142.
- van Nimwegen, J.F., Arends, S., van Zuiden, G.S., Vissink, A., Kroese, F.G.M., Bootsma, H., 2015. The impact of primary Sjögren's syndrome on female sexual function. *Rheumatology* 54, 1286–1293.

- Varin, M.M., Guerrier, T., Devauchelle-Pensec, V., Jamin, C., Youinou, P., Pers, J.O., 2012. In Sjogren's syndrome, B lymphocytes induce epithelial cells of salivary glands into apoptosis through protein kinase C delta activation. *Autoimmun. Rev.* 11, 252–258.
- Vivino, F.B., Carsons, S.E., Foulks, G., Daniels, T.E., Parke, A., Brennan, M.T., et al., 2016. New treatment guidelines for Sjogren's disease. *Rheum. Dis. Clin. N. Am.* 42, 531–+.
- Voulgarelis, M., Ziakas, P.D., Papageorgiou, A., Baimpa, E., Tzioufas, A.G., Moutsopoulos, H.M., 2012. Prognosis and outcome of non-Hodgkin lymphoma in primary Sjogren syndrome. *Medicine* 91, 1–9.
- Wakeland, E.K., Wandstrat, A.E., Liu, K., Morel, L., 1999. Genetic dissection of systemic lupus erythematosus. *Curr. Opin. Immunol.* 11, 701–707.
- Weinstein, D.A., Wolfsdorf, J.I., 2002. Glycogen storage diseases: a primer for clinicians. *Endocrinologist* 12, 531–538.
- Wells, A.U., Denton, C.P., 2014. Interstitial lung disease in connective tissue disease—mechanisms and management. *Nat. Rev. Rheumatol.* 10, 728–739.
- Wigley, F.M., 2002. Raynaud's phenomenon. *N. Engl. J. Med.* 347, 1001–1008.
- Xuan, J.X., Shen, L., Malyavantham, K., Pankewycz, O., Ambrus, J.L., Suresh, L., 2013. Temporal histological changes in lacrimal and major salivary glands in mouse models of Sjogren's syndrome. *BMC Oral Health* 13, 51.
- Yavuz, S., Asfuroglu, E., Bicakcigil, M., Toker, E., 2011. Hydroxychloroquine improves dry eye symptoms of patients with primary Sjogren's syndrome. *Rheumatol. Int.* 31, 1045–1049.
- Yokota, Y., 2001. Id and development. *Oncogene* 20, 8290–8298.
- Zhong, Y.H., Zhong, Z.G., Zhou, Z., Ma, Z.Y., Qiu, M.Y., Peng, F.H., et al., 2017. Comparisons of presentations and outcomes of neuromyelitis optica patients with and without Sjogren's syndrome. *Neurol. Sci.* 38, 271–277.
- Zhou, X., Ma, T., Zhang, Y., Zhou, N., Li, J., 2017. Rituximab maintenance therapy for patients with diffuse large B-cell lymphoma: a meta-analysis. *PLoS One* 12, e0174648.
- Zintzaras, E., Voulgarelis, M., Moutsopoulos, H.M., 2005. The risk of lymphoma development in autoimmune diseases: a meta-analysis. *Arch. Intern. Med.* 165, 2337–2344.
- Zuo, J., Williams, A.E.G., Park, Y.J., Choi, K., Chan, A.L., Reeves, W.H., et al., 2016. Muscarinic type 3 receptor autoantibodies are associated with anti-SSA/Ro autoantibodies in Sjogren's syndrome. *J. Immunol. Methods* 437, 28–36.

# Rheumatoid Arthritis

Stephan Blüml and Josef S. Smolen

Division of Rheumatology, Department of Medicine 3, Medical University of Vienna, Vienna, Austria

## OUTLINE

Introduction	659	Pathologic Effector Mechanisms	666
Clinical, Pathologic, and Epidemiologic Features	659	Autoantibodies as Potential Immunologic Markers	668
Autoimmune Features	661	Concluding Remarks—Future Prospects	668
Genetic Characteristics	662	References	669
In Vivo Models	664		

## INTRODUCTION

The joints are a target organ of many systemic autoimmune diseases, but in rheumatoid arthritis (RA), they are the preponderant and mostly sole evident focus of attack, with a large propensity to become destroyed. The pain and damage it elicits underlie the significant disability that can strike the patients and may lead to a wheelchair- or bed-ridden state. Thus RA impairs all aspects of quality of life, including the ability to work, and the consequences of the burden of the disease to the individual and society are enormous. For all these reasons, a better understanding of the pathogenesis of RA to develop new therapeutic agents interfering ideally with all pivotal events in all patients, or finding and abrogating the cause (or causes) of the disease, constitutes a major task.

## CLINICAL, PATHOLOGIC, AND EPIDEMIOLOGIC FEATURES

The *epidemiology* of RA is quite well characterized. RA is the most common chronic inflammatory joint disease, affecting 0.5%–1% of the populations in the industrialized world and women more frequently than men (2–3:1) (Silman and Pearson, 2002; Helmick et al., 2008; Eriksson et al., 2013). However, in certain Native American (Eriksson et al., 2013) populations, the prevalence is much higher (Helmick et al., 2008). The causes of the disease are unknown; however, several pieces of indirect evidence suggest that both environmental as well as genetic factors play an important role. Several environmental risk factors have been identified: (1) RA has already at the time of its description been regarded as a disease of the poor (Landre-Beauvais, 1800) and lower levels of education and upbringing under adverse socioeconomic conditions are afflicted with a more severe course of RA and/or a higher baseline inflammatory state (Callahan and Pincus, 1997; Uhlig et al., 1999; Packard et al., 2011); (2) smoking may increase the risk and possibly also the severity of RA and is associated with increased tumor necrosis factor (TNF) and autoantibody production which in turn is related to genetic factors characteristic of RA (Silman et al., 1996; Symmons et al., 1997; Uhlig et al., 1999; Mattey et al., 2002; Glossop et al., 2006), although

these associations have not been confirmed in all populations (Klareskog et al., 2011; Vesperini et al., 2013); (3) the microbiome, especially in the gastrointestinal tract, has been identified as an important regulator of autoimmunity and appears to play a major role in experimental forms of arthritis (Yoshitomi et al., 2005; Abdollahi-Roodsaz et al., 2008; Wu et al., 2010). In human RA, an association with particular bacteria, such as *Porphyromonas gingivalis*, a bacterium that produces peptidyl-arginine deiminase (PAD1), the enzyme responsible for citrullination (see below), has been found (Wegner et al., 2010; Scher et al., 2012). Recently, *Prevotella copri* has been linked to the pathogenesis of RA, as this bacterium is overexpanded in patients with RA and both humoral and cellular immune responses to this bacteria have been observed (Scher et al. 2013; Pianta et al. 2017). Beyond these aspects, hormonal factors may also play a role in the development of RA (Silman and Pearson, 2002).

The main clinical characteristics of RA are pain and swelling of the joints. The 2010 ACR/EULAR classification criteria (Aletaha et al., 2010), which are mainly used for clinical trial purposes but can also support the diagnostic process, require the presence of clinical synovitis (i.e., swelling due to synovial involvement) in at least one joint. The more joints that are affected (swollen or painful), the easier the patient can fulfill the criteria. Joints involved are primarily those of the wrists, fingers, toes, and knees (Smolen et al., 1995) but also many other joints can be affected, although some joints, such as the distal interphalangeal joints, are usually spared. Affected joints are not only painful upon motion and visibly swollen (Fig. 34.1A) or upon clinical examination but also tender to mild



**FIGURE 34.1** A. Hands of patients with RA. In the upper left image showing early RA, swelling is visible especially at the level of the proximal interphalangeal joints (arrow); in the upper right image, showing more established RA, swelling is visible at the level of the wrist, but also metacarpophalangeal joints (arrows). In the lower left image showing long-standing RA, joint damage has led to subluxation of the metacarpophalangeal joints and one can also find massive carpal joint damage (arrows). The lower right image shows a case of mutilation in virtually all joints of the hand. B. Evolution of X-ray changes from early arthritis without visible changes to severely destructive disease (bony erosions and cartilage damage seen indirectly as narrowed joint space, arrows) and ultimately mutilating disease.

pressure and stiff for many hours after rest, such as in the morning. RA synovitis leads to subchondral bone erosions and damage to cartilage and thus to the totality of the pathology of RA which can culminate in completely destroyed joints, as seen clinically (Fig. 34.1A, lower photographs) and upon imaging, especially by radiography (Fig. 34.1B).

## AUTOIMMUNE FEATURES

The immunologic hallmark of RA is the presence of autoantibodies in the circulation (and also in the synovial fluid). Indeed, the presence of autoantibodies constitutes an important aspect of the new ACR–EULAR classification criteria of RA and contributes 2 points out of a maximum of 10 if detectable and even 3 points if at high levels (Aletaha et al., 2010).

The first autoantibody ever described in RA was rheumatoid factor (RF) (Waaler, 1939), subsequently recognized to be a family of autoantibodies directed against the Fc portion of immunoglobulin (Ig)G. In clinical routine, IgM-RF is most commonly determined, but RFs can be of any isotype, and next to IgM-RF, IgG-RF and IgA-RF are frequently seen. The latter has been found to be predictive for the development of RA and is associated with worse prognosis (Houssien et al., 1997; Rantapaa-Dahlqvist et al., 2003; Nell et al., 2005). Also, IgM-RF precedes the occurrence of RA and, especially at high levels ( $>50$  IU/mL), it is associated with significant joint damage (Nielen et al., 2004; Nell et al., 2005; Aletaha et al., 2012). Moreover, the risk of developing RA for RF-positive individuals in the general population may be up to 26-fold increased (Nielsen et al., 2012). This suggests an involvement of RF in the pathways to inflammation and especially joint destruction which could be a consequence of immune complex formation and subsequent Fc receptor binding, complement activation, and consequential increase in levels of inflammatory cytokines (Winchester et al., 1970; Schur et al., 1975; Mallya et al., 2016). RFs are not specific for RA but can occur in other disorders, such as systemic autoimmune rheumatic diseases or chronic infections (Elagib et al., 1999; Bassyouni et al., 2009; Lima et al., 2013); when occurring in the course of acute infections, such as Epstein–Barr virus infection, they are usually of low level and transient (Halbert and Anken 1982). RF occurs in up to 5% of healthy individuals, with the highest frequency in elderly people, although this has recently been disputed (Nielsen et al., 2012). The physiologic role of RFs may be to enhance immune complex clearance by amplifying complement binding and increasing immune complex size and to eliminate modified IgG (Grabar, 1975; van Snick et al., 1978). RFs are found in up to 80% of RA patients and, as indicated before, a pathogenic role of RF must be assumed. Indeed, RF-positive patients exhibit the highest disease activity among all RA subsets, irrespective of the presence or absence of anti citrullinated protein antibodies (ACPA) (Aletaha et al. 2015). RF can be produced locally by B cells infiltrating the synovial membrane (Jasin, 1985; Wernick et al., 1985; Kraan et al., 1999). The fact that IgG and IgA RFs exist and the observation that they have undergone somatic mutations (Williams et al., 1999) suggest T-cell help. On the other hand, some RFs, and especially IgM-RF, may be highly conserved and coming from a B-cell population producing natural autoantibodies, so-called B1–B cells (Chen et al., 1986; Hardy et al. 1987; Martin et al., 1992; Hayakawa et al., 1999), suggesting that IgM-RF may not be derived from long-lived plasma cells. In line with the latter, RF levels change quite rapidly with changes in disease activity and decrease with effective therapy (Bohler et al., 2013).

Another very important autoantibody in RA is directed against citrullinated peptides. Originally described as antikeratin antibodies and antiperinuclear factor (Nienhuis and Mandema, 1964; Young et al., 1979; Aho et al., 1993b), the autoantibody was subsequently characterized as reactive with citrullinated peptides (Schellekens et al., 1998; Girbal-Neuhauser et al., 1999). Citrulline is an amino acid derived from deimination of arginine by PADI. A large number of proteins can undergo this posttranslational modification, including fibrin, vimentin, alpha enolase, and collagen; some of these are used in ELISAs for the purposes of autoantibody testing, including a synthetic, not naturally occurring cyclic citrullinated peptide. The anticitrullinated-protein antibodies (ACPAs) have been suggested to be more predictive of RA, associated with a bad outcome and more specific for RA than RF. However, with increasing numbers of studies, an increasing number of diseases in which ACPAs are present are reported, including chronic infections and other rheumatic diseases (Takasaki et al., 2004; Vossenaar et al., 2004; Candia et al., 2006; Bassyouni et al., 2009; Lima and Santiago, 2010; Du Toit et al., 2011; Singh et al., 2011; Lima et al., 2013). Thus while ACPAs are somewhat more specific for RA than RF, they are neither pathognomonic nor of truly high specificity; this has also been accounted for in the ACR–EULAR classification criteria for RA (Aletaha et al., 2010), where both specificities are regarded as equivalent. Importantly, ACPA and RF overlap in over 90% of the patients and it has been suggested that RA is particularly severe when both specificities are present especially with regard to erosive joint destruction (Rantapaa-Dahlqvist et al., 2003; Hecht et al. 2015).

Also *in vitro*, the proinflammatory properties of immune complexes on macrophages are potentiated when RF and ACPA are present (Anquetil et al. 2015).

In contrast to RF, ACPA levels do not rapidly change with changes of disease activity or effective therapy (De Rycke et al., 2005; Bohler et al., 2013). While ACPA positivity has been reported to be associated with the shared epitope (see below) and suggested to be elicited by smoking by virtue of activation of PADI in the respiratory tract (Klareskog et al., 2006), these data stem from particular European sites and have not been uniformly confirmed in other cohorts of patients (Lee et al., 2007; Xue et al., 2008). Overall, determining ACPA is a valuable addition to the diagnostic armamentarium, but it may be sufficient to do this primarily in RF-negative patients (National Collaborating Centre for Chronic Conditions, 2009). In general, autoantibody testing for RA should only be done when clinical synovitis is present in at least one joint. Interestingly, ACPAs appear to precede RA for even longer periods of time than RF (Nielen et al., 2004; Brink et al., 2013); this indicates that once pre-RA becomes a recognized entity due to evidence for the efficacy of preventive therapy, ACPA testing may be an important screening method. At present, however, it appears that a significant proportion (3%) of healthy individuals are ACPA or RF positive and, therefore, further studies to define “pre-RA” are needed (Jimenez-Boj et al., 2012).

As citrullination constitutes a posttranslational protein modification, the potential autoantigenic nature of other posttranslationally modified proteins was tested and, indeed, antibodies targeting carbamylated proteins and acetylated proteins have been identified, whose significance in RA is currently under investigation (Shi et al., 2013; Juarez et al. 2016).

A variety of other autoantibodies can also be found in RA, confirming the broad autoimmune nature of the disease. Among these are autoantibodies to nuclear antigens, especially the heterogeneous nuclear ribonucleoprotein (hnRNP) A2 (anti-RA33) which occurs in 30%–40% of RA patients, has some differential-diagnostic potential especially in RF/ACPA-negative patients, and appears to be associated with a more benign form of RA (Steiner et al., 1992; Nell et al., 2003). Moreover, anti-RA33 can also precede the development of RA (Aho et al., 1993a), although this occurs closer to the time of disease onset than ACPA and RF. Anticollagen antibodies are yet another interesting autoantibody subset, but their role appears more important in experimental models of RA (Trentham et al., 1977) than in the human disease (Steffen, 1970), although collagen might play some autoantigenic role in the context of its citrullination (Burkhardt et al., 2005).

The observation of the appearance of various autoantibodies prior to the onset of RA is similar to findings in other autoimmune diseases, such as type 1 diabetes and systemic lupus erythematosus (Arbuckle et al., 2003; Pietropaolo et al., 2012). This suggests that the activation of the autoimmune response occurs long before clinical manifestations become apparent and that the evolution of disease is a multistep process which initially involves a trigger eliciting autoimmunity and a second trigger activating the disease pathology in the presence of a particular autoreactivity (Smolen et al., 2006). Recently, the identification of posttranslational modifications (primarily glycosylation) of antibodies that are able to modify their effector functions has been described (Albert et al. 2008). In RA, especially ACPAs have been described to be glycosylated, and it has been shown that ACPA glycosylation shifts toward a more proinflammatory profile (Pfeifle et al. 2017; Rombouts et al. 2015). These observations allow the development of a two-step scenario in RA, with an initial breach of (self)tolerance and the occurrence of autoantibodies, which in a second step become pathogenic by acquiring a proinflammatory glycosylation pattern, leading to clinical disease.

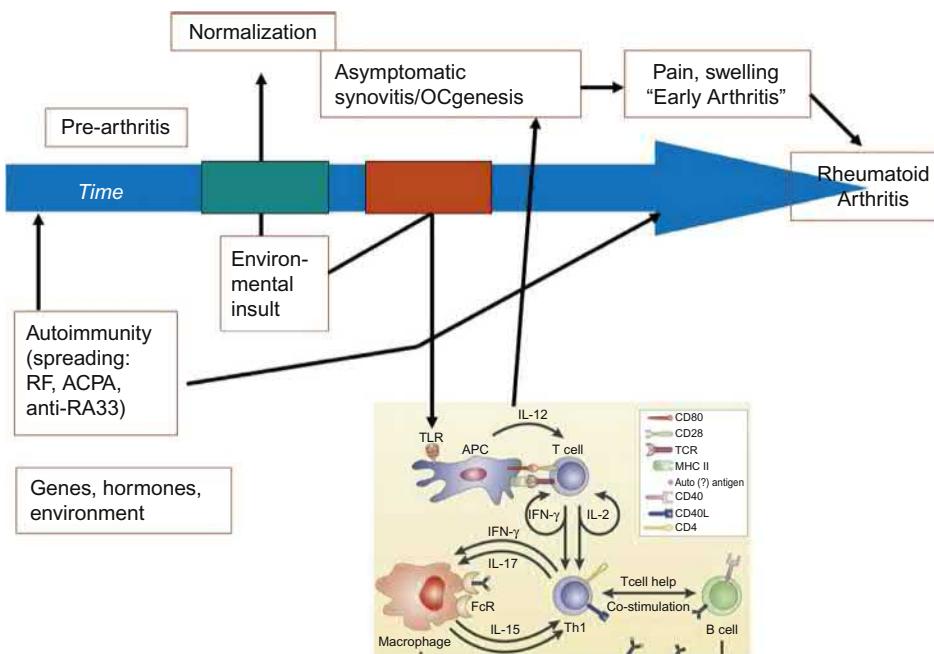
A depiction of a possible pathway from prearthritis to full-blown RA is shown in Fig. 34.2.

T cell-mediated autoimmunity in RA has been described for collagen and hnRNPA2 (Trentham et al., 1978; Fritsch et al., 2002) but not for IgG-Fc or citrullinated peptides. Whether cellular immunity to autoantigens is of pathogenetic importance is hitherto unknown. Clearly, the presence of autoantibodies is the major autoimmune characteristic of RA.

## GENETIC CHARACTERISTICS

One of the major indications for a genetic predisposition to a disease is its increased familial occurrence. While in RA the prevalence in the general population is 0.5%–1%, it rises to 2%–4% in siblings and about 15% in identical twins (Silman et al., 1993; Seldin et al., 1999); however, a quantitative genetic analysis of two European populations showed a consistent heritability of RA in the order of 60% (MacGregor et al., 2000).

The earliest observation that a particular gene may predispose to RA came from Stastny's seminal work describing the presence of human leucocyte antigen (HLA)-DRw4 in 70% of RA patients compared with 28% of controls (Stastny, 1978). The association with this major histocompatibility complex (MHC) class II gene, located



**FIGURE 34.2** A Putative Concept of Pre-arthritis and Its Evolution to Full-blown Rheumatoid Arthritis. A hostenvironment interaction (with the microbiome?) could trigger the evolution of a nonpathogenic autoimmune response which, in contrast to many similar reactions, in a genetically predisposed host may persist. Another environmental insult leads to expansion (spreading) of the autoimmune response without any clinical consequence. At some point, one of such recurring environmental insults with a potentially arthrotropic agent could trigger an asymptomatic synovitis which, in the presence of autoantibodies, could ultimately become clinically manifest and due to an overwhelming cytokine response also trigger osteoclastogenesis (OCgenesis) and thus the full RA picture.

on chromosome 6p21, was subsequently numerously confirmed to constitute the most important genetic contribution to susceptibility for the disease. It was ultimately observed that in RA patients of various ethnic backgrounds, the HLA-DRB1 alleles are frequently different, but each encodes a common sequence of amino acids at position 70–74 of the HLA-DR $\beta$  chain (QKRRA or similar, such as QRRAA or RRRAA) within the third hyper-variable region of the HLA-DRB1 molecule (Gregersen et al., 1987), a region pivotally important for peptide binding. Thus the risk contribution apparently comes from the presentation of potentially arthritogenic antigens. The respective alleles include HLA-DRB1\*0401, \*0404, \*0405, \*0408; HLA-DRB1\*0101, \*0102; HLA-DRB1\*1001; and HLA-DRB1\*1402 which occur at different frequencies in different populations but together are present in about 80% of RA patients. The overall odds ratio (OR) is about 3 with the biggest risk conveyed by HLA-DRB1\*0401 and \*0404, and especially strongly with homozygosity of the shared epitope (OR of 30). There are also protective alleles, such as HLA-DRB1\*0402. However, since HLA accounts for only about one-third of the total genetic risk in RA (Deighton et al., 1989), non-MHC genes must also contribute to susceptibility.

One of the non-MHC genes found to be associated with RA is protein tyrosine phosphatase, nonreceptor type 22 (lymphoid) gene (PTPN22) on chromosome 1p13. A single nucleotide polymorphism (SNP) in this gene is the culprit, occurring in 14% of RA patients and 9% of controls (OR B1.7) (Begovich et al., 2004). This phosphatase, which is expressed in hematopoietic cells, inhibits T-cell receptor (TCR) signal transduction and the 620W variant is associated with reduced CD4 T-cell activation.

SNPs in the complement component 5/TNF receptor-associated factor 1 (TRAF1) region at chromosome 9q33 have likewise been found to be associated with RA (Plenge et al., 2007). Complement genes, indeed, are interesting candidates given the potential involvement of complement activation in RA pathogenesis discussed previously and below. TRAF1, on the other hand, codes for a protein which acts as a negative regulator of signaling via TNF receptors 1 and 2, and in light of the apparent pivotal involvement of TNF in the pathogenesis of RA (see below), there is some logic behind this association. However, it is not clear at present which of these genes is primarily associated with RA or if the association may not even be with an adjacent gene. The OR is about 1.3.

Other proteins involved in signaling are the signal transducers and activators of transcription (STATs). STATs are activated by Janus kinases (Jaks) and recently the first Jak inhibitor was approved for the treatment of RA in the United States (Burmeister et al., 2013). Indeed, a variant allele of STAT4, located on chromosome 2q, was found associated with RA (Remmers et al., 2007). STAT4 protein mediates signaling of various cytokines, such as IL-12, IL-23, and type I interferons. The variant allele was found in 27% of patients and 22% of controls, OR B1.3. Homozygosity of the allele was associated with a 60% increased risk of RA.

Other genetic risk factors include polymorphisms in the CD40 and the TNF, alpha-induced protein 3 genes (Li and Begovich, 2009).

Thus a variety of genetic associations have been found in RA, many of them in or near genes involved in the regulation of the immune system (cytokines and their receptors, costimulatory molecules, signal transduction molecules), supporting the concept of RA being an immunologically driven disease (reviewed in Viatte et al. 2013). However, and this is the case for many genetic association studies, the functional consequences of a given single nucleotide polymorphism (SNP) are mostly not known yet (increased/decreased expression of the target gene? altered gene function?), and therefore the exact contributions to disease pathogenesis still have to be determined.

Importantly, most of these genes are primarily related to autoantibody-positive RA, indicating that immune response genes contribute their effects indeed via particular immune responses (Mattey et al., 2002; van der Helm-van Mil et al., 2006). The only genetic associations with seronegative RA that have been identified are SNPs in the DCIR gene (a C-type lectin) and IRF5 (Lorentzen et al. 2007; Sigurdsson et al. 2007). Gene–environment interactions have been postulated in terms of genetic susceptibility in smokers to develop ACPA and RA (Klareskog et al., 2011) but have not been generally confirmed (Lee et al., 2007); thus more information is clearly needed.

Apart from genetic factors, epigenetic regulators of gene expression have been identified as possible important regulators of RA pathology. Epigenetic mechanisms include DNA methylation, modification of histones (phosphorylation, acetylation), and expression of noncoding RNAs (especially microRNAs but also long noncoding RNAs) (Ospelt et al. 2017).

## IN VIVO MODELS

Based on Koch's postulate that a causal agent must be sufficient and necessary to cause infectious disease (Koch 1890; Witebsky et al., 1957) formulated postulates for autoimmune diseases: an autoantigen should be necessary and sufficient to cause an autoimmune disease in experimental models; in other words, the demonstration of an autoimmune nature of a disease requires the recognition of a particular autoantigen to which antibodies or cells are autoreactive and which elicits a similar disease in an experimental model. To date, the only such autoantigen in RA is collagen, hypothesized to be the culprit several decades ago by Steffen (1970); collagen-specific autoantibodies and autoreactive cells exist (Menzel et al., 1975; Trentham et al., 1978; Smolen et al., 1980) and type II collagen (CII) elicits a chronic, destructive arthritis (Trentham et al., 1977). This collagen-induced arthritis (CIA) meanwhile, one of the classic RA models, depends on both the activation of T cells and the production of arthritogenic autoantibodies, but the disease itself can also only be transferred with anti-CII antibodies (Holmdahl et al., 2002). However, current views do not suggest a major role for this reactivity in eliciting human RA, and interestingly, this model responds better to IL-1 than TNF-blockade, contrasting human RA (Joosten et al., 2008). Not only CII but also other cartilage antigens, such as proteoglycan, can be used to induce experimental arthritis (Glant et al., 1987, 1998), but their role in human RA is not established.

Other autoantigens used to develop experimental models, to which autoantibodies and partly cellular immune responses exist in RA, and which are even widely used diagnostically, are IgG (targeted by RFs), several citrullinated proteins including fibrinogen, vimentin, alpha enolase, and collagen (targeted by ACPA), and heterogeneous nuclear ribonucleoprotein A2 (hnRNP)-A2; targeted by anti-RA33 antibodies (Steiner et al., 1992). However, none of them elicit a chronic destructive arthritis upon immunization. At most, ACPA can be detected in occasional experimental approaches, such as a study on CIA (Kuhn et al., 2006), although other studies reported negative observations in this respect. Further, immunization with hnRNP-A2 can aggravate arthritis in TNF overexpressing mice (Hayer et al., 2005).

Several adjuvants can induce arthritis, in particular Freund's adjuvant ("adjuvant arthritis") and pristane, a mineral oil ("pristane-induced arthritis"); while they allow one to study certain aspects of the pathways to arthritis, they do not appear to be appropriate models for human RA (Holmdahl et al., 2001), although being free of known autoantigens, they support the involvement of environmental factors in the generation of arthritis.

That any type of immune complexes can induce arthritis has been shown by Dumonde and Glynn (1962). A particular form of an immune complex arthritis has been more recently described in the form of the KRN mouse model, originally developed to study diabetes; these mice express a transgenic TCR recognizing glucose-6-phosphoisomerase (G6PI), a ubiquitous cytoplasmic protein which is also present on the surface of articular cartilage. The ensuing autoantibodies, which by themselves can induce disease upon serum transfer, form intraarticular immune complexes followed by a severe inflammatory and destructive response, and expectedly the system is complement and mast cell dependent (Matsumoto et al., 2002). Interestingly, G6PI is not

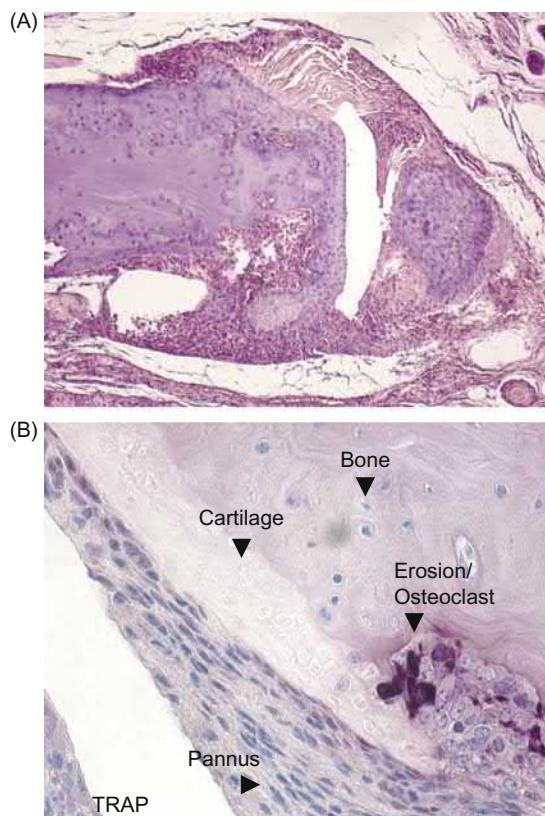
recognized as being an important autoantigen in man but nonetheless this model confirms the role of immune complexes in chronic arthritis and especially the serum transfer model can be used to investigate the role of the innate immune system selectively.

Apart from the inducible models, also spontaneous models exist. In the SKG model, which is based on a spontaneous mutation of ZAP70, an important TCR signaling adaptor molecule, mice develop a severe arthritis over the course of months with histopathological features resembling human RA. Interestingly, also high titers of RF can be found in those mice.

Another widely used model is a model in which a human TNF transgene is expressed in mice. These develop a severe inflammatory and highly destructive polyarthritis (Fig. 34.3A) (Keffer et al., 1991). This model has served particularly well in studies devoted to the development of bone and cartilage damage in arthritis (Redlich et al., 2002; Korb-Pap et al., 2012). Yet another example of the role of cytokine hyperactivity and consequential joint inflammation and damage is provided upon the deficiency of IL-1 receptor antagonist (IL-1ra) (Horai et al., 2000), although as mentioned before, IL-1 inhibition has little effect in human RA.

Thus a variety of experimental models exist which can mimic parts of the spectrum of RA allowing studies of many aspects of pathways to arthritis and joint destruction. It is interesting to note that both overactivation of the innate immune system (hTNFtg, IL-1ra deficient mice) as well as of the adaptive immune system (SKG mice) result in destructive arthritis with similar histopathological features in mice. Some of these models even appear to fulfill Witebsky's postulate; alas, the role of the respective autoantigen(s) in human RA is not confirmed.

Of note, many of these animal models do not occur under germ-free conditions and different commensal agents can activate individual forms of experimental arthritis in hosts with particular genetic backgrounds (Wu et al. 2010), further suggesting the important involvement of the environment in the induction and maintenance of chronic arthritis (Scheinecker and Smolen 2011; Scher and Abramson 2011).



**FIGURE 34.3** A. Arthritis in mice overexpressing human TNF. Note the significant inflammatory response and the dramatic destruction due to subchondral invasion of the bone by the pannus tissue. B. Bone damage is elicited by osteoclasts differentiating within the synovial membrane. The small dark blue (tartrate-resistant acid phosphatase [TRAP] positive) cells are osteoclast precursors.

## PATHOLOGIC EFFECTOR MECHANISMS

The detailed mechanisms leading to the expression of the disease are still insufficiently known. Fig. 34.4 depicts current views on the pathways to synovitis and joint damage in RA, showing a schematic representation of the events (Smolen et al., 2007) as well as histologic and immunohistochemical characteristics of the RA synovial membrane. It is assumed that an autoantigen or hitherto unrecognized foreign antigen is taken up by antigen presenting cells, prototypically dendritic cells, leading to activation of the innate immune system as well as to that of T cells (and thus also the adaptive immune response) via respective antigen presentation and costimulation. The involvement of the shared epitope (see above) suggests that either particularly arthritogenic peptides bind with high affinity to these but not other MHC molecules or that an arthritogenic T-cell repertoire is selected via the shared epitope. These activated T cells, possibly because of insufficient control by regulatory T cells (Chavele and Ehrenstein, 2011), in turn activate macrophages and provide B-cell help. However, the type of T cells involved remains enigmatic. Many have suggested that Th17 cells are pivotal in inducing autoimmunity and especially chronic inflammation, since they not only produce IL-17 which activates macrophages as well as other cells and is proosteoclastogenic but also secrete TNF (Miossec 2004). Alas, IL-17 or IL-17 receptor inhibition has been rather disappointing with respect to efficacy, especially in patients who have failed prior biologic disease modifying anti-rheumatic drug therapies (Tahir et al. 2017). Moreover, inhibition of IL-23, the major cytokine involved in Th17 differentiation, also failed to show efficacy (Smolen et al. 2017). However, also IL-12 inhibition using the anti-p40 antibody ustekinumab is not efficacious (Smolen et al., 2017). Nevertheless, as alluded to above, T cells must be involved in RA by virtue of the significant MHC association and class switch as well as affinity maturation of the autoantibodies involved. Thus one can only explain the current findings by assuming that Th1 or Th17 cells only play a role very early in the disease process and recede themselves long before signs and symptoms appear, or that a hitherto unknown T-cell population exists, that ought to be CD4 negative, since anti-CD4 is not effective in RA (van der Lubbe et al., 1995).

These events presumably occur partly centrally and partly within the synovial membrane into which the cells have migrated. Activated macrophages secrete proinflammatory cytokines. Activated RA B cells and their progeny produce autoantibodies which, after forming immune complexes, bind to Fc and complement receptors and thus increase macrophage cytokine production (Aringer and Smolen, 2012). In parallel, fibroblast-like synovial cells become activated and also produce inflammatory mediators (Lee and Firestein, 2003).

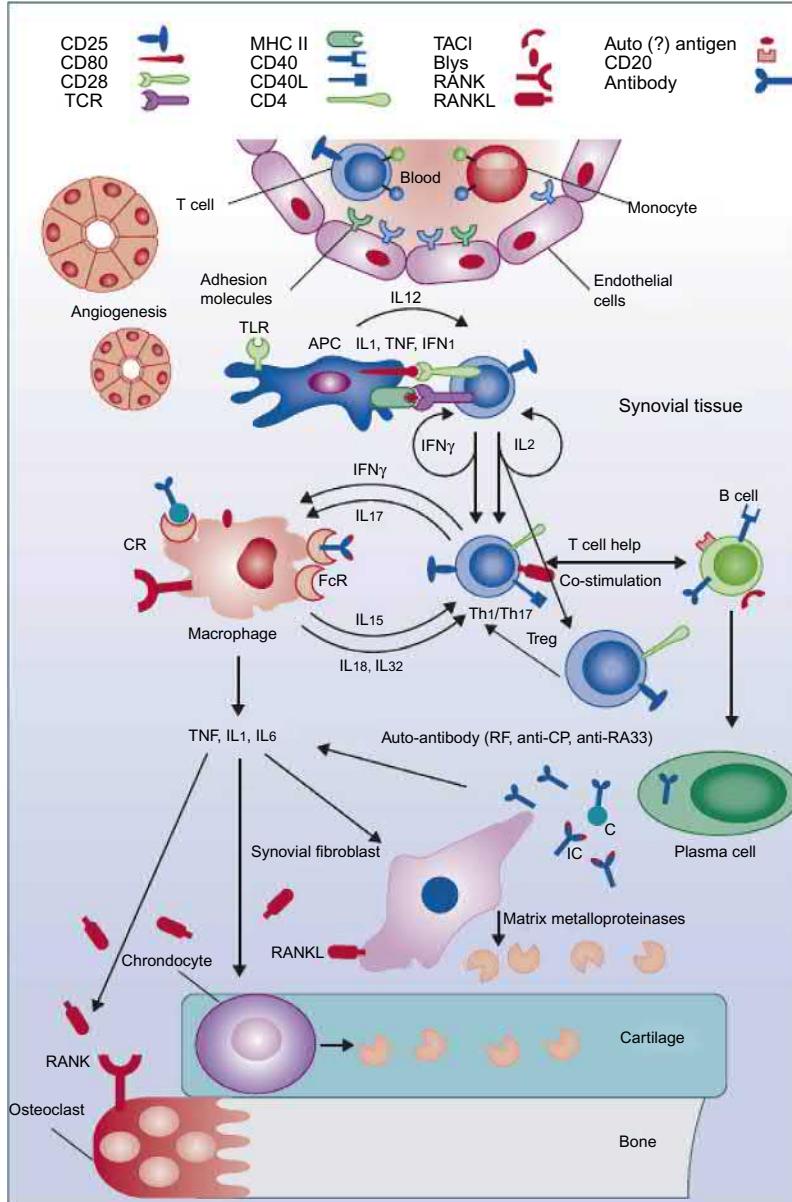
By whichever way the detailed events evolve (i.e., whether the innate immune reactivity, T or B-cell activations are dominant), the mechanisms ultimately lead to an influx of inflammatory cells into the synovial membrane; the inflamed synovial membrane transforms into an autonomous “semimalignant” tissue (pannus) leading to destruction of bone (erosions) and cartilage. Indeed, the major feature distinguishing RA from other inflammatory joint diseases is the high propensity for joint destruction.

Cartilage damage appears to arise primarily via direct action of metalloproteinases secreted within the joint on cartilage matrix and/or via the activation of chondrocytes by cytokines and subsequent matrix degradation; attachment of the synovial membrane to the cartilage appears to play an important role in these respects (Korb-Pap et al., 2012).

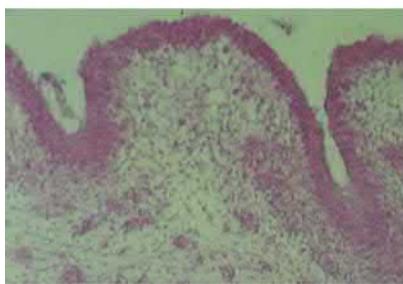
Bone destruction is mediated by osteoclasts activated within the synovial membrane at sites adjacent to bone (Fig. 34.3B) (Gravallese et al., 1998; Redlich et al., 2002). While osteoclast differentiation and activation are pivotally dependent on receptor activator of NF $\kappa$ B and its ligand, proinflammatory cytokines enhance the generation and activity of osteoclasts (Lam et al., 2000; Teitelbaum, 2000). The much stronger destructive nature of RA compared with other inflammatory joint diseases may be explained by the higher load of cytokines like TNF and IL-6 (Partsch et al., 1997) which in turn are likely to be a consequence of the presence of autoantibodies and immune complexes in the joint (Zvaifler, 1974). Indeed, the presence of RF is related to the joint damage via association with higher disease activity and also independently of disease activity (Aletaha et al., 2012).

With all due recognition of deliberations on pathogenetic pathways based on experimental models or ex vivo and in vitro studies of patient cells, tissues, or body fluids, ultimately the validation of the involvement of specific pathways must come from the efficacy of therapies targeting a presumed pathogenetic principle (sort of reverse Witebsky postulate). Due to the wealth of clinical trials in RA, both from successful ones but also (maybe even more so) from the unsuccessful ones, it is possible to redraw the concepts on the pathophysiology of human RA. The important role of TNF, IL-6, costimulatory molecules, the JAK/STAT pathway, and CD20-positive B lymphocytes has been convincingly demonstrated in clinical trials interfering with these molecules, thus validating these targets as importantly involved (although also eliciting new questions; see below). In contrast, the

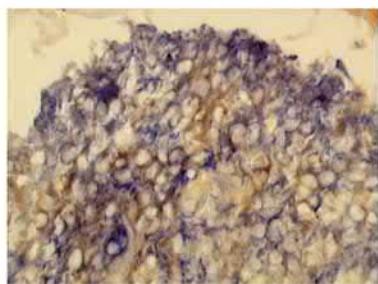
(A)



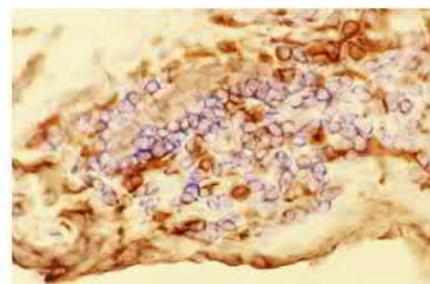
(B)



(C)



(D)



**FIGURE 34.4** A. A schematic representation of the pathogenesis of RA (Smolen et al., 2007). B. Hematoxylin-eosin stain of an RA synovial membrane section showing hyperplasia of the lining layer, hypercellularity in the sublining and hypervascularity. C. TNF expression (brown) in type A (monocyte-like, blue) and type B (fibroblast-like, unstained) synovial lining cells. D. Activated T cells in synovial membrane (CD3 in brown, HLADR in blue).

cytokines IL-12/23, IL-17, the protein kinases p38 mitogen-activated protein kinase, and spleen tyrosine kinase (Syk) all have been shown not to be important targets for the treatment of human RA and therefore very likely are not involved in its pathogenesis.

However, the fact that all currently approved, efficacious agents lead to similar clinical results, that their efficacy is not increased in patients who have failed one of the other targeted therapies, and combinations of these biological hitherto do not apparently increase efficacy although they do mostly increase serious adverse event rates suggest that all these cells and molecules have a single major final pathway in common; this bottleneck appears to be the proinflammatory cytokine system (Smolen and Aletaha, 2013).

Still some questions arise in this respect: (1) if T cells do play a major role as suggested by the efficacy of costimulation inhibition, why is anti-CD4 therapy not effective (van der Lubbe et al., 1995)? (2) If B cells play a major role, why are several B cell-directed therapies ineffective (Blumel et al., 2013)? (3) With so many similarities regarding the effects of the three major proinflammatory cytokines: TNF, IL-1, and IL-6, why is IL-1 inhibition so much less efficacious than TNF and IL-6 inhibition (Singh et al., 2009)? And, most importantly, (4) what determines the arthrotropism of the disease? Do particular antigens of the joint (cartilage components?) elicit the disease? Is it influx into the joint, with its permissive anatomical structures lacking efficient basement membranes, of nonspecific bacterial products that are of decisive nature (Toivanen, 2001)? Resolving these questions will allow better insights into the pathways leading to RA, but they simply attest that proof of concept can only come from the clinic and not from theoretical considerations or experimental models.

## AUTOANTIBODIES AS POTENTIAL IMMUNOLOGIC MARKERS

As discussed in the section on autoimmunity, the most prevalent autoantibodies in RA are RF and ACPA. They are both important components in classification criteria and therefore of diagnostic help (Aletaha et al., 2010). Their sensitivity and specificity for the diagnosis of RA are similar (sensitivity about 50%–60%, specificity about 85%–95%) (Mjaavatten et al., 2010; Neogi et al., 2010; Nicaise-Roland et al., 2013). Both of these autoantibodies fluctuate with disease activity and effective therapy, although RF appears to do so to a much greater extent (Bohler et al., 2013); indeed, RF can become negative (seroconversion) with long-standing remission which is not known for ACPA. ACPA can be tested for using several different citrullinated antigens: fibrinogen, vimentin, filaggrin, CII, enolase, or a cyclic peptide. These autoantibodies differ only slightly in terms of time of appearance and frequency (Brink et al., 2013; Nicaise-Roland et al., 2013).

Another autoantibody specificity which can be of diagnostic help is anti-RA33, especially if RF and ACPA are negative (Nell et al., 2005).

All these autoantibodies can also be present in a variety of other diseases, especially other autoimmune disorders and infectious diseases, and also notably so for ACPA (Abdel Fattah et al., 2009; Bassyouni et al., 2009; Lima and Santiago, 2010; Singh et al., 2011; Gokhan et al., 2013). They can also be present in healthy people and it has previously been suggested that RF positivity increases with age, but this was not seen in more recent series (Nielsen et al., 2012). Therefore, only in the right clinical setting, RF and ACPA can be regarded as marker autoantibodies of the disease and as having a high weighting in the classification criteria for RA (Aletaha et al., 2010).

Of particular importance, the presence of autoantibodies can partly inform in advance on response to therapy. Thus B cell-targeted therapy with rituximab is more efficacious in RF and/or ACPA positive than negative patients (Chatzidionysiou et al., 2011). This is conceptually sound since rituximab eliminates CD20-positive B cells and levels of these autoantibodies decrease with effective therapy. This observation appears to be specific for rituximab since therapies targeted against cytokines do not appear to be differently effective in seropositive and seronegative patients.

## CONCLUDING REMARKS—FUTURE PROSPECTS

RA is the prototypic autoimmune joint disease. Autoreactivity, in association with a genetic predisposition and an environmental trigger, appears to play a major role in pathways to RA and the presence of autoantibodies is associated with joint damage and thus of prognostic in addition to diagnostic value. The observations that autoimmunity precedes clinical disease onset by many years and that the presence of ACPA or RF in otherwise healthy individuals may increase the risk for developing RA more than 20-fold imply that there is a prearthritic phase. This suggests that in the future, long before the onset of disease, preventive measures might be taken for

people at risk (see Chapters 77–79). Thus the autoimmune response may at some early point in time serves as guidance toward novel therapeutic approaches. Further, the causes of RA are still unknown: which (environmental?) provocation elicits RA? And how then does the autoimmune reaction ensue?

## References

- Abdel Fattah, N.S., Hassan, H.E., Galal, Z.A., El Okda el, S.E., 2009. Assessment of anti-cyclic citrullinated peptide in psoriatic arthritis. *BMC Res Notes* 2, 44.
- Abdollahi-Roodsaz, S., Joosten, L.A., Koenders, M.I., Devesa, I., Roelofs, M.F., Radstake, T.R., et al., 2008. Stimulation of TLR2 and TLR4 differentially skews the balance of T cells in a mouse model of arthritis. *J Clin Invest* 118 (1), 205–216.
- Aho, K., von Essen, R., Kurki, P., Palusuo, T., Heliovaara, M., 1993. Antikeratin antibody and antiperinuclear factor as markers for subclinical rheumatoid disease process. *J Rheumatol*. 20, 1278–1281. 1993.
- Aho, K., Steiner, G., Kurki, P., Paimela, L., Leirisalo-Repo, M., Palusuo, T., et al., 1993. Anti-RA 33 as a marker antibody of rheumatoid arthritis in a Finnish population. *Clin Exp Rheumatol* 11, 645–647.
- Albert, H., Collin, M., Dudziak, D., Ravetch, J.V., Nimmerjahn, F., 2008. In vivo enzymatic modulation of IgG glycosylation inhibits autoimmune disease in an IgG subclass-dependent manner. *Proc Natl Acad Sci U.S.A* 105 (39), 15005–15009. available from: PM:18815375.
- Aletaha, D., Neogi, T., Silman, A., Funovits, J., Felson, D., Bingham III, C.O., et al., 2010. The American College of Rheumatology / European League Against Rheumatism Classification Criteria for Rheumatoid Arthritis. *Ann Rheum Dis* 2010 (69), 1580–1588.
- Aletaha, D., Alasti, F., Smolen, J.S., 2015. Rheumatoid factor, not antibodies against citrullinated proteins, is associated with baseline disease activity in rheumatoid arthritis clinical trials. *Arthritis Res Ther*. 17, 229. available from: PM:26307354.
- Aletaha, D., Alasti, F., Smolen, J.S., 2012. Rheumatoid factor determines structural progression of rheumatoid arthritis dependent and independent of disease activity. *Ann Rheum Dis*.
- Anquetil, F., Clavel, C., Offer, G., Serre, G., Sebbag, M., 2015. IgM and IgA rheumatoid factors purified from rheumatoid arthritis sera boost the Fc receptor- and complement-dependent effector functions of the disease-specific anti-citrullinated protein autoantibodies. *J. Immunol.* 194 (8), 3664–3674. available from: PM:25769920.
- Arbuckle, M.R., McClain, M.T., Rubertone, M.V., Scofield, R.H., Dennis, G.J., James, J.A., et al., 2003. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med* 349 (16), 1526–1533.
- Aringer, M., Smolen, J.S., 2012. Therapeutic blockade of TNF in patients with SLE—promising or crazy? *Autoimmun Rev* 11 (5), 321–325.
- Bassyouni, I.H., Ezzat, Y., Hamdy, S., Talaat, R.M., 2009. Clinical significance of anti-cyclic citrullinated peptide antibodies in Egyptian patients with chronic hepatitis C virus genotype IV infection. *Clin Chem Lab Med* 47 (7), 842–847.
- Begovich, A.B., Carlton, V.E., Honigberg, L.A., Schrödi, S.J., Chokkalingam, A.P., Alexander, H.C., et al., 2004. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. *Am J Hum Genet* 75 (2), 330–337.
- Bluml, S., McKeever, K., Ettinger, R., Smolen, J., Herbst, R., 2013. B-cell targeted therapeutics in clinical development. *Arthritis Res Ther* 15 (Suppl 1), S4.
- Bluml, S., Sahin, E., Saferding, V., Goncalves-Alves, E., Hainzl, E., Niederreiter, B., et al., 2015. Phosphatase and tensin homolog (PTEN) in antigen-presenting cells controls Th17-mediated autoimmune arthritis. *Arthritis Res Ther* 17, 230.
- Bohler, C., Radner, H., Smolen, J.S., Aletaha, D., 2013. Serological changes in the course of traditional and biological disease modifying therapy of rheumatoid arthritis. *Ann Rheum Dis* 72 (2), 241–244.
- Brink, M., Hansson, M., Mathsson, L., Jakobsson, P.J., Holmdahl, R., Hallmans, G., et al., 2013. Multiplex analyses of antibodies against citrullinated peptides in individuals prior to development of rheumatoid arthritis. *Arthritis Rheum* 65 (4), 899–910.
- Burkhardt, H.J., Sehnert, B., Bochermann, R., Engstrom, A., Kalden, J.R., Holmdahl, R., 2005. Humoral immune response to citrullinated collagen type II determinants in early rheumatoid arthritis. *Europ J Immunol* 35, 1643–1652.
- Burmester, G.R., Blanco, R., Charles-Schoeman, C., Wollenhaupt, J., Zerbini, C., Benda, B., et al., 2013. Tofacitinib (CP-690,550) in combination with methotrexate in patients with active rheumatoid arthritis with an inadequate response to tumour necrosis factor inhibitors: a randomised phase 3 trial. *Lancet* 381, 451–560.
- Callahan, L.F., Pincus, T., 1997. Education, self-care, and outcomes of rheumatic diseases: further challenges to the “biomedical model” paradigm. *Arthritis Care Res* 10 (5), 283–288.
- Candia, L., Marquez, J., Gonzalez, C., Santos, A.M., Londono, J., Valle, R., et al., 2006. Low frequency of anticyclic citrullinated peptide antibodies in psoriatic arthritis but not in cutaneous psoriasis. *J Clin Rheumatol* 12 (5), 226–229.
- Chavele, K.M., Ehrenstein, M.R., 2011. Regulatory T-cells in systemic lupus erythematosus and rheumatoid arthritis. *FEBS Lett* 585 (23), 3603–3610.
- Chen, P.P., Albrandt, K., Orida, N.K., Radoux, V., Chen, E.Y., Schrantz, R., et al., 1986. Genetic basis for the cross-reactive idiotypes on the light chains of human IgM anti-IgG autoantibodies. *Proc Natl Acad Sci U S A* 83 (21), 8318–8322.
- De Rycke, L., Verhelst, X., Kruithof, E., van den Bosch, F., Hoffman, I.E., Veys, E.M., et al., 2005. Rheumatoid factor, but not anti-cyclic citrullinated peptide antibodies, is modulated by infliximab treatment in rheumatoid arthritis. *Ann Rheum Dis* 64, 299–302.
- Deighton, C.M., Walker, D.J., Griffiths, I.D., Roberts, D.F., 1989. The contribution of HLA to rheumatoid arthritis. *Clin Genet* 36 (3), 178–182.
- Dumonde, D.C., Glynn, L.E., 1962. The production of arthritis in rabbits by an immunological reaction to fibrin. *Br J Exp Pathol* 43, 373–383.
- du, T.R., Whitelaw, D., Taljaard, J.J., du, P.L., Esser, M., 2011. Lack of specificity of anticyclic citrullinated peptide antibodies in advanced human immunodeficiency virus infection. *J Rheumatol* 38 (6), 1055–1060.
- Elagib, K.E., Borretzen, M., Jonsson, R., Haga, H.J., Thoen, J., Thompson, K.M., et al., 1999. Rheumatoid factors in primary Sjögren's syndrome (pSS) use diverse VH region genes, the majority of which show no evidence of somatic hypermutation. *Clin Exp Immunol* 117, 388–394.
- Eriksson, J.K., Neovius, M., Ernestam, S., Lindblad, S., Simard, J.F., Askling, J., 2013. Incidence of rheumatoid arthritis in sweden: a nationwide population-based assessment of incidence, its determinants, and treatment penetration. *Arthritis Care Res (Hoboken)* 65 (6), 870–878.

- Fritsch, R., Eselbeck, D., Skriner, K., Jahn-Schmid, B., Scheinecker, C., Bohle, B., et al., 2002. Characterization of autoreactive T cells to the autoantigens heterogeneous nuclear ribonucleoprotein A2 (RA33) and filaggrin in patients with rheumatoid arthritis. *J Immunol* 169 (2), 1068–1076.
- Girbal-Neuhauser, E., Durieux, J.J., Arnaud, M., Dalbon, P., Sebbag, M., Vincent, C., et al., 1999. The epitopes targeted by the rheumatoid arthritis-associated antifilaggrin autoantibodies are posttranslationally generated on various sites of (pro)filaggrin by deimination of arginine residues. *J Immunol* 162 (1), 585–594.
- Gregersen, P.K., Silver, J., Winchester, R.J., 1987. The shared epitope hypothesis: an approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis Rheum* 30, 1205–1213.
- Glant, T.T., Mikecz, K., Arzoumanian, A., Poole, A.R., 1987. Proteoglycan-induced arthritis in BALB/c mice. Clinical features and histopathology. *Arthritis Rheum* 30 (2), 201–212.
- Glant, T.T., Cs-Szabo, G., Nagase, H., Jacobs, J.J., Mikecz, K., 1998. Progressive polyarthritis induced in BALB/c mice by aggrecan from normal and osteoarthritic human cartilage. *Arthritis Rheum* 41 (6), 1007–1018.
- Glossop, J.R., Dawes, P.T., Mattey, D.L., 2006. Association between cigarette smoking and release of tumour necrosis factor alpha and its soluble receptors by peripheral blood mononuclear cells in patients with rheumatoid arthritis. *Rheumatology (Oxford)* 45 (10), 1223–1229.
- Gokhan, A., Turkeyer, I.H., Babacan, T., Pehlivan, Y., Dag, M.S., Bosnak, V.K., et al., 2013. The antibodies cyclic citrullinated peptides (anti-CCP) positivity could be a promising marker in brucellosis patients presented with peripheral arthritis. *Mod Rheumatol*.
- Gossec, L., Smolen, J.S., Ramiro, S., De, W.M., Cutolo, M., Dougados, M., et al., 2016. European League Against Rheumatism (EULAR) recommendations for the management of psoriatic arthritis with pharmacological therapies: 2015 update. *Ann Rheum Dis* 75 (3), 499–510.
- Grabar, P., 1975. The “globulines-transporteurs” theory and auto-sensitization. *Med Hypotheses* 1 (5), 172–175.
- Gravallese, E.M., Harada, Y., Wang, J.T., Gorn, A.H., Thornhill, T.S., Goldring, S.R., 1998. Identification of cell types responsible for bone resorption in rheumatoid arthritis and juvenile rheumatoid arthritis. *Am J Pathol* 152, 943–951.
- Halbert, S.P., Anken, M., 1982. Auto-antibodies in infectious mononucleosis, as determined by ELISA. *Int Arch Allergy Appl Immunol* 69 (3), 257–261.
- Hardy, R.R., Hayakawa, K., Shimizu, M., Yamasaki, K., Kishimoto, T., 1987. Rheumatoid factor secretion from human Leu-1+ B cells. *Science* 236 (4797), 81–83. available from: PM:3105057.
- Hayakawa, K., Asano, M., Shinton, S.A., Gui, M., Allman, D., Stewart, C.L., et al., 1999. Positive selection of natural autoreactive B cells. *Science* 285 (5424), 113–116.
- Hayer, S., Tohidast-Akrad, M., Haralambous, S., Jahn-Schmid, B., Skriner, K., Trembleau, S., et al., 2005. Aberrant expression of the autoantigen heterogeneous nuclear ribonucleoprotein-A2 (RA33) and spontaneous formation of rheumatoid arthritis-associated anti-RA33 autoantibodies in TNF-alpha transgenic mice. *J Immunol* 175 (12), 8327–8336.
- Hecht, C., Schett, G., Finzel, S., 2015. The impact of rheumatoid factor and ACPA on bone erosion in rheumatoid arthritis. *Ann. Rheum. Dis.* 74 (1), e4. available from: PM:25326218.
- Helmick, C.G., Felson, D.T., Lawrence, R.C., Gabriel, S., Hirsch, R., Kwoh, C.K., et al., 2008. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part I. *Arthritis Rheum* 58 (1), 15–25.
- Holmdahl, R., Lorentzen, J.C., Lu, S., Olofsson, P., Wester, L., Holmberg, J., et al., 2001. Arthritis induced in rats with nonimmunogenic adjuvants as models for rheumatoid arthritis. *Immunol Rev* 184, 184–202.
- Holmdahl, R., Bockermann, R., Backlund, J., Yamada, H., 2002. The molecular pathogenesis of collagen-induced arthritis in mice—a model for rheumatoid arthritis. *Ageing Res Rev* 1 (1), 135–147.
- Houssien, D.A., Jonsson, T., Davies, E., Scott, D.L., 1997. Clinical significance of IgA rheumatoid factor subclasses in rheumatoid arthritis. *J Rheumatol* 24, 2119–2122.
- Horai, R., Saijo, S., Tanioka, H., Nakae, S., Sudo, K., Okahara, A., et al., 2000. Development of chronic inflammatory arthropathy resembling rheumatoid arthritis in interleukin 1 receptor antagonist-deficient mice. *J Exp Med* 191 (2), 313–320.
- Jasin, H.E., 1985. Autoantibody specificities of immune complexes sequestered in articular cartilage of patients with rheumatoid arthritis and osteoarthritis. *Arthritis Rheum* 28 (3), 241–248.
- Jimenez-Boj, M.E., Bauer, R., Gaertner, M., Nell-Duxneuner, V.P., Stamm, T.A., Wagner, O., et al., 2012. Predicting rheumatoid arthritis by autoantibody testing (prera): preliminary results of a community-based investigation. *Ann Rheum Dis* 71 (Suppl 3), 338.
- Joosten, L.A., Helsen, M.M., van De Loo, F.A., van Den Berg, W.B., 2008. Anticytokine treatment of established type II collagen-induced arthritis in DBA/1 mice: a comparative study using anti-TNFalpha, anti-IL-1alpha/beta and IL-1Ra. *Arthritis Rheum* 58 (2 Suppl), S110–S122.
- Juarez, M., Bang, H., Hammar, F., Reimer, U., Dyke, B., Sahbuddin, I., et al., 2016. Identification of novel antiacetylated vimentin antibodies in patients with early inflammatory arthritis. *Ann. Rheum. Dis.* 75 (6), 1099–1107. available from: PM:26160441.
- Keffer, J., Probert, L., Cazlaris, H., Georgopoulos, S., Kaslaris, E., Kioussis, D., et al., 1991. Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. *EMBO J* 10, 4025–4031.
- Kuhn, K.A., Kulik, L., Tomooka, B., Braschler, K.J., Arend, W.P., Robinson, W.H., et al., 2006. Antibodies against citrullinated proteins enhance tissue injury in experimental autoimmune arthritis. *J Clin Invest* 116 (4), 961–973.
- Koch, R., 1890. An Address on bacteriological research. *Br Med J* 2 (1546), 380–383.
- Korb-Pap, A., Stratis, A., Muhlenberg, K., Niederreiter, B., Hayer, S., Echtermeyer, F., et al., 2012. Early structural changes in cartilage and bone are required for the attachment and invasion of inflamed synovial tissue during destructive inflammatory arthritis. *Ann Rheum Dis* 2012.
- Klareskog, L., Stolt, P., Lundberg, K., Kallberg, H., Bengtsson, C., Grunewald, J., et al., 2006. A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. *Arthritis Rheum* 54 (1), 38–46.
- Klareskog, L., Malmstrom, V., Lundberg, K., Padyukov, L., Alfredsson, L., 2011. Smoking, citrullination and genetic variability in the immunopathogenesis of rheumatoid arthritis. *Semin Immunol* 23 (2), 92–98.
- Kraan, M.C., Haringman, J.J., Post, W.J., Versendaal, J., Breedveld, F.C., Tak, P.P., 1999. Immunohistological analysis of synovial tissue for differential diagnosis in early arthritis. *Rheumatology (Oxford)* 38 (11), 1074–1080.

- Lam, J., Takeshita, S., Barker, J.E., Kanagawa, O., Ross, F.P., Teitelbaum, S.L., 2000. TNF-alpha induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand. *J Clin Invest* 106, 1481–1488.
- Landre-Beauvais, A.J. Doit-on admettre une nouvelle espece de Goutte sous la denomination de Goutte Asthenique Primitive? Paris, Brisson, An VIII; 1800.
- Lee, Z.H., Firestein, G.S., 2003. Fibroblasts. In: Smolen, J.S., Lipsky, P.L. (Eds.), *Targeted Therapies in Rheumatology*, 1st ed Martin Dunitz, London, New York, pp. 133–146.
- Lee, H.S., Irigoyen, P., Kern, M., Lee, A., Batliwalla, F., Khalili, H., et al., 2007. Interaction between smoking, the shared epitope, and anti-cyclic citrullinated peptide: a mixed picture in three large North American rheumatoid arthritis cohorts. *Arthritis Rheum* 56 (6), 1745–1753.
- Li, Y., Begovich, A.B., 2009. Unraveling the genetics of complex diseases: susceptibility genes for rheumatoid arthritis and psoriasis. *Semin Immunol* 21 (6), 318–327.
- Lima, I., Santiago, M., 2010. Antibodies against cyclic citrullinated peptides in infectious diseases—a systematic review. *Clin Rheumatol* 29 (12), 1345–1351.
- Lima, I., Oliveira, R.C., Atta, A., Marchi, S., Barbosa, L., Reis, E., et al., 2013. Antibodies to citrullinated peptides in tuberculosis. *Clin Rheumatol*. Lorentzen, J.C., Flornes, L., Eklow, C., Backdahl, L., Ribbhammar, U., Guo, J.P., et al., 2007. Association of arthritis with a gene complex encoding C-type lectin-like receptors. *Arthritis Rheum.* 56 (8), 2620–2632. available from: PM:17665455.
- van der Lubbe, P.A., Dijkmans, B.S., Markusse, H., Nassander, U., Breedveld, F.C., 1995. A randomized, double-blind, placebo-controlled study of CD4 monoclonal antibody therapy in early rheumatoid arthritis. *Arthritis Rheum* 38, 1097–1106. Ref Type: Abstract.
- MacGregor, A.J., Snieder, H., Rigby, A.S., Koskenvuo, M., Kaprio, J., Aho, K., et al., 2000. Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. *Arthritis Rheum* 43 (1), 30–37.
- Mallya, R.K., Vergani, D., Tee, D.E., Bevis, L., de Beer, F.C., Berry, H., et al., 2016. Correlation in rheumatoid arthritis of concentrations of plasma C3d, serum rheumatoid factor, immune complexes and C-reactive protein with each other and with clinical features of disease activity. *Clin Exp Immunol* 48 (3), 747–753.
- Martin, T., Duffy, S.F., Carson, D.A., Kipps, T.J., 1992. Evidence for somatic selection of natural autoantibodies. *J Exp Med* 175 (4), 983–991.
- Matsumoto, I., Maccioni, M., Lee, D.M., Maurice, M., Simmons, B., Brenner, M., et al., 2002. How antibodies to a ubiquitous cytoplasmic enzyme may provoke joint-specific autoimmune disease. *Nat Immunol.* 3 (4), 360–365.
- Mattey, D.L., Dawes, P.T., Clarke, S., Fisher, J., Brownfield, A., Thomson, W., et al., 2002. Relationship among the HLA-DRB1 shared epitope, smoking and rheumatoid factor production in rheumatoid arthritis. *Arthritis Rheum* 47, 403–407.
- Menzel, J., Steffen, C., Kolarz, G., Eberl, G., Frank, O., Thumb, N., 1975. Demonstration of antibodies to collagen and of collagen-anticollagen immune complexes in rheumatoid arthritis synovial fluids. *Ann Rheum Dis* 35 (5), 446–450.
- Miossec, P., 2004. IL-17 in rheumatoid arthritis: a new target for treatment or just another cytokine? *Joint Bone Spine* 71 (2), 87–90. available from: PM:15050191.
- Mjaavatten, M.D., van der Heijde, D., Uhlig, T., Haugen, A.J., Nygaard, H., Sidenvall, G., et al., 2010. The likelihood of persistent arthritis increases with the level of anti-citrullinated peptide antibody and immunoglobulin M rheumatoid factor: a longitudinal study of 376 patients with very early undifferentiated arthritis. *Arthritis Res Ther* 12 (3), R76.
- National Collaborating Centre for Chronic Conditions. 2009. *Rheumatoid arthritis: national clinical guideline for management and treatment in adults*. Royal College of Physicians, London.
- Nell, V.P.K., Machold, K.P., Eberl, G., Hiesberger, H., Hoefer, E., Smolen, J.S., et al., 2003. The diagnostic and prognostic significance of auto-antibodies in patients with early arthritis. *Ann Rheum Dis* 62 (Suppl 1), OP0015.
- Neogi, T., Aletaha, D., Silman, A.J., Naden, R.L., Felson, D.T., Aggarwal, R., et al., 2010. The 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for rheumatoid arthritis: Phase 2 methodological report. *Arthritis Rheum* 62 (9), 2582–2591.
- Nicaise-Roland, P., Nogueira, L., Demattei, C., de, C.L., Rincheval, N., Cornillet, M., et al., 2013. Autoantibodies to citrullinated fibrinogen compared with anti-MCV and anti-CCP2 antibodies in diagnosing rheumatoid arthritis at an early stage: data from the French ESPOIR cohort. *Ann Rheum Dis* 72 (3), 357–362.
- Nielen, M.M., van Schaardenburg, Reesink, W.H., van de Stadt, R.J., van der Horst-Bruinsma, I.E., de Koning, M.G., et al., 2004. Specific auto-antibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum* 50, 380–386.
- Nielsen, S.F., Bojesen, S.E., Schnohr, P., Nordestgaard, B.G., 2012. Elevated rheumatoid factor and long term risk of rheumatoid arthritis: a prospective cohort study. *BMJ* 345, e5244.
- Nienhuis, R.L., Mandema, E.A., 1964. NEW SERUM FACTOR IN PATIENTS WITH RHEUMATOID ARTHRITIS; THE ANTIPERINUCLEAR FACTOR. *Ann Rheum Dis* 23, 302–305.
- Ospelt, C., Gay, S., Klein, K., 2017. Epigenetics in the pathogenesis of RA. *Semin. Immunopathol.* 39 (4), 409–419. available from: PM:28324153.
- Packard, C.J., Bezlyak, V., McLean, J.S., Batty, G.D., Ford, I., Burns, H., et al., 2011. Early life socioeconomic adversity is associated in adult life with chronic inflammation, carotid atherosclerosis poorer lung function and decreased cognitive performance: a cross-sectional, population-based study. *BMC Public Health* 11, 42.
- Partsch, G., Steiner, G., Leeb, B.F., Dunky, A., Broll, H., Smolen, J.S., 1997. Highly increased levels of tumor necrosis factor-alpha and other proinflammatory cytokines in psoriatic arthritis synovial fluid. *J Rheumatol* 24, 518–523.
- Pfeifle, R., Rothe, T., Ipseiz, N., Scherer, H.U., Culemann, S., Harre, U., et al., 2017. Regulation of autoantibody activity by the IL-23-TH17 axis determines the onset of autoimmune disease. *Nat. Immunol.* 18 (1), 104–113. available from: PM:27820809.
- Pianta, A., Arvikar, S., Strle, K., Drouin, E.E., Wang, Q., Costello, C.E., et al., 2017. Evidence of the immune relevance of *Prevotella copri*, a gut microbe, in patients with rheumatoid arthritis. *Arthritis Rheumatol* 69 (5), 964–975. available from: PM:27863183.
- Pietropaolo, M., Towns, R., Eisenbarth, G.S., 2012. Humoral autoimmunity in type 1 diabetes: prediction, significance, and detection of distinct disease subtypes. *Cold Spring Harb Perspect Med* 2 (10).
- Plenge, R.M., Seielstad, M., Padyukov, L., Lee, A.T., Remmers, E.F., Ding, B., et al., 2007. TRAF1-C5 as a risk locus for rheumatoid arthritis—a genomewide study. *N Engl J Med* 357 (12), 1199–1209.

- Rantapaa-Dahlqvist, S., de Jong, B.A., Berglin, E., Hallmans, G., Wadell, G., Stenlund, H., et al., 2003. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum.* 48, 2741–2749.
- Redlich, K., Hayer, S., Ricci, R., David, J.P., Tohidast-Akrad, M., Kollias, G., et al., 2002. Osteoclasts are essential for TNF-alpha-mediated joint destruction. *J Clin Invest.* 110 (10), 1419–1427.
- Remmers, E.F., Plenge, R.M., Lee, A.T., Graham, R.R., Hom, G., Behrens, T.W., et al., 2007. STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus. *N Engl J Med.* 357 (10), 977–986.
- Rombouts, Y., Ewing, E., van de Stadt, L.A., Selman, M.H., Trouw, L.A., Deelder, A.M., et al., 2015. Anti-citrullinated protein antibodies acquire a pro-inflammatory Fc glycosylation phenotype prior to the onset of rheumatoid arthritis. *Ann. Rheum. Dis.* 74 (1), 234–241. available from: PM:24106048.
- Scheinecker, C., Smolen, J.S., 2011. Rheumatoid arthritis in 2010: from the gut to the joint. *Nat. Rev. Rheumatol.* 7 (2), 73–75. available from: PM:21289609.
- Schellekens, G.A., de Jong, B.A., van den Hoogen, F.H., van de Putte, L.B., van Venrooij, W.J., 1998. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. *J Clin Invest.* 101, 273–281.
- Scher, J.U., Abramson, S.B., 2011. The microbiome and rheumatoid arthritis. *Nat. Rev. Rheumatol.* 7 (10), 569–578. available from: PM:21862983.
- Scher, J.U., Szczesnak, A., Longman, R.S., Segata, N., Ubeda, C., Bielski, C., et al., 2013. Expansion of intestinal *Prevotella copri* correlates with enhanced susceptibility to arthritis. *Elife.* 2, e01202. available from: PM:24192039.
- Scher, J.U., Ubeda, C., Equinda, M., Khanin, R., Buischi, Y., Viale, A., et al., 2012. Periodontal disease and the oral microbiota in new-onset rheumatoid arthritis. *Arthritis Rheum.* 64 (10), 3083–3094.
- Schur, P.H., Britton, M.C., Franco, A.E., Corson, J.M., Sosman, J.L., Ruddy, S., 1975. Rheumatoid synovitis: complement and immune complexes. *Rheumatology.* 6, 34–42.
- Seldin, M.F., Amos, C.I., Ward, R., Gregersen, P.K., 1999. The genetics revolution and the assault on rheumatoid arthritis. *Arthritis Rheum.* 42 (6), 1071–1079.
- Shi, J., Willemze, A., Janssen, G.M., van Veelen, P.A., Drijfhout, J.W., Cerami, A., et al., 2013. Recognition of citrullinated and carbamylated proteins by human antibodies: specificity, cross-reactivity and the 'AMC-Senshu' method. *Ann. Rheum. Dis.* 72 (1), 148–150.
- Sigurdsson, S., Padyukov, L., Kurreeman, F.A., Liljedahl, U., Wiman, A.C., Alfredsson, L., et al., 2007. Association of a haplotype in the promoter region of the interferon regulatory factor 5 gene with rheumatoid arthritis. *Arthritis Rheum.* 56 (7), 2202–2210. available from: PM:17599733.
- Silman, A.J., MacGregor, A.J., Thomson, W., Holligan, S., Carthy, D., Farhan, A., et al., 1993. Twin concordance rates for rheumatoid arthritis: results from a nationwide study. *Br J Rheumatol.* 32 (10), 903–907.
- Silman, A.J., Pearson, J.E., 2002. Epidemiology and genetics of rheumatoid arthritis. *Arthritis Res.* 4 (Suppl 3), S265–S272.
- Singh, J.A., Christensen, R., Wells, G.A., Suarez-Almazor, M.E., Buchbinder, R., Lopez-Olivo, M.A., et al., 2009. Biologics for rheumatoid arthritis: an overview of Cochrane reviews. *Cochrane Database Syst Rev.* 4, CD007848.
- Singh, U., Singh, S., Singh, N.K., Verma, P.K., Singh, S., 2011. Anticyclic citrullinated peptide autoantibodies in systemic lupus erythematosus. *Rheumatol. Int.* 31 (6), 765–767.
- Smolen, J.S., Breedveld, F.C., Eberl, G., Jones, I., Leeming, M., Wylie, G.L., et al., 1995. Validity and reliability of the twenty-eight-joint count for the assessment of rheumatoid arthritis activity. *Arthritis Rheum.* 38 (1), 38–43.
- Smolen, J.S., Aletaha, D., Machold, K., Nell, V., Redlich, K., Schett, G., et al., 2006. Prearthritis - a concept whose time has come. *Future Rheumatol.* 1, 1–4.
- Smolen, J.S., Aletaha, D., Koeller, M., Weisman, M., Emery, P., 2007. New therapies for the treatment of rheumatoid arthritis. *Lancet.* 370, 1861–1874.
- Smolen, J.S., Agarwal, S.K., Ilivanova, E., Xu, X.L., Miao, Y., Zhuang, Y., et al., 2017. A randomised phase II study evaluating the efficacy and safety of subcutaneously administered ustekinumab and guselkumab in patients with active rheumatoid arthritis despite treatment with methotrexate. *Ann. Rheum. Dis.* 76 (5), 831–839. available from: PM:28087506.
- Smolen, J.S., Aletaha, D., Barton, A., Burmester, G.R., Emery, P., Firestein, G.S., et al., 2018. Rheumatoid arthritis. *Nat Rev Dis Primers.* 4, 18001.
- Smolen, J.S., Aletaha, D., 2013. Forget personalised medicine and focus on abating disease activity. *Ann. Rheum. Dis.* 72 (1), 3–6.
- Smolen, J.S., Menzel, E.J., Scherak, O., Kojer, M., Kolarz, G., Steffen, C., et al., 1980. Lymphocyte transformation to denatured type I collagen and B lymphocyte alloantigens in rheumatoid arthritis. *Arthritis Rheum.* 23 (4), 424–431.
- Stastny, P., 1978. Association of the B-cell alloantigen DRw4 with rheumatoid arthritis. *N Engl J Med.* 298 (16), 869–871.
- Steffen, C., 1970. Consideration of pathogenesis of rheumatoid arthritis as collagen autoimmunity. *Z Immunitsatsforsch Allerg Klin Immunol.* 139 (3), 219–227.
- Steiner, G., Hartmuth, K., Skriner, K., Maurer-Fogy, I., Sinski, A., Thalmann, E., et al., 1992. Purification and partial sequencing of the nuclear autoantigen RA33 shows that it is indistinguishable from the A2 protein of the heterogeneous nuclear ribonucleoprotein complex. *J Clin. Invest.* 90 (3), 1061–1066.
- Symmons, D.P., Bankhead, C.R., Harrison, B.J., Brennan, P., Barrett, E.M., Scott, D.G., et al., 1997. Blood transfusion, smoking, and obesity as risk factors for the development of rheumatoid arthritis: results from a primary care-based incident case-control study in Norfolk, England. *Arthritis Rheum.* 40 (11), 1955–1961.
- Takasaki, Y., Yamanaka, K., Takasaki, C., Matsushita, M., Yamada, H., Nawata, M., et al., 2004. Anticyclic citrullinated peptide antibodies in patients with mixed connective tissue disease. *Mod. Rheumatol.* 14 (5), 367–375.
- Tahir, H., Deodhar, A., Genovese, M., Takeuchi, T., Aelion, J., Van den Bosch, F., et al., 2017. Secukinumab in active rheumatoid arthritis after anti-TNFalpha therapy: a randomized, double-blind placebo-controlled phase 3 study. *Rheumatol. Ther.* 4 (2), 475–488. available from: PM:29138986.
- Teitelbaum, S.L., 2000. Bone resorption by osteoclasts. *Science.* 289, 1504–1508.
- Toivanen, P., 2001. From reactive arthritis to rheumatoid arthritis. *J Autoimmun.* 16, 369–371.

- Trentham, D.E., Townes, A.S., Kang, A.H., 1977. Autoimmunity to type II collagen an experimental model of arthritis. *J Exp Med* 146 (3), 857–868.
- Trentham, D.E., Dynesius, R.A., Rocklin, R.E., David, J.R., 1978. Cellular sensitivity to collagen in rheumatoid arthritis. *N Engl J Med* 299 (7), 327–332. 1978.
- Uhlig, T., Hagen, K.B., Kvien, T.K., 1999. Current tobacco smoking, formal education, and the risk of rheumatoid arthritis. *J Rheumatol* 26 (1), 47–54.
- van der Helm-van Mil, A.H., Verpoort, K.N., Breedveld, F.C., Huizinga, T.W., Toes, R.E., de Vries, R.R., 2006. The HLA-DRB1 shared epitope alleles are primarily a risk factor for anti-cyclic citrullinated peptide antibodies and are not an independent risk factor for development of rheumatoid arthritis. *Arthritis Rheum* 54 (4), 1117–1121.
- van Snick, J.L., Van, R.E., Markowetz, B., Cambiaso, C.L., Masson, P.L., 1978. Enhancement by IgM rheumatoid factor of in vitro ingestion by macrophages and in vivo clearance of aggregated IgG or antigen-antibody complexes. *Eur J Immunol* 8 (4), 279–285.
- Vesperini, V., Lukas, C., Fautrel, B., Le L, X., Rincheval, N., Combe, B., 2013. Association of tobacco exposure and reduction of radiographic progression in early rheumatoid arthritis: results from a French multicenter cohort. *Arthritis Care Res (Hoboken)* 65 (12), 1899–1906.
- Viatte, S., Plant, D., Raychaudhuri, S., 2013. Genetics and epigenetics of rheumatoid arthritis. *Nat. Rev. Rheumatol* 9 (3), 141–153. available from: PM:23381558.
- Vossenaar, E.R., Smeets, T.J., Kraan, M.C., Raats, J.M., van Venrooij, W.J., Tak, P.P., 2004. The presence of citrullinated proteins is not specific for rheumatoid synovial tissue. *Arthritis Rheum* 50, 3485–3494.
- Waaler, E., 1939. On the occurrence of a factor in human serum activating the specific agglutination of sheep blood corpuscles. *Acta Pathol Microbiol Immunol Scand* 17, 172–182.
- Williams, D.G., Moyes, S.P., Mageed, R.A., 1999. Rheumatoid factor isotype switch and somatic mutation variants within rheumatoid arthritis synovium. *Immunology* 98 (1), 123–136.
- Winchester, R.J., Agnello, V., Kunkel, H.G., 1970. Gamma globulin complexes in synovial fluids of patients with rheumatoid arthritis. Partial characterization and relationship to lowered complement levels. *Clin Exp Immunol* 6 (5), 689–706.
- Witebsky, E., Rose, N.R., Terplan, K., Paine, J.R., Egan, R.W., 1957. Chronic thyroiditis and autoimmunization. *J Am Med Assoc* 164 (13), 1439–1447.
- Wegner, N., Wait, R., Sroka, A., Eick, S., Nguyen, K.A., Lundberg, K., et al., 2010. Peptidylarginine deiminase from *Porphyromonas gingivalis* citrullinates human fibrinogen and alpha-enolase: implications for autoimmunity in rheumatoid arthritis. *Arthritis Rheum* 62 (9), 2662–2672.
- Wernick, R.M., Lipsky, P.E., Marban-Arcos, E., Maliakkal, J.J., Edelbaum, D., Ziff, M., 1985. IgG and IgM rheumatoid factor synthesis in rheumatoid synovial membrane cell cultures. *Arthritis Rheum* 28 (7), 742–752.
- Wu, H.J., Ivanov, I.I., Darce, J., Hattori, K., Shima, T., Umesaki, Y., et al., 2010. Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. *Immunity* 32 (6), 815–827. available from: PM:20620945.
- Xue, Y., Zhang, J., Chen, Y.M., Guan, M., Zheng, S.G., Zou, H.J., 2008. The HLA-DRB1 shared epitope is not associated with antibodies against cyclic citrullinated peptide in Chinese patients with rheumatoid arthritis. *Scand J Rheumatol* 37 (3), 183–187.
- Yoshitomi, H., Sakaguchi, N., Kobayashi, K., Brown, G.D., Tagami, T., Sakihama, T., et al., 2005. A role for fungal  $\beta$ -glucans and their receptor Dectin-1 in the induction of autoimmune arthritis in genetically susceptible mice. *J Exp Med* 201 (6), 949–960.
- Young, B.J., Mallya, R.K., Leslie, R.D., Clark, C.J., Hamblin, T.J., 1979. Anti-keratin antibodies in rheumatoid arthritis. *Br Med J* 2 (6182), 97–99.

## Juvenile Idiopathic Arthritis

Clara Malattia<sup>1,2</sup> and Alberto Martini<sup>2</sup>

<sup>1</sup>Division of Pediatric Rheumatology, Gaslini Children's Hospital, Genoa, Italy <sup>2</sup>Department of Pediatrics, University of Genoa, Genoa, Italy

### OUTLINE

Epidemiology	676	Genetics	680
<b>Clinical Features</b>	<b>676</b>	<b>Proinflammatory Mediators</b>	<b>682</b>
Systemic Arthritis	676	Interleukin-6	682
Rheumatoid Factor–Positive Polyarthritis	677	Interleukin-1	682
Enthesitis-Related Arthritis	677	Interleukin-18	683
Oligoarthritis	678	<b>Macrophage Activation Syndrome</b>	<b>683</b>
Rheumatoid Factor Negative Polyarthritis	678	Oligoarticular Juvenile Idiopathic Arthritis	684
Psoriatic Arthritis	679	<b>Treatment</b>	<b>686</b>
Undifferentiated Arthritis	679	<b>References</b>	<b>687</b>
Perspectives	679		
<b>Etiology and Pathogenesis</b>	<b>680</b>		
Systemic Juvenile Idiopathic Arthritis	680		

Juvenile idiopathic arthritis (JIA) is not a single disease, but an exclusion diagnosis that gathers together all forms of arthritis of unknown origin, with onset prior to the age of 16 years and lasting more than 6 weeks (Ravelli and Martini, 2007; Prakken et al., 2011). This heterogeneous group of chronic arthritides, we call JIA, is unified by the presence of a chronic inflammatory process, primarily targeting the synovial membrane. The persistence of synovial inflammation may lead to an increased risk of osteocartilaginous damage with subsequent physical functional disability. The etiology of JIA is still unknown. The heterogeneity of these conditions indicates that different factors are likely to contribute to the pathogenesis of the various forms of childhood chronic arthritis. According to the current classification, proposed by the International League of Associations for Rheumatology (ILAR) (Petty et al., 2004) (Table 35.1), patients are classified into mutually exclusive categories based on clinical features and laboratory results during the first 6 months of the disease. This ILAR classification, which was conceived as “work in progress,” has however been recently challenged since there is evidence that while some categories represent quite definite disease entities, others still include heterogeneous disorders (Martini, 2003; Martini, 2012a,b).

Genetic and immunological studies, as we will discuss, provide an opportunity to address this heterogeneity through classifying patients into more homogeneous subtypes based on the genetic pathways that drive disease.

**TABLE 35.1** Frequency, Age at Onset, and Sex Distribution of the International League of Associations for Rheumatology Categories of Juvenile Idiopathic Arthritis

	Frequency <sup>a</sup> (%)	Onset age	Sex ratio
Systemic arthritis	4–17	Throughout childhood	F = M
Exclusions: a, b, c, d			
Oligoarthritis	27–56	Early childhood; peak at 2–3 years	F>>>M
Exclusions: a, b, c, d, e			
Rheumatoid factor–positive polyarthritis	2–7	Late childhood or adolescence	F>>M
Exclusions: a, b, c, e			
Rheumatoid factor–negative polyarthritis	11–28	Biphasic distribution, early at 2–4 years and later peak at 6–12 years	F>>M
Exclusions: a, b, c, d, e			
Enthesitis-related arthritis	3–11	Late childhood or adolescence	M>>F
Exclusions: a, d, e			
Psoriatic arthritis	2–11	Biphasic distribution; early peak at 2–4 years and later peak at 9–11 years	F>M
Exclusions: b, c, d, e			
Undifferentiated arthritis	11–21		

<sup>a</sup>Percentage of all juvenile idiopathic arthritis cases.

Exclusion criteria: (a) Psoriasis or a history of psoriasis in the patient or first-degree relative. (b) Arthritis in an HLA-B27 positive male beginning after the sixth birthday. (c) Ankylosing spondylitis, enthesitis-related arthritis, sacroileitis with inflammatory bowel disease, Reiter's syndrome, or acute anterior uveitis, or a history of one of these disorders in a first-degree relative. (d) The presence of IgM rheumatoid factor on at least two occasions at least 3 months apart. (e) The presence of systemic JIA in the patient. The different categories are defined according to the symptoms presented during the first 6 months of disease. JIA, Juvenile idiopathic arthritis.

## EPIDEMIOLOGY

JIA as a whole is the most common chronic rheumatic condition in childhood and an important cause of short- and long-term disability. Although present across the world, its incidence and prevalence vary considerably throughout. Studies in Western populations have reported an incidence and a prevalence varying from 2 to 20 and from 16 to 150 per 100,000, respectively, with higher occurrence in northern Europe (Ravelli and Martini, 2007). The rate of occurrence of the various JIA subtypes alters considerably according with geographic and ethnic differences: oligoarthritis, which is the most widespread category in the Western countries, is, in fact, quite rare in areas such as India, South Africa, Costa Rica, and New Zealand where polyarthritis outnumbers oligoarthritis and systemic-onset disease. Although JIA is more frequent in females than in males, the proportion may vary according to JIA onset categories and geographic or racial groups.

## CLINICAL FEATURES

The group of diseases gathered under the umbrella term of JIA encompasses several different forms of chronic arthritis that, in the absence of etiologic clues, have been classified according to clinical criteria.

### Systemic Arthritis

Systemic JIA (sJIA) accounts for 10%–15% of the children with JIA, and it is among the most severe childhood inflammatory diseases. First described by Sir George Frederic Still over a century ago, sJIA is characterized by arthritis and prominent systemic features, such as high-spiking fever, an evanescent salmon pink skin rash that characteristically occurs with fever peaks, hepatosplenomegaly, generalized lymphadenopathy, and serositis. Myalgias and abdominal pain may be intense during fever peaks. Arthritis is more often symmetrical and polyarticular. It may be absent at onset but could develop during disease course. Laboratory investigations show

leukocytosis (with neutrophilia), thrombocytosis and very high erythrocyte sedimentation rate, and C-reactive protein concentration. A microcytic anemia is common.

About 5%–8% of the children with sJIA develop a life-threatening complication, named macrophage activation syndrome (MAS) (Ravelli et al., 2012). The hallmark of MAS is an uncontrolled and dysfunctional immune response involving the persistent activation and expansion of T lymphocytes and macrophages and leading to an actual cytokine storm. The syndrome is a form of reactive hemophagocytic lymphohistiocytosis (HLH) and is characterized by the sudden onset of sustained fever, pancytopenia, hepatosplenomegaly, liver insufficiency, coagulopathy with hemorrhagic manifestations, and neurological symptoms. Laboratory features include elevated triglycerides, low sodium levels, and markedly increased ferritin concentrations. Soluble CD163 and soluble interleukin (IL)-2 receptor are also greatly increased in active MAS. The demonstration of active phagocytosis of hematopoietic cells by macrophage in the bone marrow is common. The early recognition and treatment of MAS, before the development of severe multisystem involvement, is crucial for the prognosis. In this perspective, diagnostic guidelines for sJIA-associated MAS have been developed (Ravelli et al., 2016).

sJIA probably does not represent a disease but rather a syndrome, the common endpoint of several different diseases causing a marked and persistent activation of the innate immune system (Martini, 2012a,b). The potential heterogeneity of this condition is suggested by a dramatic sensitivity to IL-1 blockade occurring in a subset of patients (see below) and by differences in clinical course.

Indeed, in about half of the patients, the disease is mainly characterized by the systemic features while the arthritis usually remits when systemic features are controlled. In the other half of patients, the disease follows an unremitting course; systemic symptoms may eventually resolve, leaving chronic arthritis as the major long-term problem. There are, moreover, patients who present the same systemic features observed in sJIA but never develop arthritis and, therefore, cannot by definition be classified as sJIA. This subgroup of patients lacks nowadays any taxonomic definition. The superimposable systemic clinical and laboratory features suggest that they have a disease strongly related to sJIA despite the lack of arthritis. This type of patients is indeed included in the definition of adult-onset Still's disease, where the presence of arthritis is not required for diagnosis. It has been suggested to include these patients in the sJIA disease category (Martini, 2012a,b), but, given the absence of arthritis, the term sJIA should be changed.

## Rheumatoid Factor–Positive Polyarthritis

Rheumatoid factor (RF)–positive polyarthritis is defined as five or more joints affected during the first 6 months of the disease and by the presence of positive RF. This rare (5% of the patients with JIA) subgroup is the childhood equivalent of adult RF-positive rheumatoid arthritis (RA), both sharing similar clinical phenotype, serology, and immunogenetic profile. The shared epitope present in some HLA-DR4, DR1, and DR14 alleles is associated with an increased risk for both adult RA- and RF-positive JIA. The onset occurs usually in late childhood or adolescence with a female predominance and a symmetric polyarthritis affecting principally wrists and the small joints of the hands and feet. Rheumatoid nodules, typical of adult RA, have been also reported in about a third of RF-positive JIA. Low-grade fever, lymphadenopathy, and weight loss may occur with active disease. Acute phase reactants are usually increased, and a moderate normochromic and normocytic anemia is often associated. RF-positive polyarthritis is the only form of JIA with positive antibodies to cyclic citrullinated peptides. As in adults RA, it is a chronic erosive disease. Erosive changes, as detected by conventional radiography, occur earlier and more frequently in RF-positive polyarthritis than in the other JIA categories and are commonly detected in the hands and feet. The aggressive medical treatment of RF-positive polyarthritis is recommended due to its poor prognosis. Patients with RF-positive polyarthritis, in fact, have the lowest remission rate (5%) off-medication among children with chronic arthritis.

## Enthesitis-Related Arthritis

Enthesitis-related arthritis represents a form of undifferentiated spondyloarthropathy. It accounts for about 5%–10% of the JIA cases and usually starts after the age of 6, being more frequent in boys. Arthritis initially affects the larger joints of the lower limbs and is generally associated to enthesitis (inflammation of the point where a tendon, ligament, or fascia inserts into the bone). The most common sites of enthesitis are the calcaneal insertions of the Achilles tendon and of the plantar fascia and the tarsal area. Most patients are HLA-B27

positive. The disease course can be moderate and remitting, but a variable percentage of patients, most of whom cannot be identified at disease onset, will develop involvement of sacroiliac joints later on. Unlike other JIA categories, hip involvement is frequent at disease presentation. These patients may present a symptomatic acute uveitis characterized by red eyes, photophobia, and pain.

All the different forms of adult spondyloarthropathies can be observed in children, although a much higher proportion of undifferentiated spondyloarthropathies is present in childhood. The discrepancy in terminology between children and adults has been a source of confusion. It would be advisable to substitute the term "juvenile spondyloarthropathy" in the place of enthesitis-related arthritis, which could suggest the existence of a form peculiar to childhood, not observed in adults ([Martini, 2012a,b](#)).

## Oligoarthritis

It is defined by the involvement of four or less joints during the first 6 months of disease. In the Western countries the large majority of patients belongs to a quite well-defined disease entity observed only in children and characterized by an asymmetric arthritis, involving mainly large joints, an early onset (before 6 years of age), a female gender predilection, a high frequency of positive antinuclear antibodies (ANA), a high risk of developing chronic iridocyclitis, and consistent human leukocyte antigen (HLA) associations (HLA-DRB1\*08 in particular). The current ILAR classification distinguishes two categories of oligoarthritis based on the number of joints affected after the first 6 months of the disease: one, often with a good long-term prognosis, in which the disease remains confined to four or less joints (persistent oligoarthritis) and another in which arthritis extends to more than four joints after the first 6 months of disease (extended oligoarthritis). It has however been shown that ANA-positive patients regardless of whether they have persistent or extended oligoarthritis, share the same characteristics (e.g., age at onset, sex ratio, asymmetry of articular involvement, etc.), strongly suggesting that these two subcategories of oligoarthritis represent the same disease, varying only in the spread of arthritis ([Ravelli et al., 2005](#)). About one-third of the patients with oligoarticular JIA develop a chronic, non-granulomatous, anterior uveitis affecting the iris and the ciliary body (iritidocyclitis), which may cause severe visual impairment. The onset is usually insidious and very often entirely asymptomatic. Uveitis generally occurs at the time of diagnosis or shortly thereafter, although in less than 10% patients it precedes the onset of arthritis. Evidence of increased protein concentration and inflammatory cells in the anterior chamber of the eye suggests anterior uveitis. Though inflammatory cytokines [IL-1 $\beta$ , IL-2, IL-6, interferon- $\gamma$ , and tumor necrosis factor (TNF)- $\alpha$ ] have been reported in ocular fluids and tissues, the pathogenesis of uveitis and the link between inflammatory joint and ocular diseases is not yet clear. The course of uveitis may be relapsing or chronic and does not parallel the clinical course of arthritis. Early diagnosis and treatment are of the utmost importance in order to prevent the serious visually disabling complications such as posterior synechiae, band keratopathy, cataracts, glaucoma, and cystoid macular edema. Complications can result from uncontrolled intraocular inflammation as well as from its treatment, particularly prolonged use of high-dose topical corticosteroids ([Sen et al., 2015](#)). Since uveitis is asymptomatic at onset, children with ANA-positive JIA should be screened at least every 3 months by slit-lamp examination. ANA positivity represents a strong risk factor for developing uveitis. ANA have been found to react against different chromatin constituents and against the DEK nuclear protein, a putative oncprotein; however, none of these molecular targets have been found to be specific for oligoarticular JIA. Although uveitis is more frequent in ANA-positive oligoarticular JIA, it is also manifest in 14% of the RF-negative polyarthritis and in 10% of the psoriatic arthritis, while it is uncommon in sJIA or RF-positive polyarthritis.

## Rheumatoid Factor Negative Polyarthritis

It is defined by the involvement of 5 or more joints during the first 6 months of disease in the absence of immunoglobulin M (IgM) RF. It is probably the most heterogeneous JIA category, and at least two distinct clinical phenotypes can be identified: one, that is comparable to adult-onset RF-negative RA, is characterized by overt symmetric arthritis of large and small joints, onset at school age and negative ANA; the second resembles ANA-positive early-onset oligoarthritis in many respects but for the number of joint involved during the first 6 months of disease. The similarities between this second subset and early-onset oligoarthritis led to the hypothesis that they represent the same disease, the former being a more aggressive phenotype than the latter ([Martini, 2003](#)). This view has been confirmed by the demonstration that those features (early age at onset, positive tests for

ANA, asymmetric arthritis, female predominance, and increased risk of chronic iridocyclitis) that characterize ANA-positive oligoarthritis are also present in ANA-positive, RF-negative polyarthritis, but not in ANA-negative RF-negative polyarthritis or in ANA-negative oligoarthritis (Ravelli et al., 2005, 2011). Moreover, ANA-positive, RF-negative polyarthritis is seldom observed in those countries in which ANA-positive oligoarthritis is rare (Martini, 2003). This hypothesis has finally gained support from gene expression studies demonstrating B-cell signature (Barnes et al., 2010) and an association with HLA-DRB1 (Hollenbach et al., 2010) in both oligoarticular and polyarticular forms of diseases in patients with early-onset arthritis ( $\leq 6$  years).

## Psoriatic Arthritis

This is another inadequately defined JIA category. The diagnosis of juvenile psoriatic arthritis (JPsA) according to the ILAR criteria requires the simultaneous presence of arthritis and a typical psoriatic rash or, if the latter is absent, the presence of arthritis and two of the following: dactylitis (swelling of one or more digits/fingers that extends beyond the joint margins), nail pitting or onycholysis, and a family history of psoriasis in a first-degree relative.

JPsA does not represent a clearly defined entity and at least two subsets of patients can be identified (Martini, 2003; Stoll et al., 2006; Ravelli et al., 2011). Younger children (<5 years old), most usually female and with ANA-positive test, are likely to develop asymmetric oligoarthritis and show a greater risk of chronic iridocyclitis. This subgroup appears very similar to the early-onset oligoarticular JIA. By contrast, later onset JPsA patients exhibit a gender ratio closer to 1:1, with a propensity to enthesitis and to develop sacroiliitis during follow-up, similar to several adult patients with psoriatic arthritis, who share features with spondyloarthropathies. The ILAR classification, in which patients with enthesitis are by definition excluded, defers by default the latter group to the category of undifferentiated arthritis (Stoll et al., 2008).

## Undifferentiated Arthritis

It does not represent a separate subset, but a category in which by definition patients that do not fulfill the inclusion criteria of any of the categories, or fit criteria for more than one category, are included.

## Perspectives

Over the last 20 years, there have been substantial developments in the classification of chronic arthritis in children. However, the debate surrounding classification is far from resolved. In fact, while some JIA categories clearly identify distinct disease entities, others still include heterogeneous disorders. There is now enough evidence that some patients with ANA-positive, early-onset arthritis are in the current ILAR classification mistakenly included in the RF-negative polyarthritis and in the psoriatic arthritis categories. It would be, therefore, advisable to group all these patients together in a new category of ANA-positive, early-onset arthritis, independently from the number of joints involved or the presence of psoriasis (Martini, 2012a,b). Once these patients are removed, the RF-negative polyarthritis and the psoriatic arthritis categories would presumably mainly be represented by patients with the clinical characteristics similar to their adult counterpart. Aligning JIA classification to adult nomenclature would alter the misleading concept that JIA is a single disease and that the various categories represent just phenotypic variants.

Further advances in classification will come from immunologic, genomic, and proteomic studies in the various JIA categories, as well as from differences in the response to biological agents that, by targeting specific molecules, represent a precious opportunity of “reverse translation” (from the bed to the bench side), as it has been with the inhibition of IL-1 in sjIA. As anticipated, sjIA appears to be a syndrome which includes a group of disorders characterized by an autoinflammatory hallmark. This group should include patients with systemic features without arthritis as in adults and switching to the nomenclature used by the adult physicians (Still’s disease) could be taken into consideration. Of note, gene-expression analysis of adult-onset Still’s disease and sjIA has suggested that these two clinical phenotypes are part of the same disease continuum, with different ages of onset (Nirmala et al., 2015; Jamilloux et al., 2015).

## ETIOLOGY AND PATHOGENESIS

As discussed above, it is now clear that in children, as in adults, there are several completely different diseases which are all responsible for chronic arthritis and that are gathered under the umbrella term of JIA. With the exception of early-onset, ANA-positive arthritis, which is a well-defined entity observed only in childhood, they appear to represent the childhood counterpart of diseases observed also in adults. Therefore, in order to avoid overlaps with other chapters, only those immunological aspects that belong to sJIA, which is far more common in children, and to early-onset ANA-positive arthritis, which occurs only in children, will be discussed in detail. For the other diseases, there are not relevant, specific immunological findings, and characteristic of childhood-onset forms.

### Systemic Juvenile Idiopathic Arthritis

sJIA is etiopathogenically different from all the other forms of JIA (Mellins et al., 2011). The systemic features, the marked inflammatory response, which has been associated with dysregulation of the innate immune system, and the lack of sex bias have suggested that it may be part of the spectrum of autoinflammatory disorders. The disease is in fact unique with regard to the other JIA categories, in terms of clinical manifestations, prognosis, and response to conventional immunosuppressant therapies. Indeed, methotrexate (MTX) or anti-TNF agents are less effective than in the other JIA categories, while anti-IL-6 or anti-IL-1 drugs are of impressive efficacy.

### Genetics

sJIA is much more frequent in children than in adults (adult-onset Still's disease). This can suggest a role for some widely diffused infectious agents that are encountered early in life or represent the effect of a strong genetic predisposing background. The disease is considered multifactorial and multigenic, although it is not known if genetic predisposition is due to the combinations of common genetic variants, each providing a small contribution to inherited susceptibility, or to high-penetrance rare mutations that only account for a few cases each. Of interest, a study from Saudi Arabia (Wakil et al., 2015) has recently reported a monogenic autosomal-recessive form of sJIA. Whole-exome sequencing of 13 patients with a phenotype of sJIA from five consanguineous families identified a homoallelic missense mutation in laccase domain containing 1 (LACC1), which encodes the enzyme laccase. It is a nonspecific enzyme that catalyzes the oxidation of polyphenols, aromatic amines, and inorganic ions; however, its specific biologic function is not yet fully understood.

Consistent with the supposed autoinflammatory nature of sJIA, gene expression studies on peripheral blood mononuclear cells (PBMCs) have shown an upregulation of genes associated with the activation of monocyte/macrophage lineage and a downregulation of the gene networks involving natural killer (NK) cells, T cells, and MHC antigen-related biological processes (Pascual et al., 2005; Fall et al., 2007; Ogilvie et al., 2007; Allantaz et al., 2007; Barnes et al., 2009). Some genetic polymorphisms, particularly of cytokine genes, have been associated with sJIA (Table 35.2).

Of interest the potential role of NK in the pathogenesis of sJIA has been recently reviewed. Put et al. found that NK cells from the sJIA patients showed increased expression of innate pathways, such as Toll-like receptor (TLR) 4 (TLR-4) and S100 proteins. Although NK cells cytotoxic activity appeared intact, a decreased expression of immune-regulating genes such as granzyme K in CD56 bright NK cells and a defective IL-18-induced IFN- $\gamma$  production were demonstrated (Put et al., 2017). Of note, CD56 bright NK cells were found to be able to kill autologous-activated T cells in patients with multiple sclerosis via granzyme K. As a result, the hypothesis is that a decreased granzyme K expression in sJIA patients might influence the killing of activated autologous cells, thus supporting the concept of defective immune regulation in sJIA.

The modern notion that sJIA is more a disease of innate than adaptive immunity has been recently challenged by the results of a multicenter study that has examined the MHC locus in a large collection of patients (982 patients and 8010 healthy controls). Metaanalysis of imputed classic HLA-type associations in six study populations of Western European ancestry has identified HLA-DRB1\*11 alleles as the strongest single-risk factor for sJIA, with a pooled odds ratio (OR) of 2.3 (Ombrello et al., 2015). This study strengthens the relationship between the class II HLA region and sJIA, implicating adaptive immune molecules in the pathogenesis of sJIA.

Ombrello et al. (2017) also found that sJIA did not share heritable risk factors with the more common oligoarticular and polyarticular forms of JIA. Even within the class II MHC region, which harbors disease-associated

**TABLE 35.2** Genetic Associations With Systemic Juvenile Idiopathic Arthritis

Gene	No. of patients	No. of healthy control	Ethnicity	Genotype	Comments and OR	Reference
5'-Flanking region of the IL-6	92	383	Caucasian	-174 IL-6 CC <sup>a</sup>	Significant lower frequency of the CC genotype in patients with age of onset $\leq$ 5 years OR 0.34, $P = .04$	Fishman et al. (1998)
5'-Flanking promoter/enhancer region of TNF $\alpha$	50	575	Japanese	-1031C -863A -857T -857T/DRB1*0405	OR 1.84, $P = .015$ OR 1.83, $P = .022$ OR 1.80, $P = .016$ OR 3.84, $P = .0001$	Date et al. (1999)
5'-Flanking region of MIF	117	172	Caucasian	MIF-173*C	OR 2.3, $P = .0005$	Donn et al. (2001)
IL-18 promoter region	16 AOSD	92	Japanese	Haplotype S01 Diplootype S01/S01	OR 2.90, $P = .0072$ OR 7.81, $P = .0005$	Sugiura et al. (2002)
5'-flanking region of the MIF	136		Caucasian	MIF-173*C	Higher MIF serum and synovial fluid levels; poorer response to steroid treatment; persistence of active disease, and poor functional outcome	De Benedetti et al. (2003)
5'-flanking region of the IL-6	222 sJIA families		Caucasian	-174G IL-6	Excess transmission of the G allele to affected offspring with age at onset $>$ 5 years ( $P = .007$ )	Ogilvie et al. (2003)
IL10				IL10-1082A <sup>b</sup>	OR 1.335, $P = .031$	Fife et al. (2006)
IL20 (it is a member of IL-10 gene family)	172	473	Caucasian	IL-20 -468T IL10-1082A/IL20-468T haplotype	OR 1.507, $P = .028$ OR 2.24, $P = .0006$	
SLC26A2 Gene for diastrophic dysplasia				rs1541915 rs245056	OR 2.3, $P = .0003$ OR 2.8, $P = 0.00002$	Lamb et al. (2007)
IL-1 gene family	133	617	Caucasian	rs245055 rs245051 rs245076 rs8073	OR 2.5, $P = .004$ OR 2.3, $P = .0005$ OR 2.7, $P = .0015$ OR 2.3, $P = .04$	
IL-10	235	335	Caucasian	IL1 ligand cluster rs6712572 rs2071374 rs1688075	OR 1.32, $P = .025$ OR 1.48, $P = .002$ OR 2.04, $P = .002$	Stock et al. (2008)
				IL1 receptor cluster rs12712122 -1082A <sup>b</sup>	OR 1.29, $P = .047$ Increased frequency of -1082A ( $P < .001$ ), ACC ( $P = .01$ ) and GTC ( $P < .001$ ) haplotypes in sJIA	Möller et al. (2010)
	74	249	Caucasian	-819T -592A		

<sup>a</sup>C allele was associated with significantly lower levels of plasma-IL-6.<sup>b</sup>The IL-10 promoter polymorphism -1082A was associated with low IL-10 production.

AOSD, Adult-onset Still's disease; OR, odds ratio; IL, interleukin; sJIA, systemic juvenile idiopathic arthritis; ACC, adenine-cytosine-cytosine; GTC, guanine-thymine-cytosine.

genetic variation in each of these categories of JIA, the subtype-specific risk factors (single-nucleotide polymorphisms (SNPs), HLA alleles, and HLA haplotypes) are not shared between the subtypes. sJIA bears a unique genetic architecture, indicating that its underlying pathophysiological mechanisms are significantly divergent from other forms of JIA and arguing for a different classification framework.

## PROINFLAMMATORY MEDIATORS

Laboratory observations as well as the therapeutic efficacy of cytokine inhibitors have provided clear evidence for a major pathogenic role of phagocyte-derived cytokines, in particular of IL-6 and IL-1.

### Interleukin-6

The role of IL-6 in the pathogenesis of sJIA appears to be pivotal. Macrophages and endothelial cells are the main sources of IL-6, a cytokine that is responsible not only for the production of acute-phase proteins by hepatocytes, but also for hyperferritinemia, characterized by the uptake of free iron and ferritin synthesis. Furthermore, IL-6 is also closely related to the development of arthritis in both sJIA and adult-onset Still's disease. Circulating levels of IL-6 are markedly elevated, increase during the peak of fever, and correlate with the extent and severity of joint involvement and with platelet counts (De Benedetti et al., 1991). Synovial fluid levels of IL-6 are also markedly increased and significantly higher than those observed in patients with polyarticular and oligoarticular JIA or in RA patients (De Benedetti et al., 1997a,b). Moreover, in sJIA patients, huge quantities of IL-6 circulate bound to the soluble receptor; this bound IL-6 seems biologically active since it correlates much better with the level of C-reactive protein than free-circulating IL-6 (De Benedetti et al., 1994). At variance with findings in adult RA, anemia in sJIA is microcytic and characterized by a marked defect in iron supply for erythropoiesis, while growth of erythroid colonies is normal, and erythropoietin production is appropriate (Cazzola et al., 1996). These finding are consistent with the effect of IL-6 on the bone marrow. Indeed, IL-6 stimulates both hypoxia-induced erythropoietin production and erythroid progenitor proliferation and, by increasing ferritin expression and hepatic uptake of serum iron, causes a reticuloendothelial iron block. Moreover, IL-6 dramatically induces the liver production of hepcidin, a peptide that inhibits iron absorption in the small intestine and the release of recycled iron from the macrophages (Nemeth et al., 2004). This can well explain the oral iron malabsorption that has been observed in patients with sJIA (Martini et al., 1994).

Growth impairment is a well-known feature of sJIA since its first description and IL-6-transgenic mice with high circulating levels of IL-6 show a decreased rate of growth, attaining 50%–60% of the size of their wild-type littermates (De Benedetti et al., 1997a,b). Moreover, similar to patients with sJIA, IL-6-transgenic mice have normal growth hormone production but low levels of insulin-like growth factor 1 (IGF-1) and IGF-binding protein 3 (De Benedetti et al., 2001). All these finding led to the hypothesis that sJIA is an IL-6 and not a TNF-driven disease (De Benedetti and Martini, 1998). This hypothesis was supported 10 years later by the double-blinded controlled study with a withdrawal design that showed the marked efficacy of tocilizumab, a monoclonal antibody against the IL-6 soluble receptor (sIL-6R), which is essential for signal transduction (Yokota et al., 2008). These brilliant therapeutic results have been subsequently confirmed by a double-blind, placebo-controlled study (De Benedetti et al., 2012). Of note, it has been recently demonstrated that during treatment with tocilizumab patients with sJIA experience significant catch-up growth, normalization of IGF-1 levels, and bone balance improvement favoring bone formation (De Benedetti et al., 2015).

### Interleukin-1

The most compelling evidence for the important role of IL-1 in the pathogenesis of sJIA came from the quite serendipitous finding of the marked therapeutic efficacy of anakinra, an IL-1 inhibitor which is the recombinant version of the naturally occurring soluble IL-1 receptor antagonist (Verbsky and White, 2004; Pascual et al., 2005). In vitro exposure of healthy donor PBMCs to sera from subjects with sJIA induces transcription of genes associated with the IL-1 signaling pathway and markedly increases IL-1 $\beta$  protein secretion (Pascual et al., 2005). The role of IL-1 is not in contrast with that of IL-6, since IL-1 is upstream to IL-6. Interestingly, serum levels of myeloid-related proteins (MRPs) 8 and 14 (which are secreted by activated neutrophils and monocytes) are very high during active disease, and MRP-14 in serum of patients with sJIA has been shown to be a strong inducer of

IL-1 expression in phagocytes (Frosch et al., 2009). Moreover, MRP8/14 serum concentrations have been reported to correlate closely with response to drug treatment and disease activity and might, therefore, be useful also in monitoring treatment of individual patients with sJIA (Holzinger et al., 2012).

Of note, it has been shown that the response to anakinra can identify two different populations of sJIA patients (Gattorno et al., 2008). One (accounting for about 40% of the patients) shows a dramatic response to IL-1 blockade (similar to that observed in cryopyrin-associated autoinflammatory syndromes) leading to complete normalization of clinical as well as laboratory features in a few days. The other is resistant to treatment or shows an intermediate response. These two groups differed only in the number of joints involved, with patients with fewer joints affected, having a much higher probability of responding to anti-IL-1 therapy. The group with positive response could represent a separate entity in which the autoinflammatory component has the leading pathogenic role and where monocytes and neutrophils, rather than lymphocytes, are the predominant effector cells. The other group, characterized by the presence of an important synovitis, seems to have in addition a relevant autoimmune component. Of note, also Shimizu et al. (2013) suggested that patients with sJIA could be stratified into two subgroups, characterized respectively by very high circulating levels of IL-18 or IL 6. Interestingly, those with high levels of IL-18 had a low number of joint involved. As previously mentioned, Ombrello et al. (2015) found a relationship between the class II HLA region and sJIA, implicating adaptive immune molecules in the pathogenesis of sJIA. Although not tested, it is intriguing to speculate that this association could be limited to the sJIA subset with autoimmune features characterized by high number of joint involved, limited response to IL-1 inhibitors and high levels circulating IL-6.

A controlled trial has further supported the efficacy of Anakinra in sJIA (Quartier et al., 2011). Of interest in this trial a “de novo” type I IFN signature (which is not a feature of untreated sJIA) was observed in the majority of anakinra-treated patients regardless of the clinical response.

In a retrospective study, it has been recently suggested that a precocious treatment with Anakinra could prevent the subsequent appearance of refractory arthritis in the large majority of patients (Nigrovic et al., 2011). This hypothesis however needs to be confirmed in prospective randomized trials. More recently a high-affinity human IL-1 $\beta$  monoclonal antibody (canakinumab) has been used in the treatment of sJIA. It selectively binds to human IL-1 $\beta$ , thereby inactivating IL-1 $\beta$  signaling pathways and neutralizing its downstream effects. Clinical evidence of the efficacy and safety of canakinumab in patients with sJIA was demonstrated in two international trials that served as the basis for the approval of canakinumab for the treatment of sJIA by the United States and European regulatory authorities (Ruperto et al., 2012a,b).

## Interleukin-18

sJIA, as well as adult-onset Still’s disease, are characterized by very high levels of circulating IL-18 (Kawashima et al., 2001; Maeno et al., 2002). Of note, IL-18 is a member of the IL-1 cytokine superfamily, is stored as precursor protein, and, upon activation, is cleaved by caspase 1 in a manner similar to that of IL-1 to yield the active cytokine. It seems that in sJIA during MAS (see below), IL-18, and other proinflammatory cytokines (IFN- $\gamma$ , IL-6, TNF) are mainly produced by resident macrophages (bone marrow and liver) (Maeno et al., 2004; Billiau et al., 2005). A reversible defect in phosphorylation of IL-18 receptor has been reported in patients with sJIA (de Jager et al., 2009).

## MACROPHAGE ACTIVATION SYNDROME

It remains substantially a mystery why sJIA is so strongly associated with MAS. Of note, MAS is seldom observed in autoinflammatory diseases. It is unclear if this complication characterizes a distinct subset of the disease. Interestingly, activated macrophages or frank hemophagocytic cells have been found in 53% of the patients with sJIA who underwent bone-marrow aspiration, suggesting that subclinical MAS may be present in half of the patients (Behrens et al., 2007).

As mentioned above, MAS bears strong resemblance to a group of histiocytic disorders collectively known as HLH (Janka, 2012). Two types of HLH are recognized. The primary or familial HLH, familial haemophagocytic lymphohistiocytosis (FHLH) forms are secondary to genetic deficiency in cytolytic pathway proteins. The secondary or reactive HLH forms are associated with an identifiable infectious episode or are secondary to an autoimmune condition or to a neoplastic or a metabolic disorder.

HLH symptoms can be explained by the high concentration of inflammatory cytokines and by organ infiltration by lymphocytes and histiocytes. Fever is induced by IL-1 and IL-6, and pancytopenia is the consequence of high levels of TNF and IFN- $\gamma$  rather than of hemophagocytosis. TNF inhibits lipoprotein lipase leading to elevated triglycerides. Activated macrophages secrete ferritin and plasminogen activator which results in high plasmin levels and hyperfibrinolysis. Hepatosplenomegaly, increased liver enzymes, neurological symptoms are the consequence of organ infiltration by activated lymphocytes and histiocytes. Activated lymphocytes and macrophages are respectively the source for high concentration of circulating sIL-2 Ra and sCD163.

To date, all the described genetic defects associated with HLH appear to be related to one another in the pathway of granule-mediated cytotoxicity, with the lack of perforin being the most common. These genetic defects interrupt the mechanisms responsible for triggered apoptosis (mediated by cytotoxic cells on the target cell) or activation-induced apoptosis (putative suicide of activated T cell).

The similarities between HLH and MAS are not limited to clinical features. Similarly to patients with FHLH, patients with MAS in association with sJIA also have profoundly decreased cytolytic function, although this impairment reverts to normal after treatment (Ravelli et al., 2012).

These observations suggest that background inflammation is at least partially responsible for this functional abnormality in MAS. Indeed, IL-6 has been found to induce defective expression of perforin and decreased NK cell cytotoxic activity.

It has been recently hypothesized that cytolytic dysfunction in sJIA with MAS might be also influenced by a genetic component. In fact, a study using whole-exome sequencing reported hypomorphic mutations in primary HLH-associated genes in nearly a third of patients with sJIA and MAS (Kaufman et al., 2014).

The best experimental model of HLH is perforin-deficient mouse in which CD8 T cells produce large amounts of IFN- $\gamma$ .

It has been shown that repeated stimulation of TLR-9 produces an HLH/MAS-like syndrome on a normal genetic background, without exogenous antigen (Behrens et al., 2011). Like in perforin-deficient mice, the syndrome depends on the presence of IFN- $\gamma$ ; however, IFN- $\gamma$  is arising from different sources than CD8 T cells, including dendritic cells and NK cells. This model shows that MAS can develop during persistent innate immune activation independently from defects in cytotoxic cell function. Treatment of IL-6-transgenic mice with TLR ligands led to elevated levels of IL-1 $\beta$ , TNF, IL-6, and IL-18 and an increased fatality rate with features similar to those observed in MAS (Strippoli et al., 2012).

Of note, two reports described patients with periodic fevers and MAS-like features linked to a gain-of-function mutation in *NLRC4*, which was in turn linked to an overproduction of IL-1 $\beta$  and IL-18, in addition to an increased pyroptosis (a peculiar form of cell death). This caspase 1-mediated process is characterized by quick plasma-membrane rupture and release of proinflammatory intracellular contents. In patients with *NLRC4* mutation, MAS-like symptoms seemed to be induced by a macrophage intrinsic defect in the absence of primary cytotoxic abnormalities (Canna et al., 2014; Romberg et al., 2014).

The pivotal role of IFN- $\gamma$  pathway in sJIA patients with MAS has been recently confirmed by Bracaglia et al. who found levels of IFN- $\gamma$  and of IFN- $\gamma$ -induced chemokines (CXCL9 and CXCL10) markedly elevated during active MAS. In addition, levels of IFN- $\gamma$ , CXCL9, and CXCL10 strongly correlate with laboratory parameters of MAS severity (Bracaglia et al., 2017).

These observations suggest IFN- $\gamma$  could be targeted therapeutically in HLH; of note when this cytokine is neutralized, survival improved substantially in HLH animal models. A study to investigate the safety and efficacy of an Anti-IFN- $\gamma$  mAb in children affected by primary HLH is underway (<https://clinicaltrials.gov/ct2/show/NCT01818492>).

## Oligoarticular Juvenile Idiopathic Arthritis

As mentioned above, in the Western countries, most children with oligoarthritis have a definite disease, peculiar of childhood and characterized by an asymmetric arthritis, involving mainly large joints, an early onset (before 6 years of age), a female predilection, a high frequency of positive ANA, a high risk of developing chronic iridocyclitis. The homogeneity of this form of arthritis is also witnessed by consistent associations with HLA antigens. Positive associations include HLA-A2, HLA-DRB1\*11 (a subtype of HLA-DR5), and HLA-DRB1\*08. On the contrary, HLA-DRB1\*04 and HLA-DRB1\*07 have been found to be significantly decreased (Prahala and Glass, 2008). Evidence of the crucial role played by genetic variants in the HLA region is provided by the recent genome-wide association study (GWAS) studies of JIA (Hinks et al., 2009, 2013). In particular, the ImmunoChip

study (2816 JIA patients and 13,056 controls) found that the SNP rs7775055, located in the class II HLA region (DQ), was associated with oligoarticular and polyarticular RF-negative JIA with an OR of 6.01 (95% CI 5.30–6.81). HLA associations support an autoimmune pathogenesis, and the early onset suggests that the disease could be elicited by a very common infectious agent that can be encountered early in life. In this respect, evidence has been provided that T cells from patients with early-onset ANA-positive oligoarticular JIA are sensitized against epitopes that are shared between the disease-associated HLA antigens and proteins present on Epstein–Barr virus and other herpes viruses (Massa et al., 2002); herpes viruses represent a group of infectious agents to which the vast majority of children have already been exposed by 6 years of age.

The HLA region is estimated to explain ~8%–13% of the total variation in JIA susceptibility, suggesting that there are still many non-HLA loci to be identified. The most significant association outside the MHC region has been found with the PTPN22 gene, located on chromosome 1p13.2, and with the PTPN2 gene (Hinks et al., 2013). Both genes encode proteins tyrosine phosphatase involved in T-cell regulation. It is thought that functional mutations in these genes might lead to T-cell activation and the subsequent promotion of autoimmune disease. Mutations in PTPN22 have also been associated with multiple autoimmune diseases such as RA, systemic lupus erythematosus, autoimmune thyroid disease, and Type 1 diabetes mellitus. The ImmunoChip study has dramatically increased our knowledge of JIA susceptibility loci from non-HLA regions (PTPN22, STAT4, PTPN2, ANKRD55, IL2-IL21, TYK2, IL2RA, SH2B3-ATXN2, ERAP2-LNPEP, UBE2L3, C5orf6-IRF1, RUNX1, IL2RB, ATP8B2-IL6R, FAS, ZFP36L1 (Hinks et al., 2013)). In addition, multiple gene regions such as IL2RA, IL2/IL21, and IL2RB were confirmed to be susceptibility loci for JIA, thus highlighting the important role of the IL-2 pathway in JIA pathogenesis. This pathway plays a pivotal role in T-cell activation and development, as well as a key role in maintenance of immune tolerance through the dependence of regulatory T cells (Tregs) on IL-2. *SH2B3*, an adaptor protein involved in T-cell activation, and *STAT4*, a transcription factor important in T-cell differentiation, were also found to be related to this pathway and were confirmed to be susceptibility loci for JIA (Hinks et al., 2013).

The inflammatory synovitis observed in JIA is similar to that of adult RA. The synovium shows hyperplasia of the lining layer and infiltration of the sublining layer with mononuclear cells, including T cells, B cells, macrophages, dendritic cells, and plasma cells. The T-cell infiltrates are composed predominantly of Th-1-skewed CD4+ cells, expressing an activated memory phenotype. The inflammatory process leads to pannus formation with cartilage and bone erosions mediated by degradative enzymes such as metalloproteinases (Ravelli and Martini, 2007).

As mentioned above, a B-cell signature has been shown to characterize patients with early-onset arthritis (Barnes et al., 2010). Moreover, lymphoid aggregates and plasma cell infiltration have been found more frequently in patients with circulating ANA (Gregorio et al., 2007). These observations suggest that B cells can play a role in early-onset ANA positive arthritis. Corcione et al. found an expansion of activated switch memory B cells and of IgG-secreting plasma blasts in the SF of oligoarticular JIA patients. Memory B cells belonged to either the CD27+ or the CD27− subsets and expressed CD86, suggesting their involvement in antigen presentation to T cells (Corcione et al., 2009). Another study (Morbach et al., 2011), involving mainly patients with oligoarticular disease, has also confirmed that activated immunoglobulin class-switched CD27− and CD27+ memory B cells accumulate in the joints and might be involved in the amplification of pathogenic T-cell activation. These findings suggest that B cells play an antibody-independent immunopathologic role in oligoarthritis.

Cosmi et al. (2011) have studied Th17 cells in children with oligoarticular JIA. They showed that synovial fluid T cells could switch easily from a Th17 phenotype to a mixed Th1/Th17 phenotype, and, then, to a Th1 phenotype; the switch was linked to the presence of IL-12 in the synovial fluid. This study is in line with the findings of another study performed in JIA patients which showed that synovial fluid Th17 cells “convert” to Th17/1 in the presence of low TGFβ and high IL-12 levels, whereas Th1 cells cannot convert to Th17 (Nistala et al., 2010).

Within the joint, an inverse relationship between IL-17+ T cells and FoxP3+ Treg cells has been found (Nistala et al., 2008). In JIA, Tregs from the peripheral blood as well as from the inflamed joints are fully functional. Nevertheless, Treg-mediated suppression of cell proliferation and cytokine production by effector cells from the site of inflammation have been shown to be severely impaired (Haufe et al., 2011; Wehrens et al., 2011). This resistance to suppression has been shown to be secondary to the activation of protein kinase B/c-akt in inflammatory effector cells, since inhibition of this kinase restores responsiveness to suppression (Wehrens et al., 2011).

The fact that in oligoarticular JIA joint involvement can remain limited to four or less joints (oligoarticular persistent) or can extend to affect five or more joints after the first 6 months of disease (oligoarticular extended) provides an opportunity to study factors that are associated with disease extension.

IL-17+ T-cell numbers were found to be higher in patients with extended oligoarthritis as compared with patients with persistent oligoarthritis (Nistala et al., 2008). The ratio between SF regulatory and activated effector

CD4 cells was found to be higher in patients with persistent oligoarticular disease with respect to those in whom the disease extended to affect five or more joints (Ruprecht et al., 2005). Hunter et al. sampled patients with recent-onset oligoarticular JIA and looked at potential differences in synovial fluid cells between patients with persistent or extended disease. Synovial CCL5 levels were higher and SF CD4:CD8 ratio was lower in children whose disease extended to a more severe phenotype. Gene expression profiling revealed increased levels of genes associated with inflammation and macrophage differentiation in patients with extended disease and of genes associated with immune regulation in patients with persistent oligoarticular disease (Hunter et al., 2010). Finnegan et al. studied the immunohistochemical features of the synovial membrane in early untreated, newly diagnosed JIA in relation with disease outcome at 2 years. CD4 expression and B-cell infiltrates was significantly higher and vascularization more pronounced in patients in whom arthritis extended to involve more joints with respect to those with persistent oligoarthritis (Finnegan et al., 2011).

The fact that persistent oligoarticular JIA is self-limiting, and in about half of all cases even self-remitting, suggests an endogenous regulation. Heat shock proteins (HSPs) are endogenous proteins that are expressed upon cellular stress and are able to modulate immune responses. HSPs are highly present at sites of inflammation, such as the inflamed joints of JIA patients (Boog et al., 1992). Studies have showed in JIA the presence of antigen-specific T cells against peptides derived from two types of HSPs, HSP60, and DnaJ (Kamphuis et al., 2005; Massa et al., 2007). T-cell recognition of self-peptides or of peptides with a high degree of homology with self was associated with immune mechanisms with regulatory function, including the presence of T cells with the regulatory functional phenotype. These responses are significantly augmented in patients with persistent oligoarticular JIA, which again suggests a direct role in modulation of autoimmune inflammation. The identification of HSP tolerogenic peptides could have therapeutic implications in the future.

## TREATMENT

The management of JIA has improved greatly over the past decade with the introduction of biologic agents, tight control strategies, and early disease-modifying antirheumatic drug treatment. The implementation by the Food and Drug Administration (FDA) and the European Medicines Agency of the so-called pediatric rule has also played a pivotal role in the improvement of treatment and outcome. According to this rule, an industry that wishes to register a new treatment for a given disease in adults has to conduct studies also in children if there is a pediatric counterpart of the illness. Pediatric rheumatology was able to take a quick advantage from this rule thanks to the existence of two very large networks that have worked in a highly integrated fashion, the Pediatric Rheumatology Collaborative Study Group in North America and the Pediatric Rheumatology International Trial Organization in Europe and the rest of the world (Ruperto and Martini, 2004).

Since JIA is not a single disease, different therapeutic approaches should be considered according to the diverse disease categories. Nonetheless, general guiding principles may be adopted (Beukelman et al., 2011; Ringold et al., 2013). The widespread use of intraarticular steroid injections has played an important role in preventing deformities, for example, valgus knee, secondary to joint contractures. The long-acting steroid triamcinolone hexacetonide is used worldwide since its induced remission lasts much longer compared to other steroids. Patients who do not respond to nonsteroidal anti-inflammatory drugs (NSAIDs) and intraarticular steroid injections are treated with MTX whose maximally effective dose has been established in a randomized trial (Ruperto et al., 2004). Although a trial has shown the efficacy of leflunomide in polyarticular JIA (Silverman et al., 2005), the experience with this drug in children is still scarce. In patients who do not respond adequately to MTX, the efficacy and safety of several anti-TNF agents such as etanercept (the soluble TNF- $\alpha$  receptor), adalimumab, and golimumab (two fully humanized monoclonal antibodies to TNF- $\alpha$ ) have been demonstrated in randomized controlled trials (RCTs) in polyarticular course JIA (Lovell et al., 2000, 2008; Brunner et al., 2017). Adalimumab is the anti-TNF of choice in the case of JIA-associated uveitis (Ramanan et al., 2017). Abatacept, cytotoxic T-lymphocyte antigen 4 (CTLA-4) Ig, an inhibitor of costimulatory signals during antigen presentation, is an approved therapeutic option for patients with polyarthritis who are resistant to TNF- $\alpha$ -inhibitors (Ruperto et al., 2008). The efficacy and safety of the IL-6 receptor inhibitor (tocilizumab) for the treatment of patients with polyarticular-course JIA has been recently demonstrated in phase III controlled trial (Brunner et al., 2015).

sJIA patients are often recalcitrant to standard treatments. Recent insights into its pathophysiological mechanisms have however led to major changes in the management of this disease, especially with the development of biologic treatments. Corticosteroid use has substantially decreased; severe anemia, myocarditis, serositis,

and MAS are still considered to be absolute indications. The treatment of MAS relies on high-dose steroids and cyclosporine.

In sJIA patients who are steroid dependent anti-IL-6 and anti-IL-1 treatments, as mentioned above, have shown a dramatic efficacy. The 2013 update of the 2011 American College of Rheumatology (ACR) Recommendations outlines the indications for use of IL-1 and IL-6 inhibitors, including anakinra, canakinumab, and tocilizumab (Ringold et al., 2013).

The safety profile of biological agents appears to be good. The initial concern raised by the FDA that treatment with anti-TNF agents could be associated with an increased rate of malignancy (Diak et al., 2010) has not been confirmed by retrospective studies that have on the opposite suggested that JIA itself and not anti-TNF therapy is associated (as adult RA) with a small increased risk of neoplasia (Beukelman et al., 2012).

With the improvement in JIA care, remission has become an achievable goal for a large proportion of patients.

## References

- Allantaz, F., Chaussabel, D., Stichweh, D., Bennett, L., Allman, W., Mejias, A., et al., 2007. Blood leukocyte microarrays to diagnose systemic onset juvenile idiopathic arthritis and follow the response to IL-1 blockade. *J. Exp. Med.* 204, 2131–2144.
- Barnes, M.G., Grom, A.A., Thompson, S.D., Griffin, T.A., Pavlidis, P., Itert, L., et al., 2009. Subtype-specific peripheral blood gene expression profiles in recent-onset juvenile idiopathic arthritis. *Arthritis Rheum.* 60, 2102–2112.
- Barnes, M.G., Grom, A.A., Thompson, S.D., Griffin, T.A., Luyrink, L.K., Colbert, R.A., et al., 2010. Biologic similarities based on age at onset in oligoarticular and polyarticular subtypes of juvenile idiopathic arthritis. *Arthritis Rheum.* 62, 3249–3258.
- Behrens, E.M., Beukelman, T., Paessler, M., Cron, R.Q., 2007. Occult macrophage activation syndrome in patients with systemic juvenile idiopathic arthritis. *J. Rheumatol.* 34, 1133–1138.
- Behrens, E.M., Canna, S.W., Slade, K., Rao, S., Kreiger, P.A., Paessler, M., et al., 2011. Repeated TLR9 stimulation results in macrophage activation syndrome-like disease in mice. *J. Clin. Invest.* 121, 2264–2277.
- Beukelman, T., Patkar, N.M., Saag, K.G., Tolleson-Rinehart, S., Cron, R.Q., DeWitt, E.M., et al., 2011. American College of Rheumatology recommendations for the treatment of juvenile idiopathic arthritis: initiation and safety monitoring of therapeutic agents for the treatment of arthritis and systemic features. *Arthritis Care. Res. (Hoboken)* 2011 (63), 465–482.
- Beukelman, T., Haynes, K., Curtis, J.R., Xie, F., Chen, L., Bemrich-Stolz, C.J., et al., 2012. Rates of malignancy associated with juvenile idiopathic arthritis and its treatment. *Arthritis Rheum.* 64, 1263–1271.
- Billiau, A.D., Roskams, T., Van Damme-Lombaerts, R., Matthys, P., Wouters, C., 2005. Macrophage activation syndrome: characteristic findings on liver biopsy illustrating the key role of activated, IFN-gamma-producing lymphocytes and IL-6- and TNF-alpha-producing macrophages. *Blood* 105, 1648–1651.
- Boog, C.J., de Graeff-Meeder, E.R., Lucassen, M.A., van der Zee, R., Voorhorst-Ogink, M.M., van Kooten, P.J., et al., 1992. Two monoclonal antibodies generated against human hsp60 show reactivity with synovial membranes of patients with juvenile chronic arthritis. *J. Exp. Med.* 175, 1805–1810.
- Bracaglia, C., de Graaf, K., Pires Marafon, D., Guilhot, F., Ferlin, W., Prencipe, G., et al., 2017. Elevated circulating levels of interferon- $\gamma$  and interferon- $\gamma$ -induced chemokines characterise patients with macrophage activation syndrome complicating systemic juvenile idiopathic arthritis. *Ann. Rheum. Dis.* 76, 166–172.
- Brunner, H.I., Ruperto, N., Zuber, Z., Keane, C., Harari, O., Kenwright, A., et al., 2015. Efficacy and safety of tocilizumab in patients with polyarticular-course juvenile idiopathic arthritis: results from a phase 3, randomised, double-blind withdrawal trial. *Ann. Rheum. Dis.* 74, 1110–1117.
- Brunner, H.I., Ruperto, N., Tzaribachev, N., Horneff, G., Chasnyk, V.G., Panaviene, V., et al., 2017. Subcutaneous golimumab for children with active polyarticular-course juvenile idiopathic arthritis: results of a multicentre, double-blind, randomised-withdrawal trial. *Ann. Rheum. Dis.* Available from: <https://doi.org/10.1136/annrheumdis-2016-210456> [Epub ahead of print].
- Canna, S.W., de Jesus, A.A., Gouni, S., Brooks, S.R., Marrero, B., Liu, Y., et al., 2014. An activating NLRC4 inflammasome mutation causes autoinflammation with recurrent macrophage activation syndrome. *Nat. Genet.* 46, 1140–1146.
- Cazzola, M., Ponchio, L., de Benedetti, F., Ravelli, A., Rosti, V., Beguin, Y., et al., 1996. Defective iron supply for erythropoiesis and adequate endogenous erythropoietin production in the anemia associated with systemic-onset juvenile chronic arthritis. *Blood* 87, 4824–4830.
- Corcione, A., Ferlito, F., Gattorno, M., Gregorio, A., Pistorio, A., Gastaldi, R., et al., 2009. Phenotypic and functional characterization of switch memory B cells from patients with oligoarticular juvenile idiopathic arthritis. *Arthritis Res. Ther.* 11, R150.
- Cosmi, L., Cimaz, R., Maggi, L., Santarasci, V., Capone, M., Borriello, F., et al., 2011. Evidence of the transient nature of the Th17 phenotype of CD4 + CD161 + T cells in the synovial fluid of patients with juvenile idiopathic arthritis. *Arthritis Rheum.* 63, 2504–2515.
- Date, Y., Seki, N., Kamizono, S., Higuchi, T., Hirata, T., Miyata, K., et al., 1999. Identification of a genetic risk factor for systemic juvenile rheumatoid arthritis in the 5'-flanking region of the TNFalpha gene and HLA genes. *Arthritis Rheum.* 42, 2577–2582.
- De Benedetti, F., Martini, A., 1998. Is systemic juvenile rheumatoid arthritis an interleukin 6 mediated disease? *J. Rheumatol.* 25, 203–207.
- De Benedetti, F., Massa, M., Robbioni, P., Ravelli, A., Burgio, G.R., Martini, A., 1991. Correlation of serum interleukin-6 levels with joint involvement and thrombocytosis in systemic juvenile rheumatoid arthritis. *Arthritis Rheum.* 34, 1158–1163.
- De Benedetti, F., Massa, M., Pignatti, P., Albani, S., Novick, D., Martini, A., 1994. Serum soluble interleukin 6 (IL-6) receptor and IL-6/soluble IL-6 receptor complex in systemic juvenile rheumatoid arthritis. *J. Clin. Invest.* 93, 2114–2119.
- De Benedetti, F., Alonzi, T., Moretta, A., Lazzaro, D., Costa, P., Poli, V., et al., 1997a. Interleukin 6 causes growth impairment in transgenic mice through a decrease in insulin-like growth factor-I. A model for stunted growth in children with chronic inflammation. *J. Clin. Invest.* 99, 643–650.

- De Benedetti, F., Pignatti, P., Gerloni, V., Massa, M., Sartirana, P., Caporali, R., et al., 1997b. Differences in synovial fluid cytokine levels between juvenile and adult rheumatoid arthritis. *J. Rheumatol.* 24, 1403–1409.
- De Benedetti, F., Meazza, C., Oliveri, M., Pignatti, P., Vivarelli, M., Alonzi, T., et al., 2001. Effect of IL-6 on IGF binding protein-3: a study in IL-6 transgenic mice and in patients with systemic juvenile idiopathic arthritis. *Endocrinology* 142, 4818–4826.
- De Benedetti, F., Meazza, C., Vivarelli, M., Rossi, F., Pistorio, A., Lamb, R., et al., 2003. Functional and prognostic relevance of the -173 polymorphism of the macrophage migration inhibitory factor gene in systemic-onset juvenile idiopathic arthritis. *Arthritis Rheum.* 48, 1398–1407.
- De Benedetti, F., Brunner, H.I., Ruperto, N., Kenwright, A., Wright, S., Calvo, I., et al., 2012. Randomized trial of tocilizumab in systemic juvenile idiopathic arthritis. *N. Engl. J. Med.* 367, 2385–2395.
- De Benedetti, F., Brunner, H., Ruperto, N., Schneider, R., Xavier, R., Allen, R., et al., 2015. Catch-up growth during tocilizumab therapy for systemic juvenile idiopathic arthritis: results from a phase III trial. *Arthritis Rheumatol.* 67, 840–848.
- de Jager, W., Vastert, S.J., Beekman, J.M., Wulffraat, N.M., Kuis, W., Coffer, P.J., et al., 2009. Defective phosphorylation of interleukin-18 receptor beta causes impaired natural killer cell function in systemic-onset juvenile idiopathic arthritis. *Arthritis Rheum.* 60, 2782–2793.
- Diak, P., Siegel, J., La Grenade, L., Choi, L., Lemery, S., McMahon, A., et al., 2010. Tumor necrosis factor alpha blockers and malignancy in children: forty-eight cases reported to the Food and Drug Administration. *Arthritis Rheum.* 62, 2517–2524.
- Donn, R.P., Barrett, J.H., Farhan, A., Stopford, A., Pepper, L., Shelley, E., et al., 2001. Cytokine gene polymorphisms and susceptibility to juvenile idiopathic arthritis. British Paediatric Rheumatology Study Group. *Arthritis Rheum.* 44, 802–810.
- Fall, N., Barnes, M., Thornton, S., Luyrink, L., Olson, J., Ilowite, N.T., et al., 2007. Gene expression profiling of peripheral blood from patients with untreated new-onset systemic juvenile idiopathic arthritis reveals molecular heterogeneity that may predict macrophage activation syndrome. *Arthritis Rheum.* 56, 3793–3804.
- Fife, M.S., Gutierrez, A., Ogilvie, E.M., Stock, C.J., Samuel, J.M., Thomson, W., et al., 2006. Novel IL10 gene family associations with systemic juvenile idiopathic arthritis. *Arthritis Res. Ther.* 8, R148.
- Finnegan, S., Clarke, S., Gibson, D., McAllister, C., Rooney, M., 2011. Synovial membrane immunohistology in early untreated juvenile idiopathic arthritis: differences between clinical subgroups. *Ann. Rheum. Dis.* 70, 1842–1850.
- Fishman, D., Faulds, G., Jeffery, R., Mohamed-Ali, V., Yudkin, J.S., Humphries, S., et al., 1998. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J. Clin. Invest.* 102, 1369–1376.
- Frosch, M., Ahlmann, M., Vogl, T., Wittkowski, H., Wulffraat, N., Foell, D., et al., 2009. The myeloid-related proteins 8 and 14 complex, a novel ligand of Toll-like receptor 4, and interleukin-1beta form a positive feedback mechanism in systemic-onset juvenile idiopathic arthritis. *Arthritis Rheum.* 60, 883–891.
- Gattorno, M., Piccini, A., Lasigliè, D., Tassi, S., Brisca, G., Carta, S., et al., 2008. The pattern of response to anti-interleukin-1 treatment distinguishes two subsets of patients with systemic-onset juvenile idiopathic arthritis. *Arthritis Rheum.* 58, 1505–1515.
- Gregorio, A., Gambini, C., Gerloni, V., Parafioriti, A., Sormani, M.P., Gregorio, S., et al., 2007. Lymphoid neogenesis in juvenile idiopathic arthritis correlates with ANA positivity and plasma cells infiltration. *Rheumatology (Oxford)* 46, 308–313.
- Haufe, S., Haug, M., Schepp, C., Kuemmerle-Deschner, J., Hansmann, S., Rieber, N., et al., 2011. Impaired suppression of synovial fluid CD4 + CD25+ T cells from patients with juvenile idiopathic arthritis by CD4 + CD25+ Treg cells. *Arthritis Rheum.* 63, 3153–3162.
- Hinks, A., Barton, A., Shephard, N., Eyre, S., Bowes, J., Cargill, M., et al., 2009. Identification of a novel susceptibility locus for juvenile idiopathic arthritis by genome-wide association analysis. *Arthritis Rheum.* 60, 258–263.
- Hinks, A., Cobb, J., Marion, M.C., Prahalad, S., Sudman, M., Bowes, J., et al., 2013. Dense genotyping of immune-related disease regions identifies 14 new susceptibility loci for juvenile idiopathic arthritis. *Nat. Genet.* 45, 664–669.
- Hollenbach, J.A., Thompson, S.D., Bugawan, T.L., Ryan, M., Sudman, M., Marion, M., et al., 2010. Juvenile idiopathic arthritis and HLA class I and class II interactions and age-at-onset effects. *Arthritis Rheum.* 62, 1781–1791.
- Holzinger, D., Frosch, M., Kastrup, A., Prince, F.H., Otten, M.H., Van Suijlekom-Smit, L.W., et al., 2012. The Toll-like receptor 4 agonist MRP8/14 protein complex is a sensitive indicator for disease activity and predicts relapses in systemic-onset juvenile idiopathic arthritis. *Ann. Rheum. Dis.* 71, 974–980.
- Hunter, P.J., Nistala, K., Jina, N., Eddaoudi, A., Thomson, W., Hubank, M., et al., 2010. Biologic predictors of extension of oligoarticular juvenile idiopathic arthritis as determined from synovial fluid cellular composition and gene expression. *Arthritis Rheum.* 62, 896–907.
- Jamilloux, Y., Gerfaud-Ventin, M., Martinon, F., et al., 2015. Pathogenesis of adult onset Still's disease: new insights from the juvenile counterpart. *Immunol. Res.* 61, 53–62.
- Janka, G.E., 2012. Familial and acquired hemophagocytic lymphohistiocytosis. *Annu. Rev. Med.* 63, 233–246.
- Kamphuis, S., Kuis, W., de Jager, W., Teklenburg, G., Massa, M., Gordon, G., et al., 2005. Tolerogenic immune responses to novel T-cell epitopes from heat-shock protein 60 in juvenile idiopathic arthritis. *Lancet* 366, 50–56.
- Kaufman, K.M., Linghu, B., Szustakowski, J.D., Husami, A., Yang, F., Zhang, K., et al., 2014. Whole exome sequencing reveals overlap between macrophage activation syndrome in systemic juvenile idiopathic arthritis and familial hemophagocytic lymphohistiocytosis. *Arthritis Rheum.* 66, 3486–3495.
- Kawashima, M., Yamamura, M., Taniai, M., Yamauchi, H., Tanimoto, T., Kurimoto, M., et al., 2001. Levels of interleukin-18 and its binding inhibitors in the blood circulation of patients with adult-onset Still's disease. *Arthritis Rheum.* 44, 550–560.
- Lamb, R., Thomson, W., British Society of Paediatric and Adolescent Rheumatology, Ogilvie, E.M., Donn, R., 2007. Positive association of SLC26A2 gene polymorphisms with susceptibility to systemic-onset juvenile idiopathic arthritis. *Arthritis Rheum.* 56, 1286–1291.
- Lovell, D.J., Giannini, E.H., Reiff, A., Cawkwell, G.D., Silverman, E.D., Nocton, J.J., et al., 2000. Etanercept in children with polyarticular juvenile rheumatoid arthritis. Pediatric Rheumatology Collaborative Study Group. *N. Engl. J. Med.* 342, 763–769.
- Lovell, D.J., Ruperto, N., Goodman, S., Reiff, A., Jung, L., Jarosova, K., et al., 2008. Adalimumab with or without methotrexate in juvenile rheumatoid arthritis. *N. Engl. J. Med.* 359, 810–820.

- Maeno, N., Takei, S., Nomura, Y., Imanaka, H., Hokonohara, M., Miyata, K., 2002. Highly elevated serum levels of interleukin-18 in systemic juvenile idiopathic arthritis but not in other juvenile idiopathic arthritis subtypes or in Kawasaki disease: comment on the article by Kawashima et al. *Arthritis Rheum.* 46, 2539–2541.
- Maeno, N., Takei, S., Imanaka, H., Yamamoto, K., Kuriwaki, K., Kawano, Y., et al., 2004. Increased interleukin-18 expression in bone marrow of a patient with systemic juvenile idiopathic arthritis and unrecognized macrophage-activation syndrome. *Arthritis Rheum.* 50, 1935–1938.
- Martini, A., 2003. Are the number of joints involved or the presence of psoriasis still useful tools to identify homogeneous disease entities in juvenile idiopathic arthritis? *J. Rheumatol.* 30, 1900–1903.
- Martini, A., 2012a. It is time to rethink juvenile idiopathic arthritis classification and nomenclature. *Ann. Rheum. Dis.* 71, 1437–1439.
- Martini, A., 2012b. Systemic juvenile idiopathic arthritis. *Autoimmun. Rev.* 12, 56–59.
- Martini, A., Ravelli, A., Di Fuccia, G., Rosti, V., Cazzola, M., Barosi, G., 1994. Intravenous iron therapy for severe anaemia in systemic-onset juvenile chronic arthritis. *Lancet* 344, 1052–1054.
- Massa, M., Mazzoli, F., Pignatti, P., De Benedetti, F., Passalia, M., Viola, S., et al., 2002. Proinflammatory responses to self HLA epitopes are triggered by molecular mimicry to Epstein-Barr virus proteins in oligoarticular juvenile idiopathic arthritis. *Arthritis Rheum.* 46, 2721–2729.
- Massa, M., Passalia, M., Manzoni, S.M., Campanelli, R., Ciardelli, L., Yung, G.P., et al., 2007. Differential recognition of heat-shock protein dnaj-derived epitopes by effector and Treg cells leads to modulation of inflammation in juvenile idiopathic arthritis. *Arthritis Rheum.* 56, 1648–1657.
- Mellins, E.D., Macaubas, C., Grom, A.A., 2011. Pathogenesis of systemic juvenile idiopathic arthritis: some answers, more questions. *Nat. Rev. Rheumatol.* 7, 416–426.
- Möller, J.C., Paul, D., Ganser, G., Range, U., Gahr, M., Kelsch, R., et al., 2010. L10 promoter polymorphisms are associated with systemic onset juvenile idiopathic arthritis (SoJIA). *Clin. Exp. Rheumatol.* 28, 912–918.
- Morbach, H., Wiegering, V., Richl, P., Schwarz, T., Suffa, N., Eichhorn, E.M., et al., 2011. Activated memory B cells may function as antigen-presenting cells in the joints of children with juvenile idiopathic arthritis. *Arthritis Rheum.* 63, 3458–3466.
- Nemeth, E., Rivera, S., Gabayan, V., Keller, C., Taudorf, S., Pedersen, B.K., et al., 2004. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *J. Clin. Invest.* 113, 1271–1276.
- Nigrovic, P.A., Mannion, M., Prince, F.H., Zeft, A., Rabinovich, C.E., van Rossum, M.A., et al., 2011. Anakinra as first-line disease-modifying therapy in systemic juvenile idiopathic arthritis: report of forty-six patients from an international multicenter series. *Arthritis Rheum.* 63, 545–555.
- Nirmala, N., Brachat, A., Feist, E., Blank, N., Specker, C., Witt, M., et al., 2015. Gene-expression analysis of adult-onset Still's disease and systemic juvenile idiopathic arthritis is consistent with a continuum of a single disease entity. *Pediatr. Rheumatol. Online J.* 13, 50.
- Nistala, K., Moncrieffe, H., Newton, K.R., Varsani, H., Hunter, P., Wedderburn, L.R., 2008. Interleukin-17-producing T cells are enriched in the joints of children with arthritis, but have a reciprocal relationship to regulatory T cell numbers. *Arthritis Rheum.* 58, 875–887.
- Nistala, K., Adams, S., Cambrook, H., Ursu, S., Olivito, B., de Jager, W., et al., 2010. Th17 plasticity in human autoimmune arthritis is driven by the inflammatory environment. *Proc. Natl. Acad. Sci. U.S.A.* 107, 14751–14756.
- Ogilvie, E.M., Fife, M.S., Thompson, S.D., Twine, N., Tsoras, M., Morolodo, M., et al., 2003. The -174G allele of the interleukin-6 gene confers susceptibility to systemic arthritis in children: a multicenter study using simplex and multiplex juvenile idiopathic arthritis families. *Arthritis Rheum.* 48, 3202–3206.
- Ogilvie, E.M., Khan, A., Hubank, M., Kellam, P., Woo, P., 2007. Specific gene expression profiles in systemic juvenile idiopathic arthritis. *Arthritis Rheum.* 56, 1954–1965.
- Ombrello, M.J., Remmers, E.F., Tachmazidou, I., Grom, A., Foell, D., Haas, J.P., et al., 2015. HLADRB1\* 11 and variants of the MHC class II locus are strong risk factors for systemic juvenile idiopathic arthritis. *Proc. Natl. Acad. Sci. U.S.A.* 112, 15970–15975.
- Ombrello, M.J., Arthur, V.L., Remmers, E.F., Hinks, A., Tachmazidou, I., Grom, A.A., et al., 2017. Genetic architecture distinguishes systemic juvenile idiopathic arthritis from other forms of juvenile idiopathic arthritis: clinical and therapeutic implications. *Ann. Rheum. Dis.* 76, 906–913.
- Pascual, V., Allantaz, F., Arce, E., Punaro, M., Banchereau, J., 2005. Role of interleukin-1 (IL-1) in the pathogenesis of systemic onset juvenile idiopathic arthritis and clinical response to IL-1 blockade. *J. Exp. Med.* 201, 1479–1486.
- Petty, R.E., Southwood, T.R., Manners, P., Baum, J., Glass, D.N., Goldenberg, J., et al., 2004. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. *J. Rheumatol.* 31, 390–392.
- Prahad, S., Glass, D.N., 2008. A comprehensive review of the genetics of juvenile idiopathic arthritis. *Pediatr. Rheumatol. Online J.* 21, 6–11.
- Prakken, B., Albani, S., Martini, A., 2011. Juvenile idiopathic arthritis. *Lancet* 377, 2138–2149.
- Put, K., Vandenhoute, J., Avau, A., van Nieuwenhuijze, A., Brisse, E., Dierckx, T., et al., 2017. Inflammatory gene expression profile and defective interferon- $\gamma$  and granzyme K in natural killer cells from systemic juvenile idiopathic arthritis patients. *Arthritis Rheumatol.* 69, 213–224.
- Quartier, P., Allantaz, F., Cimaz, R., Pillet, P., Messiaen, C., Bardin, C., et al., 2011. A multicentre, randomised, double-blind, placebo-controlled trial with the interleukin-1 receptor antagonist anakinra in patients with systemic-onset juvenile idiopathic arthritis (ANAJIS trial). *Ann. Rheum. Dis.* 70, 747–754.
- Ramanan, A.V., Dick, A.D., Jones, A.P., McKay, A., Williamson, P.R., Compeyrot-Lacassagne, S., et al., 2017. Adalimumab plus methotrexate for uveitis in juvenile idiopathic arthritis. *N. Engl. J. Med.* 376, 1637–1646.
- Ravelli, A., Martini, A., 2007. Juvenile idiopathic arthritis. *Lancet* 369, 767–778.
- Ravelli, A., Felici, E., Magni-Manzoni, S., Pistorio, A., Novarini, C., Bozzola, E., et al., 2005. Patients with antinuclear antibody-positive juvenile idiopathic arthritis constitute a homogeneous subgroup irrespective of the course of joint disease. *Arthritis Rheum.* 52, 826–832.
- Ravelli, A., Varnier, G.C., Oliveira, S., Castell, E., Arguedas, O., Magnani, A., et al., 2011. Antinuclear antibody-positive patients should be grouped as a separate category in the classification of juvenile idiopathic arthritis. *Arthritis Rheum.* 63, 267–275.

- Ravelli, A., Grom, A.A., Behrens, E.M., Cron, R.Q., 2012. Macrophage activation syndrome as part of systemic juvenile idiopathic arthritis: diagnosis, genetics, pathophysiology and treatment. *Genes. Immun.* 13, 289–298.
- Ravelli, A., Minoia, F., Davì, S., Horne, A., Bovis, F., Pistorio, A., et al., 2016. 2016 Classification criteria for macrophage activation syndrome complicating systemic juvenile idiopathic arthritis. *Arthritis Rheumatol.* 68, 566–576.
- Ringold, S., Weiss, P.F., Beukelman, T., Dewitt, E.M., Ilowite, N.T., Kimura, Y., et al., 2013. 2013 update of the 2011 American College of Rheumatology recommendations for the treatment of juvenile idiopathic arthritis: recommendations for the medical therapy of children with systemic juvenile idiopathic arthritis and tuberculosis screening among children receiving biologic medications. *Arthritis Care Res. (Hoboken)* 65, 1551–1563.
- Romberg, N., Al Mousawi, K., Nelson-Williams, C., Stiegler, A.L., Loring, E., Choi, M., et al., 2014. Mutation of NLRC4 causes a syndrome of enterocolitis and autoinflammation. *Nat. Genet.* 46, 1135–1139.
- Ruperto, N., Martini, A., 2004. International research networks in pediatric rheumatology: the PRINTO perspective. *Curr. Opin. Rheumatol.* 16, 566–570.
- Ruperto, N., Murray, K.J., Gerloni, V., Wulffraat, N., de Oliveira, S.K., Falcini, F., et al., 2004. A randomized trial of parenteral methotrexate comparing an intermediate dose with a higher dose in children with juvenile idiopathic arthritis who failed to respond to standard doses of methotrexate. *Arthritis Rheum.* 50, 2191–2201.
- Ruperto, N., Lovell, D.J., Quartier, P., Paz, E., Rubio-Pérez, N., Silva, C.A., et al., 2008. Abatacept in children with juvenile idiopathic arthritis: a randomised, double-blind, placebo-controlled withdrawal trial. *Lancet* 372, 383–391.
- Ruperto, N., Quartier, P., Wulffraat, N., Woo, P., Ravelli, A., Mouy, R., et al., 2012a. A phase II, multicenter, open-label study evaluating dosing and preliminary safety and efficacy of canakinumab in systemic juvenile idiopathic arthritis with active systemic features. *Arthritis Rheum.* 64, 557–567.
- Ruperto, N., Brunner, H.I., Quartier, P., Constantin, T., Wulffraat, N., Horneff, G., et al., 2012b. Two randomized trials of canakinumab in systemic juvenile idiopathic arthritis. *N. Engl. J. Med.* 367, 2396–2406.
- Ruprecht, C.R., Gattorno, M., Ferlito, F., Gregorio, A., Martini, A., Lanzavecchia, A., et al., 2005. Coexpression of CD25 and CD27 identifies FoxP3+ regulatory T cells in inflamed synovia. *J. Exp. Med.* 201, 1793–1803.
- Sen, E.S., Dick, A.D., Ramanan, A.V., 2015. Uveitis associated with juvenile idiopathic arthritis. *Nat. Rev. Rheumatol.* 1, 338–348.
- Shimizu, M., Nakagishi, Yachie, A., 2013. Distinct subsets of patients with systemic juvenile idiopathic arthritis based on their cytokine profiles. *Cytokine* 61, 345–348.
- Silverman, E., Mouy, R., Spiegel, L., Jung, L.K., Saurenmann, R.K., Lahdenne, P., et al., 2005. Leflunomide or methotrexate for juvenile rheumatoid arthritis. *N. Engl. J. Med.* 352, 1655–1666.
- Stock, C.J., Ogilvie, E.M., Samuel, J.M., Fife, M., Lewis, C.M., Woo, P., 2008. Comprehensive association study of genetic variants in the IL-1 gene family in systemic juvenile idiopathic arthritis. *Genes. Immun.* 9, 349–357.
- Stoll, M.L., Zurakowski, D., Nigrovic, L.E., Nichols, D.P., Sundel, R.P., Nigrovic, P.A., 2006. Patients with juvenile psoriatic arthritis comprise two distinct populations. *Arthritis Rheum.* 54, 3564–3572.
- Stoll, M.L., Lio, P., Sundel, R.P., Nigrovic, P.A., 2008. Comparison of Vancouver and International League of Associations for rheumatology classification criteria for juvenile psoriatic arthritis. *Arthritis Rheum.* 59, 51–58.
- Strippoli, R., Carvello, F., Scianaro, R., De Pasquale, L., Vivarelli, M., Petrini, S., et al., 2012. Amplification of the response to Toll-like receptor ligands by prolonged exposure to interleukin-6 in mice: implication for the pathogenesis of macrophage activation syndrome. *Arthritis Rheum.* 64, 1680–1688.
- Sugiura, T., Kawaguchi, Y., Harigai, M., Terajima-Ichida, H., Kitamura, Y., Furuya, T., et al., 2002. Association between adult-onset Still's disease and interleukin-18 gene polymorphisms. *Genes. Immun.* 3, 394–399.
- Verbsky, J.W., White, A.J., 2004. Effective use of the recombinant interleukin 1 receptor antagonist anakinra in therapy resistant systemic onset juvenile rheumatoid arthritis. *J. Rheumatol.* 31, 2071–2075.
- Wakil, S.M., Monies, D.M., Abouelhoda, M., Al-Tassan, N., Al-Dusery, H., Naim, E.A., et al., 2015. Association of a mutation in LACC1 with a monogenic form of systemic juvenile idiopathic arthritis. *Arthritis Rheum.* 67, 288–295.
- Wehrens, E.J., Mijnheer, G., Duurland, C.L., Klein, M., Meerding, J., van Loosdregt, J., et al., 2011. Functional human regulatory T cells fail to control autoimmune inflammation due to PKB/c-akt hyperactivation in effector cells. *Blood* 118, 3538–3548.
- Yokota, S., Imagawa, T., Mori, M., Miyamae, T., Aihara, Y., Takei, S., et al., 2008. Efficacy and safety of tocilizumab in patients with systemic-onset juvenile idiopathic arthritis: a randomised, double-blind, placebo-controlled, withdrawal phase III trial. *Lancet* 371, 998–1006.

## 36

# Spondyloarthritides

*Uta Syrbe and Joachim Sieper*

Department of Gastroenterology, Infectious Diseases and Rheumatology, Charité - University Medicine Berlin,  
Berlin, Germany

## OUTLINE

General Introduction	691	The Role of HLA-B27 in the Pathogenesis of Spondyloarthritis	695
Historical Aspects	691	The Role of Non-MHC Genes in Spondyloarthritis	696
Epidemiology	692	Animal Models With Possible Relevance for the Pathogenesis of Spondyloarthritis	697
Clinical Features and Disease Associations	692	Treatment	698
Pathological Features	693	References	699
Autoimmune Features	693	Further Reading	701
Genetics	695		

## GENERAL INTRODUCTION

The spondyloarthritis (SpA) diseases comprise axial spondyloarthritis (axial SpA) including ankylosing spondylitis (AS), reactive arthritis (ReA), arthritis/spondylitis with inflammatory bowel disease (IBD), and arthritis/spondylitis with psoriasis. The main links between each of these are the associations with HLA-B27, similar clinical symptoms, such as inflammatory back pain, and similar patterns of peripheral joint involvement with an asymmetric arthritis predominantly of the lower limbs, and the possible occurrence of sacroiliitis, spondylitis, enthesitis, and uveitis. Most striking is the direct relationship between the prevalence of SpA and the prevalence of HLA-B27 in the general population. This strong correlation suggests that the environmental or genetic factors that are necessary in addition to HLA-B27 to get SpA must be ubiquitous (Sieper and Poddubnyy, 2017).

## HISTORICAL ASPECTS

The first most clear-cut descriptions of AS date back to the end of the 19th century. At that time, the disease was described independently by three people—in Russia by Vladimir Bechterew in 1893 (Bechterew, 1893), in Germany by Adolf von Strümpell in 1897 (Strümpell, 1897), and in 1898 by Pierre Marie in France (Marie, 1898). Therefore, *Spondylitis ankylosans* is sometimes referred to as *Morbus Bechterew* or *Bechterew–Strümpell–Marie disease*. With the availability of radiography, the involvement of the sacroiliac joints was noted in the 1930s

([Buckley, 1931](#)). In 1973, the association with HLA-B27 was described ([Brewerton et al., 1973](#)) that opened a new area of research on the pathology of the disease.

## EPIDEMIOLOGY

Axial SpA covers both nonradiographic (nr) axial SpA and AS. AS is characterized by the presence of structural changes in the bone on X-rays and is regarded as the SpA with the most severe outcome. Its prevalence has been estimated to be between 0.2% and 0.9% and the disease normally starts in the second decade of life. The male-to-female ratio has more recently been estimated to be around 2:1. HLA-B27 is found to be positive in 90%–95% of the patients, and inflammatory bowel disease, psoriasis, or preceding ReA can be found in about 10% of the AS patients.

## CLINICAL FEATURES AND DISEASE ASSOCIATIONS

Back pain is the leading clinical symptom in patients with axial SpA. Such back pain is characterized by morning stiffness and subsides by exercise. This disease starts in 90% or more cases with sacroiliitis. Further, in the course of the disease, the whole spine can be affected with spondylitis, spondylodiscitis, and arthritis of the small intervertebral joints. As a reaction to the inflammation, ankylosis occurs, which can involve the whole spine. Relapsing uveitis, peripheral asymmetric arthritis predominantly of the lower limbs, and enthesitis are the most frequent extraspinal manifestations. Diagnosis is made by a combination of clinical symptoms (such as inflammatory back pain or limitation of spinal mobility), laboratory findings, such as HLA-B27 and CRP, and positive imaging. Sacroiliitis on MRI (active inflammation) or on X-rays (structural damage) are crucial for the classification and diagnosis of axial SpA ([Rudwaleit et al., 2009a,b](#)). A new approach to an earlier diagnosis is also mandatory because of the major delay of 5–7 years between the occurrence of the first symptoms and conducting of the diagnosis.

ReA occurs after a preceding infection of the urogenital tract with *Chlamydia trachomatis* or of the gut with enterobacteria, such as *Yersinia*, *Salmonella*, *Campylobacter jejuni*, or *Shigella*, usually after a few days up to 4–6 weeks ([Sieper et al., 2000](#)). Between 30% and 60% of the patients with ReA are positive for HLA-B27; arthritis occurs in approximately 4% of the general population, but in about 25% of the HLA-B27 + carriers, after one of these infections. Arthritis is normally oligoarthritis, predominantly of the lower limbs, but in about 20%, it will manifest as polyarthritis. Other manifestations can be enthesitis, conjunctivitis/uveitis, or inflammatory back pain. Usually, the patients recover within 3–6 months; however, up to 20% can have chronic recurrence of longer than 12 months. Bacterial antigen and, in case of *Chlamydia*, DNA and RNA have also been detected in the joint, indicating that bacterial antigens persist in the joint and drive the local immune response. For conducting a diagnosis of ReA, a combination of the clinical manifestations (preceding infection, typical pattern of arthritis) and laboratory evidences of the previous or present bacterial infections is necessary ([Sieper et al., 2002](#)). While antibiotic treatment of urogenital tract infection with antibiotics prevents the occurrence of arthritis, this is not the case for bacterial enteritis. Once arthritis is established, long-term antimicrobial monotherapy does not seem to influence such arthritis ([Sieper et al., 2000](#)). However, as have been recently shown, improvement of chronic *Chlamydia*-induced arthritis can be achieved after prolonged, 6-month combined antimicrobial therapy, suggesting that antibiotic monotherapy is not effective in the eradication of the ReA-inducing microbes ([Carter et al., 2010](#)).

Arthritis with IBD often occurs concurrently with gut inflammation in approximately 10%–20% of the patients with IBD. Arthritis is often a case of transient peripheral arthritis of the lower limbs. About 5% of IBD patients, mostly those who are HLA-B27 + will develop AS. The frequency of HLA-B27 among patients with peripheral arthritis is only slightly higher but is present among 50%–70% of the patients with IBD and AS. Treatment should primarily be directed against the gut inflammation.

Up to 50% of the patients with psoriatic arthritis show a clinical picture compatible with SpA, such as oligoarthritis of the lower limbs and/or spinal inflammation. Among these patients, HLA-B27 is positive in approximately 25% (peripheral arthritis) to 60% (spinal manifestations). Treatment is similar to that for the other forms of SpA.

## PATHOLOGICAL FEATURES

Studies using MRI have shown that the most relevant inflammatory site in SpA is osteitis occurring at the bone/cartilage interface (McGonagle et al., 1999). Osteitis is detected in short-tau inversion recovery sequence and indicates a change in the homeostasis of the bone marrow. Osteitis in the sacrum and ilium adjacent to the sacroiliac joints is a primary finding in axial SpA (Rudwaleit et al., 2009a,b). Long-term disease and symptom occurrence duration may lead to structural changes in the subchondral bone, including erosions, joint space narrowing, and ankylosis, which can be detected by X-ray. Within the spine, osteitis can be detected mainly at the corners of the vertebral bodies, where at later points of time, syndesmophyte development is observed (Baraliakos et al., 2014).

Histological studies in AS are limited due to the difficulties of acquiring joint material from axial joints. The most comprehensive histological studies were performed by Cruickshank (1951, 1956). He investigated joints from autopsies of AS patients, such as hip joints, sacroiliac joints, and intervertebral joints and reported synovial proliferation, fibrosis of synovial tissue, and foci of metaplasia to cartilage and bone. Moreover, cartilage destruction and presence of a fibroblast-rich granulation tissue were commonly found. By comparing histology to radiographs, he found that the radiographic joint space narrowing was correlated with cartilaginous degeneration. The presence of the radiographic erosions correlated with the presence of the fibrous tissue within the marrow space. More recent histological analysis of biopsy specimen taken from the sacroiliac joints described that a proliferative connective tissue and inflammatory cells were present in patients with active sacroiliitis and short disease duration, whereas calcification and ossification were more often found in long-standing disease (Bollow et al., 2000). Comprehensive analysis of the facet joints, which are also often affected in AS, described the sequential features of the joint remodeling in SpA (Bleil et al., 2014). The transformation of the bone marrow into a fibrous tissue was found to precede the cartilaginous fusion of the joints. The presence of osteoclasts, which erode the subchondral bone, and the cartilage from the bone marrow site seem instrumental in the destruction of the joints. Osteoblasts are located as well at the edges of this invasive tissue that synthesize a collagen type I matrix, which is subsequently mineralized, leading to the intraarticular bony bridges promoting ankylosis of the joints (Bleil et al., 2016).

It suggests that the pathological immune reaction starts at the bone marrow sites, predominately at the regions where the ligaments attach, suggesting that the physiological–biomechanical stress contributes to the local inflammation.

## AUTOIMMUNE FEATURES

Exposure of the immune system to bacteria rather than the presence of one distinctive autoantigen seems to be important for triggering SpA. The best evidence for this comes from ReA, which is triggered usually by a genito-urinary infection with *C. trachomatis* or enteritis due to certain gram-negative enterobacteria, such as *Shigella*, *Salmonella*, *Yersinia*, or *Campylobacter*. The demonstration of microbial antigens within the synovium suggests that ReA may be related to the persistence of microbial antigens at the sites of inflammatory arthritis (Granfors et al., 1989). Approximately 20%–40% of the HLA-B27+ ReA patients develop the full clinical manifestations of AS after 10–20 years (Leirisalo-Repo, 1998). Although clinically diagnosed ReA arthritis precedes AS only in less than 10% of the occurrences, this percentage may be much higher because many of the gut or urogenital infections preceding the clinical manifestation of ReA can be asymptomatic.

The ReA-associated bacteria are obligate (such as *Chlamydia*) or facultative intracellular bacteria. T-helper 1 (Th1) cytokines, such as tumor necrosis factor alpha (TNF $\alpha$ ) and interferon gamma (IFN $\gamma$ ), are crucial for the effective elimination of these bacteria, while Th2 cytokines, such as interleukin (IL)-4, or antiinflammatory cytokines, such as IL-10, might inhibit effective elimination. Relative deficiency of Th1-cytokines was found in ReA patients, not only of TNF $\alpha$  but also of IFN $\gamma$ , both locally in synovial fluid and synovial membrane and systemically in peripheral blood (Yin et al., 1997a; Braun et al., 1999). The low TNF $\alpha$  production in peripheral blood is correlated with the longer duration of arthritic symptoms (Braun et al., 1999). Thus a relative lack of Th1-cytokines appears to be relevant for the occurrence and persistence of ReA. IL-10 was found relatively upregulated in ReA, which may contribute to bacterial persistence in ReA, possibly by down-regulation of the Th1-cytokines, IFN $\gamma$  and TNF $\alpha$  (Yin et al., 1997b). The persistence of bacterial antigens being disseminated to the joints might cause aberrant inflammation at these sites, which are also exposed to mechanical stress.

Alternatively, the persistence of antigens might cause the pathological priming of T cells cross-reacting to the autologous antigens.

The central role of bacteria in the pathogenesis of SpA is further supported by the relationship between IBD and SpA. Arthritis with IBD occurs in approximately 10%–20% of the patients with IBD. Among the AS patients, about 7% have concomitant IBD (Stolwijk et al., 2013). The presence of IBD and HLA-B27 positivity is associated with a high prevalence of AS in particular, as suggested by a study of Crohn's disease patients: in 13 out of 24 (54%) HLA-B27 + patients with Crohn's disease, AS was also diagnosed, but only in 5 out of 189 (2.6%) patients who were HLA-B27 –, AS was not diagnosed (Purmann et al., 1988). In IBD, leakage in the gut mucosa is observed as a consequence of the related inflammation, which presumably allows an interaction of the immune system with the normal gut bacteria. In addition to the high prevalence of overt IBD in AS, also in about 50% of the patients with axial SpA, macroscopic or microscopic mucosal inflammation, some of which resembling Crohn's disease, can be detected in the gut by colonoscopy (Mielants et al., 1988; Van Praet et al., 2013). This indicates that gut inflammation is a rather common feature of SpA suggesting a link between the gut and its microbiota and pathogenesis of axial SpA. This is also supported by findings from animal SpA models. HLA-B27 transgenic rats, representing the most relevant SpA model, develop gut inflammation, peripheral arthritis, and psoriasisiform skin and nail changes. The importance of environmental factors is emphasized by the observation that many of these features, including gut inflammation and arthritis, do not develop in HLA-B27 transgenic rats born and bred in a germ-free environment. Germ-free animals rapidly develop inflammatory disease on removal from the sterile environment. This can be partially prevented by treatment with antibiotics (Taurog et al., 1999).

Bacteria appear to play a crucial role as an initial event for the pathogenesis of AS; however, there is no clear evidence that they are also directly responsible for the immunopathology in AS. While bacterial antigens are found in the joints of ReA patients, no such antigen has been found so far in sacroiliac joints (Braun et al., 1997). However, considering that commensal bacteria from the gut comprise a great variety of different microorganisms, it is particularly difficult to detect distinct bacteria. As first evidence, in AS with peripheral arthritis, enhanced reactivity toward *Escherichia coli* proteins, a common mucosal bacterium, has been reported (Syrbe et al., 2012).

If such antigens persist in joints or if AS occurs as the result of bacteria-induced autoimmunity is a matter of debate. Surprisingly, given the strong association with the MHC class I molecule HLA-B27, it was in the HLA-B27 transgenic rat model that the disease induction is independent of the presence of CD8 + T cells but requires CD4 + T cells (Taurog et al., 2009). Whether these cells are activated by bacterial antigens or by autoantigens remains unclear; however, the Aggrecan G1 domain has been suggested as one source of a possible CD4 + T-cell autoantigen in AS and similar rheumatic diseases, based both on the results of animal models and from the studies conducted on patients (Zou et al., 2003). Thus in 60% of the patients, a CD4 + response against the whole G1 protein and against a set of overlapping peptides derived from this G1 protein was detectable (Zou et al., 2003). However, clinical trials suggest that classical disease modifying drugs, such as methotrexate and abatacept, targeting T-cell stimulation, are ineffective in AS. In contrast, genetic studies and studies on cytokine inhibition suggest a strong impact of the cytokines TNF $\alpha$ , IL-23, and IL-17 in AS. Thus treatment of AS patients with TNF $\alpha$  inhibitors is highly effective in improving the signs and symptoms of the disease (Braun and Sieper, 2004). Furthermore, anti-IL-17 antibody treatment has been approved for the treatment of the axial SpA patients and an anti-p40 mab (targeting the shared p40 subunit of the IL-12/IL-23 receptor) has shown some efficacy in a small clinical study of AS patients. In contrast, IL-6 blockade had no effect on axial SpA while it is effective in rheumatoid arthritis.

The cellular sources and triggers of pathogenic cytokine production are poorly defined in AS. In fact, in the blood of AS patients CD4 + and CD8 + T cells producing TNF $\alpha$  (and IFN $\gamma$ ) after mitogenic stimulation are even less abundant than in HLA-B27 – controls (Rudwaleit et al., 2001). However, in a small study where sacroiliac biopsies were acquired, abundant TNF $\alpha$  was found in the sacroiliac joints from AS patients (Braun et al., 1995).

Analyzing IL-17A expression in AS, no difference was found in the percentage of Th17 cells in the peripheral blood compared to controls while in the subchondral bone marrow of facet joints, acquired from patients with AS, an increased percentage of IL-17A + cells was found (Appel et al., 2011). Those IL-17A + cells were mostly of myeloid origin. Also, in inflamed peripheral joints of SpA patients, mononuclear and polymorphonuclear, synovial cell infiltrates were found to express IL-17 (Moran et al., 2011). This suggests that rather innate immune cells might contribute to pathogenic IL-17 production in AS than adaptive immune cells.

IL-23 is induced during the unfolded protein response triggered by protein misfolding. Stimulation of innate cells, such as macrophages with pharmacological inducers of the unfolded protein response, results in the enhanced LPS-elicited production of the inflammatory cytokines, in particular IFN $\beta$  and IL-23 (Smith et al.,

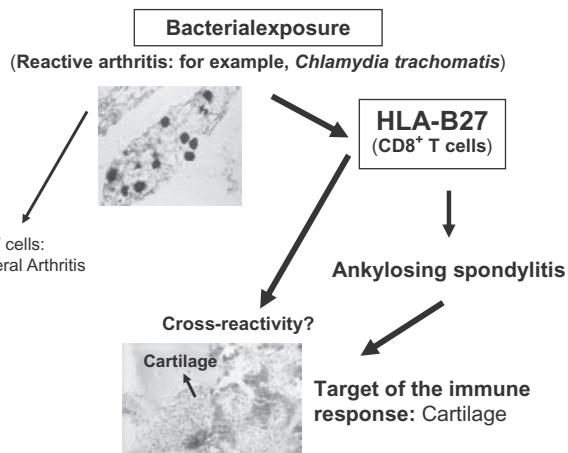
2008). A recent study shows that peripheral blood-derived macrophages from AS patients produced strikingly higher levels of IL-23 in response to the stimulation with lipopolysaccharide, even in the absence of unfolded protein response (UPR) induction (Zeng et al., 2011). Furthermore, IL-23 + cells were also found to have increased within bone marrow cells, again mostly among cells of myeloid origin, in facet joints from AS patients (Appel et al., 2013).

## GENETICS

### The Role of HLA-B27 in the Pathogenesis of Spondyloarthritis

The susceptibility to AS has been estimated to be more than 90% genetically determined, and so it has been suggested that there is a rather ubiquitous environmental factor. The most relevant genetic risk factor is HLA-B27 (Brewerton et al., 1973; Schlosstein et al., 1973). The association of HLA-B27 with SpA is the highest known MHC association for human diseases and the most relevant single factor for the pathogenesis of SpA. There are now considerable data from epidemiologic studies and transgenic animals to indicate a direct effect of HLA-B27, rather than that of a closely linked gene, in disease pathogenesis. It is also clear now that one copy of HLA-B27 (heterozygosity) is sufficient for increasing the risk of disease development. Besides HLA-B27, other MHC genes, such as HLA-B60 and HLA-DR1, seem to be associated, but are of minor importance. However, although MHC is the major susceptibility locus, it has been suggested that it contributes only approximately 36% to the overall genetic risk (Brown et al., 2002).

Although the association between HLA-B27 and SpA has been known for more than 40 years, the mechanism by which HLA-B27 confers susceptibility to SpA is still unknown. Three hypotheses are discussed that refer to the distinct properties of the HLA-B27 molecule. The arthritogenic peptide hypothesis suggests a link between the SpA induction and the main function of HLA class I molecules to present peptide antigens to cytotoxic T cells. Thus some HLA-B27 subtypes, due to their unique amino-acid residues, can bind distinct sets of peptides that are presented to CD8<sup>+</sup> T cells. In response to such exogenous peptides, T cells recognizing autoantigens with sufficient structural similarity might become activated and react toward self-peptides that are present particularly in spinal joints (Fig. 36.1). Major support for this hypothesis comes from studies in humans showing the differential association of some of the HLA-B27 subtypes with AS. While B\*2705, B\*2702, B\*2704, and B\*2707 are strongly associated with the disease, the HLA-B27 subtypes B\*2709 in Whites and B\*2706 in Southeast Asians are not at all or only rarely associated. Most interestingly, B\*2709 differs from the disease associated B\*2705 by only one amino-acid substitution, the exchange of Asp116 to His116. B\*2706 differs by only two amino-acid substitutions from the disease associated B\*2704 by exchange of His114 to Asp114 and Asp116 to Tyr116 (Khan, 2000).



**FIGURE 36.1** Hypothesis of how bacterial exposure induces a peripheral arthritis, probably via a CD4<sup>+</sup> T-cell response. However, axial manifestations might be mediated by CD8<sup>+</sup> T cells because of the high association with HLA-B27. The cartilage might become the primary target of the immune response through cross-reactivity with bacterial antigens.

As the arthritogenic peptide hypothesis suggests that a common antigen might bind to the disease associated HLA-B27 subtype, a recent study has searched for shared sequences and motifs in CD8+ T cells in HLA-B27-positive AS patients using next-generation sequencing (Faham et al., 2017). In this study, several T-cell receptor (TCR) beta motifs were identified that were enriched in B27+ AS patients as compared to B27+ healthy controls. Moreover, both bacteria-specific and autoreactive CD8+ T cells have been demonstrated in AS and ReA. For instance, in patients with *Yersinia*-induced ReA, a synovial CD8+ T-cell response to a peptide from *Yersinia* heat shock protein 60 was found and in patients with *Chlamydia*-induced ReA, an HLA-B27-restricted CD8+ T-cell response to peptides was derived from several chlamydial proteins (Kuon and Sieper, 2003). In AS patients, a CD8+ T-cell response to an Epstein–Barr virus epitope derived from the LMP2 protein was found, with cross-reactivity to a sequence-related self-peptide from the autoantigen vasoactive intestinal peptide receptor 1 (Fiorillo et al., 2000). However, the exact identity of a potentially arthritogenic peptide is yet to be determined. An oligoclonal expansion of T cells has also been demonstrated for CD4+ and CD8+ T cells in AS and for CD8+ T cells in ReA. Synovial T cells derived from different HLA-B27+ patients suffering from ReA and triggered by different bacteria revealed a high homology of the TCRs (May et al., 2002).

Besides the “classical” arthritogenic peptide theory, other hypotheses have emerged. One such concept, the HLA-B27 misfolding and UPR hypothesis, states that HLA-B27 itself is directly involved in the pathologic process of SpA. Thus HLA-B27 has a tendency for misfolding in the endoplasmatic reticulum, which might have implications for pathogenesis (Colbert, 2004). It is suggested that the misfolding is a particular feature of the HLA-B27 molecule: e.g., newly synthesized HLA-B\*2705 seems to fold and associate with β2-microglobulin more slowly compared with other MHC class I molecules. Moreover, Mear et al. (1999) observed that HLA-B\*2705 in cell lines showed a fraction of heavy chains undergoing degradation within the endoplasmic reticulum. These render the cells susceptible to the unfolded protein response, which is associated with the upregulation of IL-23, the main stabilizing factor for IL-17-producing T cells. The IL-23/IL-17 axis is intriguing because IL-23 receptor polymorphisms have been shown to be associated with SpA, psoriasis, and Crohn’s disease (Wellcome Trust Case-Control Consortium and Australo-Anglo-American Spondyloarthritis Consortium, 2007). Hence, the HLA-B27 misfolding and unfolded protein response hypothesis postulates a role for CD4+ T and NK cells in the pathogenesis of AS and other SpA. This hypothesis is also supported by the fact that disease development and severity is unimpaired in HLA-B27 transgenic rats that lack functional CD8+ T cells but require CD4+ T cells (Taurog et al., 2009). Similar results have been observed in the cell-transfer and depletion experiments (Breban et al., 1996; May et al., 2003) which challenge the arthritogenic peptide hypothesis.

A third hypothesis is related to the formation of noncanonical forms of HLA class I molecules on the surface of cells. Allen et al. (1999) reported that as a consequence of HLA-B27 misfolding, free HLA-B27 heavy chains can form abnormal heavy-chain homodimers. This homodimer formation could be facilitated by unpaired free cysteine residues at position 67 (Cys67) of the HLA-B27 heavy chain, α1 helix. Using fluorescence tagged tetramers of HLA-B27\*05 homodimers, Kollnberger et al. (2002) showed that they bind to cell surfaces of the NK inhibitory receptors, KIR3DL1, and KIR3DL2, and to leukocyte immunoglobulin-like receptor (LILR) B2, which is expressed on dendritic cells, monocytes, and macrophages. In HLA-B27+ SpA patients, KIR3DL2+ NK cells and CD4+ T cells are expanded in peripheral blood, compared with HLA-B27– SpA, rheumatoid arthritis, and other control patients and NK cells of these patients show higher levels of cytotoxicity and CD4+ T cells produce IL-17 (Chan et al., 2005). It is suggested that the binding of the HLA-B27 heavy-chain homodimers to KIR and LILR could promote inflammation by enhancing survival of the NK and T cells and by influencing the differentiation of LILR-expressing antigen-presenting cells (Kollnberger and Bowness, 2009).

## The Role of Non-MHC Genes in Spondyloarthritis

Only a small proportion of HLA-B27 carriers develop AS (1%–5% in most series), which cannot be explained by HLA-B27 subtypes. As we know from independent twin studies that the heritability of the susceptibility to AS is more than 90% (Brown et al., 1997; Petersen et al., 2006), it is likely that other genes contribute to the susceptibility. Genome-wide association studies performed in the last few years have identified definite associations of AS with the non-MHC genes ERAP1 and IL-23R, and with the gene deserts 2p15 and 21q22 [Wellcome Trust Case-Control Consortium and Australo-Anglo-American Spondyloarthritis Consortium, 2007; Australo-Anglo-American Spondyloarthritis Consortium (TASC), 2010; Australo-Anglo-American Spondyloarthritis Consortium (TASC) and Wellcome Trust Case Control Consortium 2 (WTCCC2), 2011]. Furthermore, a strong evidence to

support the association with AS has been demonstrated for the genes *IL-1R2*, *ANTXR2*, *TNFSF15*, *TNFR1*, and *STAT3* and a region on chromosome 16q including the gene *TRADD* (Wellcome Trust Case-Control Consortium and Australo-Anglo-American Spondyloarthritis Consortium, 2007; TASC, 2010; TASC and WTCCC2, 2011; Pointon et al., 2010; Zinovieva et al., 2009; Danoy et al., 2009).

The *ERAP1* gene encodes the enzyme endoplasmic reticulum aminopeptidase 1, which is involved in trimming of the peptides in the endoplasmic reticulum prior to HLA class I presentation and it may be involved in the trimming of cytokine receptors as well. The ERAP1 polymorphisms only affect the AS risk in HLA-B27 + individuals (Wellcome Trust Case-Control Consortium and Australo-Anglo-American Spondyloarthritis Consortium, 2007; TASC, 2010; TASC and WTCCC2, 2011) and the majority of evidences suggest that ERAP1 variants reducing aminopeptidase activity are associated with protection from AS (Tran and Colbert, 2015). This may suggest that HLA-B27 operates through a mechanism involving aberrant processing of antigenic peptides; however, it was also shown that ERAP1 polymorphisms may also modulate accumulation of HLA-B27 homodimers on the cell surface (Chen et al., 2015).

The protective association of the nonsynonymous single nucleotide polymorphism (Arg381Gln; rs11209026) in the *IL-23R* gene with AS was confirmed in several studies and *IL-23R* rs11209026 was also found to be associated with IBD and psoriasis (Wellcome Trust Case-Control Consortium and Australo-Anglo-American Spondyloarthritis Consortium, 2007; TASC, 2010). IL-23 signaling plays an important role in sustaining Th17 cell responses in vivo. The 381Q (Arg381Gln) variant results in reduced phosphorylation of STAT3, a downstream signaling molecule of the *IL-23R*, in response to IL-23 in T cells (Pidasheva et al., 2011). Also, STAT3 itself was found to be associated with Crohn's disease and also has suggestive association with AS (Barrett et al., 2008; Danoy et al., 2009). Thus the genetic association of *IL-23R* and *STAT3* with AS definitively suggests impact of the IL-23–Th17 axis in the pathogenesis of AS.

The association of the gene deserts 2p15 and 21q22 with AS was identified and confirmed by the TASC (2010) genome-wide association study. Gene deserts are lengthy regions without known genes. Therefore, these regions might contain regulatory elements, yet unidentified genes, or regions which are involved in epigenetic gene regulation.

Apart from these confirmed genetic associations, several other genes, such as *ANTXR2*, *IL-1R2*, *TNFSF15*, *TNFR1*, and *TRADD*, show suggestive association with AS (TASC, 2010; Pointon et al., 2010).

In earlier studies, two promoter polymorphisms of the *TNF $\alpha$*  gene at positions -308 (308.1 and 308.2) and -238 (238.1 and 238.2) have been investigated in AS. The 308.2 genotype was found significantly less frequently in AS than in controls. In some studies, the 308.2 genotype was associated with higher transcriptional activity (Hoehler et al., 1998). Thus there is some evidence that *TNF $\alpha$*  genotypes that may be associated with a low *TNF $\alpha$*  production are present in a higher percentage in patients with ReA or AS.

Also in earlier studies, which investigated the association of *TNF $\alpha$*  microsatellites, an association of ReA with a *TNF $\alpha$ 6*-allele has been described; this allele has previously been associated with a low *TNF $\alpha$*  secretion. Since, in this study from Finland, *TNF $\alpha$ 6* was also associated with HLA-B27, the association of *TNF $\alpha$ 6* with ReA was thought to be secondary to B27.

In another study analyzing *IL-10* gene polymorphism in ReA, a significant decrease in the promoter alleles G12 and G10 was found in the ReA group compared with HLA-B27 + controls, indicating that these alleles might have a protective effect against the occurrence of ReA (Kaluza et al., 2001). Although it is not yet clear whether these alleles are associated with a higher production of *IL-10*, these data suggest that the relative increase of *IL-10* found in ReA might be, at least partially, genetically determined.

## ANIMAL MODELS WITH POSSIBLE RELEVANCE FOR THE PATHOGENESIS OF SPONDYLOARTHRITIS

The most important animal model of SpA is certainly the HLA-B27 transgenic rat model (Taurog et al., 2009). In the early 1990s, several lines of HLA-B27/human  $\beta$ 2-microglobulin transgenic rats were established. The rats developed a spontaneous inflammatory disease, resembling several features of the human disease, such as peripheral arthritis, spondylitis, colitis, psoriasisiform skin and nail disease. Interestingly, the copy number of both transgenes has impact on the development of the disease. Low copy number of HLA-B27 and high transgenic expression of the human  $\beta$ 2-microglobulin resulted in a phenotype most reminiscent of AS characterized by

extensive spondylitis, with formation of syndesmophytes, sacroiliitis and peripheral arthritis, and dactylitis (Tran et al., 2006). Housing of HLA-B27 transgenic rats in germ-free isolators indicated that the regular microbiota from the gut is required for the disease induction (Taurog et al., 2009). Cell-transfer and depletion experiments clarified that the expression of the transgene on immune cells, that is, bone marrow derived hematopoietic cells rather than on mesenchymal cells matters for disease development (Taurog et al., 2009). Moreover, CD8+ T cells are not required for disease development as shown by genetic deletion of CD8+ T cells (Taurog et al., 2009). A recent study has analyzed the histopathological changes at inflamed axial sites (van Duivenvoorde et al., 2012). The authors found that spondylitis in B27/h $\beta$ (2) transgenic rats is characterized by inflammation in the connective tissue adjacent to the junction of the annulus fibrosus and vertebral bone but not by inflammation at the enthesis or by osteitis. Osteitis was found only at joints with severe inflammation where osteoclasts eroded the bone outside the cartilage end plate. Osteoproliferation was present in sections with moderate to severe inflammation and occurred at the border of inflammation, at a distance from bone destruction, which is different to what is observed in human SpA, in which osteophytes grow from the corner of the vertebral body.

Several other animal models reproduce some of the characteristic features of SpA in the absence of human HLA-B27, as recently reviewed (Vieira-Sousa et al., 2015). For instance, aging male DBA/1 mice develop spontaneous dactylitis and enthesial inflammation followed by endochondral bone formation. Manipulating the expression of cytokines, whose putative impact in AS is supported by genetic association of the gene itself or its receptors, has shed some light into the impact of these cytokines in SpA. Human TNF-transgenic mice develop a destructive polyarticular polyarthritis and sacroiliitis but lack spinal involvement. TNF $^{\Delta\text{ARE}}$  carry a 69-bp deletion of the TNF AU-rich elements (ARE), which leads to a steady-state increase in murine TNF mRNA and thereby to an overexpression of the murine TNF protein. TNF $^{\Delta\text{ARE}}$  mice also develop a spontaneous chronic peripheral arthritis as well as signs of sacroiliitis. Additionally, these mice also develop bowel inflammation, which precedes the development of joint inflammation. A third model exploring the effect of TNF is the tmTNF-transgenic mouse model. In this model, mice carry a mutant TNF gene where the cleavage site for TNF-converting enzyme-like protease is deleted, thus preventing enzymatic cleavage and generation of the soluble form of TNF. In these mice, arthritis is less severe than in the other TNF-overexpressing models, but mice also develop spondylitis. Moreover, tmTNF-transgenic mice do not show systemic inflammation.

IL-23 has attained attention through the identification of several susceptibility loci in the IL-23/IL-17 pathway associated with AS (Wellcome Trust Case-Control Consortium and Australo-Anglo-American Spondyloarthritis Consortium, 2007; TASC, 2010). In a model using hydrodynamic injection of minicircle DNA constructs encoding IL23p19, which leads to high and sustained overexpression of IL-23, Sherlock et al. (2012) reported severe paw swelling as early as 5 days after IL-23mc injection. Swelling was rather related to enthesial inflammation than to synovitis and retinoic acid receptor related orphan nuclear receptor  $\gamma$ T (ROR $\gamma$ T) + CD3 + CD4 – CD8 – enthesial resident lymphocytes were identified as the IL-23-responsive cell type.

The Curdlan-induced SKG mouse model displays features of RA as well SpA. In SKG mice, carrying the ZAP-70 W163C mutation, signaling downstream of the TCR is affected. These mice develop spontaneous autoimmune arthritis characterized by rheumatoid factor and anti-type II collagen. When these mice are immunized with the fungal component Curdlan, these develop dactylitis, deformities of the tail, and a hunched back apart from arthritis (Vieira-Sousa et al., 2015). Furthermore, mice developed ileitis, unilateral uveitis, and some dermal inflammation.

In summary, there are several valuable animal models for studying pathogenesis of SpA. However, the exact features, in particular of axial disease, are difficult to model. Upright walking of man certainly generates a distinctive biomechanical loading that might contribute to the specific occurrence of osteitis, which most of the models lack.

## TREATMENT

In the last few decades, only nonsteroidal antiinflammatory drugs (NSAIDs) were used, together with physiotherapy, as an effective mode of treatment. Rather surprisingly, disease modifying antirheumatic drugs and corticosteroids, which are highly effective in other chronic inflammatory diseases, such as rheumatoid arthritis, show only an effect in patients with predominant peripheral arthritis. On this background, the finding that the blockers of TNF are highly effective, both for patients with nr-axial SpA and AS (Sieper and Poddubnyy, 2016) means a breakthrough in the treatment of this disease (Braun and Sieper, 2004; van der Heijde et al., 2005; Inman et al., 2008). At least 50% of the active AS patients refractory to treatment with NSAIDs show a 50% or more

improvement when treated either with the monoclonal anti-TNF $\alpha$  antibody infliximab (Braun et al., 2003; van der Heijde et al., 2005) or the soluble TNF-receptor construct etanercept (Davis et al., 2003). Most recently, a similar level of response was also reported for the anti-IL-17 inhibitor secukinumab in patients with AS (Baeten et al., 2015). The relative role of the TNF-blockers and IL-17 inhibitors in the treatment of axial SpA is yet to be determined in the near future. Furthermore, whether other targeted therapies, for example, JAK inhibitors that had been proven to be effective in other chronic inflammatory diseases, are also effective in axial SpA, has to be investigated further.

## References

- Allen, R.L., O'Callaghan, C.A., McMichael, A.J., Bowness, P., 1999. Cutting edge: HLA-B27 can form a novel beta 2-microglobulin-free heavy chain homodimer structure. *J. Immunol.* 162, 5045–5048.
- Appel, H., Maier, R., Wu, P., Scheer, R., Hempfing, A., Kayser, R., et al., 2011. Analysis of IL-17(+) cells in facet joints of patients with spondyloarthritis suggests that the innate immune pathway might be of greater relevance than the Th17-mediated adaptive immune response. *Arthritis Res. Ther.* 13 (3), R95.
- Appel, H., Maier, R., Bleil, J., Hempfing, A., Loddenkemper, C., Schlichting, U., et al., 2013. In situ analysis of interleukin-23- and interleukin-12-positive cells in the spine of patients with ankylosing spondylitis. *Arthritis Rheum.* 65 (6), 1522–1529. Available from: <https://doi.org/10.1002/art.37937>.
- Australo-Anglo-American Spondyloarthritis Consortium (TASC), 2010. Genome-wide association study of ankylosing spondylitis identifies non-MHC susceptibility loci. *Nat. Genet.* 42, 123–127.
- Australo-Anglo-American Spondyloarthritis Consortium (TASC), Wellcome Trust Case Control Consortium 2 (WTCCC2), 2011. Interaction between ERAP1 and HLA-B27 in ankylosing spondylitis implicates peptide handling in the mechanism for HLA-B27 in disease susceptibility. *Nat. Genet.* 43, 761–767.
- Baeten, D., Sieper, J., Braun, J., Baraliakos, X., Dougados, M., Emery, P., et al., 2015. Secukinumab, an interleukin-17A inhibitor, in ankylosing spondylitis. *N. Engl. J. Med.* 373, 2534–2548.
- Baraliakos, X., Heldmann, F., Callhoff, J., Listing, J., Appelboom, T., Brandt, J., et al., 2014. Which spinal lesions are associated with new bone formation in patients with ankylosing spondylitis treated with anti-TNF agents? A long-term observational study using MRI and conventional radiography. *Ann. Rheum. Dis.* 73 (10), 1819–1825.
- Barrett, J.C., Hansoul, S., Nicolae, D.L., et al., 2008. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat. Genet.* 40, 955–962.
- Bechterew, W., 1893. Steifigkeit der Wirbelsaule und ihre Verkrümmung als besondere Erkrankungsform. *Neurol Centralbl.* Band 12, S.426–S.434.
- Bleil, J., Maier, R., Hempfing, A., Schlichting, U., Appel, H., Sieper, J., et al., 2014. Histomorphological and histomorphometric characteristics of zygapophyseal joint remodelling in ankylosing spondylitis. *Arthritis Rheumatol.* 66 (7), 1745–1754.
- Bleil, J., Maier, R., Hempfing, A., Sieper, J., Appel, H., Syrbe, U., 2016. Granulation tissue eroding the subchondral bone also promotes new bone formation in ankylosing spondylitis. *Arthritis Rheumatol.* 68 (10), 2456–2465.
- Bollow, M., Fischer, T., Reisshauer, H., Backhaus, M., Sieper, J., Hamm, B., et al., 2000. Quantitative analyses of sacroiliac biopsies in spondyloarthropathies: T cells and macro phages predominate in early and active sacroiliitis—cellularity correlates with the degree of enhancement detected by magnetic resonance imaging. *Ann. Rheum. Dis.* 59, 135–140.
- Braun, J., Sieper, J., 2004. Biological therapies in the spondyloarthritides—the current state. *Rheumatology (Oxford)* 43, 1072–1084.
- Braun, J., Bollow, M., Neure, L., Seipelt, E., Seyrekbasan, F., Herbst, H., et al., 1995. Use of immunohistologic and in-situ hybridization techniques in the examination of sacroiliac joint biopsy specimens from patients with ankylosing spondylitis. *Arthritis Rheum.* 38, 499–505.
- Braun, J., Tuszewski, M., Ehlers, S., Haberle, J., Bollow, M., Eggens, U., et al., 1997. Nested polymerase chain reaction strategy simultaneously targeting DNA sequences of multiple bacterial species in inflammatory joint diseases. II. Examination of sacroiliac and knee joint biopsies of patients with spondyloarthropathies and other arthritides. *J. Rheumatol.* 24 (6), 1101–1105.
- Braun, J., Yin, Z.N., Spiller, I., Siegert, S., Rudwaleit, M., Liu, L.Z., et al., 1999. Low secretion of tumor necrosis factor alpha, but no other Th1 or Th2 cytokines, by peripheral blood mononuclear cells correlates with chronicity in reactive arthritis. *Arthritis Rheum.* 42, 2039–2044.
- Braun, J., Brandt, J., Listing, J., Rudwaleit, M., Sieper, J., 2003. Biologic therapies in the spondyloarthritis: new opportunities, new challenges. *Curr. Opin. Rheumatol.* 15, 394–407.
- Breban, M., Fernandez-Sueiro, J.L., Richardson, J.A., Hadavand, R.R., Maika, S.D., Hammer, R.E., et al., 1996. T cells, but not thymic exposure to HLA-B27, are required for the inflammatory disease of HLA-B27 transgenic rats. *J. Immunol.* 156, 794–803.
- Brewerton, D.A., Hart, F.D., Nicholls, A., et al., 1973. Ankylosing spondylitis and HLA-B27. *Lancet* 1, 904–907.
- Brown, M.A., Kennedy, L.G., MacGregor, A.J., et al., 1997. Susceptibility to ankylosing spondylitis in twins: the role of genes, HLA, and the environment. *Arthritis Rheum.* 40, 1823–1828.
- Brown, M.A., Wordsworth, B.P., Reveille, J.D., 2002. Genetics of ankylosing spondylitis. *Clin. Exp. Rheumatol.* 20, S43–S49.
- Buckley, C.W., 1931. Spondylitis deformans. *BMJ i*, 1108–1112.
- Carter, J.D., Espinoza, L.R., Inman, R.D., Sneed, K.B., Ricca, L.R., Vasey, F.B., et al., 2010. Combination antibiotics as a treatment for chronic *Chlamydia*-induced reactive arthritis: a double-blind, placebo-controlled, prospective trial. *Arthritis Rheum.* 62 (5), 1298–1307.
- Chan, A.T., Kollnberger, S.D., Wedderburn, L.R., Bowness, P., 2005. Expansion and enhanced survival of natural killer cells expressing the killer immunoglobulin-like receptor KIR3DL2 in spondyloarthritis. *Arthritis Rheum.* 52, 3586–3595.
- Chen, L., Ridley, A., Hammitzsch, A., Al-Mossawi, M.H., Bunting, H., Georgiadis, D., et al., 2015. Silencing or inhibition of endoplasmic reticulum aminopeptidase 1 (ERAP1) suppresses free heavy chain expression and Th17 responses in ankylosing spondylitis. *Ann. Rheum. Dis.* 75 (5), 916–923.

- Colbert, R.A., 2004. The immunobiology of HLA-B27: variations on a theme. *Curr. Mol. Med.* 4, 21–30.
- Cruickshank, B., 1951. Histopathology of diarthrodial joints in ankylosing spondylitis. *Ann. Rheum. Dis.* 10 (4), 393–404.
- Cruickshank, B., 1956. Lesions of cartilaginous joints in ankylosing spondylitis. *J. Pathol. Bacteriol.* 71 (1), 73–84.
- Danoy, P., Pryce, K., Hadler, J., et al., 2009. Evidence for genetic overlap between ankylosing spondylitis and Crohn's disease. *Arthritis Rheum.* 60 (10, Suppl), S249.
- Davis Jr, J.C., Van Der Heijde, D., Braun, J., Dougados, M., Cush, J., Clegg, D.O., et al., 2003. Recombinant human tumor necrosis factor receptor (etanercept) for treating ankylosing spondylitis: a randomized, controlled trial. *Arthritis Rheum.* 48, 3230–3236.
- van Duivenvoorde, L.M., Dorris, M.L., Satumtira, N., van Tok, M.N., Redlich, K., Tak, P.P., et al., 2012. Relationship between inflammation, bone destruction, and osteoproliferation in the HLA-B27/human  $\beta$ 2-microglobulin-transgenic rat model of spondylarthritis. *Arthritis Rheum.* 64 (10), 3210–3219.
- Faham, M., Carlton, V., Moorhead, M., Zheng, J., Klinger, M., Pepin, F., et al., 2017. Discovery of T cell receptor  $\beta$  motifs specific to HLA-B27-positive ankylosing spondylitis by deep repertoire sequence analysis. *Arthritis Rheumatol.* 69 (4), 774–784.
- Fiorillo, M.T., Maragno, M., Butler, R., Dupuis, M.L., Sorrentino, R., 2000. CD8 + T-cell autoreactivity to an HLA-B27-restricted self-epitope correlates with ankylosing spondylitis. *J. Clin. Invest.* 106, 47–53.
- Granfors, K., Jalkanen, S., von Essen, R., Lahesmaa-Rantala, R., Isomaki, O., Pekkola-Heino, K., et al., 1989. Yersinia antigens in synovial-fluid cells from patients with reactive arthritis. *N. Engl. J. Med.* 319, 216–221.
- van der Heijde, D., Dijkmans, B., Geusens, P., Sieper, J., Dewoody, K., Williamson, P., et al., 2005. Efficacy and safety of infliximab in patients with ankylosing spondylitis: results of a randomized, placebo-controlled trial (ASSERT). *Arthritis Rheum.* 52, 582–591.
- Hoehler, T., Schaper, T., Schneider, P.M., zum Buschenfelde, K.H.M., Marker-Hermann, E., 1998. Association of different tumor necrosis factor alpha promoter allele frequencies with ankylosing spondylitis in HLA-B27 positive individuals. *Arthritis Rheum.* 41, 1489–1492.
- Inman, R.D., Davis Jr, J.C., Heijde, D., Diekman, L., Sieper, J., Kim, S.I., et al., 2008. Efficacy and safety of golimumab in patients with ankylosing spondylitis: results of a randomized, double-blind, placebo-controlled, phase III trial. *Arthritis Rheum.* 58 (11), 3402–3412.
- Kaluza, W., Leirisalo-Repo, M., Marker-Hermann, E., Westman, P., Reuss, E., Hug, R., et al., 2001. IL10.G microsatellites mark promoter haplotypes associated with protection against the development of reactive arthritis in Finnish patients. *Arthritis Rheum.* 44, 1209–1214.
- Khan, M.A., 2000. Update: the twenty subtypes of HLA-B27. *Curr. Opin. Rheumatol.* 12, 235–238.
- Kollnberger, S., Bowness, P., 2009. The role of B27 heavy chain dimmer immune receptor interactions in spondyloarthritis. *Adv. Exp. Med.* 649, 277–285.
- Kollnberger, S., Bird, L., Sun, M.Y., Retiere, C., Braud, V.M., McMichael, A., et al., 2002. Cell-surface expression and immune receptor recognition of HLA-B27 homodimers. *Arthritis Rheum.* 46, 2972–2982.
- Kuon, W., Sieper, J., 2003. Identification of HLA-B27-restricted peptides in reactive arthritis and other spondyloarthropathies: computer algorithms and fluorescent activated cell sorting analysis as tools for hunting of HLA-B27-restricted chlamydial and autologous crossreactive peptides involved in reactive arthritis and ankylosing spondylitis. *Rheum. Dis. Clin. N. Am.* 29, 595–611.
- Leirisalo-Repo, M., 1998. Prognosis, course of disease, and treatment of the spondyloarthropathies. *Rheum. Dis. Clin. N. Am.* 24, 737.
- Marie, P., 1898. Sur la spondylose rhizomélique. *Rev. Med.* 18, 285–315.
- May, E., Dulphy, N., Frauendorf, E., Duchmann, R., Bowness, P., de Castro, J.A.L., et al., 2002. Conserved TCR beta chain usage in reactive arthritis: evidence for selection by a putative HLA-B27-associated autoantigen. *Tissue Antigens* 60, 299–308.
- May, E., Dorris, M.L., Satumtira, N., Iqbal, I., Rehman, M.I., Lightfoot, E., et al., 2003. CD8 alpha beta T cells are not essential to the pathogenesis of arthritis or colitis in HLA-B27 transgenic rats. *J. Immunol.* 170, 1099–1105.
- McGonagle, D., Gibbon, W., O'Connor, P., Green, M., Pease, C., Ridgway, J., et al., 1999. An anatomical explanation for good-prognosis rheumatoid arthritis. *Lancet* 353, 123–124.
- Mear, J.P., Schreiber, K.L., Munz, C., Zhu, X., Stevanovic, S., Rammensee, H.G., et al., 1999. Misfolding of HLA-B27 as a result of its B pocket suggests a novel mechanism for its role in susceptibility to spondyloarthropathies. *J. Immunol.* 163, 6665–6670.
- Mielants, H., Veys, E.M., Cuvelier, C., Devos, M., 1988. Ileocolonoscopic findings in seronegative spondylarthropathies. *Br. J. Rheumatol.* 27, 95–105.
- Moran, E.M., Heydrich, R., Ng, C.T., Saber, T.P., McCormick, J., Sieper, J., et al., 2011. IL-17A expression is localised to both mononuclear and polymorphonuclear synovial cell infiltrates. *PLoS One* 6 (8), e24048.
- Petersen, O., Svendsen, A., Ejstrup, L., et al., 2006. Heritability estimates on ankylosing spondylitis. *Clin. Exp. Rheumatol.* 24, 463.
- Pidasheva, S., et al., 2011. Functional studies on the IBD susceptibility gene IL23R implicate reduced receptor function in the protective genetic variant R381Q. *PLoS One* 6, e25038.
- Pointon, J.J., Harvey, D., Karaderi, T., et al., 2010. The chromosome 16q region associated with ankylosing spondylitis includes the candidate gene TRADD (TNF receptor type 1-associated death domain). *Ann. Rheum. Dis.* 69 (6), 1243–1246.
- Purmann, J., Zeidler, H., Bertrams, J., Juli, E., Cleveland, S., Berges, W., et al., 1988. Hla antigens in ankylosing-spondylitis associated with Crohns-disease—increased frequency of the Hla phenotype B27, B44. *J. Rheumatol.* 15, 1658–1661.
- Rudwaleit, M., Siegert, S., Yin, Z., Eick, J., Thiel, A., Radbruch, A., et al., 2001. Low T cell production of TNF alpha and IFN gamma in ankylosing spondylitis: its relation to HLA-B27 and influence of the TNF-308 gene polymorphism. *Ann. Rheum. Dis.* 60, 36–42.
- Rudwaleit, M., van der Heijde, D., Landewé, R., Listing, J., Akkoc, N., Brandt, J., et al., 2009a. The development of Assessment of SpondyloArthritis International Society classification criteria for axial spondyloarthritis (part II): validation and final selection. *Ann. Rheum. Dis.* 68, 777–783.
- Rudwaleit, M., Jurik, A.G., Hermann, K.G., Landewe, R., van der Heijde, D., Baraliakos, X., et al., 2009b. Defining active sacroiliitis on magnetic resonance imaging (MRI) for classification of axial spondyloarthritis: a consensual approach by the ASAS/OMERACT MRI group. *Ann. Rheum. Dis.* 68 (10), 1520–1527.
- Schlosstein, L., Teasaki, P.I., Bluestone, R., et al., 1973. High association of HL-A antigen, W27, with ankylosing spondylitis. *N. Engl. J. Med.* 288, 704–706.
- Sherlock, J.P., Joyce-Shaikh, B., Turner, S.P., Chao, C.C., Sathe, M., Grein, J., et al., 2012. IL-23 induces spondyloarthropathy by acting on ROR- $\gamma$ t + CD3 + CD4 – CD8 – enthesal resident T cells. *Nat Med.* 18 (7), 1069–1076.

- Sieper, J., Poddubnyy, D., 2016. New evidence on the management of spondyloarthritis. *Nat. Rev. Rheumatol.* 12, 282–295.
- Sieper, J., Poddubnyy, D., 2017. Axial spondyloarthritis. *Lancet* 390, 73–84.
- Sieper, J., Braun, J., Kingsley, G.H., 2000. Report on the fourth international workshop on reactive arthritis. *Arthritis Rheum.* 43, 720–734.
- Sieper, J., Rudwaleit, M., Braun, J., van der Heijde, D., 2002. Diagnosing reactive arthritis—role of clinical setting in the value of serologic and microbiologic assays. *Arthritis Rheum.* 46, 319–327.
- Smith, J.A., Turner, M.J., DeLay, M.L., Klenk, E.I., Sowders, D.P., Colbert, R.A., 2008. Endoplasmatic reticulum stress and the unfolded protein response are linked to synergistic IFN- $\beta$  induction via X-box binding protein 1. *Eur. J. Immunol.* 38, 1194–1203.
- Stolwijk, C., van Tubergen, A., Castillo-Ortiz, J.D., Boonen, A., 2013. Prevalence of extra-articular manifestations in patients with ankylosing spondylitis: a systematic review and meta-analysis. *Ann. Rheum. Dis.* 74 (1), 65–73.
- Strümpell, A., 1897. Bemerkung über die chronische ankylosierende Entzündung der Wirbelsäule und der Huftgelenke. *Dtsch Z Nervenheilkd.* 11, 338–342.
- Syrbe, U., Scheer, R., Wu, P., Sieper, J., 2012. Differential synovial Th1 cell reactivity towards *Escherichia coli* antigens in patients with ankylosing spondylitis and rheumatoid arthritis. *Ann. Rheum. Dis.* 71 (9), 1573–1576.
- Taurog, J.D., Maika, S.D., Satumtira, N., Dorris, M.L., Mclean, I.L., Yanagisawa, H., et al., 1999. Inflammatory disease in HLA-B27 transgenic rats. *Immunol. Rev.* 169, 209–223.
- Taurog, J.D., Dorris, M.L., Satumtira, N., Tran, T.M., Sharma, R., Dressel, R., 2009. Spondyloarthritis in HLA-B27/human beta 2-microglobulin-transgenic rats is not prevented by lack of CD8. *Arthritis Rheum.* 60, 1977–1984.
- Tran, T.M., Colbert, R.A., 2015. Endoplasmatic reticulum aminopeptidase 1 and rheumatic diseases: functional variation. *Curr. Opin. Rheumatol.* 27, 357–363.
- Tran, T.M., Dorris, M.L., Satumtira, N., Richardson, J.A., Hammer, R.E., Shang, J., et al., 2006. Additional human beta2-microglobulin curbs HLA-B27 misfolding and promotes arthritis and spondylitis without colitis in male HLA-B27-transgenic rats. *Arthritis Rheum.* 54 (4), 1317–1327.
- Van Praet, L., Van den Bosch, F.E., Jacques, P., Carron, P., Jans, L., Colman, R., et al., 2013. Microscopic gut inflammation in axial spondyloarthritis: a multiparametric predictive model. *Ann. Rheum. Dis.* 72 (3), 414–417.
- Vieira-Sousa, E., van Duivenvoorde, L.M., Fonseca, J.E., Lories, R.J., Baeten, D.L., 2015. Review: animal models as a tool to dissect pivotal pathways driving spondyloarthritis. *Arthritis Rheumatol.* 67 (11), 2813–2827.
- Wellcome Trust Case-Control Consortium, Australo-Anglo-American Spondyloarthritis Consortium, 2007. Association scan of 14,500 non synonymous SNPs in four diseases identifies autoimmunity variants. *Nat. Genet.* 39, 1329–1337.
- Yin, Z., Braun, J., Grolms, M., Spiller, I., Radbruch, A., Sieper, J., 1997a. IFN gamma, IL-4 and IL-10 positive cells in the CD4+ and CD8+ T cell population of peripheral blood in untreated patients with early rheumatoid arthritis and early reactive arthritis. *Arthritis Rheum.* 40, 41.
- Yin, Z., Neure, L., Grolms, M., Eggens, U., Radbruch, A., Braun, J., et al., 1997b. TH1/TH2 cytokine pattern in the joint rheumatoid arthritis and reactive arthritis patients: analysis at the single cell level. *Arthritis Rheum.* 40, 40.
- Zeng, L., Lindstrom, M.J., Smith, J.A., 2011. Ankylosing spondylitis macrophage production of higher levels of interleukin-23 in response to lipopolysaccharide without induction of a significant unfolded protein response. *Arthritis Rheum.* 63, 3807–3817.
- Zinovieva, E., Bourgain, C., Kadi, A., et al., 2009. Comprehensive linkage and association analyses identify haplotype, near the TNFSF15 gene, significantly associated with spondyloarthritis. *PLoS Genet.* 5, e100528.
- Zou, J., Zhang, Y., Thiel, A., Rudwaleit, M., Shi, S.L., Radbruch, A., et al., 2003. Predominant cellular immune response to the cartilage autoantigenic G1 aggrecan in ankylosing spondylitis and rheumatoid arthritis. *Rheumatology* 42, 846–855.

## Further Reading

- Appel, H., Ruiz-Heiland, G., Listing, J., Zwerina, J., Herrmann, M., Mueller, R., et al., 2009. Altered skeletal expression of sclerostin and its link to radiographic progression in ankylosing spondylitis. *Arthritis Rheum.* 60 (11), 3257–3262.
- Boyle, L.H., Goodall, J.C., Opat, S.S., Gaston, J.S., 2001. The recognition of HLA-B27 by human CD4(+) T lymphocytes. *J. Immunol.* 167, 2619–2624.
- Boyle, L.H., Goodall, J.C., Gaston, J.S., 2004. The recognition of abnormal forms of HLA-B27 by CD4+ T cells. *Curr. Mol. Med.* 4, 51–58.
- Braun, J., Sieper, J., 2003. Spondyloarthritides and related arthritides. In: Warrel, D.A., Cox, T.M., Firth, J.D., Benz, E.J. (Eds.), *Oxford Textbook of Medicine*, fourth ed. Oxford University Press, New York, pp. 43–53.
- Heiland, G.R., Appel, H., Poddubnyy, D., Zwerina, J., Hueber, A., Haibel, H., et al., 2012. High level of functional dickkopf-1 predicts protection from syndesmophyte formation in patients with ankylosing spondylitis. *Ann. Rheum. Dis.* 71 (4), 572–574.
- Maksymowych, W.P., 2000. Ankylosing spondylitis—at the interface of bone and cartilage. *J. Rheumatol.* 27, 2295–2301.
- Miller, J.R., 2002. The Wnts. *Genome Biol.* 3 (1), REVIEW3001.
- van der Heijde, D., Landewé, R., Baraliakos, X., Houben, H., van Tubergen, A., Williamson, P., et al., 2008a. Radiographic findings following two years of infliximab therapy in patients with ankylosing spondylitis. *Arthritis Rheum.* 58 (10), 3063–3070.
- van der Heijde, D., Landewé, R., Einstein, S., Ory, P., Vosse, D., Ni, L., et al., 2008b. Radiographic progression of ankylosing spondylitis after up to two years of treatment with etanercept. *Arthritis Rheum.* 58 (5), 1324–1331.
- van der Heijde, D., Salonen, D., Weissman, B.N., Landewé, R., Maksymowych, W.P., Kupper, H., et al., 2009. Assessment of radiographic progression in the spines of patients with ankylosing spondylitis treated with adalimumab for up to 2 years. *Arthritis Res. Ther.* 11 (4), R127. Epub 2009 Aug 24.

# The Autoimmune Myopathies

Livia Casciola-Rosen and Antony Rosen

Division of Rheumatology, Johns Hopkins University School of Medicine,  
Baltimore, MD, United States

## OUTLINE

Defining Autoimmune Myopathies	703	Mechanisms of Disease	709
Clinical and Pathological Descriptions of Different Phenotypes, Including Immune-Mediated Necrotizing Myopathy	704	<i>The Association of Malignancy With Autoimmunity: Insights Into Disease Initiation</i>	709
Characteristic Pathology, but Significant Overlap Between Phenotypes	704	<i>Enhanced Expression of Myositis Autoantigens in Regenerating Muscle Cells to Focus Propagation on Muscle</i>	710
Epidemiological Clues Into Mechanism	705	<i>Modification of Autoantigen Expression or Structure by Immune Effector Pathways to Generate a Self-Sustaining Phenotype</i>	710
Specific Autoantibodies Are Strongly Associated With Phenotype, Making Them Useful Probes of Disease Mechanism	705	Therapeutic Insights	711
Myositis-Specific Autoantibodies	706	Concluding Remarks	711
3-Hydroxy-3-methylglutaryl Coenzyme A Reductase Autoantibodies in Statin-Associated Immune-Mediated Necrotizing Myopathy	707	References	711

## DEFINING AUTOIMMUNE MYOPATHIES

The autoimmune myopathies are an uncommon group of disorders, unified by autoimmune damage of skeletal muscle (Mammen, 2011). The process may occur as a distinct named disease [e.g., polymyositis (PM), dermatomyositis (DM), immune-mediated necrotizing myopathy (IMNM)], or as a feature of other systemic autoimmune diseases (e.g., systemic lupus erythematosus or scleroderma). While skeletal muscle is a primary target in the autoimmune myopathies, there are frequently other tissues that may also be affected, including skin (in DM), lung, cardiac muscle, and synovial joints. Although inclusion body myositis has features which might reflect autoimmunity (including autoantibodies such as cytosolic 5'-nucleotidase 1A; Larman et al., 2013), the pathology of this entity and the poor response to the types of immunosuppressive therapies which characteristically are highly effective in PM and DM suggest that this entity is distinct, and we have not considered it further in this chapter.

## CLINICAL AND PATHOLOGICAL DESCRIPTIONS OF DIFFERENT PHENOTYPES, INCLUDING IMMUNE-MEDIATED NECROTIZING MYOPATHY

Like most autoimmune rheumatic diseases, the autoimmune myopathies are quite heterogeneous in their clinical presentation. These diseases are frequently characterized by the subacute onset of painless weakness, predominantly affecting proximal muscles in a symmetrical way (Christopher-Stine et al., 2012; Miller, 2012; Robinson and Reed, 2011). In some cases, there may be an involvement of striated muscle of the nasopharynx and upper esophagus, with nasal regurgitation, weakness of phonation, tendency to aspiration, and difficulty in swallowing. In severe cases, the weakness of the respiratory muscles can occur, but this is infrequent. Muscle involvement is characterized by the leaking of various muscle enzymes, including creatine kinase (CK), aspartyl, and alaninyl transaminases, which are often incorrectly interpreted as arising from liver, as well as aldolase A. In addition, the inflammatory myopathies are characterized by an irritable myopathy on electromyography (EMG).

The involvement of tissues other than skeletal muscle is also frequent and may be accompanied by systemic inflammatory symptoms (malaise, fever). Skin involvement is a prominent feature in DM, with the pattern and type of skin involvement often being diagnostic features. Typical skin manifestations include (1) the characteristic heliotrope rash on the face around the eyelids; (2) Gottron's papules, inflammatory scaly papules limited to the dorsal aspect of the metacarpophalangeal and proximal interphalangeal joints; (3) a violaceous eruption involving the shawl area, the chest, the flanks, and the thighs; and (4) skin ulcers and palmar papules, which occur in a distinct subpopulation of patients with DM (Chaisson et al., 2012). Importantly, the small joints of the hands may be affected by a rheumatoid arthritis-like inflammatory synovitis; this is particularly evident in patients with the dermatopulmonary syndromes described below. Involvement of the lung is not infrequent in patients with autoimmune myopathies, with an estimated 20%–65% of patients having evidence of interstitial lung disease (ILD) (Labirua and Lundberg, 2010; Danoff and Casciola-Rosen, 2011). Johnson et al. (2016) recently assessed mortality in autoimmune myositis patients with and without associated ILD and found significantly higher mortality rates in the former, underscoring the importance of lung disease in myositis.

A distinct form of IMNM associated with an exposure to statins has recently been described (Christopher-Stine et al., 2010; Mammen et al., 2011). While statins are frequently associated with myalgias, which can sometimes necessitate the cessation of the drug, muscle pathology is generally self-limiting, and stopping the drug results in a complete resolution of the muscle process. A distinct subtype of this process presents with features of a severe autoimmune myopathy in the setting of statin exposure, where resolution does not follow a cessation of statin therapy. Patients with this statin-induced IMNM have proximal muscle weakness, very high serum CK levels, irritable changes on EMG, and a prominent necrotizing myopathy on biopsy (Grable-Esposito et al., 2010; Christopher-Stine et al., 2010; Mammen et al., 2011). Recent data from an exploratory case-control study showed that statin-exposed patients with anti-3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) antibodies have severe skeletal muscle manifestations and that atorvastatin and type 2 diabetes mellitus may be associated with an increased risk of developing this myopathy (Basharat et al., 2016).

## CHARACTERISTIC PATHOLOGY, BUT SIGNIFICANT OVERLAP BETWEEN PHENOTYPES

Distinct pathological hallmarks of the different immune-mediated myopathies have been described. While PM is characterized by intrafascicular inflammation and regeneration (with evidence of lymphocytes surrounding morphologically normal muscle cells), the classic pattern of DM shows perifascicular atrophy and regeneration, associated with striking perivascular inflammation. In contrast, there is only a limited inflammatory infiltrate in IMNM. Although necrotic muscle cells are found in both PM and DM, they are highly enriched in IMNM. There is an increasing appreciation that the pathological pattern in any specific patient is often less distinct, with features of the different entities present in mixed combinations (Pestronk, 2011). Pinal-Fernandez et al. (2015) recently investigated the association between autoantibody status and muscle pathology in DM patients; their study showed that different histopathologic features in DM muscle biopsies varied according to autoantibody status.

It is noteworthy that biopsies reflect a single moment in time, capturing a highly dynamic and integrated homeostatic system. In the case of the autoimmune myopathies, this system includes normal, damaged and repairing muscle cells, and various inflammatory cell subsets. Prominent among the infiltrating cells are cytotoxic lymphocytes and cells of the monocyte–macrophage and dendritic cell lineages. These different cells are not

isolated but rather are components of a highly interactive and reinforcing system. Indeed, the different clinical and pathological phenotypes likely represent new metastable states, reflecting a balance between pathways of damage and repair (see below).

## EPIDEMIOLOGICAL CLUES INTO MECHANISM

The epidemiology of human disease can provide important insights into underlying mechanisms. In myositis, combining epidemiological associations with the specificity of the immunological response has been particularly instructive. For example, the recent recognition that patients with IMNM associated with statin exposure (see above) have high titer autoantibodies against HMGCR has highlighted the importance of environmental exposures in initiating autoimmunity to specific, ubiquitously expressed autoantigens, while driving injury relatively focused on skeletal muscle (Christopher-Stine et al., 2010; Mammen et al., 2011).

The association of myositis and cancer has the potential to provide similar insights into pathogenesis but is significantly more complex for numerous reasons, including the presentation of cancer either before or after myositis diagnosis, as well as effects of immunosuppressive therapy. Nevertheless, the nature of this association provides an important framework for understanding pathogenesis of spontaneous disease. The initial clinical observation that myositis and cancer are associated stimulated numerous studies over the past five decades to define the nature and kinetics of this association (Sigurgeirsson et al., 1992). Although the strength of the association of cancer with DM is higher than that with PM, there is now definitive evidence that cancer is associated with both phenotypes (Hill et al., 2001). Interestingly, the overall risk and frequency, as well as the types of cancers associated with myositis, differ between PM and DM. In one large study, cancer was found in 32% of DM patients, but in 15% of PM patients (Hill et al., 2001). Overall, the elevated risk of cancer in DM has been noted in various studies to be 3–6-fold over controls for DM but increased only 1.3–2.0-fold in PM (Hill et al., 2001; Buchbinder et al., 2001; Chow et al., 1995; Stockton et al., 2001). In DM the highest risk was observed for adenocarcinomas, although any tumor type could be associated (Hill et al., 2001). The most frequent cancers associated with DM include ovarian, lung, prostate, pancreatic, stomach, and colorectal cancers. Non-Hodgkin's lymphomas are also enriched in DM. PM has been associated with an increased risk of non-Hodgkin's lymphoma, lung, and bladder cancers.

Multiple studies have demonstrated a striking temporal clustering of cancer and myositis in both phenotypes, with most cancers occurring within  $\pm 2$  years of the myositis diagnosis. Indeed, standardized incidence ratios (SIRs) appear highest in the first year after the diagnosis of myositis and decrease thereafter (Stockton et al., 2001). This temporal clustering would not be expected to occur if the cancers are the result of immunosuppression, suggesting that cancer and autoimmunity might be mechanistically related (see below). Although the incidence of cancer is increased in patients with myositis, it is noteworthy that 70%–85% of DM and PM patients never develop a cancer. Possible reasons for a cancer association in only a minority of myositis patients are discussed below.

An emerging area of research that likely will further illuminate the mechanistic link between cancer and autoimmunity comes from the emergence of autoimmune syndromes following the use of checkpoint inhibitors in cancer patients. A recent retrospective study by Touat et al. (2018) described 10 patients with metastatic cancer who developed a form of inflammatory myositis during immune checkpoint inhibitor therapy, mostly within 2 months of treatment initiation, with a rapid progression of symptom severity. The similarity of the clinical features—likely a new clinical entity—among these patients was noteworthy; these included elevated CK levels, the presence of multifocal necrotic myofibers in muscle biopsies, and the absence of myositis-specific antibodies. After immune checkpoint inhibitor cessation and treatment with corticosteroids, these patients experienced significant clinical improvement. It is presently unknown if these patients express a distinct autoimmune response not previously described. Further studies are warranted to identify those cancer patients at high risk of experiencing an adverse event after treatment with immune checkpoint inhibitors, and to understand the mechanistic underpinnings that link adverse events and cancer control/elimination.

## SPECIFIC AUTOANTIBODIES ARE STRONGLY ASSOCIATED WITH PHENOTYPE, MAKING THEM USEFUL PROBES OF DISEASE MECHANISM

While the clinical damage in autoimmune myopathies is focused on skeletal muscle and related tissues, the well-described targets of the immune response in these diseases are all notably not muscle specific

(Suber et al., 2008; Casciola-Rosen and Mammen, 2012). Rather, they are expressed ubiquitously, raising questions about the mechanisms of the tissue-specific focus of the immune response.

Several recent studies provide tantalizing evidence that there may be additional important autoantibodies against muscle-specific proteins in myositis patients. New data emerging from screens performed using myositis patient sera and a muscle complementary DNA library describes anti-four and a half LIM domains protein (FHL1) as an autoantigen in 25% of patients in this disease spectrum (Albrecht et al., 2015). What distinguishes FHL1 from the other defined myositis autoantigens is that it is a muscle-specific protein. The authors noted patchy FHL1 expression in muscle tissue from anti-FHL1-positive patients, which differed from the homogeneous expression observed in antibody-negative myositis patients and healthy controls. In another study, Cottrell et al. (2012) used cultured differentiating muscle cells as an antigen source to screen for myositis autoantibodies. They found a new 28 kDa specificity that was muscle specific and differentiation stage specific (i.e., absent in myoblasts, with peak expression at day 4 of differentiation, coincident with myogenin expression). The authors subsequently identified this as embryonic myosin light chain 4 (MYL4) using a mass spec approach (Cottrell et al., 2012). Additional studies for both FHL1 and MYL4 antibodies will be needed to define and validate these tantalizing new specificities in other myositis cohorts. Targeting of tissue-specific—and, more importantly—differentiation-stage-specific proteins are likely to be highly relevant in amplifying and sustaining injury in myositis and other autoimmune rheumatic diseases.

Among the well-defined myositis autoantigens, there is a striking association of specific autoantibodies with distinct clinical phenotypes, suggesting that the targeting of specific molecules by the immune system might either (1) participate in generating the unique phenotype or (2) that the specific immune response is stimulated by a unique series of tissue events which make that specific antigen available to drive the immune response.

A key fact remains unknown regarding the kinetics of autoimmune myopathy evolution: does the immune response to myositis-specific autoantigens precede or coincide with the onset of clinical symptoms? For most autoimmune diseases where this has been studied (e.g., systemic lupus erythematosus, rheumatoid arthritis, and type I diabetes mellitus), evidence of autoimmunity precedes clinical symptoms by several years (Arbuckle et al., 2003; Nielsen et al., 2004; van der Helm-van Mil et al., 2005; Majka et al., 2008; Baekkeskov et al., 1984; Eisenbarth, 2003). For the autoimmune myopathies, no systematic data are yet available, but there have been cases where autoantibodies to histidyl-tRNA synthetase preceded clinical myositis (Miller et al., 1990), suggesting that the generation of the immune response may precede the establishment of the clinical phenotype.

## Myositis-Specific Autoantibodies

The autoantibodies elaborated in patients with myositis recognize a family of autoantigens which have important, conserved functions in general cellular processes. Prominent among these functions are protein translation [e.g., aminoacyl-tRNA synthetases, signal recognition particle (Mathews and Bernstein, 1983; Reeves et al., 1986)], gene expression [e.g., components of the nucleosome remodeling and deacetylation complex (Targoff and Reichlin, 1985)], DNA repair machinery [e.g., double-strand break and mismatch repair machinery (Suwa et al., 1996; Casciola-Rosen et al., 1995, 2001)], posttranslational modification [e.g., small ubiquitin-like modifier-activating enzyme subunit 1 (Okuma et al., 1999)], nuclear body formation [e.g., nuclear matrix protein NXP2 (Mimura et al., 2010)], and the exosome complex. In addition, several autoantigens (e.g., Ro52 and MDA5) are induced by interferons (Rhodes et al., 2002; Sato et al., 2009).

Autoantibodies in myositis have a striking association with phenotype (see Table 37.1). For example, antibodies recognizing aminoacyl-tRNA synthetases are frequently associated with the “synthetase syndrome,” a clinical syndrome with relatively mild myositis, Raynaud’s phenomenon, inflammatory arthritis of the small joints of the hands, mechanic’s hands, and ILD of variable severity (Friedman et al., 1996). Antibodies to the signal recognition particle are associated with a particularly severe form of necrotizing myopathy, with cardiac involvement. While antibodies to Mi-2 tend to be associated with the characteristic DM skin rash (including heliotrope and truncal rash), antibodies to MDA5 have been associated with a distinct dermatopulmonary syndrome, with mild or no myositis, mild-to-severe ILD, as well as unique dermatological features (including gum pain, palmar papules, and ulceration) (Fiorentino et al., 2011; Chaisson et al., 2012). Numerous additional phenotypic features associated with the different antibodies have been described; these are summarized in several excellent recent reviews (Fujimoto et al., 2016; Satoh et al., 2017; Betteridge and McHugh, 2016; Gunawardena, 2017).

An interesting feature of many of the autoantigens targeted in myositis is their striking susceptibility to cleavage by the cytotoxic lymphocyte granule protease, granzyme B (GrB) (Casciola-Rosen et al., 1999). While GrB is a

**TABLE 37.1** Association of Myositis-Specific Autoantibodies With Disease Subsets

Autoantibody	Disease subset
Antisynthetases (anti-Jo-1, PL-7, PL-12, EJ, OJ, KS, Ha, Zo)	Antisynthetase syndrome with polymyositis or dermatomyositis
Anti-Mi-2	Dermatomyositis
Antimelanoma differentiation-associated protein (anti-MDA5, anti-CADM140)	
Antitranscription intermediary factor 1 $\gamma$ (anti-p155)	
Antinuclear matrix protein 2 (anti-NXP2, anti-MJ)	
Anti-SAE1	
Anti-SRP	Necrotizing myopathy
Anti-3-hydroxy-3-methylglutaryl coenzyme A (anti-HMGCR)	

fastidious protease which cleaves a minority of proteins across the proteome, it efficiently cleaves the majority of myositis autoantigens. This unusual enrichment of GrB substrates among myositis autoantigens, together with the demonstrated activity of cytotoxic cells against muscle cells in myositis patients, possibly identifies an important amplification loop in this group of diseases (see below).

New studies suggest that the autoantibodies found in myositis patients with cancer may be distinct from those found in patients where cancer does not appear. Thus, recent studies suggest that antibodies to TIF1 $\gamma$  (TRIM33) may be enriched in DM patients with cancer, while cancer is very uncommon in patients with antibodies against Mi-2 or aminoacyl-tRNA synthetases (Fujimoto et al., 2012; Hoshino et al., 2010; Trallero-Araguas et al., 2012).

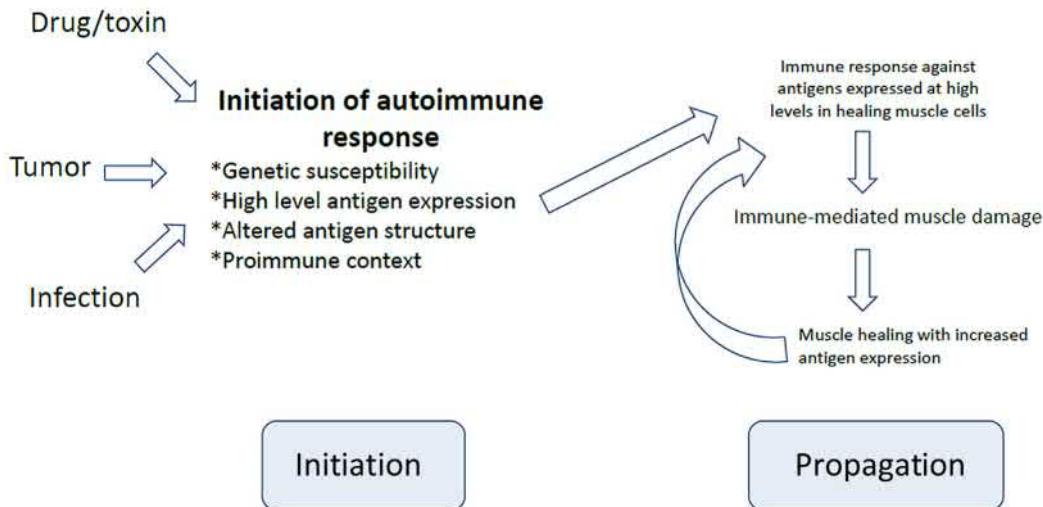
Since the majority of myositis autoantigens function in pathways of general relevance to cellular function and homeostasis, it has been reasonable to assume that these autoantigens are ubiquitously expressed. This remains true, with the noteworthy exceptions of the two muscle-specific autoantigens described to date (discussed above). Experiments to directly address the expression of ubiquitous autoantigens in normal and affected target tissues have shown that myositis autoantigens are expressed at very low levels in normal muscle but are robustly expressed in myositis muscle, with the highest levels of autoantigen expression being found in regenerating muscle cells (Casciola-Rosen et al., 2005). This restriction of high level autoantigen expression to cells repairing muscle injury strongly suggests that normal muscle is unlikely to be the source of antigen to initiate and drive autoimmunity to these molecules in myositis and focuses attention on other antigen sources (including cancer and repairing muscle cells) as more relevant in this regard (see Fig. 37.1 and text below). Consistent with this, aldolase A expression in the absence of CK in undifferentiated muscle cells (and those early in the differentiation process) may reflect preferential immune-mediated damage of early regenerating cells and may explain the occurrence of elevated serum aldolase A in the absence of CK in a subset of myositis patients (Casciola-Rosen et al., 2012).

Important insights have emerged from studies addressing myositis autoantigen expression in normal tissues and the relevant myositis-associated cancers. These demonstrated that myositis autoantigen expression in normal tissues is very low but is elevated in multiple malignancies, including lung and breast (Casciola-Rosen et al., 2005). It is possible that high level autoantigen expression in the tumor can induce an immune response which cross-reacts with muscle cells. In patients with a malignancy, this reflects an effective anticancer response, or perhaps selection of this immune target via distinct mechanisms.

### 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase Autoantibodies in Statin-Associated Immune-Mediated Necrotizing Myopathy

Statin treatment is very frequent in the population, with an estimated 25 million users worldwide. Muscle-related complications related to statin therapy are widely recognized, with a spectrum from self-limited myalgias to statin-associated rhabdomyolysis (Bruckert et al., 2005; Franc et al., 2003; Graham et al., 2004). The vast majority of these cases resolve fully after stopping the statin. However, in a small group of patients, statin exposure appears to induce a self-sustaining, immune-mediated myopathy that persists despite statin discontinuation. In a recent series of studies, a novel autoantibody specificity recognizing an autoantigen migrating as a doublet of

## Autoimmunity in myositis—a simplified model



**FIGURE 37.1** The role of autoimmunity in myositis. Myositis arises when an immune response against myositis-specific autoantigens is initiated. The initiation phase may be separated spatially and temporally from propagation events, which are focused on muscle. In the absence of a stimulus inducing muscle injury and repair, it is possible that the autoimmune response may remain silent. In the case of cancer-associated myositis, it is possible that an unrelated muscle injury is the second hit which focuses the anticancer immune response onto repairing muscle and propagates the injury there. In statin-induced necrotizing myopathy, the site of immunization with HMGCR may be muscle. The autoantigens targeted are characterized by their shared expression patterns in immunizing tissue and regenerating muscle, and by their susceptibility to modification by immune effector pathways.

100k/200k bands was defined in a group of immunosuppression-responsive patients with a necrotizing myopathy and very high CK levels ([Christopher-Stine et al., 2010](#)).

Interestingly, the majority of patients in that study were statin-exposed. Since the target of statins is a 100 kDa protein called HMGCR, the key enzyme in the de novo cholesterol synthesis pathway, the association with statin exposure provided an important clue for identifying the autoantigen targeted in this syndrome. While HMGCR levels are low in most cells, they are dramatically upregulated by statin exposure ([Goldstein and Brown, 1990](#)). Antibodies from INNM patients recognized higher levels of the 100k/200k antigen in statin-treated cells, leading Mammen et al. to demonstrate that the intracellular C-terminal domain containing the HMGCR active site is indeed the target of these autoantibodies ([Mammen et al., 2011; Christopher-Stine et al., 2010](#)).

As has been noted with other myositis autoantigens, a high level expression of HMGCR was detected in regenerating muscle cells in biopsies of patients with HMGCR antibodies ([Mammen et al., 2011; Casciola-Rosen et al., 2005](#)). This suggests that ongoing immune effector pathways are focused onto cells attempting to repair muscle injury, thereby creating a feedforward cycle of damage and repair ([Casciola-Rosen et al., 2005](#)). The patients with HMGCR antibodies did not have the genetic polymorphism in the anion transporter frequently associated with statin myopathy, suggesting a novel mechanism underlying the targeting of this molecule in this patient subgroup. Since the majority of patients exposed to statins do not generate immune responses to HMGCR ([Mammen et al., 2012b](#)), [Mammen et al. \(2012a\)](#) examined whether there might be any major histocompatibility complex (MHC) associations with the HMGCR subgroup and showed a striking association of HMGCR antibodies with human leukocyte antigen (HLA) class II DRB1\*11:01. This magnitude of this association is one of the largest ones described to date, suggesting that the immune response to HMGCR might be restricted by this HLA molecule and that patients with this HLA molecule might be particularly susceptible to this syndrome.

[Tiniakou et al. \(2017\)](#) recently studied disease severity and response to therapy in a large cohort of anti-HMGCR antibody-positive myositis patients. In this subgroup, they found that younger patients had more severe disease and a worse prognosis than those who were older. Interestingly, this persistent muscle weakness could not be explained by coexisting muscular dystrophy since this was not detected in these patients. In another study

from this group, the severity of weakness in IMNM patients with anti-HMGCR or antisignal recognition particle antibodies was compared (Pinal-Fernandez et al., 2017). Overall, patients with the latter specificity were significantly weaker than anti-HMGCR-positive patients ( $P = .001$ ), indicating that these antibodies are likely associated with distinct forms of IMNM.

## MECHANISMS OF DISEASE

Autoimmune myopathies represent a chronic, self-sustaining process, characterized by inflammation, muscle damage, and muscle repair. While the mechanisms of disease are likely heterogeneous in different individuals, with distinct pathways playing different relative roles in different patients, recent data enables the proposal of a unifying model which incorporates the essential features of the disease spectrum described above. This model will include the following components: (1) the important association of malignancy with myositis as a potential initiating force; (2) the enhanced expression of myositis autoantigens in regenerating muscle cells to focus propagation on muscle; and (3) the modification of autoantigen expression or structure by immune effector pathways to generate a self-sustaining phenotype. Each of these components is discussed in detail below (see Fig. 37.1).

### The Association of Malignancy With Autoimmunity: Insights Into Disease Initiation

In 10%–20% of myositis patients who also have cancer, the two processes cluster together temporally (Chow et al., 1995; Hill et al., 2001). This kinetic clustering suggests strongly that the two processes are mechanistically related. Recent studies at this interface have been especially informative in the scleroderma disease spectrum; the emerging principles (reviewed below) are highly applicable to the myositis–cancer interface.

Shah et al. (2010) investigated whether autoantibodies could define subsets of patients with scleroderma who might have an associated cancer. This study showed an unexpectedly close temporal relationship between disease onset and malignancy detection in patients with RNA polymerase III antibodies. These authors and other collaborators (Joseph et al., 2014) subsequently sequenced tumors from 16 scleroderma patients, 8 with anti-RNA polymerase III antibodies, and 8 with other scleroderma antibody specificities. They found genetic alterations in the *POLR3A* gene locus in 6/8 cancers for the anti-RNA polymerase III-positive patients, but not in tumors from the scleroderma patients with other antibody specificities. Since *POLR3A* gene mutations are very rare in cancer, it is conceivable that they play a role in initiating the immune response when presented to the immune system in the context of the appropriate MHC (see below for further details in the context of myositis; also reviewed in Shah et al., 2015). Interestingly, ~85% of patients with scleroderma and anti-RNA polymerase III antibodies do not manifest a cancer. In these patients, it is possible that a potent antitumor immune response successfully eradicates malignancy.

Similar tantalizing connections between myositis autoantibodies and cancer susceptibility have been made (Fiorentino et al., 2013). Fiorentino et al. demonstrated a higher prevalence of cancers within 3 years of myositis diagnosis in myositis patients with antibodies against TIF1 $\gamma$  or NXP2. Interestingly, even in patients with these antibodies, ~80% did not manifest any cancer—consistent with the anti-RNA polymerase III antibody findings in scleroderma. Given these observations, and in the context of the recent cancer scleroderma findings, it is reasonable to assume that similar principles operate at the cancer–myositis interface.

A likely model for myositis initiation would be as follows. Mutations in myositis-specific autoantigens (TIF1 $\gamma$  and NXP2 are attractive candidates) in cancers initiate an autoimmune response. The initial mutation-specific immune response spreads to the wild-type protein. In turn, this induces tissue damage, notably focused on tissues in which there is a prominent expression of the autoantigen. For example, regenerating cells would be prominent targets in muscle. In some patients, the immune response is robust enough to challenge the cancer, effectively maintaining it in equilibrium or eliminating it. Myositis in the absence of cancer would manifest in this scenario. In others, the cancer might escape from this immune response through genetic changes such as loss of heterozygosity; in this subset of myositis patients, cancer emerges.

Further studies to define the critical immune effector pathways will be insightful for understanding the observed damage and dysfunction of normal tissue. Therapies that effectively kill cancers may eliminate the driving immune stimulus in affected individuals. In turn, this might enable the peripheral immune-mediated damage to resolve. Such approaches highlight the immune response in autoimmune diseases as a positive force.

The regulation of antigen expression in the target tissue(s) may allow beneficial anticancer effects of the immune system to focus exclusively on the cancer, thus avoiding damage to self-tissues.

That cancer can both precede and follow the diagnosis of myositis is important, as it reinforces that cancer is not emerging exclusively due to immunosuppressive therapy once myositis is diagnosed. The short interval separating the two diagnoses, and the occurrence of cancers in tissues often unaffected in myositis, also makes it unlikely that the inflammatory microenvironment enhances transformation and cancer growth. The available data is consistent with a mechanism in which shared antigen expression patterns in cancers and regenerating muscle cells may explain numerous features of the disease, including the kinetics noted above. In this model, natural anticancer immunity (including CD4 T cell and cytotoxic lymphocyte responses) develops in response to an incipient cancer.

### Enhanced Expression of Myositis Autoantigens in Regenerating Muscle Cells to Focus Propagation on Muscle

The evidence showing that myositis antigens are expressed at high levels in cancers and also in myositis tissue, where robust expression is noted in regenerating muscle cells, suggests a possible mechanism whereby an initial anticancer immune response targeting one of the myositis autoantigens becomes secondarily focused on injured muscle, thus beginning the propagation of a self-sustaining cycle at that site (Casciola-Rosen et al., 2005). The reasons for the unusual restriction of immune responses in myositis to such a limited group of antigens remain uncertain but may reflect the ability of only a very limited group of molecules to participate in a feedforward cycle (due to the need to both respond to and stimulate additional immune response—see below). It is possible that anticancer immune responses directed even at myositis antigens might be silent, since the number of regenerating cells in unperturbed muscle is very low. Very few cells in normal muscle are therefore susceptible to immune injury focused on these “regenerating cell” antigens. In the setting of a second insult which damages muscle (strenuous exercise, drugs, viral infection), the preformed anticancer immune response could respond to antigens now expressed in regenerating muscle cells. When these cells are damaged, a self-sustaining damage-healing cycle is generated, driven by immune-mediated cytolysis and the need to continuously provide additional precursors to accomplish tissue repair. This model explains several additional features of the disease, including the patchy nature of pathology, the inexact kinetic relationships between cancer and myositis, and the antigen expression patterns.

### Modification of Autoantigen Expression or Structure by Immune Effector Pathways to Generate a Self-Sustaining Phenotype

A central feature of any feedforward loop is that the activity of one component augments the amplitude of the other, which in turn augments the first. Several immune effector pathways (discussed below) are highly represented in myositis muscle. These dramatically influence antigen expression and structure and may play important roles in driving ongoing immune responses.

Several prominent myositis autoantigens (MDA5 and Ro52) are strongly interferon-regulated, and interferon signatures are prominent in the muscle (PM and DM) and skin (DM) in myositis patients. In addition, cells with the capacity to secrete large amounts of both type I (plasmacytoid DC, pDCs) and type II interferons (activated CD8 T cells) are enriched in DM and PM muscle (Greenberg et al., 2005; Goebels et al., 1996). This capacity of effector pathways to augment antigen expression and thus create additional targets for cytolytic damage and additional interferon secretion may be important pathogenically and might be particularly amenable to therapeutic manipulation.

A second potential intersection of the immune effector and antigen arms centers on the susceptibility of most myositis autoantigens to cleavage by GrB (Casciola-Rosen et al., 1999). It is noteworthy that the expression of this protease is highly restricted to the cytolytic effector arm of the innate and adaptive immune systems (Darrah and Rosen, 2010). Furthermore, since GrB has a unique specificity among proteases, most antigens will not have been cleaved by GrB during the development of tolerance. The potential for GrB-mediated cleavage to generate fragments that have not been tolerized would provide another feedforward loop to contribute to driving myositis. In such a model, immune effector pathways generate forms of antigens not previously seen and tolerized by the immune system, thus driving the immune response, which further drives antigen generation. Multiple studies suggest that such effects are likely to be driven at the level of CD4 T cells, but this remains to be demonstrated directly.

## THERAPEUTIC INSIGHTS

The therapy of myositis currently focuses entirely on the modification of the immune side of the pathogenic cycle. Since myositis is a rare autoimmune phenotype, there is little randomized trial-derived data about the efficacy of specific agents, but clinical experience suggests efficacy with multiple immunosuppressive agents. As with all unfocused immunosuppressive strategies, the major limitations are infectious side effects. Since autoantigen expression in cells which are repairing tissue injury may be an important partner in driving the ongoing immune response and tissue damage in myositis, it is possible that focusing attention on modifying pathways of antigen expression in the target cell may be an effective strategy. If cancer is an important driver of the initial immune response, perhaps early anticancer treatment strategies might be of benefit (see above).

## CONCLUDING REMARKS

Although the autoimmune myopathies are uncommon, their interesting presentations and associations provide unique insights into the mechanisms of human autoimmunity, which are potentially of broad relevance, and may be of importance therapeutically. The highly focused immune response on a limited number of antigens which function in pathways of general relevance is of great interest. The association of myositis with cancer and the shared antigen expression patterns between cancer and regenerating muscle cells may be of importance in disease propagation. The participation of multiple interacting pathways in augmenting the amplitude of response of other partners may be critical in generating a self-sustaining feedforward loop which characterizes these diseases and may provide novel therapeutic opportunities. Of particular importance are the reinforcing interactions between immune effector pathways, antigen, and the target tissue.

## References

- Albrecht, I., Wick, C., Hallgren, A., Tjarnlund, A., Nagaraju, K., Andrade, F., et al., 2015. Development of autoantibodies against muscle-specific FHL1 in severe inflammatory myopathies. *J. Clin. Invest.* 125 (12), 4612–4624.
- Arbuckle, M.R., McClain, M.T., Rubertone, M.V., Scofield, R.H., Dennis, G.J., James, J.A., et al., 2003. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N. Engl. J. Med.* 349 (16), 1526–1533.
- Baekkeskov, S., Dyrberg, T., Lernmark, A., 1984. Autoantibodies to a 64-kilodalton islet cell protein precede the onset of spontaneous diabetes in the BB rat. *Science* 224 (4655), 1348–1350.
- Basharat, P., Lahouti, A.H., Paik, J.J., Albayda, J., Pinal-Fernandez, I., Bichile, T., et al., 2016. Statin-induced anti-HMGCR-associated myopathy. *J. Am. Coll. Cardiol.* 68 (2), 234–235.
- Betteridge, Z., McHugh, N., 2016. Myositis-specific autoantibodies: an important tool to support diagnosis of myositis. *J. Intern. Med.* 280 (1), 8–23.
- Bruckert, E., Hayem, G., Dejager, S., Yau, C., Begaud, B., 2005. Mild to moderate muscular symptoms with high-dosage statin therapy in hyperlipidemic patients—the PRIMO study. *Cardiovasc. Drugs Ther.* 19 (6), 403–414.
- Buchbinder, R., Forbes, A., Hall, S., Dennett, X., Giles, G., 2001. Incidence of malignant disease in biopsy-proven inflammatory myopathy. A population-based cohort study. *Ann. Intern. Med.* 134 (12), 1087–1095.
- Casciola-Rosen, L., Mammen, A.L., 2012. Myositis autoantibodies. *Curr. Opin. Rheumatol.* 24 (6), 602–608.
- Casciola-Rosen, L.A., Anhalt, G.J., Rosen, A., 1995. DNA-dependent protein kinase is one of a subset of autoantigens specifically cleaved early during apoptosis. *J. Exp. Med.* 182 (6), 1625–1634.
- Casciola-Rosen, L., Andrade, F., Ulanet, D., Wong, W.B., Rosen, A., 1999. Cleavage by granzyme B is strongly predictive of autoantigen status: implications for initiation of autoimmunity. *J. Exp. Med.* 190 (6), 815–826.
- Casciola-Rosen, L.A., Pluta, A.F., Plotz, P.H., Cox, A.E., Morris, S., Wigley, F.M., et al., 2001. The DNA mismatch repair enzyme PMS1 is a myositis-specific autoantigen. *Arthritis Rheum.* 44 (2), 389–396.
- Casciola-Rosen, L., Nagaraju, K., Plotz, P., Wang, K., Levine, S., Gabrielson, E., et al., 2005. Enhanced autoantigen expression in regenerating muscle cells in idiopathic inflammatory myopathy. *J. Exp. Med.* 201 (4), 591–601.
- Casciola-Rosen, L., Hall, J.C., Mammen, A.L., Christopher-Stine, L., Rosen, A., 2012. Isolated elevation of aldolase in the serum of myositis patients: a potential biomarker of damaged early regenerating muscle cells. *Clin. Exp. Rheumatol.* 30 (4), 548–553.
- Chaisson, N.F., Paik, J., Orbai, A.M., Casciola-Rosen, L., Fiorentino, D., Danoff, S., et al., 2012. A novel dermato-pulmonary syndrome associated with MDA-5 antibodies: report of 2 cases and review of the literature. *Medicine (Baltimore)* 91 (4), 220–228.
- Chow, W.H., Gridley, G., Mellemkjaer, L., McLaughlin, J.K., Olsen, J.H., Fraumeni Jr., J.F., 1995. Cancer risk following polymyositis and dermatomyositis: a nationwide cohort study in Denmark. *Cancer Causes Control* 6 (1), 9–13.
- Christopher-Stine, L., Casciola-Rosen, L.A., Hong, G., Chung, T., Corse, A.M., Mammen, A.L., 2010. A novel autoantibody recognizing 200-kd and 100-kd proteins is associated with an immune-mediated necrotizing myopathy. *Arthritis Rheum.* 62 (9), 2757–2766.
- Christopher-Stine, L., Robinson, D.R., Wu, C.C., Mark, E.J., 2012. Case records of the Massachusetts General Hospital. Case 37-2012. A 21-year-old man with fevers, arthralgias, and pulmonary infiltrates. *N. Engl. J. Med.* 367 (22), 2134–2146.

- Cottrell, T.R., Hall, J.C., Rosen, A., Casciola-Rosen, L., 2012. Identification of novel autoantigens by a triangulation approach. *J. Immunol. Methods* 385 (1–2), 35–44.
- Danoff, S.K., Casciola-Rosen, L., 2011. The lung as a possible target for the immune reaction in myositis. *Arthritis Res. Ther.* 13 (4), 230.
- Darrah, E., Rosen, A., 2010. Granzyme B cleavage of autoantigens in autoimmunity. *Cell Death Differ.* 17 (4), 624–632.
- Eisenbarth, G.S., 2003. Insulin autoimmunity: immunogenetics/immunopathogenesis of type 1A diabetes. *Ann. N.Y. Acad. Sci.* 1005, 109–118.
- Fiorentino, D., Chung, L., Zwerner, J., Rosen, A., Casciola-Rosen, L., 2011. The mucocutaneous and systemic phenotype of dermatomyositis patients with antibodies to MDA5 (CADM-140): a retrospective study. *J. Am. Acad. Dermatol.* 65 (1), 25–34.
- Fiorentino, D.F., Chung, L.S., Christopher-Stine, L., Zaba, L., Li, S., Mammen, A.L., et al., 2013. Most patients with cancer-associated dermatomyositis have antibodies to nuclear matrix protein NXP-2 or transcription intermediary factor 1gamma. *Arthritis Rheum.* 65 (11), 2954–2962.
- Franc, S., Dejager, S., Bruckert, E., Chauvenet, M., Giral, P., Turpin, G., 2003. A comprehensive description of muscle symptoms associated with lipid-lowering drugs. *Cardiovasc. Drugs Ther.* 17 (5–6), 459–465.
- Friedman, A.W., Targoff, I.N., Arnett, F.C., 1996. Interstitial lung disease with autoantibodies against aminoacyl-tRNA synthetases in the absence of clinically apparent myositis. *Semin. Arthritis Rheum.* 26 (1), 459–467.
- Fujimoto, M., Hamaguchi, Y., Kaji, K., Matsushita, T., Ichimura, Y., Kodera, M., et al., 2012. Myositis-specific anti-155/140 autoantibodies target transcription intermediary factor 1 family proteins. *Arthritis Rheum.* 64 (2), 513–522.
- Fujimoto, M., Watanabe, R., Ishitsuka, Y., Okiyama, N., 2016. Recent advances in dermatomyositis-specific autoantibodies. *Curr. Opin. Rheumatol.* 28 (6), 636–644.
- Goebels, N., Michaelis, D., Engelhardt, M., Huber, S., Bender, A., Pongratz, D., et al., 1996. Differential expression of perforin in muscle-infiltrating T cells in polymyositis and dermatomyositis. *J. Clin. Invest.* 97 (12), 2905–2910.
- Goldstein, J.L., Brown, M.S., 1990. Regulation of the mevalonate pathway. *Nature* 343 (6257), 425–430.
- Grable-Esposito, P., Katzberg, H.D., Greenberg, S.A., Srinivasan, J., Katz, J., Amato, A.A., 2010. Immune-mediated necrotizing myopathy associated with statins. *Muscle Nerve* 41 (2), 185–190.
- Graham, D.J., Staffa, J.A., Shatin, D., Andrade, S.E., Schech, S.D., La Grenade, L., et al., 2004. Incidence of hospitalized rhabdomyolysis in patients treated with lipid-lowering drugs. *JAMA* 292 (21), 2585–2590.
- Greenberg, S.A., Pinkus, J.L., Pinkus, G.S., Burleson, T., Sanoudou, D., Tawil, R., et al., 2005. Interferon-alpha/beta-mediated innate immune mechanisms in dermatomyositis. *Ann. Neurol.* 57 (5), 664–678.
- Gunawardena, H., 2017. The clinical features of myositis-associated autoantibodies: a review. *Clin. Rev. Allergy Immunol.* 52 (1), 45–57.
- Hill, C.L., Zhang, Y., Sigurgeirsson, B., Pukkala, E., Mellemkjaer, L., Airio, A., et al., 2001. Frequency of specific cancer types in dermatomyositis and polymyositis: a population-based study. *Lancet* 357 (9250), 96–100.
- Hoshino, K., Muro, Y., Sugiura, K., Tomita, Y., Nakashima, R., Mimori, T., 2010. Anti-MDA5 and anti-TIF1-gamma antibodies have clinical significance for patients with dermatomyositis. *Rheumatology (Oxford)* 49 (9), 1726–1733.
- Johnson, C., Pinal-Fernandez, I., Parikh, R., Paik, J., Albayda, J., Mammen, A.L., et al., 2016. Assessment of mortality in autoimmune myositis with and without associated interstitial lung disease. *Lung* 194 (5), 733–737.
- Joseph, C.G., Darrah, E., Shah, A.A., Skora, A.D., Casciola-Rosen, L.A., Wigley, F.M., et al., 2014. Association of the autoimmune disease scleroderma with an immunologic response to cancer. *Science* 343 (6167), 152–157.
- Labirua, A., Lundberg, I.E., 2010. Interstitial lung disease and idiopathic inflammatory myopathies: progress and pitfalls. *Curr. Opin. Rheumatol.* 22 (6), 633–638.
- Larman, H.B., Salajegheh, M., Nazareno, R., Lam, T., Sauld, J., Steen, H., et al., 2013. Cytosolic 5'-nucleotidase 1A autoimmunity in sporadic inclusion body myositis. *Ann. Neurol.* 73 (3), 408–418.
- Majka, D.S., Deane, K.D., Parrish, L.A., Lazar, A.A., Baron, A.E., Walker, C.W., et al., 2008. Duration of preclinical rheumatoid arthritis-related autoantibody positivity increases in subjects with older age at time of disease diagnosis. *Ann. Rheum. Dis.* 67 (6), 801–807.
- Mammen, A.L., 2011. Autoimmune myopathies: autoantibodies, phenotypes and pathogenesis. *Nat. Rev. Neurol.* 7 (6), 343–354.
- Mammen, A.L., Chung, T., Christopher-Stine, L., Rosen, P., Rosen, A., Doering, K.R., et al., 2011. Autoantibodies against 3-hydroxy-3-methylglutaryl-coenzyme A reductase in patients with statin-associated autoimmune myopathy. *Arthritis Rheum.* 63 (3), 713–721.
- Mammen, A.L., Gaudet, D., Brisson, D., Christopher-Stine, L., Lloyd, T.E., Leffell, M.S., et al., 2012a. Increased frequency of DRB1\*11:01 in anti-hydroxymethylglutaryl-coenzyme A reductase-associated autoimmune myopathy. *Arthritis Care Res. (Hoboken)* 64 (8), 1233–1237.
- Mammen, A.L., Pak, K., Williams, E.K., Brisson, D., Coresh, J., Selvin, E., et al., 2012b. Rarity of anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase antibodies in statin users, including those with self-limited musculoskeletal side effects. *Arthritis Care Res. (Hoboken)* 64 (2), 269–272.
- Mathews, M.B., Bernstein, R.M., 1983. Myositis autoantibody inhibits histidyl-tRNA synthetase: a model for autoimmunity. *Nature* 304 (5922), 177–179.
- Miller, F.W., 2012. New approaches to the assessment and treatment of the idiopathic inflammatory myopathies. *Ann. Rheum. Dis.* 71 (Suppl. 2), i82–i85.
- Miller, F.W., Twitty, S.A., Biswas, T., Plotz, P.H., 1990. Origin and regulation of a disease-specific autoantibody response. Antigenic epitopes, spectrotype stability, and isotype restriction of anti-Jo-1 autoantibodies. *J. Clin. Invest.* 85 (2), 468–475.
- Mimura, Y., Takahashi, K., Kawata, K., Akazawa, T., Inoue, N., 2010. Two-step colocalization of MORC3 with PML nuclear bodies. *J. Cell. Sci.* 123 (Pt 12), 2014–2024.
- Nielen, M.M., van Schaardenburg, D., Reesink, H.W., van de Stadt, R.J., van der Horst-Bruinsma, I.E., de Koning, M.H., et al., 2004. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum.* 50 (2), 380–386.
- Okuma, T., Honda, R., Ichikawa, G., Tsumagari, N., Yasuda, H., 1999. In vitro SUMO-1 modification requires two enzymatic steps, E1 and E2. *Biochem. Biophys. Res. Commun.* 254 (3), 693–698.
- Pestronk, A., 2011. Acquired immune and inflammatory myopathies: pathologic classification. *Curr. Opin. Rheumatol.* 23 (6), 595–604.

- Pinal-Fernandez, I., Casciola-Rosen, L.A., Christopher-Stine, L., Corse, A.M., Mammen, A.L., 2015. The prevalence of individual histopathologic features varies according to autoantibody status in muscle biopsies from patients with dermatomyositis. *J. Rheumatol.* 42 (8), 1448–1454.
- Pinal-Fernandez, I., Parks, C., Werner, J.L., Albayda, J., Paik, J., Danoff, S.K., et al., 2017. Longitudinal course of disease in a large cohort of myositis patients with autoantibodies recognizing the signal recognition particle. *Arthritis Care Res. (Hoboken)* 69 (2), 263–270.
- Reeves, W.H., Nigam, S.K., Blobel, G., 1986. Human autoantibodies reactive with the signal-recognition particle. *Proc. Natl. Acad. Sci. U.S.A.* 83 (24), 9507–9511.
- Rhodes, D.A., Ihrke, G., Reinicke, A.T., Malcherek, G., Towey, M., Isenberg, D.A., et al., 2002. The 52 000 MW Ro/SS-A autoantigen in Sjögren's syndrome/systemic lupus erythematosus (Ro52) is an interferon-gamma inducible tripartite motif protein associated with membrane proximal structures. *Immunology* 106 (2), 246–256.
- Robinson, A.B., Reed, A.M., 2011. Clinical features, pathogenesis and treatment of juvenile and adult dermatomyositis. *Nat. Rev. Rheumatol.* 7 (11), 664–675.
- Sato, S., Hoshino, K., Satoh, T., Fujita, T., Kawakami, Y., Fujita, T., et al., 2009. RNA helicase encoded by melanoma differentiation-associated gene 5 is a major autoantigen in patients with clinically amyopathic dermatomyositis: association with rapidly progressive interstitial lung disease. *Arthritis Rheum.* 60 (7), 2193–2200.
- Satoh, M., Tanaka, S., Ceribelli, A., Calise, S.J., Chan, E.K., 2017. A comprehensive overview on myositis-specific antibodies: new and old biomarkers in idiopathic inflammatory myopathy. *Clin. Rev. Allergy Immunol.* 52 (1), 1–19.
- Shah, A.A., Rosen, A., Hummers, L., Wigley, F., Casciola-Rosen, L., 2010. Close temporal relationship between onset of cancer and scleroderma in patients with RNA polymerase I/III antibodies. *Arthritis Rheum.* 62 (9), 2787–2795.
- Shah, A.A., Casciola-Rosen, L., Rosen, A., 2015. Review: cancer-induced autoimmunity in the rheumatic diseases. *Arthritis Rheumatol.* 67 (2), 317–326.
- Sigurgeirsson, B., Lindelof, B., Edhag, O., Allander, E., 1992. Risk of cancer in patients with dermatomyositis or polymyositis. A population-based study. *N. Engl. J. Med.* 326 (6), 363–367.
- Stockton, D., Doherty, V.R., Brewster, D.H., 2001. Risk of cancer in patients with dermatomyositis or polymyositis, and follow-up implications: a Scottish population-based cohort study. *Br. J. Cancer* 85 (1), 41–45.
- Suber, T.L., Casciola-Rosen, L., Rosen, A., 2008. Mechanisms of disease: autoantigens as clues to the pathogenesis of myositis. *Nat. Clin. Pract. Rheumatol.* 4 (4), 201–209.
- Suwa, A., Hirakata, M., Takeda, Y., Okano, Y., Mimori, T., Inada, S., et al., 1996. Autoantibodies to DNA-dependent protein kinase. Probes for the catalytic subunit. *J. Clin. Invest.* 97 (6), 1417–1421.
- Targoff, I.N., Reichlin, M., 1985. The association between Mi-2 antibodies and dermatomyositis. *Arthritis Rheum.* 28 (7), 796–803.
- Tiniakou, E., Pinal-Fernandez, I., Lloyd, T.E., Albayda, J., Paik, J., Werner, J.L., et al., 2017. More severe disease and slower recovery in younger patients with anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase-associated autoimmune myopathy. *Rheumatology (Oxford)* 56 (5), 787–794.
- Touat, M., Maisonobe, T., Knauss, S., Ben Hadj Salem, O., Hervier, B., Aure, K., et al., 2018. Immune checkpoint inhibitor-related myositis and myocarditis in patients with cancer. *Neurology* 91, e985–e994. Epub ahead of print, PMID 30089619.
- Trallero-Araguas, E., Rodrigo-Pendas, J.A., Selva-O'Callaghan, A., Martinez-Gomez, X., Bosch, X., Labrador-Horillo, M., et al., 2012. Usefulness of anti-p155 autoantibody for diagnosing cancer-associated dermatomyositis: a systematic review and meta-analysis. *Arthritis Rheum.* 64 (2), 523–532.
- van der Helm-van Mil, A.H., Verpoort, K.N., Breedveld, F.C., Toes, R.E., Huizinga, T.W., 2005. Antibodies to citrullinated proteins and differences in clinical progression of rheumatoid arthritis. *Arthritis Res. Ther.* 7 (5), R949–R958.

# Immunoglobulin G4-Related Disease

John H. Stone<sup>1,2</sup>

<sup>1</sup>Harvard Medical School, Boston, MA, United States <sup>2</sup>Rheumatology Clinic, Massachusetts General Hospital, Boston, MA, United States

## OUTLINE

General Introduction	715	Clinical Features and Disease Associations	720
Historical Aspects	716	Lymphadenopathy	720
Epidemiology	716	Lacrimal and Salivary Glands	720
Pathology	716	Orbits	720
Histological Features	716	Ear/Nose/Throat	720
Immunostaining	717	Pancreas	721
Pathophysiology	717	Immunoglobulin G4-Related Sclerosing Cholangitis and Cholecystitis	721
B Cells	718	Lung	721
T Cells	718	Kidney	721
Serum Immunoglobulin G4 Concentrations	718	Thoracic Aorta and Coronary Lesions	722
Autoimmune Features	719	Chronic Periaortitis and Retroperitoneal Fibrosis	722
Does the Immunoglobulin G4 Molecule Participate in Autoimmunity?	719	Nervous System	722
Evidence for Autoimmunity in Immunoglobulin G4-Related Disease	719	Other Immunoglobulin G4-Related Disease Lesions	723
Genetics	719	Classification Criteria	723
An Animal Model	720	Treatment	723
	720	B Cell—Targeted Treatments	724
		Costimulatory Blockade	724
		CD4+ Cytotoxic T Lymphocytes	724
		Perspectives	725
		References	725

## GENERAL INTRODUCTION

Immunoglobulin G4-related disease (IgG4-RD) is a multiorgan immune-mediated condition recognized only within the past 15 years to be a distinct disorder (Stone et al., 2012; Mahajan et al., 2014). The expanding knowledge of this disease has linked numerous conditions once regarded as isolated, single-organ diseases, including “Küttner’s tumor” (bilateral submandibular gland enlargement), autoimmune pancreatitis (AIP), retroperitoneal fibrosis (RPF), Riedel’s thyroiditis, and many more. Moreover, understanding the immune dysregulation

associated with IgG4-RD has provided important insights into the processes of B and T lymphocyte cross talk, the impact of B cell–directed therapies on the T-cell compartment, and fibrosis (Perugino et al., 2017).

Observations of restricted repertoires of both plasmablasts and of a particular CD4+ cytotoxic T lymphocyte (CD4+ CTL) suggest that IgG4-RD is an antigen-driven disease (Mattoo et al., 2014a; Mattoo et al., 2016). Galectin-3 has recently been identified as a candidate antigenic trigger in approximately 30% of patients with this disease in one cohort (Perugino et al., 2019). It appears likely that multiple antigens can trigger this disease, but the precise identities of other disease-associated antigens remain unknown.

## HISTORICAL ASPECTS

Although “discovered” only in the early years of this century, IgG4-RD is clearly not a new disease. Mikulicz appears to have described the first case in the medical literature in 1892, when he wrote of a 44-year-old German farmer with massive enlargements of lacrimal, parotid, and submandibular glands (Mikulicz, 1892). The disorder termed “multifocal fibrosclerosis” in the 1960s is also clearly part of the spectrum of what we now consider to be IgG4-RD (Comings et al., 1967). The concept of AIP was also formulated in the 1960s (Sarles et al., 1961). In 2001 Japanese investigators reported the relationship between “sclerosing pancreatitis” and high serum concentrations of IgG4 (Hamano et al., 2001). In 2003 other Japanese investigators recognized that the histopathological findings characteristic of sclerosing pancreatitis (i.e., type 1 AIP in the current parlance) could also be found in extrapancreatic organs such as the submandibular glands and lungs (Kamisawa et al., 2003a, b). Between 2003 and the present time, IgG4-RD was described in virtually every organ and the concept of a multiorgan inflammatory disease characterized by histopathological features consistent across all organs was established (Kamisawa et al., 2015).

## EPIDEMIOLOGY

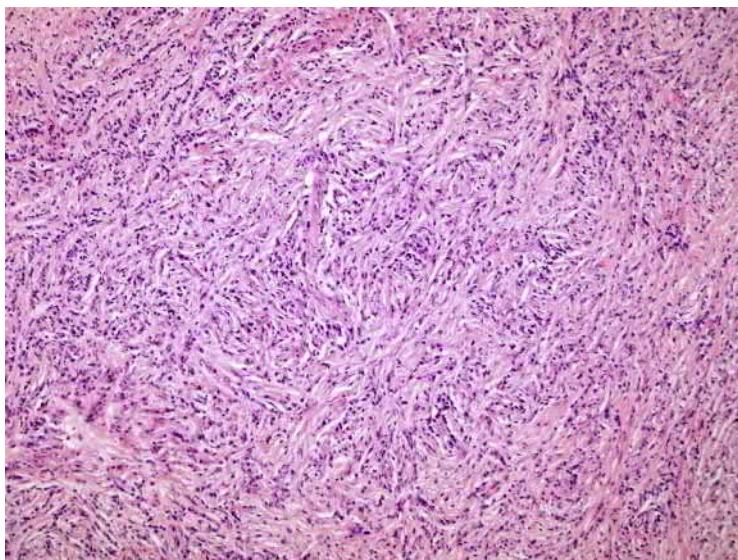
The typical patient with IgG4-RD is a middle-aged to elderly male. In AIP the mean age at diagnosis is on the order of 67 years, and the male:female ratio is approximately 3:1 (Kanno et al., 2012). When all types of IgG4-RD organ involvement are considered, however, this ratio may be closer to 3:2. Although the disease is somewhat less common in women—in contrast to the situation with classic autoimmune diseases—it does not appear to be less severe in women (Wallace et al., 2015).

IgG4-RD was recognized first in the pancreas. The overall prevalence of type 1 (IgG4-related) AIP was estimated to be 2.2 cases per 100,000 population in Japan (Kanno et al., 2012), but because the study was conducted only shortly after the first publications about the disease, this figure clearly underestimates the true prevalence of AIP. Moreover, the pancreas is only one of more than a dozen organs affected by IgG4-RD. Although likely still an orphan disease, IgG4-RD appears to be more common than autoimmune conditions such as vasculitis associated with antineutrophil cytoplasmic antibodies and systemic sclerosis. IgG4-RD has a clinical phenotype that is now increasingly recognized as the knowledge of this condition grows. This is emphasized by the recently completed 2018 Classification Criteria for IgG4-RD, a worldwide effort funded by the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) (Wallace et al., in press) (see below). The numbers of cases recognized and reported will undoubtedly grow substantially in the near future.

## PATHOLOGY

### Histological Features

The hallmark morphologic findings of IgG4-RD include a dense lymphoplasmacytic infiltrate rich in IgG4+ plasma cells (Deshpande et al., 2012). This lymphoplasmacytic infiltrate is enmeshed by an irregularly whorled fibrotic process that is termed “storiform” fibrosis (Fig. 38.1). Obliterative phlebitis, resulting in destruction of the venous lumen, is another characteristic lesion observed in the majority of cases. Obliterative arteritis is also observed in some organs, particularly the lung. Eosinophilic infiltration occurs in approximately 50% of cases, regardless of the organ involved.



**FIGURE 38.1** Lymphoplasmacytic infiltrate with storiform fibrosis. This pancreatic tissue has a swirling pattern, characteristic of the type of fibrosis that is classic for IgG4-related disease. IgG4, Immunoglobulin G4.

### Immunostaining

The preponderance of plasma cells within tissue lesions stain for IgG4. Even in the presence of an increase in the number of IgG4+ plasma cells/high power field (hpf) and an elevated IgG4+/IgG+ plasma cell ratio, however, careful clinicopathologic correlation and sound clinical judgment must be exercised before rendering an IgG4-RD diagnosis. Neither the number of IgG4+ plasma cells per hpf nor the IgG4+/IgG+ plasma cell ratio is specific for the diagnosis of IgG4-RD.

The IgG4+/IgG+ plasma cell ratio can also be a useful metric to aid in diagnosis. Most cases of documented IgG4-RD have an IgG4+/IgG+ plasma cell ratio >40%. The IgG4+/IgG+ plasma cell ratio can be particularly helpful in the setting of advanced fibrosis (e.g., in RPF), when the paucity of cells makes large concentrations of IgG4+ plasma cells unlikely.

## PATHOPHYSIOLOGY

IgG4-RD involves antigen-driven interactions among cells of the B lymphocyte lineage (plasmablasts, activated B cells) and at least two CD4+ T lymphocytes, and CD4+ T follicular helper cells and a CD4+ CTL (Perugino et al., 2017). The CD4+ CTL is currently regarded as the linchpin of this disease. These CD4+ CTLs are clonally restricted, abundant in IgG4-RD tissues, and likely contribute to fibrosis.

At present, activated effector CD4+ CTLs have been identified as the dominant T-cell driving IgG4-RD (Mattoo et al., 2016). CD4+ CTLs often represent up to 80% of all infiltrating CD4+ T cells within tissues affected by IgG4-RD and appear to be universally present among IgG4-RD patients. These cells secrete several profibrotic cytokines, including interleukin (IL)-1 $\beta$ , transforming growth factor-beta (TGF- $\beta$ ), and interferon-gamma (IFN- $\gamma$ ). Moreover, they express perforin and granzymes, and their cytolytic capacity has been demonstrated in vitro using Epstein-Barr virus (EBV)-transformed B cells.

This CD4+ CTLs may contribute to fibrosis *in situ* by multiple mechanisms, including profibrotic cytokine secretion and via the induction of apoptosis in targeted cells. Because B-cell depleting therapy with the anti-CD20 monoclonal antibody, rituximab, leads to both profound clinical responses and to declines in CD4+ CTLs, a plausible hypothesis is that activated B cells drive the activation of CD4+ CTLs at the sites of disease through antigen presentation (Perugino et al., 2017).

SLAMF7 (Signaling Lymphocytic Activation Molecule Family), an antigen previously known to be expressed only on cells of the B lymphocyte lineage, is also found on CD4+ CTLs in IgG4-RD. SLAMF7 is one of a family of six surface receptors expressed by hematopoietic cells. Nonlymphoid cell types lack SLAMF7 expression altogether (Hsi et al., 2008). SLAM receptors act via homotypic interactions; that is, SLAMF7 on one cell binds to SLAMF7 on another. It is conceivable that such homotypic SLAMF7 interactions between infiltrating B and T cells in IgG4-RD are critical to the disease process.

The effect on the cell of binding SLAMF7 depends on the type of intracellular adaptor molecule expressed. For example, natural killer (NK) cells express Ewing's sarcoma-associated transcript 2 (EAT-2), which mediates intracellular calcium influx via phospholipase C- $\gamma$ , resulting in granule polarization, enhanced exocytosis, and NK cell activation (Pérez-Quintero et al., 2014).

## B Cells

B cells and the cells of their lineage play an important role in IgG4-RD (Mattoo et al., 2014a; Wallace et al., 2014). These cells play a variety of roles in IgG4-RD, including the production of IgG4 (albeit the centrality of IgG4 to disease pathophysiology is debated); B cells and plasmablasts are speculated to play important roles in antigen presentation to T cells. The cells making most of the serum IgG4 are short-lived plasmablasts and plasma cells. Plasmablasts are found in high concentrations in IgG4-RD, regardless of the serum IgG4 concentration (Mattoo et al., 2014a; Wallace et al., 2014). Both total plasmablasts and IgG4+ plasmablasts are useful biomarkers for disease activity in IgG4-RD.

## T Cells

CD4+ T cells are typically dispersed throughout localized IgG4-RD lesions and are the most abundant cell found within affected tissues. Next-generation sequencing studies of the effector T-cell V $\beta$  repertoire have identified a CD4+ CTL that is believed to be the linchpin of IgG4-RD. Four strong pieces of evidence support CD4+ CTLs as principal drivers of IgG4-RD. These cells (1) undergo large clonal expansions; (2) infiltrate tissues affected by the disease in large numbers; (3) actively secrete cytokines in these tissues, indicating recent antigen activation; and (4) decline with rituximab-induced disease remission (Mattoo et al., 2016; Maehara et al., 2017).

The gene signature of the CD4+ CTL found in IgG4-RD consists of both cytolytic (e.g., granzyme, granulysin, and perforin) and myeloid (e.g., IL-1 $\beta$ ) features. In addition, these cells were found to secrete two other profibrotic cytokines, TGF- $\beta$ , and IFN- $\gamma$ .

One appealing way to link the findings pertaining to both the B- and T-cell lineages is to presume that the CD4+ T cells orchestrating the disease are sustained by continuous antigen presentation by B cells. A T-follicular helper cell response that is separate from the CD4+ CTLs is likely to be responsible for the development of germinal centers within lymph nodes (and involved organs) (Maehara et al., 2018). In theory, such T-follicular helper cells could produce the cytokines (e.g., IL-4) that drive the IgG4 class-switch, culminating in the creation of IgG4-secreting plasmablasts and long-lived plasma cells. The fact that B-cell depletion does not lead to the complete normalization of serum IgG4 concentrations implies the presence of long-lived plasma cells that continue to make this immunoglobulin.

## SERUM IMMUNOGLOBULIN G4 CONCENTRATIONS

Serum IgG4 concentrations are known to increase over the professional lives of beekeepers, presumably as a guard against anaphylaxis (Aalberse et al., 2009). The same observations have been made in patients treated with desensitizing immunotherapy, following which the induction of IgG4 responses corresponds to reduced allergic symptoms. For these reasons, IgG4 has traditionally been considered a noninflammatory, perhaps antiinflammatory, and antibody, especially in conditions mediated by IgE.

Nevertheless, serum IgG4 measurements play an important role in the diagnosis and management of IgG4-RD, and there remains a possibility that IgG4 contributes to the pathophysiology of tissue injury in this disease either directly or indirectly. Patients with IgG4-RD can have elevations of all IgG subclasses, but the elevations of IgG4 are usually disproportionate to those of IgG1, -2, and -3. Patients with multiorgan disease can have dramatic elevations in serum IgG4 concentrations, occasionally exceeding 4 mg/dL.

Large elevations of serum IgG4 concentrations, for example, on the order of six to eight times, the upper limit of normal for a given assay strongly suggests the diagnosis but are not confirmatory in and of themselves.

---

## AUTOIMMUNE FEATURES

---

### Does the Immunoglobulin G4 Molecule Participate in Autoimmunity?

IgG4 normally accounts for approximately 4% of the total immunoglobulins among healthy individuals and is usually the least abundant of all of the subclasses. Despite the substantial elevations in serum IgG4 concentrations observed in most patients with IgG4-RD, IgG4 itself is unlikely to drive the pathogenesis of this disorder. IgG4 demonstrates weak binding to C1q and to Fc-gamma receptors because of a critical few amino acid differences in its CH2 domain (Bruhns et al., 2009; Vidarsson et al., 2014). The ability of IgG4 to activate the classical complement pathway and participate in antibody-dependent cell-mediated cytotoxicity is therefore substantially less than that of other IgG subclasses, for example, IgG1 (Vidarsson et al., 2014).

IgG4 also has the unique ability to form “half-antibodies” through the process of Fab-arm exchange. Fab-arm exchanges result in the formation of IgG molecules with two different binding specificities (Vidarsson et al., 2014). Amino acid variation at the hinge region of IgG4 permits the reduction of the disulfide bonds that join the two halves of an IgG4 molecule. Recombination of the dissociated arms leads to the random formation of “asymmetric antibodies” composed of half-antibody fragments directed against different antigens (Bruhns et al., 2009; Vidarsson et al., 2014). The consequence is a reduced ability to cross-link antigens and form immune complexes. IgG4 may therefore play the role of a noninflammatory “antigen sink,” the purpose of which is to “mop up” antigen through its monovalent binding in a process that tends to downregulate inflammation.

It remains possible that a “disease-causing” IgG fraction produced by a subset of antibody-secreting cells contributes to tissue injury, perhaps through immune complexes or Fc-receptor engagement. There are certain diseases, for example, pemphigus vulgaris and idiopathic membranous glomerulonephropathy, in which IgG4 antibodies themselves appear to be pathogenic. Many IgG4-RD patients have concurrent elevations in other IgG subclasses (e.g., IgG1) that are more likely than IgG4 to form immune complexes, engage Fc receptors, and incite or participate in an ongoing immune response.

### Evidence for Autoimmunity in Immunoglobulin G4-Related Disease

Single-cell-sorted plasmablasts from patients with IgG4-RD, cloned and used to express monoclonal antibodies from dominantly expanded plasmablasts, are self-reactive to eukaryotic cells (Perugini et al., 2019). The passive transfer model of purified human IgG to neonatal mice also suggests autoreactivity (Shiokawa et al., 2016). These observations provided reasonably compelling evidence that IgG4-RD might be driven by a self-antigen. More recently, one such an antigen has been identified.

#### **Galectin-3**

Antigalectin-3 autoantibodies are present in a sizeable subset of IgG4-RD patients. These autoantibodies are predominantly of the IgG4 subclass and correlate with galectin-3 levels in plasma, suggesting that overexpression of this protein leads to a breach of immune tolerance for this autoantigen. The findings also suggest that the marked elevations of circulating IgG4 and IgE observed in this disease are, at least in part, due to the development of IgG4- and IgE-specific autoantibody responses. These findings were based on a series of experiments summarized below.

Using single-cell clones of dominantly expanded plasmablasts sorted from a patient with IgG4-RD, recombinant human monoclonal antibodies were generated. Paired heavy- and light-chain cDNAs from the top two dominant clones, identified by identical heavy-chain variable/diversity/joining gene segments (V) (VDJ), light-chain variable/joining gene segments (VJ), and predicted complementarity determining region 3 (CDR3) amino acid sequences, were expressed as monoclonal antibodies. The monoclonal antibodies were then used to purify relevant antigens from a pancreatic cell line using immunoaffinity chromatography. Mass spectrometry was used to identify the affinity-purified antigens of interest, and findings were validated by enzyme immunoassay.

The two most frequent clones accounted for 77% of all the sorted cells. The two monoclonal antibodies expressed from these dominant clones stained human pancreatic tissue sections and permeabilized pancreatic cancer cell lines. Galectin-3 was identified as the antigen, specifically recognized by both antibodies. Finally, antigalectin-3 autoantibody responses were identified in the plasma of nearly 40% of IgG4-RD patients in a large cohort. Total 28% of the antibodies were of the IgG4 isotype and 11% were IgE. Responses involving IgG1, IgG2, or IgG3 isotypes were not observed.

## GENETICS

Human leukocyte antigen (HLA) associations and other potential genetic links of IgG4-RD remain incompletely characterized. Familial cases are very rare, but detailed genetic studies have not yet been undertaken in populations of sufficient size.

## AN ANIMAL MODEL

An animal model that recapitulates some aspects of human IgG4-RD has been developed in mice (Shiokawa et al., 2016). Purified IgG subclass molecules from the serum of patients with IgG4-RD and controls were transferred to neonatal mice, inducing pathologic changes in both the pancreas and submandibular glands. Transfer of either purified IgG1 or IgG4 alone produced the disease phenotype, yet the transfer of both molecules together led to reduced IgG1 deposition and a decrease in the histopathologic severity of disease within affected organs.

The induction of disease by IgG4 transfer in this model appears contrary to the concept of IgG4 as an antiinflammatory molecule. It is possible, however, that a subset of IgG4 antibodies not subject to Fab-arm exchange might have been responsible for the findings observed (the extent to which Fab-arm exchange occurs *in vivo* is unknown). It is also possible that under some conditions, IgG4 participates in the formation of immune complexes, which contribute to tissue injury. For example, there is clear evidence of IgG4 immune complex deposition in IgG4-related tubulointerstitial nephritis (TIN) and some suggestion that a similar process contributes to IgG4-related AIP.

## CLINICAL FEATURES AND DISEASE ASSOCIATIONS

### Lymphadenopathy

The lymphadenopathy associated with IgG4-RD is typically either a generalized lymphadenopathy or localized disease adjacent to an affected organ. The lymph nodes involved are generally 1–3 cm in diameter and non-tender. The disease does not have a predilection for any particular set of lymph nodes. Establishing the diagnosis of IgG4-RD through lymph node biopsy is often difficult because it is unusual for lymph nodes to undergo the degree of fibrosis observed in other organs. In addition, the histopathology of lymph nodes in IgG4-RD spans a broad range of findings. One form, for example, strongly resembles the lymph node findings in Castleman's disease.

### Lacrimal and Salivary Glands

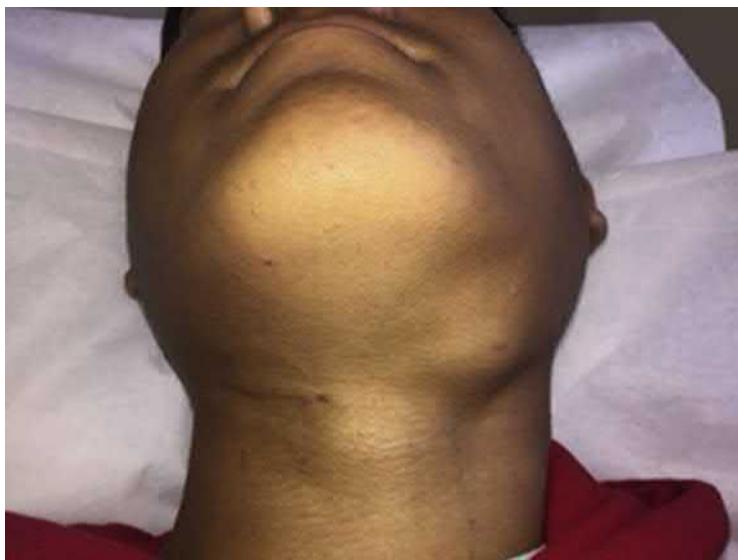
Lacrimal gland enlargement (dacryoadenitis) is the most common feature of ophthalmic disease in IgG4-RD. For more than 100 years, the triad of dacryoadenitis and enlargement of both the parotid and submandibular glands was referred to as "Mikulicz' disease" (Fig. 38.2). It is now known to be a classic feature of many patients with IgG4-RD. Fine-needle aspirates of major salivary glands are useful for excluding malignancy, but excisional biopsies are usually required for diagnostic confirmation.

### Orbits

Proptosis can result from the combinations of orbital lesions, such as pseudotumors that do not affect the lacrimal gland and inflammation and thickening of the extraocular muscles involvement.

### Ear/Nose/Throat

Mild-to-moderate peripheral eosinophilia and serum IgE concentration elevations that sometimes exceed 10 times the upper limit of normal are not unusual in IgG4-RD. Allergic rhinitis, nasal polyps, chronic sinusitis, nasal obstruction, and rhinorrhea are common in IgG4-RD. A subset of nonatopic IgG4-RD patients has peripheral blood eosinophilia and elevated IgE levels (Della Torre et al., 2014). This suggests that processes inherent to IgG4-RD rather than to atopy per se contribute to the eosinophilia and IgE elevation. Circulating



**FIGURE 38.2** Nontender enlargement of the left submandibular gland in a patient with IgG4-related sialadenitis. The right submandibular gland has been removed for diagnostic purposes. IgG4, Immunoglobulin G4.

T helper cell 2 (Th2) memory cells are not generally detected in IgG4-RD but can be found in IgG4-RD patients with preexisting histories of atopy (Mattoo et al., 2014b).

Mass lesions can occur in the sinuses and destructive lesions in the middle ear and facial bones have been reported. IgG4-RD can also lead to diffuse inflammation in the pharynx, hypopharynx, and Waldeyer's ring.

## Pancreas

Type 1 AIP, which demonstrates the classic histopathological findings of lymphoplasmacytic sclerosing pancreatitis, is a major complication of IgG4-RD. The most common clinical presentation of type 1 AIP is obstructive jaundice, induced by concomitant IgG4-related sclerosing cholangitis. Secondary diabetes mellitus commonly results, but exocrine pancreatic failure leading to massive weight loss is an even bigger problem. Computed tomographic features of AIP include diffuse pancreatic enlargement with delayed enhancement and a capsule-like low-density rim (Fig. 38.3). Diffuse, irregular narrowing of the main pancreatic duct on endoscopic retrograde, and magnetic resonance cholangiopancreatography is also highly specific for AIP.

## Immunoglobulin G4-Related Sclerosing Cholangitis and Cholecystitis

IgG4-related sclerosing cholangitis frequently occurs with type 1 AIP. IgG4-related sclerosing cholangitis must be differentiated from both primary sclerosing cholangitis and hilar cholangiocarcinoma. Neither serum IgG4 concentrations nor cholangiographic or cholangioscopic findings differentiate these diseases from each other with clarity. Moreover, the superficial nature of endoscopic biopsies limits their utility for diagnosing IgG4-related sclerosing cholangitis.

## Lung

IgG4-RD demonstrates multiple findings in the lung. Thickening of the bronchovascular bundle, best appreciated on computed tomographic examination, underscores the tendency of IgG4-RD to track along bronchi and blood vessels, which course together. Other radiologic features of IgG4-RD include pulmonary nodules, ground-glass opacities, pleural thickening, and interstitial lung disease. The interstitial lung disease mimics nonspecific interstitial pneumonitis and other forms of interstitial fibrosis.

## Kidney

TIN is the most characteristic form of IgG4-related kidney disease. Profound hypocomplementemia is often found in patients with IgG4-related TIN. The basis of this hypocomplementemia remains poorly understood, but it could result from the formation of immune complexes that contain IgG1 or IgG3, subclasses that bind



**FIGURE 38.3** Dacryoadenitis (inflammation and enlargement of the right lacrimal gland).

complement more effectively and are often elevated to a lesser degree. IgG4 appears unlikely to explain the hypocomplementemia by itself because the molecule does not bind complement effectively in most settings.

CT scanning in IgG4-related TIN can reveal substantial renal enlargement and hypodense lesions within the renal parenchyma. Advanced renal dysfunction and even end-stage renal disease can result. Proteinuria can develop, but the levels are usually subnephrotic. Kidneys affected by IgG4-RD can undergo atrophy, even in the setting of good clinical responses to therapy.

Membranous glomerulonephropathy also occurs in IgG4-RD, perhaps through a different pathophysiology. The anti-phospholipase A2 receptor antibody now linked to “idiopathic” membranous glomerulonephropathy in a high percentage of cases is not found in IgG4-related membranous glomerulonephropathy, even though that antibody is generally an IgG4 molecule.

### Thoracic Aorta and Coronary Lesions

IgG4-related aortitis, often suspected on the basis of an incidental radiological finding, is also sometimes an unexpected finding at surgery. IgG4-related aortitis can lead to aneurysms or dissections in the thoracic aorta. Coronary artery lesions in IgG4-RD, sometimes associated with aneurysm formation, are documented. Definitive histopathological investigations of primary aortic branch vessels have not been undertaken.

### Chronic Periaortitis and Retroperitoneal Fibrosis

IgG4-RD is responsible for up to two-thirds of the cases of “idiopathic,” RPF which is now considered to belong to a larger disease spectrum known as chronic periaortitis. The three major components of chronic periaortitis are IgG4-related RPF, IgG4-related abdominal aortitis, and IgG4-related perianeurysmal fibrosis.

IgG4-related RPF and chronic periaortitis commonly present with poorly localized pain in the back, flanks, lower abdomen, or thighs; lower extremity edema; and hydronephrosis from ureteral involvement. The disease targets three sites: (1) periaortic/arterial regions, involving connective tissue around the abdominal aorta or its first branches (Fig 38.4); (2) periureteral areas, leading to ureteral obstruction and hydronephrosis; and (3) a plaque-like mass that broadly involves the retroperitoneum.

### Nervous System

Involvement of the brain parenchyma by IgG4-RD is extremely rare, but this condition is among the most common causes of “idiopathic” hypertrophic pachymeningitis. IgG4-RD is also an established cause of hypophysitis. IgG4-related hypophysitis can lead to hormone deficiencies from both the anterior and posterior pituitary. Magnetic resonance imaging (MRI) shows sellar enlargement and thickening of the pituitary stalk. Peripheral



**FIGURE 38.4** Type 1 (IgG4-related) autoimmune pancreatitis. Computed tomographic study showing an enlarged, sausage-shaped pancreas that has lost its normal markings. IgG4, Immunoglobulin G4.

nerve lesions in the area of the orbit also occur commonly, typically observed on MRI in the absence of overt clinical manifestations. The trigeminal and infraorbital nerves are affected most commonly.

### Other Immunoglobulin G4-Related Disease Lesions

The clinical entity long known as “Riedel’s thyroiditis” is in fact IgG4-RD of the thyroid gland. Riedel’s thyroiditis is emblematic of organ manifestations of IgG4-RD that tend to present with overwhelmingly fibrotic lesions, described back in the 1960s as “multicentric fibrosclerosis” (Comings et al., 1967). Sclerosing lesions of both the mesentery and mediastinum are described. Sclerosing mesenteritis often appears to originate at the mesenteric root. The ensuing process often merges imperceptibly with RPF and sometimes encases vital organs and blood vessels, making surgical resection difficult or impossible. Fibrosing mediastinitis can lead to the compression of vital mediastinal structures from proliferation of invasive fibrous tissue. The relationship between these cases and antecedent histoplasmosis infections, if any, remains unclear.

Several clinical presentations of IgG4-related skin disease have been reported. The most common cutaneous manifestation is the presence of erythematous papules. These lesions typically affect the region of the head and neck but have also been described on the trunk and extremities. Biopsy-proven IgG4-related prostatic disease leading to prostatic enlargement has been reported. The diagnosis of IgG4-related prostate disease is often made presumptively when the initiation of treatment for IgG4-RD in other organs mediates abrupt symptomatic relief of “benign prostatic hyper trophy.”

## CLASSIFICATION CRITERIA

The 2018 ACR/EULAR Classification Criteria for IgG4-RD incorporate clinical, serological, radiological, and pathological features of IgG4-RD (Wallace et al., 2018). These criteria, which feature both exclusion and inclusion criteria, have a specificity of 98% and a sensitivity of 83%.

## TREATMENT

Glucocorticoids are currently the first-line treatment for IgG4-RD. Most data on the use of glucocorticoids are derived from the treatment of AIP. Treatment of IgG4-RD with glucocorticoids typically results in a symptomatic response within 2 weeks and the absence of clinically evident disease within 2–3 months (Khosroshahi et al., 2015). Unfortunately, many patients relapse as glucocorticoids are tapered or discontinued, and the optimal approach to remission maintenance remains unclear (Khosroshahi et al., 2015). The consequences of prolonged or

recurrent glucocorticoid treatment regimens are substantial in this disease that tends to affect a middle-aged to elderly population and often targets the pancreas.

## B Cell–Targeted Treatments

Anti-CD20-targeted therapy is an effective approach to treating IgG4-RD ([Khosroshahi et al., 2010; Carruthers et al., 2015](#)). Rituximab is an appealing alternative to glucocorticoids both for the induction and maintenance of remission in IgG4-RD. B-cell depletion leads to swift clinical improvement and steady declines in serum IgG4 concentrations over a period of a year or longer. Blood concentrations of the SLAMF7 + CD4+ CTL also decline overtime. Clinical responses have a variable duration from months to sometimes greater than a year, but relapses are the rule for most patients.

XmAb5871 is a novel molecule with an antigen-binding site specific for CD19, thereby also targeting cells of the B-cell lineage. The mechanism of XmAb5871 differs, however, from the antibody-dependent cellular cytotoxicity (ADCC) mechanism of anti-CD20-directed therapy. XmAb5871 capitalizes upon the natural inhibitory mechanism of Fc $\gamma$ RIIb, the only Fc receptor expressed by B cells, which acts as a negative regulator in conditions of antigen excess and immune complex formation ([Chu et al., 2014](#)). When Fc $\gamma$ RIIb is colligated with CD19, B-cell function is inhibited and plasmablasts are rendered dormant.

Preliminary analyses of this approach are promising and this medication is now being tested in a large phase 3 trial.

## Costimulatory Blockade

Abatacept, a fusion protein composed of the extracellular domain of CTL-associated protein 4 and the IgG1 constant domain, ameliorates autoimmune disease by binding to CD80/86 on antigen-presenting cells, thereby interfering with ligation to the costimulatory CD28 molecule on T cells. This treatment approach has been reported effective in a small number of patients and is now being tested in a larger phase 2 study.

## CD4+ Cytotoxic T Lymphocytes

CD4+ CTLs have strong appeal as a potential therapeutic target. Elotuzumab, an immunostimulatory mAb directed against SLAMF7, is approved for the treatment of refractory multiple myeloma. The proposed mode of action of elotuzumab is via the activation of NK cells and resultant ADCC of the SLAMF7-expressing cellular target.

**TABLE 38.1** Names of Previously Recognized Conditions Now Classified Frequently as Immunoglobulin G4 (IgG4)-Related Disease

---

Autoimmune pancreatitis (lymphoplasmacytic sclerosing pancreatitis)
Sclerosing cholangitis (a subset is in fact IgG4-related sclerosing cholangitis)
Mikulicz's disease (affecting the salivary and lacrimal glands)
Küttner's tumor (affecting the submandibular glands)
Multifocal fibrosclerosis (commonly affecting the orbits, thyroid gland, retroperitoneum, mediastinum, and other tissues/organs)
Riedel's thyroiditis
Inflammatory pseudotumor (orbits, lungs, kidneys, and other organs)
Mediastinal fibrosis
Retroperitoneal fibrosis
Sclerosing mesenteritis
Periaortitis/periarteritis
Inflammatory aortic aneurysm
Hypertrophic pachymeningitis
Eosinophilic angiocentric fibrosis (affecting the orbits and upper respiratory tract)
Idiopathic hypocomplementemic tubulointerstitial nephritis with extensive tubulointerstitial deposits

---

## PERSPECTIVES

IgG4-RD was recognized as a discrete disease entity only during the first years of this century, yet a remarkable amount has been learned about this condition in a short period of time. Its generally slow-moving course makes it an excellent disorder in which to study human immunology from a clinical and translational perspective. The ability to bring clinical samples from well-characterized patients together with cutting-edge laboratory techniques may lead to substantial progress in the next few years, with major implications for processes such as fibrosis and diseases beyond the IgG4-RD spectrum (Table 38.1).

## References

- Alberse, R.C., Stapel, S.O., Schuurman, J., Rispens, T., 2009. Immunoglobulin G4: an odd antibody. *Clin. Exp. Allergy* 39, 469–477.
- Bruhns, P., Iannascoli, B., England, P., Mancardi, D.A., Fernandez, N., Jorieux, S., et al., 2009. Specificity and affinity of human Fc $\gamma$  receptors and their polymorphic variants for human IgG subclasses. *Blood* 113, 3716–3725.
- Carruthers, M.N., Topazian, M.D., Khosroshahi, A., Witzig, T.E., Wallace, Z.S., Hart, P.H., et al., 2015. Rituximab for IgG4-related disease: a prospective, open-label trial. *Ann. Rheum. Dis.* 74, 1171–1177.
- Chu, S.Y., Yeter, K., Kotha, R., Pong, E., Miranda, Y., Phung, S., et al., 2014. Suppression of rheumatoid arthritis B-cells by XmAb5871, an anti-CD19 antibody that coengages B-cell antigen receptor complex and Fc $\gamma$  receptor IIb inhibitory receptor. *Arthritis Rheumatol.* 66, 1153–1164.
- Comings, D.E., Skubi, K.B., Van Eyes, J., Motulsky, A.G., 1967. Familial multifocal fibrosclerosis. Findings suggesting that retroperitoneal fibrosis, mediastinal fibrosis, sclerosing cholangitis, Riedel's thyroiditis, and pseudotumor of the orbit may be different manifestations of a single disease. *Ann. Intern. Med.* 66 (5), 884–892.
- Della Torre, E., Mattoo, H., Mahajan, V.S., Carruthers, M., Pillai, S., Stone, J.H., 2014. Prevalence of atopy, eosinophilia, and IgE elevation in IgG4-related disease. *Allergy* 69, 269–272.
- Deshpande, V., Zen, Y., et al., 2012. Consensus statement on the pathology of IgG4-related disease. *Mod. Pathol.* 25, 1181–1192.
- Hamano, H., Kawa, S., Horiuchi, A., Unno, H., Furuya, N., Akamatsu, T., et al., 2001. High serum IgG4 concentrations in patients with sclerosing pancreatitis. *N. Engl. J. Med.* 344 (10), 732–738.
- Hsi, E.D., et al., 2008. CS1, a potential new therapeutic antibody target for the treatment of multiple myeloma. *Clin. Cancer Res.* 14, 2775–2784.
- Kamisawa, T., Funata, N., Hayashi, Y., et al., 2003a. A new clinicopathological entity of IgG4-related autoimmune disease. *J. Gastroenterol.* 38, 982–984.
- Kamisawa, T., Egawa, N., Nakajima, H., 2003b. Autoimmune pancreatitis is a systemic autoimmune disease. *Am. J. Gastroenterol.* 98, 2811–2812.
- Kamisawa, T., Zen, Y., Pillai, S., Stone, J.H., 2015. IgG4-related disease. *Lancet* 385, 1460–1471.
- Kanno, A., Nishimori, I., Masamune, A., the Research Committee on Intractable Diseases of Pancreas, et al., 2012. Nationwide epidemiological survey of autoimmune pancreatitis in Japan. *Pancreas* 41, 835–839.
- Khosroshahi, A., Bloch, D., Deshpande, V., Stone, J.H., 2010. Rituximab therapy leads to rapid decline of serum IgG4 levels and prompt clinical improvement in IgG-related systemic disease. *Arthritis Rheum.* 62, 1755–1762.
- Khosroshahi, A., Wallace, Z.A., Crow, J.L., Akamizu, T., Azumi, A., Carruthers, M.N., et al., 2015. International consensus guidance statement on the management and treatment of IgG4-related disease. *Arthritis Rheum.* 67, 1688–1699.
- Maehara, T., Mattoo, H., Ohta, M., Mahajan, V., Moriyama, M., Yamauchi, M., et al., 2017. Lesional CD4+ IFN- $\gamma$  + cytotoxic T lymphocytes in IgG4-related dacryoadenitis and sialoadenitis. *Ann. Rheum. Dis.* 76, 377–385.
- Maehara, T., Mattoo, H., Mahajan, V., Ishiguro, N., Ohta, M., Moriyama, M., et al., 2018. The expansion in lymphoid organs of IL-4 + BATF + T follicular helper cells is linked to IgG4 class switching in vivo. *Ann. Rheum. Dis.* 1, e201800050.
- Mahajan, V.S., Mattoo, H., Deshpande, V., Pillai, S.S., Stone, J.H., 2014. IgG4-related disease. *Annu. Rev. Pathol.* 9, 315–347.
- Mattoo, H., Mahajan, V.S., Maehara, T., Deshpande, V., Della-Torre, E., Wallace, Z.S., et al., 2016. Clonal expansion of CD4(+) cytotoxic T lymphocytes in patients with IgG4-related disease. *J. Allergy Clin. Immunol.* 138 (3), 825–838.
- Mattoo, H., Mahajan, V.S., Della-Torre, E., et al., 2014a. De novo oligoclonal expansions of circulating plasmablasts in active and relapsing IgG4-related disease. *J. Allergy Clin. Immunol.* Available from: <https://doi.org/10.1016/j.jaci.2014.03.034>. Published online May 6.
- Mattoo, H., Della-Torre, E., Mahajan, V., Stone, J., Pillai, S., 2014b. Circulating Th2 memory cells in IgG4 Related Disease are restricted to a defined subset of subjects with atopy. *Allergy* 69, 399–402.
- Mikulicz, J., 1892. Über eine eigenartige symmetrische erkrankung der tränen und mundspeicheldrüsen. Stuttgart: Beitr. z. Chir. Festschr. f. Theodor Billroth. pp. 610–630.
- Pérez-Quintero, L.-A., Roncagalli, R., Guo, H., Latour, S., Davidson, D., Veillette, A., 2014. EAT-2, a SAP-like adaptor, controls NK cell activation through phospholipase C $\gamma$ , Ca $^{++}$ , and Erk, leading to granule polarization. *J. Exp. Med.* 211 (4), 727–742.
- Perugino, C.A., AlSalem, S.B., Mattoo, H., Della-Torre, E., Mahajan, V., Ganesh, G., et al., 2019. Identification of galectin-3 as an autoantigen in patients with IgG4-related disease. *J. Allergy Clin. Immunol.* 143 (2), 736–745.e6. Available from: <https://doi.org/10.1016/j.jaci.2018.05.011>.
- Perugino, C.A., Mattoo, H., Mahajan, V.S., Maehara, T., Wallace, Z.S., Pillai, S., et al., 2017. IgG4-related disease: insights into human immunology and targeted therapies. *Arthritis Rheumatol.* 69 (9), 1722–1732.
- Sarles, H., Sarles, J.-C., Muratore, R., Guien, C., 1961. Chronic inflammatory sclerosis of the pancreas: an autonomous pancreatic disease? *Am. J. Dig. Dis.* 6, 688–698.
- Shiokawa, M., Kodama, Y., Kuriyama, K., Yoshimura, K., Tomono, T., Morita, T., et al., 2016. Pathogenicity of IgG in patients with IgG4-related disease. *Gut* 65, 1322–1332.

- Stone, J.H., Zen, Y., Deshpande, V., 2012. IgG4-related disease. *N. Engl. J. Med.* 366, 539–551.
- Vidarsson, G., Dekkers, G., Rispens, T., 2014. IgG subclasses and allotypes: from structure to effector functions. *Front. Immunol* 5, 520.
- Wallace, Z.S., Mattoo, H., Carruthers, M.N., et al., 2014. Plasmablasts as a biomarker for IgG4-related disease, independent of serum IgG4 concentrations. *Ann. Rheum. Dis.* Available from: <https://doi.org/10.1136/annrheumdis-2014-205233>. published online May 9.
- Wallace, Z.S., Deshpande, V., Mattoo, H., Mahajan, V., Kulikova, M., Pillai, S., et al., 2015. IgG4-related disease. clinical and laboratory features in one hundred twenty-five patients. *Arthritis Rheum.* 67, 2466–2475.
- Wallace, Z.S., Naden, R.P., Choi, H., Dellatorre, E., Dicaire, J.-F., Hart, P., et al. The 2019 ACR/EULAR classification criteria of IgG4-RD (in press).

## Polyendocrine Syndromes

Pärt Peterson<sup>1</sup> and Eystein S. Husebye<sup>2,3</sup>

<sup>1</sup>Molecular Pathology, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia

<sup>2</sup>Department of Clinical Science, K.G. Jebsen Center for Autoimmune Disorders, University of Bergen, Bergen, Norway <sup>3</sup>Department of Medicine, Haukeland University Hospital, Bergen, Norway

### O U T L I N E

Historic Background	732	Spontaneous Animal Models	739
Clinical, Pathologic, and Epidemiologic Features	732	Thymectomy Animal Model	739
Autoimmune Polyendocrine Syndrome Type 1	732	Pathogenic Mechanisms	740
Autoimmune Polyendocrine Syndrome Type 2	734	Immunologic Markers in Diagnosis	741
Autoimmune Features	735	Treatment and Outcome	741
Genetic Features	736	Concluding Remarks—Future Prospects	742
Environmental Features	738	Acknowledgment	742
Animal Models	739	References	742
Aire-Deficient Mouse as a Model for Autoimmune Polyendocrine Syndrome Type 1	739		

Autoimmune polyendocrinopathies denote syndromes characterized by immune-mediated destruction of two or more endocrine glands. Often many nonendocrine organs and tissues are also affected. They can be broadly divided into the ultrarare monogenic autoimmune polyendocrine syndrome (APS) type 1 (APS-1) and the common APS type 2 (APS-2) with complex inheritance. The former is caused by mutations in the *Autoimmune Regulator (AIRE)* gene, and the latter is associated with certain major histocompatibility complex genotypes and variants in a range of other genes, most of which are involved in the adaptive and innate immune system. In both conditions numerous autoantibodies can be detected, which correlates with clinical components, and can be utilized for diagnosis and prognosis.

Recent years have provided significant progress in our knowledge about disease genes and immunopathology of APS. We have come to appreciate that AIRE has a dual role in the thymus, both to promote expression of tissue-specific antigens and to facilitate the generation of a specific subset of regulatory T cells (Tregs). Moreover, several autoantibodies with unique specificities that can be utilized for diagnostic purposes have been identified.

In this overview, we summarize recent developments in the understanding of APS-1 and APS-2, and how these findings translate into improved diagnosis and management.

## HISTORIC BACKGROUND

In 1855 Thomas Addison published observations linking the clinical features of “general languor and disability, feebleness of the heart’s action” and hyperpigmentation of the skin to disease in the suprarenal capsules (adrenals) (Addison, 1855). This disorder has since been known as Addison’s disease (Wilks, 1862). Previously Schmidt (1926) noticed the propensity for polyendocrinopathy by demonstrating lymphocytic infiltration in both the adrenal and thyroid glands (Schmidt syndrome, OMIM 269200); later, Carpenter et al. (1964) added insulin-dependent diabetes mellitus to this list. The first clinical reports on patients with Addison’s disease presenting with chronic *Candida* infection and hypoparathyroidism appeared in the 1950s (Hetzl and Robson, 1958), although Thorpe and Handley (1929) reported a case with hypoparathyroidism and candidiasis. In the 1960s several reports pointed to the clinical and genetic heterogeneity among patients with Addison’s disease (Blizzard and Gibbs, 1968; Spinner et al., 1968), proposing that, depending on the associated disorders, Addison’s disease could be part of two separate and distinct clinical syndromes; APS types 1 and 2 (Neufeld et al., 1981). In addition, APS type 3 was defined as a disorder with autoimmune thyroid disease combined with polyglandular disorders.

A major breakthrough in separating APS-1 and APS-2 was the cloning and characterization of the AIRE gene in 1997 (Finnish-German-Consortium, 1997; Nagamine et al., 1997), which has helped to uncover how central immunological tolerance is developed and how it is compromised in autoimmune diseases. Subsequent use of animal models and advances in genomics and single-cell technologies have helped researchers to unravel many of the molecular mechanisms involved in the break of tolerance, summarized in this chapter.

## CLINICAL, PATHOLOGIC, AND EPIDEMIOLOGIC FEATURES

### Autoimmune Polyendocrine Syndrome Type 1

APS-1 (OMIM 240300) has a broad spectrum of clinical manifestations (Table 39.1, Fig. 39.1). The disease is also referred to as autoimmune–polyendocrinopathy–candidiasis–ectodermal dystrophy or polyglandular autoimmune syndrome type 1. The most frequent clinical entities include chronic mucocutaneous candidiasis (CMC), hypoparathyroidism, and Addison’s disease. Traditionally, two of the three major components should be present in order to make the diagnosis clinically. However, a phenotypic variability and delay in appearance of these main manifestations for many years make the diagnosis difficult in many instances. Typically, APS-1 starts in childhood with CMC as the first manifestation followed by hypoparathyroidism and Addison’s disease (Ahonen et al., 1990; Orlova et al., 2017). As more patient cohorts have been published, it has become apparent that the phenotypic variation is large, even within the same family. Some patients develop manifestations late and display only a few components and never develop two of the main components (Perheentupa, 2002, 2006). Others are severely sick and can harbor more than 10 manifestations. Some of the minor components such as periodic fever with rash, nail dystrophy, and keratoconjunctivitis can be the first to appear and may not be recognized as APS-1 (Ferre et al., 2016).

The majority of patients develop CMC either with intermittent bouts of infection or more as a chronic infection with white thrush on the tongue. The infection may spread to nails and in some cases even to skin of the hands and face. Candidal esophagitis is often present but asymptomatic until stricture and dysphagia develops. Several cases of carcinoma in the oral cavity and esophagus have been reported, suggesting that oral candidiasis might be carcinogenic, especially in the combination with cigarette smoking (Perheentupa, 2002). CMC is associated with reduced numbers of IL-22 and IL-17F-producing T cells and the presence of autoantibodies against the Th17 cell mediators IL-17F, IL-17A, and IL-22 (Kisand et al., 2010; Puel et al., 2010), which seem to be the main cause for the increased propensity for CMC. In addition, patient-derived anti-IL-22 increased the susceptibility to CMC in mouse model (Bichele et al., 2017). APS-1 patients were also shown to have reduced expression of STAT1 in peripheral blood (Zimmerman et al., 2017).

Hypoparathyroidism is the most frequent and sometimes only endocrine component in APS-1 (Li et al., 2017) followed by adrenal insufficiency seen in about half of the patients (Meloni et al., 2012; Perheentupa, 2006; Wolff et al., 2007). Gonadal insufficiency is very common in females (female/male ratio, 3:1) and manifests itself in teenage and early twenties (Reato et al., 2011). Other endocrinopathies in falling frequencies are type 1 diabetes,

**TABLE 39.1** Manifestations and Organ-Specific Autoantibodies in APS-1

Main disease component		Autoantigen	Characteristics/Prevalence
APS-1			
Main triad	Hypoparathyroidism	IFN $\omega$ , IFN $\alpha$	Almost all patients are positive
	Addison's disease	NALP5	70%–80%, childhood
	Candidiasis	P450c21	65%–85%, childhood
Other endocrine manifestations	Ovarian failure	IL-22, IL-17F, IL-17A P450scc, P450c17, NALP5	80%–100%, childhood 60%
	Testicular failure	TSGA10	Adult prevalence up to 60%
	Autoimmune thyroid disease	TPO, TG	~10%, relatively rare
	Type 1 diabetes	IA-2	0%–25%
	Hypophysitis	TDRD6	Rare
Gastrointestinal	Malabsorption	TPH	Common, diarrhea, severe constipation, fatty stools
	Autoimmune gastritis		10%–30%
	Autoimmune hepatitis	AADC, CYP1A2	5%–20%, childhood, severe sometimes fatal
	Exocrine pancreatitis	n.d.	Rare
Hematological	Hypo/asplenia		10%–20%, risk of septicemia
	Hypergammaglobulinemia		5%
	Pure red cell anemia		Rare
Nephrological	Mineralocorticoid excess with hypertension		Rare
	Interstitial nephritis		Rare
Lung	Bronchiolitis obliterans	KCNRG	Rare, can be fatal
Ectodermal	Keratoconjunctivitis	BPIFB1	Prevalence approx. 10% may result in blindness
	Retinitis		Rare
	Alopecia	SOX9	Common, prevalence 30%–40%
	Vitiligo	TH	Prevalence ~20%
	Periodic rash with fever		Rare
	Squamous cell carcinoma		Associated with candidosis and smoking, can be fatal
	Enamel dysplasia		Prevalence 50%–70%
Muscular, neurological	Metaphysial dysplasia		Rare
	Polyarthritits		Rare
	Myopathy		Rare
	Neuropathy		Rare
	Ptosis		Rare

AADC, Aromatic L-amino acid decarboxylase; APS, autoimmune polyendocrine syndrome; IFN $\alpha$ , interferon alfa; IFN $\omega$ , interferon omega; IA-2, islet antigen-2; NALP5, NACHT-LLR-PYD-containing domain 5; KCNRG, putative potassium channel regulatory protein; SOX9, sex-determining region Y-box 9; TDRD6, tudor domain-containing 6; TG, thyroglobulin; TH, tyrosine hydroxylase; TPH, tryptophan hydroxylase; TPO, thyroperoxidase; TSGA10, testis-specific gene 10 protein.



**FIGURE 39.1** Clinical manifestations of autoimmune polyendocrine syndrome type 1. Carpal spasm caused by hypocalcemia (Trousseau's sign; upper left); vitiligo (middle right); enamel hypoplasia (middle left); *Candida* infection of the nail (middle right); alopecia areata; candidiasis of the tongue. Courtesy of Dr. Martina Erichsen.

autoimmune thyroid disease and hypophysitis. Many patients suffer from gastrointestinal autoimmunity, notably enteropathy with malabsorption and/or obstipation, hepatitis, autoimmune gastritis with and without vitamin B12 and iron deficiencies, and exocrine pancreatitis.

APS-1 patients often display a number of organ-specific lesions affecting ectodermal structures, namely enamel dysplasia, vitiligo, alopecia, keratoconjunctivitis, and nail dystrophy. Hyposplenism or splenic atrophy has been reported (Perheentupa, 2002, 2006), which render them susceptible to serious bacterial infections. Hypertension and increased mineralocorticoid sensitivity (Ahonen et al., 1990), interstitial nephritis (Landegren et al., 2016a), pneumonitis (Alimohammadi et al., 2009), and pure red cell aplasia are rare but serious complications (Orlova et al., 2017). Finally, a number of rare components associated to APS-1 have recently been reported, including retinitis (Orlova et al., 2010), lipodystrophy (Sorkina et al., 2016), ptosis (Orlova et al., 2017), polyarthritis (Gutierrez et al., 2017), peripheral neuropathy (Valenzise et al., 2009), and metaphyseal dysplasia (Harris et al., 2003).

On average each patient has about 4–5 different diseases, but up to 20 have been reported. While CMC, hypoparathyroidism, periodic fever with rash, and keratitis commence early, other manifestations like autoimmune thyroid disease, vitamin B12 deficiency, testicular failure, interstitial nephritis, and hyposplenism usually appear in adult age (Ferre et al., 2016; Orlova et al., 2017).

## Autoimmune Polyendocrine Syndrome Type 2

APS-2 is a genetically complex disease with a multifactorial etiology. The clinical onset usually occurs in adulthood, although it can start at any time during the life span, though seldom before puberty. APS-2 affects more often middle-aged Caucasian women with a male/female ratio of 1:2–3. The classical definition of APS-2 (Neufeld et al., 1981) comprises a combination of Addison's disease with autoimmune thyroid disease and/or

type 1 diabetes. Probably any combinations of autoimmune disorders within the complex represent the same disorder with an inherited propensity to develop organ-specific autoimmunity (Schatz and Winter, 2002). Type 1 diabetes can develop before and after Addison's disease (Erichsen et al., 2009). In common with APS-1, manifestations such as vitiligo, alopecia, hypergonadotropic hypogonadism, and autoimmune gastritis with or without pernicious anemia are prevalent although less common and usually not present in childhood.

At least half of the patients with autoimmune Addison's disease develop another autoimmune disorder (Betterle and Morlin, 2011; Dalin et al., 2017; Erichsen et al., 2010). Using the prevalence numbers for Addison's disease, which are most recently found to be between 16 and 22 per 100,000 inhabitants (Olafsson and Sigurjonsdottir, 2016), the prevalence of APS-2 is about 8–11 per 100,000 inhabitants. A recent analysis of heritability using the Swedish twin registry found Addison's disease to have an exceptionally high heritability, even higher than celiac disease (Skov et al., 2017). Interestingly, when monozygotic twins developed APS-2, the second organ-specific manifestation was often the same, as opposed to dizygotic twins where they often differed. Unfortunately, studies of epidemiology and inheritance in other populations are largely lacking.

## AUTOIMMUNE FEATURES

Each of the major organ manifestations in APS-1 and APS-2 has corresponding autoantibodies correlating to the presence of their particular components. Thus reactivities to thyroid peroxidase, thyroglobulin, and the TSH receptor are typical for autoimmune thyroid disease. In type 1 diabetes the main autoantigens are glutamic acid decarboxylase-65 (GAD65), islet antigen-2 (IA-2), and the beta-cell-specific zinc transporter Zn8 (ZnT8). In Addison's disease the steroid 21-hydroxylase (P450c21 or CYP21) (Winqvist et al., 1992) is the major autoantigen with similar frequencies of positivity in APS-1 and APS-2 patients (Bruserud et al., 2016; Erichsen et al., 2009; Eriksson et al., 2017). The main autoantigen in hypoparathyroidism was identified as NALP5, a protein highly expressed in the parathyroid gland (Alimohammadi et al., 2008). Autoantibodies against gonadal tissues target side-chain cleavage enzyme (P450ccc or CYP11A1) and steroid 17-alpha-hydroxylase (P450c17 or CYP17) (Brozzetti et al., 2010a; Krohn et al., 1992; Reato et al., 2011; Uibo et al., 1994; Winqvist et al., 1993, 1995). It remains perplexing as to why certain diseases of APS coexist. Initially antigenic epitope sharing in the affected glands was thought to provide an explanation for the involvement of certain organs, but with the identification of large number of autoantigens, other mechanisms interrupting immune tolerance to specific organs should be considered.

Autoantibodies to several other organ-specific self-antigens have been reported in APS-1 (Table 39.1). One group is of decarboxylases and hydroxylases involved in neurotransmitter biosynthesis. Examples are autoantibodies against aromatic L-amino acid decarboxylase correlated to type 2 autoimmune hepatitis (Husebye et al., 1997), tryptophan hydroxylase connected to autoimmune enteropathy (Ekwall et al., 1998), and tyrosine hydroxylases correlated to alopecia (Hedstrand et al., 2000). Patients also react with glutamic acid decarboxylase 2 (GAD2/GAD65) (Skoldberg et al., 2004; Soderbergh et al., 2004) and with GAD1/GAD67 (Tuomi et al., 1996). The other group is of cytochrome P450 enzymes, the steroidogenic enzymes mentioned above. In addition, autoantibodies against CYP1A2 are associated with autoimmune hepatitis in APS-1 (Gebre-Medhin et al., 1997).

While these established autoimmune biomarkers were identified with the traditional techniques of Western blot, screening of expression libraries or by the use of bioinformatics (ZnT8), several novel autoantigens have recently been identified by the screening of protein arrays (Fishman et al., 2017; Landegren et al., 2016b). These studies have confirmed many, but not all the traditional autoantigens, a discrepancy that can be explained by differences in the techniques that affect exposure of epitopes. Among novel antigens identified by protein array screening is transglutaminase-4 (TGM4), a prostate-specific autoantigen. TGM4 is the first sex-specific autoantigen since females normally do not express this protein (Landegren et al., 2015). Furthermore, the reactivity toward a number of cancer-testis autoantigens was found, including PDILT and different MAGE proteins (Fishman et al., 2017; Landegren et al., 2016b). Another interesting finding was the large spectrum of autoantigens identified and that only a handful of patients reacted with a particular protein (Fishman et al., 2017; Meyer et al., 2016). In addition to AIRE-regulated tissue-specific transcripts, the genes for many of the identified autoantigens were not regulated by AIRE (Fishman et al., 2017). From those, many were intracellular phosphoproteins and proteins expressed in lymphoid cells. Although the patient sample was limited, there was a trend to have more autoantibodies with time and more autoantibodies if there was a nonsense mutation as opposed to a missense mutation (Fishman et al., 2017).

In addition to the organ-specific autoantibodies, APS-1 patients display autoantibodies to a number of interferons and interleukins. These are targeting type 1 interferon alpha and omega (IFN- $\alpha$  and IFN- $\omega$ ) IL-17A, IL-17F, and IL-22 (Meager et al., 2006; Kisand et al., 2010). Autoantibodies to various isoforms of IFN- $\alpha$  and IFN- $\omega$  are present in almost all patients, are neutralizing, occur in extremely high titers up to 1–10<sup>6</sup>, are detected at very early stage of APS-1, and persist for decades. Such high frequencies of positivity render assay of IFN- $\alpha$  and IFN- $\omega$  antibodies a very useful tool to identify patients with APS-1 (Meloni et al., 2008; Oftedal et al., 2008). Anti-IFN- $\alpha$  antibodies-reduced interferon-stimulated gene expression in vivo and high levels of these autoantibodies were related to the protection against type 1 diabetes (Kisand et al., 2008; Meyer et al., 2016). Other interleukins are targeted albeit at lower frequencies including IFN- $\lambda$ 1, -2, -3, IL-5, IL-6, and IL-32 (Karner et al., 2016; Meyer et al., 2016). The prevalent autoantibodies against interleukins IL-17A, IL-17F, and IL-22 were found to correlate with the presence of candidiasis (Kisand et al., 2010; Puel et al., 2010), although the mechanism by which these autoantibodies could trigger CMC remains elusive.

Type 1 interferon autoantibodies are highly specific but not entirely exclusive for APS-1 as they are also found in patients with myasthenia gravis, particularly in association with the late-onset form of thymoma (Meager et al., 2006). Recently patients with mild RAG mutations and Omenn syndrome were found to display such autoantibodies (Walter et al., 2016). There is also a considerable overlap between APS-1 and APS-2 for some of the organ-specific autoantibodies, in particular autoantibodies to P450c21, P450c17, and P450scc (Betterle et al., 2002; Hoek et al., 1997; Soderbergh et al., 2004). APS-2 patients with type 1 diabetes frequently have autoantibodies to GAD65 and/or IA-2 protein, while anti-GAD65 autoantibodies do not correlate to type 1 diabetes in APS-1 patients; instead an association to malabsorption (Soderbergh et al., 2004) and vitiligo was reported (Fishman et al., 2017). Taken together these new discoveries have provided clinicians with a useful toolkit of assays to diagnose both APS-1 and APS-2, with high sensitivity and specificity.

## GENETIC FEATURES

APS-1 is inherited in an autosomal recessive manner and occurs as a defect in the *AIRE* gene. The prevalence worldwide is very low and population dependent. The frequency is higher among certain populations such as Finns (1/25,000) (Aaltonen et al., 1994), Persian Jews (1/9000) (Zlotogora and Shapiro, 1992), and among Sardinians (1/14,400) (Rosatelli et al., 1998). The prevalence, reported from Norway at 1/80,000 (Wolff et al., 2007), probably reflects the frequency in most countries.

*AIRE* was identified by positional cloning on chromosome 21q22.3 (Finnish-German-Consortium, 1997; Nagamine et al., 1997), and to date, more than 120 mutations (<http://www.hgmd.cf.ac.uk/ac/gene.php?gene=AIRE> and about 500 genetic variants ([https://www.ncbi.nlm.nih.gov/SNP/snp\\_ref.cgi?locusId=326](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=326)) have been identified. The mutations are spread throughout the coding region with two mutational hotspots. From these, the most common mutation is R257X in exon 6, which is found in 83% of the Finnish APS-1 chromosomes (Björres et al., 2000) but is also common in APS-1 patients of other ethnic origins (Scott et al., 1998). Another frequently occurring mutation is 967-979del13bp, which has been reported in patients from different populations (Wang et al., 1998; Pearce et al., 1998; Heino et al., 1999; Proust-Lemoine et al., 2010). Furthermore, R139X mutation is common among Sardinian (Rosatelli et al., 1998), R203X among Sicilian (Giordano et al., 2011), and Y85C among Persian Jews (Björres et al., 2000; Heino et al., 1999; Pearce et al., 1998; Wang et al., 1998). Many *AIRE* mutations are nonsense mutations or deletion/insertions leading to nonsense-regulated mRNA decay and no protein translation (Huang and Wilkinson, 2012). Most of the missense mutations occur in the N-terminus of *AIRE*, a region responsible for the homodimerization and correct intracellular localization of the protein. Recent reports on the critical role of NF- $\kappa$ B containing enhancer in regulation of *AIRE* expression (Haljasorg et al., 2015; LaFlam et al., 2015) suggest that the mutations in this upstream regulatory region may be involved in APS-1.

APS-1 patients have considerable phenotypic variation but no clear correlation with the genotype. Among Iranian Jews, the Y85C mutation has much lower frequencies of candidiasis and Addison's disease than other cohorts (Zlotogora and Shapiro, 1992). Moreover, in a study of 160 Finnish patients with the three most prevalent mutations, candidiasis was found more often in patients with homozygous R139X and R257X mutations (i.e., truncating *AIRE* before SAND domain) than with homozygous 967-979del13bp mutation (Kisand et al., 2010; Kisand and Peterson, 2011).

APS-1 was until recently considered to be mainly an autosomal recessive disease requiring two disease-causing mutation in order to elicit clinical manifestations. Recent studies have revealed certain heterozygous

missense mutations giving rise to APS-1 with dominant inheritance (Oftedal et al., 2015). Most of these mutations are located in the first plant homeodomain (PHD1) and in two cases, in the SAND domain (Cetani et al., 2001; Abbot et al., 2017). In many cases, these patients display milder disease with clinical overlap with APS-2 and isolated organ-specific autoimmune disease. Intriguingly, PHD1 mutations seem to predispose for vitamin B12 deficiency/pernicious anemia and vitiligo while the SAND-domain mutation G228W has a propensity for autoimmune thyroid disease (Cetani et al., 2001). Based on the frequencies at around 0.001 of these variants in public data bases, the dominant nonclassic APS-1 can be much more common than we believe.

APS-1 association with Human leukocyte antigen (HLA) has remained controversial, although some components of the disease seem to correlate with certain HLA alleles (Halonen et al., 2002; Gylling et al., 2003). In contrast to monogenic inheritance of APS-1, APS-2 is a polygenic disease. As for most autoimmune diseases, APS-2 is strongly associated with the HLA gene locus in chromosome 6p21 (Table 39.2). Genetic studies have demonstrated a consistent association of APS-2 with HLA DRB1\*03:01 (DR3) DQA1\*05:01 DQB1\*02:01 (DQ2) and DRB1\*04:01 (DR4) DQA1\*03:01 DQB1\*03:02 (DQ8) haplotypes, which both are in strong linkage equilibrium with each other (Falorni et al., 2008; Flesch et al., 2014; MacLaren and Riley, 1986; Partanen et al., 1994). The haplotype is associated with isolated disease components of APS-2; type 1 diabetes, Graves' disease, autoimmune

**TABLE 39.2** Autoimmune Polyendocrine Syndrome Type 2-Associated HLA Alleles and Non-HLA Genes

Genes	Details	Reference
MHC class II	DR3-DQ2 DR4(0404)-DQ8	Antigen presentation; DR3/DR4(0404) heterozygotes have OR ~30 for AAD
	DQ5	Protective; OR 0.4 for AAD
MHC class I	HLA-B8	Antigen presentation, DR3-B8 haplotype associated with familial AAD
	HLA-B15 (protective)	Protective for progression to overt AAD
Other genes in the MHC region	MICA5.1	Involved in NK and T-cell activation, OR 18 for homozygotes with AAD
	21-Hydroxylase	Steroidogenic enzyme and main autoantigen in AAD; effect could be secondary due to LD
	Tumor necrosis factor	Inflammatory cytokine; effect could be secondary due to LD
Non-MHC genes	CTLA-4	Involved in downregulation of T-cell responses; A to G SNP at position 49; 3' untranslated region microsatellite
	PTPN22	Involved in T-cell activation; AAD associate to the 1858T allele and rare variants
	NALP1	Involved in inflammatory responses; OR 1.15 for AAD
	CLEC16A	C-type lectin of unknown function; OR 0.76 for AAD
	CIITA	Govern MHC class II expression; OR 1.7 for AAD
	PD-LI	Downregulate cytokine production and T-cell responses (CTLA-4/CD28 family of costimulatory receptors); OR 1.33 for AAD
	CYP27B1	25 hydroxyvitamin D3-1-alpha hydroxylase; OR 1.1-1.7 for AAD
	FCRL3	Immune regulatory; OR 1.6 for AAD
	BACH2	OR 2.01 for AAD

AAD, Autoimmune Addison's disease; CIITA, class II, major histocompatibility complex, transactivator; CLEC16A, C-type lectin domain family 16; CTLA-4, cytotoxic T lymphocyte antigen 4; CYP27B1, 25 hydroxyvitamin D3-1-alpha hydroxylase; FCRL3, Fc receptor like 3; HLA, human leukocyte antigen; MHC, major histocompatibility complex; MICA5.1, major histocompatibility complex class I chain-related MIC-A polymorphisms in exon 5; NALP1, NACHT-LRR-PYD-containing protein 1; OR, odds ratio; PTPN22, protein tyrosine phosphatase, nonreceptor type 22.

hypothyroidism, and Addison's disease. The increased risk of DR4 was attributed to DRB\*0404 in Addison's disease, and not DR4\*0401 in variance with type 1 diabetes (Erichsen et al., 2009; Falorni et al., 2008; Gambelunghe et al., 2005). The association was strongest for the DRB1 locus alleles DRB1\*03:01 and DRB1\*04:04 (Skinningsrud et al., 2010). Conditioning for DRB1-indicated independent effects of HLA-B and MICA5.1 (Falorni et al., 2008; Skinningsrud et al., 2010), a positive association to HLA-B8 (Baker et al., 2010), while HLA-B15 was not protective to the formation of P450c21 antibodies, but to the progression to overt disease (Baker et al., 2011). Conversely, the haplotypes DRB1\*01 (DR1) DQA1\*01:01 DQB1\*05:01 (DQ5), DRB1\*13:01 DQB1\*06:03 DQA1\*01:03, DRB1\*13:02 DQB1\*06:04 DQA1\*01:02, and DRB1\*07 DQB1\*02:01 DQA1\*02:01 are protective against Addison's disease (Erichsen et al., 2009). Alleles of other genes located in HLA region, such as TNF and P450c21 have been associated with APS-2 (Falorni et al., 2008; Partanen et al., 1994; Peterson et al., 1995), but these associations could be secondary to the strong linkage disequilibrium in the HLA region.

The cytotoxic T-lymphocyte-associated protein 4 (*CTLA-4*) gene on chromosome 2q33 is associated with autoimmune endocrinopathies from different population (Brozzetti et al., 2010b). In particular the allelic variants of an A–G single-nucleotide polymorphism at position 49 (*rs231775*) and microsatellite marker in the 3' untranslated region of the *CTLA-4* gene are well known to associate with type 1 diabetes and thyroiditis (Levin and Tomer, 2003). These two variants of the *CTLA-4* gene as well as *rs231727* are increased among Addison's disease and APS-2 patients of European origins (Brozzetti et al., 2010b; Kemp et al., 1998; Wolff et al., 2015). The associated allelic variation was correlated with lower messenger RNA levels of the soluble alternative splice form of *CTLA-4* (Ueda et al., 2003); however, the mechanism by which *CTLA-4* confers susceptibility remains incompletely understood (Esensten et al., 2016).

In addition, a number of other susceptibility genes have been characterized in Addison's disease and APS-2 (Table 39.2). The majority of these associations are to genes involved in immunity and inflammation, and none of the associations are specific for Addison's disease or APS-2. These include PTPN22 (Rycroft et al., 2009; Skinningsrud et al., 2008a), where both association to the 1858T allele and to rare variants were found; NALP1 that could be involved in inflammation (Magitta et al., 2009); the class II, major histocompatibility complex transactivator (Ghaderi et al., 2006; Skinningsrud et al., 2008b), the programmed death ligand 1 (Mitchell et al., 2009), the lymphocyte cell surface molecule FCRL3 (Owen et al., 2007), and BACH2 (Eriksson et al., 2016).

An influence of *AIRE* gene polymorphisms in APS-2 or other major autoimmune diseases has been suggested. The *AIRE* gene was described as a susceptible locus for the predisposition to rheumatoid arthritis in the Japanese population (Terao et al., 2011), but neither *AIRE* mutations R257X and 967-979del13bp nor SNP analyses across the *AIRE* gene contributed to the susceptibility to type 1 diabetes, Graves' disease, autoimmune hepatitis, or Addison's disease (Boe Wolff et al., 2008; Meyer et al., 2001; Nithiyanthan et al., 2000; Turunen et al., 2006; Vaidya et al., 2000). Nevertheless, a meta-analysis of *rs2075876* and *rs760426* in the *AIRE* gene confirmed their association with an increased risk of rheumatoid arthritis among Asian populations (Bérczi et al., 2017).

## ENVIRONMENTAL FEATURES

The associations with environmental factors such as vitamin D, smoking, socioeconomic status, infections, or vaccines are less clear than with genetic factors (Anaya et al., 2016). An increase in the incidence of type 1 diabetes mellitus and other autoimmune endocrine diseases in most of the developed countries points to the novel environmental influences as the genetic background have largely remained unchanged. The role of environment has been best studied in patients with type 1 diabetes [see Chapter 41: Autoimmune (Type 1) Diabetes] in which analysis of twins shows that the genetic background cannot be the only reason for the disease (Hyttinen et al., 2003). National prosperity and good hygiene level seem to correlate in uncertain ways with type 1 diabetes (Patterson et al., 2001). The role of viral infections in polyendocrinopathies remains unclear, and no clear inciting agent has been identified. Studies on the interaction of the intestinal microbiome with the immune system showed that segmented filamentous bacteria seems to induce the generation of Th17 cells involved in the early phases of the immune response and linked to autoimmunity (Ivanov et al., 2009). Thus environmental factors could mediate their effect via the intestinal microbiome (see Chapter 25: Epigenetics of Autoimmune Diseases).

## ANIMAL MODELS

### Aire-Deficient Mouse as a Model for Autoimmune Polyendocrine Syndrome Type 1

Aire-deficient mice present the autoimmune features such as multiorgan lymphocytic infiltration and circulating autoantibodies (Anderson et al., 2002; Ramsey et al., 2002). The disease severity is dependent on mouse genetic background with C57Bl/6 having very mild and nonobese diabetes (NOD) mouse presenting exocrine (but no endocrine) pancreatitis with wasting disease. Studies on Aire mouse have been critical in elucidation of APS-1 pathogenesis and thymic tolerance (for review, see Anderson and Su, 2016). The studies in these experimental models have established that Aire promotes the expression of self-antigens in thymic medullary epithelial cells needed for efficient negative selection of autoreactive T cells (Anderson et al., 2005; Liston et al., 2003). Aire is also involved in the presentation of self-proteins (Kuroda et al., 2005) as it enhances the capacity of medullary thymic epithelial cells (mTECs) to cross-present antigen to thymic dendritic cells (Hubert et al., 2011; Koble and Kyewski, 2009) and has been implicated in the differentiation of mTECs (Nishikawa et al., 2010; Wang et al., 2012) and migration of thymocytes (Laan et al., 2009). More recent studies have shown defect in the neonatal output of Tregs (Yang et al., 2015), thymic  $\gamma\delta$  T cells (Fujikado et al., 2016) and B cells (Gavanescu et al., 2008). Although Aire-deficient mice have been central in understanding of AIRE function in thymic medullary epithelial cells, the mice are also at variance with APS-1 patients in several aspects, for example, the Aire-deficient mice do not share autoantibody targets seen in humans (Kekalainen et al., 2007a). Furthermore, none of the Aire-deficient mouse models spontaneously display the three main components of the APS-1, although autoimmunity toward liver, gastric mucosa, and ovaries are shared. Clearly, more human-like experimental models are needed to understand the pathologies in the patients.

### Spontaneous Animal Models

Most studied spontaneous animal models for polyendocrine autoimmunity are biobreeding (BB) rat and NOD mouse. These rodent models indicate that MHC genes are major genetic factors in disease development, and that other non-MHC genes are also involved. BB rat and NOD mouse also have impaired regulation of immune responses, including tolerance defects and abnormal function of Tregs. The NOD mouse is mostly studied as a model for type 1 diabetes [see Chapter 41: Autoimmune (Type 1) Diabetes]. The BB rat develops type 1 diabetes, lymphocytic infiltration in thyroid glands (Awata et al., 1995), and has autoantibodies to gastric parietal cells and smooth muscle (Crisa et al., 1992); however, no lymphocytic infiltration to adrenal gland has been observed. They have severe T-cell lymphopenia which is strongly associated with the diabetes development. The T-cell lymphopenia is due to a single-nucleotide deletion in *Gimap5* gene (Hornum et al., 2002; MacMurray et al., 2002). A number of breeds such as the standard poodle and Portuguese water dog are susceptible to the development of Addison's disease (Van Lanen and Sande, 2014).

### Thymectomy Animal Model

A well-studied mouse model for polyendocrinopathy is thymectomy at day 3 in the Balb/C strain, which leads to multiorgan autoimmunity characterized by gastritis, thyroiditis, oophoritis, and other infiltrations. The infiltrations are T-cell predominant, and the mice develop autoantibodies to affected tissues. Thymectomy-induced autoimmunity depends on time of thymectomy, that is, between the second and fifth day after birth (Bonomo et al., 1995). The lack of CD4 + CD25 + Tregs was proposed to cause autoimmunity, and the injection of purified Tregs into the thymectomized mice was used to prevent autoimmunity (Asano et al., 1996). However, in adult age, the Treg numbers in thymectomized mice are restored. Thymectomy causes lymphopenia in which development of autoimmune diabetes rather appears to be linked to effector and not to Tregs (Gagnerault et al., 2009), and in this respect, the prolonged neonatal lymphopenia and homeostatic expansion of peripheral T cells in Aire-deficient mouse resemble thymectomized model (Kisand et al., 2014). Further implication of this model in endocrine autoimmunity was demonstrated with NALP5 (MATER), an ovarian autoantigen in APS-1, which, when transgenically expressed in MHC class II cells in thymus and secondary lymphoid system, was sufficient to mediate a significant reduction in autoimmune oophoritis after thymectomy (Otsuka et al., 2011).

## PATHOGENIC MECHANISMS

The endocrine glands in APS are gradually destroyed, resulting in atrophy or parenchymal cells are replaced by fat cells (Perheentupa, 2002). Adrenalitis in APS patients appears to be diffuse, affecting all three layers of the cortex. As an indication of T-cell-mediated autoimmunity, it mainly consists of lymphocytes, but macrophages and plasma cells have also been detected (Carpenter et al., 1964; Muir et al., 1993). The infiltrating lymphocytes are of both B and T lineages, while the T-cell population includes both the CD4 + and CD8 + subsets. Strikingly, adjacent nontargeted tissue is spared and intact. At the end of the disease, atrophy and fibrosis become prominent in affected glands.

Only few studies have addressed the autoreactive T cells in APS patients, partly due to the lack of specific and sensitive methods, and to the fact that T cells in contrast to autoantibodies are sequestered in the specific tissue lesions and are thus difficult to access. Recombinant P450c21 or corresponding peptides have been demonstrated to elicit CD4 + or CD8 + T-cell responses; and CD8 + T cells could lyse 21-hydroxylase-expressing target cells (Bratland et al., 2009; Dawoodji et al., 2014). Autoantibodies have long been regarded as having a bystander role, yet they still could have an important role as amplifiers of the immune response toward the adrenal cortex. Increased serum levels of proinflammatory CXCL10 chemokine have been found in patients with isolated Addison's disease, APS-2 (Rotondi et al., 2005), and APS-1 (Kisand et al., 2008). The adrenocortical cell is likely to be an active participant in the autoimmune process as of itself it can produce CXCL10 in response to interferon gamma (Bratland et al., 2013; Rotondi et al., 2005).

APS-1 patients have an increased frequency of highly differentiated CD8 + effector T cells (Laakso et al., 2011). Although the specificity of these cells is unknown they may represent autoreactive population (Arstila and Jarva, 2013). CD4 + T-cell numbers and proportion of memory or activated T cells have been reported to be increased or normal (Heikkilä et al., 2016; Perniola et al., 2005; Tuovinen et al., 2009) and controversial results have been found on iNKT and monocyte cell numbers (Hong et al., 2009; Lindh et al., 2010; Perniola et al., 2008). IL-17F and IL-22 responses are lower, while IL-17A responses are normal (Kisand et al., 2010; Laakso et al., 2014; Ng et al., 2010). Most consistent findings in APS-1 patients are in Treg compartment. Tregs in APS-1 patients are decreased in number, have impaired function, and have decreased expression of FoxP3 (Kekäläinen et al., 2007b; Ryan et al., 2008; Wolff et al., 2010). TCR repertoire spectratyping of CD4 + and CD8 + T cells did not reveal abnormalities in CDR3 length (Niemi et al., 2015); however, longer CDR3 was found in naive but not in activated/memory Tregs, suggesting failure of thymic selection (Koivula et al., 2017). In addition, dysregulated responses to commensal antigens suggest a failure in intestinal tolerance and defect in Treg functions (Hatemaki et al., 2016).

Autoimmunity in APS-1 has been postulated, based on studies in Aire-deficient mice, to be a consequence of defective central tolerance to the autoantigens. The lack of AIRE expression in thymus may thus lead to a defect in clonal deletion of autoreactive T cells and ultimately to autoimmunity (Liston et al., 2003). However, direct data are lacking on the clonal deletion in human APS-1 patients. In contrast, a growing number of studies on Aire-deficient mouse and APS-1 patients point to the defective selection or function of Treg. A decrease in the number of Tregs of thymic origin is evident already in the neonatal period (Yang et al., 2015), and lower numbers have been also reported in adult mice (Malchow et al., 2013). Aire seems to affect a certain subset of Treg clones as their development is Aire dependent (Malchow et al., 2013), whereas most frequent Treg clones seem to be independent of Aire (Perry et al., 2014). A scenario where Aire deficiency may cause the diversion of Tregs into pathogenic conventional T cells has been proposed (Malchow et al., 2016).

The organs targeted in APS-1 patients are limited, and the syndrome typically starts with CMC, followed by hypoparathyroidism and Addison's disease indicating the abovementioned phenotypic differences between human patients and Aire mouse models. Whereas the candidiasis is associated with the decrease in Th17 responses and early generation of anti-IL-17 and IL-22 autoantibodies, the reason for the high prevalence of autoimmune hypoparathyroidism and Addison's disease remains unclear. The autoantibodies against type 1 IFN and Th17 cytokines in APS-1 have implications similar to those of thymoma patients where autoantibodies to type 1 IFN and other cytokines have also been found. The defects in central T-cell tolerance in APS-1 provoke wide-ranging deviation of B-cell tolerance (Meyer et al., 2016); nevertheless, the mechanism how AIRE mutations affect B-cell tolerance and how much T- and B-cell reactivities overlap remains elusive. An active autoimmunization process within the thymus tissue has been proposed to occur in both APS-1 and thymoma patients as both diseases are associated with thymus dysfunction (Kisand et al., 2011; Meager et al., 2008) and share similarities in

autoantibody profiles (Wolff et al., 2014). According to this hypothesis, the thymus in AIRE deficiency exports abnormal and already activated T cells capable of helping autoantibody production of B cells (Kisand and Peterson, 2011).

## IMMUNOLOGIC MARKERS IN DIAGNOSIS

Autoantibodies are often diagnostic or even predictive markers for the clinical disease. The presence of high titer and persistent anti-IFN- $\alpha$  and  $\omega$  autoantibodies in almost 100% of APS-1 patients provides an easy, early, and cost-efficient way to screen for APS-1. Positive tests should be confirmed by sequencing AIRE gene since these antibodies can be found in other conditions.

In addition, autoantibodies to the steroidogenic enzymes (P450c21, P450c17, and P450scc) are significant and specific markers for onset of Addison's disease. Antibodies against at least one of the three antigens are found in 84% of the patients with Addison's disease (Brozzetti et al., 2010a; Soderbergh et al., 2004). Furthermore, hypogonadism is well associated with the presence of autoantibodies to P450scc protein and recently against NALP5 (Brozzetti et al., 2015). For type 1 diabetes, autoantibodies against GAD65, IA-2, and Zn8T are used. The presence of anti-P450c21 antibodies in patients with autoimmune thyroid disease or type 1 diabetes, but without Addison's disease (Barker et al., 2005), would indicate risk for later development of overt Addison's disease, although the progression to overt Addison's disease may take years. Among relatives with organs-specific autoimmunity, individuals with normal adrenal function and P450c21 autoantibodies, about 15%, progressed to adrenal failure within 5 years (Coco et al., 2006).

## TREATMENT AND OUTCOME

In patients with APS-1, anticandidal drugs such as amphotericin B, ketoconazole, or fluconazole are used in treatment of the candidiasis. Itraconazole has been reported to be effective for nail candidiasis; however, 4–6-month treatment is needed to eliminate the infection (Rautemaa et al., 2007; Rautemaa et al., 2008a; Rautemaa et al., 2008b). Clinical follow-up of oral candidal infection is needed at least once or twice per year, and, because of risk of cancer, attention should be paid to suppression of the oral infection. Smokers are at particular risk.

Replacement therapy with hormones has been efficiently used for Addison's disease and hypoparathyroidism. Patients with Addison's disease are treated with hydrocortisone at the smallest dose that relieves symptoms, usually 10 + 5 + 5 mg a day (the corresponding doses of cortisone acetate is 12.5 + 6.25 + 6.25 mg). A dual release formulation of hydrocortisone is now available, given once daily. Fludrocortisone is used to replace aldosterone, 0.05–0.02 mg once daily.

The therapy of hypoparathyroidism is aimed at maintaining normal calcium levels, and serum calcium and phosphate levels should be monitored regularly. Therapies are calciferol sterols (vitamin D hydroxylated forms, calcidiol, or calcitriol) and calcium salt preparations, preferably calcium carbonate, but these, however, do not efficiently substitute for parathyroid hormone and are difficult to regulate. As the calcium levels may vary significantly over a short time, there are significant risks for both hypo- and hypercalcemia. Recently available recombinant parathyroid hormone may improve the treatment of the hypoparathyroidism in APS-1 patients (Winer et al., 2012) but is currently not recommended due to the risk of osteosarcoma in children and lack of studies proving efficiency in relation to cost. The patients should receive advice and written information about the symptoms complications and risk elements of the disease. APS-1 may cause great psychosocial burden as the persistent risk of developing new disease components can be a source of continuous distress (Perheentupa, 2006).

There is unfortunately no established immune modulation treatment of APS. Treatment with immunosuppressive drugs, cyclosporine A, directed against T cells, produce temporary remission of the disease in APS-2 patients (Csaszar and Patakfalvi, 1992). In a pilot trial, one out of six patients with recent-onset autoimmune Addison's disease regained adrenocortical function after treatment with rituximab (Pearce et al., 2012). Immunotherapy of type 1 diabetes with anti-CD3 monoclonal drugs might be considered a treatment option. In APS-1, immunotherapy is indicated for some of the components. Autoimmune hepatitis can be successfully treated with steroid and

azathioprine (Perheentupa, 2006), interstitial lung disease could respond to rituximab and cyclosporine A (Popler et al., 2012), and cyclosporine A and mycophenolate mofetil have shown beneficial effects on malabsorption in individual patients (Ulinski et al., 2006; Ward et al., 1999). For keratitis topical treatment with steroids and cyclosporine can induce remission if started early. Unfortunately, systematic studies of the effect of immunotherapy is lacking in APS-1.

## CONCLUDING REMARKS—FUTURE PROSPECTS

Recent years have provided remarkable progress in our knowledge about the mechanisms of autoimmune polyendocrinopathies in APS-1 and APS-2. The autoimmune reaction destroys with high precision the endocrine organs, which often express tissue-specific proteins. The identification of the autoantibody targets in APS has revealed that the autoantigens often belong to related protein families, raising the question of exactly how the immune system selects specific targets for autoimmune reactivity. Clinically, assay of autoantibodies can be used for diagnostic purposes. The presence of antibodies against IFN- $\alpha$  and IFN- $\omega$  is almost diagnostic for APS-1 if myasthenia gravis and thymoma has been ruled out. The pattern of autoantibodies is an early phenomenon which correlates to organ manifestations and could, therefore, predict future disease, for instance oophoritis.

Much effort is being put into the identification of genes involved in APS-2. Clearly, MHC genes have the dominant role, but whether an Addison-specific gene association exists is not yet clear. Environmental factors in APS-2 certainly remain to be clarified and deserve more attention. Although a monogenic disease, APS-1 seems to be influenced by environmental, stochastic, and/or other genetic factors to account for the variety of clinical symptoms and disease expressions. The possibility that heterozygous deficiency of *AIRE* in combination with other genetic defects might contribute to the occurrence of autoimmunity is worthy of further investigations.

### Acknowledgment

The authors are grateful to Dr. Kai Kisand for valuable comments to the manuscript.

### References

- Altonen, J., Bjorses, P., Sandkuyl, L., Perheentupa, J., Peltonen, L., 1994. An autosomal locus causing autoimmune disease: autoimmune poly-glandular disease type I assigned to chromosome 21. *Nat. Genet.* 8, 83–87.
- Addison, T., 1855. On the Constitutional and Local Effects of Disease of the Suprarenal Capsules. New Sydenham Society, London (1868).
- Ahonen, P., Myllarniemi, S., Sipila, I., Perheentupa, J., 1990. Clinical variation of autoimmune polyendocrinopathy-candidiasis-ectodermal dys-trophy (APECED) in a series of 68 patients. *N. Engl. J. Med.* 322, 1829–1836.
- Alimohammadi, M., Bjorklund, P., Hallgren, A., Pontynen, N., Szinnai, G., Shikama, N., et al., 2008. Autoimmune polyendocrine syndrome type 1 and NALP5, a parathyroid autoantigen. *N. Engl. J. Med.* 358, 1018–1028.
- Alimohammadi, M., Dubois, N., Skoldberg, F., Hallgren, A., Tardivel, I., Hedstrand, H., et al., 2009. Pulmonary autoimmunity as a feature of autoimmune polyendocrine syndrome type 1 and identification of KCNRG as a bronchial autoantigen. *Proc. Natl. Acad. Sci. U.S.A.* 106, 4396–4401.
- Anaya, J.M., Ramirez-Santana, C., Alzate, M.A., Molano-Gonzalez, N., Rojas-Villarraga, A., 2016. The autoimmune ecology. *Front. Immunol.* 7, 139.
- Anderson, M.S., Su, M.A., 2016. AIRE expands: new roles in immune tolerance and beyond. *Nat. Rev. Immunol.* 16, 247–258.
- Anderson, M.S., Venanzio, E.S., Klein, L., Chen, Z., Berzins, S.P., Turley, S.J., et al., 2002. Projection of an immunological self-shadow within the thymus by the aire protein. *Science (New York, NY)* 298, 1395–1401.
- Anderson, M.S., Venanzio, E.S., Chen, Z., Berzins, S.P., Benoist, C., Mathis, D., 2005. The cellular mechanism of Aire control of T cell tolerance. *Immunity* 23, 227–239.
- Arstila, T.P., Jarva, H., 2013. Human APECED; a sick thymus syndrome? *Front. Immunol.* 4, 313.
- Asano, M., Toda, M., Sakaguchi, N., Sakaguchi, S., 1996. Autoimmune disease as a consequence of developmental abnormality of a T cell sub-population. *J. Exp. Med.* 184, 387–396.
- Awata, T., Guberski, D.L., Like, A.A., 1995. Genetics of the BB rat: association of autoimmune disorders (diabetes, insulitis, and thyroiditis) with lymphopenia and major histocompatibility complex class II. *Endocrinology* 136, 5731–5735.
- Barker, J.M., Yu, J., Yu, L., Wang, J., Miao, D., Bao, F., et al., 2005. Autoantibody “subspecificity” in type 1 diabetes: risk for organ-specific autoimmunity clusters in distinct groups. *Diabetes Care* 28, 850–855.
- Baker, P.R., Baschal, E.E., Fain, P.R., Triolo, T.M., Nanduri, P., Siebert, J.C., et al., 2010. Haplotype analysis discriminates genetic risk for DR3-associated endocrine autoimmunity and helps define extreme risk for Addison’s disease. *J. Clin. Endocrinol. Metab.* 95, E263–E270.
- Baker, P.R., Baschal, E.E., Fain, P.R., Nanduri, P., Triolo, T.M., Siebert, J.C., et al., 2011. Dominant suppression of Addison’s disease associated with HLA-B15. *J. Clin. Endocrinol. Metab.* 96, 2154–2162.

- Bérczi, B., Gerencsér, G., Farkas, N., Hegyi, P., Veres, G., Bajor, J., et al., 2017. Association between AIRE gene polymorphism and rheumatoid arthritis: a systematic review and meta-analysis of case-control studies. *Sci. Rep.* 7, 14096.
- Betterle, C., Morlin, L., 2011. Autoimmune Addison's disease. *Endocr. Dev.* 20, 161–172.
- Betterle, C., Dal Pra, C., Mantero, F., Zanchetta, R., 2002. Autoimmune adrenal insufficiency and autoimmune polyendocrine syndromes: auto-antibodies, autoantigens, and their applicability in diagnosis and disease prediction. *Endocr. Rev.* 23, 327–364.
- Bichele, R., Kärner, J., Truuusalu, K., Smidt, I., Mändar, R., Conti, H.R., et al., 2017. IL-22 neutralizing autoantibodies impair fungal clearance in murine oropharyngeal candidiasis model. *Eur. J. Immunol.* 48, 464–470.
- Björkes, P., Halonen, M., Palvimo, J.J., Kolmer, M., Aaltonen, J., Ellonen, P., et al., 2000. Mutations in the AIRE gene: effects on subcellular location and transactivation function of the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy protein. *Am. J. Hum. Genet.* 66, 378–392.
- Blizzard, R.M., Gibbs, J.H., 1968. Candidiasis: studies pertaining to its association with endocrinopathies and pernicious anemia. *Pediatrics* 42, 231–237.
- Blomhoff, A., Lie, B.A., Myhre, A.G., Kemp, E.H., Weetman, A.P., Akselsen, H.E., et al., 2004. Polymorphisms in the cytotoxic T lymphocyte antigen-4 gene region confer susceptibility to Addison's disease. *J. Clin. Endocrinol. Metab.* 89, 3474–3476.
- Boe Wolff, A.S., Oftedal, B., Johansson, S., Bruland, O., Lovas, K., Meager, A., et al., 2008. AIRE variations in Addison's disease and autoimmune polyendocrine syndromes (APS): partial gene deletions contribute to APS I. *Genes Immun.* 9, 130–136.
- Bonomo, A., Kehn, P.J., Shevach, E.M., 1995. Post-thymectomy autoimmunity: abnormal T-cell homeostasis. *Immunol. Today* 16, 61–67.
- Bratland, E., Skinningsrud, B., Undlien, D.E., Mozes, E., Husebye, E.S., 2009. T cell responses to steroid cytochrome P450 21-hydroxylase in patients with autoimmune primary adrenal insufficiency. *J. Clin. Endocrinol. Metab.* 94, 5117–5124.
- Bratland, E., Hellesen, A., Husebye, E.S., 2013. Induction of CXCL10 chemokine in adrenocortical cells by stimulation through Toll-like receptor 3. *Mol. Cell. Endocrinol.* 365, 75–83.
- Brozzetti, A., Marzotti, S., La Torre, D., Bacosi, M.L., Morelli, S., Bini, V., et al., 2010a. Autoantibody responses in autoimmune ovarian insufficiency and in Addison's disease are IgG1 dominated and suggest a predominant, but not exclusive, Th1 type of response. *Eur. J. Endocrinol.* 163, 309–317.
- Brozzetti, A., Marzotti, S., Tortoioli, C., Bini, V., Giordano, R., Dotta, F., et al., 2010b. Cytotoxic T lymphocyte antigen-4 Ala17 polymorphism is a genetic marker of autoimmune adrenal insufficiency: Italian association study and meta-analysis of European studies. *Eur. J. Endocrinol.* 162, 361–369.
- Brozzetti, A., Alimohammadi, M., Morelli, S., Minarelli, V., Hallgren, Å., Giordano, R., et al., 2015. Autoantibody response against NALP5/MATER in primary ovarian insufficiency and in autoimmune Addison's disease. *J. Clin. Endocrinol. Metab.* 100, 1941–1948.
- Bruserud, Ø., Oftedal, B.E., Landegren, N., Erichsen, M.M., Bratland, E., Lima, K., et al., 2016. A longitudinal follow-up of autoimmune poly-endocrine syndrome type 1. *J. Clin. Endocrinol. Metab.* 101, 2975–2983.
- Carpenter, C.C., Solomon, N., Silverberg, S.G., Bledsoe, T., Northcutt, R.C., Klinenberg, J.R., et al., 1964. Schmidt's syndrome (thyroid and adrenal insufficiency). A review of the literature and a report of fifteen new cases including ten instances of coexistent diabetes mellitus. *Medicine* 43, 153–180.
- Cetani, F., Barbesino, G., Borsari, S., Pardi, E., Cianferotti, L., Pinchera, A., et al., 2001. A novel mutation of the autoimmune regulator gene in an Italian kindred with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy, acting in a dominant fashion and strongly cosegregating with hypothyroid autoimmune thyroiditis. *J. Clin. Endocrinol. Metab.* 86, 4747–4752.
- Coco, G., Dal Pra, C., Presotto, F., Albergoni, M.P., Canova, C., Pedini, B., et al., 2006. Estimated risk for developing autoimmune Addison's disease in patients with adrenal cortex autoantibodies. *J. Clin. Endocrinol. Metab.* 91, 1637–1645.
- Crisi, L., Mordes, J.P., Rossini, A.A., 1992. Autoimmune diabetes mellitus in the BB rat. *Diabetes/Metab. Rev.* 8, 4–37.
- Csaszar, T., Patakfalvi, A., 1992. Treatment of polyglandular autoimmune syndrome with cyclosporin-A. *Acta Med. Hung.* 49, 187–193.
- Dalin, F., Nordling Eriksson, G., Dahlqvist, P., Hallgren, Å., Wahlberg, J., Ekwall, O., et al., 2017. Clinical and immunological characteristics of autoimmune Addison disease: a nationwide Swedish multicenter study. *J. Clin. Endocrinol. Metab.* 102, 379–389.
- Dawoodji, A., Chen, J.L., Shepherd, D., Dalin, F., Tarlton, A., Alimohammadi, M., et al., 2014. High frequency of cytolytic 21-hydroxylase-specific CD8+ T cells in autoimmune Addison's disease patients. *J. Immunol.* 193, 2118–2126.
- Ekwall, O., Hedstrand, H., Grimalius, L., Haavik, J., Perheentupa, J., Gustafsson, J., et al., 1998. Identification of tryptophan hydroxylase as an intestinal autoantigen. *Lancet* 352, 279–283.
- Erichsen, M.M., Lovas, K., Skinningsrud, B., Wolff, A.B., Undlien, D.E., Svartberg, J., et al., 2009. Clinical, immunological, and genetic features of autoimmune primary adrenal insufficiency: observations from a Norwegian registry. *J. Clin. Endocrinol. Metab.* 94, 4882–4890.
- Erichsen, M.M., Husebye, E.S., Michelsen, T.M., Dahl, A.A., Lovas, K., 2010. Sexuality and fertility in women with Addison's disease. *J. Clin. Endocrinol. Metab.* 95, 4354–4360.
- Eriksson, D., Bianchi, M., Landegren, N., Nordin, J., Dalin, F., Mathioudaki, A., et al., 2016. Extended exome sequencing identifies BACH2 as a novel major risk locus for Addison's disease. *J. Intern. Med.* 280, 595–608.
- Eriksson, D., Dalin, F., Eriksson, G.N., Landegren, N., Bianchi, M., Hallgren, Å., et al., 2017. Cytokine autoantibody screening in the Swedish Addison Register identifies patients with undiagnosed APS1. *J. Clin. Endocrinol. Metab.* 103, 179–186.
- Esensten, J.H., Helou, Y.A., Chopra, G., Weiss, A., Bluestone, J.A., 2016. CD28 costimulation: from mechanism to therapy. *Immunity* 44, 973–988.
- Falorni, A., Brozzetti, A., Torre, D.L., Tortoioli, C., Gambelunghe, G., 2008. Association of genetic polymorphisms and autoimmune Addison's disease. *Expert Rev. Clin. Immunol.* 4, 441–456.
- Ferre, E.M., Rose, S.R., Rosenzweig, S.D., Burbelo, P.D., Romito, K.R., Niemela, J.E., et al., 2016. Redefined clinical features and diagnostic criteria in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *JCI Insight* 1, e88782.
- Finnish-German-Consortium, 1997. An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. *Nat. Genet.* 17, 399–403.
- Fishman, D., Kisand, K., Hertel, C., Rothe, M., Remm, A., Pihlap, M., et al., 2017. Autoantibody repertoire in APECED patients targets two distinct subgroups of proteins. *Front. Immunol.* 8, 976.

- Flesch, B.K., Matheis, N., Alt, T., Weinstock, C., Bux, J., Kahaly, G.J., 2014. HLA class II haplotypes differentiate between the adult autoimmune polyglandular syndrome types II and III. *J. Clin. Endocrinol. Metab.* 99, E177–E182.
- Fujikado, N., Mann, A.O., Bansal, K., Romito, K.R., Ferre, E.M.N., Rosenzweig, S.D., et al., 2016. Aire inhibits the generation of a perinatal population of interleukin-17A-producing  $\gamma\delta$  T cells to promote immunologic tolerance. *Immunity* 45, 999–1012.
- Gagnerault, M.C., Lanvin, O., Pasquier, V., Garcia, C., Damotte, D., Lucas, B., et al., 2009. Autoimmunity during thymectomy-induced lymphopenia: role of thymus ablation and initial effector T cell activation timing in nonobese diabetic mice. *J. Immunol.* 183, 4913–4920.
- Gambelunghe, G., Falorni, A., Ghaderi, M., Laureti, S., Tortozioli, C., Santeusanio, F., et al., 1999. Microsatellite polymorphism of the MHC class I chain-related (MIC-A and MIC-B) genes marks the risk for autoimmune Addison's disease. *J. Clin. Endocrinol. Metab.* 84, 3701–3707.
- Gambelunghe, G., Kockum, I., Bini, V., De Giorgi, G., Celi, F., Betterle, C., et al., 2005. Retrovirus-like long-terminal repeat DQ-LTR13 and genetic susceptibility to type 1 diabetes and autoimmune Addison's disease. *Diabetes* 54, 900–905.
- Gavanescu, I., Benoist, C., Mathis, D., 2008. B cells are required for Aire-deficient mice to develop multi-organ autoinflammation: a therapeutic approach for APECED patients. *Proc. Natl. Acad. Sci. U.S.A.* 105, 13009–13014.
- Gebre-Medhin, G., Husebye, E.S., Gustafsson, J., Winqvist, O., Goksoyr, A., Rorsman, F., et al., 1997. Cytochrome P450IA2 and aromatic L-amino acid decarboxylase are hepatic autoantigens in autoimmune polyendocrine syndrome type I. *FEBS Lett.* 412, 439–445.
- Ghaderi, M., Gambelunghe, G., Tortozioli, C., Brozzetti, A., Jatta, K., Gharizadeh, B., et al., 2006. MHC2TA single nucleotide polymorphism and genetic risk for autoimmune adrenal insufficiency. *J. Clin. Endocrinol. Metab.* 91, 4107–4111.
- Giordano, C., Modica, R., Allotta, M.L., Guarnera, V., Cervato, S., Masiero, S., et al., 2011. Autoimmune polyendocrinopathy-candidiasis-ectodermal-dystrophy (APECED) in Sicily: confirmation that R203X is the peculiar AIRE gene mutation. *J. Endocrinol. Invest.* 35, 384–388.
- Gutierrez, M.J., Gilson, J., Zacharias, J., Ishmael, F., Bingham, C.A., 2017. Childhood polyarthritis as early manifestation of autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy syndrome. *Front. Immunol.* 8, 377.
- Gylling, M., Kaariainen, E., Vaisanen, R., Kerosuo, L., Solin, M.L., Halme, L., et al., 2003. The hypoparathyroidism of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy protective effect of male sex. *J. Clin. Endocrinol. Metab.* 88, 4602–4608.
- Haljasorg, U., Bichele, R., Saare, M., Guha, M., Maslovskaja, J., Kond, K., et al., 2015. A highly conserved NF- $\kappa$ B-responsive enhancer is critical for thymic expression of Aire in mice. *Eur. J. Immunol.* 45, 3246–3256.
- Halonen, M., Eskelin, P., Myhre, A.G., Perheentupa, J., Husebye, E.S., Kampe, O., et al., 2002. AIRE mutations and human leukocyte antigen genotypes as determinants of the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy phenotype. *J. Clin. Endocrinol. Metab.* 87, 2568–2574.
- Harris, M., Kecha, O., Deal, C., Howlett, C.R., Deiss, D., Tobias, V., et al., 2003. Reversible metaphyseal dysplasia, a novel bone phenotype, in two unrelated children with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy: clinical and molecular studies. *J. Clin. Endocrinol. Metab.* 88, 4576–4585.
- Hedstrand, H., Ekwall, O., Haavik, J., Landgren, E., Betterle, C., Perheentupa, J., et al., 2000. Identification of tyrosine hydroxylase as an autoantigen in autoimmune polyendocrine syndrome type I. *Biochem. Biophys. Res. Commun.* 267, 456–461.
- Heikkilä, N., Laakso, S.M., Mannerström, H., Kekäläinen, E., Saavalainen, P., Jarva, H., et al., 2016. Expanded CD4(+) effector/memory T cell subset in APECED produces predominantly interferon gamma. *J. Clin. Immunol.* 36, 555–563.
- Heino, M., Peterson, P., Kudoh, J., Nagamine, K., Lagerstedt, A., Ovod, V., et al., 1999. Autoimmune regulator is expressed in the cells regulating immune tolerance in thymus medulla. *Biochem. Biophys. Res. Commun.* 257, 821–825.
- Hetemaki, I., Jarva, H., Kluger, N., Baldauf, H.-M., Laakso, S., Bratland, E., et al., 2016. Antimicrobial responses are associated with regulatory T cell defect in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients. *J. Immunol.* 196, 2955–2964.
- Hetzell, B.S., Robson, H.N., 1958. The syndrome of hypoparathyroidism, Addison's disease and moniliasis. *Australas. Ann. Med.* 7, 27–33.
- Hoek, A., Schoemaker, J., Drexhage, H.A., 1997. Premature ovarian failure and ovarian autoimmunity. *Endocrine Rev.* 18, 107–134.
- Hong, M., Ryan, K.R., Arkwright, P.D., Genneyer, A.R., Costigan, C., Dominguez, M., et al., 2009. Pattern recognition receptor expression is not impaired in patients with chronic mucocutaneous candidiasis with or without autoimmune polyendocrinopathy candidiasis ectodermal dystrophy. *Clin. Exp. Immunol.* 156, 40–51.
- Hornum, L., Romer, J., Markholst, H., 2002. The diabetes-prone BB rat carries a frameshift mutation in Ian4, a positional candidate of Iddm1. *Diabetes* 51, 1972–1979.
- Huang, L., Wilkinson, M.F., 2012. Regulation of nonsense-mediated mRNA decay. *Wiley Interdiscip. Rev. RNA* 3, 807–828.
- Hubert, F.X., Kinkel, S.A., Davey, G.M., Phipson, B., Mueller, S.N., Liston, A., et al., 2011. Aire regulates the transfer of antigen from mTECs to dendritic cells for induction of thymic tolerance. *Blood* 118, 2462–2472.
- Husebye, E.S., Gebre-Medhin, G., Tuomi, T., Perheentupa, J., Landin-Olsson, M., Gustafsson, J., et al., 1997. Autoantibodies against aromatic L-amino acid decarboxylase in autoimmune polyendocrine syndrome type I. *J. Clin. Endocrinol. Metab.* 82, 147–150.
- Hyttinen, V., Kaprio, J., Kinnunen, L., Koskenvuo, M., Tuomilehto, J., 2003. Genetic liability of type 1 diabetes and the onset age among 22,650 young Finnish twin pairs: a nationwide follow-up study. *Diabetes* 52, 1052–1055.
- Ivanov, I.I., Atarashi, K., Manel, N., Brodie, E.L., Shima, T., Karaoz, U., et al., 2009. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 139, 485–498.
- Jennings, C.E., Owen, C.J., Wilson, V., Pearce, S.H., 2005. A haplotype of the CYP27B1 promoter is associated with autoimmune Addison's disease but not with Graves' disease in a UK population. *J. Mol. Endocrinol.* 34, 859–863.
- Karner, J., Pihlap, M., Ranki, A., Krohn, K., Podkrajsek, K.T., Bratanic, N., et al., 2016. IL-6-specific autoantibodies among APECED and thymoma patients. *Immunity Inflamm. Dis.* 4, 235–243.
- Kekäläinen, E., Miettinen, A., Arstila, T.P., 2007a. Does the deficiency of Aire in mice really resemble human APECED? *Nat. Rev.* 7, 1.
- Kekäläinen, E., Tuovinen, H., Joensuu, J., Gylling, M., Franssila, R., Pontynen, N., et al., 2007b. A defect of regulatory T cells in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *J. Immunol.* 178, 1208–1215.
- Kemp, E.H., Ajjan, R.A., Husebye, E.S., Peterson, P., Uibo, R., Imrie, H., et al., 1998. A cytotoxic T lymphocyte antigen-4 (CTLA-4) gene polymorphism is associated with autoimmune Addison's disease in English patients. *Clin. Endocrinol. (Oxf.)* 49, 609–613.

- Kisand, K., Peterson, P., 2011. Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy: known and novel aspects of the syndrome. *Ann. N.Y. Acad. Sci.* 1246, 77–91.
- Kisand, K., Link, M., Wolff, A.S., Meager, A., Tserel, L., Org, T., et al., 2008. Interferon autoantibodies associated with AIRE deficiency decrease the expression of IFN-stimulated genes. *Blood* 112, 2657–2666.
- Kisand, K., Bøe Wolff, A.S., Podkrajsek, K.T., Tserel, L., Link, M., Kisand, K.V., et al., 2010. Chronic mucocutaneous candidiasis in APECED or thymoma patients correlates with autoimmunity to Th17-associated cytokines. *J. Exp. Med.* 207, 299–308.
- Kisand, K., Lilic, D., Casanova, J.L., Peterson, P., Meager, A., Willcox, N., 2011. Mucocutaneous candidiasis and autoimmunity against cytokines in APECED and thymoma patients: clinical and pathogenetic implications. *Eur. J. Immunol.* 41, 1517–1527.
- Kisand, K., Peterson, P., Laan, M., 2014. Lymphopenia-induced proleation in Aire-deficient mice helps to explain their autoimmunity and differences from human patients. *Front. Immunol.* 5, 51.
- Koble, C., Kyewski, B., 2009. The thymic medulla: a unique microenvironment for intercellular self-antigen transfer. *J. Exp. Med.* 206, 1505–1513.
- Koivula, T.T., Laakso, S.M., Niemi, H.J., Kekäläinen, E., Laine, P., Paulin, L., et al., 2017. Clonal analysis of regulatory T cell defect in patients with autoimmune polyendocrine syndrome type 1 suggests intrathymic impairment. *Scand. J. Immunol.* 86, 221–228.
- Krohn, K., Uibo, R., Aavik, E., Peterson, P., Savilahti, K., 1992. Identification by molecular cloning of an autoantigen associated with Addison's disease as steroid 17 alpha-hydroxylase. *Lancet* 339, 770–773.
- Kuroda, N., Mitani, T., Takeda, N., Ishimaru, N., Arakaki, R., Hayashi, Y., et al., 2005. Development of autoimmunity against transcriptionally unexpressed target antigen in the thymus of Aire-deficient mice. *J. Immunol.* 174, 1862–1870.
- LaFlam, T.N., Seumois, G., Miller, C.N., Lwin, W., Fasano, K.J., Waterfield, M., et al., 2015. Identification of a novel cis-regulatory element essential for immune tolerance. *J. Exp. Med.* 212, 1993–2002.
- Laakso, S.M., Kekäläinen, E., Rossi, L.H., Laurinolli, T.T., Mannerström, H., Heikkila, N., et al., 2011. IL-7 dysregulation and loss of CD8 + T cell homeostasis in the monogenic human disease autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *J. Immunol.* 187, 2023–2030.
- Laakso, S.M., Kekäläinen, E., Heikkilä, N., Mannerström, H., Kisand, K., Peterson, P., et al., 2014. In vivo analysis of helper T cell responses in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy provides evidence in support of an IL-22 defect. *Autoimmunity* 47, 556–562.
- Laan, M., Kisand, K., Kont, V., Moll, K., Tserel, L., Scott, H.S., et al., 2009. Autoimmune regulator deficiency results in decreased expression of CCR4 and CCR7 ligands and in delayed migration of CD4 + thymocytes. *J. Immunol.* 183, 7682–7691.
- Landegren, N., Sharon, D., Shum, A.K., Khan, I.S., Fasano, K.J., Hallgren, Å., et al., 2015. Transglutaminase 4 as a prostate autoantigen in male subfertility. *Sci. Transl. Med.* 7, 292ra101.
- Landegren, N., Pourmousa Lindberg, M., Skov, J., Hallgren, Å., Eriksson, D., Lisberg Toft-Bertelsen, T., et al., 2016a. Autoantibodies targeting a collecting duct-specific water channel in tubulointerstitial nephritis. *J. Am. Soc. Nephrol.* 27, 3220–3228.
- Landegren, N., Sharon, D., Freyhult, E., Hallgren, Å., Eriksson, D., Edqvist, P.H., et al., 2016b. Proteome-wide survey of the autoimmune target repertoire in autoimmune polyendocrine syndrome type 1. *Sci. Rep.* 6, 20104.
- Levin, L., Tomer, Y., 2003. The etiology of autoimmune diabetes and thyroiditis: evidence for common genetic susceptibility. *Autoimmun. Rev.* 2, 377–386.
- Li, D., Streten, E.A., Chan, A., Lwin, W., Tian, L., Pellegrino da Silva, R., et al., 2017. Exome sequencing reveals mutations in AIRE as a cause of isolated hypoparathyroidism. *J. Clin. Endocrinol. Metab.* 102, 1726–1733.
- Lindh, E., Rosmaraki, E., Berg, L., Brauner, H., Karlsson, M.C., Peltonen, L., et al., 2010. AIRE deficiency leads to impaired iNKT cell development. *J. Autoimmun.* 34, 66–72.
- Liston, A., Lesage, S., Wilson, J., Peltonen, L., Goodnow, C.C., 2003. Aire regulates negative selection of organ-specific T cells. *Nat. Immunol.* 4, 350–354.
- Lopez, E.R., Zwermann, O., Segni, M., Meyer, G., Reincke, M., Seissler, J., et al., 2004. A promoter polymorphism of the CYP27B1 gene is associated with Addison's disease, Hashimoto's thyroiditis, Graves' disease and type 1 diabetes mellitus in Germans. *Eur. J. Endocrinol.* 151, 193–197.
- MacMurray, A.J., Moralejo, D.H., Kwitek, A.E., Rutledge, E.A., Van Yserloo, B., Gohlke, P., et al., 2002. Lymphopenia in the BB rat model of type 1 diabetes is due to a mutation in a novel immune-associated nucleotide (Ian)-related gene. *Genome Res.* 12, 1029–1039.
- Maclarens, N.K., Riley, W.J., 1986. Inherited susceptibility to autoimmune Addison's disease is linked to human leukocyte antigens-DR3 and/or DR4, except when associated with type I autoimmune polyglandular syndrome. *J. Clin. Endocrinol. Metab.* 62, 455–459.
- Magitta, N.F., Boe Wolff, A.S., Johansson, S., Skinningsrud, B., Lie, B.A., Myhr, K.M., et al., 2009. A coding polymorphism in NALP1 confers risk for autoimmune Addison's disease and type 1 diabetes. *Genes Immun.* 10, 120–124.
- Malchow, S., Leventhal, D.S., Nishi, S., Fischer, B.I., Shen, L., Paner, G.P., et al., 2013. Aire-dependent thymic development of tumor-associated regulatory T cells. *Science* 339, 1219–1224.
- Malchow, S., Leventhal, D.S., Lee, V., Nishi, S., Soccia, N.D., Savage, P.A., 2016. Aire enforces immune tolerance by directing autoreactive T cells into the regulatory T cell lineage. *Immunity* 44, 1102–1113.
- Meager, A., Visvalingam, K., Peterson, P., Moll, K., Murumagi, A., Krohn, K., et al., 2006. Anti-interferon autoantibodies in autoimmune polyendocrinopathy syndrome type 1. *PLoS Med.* 3, e289.
- Meager, A., Peterson, P., Willcox, N., 2008. Hypothetical review: thymic aberrations and type-I interferons: attempts to deduce autoimmunizing mechanisms from unexpected clues in monogenic and paraneoplastic syndromes. *Clin. Exp. Immunol.* 154, 141–151.
- Meloni, A., Furcas, M., Cetani, F., Marcocci, C., Falorni, A., Perniola, R., et al., 2008. Autoantibodies against type I interferons as an additional diagnostic criterion for autoimmune polyendocrine syndrome type I. *J. Clin. Endocrinol. Metab.* 93, 4389–4397.
- Meloni, A., Willcox, N., Meager, A., Atzeni, M., Wolff, A.S., Husebye, E.S., et al., 2012. Autoimmune polyendocrine syndrome type 1: an extensive longitudinal study in sardinian patients. *J. Clin. Endocrinol. Metab.* 97, 1114–1124.

- Meyer, G., Donner, H., Herwig, J., Bohles, H., Usadel, K.H., Badenhoop, K., 2001. Screening for an AIRE-1 mutation in patients with Addison's disease, type 1 diabetes, Graves' disease and Hashimoto's thyroiditis as well as in APECED syndrome. *Clin. Endocrinol.* 54, 335–338.
- Meyer, S., Woodward, M., Hertel, C., Vlaicu, P., Haque, Y., Kärner, J., et al., 2016. AIRE-deficient patients harbor unique high-affinity disease-ameliorating autoantibodies. *Cell* 166, 582–595.
- Mitchell, A.L., Cordell, H.J., Soemedi, R., Owen, K., Skinningsrud, B., Wolff, A.B., et al., 2009. Programmed death ligand 1 (PD-L1) gene variants contribute to autoimmune Addison's disease and Graves' disease susceptibility. *J. Clin. Endocrinol. Metab.* 94, 5139–5145.
- Muir, A., Schatz, D.A., Maclarens, N.K., 1993. Autoimmune Addison's disease. *Springer Semin. Immunopathol.* 14, 275–284.
- Nagamine, K., Peterson, P., Scott, H.S., Kudoh, J., Minoshima, S., Heino, M., et al., 1997. Positional cloning of the APECED gene. *Nat. Genet.* 17, 393–398.
- Neufeld, M., Maclarens, N.K., Blizzard, R.M., 1981. Two types of autoimmune Addison's disease associated with different polyglandular autoimmune (PGA) syndromes. *Medicine* 60, 355–362.
- Ng, W.F., von Delwig, A., Carmichael, A.J., Arkwright, P.D., Abinun, M., Cant, A.J., et al., 2010. Impaired T(H)17 responses in patients with chronic mucocutaneous candidiasis with and without autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *J. Allergy Clin. Immunol.* 126, 1006–1015. 1015.e1001–1004.
- Niemi, H.J., Laakso, S., Salminen, J.T., Arstila, T.P., Tuulasvaara, A., 2015. A normal T cell receptor beta CDR3 length distribution in patients with APECED. *Cell Immunol.* 295, 99–104.
- Nishikawa, Y., Hirota, F., Yano, M., Kitajima, H., Miyazaki, J., Kawamoto, H., et al., 2010. Biphasic Aire expression in early embryos and in medullary thymic epithelial cells before end-stage terminal differentiation. *J. Exp. Med.* 207, 963–971.
- Nithiyanthan, R., Heward, J.M., Allahabadia, A., Barnett, A.H., Franklyn, J.A., Gough, S.C., 2000. A heterozygous deletion of the autoimmune regulator (AIRE1) gene, autoimmune thyroid disease, and type 1 diabetes: no evidence for association. *J. Clin. Endocrinol. Metab.* 85, 1320–1322.
- Oftedal, B.E., Wolff, A.S., Bratland, E., Kampe, O., Perheentupa, J., Myhre, A.G., et al., 2008. Radioimmunoassay for autoantibodies against interferon omega; its use in the diagnosis of autoimmune polyendocrine syndrome type I. *Clin. Immunol.* 129, 163–169.
- Oftedal, B.E., Hellesen, A., Erichsen, M.M., Bratland, E., Vardi, A., Perheentupa, J., et al., 2015. Dominant mutations in the autoimmune regulator AIRE are associated with common organ-specific autoimmune diseases. *Immunity* 42, 1185–1196.
- Olafsson, A.S., Sigurjonsdottir, H.A., 2016. Increasing prevalence of Addison disease: results from a nationwide study. *Endocr. Pract.* 22, 30–35.
- Orlova, E.M., Bukina, A.M., Kuznetsova, E.S., Kareva, M.A., Zakhарова, Е.У., Peterkova, V.A., et al., 2010. Autoimmune polyglandular syndrome type 1 in Russian patients: clinical variants and autoimmune regulator mutations. *Horm. Res. Paediatr.* 73, 449–457.
- Orlova, E.M., Sozaeva, L.S., Kareva, M.A., Oftedal, B.E., Wolff, A.S.B., Breivik, L., et al., 2017. Expanding the phenotypic and genotypic landscape of autoimmune polyendocrine syndrome type 1. *J. Clin. Endocrinol. Metab.* 102, 3546–3556.
- Otsuka, N., Tong, Z.B., Vanevski, K., Tu, W., Cheng, M.H., Nelson, L.M., 2011. Autoimmune oophoritis with multiple molecular targets mitigated by transgenic expression of mater. *Endocrinology* 152, 2465–2473.
- Owen, C.J., Kelly, H., Eden, J.A., Merriman, M.E., Pearce, S.H., Merriman, T.R., 2007. Analysis of the Fc receptor-like-3 (FCRL3) locus in Caucasians with autoimmune disorders suggests a complex pattern of disease association. *J. Clin. Endocrinol. Metab.* 92, 1106–1111.
- Partanen, J., Peterson, P., Westman, P., Aranko, S., Krohn, K., 1994. Major histocompatibility complex class II and III in Addison's disease. MHC alleles do not predict autoantibody specificity and 21-hydroxylase gene polymorphism has no independent role in disease susceptibility. *Hum. Immunol.* 41, 135–140.
- Patterson, C.C., Dahlquist, G., Soltesz, G., 2001. Maternal age and risk of type 1 diabetes in children. Relative risks by maternal age are biased. *BMJ.* 322, 1489–1490. Clinical research ed.; author reply 1490–1481.
- Pearce, S.H., Cheetham, T., Imrie, H., Vaidya, B., Barnes, N.D., Bilous, R.W., et al., 1998. A common and recurrent 13-bp deletion in the autoimmune regulator gene in British kindreds with autoimmune polyendocrinopathy type 1. *Am. J. Hum. Genet.* 63, 1675–1684.
- Pearce, S.H., Mitchell, A.L., Bennett, S., King, P., Chandran, S., Nag, S., et al., 2012. Adrenal steroidogenesis after B lymphocyte depletion therapy in new-onset Addison's disease. *J. Clin. Endocrinol. Metab.* 97, E1927–E1932.
- Perheentupa, J., 2002. APS-I/APECED: the clinical disease and therapy. *Endocrinol. Metab. Clin. N. Am.* 31, 295–320. vi.
- Perheentupa, J., 2006. Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *J. Clin. Endocrinol. Metab.* 91, 2843–2850.
- Perniola, R., Lobreglio, G., Rosatelli, M.C., Pitotti, E., Accogli, E., De Rinaldis, C., 2005. Immunophenotypic characterisation of peripheral blood lymphocytes in autoimmune polyglandular syndrome type 1: clinical study and review of the literature. *J. Pediatr. Endocrinol. Metab.* 18, 155–164.
- Perniola, R., Congedo, M., Rizzo, A., Sticchi Damiani, A., Faneschi, M.L., Pizzolante, M., et al., 2008. Innate and adaptive immunity in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *Mycoses* 51, 228–235.
- Perry, J.S., Lio, C.W., Kau, A.L., Nutsch, K., Yang, Z., Gordon, J.I., et al., 2014. Distinct contributions of Aire and antigen-presenting-cell subsets to the generation of self-tolerance in the thymus. *Immunity* 41, 414–426.
- Peterson, P., Partanen, J., Aavik, E., Salmi, H., Pelkonen, R., Krohn, K.J., 1995. Steroid 21-hydroxylase gene polymorphism in Addison's disease patients. *Tissue Antigens* 46, 63–67.
- Popler, J., Alimohammadi, M., Kämpe, O., Dalin, F., Dishop, M.K., Barker, J.M., et al., 2012. Autoimmune polyendocrine syndrome type 1: Utility of KCNRG autoantibodies as a marker of active pulmonary disease and successful treatment with rituximab. *Pediatr. Pulmonol.* 47, 84–87.
- Proust-Lemoine, E., Saugier-Véber, P., Lefranc, D., Dubucquoi, S., Ryndak, A., Buob, D., et al., 2010. Autoimmune polyendocrine syndrome type 1 in north-western France: AIRE gene mutation specificities and severe forms needing immunosuppressive therapies. *Horm. Res. Paediatr.* 74, 275–284.
- Puel, A., Doffinger, R., Natividad, A., Chrabieh, M., Barcenas-Morales, G., Picard, C., et al., 2010. Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. *J. Exp. Med.* 207, 291–297.

- Ramsey, C., Winqvist, O., Puhakka, L., Halonen, M., Moro, A., Kampe, O., et al., 2002. Aire deficient mice develop multiple features of APECED phenotype and show altered immune response. *Hum. Mol. Genet.* 11, 397–409.
- Rautemaa, R., Richardson, M., Pfaller, M., Koukila-Kahkola, P., Perheentupa, J., Saxen, H., 2007. Decreased susceptibility of *Candida albicans* to azole antifungals: a complication of long-term treatment in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) patients. *J. Antimicrob. Chemother.* 60, 889–892.
- Rautemaa, R., Richardson, M., Pfaller, M., Perheentupa, J., Saxen, H., 2008a. Reduction of fluconazole susceptibility of *Candida albicans* in APECED patients due to long-term use of ketoconazole and miconazole. *Scand. J. Infect. Dis.* 40, 904–907.
- Rautemaa, R., Richardson, M., Pfaller, M.A., Perheentupa, J., Saxen, H., 2008b. Activity of amphotericin B, anidulafungin, caspofungin, micafungin, posaconazole, and voriconazole against *Candida albicans* with decreased susceptibility to fluconazole from APECED patients on long-term azole treatment of chronic mucocutaneous candidiasis. *Diagn. Microbiol. Infect. Dis.* 62, 182–185.
- Reato, G., Morlin, L., Chen, S., Furmaniak, J., Smith, B.R., Masiero, S., et al., 2011. Premature ovarian failure in patients with autoimmune Addison's disease: clinical, genetic, and immunological evaluation. *J. Clin. Endocrinol. Metab.* 96, E1255–E1261.
- Rosatelli, M.C., Meloni, A., Meloni, A., Devoto, M., Cao, A., Scott, H.S., et al., 1998. A common mutation in Sardinian autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients. *Hum. Genet.* 103, 428–434.
- Rotondi, M., Falorni, A., De Bellis, A., Laureti, S., Ferruzzi, P., Romagnani, P., et al., 2005. Elevated serum interferon-gamma-inducible chemokine-10/CXC chemokine ligand-10 in autoimmune primary adrenal insufficiency and in vitro expression in human adrenal cells primary cultures after stimulation with proinflammatory cytokines. *J. Clin. Endocrinol. Metab.* 90, 2357–2363.
- Roycroft, M., Fichna, M., McDonald, D., Owen, K., Zurawek, M., Gryczynska, M., et al., 2009. The tryptophan 620 allele of the lymphoid tyrosine phosphatase (PTPN22) gene predisposes to autoimmune Addison's disease. *Clin. Endocrinol.* 70, 358–362.
- Ryan, K.R., Hong, M., Arkwright, P.D., Gennery, A.R., Costigan, C., Dominguez, M., et al., 2008. Impaired dendritic cell maturation and cytokine production in patients with chronic mucocutaneous candidiasis with or without APECED. *Clin. Exp. Immunol.* 154, 406–414.
- Schatz, D.A., Winter, W.E., 2002. Autoimmune polyglandular syndrome. II: Clinical syndrome and treatment. *Endocrinol. Metab. Clin. N. Am.* 31, 339–352.
- Schmidt, M., 1926. Eine biglanduläre erkrankung (Nebennieren und schilddrüse) bei morbus Addisonii. *Verh. Dtsch. Ges. Pathol.* 21, 212–221.
- Scott, H.S., Heino, M., Peterson, P., Mittaz, L., Lalioti, M.D., Betterle, C., et al., 1998. Common mutations in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients of different origins. *Mol. Endocrinol. (Baltimore, MD)* 12, 1112–1119.
- Skinningsrud, B., Husebye, E.S., Gervin, K., Lovas, K., Blomhoff, A., Wolff, A.B., et al., 2008a. Mutation screening of PTPN22: association of the 1858T-allele with Addison's disease. *Eur. J. Hum. Genet.* 16, 977–982.
- Skinningsrud, B., Husebye, E.S., Pearce, S.H., McDonald, D.O., Brandal, K., Wolff, A.B., et al., 2008b. Polymorphisms in CLEC16A and CIITA at 16p13 are associated with primary adrenal insufficiency. *J. Clin. Endocrinol. Metab.* 93, 3310–3317.
- Skinningsrud, B., Lie, B.A., Lavant, E., Carlson, J.A., Erlich, H., Akselsen, H.E., et al., 2010. Multiple loci in the HLA complex are associated with Addison's disease. *J. Clin. Endocrinol. Metab.* 96, E1703–E1708.
- Skoldberg, F., Rorsman, F., Perheentupa, J., Landin-Olsson, M., Husebye, E.S., Gustafsson, J., et al., 2004. Analysis of antibody reactivity against cysteine sulfenic acid decarboxylase, a pyridoxal phosphate-dependent enzyme, in endocrine autoimmune disease. *J. Clin. Endocrinol. Metab.* 89, 1636–1640.
- Skov, J., Höijer, J., Magnusson, P.K.E., Ludvigsson, J.F., Kämpe, O., Bensing, S., 2017. Heritability of Addison's disease and prevalence of associated autoimmunity in a cohort of 112,100 Swedish twins. *Endocrine* 58, 521–527.
- Soderbergh, A., Myhre, A.G., Ekwall, O., Gebre-Medhin, G., Hedstrand, H., Landgren, E., et al., 2004. Prevalence and clinical associations of 10 defined autoantibodies in autoimmune polyendocrine syndrome type I. *J. Clin. Endocrinol. Metab.* 89, 557–562.
- Sorkina, E., Frolova, E., Rusinova, D., Polyakova, S., Roslavtseva, E., Vasilyev, E., et al., 2016. Progressive generalized lipodystrophy as a manifestation of autoimmune polyglandular syndrome type 1. *J. Clin. Endocrinol. Metab.* 101, 1344–1347.
- Spinner, M.W., Blizzard, R.M., Childs, B., 1968. Clinical and genetic heterogeneity in idiopathic Addison's disease and hypoparathyroidism. *J. Clin. Endocrinol. Metab.* 28, 795–804.
- Terao, C., Yamada, R., Ohmura, K., Takahashi, M., Kawaguchi, T., Kochi, Y., et al., 2011. The human AIRE gene at chromosome 21q22 is a genetic determinant for the predisposition to rheumatoid arthritis in Japanese population. *Hum. Mol. Genet.* 20, 2680–2685.
- Thorpe, E., Handley, H., 1929. Chronic tetany and chronic mycelial stomatitis in a child aged four and one-half years. *Am. J. Dis. Child.* 38, 228–238 (1960).
- Tuomi, T., Björkes, P., Falorni, A., Partanen, J., Perheentupa, J., Lernmark, A., et al., 1996. Antibodies to glutamic acid decarboxylase and insulin-dependent diabetes in patients with autoimmune polyendocrine syndrome type I. *J. Clin. Endocrinol. Metab.* 81, 1488–1494.
- Tuovinen, H., Pontynen, N., Gylling, M., Kekäläinen, E., Perheentupa, J., Miettinen, A., et al., 2009. gammadelta T cells develop independently of Aire. *Cell. Immunol.* 257, 5–12.
- Turunen, J.A., Wessman, M., Forsblom, C., Kilpikari, R., Parkkonen, M., Pontynen, N., et al., 2006. Association analysis of the AIRE and insulin genes in Finnish type 1 diabetic patients. *Immunogenetics* 58, 331–338.
- Ueda, H., Howson, J.M., Esposito, L., Heward, J., Snook, H., Chamberlain, G., et al., 2003. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* 423, 506–511.
- Uibo, R., Perheentupa, J., Ovod, V., Krohn, K.J., 1994. Characterization of adrenal autoantigens recognized by sera from patients with autoimmune polyglandular syndrome (APS) type I. *J. Autoimmun.* 7, 399–411.
- Ulinski, T., Perrin, L., Morris, M., Houang, M., Cabrol, S., Grapin, C., et al., 2006. Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome with renal failure: impact of posttransplant immunosuppression on disease activity. *J. Clin. Endocrinol. Metab.* 91, 192–195.
- Vaidya, B., Pearce, S., Kendall-Taylor, P., 2000. Recent advances in the molecular genetics of congenital and acquired primary adrenocortical failure. *Clin. Endocrinol. (Oxf.)* 53, 403–418.
- Valenzise, M., Meloni, A., Betterle, C., Giometto, B., Autunno, M., Mazzeo, A., et al., 2009. Chronic inflammatory demyelinating polyneuropathy as a possible novel component of autoimmune poly-endocrine-candidiasis-ectodermal dystrophy. *Eur. J. Pediatr.* 168, 237–240.
- Van Lanen, K., Sande, A., 2014. Canine hypoadrenocorticism: pathogenesis, diagnosis, and treatment. *Top Companion Anim. Med.* 29, 88–95.

- Walter, J.E., Rosen, L.B., Csomas, K., Rosenberg, J.M., Mathew, D., Keszei, M., et al., 2016. Broad-spectrum antibodies against self-antigens and cytokines in RAG deficiency. *J. Clin. Invest.* 126, 4389.
- Wang, C.Y., Davoodi-Semiroomi, A., Huang, W., Connor, E., Shi, J.D., She, J.X., 1998. Characterization of mutations in patients with autoimmune polyglandular syndrome type 1 (APS1). *Hum. Genet.* 103, 681–685.
- Wang, X., Laan, M., Bichele, R., Kisand, K., Scott, H.S., Peterson, P., 2012. Post-Aire maturation of thymic medullary epithelial cells involves selective expression of keratinocyte-specific autoantigens. *Front. Immunol.* 3, 19.
- Ward, L., Paquette, J., Seidman, E., Huot, C., Alvarez, F., Crock, P., et al., 1999. Severe autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy in an adolescent girl with a novel AIRE mutation: response to immunosuppressive therapy. *J. Clin. Endocrinol. Metab.* 84, 844–852.
- Wilks, S., 1862. On diseases of the suprarenal capsule or morbus addisonii. *Guy's Hosp Rep* 8, 1.
- Winer, K.K., Zhang, B., Shrader, J.A., Peterson, D., Smith, M., Albert, P.S., et al., 2012. Synthetic human parathyroid hormone 1-34 replacement therapy: a randomized crossover trial comparing pump versus injections in the treatment of chronic hypoparathyroidism. *J. Clin. Endocrinol. Metab.* 97, 391–399.
- Winqvist, O., Karlsson, F.A., Kampe, O., 1992. 21-Hydroxylase, a major autoantigen in idiopathic Addison's disease. *Lancet* 339, 1559–1562.
- Winqvist, O., Gustafsson, J., Rorsman, F., Karlsson, F.A., Kampe, O., 1993. Two different cytochrome P450 enzymes are the adrenal antigens in autoimmune polyendocrine syndrome type I and Addison's disease. *J. Clin. Invest.* 92, 2377–2385.
- Winqvist, O., Gebre-Medhin, G., Gustafsson, J., Ritzen, E.M., Lundkvist, O., Karlsson, F.A., et al., 1995. Identification of the main gonadal auto-antigens in patients with adrenal insufficiency and associated ovarian failure. *J. Clin. Endocrinol. Metab.* 80, 1717–1723.
- Wolff, A.S., Erichsen, M.M., Meager, A., Magitta, N.F., Myhre, A.G., Bollerslev, J., et al., 2007. Autoimmune polyendocrine syndrome type 1 in Norway: phenotypic variation, autoantibodies, and novel mutations in the autoimmune regulator gene. *J. Clin. Endocrinol. Metab.* 92, 595–603.
- Wolff, A.S., Oftedal, B.E., Kisand, K., Ersvaer, E., Lima, K., Husebye, E.S., 2010. Flow cytometry study of blood cell subtypes reflects autoimmune and inflammatory processes in autoimmune polyendocrine syndrome type I. *Scand. J. Immunol.* 71, 459–467.
- Wolff, A.S., Kärner, J., Owe, J.F., Oftedal, B.E., Gilhus, N.E., Erichsen, M.M., et al., 2014. Clinical and serologic parallels to APS-I in patients with thymomas and autoantigen transcripts in their tumors. *J. Immunol.* 193, 3880–3890.
- Wolff, A.S., Mitchell, A.L., Cordell, H.J., Short, A., Skinningsrud, B., Ollier, W., et al., 2015. CTLA-4 as a genetic determinant in autoimmune Addison's disease. *Genes Immun.* 16, 430–436.
- Yang, S., Fujikado, N., Kolodin, D., Benoist, C., Mathis, D., 2015. Immune tolerance. Regulatory T cells generated early in life play a distinct role in maintaining self-tolerance. *Science* 348, 589–594.
- Zimmerman, O., Rosen, L.B., Swamydas, M., Ferre, E.M.N., Natarajan, M., van de Veerdonk, F., et al., 2017. Autoimmune regulator deficiency results in a decrease in STAT1 levels in human monocytes. *Front. Immunol.* 8, 820.
- Zlotogora, J., Shapiro, M.S., 1992. Polyglandular autoimmune syndrome type I among Iranian Jews. *J. Med. Genet.* 29, 824–826.

# Thyroid Disease

Anthony P. Weetman

The Medical School University of Sheffield, Sheffield, United Kingdom

## OUTLINE

<b>Autoimmune Thyroiditis</b>	<b>749</b>	Clinical, Pathologic, and Epidemiologic	757
Historic Background	749	Features	757
Clinical, Pathologic, and Epidemiologic		Autoimmune Features	758
Features	749	Genetic Features	759
Autoimmune Features	750	Environmental Influences	759
Genetic Features	752	In Vivo Models	760
Environmental Influences	753	Pathologic Effector Mechanisms	761
In Vivo Models	754	Autoantibodies as Potential Immunological	
Pathologic Effector Mechanisms	755	Markers	761
Autoantibodies as Potential Immunological		Treatment and Outcome	761
Markers	756	Thyroid-Associated Ophthalmopathy and	
Treatment and Outcome	756	Dermopathy	762
Concluding Remarks—Future Prospects	757	Concluding Remarks—Future Prospects	763
<b>Graves' Disease</b>	<b>757</b>	References	763
Historic Background	757	Further Reading	767

## AUTOIMMUNE THYROIDITIS

### Historic Background

The clinical features of myxedema, the end stage of autoimmune thyroiditis (AT), were defined in 1874 by Gull, and Murray, in Newcastle-upon-Tyne, was the first to give thyroid extract as treatment in 1891. The characteristic lymphocytic infiltration (struma lymphomatosa) was first noted by Hashimoto in 1912. Proof that AT was due to the loss of self-tolerance came from Rose and Witebsky (1956) who showed that rabbits immunized with homologous thyroid extract and adjuvant developed a thyroid lymphocytic infiltrate and thyroglobulin (TG) antibodies. The latter were detected in Hashimoto's thyroiditis during the same year by Roitt et al. (1956).

### Clinical, Pathologic, and Epidemiologic Features

Several types of AT have been described (Table 40.1). Two in particular, goitrous (Hashimoto's) thyroiditis and atrophic thyroiditis (primary myxedema), result in hypothyroidism (Pearce et al., 2003). The goiter in Hashimoto's thyroiditis is usually firm and painless, with an irregular surface. Patients are often euthyroid at

**TABLE 40.1** Types of Autoimmune Thyroiditis

Type	Course	Features
Goitrous (Hashimoto's) thyroiditis	Chronic: leads to hypothyroidism	Goiter: moderate-to-extensive lymphocytic infiltration and variable fibrosis
Atrophic thyroiditis (primary myxedema)	Chronic hypothyroidism	Atrophy: fibrosis and variable lymphocytic infiltrate
Juvenile thyroiditis	Chronic but may remit	Small goiter with moderate lymphocytic infiltrate
Postpartum thyroiditis	Transient thyrotoxicosis and/or hypothyroidism 3–6 months after delivery	Small goiter with some lymphocytic infiltrate
Silent thyroiditis	Transient thyrotoxicosis and/or hypothyroidism	Small goiter with some lymphocytic infiltrate
Focal thyroiditis	Progressive in some patients	Occurs in 20%–40% thyroid specimens at autopsy: associated with thyroid carcinoma

presentation but serum thyroid-stimulating hormone (TSH) levels can be elevated even if thyroxine (T4) levels are within the reference range, representing subclinical thyroid failure. Primary myxedema is typically identified when hypothyroidism is apparent clinically and biochemically.

Autoimmunity accounts for over 90% of the noniatrogenic hypothyroidism in iodine-sufficient countries. Women are 5–10 times more likely to be affected, with a peak incidence at 50–60 years of age. The prevalence of AT in the general Caucasian population is 0.5 per 1000 (but only half this in black people), whereas thyroid autoantibodies can be found in up to 20%, reflecting the presence of focal thyroiditis (Eaton et al., 2010).

Pathological changes in AT range from mild focal thyroiditis to extensive lymphocytic infiltration and scarring (Fig. 40.1). In Hashimoto's thyroiditis, there is a dense infiltration by lymphocytes, plasma cells, and macrophages, and germinal center formation. Thyroid follicles are progressively destroyed and, in the process, the cells undergo hyperplasia and oxyphil metaplasia to become Hürthle cells. In rare cases, there are concurrent changes of Graves' disease, so-called hashitoxicosis. There is a variable degree of fibrosis and, when this is extensive, the picture may resemble primary myxedema, in which the gland is atrophic and there is extensive fibrosis with the loss of normal lobular architecture and minimal or modest lymphocytic infiltration. Patients with high serum IgG<sub>4</sub> levels and increased IgG<sub>4</sub>-positive plasma cells in the thyroid have more stromal fibrosis, lymphoplasmacytic infiltration, and hypothyroidism (Kottahachchi and Topliss, 2016). The histology in postpartum and silent thyroiditis resembles Hashimoto's thyroiditis.

## Autoimmune Features

### Autoantibodies

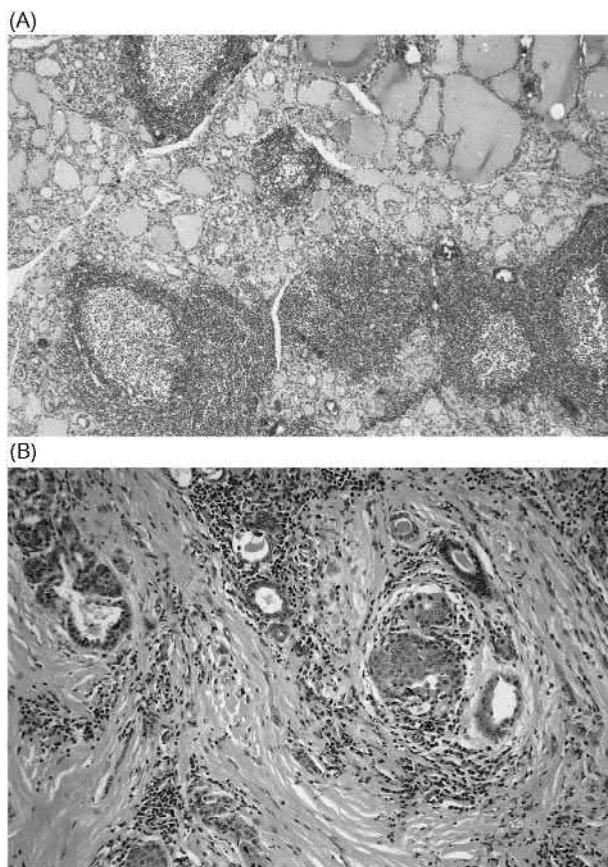
Circulating autoantibodies against TG and thyroid peroxidase (TPO) are found, often at very high levels, in most patients with AT. These antibodies are common, albeit at low levels, in association with focal thyroiditis.

### THYROGLOBULIN ANTIBODIES

TG is a 660-kDa homodimeric glycoprotein secreted by thyroid follicular cells (TFC) and stored in the luminal colloid. At 4–8 hormonogenic sites, iodinated tyrosine residues couple to form T4 or triiodothyronine (T3). The iodination state of TG alters its immunogenicity in animals and in man (Saboori et al., 1999). There are two major and one minor antibody epitopes on each 330-kDa subunit, and the wide spacing of these prevents IgG cross-linking and therefore complement fixation. The restriction of the response to three epitopes is only relative, as TG antibodies recognize an increasing number of determinants as their titer rises and somatic hypermutation occurs (McIntosh et al., 1998). Antibody reactivity is predominantly in the IgG<sub>1</sub> and IgG<sub>4</sub> subclasses but not light chain-restricted.

### THYROID PEROXIDASE ANTIBODIES

TPO is a 100–105-kDa apical membrane protein responsible for tyrosine iodination and coupling in the formation of thyroid hormones. The molecular characterization of this autoantigen has been reviewed extensively



**FIGURE 40.1** Pathology of autoimmune hypothyroidism (A) Hashimoto's thyroiditis showing germinal center formation; (B) primary myxedema with extensive fibrosis. (Original magnification:  $\times 100$ ). Photo courtesy of Dr. Judith Channer, Northern General Hospital, Sheffield.

(McLachlan and Rapoport, 2007). Antibodies to TPO have a similar IgG subclass distribution to TG but are kappa light chain-restricted (McIntosh et al., 1998). A total of 80% of the TPO antibodies recognize an immunodominant region involving overlapping, conformational epitopes in two extracellular domains: specific patterns of TPO autoantibody recognition are stable in an individual and genetically transmitted in AT families (Jaume et al., 1999). In vitro, TPO can bind C4 complement component, which may contribute to the susceptibility of TFC to destruction (Blanchin et al., 2003).

#### OTHER AUTOANTIBODIES

TSH-receptor (TSH-R) blocking antibodies (TBAb) are found in around 20% of the patients with AT (Diana et al., 2017). They contribute significantly to hypothyroidism in some patients, but there is no close correlation with the presence or absence of a goiter (Feingold et al., 2009). Other thyroid-specific autoantibodies, found in 10%–20% of the patients, recognize the  $\text{Na}^+/\text{I}^-$  transporter and pendrin, but any importance of these in pathogenesis is unknown (Brix et al., 2014). Autoantibodies against T4 and T3 occur in 15%–35% of the patients with AT. AT occurs in association with many other autoimmune disorders (Boelaert et al., 2010), and the respective autoantibodies may help in diagnosing these associated conditions.

#### T-Cell Responses

A major site of autoreactivity is within the thyroid itself, although autoimmune responses can also be detected within the draining lymph nodes and bone marrow (Weetman et al., 1984). The recruitment of lymphocytes to the thyroid requires upregulation, on endothelial cells, of various adhesion molecules and selectins, and the infiltrating lymphocytes express the reciprocal adhesion molecules, including CD11a, CD18, CD29, CD49a, and CD49e (Marazuela, 1999). The local infiltrate produces an array of chemokines which aid homing, including CXCL12, CXCL13, and CCL22, particularly when lymphoid follicles are present, and the TFC contribute to

the chemokine pool, exacerbating the autoimmune process (Liu et al., 2008). Most phenotyping and functional studies on T cells have used the readily sampled peripheral blood: this source may reflect poorly (if at all) the responses within the autoimmune target.

### STUDIES OF T-CELL PHENOTYPES

The number of circulating HLA-DR<sup>+</sup> (activated) T cells is elevated and CD8<sup>+</sup> T cells are reduced, but only in active thyroiditis (Iwatani et al., 1992). CD4<sup>+</sup> T cells predominate in the thyroid infiltrate and many of these are activated (Aichinger et al., 1985). The majority of T cells express the  $\alpha\beta$  receptor and no obvious bias in T-cell receptor usage is apparent (McIntosh et al., 1997). Although a pauciclonal T-cell response seems likely in early AT, by the time of clinical presentation, there is spreading of the immune response to produce a polyclonal T-cell response, directed against an array of autoantigens and epitopes.

#### **Functional Studies**

Weak T-cell proliferative responses against TG and TPO are found in many patients and can be enhanced by IL-2 supplementation (Butscher et al., 2001). To identify T-cell epitopes for TPO, circulating T cells from patients with AT have been stimulated with overlapping synthetic peptides (Tandon et al., 1991). No dominant epitope has been identified; instead there is considerable heterogeneity both within and between individual patients. A T-cell epitope on TG has been identified as a strong and specific binder to the major histocompatibility complex (MHC) class II disease susceptibility HLA-DR $\beta$ 1-Arg74 molecule, and this stimulates T cells from both mice and humans with AT (Menconi et al., 2010). Such an epitope could initiate an immune response that then spreads to involve other autoantigens and fits with observations in an animal model of AT that self-tolerance may be broken first for TG and subsequently for TPO (Chen et al., 2010). Impaired activity in T regulatory cells (Tregs) has been demonstrated in patients with AT and with Graves' disease (Glick et al., 2013).

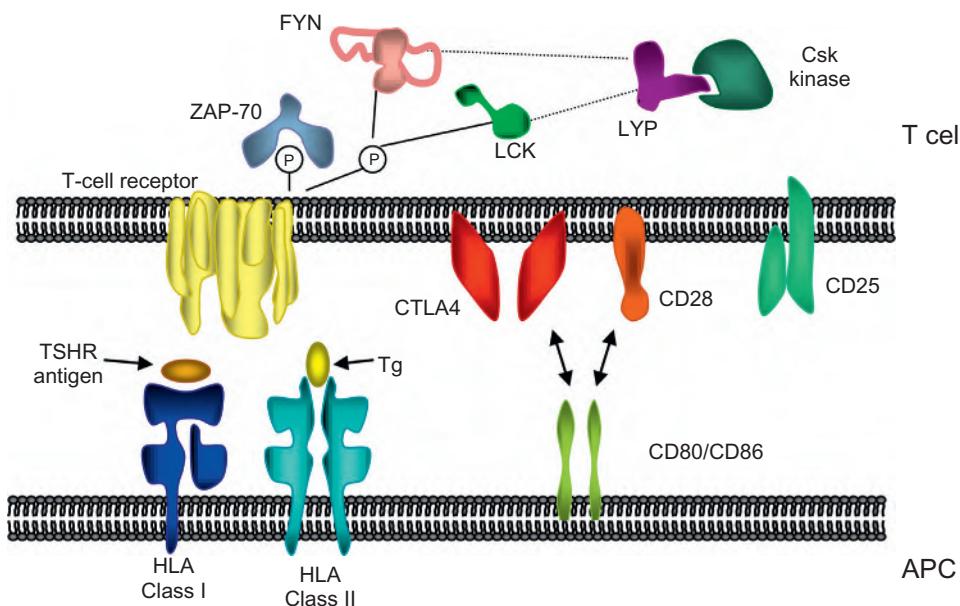
Reverse transcription of cytokine mRNA and cDNA amplification has revealed a mixed Th1 and Th2 response in Hashimoto's thyroiditis (Aijan et al., 1996). The demonstration of MHC class II expression on TFC in Hashimoto's thyroiditis and Graves' disease gave rise to the concept that such "aberrant" expression could initiate or perpetuate the autoimmune response by converting the TFC into antigen-presenting cells (Bottazzo et al., 1983). However, class II expression is restricted to TFC adjacent to T cells producing IFN- $\gamma$  (Hamilton et al., 1991), and only this cytokine is capable of initiating class II expression in vitro. MHC class II expression is found on TFC in experimental AT (EAT) induced by neonatal thymectomy (see below) but always follows the appearance of a lymphocytic infiltrate. TFC do not express costimulatory molecules even after cytokine exposure (Marelli-Berg et al., 1997). Together these results indicate that TFC are unlikely to initiate the autoimmune response.

T-cell lines and clones derived from the thyroids of patients can be activated by class II-expressing TFC (Londei et al., 1985). Such T cells are likely to have been previously activated by classic antigen-presenting cells and are no longer dependent on B7 costimulation (Marelli-Berg et al., 1997). Anergy can be induced in B7-dependent, naïve T cells by class II-positive TFC, and this type of peripheral tolerance may be important in regulating autoreactive T cells within the normal thyroid. In established AT, however, T cells are resistant to tolerance induction (Dayan et al., 1993) and class II-expressing TFC may then help to perpetuate the autoimmune response. TFC may participate in the autoimmune process in other ways besides MHC class II expression, through the expression of other immunologically active molecules such as CD40 and CD56.

### Genetic Features

The role of genetic factors in AT (Fig. 40.2) is suggested by the frequent presence of thyroid autoantibodies in other family members and the association of thyroiditis with other endocrinopathies as part of the type 2 autoimmune polyglandular syndrome. Twin studies show a 0.55 concordance rate in monozygotic, but not dizygotic, twins, and similar findings have been reported for the aggregation of thyroid autoantibodies in the euthyroid twins of individuals with AT (Brix et al., 2000, 2004).

As with most autoimmune disorders, associations with HLA alleles have been extensively investigated, producing conflicting results (Tomer and Davies, 2003). Initially it appeared that primary myxedema and Hashimoto's thyroiditis in Caucasians had distinct associations with HLA-DR3 and -DR5, respectively, but subsequent studies have shown that Hashimoto's thyroiditis is associated with HLA-DR3 and to a lesser extent HLA-DR4. Postpartum thyroiditis has a weak association with HLA-DR5.



**FIGURE 40.2** Genetic associations in autoimmune thyroid disease. HLA classes I and II polymorphisms on the APC interact with presentation of the polymorphic thyroid antigens TSH-R and Tg. The binding of CTLA-4 and CD28 with CD80/CD86 (B7), IL-2 signaling via CD25, and *PTPN22*-encoded signaling all regulate T-cell activation. APC, Antigen-presenting cell; Tg, thyroglobulin; TSH-R, thyroid-stimulating hormone receptor. From Zeitlin, A.A., et al., 2008. *Genetic developments in autoimmune thyroid disease: an evolutionary process*. *Clin. Endocrinol. (Oxf.)* 68, 671–682, with permission.

Polymorphism in *CTLA-4* confers a relative risk of around 1.3–1.5 (Ji et al., 2013). The existence of many genes with individual small effects, gene–gene interactions, and subset effects may explain why it has been more difficult to show the consistent effects of other individual genes in patient populations than would be expected from the twin data on genetic susceptibility (Tomer, 2010). Genome-wide association studies are beginning to reveal novel loci such as *LPP* and *BACH2* which are implicated in other autoimmune disease as well as AT (Cooper et al., 2012), and polymorphisms in *MAGI3* are associated with progression from TPO antibody-positivity to full-blown AT (Medici et al., 2014). Around 45% of the Turner's syndrome patients have TPO antibodies and a third become hypothyroid (Mortensen et al., 2009); there is a similar propensity in Down's syndrome, and in both cases, there may be progression to Graves' disease (Aversa et al., 2014).

## Environmental Influences

The female preponderance of AT may be due to the influence of sex steroids, although skewed X chromosome inactivation may be a further factor. Certainly in EAT, estrogens or progesterone exacerbates thyroiditis, and this is reversed by testosterone (Okayasu et al., 1981; Ansar-Ahmed et al., 1983). Pregnancy ameliorates AT, but there is an exacerbation in the year after delivery, reflected by a rise in TPO antibody levels. In some women with a previously mild thyroiditis, the enhanced autoimmune response is sufficient to cause biochemical or clinical thyroid dysfunction, and this is termed postpartum thyroiditis (Alexander et al., 2017). These changes may be related to the fluctuations in regulatory factors operating to maintain fetal tolerance during pregnancy. Although usually a transient phenomenon, around a quarter of such women develop permanent hypothyroidism within 10 years, so that pregnancy is a risk factor which precipitates clinical disease in predisposed subjects. Fetal microchimerism may play a pathogenic role via intrathyroidal chimeric cells breaking immunological tolerance, and such a possibility is supported by the increase in the prevalence of high titer TPO antibodies with advancing parity (Greer et al., 2011).

A strong influence of exogenous environmental factors is suggested by epidemiological data, such as the 10-fold rise in AT prevalence over three decades in Italy (Benvenega and Trimarchi, 2008). There is no convincing evidence for a direct role of infection in etiology (Tomer and Davies, 1993). AT is more prevalent in the areas of increased prosperity and hygiene, compatible with the 'hygiene hypothesis' which suggests that exposure to microbial antigens may skew the immune response to protect against autoimmunity (Kondrashova

et al., 2008). Indirect evidence for dietary iodine-induced injury is provided by studies of iodine-deficient regions, and an increase in thyroid autoantibodies and lymphocytic thyroiditis shortly after iodine supplementation has generally been reported. As well as causing thyroid injury through the generation of reactive oxygen metabolites (Bagchi et al., 1995), iodine enhances the immunogenicity of TG (Barin et al., 2005). Deficiency of both vitamin D and selenium has also been associated with AT, but trials of selenium supplementation have shown only a modest effect on thyroid autoantibody levels (Wichman et al., 2016).

Smoking is associated with a lower risk of AT and smoking cessation temporarily increases the risk (Wiersinga, 2013); alcohol consumption is also a protective influence (Effraimidis et al., 2012). The administration of lithium may exacerbate AT both immunologically and biochemically, but the adverse effect of the cytokine IFN- $\alpha$  and other antineoplastic drugs on autoimmune thyroid dysfunction is more striking (Hamnvik et al., 2011).

## In Vivo Models

AT can be induced experimentally in animals, and also occurs spontaneously, with features most closely resembling Hashimoto's thyroiditis.

### **Immunization-Induced Thyroiditis**

In mice and rats, the strength of the autoimmune response to immunization with TG in adjuvant is strain dependent. Murine EAT susceptibility (as shown by the severity of lymphocytic thyroiditis) is governed by the class II I-A subregion of the H-2 major histocompatibility complex (Vladutiu and Rose, 1971). H-2<sup>k,s</sup> strains may even develop EAT with syngeneic TG immunization alone, demonstrating that untolerized autoreactive T cells exist in normal animals, in which they are usually under active regulation (ElRehewy et al., 1981). The influence of I-E is strain dependent but less clear. MHC classes I, K, and D alleles also influence susceptibility, presumably by determining the strength of effector cytotoxic T-cell interaction with the thyroid cell target. Transgenic mice expressing HLA-DR3, but not HLA-DR2, develop EAT after TG immunization, confirming a role for this HLA specificity in thyroiditis (Kong et al., 1996).

Female animals have worse EAT than males, and this is dependent on sex hormones, estrogen excess worsening thyroiditis, and testosterone ameliorating it (Okayasu et al., 1981). Another important influence in mice is the level of circulating TG, which can induce Tregs (Kong et al., 2009). The thyroiditis after immunization consists of CD4<sup>+</sup> and CD8<sup>+</sup> T cells and macrophages, with only a small percentage of B cells. Disease can be transferred by T cells but not by TG antibodies, and the critical effector cells are CD8<sup>+</sup> cytotoxic T cells which require specific CD4<sup>+</sup> T cells for their induction (Creemers et al., 1983). Immunization with the immunodominant T-cell epitope of murine TPO will also induce EAT (Ng and Kung, 2006).

### **Experimental Autoimmune Thyroiditis Resulting From Immune Modulation**

Neonatal thymectomy in certain strains of mice or rats, or thymectomy plus sublethal irradiation in certain strains of rats, results in severe EAT (Penhale et al., 1973; Kojima et al., 1976), as well as other autoimmune endocrinopathies. From these initial observations came the understanding of the crucial role of CD4<sup>+</sup>, CD25<sup>+</sup> Tregs in preventing autoimmunity (Sakaguchi et al., 2001). Other maneuvers affecting T cells, such as treatment of neonatal mice with cyclosporin A or T-cell depletion and reconstitution, can induce EAT (Sakaguchi and Sakaguchi, 1989). The depletion of CD7/CD28 in knock-out mice also prevents generation of CD4<sup>+</sup>, CD25<sup>+</sup> Tregs and results in EAT (Sempowski et al., 2004). The induction of EAT by T-cell modulation elegantly demonstrates the presence of thyroid-reactive T cells in the normal newborn repertoire. These CD4<sup>+</sup> T cells may be destined for deletion after birth, but there is now compelling evidence for incomplete tolerance and active suppression by Tregs even later.

In addition to MHC and non-MHC genes, environmental factors play an important role in susceptibility. Rats raised under specific pathogen-free conditions until weaning are resistant to EAT following thymectomy and irradiation, but the transfer of normal gut microflora results in EAT in the germ-free animals (Penhale and Young, 1988). It is unclear whether radiation-induced damage to the intestine is involved in this effect of gut microflora.

### **Spontaneous Autoimmune Thyroiditis**

Several species develop spontaneous thyroiditis. Lymphocytic infiltration of the thyroid and TG antibodies occur in around 60% of the diabetic and 10% of the nondiabetic BioBreeding (BB) rats, but diabetes-prone

sublines have a range of prevalence, from 100% in the NB line to 5% in the BE line, suggesting that diabetes is not tightly linked genetically to EAT (Crisa et al., 1992). The Buffalo strain rat has a low spontaneous incidence of thyroiditis, reaching a maximum of 25% in old, multiparous females (Noble et al., 1976). Nonobese diabetic (NOD) mice infrequently develop thyroiditis, but this trait is greatly enhanced by deficiency of the chemokine receptor CCR7, possibly related to the roles of CCR7 in negative selection of autoreactive T cells in the thymus and Treg control (Martin et al., 2009). CD28-knockout NOD mice have a deficiency of Tregs and develop severe thyroiditis, with thyroid fibrosis and hypothyroidism (Ellis et al., 2013).

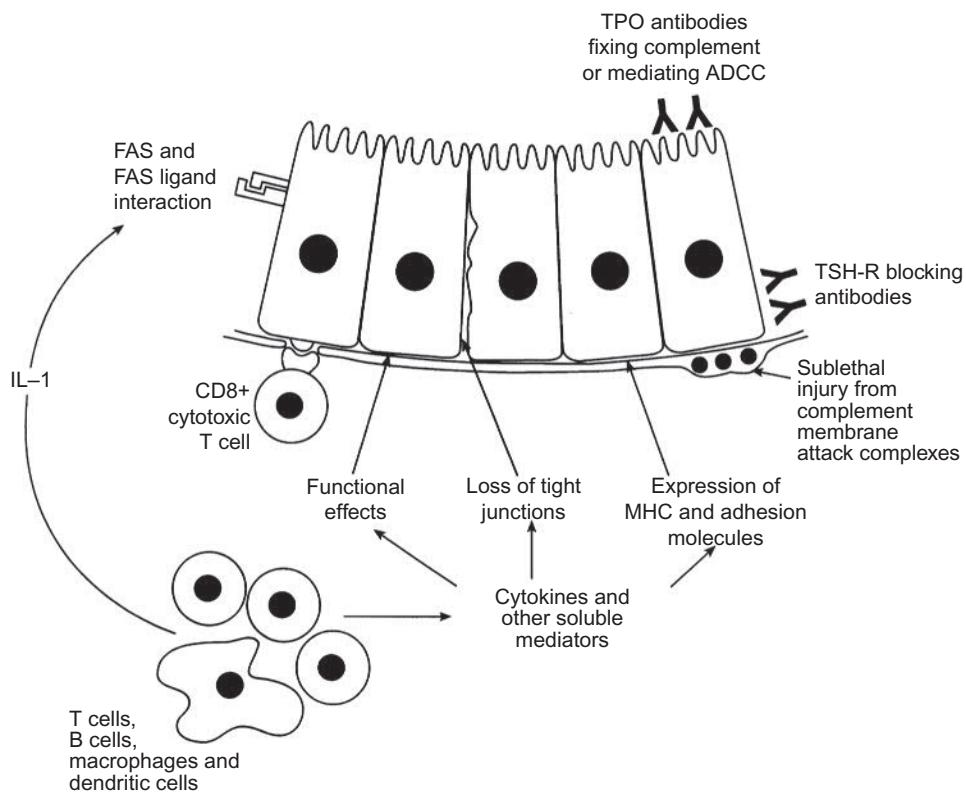
Spontaneous AT in the Obese strain (OS) chicken is the closest animal model of Hashimoto's thyroiditis. The birds were originally bred from a White Leghorn flock of Cornell strain chickens for phenotypic features of hypothyroidism. Over time, the factors influencing disease development have changed: the importance of MHC genes and sex has diminished and the main genetic determinants in current OS chickens govern target organ susceptibility, T-cell hyperreactivity, and corticosteroid responses (Wick et al., 2006). Unlike other models, OS chickens develop severe hypothyroidism as well as a lymphocytic thyroiditis and TG antibodies, and require T4 supplementation to thrive. The disease is T-cell dependent as thymectomy at birth prevents disease, although later thymectomy exacerbates thyroiditis, presumably by altering the balance of T cell-mediated regulation.

## Pathologic Effector Mechanisms

Several antibody-dependent and cell-mediated mechanisms contribute to thyroid injury in autoimmune hypothyroidism, and differences in the relative importance of each may determine some of the clinical and pathological variants described above (Fig. 40.3).

### Antibody-Mediated Injury

Immune complexes are deposited in the basement membrane around the thyroid follicles in Hashimoto's thyroiditis (Pfaltz and Hedinger, 1986), and terminal complement complexes are also present at this location indicating the formation of membrane attack complexes (Weetman et al., 1989). TFC are relatively resistant to lysis,



**FIGURE 40.3** Mechanisms of thyroid destruction in autoimmune hypothyroidism. From Weetman, A.P., 2002. *Autoimmune thyroid disease*. In: Wass, J.A.H., Shalet, S.M. (Eds.), *Oxford Textbook of Endocrinology and Diabetes*. Oxford University Press, pp. 392–408, with permission.

through enhanced expression multiple regulators of complement activation, especially CD59, in response to cytokines derived from the infiltrating lymphocytes and macrophages (Tandon et al., 1994). After sublethal complement attack, TFC are less able to respond to TSH stimulation and also release cytokines (IL-1, IL-6), prostaglandin E<sub>2</sub>, and reactive oxygen metabolites, which may have proinflammatory effects (Weetman et al., 1992).

TPO antibodies are generally assumed to be the major mediators of complement fixation and activation within the thyroid and may also provoke damage by antibody-dependent cell-mediated cytotoxicity (Rebuffat et al., 2008). Cytokines such as IL-1 may be critical in dissociating the junctional complex and thus allowing access of autoantibodies to apically expressed TPO and other such antigens (Nilsson et al., 1998), in turn implying a secondary role for these antibodies in pathogenesis and explaining why neonates born to mother with TPO antibodies have normal thyroid function. TBAb directly impair thyroid cell function, as shown by their placental transfer which causes transient neonatal hypothyroidism (Matsuura et al., 1980).

### **T-Cell Mediated Injury**

Despite the strong lead provided by studies in EAT, there is only modest direct evidence that T-cell mediated injury is important in autoimmune hypothyroidism. Two groups have derived CD8<sup>+</sup> T-cell clones and lines from patients with Hashimoto's thyroiditis which lyse autologous TFC in a MHC class I-restricted fashion (MacKenzie et al., 1987; Sugihara et al., 1995). The autoantigen specificity of these T cells has not been elucidated. Indirect evidence for the importance of cytotoxic T cells in pathogenesis is provided by the demonstration of frequent perforin-containing T cells in the intrathyroidal CD8<sup>+</sup> T-cell population in Hashimoto's thyroiditis (Wu et al., 1994). T cells may also provoke thyroid dysfunction by release of cytokines. There is an increase in intrathyroidal Th17 lymphocytes and enhanced synthesis of Th17 cytokines in AT, implying a role for these proinflammatory cells in pathogenesis (Figueroa-Vega et al., 2010). Circulating platelet-derived microvesicles are increased in AT patients and can inhibit the differentiation of Tregs and induce Th17 cell differentiation (Rodríguez-Muñoz et al., 2015). Hürthle cell formation results from the overexpression of immunoproteasome subunits secondary to chronic inflammation (Kimura et al., 2009).

Attention has also focused on the expression of death receptor-mediated apoptosis as a major pathway for TFC destruction, based on the finding of both Fas (CD95) and Fas ligand (CD95L) on TFC in Hashimoto's thyroiditis (Giordano et al., 1997). Fas expression by TFC was upregulated by IL-1 $\beta$ , leading to the suggestion that cytokines could induce cytotoxicity through this pathway by suicide or fratricide as Fas interacted with FasL. Moreover, it has been proposed that FasL on TFC induces apoptosis in the infiltrating lymphocytes, suggesting that a T cell-mediated cytotoxic mechanism for thyroid destruction is less important than autocrine/paracrine Fas–FasL interaction (Stassi et al., 1999). Other interpretations have been put on the role of such apoptosis, not least because of the possible role of other decoy death receptors and regulators of apoptosis signaling, and these multiple complex pathways may explain why induction of apoptosis in AT results in the destruction of thyroid cells, while apoptosis in the graves' disease leads to the damage of thyroid-infiltrating lymphocytes (Wang and Baker, 2007).

### **Autoantibodies as Potential Immunological Markers**

The diagnosis of autoimmune hypothyroidism is usually straightforward, patients having biochemical evidence of hypothyroidism plus TG and/or TPO antibodies which can easily be measured by passive hemagglutination or immunoassays. An abnormal thyroid ultrasound pattern is also highly predictive of AT (Raber et al., 2002). Fine-needle aspiration biopsy is used in difficult cases, especially to exclude an associated lymphoma.

### **Treatment and Outcome**

Treatment consists of T4 replacement (Jonklaas et al., 2014), which is generally straightforward, and future attempts at immunomodulation seem unlikely to improve outcome. In around 10% of the patients, there may be a spontaneous remission 4–8 years after starting T4, and this is associated with the disappearance of TBAb (Takasu et al., 1992). However, the permanence of such remissions has not been established. Lymphoma is a rare complication of AT.

## Concluding Remarks—Future Prospects

Considerable progress in understanding the pathogenesis of AT has been made in the half century since the first demonstration of EAT and the realization that a similar process accounts for Hashimoto's thyroiditis. Additional work is needed to determine the exact genetic basis for AT and to clarify the relationships between these disorders and Graves' disease, which are associated together in families and sometimes in the same individual. It may also be possible in future to use autoantibody patterns to predict which patients are at most risk of developing hypothyroidism. Determining the exact role for T cell-mediated, thyroid-specific immunoregulation in preventing AT remains complex. There is no doubt that Tregs are important, but so far, the precise mechanisms that control thyroid-specific T cells that have escaped central tolerance in human AT are elusive and not yet able to be exploited therapeutically.

## GRAVES' DISEASE

### Historic Background

The first description of this disorder was by Caleb Parry in 1825, but it was Robert Graves whose name became attached to the disease through his report of four cases published in 1835. Basedow was the first to highlight the association with exophthalmos in 1840. Originally believed to have a cardiac and then neurological origin, the role of the thyroid in Graves' disease became established in the 1890s as thyroidectomy for apparently coincidental goiter improved the other manifestations. The cause remained obscure until [Adams and Purves \(1956\)](#) showed that the serum from Graves' patients contained a long-acting thyroid stimulator (LATS) which was distinct from TSH: working separately, Kriss and McKenzie went on to show this stimulator was an IgG.

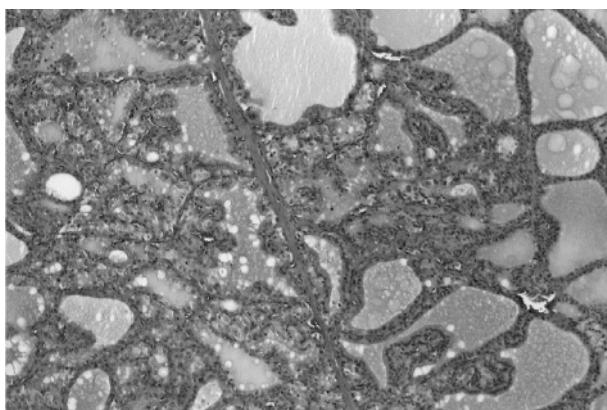
### Clinical, Pathologic, and Epidemiologic Features

Although sharing many immunological features with AT, it is the production of TSH-R-stimulating antibodies (TSAb), which characterizes Graves' disease ([Smith and Hegedüs, 2016](#)). It is the commonest cause of hyperthyroidism, accounting for 60%–80% of the cases. The prevalence is around four per 1000, with a five- to 10-fold higher frequency in women, and it is more common in black and Asian/Pacific Island people ([Eaton et al., 2010](#)). Over 70% of the patients with Graves' disease have thyroid-associated ophthalmopathy (TAO), which can be revealed by scanning techniques showing enlarged extraocular muscles ([Bahn, 2010](#)). Clinically obvious eye disease is apparent in around 50% of the patients ([Fig. 40.4](#)). TAO is not exclusive to Graves' disease, as around 5% of the patients have AT, and another 5% have little evidence of thyroid dysfunction. Thyroid dermopathy (or pretibial myxedema, reflecting the usual site for this complication) occurs in only around 1% of the patients, who typically also have marked TAO ([Fatourechi, 2012](#)).

In the untreated state, there is both hypertrophy and hyperplasia of the thyroid follicles; the epithelium becomes columnar and folded into the follicular lumen, new small follicles form, and there is little colloid ([Fig. 40.5](#)). A variable degree of lymphocytic infiltration occurs, and germinal centers may form. Antithyroid



**FIGURE 40.4** Eye signs in a patient with thyroid-associated ophthalmopathy showing exophthalmos, scleral injection, and periorbital edema.



**FIGURE 40.5** Pathology of Graves' disease showing columnar and folded thyroid epithelium, small new follicles, and active colloid resorption, with "scalloping" of the colloid. A lymphocytic infiltrate is not prominent in this specimen. (Original magnification:  $\times 100$ .) Photo courtesy of Dr. Judith Channer, Northern General Hospital, Sheffield.

dugs diminish the lymphocytic infiltrate and the epithelium reverts to a normal appearance. Lymphoid hyperplasia may occur in the lymph nodes, thymus, and spleen, but reverses with antithyroid drugs.

## Autoimmune Features

### Autoantibodies

TG and TPO autoantibodies occur up to 80% of the patients. TSAb can be detected in over 95% of the patients with current assays. The TSH-R consists of a 398 amino acid extracellular domain with a series of leucine-rich repeats and a hinge region, a 266 amino acid transmembrane spanning domain with seven hydrophobic regions, and an 83 amino acid intracellular domain (Rapoport and McLachlan, 2007). The receptor undergoes complex posttranslational processing at the hinge region to form A- and B-subunits linked by a disulfide bond. Thyroid cells shed the A-subunit, which binds TSAb more strongly than the holoreceptor, and this is an important factor in the induction and affinity maturation of TSAb (Rapoport and McLachlan, 2016). TSAb-induced activation of the TSH-R typically causes a rise in cAMP, but TSH-R antibodies may also cause activation of other intracellular signaling pathways.

The original bioassay method measured release of radioiodine from preloaded thyroid glands after injection of serum or IgG into intact animals, and this activity was termed LATS (Adams and Purves, 1956). Other early assays measured cAMP release from primary TFC cultures or thyroid cell lines (such as FRTL-5); more recently TSH-R-transfected eukaryotic cells have been utilized (Ajjan and Weetman, 2008). A separate type of assay measures displacement of TSH from solubilized or recombinant TSH-R by TSH-R antibodies in a radiolabeled or chemiluminescent format; these antibodies are called TSH-binding inhibiting immunoglobulins or TBII. Current TBII assays have a sensitivity and specificity which exceeds 95% but measure both TSAb and TBAb (which inhibit TSH-induced cAMP release). Therefore the level of TBII gives no direct information on functional activity, but in practice these assays are very useful when interpreted in the clinical context (McLachlan and Rapoport, 2013). Unlike TG and TPO autoantibodies, TSAb are IgG<sub>1</sub> subclass restricted and are often  $\lambda$  light chain-restricted (Zakarija, 1983), suggesting a pauciclonal origin for TSAb in some patients.

The majority of TSH-R B-cell epitopes appear to be conformational and overlap with the binding site for TSH (Rapoport and McLachlan, 2007). Heterogeneity between patients in their antibody binding sites is apparent, for instance by studies using chimeric TSH/luteinizing hormone receptors or monoclonal TSH-R antibodies, and mutated receptors have revealed that a component of the epitope for TSAb is on the N terminus. The epitopes for TBAb overlap with those for TSAb but are more focused on the C terminus and recognize holoreceptor more efficiently. TSH-R neutral antibodies have also been identified which do not stimulate cAMP production but can induce thyroid cell apoptosis in vitro (Morshed et al., 2010).

### T-Cell Responses

Many studies have found a reduction in circulating CD8<sup>+</sup> T cells and an increase in HLA-DR<sup>+</sup> T cells in active Graves' disease (Weetman and McGregor, 1994). Intrathyroidal T cells are CD4<sup>+</sup> and CD8<sup>+</sup> in varying

proportions; similar homing mechanisms to AT account for their localization (Marazuela, 1999). Using overlapping TSH-R peptides covering the extracellular domain, a heterogeneous response was obtained using circulating or intrathyroidal T cells in proliferation assays, with multiple peptides stimulating different patients' cells (Tandon et al., 1992). There is no distinct Th1 or Th2 pattern of response by the stage of disease that specimens are available for study (Okumura et al., 1999). Cytokines such as IL-1, IL-6, IL-8, and IL-10 are strongly expressed, with some cytokines being in part derived from the TFC themselves. As in AT, there are plentiful Tregs in the thyroid in Graves' disease, apparently without the ability to halt disease (Marazuela et al., 2006). This Treg defect may at least in part be the result of impaired plasmacytoid dendritic cell function and elevated levels of thyroid hormone (Mao et al., 2011).

## Genetic Features

Monozygotic twins are around 20%–30% concordant for Graves' disease, at least 10-fold higher than for dizygotic twins (Brix et al., 1998). A consistent but weak association exists between the serologically defined specificity HLA-DR3 and Graves' disease in Caucasians, with a relative risk of 2–3 (Tomer and Davies, 2003). The relative weakness of the contribution of HLA genes is underlined by the low concordance (7%) for Graves' disease in HLA-identical siblings (Stenszky et al., 1985). Many candidate gene polymorphisms (encoding immune receptors, immunoglobulins, and cytokines) have been tested for associations with Graves' disease with inconsistent results (Fig. 40.2). Polymorphisms in *CTLA-4* confer a relative risk of around 2–3 (Yanagawa et al., 1995), and polymorphism in other genes affecting B and T-cell responses, *PTPN22*, *CD25*, *FCRL3*, and *CD226*, have also been associated with Graves' disease (Tomer, 2010). The sharing of these genetic associations with many other autoimmune diseases probably accounts for the frequent concurrence of other autoimmune diseases with Graves' disease and AT.

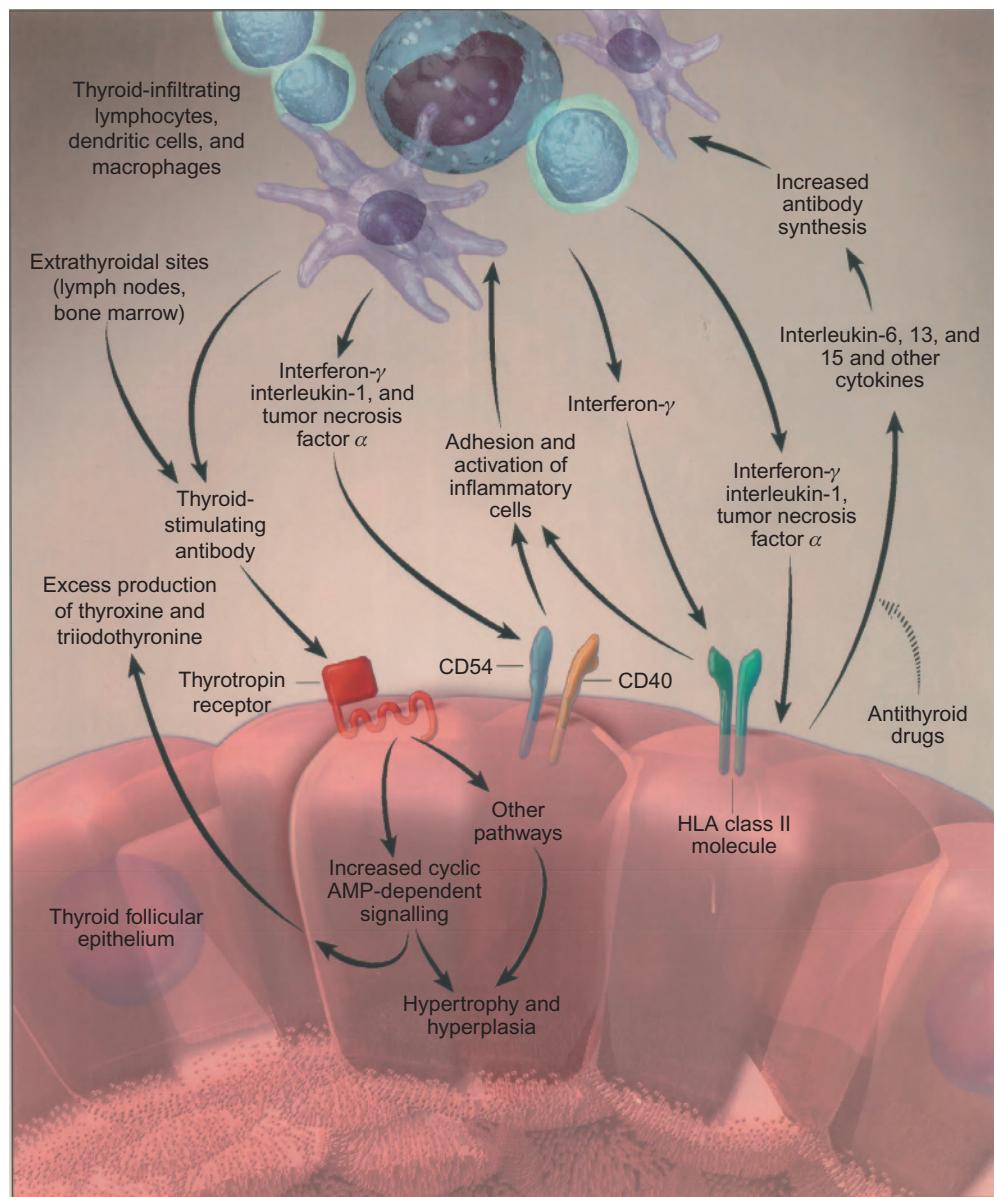
Polymorphisms in the *TSH-R* gene confer susceptibility to Graves' disease, but not autoimmune hypothyroidism, and may be an important factor in determining which members of families with a predisposition to develop thyroid autoimmunity actually get Graves' disease (Brand et al., 2009). Genome-wide association studies using thousands of patients with Graves' disease have revealed many new susceptibility loci including the *RNASET2-FGFR1OP-CCR6* region at 6q27, *MMEL1*, *LPP*, *BACH2*, *FGFR1OP*, and *PRICKLE1*, as well as the gene encoding TG (Chu et al., 2011; Cooper et al., 2012; Zhao et al., 2013).

## Environmental Influences

Women are predisposed to Graves' disease and, as with AT, parity increases the risk by around 10% (Jørgensen et al., 2012). Retrospective studies have shown a significantly higher number of adverse life events during the year preceding the recognition of Graves' disease when patients are compared to matched controls, and stress is also associated with a worse outcome after antithyroid drug treatment (Falgarone et al., 2013). Presumably this effect of stress operates through the interactions between the nervous, endocrine, and immune systems. A high iodine intake increases the risk of developing an autoimmune response against the thyroid, the type being determined by genetic factors. However, in an animal model of Graves' disease produced by immunizing NOD mice with adenovirus expressing the TSH-R A-subunit, increased iodine intake increased the severity of thyroiditis, but not hyperthyroidism, suggesting a dichotomy in these two autoimmune responses (McLachlan et al., 2005).

Evidence for a role of infections is circumstantial (Tomer and Davies, 1993). It remains possible that a variety of infections could precipitate Graves' disease either specifically by molecular mimicry or modulation of TFC behavior, or nonspecifically, by enhancing any ongoing immune responses. Such nonspecific enhancement, presumably mediated by cytokines, would explain the association between attacks of allergic rhinitis and recurrence of Graves' disease (Hidaka et al., 1993).

Smoking is strongly associated with the development of ophthalmopathy and weakly with the development of Graves' disease, although negatively associated with the presence of TG and TPO antibodies in Graves' patients (Bartalena et al., 1995; Hou et al., 2011). Cytokine treatment is sometimes complicated by the development of Graves' disease, although this is less frequent than AT (Hamnvik et al., 2011). Perhaps the most striking example of Graves' disease caused by an obvious external agent is its appearance during the period of immune reconstitution following alemtuzumab or highly active antiretroviral treatments (Weetman, 2009). This type of response may be the result of alteration levels of Tregs as the lymphocyte population is recovering.



**FIGURE 40.6** Pathogenesis of Graves' disease. Hyperthyroidism is the result of TSH-R activation by thyroid-stimulating antibodies. The intrathyroidal inflammatory response is enhanced by cytokines, some of which are derived from the thyroid cells themselves: antithyroid drugs interfere with this step in the pathway. Thyroid cells produce other molecules such as CD54, CD40, and HLA class II which help perpetuate the autoimmune response. TSH-R, Thyroid-stimulating hormone receptor. From Weetman, A.P., 2000. Graves' disease. *N. Engl. J. Med.* 343, 1236–1248, with permission.

## In Vivo Models

There is still no entirely satisfactory animal model of Graves' disease. All are induced rather than spontaneous, and the likely absence of Graves' disease even in great apes suggests that there is something unique about the susceptibility factors for Graves' disease in man (Nagayama, 2007; McLachlan et al., 2011). In the first attempts to produce an animal model, immunization of BALB/c mice with the extracellular domain of TSH-R and adjuvant containing *Bordetella pertussis* induced TBAb, but not TSAb, and a severe thyroiditis (Costagliola et al., 1994), while genetic immunization with TSH-R cDNA produced thyroiditis but without TSAb production (Costagliola et al., 1998). Immunizing AKR/N mice with fibroblasts double transfected with human TSH-R and haploidentical MHC class II genes led to hyperthyroidism caused by TSAb, although without thyroiditis (Yamaguchi et al., 1997).

Subsequently, immunization experiments with adenovirus encoding the TSH-R, especially the A-subunit alone, have been successful in producing a high frequency of disease in mice (Chen et al., 2004). Such immunization in transgenic mice expressing human TSH-R A-subunit has provided an insight into the role for Tregs in determining the disease outcome; the depletion of these cells led to thyroiditis, hypothyroidism, and spreading of the autoimmune response from TSH-R to involve TPO and TG (McLachlan and Rapoport, 2007). This model has also been used to show that pretreatment with the TSH-R A-subunit can deviate the immune response from pathogenic epitopes to the production of neutral TSH-R antibodies (Misharin et al., 2009). NOD mice made transgenic for the human TSH-R develop pathogenic antibodies to the human receptor, especially in females with iodine excess (Rapoport et al., 2015).

Reproducing ophthalmopathy has proved even more challenging. Genetic immunization by electroporation with the TSH-R A-subunit leads to the production of antibodies against the IGF-1 receptor, a possible autoantigen in ophthalmopathy, and orbital fibrosis, in addition to TSAb and thyroiditis (Zhao et al., 2011). However, this was not a consistent response in all animals. Deep-muscle electroporation with plasmid encoding the A-subunit produced either extraocular muscle inflammation or adipogenesis, with associated proptosis, in some, but not all, animals, and this was accompanied by TBAb rather TSAb production (Moshkelgosha et al., 2013). Equally inconsistent results between animals have been obtained using immunization with a different A-subunit vector, with a very little evidence of orbital muscle inflammation or proptosis, although these animals had features of hyperthyroidism (Xia et al., 2017).

## Pathologic Effector Mechanisms

Clearly the main effector mechanism in Graves' disease is the production of TSAb (Fig. 40.6), but the circulating levels of these do not correlate closely with the level of thyroid hormones or the clinical severity of disease, because of a variable, concurrent response against other autoantigens and, in some patients, production of TBAb, which reduce the effects of TSAb. Women with the highest levels of TSAb who become pregnant give birth to babies with transient neonatal thyrotoxicosis.

## Autoantibodies as Potential Immunological Markers

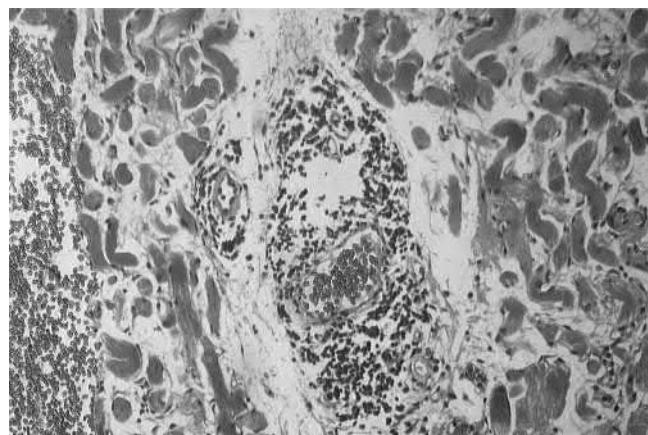
TG and TPO antibodies provide readily available evidence for Graves' disease; although not specific, their presence in a patient with hyperthyroidism strongly suggests the diagnosis. These antibodies are present in 20%–30% of the cases 5–7 years before diagnosis (Hutfless et al., 2011). Although TSAb are not generally measured because present bioassays are laborious, TBII estimation is a cheap, commercially available surrogate which is very useful in confirming the diagnosis of Graves' disease in the absence of clinical evidence such as ophthalmopathy. TSAb or TBII levels have been investigated as predictive markers for the success of antithyroid drug treatment in Graves' disease. Although patients with the highest levels tend to relapse the most, the results are insufficiently accurate for clinical use (Aijan and Weetman, 2008). The most important indication for TBII assay in Graves' disease is in women with known Graves' disease during pregnancy, when a level more than threefold higher than normal predicts the risk of neonatal thyrotoxicosis (Alexander et al., 2017).

## Treatment and Outcome

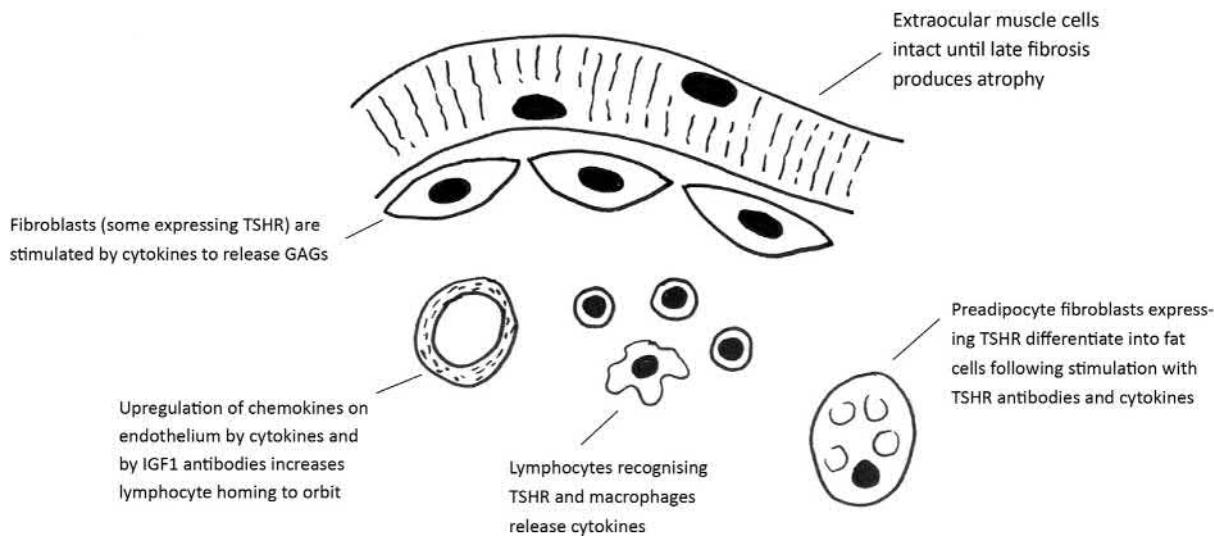
Treatment consists of antithyroid drugs (carbimazole, methimazole, or propylthiouracil), radioiodine, or thyroidectomy (Ross et al., 2016). Around 40% of the patients treated with antithyroid drugs achieve a permanent remission. These drugs ameliorate EAT and TSAb levels fall during treatment due to a decrease in the expression of proinflammatory molecules by TFC (Weetman et al., 1992). Relapse after antithyroid drugs is particularly likely in younger patients with severe hyperthyroidism or a large goiter, and those who smoke or have evidence of a strong Th2 response (Hidaka et al., 1993, Komiya et al., 2001). Radioiodine treatment is followed by a striking rise in TSH-R (and other thyroid) autoantibodies at 3–6 months, whereas TSH-R antibody levels gradually fall in most, but not all, patients over the year following subtotal thyroidectomy (Laurberg et al., 2008). The complete ablation of thyroid tissue results in disappearance of all thyroid autoantibodies, confirming the need for these autoantigens to maintain antibody production (Chiavato et al., 2003).

## Thyroid-Associated Ophthalmopathy and Dermopathy

These complications of Graves' disease are most likely due to an autoimmune response, which stimulates fibroblasts localized within the extraocular muscle or dermis, causing glycosaminoglycan release and edema, and accompanied by fat expansion (Bahn, 2010). The extraocular muscles and dermis are infiltrated by activated T cells and local cytokine production (IFN- $\gamma$ , IL-1, and tumor necrosis factor) can be demonstrated: these cytokines stimulate glycosaminoglycan synthesis by fibroblasts in vitro (Figs. 40.7 and 40.8). The main target of the autoimmune response appears to be the TSH-R which is expressed on a subset of fibroblasts, but the response may be enhanced by antibodies against the IGF-1 receptor (Wang and Smith, 2014). The treatment of mild TAO usually consists of supportive measures, and in many the condition spontaneously becomes stable or regresses; in moderate-to-severe disease, corticosteroids or other immunosuppressive agents are useful (Wiersinga, 2017).



**FIGURE 40.7** Photomicrograph of an extraocular muscle from a patient with thyroid-associated ophthalmopathy. There is an extensive lymphocytic infiltrate and edema: the muscle fibers are intact. (Original magnification:  $\times 200$ .)



**FIGURE 40.8** Pathogenic mechanisms in thyroid-associated ophthalmopathy. The inflammatory infiltrate in the orbit releases a variety of cytokines that stimulate orbital fibroblasts to secrete GAGs, which trap water and lead to edema; later fibroblast activation leads to fibrosis. A subset of orbital fibroblasts differentiates into adipocytes as a result of cytokine signaling, leading to fat expansion within the orbit. GAGs, Glycosaminoglycans. From Weetman, A.P., 2016. Autoimmune thyroid disease. In: Ratcliffe, M.J.H. (Editor-in-Chief), *Encyclopedia of Immunobiology*, vol. 5. Academic Press, pp. 150–158 with permission.

## Concluding Remarks—Future Prospects

Graves' disease shares many features with AT, and what determines the type of disorder is a critical issue. Apart from *TSH-R* polymorphism, genetic susceptibility factors have proven relatively nonspecific, and more detailed analysis should reveal specific genotypes for Graves' disease. Future studies will clarify the relative importance of stress, iodine intake, smoking, and other environmental factors, as well as how these interact with each other and with genetic factors. It will be particularly important to study mechanisms at the earliest possible stage of disease, so that an initiating rather than an enhancing role for these factors can be assessed. Experiments of nature, such as the appearance of Graves' disease after immunological treatments, may provide fresh insights into regulatory mechanisms and the early phase of disease.

The recent development of animal models of TAO will be useful in clarifying the relative roles of TSH-R, the IGF-1 receptor, and other putative orbital antigens in pathogenesis and may improve treatment options. Our current therapies for Graves' disease are effective, but not ideal, with many patients having recurrences or exchanging hyperthyroidism for permanent hypothyroidism. Immunologically based treatment offers considerable potential advantages but will need to be specific and innocuous: the recent description of a small molecular antagonist of TSAb suggests one possible approach (Neumann et al., 2011).

## References

- Adams, D.D., Purves, H.D., 1956. Abnormal responses in the assay of thyrotrophin. *Proc. Univ. Otago Med. School* 34, 11–12.
- Aichinger, G., Fill, H., Wick, G., 1985. In situ immune complexes, lymphocyte subpopulations, and HLA-DR-positive epithelial cells in Hashimoto thyroiditis. *Lab. Invest.* 52, 132–140.
- Aijan, R.A., Weetman, A.P., 2008. Techniques to quantify TSH receptor antibodies. *Nat. Clin. Pract. Endocrinol. Metab.* 4, 461–468.
- Aijan, R.A., Watson, P.F., McIntosh, R.S., Weetman, A.P., 1996. Intrathyroidal cytokine gene expression in Hashimoto's thyroiditis. *Clin. Exp. Immunol.* 105, 523–528.
- Alexander, E.K., Pearce, E.N., Brent, G.A., Brown, R.S., Chen, H., Dosio, C., et al., 2017. Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and the postpartum. *Thyroid* 27, 315–389.
- Ansar-Ahmed, S., Young, P.R., Penhale, W.J., 1983. The effects of female sex steroids on the development of autoimmune thyroiditis in thyroidectomized and irradiated rats. *Clin. Exp. Immunol.* 54, 351–358.
- Aversa, T., Lombardo, F., Corrias, A., Salerno, M., De Luca, F., Wasniewska, M., 2014. In young patients with Turner or Down syndrome, Graves' disease presentation is often preceded by Hashimoto's thyroiditis. *Thyroid* 24, 744–747.
- Bagchi, N., Brown, T.R., Sundick, R.S., 1995. Thyroid cell injury is an initial event in the induction of autoimmune thyroiditis by iodine in obese strain chickens. *Endocrinology* 136, 5054–5060.
- Bahn, R.S., 2010. Graves' ophthalmopathy. *N. Engl. J. Med.* 362, 726–738.
- Barin, J.G., Talor, M.V., Sharma, R.B., Rose, N.R., Burek, C.L., 2005. Iodination of murine thyroglobulin enhances autoimmune reactivity in the NOD.H2 mouse. *Clin. Exp. Immunol.* 142, 251–259.
- Bartalena, L., Bogazzi, F., Tanda, M.L., Manetti, L., Dell'Unto, E., Martino, E., 1995. Cigarette smoking and the thyroid. *Eur. J. Endocrinol.* 133, 507–512.
- Benvenga, S., Trimarchi, F., 2008. Changed presentation of Hashimoto's thyroiditis in North-Eastern Sicily and Calabria (Southern Italy) based on a 31-year experience. *Thyroid* 18, 429–441.
- Blanchin, S., Estienne, V., Durand-Gorde, J.M., Carayon, P., Ruf, J., 2003. Complement activation by direct C4 binding to thyroperoxidase in Hashimoto's thyroiditis. *Endocrinology* 144, 5422–5429.
- Boelaert, K., Newby, P.R., Simmonds, M.J., Holder, R.L., Carr-Smith, J.D., Heward, J.M., et al., 2010. Prevalence and relative risk of other autoimmune diseases in subjects with autoimmune thyroid disease. *Am. J. Med.* 123, 183.e1–183.e9.
- Bottazzo, G.F., Pujol-Borrell, R., Hanafusa, T., Feldmann, M., 1983. Role of aberrant HLA-DR expression and antigen presentation in induction of endocrine autoimmunity. *Lancet* 2, 1115–1119.
- Brand, O.J., Barrett, J.C., Simmonds, M.J., Newby, P.R., McCabe, C.J., Bruce, C.K., et al., 2009. Association of the thyroid stimulating hormone receptor gene (TSHR) with Graves' disease. *Hum. Mol. Genet.* 18, 1704–1713.
- Brix, T.H., Kyvik, K.O., Hegedüs, L., 1998. What is the evidence of genetic factors in the etiology of Graves' disease? A brief review. *Thyroid* 8, 727–734.
- Brix, T.H., Kyvik, K.O., Hegedüs, L., 2000. A population-based study of chronic autoimmune hypothyroidism in Danish twins. *J. Clin. Endocrinol. Metab.* 85, 536–539.
- Brix, T.H., Hansen, P.S., Kyvik, K.O., Hegedüs, L., 2004. Aggregation of thyroid autoantibodies in first-degree relatives of patients with autoimmune thyroid disease is mainly due to genes: a twin study. *Clin. Endocrinol. (Oxf.)* 60, 329–334.
- Brix, T.H., Hegedüs, L., Weetman, A.P., Kemp, H., 2014. Pendrin and NIS antibodies are absent in healthy individuals and are rare in autoimmune thyroid disease: evidence from a Danish twin study. *Clin. Endocrinol. (Oxf.)* 81, 440–444.
- Butscher, W.G., Ladenson, P.W., Burek, C.L., 2001. Whole-blood proliferation assay for autoimmune thyroid disease: comparison to density-gradient separated-peripheral blood lymphocytes. *Thyroid* 11, 531–537.
- Chen, C.R., Pichurin, P., Chazebalk, G.D., Aliesky, H., Nagayama, Y., McLachlan, S.M., et al., 2004. Low-dose immunization with adenovirus expressing the thyroid-stimulating hormone receptor A-subunit deviates the antibody response toward that of autoantibodies in human Graves' disease. *Endocrinology* 145, 228–233.

- Chen, C.R., Hamidi, S., Braley-Mullen, H., Nagayama, Y., Bresee, C., Aliesky, H.A., et al., 2010. Antibodies to thyroid peroxidase arise spontaneously with age in NOD.H-2h4 mice and appear after thyroglobulin antibodies. *Endocrinology* 151, 4583–4593.
- Chiovato, L., Latrofa, F., Braverman, L.E., Pacini, F., Capezzzone, M., Masserini, L., et al., 2003. Disappearance of humoral thyroid autoimmunity after complete removal of thyroid antigens. *Ann. Int. Med.* 139, 346–351.
- Chu, X., Pan, C.M., Zhao, S.X., Liang, J., Gao, G.Q., Zhang, X.M., et al., 2011. A genome-wide association study identifies two new risk loci for Graves' diseaseChina Consortium for Genetics of Autoimmune Thyroid Disease *Nat. Genet.* 43, 897–901.
- Cooper, J.D., Simmonds, M.J., Walker, N.M., Burren, O., Brand, O.J., Guo, H., et al., 2012. Seven newly identified loci for autoimmune thyroid disease. *Hum. Mol. Genet.* 21, 5202–5208.
- Costagliola, S., Many, M.C., Stalmans-Falys, M., Tonacchera, M., Vassart, G., Ludgate, M., 1994. Recombinant thyrotropin receptor and the induction of autoimmune thyroid disease in BALB/c mice: a new animal model. *Endocrinology* 135, 2150–2159.
- Costagliola, S., Rodien, P., Many, M.C., Ludgate, M., Vassart, G., 1998. Genetic immunization against the human thyrotropin receptor causes thyroiditis and allows production of monoclonal antibodies recognizing the native receptor. *J. Immunol.* 160, 1458–1465.
- Creemers, P., Rose, N.R., Kong, Y.M., 1983. Experimental autoimmune thyroiditis. In vitro cytotoxic effects of T lymphocytes on thyroid monolayers. *J. Exp. Med.* 157, 559–571.
- Crisa, L., Mordes, J.P., Rossini, A.A., 1992. Autoimmune diabetes mellitus in the BB rat. *Diabetes Metab. Rev.* 8, 4–37.
- Dayan, C.M., Chu, N.R., Londei, M., Rapoport, B., Feldmann, M., 1993. T cells involved in human autoimmune disease are resistant to tolerance induction. *J. Immunol.* 151, 1606–1613.
- Diana, T., Krause, J., Olivo, P.D., König, J., Kanitz, M., Decallonne, B., et al., 2017. Prevalence and clinical relevance of thyroid stimulating hormone receptor-blocking antibodies in autoimmune thyroid disease. *Clin. Exp. Immunol.* 189, 304–309.
- Eaton, W.W., Pedersen, M.G., Atladóttir, H.O., Gregory, P.E., Rose, N.R., Mortensen, P.B., 2010. The prevalence of 30 ICD-10 autoimmune diseases in Denmark. *Immunol. Res.* 47, 228–231.
- Effraimidis, G., Tijssen, J.G., Wiersinga, W.M., 2012. Alcohol consumption as a risk factor for autoimmune thyroid disease: a prospective study. *Eur. Thyroid J.* 1, 99–104.
- ElRehewy, M., Kong, Y.M., Giraldo, A.A., Rose, N.R., 1981. Syngeneic thyroglobulin is immunogenic in good responder mice. *Eur. J. Immunol.* 11, 146–151.
- Ellis, J.S., Hong, S.H., Zaghouani, H., Braley-Mullen, H., 2013. Reduced effectiveness of CD4 + Foxp3 + regulatory T cells in CD28-deficient NOD.H-2h4 mice leads to increased severity of spontaneous autoimmune thyroiditis. *J. Immunol.* 191, 4940–4949.
- Falgarone, G., Heshmati, H.M., Cohen, R., Reach, G., 2013. Mechanisms in endocrinology. Role of emotional stress in the pathophysiology of Graves' disease. *Eur. J. Endocrinol.* 168, R13–R18.
- Fatourechi, V., 2012. Thyroid dermopathy and acropachy. *Best Pract. Res. Clin. Endocrinol. Metab.* 26, 553–565.
- Feingold, S.B., Smith, J., Houtz, J., Popovsky, E., Brown, R.S., 2009. Prevalence and functional significance of thyrotropin receptor blocking antibodies in children and adolescents with chronic lymphocytic thyroiditis. *J. Clin. Endocrinol. Metab.* 94, 4742–4748.
- Figueroa-Vega, N., Alfonso-Pérez, M., Benedicto, I., Sánchez-Madrid, F., González-Amaro, R., Marazuela, M., 2010. Increased circulating pro-inflammatory cytokines and Th17 lymphocytes in Hashimoto's thyroiditis. *J. Clin. Endocrinol. Metab.* 95, 953–962.
- Giordano, C., Stassi, G., De Maria, R., Todaro, M., Richiusa, P., Papoff, G., et al., 1997. Potential involvement of Fas and its ligand in the pathogenesis of Hashimoto's thyroiditis. *Science* 275, 960–963.
- Glick, A.B., Wodzinski, A., Fu, P., Levine, A.D., Wald, D.N., 2013. Impairment of regulatory T cell function in autoimmune thyroid disease. *Thyroid* 23, 871–878.
- Greer, L.G., Casey, B.M., Halvorson, L.M., Spong, C.Y., McIntire, D.D., Cunningham, F.G., 2011. Antithyroid antibodies and parity: further evidence for microchimerism in autoimmune thyroid disease. *Am. J. Obstet. Gynecol.* 205, 471.e1–471.e4.
- Hamilton, F., Black, M., Farquharson, M.A., Stewart, C., Foulis, A.K., 1991. Spatial correlation between thyroid epithelial cells expressing class II MHC molecules and interferon-gamma-containing lymphocytes in human thyroid autoimmune disease. *Clin. Exp. Immunol.* 83, 64–68.
- Hamnvik, O.P., Larsen, P.R., Marqusee, E., 2011. Thyroid dysfunction from antineoplastic agents. *J. Natl. Cancer Inst.* 103, 1572–1587.
- Hidaka, Y., Amino, N., Iwatani, Y., Itoh, E., Matsunaga, M., Tamaki, H., 1993. Recurrence of thyrotoxicosis after attack of allergic rhinitis in patients with Graves' disease. *J. Clin. Endocrinol. Metab.* 77, 1667–1670.
- Hou, X., Li, Y., Li, J., Wang, W., Fan, C., Wang, H., et al., 2011. Development of thyroid dysfunction and autoantibodies in Graves' multiplex families: an eight-year follow-up study in Chinese Han pedigrees. *Thyroid* 21, 353–358.
- Huttfless, S., Matos, P., Talor, M.V., Caturegli, P., Rose, N.R., 2011. Significance of prediagnostic thyroid antibodies in women with autoimmune thyroid disease. *J. Clin. Endocrinol. Metab.* 96, E1466–E1471.
- Iwatani, Y., Amino, N., Hidaka, Y., Kaneda, T., Ichihara, K., Tamaki, H., et al., 1992. Decreases in alpha beta T cell receptor negative T cells and CD8 cells, and an increase in CD4 + CD8 + cells in active Hashimoto's disease and subacute thyroiditis. *Clin. Exp. Immunol.* 87, 444–449.
- Jaume, J.C., Guo, J., Pauls, D.L., Zakarija, M., McKenzie, J.M., Egeland, J.A., et al., 1999. Evidence for genetic transmission of thyroid peroxidase autoantibody epitopic "fingerprints". *J. Clin. Endocrinol. Metab.* 84, 1424–1431.
- Ji, R., Feng, Y., Zhang, W.W., 2013. Updated analysis of studies on the cytotoxic T-lymphocyte-associated antigen-4 gene polymorphism A49G and Hashimoto's thyroiditis risk. *Genet. Mol. Res.* 12, 1421–1430.
- Jonklaas, J., Bianco, A.C., Bauer, A.J., Burman, K.D., Cappola, A.R., Celi, F.S., et al., 2014. Guidelines for the treatment of hypothyroidism: prepared by the American Thyroid Association task force on thyroid hormone replacement. *Thyroid* 24, 1670–1751.
- Jørgensen, K.T., Pedersen, B.V., Nielsen, N.M., Jacobsen, S., Frisch, M., 2012. Childbirths and risk of female predominant and other autoimmune diseases in a population-based Danish cohort. *J. Autoimmun.* 38, J81–J87.
- Kimura, H.J., Chen, C.Y., Tzou, S.C., Rocchi, R., Landek-Salgado, M.A., Suzuki, K., et al., 2009. Immunoproteasome overexpression underlies the pathogenesis of thyroid oncocytes and primary hypothyroidism: studies in humans and mice. *PLoS One* 4, e7857.
- Kojima, A., Tanaka-Kojima, Y., Sakakura, T., Nishizuka, Y., 1976. Spontaneous development of autoimmune thyroiditis in neonatally thymectomized mice. *Lab. Invest.* 34, 550–557.
- Komiya, I., Yamada, T., Sato, A., Kouki, T., Nishimori, T., Takasu, N., 2001. Remission and recurrence of hyperthyroid Graves' disease during and after methimazole treatment when assessed by IgE and interleukin 13. *J. Clin. Endocrinol. Metab.* 86, 3540–3544.

- Kondrashova, A., Viskari, H., Haapala, A.M., Seiskari, T., Kulmala, P., Ilonen, J., et al., 2008. Serological evidence of thyroid autoimmunity among schoolchildren in two different socioeconomic environments. *J. Clin. Endocrinol. Metab.* 93, 729–734.
- Kong, Y.C., Lomo, L.C., Motte, R.W., Giraldo, A.A., Baisch, J., Strauss, G., et al., 1996. HLA-DRB1 polymorphism determines susceptibility to autoimmune thyroiditis in transgenic mice: definitive association with HLA-DRB1\*0301 (DR3) gene. *J. Exp. Med.* 184, 1167–1172.
- Kong, Y.C., Morris, G.P., Brown, N.K., Yan, Y., Flynn, J.C., David, C.S., 2009. Autoimmune thyroiditis: a model uniquely suited to probe regulatory T cell function. *J. Autoimmun.* 33, 239–246.
- Kottahachchi, D., Topliss, D.J., 2016. Immunoglobulin G4-related thyroid diseases. *Eur. Thyroid J.* 5, 231–239.
- Laurberg, P., Wallin, G., Tallstedt, L., Abraham-Nordling, M., Lundell, G., Tørring, O., 2008. TSH-receptor autoimmunity in Graves' disease after therapy with anti-thyroid drugs, surgery, or radiiodine: a 5-year prospective randomized study. *Eur. J. Endocrinol.* 158, 69–75.
- Liu, C., Papewalis, C., Domberg, J., Scherbaum, W.A., Schott, M., 2008. Chemokines and autoimmune thyroid diseases. *Horm. Metab. Res.* 40, 361–368.
- Londei, M., Bottazzio, G.F., Feldmann, M., 1985. Human T-cell clones from autoimmune thyroid glands: specific recognition of autologous thyroid cells. *Science* 228, 85–89.
- MacKenzie, W.A., Schwartz, A.E., Friedman, E.W., Davies, T.F., 1987. Intrathyroidal T cell clones from patients with autoimmune thyroid disease. *J. Clin. Endocrinol. Metab.* 64, 818–824.
- Mao, C., Wang, S., Xiao, Y., Xu, J., Jiang, Q., Jin, M., et al., 2011. Impairment of regulatory capacity of CD4 + CD25 + regulatory T cells mediated by dendritic cell polarization and hyperthyroidism in Graves' disease. *J. Immunol.* 186, 4734–4743.
- Marazuela, M., 1999. Lymphocyte traffic and homing in autoimmune thyroid disorders. *Eur. J. Endocrinol.* 140, 287–290.
- Marazuela, M., García-López, M.A., Figueiro-Vega, N., de la Fuente, H., Alvarado-Sánchez, B., Monsiváis-Urenda, A., et al., 2006. Regulatory T cells in human autoimmune thyroid disease. *J. Clin. Endocrinol. Metab.* 91, 3639–3646.
- Marelli-Berg, F.M., Weetman, A., Frasca, L., Deacock, S.J., Imami, N., Lombardi, G., et al., 1997. Antigen presentation by epithelial cells induces anergic immunoregulatory CD45R0 + T cells and deletion of CD45RA + T cells. *J. Immunol.* 159, 5853–5861.
- Martin, A.P., Marinkovic, T., Canasto-Chibuque, C., Latif, R., Unkeless, J.C., Davies, T.F., et al., 2009. CCR7 deficiency in NOD mice leads to thyroiditis and primary hypothyroidism. *J. Immunol.* 183, 3073–3080.
- Matsuura, N., Yamada, Y., Nohara, Y., Konishi, J., Kasagi, K., Endo, K., et al., 1980. Familial neonatal transient hypothyroidism due to maternal TSH-binding inhibitor immunoglobulins. *N. Engl. J. Med.* 303, 738–741.
- McIntosh, R., Watson, P., Weetman, A., 1998. Somatic hypermutation in autoimmune thyroid disease. *Immunol. Rev.* 162, 219–231.
- McIntosh, R.S., Watson, P.F., Weetman, A.P., 1997. Analysis of the T cell receptor V alpha repertoire in Hashimoto's thyroiditis: evidence for the restricted accumulation of CD8 + T cells in the absence of CD4 + T cell restriction. *J. Clin. Endocrinol. Metab.* 82, 1140–1146.
- McLachlan, S.M., Rapoport, B., 2007. Thyroid peroxidase as an autoantigen. *Thyroid* 17, 939–948.
- McLachlan, S.M., Rapoport, B., 2013. Thyrotropin-blocking autoantibodies and thyroid stimulating autoantibodies: potential mechanisms involved in the pendulum swinging from hypothyroidism to hyperthyroidism or vice versa. *Thyroid* 23, 14–24.
- McLachlan, S.M., Braley-Mullen, H., Chen, C.R., Aliesky, H., Pichurin, P.N., Rapoport, B., 2005. Dissociation between iodide-induced thyroiditis and antibody-mediated hyperthyroidism in NOD-H-2h4 mice. *Endocrinology* 146, 294–300.
- McLachlan, S.M., Alpi, K., Rapoport, B., 2011. Review and hypothesis: does Graves' disease develop in non-human great apes? *Thyroid* 21, 1359–1366.
- Medici, M., Porcu, E., Pistis, G., Teumer, A., Brown, S.J., Jensen, R.A., et al., 2014. Identification of novel genetic loci associated with thyroid peroxidase antibodies and clinical thyroid disease. *PLoS Genet.* 10, e1004123.
- Menconi, F., Huber, A., Osman, R., Concepcion, E., Jacobson, E.M., Stefan, M., et al., 2010. Tg.2098 is a major human thyroglobulin T-cell epitope. *J. Autoimmun.* 35, 45–51.
- Misharin, A.V., Nagayama, Y., Aliesky, H.A., Mizutori, Y., Rapoport, B., McLachlan, S.M., 2009. Attenuation of induced hyperthyroidism in mice by pretreatment with thyrotropin receptor protein: deviation of thyroid-stimulating to nonfunctional antibodies. *Endocrinology* 150, 3944–3952.
- Morshed, S.A., Ando, T., Latif, R., Davies, T.F., 2010. Neutral antibodies to the TSH receptor are present in Graves' disease and regulate selective signaling cascades. *Endocrinology* 151, 5537–5549.
- Mortensen, K.H., Cleemann, L., Hjerrild, B.E., Nexo, E., Locht, H., Jeppesen, E.M., et al., 2009. Increased prevalence of autoimmunity in Turner syndrome—fluence of age. *Clin. Exp. Immunol.* 156, 205–210.
- Moshkelgosha, S., So, P.W., Deasy, N., Diaz-Cano, S., Banga, J.P., 2013. Cutting edge: retrobulbar inflammation, adipogenesis, and acute orbital congestion in a preclinical female mouse model of Graves' orbitopathy induced by thyrotropin receptor plasmid-in vivo electroporation. *Endocrinology* 154, 3008–3015.
- Nagayama, Y., 2007. Graves' animal models of Graves' hyperthyroidism. *Thyroid* 17, 981–988.
- Neumann, S., Eliseeva, E., McCoy, J.G., Napolitano, G., Giuliani, C., Monaco, F., et al., 2011. A new small-molecule antagonist inhibits Graves' disease antibody activation of the TSH receptor. *J. Clin. Endocrinol. Metab.* 96, 548–554.
- Ng, H.P., Kung, A.W., 2006. Induction of autoimmune thyroiditis and hypothyroidism by immunization of immunoactive T cell epitope of thyroid peroxidase. *Endocrinology* 147, 3085–3092.
- Nilsson, M., Husmark, J., Bjorkman, U., Ericson, L.E., 1998. Cytokines and thyroid epithelial integrity: interleukin-1 alpha induces dissociation of the junctional complex and paracellular leakage in filter-cultured human thyrocytes. *J. Clin. Endocrinol. Metab.* 83, 945–952.
- Noble, B., Yoshida, T., Rose, N.R., Bigazzi, P.E., 1976. Thyroid antibodies in spontaneous autoimmune thyroiditis in the Buffalo rat. *J. Immunol.* 117, 1447–1455.
- Okayasu, I., Kong, Y.M., Rose, N.R., 1981. Effect of castration and sex hormones on experimental autoimmune thyroiditis. *Clin. Immunol. Immunopathol.* 20, 240–245.
- Okumura, M., Hidaka, Y., Matsuzuka, F., Takeoka, K., Tada, H., Kuma, K., et al., 1999. CD30 expression and interleukin-4 and interferon-gamma production of intrathyroidal lymphocytes in Graves' disease. *Thyroid* 9, 333–339.
- Pearce, E.N., Farwell, A.P., Braverman, L.E., 2003. Thyroiditis. *N. Engl. J. Med.* 348, 2646–2655.

- Penhale, W.J., Young, P.R., 1988. The influence of the normal microbial flora on the susceptibility of rats to experimental autoimmune thyroiditis. *Clin. Exp. Immunol.* 72, 288–292.
- Penhale, W.J., Farmer, A., McKenna, R.P., Irvine, W.J., 1973. Spontaneous thyroiditis in thymectomized and irradiated Wistar rats. *Clin. Exp. Immunol.* 15, 225–236.
- Pfaltz, M., Hedinger, C.E., 1986. Abnormal basement membrane structures in autoimmune thyroid disease. *Lab. Invest.* 55, 531–539.
- Raber, W., Gessl, A., Nowotny, P., Vierhapper, H., 2002. Thyroid ultrasound versus antithyroid peroxidase antibody determination: a cohort study of four hundred fifty-one subjects. *Thyroid* 12, 725–731.
- Rapoport, B., McLachlan, S.M., 2007. The thyrotropin receptor in Graves' disease. *Thyroid* 17, 911–922.
- Rapoport, B., McLachlan, S.M., 2016. TSH receptor cleavage into subunits and shedding of the A-subunit; a molecular and clinical perspective. *Endocr. Rev.* 37, 114–134.
- Rapoport, B., Aliesky, H.A., Banuelos, B., Chen, C.R., McLachlan, S.M., 2015. A unique mouse strain that develops spontaneous, iodine-accelerated, pathogenic antibodies to the human thyrotrophin receptor. *J. Immunol.* 194, 4154–4161.
- Rebuffat, S.A., Nguyen, B., Robert, B., Castex, F., Peraldi-Roux, S., 2008. Antithyroperoxidase antibody-dependent cytotoxicity in autoimmune thyroid disease. *J. Clin. Endocrinol. Metab.* 93, 929–934.
- Rodríguez-Muñoz, A., Martínez-Hernández, R., Ramos-Leví, A., Serrano-Somavilla, A., González-Amaro, R., Sánchez-Madrid, F., et al., 2015. Circulating microvesicles regulate Treg and Th17 differentiation in human autoimmune thyroid disorders. *J. Clin. Endocrinol. Metab.* 100, E1531–E1539.
- Roitt, I.M., Doniach, D., Campbell, P.N., Hudson, R.V., 1956. Auto-antibodies in Hashimoto's disease (lymphadenoid goitre). *Lancet* 271, 820–821.
- Rose, N.R., Witebsky, E., 1956. Studies in organ specificity. V. Changes in the thyroid glands of rabbits following active immunization with rabbit thyroid extracts. *J. Immunol.* 76, 417–427.
- Ross, D.S., Burch, H.B., Cooper, D.S., Greenlee, M.C., Laurberg, P., Maia, A.L., et al., 2016. American Thyroid Association guidelines for diagnosis and management of hyperthyroidism and other causes of thyrotoxicosis. *Thyroid* 26, 1343–1421.
- Saboori, A.M., Rose, N.R., Yuhasz, S.C., Amzel, L.M., Burek, C.L., 1999. Peptides of human thyroglobulin reactive with sera of patients with autoimmune thyroid disease. *J. Immunol.* 163, 6244–6250.
- Sakaguchi, S., Sakaguchi, N., 1989. Organ-specific autoimmune disease induced in mice by elimination of T cell subsets. V. Neonatal administration of cyclosporin A causes autoimmune disease. *J. Immunol.* 142, 471–480.
- Sakaguchi, S., Sakaguchi, N., Shimizu, J., Yamazaki, S., Sakihama, T., Itoh, M., et al., 2001. Immunologic tolerance maintained by CD25+ CD4+ regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. *Immunol. Rev.* 182, 18–32.
- Sempowski, G.D., Cross, S.J., Heinly, C.S., Scearce, R.M., Haynes, B.F., 2004. CD7 and CD28 are required for murine CD4+ CD25+ regulatory T cell homeostasis and prevention of thyroiditis. *J. Immunol.* 172, 787–794.
- Smith, T.J., Hegedüs, L., 2016. Graves' disease. *N. Engl. J. Med.* 375, 1552–1565.
- Stassi, G., Todaro, M., Buccieri, F., Stoppacciaro, A., Farina, F., Zummo, G., et al., 1999. Fas/Fas ligand-driven T cell apoptosis as a consequence of ineffective thyroid immunoprivilege in Hashimoto's thyroiditis. *J. Immunol.* 162, 263–267.
- Stenszky, V., Kozma, L., Balazs, C., Rochlitz, S., Bear, J.C., Farid, N.R., 1985. The genetics of Graves' disease: HLA and disease susceptibility. *J. Clin. Endocrinol. Metab.* 61, 735–740.
- Sugihara, S., Fujiwara, H., Niimi, H., Shearer, G.M., 1995. Self-thyroid epithelial cell (TEC)-reactive CD8+ T cell lines/clones derived from autoimmune thyroiditis lesions. They recognize self-thyroid antigens directly on TEC to exhibit T helper cell 1-type lymphokine production and cytotoxicity against TEC. *J. Immunol.* 155, 1619–1628.
- Takasu, N., Yamada, T., Takasu, M., Komiya, I., Nagasawa, Y., Asawa, T., et al., 1992. Disappearance of thyrotropin-blocking antibodies and spontaneous recovery from hypothyroidism in autoimmune thyroiditis. *N. Engl. J. Med.* 326, 513–518.
- Tandon, N., Freeman, M., Weetman, A.P., 1991. T cell responses to synthetic thyroid peroxidase peptides in autoimmune thyroid disease. *Clin. Exp. Immunol.* 86, 56–60.
- Tandon, N., Freeman, M.A., Weetman, A.P., 1992. T cell responses to synthetic TSH receptor peptides in Graves' disease. *Clin. Exp. Immunol.* 89, 468–473.
- Tandon, N., Yan, S.L., Morgan, B.P., Weetman, A.P., 1994. Expression and function of multiple regulators of complement activation in autoimmune thyroid disease. *Immunology* 81, 643–647.
- Tomer, Y., 2010. Genetic susceptibility to autoimmune thyroid disease: past, present, and future. *Thyroid* 20, 715–725.
- Tomer, Y., Davies, T.F., 1993. Infection, thyroid disease, and autoimmunity. *Endocrine Rev.* 14, 107–120.
- Tomer, Y., Davies, T.F., 2003. Searching for the autoimmune thyroid disease susceptibility genes: from gene mapping to gene function. *Endocrine Rev.* 24, 694–717.
- Vladutiu, A.O., Rose, N.R., 1971. Autoimmune murine thyroiditis relation to histocompatibility (H-2) type. *Science* 174, 1137–1139.
- Wang, S.H., Baker, J.R., 2007. The role of apoptosis in thyroid autoimmunity. *Thyroid* 17, 975–979.
- Wang, Y., Smith, T.J., 2014. Current concepts in the molecular pathogenesis of thyroid-associated ophthalmopathy. *Invest. Ophthalmol. Vis. Sci.* 55, 1735–1748.
- Weetman, A., 2009. Immune reconstitution syndrome and the thyroid. *Best Pract. Res. Clin. Endocrinol. Metab.* 23, 693–702.
- Weetman, A.P., McGregor, A.M., 1994. Autoimmune thyroid disease: further developments in our understanding. *Endocrine Rev.* 15, 788–830.
- Weetman, A.P., McGregor, A.M., Wheeler, M.H., Hall, R., 1984. Extrathyroidal sites of autoantibody synthesis in Graves' disease. *Clin. Exp. Immunol.* 56, 330–336.
- Weetman, A.P., Cohen, S.B., Oleesky, D.A., Morgan, B.P., 1989. Terminal complement complexes and C1/C1 inhibitor complexes in autoimmune thyroid disease. *Clin. Exp. Immunol.* 77, 25–30.
- Weetman, A.P., Tandon, N., Morgan, B.P., 1992. Antithyroid drugs and release of inflammatory mediators by complement-attacked thyroid cells. *Lancet* 340, 633–636.

- Wichman, J., Winther, K.H., Bonnema, S.J., Hegedüs, L., 2016. Selenium supplementation significantly reduces thyroid autoantibody levels in patients with chronic autoimmune thyroiditis: a systematic review and meta-analysis. *Thyroid* 26, 1681–1692.
- Wick, G., Andersson, L., Hala, K., Gershwin, M.E., Selmi, C., Erf, G.F., et al., 2006. Avian models with spontaneous autoimmune diseases. *Adv. Immunol.* 92, 71–117.
- Wiersinga, W.M., 2013. Smoking and thyroid. *Clin. Endocrinol. (Oxf.)* 79, 145–151.
- Wiersinga, W.M., 2017. Advances in treatment of active, moderate-to-severe Graves' ophthalmopathy. *Lancet Diabetes Endocrinol.* 5, 134–142.
- Wu, Z., Podack, E.R., McKenzie, J.M., Olsen, K.J., Zakarija, M., 1994. Perforin expression by thyroid-infiltrating T cells in autoimmune thyroid disease. *Clin. Exp. Immunol.* 98, 470–477.
- Xia, N., Ye, X., Hu, X., Song, S., Xu, H., Niu, M., et al., 2017. Simultaneous induction of Graves' hyperthyroidism and Graves' ophthalmopathy by TSHR genetic immunization in BALB/c mice. *PLoS One* 12, e0174260.
- Yamaguchi, K., Shimojo, N., Kikuoka, S., Hoshioka, A., Hirai, A., Tahara, K., et al., 1997. Genetic control of anti-thyrotropin receptor antibody generation in H-2K mice immunized with thyrotropin receptor-transfected fibroblasts. *J. Clin. Endocrinol. Metab.* 82, 4266–4269.
- Yanagawa, T., Hidaka, Y., Guimaraes, V., Soliman, M., DeGroot, L.J., 1995. CTLA-4 gene polymorphism associated with Graves' disease in a Caucasian population. *J. Clin. Endocrinol. Metab.* 80, 41–45.
- Zakarija, M., 1983. Immunochemical characterization of the thyroid-stimulating antibody (TSAb) of Graves' disease: evidence for restricted heterogeneity. *J. Clin. Lab. Immunol.* 10, 77–85.
- Zhao, S.X., Tsui, S., Cheung, A., Douglas, R.S., Smith, T.J., Banga, J.P., 2011. Orbital fibrosis in a mouse model of Graves' disease induced by genetic immunization of thyrotropin receptor cDNA. *J. Endocrinol.* 210, 369–377.
- Zhao, S.X., Xue, L.Q., Liu, W., Gu, Z.H., Pan, C.M., Yang, S.Y., et al., 2013. Robust evidence for five new Graves' disease risk loci from a staged genome-wide association analysis. *China Consortium for the Genetics of Autoimmune Thyroid Disease Hum. Mol. Genet.* 22, 3347–3362.

## Further Reading

- Akamizu, T., Ueda, Y., Hua, L., Okuda, J., Mori, T., 1995. Establishment and characterization of an antihuman thyrotropin (TSH) receptor-specific CD4+ T cell line from a patient with Graves' disease: evidence for multiple T cell epitopes on the TSH receptor including the transmembrane domain. *Thyroid* 5, 259–264.
- Bogner, U., Hegedüs, L., Hansen, J.M., Finke, R., Schleusener, H., 1995. Thyroid cytotoxic antibodies in atrophic and goitrous autoimmune thyroiditis. *Eur. J. Endocrinol.* 132, 69–74.
- Many, M.C., Costagliola, S., Detrait, M., Denef, F., Vassart, G., Ludgate, M.C., 1999. Development of an animal model of autoimmune thyroid eye disease. *J. Immunol.* 162, 4966–4974.

# Autoimmune (Type 1) Diabetes

*Ida Lindbladh, Agnes Andersson Svärd and Åke Lernmark*

Department of Clinical Sciences, Lund University CRC, Skåne University Hospital, Malmö, Sweden

## OUTLINE

<b>Introduction</b>	<b>769</b>	<i>Environmental Factors</i>	778
<b>Epidemiology</b>	<b>770</b>	<i>Cellular Mechanisms</i>	779
<i>Asymptomatic Islet Autoimmunity</i>	770	<i>Humoral Biomarkers</i>	779
<i>Symptomatic Autoimmune (Type 1) Diabetes</i>	771	<i>Pathology</i>	779
<b>Diagnostic Criteria and Classification of Diabetes</b>	<b>771</b>	<b>In Vivo and In Vitro Models</b>	780
<b>Etiology</b>	<b>772</b>	<b>Primary Prevention</b>	780
<i>Genetic Etiology of Islet Autoimmunity</i>	772	<b>Secondary Prevention</b>	781
<i>Environmental Factors Associated With First Appearing Autoantibodies</i>	774	<b>Intervention</b>	781
<b>Pathogenesis</b>	<b>775</b>	<b>Conclusion and Future Directions</b>	781
<i>Pathophysiology</i>	776	<b>References</b>	782
<i>Genetic Factors</i>	777		

## INTRODUCTION

Autoimmune diabetes, commonly referred to as type 1 diabetes mellitus (an older synonym is insulin-dependent diabetes mellitus), is a chronic immune-mediated disease characterized by insulin deficiency due to pancreatic islet beta-cell destruction with increasing blood glucose levels (Table 41.1) (Katsarou et al., 2017; Atkinson et al., 2014). Type 1 diabetes is a disease of unidentified etiology and mode of inheritance but strongly associated with essentially two different human leukocyte antigen (HLA) haplotypes on chromosome 6. Young genetically predisposed individuals are exposed to hypothetical environmental factors which trigger an aggressive and selective autoimmune response against the pancreatic islet beta cells. The beta-cell destruction is thought to be an immune-mediated attack, often referred to a T cell–mediated disease and results eventually in insulitis and finally to a lost production of insulin (Richardson et al., 2016; Kaddis et al., 2015; In't Veld et al., 2007; Krogvold et al., 2016). The insulitis appears to be a late event during the prolonged asymptomatic prodrome but may be associated with the actual killing and major loss of beta cells (In't Veld et al., 2007; Pipeleers et al., 2008). The patient becomes dependent on lifelong exogenous insulin treatment.

Autoimmune diabetes is increasing worldwide and has been increasing 3%–5% per year in the last few decades, doubling approximately every 20 years (Group, 2008). The incidence rate varies dramatically between countries and within Europe, with the exception of Sardinia, it shows a north–south gradient, with a high incidence in the northern latitude (Visalli et al., 2003). Finland is the country with the highest annual incidence of autoimmune diabetes with 57.6 patients/100,000 people aged <15 years. The genetic risk is more important than the

**TABLE 41.1** Criteria for the Diagnosis of Diabetes

FPG $\geq 126$ mg/dL (7.0 mmol/L)
Fasting 8 h
or
OGTT: 2-h plasma glucose $\geq 200$ mg/dL (11.1 mmol/L)
or
HbA1C $\geq 6.5\%$ (48 mmol/mol)
or
Symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose $\geq 200$ mg/dL (11.1 mmol/L)

FPG, Fasting plasma glucose; OGTT, oral glucose tolerance test.

By American Diabetes Association, 2017. *Standards of medical care in diabetes—2017 abridged for primary care providers*. Clin. Diabetes 35 (1), 5–26.

geographical location as Sardinia has an incidence rate close to that of Finland (Tuomilehto, 2013; Soltesz et al., 2007); Saudi Arabia has a marked increase in incidence (Abdullah, 2005; Alotaibi et al., 2017), but in Asia, autoimmune diabetes seems yet to be a rare disease (Patterson et al., 2014).

Our understanding of autoimmune diabetes has improved, although the etiology is not completely understood (Katsarou et al., 2017). It is generally assumed that the disease is hereditary, but most patients do not have a family history of diabetes; and the proportion of children with an affected first-degree relative (FDR) is only about 10%–12% (Parkkola et al., 2013; Harjutsalo et al., 2008). The HLA class II genes in the major histocompatibility complex (MHC) are the most important susceptibility genes for autoimmune diabetes, accounting for approximately 50% of the genetic contribution to the disease (Pociot and Lernmark, 2016; Rich et al., 2006). The triggering event is unknown, but antigen presentation by dendritic cells is expected to activate a T and B lymphocyte-mediated autoimmune reaction targeting specific beta-cell autoantigen. It is still not clear whether the triggering antigen presentation is with an antigen from an infectious agent or with an autoantigen. It cannot be excluded that an immune response to an infectious agent antigen may turn itself onto autoimmunity due to a mechanism of molecular mimicry (Srinivasappa et al., 1986; Shoenfeld and Aron-Maor, 2000).

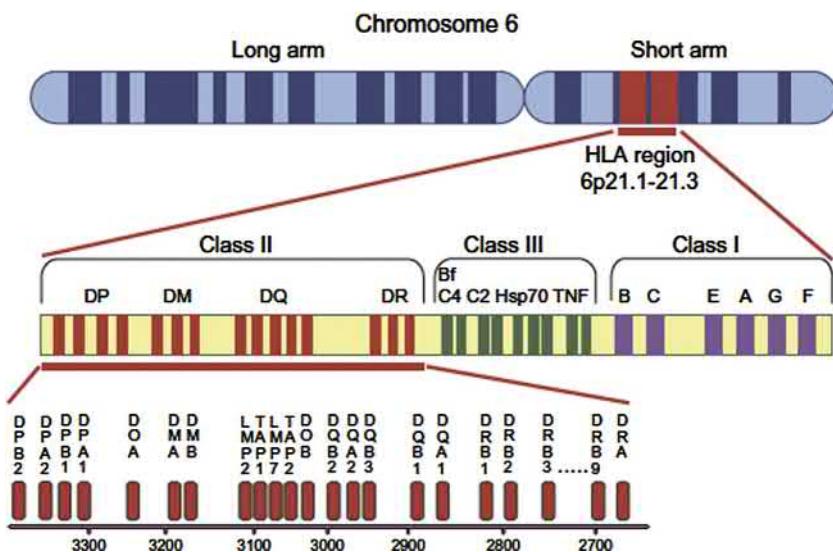
Autoimmune diabetes is characterized by a complex and prolonged subclinical phase (prodrome or prediabetes) with asymptomatic subclinical stages lasting month to years before the clinical onset. The prodrome is initiated by an autoimmune reaction against either insulin in HLA DR4-DQ8 children or glutamic acid decarboxylase (GAD65) in HLA DR3-DQ2 children (Krischer et al., 2015, 2017a,b.; Lynch et al., 2018). Within 12 months, 60% of the children with a first autoantibody may develop a second autoantibody perhaps to be followed by a third or fourth against either insulin (IAA), GADA, islet antigen-2 (IA-2A), or the zinc transporter 8 (ZnT8A).

The number of positive islet autoantibodies correlates to the risk of progressing to clinical disease (Ziegler et al., 2013). The cumulative risk of diabetes varies according to age and younger age at seroconversion, positivity for multiple autoantibodies, high antibody levels, and persistent positivity for IAA (Steck et al., 2015). The combination of genetic analysis—primarily of HLA—in combination with analyses of standardized islet autoantibodies has made it possible to dissect both the etiology and the pathogenesis as well as to predict autoimmune diabetes (Katsarou et al., 2017; Pociot and Lernmark, 2016; Todd et al., 2011).

## EPIDEMIOLOGY

### Asymptomatic Islet Autoimmunity

Recent efforts to develop and standardize reliable islet autoantibody tests of high throughput have made it possible to test to what extent these biomarkers are present in the general population. Incidence rate of first appearing autoantibody has been reported in studies of children followed since birth (Krischer et al., 2015, 2017a; Ziegler et al., 1999; Ziegler and Bonifacio, 2012; Ilonen et al., 2013). The incidence rate in TEDDY (The Environmental Determinants of Diabetes in the Young) showed a difference between IAA first and GADA first. The IAA first peaked at 1–3 years of age followed by a decline, and it did occur primarily in children with the HLA DR4-DQ8 haplotype (Fig. 41.1). In contrast, GADA first developed at 3 years of age and reached a plateau which did not decline (Krischer et al., 2015; Aronsson et al., 2015). In these high-risk children, the prevalence of the first appearing autoantibody was IAA first 43%, GADA first 38%, and both IAA and GADA first 14%. IA-2A first was only found in 4% (Krischer et al., 2015, 2017a).



**FIGURE 41.1** The HLA region on the short arm of chromosome 6. The genes at the DRB3', DRB4' and DRB5' along with DRB1\* as well as DQA1\*-B1\* are the strongest alleles which affect both the risk for a first appearing autoantibody as well as progression to clinical onset. *HLA*, Human leukocyte antigen.

A true incidence rate would require a study of all children born to be followed from birth regardless of genetic risk. This type of incidence study would be of interest in high incidence countries such as Finland and Sweden as data would reflect environmental exposures associated with the triggering of a first appearing autoantibody be it IAA or GADA.

The prevalence of islet autoantibodies regardless of genetic risk was determined in 2–5 year olds (two autoantibodies in 0.39%) in the Fr1da study (Raab et al., 2016), in school children (one autoantibody in 2.4% and two autoantibodies in 0.5%) in the Karlsburg School Children study (Schlosser et al., 2002), in school children (one autoantibody 0.3%) in the Washington School Children study (LaGasse et al., 2002) or in Australia (Colman et al., 2000), and in nondiabetes 30–60 year olds (one autoantibody 1%) in the Swedish Västerbotten study (Rolandsson et al., 1999). The possibility to develop (Raab et al., 2016) tests that simultaneously detect multiple autoantigen autoantibodies should prove useful for future screening efforts (Wasserfall et al., 2016; Ankelo et al., 2007; Amoroso et al., 2016).

## Symptomatic Autoimmune (Type 1) Diabetes

According to the International Diabetes Federation, 8.8% of the adult population worldwide has diabetes. Only 10%–15% of the adult diabetes population have autoimmune diabetes; but in children <15 years of age, autoimmune (type 1) diabetes is the most common form of diabetes, and 90,000 children are diagnosed every year. The highest incidence of autoimmune diabetes is in Scandinavia, Europe, and North America. In the Asian countries, the incidence is still low. Saudi Arabia seems to be the country with the fastest growing incidence rate ([Abduljabbar et al., 2010](#); [Habeb et al., 2011](#)). In resource-poor countries, autoimmune diabetes may not be diagnosed correctly.

High-risk genotypes vary between countries. Genotypes conferring a low risk in one country may go on to confer a risk in children born to parents who immigrate to a high-risk country such as Sweden (Katsarou et al., 2017; Delli et al., 2010; Hussen et al., 2015).

## DIAGNOSTIC CRITERIA AND CLASSIFICATION OF DIABETES

The diagnosis of diabetes is based on blood glucose levels according to combined recommendations of the WHO and the American Diabetes Association (2017) ([Table 41.1](#)).

The HbA1c value reflects 90 days moving average of blood glucose concentrations and has been proposed as an alternative criterion for the diagnosis of diabetes. The recommended threshold is 5.7% or higher for impaired glucose tolerance and 6.5% or higher in two separate tests for the diagnosis of diabetes. These values of HbA1c are based on studies on adults with type 2 diabetes, and according to recent studies, HbA1c level on 6.5% or higher is not an adequate alternative criterion for diagnosing early type 1 diabetes in high-risk asymptomatic

subjects <21 years of age. Oral glucose tolerance test (OGTT) is a sensitive indicator of diabetes and early impaired glucose homeostasis and type 1 diabetes (Vehik et al., 2012). The utility of HbA1c with or without continuous glucose monitoring to diagnose diabetes in children is still uncertain (Xu and Krischer, 2016; Danne et al., 2017; Buysschaert et al., 2016).

To properly classify autoimmune diabetes mellitus the presence of beta-cell autoantibodies must be confirmed in combination with elevated blood glucose (Leslie et al., 2008).

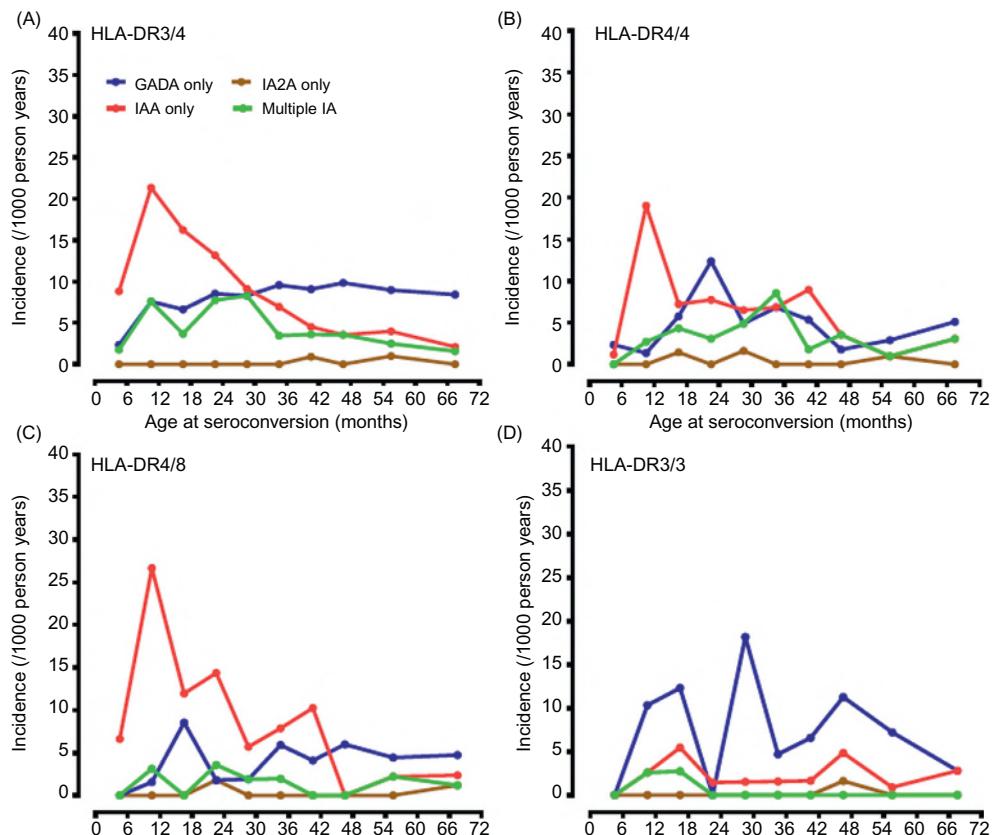
The classification of autoimmune (type 1) diabetes has been updated by the American Diabetes Association (Insel et al., 2015) to include Stage I and II as subclinical stages with two or more islet autoantibodies and Stage III as the clinical stage (Table 41.3).

## ETIOLOGY

### Genetic Etiology of Islet Autoimmunity

Genetic factors for the risk of a first appearing biomarker for autoimmune diabetes require longitudinal studies from birth. Such studies have either followed all newborns (Ludvigsson et al., 2017), utilized HLA-DR-DQ as inclusion criteria for follow-up (Nejentsev et al., 1999; Rewers et al., 1996; Group, 2007), or followed children born in families already affected by autoimmune diabetes (Ziegler et al., 1999; The Environmental, 2008).

In the TEDDY study (Fig. 41.2), IAA first was associated with HLA DR4-DQ8 while GADA first was associated with DR3-DQ2 (Krischer et al., 2015, 2017a,b; Lynch et al., 2018). Non-HLA genetic factors were differently associated with either IAA first or GADA first (Table 41.2). For example, the genetic polymorphism at the *INS* gene was strongly associated with the risk for IAA first but not for GADA first. Conversely, the risk for GADA first was associated with *CTLA4*, *ERBB3*, and *BACH2* but not with IAA first.



**FIGURE 41.2** Incidence rate of GADA only, IAA only, IA-2A only, and multiple autoantibodies as first appearing islet autoantibody at the time of seroconversion. The incidence is shown by HLA type with HLA DR3/4 in (A), DR4/4 in (B), DR4/8 in (C), and DR3/3 in (D). Source: The figure is reproduced by permission from the Krischer, J.P., Lynch, K.F., Lernmark, A., Hagopian, W.A., Rewers, M.J., She, J.X., et al., 2017a. Genetic and environmental interactions modify the risk of diabetes-related autoimmunity by 6 years of age: the TEDDY study. *Diabetes Care* 40 (9), 1194–1202.

**TABLE 41.2** Association Between Human Leucocyte Antigen (HLA) and Non-HLA Genetic Factors and the First Appearing Autoantibody

First appearing autoantibody	IAA	GADA
<b>GENETIC FACTORS</b>		
HLA DR3-DQ2	No	Yes
HLA DR4-DQ8	Yes	No
INS	Yes	No
CTLA-4	No	Yes
PTPN22	Yes	Yes
ERBB3	Yes	Yes
SH2B3	No	Yes
BACH2	No	Yes

From Krischer, J.P., Lynch, K.F., Lernmark, A., Hagopian, W.A., Rewers, M.J., She, J.X., et al., 2017a. Genetic and environmental interactions modify the risk of diabetes-related autoimmunity by 6 years of age: the TEDDY study. *Diabetes Care* 40 (9), 1194–1202; Lynch, K.F., Lee, H.S., Torn, C., Vehik, K., Krischer, J.P., Larsson, H.E., et al., 2018. Gestational respiratory infections interacting with offspring HLA and CTLA-4 modifies incident beta-cell autoantibodies. *J. Autoimmun.* 86, 93–103.

The HLA class II genes in the MHC region on chromosome 6 (6p21) (Fig. 41.1) (Group, 2008) are directly involved in autoimmune diabetes (Fig. 41.1) (Group, 2008). HLA genes are necessary but not sufficient causal factors for the triggering mechanisms as well as for progression to clinical disease, and their penetration is low. Concordance in monozygotic twins is only 30% (Peakman et al., 1993). The development of autoimmunity is a multifactorial process and the major genetic risk factors are the HLA class II haplotype, HLA DR3-DQ2 and HLA DR4-DQ8. The haplotypes vary between countries, and the risk for autoimmune diabetes, for example, in Japan, is associated with DRB1\*04:05-DQB1\*04:01 (DR4-DQ4), DRB1\*08:02-DQB1\*03:02 (DR8-DQ8), and DRB1\*09:01-DQB1\*03:03 (DR9-DQ9) (Kawabata et al., 2009).

There is a large number of HLA-DRB1 alleles in humans, and some alleles have protective effects on both the appearance of a first autoantibody as well as clinical onset (e.g., DRB1\*04:03, 04:07, and 04:10 are protective alleles), whereas others are associated with greater risk of both the trigger and the subsequent disease process (e.g., DRB1\*04:01 and DRB1\*04:04). The HLA region is subject to further analysis by direct sequencing and HLA typing referred to as next-generation sequencing to resolve all alleles at the DRB\*3, 4, and 5 loci as well as DRB1\*, DQA1-B1, and DPA1-B1 (Erlich et al., 2013; Smith et al., 2014; Zhao et al., 2016). Understanding which alleles, DRB and DQ, are necessary for the triggering of an islet autoantigen autoimmunity reflected in the appearance of a first islet autoantibody should make it possible to identify the initial cellular mechanisms resulting in self-reactive T and B lymphocytes as well as autoantibodies.

Normally, the HLA–autoantigen complex in the thymus presents weak- and low-affinity signals to T lymphocytes, which will be educated (positive selection) to identify self-antigen as “self.” If these signals were deficient or were too strong, these T lymphocytes will be deleted (negative selection) as part of central tolerance induction (Ohashi, 2003). T cells play a central role in controlling the acquired immune response, and there is strong evidence for the association between defects in thymic T cell–negative selection related to insulin reactivity (Bluestone et al., 2010). The central role in autoimmune responses against islet autoantigens is related to the structural features of the HLA class II molecules and their interaction with T lymphocytes (Eerlich et al., 2011; van Lummel et al., 2012). Some of the HLA alleles coding these molecules may modify the timing, intensity, and rate of an autoimmune response and thereby affect subsequent pathogenesis.

The DQA and DQB loci code for the alpha and beta subunits, the two chains of the HLA-DQ heterodimer molecules. These molecules are expressed on the cell surface of antigen-presenting cells (APC) and allow the presentation of peptide antigens to T lymphocytes through the T-lymphocyte receptor. The binding of islet autoantigen peptides to HLA-DQ molecules was found to modulate the autoimmune response (van Lummel et al., 2012; Eerlich et al., 2011; Bach et al., 1997; Ge et al., 2011). The transheterodimer between HLA DQ2 and DQ8 may be of particular interest in antigen presentation as it is increasing the number of potential antigen-presenting

heterodimers (van Lummel et al., 2013). Binding insulin peptides to DQ8 molecules induced proinflammatory responses while binding to DQ6 molecules induced regulatory T (Treg) lymphocytes responses (Erlich et al., 2011). These differences may be related to the structural differences in the DQ molecules and the affinity of these molecules to accommodate certain antigens and thereby facilitate thymic recognition of such antigens as “self” or propagate proinflammatory response leading to the activation of autoreactive T lymphocytes.

Structural studies of autoantigen peptide binding to HLA DQ2, DQ8, and DQ6.4 have suggested differences that may be related to the features of autoimmune diabetes. It was proposed that the DQ2 heterodimer has the ability to bind multiple peptides due to a wider binding groove compared to DQ8 which is less accommodating (Lee et al., 2001; Suri et al., 2005). Structural differences between the risk-conferring alleles such as DQ8 and DQ2 and also the protective allele DQ6 were suggested to modify their binding properties through modification of the volume and polarity of binding grooves (Jones et al., 2006). Similarly, HLA-DQ6.4, which is strongly associated with ZnT8 autoantibodies, showed an epitope-binding pattern that would be consistent with a reduction in ZnT8 peptide presentation in the thymus. It has been speculated that a reduction in thymic presentation increases the risk for autoimmunity (Pugliese et al., 1997; Delli et al., 2012). As both IAA first and GADA first may be related to virus infection (Lynch et al., 2018; Lee et al., 2013; Lonnrot et al., 2017), it will be important to determine how virus antigen peptides are presented by DR and DQ heterodimers and how presentation of virus may be related to the subsequent induction of autoimmunity. It needs to be resolved by which mechanisms either a virus or a vaccine such as Pandemrix in DQ6.2 subjects afflicted with narcolepsy (Tafti et al., 2014; Ollila et al., 2015) induced cell-specific autoimmunity. Other mechanisms may prevail as gestational respiratory infections and the risk for IAA first or GADA first may depend on offspring HLA and CTLA-4 alleles (Lynch et al., 2018). These observations support a bidirectional trigger for IAA or GADA as a first appearing beta-cell autoantibody in early life.

Genetic factors have also been shown to alter the risk of autoimmune diseases. The HLA molecules largely determine the distinction between “self” and “nonself” antigens, and HLA compatibility between the mother and fetus could therefore affect autoimmune reactions in the offspring. Microchimerism refers to a small number of cells or DNA in one individual which derives from another genetically distinct individual. Microchimerism takes place during pregnancy through the maternal–fetal cell trafficking which is bidirectional through the placenta. During pregnancy, the fetus would be exposed to the noninherited maternal antigen which may modify the negative selection of T cells in the thymus and alter the generation of specific T cells, inducing central lasting tolerance (Nelson et al., 1998; Bronson et al., 2009). The development of central tolerance is important in allotransplantation and may also play a role in development of autoimmune disorders. Interest in chimerism has been increased due to the development of DNA technology, now using short tandem repeats of DNA or single-nucleotide polymorphism (SNP) to resolve the role of microchimerism and its possible role in autoimmunity (Bluestone et al., 2010; AKMN, 2004; Yunis et al., 2007). Other mechanisms of triggering islet autoimmunity are yet to be elucidated.

## Environmental Factors Associated With First Appearing Autoantibodies

Dietary exposure, such as maternal diet during pregnancy, breastfeeding, and diet in early childhood, may be related to triggering islet autoimmunity (Virtanen, 2016; Lamb et al., 2008; Sorkio et al., 2010). Earlier studies have examined the association between age at first exposure to cow’s milk and type 1 diabetes, resulting in inconsistent findings (Akerblom et al., 2005; Knip et al., 2010, 2014). However, TEDDY study reported that early weaning to an extensively hydrolyzed cow’s milk-based formula was rather associated with an increased risk for islet autoimmunity (Hummel et al., 2017). TEDDY also found higher plasma 25(OH)D concentrations in the child to be associated with a decreased risk of islet autoimmunity (Norris et al., 2018), and an early exposure to probiotics was shown to be protective (Uusitalo et al., 2016). The role of dietary factors associated with triggering beta-cell autoimmunity as marked by a first appearing autoantibody will require further studies.

A major question to understand the etiology of autoimmune diabetes is whether either insulin or GAD65 autoimmunity may be induced by virus. The mechanism may involve a common virus infection associated with a poor immune response that tends to involve the appearance of low-affinity, multiple reactive IgM (Chen et al., 1996; Zhou et al., 2007). Subsequent isotype shift may favor the formation of autoantigen IgG dependent on T-cell help and genetic propensity. Recently, it was reported in the diabetes prediction and prevention (DIPP) study (Honkanen et al., 2017; Sioofy-Khojine et al., 2018) that Coxsackie B1 virus infections may contribute to IAA first but not GADA first.

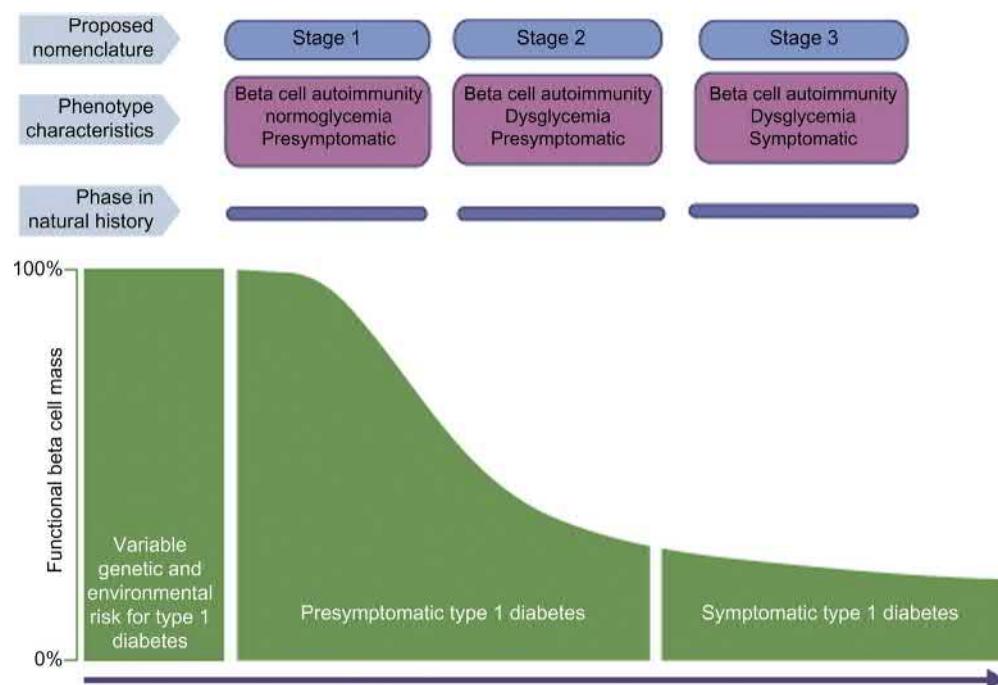
In Russia, children with a history of several infections had lower risk of autoimmunity compared to children with the same genetic risk but fever infections (Katsarou et al., 2017; Knip et al., 2005). It cannot be excluded that molecular mimicry may contribute. Coxsackie virus shares the sequence PEVKEK with GAD65, and it has been proposed that this type of molecular mimicry may explain the association between the virus and first appearing autoantibody (Sioofy-Khojine et al., 2018; Coppieters et al., 2012; Atkinson et al., 1994).

Other possible mechanisms are vaccinations early in life. Vaccinations may alter the immune response to infections and modulate the identification of foreign and self-antigens and thereby increase the risk of autoimmune diseases. A unique instance is the trigger of autoimmunity against hypocretin neurons and development of narcolepsy in only HLA-DQ6.2 subjects after vaccination with Pandemrix (Tafti et al., 2014; Ollila et al., 2015). The vaccine was the trigger, which may help to dissect the mechanisms by which this environmental factor was able to induce disease. However, the research effort is hampered by the fact that an autoantigen is yet to be identified (Lind et al., 2014). It is noted that Pandemrix vaccination did not increase the risk for islet autoantibodies in children with increased genetic risk for autoimmune diabetes (Elding Larsson et al., 2018a). Rather in children from Finland, Pandemrix was negatively associated with the risk for a first appearing islet autoantibody.

Further studies on the possible relation between a common virus infection and the subsequent appearance of a first islet autoantibody as a biomarker of islet autoimmunity will be needed.

## PATHOGENESIS

The pathogenesis of autoimmune type 1 diabetes is currently divided into three stages that relate to the detection of autoantibodies indicating autoimmunity, level of beta-cell destruction, and clinical symptoms of disease (Fig. 41.3; Table 41.3). The prodrome stages may last for weeks up to years before the onset of clinical symptoms of disease. The autoantibodies circulating in the blood are not pathognomonic for autoimmune type 1 diabetes but serve as biomarkers of autoimmunity (Stage 1). Two or more islet autoantibodies were selected to define



**FIGURE 41.3** Staging of autoimmune type 1 diabetes. Variable genetic and environmental risk represents the etiology of type 1 diabetes. The etiological stage is likely to be short term and is concluded by the appearance of a first islet autoantibody as the biomarker of islet autoimmunity. In Stage I, two or more islet autoantibodies have been formed but normoglycemia prevails, and there are no symptoms. In Stage II, islet autoantibodies remain, dysglycemia has developed, but there are no symptoms while in Stage III, diabetes has been diagnosed and there may be symptoms. The staging of type 1 diabetes pathogenesis was proposed by Insel et al. (2015). Source: The figure was reproduced by permission from Regnell, S.E., Lernmark, A., 2017. Early prediction of autoimmune (type 1) diabetes. *Diabetologia* 60, 1370–1381 (Regnell and Lernmark, 2017).

**TABLE 41.3** Classification of Autoimmune Diabetes

Stages of autoimmune diabetes mellitus (type 1 diabetes)

- Stage I: Subclinical stage. Autoimmunity: two or more islet autoantibodies: IAA, GADA, IA-2A, or ZnT8A. Normoglycemia
- Stage II: Subclinical. Autoimmunity and dysglycemia some indication of islet beta-cell loss. Rising glucose levels detectable with OGTT
- Stage III: Clinical disease. Autoimmunity and beta-cell destruction with insulin deficiency, dysglycemia, and hyperglycemia-related symptoms

*Insel, R.A., Dunne, J.L., Atkinson, M.A., Chiang, J.L., Dabelea, D., Gottlieb, P.A., et al., 2015. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. Diabetes Care 38 (10), 1964–1974.*

Stage I as the progression to clinical onset is slow in single autoantibody-positive subjects (Ziegler et al., 2013; Vehik et al., 2016). Islet autoantibodies serve as biomarkers of presymptomatic or subclinical disease (Table 41.3). Major islet autoantibodies target insulin (IAA), the 65 kDa GADA, insulinoma-associated protein 2 (IA-2A), or ZNT8As are found in a blood sample. ZnT8As are complicated as there are three variants, amino acid tryptophan (W), arginine (R), or glutamine (Q) at position 325 (Wenzlau et al., 2008b). Subjects may develop ZnT8As which are either specific to one of these variants or positive to two or all three (Skarstrand et al., 2015).

In young children, the first autoantibody detected was found to be IAA, possibly in combination with GAD (Katsarou et al., 2017; Krischer et al., 2015; Ilonen et al., 2013; Sioofy-Khojine et al., 2018; Ilonen et al., 2018). The appearance of IAA may be preceded by an enterovirus infection (Sioofy-Khojine et al., 2018). A second autoantibody may appear within 12 months in 60% of the children developing a first autoantibody (Iilonen et al., 2013; Vehik et al., 2016). In the first subclinical stage (Stage I, Table 41.3), the individual has developed autoimmunity, defined by the presence of two or more islet autoantibodies, but is still asymptomatic and normoglycemic. The beta-cell function is normal. Despite that two or more autoantibodies are detectable in the blood, there is no indication of insulitis (In't Veld et al., 2007; Pugliese, 2014; Diedisheim et al., 2016). In the second subclinical stage (Stage II, Table 41.3), the individual has developed dysglycemia but is still asymptomatic. Signs of insulitis may be detected during late Stage II (In't Veld et al., 2007) although not as common as expected (Reddy et al., 2015; Wiberg et al., 2015; Lundberg et al., 2017). In this stage, rising glucose intolerance is often detected in an OGTT (Lundberg et al., 2017; Sosenko et al., 2015). In the third stage of disease, the individual has autoimmunity with islet autoantibodies, and beta cells have been lost leading to insulin deficiency, dysglycemia, and symptoms of hyperglycemia such as polyuria and thirst. Lifelong treatment with exogenous insulin is needed for survival and the majority of these patients develop secondary micro- and macrovascular complications later in life (Katsarou et al., 2017; Insel et al., 2015; Hammond and Kronenberg, 2003).

## Pathophysiology

When 80%–90% of the beta-cell mass has been lost, clinical symptoms of hyperglycemia and overt diabetes are prevalent. The rate of progression from an asymptomatic, autoimmune stage to symptomatic disease varies with age. Young children lose proportionally more beta cells compared to teenagers, young adults, and adults (Wallensteen et al., 1988). The clinical onset is therefore not only a function of a reduced beta-cell mass but also of beta-cell function and insulin resistance (Fourlanos et al., 2004). The risk of progression to Stage III disease is associated with the number of autoantibodies, age at seroconversion of the first presenting autoantibody, autoantibody type, affinity, and autoantibody titer (Katsarou et al., 2017; Krischer et al., 2017b). Progression is often faster in girls than in boys and in young children <3 years of age (Katsarou et al., 2017). Young patients may show classic symptoms such as weight loss, thirst, frequent urination, and hunger. Adult subjects may develop diabetes symptoms that would be consistent with type 2 diabetes (Insel et al., 2015; Achenbach et al., 2013).

Islet autoantibodies are important indicator of progression to diabetes as well as the disease outcome (Harel and Shoenfeld, 2006). The type and number of these autoantibodies signify the advancement of islet autoimmunity, therefore predict autoimmune type 1 diabetes not only in FDRs (Verge et al., 1996) but also in subjects from the general population (Ziegler et al., 2013; Bingley et al., 1997).

Islet cell autoantibodies have been standardized in international workshops since 1985 (Gleichmann and Bottazzo, 1987). A WHO standard for autoantibodies against GAD65 and IA-2 was established by the Immunology of Diabetes Workshops (Mire-sluis et al., 2000) and further developed by the Diabetes Autoantibody and Islet Autoantibody Standardization Programs (Torn et al., 2008; Schlosser et al., 2010; Lampasona et al., 2011). A DK standard was developed to be used in NIH-sponsored research (Bonifacio et al.,

2010). The interlaboratory variation in analyzing autoantibodies to insulin, GAD65, IA-2, and ZnT8 has been reduced through the use of common standards in several international workshops.

The risk of developing autoimmune type 1 diabetes increases with increasing number of autoantibodies (Ziegler et al., 2013; Krischer et al., 2015). Autoantibodies against all four autoantigens are used to follow children at increased genetic risk from birth to determine triggers of islet autoimmunity (Ziegler et al., 2013; Hagopian et al., 2011) randomize subjects to secondary prevention trials (Yu et al., 2012) as well as to improve clinical classification (Delli et al., 2012). Interestingly, nearly half of younger patients who were autoantibody negative at the time of diagnosis showed later seroconversion (Hameed et al., 2011) indicating that islet autoantibodies may exist invariably in pre- and postdiabetes diagnosis as well as at the time of diagnosis. Islet autoantibodies, therefore, remain robust markers of autoimmune type 1 diabetes and should prove useful to assist progress toward assays that better reflect environmental exposures as well as seroconversion.

The mechanisms responsible for the variable progress to clinical onset during Stage III are poorly understood. Parallel testing for autoantibodies against GAD65 and IA-2 followed by insulin autoantibodies was found to identify 50% of the patients younger than 20 years of age and was associated with a 71% risk within 10 years (Bingley et al., 1999). In FDR, being 20–39 years of age, this strategy conferred 51% risk. Primary screening for IA-2 and GAD65 autoantibodies followed by testing for insulin autoantibodies conferred a 63% risk to develop diabetes. Further studies including subjects also from the general population will be needed to establish the positive predictive value of an islet autoantibody test to predict autoimmune type 1 diabetes. Studies of children followed since birth because of increased genetic risk for autoimmune (type 1) diabetes are of particular interest in this regard (Rewers et al., 1996; Ziegler et al., 2013; Hagopian et al., 2011).

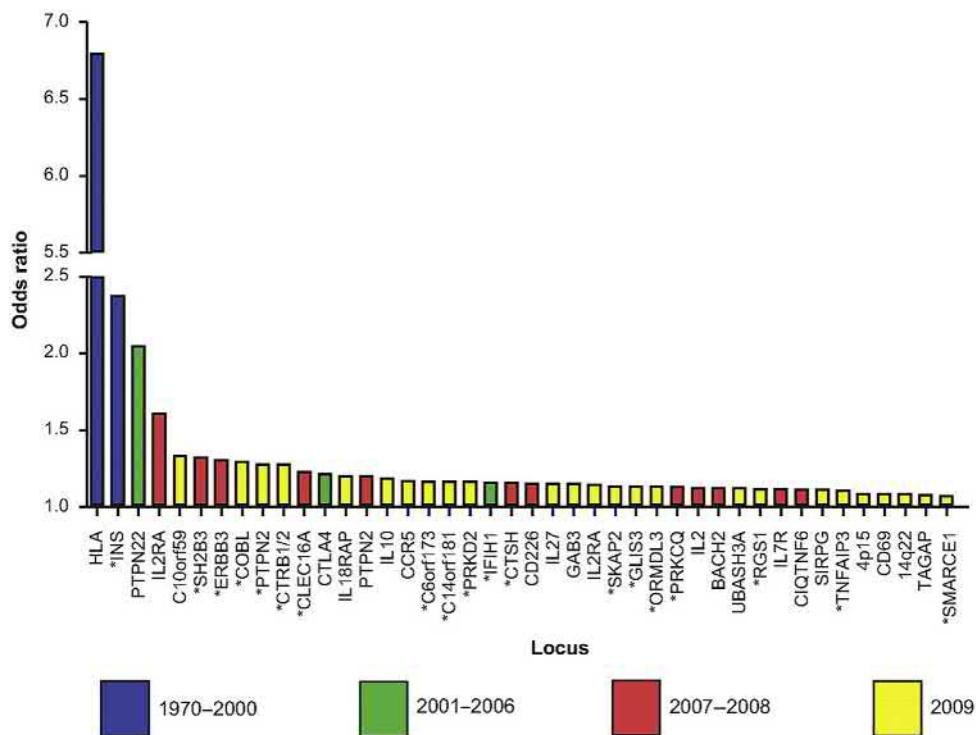
## Genetic Factors

The genetic predisposition of HLA-DR-DQ confers the highest risk for type 1 diabetes but is not enough to cause the autoimmune reaction to the insulin-producing beta cells. It is important to determine the mechanism by which antigens are presented to immune cells on HLA class II molecules, expressed on APC. It is speculated that an etiological trigger such as a common virus infecting beta cells resulting in reactivity with specific autoantigens. Once autoimmunity has been triggered against one autoantigen, be it insulin or GAD65, the pathogenesis is initiated as reflected by the appearance of a second, third, etc. autoantibody. Data suggest that after the appearance of a first autoantibody, 60% develop a second autoantibody within 12 months (Krischer et al., 2017b). However, it is important to note that the appearance of a second islet autoantibody was not associated with HLA (Ilonen et al., 2013). Other mechanisms promoting spreading to other autoantigens will need to be identified. Other possible mechanisms during pathogenesis may include genetic factors that are associated with apoptosis (Cnop et al., 2005; Grieco et al., 2014), autophagy (Rojas et al., 2018), or endoplasmic reticulum stress (Cnop et al., 2005; Eizirik et al., 2013).

Notwithstanding the major importance of HLA in the association with the etiology of diabetes, it cannot be excluded that HLA also is related to the pathogenesis of the disease. Major efforts have been made through genome-wide association studies to identify non-HLA genetic factors associated with type 1 diabetes and more than 60 loci have been identified (Pociot and Lernmark, 2016; Rich et al., 2009; Ram et al., 2016; Pociot et al., 2010) (Fig. 41.4).

Apart from the recent rapid progress in detecting genes contributing to type 1 diabetes risk, the data also illustrate that the majority of the genes have a low risk.

It is possible to distinguish genes that contribute to the risk of developing the first autoantibody from genes that contribute to the risk of progressing to clinical onset (Krischer et al., 2017b; Torn et al., 2015). In children with two or more islet autoantibodies, there may be rapid as well as slow progressors. The two groups may be distinguished by the combined presence or absence of type 1 diabetes risk alleles of non-HLA genes, most notably IL2, CD25, INS VNTR, IL18RAP, IL10, IFIH1, and PTPN22 (Achenbach et al., 2013). The T1DBase (<http://www.t1dbase.org>) lists risk genes and their functions. It is noticed that the majority of the type 1 diabetes risk genes are related to the function of the immune system. Moreover, in analyzing type 1 diabetes, rheumatoid arthritis, celiac disease, and multiple sclerosis, there were 90 regions associated with at least one disease. A total of 33 (37%) regions were associated with two or more disorders. Most of these gene regions are related to the pathogenesis and recent approaches are to employ the genetic information to develop genetic risk scores (GRSs) (Oram et al., 2016). One GRS based on the TEDDY study identified infants without a family history of type 1



**FIGURE 41.4** HLA and non-HLA genetic factors detected by comparing the frequency of genetic polymorphisms in type 1 diabetes patients with healthy controls to calculate odds ratio. The color code indicates that year span of the observations was made. It is noticed that the progress to detect novel genetic factors that increase the risk for type 1 diabetes has accelerated after year 2000. HLA, Human leucocyte antigen. Source: Reproduced with permission from Pociot, F., Akolkar, B., Concannon, P., Erlich, H.A., Julier, C., Morahan, G., et al., 2010. Genetics of type 1 diabetes: what's next? *Diabetes* 59, 1561–1571.

diabetes who had a >10% risk for islet autoimmunity (pre-Stage 1 in Fig. 41.3) (Bonifacio et al., 2018). This GRS may be used to enroll children in primary prevention studies (Ziegler et al., 2016). Another GRS may be used to better detect autoimmune type 1 diabetes in the adult population (Thomas et al., 2018). Taken together, combining the genetic information from HLA and non-HLA genes or genetic regions into GRS may prove useful for prediction of risk of etiology as well as pathogenesis to better identify and delineate progression to clinical onset. However, the mechanisms that explain the contribution of individual genes remain to be determined also in relation to the possible gene–environment interactions that may take place during years of Stage I and II islet autoimmunity.

## Environmental Factors

Many environmental factors, most importantly viral infections, have been assumed to either trigger an autoimmune response (Honkanen et al., 2017; Sioofy-Khojine et al., 2018) or affect the pathogenesis to alter the rate of progression to clinical onset (Stene et al., 2010). Viral infection of remaining beta cells may induce local inflammatory mechanisms, secretion of proinflammatory cytokines, and involvement of APC to accelerate pathogenesis. This activation may recruit additional autoreactive T lymphocytes. It has been suggested that these T lymphocytes need to be provoked by molecular mechanisms before being able to propagate autoimmunity (von Herrath et al., 2003), but it cannot be excluded that virus infections may contribute to epitope spreading and the appearance of additional autoantibodies.

There is limited information of the extent by which virus infection of islet autoantibody-positive subjects may result in the beta cells being infected (Stene et al., 2010).

Dietary factors that may affect the pathogenesis in islet autoantibody-positive subjects may include gluten (Bosi et al., 2005) and vitamin D (Norris et al., 2018; Raab et al., 2014). Gluten-free diet was tested in subjects with islet autoantibodies but did not affect the frequency or levels of autoantibodies (Hummel et al., 2002). Beta-cell function after 6 months of gluten-free diet in islet autoantibody-positive subjects was improved (Pastore et al.,

2003). Further studies on possible effects of environmental factors, such as diet, in subjects in Stage I and Stage II (Fig. 41.3) on the progression to clinical onset (Stage III) will be needed.

## Cellular Mechanisms

In the preclinical prodromal phase of autoimmune type 1 diabetes, there is initially no sign of reduced beta-cell function (Stage I). A selective loss of pancreatic islet beta cells seems to appear late and it cannot be excluded that Stage II dysglycemia is associated with insulitis that escalates and involves T cell-mediated destruction (Bluestone et al., 2010; Bronson et al., 2009). T lymphocytes play a central role in controlling the acquired immune response. There is a strong evidence for association between defects in thymic T cell-negative selection related to insulin reactivity (Bluestone et al., 2010). Normally, the HLA-autoantigen complex in the thymus presents weak and low-affinity signals to T lymphocytes, which will be educated (positive selection) to identify self-antigen as "self." If these signals were deficient or were too strong, these T lymphocytes will be deleted (negative selection) as part of central tolerance induction (Woo et al., 2017). In the periphery, Treg cells help to maintain normal response to "self"-antigens through eliminating autoreactive T lymphocytes that escape negative selection by a process called "clonal deletion," which is part of peripheral tolerance. In Stage I and II autoimmune diabetes (Fig. 41.3), there is likely a loss of the normal regulatory immune mechanisms. An imbalance between Treg cells and effector T lymphocytes has been described and the current view is that both negative and positive selection and Treg induction in the thymus are important for effective control of autoreactivity in peripheral tissue (Woo et al., 2017; Morran et al., 2008). Cellular studies of subjects in Stage I and II are rare. Using HLA Class II tetramers, GAD65 peptide-specific CD4+ T cells were observed in GADA positive but not negative subjects with increased genetic risk for type 1 diabetes (Oling et al., 2012). Stage I subjects compared to controls did not show quantitative or functional differences in Treg cells. Children with multiple autoantibodies compared to controls showed decreased mRNA expression levels of T-cell subtype markers. This may reflect an exhaustion of the immune system after the strong immune activation during a prolonged autoimmune process (Hamari et al., 2016). T-cell exhaustion may play a central role in determining outcome in autoimmune disease (McKinney et al., 2015).

## Humoral Biomarkers

Four major autoantigens have been identified in type 1 diabetes along with a growing list of minor autoantigens (Hirai et al., 2008). Proinsulin is the exclusive beta cell-specific antigen (Pugliese et al., 2001) and insulin was described as a major target for the T-lymphocyte attack especially in young children (Arif et al., 2004). GAD65 is specific for beta cells but is expressed in other cells as well (Karlsen et al., 1991). The IA-2 and the isoform IA-2 $\beta$  are important antigens especially in carriers of the HLA-DQ8 haplotype (Delli et al., 2010). The development of persistent ( $>3$  months) single or multiple ( $\geq 2$ ) islet autoantibodies is thought to occur shortly following the appearance of a first islet autoantibody but this seems to occur regardless of insulitis (In't Veld et al., 2007). A large group of minor autoantigens have also been proposed; however, the role of T- and B-lymphocyte reactivity as well as autoantibodies against most of these autoantigens has not been fully determined. Among these autoantigens are the secretory vesicle-associated proteins, chromogranin A, VAMP2 and NPY, HSP-60 and HSP-70, IGRP, Glima-38, and many others (detailed list is given in Wenzlau et al., 2008a). It cannot be excluded that the autoimmunity against these minor autoantigens reflects antigen and epitope spreading (Morran et al., 2008) (presentation of new antigen to inflammatory cells of the immune system leading to activation of new T lymphocytes). Further studies are needed to determine if the autoreactivity to minor autoantigens affects the progression from Stage I to II and reaching Stage III (Fig. 41.3).

## Pathology

Historically, the involvement of immune cells in autoimmune type 1 diabetes was described when inflammatory cell infiltrate, fibrosis and atrophy of the islets were demonstrated in postmortem pancreatic tissues obtained from some children who died soon following diagnosis (Gepts, 1965). The pathological feature of type 1 diabetes is the conspicuous loss of the pancreatic islet beta cells (Pipeleers et al., 2008). An infiltration of mononuclear cells in islets is often but not always observed (In't Veld, 2011). Insulitis ( $\geq 2$  mononuclear immune cells per islets) appears as a late manifestation and is observed primarily in multiple autoantibody-positive subjects prior to clinical diagnosis (In't Veld et al., 2007). Quantitative immunocytochemistry revealed a specific loss of beta cells and

that the neighboring cells producing glucagon, somatostatin, pancreatic polypeptide, or ghrelin were not affected. In subjects with insulitis, the islets of Langerhans may be infiltrated by T and B lymphocytes as well as monocytes and dendritic cells supporting a state of chronic inflammation (Eizirik et al., 2009). In some insulitis-positive islets, it has been possible to demonstrate markers of inflammation along with viral antigens (Foulis, 2008; Foulis et al., 1991).

The possible role of subclinical pancreatitis and infiltration of immune cells, in particular dendritic cells, needs to be explored (Skog et al., 2013).

The known insulitis characteristics are derived from specimens that mostly reflect an advanced stage of the disease or more extensive form of it when obtained from postmortem autopsies. Little is understood about early stages of propagation of autoimmune pathological features in the prolonged phases of Stage I and II. T lymphocytes, especially the CD4+ and CD8+ cell subsets, dominate the insulitis (Pinkse et al., 2005), compared to B lymphocytes, and may be found in larger populations in the islets (Kent et al., 2005). Therefore the infiltration of pancreatic islets by inflammatory cells, beta-cell destruction, and the resulting insulitis is a multistep process, which may vary widely in duration and intensity before diabetes becomes clinically manifested. Furthermore, in recent-onset type 1 diabetes, residual beta-cell function was temporarily preserved through the use of monoclonal antibodies targeting CD3 on T lymphocytes, CD20 on B lymphocytes, or drugs such as cyclosporine targeting monocyte/macrophage populations (Pescovitz et al., 2009; Herold et al., 2002; Bougnères et al., 1988). These observations in addition to the significant role of cellular immunological pathway, predominantly CD8+ lymphocytes, suggest that type 1 diabetes is primarily a cell-mediated autoimmune disease (Notkins and Lernmark, 2001; Willcox et al., 2009).

## IN VIVO AND IN VITRO MODELS

Several animal species were studied as models for autoimmune type 1 diabetes, although their diabetes phenotype was found to differ from the human type. Nevertheless, research on these animals yielded valuable guidance to human diabetes, where ethical issues or difficulties in obtaining human pancreatic samples may limit research. Type 1 diabetes-like syndromes develop spontaneously in the BioBreeding (BB) (Mordes et al., 2004), the LEW.1AR1-iddm (Jorns et al., 2010), and the Komeda diabetes-prone rat (Yokoi et al., 2003). All three types of rats have features similar to human type 1 diabetes; however, the trigger is due to mutations in different genes. Diabetes in all three rats is RT1.u, an ortholog of HLA DQ2. None of the rats develop islet autoantibodies that predict diabetes. The nonobese diabetic (NOD) mouse, which also develops diabetes with features comparable to human type 1 diabetes, has been studied extensively (Thayer et al., 2010; Mathis et al., 2001; Driver et al., 2011). The NOD mouse and its many congenic lines may be useful to dissect the genetic and pathogenic basis for T lymphocyte-mediated diabetes. However, while sharing many similarities, it is becoming increasingly clear that there are major differences in the immunopathogenesis between humans and NOD mice. Combination therapy with rapamycin and interleukin-2 prevented NOD mouse diabetes (Rabinovich et al., 2003) but accelerated the loss of residual beta-cell function in newly diagnosed type 1 diabetes patients (Long et al., 2012). Wild bank voles (*Myodes glareolus*) were reported to develop diabetes in laboratory captivity in association with autoantibodies against GAD65, IA-2, and insulin in standardized radioligand-binding assays as well as antibodies to in vitro transcribed and translated Ljungan virus antigens. It was speculated that bank voles may have a possible zoonotic role as a reservoir and vector for a virus that may contribute to type 1 diabetes (Niklasson et al., 2003).

## PRIMARY PREVENTION

The association between age at first exposure to cow's milk and type 1 diabetes raised the question if hydrolyzed cow's milk-based formula would prevent the appearance of islet autoantibodies. The clinical trials resulted in inconsistent findings (Akerblom et al., 2005; Knip et al., 2010, 2014). Also the TEDDY study reported that early weaning to an extensively hydrolyzed cow's milk-based formula was rather associated with an increased risk for islet autoimmunity (Hummel et al., 2017).

The possibility to identify newborn children with increased genetic risk to develop either IAA or GADA as the first appearing autoantibody (Krischer et al., 2015; Ziegler et al., 1999) raised the question of primary prevention through antigen exposure to induce immunological tolerance. A first study with oral insulin to prevent IAA first showed high safety and sign of immune activation (Bonifacio et al., 2015). A phase 2 study, Primary Oral

Insulin Trial (NCT03364868), has been initiated using GRS to screen newborns to identify children with 10% risk of developing IAA first (Bonifacio et al., 2018) and treat them with 67.5 mg insulin per day or placebo (Ziegler et al., 2016).

## SECONDARY PREVENTION

A large number of secondary prevention studies have been carried out to test if progression to clinical onset of type 1 diabetes can be prevented. The study of parenteral injections of insulin to autoantibody-positive individuals did not show protection (Effects, 2002). In subjects at lesser risk given oral insulin, there was no protection (Skyler et al., 2005). However, an ad hoc analysis suggested that a subgroup representing high titer IAA showed a delay in the progression to clinical diagnosis (Skyler et al., 2005). These results encouraged TrialNet to carry out a controlled clinical trial in a large number of IAA-positive subjects (Writing Committee for the Type 1 Diabetes TrialNet Oral Insulin Study Group et al., 2017). Overall, there was no benefit with oral insulin to prevent or delay the onset of diabetes; however, in one predetermined subgroup, there was a significant delay in the progression to diabetes. Further studies will be needed to better define treatment regimen and dosage of oral insulin.

A prevention study with GAD65-alum, Diabetes Prevention-Immune Tolerance, showed safety but no effect on the progression to diabetes (Elding Larsson et al., 2018b).

## INTERVENTION

The very first intervention studies with immune suppression were carried out already in 1975 shortly after the first description of islet cell antibodies (ICA). These early attempts to intervene with the autoimmune process have been followed by a very large number of both open and controlled studies using essentially every immune suppressive agent reaching the market [comprehensive reviews are available by Sherr et al. (2008), Skyler (2011), and Skyler (2013)]. None of the efforts have prevented the loss of beta cells and preserved residual beta-cell function long term.

## CONCLUSION AND FUTURE DIRECTIONS

Our understanding of the etiology and pathogenesis of autoimmune (type 1) diabetes mellitus is in rapid progress through several major efforts. The sequencing of the human genome has made it possible to better define the genetic contribution to the risk of islet autoimmunity and diabetes. Further studies are needed to better understand the genetic propensity for type 1 diabetes when moving between countries. Second, studies from birth in genetically susceptible individuals may uncover triggers that launch seroconversion. A current challenge is to better uncover the series of events that contribute to islet autoimmunity. It will also be a challenge to define the APC, T and B lymphocytes during the chronic stage of islet autoimmunity, and their contribution of to the beta-cell destruction. More than 50 non-HLA genes and possible SNPs should be examined one-by-one to reveal their possible contribution to the variable progression. These non-HLA genetic factors may also represent potential drug targets to secondary prevention or intervention. The limited success in secondary prevention and intervention studies with immunosuppressive agents suggests that novel approaches perhaps in combination trials with islet autoantigens will be required to successfully halt progression to the clinical onset or the loss of endogenous beta-cell function that invariably takes place after clinical diagnosis.

At present, the number of positive islet autoantibodies correlates to the risk of developing clinical disease. The cumulative risk of diabetes varies according to age and, the younger age at seroconversion, positivity for multiple autoantibodies, high antibody levels and persistent positivity for IAA, the greater the risk for type 1 diabetes.

Recent data from the TEDDY study show that seroconversion tends to occur during the first years of life, suggesting that environmental exposures in early in life may have a unique impact on children with increased genetic risk for diabetes. Large cohort studies on genetically susceptible individuals are needed to better uncover environmental and genetic triggers and/or protectors, to answer the question: Why only a minor part of individuals with genetic susceptibility develop autoimmune diabetes, while others with the same genetic risk do not?

## References

- AKMN, J.L., 2004. Microchimerism: an investigative frontier in autoimmunity and transplantation. *JAMA* 291 (9), 1127–1131.
- Abduljabbar, M.A., Aljubeh, J.M., Amalraj, A., Cherian, M.P., 2010. Incidence trends of childhood type 1 diabetes in eastern Saudi Arabia. *Saudi Med. J.* 31 (4), 413–418.
- Abdullah, M.A., 2005. Epidemiology of type I diabetes mellitus among Arab children. *Saudi Med. J.* 26 (6), 911–917.
- Achenbach, P., Hummel, M., Thumer, L., Boerschmann, H., Hofelmann, D., Ziegler, A.G., 2013. Characteristics of rapid vs slow progression to type 1 diabetes in multiple islet autoantibody-positive children. *Diabetologia* 56 (7), 1615–1622.
- Akerblom, H.K., Virtanen, S.M., Ilonen, J., Savilahti, E., Vaarala, O., Reunanan, A., et al., 2005. Dietary manipulation of beta cell autoimmunity in infants at increased risk of type 1 diabetes: a pilot study. *Diabetologia* 48 (5), 829–837.
- Alotaibi, A., Perry, L., Gholizadeh, L., Al-Ganmi, A., 2017. Incidence and prevalence rates of diabetes mellitus in Saudi Arabia: an overview. *J. Epidemiol. Glob. Health* 7 (4), 211–218.
- American Diabetes Association, 2017. Standards of medical care in diabetes—2017 abridged for primary care providers. *Clin. Diabetes* 35 (1), 5–26.
- Amoroso, M., Achenbach, P., Powell, M., Coles, R., Chlebowska, M., Carr, L., et al., 2016. 3 Screen islet cell autoantibody ELISA: a sensitive and specific ELISA for the combined measurement of autoantibodies to GAD65, to IA-2 and to ZnT8. *Clin. Chim. Acta* 462, 60–64.
- Ankelo, M., Westerlund, A., Blomberg, K., Knip, M., Ilonen, J., Hinkkanen, A.E., 2007. Time-resolved immunofluorometric dual-label assay for simultaneous detection of autoantibodies to GAD65 and IA-2 in children with type 1 diabetes. *Clin. Chem.* 53 (3), 472–479.
- Arif, S., Tree, T.I., Astill, T.P., Tremble, J.M., Bishop, A.J., Dayan, C.M., et al., 2004. Autoreactive T cell responses show proinflammatory polarization in diabetes but a regulatory phenotype in health. *J. Clin. Invest.* 113 (3), 451–463.
- Aronsson, C.A., Lee, H.S., Liu, E., Uusitalo, U., Hummel, S., Yang, J., et al., 2015. Age at gluten introduction and risk of celiac disease. *Pediatrics* 135 (2), 239–245.
- Atkinson, M.A., Bowman, M.A., Campbell, L., Darrow, B.L., Kaufman, D.L., McLaren, N.K., 1994. Cellular immunity to a determinant common to glutamate decarboxylase and coxsackie virus in insulin-dependent diabetes. *J. Clin. Invest.* 94, 2125–2129.
- Atkinson, M.A., Eisenbarth, G.S., Michels, A.W., 2014. Type 1 diabetes. *Lancet* 383 (9911), 69–82.
- Bach, J.M., Otto, H., Nepom, G.T., Jung, G., Cohen, H., Timsit, J., et al., 1997. High affinity presentation of an autoantigenic peptide in type I diabetes by an HLA class II protein encoded in a haplotype protecting from disease. *J. Autoimmun.* 10 (4), 375–386.
- Bingley, P.J., Bonifacio, E., Williams, A.J., Genovese, S., Bottazzo, G.F., Gale, E.A., 1997. Prediction of IDDM in the general population: strategies based on combinations of autoantibody markers. *Diabetes* 46 (11), 1701–1710.
- Bingley, P.J., Williams, A.J., Gale, E.A., 1999. Optimized autoantibody-based risk assessment in family members. Implications for future intervention trials. *Diabetes Care* 22 (11), 1796–1801.
- Bluestone, J.A., Herold, K., Eisenbarth, G., 2010. Genetics, pathogenesis and clinical interventions in type 1 diabetes. *Nature* 464, 1293–1300.
- Bonifacio, E., Yu, L., Williams, A.K., Eisenbarth, G.S., Bingley, P.J., Marcovina, S.M., et al., 2010. Harmonization of glutamic acid decarboxylase and islet antigen-2 autoantibody assays for national institute of diabetes and digestive and kidney diseases consortia. *J. Clin. Endocrinol. Metab.* 95 (7), 3360–3367.
- Bonifacio, E., Ziegler, A.G., Klingensmith, G., Schober, E., Bingley, P.J., Rottenkolber, M., et al., 2015. Effects of high-dose oral insulin on immune responses in children at high risk for type 1 diabetes: the Pre-POINT randomized clinical trial. *JAMA* 313 (15), 1541–1549.
- Bonifacio, E., Beyerlein, A., Hippich, M., Winkler, C., Vehik, K., Weedon, M.N., et al., 2018. Genetic scores to stratify risk of developing multiple islet autoantibodies and type 1 diabetes: a prospective study in children. *PLoS Med.* 15 (4), e1002548.
- Bosi, E., Pastore, M.R., Molteni, L., Bazzigaluppi, E., Bonifacio, E., Piemonti, L., 2005. Gluten-free diet in subjects at risk for type 1 diabetes: a tool for delaying progression to clinical disease? *Adv. Exp. Med. Biol.* 569, 157–158.
- Bougnères, P.F., Carel, J.C., Castano, L., Boitard, C., Gardin, J.P., Landais, P., et al., 1988. Factors associated with early remission of type I diabetes in children treated with cyclosporine. *N. Eng. J. Med.* 318, 663–670.
- Bronson, P.G., Ramsay, P.P., Thomson, G., Barcellos, L.F., Diabetes Genetics Consortium, 2009. Analysis of maternal-offspring HLA compatibility, parent-of-origin and non-inherited maternal effects for the classical HLA loci in type 1 diabetes. *Diabetes Obes. Metab.* 11 (Suppl. 1), 74–83.
- Buysschaert, M., Medina, J.L., Buysschaert, B., Bergman, M., 2016. Definitions (and current controversies) of diabetes and prediabetes. *Curr. Diabetes Rev.* 12 (1), 8–13.
- Chen, Z.J., Shimizu, F., Wheeler, J., Notkins, A.L., 1996. Polyreactive antigen-binding B cells in the peripheral circulation are IgD+ and B7. *Eur. J. Immunol.* 26 (12), 2916–2923.
- Cnop, M., Welsh, N., Jonas, J.C., Jorns, A., Lenzen, S., Eizirik, D.L., 2005. Mechanisms of pancreatic beta-cell death in type 1 and type 2 diabetes: many differences, few similarities. *Diabetes* 54 (Suppl. 2), S97–S107.
- Colman, P.G., McNair, P., King, J., Caudwell, J., Jankulovski, C., Tait, B.D., et al., 2000. Screening for preclinical type 1 diabetes in a discrete population with an apparent increased disease incidence. *Pediatr. Diabetes* 1 (4), 193–198.
- Coppelters, K.T., Wiberg, A., von Herrath, M.G., 2012. Viral infections and molecular mimicry in type 1 diabetes. *APMIS* 120 (12), 941–949.
- Danne, T., Nimri, R., Battelino, T., Bergenfelz, R.M., Close, K.L., DeVries, J.H., et al., 2017. International consensus on use of continuous glucose monitoring. *Diabetes Care* 40 (12), 1631–1640.
- Delli, A.J., Lindblad, B., Carlsson, A., Forsander, G., Ivarsson, S.A., Ludvigsson, J., et al., 2010. Type 1 diabetes patients born to immigrants to Sweden increase their native diabetes risk and differ from Swedish patients in HLA types and islet autoantibodies. *Pediatr. Diabetes* 11 (8), 513–520.
- Delli, A.J., Vaziri-Sani, F., Lindblad, B., Elding-Larsson, H., Carlsson, A., Forsander, G., et al., 2012. Zinc transporter 8 autoantibodies and their association with SLC30A8 and HLA-DQ genes differ between immigrant and Swedish patients with newly diagnosed type 1 diabetes in the Better Diabetes Diagnosis study. *Diabetes* 61 (10), 2556–2564.
- Diedrichsheim, M., Mallone, R., Boitard, C., Larger, E., 2016. Beta-cell mass in nondiabetic autoantibody-positive subjects: an analysis based on the network for pancreatic organ donors database. *J. Clin. Endocrinol. Metab.* 101 (4), 1390–1397.

- Driver, J.P., Serreze, D.V., Chen, Y.G., 2011. Mouse models for the study of autoimmune type 1 diabetes: a NOD to similarities and differences to human disease. *Semin. Immunopathol.* 33 (1), 67–87.
- Diabetes Prevention Trial—Type 1 Diabetes Study Group, 2002. Effects of insulin in relatives of patients with type 1 diabetes mellitus. *N. Engl. J. Med.* 346 (22), 1685–1691.
- Eerligh, P., van Lummel, M., Zaldumbide, A., Moustakas, A.K., Duinkerken, G., Bondinas, G., et al., 2011. Functional consequences of HLA-DQ8 homozygosity versus heterozygosity for islet autoimmunity in type 1 diabetes. *Genes Immun.* 12 (6), 415–427.
- Eizirik, D.L., Colli, M.L., Ortis, F., 2009. The role of inflammation in insulitis and beta-cell loss in type 1 diabetes. *Nat. Rev. Endocrinol.* 5 (4), 219–226.
- Eizirik, D.L., Miani, M., Cardozo, A.K., 2013. Signalling danger: endoplasmic reticulum stress and the unfolded protein response in pancreatic islet inflammation. *Diabetologia* 56 (2), 234–241.
- Elding Larsson, H., Lynch, K.F., Lonnrot, M., Haller, M.J., Lernmark, A., Hagopian, W.A., et al., 2018a. Pandemrix(R) vaccination is not associated with increased risk of islet autoimmunity or type 1 diabetes in the TEDDY study children. *Diabetologia* 61 (1), 193–202.
- Elding Larsson, H., Lundgren, M., Jonsdottir, B., Cuthbertson, D., Krischer, J., Di A-ITSG, 2018b. Safety and efficacy of autoantigen-specific therapy with 2 doses of alum-formulated glutamate decarboxylase in children with multiple islet autoantibodies and risk for type 1 diabetes: a randomized clinical trial. *Pediatr. Diabetes* 19 (3), 410–419.
- Erlich, H.A., Valdes, A.M., McDevitt, S., Simen, B.B., Blake, L.A., McGowan, K.R., et al., 2013. Next generation sequencing reveals the association of DRB3\*02:02 with type I diabetes. *Diabetes*. 62, 2618–2622.
- Foulis, A.K., 2008. Pancreatic pathology in type 1 diabetes in human. *Novartis Found Symp.* 292, 2–13 (discussion 13–8, 122–129, 202–203).
- Foulis, A.K., McGill, M., Farquharson, M.A., 1991. Insulitis in type 1 (insulin-dependent) diabetes mellitus in man—macrophages, lymphocytes, and interferon-gamma containing cells. *J. Pathol.* 165 (2), 97–103.
- Fourlanos, S., Narendran, P., Byrnes, G.B., Colman, P.G., Harrison, L.C., 2004. Insulin resistance is a risk factor for progression to type 1 diabetes. *Diabetologia* 47 (10), 1661–1667.
- Ge, X., James, E.A., Reijonen, H., Kwok, W.W., 2011. Differences in self-peptide binding between T1D-related susceptible and protective DR4 subtypes. *J. Autoimmun.* 36 (2), 155–160.
- Gepts, W., 1965. Pathologic anatomy of the pancreas in juvenile diabetes mellitus. *Diabetes* 14, 619–633.
- Gleichmann, H., Bottazzo, G.F., 1987. Progress toward standardization of cytoplasmic islet cell antibody assay. *Diabetes* 36 (5), 578–584.
- Grieco, F.A., Moore, F., Vigneron, F., Santin, I., Villate, O., Marselli, L., et al., 2014. IL-17A increases the expression of proinflammatory chemokines in human pancreatic islets. *Diabetologia* 57 (3), 502–511.
- Group TS, 2007. The Environmental Determinants of Diabetes in the Young (TEDDY) study: study design. *Pediatr. Diabetes* 8 (5), 286–298.
- Group TS, 2008. The Environmental Determinants of Diabetes in the Young. *Ann. N.Y. Acad. Sci.* 1150, 1–13.
- Group TS, 2008. The Environmental Determinants of Diabetes in the Young (TEDDY) study. *Ann. N.Y. Acad. Sci.* 1150 (December), 1–13.
- Habeb, A.M., Al-Magamsi, M.S., Halabi, S., Eid, I.M., Shalaby, S., Bakoush, O., 2011. High incidence of childhood type 1 diabetes in Al-Madinah, North West Saudi Arabia (2004–2009). *Pediatr. Diabetes* 12 (8), 676–681.
- Hagopian, W.A., Erlich, H., Lernmark, A., Rewers, M., Ziegler, A.G., Simell, O., et al., 2011. The Environmental Determinants of Diabetes in the Young (TEDDY): genetic criteria and international diabetes risk screening of 421 000 infants. *Pediatr. Diabetes* 12 (8), 733–743.
- Hamari, S., Kirveskoski, T., Glumoff, V., Kulmala, P., Simell, O., Knip, M., et al., 2016. Analyses of regulatory CD4+ CD25+ FOXP3+ T cells and observations from peripheral T cell subpopulation markers during the development of type 1 diabetes in children. *Scand. J. Immunol.* 83 (4), 279–287.
- Hameed, S., Ellard, S., Woodhead, H.J., Neville, K.A., Walker, J.L., Craig, M.E., et al., 2011. Persistently autoantibody negative (PAN) type 1 diabetes mellitus in children. *Pediatr. Diabetes* 12 (3 Pt 1), 142–149.
- Hammond, K.J., Kronenberg, M., 2003. Natural killer T cells: natural or unnatural regulators of autoimmunity? *Curr. Opin. Immunol.* 15 (6), 683–689.
- Harel, M., Shoenfeld, Y., 2006. Predicting and preventing autoimmunity, myth or reality? *Ann. N.Y. Acad. Sci.* 1069, 322–345.
- Harjutsalo, V., Sjöberg, L., Tuomilehto, J., 2008. Time trends in the incidence of type 1 diabetes in Finnish children: a cohort study. *Lancet* 371 (9626), 1777–1782.
- Herold, K.C., Hagopian, W., Auger, J.A., Poumian-Ruiz, E., Taylor, L., Donaldson, D., et al., 2002. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N. Engl. J. Med.* 346 (22), 1692–1698.
- von Herrath, M.G., Fujinami, R.S., Whitton, J.L., 2003. Microorganisms and autoimmunity: making the barren field fertile? *Nat. Rev. Microbiol.* 1, 151–157.
- Hirai, H., Miura, J., Hu, Y., Larsson, H., Larsson, K., Lernmark, A., et al., 2008. Selective screening of secretory vesicle-associated proteins for autoantigens in type 1 diabetes: VAMP2 and NPY are new minor autoantigens. *Clin. Immunol.* 127 (3), 366–374.
- Honkanen, H., Oikarinen, S., Nurminen, N., Laitinen, O.H., Huhtala, H., Lehtonen, J., et al., 2017. Detection of enteroviruses in stools precedes islet autoimmunity by several months: possible evidence for slowly operating mechanisms in virus-induced autoimmunity. *Diabetologia* 60 (3), 424–431.
- Hummel, M., Bonifacio, E., Naserke, H.E., Ziegler, A.G., 2002. Elimination of dietary gluten does not reduce titers of type 1 diabetes-associated autoantibodies in high-risk subjects. *Diabetes Care* 25 (7), 1111–1116.
- Hummel, S., Beyerlein, A., Tamura, R., Uusitalo, U., Andren Aronsson, C., Yang, J., et al., 2017. First infant formula type and risk of islet autoimmunity in The Environmental Determinants of Diabetes in the Young (TEDDY) study. *Diabetes Care* 40 (3), 398–404.
- Hussen, H.I., Moradi, T., Persson, M., 2015. The risk of type 1 diabetes among offspring of immigrant mothers in relation to the duration of residency in Sweden. *Diabetes Care* 38 (5), 934–936.
- Ilonen, J., Hammais, A., Laine, A.P., Lempainen, J., Vaarala, O., Veijola, R., et al., 2013. Patterns of beta-cell autoantibody appearance and genetic associations during the first years of life. *Diabetes* 62 (10), 3636–3640.
- Ilonen, J., Lempainen, J., Hammais, A., Laine, A.P., Harkonen, T., Toppari, J., et al., 2018. Primary islet autoantibody at initial seroconversion and autoantibodies at diagnosis of type 1 diabetes as markers of disease heterogeneity. *Pediatr. Diabetes* 19 (2), 284–292.

- Insel, R.A., Dunne, J.L., Atkinson, M.A., Chiang, J.L., Dabelea, D., Gottlieb, P.A., et al., 2015. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. *Diabetes Care* 38 (10), 1964–1974.
- In't Veld, P., 2011. Insulitis in the human endocrine pancreas: does a viral infection lead to inflammation and beta cell replication? *Diabetologia* 54 (9), 2220–2222.
- In't Veld, P., Lievens, D., De Grijse, J., Ling, Z., Van der Auwera, B., Pipeleers-Marichal, M., et al., 2007. Screening for insulitis in adult autoantibody-positive organ donors. *Diabetes* 56 (9), 2400–2404.
- Jones, E.Y., Fugger, L., Strominger, J.L., Siebold, C., 2006. MHC class II proteins and disease: a structural perspective. *Nat. Rev. Immunol.* 6 (4), 271–282.
- Jorns, A., Rath, K.J., Terbish, T., Arndt, T., Meyer Zu Vilsendorf, A., Wedekind, D., et al., 2010. Diabetes prevention by immunomodulatory FTY720 treatment in the LEW.1AR1-IDDM rat despite immune cell activation. *Endocrinology* 151 (8), 3555–3565.
- Kaddis, J.S., Pugliese, A., Atkinson, M.A., 2015. A run on the biobank: what have we learned about type 1 diabetes from the nPOD tissue repository? *Curr. Opin. Endocrinol. Diabetes Obes.* 22 (4), 290–295.
- Karlsen, A.E., Hagopian, W.A., Grubin, C.E., Dube, S., Disteche, C.M., Adler, D.A., et al., 1991. Cloning and primary structure of a human islet isoform of glutamic acid decarboxylase from chromosome 10. *Proc. Natl. Acad. Sci. U.S.A.* 88 (19), 8337–8341.
- Katsarou, A., Gudbjornsdottir, S., Rawshani, A., Dabelea, D., Bonifacio, E., Anderson, B.J., et al., 2017. Type 1 diabetes mellitus. *Nat. Rev. Dis. Primers* 3, 17016.
- Kawabata, Y., Ikegami, H., Awata, T., Imagawa, A., Maruyama, T., Kawasaki, E., et al., 2009. Differential association of HLA with three subtypes of type 1 diabetes: fulminant, slowly progressive and acute-onset. *Diabetologia* 52 (12), 2513–2521.
- Kent, S.C., Chen, Y., Bregoli, L., Clemmings, S.M., Kenyon, N.S., Ricordi, C., et al., 2005. Expanded T cells from pancreatic lymph nodes of type 1 diabetic subjects recognize an insulin epitope. *Nature* 435, 224–228.
- Knip, M., Veijola, R., Virtanen, S.M., Hyöty, H., Vaarala, O., Åkerblom, H.K., 2005. Environmental triggers and determinants of type 1 diabetes. *Diabetes* 54 (2), 125–136.
- Knip, M., Virtanen, S.M., Seppa, K., Ilonen, J., Savilahti, E., Vaarala, O., et al., 2010. Dietary intervention in infancy and later signs of beta-cell autoimmunity. *N. Engl. J. Med.* 363 (20), 1900–1908.
- Knip, M., Åkerblom, H.K., Becker, D., Dosch, H.M., Dupre, J., Fraser, W., et al., 2014. Hydrolyzed infant formula and early beta-cell autoimmunity: a randomized clinical trial. *JAMA* 311 (22), 2279–2287.
- Krischer, J.P., Lynch, K.F., Schatz, D.A., Ilonen, J., Lernmark, A., Hagopian, W.A., et al., 2015. The 6 year incidence of diabetes-associated auto-antibodies in genetically at-risk children: the TEDDY study. *Diabetologia* 58 (5), 980–987.
- Krischer, J.P., Lynch, K.F., Lernmark, A., Hagopian, W.A., Rewers, M.J., She, J.X., et al., 2017a. Genetic and environmental interactions modify the risk of diabetes-related autoimmunity by 6 years of age: the TEDDY study. *Diabetes Care* 40 (9), 1194–1202.
- Krischer, J.P., Liu, X., Lernmark, A., Hagopian, W.A., Rewers, M.J., She, J.X., et al., 2017b. The influence of type 1 diabetes genetic susceptibility regions, age, sex, and family history on the progression from multiple autoantibodies to type 1 diabetes: a TEDDY study report. *Diabetes* 66 (12), 3122–3129.
- Krogvold, L., Wiberg, A., Edwin, B., Buanes, T., Jahnsen, F.L., Hanssen, K.F., et al., 2016. Insulitis and characterisation of infiltrating T cells in surgical pancreatic tail resections from patients at onset of type 1 diabetes. *Diabetologia* 59 (3), 492–501.
- LaGasse, J.M., Brantley, M.S., Leech, N.J., Rowe, R.E., Monks, S., Palmer, J.P., et al., 2002. Successful prospective prediction of type 1 diabetes in schoolchildren through multiple defined autoantibodies: an 8-year follow-up of the Washington State Diabetes Prediction Study. *Diabetes Care* 25 (3), 505–511.
- Lamb, M.M., Myers, M.A., Barriga, K., Zimmet, P.Z., Rewers, M., Norris, J.M., 2008. Maternal diet during pregnancy and islet autoimmunity in offspring. *Pediatr. Diabetes* 9 (2), 135–141.
- Lampasona, V., Schlosser, M., Mueller, P.W., Williams, A.J., Wenzlau, J.M., Hutton, J.C., et al., 2011. Diabetes antibody standardization program: first proficiency evaluation of assays for autoantibodies to zinc transporter 8. *Clin. Chem.* 57 (12), 1693–1702.
- Lee, K.H., Wucherpfennig, K.W., Wiley, D.C., 2001. Structure of a human insulin peptide-HLA-DQ8 complex and susceptibility to type 1 diabetes. *Nat. Immunol.* 2 (6), 501–507.
- Lee, H.S., Briese, T., Winkler, C., Rewers, M., Bonifacio, E., Hyoty, H., et al., 2013. Next-generation sequencing for viruses in children with rapid-onset type 1 diabetes. *Diabetologia* 56 (8), 1705–1711.
- Leslie, R.D., Kolb, H., Schloot, N.C., Buzzetti, R., Mauricio, D., De Leiva, A., et al., 2008. Diabetes classification: grey zones, sound and smoke: action LADA 1. *Diabetes Metab. Res. Rev.* 24 (7), 511–519.
- Lind, A., Ramelius, A., Olsson, T., Arnheim-Dahlstrom, L., Lamb, F., Khademi, M., et al., 2014. A/H1N1 antibodies and TRIB2 autoantibodies in narcolepsy patients diagnosed in conjunction with the Pandemrix vaccination campaign in Sweden 2009–2010. *J. Autoimmun.* 50, 99–106.
- Long, S.A., Rieck, M., Sanda, S., Bollyky, J.B., Samuels, P.L., Goland, R., et al., 2012. Rapamycin/IL-2 combination therapy in patients with type 1 diabetes augments Tregs yet transiently impairs beta-cell function. *Diabetes*. 61 (September), 2340.
- Lonnrot, M., Lynch, K.F., Elding Larsson, H., Lernmark, A., Rewers, M.J., Torn, C., et al., 2017. Respiratory infections are temporally associated with initiation of type 1 diabetes autoimmunity: the TEDDY study. *Diabetologia* 60 (10), 1931–1940.
- Ludvigsson, J., Jones, M.P., Faresjo, A., 2017. Worm infestations and development of autoimmunity in children—the ABIS study. *PLoS One* 12 (3), e0173988.
- van Lummel, M., van Veelen, P.A., Zaldumbide, A., de Ru, A., Janssen, G.M., Moustakas, A.K., et al., 2012. Type 1 diabetes-associated HLA-DQ8 transdimer accommodates a unique peptide repertoire. *J. Biol. Chem.* 287 (12), 9514–9524.
- van Lummel, M., Duinkerken, G., van Veelen, P.A., de Ru, A., Cordfunke, R., Zaldumbide, A., et al., 2013. Post-translational modification of Hla-Dq binding islet-autoantigens in type 1 diabetes. *Diabetes* 63 (1).
- Lundberg, M., Seiron, P., Ingvast, S., Korsgren, O., Skog, O., 2017. Insulitis in human diabetes: a histological evaluation of donor pancreases. *Diabetologia* 60 (2), 346–353.
- Lynch, K.F., Lee, H.S., Torn, C., Vehik, K., Krischer, J.P., Larsson, H.E., et al., 2018. Gestational respiratory infections interacting with offspring HLA and CTLA-4 modifies incident beta-cell autoantibodies. *J. Autoimmun.* 86, 93–103.

- Mathis, D., Vence, L., Benoist, C., 2001. B-cell death during progression to diabetes. *Nature* 414, 792–798.
- McKinney, E.F., Lee, J.C., Jayne, D.R., Lyons, P.A., Smith, K.G., 2015. T-cell exhaustion, co-stimulation and clinical outcome in autoimmunity and infection. *Nature* 523 (7562), 612–616.
- Mire-sluis, A.R., Gaines Das, R., Lernmark, A., 2000. The World Health Organization International Collaborative Study for islet cell antibodies. *Diabetologica* 43, 1282–1292.
- Mordes, J.P., Bortell, R., Blankenhorn, E.P., Rossini, A.A., Greiner, D.L., 2004. Rat models of type 1 diabetes: genetics, environment, and autoimmunity. *ILAR* 45 (3), 278–291.
- Morran, M.P., McInerney, M.F., Pietropaolo, M., 2008. Innate and adaptive autoimmunity in type 1 diabetes. *Pediatr. Diabetes* 9 (2), 152–161.
- Nejentsev, S., Sjoroos, M., Soukka, T., Knip, M., Simell, O., Lovgren, T., et al., 1999. Population-based genetic screening for the estimation of Type 1 diabetes mellitus risk in Finland: selective genotyping of markers in the HLA-DQB1, HLA-DQA1 and HLA-DRB1 loci. *Diabet. Med.* 16 (12), 985–992.
- Nelson, J.L., Furst, D.E., Maloney, S., Gooley, T., Evans, P.C., Smith, A., et al., 1998. Microchimerism and HLA-compatible relationships of pregnancy in scleroderma. *Lancet* 351 (9102), 559–562.
- Niklasson, B., Hornfeldt, B., Nyholm, E., Niedrig, M., Donoso-Mantke, O., Gelderblom, H.R., et al., 2003. Type 1 diabetes in Swedish bank voles (*Clethrionomys glareolus*): signs of disease in both colonized and wild cyclic populations at peak density. *Ann. N.Y. Acad. Sci.* 1005, 170–175.
- Norris, J.M., Lee, H.S., Frederiksen, B., Erlund, I., Uusitalo, U., Yang, J., et al., 2018. Plasma 25-hydroxyvitamin D concentration and risk of islet autoimmunity. *Diabetes* 67 (1), 146–154.
- Notkins, A.L., Lernmark, A., 2001. Autoimmune type 1 diabetes: resolved and unresolved issues. *J. Clin. Invest.* 108 (9), 1247–1252.
- Ohashi, P., 2003. Negative selection and autoimmunity. *Curr. Opin. Immunol.* 15 (6), 668–676.
- Oling, V., Reijonen, H., Simell, O., Knip, M., Ilonen, J., 2012. Autoantigen-specific memory CD4+ T cells are prevalent early in progression to Type 1 diabetes. *Cell Immunol.* 273 (2), 133–139.
- Ollila, H.M., Ravel, J.M., Han, F., Faraco, J., Lin, L., Zheng, X., et al., 2015. HLA-DPB1 and HLA class I confer risk of and protection from narcolepsy. *Am. J. Hum. Genet.* 96 (1), 136–146.
- Oram, R.A., Patel, K., Hill, A., Shields, B., McDonald, T.J., Jones, A., et al., 2016. A type 1 diabetes genetic risk score can aid discrimination between type 1 and type 2 diabetes in young adults. *Diabetes Care* 39 (3), 337–344.
- Parkkola, A., Härkönen, T., Ryhänen, S.J., Ilonen, J., Knip, M., 2013. Extended family history of type 1 diabetes and phenotype and genotype of newly diagnosed children. *Diabetes Care* 36, 348–354.
- Pastore, M.R., Bazzigaluppi, E., Belloni, C., Arcovio, C., Bonifacio, E., Bosi, E., 2003. Six months of gluten-free diet do not influence autoantibody titers, but improve insulin secretion in subjects at high risk for type 1 diabetes. *J. Clin. Endocrinol. Metab.* 88 (1), 162–165.
- Patterson, C., Guariguata, L., Dahlquist, G., Soltesz, G., Ogle, G., Silink, M., 2014. Diabetes in the young—a global view and worldwide estimates of numbers of children with type 1 diabetes. *Diabetes Res. Clin. Pract.* 103 (2), 161–175.
- Peakman, M., Leslie, R.D., Vergani, D., 1993. Immunological studies on type 1 diabetes in identical twins. *Arch. Dis. Child.* 69 (1), 97–99.
- Pescovitz, M.D., GCJ, Krause-Steinrauf, H., Becker, D.J., Gittleman, S.E., Goland, R., et al., 2009. Rituximab, B-lymphocyte depletion, and preservation of beta-cell function. *N. Eng. J. Med.* 361, 2143–2152.
- Pinkse, G.G.M., TOHM, Bergen, C.A.M., Kester, M.G.D., Ossendorp, F., van Veelen, P.A., et al., 2005. Autoreactive CD8 T cells associated with beta cell destruction in type 1 diabetes. *Proc. Natl. Acad. Sci. U.S.A.* 102 (51), 18425–18430.
- Pipeleers, D., In't Veld, P., Pipeleers-Marichal, M., Gorus, F., 2008. The beta cell population in type 1 diabetes. *Novartis Found Symp.* 292, 19–31 (122-9, 202-3).
- Pociot, F., Lernmark, A., 2016. Genetic risk factors for type 1 diabetes. *Lancet* 387 (10035), 2331–2339.
- Pociot, F., Akolkar, B., Concannon, P., Erlich, H.A., Julier, C., Morahan, G., et al., 2010. Genetics of type 1 diabetes: what's next? *Diabetes* 59 (7), 1561–1571.
- Pugliese, A., 2014. Advances in the etiology and mechanisms of type 1 diabetes. *Discov. Med.* 18 (98), 141–150.
- Pugliese, A., Zeller, M., Fernandez Jr, A., Zalcberg, L.J., Bartlett, R.J., Ricordi, C., et al., 1997. The insulin gene is transcribed in the human thymus and transcription levels correlated with allelic variation at the INS VNTR-IDDM2 susceptibility locus for type 1 diabetes. *Nat. Genet.* 15 (3), 293–297.
- Pugliese, A., Brown, D., Garza, D., Murchison, D., Zeller, M., Redondo, M.J., et al., 2001. Self-antigen-presenting cells expressing diabetes-associated autoantigens exist in both thymus and peripheral lymphoid organs. *J. Clin. Invest.* 107 (5), 555–564.
- Raab, J., Giannopoulou, E.Z., Schneider, S., Warncke, K., Krasmann, M., Winkler, C., et al., 2014. Prevalence of vitamin D deficiency in pre-type 1 diabetes and its association with disease progression. *Diabetologia* 57 (5), 902–908.
- Raab, J., Haupt, F., Scholz, M., Matzke, C., Warncke, K., Lange, K., et al., 2016. Capillary blood islet autoantibody screening for identifying pre-type 1 diabetes in the general population: design and initial results of the Fr1da study. *BMJ Open* 6 (5), e011144.
- Rabinovich, A., Suarez-Pinzon, W.L., Shapiro, A.M.J., Rajotte, R.V., 2003. Combination therapy with sirolimus and interleukin-2 prevents spontaneous and recurrent autoimmune diabetes in NOD mice. *Diabetes* 51 (March), 638.
- Ram, R., Mehta, M., Nguyen, Q.T., Larma, I., Boehm, B.O., Pociot, F., et al., 2016. Systematic evaluation of genes and genetic variants associated with type 1 diabetes susceptibility. *J. Immunol.* 196 (7), 3043–3053.
- Reddy, S., Zeng, N., Al-Diery, H., Jung, D., Yeu, C., Joret, M.O., et al., 2015. Analysis of peri-islet CD45-positive leucocytic infiltrates in long-standing type 1 diabetic patients. *Diabetologia* 58 (5), 1024–1035.
- Regnall, S.E., Lernmark, A., 2017. Early prediction of autoimmune (type 1) diabetes. *Diabetologia* 60, 1370–1381.
- Rewers, M., Bugawan, T.L., Norris, J.M., Blair, A., Beaty, B., Hoffman, M., et al., 1996. Newborn screening for HLA markers associated with IDDM: diabetes autoimmunity study in the young (DAISY). *Diabetologia* 39 (7), 807–812.
- Rich, S.S., Concannon, P., Erlich, H., Julier, C., Morahan, G., Nerup, J., et al., 2006. The type 1 diabetes genetics consortium. *Ann. N.Y. Acad. Sci.* 1079, 1–8.
- Rich, S.S., Akolkar, B., Concannon, P., Erlich, H., Hilner, J.E., Julier, C., et al., 2009. Current status and the future for the genetics of type 1 diabetes. *Genes Immun.* 10 (Suppl. 1), S128–S131.

- Richardson, S.J., Rodriguez-Calvo, T., Gerling, I.C., Mathews, C.E., Kaddis, J.S., Russell, M.A., et al., 2016. Islet cell hyperexpression of HLA class I antigens: a defining feature in type 1 diabetes. *Diabetologia* 59, 2448–2458.
- Rojas, J., Bermudez, V., Palmar, J., Martinez, M.S., Olivar, L.C., Nava, M., et al., 2018. Pancreatic beta cell death: novel potential mechanisms in diabetes therapy. *J. Diabetes Res.* 2018, 9601801.
- Rolandsson, O., Hagg, E., Hampe, C., Sullivan Jr., E.P., Nilsson, M., Jansson, G., et al., 1999. Glutamate decarboxylase (GAD65) and tyrosine phosphatase-like protein (IA-2) autoantibodies index in a regional population is related to glucose intolerance and body mass index. *Diabetologia* 42 (5), 555–559.
- Schlosser, M., Strelbow, M., Wassmuth, R., Arnold, M.L., Breunig, I., Rjasanowski, I., et al., 2002. The Karlsburg type 1 diabetes risk study of a normal schoolchild population: association of beta-cell autoantibodies and human leukocyte antigen-DQB1 alleles in antibody-positive individuals. *J. Clin. Endocrinol. Metab.* 87 (5), 2254–2261.
- Schlosser, M., Mueller, P.W., Torn, C., Bonifacio, E., Bingley, P.J., Participating, L., 2010. Diabetes Antibody Standardization Program: evaluation of assays for insulin autoantibodies. *Diabetologia* 53 (12), 2611–2620.
- Sherr, J., Sosenko, J., Skyler, J.S., Herold, K.C., 2008. Prevention of type 1 diabetes: the time has come. *Nat. Clin. Pract. Endocrinol. Metab.* 4 (6), 334–343.
- Shoenfeld, Y., Aron-Maor, A., 2000. Vaccination and autoimmunity—‘vaccinosis’: a dangerous liaison? *J. Autoimmun.* 14 (1), 1–10.
- Sioof-Khojine, A.B., Lehtonen, J., Nurminen, N., Laitinen, O.H., Oikarinen, S., Huhtala, H., et al., 2018. Coxsackievirus B1 infections are associated with the initiation of insulin-driven autoimmunity that progresses to type 1 diabetes. *Diabetologia* 61, 1193–1202.
- Skarstrand, H., Krupinska, E., Haataja, T.J., Vaziri-Sani, F., Lagerstedt, J.O., Lernmark, A., 2015. Zinc transporter 8 (ZnT8) autoantibody epitope specificity and affinity examined with recombinant ZnT8 variant proteins in specific ZnT8R and ZnT8W autoantibody-positive type 1 diabetes patients. *Clin. Exp. Immunol.* 179 (2), 220–229.
- Skog, O., Korsgren, S., Melhus, A., Korsgren, O., 2013. Revisiting the notion of type 1 diabetes being a T-cell-mediated autoimmune disease. *Curr. Opin. Endocrinol. Diabetes Obes.* 20 (2), 118–123.
- Skyler, J.S., 2011. Immune intervention for type 1 diabetes mellitus. *Int. J. Clin. Pract. Suppl.* 170, 61–70.
- Skyler, J.S., 2013. The year in immune intervention for type 1 diabetes. *Diabetes Technol. Ther.* 15 (Suppl. 1), S88–S95.
- Skyler, J.S., Krischer, J.P., Wolfsdorf, J., Cowie, C., Palmer, J.P., Greenbaum, C., et al., 2005. Effects of oral insulin in relatives of patients with type 1 diabetes: The Diabetes Prevention Trial—Type 1. *Diabetes Care* 28 (5), 1068–1076.
- Smith, A.G., Pyo, C.W., Nelson, W., Gow, E., Wang, R., Shen, S., et al., 2014. Next generation sequencing to determine HLA class II genotypes in a cohort of hematopoietic cell transplant patients and donors. *Hum. Immunol.* 75 (10), 1040–1046.
- Soltesz, G., Patterson, C.C., Dahlquist, G., 2007. Worldwide childhood type 1 diabetes incidence—what can we learn from epidemiology? *Pediatr. Diabetes* 8 (Suppl. 6), 6–14.
- Sorkio, S., Cuthbertson, D., Barlund, S., Reunanen, A., Nucci, A.M., Berseth, C.L., et al., 2010. Breastfeeding patterns of mothers with type 1 diabetes: results from an infant feeding trial. *Diabetes Metab. Res. Rev.* 26 (3), 206–211.
- Sosenko, J.M., Skyler, J.S., DiMeglio, L.A., Beam, C.A., Krischer, J.P., Greenbaum, C.J., et al., 2015. A new approach for diagnosing type 1 diabetes in autoantibody-positive individuals based on prediction and natural history. *Diabetes Care* 38 (2), 271–276.
- Srinivasappa, J., Saegusa, J., Prabhakar, B.S., Gentry, M.K., Buchmeier, M.J., Wiktor, T.J., et al., 1986. Molecular mimicry: frequency of reactivity of monoclonal antiviral antibodies with normal tissues. *J. Virol.* 57 (1), 397–401.
- Steck, A.K., Vehik, K., Bonfacio, E., Lernmark, Å., Ziegler, A.-G., Hagopian, W.A., et al., 2015. Predictors of progression from the appearance of islet autoantibodies to early childhood diabetes: The Environmental Determinants of Diabetes in the Young (TEDDY). *Diabetes Care* February 9, 1–6.
- Stene, L.C., Oikarinen, S., Hyoty, H., Barriga, K.J., Norris, J.M., Klingensmith, G., et al., 2010. Enterovirus infection and progression from islet autoimmunity to type 1 diabetes: the Diabetes and Autoimmunity Study in the Young (DAISY). *Diabetologia* 53 (12), 3174–3180.
- Suri, A., Walters, J.J., Gross, M.L., Unanue, E.R., 2005. Natural peptides selected by diabetogenic DQ8 and murine I-A(g7) molecules show common sequence specificity. *J. Clin. Invest.* 115 (8), 2268–2276.
- Tafti, M., Hor, H., Dauvilliers, Y., Lammers, G.J., Overeem, S., Mayer, G., et al., 2014. DQB1 locus alone explains most of the risk and protection in narcolepsy with cataplexy in Europe. *Sleep* 37 (1), 19–25.
- Thayer, T.C., Wilson, S.B., Mathews, C.E., 2010. Use of nonobese diabetic mice to understand human type 1 diabetes. *Endocrinol. Metab. Clin. North Am.* 39 (3), 541–561.
- TEDDY Study Group, 2008. The Environmental Determinants of Diabetes in the Young (TEDDY) Study. *Ann. N.Y. Acad. Sci.* 1150, 1–13.
- Thomas, N.J., Jones, S.E., Weedon, M.N., Shields, B.M., Oram, R.A., Hattersley, A.T., 2018. Frequency and phenotype of type 1 diabetes in the first six decades of life: a cross-sectional, genetically stratified survival analysis from UK Biobank. *Lancet Diabetes Endocrinol.* 6 (2), 122–129.
- Todd, J.A., Knip, M., Mathieu, C., 2011. Strategies for the prevention of autoimmune type 1 diabetes. *Diabet. Med.* 28 (10), 1141–1143.
- Torn, C., Mueller, P.W., Schlosser, M., Bonifacio, E., Bingley, P.J., Participating, L., 2008. Diabetes Antibody Standardization Program: evaluation of assays for autoantibodies to glutamic acid decarboxylase and islet antigen-2. *Diabetologia* 51 (5), 846–852.
- Torn, C., Hadley, D., Lee, H.S., Hagopian, W., Lernmark, A., Simell, O., et al., 2015. Role of type 1 diabetes-associated SNPs on risk of autoantibody positivity in the TEDDY study. *Diabetes* 64 (5), 1818–1829.
- Tuomilehto, J., 2013. The emerging global epidemic of type 1 diabetes. *Curr. Diab. Rep.* 13 (6), 795–804.
- Uusitalo, U., Liu, X., Yang, J., Aronsson, C.A., Hummel, S., Butterworth, M., et al., 2016. Association of early exposure of probiotics and islet autoimmunity in the TEDDY study. *JAMA Pediatr.* 170 (1), 20–28.
- Vehik, K., Cuthbertson, D., Boulware, D., Beam, C.A., Rodriguez, H., Legault, L., et al., 2012. Performance of HbA1c as an early diagnostic indicator of type 1 diabetes in children and youth. *Diabetes Care* 1–5.
- Vehik, K., Lynch, K.F., Schatz, D.A., Akolkar, B., Hagopian, W., Rewers, M., et al., 2016. Reversion of beta-cell autoimmunity changes risk of type 1 diabetes: TEDDY study. *Diabetes Care* 39 (9), 1535–1542.
- Verge, C.F., Gianani, R., Kawasaki, E., Yu, L., Pietropaolo, M., Jackson, R.A., et al., 1996. Prediction of type I diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-2 autoantibodies. *Diabetes* 45 (7), 926–933.

- Virtanen, S.M., 2016. Dietary factors in the development of type 1 diabetes. *Pediatr. Diabetes* 17 (Suppl. 22), 49–55.
- Visalli, N., Sebastiani, L., Adorisio, E., Conte, A., De Cicco, A.L., DELia, R., et al., 2003. Environmental risk factors for type 1 diabetes in Rome and province. *Arch. Dis. Child.* 88, 695–698.
- Wallensteen, M., Dahlquist, G., Persson, B., Landin-Olsson, M., Lernmark, A., Sundkvist, G., et al., 1988. Factors influencing the magnitude, duration, and rate of fall of B-cell function in type 1 (insulin-dependent) diabetic children followed for two years from their clinical diagnosis. *Diabetologia* 31 (9), 664–669.
- Wasserfall, C., Montgomery, E., Yu, L., Michels, A., Gianani, R., Pugliese, A., et al., 2016. Validation of a rapid type 1 diabetes autoantibody screening assay for community-based screening of organ donors to identify subjects at increased risk for the disease. *Clin. Exp. Immunol.* 185 (1), 33–41.
- Wenzlau, J.M., Hutton, J.C., Davidson, H.W., 2008a. New antigenic targets in type 1 diabetes. *Curr. Opin. Endocrinol. Diabetes Obes.* 15 (4), 315–320.
- Wenzlau, J.M., Liu, Y., Yu, L., Moua, O., Fowler, K.T., Rangasamy, S., et al., 2008b. A common nonsynonymous single nucleotide polymorphism in the SLC30A8 gene determines ZnT8 autoantibody specificity in type 1 diabetes. *Diabetes* 57 (10), 2693–2697.
- Wiberg, A., Granstam, A., Ingvast, S., Harkonen, T., Knip, M., Korsgren, O., et al., 2015. Characterization of human organ donors testing positive for type 1 diabetes-associated autoantibodies. *Clin. Exp. Immunol.* 182 (3), 278–288.
- Willcox, A., Richardson, S.J., Bone, A.J., Foulis, A.K., Morgan, N.G., 2009. Analysis of islet inflammation in human type 1 diabetes. *Clin. Exp. Immunol.* 155 (2), 173–181.
- Woo, H.J., Yu, C., Reifman, J., 2017. Collective genetic interaction effects and the role of antigen-presenting cells in autoimmune diseases. *PLoS One* 12 (1), e0169918.
- Writing Committee for the Type 1 Diabetes TrialNet Oral Insulin Study Group, Krischer, J.P., Schatz, D.A., Bundy, B., Skyler, J.S., Greenbaum, C.J., 2017. Effect of oral insulin on prevention of diabetes in relatives of patients with type 1 diabetes: a randomized clinical trial. *JAMA* 318 (19), 1891–1902.
- Xu, P., Krischer, J.P., 2016. Prognostic classification factors associated with development of multiple autoantibodies, dysglycemia, and type 1 diabetes—a recursive partitioning analysis. *Diabetes Care* 39 (6), 1036–1044.
- Yokoi, N., Namae, M., Fuse, M., Wang, H., 2003. Establishment and characterization of the Komeda diabetes-prone rat as a segregating inbred strain. *Exp. Anim.* 52 (4), 295–301.
- Yu, L., Boulware, D.C., Beam, C.A., Hutton, J.C., Wenzlau, J.M., Greenbaum, C.J., et al., 2012. Zinc transporter-8 autoantibodies improve prediction of type 1 diabetes in relatives positive for the standard biochemical autoantibodies. *Diabetes Care* 35 (6), 1213–1218.
- Yunis, E.J., Zuniga, J., Romero, V., Yunis, E.J., 2007. Chimerism and tetragametic chimerism in humans: implications in autoimmunity, allorecognition and tolerance. *Immunol. Res.* 38 (1–3), 213–236.
- Zhao, L.P., Alshiekh, S., Zhao, M., Carlsson, A., Elding Larsson, H., Forsander, G., et al., 2016. Next-generation sequencing reveals that HLA-DRB3, -DRB4, and -DRB5 may be associated with islet autoantibodies and risk for childhood type 1 diabetes. *Diabetes* 65 (3), 710–718.
- Zhou, Z.H., Zhang, Y., Hu, Y.F., Wahl, L.M., Cisar, J.O., Notkins, A.L., 2007. The broad antibacterial activity of the natural antibody repertoire is due to polyreactive antibodies. *Cell Host Microbe.* 1 (1), 51–61.
- Ziegler, A.G., Bonifacio, E., 2012. Age-related islet autoantibody incidence in offspring of patients with type 1 diabetes. *Diabetologia* 55 (7), 1937–1943.
- Ziegler, A.G., Hummel, M., Schenker, M., Bonifacio, E., 1999. Autoantibody appearance and risk for development of childhood diabetes in offspring of parents with type 1 diabetes: the 2-year analysis of the German BABYDIAB study. *Diabetes* 48 (3), 460–468.
- Ziegler, A.G., Rewers, M., Simell, O., Simell, T., Lempainen, J., Steck, A., et al., 2013. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. *JAMA* 309 (23), 2473–2479.
- Ziegler, A.G., Danne, T., Dunger, D.B., Berner, R., Puff, R., Kiess, W., et al., 2016. Primary prevention of beta-cell autoimmunity and type 1 diabetes—The Global Platform for the Prevention of Autoimmune Diabetes (GPPAD) perspectives. *Mol. Metab.* 5 (4), 255–262.

## Adrenalitis

Corrado Betterle<sup>1</sup>, Fabio Presotto<sup>2</sup> and Renato Zanchetta<sup>1</sup>

<sup>1</sup>Unit of Endocrinology, Department of Medicine, University of Padova, Padua, Italy <sup>2</sup>Unit of Internal Medicine, Department of Medicine, Ospedale dell'Angelo, Mestre-Venezia, Italy

### OUTLINE

Introduction	789	Other Autoantibodies Detected in Patients With Autoimmune Addison's Disease	797
Anatomy and Physiology of the Adrenals	790	Natural History of Autoimmune Addison's Disease	798
Epidemiology of Addison's Disease and Autoimmune Adrenalitis	791	Diagnosis of Autoimmune Addison's Disease	800
Autoimmune Addison's Disease	792	Clinical Manifestations	800
Focal Lymphocytic Adrenalitis	792	General Biochemical Indices	800
Diffuse Lymphocytic Adrenalitis	793	Hormonal Tests	801
Induced Immunity in Animal Models	793	Imaging	802
Spontaneous Animal Models	793	Different Clinical Presentations of Autoimmune Addison's Disease	802
Family History of Autoimmune Addison's Disease and Genetic Predisposition	793	Association With Other Autoimmune Disorders	802
Cellular Immunity	794	Therapy	804
Humoral Immunity	795	Acute Adrenal Failure (Adrenal Crisis)	806
Identification of Autoantigens of Adrenal Cortex Autoantibodies	796	Emergency Care	807
Identification of Autoantigens of Steroid-Producing Cells Autoantibodies	796	Quality of Life	807
Techniques for Identification of Autoantibodies to 21-Hydroxylase	797	Mortality	807
Techniques for Identification of Autoantibodies to Other Steroidogenic Enzymes	797	Osteoporosis	807
		Acknowledgments	808
		References	808

### INTRODUCTION

Bartolomeo Eustachius was the first to describe the existence of the adrenals as "de glandulis quae renibus incumbent" in the *Opuscula Anatomica* published in Venice on 1563. Subsequently, Casserius (1561–1616) validated the discovery, depicting and naming them as "corpuscola reni incumbentia sive renes succenturiati" (Hiatt and Hiatt, 1997). In 1855 Thomas Addison first proved that the adrenals were vital organs when he described the symptoms and signs of patients with "anaemia, feebleness of the heart action, a peculiar change

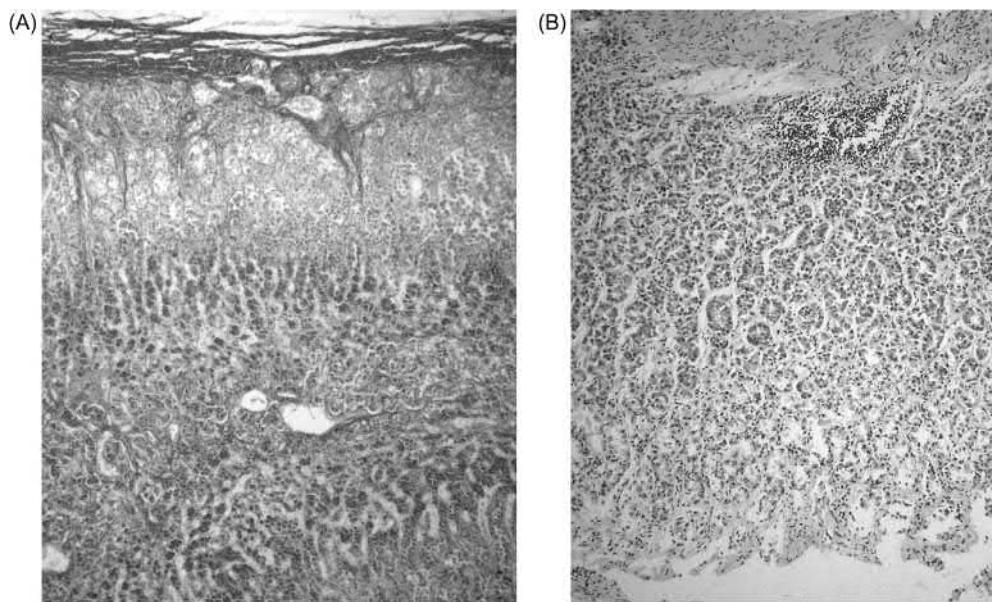
of colour in the skin occurring in connection with a diseased condition of the suprarenal capsules." He called this disorder "melasma suprarenale," postulating that it might be due to abnormal lesions in the adrenal glands. In this first description from the autopsies of 11 patients, he found 6 cases with tuberculosis, 3 with malignancies, 1 with adrenal hemorrhage, and 1 with adrenal fibrosis of an unknown origin. In this case, Addison (1855) reported, "The two adrenals together weighed 49 grains, they appeared exceedingly small and atrophied, so that the diseased condition did not result as usual from a deposit either of a strumous or malignant character, but appears to have been occasioned by an actual inflammation, that inflammation having destroyed the integrity of the organs, which finally led to their contraction and atrophy." Probably, this last case was the first description of an autoimmune adrenalitis. In 1856 the adrenal insufficiency was named "Addison's disease" by Troussseau, 1856. In the same year, Brown-Séquard (1856) demonstrated that dogs, cats, and guinea pigs subjected to bilateral adrenalectomy died, and adrenal glands were recognized as "organs essential for life." In 1896 William Osler administered the extracts of adrenal gland to treat a patient with Addison disease, but only from 1937 to 1955 corticosteroid hormones were isolated, their structures identified and synthesized (Medvei, 1993).

## ANATOMY AND PHYSIOLOGY OF THE ADRENALS

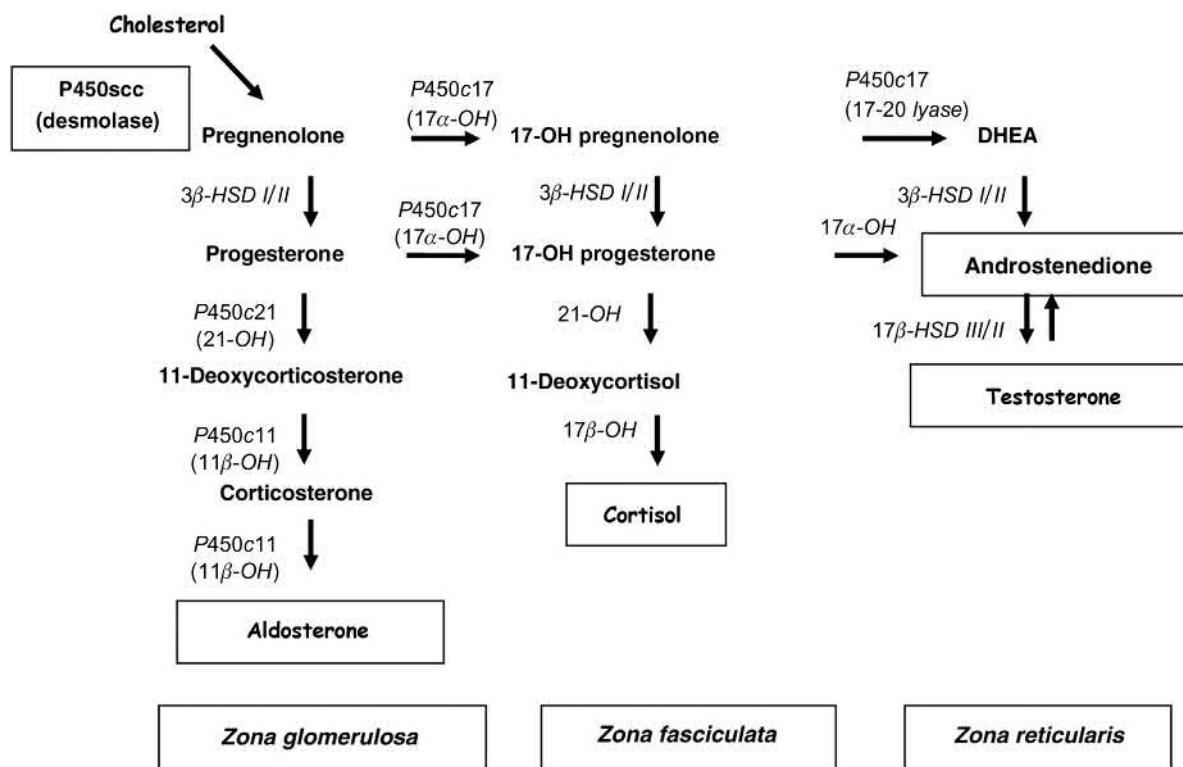
The adrenal glands develop from the mesenchyme the outer part (cortex) from the mesoderm and the central part (medulla) from the neuroectoderm, which comprises part of the chromaffin system (Kannan, 1988). The adult adrenal gland has a pyramidal configuration and is approximately 4 g in weight, 2 cm wide, 5 cm long, and 1 cm thick that lies immediately over the kidney on its posteromedial surface. The vasculature of the adrenal cortex is complex. Adrenal blood supply derives from 3 main suprarenal arteries that pierce the gland surface, dividing into 50–60 small branches and forming a subcapsular plexus which embraces the cell clusters in the zona glomerulosa and then run among the cellular cords in the zona fasciculata. The capillary branching from the arterial system forms a microvascular network around the zona reticularis which drains into the medullary sinusoids by relatively few small venules that eventually form a large central vein. The right adrenal vein is short and empties directly into the inferior vena cava, while the longer left adrenal vein usually empties into the left renal vein (Stewart and Newell-Price, 2016). The adrenal cortex is divided into three layers. The *zona glomerulosa* (5%–15% of the cortex) comprises discontinuous subcapsular aggregates of small cells, containing less cytoplasm than the other cortical cells. The middle zone or *zona fasciculata* (70%–75% of the cortex) is formed by radial cords of large cells arranged in columns with abundant lipid-filled cytoplasm. The inner cortical zone or *zona reticularis* is composed of cells arranged in cords with compact, finely granular, eosinophilic cytoplasm (Fig. 42.1A). The maintenance of normal adrenal size probably involves a progenitor cell population lying between the *zona glomerulosa* and the *zona fasciculata*. Cell migration and differentiation occur within the *fasciculata*, and senescence occurs within the *zona reticularis*, but the factors which modulate adrenal regeneration are unknown. ACTH administration results in *glomerulosa* cells acquiring a *fasciculata* phenotype and, in turn, the deepest *fasciculata* cells acquire a *reticularis* phenotype that is reversible on withdrawal of ACTH (Stewart and Newell-Price, 2016).

The adrenal cortex synthesizes three main groups of hormones (glucocorticoids, mineralocorticoids, and adrenal androgens) (Auchus and Miller, 2001). The homeostasis of glucocorticoids is regulated by a feedback mechanism through the hypothalamus by means of corticotropin-releasing hormone, the pituitary gland by ACTH, and the adrenal cortex by cortisol (Koch, 2004). The main steps in the synthesis of the adrenal cortex hormones and the enzymes involved are described in Fig. 42.2.

By immunocytochemical techniques, the normal adrenal cortex shows the presence of many proteins (Thiebaut et al., 1987; Henzen-Logmans et al., 1988; Sasano et al., 1989; Muscatelli et al., 1994; Fogt et al., 1998; Pelkey et al., 1998). Immunopositivity has been demonstrated for the class II major histocompatibility complexes (MHCs) (found in 10%–20% of the cortical cells) including the human antigen D-related leukocyte (HLA-DR) (McNicol, 1986; Jackson et al., 1988) and interleukin-6 (Gonzalez-Hernandez et al., 1994), which is involved in the communication process between the immune and endocrine system. Using specific antibodies against the steroidogenic enzymes and cytochrome P450 (Sasano et al., 1994), it was demonstrated that cytochrome P450 is involved in the adrenal steroid biosynthesis process, while the AdBP/SF-1 transcription factor regulates the expression of the CYP genes (Orth and Kavacs, 1998).



**FIGURE 42.1** (A) Histopathology of the normal adrenal cortex showing the typical three layers. (B) Adrenal cortex from a patient with autoimmune Addison's disease showing atrophy of the cortex and a diffuse lymphocytic infiltration and, at top, a lymphoid follicle.



**FIGURE 42.2** Main steps of the pathway of adrenal cortex hormone synthesis and the respective enzymes.

## EPIDEMIOLOGY OF ADDISON'S DISEASE AND AUTOIMMUNE ADRENALITIS

Addison's disease (AD) is a very rare disorder and formerly tuberculosis was the most frequent cause. Of the 11 cases originally described by Addison (1855), 6 (55%) had tuberculosis and only 1 (9%) had an idiopathic fibrotic form probably the first case of an autoimmune AD (AAD). For many years, tuberculosis was the most

frequent form of adrenal insufficiency. **Guttman (1930)** examined 566 autopsied patients with AD and found 70% with tuberculous adrenalitis. **Dunlop (1963)** reviewed 86 cases of AD and reported 79% with tuberculous adrenalitis. In the following years the frequency of tuberculosis AD progressively decreased. On the contrary, the prevalence of the autoimmune form in the past was reported to be 31% in London (**Mason et al., 1968**), 17% in Denmark (**Nerup, 1974**), and 21%–33% in Italy (**Betterle et al., 1989; De Rosa et al., 1987**) of all patients with AD.

During the last four decades, AAD progressively increased and has now become the most frequent cause of AD. From 1974 to 2002, 1557 patients with AD were evaluated in Europe, and the prevalence of AAD ranged from 44.5% to 94% (**Betterle et al., 2002**). On 633 patients with AD collected in Padua from 1960 to 2012, AAD was found to be 54% of the cases in the decade from 1960 to 1970, but its prevalence increased to 83.7% in the years from 2000 to 2012 (**Betterle et al., 2013**). In spite of the reduction of the AD due to tuberculous adrenalitis, the total frequency of AD was increasing. The prevalence of AD was estimated to be of 39 cases per million in London area in 1968 (**Mason et al., 1968**), 60 cases per million in Denmark in 1974 (**Nerup, 1974**), 93 cases per million in Coventry in 1997 (**Willis and Vince, 1997**), 110 cases per million in Nottingham in 1994 (**Kong and Jeffcoate, 1994**), 117 cases per million in Italy in 1999 (**Laureti et al., 1999**), and 144 cases per million in Norway in 2009 (**Erichsen et al., 2009a**). Moreover, a very high prevalence has been recently found in Iceland with 221 cases per million (**Olafsson and Sigurjonsdottir, 2016**). The incidence of AD in Europe has been estimated to be of 5–6 new cases per 1000,000 inhabitants per year (**Kong and Jeffcoate, 1994; Erichsen et al., 2009b**). However, based on the recent epidemiological data, the real incidence of AD in Europe could be more elevated.

The prevalence of AD varies also in relation to different geographic areas: in New Zealand (**Eason et al., 1982**) 4.5 cases per million, in Japan 5 cases per million (**Takayanagi et al., 2000**), in the United States 50 cases per million (**Jacobson et al., 1997**), and in Europe 110–221 cases per million, as mentioned above. Despite the constant decrease of tuberculous forms of AD, the overall prevalence of AD has progressively increased, and this may be due to an absolute increase of AAD.

## AUTOIMMUNE ADDISON'S DISEASE

AAD depends on a combination of genetic, environmental, and endogenous factors able to both induce a break of immune tolerance and initiate an autoimmune attack on the adrenal cortex, as for other autoimmune diseases (**Kamradt and Mitchinson, 2001; Bratland and Husebye, 2011**).

### Focal Lymphocytic Adrenalitis

**Duff and Bernstein (1933)** and **Kiaer and Rytter Norgaard (1969)** described a focal adrenalitis in patients without signs or symptoms of AD. **Petri and Nerup (1971)**, studying 2 groups of patients (413 and 161 miscellaneous cases), reported the presence of very small numbers of lymphocytes in 15% and 18.6% of the adrenal glands, respectively. Subsequently, in up to half of the autopsied patients without AD, a focal accumulation of lymphocytes and plasma cells was demonstrated in the adrenal cortex, associated with chronic inflammatory diseases in the retroperitoneum (**Fidler, 1977; Orth and Kavacs, 1998**). The percentage of these infiltrates was similar to that reported in focal thyroiditis. In 1989 Hayashi et al. evaluated 174 cases at autopsy and demonstrated that the mononuclear cell infiltration in the adrenal cortex increased with age, being present in about 7.4% of those aged over 49 years and 63% aged over 60 years. Immunohistochemical studies also revealed that the infiltrating mononuclear cells were mainly composed of CD3+ T cells. The major proportion of CD3+ T cells expresses the CD4 phenotype, whereas CD8+ T cells were fewer. A proportion of the CD4+ T cells was activated (**Hayashi et al., 1989**). These findings indicate that the focal lymphocytic infiltration of the adrenal glands is not rare and may represent a latent adrenalitis which, however, rarely reaches clinical expression since adrenal cortex antibodies and symptomatic AAD are extremely rare in the population.

A comprehensive investigation of the cellular and molecular components of the infiltrate has never been performed on the adrenal glands in patients with AAD so that the mechanisms by which the adrenal cortex is destroyed remain hypothetical (**Bratland and Husebye, 2011**).

## Diffuse Lymphocytic Adrenalitis

The pathologic findings initially described by Addison (1855) as "idiopathic" are constantly present in the adrenal glands of patients affected by AAD. On macroscopic examination, both the adrenals are small (weight 1–2 g), and sometimes their identification is difficult in the retroperitoneal tissue. The capsule is fibrous so that the adrenal glands are not detectable macroscopically and adrenal tissue is not visible in multiple sections (Drury et al., 1979).

On microscopic examination, there is complete destruction of the three-layer architecture. The adrenal cortical cells are single, enlarged, or pleomorphic, with increased eosinophilia, depleted in lipids, or present as part of a cluster. Residual cortical nodules are seen as the disease progresses. The tissue is diffusely infiltrated by small lymphocytes, plasma cells, and macrophages (McNicol and Laidler, 1996). The histopathologic finding of infiltrating lymphocytes sometimes is associated with follicle formation and fibrosis (Fig. 42.1B). The medulla in AAD remains normal. This pattern is present in patients with AAD, either isolated or associated in the context of autoimmune polyendocrine syndromes (McIntyre Gass, 1962; Irvine and Barnes 1975; Betterle et al., 2002).

## Induced Immunity in Animal Models

Colover and Glynn (1958) reported isoimmunization in guinea pigs by injection of adrenal antigens and complete Freund's adjuvant, with distinctive and "specific" lesions of the adrenals. Subsequently, some authors (Steiner et al., 1960; Barnett et al., 1963) reported examples of experimental autoimmune adrenalitis (EAA) in different species (rabbits, guinea pigs, rats, and monkeys), including an antibody reaction in the guinea pigs and a lymphocytic infiltration in the rabbit adrenal tissue (Witebsky and Milgrom, 1962; Barnett et al., 1963). The adrenal lesions in rabbits were characterized by foci of lymphocytes and histiocytes, with a small number of plasma cells and eosinophils, and degenerative changes in adrenal cells. Andrade et al. (1968) and Werdelin and Witebsky (1970) demonstrated that EAA in a Lewis rat model was induced at day 7 after immunization. The histology was studied by autoradiographic tracing of H<sub>3</sub> thymidine and H<sub>3</sub> adenosine-labeled cells and demonstrated that adrenalitis was initiated with the appearance of a few specifically reactive lymphocytes, followed by an infiltration of mononuclear cells, mainly lymphocytes and plasma cells, throughout the adrenal cortex. Eosinophilia, cytoplasmic vacuolization, and a loss of nuclear definition were evident in cortical cells.

Using electron microscopy, Hoenig et al. (1970) studied the inflammatory lesions in paraffin-embedded tissues; 5 days after immunization, lymphocytes were present in sinusoids and adrenal parenchyma where the damage was in the vicinity of lymphocytes, with enlargement of intercellular spaces and ischemic areas with inflammatory cells and fibrin.

Fujii et al. (1992) demonstrated that in mice repeated immunizations caused a delayed type of hypersensitivity to adrenal antigens and that transfer of adrenalitis from an affected to a healthy animal was not possible by serum but only by using spleen cells, confirming the previous reports (Levine and Wenk, 1968; Werdelin et al., 1971) in which the disease was passively transferred by immunocytes derived from lymph nodes.

## Spontaneous Animal Models

Spontaneous AD occurs in dogs (Harlton, 1976; Kaufman, 1984; Little et al., 1989; Kintzer and Peterson, 1994; Sadek and Schaer, 1996; Dunn and Herrtage, 1998) and cats (Kaufman, 1984; Peterson et al., 1989; Tasker et al., 1999; Stonehewer and Tasker, 2001) with hypoadrenocorticism due to immune-mediated destruction of the adrenal glands. While biochemical laboratory data were reported, none of the authors described positivity for the adrenal cortex autoantibodies (ACAs), but only the presence of lymphocytic infiltration in the adrenal glands, at autopsy. Beales et al. (2002) found the adrenal glands of a nonobese diabetic (NOD) mouse to contain a mononuclear cell infiltration in the adrenal cortex but without signs of hypoadrenalinism. Thus the NOD mouse was proposed as a spontaneous model suitable for investigating mechanisms involved in diffuse lymphocytic infiltration of the adrenal glands.

## Family History of Autoimmune Addison's Disease and Genetic Predisposition

In Norwegian patients with AAD, 10% of patients had another family member with AAD (Erichsen et al., 2009a). In our studies a family history for AAD was found in 2% of the patients with AAD in the context of autoimmune polyglandular syndrome (APS) type 2, Type 4 or isolated AAD, whereas this association was present in

25% of the patients with AAD in the context of APS type 1 (Betterle et al., 2013). In a Swedish study a family history for AAD was found in 6.4% of the patients with AAD without APS-1 (Dalin et al., 2017). The majority of identical twins with an autoimmune disease have unaffected twins, that is, they are discordant for the disease (Salvetti et al., 2000). However, there are only limited case reports with concordance or discordance of the disease in twins with AAD (Simmonds and Lister, 1978; Emy, 1998). As regards the genetic predisposition of AAD, the majority of the studies were performed in patients with isolated AAD or in the context of APS type 2. In these patients the association was primarily related to class II HLA alleles in the MHC (Myhre et al., 2002). Reports from several different populations have demonstrated that the risk of AAD is significantly increased in the presence of *HLA-DRB1\*03-DQA1\*0501-DQB1\*0201* (*DR3/DQ2*) and *DRB1\*0404-DQA1\*0301-DQB1\*0302* (*DR4/4/DQ8*). *DRB1\*0404* is much more frequent and indicates that peptides from 21-hydroxylase (21-OH) are well presented to autoreactive T lymphocytes in the presence of *DR4/4*. In addition to the abovementioned alleles, it has been demonstrated that *HLA-B8* was significantly increased independently of *DR3* (Bratland and Husebye, 2011). Furthermore, an association between AAD and the 5.1 allele of MHC class I chain-related A (MIC-A) has been demonstrated. Homozygosity for the 5.1 allele of *MIC-A* in the presence of high-risk HLA genotype (*DR3-DQ2/DR4/4-DQ8*) may define subjects at very high risk of AAD. *MIC-A* is not involved in the antigen presentation but as a mediator of activating natural killer cells and T cytotoxic lymphocytes (Bratland and Husebye, 2011). A number of other genes outside the MHC have also been reported such as the cytotoxic T lymphocyte (CTL)-associated protein-4, a key negative regulator in adaptive immunity, the MHC class II transactivator (*MHC2TA*) which regulates the expression of class II molecules, the tyrosine-protein phosphatase nonreceptor type 22 (*PTPN22*) involved in the regulation of T-cell receptor signaling, and the programmed death ligand 1 (PD-L1) which is an inhibitory molecule expressed on activated T cells. In addition to the abovementioned genes, AAD is associated with genes involved in the metabolism of vitamin D with the gene encoding NACHT leucine-rich-repeat protein 1 (*NLRP1*) (Bratland and Husebye, 2011). Other involved genes are *STAT4*, *FCRL3*, *GPR174*, *GATA3*, and *CYP27B1* (Mitchell et al., 2014; Falorni et al., 2016). A study of multiplex AAD in families comprising two or more individuals with the disease from the United Kingdom and Norway reported that the non-HLA locus *NFATC1* (nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 1) was a possible susceptibility gene for AAD. *NFATC1* is a candidate gene as it is expressed in adrenal cortex and encodes a transcriptional factor which plays a central role in gene transcription during the immune response (Mitchell et al., 2015).

Finally, it is worthwhile remembering that in patients with APS-1, where AAD is one of the major components, there are mutations of the autoimmune regulator (*AIRE*) gene (Cervato et al., 2009; Bratland and Husebye, 2011). So far, about 100 *AIRE* mutations have been identified (Bruserud et al., 2016). This gene is involved in the presentation of autoantigen to autoreactive T lymphocytes in order to induce tolerance, and the presence of the *AIRE* gene with mutated proteins may inhibit the apoptosis of autoreactive T lymphocytes at the thymic level. These cells can migrate at the peripheral level where they can initiate an autoimmune aggression in a very young age in these patients. In Scandinavian patients with type 1 APS, AAD seems to be associated with *HLA-DRB1\*03*, alopecia with *HLA-DRB1\*04-DQB1\*0302*, whereas *HLA-DRB1\*15-DQB1\*0602* alleles are protective for type 1 diabetes (Halonen et al., 2002). However, in APS-1 the association with HLA alleles is limited or lacking (Cervato et al., 2009; Bratland and Husebye, 2011).

## Cellular Immunity

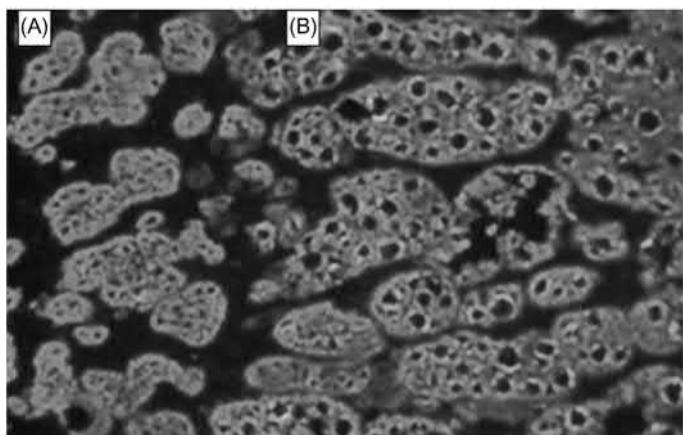
Although autoantibodies are strong markers of autoimmune adrenalitis and may identify individuals at high disease risk or have a cytotoxic effect *in vitro*, they have not been demonstrated to display pathogenic effects *in vivo* (Boscaro et al., 1996). The early studies on AD, using the assay for migration inhibition factor, reported cell-mediated immunity in affected patients, with claims for organ-specific hypersensitivity (Nerup and Bendixen, 1969). Subsequent studies, by means of an intracutaneous test with adrenal extracts, showed a delayed-type hypersensitivity reaction but no collateral evidence based on blast transformation experiments (Nerup et al., 1970). Other studies reported a decrease in suppressor T-cell function (Vergheze et al., 1980) and an increase in circulating Ia-positive T lymphocytes (Rabinowe et al., 1984), which indicated the involvement of cellular immunity. Initially, studies of cellular immunity in AAD were carried when a proliferative T-cell response to an adrenal-specific protein fraction of 18–24 kDa molecular weight was described out (Freeman and Weetman, 1992). Additionally, and an impaired suppressive function of CD4+CD25+ regulatory T cells were described in APS 2 patients (Kriegel et al., 2004). Subsequently, in order to identify the autoepitopes recognized by T lymphocytes, BALB/c and SJL

inbred mouse strains were immunized with recombinant 21-OH. T lymphocytes of the immunized animals were stimulated by the peptide 342–361 of 21-OH. This region may be involved in the pathogenesis of AAD (Husebye et al., 2006). It was also demonstrated that patients with AAD have circulating 21-OH-specific T cells recognizing the amino acidic fragment 342–361 of 21-OH, who constitute a disease-specific epitope presented by *HLA-DRB1\*0404*. Furthermore, cellular proliferation and secretion of interferon- $\gamma$  in response to 21-OH were significantly higher in patients with AAD respect to controls (Bratland et al., 2009). It was recently demonstrated that 21-OH-specific T cells are frequently detectable in patients with AAD. Immunodominant CD8+ and CD4+ T-cell responses were detectable in a large proportion of AAD, both *in vivo* and *in vitro*. *HLA* class I-guided isolation of 21-OH-specific CD8+ T cells showed the ability to lyse 21-OH-positive target cells, consistent with a potential mechanism for disease pathogenesis (Dawoodji et al., 2014). These data indicate that strong CTL responses to 21-OH often occur *in vivo* and that reactive CTLs have substantial proliferative and cytolytic potential.

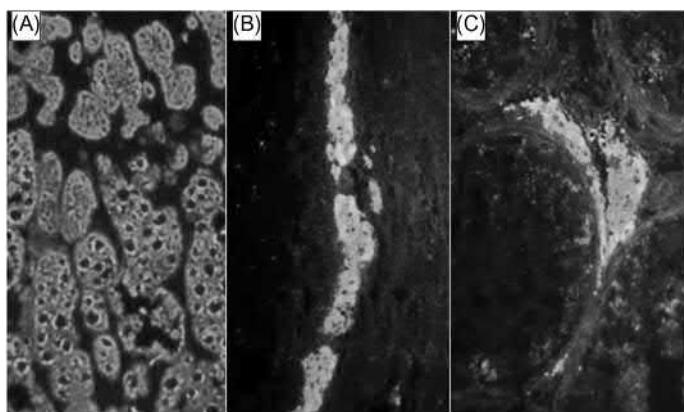
## Humoral Immunity

Anderson et al. (1957), using a complement fixation test with a homogenate of adrenal cortex tissue, first demonstrated that two out of eight (i.e., 25%) patients with “idiopathic” AD had complement-fixing antibodies.

Subsequently, Blizzard and Kyle (1963), using the indirect immunofluorescence (IIF) on animal or human cryostatic sections of adrenal gland, demonstrated that ACAs are organ-specific, but not species-specific, antibodies reacting with all the three layers of the adrenal cortex and producing a homogeneous cytoplasmic staining pattern (Fig. 42.3). Sometimes, there is a reactivity against one or two of the three layers of the cortex (Irvine and Barnes, 1975; Sotsiou et al., 1980). The autoantibodies also reacted with the surface of living cortical cells in culture (Khoury et al., 1981), indicating that microsomal antigens are also expressed on the surface of adrenal cortical cells. Between 1963 and 2002, using IIF, ACAs were found to be positive in 61% of 1637 patients with AAD and in 6.7% of 267 with tuberculous AD, as reviewed by Betterle (2002). The prevalence of ACA has varied considerably between different laboratories because of differences in the substrates used (animal or human), time of incubation, geographic or racial origins of individuals, gender, age of onset, duration of the disease, or whether other associated diseases were present (Rees Smith and Furmaniak, 1995; Betterle et al., 2002; Nigam et al., 2003). Two studies (Kendall-Taylor et al., 1988; Wulfraat et al., 1989) reported the presence of autoantibodies blocking the ACTH receptor in 90% of the patients affected by AAD, but these data could not be confirmed (Wardle et al., 1993). Finally, autoantibodies to hydrocortisone were detected in patients with an Addison-like syndrome and AIDS (Salim et al., 1988). In 1968 the steroid-producing cell antibodies (StCA) were first described (Anderson et al., 1968) in two males affected by AAD without gonadal failure (Fig. 42.4). Subsequently, StCA were detected in 60%–80% of the females affected by type 1 APS, in 25%–40% of those with type 2 APS, and in 18% of those with isolated AAD (Betterle et al., 2002, 2005). The presence of StCA correlates with premature ovarian failure (POF) characterized by lymphocytic oophoritis (Betterle et al., 1993; Hoek et al., 1997; Betterle and Volpato, 1998). A study in Norway demonstrated that 6.7% of females with AAD had POF (Erichsen et al., 2009a). An Italian study performed on 258 females with AAD revealed a POF in 20% of the patients. Particularly, POF was found in 40.8% of patients with APS-1, in 33.3% with APS-4, and in 16% with APS-2. StCA were detected in 72% of AAD with POF of various duration, but with a higher prevalence (92%) in patients with POF duration of less



**FIGURE 42.3** Immunofluorescence pattern on normal human adrenal cortex given by serum of a patient with autoimmune Addison's disease. This serum diffusely reacted with the cytoplasm of the cells in all the three layers of the cortex, but shown here are only *glomerulosa* (A) and *fasciculata* (B) zones.



**FIGURE 42.4** Immunofluorescence pattern given by serum of a patient with autoimmune Addison's disease (AAD) and premature ovarian failure. This serum reacted against adrenal cortex (A), follicular theca of the ovary (B), and Leydig cells of the testis (C) and reflects autoantibodies to steroid-producing cells (StCA).

than 5 years. StCa were also found in 25.7% of patients without POF. However, a follow-up study on young females with AAD without POF but positive for StCA revealed that 38% developed POF at a mean age of 23 years, thus demonstrating that steroidogenic autoantibodies are predictive markers of future clinical POF (Reato et al., 2011). We also searched for StCA in 154 Italian males with AAD (mean age 34 years) and they were found in 18.8% of the patients (60.7% with APS-1 and 10% with other APS types or isolated AAD). Differently from females, all males with StCA had a normal gonadal function, and during a mean follow-up of 7 years, none of the positive patients developed hypergonadotropic hypogonadism (Dalla Costa et al., 2014).

### Identification of Autoantigens of Adrenal Cortex Autoantibodies

Furmaniak et al. (1988) described a specific 55 kDa protein in human adrenal microsomes which was reactive with ACA. In 1992 two independent laboratories (Baumann-Antczak et al., 1992; Bednarek et al., 1992; Winqvist et al., 1992) demonstrated by means of purification of native 21-OH that this adrenal enzyme is a major antigen of adrenal cortical cells. Subsequently, this was confirmed in experiments with specific absorption with purified human 21-OH, using sera from six patients with different forms of AAD (Morgan et al., 2000). 21-OH is an adrenal-specific enzyme of the cytochrome P450 family and plays a key role in the synthesis of the cortical hormones (Furmaniak et al., 1999; Furmaniak and Rees Smith, 2002). 21-OH is encoded by the CYP21B gene, whereas the CYP21A gene is inactive (Wilson et al., 1995). 21-OH catalyzes the conversion of progesterone and 17-hydroxyprogesterone into 11-deoxycorticosterone and 11-deoxycortisol (see Fig. 42.2). It is a 55 kDa microsomal protein containing a heme group, located in the active site of the C-terminal end of the molecule, which is important for antibody binding (Wedlock et al., 1993; Asawa et al., 1994) and in oxidation-reduction reactions (Picado-Leonard and Miller, 1987; Lin et al., 1994). Analysis of antibody-binding sites on 21-OH indicated that the epitopes on 21-OH were conformational (Wedlock et al., 1993) and confirmed the participation of both the central and C-terminal parts of the molecule. These studies identified the presence of three different, short 5-, 6-, and 15-amino acid sequences in the C-terminal part of the 21-OH involved in the binding of antibodies to 21-OH (Chen et al., 1998). These observations are important in relation to effects of 21-OH antibodies on 21-OH enzyme activity. In fact, in studies *in vitro*, using sera positive for 21-OH Abs from patients with AAD, a dose-dependent blocking activity was identified (Furmaniak et al., 1994), although this is not usually evident *in vivo* (Boscaro et al., 1996). Furthermore, maternal 21-OH Abs of IgG class crossing the placenta during pregnancy do not induce hypoadrenalinism in newborns (Betterle 2014a). On the basis of these data we cannot consider that these autoantibodies had a pathogenetic role *in vivo*.

### Identification of Autoantigens of Steroid-Producing Cells Autoantibodies

With regard to StCa autoantigens, in addition to 21-OH, Krohn et al. (1992) reported that screening of a human fetal adrenal cDNA expression library with sera from patients with APS type 1 identified a protein with high homology to 17 $\alpha$ -hydroxylase (17 $\alpha$ -OH) reacting also with a fragment of recombinant 17 $\alpha$ -OH expressed in bacteria. 17 $\alpha$ -OH, coded by a single gene on the human chromosome 10, showed 30% homology with 21-OH antigen. Winqvist et al. (1992), using immunoblotting and immunoprecipitation studies, also observed a reactivity of sera from APS-1 patients with a cytochrome P450 side-chain cleavage enzyme (P450scc), a heme-binding protein coded by a single gene on human chromosome 15 that showed 20%

homology with 21-OH sequence (Chung et al., 1986). The binding sites of these two antigens and their enzyme activity have not been studied, even though Peterson and Krohn (1994) reported the presence of four distinct reactive regions in the 17 $\alpha$ -OH molecule and an inhibiting effect *in vivo* of the P450sccAbs in the APS-1 patients' serum (Winqvist et al., 1993).

### Techniques for Identification of Autoantibodies to 21-Hydroxylase

Following the discovery that 21-OH is the major adrenal cortex autoantigen, a specific and sensitive technique was described, by labeling the protein with  $^{35}\text{S}$ -methionine in an *in vitro* transcription translation system, and using a radioimmunoprecipitation assay (RIA), for the detection of antibodies (Colls et al., 1995; Falorni et al., 1995; Chen et al., 1996). Thereafter, because of certain limitations of this technique, a more convenient assay to measure 21-OH Abs was developed based on the use of  $^{125}\text{I}$ -labeled recombinant human 21-OH and the precipitation of the immunocomplexes using solid-phase protein A (RIA) (Tanaka et al., 1997). Using these techniques, from 1995 to 2002, a group of 572 patients with AAD and 76 with tuberculosis was studied; 78% and 1.9%, respectively, were 21-OH Abs positive, reviewed by Betterle (2004).

In order to compare techniques for ACA and 21-OH Abs determinations, we studied 165 patients with AD, with different duration of disease, and found that 81% of those with AAD were positive by both techniques, whereas none with non-AAD was positive (Betterle et al., 1999). The prevalence varied in relation to both the clinical presentation (type 1 or 2 APS or isolated AAD) and the length of the disease, being higher in patients with recent (100%) onset than in those with long-standing disease (79%), and in those with type 2 APS than in those with isolated AAD (Betterle et al., 2002). In these studies, results using IIF for ACA and RIA for 21-OH Abs were in good agreement. Some discrepancies were reported by Falorni et al. (1997) and Betterle et al. (1999) on the relationships between ACA measured using IIF and 21-OH Abs measured by RIA. For example, a few patient samples were positive for 21-OH Abs while negative for ACA, and this may reflect greater sensitivity of 21-OH Abs IPA. In contrast, a small number of sera were positive for ACA while negative for 21-OH Abs by RIA. This discrepancy may be related to serum reactivity with adrenal cortex antigens in the IIF that are distinct from 21-OH.

An international standardization program for 21-OH Abs detection was first carried out in 2010 to compare the measurements from four laboratories with different methods and perform an interlaboratory concordance study. This investigation showed a very good agreement among the laboratories on reporting 21-OH Abs-positive/negative samples. The sensitivity for AAD was greater than 80% in all participating laboratories. However, the study highlighted the need to produce the international standard preparation to enable the harmonized expression of the results (Falorni et al., 2011). Another standardization study for 21-OH Abs measurement was organized by the EurAdrenal Consortium in 2014, and 13 different laboratories across Europe and one from the United States participated in this program. The results demonstrated good diagnostic sensitivity, specificity, and accuracy for "in-house" and commercial assays (Falorni et al., 2015). In the future the standardization program is needed to identify common standard sera and common measuring units.

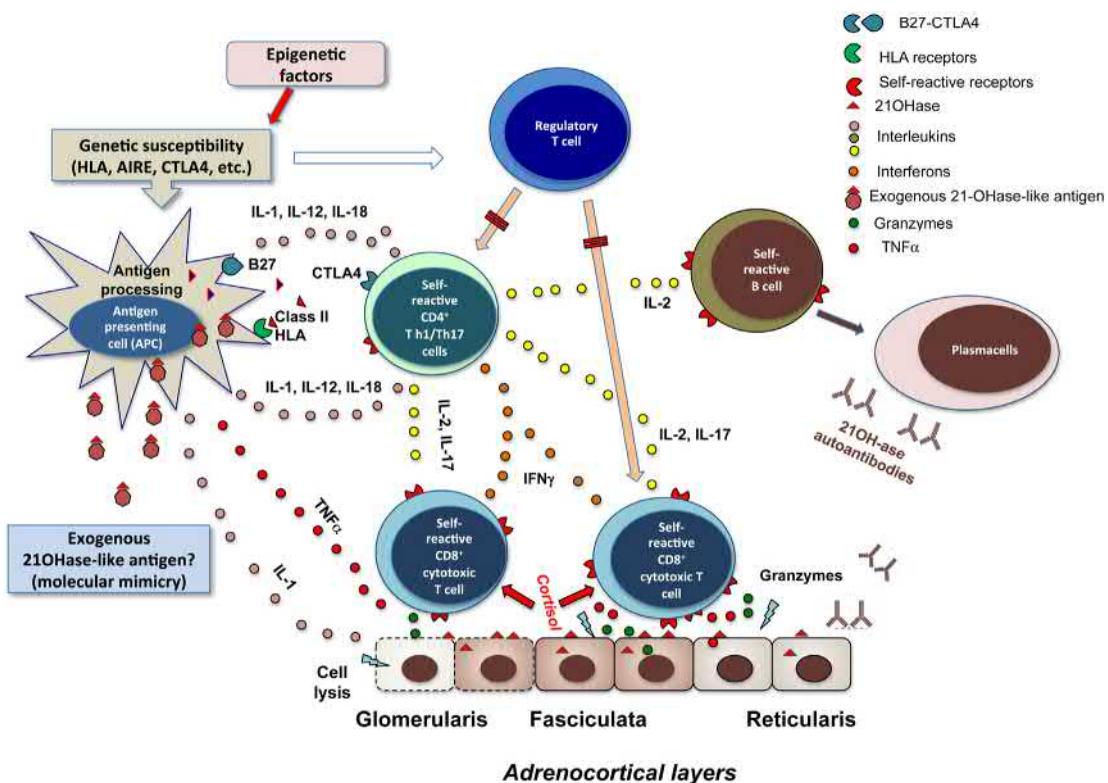
### Techniques for Identification of Autoantibodies to Other Steroidogenic Enzymes

From 1996 the 17 $\alpha$ -OH Abs and P450sccAbs are detectable by a RIA kit, using recombinant human antigens (Chen et al., 1996), and the results are correlated with StCA detected by IIF (Betterle et al., 1999, 2002; Reato et al., 2011; Dalla Costa et al., 2014). A hypothetical mechanism of autoimmune aggression against the adrenal cortex is shown in the Fig. 42.5.

A hypothetical mechanism of autoimmune aggression against the adrenal cortex is shown in the Figure 42.5.

### Other Autoantibodies Detected in Patients With Autoimmune Addison's Disease

In addition to the main autoantibodies previously mentioned (ACA, 21-OH Abs, StCA, 17 $\alpha$ -OH Abs, and P450sccAbs), other autoantibodies have also been discovered that are characteristic of patients with APS-1, where the AAD represents one of the principal diseases (Husebye et al., 2009). Winqvist et al. (1996) identified an autoantibody of 51 kDa, recognizing the aromatic l-amino acid decarboxylase (AADCAbs) involved in the generation of serotonin and dopamine. AADCAbs was reported in the sera of patients in association with chronic hepatitis, vitiligo, and type 1 diabetes mellitus (Husebye et al., 1997), and also with autoimmune gastrointestinal



**FIGURE 42.5** Hypothetical pathogenesis of autoimmune adrenalitis. A currently unknown exogenous antigen (viruses, bacteria, chemicals) may activate antigen presenting cells (APCs). After antigen uptake, APCs process and present antigens to CD4 + T-helper 1 and T-helper 17 (Th1/Th17). In turn, T-helper cells promote activation and clonal expansion of cytotoxic T lymphocytes to exogenous antigen but also of auto-reactive cytotoxic T lymphocytes (CD8 +) and autoreactive B cells which release self-destructive cytokines and steroid 21-hydroxylase autoantibodies (21-OHAbs), respectively. This self-reactive process might be allowed by deficiency of T-regulatory (T-reg) cells. The progressive destruction of glomerular, fascicular, and reticular cells of adrenal cortex is mediated by cytotoxic T cells through local production of cytokines. 21-OHAbs may also activate the complement system and antibody-dependent cellular cytotoxicity. Yet, these mechanisms of damage have been demonstrated in vitro but not in vivo. Local release of cortisol by zona fasciculata may hamper or at least delay this process. Source: Modified with permission from Malattie autoimmuni del surrene. In C. Betterle Le Malattie Autoimmuni, Second Edition, 2017. Piccin Editore, Padova, Italy.

dysfunction (Kluger et al., 2015). Autoantibodies against a 230 kDa enzyme, identified as tryptophan hydroxylase (TPHAbs) (Ekwall et al., 1998; Dal Pra et al., 2004), and against histidine decarboxylase (HDAbs) (Skoldberg et al., 2003) were demonstrated in patients who had a gastrointestinal dysfunction (Ekwall et al., 1998; Scarpa et al., 2013; Kluger et al., 2015). Autoantibodies to cytochromes P450, CYPIA2, and CYP2A6 were associated to patients with autoimmune hepatitis (Clemente et al., 1997, 1998). Autoantibodies to SOX9 (SOX9Abs) and SOX10 (SOX10Abs) were reported in patients with vitiligo (Hedstrand et al., 2001), while autoantibodies to tyrosine hydroxylase (THAbs) in those with alopecia areata (Hedstrand et al., 2000). Autoantibodies to NALP5 (NALP5Abs) were found in patients with type 1 APS and chronic hypoparathyroidism (Alimohammadi et al., 2008), while autoantibodies to a potassium channel regulator (KCNRGAbs) in those with autoimmune bronchiolitis (Alimohammadi et al., 2009). In addition, autoantibodies to various interferon types (IFNAb) were demonstrated to be markers of APS-1 independently of the major or minor clinical manifestations (Larosa et al., 2017). Analysis of these autoantibodies in APS-1 patients is a useful tool for establishing autoimmune manifestations of the disease, as well as providing diagnosis in patients with suspected disease and predicting future clinical manifestations (Söderbergh et al., 2004).

## NATURAL HISTORY OF AUTOIMMUNE ADDISON'S DISEASE

ACA can be detected also in patients without AAD and specifically in 0.2% of normal controls, in 4% of first-degree relatives of AAD patients, in 4% of hospitalized patients, and in 1.3% of patients with organ-specific autoimmune disease, with a much higher prevalence in POF (2.5%–20%), in chronic hypoparathyroidism and/or

chronic candidiasis (Nerup, 1974; De Bellis et al., 1993; Betterle et al., 1997a, 1997b). AAD is a chronic disease with a long silent period marked by the presence of the ACA. The natural history of the disease could entail five main functional phases: one potential, three subclinical, and one clinical (Betterle et al., 1988, 2002, 2016; Coco et al., 2006). To recognize these different phases, it is necessary to measure basal plasma levels of cortisol, ACTH, plasma renin activity or concentration, aldosterone, cortisol 30 and 60 minutes after an intravenous injection of 250 µg of cosyntropin ( $\alpha$ 1-24-corticotropin) (Synacthen) (ACTH test) (Stewart et al., 1988; Grinspoon and Biller, 1994). Stage 0 is characterized by the presence of adrenal-specific autoantibodies and a normal adrenal cortical mass, in the absence of any detectable dysfunction of the adrenal glands by ACTH test, representing the “potential” phase of chronic adrenalitis. Subclinical Stage 1 is characterized by an increase in plasma renin activity, together with normal/decreased levels of aldosterone (reduced production of mineralocorticoids); subclinical Stage 2 by normal ACTH values with normal basal cortisol but a low cortisol peak (low reserve of glucocorticoids); and subclinical Stage 3 by an increase in ACTH and low level of basal cortisol, indicating reduced glucocorticoid production. Stage 4, the clinical stage, is when the ACTH is significantly increased and the basal cortisol is very low (Table 42.1).

Stage 1 indicates that the *zona glomerulosa* of the adrenal glands is first affected by the autoimmune attack probably because it is smaller and thus exhibits greater sensitivity to diffuse lymphocyte infiltration, or because the deeper zones are immunologically protected by local production of glucocorticoids (Betterle et al., 2002) or for their greater ability to self-regenerate. The adrenal cortex is indeed one of the most plastic tissues in the human body. For more than 60 years, it has been known that bilateral subcapsular enucleation of the adrenals in rodents leads to the regeneration of adrenocortical cell mass within 4–6 weeks (Gan et al., 2017). For the study of patients with ACA and/or 21-OHAbs, it was proposed to use also the ACTH test with a low dose (1 µg) of ACTH (Laureti et al., 2000). It has been found that this low-dose test has a diagnostic accuracy as high as that of the classical high-dose intravenous 250 µg test, but it remains unclear whether “low-dose” ACTH (Synacthen) tests have better sensitivity for detecting adrenal failure compared to standard test.

Various previous follow-up studies carried out in ACA/21-OHAbs-positive patients without clinical AD have allowed to understand the importance of these autoantibodies as markers for defining the natural history of AAD (Betterle et al., 1997a,b, 1983; Ahonenm et al., 1987; Peterson et al., 1997; Laureti et al., 1998; Yu et al., 1999; Coco et al., 2006), but the positive predictive value greatly varied (range 0–90). In one of these follow-up studies (Coco et al., 2006), 100 patients were investigated for a mean period of 6 years and for a maximum period of 21 years. A multivariate analysis demonstrated that the occurrence of AAD was significantly correlated to four main conditions: gender, ACAs titers, adrenal cortex functional status at enrollment, and type of autoimmune preexisting disease. The study allowed to identify the *point of no return* toward AAD on Stage 1 of subclinical hypoadrenalinism and that the development of AAD can occur after a maximum period of 11 years from the detection of autoantibodies (Coco et al., 2006; Betterle et al., 2016). In a recent paper 143 patients with autoimmune diseases (29 patients with APS-1 and 114 patients with APS-2 or APS-4) being positive for adrenal cortex autoantibodies, were followed-up for a median of 10 years (range 6 months–33 years) and assessed by ACTH test. The risk of AAD was estimated according to age, gender, stage of adrenal dysfunction, associated diseases and antibody titer. Univariate and multivariate Cox proportional hazard models were used for statistical analysis. We found that the cumulative risk (CR) of developing AAD was higher in APS-1 patients (94.2%) compared to

**TABLE 42.1** ACTH Test: Stages of Adrenal Cortical Function in ACA/21-OHAbs-Positive Patients

Adrenocortical function	Stage	Autoimmune Addison's	ACA and/or 21-OHAb	Basal PRA	Basal aldosterone	Basal ACTH	Basal cortisol	Cortisol response 60 min after ACTH i.v.	Clinical manifestations
Normal	0	Potential	+	Normal	Normal	Normal	Normal	Normal	Absent
Reduced production of mineralocorticoids	1	Subclinical	+	High	Normal or low	Normal	Normal		Absent
Reduced reserve of glucocorticoids	2	Subclinical	+	High	Low	Normal	Normal	Reduced	Absent
Reduced production of glucocorticoids	3	Subclinical	+	High	Low	High	Low	Absent	Absent
Important mineralocorticoids and glucocorticoids deficiency	4	Clinical	+	Very high	Very low	Very high	Very low	Absent	Absent

patients with APS-2/APS-4 (38.7%). The CR was high in both males and females with APS-1 patients, while in patients with APS-2/APS-4 it was high only in males. *Stage 1* (increased plasma renin) for patients with APS-1 and *Stage 2* (no response of cortisol to ACTH-test) for patients with APS-2/APS-4 were established as the *points of no return* in the progression to AAD. Adjusted hazard ratio analyses by multivariate Cox model for AAD showed that gender, diseases, adrenal function were independent risk factors for developing clinical AAD. The risk of developing clinical AAD appears to subside after 19 years of follow up. On the basis of these data a model for estimating the probability to survive free of AAD has been developed and should be a useful tool in designing appropriate follow-up intervals and future therapeutic strategies ([Naletto et al. 2019](#)).

## DIAGNOSIS OF AUTOIMMUNE ADDISON'S DISEASE

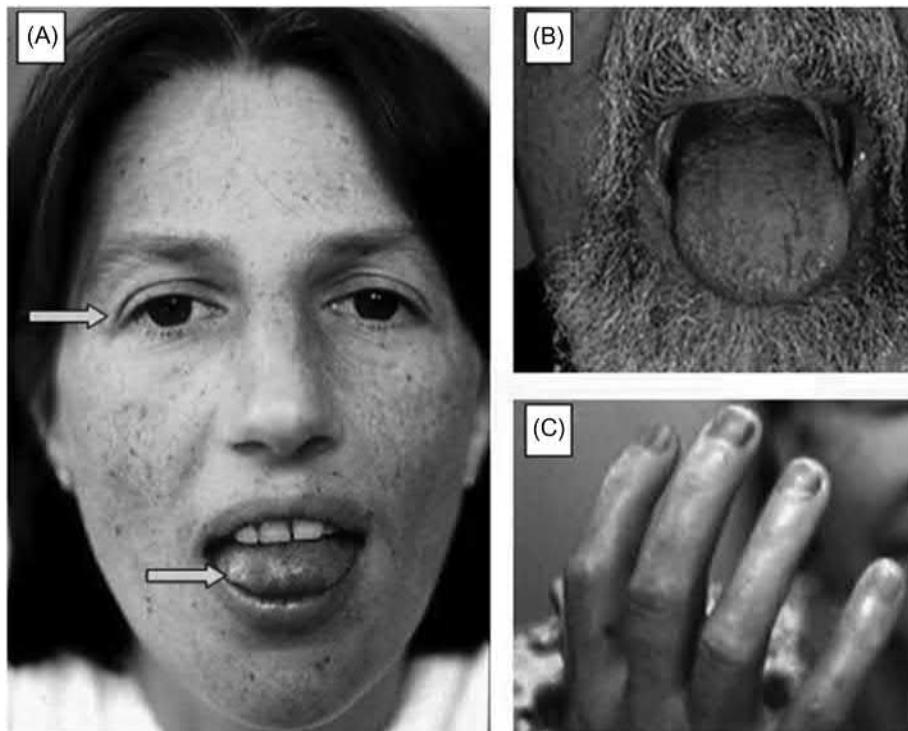
### Clinical Manifestations

AAD has a long preclinical period and clinical features do not appear until 80%–90% or more of the adrenal cortex is destroyed. The main clinical signs at onset are general malaise, fatigue, weakness (99%), anorexia, nausea and vomiting (90%), weight loss (97%), cutaneous and mucosal hyperpigmentation ([Fig. 42.6](#)) caused by the enhanced stimulation of the skin MC1-receptor by ACTH and other proopiomelanocortin-related peptides (98%), and severe hypotension (87%). Other signs (abdominal pain, salt craving, diarrhea, constipation, and syncope) have a variable frequency (34%–39%) ([Williams and Dluhy, 1998](#)). In women a loss of axillary and pubic hair, dry skin, reduced libido and an impairment of well-being also occur.

### General Biochemical Indices

In AAD at diagnosis, the serum levels of sodium, chloride, and bicarbonate are reduced, while those of potassium are elevated. The hyponatremia (in 100% of patients) is due to loss of sodium in urine and increase in both plasma vasopressin and angiotensin II which impair free water clearance; hyperkalemia (in 50%–70%) is due to aldosterone deficiency, impaired glomerular filtration, and metabolic acidosis.

A percentage of 10–20 of patients have a mild or moderate hypercalcemia for unknown reasons ([Williams and Dluhy, 1998](#)). Anemia is present in 40%–50%, and eosinophilia and lymphocytosis in 10%–15% of the cases.



**FIGURE 42.6** Clinical manifestations of autoimmune Addison's disease at onset. (A) Hyperpigmentation of the skin, melanosis of the tongue, and exophthalmos as a sign of dehydration. (B) Male showing a melanosis of the tongue. (C) Hyperpigmentation of the nails.

## Hormonal Tests

The association between a morning (0800 hours) immunometric determination of both plasma ACTH and basal cortisol levels differentiates cases of primary adrenal failure from both a healthy status and other type of adrenal disease (Oelkers et al., 1992).

A recent Endocrine Society Clinical Practice Guideline on diagnosis and treatment of primary adrenal insufficiency suggests that a decrease (below 140 nmol/L or 5 µg/dL) in basal morning cortisol with an increase in levels of ACTH (>twofold the upper limit of the reference range 22.0 pmol/L) indicate primary adrenal failure (Bornstein et al., 2016). The simultaneous measurement of plasma renin and aldosterone is recommended to determine the presence of mineralocorticoid deficiency, an elevated plasma renin activity or concentration in combination with a normal or low serum aldosterone concentration is suggestive of AD (Bornstein et al., 2016). In relation to differential diagnosis, in the case of secondary adrenal failure, levels of both ACTH and cortisol are low and, in general, aldosterone and plasma renin activity are normal. Dehydroepiandrosterone (DHEA), which is the major precursor of sex steroid synthesis, is involved in the adrenal failure, causing a pronounced androgen deficiency in women, with the loss of both axillary and pubic hair, dry skin, reduced libido, and, frequently, an impairment in well-being (Arlt and Allolio, 2003). In cases in which there are no clear clinical manifestations of AD, and/or in the presence of ACA, it may be necessary to execute the ACTH stimulation test. The standard dose of synthetic corticotropin to establish the diagnosis of adrenal insufficiency is 250 µg (one ampule) by the intravenous injection in adults and in children ≥ 2 years of age, 125 µg for children (<2 years of age) testing plasma cortisol after 30 or 60 minutes. Peak cortisol levels below 500 nmol/L (18 µg/dL) (assay dependent) at 30 or 60 minutes lead to the diagnosis of adrenal insufficiency (Bornstein et al., 2016). Once AD is diagnosed,



**FIGURE 42.7** Adrenal imaging. Computed tomography of the adrenal glands of two patients with autoimmune Addison's disease at diagnosis. The adrenals are small (see *white arrows* in the figures).

thyroid stimulating hormone (TSH) levels are increased in 30% of patients, because of the lack of the inhibiting effect of cortisol on TSH production or the presence of coexisting autoimmune hypothyroidism (Orth and Kavacs, 1998). It has been demonstrated that the measurement of salivary and serum cortisol in the morning has a low sensitivity and specificity for detecting primary adrenal insufficiency (Raff, 2009).

## Imaging

Computed tomography or nuclear magnetic resonance shows the adrenals with optimal resolution and clarity and greatly facilitates the diagnosis and characterization of adrenal insufficiency. In patients with AAD, either isolated or as a component of APS syndromes, the adrenal glands appear bilaterally minuscule without calcifications (Doppman, 2001) (Fig. 42.6).

The imaging of the adrenal glands was performed in 250 patients with AAD. Normal adrenal glands were found in 76% of the patients. In 23.6% of the cases the adrenals were reduced in size/volume or undetectable, and only in one patient (0.4%) there was a modest increase in adrenal volume. None of the examined adrenal glands showed calcifications. There were no differences in the adrenal imaging between different forms of AAD in the context of the various APS (Betterle et al., 2013). For these reasons, in case of ACA/21-OHAbs positivity, it is not necessary to perform adrenal imaging, that is otherwise required in cases without ACA (Falorni et al., 2004; Betterle et al., 2013; Bornstein et al., 2016) (Fig. 42.7).

## DIFFERENT CLINICAL PRESENTATIONS OF AUTOIMMUNE ADDISON'S DISEASE

Neufeld and Blizzard (1980) first proposed the classification of autoimmune syndromes into four main groups, involving different endocrine glands, and named them "autoimmune polyglandular syndromes" (APS) (see Box 42.1). According to this classification, AAD can be associated with type 1, type 2 APS.

Betterle and Presotto (2008) proposed the existence of three different types of APS involving AAD (APS-1 associated with chronic candidiasis and/or chronic hypoparathyroidism; APS-2 associated with autoimmune thyroid diseases and/or type 1 diabetes mellitus; and APS-4 associated with other autoimmune diseases not included in the previous list). So, AAD can present in four main clinical forms: isolated or composite in the context of APS-1, APS-2, or APS-4.

## Association With Other Autoimmune Disorders

In a cohort study on 426 Norwegian patients with AAD, the presence of one or more clinical, subclinical, or latent autoimmune diseases was found in 88% of the patients (Erichsen et al., 2009). Another study from United Kingdom on 48 patients with AAD revealed that 73% had associated autoimmune comorbidities (Leelarathna et al., 2010). In an Italian study on 524 patients with AAD, 82.4% had one or more clinical, subclinical, or potential autoimmune diseases, and only 17.6% was isolated AAD (Betterle et al., 2013). In studying a cohort of 660 Swedish patients with AAD, it was demonstrated that 97% presented with at least one additional autoimmune disease or tested positive for any of the autoantibodies analyzed

### BOX 42.1

#### CLINICAL CLASSIFICATION OF APS ACCORDING TO NEUFELD AND BLIZZARD (1980) (MODIFIED)

- APS-1 Chronic candidiasis, chronic hypoparathyroidism, Addison's disease (*at least two diseases must be present*)
- APS-2 Addison's disease (*always present*) 1 thyroid autoimmune diseases and/or type 1 diabetes mellitus
- APS-3 Thyroid autoimmune diseases 1 other autoimmune diseases (*excluding Addison's disease and/or hypoparathyroidism*)
- APS-4 Combinations of other autoimmune diseases not included in previous classifications

(Dalin et al., 2017). The most frequent concomitant diseases were hypothyroidism, type 1 diabetes mellitus, pernicious anemia, POF, hyperthyroidism, in agreement with the previous studies from Norway, Italy, and United Kingdom. These data confirm that AAD is the autoimmune disease with the highest association with other autoimmune disorders. For these reasons, we suggest that every patient with a diagnosis of AAD is investigated for personal and family history of thyroid and/or gastric diseases, gonadal failure, type 1 diabetes mellitus, chronic hypoparathyroidism, chronic candidiasis, vitiligo, alopecia, hepatitis, celiac disease, and other minor autoimmune disease. In addition, in the absence of clinical manifestations of these disorders, it is also suggested to detect periodically (annually) an organ- and nonorgan-specific autoantibody screening (Husebye et al., 2014; Bornstein et al., 2016). This is because a proportion of patients with apparently isolated AAD can be positive for one or more autoantibodies which can recognize patients at risk for future target organ dysfunction (Betterle et al., 2013). Any associated autoimmune disease contributes to classify the patients with AAD in four subtypes of the disease (i.e., APS-1, APS-2, APS-4, and isolated), and every subtype of AAD has some common (e.g., family history for AAD and for other autoimmune diseases, autoantibodies to adrenal cortex, adrenal imaging) and some different features (e.g., age at onset, female/male ratio, adults/children ratio, genetic predisposition, association with the minor autoimmune diseases, and association with cancer) (Betterle et al., 2013). The typical profiles of the four different forms of AAD are summarized in Table 42.2.

**TABLE 42.2** Four Different Clinical Presentations of Autoimmune Adrenalitis [Number of Autoimmune Addison's Disease (AAD) Patients = 524]

Subgroups	APS-2	APS-1	APS-4	Isolated
Frequency	61.6%	13.6%	8%	17.5%
F/M	2.3	2.1	1	0.6
Adults/children	16	0.8	5.3	4
Mean age (in years) at onset of AAD	34.6	15	32	32
Range	1–85	2–41	6–62	1–84
Family history for autoimmune diseases	Frequent	Frequent	Frequent	Frequent
Family history for AAD	Rare	Frequent	Rare	Rare
Genetic	HLA-DR3 and/or DR4	AIRE gene Mutations	HLA (?)	HLA (?)
Autoimmune Addison's disease (%)	100	80	100	100
Chronic candidiasis	Absent	76%	Absent	Absent
Chronic hypoparathyroidism	Absent	90%	Absent	Absent
Thyroid autoimmune diseases	93.7%	30%	Absent	Absent
Type 1 diabetes mellitus	15.5%	6%	Absent	Absent
Other autoimmune diseases (vitiligo, alopecia, autoimmune gastritis, pernicious anemia, celiac disease, myasthenia gravis, Sjögren's syndrome, Werlhof's syndrome, multiple sclerosis, etc.)	Until 43%	Until 57%	100%	Absent
Ectodermal dystrophy	Absent	Present	Absent	Absent
Cancer	5.3%	9.9%	2.6%	4.3%
ACA at onset of AD	88%	91%	95%	69%
21-OHAb at onset of AD	94%	92%	100%	86%
Adrenals imaging	Normal/ Atrophic	Normal/Atrophic	Normal/Atrophic	Normal/Atrophic

AD, Addison's disease; ACA, adrenal cortex autoantibody; APS, autoimmune polyglandular syndrome.

## Therapy

### Conventional Therapy

Glucocorticoids are secreted into the bloodstream in a circadian fashion, with a peak at 0800 hours, and a nadir at 2400 hours. Normal adults produce 9–11 mg of cortisol daily per square meter of body surface, corresponding to about 15–19 mg/day (Esteban et al., 1991; Kraan et al., 1998). Life-saving therapy for patients with chronic adrenal insufficiency is two or three daily doses of a specific hormone replacement (Groves et al., 1988; Howlett, 1997; Williams and Dluhy, 1998; Arlt and Allolio, 2003). All patients with confirmed aldosterone deficiency need to receive mineralocorticoid replacement with fludrocortisone recommended at 50–200 µg in adults at awakening (Husebye et al., 2014) or 50–100 µg and not restrict their salt intake (Bornstein et al., 2016). During the summer period, especially in the Mediterranean or tropical climates, the doses of mineralocorticoids should be increased (Arlt, 2009). The new formulation instructions (e.g., for Florinef) require patients to keep the medication refrigerated. The actual decay rate is, however, only 0.1% in the first 6 months at room temperature (Husebye et al., 2014). It was recommended monitoring of mineralocorticoid replacement deficiency or over therapy primarily based on the clinical assessment (i.e., salt craving, postural hypotension, or edema), and blood electrolyte measurements (Bornstein et al., 2016). If essential hypertension develops in patients with mineralocorticoid replacement therapy, the dose may be first slightly reduced. If hypertension persists, antihypertensive drugs need to be started (Arlt, 2009; Husebye et al., 2014; Bornstein et al., 2016), favoring calcium-channel blockers or adrenergic receptor antagonists because they less interfere with the renin–angiotensin–aldosterone system.

DHEA (dehydroepiandrosterone) is the most important source of androgens in females; replacement therapy can enhance well-being, mood, subjective health status, energy levels, and libido in women with adrenal insufficiency. Therefore the therapy should be reserved for patients with significant impairment in well-being despite the optimized classical replacement therapy. Doses vary from 10 to 50 mg/day in a single dose in the morning guided by serum DHEA sulfate (DHEAS), androstenedione, and testosterone levels, which should be maintained in the normal range when measured in the morning prior to DHEA ingestion. There is only limited objective evidence of clinical benefit from large studies (Arlt, 2009; Husebye et al., 2014). If the female patient does not report a beneficial effect after 6 months of replacement therapy, DHEA should be discontinued. (Husebye et al., 2014; Bornstein et al., 2016).

### New Therapies

In order to mimic the physiological circadian biorhythm of cortisol, a once-daily dual-release hydrocortisone tablet was produced based on an immediate-release coating together with an extended-release core (Johannsson et al., 2009). In an open randomized, two-period 12-week crossover trial, the once-daily dual-release hydrocortisone tablet, compared to thrice-daily dose of conventional hydrocortisone tablets, produces a more circadian-based serum cortisol profile, reduces body weight, blood pressure, and improves glucose metabolism in patients with AD and concomitant diabetes mellitus (Johannsson et al., 2012).

Subsequent studies confirmed the beneficial effect of a dual-release tablet on glucose metabolism by demonstrating that modified release hydrocortisone reduced significantly either body mass index or glycated hemoglobin in the subgroup of diabetic patients when compared with conventional treatment, although absolute changes were small (Quinkler et al., 2015b). Recent studies, performed on a limited number of patients with AD, showed that the dual-release hydrocortisone preparation is effective in reducing central adiposity, improving glucose and lipids metabolism, as well as the quality of life (QoL) (Giordano et al., 2016). The total doses of hydrocortisone administered by the modified-release and conventional tablets are equivalent. Therefore it has been suggested that a more physiological daily cortisol profile (avoiding absorption peaks) related to dual-release delivery may explain the reduction in waist circumference, glycemic, and lipid profiles. However, the treatment with dual-release hydrocortisone preparations has not shown beneficial effect on blood pressure compared to the conventional tablets (Giordano et al., 2016).

Another oral formulation containing multi-particulate, modified-release hydrocortisone administered twice-daily for replacement therapy has become available (Whitaker et al., 2014). The formulation is intended to be taken at 2300 hours with drug release starting approximately 4 hours after administration, resulting in cortisol increase after midnight and very early in the morning with the maximum concentrations approximately 8 hours after administration (Whitaker et al., 2014). However, this formulation is not enough to cover the need for cortisol the day after the administration, and another dose should be taken in the morning. Twice-daily administration of this novel hydrocortisone formulation at 2300 and 0700 hours resulted in more effective decrease of ACTH levels and androgen secretion and may be specially of advantage for therapy of adults with congenital adrenal

hyperplasia (CAH) (Mallappa et al., 2015; Jones et al., 2017). In conclusion, these novel hydrocortisone formulations should be considered in selected categories of AD patients, also because their costs still remain elevated. Moreover, case-controlled studies designed for long follow-up in larger cohorts of patients with AD, either isolated or associated with diabetes mellitus, premature ovarian failure or other main diseases are necessary to verify their promising role in adrenal replacement therapy. Until recently there were no licensed, dose-appropriate formulations of hydrocortisone for neonates, infants and children with adrenal insufficiency. The dosing relied either on parents crushing tablets or pharmacists making up appropriate preparations from adult medicines. This unsatisfactory situation has been improved with the availability of an immediate-release hydrocortisone preparation with the additional advantage of taste masking based on multilayered technology (Whitaker et al., 2015). This multi particulate granule formulation has the maximum granule diameter controlled by passing through a 0.8 mm sieve. The size of the granules is well below FDA guidance for industry on sprinkle formulation limits of 2.5 mm diameter and permits swallowing of granules even by neonates. The granules are presented within a transparent capsule that is opened for dosing, thus allowing accurate pediatric dosing of 0.5, 1.0, 2.0, and 5.0 mg (Whitaker et al., 2015). A phase 3 open-label single-dose study has been performed in three children cohorts with AD; aged 2 to less than 6 years, 28 days to less than 2 years and neonates aged 1 to less than 28 days. This study demonstrated that the formulation is well tolerated, easy to administer to neonates, infants and children, and shows good absorption with cortisol levels at 60 minutes after administration similar to physiological cortisol levels of healthy children (Neumann et al., 2018).

Continuous subcutaneous hydrocortisone infusion (CSHI) is an experimental treatment that could be tested on selected patients not responding properly to regular oral treatment (Løvås et al., 2010; Løvås and Husebye, 2007). A recent phase 2 study performed on eight adult patients with difficult-to-treat classic CAH compared CSHI to conventional oral glucocorticoid treatment. CSHI resulted a safe and well-tolerated modality of cortisol replacement that effectively approximates physiologic cortisol secretion in patients with classic CAH poorly controlled on conventional therapy (Nella et al., 2016). Improved adrenal steroid control and positive effects on health-related QoL suggest that CSHI should be considered as a treatment option for adrenal insufficiency due to classic CAH.

Recent advances in immune intervention therapies allow developing novel strategies to prevent progression to overt AAD. Targeting autoimmune responses have shown some promise in prevention or treatment of type 1 diabetes mellitus, Graves' disease, multiple sclerosis, and other autoimmune conditions. Rituximab is a monoclonal antibody able to block CD20 molecules on the B-lymphocytes and most likely affects the ability of B cells to cooperate in antigen presentation and cytokine secretion. Rituximab was used in six patients with new onset AAD (Pearce et al., 2012). Although serum cortisol and aldosterone concentrations remained low in five throughout the follow-up period, one patient revealed a steady improvement in both serum cortisol and aldosterone, allowing discontinuation of steroids at 15 months after rituximab therapy. This patient remained well without medication for 12 months thereafter (Pearce et al., 2012). However, these preliminary results need to be confirmed in studies with a greater number of patients at the onset of clinical AAD or in high-risk patients with ACA/21-OHAbs at Stage 2 of subclinical AAD.

### **Regenerative Therapy**

Stimulation with ACTH may lead to increased regeneration of glucocorticoid secreting cells. A 20-week trial of regular administration of tetracosactide (ACTH<sub>1–24</sub>) in 13 patients with AAD of more than 1 year duration (Gan et al., 2014) showed an improvement in cortisol and aldosterone concentrations in 2, 1 remained well without steroids for 28 months while the other initial responder needed to resume glucocorticoid therapy after 7 months. It should be noted that this approach may only be effective if there would be sufficient residual adrenal cell function for achieving the sustained regeneration, and it is probably useless in patients with already high levels of ACTH. The recent advances in the understanding adrenocortical stem cell biology and adrenal plasticity will be helpful to comprehend the new therapeutic approaches addressed to modify the natural history of AAD (Gan and Pearce, 2017). Asymptomatic patients at high-risk of developing overt disease (i.e., preclinical adrenal impairment) may be offered an opportunity to participate in prevention trials with immunomodulation and/or regenerative therapeutical strategies.

### **Steroid Replacement During Surgery, Other Illness, Medical Procedures, Physical Activity, and Pregnancy**

Patients with AD need to increase their steroid doses during surgery and medical procedures according to the stress induced. In general, surgery, fever, gastrointestinal diseases, major dental procedures, and child delivery

require parenteral hydrocortisone followed by double oral doses being tapered to replacement doses over subsequent days.

Patients undergoing regular and time-limited physical activity do not generally need to make a dose adjustment. However, in case of intense or prolonged exercise, an increase in hydrocortisone and salt intake may be necessary. Stress dosing of 10 mg hydrocortisone 1 hour prior to exercise is commonly given. However, a recent double-blind study revealed that patients with AD did not find any benefit from a supplemental dose of hydrocortisone in short strenuous exercise evaluated by ergometer test with cardiorespiratory assessment (Simunkova et al., 2016). Yet, the number of investigated patients was limited, and all were females. Whether stress dosing is beneficial if given otherwise and during longer lasting or more strenuous physical activity will have to be investigated further.

Pregnancy is associated with a gradual but pronounced physiological increase in corticosteroid-binding globulin and total serum cortisol. Free cortisol levels rise during the third trimester, and some pregnant patients have a small increase in hydrocortisone requirement (by 2.5 or 5 mg daily) during the third trimester. Serum progesterone has antimineralcorticoid effects, and hence, the fludrocortisone dose may need to be increased during late pregnancy. Plasma renin activity normally increases during pregnancy (Husebye et al., 2014). Usually, the required additional hydrocortisone dose during the third trimester is of 5–10 mg (Lebbe and Aarlt, 2013). The Endocrine Society Clinical Practice Guideline recommends increasing hydrocortisone dose from 10% to 40% in the third trimester, in order to reproduce the physiological rise in free cortisol (Bornstein et al., 2016). Glucocorticoid preparation that should be used in pregnancy is hydrocortisone. Cortisone acetate remains a second choice, while corticosteroids with longer activity or half-life (i.e., prednisolone, prednisone, methylprednisolone, and betamethasone) should not be used (Bornstein et al., 2016). However, some authors believe that pregnancy does not normally require major adjustment of the glucocorticoid substitutive therapy because also in women with primary adrenal failure, there are physiological adaptations in cortisol clearance (Falorni et al., 2013).

At delivery, a bolus dose of 100 mg hydrocortisone should be given and repeated if necessary. The oral dose should be doubled for 24–48 hours postpartum followed by rapid tapering and returning back to oral administration at prepregnancy doses (Husebye et al., 2014; Falorni et al., 2013; Bornstein et al., 2016).

## Acute Adrenal Failure (Adrenal Crisis)

The risk of adrenal crisis is a possible life-threatening event in every Addisonian patient. A study evaluated frequency, causes, and risk factors of adrenal crisis in patients with chronic adrenal failure demonstrating 6.3 crises/100 patient-years. Precipitating causes were mainly gastrointestinal infection and fever (45%) but also other stressful events (e.g., major pain, surgery, psychic distress, heat, and pregnancy). Patients with comorbidities had a higher risk compared to patients with AD alone (Hahner et al., 2009). Adrenal crisis has been observed significantly more frequently in patients with primary (7.6/100 patient-years) than in patients with secondary adrenal insufficiency (3.2/100 patient-years) (Meyer et al., 2016). In addition, a higher prevalence of acute adrenal decompensation has been reported in patients with APS (10.9/100 patient-years) and in those with type 1 diabetes (12.5/100 patient-years) (Meyer et al., 2016). Other studies in patients on a long-term substitutive therapy demonstrated an annual incidence of adrenal crisis from 5 to 10 of 100 patients per year with a mortality rate of 0.5/100 patients/year (Husebye et al., 2014; Allolio, 2015). The crisis can be due to unnecessary reduction of the glucocorticoid therapy or to lack of stress-related glucocorticoid dose adjustment by patients or medical practitioners. Generally, oral glucocorticoid therapy should be increased or doubled during infections with fever, or during any other major stress-producing event, and, in general, needs to be orally increased but parenteral injected in case of gastrointestinal infections with diarrhea, surgical procedures, delivery, vomiting, or major traumas (Arlt, 2009). The Consensus Statement of Euradrenal Consortium (Husebye et al., 2014) recommends to treat adrenal crisis with rapid infusion of 100 mg of hydrocortisone and 1 L/h of i.v. isotonic saline solution. During the following 2 days, 100 mg of hydrocortisone must be given intravenously every 6 hours and 1 L of saline solution every 6 hours. On the third day, if precipitating events improve, the treatment may be continued using oral medications. Mineralocorticoid therapy is not necessary during infusion of hydrocortisone. However, it may be restored when hydrocortisone replacement falls below 50 mg/day. Admission to intensive care unit depends on the severity of underlying or occurring diseases. The Endocrine Society Clinical Practice Guideline recommends that patients with suspected adrenal crisis should be treated with an immediate parenteral injection of 100 mg hydrocortisone (50 mg/m<sup>2</sup> for children), followed by appropriate fluid resuscitation and 200 mg (50–100 mg/m<sup>2</sup> for children) of hydrocortisone in 24 hours via continuous i.v. therapy or 6 hourly injection (Bornstein et al., 2016).

## Emergency Card

The best strategy for prevention of adrenal crisis is the continuous improvement in the education of patients and health professionals (Napier and Pearce, 2012; Wass and Arlt, 2012). Patients with AD should be informed when and how to increase steroid therapy during concurrent illnesses or injury. Furthermore, the training in intramuscular and/or subcutaneous administration of glucocorticoid may be very helpful for the effective prevention of acute adrenal crisis by the patients themselves (Hahner et al., 2013). In any case, patients with AD should carry a medical identification card (or a bracelet), stating the current therapy and recommendations for emergency situations such as febrile illnesses, injury, vomiting, surgical interventions, dental extractions, or pregnancies, when the intake of glucocorticoids must be doubled or tripled (Oelkers, 1996). The Euradrenal Consortium proposed a standard emergency card which has been now produced in collaboration with the National Associations of the patients with AD and made available for distribution in different European countries (Husebye, 2014). In this card, there are specific instructions on management of adrenal crisis in the national language on one side, while in English on the reverse side. This card is a simple and effective strategy to save lives of patients with AD (Quinkler et al., 2015a).

## Quality of Life

The European Euradrenal Consortium developed a Disease-Specific Quality of Life Questionnaire of AD (AddiQoL) with the purpose to quantify altered well-being and treatment effects (Løvås et al., 2010). The original English version of AddiQoL questionnaire translated into various languages had been distributed to all the members participating to the European Consortium. This approach enabled collection of information in a large group of European patients with AD (Øksnes et al., 2012). AddiQoL questionnaire is expected to be a useful tool to evaluate the QoL in patients with AD in clinical practice in the future.

## Mortality

Three Scandinavian studies have made a contribution to the rate of mortality of AD (Berghorsdottir et al., 2006; Bensing et al., 2008; Erichsen et al., 2009a). The two registry studies from Sweden (Berghorsdottir et al., 2006; Bensing et al., 2008) showed that the mortality was more than double that in the control population and particularly elevated in patients with APS-1 with an odds ratio of 4.6. The causes of death were endocrine diseases and cancer (Bensing et al., 2008). It must be said that the two Swedish registries referred exclusively to hospitalize patients were not representative of the entire population of patients and were both based on the codification of the disease without a definitive confirmation of the diagnosis. Potential assignment mistakes may have greatly influenced the outcome. More specifically, it is known that secondary adrenal insufficiency due to ACTH deficiency has an increased risk for mortality (Filipsson et al., 2006) because of the concurrent deficiency of other pituitary hormones, and it is not sure that such patients have been excluded from the analysis. A Norwegian case-control study did not report an overall increase in mortality and also identified a subgroup of young males with doubled mortality rate, the causes of death being adrenal crisis, infections, and sudden death, while cardiovascular mortality was not increased (Erichsen et al., 2009b).

In an Italian study, we investigated the mortality in over 700 patients with AAD followed for a mean period of 15 years and demonstrated the existence of a normal survival rate in patients with AAD, with the exclusion of those with APS-1. Patients with APS-1 revealed an increased mortality rate compared to the general population matched for gender and age with the patients (Betterle et al., personal data).

## Osteoporosis

It is a constant concern that overtreatment with glucocorticoids may have negative health effects on bone mineral density (BMD). An increased incidence of osteoporosis has been reported in patients receiving daily replacement doses of 30 mg of hydrocortisone or higher, whereas replacement doses of 20–25 mg of hydrocortisone do not affect BMD (Arlt, 2009). Earlier studies have been hampered by low numbers of participants. In patients with AD, a reduced BMD was reported along with polymorphisms in genes regulating steroid action that influences BMD (Løvås et al., 2009). BMD revealed an inverse correlation to steroid dose and was especially low in those taking synthetic potent glucocorticoids such as prednisolone. Despite reduced BMD, vertebral fractures were not more prevalent than in the background population. In contrast, a follow-up registry study

from Sweden reported significantly increased frequencies of hip fractures in AD patients with an excess risk about 50% (Björnsdottir et al., 2011). A study in 81 German patients who received low replacement doses of glucocorticoids found no reduction in BMD (Koetz et al., 2012). Thus clinicians should aim to keep the glucocorticoid replacement dose as low as is compatible with normal well-being.

## Acknowledgments

We would like to thank all those who have cooperated with us over the years regarding the study of the clinical, genetic, immunologic, morphologic, and endocrine aspects of AD.  
This study was supported in part by a grant from the European Union Seventh Framework Programme, the Euradrenal project: Pathophysiology and Natural Course of Autoimmune Adrenal Failure in Europe. Grant Agreement No. 2008-201167 (EURADRENAL website: [www.euradrenal.org/](http://www.euradrenal.org/)).

## References

- Addison, T., 1855. On the Constitutional and Local Effects of Disease of the Suprarenal Capsules. Samuel Highley, London.
- Ahonenm, P., Miettinen, A., Perheentupa, J., 1987. Adrenal and steroid cell antibodies in patients with autoimmune polyglandular disease type I and risk of adrenocortical and ovarian failure. *J. Clin. Endocrinol. Metab.* 64, 494–500.
- Alimohammadi, M., Björklund, P., Hallgren, A., Pöntynen, N., Szinnai, G., Shikama, N., et al., 2008. Autoimmune polyendocrine syndrome type 1 and NALP5, a parathyroid autoantigen. *N. Engl. J. Med.* 358, 1018–1028.
- Alimohammadi, M., Dubois, N., Sköldberg, F., Hallgren, A., Tardivel, I., Hedstrand, H., et al., 2009. Pulmonary autoimmunity as a feature of autoimmune polyendocrine syndrome type 1 and identification of KCNRG as a bronchial autoantigen. *Proc. Natl. Acad. Sci. U.S.A.* 106, 4396–4401.
- Allolio, B., 2015. Extensive expertise in endocrinology: adrenal crisis. *Eur. J. Endocrinol.* 172, R115–R124.
- Anderson, J.R., Goudie, R.B., Gray, K.G., Timbury, G.C., 1957. Autoantibodies in Addison's disease. *Lancet* 1, 1123–1124.
- Anderson, J.U., Goudie, R.B., Gray, K., Stuart Smith, D.A., 1968. Immunological features of idiopathic Addison's disease: an antibody to cells producing steroid hormones. *Clin. Exp. Immunol.* 3, 107–117.
- Andrade, J.A., Skelton, F.R., Andrade, E.C., Milgrom, F., Witebsky, E., 1968. Experimental autoimmune adrenalitis in rats. *Lab. Invest.* 19, 460–465.
- Arlt, W., 2009. The approach to the adult with newly diagnosed adrenal insufficiency. *J. Clin. Endocrinol. Metab.* 94, 1059–1067.
- Arlt, W., Allolio, B., 2003. Adrenal insufficiency. *Lancet* 361, 1881–1893.
- Asawa, T., Wedlock, N., Baumann-Antczak, A., Rees Smith, B., Furmaniak, J., 1994. Naturally occurring mutations in human steroid 21-hydroxylase influence adrenal autoantibody binding. *J. Clin. Endocrinol. Metab.* 79, 372–376.
- Auchus, R.J., Miller, W.L., 2001. The principles, pathways and enzymes of human steroidogenesis. In: fourth ed DeGroot, L.J., Jameson, J.L. (Eds.), *Endocrinology*, vol. 2. W.B. Saunders.
- Barnett, E.V., Dumonde, D.C., Glynn, L.E., 1963. Induction of autoimmunity to adrenal gland. *Immunology* 6, 382.
- Baumann-Antczak, A., Wedlock, N., Bednarek, J., Kiso, Y., Krishnan, H., Fowler, S., et al., 1992. Autoimmune Addison's disease and 21-hydroxylase. *Lancet* 340, 429–430.
- Beales, P.E., Castri, F., Valiani, A., Rosignoli, G., Buckley, L., Pozzilli, P., 2002. Adrenalitis in the non-obese diabetic mouse. *Autoimmunity* 35, 329–333.
- Bednarek, J., Furmaniak, J., Wedlock, N., Kiso, Y., Baumann-Antczak, A., Fowler, S., et al., 1992. Steroid 21-hydroxylase is a major auto-antigen involved in adult onset autoimmune Addison's disease. *FEBS Lett.* 309, 51–55.
- Bensing, S., Brandt, L., Tabaroi, F., Sjöberg, O., Nilsson, B., Ekbom, A., et al., 2008. Increased death risk and altered cancer incidence pattern in patients with isolated or combined autoimmune primary adrenocortical insufficiency. *Clin. Endocrinol. (Oxf.)* 69, 697–704.
- Berghorsdottir, R., Leonsson-Zachrisson, M., Odén, A., Johansson, G., 2006. Premature mortality in patients with Addison's disease: a population-based study. *J. Clin. Endocrinol. Metab.* 91, 4849–4853.
- Betterle, C., 2004b. Addison's disease and autoimmune polyglandular syndromes. In: Geenen, V., Chrosus, G. (Eds.), *Immunoendocrinology in Health and Disease*. Dekker, New York, pp. 491–536.
- Betterle, C., Dal Pra, C., Pedini, B., Zanchetta, R., Albergoni, M.P., Chen, S., et al., 2014a. Assessment of adrenocortical function and autoantibodies in a baby born to a mother with autoimmune polyglandular syndrome Type 2. *J. Endocrinol. Invest.* 27, 618–621.
- Betterle, C., Presotto, F., 2008. Autoimmune polyendocrine syndromes (APS) or multiple autoimmune syndromes (MAS). In: Walker, S., Jara, L.J. (Eds.), *Handbook of Systemic Autoimmune Diseases*, vol. 9. Elsevier, Amsterdam, pp. 135–148.
- Betterle, C., Volpati, M., 1998. Adrenal and ovarian autoimmunity. *Eur. J. Endocrinol.* 138, 16–25.
- Betterle, C., Zanette, F., Zanchetta, R., Pedini, B., Trevisan, A., Mantero, F., et al., 1983. Complement-fixing adrenal autoantibodies as a marker for predicting onset of idiopathic Addison's disease. *Lancet* 1, 1238–1241.
- Betterle, C., Scalici, C., Presotto, F., Pedini, B., Moro, L., Rigon, F., et al., 1988. The natural history of adrenal function in autoimmune patients with adrenal autoantibodies. *J. Endocrinol.* 117, 467–475.
- Betterle, C., Scalici, C., Pedini, B., Mantero, F., 1989. Morbo di Addison: principali associazioni cliniche e descrizione della storia naturale della malattia. *Ann. Ital. Med. Int.* 4, 195–206.
- Betterle, C., Rossi, A., Dalla Pria, S., Artifoni, L., Pedini, B., Gavasso, S., et al., 1993. Premature ovarian failure: autoimmunity and natural history of the disease. *Clin. Endocrinol.* 39, 35–43.

- Betterle, C., Volpato, M., Rees Smith, B., Furmaniak, J., Chen, S., Greggio, N.A., et al., 1997a. I. Adrenal cortex and steroid 21-hydroxylase autoantibodies in adult patients with organ-specific autoimmune diseases: markers of low progression to clinical Addison's disease. *J. Clin. Endocrinol. Metab.* 82, 932–938.
- Betterle, C., Volpato, M., Rees Smith, B., Furmaniak, J., Chen, S., Zanchetta, R., et al., 1997b. II. Adrenal cortex and steroid 21-hydroxylase autoantibodies in children with organ-specific autoimmune diseases: markers of high progression to clinical Addison's disease. *J. Clin. Endocrinol. Metab.* 82, 939–942.
- Betterle, C., Volpato, M., Pedini, B., Chen, S., Rees Smith, B., Furmaniak, J., 1999. Adrenal-cortex autoantibodies and steroid producing cells autoantibodies in patients with Addison's disease: comparison of immunofluorescence and immunoprecipitation assays. *J. Clin. Endocrinol. Metab.* 84, 618–622.
- Betterle, C., Dal Pra, C., Mantero, F., Zanchetta, R., 2002. Autoimmune adrenal insufficiency and autoimmune polyendocrine syndromes: autoantibodies, autoantigens, and their applicability in diagnosis and disease prediction. *Endocr. Rev.* 23, 327–364.
- Betterle, C., Coco, G., Zanchetta, R., 2005. Adrenal cortex autoantibodies in subjects with normal adrenal function. *Best Pract. Res. Clin. Endocrinol. Metab.* 19, 85–99.
- Betterle, C., Scarpa, R., Morlin, L., Garelli, S., Lazzarotto, F., Presotto, F., et al., 2013. Addison's disease: a survey on 633 patients in Padova. *Eur. J. Endocrinol.* 169, 773–784.
- Betterle, C., Garelli, S., Presotto, F., Furmaniak, J., 2016. From appearance of adrenal autoantibodies to clinical symptoms of Addison's disease: natural history. In: Arvat, E., Falorni, A. (Eds.), *Cortisol Excess and Insufficiency*, vol 46. Karger, Horm Res. Basel, pp. 133–145.
- Björnsdottir, S., Sääf, M., Bensing, S., Kämpe, O., Michaëlsson, K., Ludvigsson, J.F., 2011. Risk of hip fracture in Addison's disease: a population-based cohort study. *J. Intern. Med.* 270, 187–195.
- Blizzard, R.M., Kyle, M., 1963. Studies on adrenal antigens and autoantibodies in Addison's disease. *J. Clin. Invest.* 42, 1653–1660.
- Bornstein, S.R., Allolio, B., Arlt, W., Barthel, A., Don-Wauchope, A., Hammer, G.D., et al., 2016. Diagnosis and treatment of primary adrenal insufficiency: an endocrine society clinical practice guideline. *J. Clin. Endocrinol. Metab.* 101, 364–389.
- Boscaro, M., Betterle, C., Volpato, M., Fallo, F., Furmaniak, J., Rees Smith, B., et al., 1996. Hormonal responses during various phases of autoimmune adrenal failure: no evidence for 21-hydroxylase enzyme activity inhibition in vivo. *J. Clin. Endocrinol Metab.* 81, 2801–2804.
- Bratland, E., Husebye, E.S., 2011. Cellular immunity and immunopathology in autoimmune Addison's disease. *Mol. Cell. Endocrinol.* 336, 180–190.
- Bratland, E., Skinningsrud, B., Undlien, D.E., Mozes, E., Husebye, E.S., 2009. T cell responses to steroid cytochrome P450 21-hydroxylase in patients with autoimmune primary adrenal insufficiency. *J. Clin. Endocrinol. Metab.* 94, 5117–5124.
- Brown-Séquard, C.E., 1856. Recherches experimentales sur la physiologie et la pathologie des capsules surrenales. *Arch. Gen. Med.* 5, 385–401.
- Bruserud, Ø., Oftedal, B.E., Wolff, A.B., Husebye, E.S., 2016. AIRE-mutations and autoimmune disease. *Curr. Opin. Immunol.* 43, 8–15.
- Cervato, S., Mariniello, B., Lazzarotto, F., Morlin, L., Zanchetta, R., Radetti, G., et al., 2009. Evaluation of the autoimmune regulator (AIRE) gene mutations in a cohort of Italian patients with autoimmune-poly-endocrinopathy-candidiasis-ectodermal-dystrophy (APECED) and in their relatives. *Clin. Endocrinol. (Oxf.)* 70, 421–428.
- Chen, S., Sawicka, J., Betterle, C., Powell, M., Prentice, L., Volpato, M., et al., 1996. Autoantibodies to steroidogenic enzymes in autoimmune polyglandular syndrome, Addison's disease, and premature ovarian failure. *J. Clin. Endocrinol. Metab.* 83, 2977–2986.
- Chen, Q.J., Lan, M.S., She, J.X., MacLaren, N.K., 1998. The gene responsible for autoimmune polyglandular syndrome type 1 maps to chromosome 21q22.3 in US patients. *J. Autoimmun.* 11, 117–183.
- Chung, B.C., Matteson, K.J., Voutilainen, R., Mohandas, T.K., Miller, W.L., 1986. Human cholesterol side-chain cleavage enzyme, P450scC: cDNA cloning, assignment of the gene to chromosome 15, and expression in the placenta. *Proc. Natl. Acad. Sci. U.S.A.* 83, 8962–8966.
- Clemente, M.G., Obermyer-Straub, P., Meloni, A., Strassburg, C.P., Arangino, V., et al., 1997. Cytochrome P450 IA2 is a hepatic autoantigen in autoimmune polyglandular syndrome type 1. *J. Clin. Endocrinol. Metab.* 82, 1353–1361.
- Clemente, M.G., Meloni, A., Obermyer-Straub, P., Frau, F., Manns, M.P., De Virgilis, S., 1998. Two cytochrome P450 are major hepatocellular autoantigens in autoimmune polyglandular syndrome type 1. *Gastroenterology* 114, 324–328.
- Coco, G., Dal Pra, C., Presotto, F., Albergoni, M.P., Canova, C., Pedini, B., et al., 2006. Estimated risk for developing autoimmune Addison's disease in patients with adrenal cortex autoantibodies. *J. Clin. Endocrinol. Metab.* 91, 1637–1645.
- Colls, J., Betterle, C., Volpato, M., Rees Smith, B., Furmaniak, J., 1995. A new immunoprecipitation assay for autoantibodies to steroid 21-hydroxylase in Addison's disease. *Clin. Chem.* 41, 375–380.
- Colover, J., Glynn, L.E., 1958. Experimental isoimmune adrenalitis. *Immunology* 2, 172–178.
- Dal Pra, C., Chen, S., Betterle, C., Zanchetta, R., McGrath, V., Furmaniak, J., et al., 2004. Autoantibodies to human tryptophan hydroxylase and aromatic L-amino acid decarboxylase. *Eur. J. Endocrinol.* 150, 313–321.
- Dalin, F., Nordling, Eriksson, G., Dahlqvist, P., Hallgren, Å., Wahlberg, J., et al., 2017. Clinical and immunological characteristics of autoimmune Addison's disease: a nationwide Swedish multicenter study. *J. Clin. Endocrinol. Metab.* 102, 379–389.
- Dalla Costa, M., Bonanni, G., Masiero, S., Faggian, D., Chen, S., Furmaniak, J., et al., 2014. Gonadal function in males with autoimmune Addison's disease and autoantibodies to steroidogenic enzymes. *Clin. Exp. Immunol.* 176, 373–379.
- Dawoodji, A., Chen, J.L., Shepherd, D., Dalin, F., Tarlton, A., Alimohammadi, M., et al., 2014. High frequency of cytolytic 21-hydroxylase-specific CD8+ T cells in autoimmune Addison's disease patients. *J. Immunol.* 193, 2118–2126.
- De Bellis, A., Bizzarro, A., Rossi, R., Paglionico, V.A., Criscuolo, T., Lombardi, G., et al., 1993. Remission of subclinical adrenocortical failure in subjects with adrenal autoantibodies. *J. Clin. Endocrinol. Metab.* 76, 1002–1007.
- De Rosa, G., Corsello, S.M., Cecchini, L., Della Casa, S., Testa, A., 1987. A clinical study of Addison's disease. *Exp. Clin. Endocrinol.* 90, 232–242.
- Doppman, J.L., 2001. Adrenal imaging. In: fourth ed De Groot, L.J., Jameson, J.L. (Eds.), *Endocrinology*, vol. 2. W.B. Saunders Co, Philadelphia, PA.
- Drury, M.J., Keelan, D.M., Timoney, F.J., Irvine, W.J., 1979. Case report. Juvenile familial endocrinopathy. *Clin. Exp. Immunol.* 7 (1), 25–132.
- Duff, G.L., Bernstein, C., 1933. Five cases of Addison's disease with so-called atrophy of the adrenal cortex. *Bull. Johns Hopkins Hosp.* 52, 67.

- Dunlop, D., 1963. Eighty-six cases of Addison's disease. *Br. Med. J.* 3, 887–891.
- Dunn, K.J., Herrtage, M.E., 1998. Hypocortisolaemia in a Labrador retriever. *J. Small Anim. Pract.* 39, 90–93.
- Eason, R.J., Croxon, M.S., Perry, M.C., Somerfield, S.D., 1982. Addison's disease, adrenal autoantibodies and computerized adrenal tomography. *N.Z. Med. J.* 95, 569–573.
- Ekwall, O., Hedstrand, H., Grimelius, L., Haavik, J., Perheentupa, J., Gustafsson, J., et al., 1998. Identification of tryptophan hydroxylase as an intestinal autoantigen. *Lancet* 352, 279–283.
- Emy, J.K., 1998. Medical mystery. The answer revealed. *N. Engl. J. Med.* 338, 266–268.
- Erichsen, M.M., Løvås, K., Skinningsrud, B., Wolff, A.B., Undlien, D.E., Svartberg, J., et al., 2009a. Clinical, immunological, and genetic features of autoimmune primary adrenal insufficiency: observations from a Norwegian registry. *J. Clin. Endocrinol. Metab.* 94, 4882–4890.
- Erichsen, M.M., Løvås, K., Fougnier, K.J., Svartberg, J., Hauge, E.R., Bollerslev, J., et al., 2009b. Normal overall mortality rate in Addison's disease, but young patients are at risk of premature death. *Eur. J. Endocrinol.* 160, 233–237.
- Esteban, N.V., Louglin, T., Yergey, A.L., Zawadzki, J.K., Booth, J.D., Winterer, J.C., et al., 1991. Daily cortisol production rate in man determined by stable isotope dilution/mass spectrometry. *J. Clin. Endocrinol. Metab.* 72, 39–45.
- Falorni, A., Nikoshkov, A., Laureti, S., Grenbäck, E., Hulting, A.L., Casucci, G., 1995. High diagnostic accuracy for idiopathic Addison's disease with a sensitive radiobinding assay for autoantibodies against recombinant human 21-hydroxylase. *J. Clin. Endocrinol. Metab.* 80, 2752–2755.
- Falorni, A., Laureti, S., Nikoshkov, A., For the Belgian Diabetes Registry, et al., 1997. 21-Hydroxylase autoantibodies in adult patients with endocrine autoimmune diseases are highly specific for Addison's disease. *Clin. Exp. Immunol.* 107, 341–346.
- Falorni, A., Laureti, S., De Bellis, A., Zanchetta, R., Tiberti, C., Arnaldi, G., et al., 2004. Italian Addison network study: update of diagnostic criteria for the etiological classification of primary adrenal insufficiency. *J. Clin. Endocrinol. Metab.* 89, 1598–1604.
- Falorni, A., Chen, S., Zanchetta, R., Yu, L., Tiberti, C., Bacosi, M.L., et al., 2011. Measuring adrenal autoantibody response: interlaboratory concordance in the first international serum exchange for the determination of 21-hydroxylase autoantibodies. *Clin. Immunol.* 140, 291–299.
- Falorni, A., Minarelli, V., Morelli, S., 2013. Therapy of adrenal insufficiency: an update. *Endocrine* 43, 514–528.
- Falorni, A., Bini, V., Betterle, C., Brozzetti, A., Castaño, L., Fichna, M., et al., 2015. Determination of 21-hydroxylase autoantibodies: inter-laboratory concordance in the Euradrenal International Serum Exchange Program. *Clin. Chem. Lab. Med.* 53, 1761–1770.
- Falorni, A., Brozzetti, A., Perniola, R., 2016. From genetic predisposition to molecular mechanisms of autoimmune primary adrenal insufficiency. *Front. Horm. Res.* 46, 115–132.
- Fidler, W.J., 1977. Ovarian thecal metaplasia in adrenal glands. *Am. J. Clin. Pathol.* 67, 318–322.
- Filipsson, H., Monson, J.P., Koltowska-Hagstrom, M., Mattsson, A., Johannsson, G., 2006. The impact of glucocorticoid replacement regimens on metabolic outcome and comorbidity in hypopituitary patients. *J. Clin. Endocrinol. Metab.* 92, 110–116.
- Fogt, F., Vortmeyer, A.O., Poremba, C., Minda, M., Harris, C.A., Tomaszewski, J.E., 1998. Bcl2 expression in normal adrenal glands and in adrenal neoplasms. *Mod. Pathol.* 11, 716–720.
- Freeman, M., Weetman, A.P., 1992. T and B cell reactivity to adrenal antigens in autoimmune Addison's disease. *Clin. Exp. Immunol.* 88, 275–279.
- Fujii, Y., Kato, N., Kito, J., Asai, J., Yokochi, T., 1992. Experimental autoimmune adrenalitis: a murine model for Addison's disease. *Autoimmunity* 12, 47–52.
- Furmaniak, J., Rees Smith, B., 2002. Addison's disease. In: Gill, R.G. (Ed.), *Immunologically Mediated Endocrine Diseases*. Lippincott Williams & Williams, Philadelphia, PA, pp. 431–451.
- Furmaniak, J., Talbot, D., Reinwein, D., Benker, G., Creag, F.M., Rees Smith, B., 1988. Immunoprecipitation of human adrenal microsomal antigen. *FEBS Lett.* 232, 25–28.
- Furmaniak, J., Komani, S., Asawa, T., Wedlock, N., Colls, J., Rees Smith, B., 1994. Autoimmune Addison's disease. Evidence for a role of steroid 21-hydroxylase autoantibodies in adrenal insufficiency. *J. Clin. Endocrinol. Metab.* 79, 1517–1521.
- Furmaniak, J., Sanders, J., Rees Smith, B., 1999. Autoantigens in the autoimmune endocrinopathies. In: Volpé, R. (Ed.), *Contemporary Endocrinology: Autoimmune Endocrinopathies*. Springer, Totowa, NY, pp. 183–216.
- Gan, E.H., Pearce, S.H., 2017. Management of endocrine disease: regenerative therapies in autoimmune Addison's disease. *Eur. J. Endocrinol.* 176, R123–R135.
- Gan, E.H., MacArthur, K., Mitchell, A.L., Hughes, B.A., Perros, P., Ball, S.G., et al., 2014. Residual adrenal function in autoimmune Addison's disease: improvement after tetracosactide (ACTH1-24) treatment. *J. Clin. Endocrinol. Metab.* 99, 111–118.
- Giordano, R., Guaraldi, F., Marinazzo, E., Fumarola, F., Rampino, A., Berardelli, R., et al., 2016. Improvement of anthropometric and metabolic parameters, and quality of life following treatment with dual-release hydrocortisone in patients with Addison's disease. *Endocrine* 51, 360–368.
- Gonzalez-Hernandez, J.A., Bornstein, S.R., Ehrhart-Bornstein, M., Spath-Schwalbe, E., Jirikowski, G., Scherbaum, W.A., 1994. Interleukin-6 messenger ribonucleic acid expression in human adrenal gland in vivo: new clue to a pancreatic or autocrine regulation of adrenal function. *J. Clin. Endocrinol. Metab.* 79, 1492–1497.
- Grinspoon, S.K., Biller, B.M., 1994. Clinical review 62: laboratory assessment of adrenal insufficiency. *J. Clin. Endocrinol. Metab.* 79, 923–931.
- Groves, R.W., Toms, G.C., Houghton, B.J., Monson, J.P., 1988. Corticosteroid replacement therapy: twice or thrice daily? *J. R. Soc. Med.* 81, 514–516.
- Guttman, P.H., 1930. Addison's disease: statistical analysis of 566 cases and study of the pathology. *Arch. Pathol.* 10, 742–895.
- Hahner, S., Loeffler, M., Bleicken, B., Drechsler, C., Milovanovic, D., Fassnacht, M., et al., 2009. Epidemiology of adrenal crisis in chronic adrenal insufficiency: the need for new prevention strategies. *Eur. J. Endocrinol.* 162, 597–602.
- Hahner, S., Burger-Stritt, S., Allolio, B., 2013. Subcutaneous hydrocortisone administration for emergency use in adrenal insufficiency. *Eur. J. Endocrinol.* 169, 147–154.
- Halonen, M., Eskelin, P., Myhre, A.G., Perheentupa, J., Husebye, E.S., Kampe, O., et al., 2002. AIRE mutations and human leukocyte antigen genotypes as determinants of the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy phenotype. *J. Clin. Endocrinol. Metab.* 87, 2568–2574.

- Harlton, B.W., 1976. Addison's disease in a dog. *Vet. Med. Small Anim. Clin.* 71, 285–288.
- Hayashi, Y., Hiyoshi, T., Takemura, T., Kurashima, C., Hirokawa, K., 1989. Focal lymphocytic infiltration in the adrenal cortex of the elderly: immunohistochemical analysis of infiltrating lymphocytes. *Clin. Exp. Immunol.* 77, 101–105.
- Hedstrand, H., Ekwall, O., Haavik, J., Landgren, E., Betterle, C., Perheentupa, J., et al., 2000. Identification of tyrosine hydroxylase as an autoantigen in autoimmune polyendocrine syndrome Type 1. *Biochem. Biophys. Res. Commun.* 267, 456–461.
- Hedstrand, H., Ekwall, O., Olsson, M.J., Landgren, E., Kemp, H.E., Weetman, A., et al., 2001. The transcription factors SOX9 and SOX10 are vitiligo autoantigens in autoimmune polyendocrine syndrome type 1. *J. Biol. Chem.* 276, 35390–35395.
- Henzen-Lohmans, S.C., Stel, H.V., Van Muijen, G.N., Mullink, H., Meijer, C.J., 1988. Expression of intermediate filament proteins in adrenal cortex and related tumors. *Histopathology* 12, 359–372.
- Hiatt, J.R., Hiatt, N., 1997. The conquest of Addison's disease. *Am. J. Surg.* 174, 280–283.
- Hoek, A., Schoemaker, J., Drexhage, H.A., 1997. Premature ovarian failure and ovarian autoimmunity. *Endocr. Rev.* 18, 107–134.
- Hoening, E.M., Hirano, A., Levine, A., Ghatak, N.R., 1970. The early development and fine structure of allergic adrenalitis. *Lab. Invest.* 22, 198–205.
- Howlett, T.A., 1997. An assessment of optimal hydrocortisone replacement therapy. *Clin. Endocrinol. (Oxf.)* 46, 263–268.
- Husebye, E.S., Gebre-Medhin, G., Thuomi, T., Perheentupa, J., Landin-Olsson, M., Gustafsson, J., et al., 1997. Autoantibodies against aromatic L-amino acid decarboxylase in autoimmune polyendocrine syndrome type I. *J. Clin. Endocrinol. Metab.* 82, 147–150.
- Husebye, E.S., Bratland, E., Bredholt, G., Fridkin, M., Dayan, M., Mozes, E., 2006. The substrate-binding domain of 21-hydroxylase, the main autoantigen in autoimmune Addison's disease, is an immunodominant T cell epitope. *Endocrinology* 147, 2411–2416.
- Husebye, E.S., Perheentupa, J., Rautemaa, R., Kampe, O., 2009. Clinical manifestations and management of patients with autoimmune polyendocrine syndrome type I. *J. Internal. Med.* 265, 514–529.
- Husebye, E.S., Løvås, K., Allolio, B., Arlt, W., Badenhoop, K., Bensing, S., et al., 2014. Consensus statement on the diagnosis, treatment and follow-up of patients with primary adrenal insufficiency. *J. Internal. Med.* 275, 104–115.
- Irvine, W.J., Barnes, E.W., 1975. Addison's disease, ovarian failure and hypoparathyroidism. *Clin. Endocrinol. Metab.* 4, 379–434.
- Jackson, R., McNicol, A.M., Farquharson, M., Foulis, A.K., 1988. Class II MHC expression in normal adrenal cortex and cortical cell in autoimmune Addison's disease. *J. Pathol.* 155, 113–120.
- Jacobson, D.L., Gange, S.J., Rose, N.R., Graham, N.M.H., 1997. Epidemiology and estimated population burden of selected autoimmune diseases in the United States. *Clin. Immunol. Immunopathol.* 84, 223–243.
- Johannsson, G., Bergthorsdottir, R., Nilsson, A.G., Lennernas, H., Hedner, T., Skrtic, S., 2009. Improving glucocorticoid replacement therapy using a novel modified-release hydrocortisone tablet: a pharmacokinetic study. *Eur. J. Endocrinol.* 161, 119–130.
- Johannsson, G., Nilsson, A.G., Bergthorsdottir, R., Burman, P., Dahlqvist, P., Ekman, B., et al., 2012. Improved cortisol exposure-time profile and outcome in patients with adrenal insufficiency: a prospective randomized trial of a novel hydrocortisone dual-release formulation. *J. Clin. Endocrinol. Metab.* 97, 473–481.
- Jones, C.M., Mallappa, A., Reisch, N., Nikolaou, N., Krone, N., Hughes, A.N., et al., 2017. Modified release and conventional glucocorticoids and diurnal androgen excretion in congenital adrenal hyperplasia. *J. Clin. Endocrinol. Metab.* 102, 1797–1806.
- Kamradt, T., Hutchinson, N.A., 2001. Tolerance and autoimmunity. *N. Engl. J. Med.* 344, 655–664.
- Kannan, C.R., 1988. Addison's disease. In: Kannan, C.R. (Ed.), *The Adrenal Gland*. Plenum, London, pp. 31–96.
- Kaufman, J., 1984. Diseases of the adrenal cortex of dogs and cats. *Mod. Vet. Pract.* 65, 513–516.
- Kendall-Taylor, P., Lambert, A., Mitchell, R., Robertson, W.R., 1988. Antibody that blocks stimulation of cortisol secretion by adrenocorticotropic hormone in Addison's disease. *Br. Med. J.* 296, 1489–1491.
- Khoury, E.L., Hammond, L., Bottazzo, G.F., Doniach, D., 1981. Surface-reactive antibodies to human adrenal cells in Addison's disease. *Clin. Exp. Immunol.* 45, 48–55.
- Kiaer, W., Rytter Norgaard, J.O., 1969. Granulomatous hypophysitis and thyroiditis with lymphocytic adrenalitis. *Acta Pathol. Microbiol. Scand.* 76, 229.
- Kintzer, P.P., Peterson, M.E., 1994. Diagnosis and management of primary spontaneous hypoadrenocorticism (Addison's disease) in dogs. *Sem. Vet. Med. Surg.* 9 (3), 148–152.
- Kluger, N., Jokinen, M., Lintulahti, A., Krohn, K., Ranki, A., 2015. Gastrointestinal immunity against tryptophan hydroxylase-1, aromatic L-amino-acid decarboxylase, AIE-75, villin and Paneth cells in APECED. *Clin. Immunol.* 158, 212–220.
- Kriegel, M.A., Lohmann, T., Gabler, C., Blank, N., Kalden, J.R., Lorenz, H.M., 2004. Defective suppressor function of human CD4+ CD25+ regulatory T cells in autoimmune polyglandular syndrome type II. *J. Exp. Med.* 199, 1285–1291.
- Koch, C.A., 2004. Adrenal cortex physiology. In: Martini, L. (Ed.), *Encyclopedia of Endocrine Disease*. Academic Press, San Diego, CA, pp. 68–74.
- Koetz, K.R., Ventz, M., Diederich, S., Quinkler, M., 2012. Bone mineral density is not significantly reduced in adult patients on low-dose glucocorticoid replacement therapy. *J. Clin. Endocrinol. Metab.* 97, 85–92.
- Kong, M.F., Jeffcoate, W., 1994. Eighty-six cases of Addison's disease. *Clin. Endocrinol.* 41, 757–761.
- Kraan, G.P., Dullaart, R.P., Pratt, J.J., Wolthers, B.G., Drayer, N.M., De Bruin, R., 1998. The daily cortisol production reinvestigated in healthy men. The serum and urinary cortisol production rates are not significantly different. *J. Clin. Endocrinol. Metab.* 83, 1247–1252.
- Krohn, K., Uibo, R., Aavik, E., Peterson, P., Savilhati, K., 1992. Identification by molecular cloning of an autoantigen associated with Addison's disease as steroid 17 $\alpha$ -hydroxylase. *Lancet* 339, 770–773.
- Larosa, M.D., Mackenzie, R., Burne, P., Garelli, S., Barollo, S., Masiero, S., et al., 2017. Assessment of autoantibodies to interferon- $\omega$  in patients with autoimmune polyendocrine syndrome type 1: using a new immunoprecipitation assay. *Clin. Chem. Lab. Med.* Available from: <https://doi.org/10.1515/cclm-2016-0615>.
- Laureti, S., De Bellis, A.M., Muccitelli, V.I., Calcinaro, F., Bizzarro, A., Rossi, R., et al., 1998. Levels of adrenocortical autoantibodies correlate with the degree of adrenal dysfunction in subjects with pre-clinical Addison's disease. *J. Clin. Endocrinol. Metab.* 83, 3507–3511.
- Laureti, S., Vecchi, L., Santeusanio, F., Falorni, A., 1999. Is the prevalence of Addison's disease underestimated? *J. Clin. Endocrinol. Metab.* 84, 1762.

- Laureti, S., Arvat, E., Candeloro, P., Di Vito, L., Ghigo, E., Santus, F., et al., 2000. Low dose (1 g) ACTH test in the evaluation of adrenal dysfunction in pre-clinical Addison's disease. *Clin. Endocrinol.* 53, 107–115.
- Lebbe, M., Aarlt, W., 2013. What is the best diagnostic and therapeutic management strategy for an Addison patient during pregnancy? *Clin. Endocrinol.* 78, 497–502.
- Leelarathna, L., Breen, L., Powrie, J.K., Thomas, S.M., Guzder, R., McGowan, B., et al., 2010. Co-morbidities, management and clinical outcome of autoimmune Addison's disease. *Endocrine* 38, 113–117.
- Levine, S., Wenk, E.J., 1968. The production and passive transfer of allergic adrenalitis. *Am. J. Pathol.* 52, 41–53.
- Lin, D., Zhang, L., Chiao, E., Miller, W.L., 1994. Modeling and mutagenesis of the active site of human P450c17. *Mol. Endocrinol.* 8, 392–402.
- Little, C., Marshall, C., Downs, J., 1989. Addison's disease in the dog. *Vet. Rec.* 124, 469–470.
- Løvås, K., Curran, S., Oksnes, M., Husebye, E.S., Huppert, F.A., Chatterjee, V.K., 2010. Development of a disease-specific quality of life questionnaire in Addison's disease. *J. Clin. Endocrinol. Metab.* 95, 545–551.
- Løvås, K., Husebye, E.S., 2007. Continuous subcutaneous hydrocortisone infusion in Addison's disease. *Eur. J. Endocrinol.* 157, 109–112.
- Løvås, K., Gjesdal, C.G., Christensen, M., Wolff, A.B., Almås, B., Svartberg, J., et al., 2009. Glucocorticoid replacement therapy and pharmacogenetics in Addison's disease: effects on bone. *Eur. J. Endocrinol.* 160, 993–1002.
- Mallappa, A., Sinaii, N., Kumar, P., Whitaker, M.J., Daley, L.A., Digweed, D., et al., 2015. A phase 2 study of Chronocort, a modified-release formulation of hydrocortisone, in the treatment of adults with classic congenital adrenal hyperplasia. *J. Clin. Endocrinol. Metab.* 100, 1137–1145.
- Mason, A.S., Meade, T.W., Lee, J.A., Morris, J.N., 1968. Epidemiological and clinical picture of Addison's disease. *Lancet* 2, 744–747.
- McIntyre Gass, J.D., 1962. The syndrome of keratoconjunctivitis, superficial moniliasis, idiopathic hypoparathyroidism and Addison's disease. *Am. J. Ophthalmol.* 54, 660–674.
- McNicol, A.M., 1986. Class II MHC expression in the adrenal cortex. *Lancet* 2, 1282.
- McNicol, A.M., Laidler, P., 1996. The adrenal gland and extra-adrenal paraganglia. In: Lewis, P.D. (Ed.), *Endocrine System*. Churchill Livingstone, Edinburgh.
- Medvei, V.C., 1993. *A History of Clinical Endocrinology*. Parthenon, Pear River, NY.
- Meyer, G., Badenhoop, K., Linder, R., 2016. Addison's disease with polyglandular autoimmunity carries a more than 2·5-fold risk for adrenal crises: German health insurance data 2010–2013. *Clin. Endocrinol. (Oxf.)* 85, 347–353.
- Mitchell, A.L., MacArthur, K.D., Gan, E.H., Baggott, L.E., Wolff, A.S., Skinningsrud, B., et al., 2014. Association of autoimmune Addison's disease with alleles of STAT4 and GATA3 in European cohorts. *PLoS One* 9, e88991. Available from: [https://doi.org/10.1371/journal.pone.0088991.eCollection](https://doi.org/10.1371/journal.pone.0088991).
- Mitchell, A.L., Bøe, Wolff, A., MacArthur, K., Weaver, J.U., Vaidya, B., et al., 2015. Correction: linkage analysis in autoimmune Addison's disease: NFATC1 as a potential novel susceptibility locus. *PLoS One* 10, e0138844. Available from: <https://doi.org/10.1371/journal.pone.0138844>.
- Morgan, J., Betterle, C., Zanchetta, R., Dal Prà, C., Chen, S., Rees Smith, B., et al., 2000. Direct evidence that steroid 21-hydroxylase (21-OH) is the major antigen recognized by adrenal cortex autoantibodies (ACA). *J. Endocrinol.* 167, OC19.
- Muscatelli, F., Strom, T.M., Walker, A.P., Zanaria, E., Recan, D., Meindl, A., et al., 1994. Mutations in the DAX-1 gene give rise to both x-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. *Nature* 372, 672–676.
- Myhre, A.G., Undelien, D.A., Lovas, K., Uhlving, S., Nedrebo, B.G., Fougnier, K.J., et al., 2002. Autoimmune adrenocortical failure in Norway autoantibodies and human leukocyte antigen class II association related to clinical features. *J. Clin. Endocrinol. Metab.* 87, 618–623.
- Napier, C., Pearce, S.H.S., 2012. Autoimmune Addison's disease. *La Presse Médicale* 41, e626–e635.
- Naletto, L., Frigo, A., Ceccato, F., Sabbadin, C., Scarpa, R., Presotto, F., et al., 2019. The natural history of autoimmune Addison's disease from the detection of autoantibodies to development of the disease: a long follow-up study on 143 patients. *Eur. J. Endocrinol. Pii, EJE-18-0313.R3*.
- Nella, A.A., Mallappa, A., Perritt, A.F., Gounden, V., Kumar, P., Ninet Sinaii, N., et al., 2016. A phase 2 study of continuous subcutaneous hydrocortisone infusion in adults with congenital adrenal hyperplasia. *J. Clin. Endocrinol. Metab.* 101, 4690–4698.
- Nerup, J., 1974. Addison's disease—clinical studies. A report of 108 cases. *Acta Endocrinol.* 76, 121–141.
- Nerup, J., Bendixen, G., 1969. Anti-adrenal cellular hypersensitivity in Addison's disease. 2. Correlation with clinical and serological findings. *Clin. Exp. Immunol.* 5, 341–353.
- Nerup, J., Andersen, V., Bendixen, G., 1970. Antiadrenal cellular hyper-sensitivity in Addison's disease. IV. In vivo and in vitro investigations of the mitochondrial fraction. *Clin. Exp. Immunol.* 6, 733.
- Neufeld, M., Blizzard, R.M., 1980. Polyglandular autoimmune diseases. In: Pinchera, A., Doniach, D., Fenzi, G.F., Baschieri, L. (Eds.), *Symposium on Autoimmune Aspects of Endocrine Disorders*. Academic Press, New York, pp. 357–365.
- Nigam, R., Bhatia, E., Miao, D., Brozzetti, A., Eisenbarth, G.S., Falorni, A., 2003. Prevalence of adrenal antibodies in Addison's disease among North Indian Caucasians. *Clin. Endocrinol.* 59, 593–598.
- Neumann, U., Whitaker, M.J., Wiegand, S., Krude, H., Porter, J., Davies, M., et al., 2018. Absorption and tolerability of taste-masked hydrocortisone granules in neonates, infants and children under 6 years of age with adrenal insufficiency. *Clin. Endocrinol. (Oxf.)* 88, 21–29.
- Oelkers, W., 1996. Adrenal insufficiency. *N. Engl. J. Med.* 335, 1206–1212.
- Oelkers, W., Diederich, S., Bahr, V., 1992. Diagnosis and therapy surveillance in Addison's disease: rapid adrenocorticotropin (ACTH) test and measurement of plasma ACTH, renin activity and aldosterone. *J. Clin. Endocrinol. Metab.* 75, 259–264.
- Øksnes, M., Bensing, S., Hulting, A.L., Kämpe, O., Hackemann, A., Meyer, G., et al., 2012. Quality of life in European patients with Addison's disease: validity of the disease-specific questionnaire AddiQoL. *J. Clin. Endocrinol. Metab.* 97, 568–576.
- Olafsson, A.S., Sigurjonsdottir, H.A., 2016. Increasing prevalence of Addison's disease. Results from a Nationwide study. *Endocr. Pract.* 22, 30–35.
- Orth, D.N., Kavacs, W.J., 1998. The adrenal cortex. In: Wilson, J.D., Foster, D.W., Kronenberg, H.M., Larse, P.R. (Eds.), *Williams Textbook of Endocrinology*, ninth ed. W.B. Saunders, Philadelphia, PA, pp. 517–664.
- Pearce, S.H., Mitchell, A.L., Bennett, S., King, P., Chandran, S., Nag, S., et al., 2012. Adrenal steroidogenesis after B lymphocyte depletion therapy in new-onset Addison's disease. *J. Clin. Endocrinol. Metab.* 97, E1927–E1932.

- Pelkey, T.J., Frierson Jr., H.F., Mills, S.E., Stoler, M.H., 1998. The alpha subunit of inhibin in adrenal cortical neoplasia. *Mod. Pathol.* 11, 516–524.
- Peterson, M.E., Greco, D.S., Orth, D.N., 1989. Primary hypoadrenocorticism in ten cats. *J. Vet. Intern. Med.* 3, 55–58.
- Peterson, P., Krohn, K.J.E., 1994. Mapping of B cell epitopes on steroid 17- $\alpha$ -hydroxylase, an autoantigen in autoimmune polyglandular syndrome type 1. *Clin. Exp. Immunol.* 98, 104–109.
- Peterson, P., Salmi, H., Hyöty, H., Miettinen, A., Ilonen, J., Reijonen, H., et al., 1997. Steroid 21-hydroxylase autoantibodies in insulin-dependent diabetes mellitus. Childhood Diabetes in Finland (DiMe) Study Group. *Clin. Immunol. Immunopathol.* 82, 37–42.
- Petri, M., Nerup, J., 1971. Addison's adrenalitis. *Acta Path. Microbiol. Scand.* 79, 381–388.
- Picado-Leonard, J., Miller, W.L., 1987. Cloning and sequence of the human gene for P450c17 (steroid 17 $\alpha$ -hydroxylase/17,20 lyase): similarity with the gene for P450c21. *DNA Cell Biol.* 6, 439–448.
- Quinkler, M., Dahlqvist, P., Husebye, E.S., Kämpe, O., 2015a. A European Emergency Card for adrenal insufficiency can save lives. *Eur. J. Intern. Med.* 26, 75–76.
- Quinkler, M., Miodini, Nilsen, R., Zopf, K., Ventz, M., Øksnes, M., 2015b. Modified-release hydrocortisone decreases BMI and HbA1c in patients with primary and secondary adrenal insufficiency. *Eur. J. Endocrinol.* 172, 619–626.
- Rabinowicz, S.L., Jackson, R.A., Dluhy, R.G., Williams, G.H., 1984. Ia-positive T lymphocytes in recently diagnosed idiopathic Addison's disease. *Am. J. Med.* 77, 597–601.
- Raff, H., 2009. Utility of salivary cortisol measurements in Cushing's syndrome and adrenal insufficiency. *J. Clin. Endocrinol. Metab.* 94, 3647–3655.
- Reato, G., Morlin, L., Chen, S., Furmaniak, J., Smith, B.R., Masiero, S., et al., 2011. Premature ovarian failure in patients with autoimmune Addison's disease: clinical, genetic, and immunological evaluation. *J. Clin. Endocrinol. Metab.* 96, E1255–E1261.
- Rees Smith, B., Furmaniak, J., 1995. Editorial: adrenal and gonadal autoimmune diseases. *J. Clin. Endocrinol. Metab.* 80, 1502–1505.
- Sadek, D., Schaer, M., 1996. Atypical Addison's disease in the dog: a retrospective survey of 14 cases. *J. Am. Anim. Hosp. Assoc.* 32, 159–163.
- Salim, Y.S., Faber, V., Wiik, A., Andersewn, P.L., Hoier-Madsen, M., Mouritsen, S., 1988. Anti-corticosteroid antibodies in AIDS patients. *Acta Pathol. Microbiol. Immunol. Scand.* 96, 889–894.
- Salvetti, M., Ristori, G., Bompuzzi, R., Pozzilli, P., Leslie, R.D.G., 2000. Twins: mirrors of the immune system. *Immunol. Today* 21, 342–347.
- Sasano, H., Nose, M., Sasano, N., 1989. Lectin histochemistry in adrenocortical hyperplasia and neoplasm with emphasis on carcinoma. *Arch. Pathol. Lab. Med.* 113, 68–72.
- Sasano, H., Suzui, T., Shizawa, S., Kato, K., Natura, H., 1994. Transforming growth factor alpha, epidermal growth factor and epidermal growth factor receptor in normal and diseased human adrenal cortex by immunohistochemistry and in situ hybridization. *Mod. Pathol.* 7, 741–746.
- Scarpa, R., Alaggio, R., Norberto, L., Furmaniak, J., Chen, S., Smith, B.R., et al., 2013. Tryptophan hydroxylase autoantibodies as markers of a distinct autoimmune gastrointestinal component of autoimmune polyendocrine syndrome type 1. *J. Clin. Endocrinol. Metab.* 98, 704–712.
- Simmonds, J.P., Lister, J., 1978. Auto-immune Addison's disease in identical twins. *Postgrad. Med. J.* 54, 552–554.
- Simunkova, K., Jovanovic, N., Rostrup, E., Methlie, P., Øksnes, M., Roy Nilsen, R.M., et al., 2016. Effect of a pre-exercise hydrocortisone dose on short-term physical performance in female patients with primary adrenal failure. *Eur. J. Endocrinol.* 174, 97–105.
- Skoldberg, F., Portela-Gomes, G.M., Grimelius, L., Nilsson, G., Perheentupa, J., Betterle, C., et al., 2003. Histidine decarboxylase is a novel autoantigen of enterochromaffin-like cells in autoimmune polyendocrine syndrome type 1. *J. Clin. Endocrinol. Metab.* 88, 1445–1452.
- Söderbergh, A., Myhre, A.G., Ekwall, O., Gebre-Medhin, G., Hedstrand, H., Landgren, E., et al., 2004. Prevalence and clinical associations of 10 defined autoantibodies in autoimmune polyendocrine syndrome type I. *J. Clin. Endocrinol. Metab.* 89, 557–562.
- Sotsiou, F., Bottazzo, G.F., Doniach, D., 1980. Immunofluorescence studies on autoantibodies to steroid-producing cells, and to germline cells in endocrine disease and infertility. *Clin. Exp. Immunol.* 39, 97–111.
- Steiner, J.W., Langer, B., Schatz, D.L., Volpé, R., 1960. Experimental immunologic adrenal injury: a response to injections of autologous and homologous adrenal antigens in adjuvant. *J. Exp. Med.* 112, 187.
- Stewart, P.M., Newell-Price, J.D.C., 2016. The adrenal cortex. *Williams Textbook of Endocrinology*. Elsevier Inc.
- Stewart, P.M., Corrie, J., Seckl, J.R., Edwards, C.R., Padfield, P.L., 1988. A rational approach for assessing the hypothalamo-pituitary-adrenal axis. *Lancet* 1, 1208–1210.
- Stonehewer, J., Tasker, S., 2001. Hypoadrenocorticism in a cat. *J. Small Anim. Pract.* 42, 186–190.
- Takayanagi, R., Miura, K., Nakagawa, H., Nawata, H., 2000. Epidemiological study of adrenal gland disorders in Japan. *Biomed. Pharmacother.* 54, 164–168.
- Tanaka, H., Perez, M.S., Powell, M., Sandres, J.F., Sawicka, J., Chen, S., et al., 1997. Steroid 21-hydroxylase autoantibodies: measurements with a new immunoprecipitations assay. *J. Clin. Endocrinol. Metab.* 82, 1440–1446.
- Tasker, S., MacKay, A.D., Sparkes, A.H., 1999. Case report. A case of feline primary hypoadrenocorticism. *J. Felin. Med. Surg.* 1, 257–260.
- Thiebaut, F., Tsuruo, T., Hamada, H., Gottesman, M.M., Pastan, I., Willingham, M.C., 1987. Cellular localization of the multidrug resistance gene product P-glycoprotein in normal human tissues. *Proc. Natl. Acad. Sci. U.S.A.* 84, 7735–7738.
- Vergheese, M.V., Ward, F.E., Eisenbarth, G.S., 1980. Decreased suppressor cell activity in patients with polyglandular failure. *Clin. Res.* 28, 270A.
- Trousseau, A., 1856. Bronze Addison's disease. *Arch. Gen. Med.* 8, 478–485.
- Wardle, C.A., Weetman, A.P., Mitchell, R., Peers, N., Robertson, W.R., 1993. Adrenocorticotrophic hormone receptor-blocking immunoglobulins in serum from patients with Addison's disease: a re-examination. *J. Clin. Endocrinol. Metab.* 77, 750–753.
- Wass, J.A.H., Arlt, W., 2012. How to avoid precipitating an acute adrenal crisis. *Br. Med. J.* 345, e6333.
- Wedlock, N., Asawa, T., Baumann-Antczak, A., Rees Smith, B., Furmaniak, J., 1993. Autoimmune Addison's disease. Analysis of autoantibody sites on human steroid 21-hydroxylase. *FEBS Lett.* 332, 123–126.
- Werdelin, O., Witebsky, E., 1970. Experimental allergic rat adrenalitis, a study on its elicitation and lymphokinetics. *Lab. Invest.* 23, 136–143.
- Werdelin, O., Wick, G., McCluskey, R.T., 1971. The fate of newly formed lymphocytes migration from an antigen-stimulated lymph node in rats with allergic adrenalitis. *Lab. Invest.* 25, 279–286.

- Whitaker, M.J., Debono, M., Huatan, H., Merke, D.P., Arlt, W., Ross, R.J., 2014. An oral multiparticulate, modified-release, hydrocortisone replacement therapy that provides physiological cortisol exposure. *Clin. Endocrinol. (Oxf.)* 80, 554–561.
- Whitaker, M.J., Spielmann, S., Digweed, D., Huata, H., Eckland, D., Johnson, T.N., et al., 2015. Development and testing in healthy adults of oral hydrocortisone granules with taste masking for the treatment of neonates and infants with adrenal insufficiency. *J. Clin. Endocrinol. Metab.* 100, 1681–1688.
- Williams, G.H., Dluhy, R.G., 1998. Disease of the adrenal cortex. In: Fauci, A.S., Braunwald, E., Isselbacher, K.J. (Eds.), *Harrison's Principles of Internal Medicine*, 14th ed. McGraw-Hill, New York.
- Willis, A.C., Vince, F.P., 1997. The prevalence of Addison's disease in Coventry, UK. *Postgrad. Med. J.* 73, 286–288.
- Wilson, R.C., Mercado, A.B., Cheng, K.C., New, M.I., 1995. Steroid 21-hydroxylase deficiency: genotype may not predict phenotype. *J. Clin. Endocrinol. Metab.* 80, 2322–2329.
- Winqvist, O., Soderbergh, A., Kampe, O., 1996. The autoimmune basis of adrenal cortical destruction in Addison's disease (review). *Mol. Med. Today* 2, 282–289.
- Winqvist, O., Karlsson, F.A., Kampe, O., 1992. 21-hydroxylase, a major autoantigen in idiopathic Addison's disease. *Lancet* 339, 1559–1562.
- Winqvist, O., Gustafsson, J., Rorsman, F., Karlsson, F.A., Kampe, O., 1993. Two different cytochrome P450 enzymes are the adrenal anti-gens in autoimmune polyendocrine syndrome type I and Addison's disease. *J. Clin. Invest.* 92, 2377–2385.
- Witebsky, E., Milgrom, F., 1962. Immunological studies on adrenal glands: II. Immunization with adrenals of the same species. *Immunology* 5, 67–78.
- Wulffraat, N.M., Drexhage, H.A., Bottazzo, G.F., Wiersinga, W.M., Jeucken, P., Van der Gaag, R., 1989. Immunoglobulins of patients with idiopathic Addison's disease block the in vitro action of adrenocortropin. *J. Clin. Endocrinol. Metab.* 69, 231–238.
- Yu, L., Brewer, K.W., Gates, S., Wu, A., Wang, T., Babu, S.R., et al., 1999. DRB1\*04 and DQ alleles: expression of 21-hydroxylase autoantibodies and risk of progression to Addison's disease. *J. Clin. Endocrinol. Metab.* 84, 328–335.

## Autoimmune Hypophysitis

*Giulia Di Dalmazi, Patrizio Caturegli and Paulina Chalan*

Department of Pathology, The Johns Hopkins University, School of Medicine, Baltimore, MD, United States

### OUTLINE

Definition and Classification	815	Diagnosis	825
Historical Background	816	Treatment	825
Epidemiology and Body of Literature	816	Outcome	826
Clinical Features	818	Hypophysitis Secondary to Cytotoxic T Lymphocyte Antigen 4 Blockade	826
Pathological Features	822	Concluding Remarks—Future Perspectives	828
Autoimmune Features	823	Acknowledgments	828
Genetic and Environmental Influences	824	References	828
Animal Models	824		

### DEFINITION AND CLASSIFICATION

Autoimmune hypophysitis is a chronic inflammation of the pituitary gland that can be classified according to anatomic location, histopathology, or cause (Leporati et al., 2011).

Location distinguishes hypophysitis into adenohypophysitis, infundibulo-neurohypophysitis, or panhypophysitis depending on whether the clinical and radiological signs (and pathological findings if available) involve the anterior lobe, the posterior lobe and the stalk, or both structures.

Histopathology identifies two main forms of hypophysitis, lymphocytic and granulomatous, as well as xanthomatous, IgG4 plasmacytic, necrotizing, and mixed variants.

Etiology distinguishes primary and secondary hypophysitis. The primary hypophysitis refers to the cases that do not currently have an identifiable cause. It is the most common form of hypophysitis, has an autoimmune pathogenesis, and occurs in isolation or associated with other well-characterized autoimmune diseases. The secondary hypophysitis includes the cases where a clear etiological agent can be identified [e.g., the administration of immunomodulatory drugs such as cytotoxic T lymphocyte antigen 4 (CTLA-4) blocking antibody], the cases where the inflammation of the pituitary is considered a reaction to sellar diseases (Rathke's cleft cyst, craniopharyngioma, germinoma, and pituitary adenomas), and the cases where hypophysitis is part of a multiorgan systemic involvement (such as Wegener's granulomatosis, tuberculosis, sarcoidosis, or syphilis).

In the past few years, an explosion of cases of hypophysitis secondary to the pharmacological treatment has been reported in cancer patients, especially those treated with immune-checkpoint inhibitors such as the CTLA-4 blocking antibody. These treatments can cause hypophysitis as a side effect and have transformed a traditionally rare disease into a common endocrinological abnormality.

## HISTORICAL BACKGROUND

Autoimmune hypophysitis of the anterior lobe (lymphocytic adenohypophysitis, LAH) was first described by [Goudie and Pinkerton \(1962\)](#) in Glasgow. The authors reported a 22-year-old woman who died 14 months after her second delivery, probably because of adrenal insufficiency. Two months before the admission, the patient felt increasingly tired and noticed a neck enlargement; she then developed severe lower abdominal pain, radiating to the right iliac fossa, associated with vomiting and diarrhea. She was brought to the operating room for suspected appendicitis. Surgery revealed an acutely inflamed, gangrenous appendix that, however, had not ruptured. The appendix was removed but 8 hours later the patient went into peripheral circulatory shock and died. The autopsy revealed a firm, enlarged thyroid gland, infiltrated by lymphocytes, atrophic adrenal glands, and a small pituitary. Surprisingly, for that time, the adenohypophysis was extensively infiltrated by lymphocytes and few plasma cells, aggregating in some areas into true lymphoid follicles. The neurohypophysis was normal. Noting the presence of Hashimoto's thyroiditis, a more extensively characterized autoimmune disease, the authors concluded their discussion by writing: "It seems reasonable to assume that the coexistence of Hashimoto's disease and the mononuclear cells infiltration of the anterior pituitary is not fortuitous. Both may be explained by the onset of autoimmune reaction to thyroid and pituitary antigens released during the puerperal involution of these glands." There is no doubt that Goudie and Pinkerton were the first to postulate the autoimmune nature of this condition, at a time when the field of autoimmunity had just begun. Earlier cases, however, are probably hidden in hospital archives or published without the recognition of the disease ([Duff and Bernstein, 1933](#); [Rupp and Paschkis, 1953](#)).

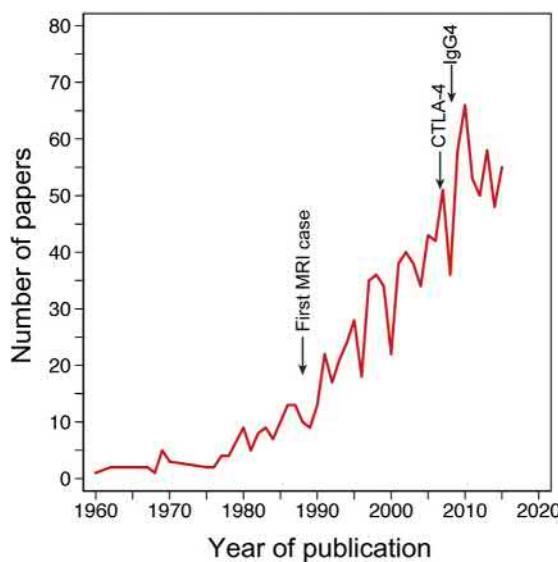
Autoimmune hypophysitis of the posterior lobe and infundibulum (lymphocytic infundibuloneurohypophysitis, LINH) was first described by [Saito et al. \(1970\)](#) in Tokyo. The authors reported a 66-year-old asthmatic woman with a 1-month history of severe dehydration that responded strikingly to the administration of pitressin. Two months after the discharge, however, she developed a severe attack of bronchial asthma and died. Autopsy revealed a marked infiltration of the neurohypophysis and the infundibular stem with lymphocytes and plasma cells, aggregating in some areas in lymphoid follicles. The adenohypophysis was normal, except for vacuolar degeneration of the basophilic cells, likely due to the prolonged use of steroids for asthma.

Autoimmune hypophysitis involving both the anterior and posterior lobe (lymphocytic panhypophysitis, LPH) was first described histologically by [Nussbaum et al. \(1991\)](#) in New York. It was a 40-year-old male with a 3-month history of headache, impotence, polyuria and polydipsia, and a sellar mass abutting the optic chiasm. Transsphenoidal surgery showed that the sella turcica was filled with whitish, fibrous tissue that was almost completely removed. Histology revealed extensive infiltration of the adenohypophysis and the neurohypophysis by lymphocytes, plasma cells, and histiocytes.

Hypophysitis secondary to blockade of CTLA-4 was first reported in 2003 by Phan et al. in Bethesda. It was a 54-year-old male with advanced melanoma metastatic to the lungs and brain who was treated with the CTLA-4 blocking antibody ipilimumab and vaccination with melanoma peptides ([Phan et al., 2003](#)).

## EPIDEMIOLOGY AND BODY OF LITERATURE

Very limited data exist to estimate the incidence of primary autoimmune hypophysitis. Sautner and Fehn analyzed 2500 surgical pituitary specimens collected at Hamburg, Germany, from 1970 to 1996 and found 6 cases (0.24%) ([Fehn et al., 1998](#); [Sautner et al., 1995](#)). Honegger et al. (1997) analyzed 2362 specimens collected from 1982 to 1995 in Erlangen, Germany, and found 7 cases (0.3%). [Leung et al. \(2004\)](#) reported in Charlottesville Virginia 13 cases of autoimmune hypophysitis among 2000 patients who underwent transsphenoidal surgery for pituitary mass lesions from 1992 to 2003 (0.65%). [Buxton and Robertson \(2001\)](#) analyzed 619 consecutive pituitary surgeries performed over 15 years at Nottingham, United Kingdom, and found 5 cases (0.8%). Considering that their hospital served a population of approximately 3 million and that all surgery for pituitary masses was dealt there, the yearly incidence in Nottingham can be estimated to be one case every 10 million people. We recently reviewed all the pituitary surgeries performed at the Johns Hopkins Hospital, from the first US operation performed by Harvey Cushing on March 25, 1909 to July 2017 ([Corsello et al., 2017](#)) and then updated the last year for this chapter. Of the total 3312 pituitary surgeries performed at Johns Hopkins in 109 years, there were 38 cases of hypophysitis (1.1%). Overall, the incidence is likely an underestimate of today's incidence, also considering that some cases of autoimmune hypophysitis cases go undiagnosed because of their indolent, subclinical course.



**FIGURE 43.1** Yearly trend of papers published on hypophysitis and pituitary autoimmunity.

At the Department of Pathology of the Johns Hopkins Hospital, we maintain a database of the papers published on hypophysitis, and pituitary autoimmunity in general, as well as of clinical characteristics of the featured patients (<http://pathology2.jhu.edu/hypophysitis>). A total of 1286 papers have been published as of September 2018, featuring 3175 different authors and 12 languages. The top two publishing authors currently are Patrizio Caturegli (36 articles, The Johns Hopkins University, Baltimore, MD) and Annamaria De Bellis (35 articles, Federico II University of Naples, Naples, Italy). Of the languages, 1075 articles (84%) were written in English, 75 (6%) in Japanese, 34 (3%) in French, 33 (3%) in Spanish, and the remaining 69 articles in 11 other languages. The number of hypophysitis papers published in each calendar year has increased significantly over time, reaching a peak of 66 in 2010 (Fig. 43.1). The causes underlying this increase are multifactorial: in part they relate to the widespread introduction of noninvasive imaging techniques of the sella turcica (mainly MRI); in part to the expansion of the spectrum of pituitary autoimmunity, which now includes forms of hypophysitis that were not existing a few years ago: for example, the IgG4-related form hypophysitis and the form secondary to blockade of CTLA-4; and in part to the increased awareness of hypophysitis in the medical community.

This body of literature describes over 1000 cases with primary hypophysitis, where the diagnosis was established by either surgical pathology (No. = 631), clinical, and imaging criteria (No. = 331), or autopsy (Table 43.1). Patients have been reported in 42 of the 193 countries in the world, but most prominently in Japan and United States (Fig. 43.2).

LAH is more common in women (F:M ratio of 3:1), who present at a younger age ( $38 \pm 14$ ) than men ( $50 \pm 17$ ). In approximately half of the women (49%), LAH manifests during late pregnancy or early postpartum (Fig. 43.3). A history of previous pregnancies does not increase the risk of developing LAH in subsequent pregnancies. This striking temporal association is one of the most interesting features of autoimmune hypophysitis and, at the moment, remains unexplained. LINH affects equally males and females (F:M ratio of 1:1), has a mean age at presentation of 42 ( $\pm 20$ ) years, and is not associated with pregnancy. LPH is slightly more common in women (F:M ratio of 1.4), has similar age at presentation in both sexes ( $43 \pm 17$  years), and does not show association with pregnancy.

Hypophysitis secondary to CTLA-4 blockade reflects the epidemiology of the underlying oncologic populations where the treatment is used. Most commonly, these are patients with not only metastatic melanoma but also renal cell carcinoma, nonsmall cell lung cancer, prostate cancer, and pancreatic cancer. These patients, therefore, are mainly men (80%) and of older ages ( $58 \pm 12$  years) than those with primary hypophysitis. With one notable exception (Caturegli et al., 2016), the diagnosis of secondary hypophysitis has been established by clinical and imaging criteria (rather than by pathology). The single case where pathology was available showed a remarkable destruction of the pituitary gland, as well as the expression of CTLA-4 on the few remaining endocrine cells (Caturegli et al., 2016).

**TABLE 43.1** Key Clinical Features of Primary Hypophysitis and Hypophysitis Secondary to CTLA-4 Blockade

Features	Primary hypophysitis	Hypophysitis secondary to CTLA-4 blockade	P value
No. of published patients: <sup>a</sup>	1005 <sup>a</sup>	148 <sup>a</sup>	
Diagnosis established by:			
Pathology: surgical pathology	631	0	
Autopsy	43	1	
Clinical and imaging criteria	331	147	
F:M numbers (ratio):	718:287 (2.5:1)	30:118 (1:4)	<.001
Mean age at onset (in years):	41 ± 16	59 ± 13	<.001
Time after the initiating event: (mean ± SD)	Unknown, likely years	10 ± 5 weeks after first antibody injection	
Symptoms at presentation:			
Headache	47% (397 of 852)	60% (89 of 148)	
Due to low cortisol	35% (288 of 824)	72% (106 of 148)	.002
Polydipsia and polyuria	35% (297 of 845)	0.9% (1 of 116)	<.001
Visual disturbances	31% (264 of 861)	3% (4 of 117)	<.001
Due to low sex steroids	20% (168 of 834)	15% (17 of 112)	<.001
Due to low thyroxine	16% (132 of 824)	20% (22 of 112)	

<sup>a</sup>Meeting abstracts and pooled patients from clinical trials are not included.

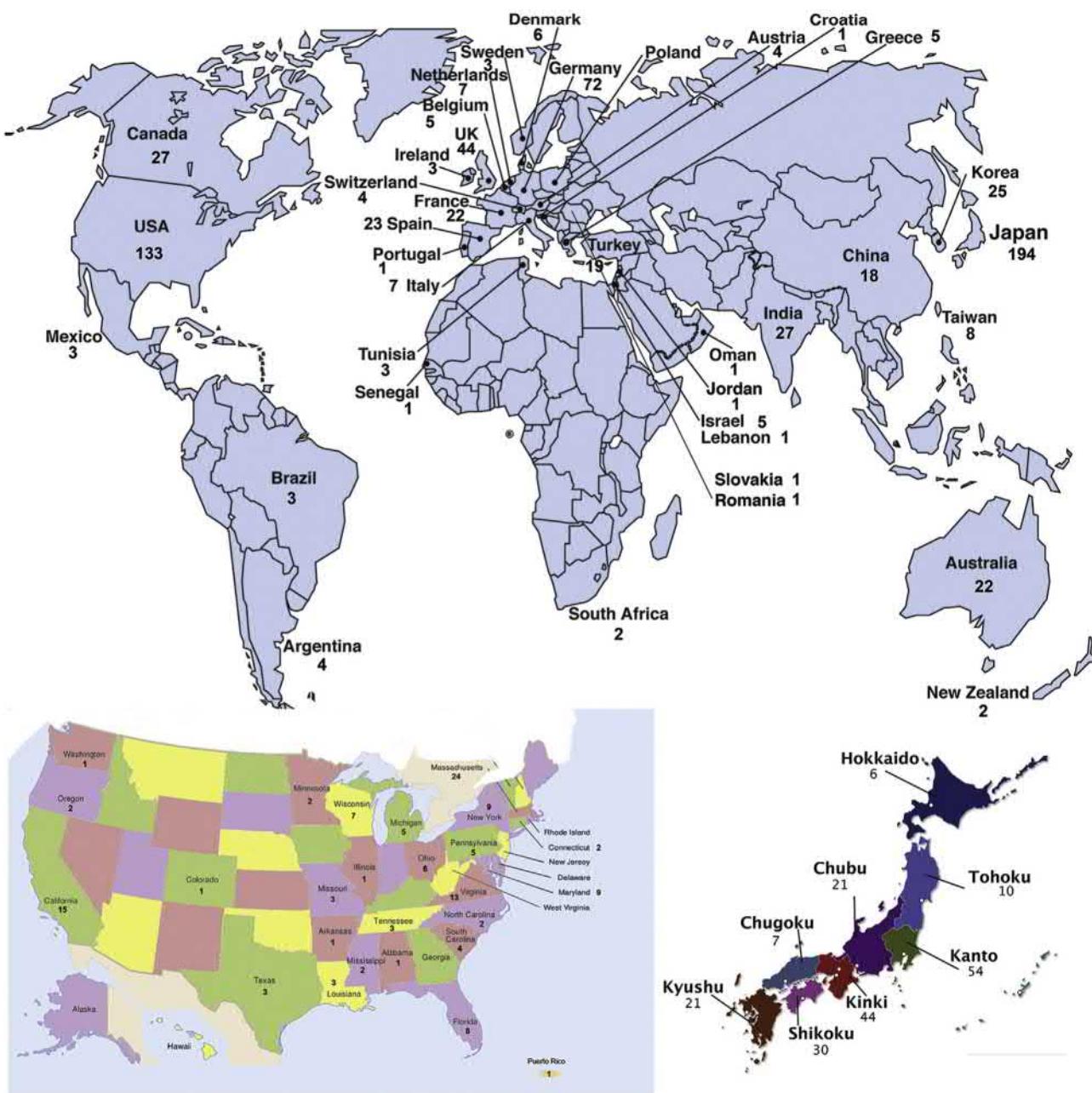
CTLA-4, Cytotoxic T lymphocyte antigen 4.

## CLINICAL FEATURES

The clinical presentation of autoimmune hypophysitis includes four categories of symptoms.

The most common symptoms are those related to the mass effect, that is, to the compression the enlarged pituitary exerts upon the nearby sellar structures. In fact, a pituitary gland that is infiltrated by autoreactive lymphocytes initially enlarges, forming a sellar mass that expands usually upward, impinging upon the optic chiasm and the dura mater, or laterally to invade the cavernous sinuses or temporal fossae. Headache is the most common presenting symptom of hypophysitis and occurs in about 50% of the patients (Table 43.1) (Caturegli et al., 2016; Honegger et al., 2015; Wang et al., 2017). It is sudden, severe, and often generalized. Visual disturbances originate from the compression of the optic chiasm and typically include defects in the temporal quadrants of the visual fields and occasionally deterioration of visual acuity. The encroachment of the cavernous sinus can cause diplopia and pupillary abnormalities from compression of the third, fourth, and sixth cranial nerves. The involvement of the branches of the trigeminal nerve can result in orbital pain or facial paresthesias. Bilateral intracavernous carotid artery occlusion is a rare complication of aggressive lymphocytic hypophysitis (Katsiveli et al., 2016). Although the onset of the mass effect symptoms is often sudden, it can vary greatly from insidious to super acute, mimicking pituitary apoplexy (Hasegawa et al., 2018) or meningitis (Suzuki et al., 2011).

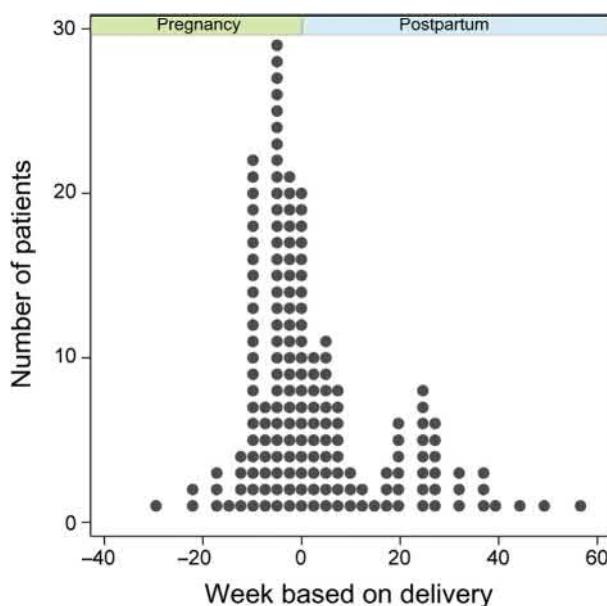
The second most common symptoms are those originating from the defective production of the anterior pituitary hormones and usually present more insidiously. These defects are considered the direct consequence of the attack of the patient's autoreactive lymphocytes onto the hormone-producing cells, although they can also originate from mass effect. The defects can be completed (pan-hypopituitarism) when all five adenohypophyseal axes (corticotroph, thyrotroph, gonadotroph, growth hormone, and prolactin) are impaired, partial when more than one axis is involved, or isolated when only one axis is involved. Corticotropin deficiency is the most common endocrine abnormality (Table 43.2) (Caturegli et al., 2016), causing symptoms such as general malaise, hypotension, nausea, vomiting, dizziness, and loss of pubic and axillary hair. The resulting hypocortisolism is often severe and can be life-threatening if not promptly recognized and treated. Gonadotropin (decreased libido, impotence, and amenorrhea) and thyrotropin (fatigue, lethargy, cold intolerance, and weight gain) deficiencies are also frequently reported in recent large series (Honegger et al., 2015; Wang et al., 2017). The defects of the growth hormone/IGF-1 axis are considered clinically silent in adults, and therefore it is not surprising that this is the



**FIGURE 43.2** Geographical distribution of published patients with the primary hypophysitis.

least studied axis in this patient population. In many of the published patients, there was no information about the growth hormone axis, so it remains to be established whether somatotrophs cells are not frequently targeted by the patient immune system or rather their involvement has not been systematically investigated. Prolactin deficiency, manifested in women as inability to lactate after delivery, is rare but when present should alert the astute clinician on a possible diagnosis of hypophysitis (Iwama et al., 2013).

The third group of symptoms are those due to not only deficit of the posterior pituitary (polyuria and polydipsia), which can be attributed to direct immune destruction of the neurohypophysis and infundibulum, but also compression of these structures. Diabetes insipidus is the defining symptom of LINH, but it can also be observed in about a third of the patients who have defects in the anterior pituitary axes. Diabetes insipidus has been recently identified as a negative prognostic factor in patients with primary autoimmune hypophysitis (Lupi et al., 2017).



**FIGURE 43.3** Distribution of symptom appearance in relation to delivery (indicated as week 0).

**TABLE 43.2** Endocrine Abnormalities Reported at Diagnosis in Patients With Primary and Secondary Hypophysitis

	Primary hypophysitis (%)	Secondary hypophysitis (%)
Secondary hypocortisolism	60	91
Secondary hypogonadism	55	83
Secondary hypothyroidism	52	84
Central diabetes insipidus	39	1
Hyperprolactinemia	37	9
Decreased growth hormone	38	43

Last are the signs of hyperprolactinemia (mainly amenorrhea/oligomenorrhea and galactorrhea), which is due to compression and/or deviation of the infundibular stalk. Hyperprolactinemia is observed in about a third of the patients (Table 43.1).

Autoimmune hypophysitis frequently occurs in association with the other autoimmune diseases, such as Hashimoto's thyroiditis, Graves' disease, or Sjögren's syndrome (Angelousi et al., 2018; Caturegli et al., 2005; Chiloiro et al., 2017; Honegger et al., 2015; Wang et al., 2017), whose specific signs and symptoms enrich and diversify the clinical presentation of hypophysitis.

Primary hypophysitis is uncommon in children, with less than 100 cases reported in literature as reviewed by Kalra et al. (2011). Juvenile hypophysitis differs markedly from adult hypophysitis in the clinical features. Headache is reported much more infrequently (17%) in children than in adults (50%), although an improper communication of this symptoms may be partly responsible for the difference. Diabetes insipidus and growth hormone deficiency are the most common endocrine disorders in children, whereas visual disturbances and hypocortisolism are rare (Kalra et al., 2011).

The clinical features of autoimmune hypophysitis are indistinguishable from those caused by the other non-hormone secreting masses arising in the sella turcica (Table 43.3). Thus although per se rare, hypophysitis enters in the differential diagnosis of about 30 conditions, all characterized by the presence of a sellar mass. Currently, hypophysitis can only be diagnosed with certainty by pituitary biopsy. However, a pathological specimen is not always necessary since a presumptive diagnosis of hypophysitis can often be made on the basis of a combination of clinical and radiological features. Clinical features suggestive of hypophysitis include the association with

**TABLE 43.3** Classification of Sellar Masses With Approximate Frequency Distribution

Disease	% of all sellar masses
Hormone-secreting pituitary adenomas:	55
PRL-secreting adenoma	42
ACTH-secreting adenoma	8
GH-secreting adenoma	5
TSH-secreting adenoma	0.25
Nonhormone-secreting sellar masses:	45
Developmental lesions:	
Rathke's cleft cyst	3.95
Arachnoid cyst	0.50
Dermoid and epidermoid cysts	0.40
Vascular lesions:	
Pituitary apoplexy (caused by hemorrhagic necrosis)	0.08
Intrasellar aneurysm	0.06
Intrasellar angioma or hemangiopericytoma	0.02
Infectious lesions:	
Tuberculosis	0.14
Pituitary abscess	0.13
Fungal infections	0.12
Autoimmune and inflammatory lesions:	
Hypophysitis	0.85
Sphenoid sinus mucocele	0.13
Sarcoidosis	0.12
Wegener's granulomatosis	0.06
Langerhans cell histiocytosis	0.06
Benign tumors:	
Nonfunctioning (null cell) pituitary adenoma	32.0
Craniopharyngioma	2.00
Chordoma	1.30
Meningioma	1.00
Glioma	0.05
Pituicytoma	0.02
Spindle cell oncocytoma	0.02
Malignant tumors:	
Metastasis (mainly from breast and lung cancer)	1.20
Germinoma	0.24
Lymphoma, plasmacytoma	0.12
Astrocytoma	0.15
Pituitary carcinoma	0.02
Teratoma	0.02

TSH, thyroid stimulating homorne; GH, growth hormone; PRL, prolactin; ACTH, adreno-corticotroph homone.

pregnancy or postpartum, the presence of other autoimmune conditions in the same patient (comorbidity) or family (familial aggregation), and a relatively rapid development of hypopituitarism, which is often disproportionate to the size of the pituitary mass. MRI remains the most powerful tool currently available to aid in the differential diagnosis and will be described in details below.

## PATHOLOGICAL FEATURES

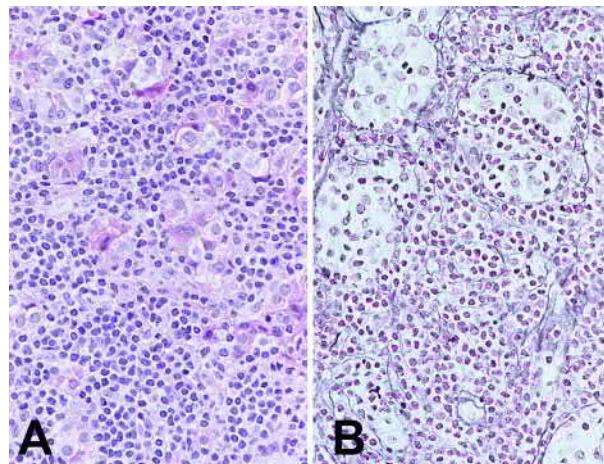
Primary hypophysitis comprises five pathological variants: lymphocytic, granulomatous, xanthomatous, IgG4 plasmacytic, and necrotizing (Table 43.1).

Lymphocytic hypophysitis, first reported by Goudie and Pinkerton in 1962, is the most common form of hypophysitis. It is characterized by the infiltration of the pituitary gland with lymphocytes (Fig. 43.4A), sometimes aggregating into lymphoid follicles with germinal centers. Immunohistochemistry reveals a mixture of T and B lymphocytes (Gutenberg et al., 2005), without a dominant subset, as is seen in other autoimmune diseases. The additional hematopoietic cell types that are found in the infiltrate include plasma cells, eosinophils, macrophages, histiocytes and neutrophils, and mast cells (Vidal et al., 2002). Fibrosis is common and often severe, explaining the toughness and adherence the surgeon finds upon entering the sella turcica. Necrosis is rare and usually of modest and focal nature. Little is known on the mechanisms by which the infiltrate causes the loss of function/destruction of the endocrine cells. Staining with reticulin usually shows that the delicate connective tissue framework is conserved (Fig. 43.4B), whereas in pituitary adenomas is destroyed.

Granulomatous hypophysitis was first described by Simmonds (1917). He analyzed 2000 pituitary glands at autopsy and found four cases not related to tuberculosis or syphilis, characterized by numerous, scattered multinucleated giant cells, histiocytes, and surrounding lymphocytes and plasma cells. The first antemortem patient was reported in 1980 (Taylor and Duff, 1980). The disease can occur together with lymphocytic hypophysitis (Madsen and Karluk, 2000; McKeel, 1983; Miyamoto et al., 1988) and has been reviewed most recently by Hunn et al. (2014). It is significantly more common in females (F:M ratio of 4:1) and has a mean ( $\pm$  SD) age at presentation of 44 ( $\pm$  15) years.

Xanthomatous hypophysitis, originally described by Folkerth et al. (1998), features an infiltration of the pituitary with foamy histiocytes and lymphocytes. The histiocytes, also called xanthoma cells, have a cytosol loaded with lipids, which confer the characteristic foamy appearance. Cystic areas can be observed in the pathological specimen and also on the MRI images. The pathogenesis of xanthomatous hypophysitis remains unknown, with autoimmune, infectious, and localized endothelial dysfunctions as postulated etiologies. Xanthomatous hypophysitis is more common in women (12 of the 17 cases reported thus far, 70%) and impairs more frequently the gonadal axis.

IgG4 plasmacytic hypophysitis is the form of most recent description. It was first reported on clinical grounds in 2004 in a 66-year-old woman with multiple pseudotumors of salivary glands, pancreas, and retroperitoneum



**FIGURE 43.4** Histopathology of primary lymphocytic hypophysitis. (A) Hematoxylin and eosin stain showing the infiltration of lymphocytes among the endocrine cells. (B) Reticulin stain showing the conservation of the connective tissue framework.

(van der Vliet and Perenboom, 2004) and then pathologically proven in 2007 in a 77-year-old man with blurred vision, hypogonadism, and a history of autoimmune pancreatitis and sclerosing cholangitis (Wong et al., 2007). This form is more common in men (13 of the 16 reported cases are men, 81%) and in advanced ages (mean age at presentation is  $69 \pm 6$  years). It is characterized pathologically by a mononuclear infiltrate of the pituitary gland that is rich in IgG4-producing plasma cells. We suggested a number of 10 or more IgG4-producing plasma cells in a high-power microscopic field for establishing this diagnosis (Leporati et al., 2011). The other diagnostic criteria are the increased levels of IgG4 in the serum ( $>140$  mg/dL) and the association with IgG4-positive lesions in other organs. The disease responds extremely well to glucocorticoids and therefore a correct preoperative diagnosis is crucial, since it can spare the patient an invasive pituitary surgery.

Necrotizing hypophysitis, first reported by Ahmed in 1993 (Ahmed et al., 1993), is the rarest form, with only five published cases, four biopsy-proven (Ahmed et al., 1993; Gutenberg et al., 2012; Nater et al., 2012), and one clinically suspected (Ogawa, 1995). Patients present suddenly with severe headache and develop a long-lasting hypopituitarism, features that, however, can also be seen with the other forms of hypophysitis, as well as with other nonhormone-secreting sellar masses. Histologically, the pituitary appears destroyed by diffuse necrosis with surrounding lymphocytes, plasma cells, and a few eosinophils. MRI shows the symmetric enlargement of the pituitary without signs of hemorrhage, and a thickened stalk, features that again are not specific enough to establish a diagnosis before surgery.

## AUTOIMMUNE FEATURES

That autoimmune hypophysitis is indeed an autoimmune disease that is indicated by the availability of animal models and circumstantial evidence (Rose and Bona, 1993). The latter include the association with other diseases of known autoimmune nature, such as Hashimoto's thyroiditis (7% of the patients) and autoimmune polyglandular syndrome type 2 (2%), the improvement of symptoms upon usage of immunosuppressive drugs such as glucocorticoids, and the induction of the disease by drugs that promote immune activation like ipilimumab.

The autoantigen(s) recognized by the autoimmune attack await identification. Kobayashi's laboratory first described sera from patients with pituitary disorders that, when reacted with pituitary cytosolic extracts, recognized a 22 kDa protein (Kikuchi et al., 2000; Yabe et al., 1995), later identified by Takao et al. (2001) as growth hormone. Crock's laboratory subsequently reported that 7 of 10 patients with biopsy-proven lymphocytic hypophysitis and 12 of 22 patients with suspected hypophysitis had a low titer antibody that recognized a 49 kDa cytosolic pituitary protein (Crock, 1998), later identified as alpha-enolase (O'Dwyer et al., 2002b). The authors concluded that alpha-enolase is the autoantigen targeted by the immune system in autoimmune hypophysitis and, considering its coexpression in the placenta, the basis to explain the strong association between autoimmune hypophysitis and pregnancy (O'Dwyer et al., 2002a). Antibodies recognizing alpha-enolase, however, have been reported in many other diseases, ranging from endometriosis to discoid lupus and Wegener's granulomatosis (Crock, 1998). In addition, Tanaka et al. (2003) have shown that the antibody is present in 7 of 17 patients (41%) with autoimmune hypophysitis, but similarly in 6 of 13 (46%) patients with nonfunctioning pituitary adenoma, and in 4 of 17 (24%) patients with other pituitary diseases, making the use of the alpha-enolase antibody not adequate as diagnostic marker of autoimmune hypophysitis. Finally, Nishiki et al. (2001) reported that 5 of 13 patients with LAH and 1 of 12 patients with LINH had antibodies that recognized 68, 49, and 43 kDa proteins in pituitary membrane extracts.

In addition to growth hormone and alpha-enolase, five candidate autoantigens have been reported in recent years: pituitary gland specific factor 1a and 2 (Tanaka et al., 2002), secretogranin 2 (Bensing et al., 2007), chorionic somatotrophic hormone (Lupi et al., 2008), TPIT (Smith et al., 2012), and PIT-1 (Yamamoto et al., 2011). None of them, however, has yet proven to be pathogenic in animal models, useful in clinical practice, or confirmed by independent investigators.

Antibodies to pituitary antigens have been measured occasionally in the published patients (98 of 857, 11%), mainly by indirect immunofluorescence (Bottazzo et al., 1975) or western blotting (Crock et al., 1993). They were found positive in a minority of patients, yielding an extremely poor sensitivity (37%) (Caturegli et al., 2005). They also seem to be not specific for autoimmune hypophysitis, since they have been described in type 1 diabetes (Mirakian et al., 1982), Hashimoto's thyroiditis (Kobayashi et al., 1988), Graves' disease (Hansen et al., 1989), and normal women during postpartum (Engelberth and Jezkova, 1965). This lack of sensitivity and specificity may have several explanations. For example, patients with autoimmune hypophysitis may come to medical attention long after the onset of disease, and it is known that antibodies against endocrine glands disappear over the years.

## GENETIC AND ENVIRONMENTAL INFLUENCES

Insufficient data are available to establish associations between the genes that are classically thought to influence autoimmunity such as the major histocompatibility complex (MHC) locus and CTLA-4, and autoimmune hypophysitis. Also, no environmental agent has been involved with primary hypophysitis, with the exception of four cases presenting after a viral infection of the meninges (Honegger et al., 1997; Matta et al., 2002; Sandler et al., 1998; Vanneste and Kamphorst, 1987).

## ANIMAL MODELS

The first animal model for autoimmune hypophysitis was published in 1964 by Beutner and Witebsky. The authors immunized 16 rabbits with rabbit anterior pituitary extracts, emulsified in complete Freund's adjuvant, and were able to induce specific antibody responses but no pituitary pathology (Beutner et al., 1964). Levine (1967) immunized rats with a single intracutaneous injection of rat pituitary tissue, emulsified in complete Freund's adjuvant, showing that 2–3 weeks after the injection, 6 of the 14 rats (43%) developed infiltration of the adenohypophysis with mononuclear cells, mainly lymphocytes, monocytes, and occasional epithelioid cells. A few posterior and intermediate lobes had minimal inflammation. Disease incidence could be increased to 75% (15 out of 20 rats) by addition of a second immunologic adjuvant, pertussis toxin. He subsequently showed that pituitary extracts from guinea pig were the most potent inducer of experimental autoimmune hypophysitis (six of six rat recipients), whereas human and cow extracts were poorly effective, and dog and rabbit extracts not effective at all (Levine, 1969). In 1970 Beck and Melvin induced experimental autoimmune hypophysitis in one rhesus monkey by injecting her multiple times, over the course of 3 years, with human placental extracts and human chorionic gonadotropin, both emulsified with Freund's adjuvant. Histology showed infiltration of the adenohypophysis with lymphocytes and scattered plasma cells; the neurohypophysis was normal.

In 1982 Klein induced lympho-plasmacytic infiltration of the anterior pituitary by injecting 12 rabbits (seven cases and five controls) 3 times, at 2-week interval, with rabbit pituitary tissue, emulsified in complete Freund's adjuvant. Eight weeks (10 rabbits) or 16 weeks (two rabbits) after the first injection, five of the seven experimental rabbits showed focal infiltration of the adenohypophysis with lymphocytes, some plasma cells, and a few eosinophils and fibrosis. None of the five controls showed histological abnormalities. In 1992 Yoon et al. immunized over 100 hamsters by injecting intradermally 3 times, at 1-week interval, recombinant rubella virus E1 and E2 glycoproteins. Three weeks after the first injection, specific antibodies against the adenohypophysis were found in 95% of the hamsters. Eleven weeks after the first injection, a diffuse lymphocytic infiltration throughout the adenohypophysis was seen. None of the hamsters that had received the control protein (nonglycosylated rubella nucleoprotein C) developed such lesions. The disease could be prevented by neonatal thymectomy and could not be produced by passive transfer of the auto-antibodies, thus indicating that T cells are critical for disease induction and that antibodies are more important as markers of disease rather than as a pathogenic player (Yoon et al., 1992). Finally, Watanabe et al. in 2001 immunized 12 Lewis rats twice, at 1-week interval, with rat pituitary extract emulsified in complete Freund's adjuvant. Three ( $N = 6$ ) or 6 weeks ( $N = 6$ ) after the first immunization, rats showed minimal lymphocytic infiltration in the adenohypophysis and developed antibodies directed against the growth hormone, thyroid-stimulating hormone, and luteinizing hormone (Watanabe et al., 2001). It is unclear, however, whether these hormones represent the initiating autoantigens is rather the natural response of the immune system to the injection of hormone rich pituitary extracts.

A somewhat different experimental approach was taken by Stockinger's laboratory (De Jersey et al., 2002, 2004). The authors have made a transgenic mouse that expresses specifically in the anterior pituitary (because under the transcriptional control of the growth hormone promoter) a nucleoprotein from the influenza virus, thus a foreign antigen. This influenza nucleoprotein contains a peptide that binds to the  $D^b$  allele of the mouse MHC locus and is recognized by specific CD8 T cells. When the authors crossed the nucleoprotein transgenic mice to T cell receptor (TCR) transgenic mice that have CD8 T cells specific for that nucleoprotein, they observe the growth hormone defect due to destruction of the growth hormone cells expressing the viral antigen. Although informative about accessibility and homing of CD8 T lymphocytes to the pituitary, this transgenic model is likely remote from the human disease where CD8 T lymphocytes are rarely seen in the infiltrate, and somatotrophs are usually spared by the autoimmune attack.

In 2008 we published a comprehensive model of autoimmune hypophysitis. It is induced in SJL female mice by immunization with pituitary proteins emulsified in complete Freund's adjuvant (Tzou et al., 2008). The model

mimics closely the human counterpart, featuring a female bias, a diffuse infiltration of the anterior lobe with a mixture of T and B lymphocytes, and panhypopituitarism. The model has provided useful insights into the human disease. It has shown that hypophysitis is characterized initially by an expansion of the pituitary gland but that later evolves into an atrophic structure that reflect the human empty sella condition (Lupi et al., 2011b). The model is being used to identify novel pituitary autoantigens with the intent of translating the findings to the human disease.

## DIAGNOSIS

As indicated above, hypophysitis is a relatively rare condition, but it enters in the differential diagnosis of other more common lesions, such as the pituitary adenomas and other masses arising in the sella turcica (classified in Table 43.3). All sellar masses share similar clinical and radiological findings so that a diagnosis of certainty can only be achieved by examining under the microscope the pituitary tissue obtained from pituitary surgery. Clinical and immunological markers have, at the moment, low predictive values in establishing a differential diagnosis without pathology. Currently, the most powerful tool to differentiate autoimmune hypophysitis from other sellar mass is MRI. We have reviewed systematically the MRI features of the published patients with the primary hypophysitis and identified a set of seven features that can orient the clinician toward a diagnosis of hypophysitis: pituitary volume, symmetry of the sellar mass, stalk diameter, status of the normal posterior pituitary bright spot, status of the mucosa lining the sphenoidal sinus, intensity of the signal after contrast, and heterogeneity of the signal after contrast (Gutenberg et al., 2009). Hypophysitis induces initially an enlargement of the pituitary gland, thus a measurable increased volume by MRI. This volume increase, however, rarely surpasses 6 mm<sup>3</sup>, as is instead more commonly seen in nonsecreting pituitary adenomas. The increase in volume is also typically symmetric in hypophysitis, with the gland assuming a sort of pear-shaped appearance, whereas in adenomas the mass typically displaces the normal (nontumorous) pituitary tissue. The stalk (or infundibulum) is composed of the median eminence of the hypothalamus, the infundibular stem that arises from it, and the pars tuberalis of the anterior hypophysis that surrounds the stem. The normal stalk has an average anteroposterior diameter (sagittal sections) of 3.25 mm at the level of the optic chiasm and 2.32 mm at the pituitary insertion (Satogami et al., 2009). In hypophysitis the stalk thickens above 4 mm, because the inflammation either involves directly the posterior pituitary or extends to it from the involved anterior pituitary. In coronal sections the normal stalk has a transverse diameter of 3.35 mm at the optic chiasm level and 2.16 mm at the pituitary insertion and a uniform cylindrical appearance. In hypophysitis instead the thickened stalk assumes a V-shaped or round appearance (Turcu et al., 2013). The stalk is of normal size and shape in pituitary adenoma. On precontrast images the anterior pituitary has a signal intensity that is approximately identical to that of the gray matter whereas the stalk is hyperintense. This hyperintensity, commonly known as posterior pituitary bright spot, is believed to reflect the high phospholipid content of the anti-diuretic hormone (ADH) and oxytocin neurosecretory granules. In hypophysitis the normal posterior pituitary bright spot is lost, whereas in adenomas is conserved. After injection of the contrast a pituitary gland affected by hypophysitis enhances the signal strongly (similar to the intensity of the cavernous sinus) and homogeneously, whereas in adenomas the enhancement is less intense and more heterogeneous. Finally, the mucosa lining the sphenoidal sinus is typically normal in hypophysitis whereas is swollen with pituitary adenomas. None of these signs is individually specific enough to diagnose with certainty hypophysitis, but in aggregate the signs classified correctly 97% of the patients, with a sensitivity of 92% and a specificity of 99% (Gutenberg et al., 2009).

More recently, Nakata et al. (2010) described an additional MRI feature that was considered characteristic of hypophysitis and capable of distinguishing hypophysitis from adenoma with certainty: the presence in hypophysitis of a dark signal intensity area around the pituitary and in the cavernous sinus on T2-weighted images.

## TREATMENT

The treatment of autoimmune hypophysitis is, at the moment, only symptomatic and aimed at reducing the size of the pituitary mass and replacing the defective hormones.

Mass reduction treatment comprises lympholytic drugs (mainly glucocorticoids), surgery, and radiotherapy. The first line of the treatment is typically the use of glucocorticoids to reduce the pituitary inflammation (reviewed in Lupi et al., 2011a). They are typically given in the form of high-dose prednisone or prednisolone

that is then tapered over a period of weeks to months, depending on the clinical response. Some patients, however, do not respond to glucocorticoids or experience relapses after an initial improvement. In these instances the other immunosuppressive medications such as cyclosporine (Ward et al., 1999), methotrexate (Tubridy et al., 2001), azathioprine (Lecube et al., 2003), and rituximab (Schreckinger et al., 2012) have been reported with success.

Surgery has historically been the most used way to reduce the pituitary mass in patients with primary hypophysitis. It also has the advantage of providing a tissue sample for pathological examination, which establishes a diagnosis of certainty and also expands our understanding of the disease. Nowadays, surgery is recommended for those patients who have significant and progressive visual loss, for those who do not respond to the various options of medical treatment outlined above, and for those with intractable symptoms. Modern pituitary surgery is typically done through the endoscopic transsphenoidal approach, an approach that is relatively safe and effective. The goal of the surgery is to debulk the pituitary mass as to relieve the compression of the cranial nerves or relieve the pressure responsible for headache. The excision of one-third to half of the pituitary mass is typically sufficient, and there is no need to perform a total excision (Iuliano and Laws, 2011).

Stereotactic radiotherapy (Selch et al., 2003) and gamma knife radiosurgery (Ray et al., 2010) have been employed effectively in a few patients who failed medical and surgical treatments and experienced a disease recurrence.

The replacement of the defective pituitary hormones is the main form of long-term therapy in patients with hypophysitis. It is carried out similarly to the replacement performed for the other causes of hypopituitarism. The hormones most commonly replaced are hydrocortisone for a defective corticotroph axis, thyroxine for the thyrotroph axis, desmopressin for the defective adreno-corticotrophic hormone (ACTH) secretion, and testosterone or estradiol for a defective gonadotroph axis. The growth hormone axis, as indicated above, is not assessed systematically in patients with hypophysitis. However, a few adult patients have also been replaced with recombinant growth hormone (Gachoud et al., 2002; Hindocha et al., 2013; Leung et al., 2004). Prolactin deficiency, if diagnosed and clinically significant, can be corrected with administration of recombinant prolactin (Powe et al., 2010).

## OUTCOME

After a diagnosis of hypophysitis is made, either by pathology or on clinical grounds, there are five main outcomes. The majority of patients (68%) improve after the initial mass reductive treatment but the pituitary hormone deficiencies remain and require prolonged replacement. In some of these patients a detailed imaging follow-up has shown that the pituitary gland becomes atrophic with time, yielding on the MRI the appearance of an empty sella (Karaca et al., 2009). In about a fifth of the patients (18%), the disease improves markedly after the initial mass reductive treatment and no further therapy is necessary. In a few patients (2%) the disease regresses spontaneously without any treatment. In a significant minority of patients (6%), the disease progresses and recurs despite medical and surgical attempts to contain it. In the remaining 6%, hypophysitis kills the patient, likely through the development of an irreversible adrenal insufficiency. These autopsy cases have been published sporadically but consistently throughout the years, the most recent one in 2009 (Gonzalez-Cuyar et al., 2009), reminding us that autoimmune hypophysitis can be fatal if unrecognized.

## HYPOPHYSITIS SECONDARY TO CYTOTOXIC T LYMPHOCYTE ANTIGEN 4 BLOCKADE

The introduction in the early 2000s of cancer drugs that act by blocking proteins expressed on immune cells (so-called immune checkpoint inhibitors) has changed dramatically the significance of hypophysitis: from being a rarity, it has now become a disease that most clinicians and practitioners involved in immunotherapy are familiar with. As of September 2018, immune checkpoint inhibitors approved by the FDA for clinical use include the following: monoclonal antibody blocking CTLA-4: ipilimumab (an IgG1, Yervoy, Bristol-Myers Squibb), monoclonal antibodies blocking PD-1: nivolumab (an IgG4, Opdivo, Bristol-Myers Squibb and Ono Pharmaceutical) and pembrolizumab (an IgG4, Keytruda, Merck), monoclonal antibodies against PD-L1: atezolizumab (an IgG1, Tecentriq, Genentech), avelumab (an IgG1, Bavencio, EMD Serono and Pfizer), durvalumab (an IgG1, Imfinzi, Medimmune and AstraZeneca). The highest incidence of hypophysitis has been reported in clinical trials using

ipilimumab alone (up to 17%), combination of ipilimumab and cancer vaccine GVAX (up to 25%) (van den Eertwegh et al., 2012), or combination of ipilimumab and nivolumab (Prete and Salvatori, 2000).

Ipilimumab binds to CTLA-4, a protein expressed mainly by T cells, found primarily in intracellular granules. Within 2–4 hours of the activation of conventional effector T cells, CTLA-4 is transported to the cell surface where it remains for about 48 hours (Jago et al., 2004; Valk et al., 2008). CTLA-4 is expressed constitutively on the surface of CD4 + Foxp3 + regulatory T cells and is required for maintenance of immune tolerance, as conditional CTLA-4 knockout in Tregs leads to fatal autoimmune reactions (Wing et al., 2008). Recent reports show that CTLA-4 expression is not only limited to lymphoid cells but also found on various tumor cells (Contardi et al., 2005; Laurent et al., 2013). CTLA-4 binds to B7 molecules on antigen-presenting cells, initiating a negative cascade that ultimately results in T-cell inhibition. Ipilimumab blocks the physiological binding of CTLA-4 to B7 and therefore prolongs T-cell activation, inducing unrestrained immune responses not only against tumor cells but also against normal self-antigens. Ipilimumab was approved in 2011 by the FDA and the European Medicines Agency for the treatment of metastatic or unresectable melanoma and is currently tested in several other types of cancer, including renal cell carcinoma, pancreatic cancer, prostate cancer, non–small cell lung cancer, and liver cancer. Patients undergo an induction course of ipilimumab consisting of one intravenous infusion every 3 weeks for a total of four infusions (Culver et al., 2011). The dose is usually 3 mg/kg and the price set by Bristol-Myers Squibb is \$30,000 per injection, which translate to a cost of \$120,000 for a course of therapy.

Patients treated with ipilimumab experience an array of autoimmune reactions collectively referred to as immune-related adverse events. As properly stated by Voskens et al. (2013), these adverse events represent “the price of tumor control”. They occur in about 75% of the patients and are clinically significant (grade 3 or 4) in about 15% (Culver et al., 2011). The most common adverse events are dermatitis, enterocolitis, hepatitis, and hypophysitis, thus differing from adverse events typically reported with chemotherapy. It is unclear why hypophysitis, traditionally considered a rare autoimmune disease, is seen at increased frequency in cancer patients treated with ipilimumab. Approximately 66 clinical trials using CTLA-4 blocking antibodies (ipilimumab or tremelimumab, as monotherapy or in combination with anti-PD-1 blocking antibody, chemotherapy or cancer vaccine) have been published between 2003 and 2018 (Chang et al., 2019). Of them, 25 reported hypophysitis as an adverse event. These 25 trials included 2417 patients with advanced cancer, 116 of whom developed hypophysitis, for an average incidence of about 7% (0.4%–25%). Additional patients developing hypophysitis after the treatment with immune checkpoint inhibitors have been published as individual case reports.

The clinical presentation of hypophysitis secondary to the administration of immune checkpoint inhibitors, such as CTAL-4 blocking antibodies, originates mainly from pituitary gland enlargement and multiple hormonal deficiencies. Headache and fatigue are the most common presenting symptoms, occurring in 60%–80% of the patients treated with CTLA-4 blockade (Caturegli et al., 2016; Faje et al., 2018; Wang et al., 2017). In contrast to primary hypophysitis, visual disturbances are usually rare, likely due to the mild degree of pituitary gland enlargement in ipilimumab-treated patients (Bertrand et al., 2015). Corticotroph, thyrotroph, and gonadotroph axes are commonly affected, occurring in more than 80% of patients. Central adrenal insufficiency may be life-threatening if prompt treatment with corticosteroid is not initiated. Few cases of isolated adrenocorticotropin deficiency due to nivolumab-induced hypophysitis have been reported (Ohara et al., 2018). As for the primary hypophysitis, growth hormone deficiency is less frequently reported. The growth hormone/IGF-1 axis is less systematically assessed because the growth hormone replacement therapy is contraindicated in the setting of active malignancies. Prolactin deficiency is common (Chang and Yialamas, 2018), whereas hyperprolactinemia due to stalk-effect is uncommon. Diabetes insipidus is extremely rare in ipilimumab-treated patients (less than 1%) (Caturegli et al., 2016). The other symptoms, such as nausea, weakness, anorexia, weight loss, decreased libido, confusions, dizziness, and hallucinations, have been reported (Chang and Yialamas, 2018). However, these symptoms are nonspecific and may be related to both cancer disease process and nonendocrine-related adverse events.

The recommended treatment for grade 3 or 4 secondary hypophysitis is a course of high-dose glucocorticoids (i.e., prednisone 50–60 mg/day), gradually tapered over a month to a physiological replacement dose (hydrocortisone 15–20 mg/day). It is unclear whether initial high doses are truly needed or if instead the patients could be started on physiological doses from the time of diagnosis. Thyroid hormone and sex steroids are then added as needed. No information about the growth hormone replacement is available. It is now considered not necessary to discontinue ipilimumab for the treatment of CTLA4-induced hypophysitis, although initial cases did. Pituitary deficits seem to persist after glucocorticoid treatment, and long-term hormonal replacement has to be anticipated for most patients.

## CONCLUDING REMARKS—FUTURE PERSPECTIVES

Autoimmune hypophysitis is a rare but increasingly recognized disease that has exploded on the medical arena due to its association with cancer immunotherapies. Its spectrum continues to expand and increase in complexity. The field will advance when a reliable serological test that identifies the autoimmune nature of hypophysitis becomes available.

### Acknowledgments

We dedicate this chapter to the memory of Breanna, a patient with primary autoimmune hypophysitis who, after delivery of her daughter, developed the inability to lactate and adrenal insufficiency that went unrecognized. Several months later, she developed an acute adrenal crisis and died on June 18, 2010.

### References

- Ahmed, S.R., et al., 1993. Necrotizing infundibulo-hypophysitis: a unique syndrome of diabetes insipidus and hypopituitarism. *J. Clin. Endocrinol. Metab.* 76, 1499–1504.
- Angelousi, A., et al., 2018. Clinical, endocrine and imaging characteristics of patients with primary hypophysitis. *Horm. Metab. Res.* 50, 296–302.
- Bensing, S., et al., 2007. Lymphocytic hypophysitis: report of two biopsy-proven cases and one suspected case with pituitary autoantibodies. *J. Endocrinol. Invest.* 30, 153–162.
- Bertrand, A., et al., 2015. Immune related adverse events associated with anti-CTLA-4 antibodies: systematic review and meta-analysis. *BMC Med.* 13, 211.
- Beutner, E.H., et al., 1964. Serological studies on rabbit antibodies to the rabbit anterior pituitary. *Immunology* 7, 172–181.
- Bottazzo, G.F., et al., 1975. Autoantibodies to prolactin-secreting cells of human pituitary. *Lancet* 2, 97–101.
- Buxton, N., Robertson, I., 2001. Lymphocytic and granulocytic hypophysitis: a single centre experience. *Br. J. Neurosurg.* 15, 242–245. discussion 245–6.
- Caturegli, P., et al., 2005. Autoimmune hypophysitis. *Endocr. Rev.* 26 (5), 599–614.
- Caturegli, P., et al., 2016. Hypophysitis secondary to cytotoxic T-lymphocyte-associated protein 4 blockade: insights into pathogenesis from an autopsy series. *Am. J. Pathol.* 186, 3225–3235.
- Chang, L.S., et al., 2019. Endocrine toxicity of cancer immunotherapy targeting immune checkpoints. *Endocr. Rev.* 40 (1), 17–65.
- Chang, L.S., Yialamas, M.A., 2018. Checkpoint inhibitor-associated hypophysitis. *J. Gen. Intern. Med.* 33, 125–127.
- Chiloiro, S., et al., 2017. An overview of diagnosis of primary autoimmune hypophysitis in a prospective single-center experience. *Neuroendocrinology* 104 (3), 280–290.
- Contardi, E., et al., 2005. CTLA-4 is constitutively expressed on tumor cells and can trigger apoptosis upon ligand interaction. *Int. J. Cancer* 117, 538–550.
- Corsello, A., et al., 2017. Walter E. Dandy: his contributions to pituitary surgery in the context of the overall Johns Hopkins Hospital experience. *Pituitary* 20, 683–691.
- Crock, P.A., 1998. Cytosolic autoantigens in lymphocytic hypophysitis. *J. Clin. Endocrinol. Metab.* 83, 609–618.
- Crock, P.A., et al., 1993. Detection of anti-pituitary antibodies by immunoblotting. *J. Immunol. Methods* 162, 31–40.
- Culver, M.E., et al., 2011. Ipilimumab: a novel treatment for metastatic melanoma. *Ann. Pharmacother.* 45, 510–519.
- De Jersey, J., et al., 2002. Activation of CD8 T cells by antigen expressed in the pituitary gland. *J. Immunol.* 169, 6753–6759.
- De Jersey, J., et al., 2004. Factors affecting the susceptibility of the mouse pituitary gland to CD8 T-cell-mediated autoimmunity. *Immunology* 111, 254–261.
- Duff, G.L., Bernstein, C., 1933. Five cases of Addison's disease with so-called atrophy of the adrenal gland. *Bull. Johns Hopkins Hosp.* 52, 67–83.
- Engelberth, O., Jezkova, Z., 1965. Autoantibodies in Sheehan's syndrome. *Lancet* 285, 1075.
- Faje, A.T., et al., 2018. High-dose glucocorticoids for the treatment of ipilimumab-induced hypophysitis is associated with reduced survival in patients with melanoma. *Cancer* 124, 3706–3714.
- Fehr, M., et al., 1998. Lymphocytic hypophysitis: light and electron microscopic findings and correlation to clinical appearance. *Endocr. Pathol.* 9, 71–78.
- Folkert, R.D., et al., 1998. Xanthomatous hypophysitis. *Am. J. Surg. Pathol.* 22, 736–741.
- Gachoud, D., et al., 2002. [Infundibulitis, an unusual case of central diabetes insipidus]. *Rev. Med. Suisse Romande* 122, 549–551.
- Gonzalez-Cuyar, L.F., et al., 2009. Sudden unexpected death in lymphocytic hypophysitis. *Am. J. Forensic. Med. Pathol.* 30, 61–63.
- Goudie, R.B., Pinkerton, P.H., 1962. Anterior hypophysitis and Hashimoto's disease in a woman. *J. Pathol. Bacteriol.* 83, 584–585.
- Gutenberg, A., et al., 2005. Immunopathology of primary hypophysitis: implications for pathogenesis. *Am. J. Surg. Pathol.* 29, 329–338.
- Gutenberg, A., et al., 2009. A radiologic score to distinguish autoimmune hypophysitis from nonsecreting pituitary adenoma preoperatively. *AJNR Am. J. Neuroradiol.* 30, 1–8.
- Gutenberg, A., et al., 2012. Necrotizing infundibulo-hypophysitis: an entity too rare to be true? *Pituitary* 15, 202–208.
- Hansen, B.L., et al., 1989. Pituitary-cell autoantibody diversity in sera from patients with untreated Graves' disease. *Autoimmunity* 5, 49–57.
- Hasegawa, T., et al., 2018. Cystic lymphocytic hypophysitis mimicking pituitary apoplexy: daunting challenge. *World Neurosurg.* 118, 1–4.
- Hindocha, A., et al., 2013. Lymphocytic hypophysitis in males. *J. Clin. Neurosci.* 20, 743–745.
- Honegger, J., et al., 1997. Lymphocytic and granulomatous hypophysitis: experience with nine cases. *Neurosurgery* 40, 713–722.

- Honegger, J., et al., 2015. Diagnosis of primary hypophysitis in Germany. *J. Clin. Endocrinol. Metab.* 100, 3841–3849.
- Hunn, B.H., et al., 2014. Idiopathic granulomatous hypophysitis: a systematic review of 82 cases in the literature. *Pituitary* 17 (4), 357–365.
- Iuliano, S.L., Laws, E.R., 2011. The diagnosis and management of lymphocytic hypophysitis. *Expert Rev. Endocrinol. Metab.* 6, 777–783.
- Iwama, S., et al., 2013. Isolated prolactin deficiency associated with serum autoantibodies against prolactin-secreting cells. *J. Clin. Endocrinol. Metab.* 98, 3920–3925.
- Jago, C.B., et al., 2004. Differential expression of CTLA-4 among T cell subsets. *Clin. Exp. Immunol.* 136, 463–471.
- Kalra, A.A., et al., 2011. Lymphocytic hypophysitis in children: a novel presentation and literature review. *J. Child Neurol.* 26, 87–94.
- Karaca, Z., et al., 2009. Empty sella may be the final outcome in lymphocytic hypophysitis. *Endocr. Res.* 34, 10–17.
- Katsivelis, P., et al., 2016. A complicated case of primary hypophysitis with bilateral intracavernous carotid artery occlusion. *Hormones (Athens)* 15, 291–296.
- Kikuchi, T., et al., 2000. Antipituitary antibodies as pathogenetic factors in patients with pituitary disorders. *Endocr. J.* 47, 407–416.
- Kobayashi, I., et al., 1988. Anterior pituitary cell antibodies detected in Hashimoto's thyroiditis and Graves' disease. *Endocrinol. Jpn.* 35, 705–708.
- Laurent, S., et al., 2013. The engagement of CTLA-4 on primary melanoma cell lines induces antibody-dependent cellular cytotoxicity and TNF-alpha production. *J. Transl. Med.* 11, 108.
- Lecube, A., et al., 2003. Lymphocytic hypophysitis successfully treated with azathioprine: first case report. *J. Neurol. Neurosurg. Psychiatry* 74, 1581–1583.
- Leporati, P., et al., 2011. IgG4-related hypophysitis: a new addition to the hypophysitis spectrum. *J. Clin. Endocrinol. Metab.* 96, 1971–1980.
- Leung, G.K., et al., 2004. Primary hypophysitis: a single-center experience in 16 cases. *J. Neurosurg.* 101, 262–271.
- Levine, S., 1967. Allergic adenohypophysitis: new experimental disease of the pituitary gland. *Science* 158, 1190–1191.
- Levine, S., 1969. Allergic adrenalitis and adenohypophysitis: further observations on production and passive transfer. *Endocrinology* 84, 469–475.
- Lupi, I., et al., 2008. Novel autoantigens in autoimmune hypophysitis. *Clin. Endocrinol.* 69 (2), 269–278.
- Lupi, I., et al., 2017. Diabetes insipidus is an unfavorable prognostic factor for response to glucocorticoids in patients with autoimmune hypophysitis. *Eur. J. Endocrinol.* 177, 127–135.
- Lupi, I., et al., 2011a. Diagnosis and treatment of autoimmune hypophysitis: a short review. *J. Endocrinol. Invest.* 34, e245–e252.
- Lupi, I., et al., 2011b. From pituitary expansion to empty sella: disease progression in a mouse model of autoimmune hypophysitis. *Endocrinology* 152, 4190–4198.
- Madsen, J.R., Karluk, D., 2000. Case records of the Massachusetts General Hospital, case 34-2000: a 71-year-old woman with an enlarging pituitary mass. *N. Engl. J. Med.* 343, 1399–1406.
- Matta, M.P., et al., 2002. A relapsing remitting lymphocytic hypophysitis. *Pituitary* 5, 37–44.
- McKeel, D.W., 1983. Common histopathologica and ultrastructural features in granulomatous and lymphoid adenohypophysitis. *Endocrinology* 112 (Suppl), 190.
- Mirakian, R., et al., 1982. Autoimmunity to anterior pituitary cells and the pathogenesis of insulin-dependent diabetes mellitus. *Lancet* 1, 755–759.
- Miyamoto, M., et al., 1988. A case of hypopituitarism due to granulomatous and lymphocytic adenohypophysitis with minimal pituitary enlargement: a possible variant of lymphocytic adenohypophysitis. *Endocrinol. Jpn.* 35, 607–616.
- Nakata, Y., et al., 2010. Parasellar T2 dark sign on MR imaging in patients with lymphocytic hypophysitis. *AJNR Am. J. Neuroradiol.* 31, 1944–1950.
- Nater, A., et al., 2012. Necrotizing infundibuloneurohypophysitis: case report and literature review. *Endocr. Pathol.* 23, 205–211.
- Nishiki, M., et al., 2001. Serum antibodies to human pituitary membrane antigens in patients with autoimmune lymphocytic hypophysitis and infundibuloneurohypophysitis. *Clin. Endocrinol. (Oxf.)* 54, 327–333.
- Nussbaum, C.E., et al., 1991. Lymphocytic hypophysitis with involvement of the cavernous sinus and hypothalamus. *Neurosurgery* 28, 440–444.
- O'Dwyer, D.T., et al., 2002a. Pituitary Autoantibodies in lymphocytic hypophysitis target both gamma- and alpha-enolase—a link with pregnancy? *Arch. Physiol. Biochem.* 110, 94–98.
- O'Dwyer, D.T., et al., 2002b. Identification of the 49-kDa autoantigen associated with lymphocytic hypophysitis as alpha-enolase. *J. Clin. Endocrinol. Metab.* 87, 752–757.
- Ogawa, R., 1995. A child with necrotizing infundibulo-neurohypophysitis. *Horm. Rinsho* 43, 33–36.
- Ohara, N., et al., 2018. Isolated adrenocorticotropin deficiency due to nivolumab-induced hypophysitis in a patient with advanced lung adenocarcinoma: a case report and literature review. *Intern. Med.* 57, 527–535.
- Phan, G.Q., et al., 2003. Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. *Proc. Natl. Acad. Sci. U.S.A.* 100, 8372–8377.
- Powe, C.E., et al., 2010. Recombinant human prolactin for the treatment of lactation insufficiency. *Clin. Endocrinol. (Oxf.)* 73, 645–653.
- Prete, A., Salvatori, R., 2000. Hypophysitis. In: DeGroot, L.J., et al., (Eds.), *Endotext*. MDText.com, Inc, South Dartmouth (MA).
- Ray, D.K., et al., 2010. Gamma knife surgery for lymphocytic hypophysitis. *J. Neurosurg.* 112 (1), 118–121.
- Rose, N.R., Bona, C., 1993. Defining criteria for autoimmune diseases (Witebsky's postulates revisited). *Immunol. Today* 14, 426–430.
- Rupp, J.J., Paschkis, K.E., 1953. Panhypopituitarism and hypocalcemic tetany in a male: case presentation. *Ann. Intern. Med.* 39, 1103–1106.
- Saito, T., et al., 1970. Chronic hypernatremia associated with inflammation of the neurohypophysis. *J. Clin. Endocrinol. Metab.* 31, 391–396.
- Sandler, R., et al., 1998. The widening spectrum of lymphocytic hypophysitis. *J. Ark. Med. Soc.* 95, 197–200.
- Satogami, N., et al., 2009. Normal pituitary stalk: high-resolution MR imaging at 3T. *AJNR Am. J. Neuroradiol.* 31, 355–359.
- Sautner, D., et al., 1995. Hypophysitis in surgical and autoptical specimens. *Acta Neuropathol.* 90, 637–644.
- Schreckinger, M., et al., 2012. Novel strategy to treat a case of recurrent lymphocytic hypophysitis using rituximab. *J. Neurosurg.* 116 (6), 1318–1323.

- Selch, M.T., et al., 2003. Stereotactic radiotherapy for the treatment of lymphocytic hypophysitis. Report of two cases. *J. Neurosurg.* 99, 591–596.
- Simmonds, M., 1917. Über das Vorkommen von Riesenzelle in der Hypophyse. *Virchows. Arch.* 223, 281–290.
- Smith, C.J., et al., 2012. Identification of TPIT and other novel autoantigens in lymphocytic hypophysitis; immunoscreening of a pituitary cDNA library and development of immunoprecipitation assays. *Eur. J. Endocrinol.* 166, 391–398.
- Suzuki, K., et al., 2011. Lymphocytic hypophysitis accompanied by aseptic meningitis mimics subacute meningoencephalitis. *Intern. Med.* 50, 2025–2030.
- Takao, T., et al., 2001. Antipituitary antibodies in patients with lymphocytic hypophysitis. *Horm. Res.* 55, 288–292.
- Tanaka, S., et al., 2002. Detection of autoantibodies against the pituitary-specific proteins in patients with lymphocytic hypophysitis. *Eur. J. Endocrinol.* 147, 767–775.
- Tanaka, S., et al., 2003. Anti-alpha-enolase antibodies in pituitary disease. *Endocr. J.* 50, 697–702.
- Taylor, C., Duff, T.A., 1980. Giant cell granuloma involving the pituitary gland. *J. Neurosurg.* 52, 584–857.
- Tubridy, N., et al., 2001. Infundibulohypophysitis in a man presenting with diabetes insipidus and cavernous sinus involvement. *J. Neurol. Neurosurg. Psychiatry* 71, 798–801.
- Turcu, A.F., et al., 2013. Pituitary stalk lesions: the Mayo clinic experience. *J. Clin. Endocrinol. Metab.* 98, 1812–1818.
- Tzou, S.C., et al., 2008. Autoimmune hypophysitis of SJL mice: clinical insights from a new animal model. *Endocrinology* 149, 3461–3469.
- Valk, E., et al., 2008. CTLA-4 trafficking and surface expression. *Trends Immunol.* 29, 272–279.
- van den Eertwegh, A.J., et al., 2012. Combined immunotherapy with granulocyte-macrophage colony-stimulating factor-transduced allogeneic prostate cancer cells and ipilimumab in patients with metastatic castration-resistant prostate cancer: a phase 1 dose-escalation trial. *Lancet Oncol.* 13, 509–517.
- van der Vliet, H.J., Perenboom, R.M., 2004. Multiple pseudotumors in IgG4-associated multifocal systemic fibrosis. *Ann. Intern. Med.* 141, 896–897.
- Vanneste, J.A., Kamphorst, W., 1987. Lymphocytic hypophysitis. *Surg. Neurol.* 28, 145–149.
- Vidal, S., et al., 2002. Immunocytochemical localization of mast cells in lymphocytic hypophysitis. *Am. J. Clin. Pathol.* 117, 478–483.
- Voskens, C.J., et al., 2013. The price of tumor control: an analysis of rare side effects of anti-CTLA-4 therapy in metastatic melanoma from the ipilimumab network. *PLoS One* 8, e53745.
- Wang, S., et al., 2017. Primary lymphocytic hypophysitis: clinical characteristics and treatment of 50 cases in a single centre in China over 18 years. *Clin. Endocrinol. (Oxf.)* 87, 177–184.
- Ward, L., et al., 1999. Severe autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy in an adolescent girl with a novel AIRE mutation: response to immunosuppressive therapy. *J. Clin. Endocrinol. Metab.* 84, 844–852.
- Watanabe, K., et al., 2001. Characteristics of experimental autoimmune hypophysitis in rats: major antigens are growth hormone, thyrotropin, and luteinizing hormone in this model. *Autoimmunity* 33, 265–274.
- Wing, K., et al., 2008. CTLA-4 control over Foxp3<sup>+</sup> regulatory T cell function. *Science* 322, 271–275.
- Wong, S., et al., 2007. Hypophysitis presented as inflammatory pseudotumor in immunoglobulin G4-related systemic disease. *Hum. Pathol.* 38, 1720–1723.
- Yabe, S., et al., 1995. Western blot analysis of rat pituitary antigens recognized by human antipituitary antibodies. *Endocr. J.* 42, 115–119.
- Yamamoto, M., et al., 2011. Adult combined GH, prolactin, and TSH deficiency associated with circulating PIT-1 antibody in humans. *J. Clin. Invest.* 121, 113–119.
- Yoon, J.W., et al., 1992. Induction of an organ-specific autoimmune disease, lymphocytic hypophysitis, in hamsters by recombinant rubella virus glycoprotein and prevention of disease by neonatal thymectomy. *J. Virol.* 66, 1210–1214.

# Autoimmune Gastritis and Pernicious Anemia

Ban-Hock Toh

Centre for Inflammatory Diseases, Department of Medicine, School of Clinical Sciences at Monash Health, Faculty of Medicine, Nursing and Health Sciences, Monash University, Clayton, VIC, Australia

## OUTLINE

Clinical, Pathologic, and Epidemiologic Features	834	Pathologic Effector Mechanisms	840
<b>Autoimmune Features</b>	<b>836</b>	<b>Autoantibodies as Potential Immunologic Markers</b>	<b>841</b>
Autoantibodies	836		
T-Cell Immunity	838	Laboratory Diagnosis	843
Genetic Features	838	Concluding Remarks—Future Prospects	843
In Vivo and In Vitro Models	839	References	843
Pathogenesis and Complications of Autoimmune Gastritis	840	Further Reading	847

Pernicious anemia is the result of advanced autoimmune gastritis, which in its initial stages is an asymptomatic autoimmune disease. First reported by Addison in 1849, the link between the anemia and gastric degeneration was realized by Flint in 1860 and histologic evidence of the gastric atrophy was provided by Fenwick in 1870. In 1871, a fatal anemia was termed perniciosa by Biermer. Subacute combined degeneration was later applied to the posterolateral spinal cord lesions that can be associated with the anemia (Pearce, 2008). In 1926, Minot and Murphy discovered that feeding patients large meals of cooked liver led to a reticulocyte response and reversal of anemia, which earned them a Nobel Prize in 1934. At first, the causal connection between pernicious anemia and chronic gastritis was incomprehensible. Castle (1953) showed that the anemia was a result of a combined deficiency of an “extrinsic factor” subsequently identified as vitamin B12 and present in the liver (Smith, 1948; Rickes et al., 1948), and an “intrinsic factor” (IF) in gastric juice (Highley et al., 1967). Oral treatment with extracts of hog stomach (Sharp, 1929; Sturgis and Isaacs, 1929; Renshaw, 1930) resulted in remission for several years (Wilkinson, 1949). However, relapses tended to occur and some cases became refractory to increasing amounts of the extract (Berlin et al., 1958a,b). This refractory state was due to a serum factor that inhibited the effectiveness of IF (Schwartz, 1958). Subsequently, sera from patients with pernicious anemia were shown to contain autoantibodies to IF (Jeffries et al., 1962; Jacob and Schilling, 1966) and to gastric parietal cells (Irvine et al., 1962; Jeffries et al., 1962; Markson and Moore, 1962). However, little progress was made in understanding the pathology of the gastric lesion until a flexible biopsy tube was designed that permitted the taking of samples of the gastric mucosa for histologic examination (Wood et al., 1949).

Pernicious anemia was observed to cluster in families (McIntyre et al., 1959; Whittingham et al., 1969) and to coexist with autoimmune thyroid diseases (Tudhope and Wilson, 1960). These observations suggested a genetic component to the disorder, a suggestion further strengthened by an association with another endocrine autoimmune disease, type 1 diabetes mellitus (Ungar et al., 1968). The suggestion by Taylor (Taylor, 1959; Taylor and

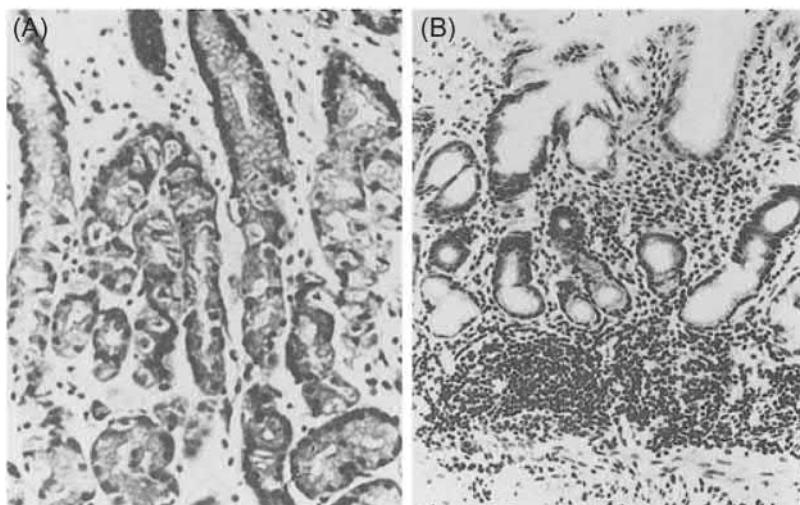
Morton, 1959) that the inhibitory substance to gastric IF in the serum of patients with pernicious anemia was antibody and possibly autoantibody and the recognition that pernicious anemia fulfilled the markers of autoimmune disease put forward by Mackay and Burnet (1963) led to its acceptance as an autoimmune disease of the stomach. The historical antecedents of pernicious anemia have recently been recapitulated as the “dawn of molecular medicine” (Bunn, 2014).

## CLINICAL, PATHOLOGIC, AND EPIDEMIOLOGIC FEATURES

Autoimmune gastritis in the early stages is asymptomatic and demonstrable only by the serologic detection of antibodies to gastric parietal cells. Pernicious anemia only manifests when stores of vitamin B12 are depleted and only 10%–15% of the patients with autoimmune gastritis develop pernicious anemia (Strickland and Mackay, 1973; Irvine et al., 1974) after a latent period of 20–30 years (Toh et al., 1997). Nonetheless, at an estimated prevalence of about 2% in Western adult populations at or over the age of 60 years, pernicious anemia represents the most common cause of vitamin B12 deficiency (Carmel, 1996). Although “silent” until the end stage, the gastric lesion can be predicted years before clinical presentation by immunologic markers specific for gastric autoimmunity. Terminally, the gastritis results in the deficiency of IF, a protein that binds avidly to dietary vitamin B12 (Glass, 1963) and promotes its transport to the terminal ileum for absorption (Donaldson et al., 1967) by ileal cubilin receptors, a multifunctional endocytic receptor (Lindblom et al., 1999). Consequently, the gastritis is expressed clinically as vitamin B12 deficiency associated with megaloblastic anemia arising as a consequence of the requirement of the vitamin for DNA synthesis. The megaloblastic anemia is demonstrated by examination of the blood and bone marrow (Chanarin, 1979) and is now readily controlled by vitamin B12 treatment (Carmel, 2008). Pernicious anemia usually results in low serum pepsinogen I levels, a low serum pepsinogen I/II ratio, and increased serum gastrin levels, which also can be diagnostically useful (Varis et al., 1979; Samloff et al., 1982; Carmel, 1988).

Pernicious anemia is uncommon before the age of 30. Reportedly more common in individuals of northern European decent, the disease has been reported in Blacks and in Latin Americans (Carmel and Johnson, 1978). Patients with advanced pernicious anemia are usually women of late middle age, who appear pale, tired, and depressed and may complain of a sore tongue and abdominal discomfort. Vitamin B12 deficiency can lead to neuropsychiatric syndromes, such as sensory impairment, abnormal reflexes, motor impairment, and spastic paraparesis, and possibly mental or psychiatric disturbances (Savage and Lindenbaum, 1995). Patients with pernicious anemia have also been reported to have a 3–5-fold higher risk of developing gastric cancer and a 13-fold increased risk of developing gastric carcinoids (Hsing et al., 1993; Fuchs and Mayer, 1995; Kokkola et al., 1998).

Strickland and Mackay (1973) proposed a classification of gastritis based on histologic findings of the gastric mucosa, the presence of gastric parietal cell antibody, and serum levels of gastrin. Type A gastritis, the “pernicious anemia type,” is restricted to the fundus and body of the stomach. Early lesions are characterized by chronic inflammation in the submucosa that extends into the lamina propria of the mucosa between gastric glands with accompanying loss of gastric and zymogenic cells (Fig. 44.1). In advanced disease, gastric atrophy is



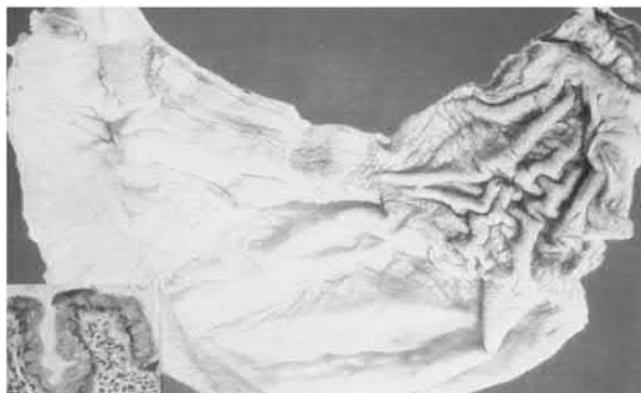
**FIGURE 44.1** The early lesion of autoimmune gastritis. (A) The normal mucosa of the body of the stomach showing gastric glands and the absence of a chronic inflammatory infiltrate in the lamina propria. (B) An early lesion of autoimmune gastritis showing a dense chronic inflammatory infiltrate in the gastric submucosa that extends into the lamina propria with the accompanying loss of gastric parietal and zymogenic cells.

readily recognized macroscopically and microscopically (Figs. 44.2 and 44.3). The wall of the fundus and body of the stomach becomes paper-thin because the gastric glands are markedly reduced or absent. In particular, parietal cells and zymogenic (chief) cells are absent from the gastric mucosa and replaced by mucus containing cells resembling those of the intestine (intestinal metaplasia) (Fig. 44.3).

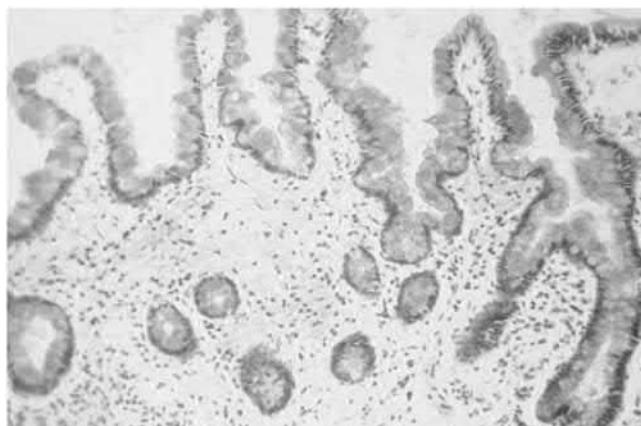
Type A gastritis is characterized by circulating antibodies to gastric parietal cells (Fig. 44.4) that have subsequently been shown to be directed to the gastric H<sup>+</sup>/K<sup>+</sup> ATPase (Burman et al., 1989; Goldkorn et al., 1989; Toh et al., 1990, 1997), achlorhydria, and high levels of serum gastrin secreted by the intact antral glands. Strickland and Mackay observed that 5 out of 30 patients with type A gastritis (16%) developed overt or latent pernicious anemia during a follow-up period of 3–24 years. Type A gastritis is also the gastritis characteristic of families in whom pernicious anemia predominates (Varis, 1981; Kekki et al., 1983).

Type B gastritis, the nonpernicious anemia type, involves the antrum initially but can extend to the fundus and body of the stomach and shows incomplete failure of acid secretion and low levels of serum gastrin because of the antral gastritis. Type B gastritis is usually associated with *Helicobacter pylori* infection; type A is not (Fong et al., 1991).

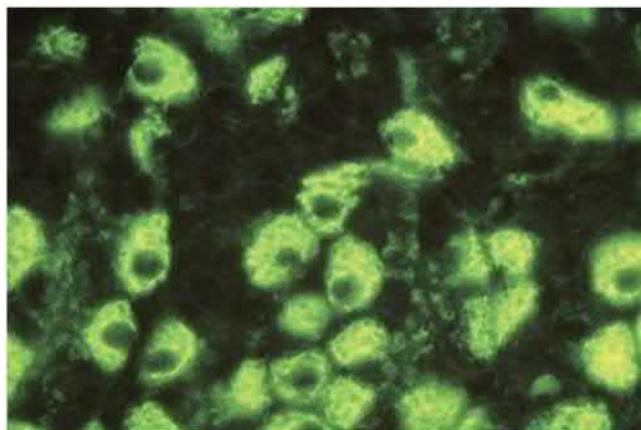
Pernicious anemia in patients with the common variable type of immunodeficiency associated with low levels of serum immunoglobulins can be distinguished from classic pernicious anemia on the basis of this classification. This former type of pernicious anemia usually occurs in a younger age group, is histologically type B, is associated with a negative test for antibodies to gastric parietal cells and IF, and shows a low level of serum gastrin (Twomey et al., 1969; Hughes et al., 1972; Cowling et al., 1974). Pernicious anemia in childhood is not associated with gastritis or achlorhydria and is the result of inadequate IF production.



**FIGURE 44.2** Macroscopic appearance of advanced autoimmune gastritis in a patient with pernicious anemia showing the extreme atrophy of the mucosa of the fundus and body of the stomach with loss of rugal folds contrasted with the healthy mucosa of the gastric antrum. Inset is the microscopic appearance of the mucosa.



**FIGURE 44.3** Microscopic appearance of the gastric mucosa of advanced autoimmune gastritis showing a chronic inflammatory infiltrate in the gastric mucosa and the loss of parietal and zymogenic cells and replacement with mucus containing cells.



**FIGURE 44.4** Indirect immunofluorescent staining of gastric parietal cells in a mouse stomach reactive with serum from a patient with autoimmune gastritis.

The evolution of gastric atrophy in most cases of pernicious anemia probably spans 20–30 years but this is difficult to assess in individual cases. The presence of gastric parietal cell antibody in the serum is predictive of autoimmune gastritis (Irvine et al., 1965; Serafini et al., 1970). Conversely, gastric parietal cell antibody is not observed when gastritis is due to diseases affecting the body of the stomach that are not autoimmune.

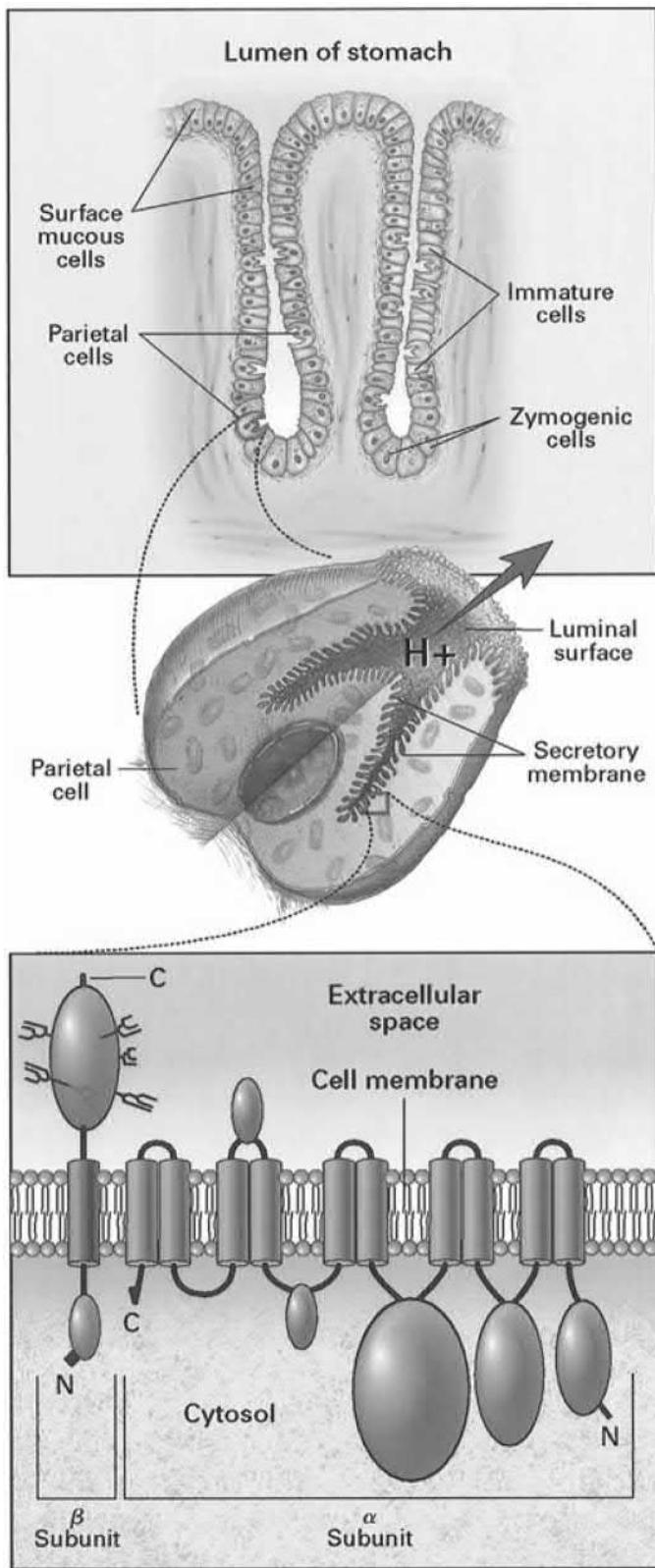
There are many reports of regeneration of gastric parietal cells, improvement in gastric function, and hematologic remission after treatment with corticosteroids (Doig et al., 1957; Gordin 1959; Ardeman and Chanarin, 1965; Jeffries et al., 1966; Rodbro et al., 1967; Strickland, 1969) or azathioprine (Jorge and Sanchez, 1973). This suggests that the gastric mucosa is the direct target of an autoimmune process that can be checked by immunosuppressive drugs. The observations also suggest that precursor stem cells capable of differentiating into parietal cells and zymogenic cells are present in stomachs of patients with autoimmune gastritis and that these are responsible for the regeneration of the differentiated cells when the destructive autoimmune process is controlled. The suggestion is supported by observations in experimental models of autoimmune gastritis where there is evidence of the persistence, and even expansion, of these precursor stem cells in gastritis stomachs (see below). It is also of interest and relevance that reversal of recovery with degeneration of the gastric mucosa recurred once immunosuppressive treatment was halted (Wall et al., 1968). This highlights the persistence of autoreactive T cells that cause this disease, even in the face of immunosuppression.

Pernicious anemia associates predominantly with the autoimmune endocrinopathies and the antireceptor autoimmune diseases. The associated diseases include Hashimoto's thyroiditis, insulin-dependent type 1 diabetes mellitus, primary Addison's disease, primary ovarian failure, primary hypoparathyroidism, premature graying of the hair, vitiligo, thyrotoxicosis, myasthenia gravis, and the Lambert–Eaton syndrome. These organ-specific “thyrogastric” autoimmune diseases may occur in the same patient with pernicious anemia but aggregate in “pernicious anemia families” (Ardeman et al., 1966; Wangel et al., 1968a; Whittingham et al., 1969).

## AUTOIMMUNE FEATURES

### Autoantibodies

Autoimmune gastritis is associated with autoantibodies to gastric parietal cells and to their secreted product, IF. Like most autoantibodies, these autoantibodies are polyclonal but are predominantly of the IgG isotype (Serafini et al., 1970). IgA antibodies to gastric IF have been demonstrated in gastric juice (Goldberg and Bluestone, 1970). Autoantibodies to gastric parietal cells are routinely detected by immunofluorescence (see Fig. 44.4). These autoantibodies are directed toward both the catalytic 100 kDa  $\alpha$  subunit and the 60–90 kDa glycoprotein  $\beta$ -subunit of the gastric  $H^+/K^+$  ATPase (Fig. 44.5) (Karlsson et al., 1988; Burman et al., 1989; Toh et al., 1990; Callaghan et al., 1993; Ma et al., 1994). The gastric  $H^+/K^+$  ATPase is the enzyme responsible for acidification of gastric luminal contents (Forte et al., 1989; Rabon and Reuben, 1990; Prinz et al., 1992). It belongs to a family of ion-motive P-type ATPases and is most closely related to the ubiquitous  $Na^+/K^+$  ATPase. The gastric  $H^+/K^+$  ATPase is located on specialized secretory membranes of gastric parietal cells (DiBona et al., 1979;



**FIGURE 44.5** Gastric parietal cell H<sup>+</sup>/K<sup>+</sup> ATPase as the target in autoimmune gastritis associated with pernicious anemia. Top panel represents a gastric gland showing location of parietal cells in relation to zymogenic cells, immature cells, and surface mucus cells. Middle panel represents a stimulated gastric parietal cell showing the lining membrane of the secretory canalculus on which gastric H<sup>+</sup>/K<sup>+</sup> ATPase is located. Bottom panel represents the catalytic  $\alpha$  and the glycoprotein  $\beta$  subunits of the gastric H/K ATPase showing their orientation in the membrane of the secretory canalculus of the parietal cells. N denotes the N-terminal of protein and C the C-terminal of protein. The role of the gastric H/K ATPase in the initiation of autoimmune gastritis is supported by the development of the gastritis following immunization with the gastric H/K ATPase (Scarff et al., 1997). Source: Reproduced with kind permission of the New England Journal of Medicine.

Callaghan et al., 1990; Toh et al., 1990; Pettitt et al., 1995). Parietal cell antibodies have been shown to deplete H<sup>+</sup>/K<sup>+</sup> ATPase activity from parietal cell membranes in vitro (Burman et al., 1989). Antibody reactivity with the  $\alpha$  subunit of the H<sup>+</sup>/K<sup>+</sup> ATPase includes an epitope on the cytosolic side of the secretory membrane

(Song et al., 1994). The H<sup>+</sup>/K<sup>+</sup> ATPase β subunit extracellular domain has three disulfide bonds and is highly glycosylated and these structural features are required for binding of autoantibodies (Goldkorn et al., 1989; Callaghan et al., 1993). These observations have led to the development of diagnostic ELISAs to gastric H/K ATPase that is more sensitive than the immunofluorescence test for parietal cell antibody (Toh et al., 2012b).

Human IF is a glycoprotein with a molecular mass of 44,000. Each molecule of IF has the capacity to bind to one molecule of vitamin B12 (Chanarin, 1979). Two distinct antibody activities are detected by radioimmunoassay; one reacts with the binding site for vitamin B12 and blocks subsequent binding of IF with free vitamin, and the other reacts with an antigenic determinant remote from this site (Samloff et al., 1968; Rothenberg et al., 1971).

## T-Cell Immunity

Studies of animal models suggest that CD4<sup>+</sup> T cells mediate the gastric lesion (see below). In humans, a significant increase in CD4<sup>+</sup> and CD8<sup>+</sup> T cells and a sixfold increase in non-T cells (probably B cells) have been observed in the cellular infiltrate of stomachs of patients with pernicious anemia (Irvine et al., 1965; Kaye et al., 1983). Electron microscopy has shown that lymphocytes line up against the membranes of gastric parietal cells and zymogenic cells in the gastric mucosa. Phenotypic analysis of the peripheral blood T cells from patients with pernicious anemia have not shown any significant differences compared to age matched controls (Vargas et al., 1995); although, in humans, no studies have been made on the gastric lymph nodes, which are the sites appropriate for such studies.

A number of early studies suggested a T cell response to IF and gastric extracts (Fisher et al., 1965, 1966; Tai and McGuigan, 1969; Rose et al., 1970; Fixa et al., 1972; Goldstone et al., 1973; MacCuish et al., 1974; Whittingham et al., 1975). However, the identification of the gastric H<sup>+</sup>/K<sup>+</sup> ATPase as the major gastric autoantigen that was targeted by antibodies in humans and mice and T cells in mice led to the isolation of human H<sup>+</sup>/K<sup>+</sup> ATPase-specific CD4<sup>+</sup> T cell clones from the gastric mucosa of patients with autoimmune gastritis. The clones were shown to recognize a number of epitopes in the H<sup>+</sup>/K<sup>+</sup> ATPase α and β subunits (Bergman et al., 2003) and were biased toward the production of IFN-γ and TNF-α although some also produced IL-4 and stimulated immunoglobulin production by B cells (D'elios et al., 2001). Most clones also displayed cytotoxic activity that was either perforin or FAS-dependent.

Studies in mice have shown that a T cell immune response to the gastric H<sup>+</sup>/K<sup>+</sup> ATPase is essential for the initiation and development of autoimmune gastritis. These observations include the resistance to autoimmune gastritis in mice expressing the H<sup>+</sup>/K<sup>+</sup> ATPase β subunit in the thymus and mice deficient in the gastric H<sup>+</sup>/K<sup>+</sup> ATPase, a high incidence of disease in mice with repertoires enriched in H<sup>+</sup>/K<sup>+</sup> ATPase reactive T cells, and mice immunized with purified H<sup>+</sup>/K<sup>+</sup> ATPase. Initial findings suggested that the H<sup>+</sup>/K<sup>+</sup> ATPase β subunit may be the primary initiating autoantigen (Alderuccio et al., 1993). However, subsequent studies indicated that both subunits are targeted in this disease and that the expression of the H<sup>+</sup>/K<sup>+</sup> ATPase β subunit is required for presentation of the α subunit to CD4<sup>+</sup> T cells, which explained earlier findings on antigenic hierarchy (Allen et al., 2005).

The pathways that lead to immunological tolerance to the gastric H<sup>+</sup>/K<sup>+</sup> ATPase have been extensively investigated. High avidity H<sup>+</sup>/K<sup>+</sup> ATPase-specific T cells exit the thymus and the H<sup>+</sup>/K<sup>+</sup> ATPase-specific T cells in the periphery represent the residue of a T cell repertoire that has been subjected to partial extrathymic deletion (Read et al., 2007). This depletion occurs following antigen-specific activation of H<sup>+</sup>/K<sup>+</sup> ATPase-specific CD4<sup>+</sup> T cells in the stomach draining paragastric lymph node. Additional mechanisms of peripheral tolerance, mediated by dendritic cells and Foxp3<sup>+</sup> regulatory T cells (Tregs), play important roles in influencing the activation of self-reactive T cells that remain after clonal deletion (Scheinecker et al., 2002; Zwar et al., 2006; Dipaolo et al., 2007; Read et al., 2007; Hogan et al., 2008; Stummvoll et al., 2008). Therefore, protection from gastritis is mediated almost exclusively by tolerogenic mechanisms in the local tissue environment.

## GENETIC FEATURES

Predisposition to pernicious anemia appears to be genetically determined, at least in part. Evidence for genetic factors influencing the expression of pernicious anemia includes clustering of this disease in families and with other autoimmune diseases as well as a racial predilection for northern Europeans. Pernicious anemia is rare

among southern Europeans and earlier reports suggest that it is almost nonexistent among Black and Asian people (Jayaratnam et al., 1967; Irvine et al., 1969). In keeping with ethnic differences, pernicious anemia is associated with phenotypic markers that are absent or occur with low frequency in these racial groups. These markers include blue eyes, fair skin, and blood group A (Callender et al., 1957). However, a more recent study by Carmel (1992) reported a higher prevalence in Black people, particularly among Black women.

There have been reports of a number of Caucasian families with a high frequency of pernicious anemia over several generations (Callender et al., 1957; Callender and Denborough, 1957; McIntyre et al., 1959; Doniach et al., 1965; Ardeman et al., 1966; Wangel et al., 1968a,b; Whittingham et al., 1969). A higher but not absolute concordance of pernicious anemia has been observed in monozygotic twins (Delva et al., 1965; Balcerzak et al., 1968). While associations with susceptibility to pernicious anemia and HLA molecules have been reported (Ungar et al., 1977), such findings were not substantiated in other studies (Whittingham et al., 1991). NALP1, a component of the inflammasome (Faustin et al., 2007), has been reported to be associated with vitiligo and multiple autoimmune diseases including pernicious anemia.

Studies in mice have also substantiated the role of specific genes in predisposing to autoimmune gastritis. BALB/c mouse strains are highly susceptible to autoimmune gastritis whereas C57BL/6 mice are very resistant (Kojima and Prehn, 1981; Silveira et al., 1999). To identify genes conferring susceptibility to the disease, linkage analysis was performed on (BALB/cCrSlc × C57BL/6)F2 mice. Two major genes on chromosome 4 that confer susceptibility to autoimmune gastritis, termed Gasa1 and Gasa2, were identified (Silveira et al., 1999). Further analysis found Gasa3 on chromosome 6, coincident with the MHC locus, and Gasa4 on chromosome 17 as minor gastritis susceptibility genes (Silveira et al., 2001). Experimental evidence for the Gasa1 and Gasa2 loci was obtained using congenic mouse strains that also proved that Gasa1 and Gasa2 can act independently to cause full expression of susceptibility to autoimmune disease (Ang et al., 2007).

## IN VIVO AND IN VITRO MODELS

Mice can readily develop high incidence of autoimmune gastritis, either spontaneously or after manipulation. Mouse models have proven to be a very reliable and robust model of autoimmune gastritis that shares key features with human disease including antigen and epitope commonality, cytokine secretion, and pathogenic mechanisms. As a result, mouse autoimmune gastritis has been used extensively to investigate basic mechanisms of immune tolerance and the cellular and molecular basis for autoimmune disease.

Spontaneous gastritis has been reported in C3H/He mice with an incidence of about 20% (Alderuccio and Toh, 1998). A variety of organ-specific autoimmune diseases can be induced by lymphopenic conditions and the particular organ affected is determined by the genetic background of the mouse strain. BALB/c mice are the most susceptible mouse strain to lymphopenia induced autoimmune gastritis whereas C57BL/6 mice are resistant to the induction of disease (Ahmed and Penhale, 1981; Kojima and Prehn, 1981). Thymectomy induced autoimmune gastritis is historically one of the most commonly used gastritis models. BALB/c mice thymectomized at approximately day 3 after birth develop autoimmune gastritis at 30%–90%. Adult mice did not develop autoimmune gastritis followed by thymectomy unless the mice were treated with cyclophosphamide or irradiation (Ahmed and Penhale, 1981; Barrett et al., 1995). It was previously believed that induction of autoimmune gastritis by neonatal thymectomy was the result of a block in Tregs being seeded into the periphery from the thymus; thus gastritogenic T cells were not suppressed (Asano et al., 1996). However, this proposal was not supported by findings that Tregs are able to repopulate in the periphery after neonatal thymectomy and these Tregs are fully suppressive in vivo (Asano et al., 1996; Dujardin et al., 2004; Ang et al., 2007; Samy et al., 2008).

Comparatively recently, it has been demonstrated that neonatal thymectomy results in a lymphopenic environment that promotes homeostatic proliferation of effector T cells, including expansion of gastritogenic T cell clones (Monteiro et al., 2008). Therefore, autoimmune gastritis induced by neonatal thymectomy is probably the result of a reduced Treg: effector T-cell ratio that does not favor suppression by Treg, rather than the depletion of Tregs per se or intrinsic defects of Tregs.

Murine autoimmune gastritis can also be induced by immunization of adult BALB/c mice with purified gastric H<sup>+</sup>/K<sup>+</sup> ATPase in complete Freund's adjuvant (Scarff et al., 1997). However, the disease induced is transient and is reversible after cessation of immunization, perhaps due to regeneration of parietal cells and zymogenic cells from the expanded stem cell population.

Autoimmune gastritis may occur as the result of the immune system being dominated by self-reactive T cells. T-cell receptor (TCR) transgenic mouse lines that constitutively express H<sup>+</sup>/K<sup>+</sup> ATPase-specific TCR on their T cells have been generated. A23 TCR transgenic mice contain CD4<sup>+</sup> T cells that target residues 630–641 of the H<sup>+</sup>/K<sup>+</sup> ATPase  $\alpha$  subunit and display a Th1 phenotype (McHugh et al., 2001), whereas A51 TCR transgenic mice contain CD4<sup>+</sup> T cells that target residues 889–899 of the H<sup>+</sup>/K<sup>+</sup> ATPase  $\alpha$  subunit and reveal a Th2 phenotype (Candon et al., 2004). Both A23 and A51 T cell clones were isolated from the paragastric lymph node of mice that developed autoimmune gastritis following neonatal thymectomy (Suri-Payer et al., 1999). A23 mice develop more severe autoimmune gastritis spontaneously with higher penetrance than in A51 mice due to difference in the availability of their respective autoantigenic epitopes (Levin et al., 2008). IE4 TCR transgenic mice were created using TCR genes isolated from a T cell hybridoma, which was derived by immunization of a BALB/c mouse with residues 253–277 of the H<sup>+</sup>/K<sup>+</sup> ATPase  $\beta$  subunit (Alderuccio et al., 2000; De Silva et al., 2001). Autoimmune gastritis only occurred in 20% of the mice, even though transgenic T cells in the periphery responded well to the antigenic peptide in vitro.

Constitutive expression of the cytokine granulocyte macrophage colony stimulating factor in the stomach induces autoimmune gastritis with very similar antigenic and pathological features to those of other models (Biondo et al., 2001). It is not clear which of the proinflammatory activities of granulocyte macrophage colony stimulating factor are responsible for the disease initiation.

T cells from mice deficient in gastric H<sup>+</sup>/K<sup>+</sup> ATPase autoantigens can induce autoimmune gastritis in sublethally irradiated mice, presumably due to the preponderance of H<sup>+</sup>/K<sup>+</sup> ATPase-specific T cells that result from a lack of autoantigen-specific tolerance (Tu et al., 2011). This recent model is attractive for the study of many aspects of organ-specific autoimmunity because of a high degree of consistency of disease severity, the use of polyclonal T cells, and a specific T cell response to a bona fide autoantigen (Fig. 44.6). The cardinal features of mouse autoimmune gastritis are illustrated in this disease. The gastric mucosa becomes heavily infiltrated by mononuclear cells, parietal and zymogenic cells are severely depleted, and there is overgrowth by immature cell types resulting in gastric hypertrophy visible at both microscopic and macroscopic levels (Fig. 44.6A–D). The depletion of parietal cells leads to an increase in gastric pH (Fig. 44.6E). High titer anti-H<sup>+</sup>/K<sup>+</sup> ATPase autoantibodies develop within 4–6 weeks (Fig. 44.6F and G).

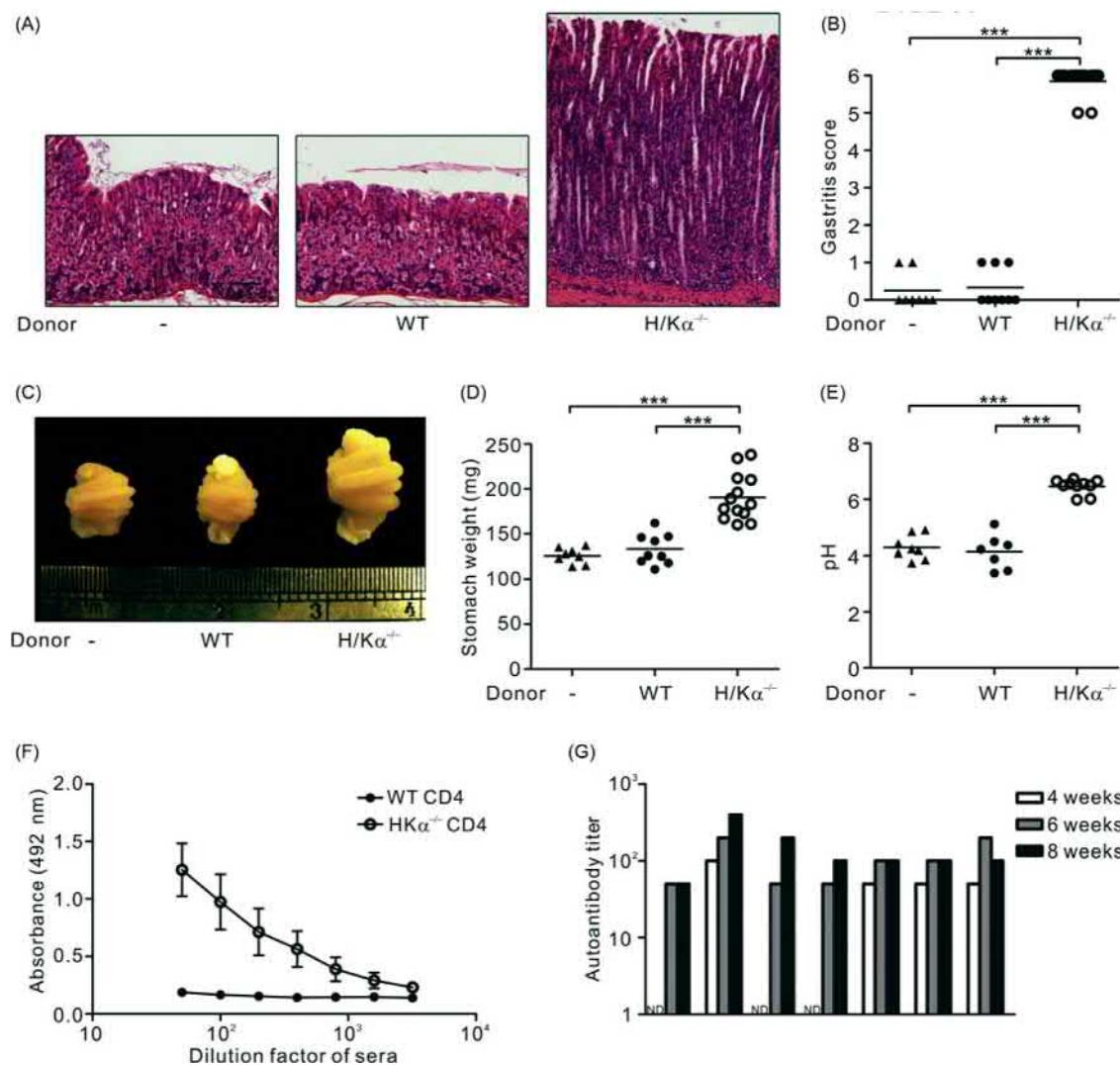
## PATHOGENESIS AND COMPLICATIONS OF AUTOIMMUNE GASTRITIS

### Pathologic Effector Mechanisms

Studies in mice have clearly indicated that autoimmune gastritis is initiated by CD4<sup>+</sup> T cells. First, gastritis can be transferred to immunocompromised hosts by CD4<sup>+</sup> T lymphocytes but not by sera from animals with autoimmune gastritis (van Driel et al., 1984, 2005; Gleeson et al., 1996; Toh et al., 1997; van Driel and Ang, 2008). In addition, histopathologic features of gastritis occur in mice before the appearance of autoantibodies. The early gastric lesion is composed predominantly of CD4<sup>+</sup> T cells and macrophages with production of a mix of Th1- and Th2-type cytokines but not interleukin-4, suggesting a key role for these cells and their cytokines in initiation of the disease (Martinelli et al., 1996; Katakai et al., 1998). Interferon- $\gamma$ , probably produced by the Th1 cells, appears to be crucial for the induction of gastric lesion, as a single injection of antibodies to interferon- $\gamma$  immediately following neonatal thymectomy prevents autoimmune gastritis (Barrett et al., 1996) and T cells from mice deficient in interferon- $\gamma$  or interleukin-12 have a reduced pathogenicity in causing autoimmune gastritis (Suri-Payer and Cantor, 2001). CD81 T cells do not seem to have a role in this disease since depletion of this T cell population by treatment with anti-CD8 antibody did not reduce the capacity of the remaining T cells to transfer disease (De Silva et al., 1998). Recent data suggest that interleukin-17 may play a role in assisting the development of severe late stage autoimmune gastritis. B lymphocytes tend to accumulate in gastric lesions and aggregate in follicular-like structures and it is not known if they play a functional role at these sites (Martinelli et al., 1996) (Fig. 44.7).

There is evidence for various mechanisms whereby T cells could cause the lesion in the gastric mucosa including direct cytotoxicity and cytokine mediated disruption of developmental pathways in the stomach (Judd et al., 1999; Marshall et al., 2002; Kang et al., 2005).

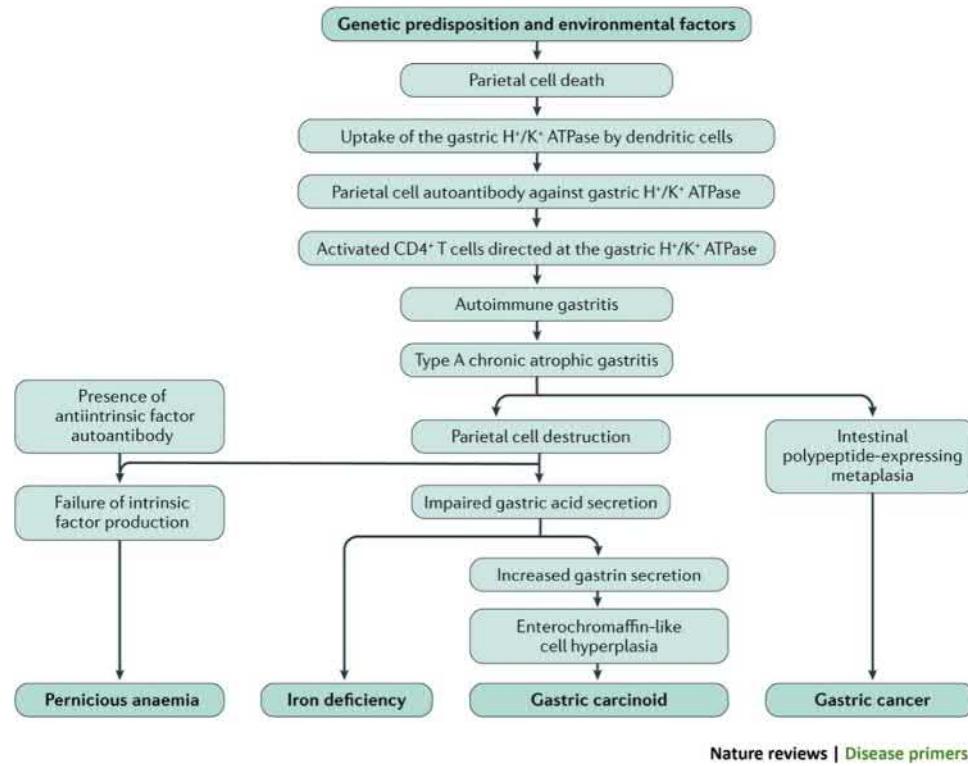
As described above, CD4<sup>+</sup> T cell clones specific for the gastric H<sup>+</sup>/K<sup>+</sup> ATPase and with activities that support a pathogenic role have also been isolated from patients with pernicious anemia (D'elios et al., 2001).



**FIGURE 44.6** A mouse model of autoimmune gastritis. Irradiated wild-type mice that received  $5 \times 10^7$  CD4 $^+$  T cells from either wild-type (WT) or H $^+$ /K $^+$  ATPase  $\alpha$  subunit-deficient mice (H/K $\alpha^{-/-}$ ), were killed 8 weeks after transfer and compared to nonmanipulated mice (−). (A) Hematoxylin and eosin stained sections of stomachs. (B) Gastritis scores. (C) Macroscopic views of stomachs. (D) Stomach weights after stomach contents were removed. (E) Gastric pH. Mice were starved overnight and their stomachs were rinsed in 1 mL saline which were then collected and measured by a pH meter. (F) H $^+$ /K $^+$  ATPase-specific autoantibodies in serum were detected by ELISA using serial twofold dilutions of mouse sera, with a starting dilution factor of 50. (G) Gastric H $^+$ /K $^+$  ATPase autoantibody levels in mice at various times after cell transfer. Serum samples were collected at the indicated time points from each mouse after transfer of CD4 $^+$  T cells from H/K $\alpha^{-/-}$  mice. Autoantibody titer was represented by the maximum dilution factor that had an absorbance reading above 50% maximum. ND = not detectable. Data pooled from four independent experiments. In (B), (D), and (E), each circle represents the data from one mouse. Mann–Whitney U test was used; bars, median, and \*\*\*, P < .001.

## AUTOANTIBODIES AS POTENTIAL IMMUNOLOGIC MARKERS

Autoimmune gastritis and pernicious anemia are typically associated with autoantibodies to parietal cells, directed to gastric H $^+$ /K $^+$  ATPase and to IF. For the diagnosis of pernicious anemia, IF antibodies have a much higher disease specificity (> 95%), albeit lower sensitivity (50%–70%), than antibodies to gastric parietal cells. A recent study suggests that assaying autoantibodies to both IF and the H $^+$ /K $^+$  ATPase results in an increased specificity and sensitivity and would aid in the selection of patients for follow-up gastroscopic procedures (Lahner et al., 2009; Toh et al., 2012b). Since the Schilling test is no longer available as a diagnostic procedure, there has been increased dependence on IF antibodies for diagnosis. Antibodies to the vitamin B12 binding site of IF are demonstrable in serum of ~70% of the patients and to a second site in ~50%. These frequencies are



**FIGURE 44.7** Pathogenesis and complications of autoimmune gastritis. Autoimmune gastritis and type A chronic atrophic gastritis can develop in genetically predisposed individuals. Environmental risk factors (such as *Helicobacter pylori* infection) might also have a role. Apoptosis of parietal cells might result in the release of gastric H<sup>+</sup>/K<sup>+</sup> ATPase constituents, which are taken up by gastric dendritic cells that then migrate to the draining gastric lymph nodes to activate naïve CD4<sup>+</sup> T cells. The gastric H<sup>+</sup>/K<sup>+</sup> ATPase-activated CD4<sup>+</sup> T cells then migrate to the gastric mucosa to initiate tissue damage by binding to MHC class II molecules and by activating FAS-dependent mechanisms. Gastritis is marked by the presence of autoantibodies to the gastric H<sup>+</sup>/K<sup>+</sup> ATPase. Impaired acid secretion leads to iron deficiency anemia that can precede the onset of pernicious anemia by up to 20 years (Hershko et al., 2006). Chronic atrophic gastritis also represents a risk factor for gastric cancer arising from polypeptide expressing intestinal metaplasia, as well as enterochromaffin-like cell hyperplasia arising from gastrin hypersecretion by antral G cells predisposing to gastric carcinoid. Pernicious anemia arises from intrinsic factor deficiency as a result of loss of intrinsic factor producing gastric parietal cells and the presence of intrinsic factor autoantibodies (Green et al., 2017; Toh, 2017).

greater if gastric juice is assayed. There may be coexisting autoantibodies specific for the various other autoimmune diseases in the thyrogastriac cluster. The incidence of these antibodies rises with increasing duration of disease, almost doubling after 10 years (Ungar et al., 1967).

Parietal cell antibodies to gastric H<sup>+</sup>/K<sup>+</sup> ATPase are diagnostic of the underlying pathologic lesion of autoimmune gastritis, as gastric biopsies carried out in asymptomatic patients have revealed the presence of type A gastritis (Uibo et al., 1984). These antibodies are routinely detected by indirect immunofluorescence or, to gastric H<sup>+</sup>/K<sup>+</sup>ATPase, by ELISA (Chuang et al., 1992; Lahner et al., 2009). Antibodies to gastric parietal cells can be demonstrated by serum reactivity with the cytoplasm of gastric parietal cells in unfixed, air-dried, and frozen sections of mouse stomach (see Fig. 44.4). Mouse stomach is preferable to rat stomach because of a lower frequency of heterophile reactions (Muller et al., 1971) that could be misinterpreted as antibody to parietal cells. The antibodies are detected by immunofluorescence in 90% of the patients with pernicious anemia, with a prevalence of 2%–5% in the general population, and in 30% of the patients with other thyrogastriac autoimmune diseases, including type 1 diabetes mellitus (De Block et al., 2000, 2001a,b). Diagnostic ELISAs to gastric H/K ATPase are more sensitive than the immunofluorescence test for parietal cell antibody and can be used instead of the immunofluorescence test (Toh et al., 2012b). The prevalence of gastric autoantibodies increases with age and correlate with rising serum gastrin levels (Jassel et al., 1999; De Block et al., 2001a). Parietal cell mass and hence the availability of autoantigens decreases as autoimmune gastritis progresses to pernicious anemia and this may explain the observation that the prevalence of autoantibodies decreases with progression (Davidson et al., 1989) and may also explain the lower antibody prevalence in one study of pernicious anemia (Carmel, 1992).

## LABORATORY DIAGNOSIS

Laboratory diagnosis of autoimmune gastritis rests on serum biomarkers of antibody to parietal cell H/K ATPase and IF and corpus atrophy identified by serum biomarkers of gastrin and pepsinogen levels. Subjects with asymptomatic parietal cell antibody should be regularly assessed for serum biomarkers for progression to corpus atrophy, development of iron and B12 deficiency anemia, and for associated autoimmune thyroiditis and type 1 diabetes mellitus (Toh, 2014).

## CONCLUDING REMARKS—FUTURE PROSPECTS

As one of the most common autoimmune diseases, autoimmune gastritis has been intensively investigated and a great deal has been discovered concerning its immunological pathogenesis. The availability of excellent animal models has meant that the identity of the gastric autoantigens is well defined, and the pathway that leads to immunological tolerance in normal individuals is well understood. On the other hand, in common with most other autoimmune diseases, the reason why the immune system begins to react to gastric autoantigens to the detriment of patients is still not clear. While we know some level of detail of the genetics of autoimmune gastritis in mice, such data have not as yet been translated into the human setting. Environmental triggers for autoimmune gastritis are also unclear. While some data suggest a role for *Helicobacter* infection, this hypothesis is not supported by other epidemiological information and the question of its role remains open (Toh et al., 2012a).

From a clinical standpoint, pernicious anemia is now routinely treatable to reverse hematologic manifestations and to prevent neurological complications of the disease by vitamin B12 replacement. High-dose oral vitamin B12 tablets (1000–2000 µg) taken daily are as effective as intramuscular monthly injections in correcting blood and neurologic abnormalities (Stabler, 2013). However, this therapy does not cure the underlying cause of the disease, namely the decimation of the gastric mucosa by the autoimmune response, which increases the risk of gastric cancer. In mice, ectopic expression of the gastric autoantigen (Murphy et al., 2003) and treatment with Tregs (Nguyen et al., 2011) lead to long-term reversal of gastric lesions. Whether such treatments will become feasible or practical in humans remains to be seen. The challenge is to restore antigen-specific T cell tolerance to the gastric H/K ATPase (Anderson and Jabri, 2013).

## References

- Ahmed, S.A., Penhale, W.J., 1981. Pathological changes in inbred strains of mice following early thymectomy and irradiation. *Experientia* 37, 1341–1343.
- Alderuccio, F., Toh, B.H., 1998. Spontaneous autoimmune gastritis in C3H/He mice: a new mouse model for gastric autoimmunity. *Am. J. Pathol.* 153, 1311–1318.
- Alderuccio, F., Toh, B.H., Tan, S.S., Gleeson, P.A., van Driel, I.R., 1993. An autoimmune disease with multiple molecular targets abrogated by the transgenic expression of a single autoantigen in the thymus. *J. Exp. Med.* 178, 419–426.
- Alderuccio, F., Cataldo, V., van Driel, I.R., Gleeson, P.A., Toh, B.H., 2000. Tolerance and autoimmunity to a gastritogenic peptide in TCR transgenic mice. *Int. Immunol.* 12, 343–352.
- Allen, S., Read, S., DiPaolo, R., McHugh, R.S., Shevach, E.M., Gleeson, P.A., et al., 2005. Promiscuous thymic expression of an autoantigen gene does not result in negative selection of pathogenic T cells. *J. Immunol.* 175, 5759–5764.
- Anderson, R.P., Jabri, B., 2013. Vaccine against autoimmune disease: antigen-specific immunotherapy. *Curr. Opin. Immunol.* 25, 410–417.
- Ang, D.K.Y., Brodnicki, T.C., Jordan, M.A., Wilson, W.E., Silveira, P., Gliddon, B.L., et al., 2007. Two genetic loci independently confer susceptibility to autoimmune gastritis. *Int. Immunol.* 19, 1135–1144.
- Ardeman, S., Chanarin, I., 1965. Steroids and Addisonian pernicious anemia. *N. Engl. J. Med.* 273, 1352–1355.
- Ardeman, S., Chanarin, I., Jacobs, A., Griffiths, L., 1966. Family study in Addisonian pernicious anemia. *Blood* 27, 599–610.
- Asano, M., Toda, M., Sakaguchi, N., Sakaguchi, S., 1996. Autoimmune disease as a consequence of developmental abnormality of a T cell subpopulation. *J. Exp. Med.* 184, 387–396.
- Balcerzak, S.P., Westerman, M.P., Heinle, E.W., 1968. Discordant occurrence of pernicious anemia in identical twins. *Blood* 32, 701–710.
- Barrett, S.P., Toh, B.H., Alderuccio, F., van Driel, I.R., Gleeson, P.A., 1995. Organ-specific autoimmunity induced by adult thymectomy and cyclophosphamide-induced lymphopenia. *Eur. J. Immunol.* 25, 238–244.
- Barrett, S.P., Gleeson, P.A., Desilva, H., Toh, B.H., van Driel, I.R., 1996. Interferon- $\gamma$  is required during the initiation of an organ-specific autoimmune disease. *Eur. J. Immunol.* 26, 1652–1655.
- Bergman, M., Amedei, A., D'Ellos, M., Azzurri, A., Benagiano, M., Tamburini, C., et al., 2003. Characterization of H 1,K1-ATPase T cell epitopes in human autoimmune gastritis. *Eur. J. Immunol.* 33, 539–545.
- Berlin, R., Berlin, H., Brante, G., Sjoberg, S.G., 1958a. Failures in long-term oral treatment of pernicious anemia with B12-intrinsic factor preparations. *Acta Med. Scand.* 161, 143–150.

- Berlin, R., Berlin, H., Brante, G., Sjoberg, S.G., 1958b. Refractoriness to intrinsic factor-B12 preparations abolished by massive doses of intrinsic factor; preliminary report. *Acta Med. Scand.* 162, 317–319.
- Biondo, M., Nasa, Z., Marshall, A., Toh, B.H., Alderuccio, F., 2001. Local transgenic expression of granulocyte macrophage-colony stimulating factor initiates autoimmunity. *J. Immunol.* 166, 2090–2099.
- Bunn, H.F., 2014. Vitamin B12 and pernicious anemia—the dawn of molecular medicine. *N. Engl. J. Med.* 370, 773–776.
- Burman, P., Mardh, S., Norberg, L., Karlsson, F.A., 1989. Parietal cell antibodies in pernicious anaemia inhibit H,K-adenosine triphosphatase, the proton pump of the stomach. *Gastroenterology* 96, 1434.
- Callaghan, J.M., Toh, B.H., Pettitt, J.M., Humphris, D.C., Gleeson, P.A., 1990. Poly-N-acetyllactosamine-specific tomato lectin interacts with gastric parietal cells: identification of a tomato-lectin binding  $60\text{--}90 \times 10^3$  Mr membrane glycoprotein of tubulovesicles. *J. Cell Sci.* 95, 563–576.
- Callaghan, J.M., Khan, M.A., Alderuccio, F., van Driel, I.R., Gleeson, P.A., Toh, B.H., 1993. Alpha and beta subunits of the gastric H1/K(1)-ATPase are concordantly targeted by parietal cell autoantibodies associated with autoimmune gastritis. *Autoimmunity* 16, 289–295.
- Callender, S.T., Denborough, M.A., 1957. A family study of pernicious anaemia. *Br. J. Haematol.* 3, 88–106.
- Callender, S.T., Denborough, M.A., Sneath, J., 1957. Blood groups and other inherited characters in pernicious anaemia. *Br. J. Haematol.* 3, 107–114.
- Candon, S., McHugh, R.S., Foucras, G., Natarajan, K., Shevach, E.M., Margulies, D.H., 2004. Spontaneous organ-specific Th2-mediated autoimmunity in TCR transgenic mice. *J. Immunol.* 172, 2917–2924.
- Carmel, R., 1988. Pepsinogens and other serum markers in pernicious anemia. *Am. J. Clin. Pathol.* 90, 442–445.
- Carmel, R., 1992. Reassessment of the relative prevalences of antibodies to gastric parietal cell and to intrinsic factor in patients with pernicious anaemia—fluence of patient age and race. *Clin. Exp. Immunol.* 89, 74–77.
- Carmel, R., 1996. Prevalence of undiagnosed pernicious anemia in the elderly. *Arch. Intern. Med.* 156, 1097–1100.
- Carmel, R., 2008. How I treat cobalamin (vitamin B12) deficiency. *Blood* 112, 2214–2221.
- Carmel, R., Johnson, C.S., 1978. Racial patterns in pernicious anemia. Early age at onset and increased frequency of intrinsic-factor antibody in black women. *N. Engl. J. Med.* 298, 647–650.
- Castle, W.B., 1953. Development of knowledge concerning the gastric intrinsic factor and its relation to pernicious anemia. *N. Engl. J. Med.* 249, 603–614.
- Chanarin, I., 1979. The Megaloblastic Anaemias. Blackwell, Oxford.
- Chuang, J.S., Callaghan, J.M., Gleeson, P.A., Toh, B.H., 1992. Diagnostic ELISA for parietal cell autoantibody using tomato lectin-purified gastric H<sup>+</sup>/K<sup>+</sup>-ATPase (proton pump). *Autoimmunity* 12, 1–7.
- Cowling, D.C., Strickland, R.G., Ungar, B., Whittingham, S., Rose, W.M., 1974. Pernicious-anaemia-like syndrome with immunoglobulin deficiency. *Med. J. Aust.* 1, 15–17.
- Davidson, R.J., Atrah, H.I., Sewell, H.F., 1989. Longitudinal study of circulating gastric antibodies in pernicious anaemia. *J. Clin. Pathol.* 42, 1092–1095.
- De Block, C.E., De Leeuw, I.H., Decochez, K., Winnock, F., Van Autreve, J., Van Campenhout, C.M., et al., 2001a. The presence of thyrogastric antibodies in first degree relatives of type 1 diabetic patients is associated with age and proband antibody status. *J. Clin. Endocrinol. Metab.* 86, 4358–4363.
- De Block, C.E., De Leeuw, I.H., Vertommen, J.J., Rooman, R.P., Du Caju, M.V., Van Campenhout, C.M., et al., 2001b. Beta-cell, thyroid, gastric, adrenal and coeliac autoimmunity and HLA-DQ types in type 1 diabetes. *Clin. Exp. Immunol.* 126, 236–241.
- De Block, C.E.M., De Leeuw, I.H., Rooman, R.P.A., Winnock, F., Du Caju, M.V.L., Van Gaal, L.F., 2000. Gastric parietal cell antibodies are associated with glutamic acid decarboxylase-65 antibodies and the HLA DQA1\*0501-DQB1\*0301 haplotype in type 1 diabetes mellitus. *Diabet. Med.* 17, 618–622.
- De Silva, H.D., Van Driel, I.R., La Gruta, N., Toh, B.H., Gleeson, P.A., 1998. CD4 + T cells, but not CD8 + T cells, are required for the development of experimental autoimmune gastritis. *Immunology* 93, 405–408.
- De Silva, H.D., Alderuccio, F., Toh, B.H., Van Driel, I.R., Gleeson, P.A., 2001. Defining T cell receptors which recognise the immunodominant epitope of the gastric autoantigen, the H/K ATPase beta-subunit. *Autoimmunity* 33, 1–14.
- Delva, P.L., MacDonald, J.E., Macintosh, D.C., 1965. Megaloblastic anaemia occurring simultaneously in white female monozygotic twins. *Can. Med. Assoc. J.* 92, 1129–1131.
- DiBona, D.R., Ito, S., Berglindh, T., Sachs, G., 1979. Cellular site of gastric acid secretion. *Proc. Natl. Acad. Sci. U.S.A.* 76, 6689–6693.
- Dipaolo, R.J., Brinster, C., Davidson, T.S., Andersson, J., Glass, D., Shevach, E.M., 2007. Autoantigen-specific TGFbeta-induced Foxp3 + regulatory T cells prevent autoimmunity by inhibiting dendritic cells from activating autoreactive T cells. *J. Immunol.* 179, 4685–4693.
- Doig, A., Girdwood, R.H., Duthie, J.J., Knox, J.D., 1957. Response of megaloblastic anaemia of prednisolone. *Lancet* 273, 966–972.
- Donaldson, R.M., Mackenzie, I.L., Trier, J.S., 1967. Intrinsic factor-mediated attachment of vitamin B12 to brush borders and microvillous membranes of hamster intestine. *J. Clin. Invest.* 46, 1215–1228.
- Doniach, D., Roitt, I.M., Taylor, K.B., 1965. Autoimmunity in pernicious anemia and thyroiditis: a family study. *Ann. N.Y. Acad. Sci.* 124, 605–625.
- van Driel, I.R., Ang, D.K.Y., 2008. Role of regulatory T cells in gastro-intestinal inflammatory disease. *J. Gastroenterol. Hepatol.* 23, 171–177.
- van Driel, I.R., Stearne, P.A., Grego, B., Simpson, R.J., Goding, J.W., 1984. The receptor for transferrin on murine myeloma cells: one-step purification based on its physiology, and partial amino acid sequence. *J. Immunol.* 133, 3220–3224.
- van Driel, I.R., Read, S., Zwar, T.D., Gleeson, P.A., 2005. Shaping the T cell repertoire to a bona fide autoantigen: lessons from autoimmune gastritis. *Curr. Opin. Immunol.* 17, 570–576.
- Dujardin, H.C., Burlen-Defranoux, O., Boucontet, L., Vieira, P., Cumano, A., Bandeira, A., 2004. Regulatory potential and control of Foxp3 expression in newborn CD4 + T cells. *Proc. Natl. Acad. Sci. U.S.A.* 101, 14473–14478.
- D'elios, M.M., Bergman, M.P., Azzurri, A., Amedei, A., Benagiano, M., De Pont, J.J., et al., 2001. H<sup>+</sup>, K<sup>+</sup>-ATPase (proton pump) is the target autoantigen of Th1-type cytotoxic T cells in autoimmune gastritis. *Gastroenterology* 120 (2), 377–386.

- Faustin, B., Lartigue, L., Bruey, J.M., Luciano, F., Sergienko, E., Bailly-Maitre, B., et al., 2007. Reconstituted NALP1 inflammasome reveals two-step mechanism of caspase-1 activation. *Mol. Cell.* 25, 713–724.
- Fisher, J.M., Rees, C., Taylor, K.B., 1965. Antibodies in gastric juice. *Science* 150, 1467–1469.
- Fisher, J.M., Rees, C., Taylor, K.B., 1966. Intrinsic-factor antibodies in gastric juice of pernicious-anemia patients. *Lancet* 2, 88–89.
- Fixa, B., Thiele, H.G., Komárková, O., Nozicka, Z., 1972. Gastric auto-antibodies and cell-mediated immunity in pernicious anaemia—a comparative study. *Scand. J. Gastroenterol.* 7, 237–240.
- Fong, T.L., Dooley, C.P., Dehesa, M., Cohen, H., Carmel, R., Fitzgibbons, P.L., et al., 1991. *Helicobacter pylori* infection in pernicious anemia: a prospective controlled study. *Gastroenterology* 100, 328–332.
- Forte, J.G., Hanzel, D.K., Urushidani, T., Wolosin, J.M., 1989. Pumps and pathways for gastric HCl secretion. *Ann. N.Y. Acad. Sci.* 574, 145–158.
- Fuchs, C.S., Mayer, R.J., 1995. Gastric carcinoma. *N. Engl. J. Med.* 333, 32–41.
- Glass, G.B., 1963. Gastric intrinsic factor and its function in the metabolism of vitamin B12. *Physiol. Rev.* 43, 529–849.
- Gleeson, P.A., Toh, B.H., van Driel, I.R., 1996. Organ-specific autoimmunity induced by lymphopenia. *Immunol. Rev.* 149, 97–125.
- Goldberg, L.S., Bluestone, R., 1970. Hidden gastric autoantibodies to intrinsic factor in pernicious anemia. *J. Lab. Clin. Med.* 75, 449–456.
- Goldkorn, I., Gleeson, P.A., Toh, B.H., 1989. Gastric parietal cell antigens of 60–90 kDa, 92 kDa and 100–120 kDa associated with autoimmune gastritis and pernicious anaemia. Role of N-glycans in the structure and antigenicity of the 60–90 kDa component. *J. Biol. Chem.* 264, 18768–18774.
- Goldstone, A.H., Calder, E.A., Barnes, E.W., Irvine, W.J., 1973. The effect of gastric antigens on the in vitro migration of leucocytes from patients with atrophic gastritis and pernicious anaemia. *Clin. Exp. Immunol.* 14, 501–508.
- Gordin, R., 1959. Vitamin B12 absorption in corticosteroid-treated pernicious anaemia. *Acta Med. Scand.* 164, 159–165.
- Green, R., Allen, L.H., BJORKE-MONSEN, A.L., Brito, A., Gueant, J.L., Miller, J.W., et al., 2017. Vitamin B12 deficiency. *Nat. Rev. Dis. Primers.* 3, 17040.
- Hershko, C., Ronson, A., Souroujon, M., Maschler, I., Heyd, J., Patz, J., 2006. Variable hematologic presentation of autoimmune gastritis: age-related progression from iron deficiency to cobalamin depletion. *Blood* 107, 1673–1679.
- Highley, D.R., Davies, M.C., Ellenbogen, L., 1967. Hog intrinsic factor. II. Some physicochemical properties of vitamin B12-binding fractions from hog pylorus. *J. Biol. Chem.* 242, 1010–1015.
- Hogan, T.V., Ang, D.K.Y., Gleeson, P.A., van Driel, I.R., 2008. Extrathymic mechanisms of T cell tolerance: Lessons from autoimmune gastritis. *J. Autoimmun.* 31, 268–273.
- Hsing, A.W., Hansson, L.E., McLaughlin, J.K., Nyren, O., Blot, W.J., Ekbom, A., et al., 1993. Pernicious anemia and subsequent cancer—a population-based cohort study. *Cancer* 71, 745–750.
- Hughes, W.S., Brooks, F.P., Conn, H.O., 1972. Serum gastrin levels in primary hypogammaglobulinemia and pernicious anemia. Studies in adults. *Ann. Intern. Med.* 77, 746–750.
- Irvine, W.J., Davies, S.H., Teitelbaum, S., Delamore, I.W., Williams, A.W., 1962. Immunological relationship between pernicious anaemia and thyroid disease. *Br. Med. J.* 2, 454–456.
- Irvine, W.J., Davies, S.H., Teitelbaum, S., Delamore, I.W., Williams, A.W., 1965. The clinical and pathological significance of gastric parietal cell antibody. *Ann. N.Y. Acad. Sci.* 124, 657–691.
- Irvine, W.J., McFadzean, A.J.S., Todd, D., Tso, S.C., Yeung, R.T.T., 1969. Pernicious anaemia in the Chinese: a clinical and immunological study. *Clin. Exp. Immunol.* 4, 375–386.
- Irvine, W.J., Cullen, D.R., Mawhinney, H., 1974. Natural history of autoimmune achlorhydric atrophic gastritis: a 1–15 year follow-up study. *Lancet* 2, 482.
- Jacob, E., Schilling, R.F., 1966. An in vitro test for the detection of serum antibody to intrinsic factor-vitamin B12 complex. *J. Lab. Clin. Med.* 67, 510–515.
- Jassel, S.V., Ardill, J.E., Fillmore, D., Bamford, K.B., O'Connor, F.A., Buchanan, K.D., 1999. The rise in circulating gastrin with age is due to increases in gastric autoimmunity and *Helicobacter pylori* infection. *QJM* 92, 373–377.
- Jayaratnam, F.J., Seah, C.S., Da Costa, J.L., Tan, K.K., O'Brien, W., 1967. Pernicious anaemia among Asians in Singapore. *Br. Med. J.* 3, 18–25.
- Jeffries, G.H., Hoskins, D.W., Slesinger, M.H., 1962. Antibody to intrinsic factor in serum from patients with pernicious anemia. *J. Clin. Invest.* 41, 1106–1115.
- Jeffries, G.H., Todd, J.E., Slesinger, M.H., 1966. The effect of prednisolone on gastric mucosal histology, gastric secretion, and vitamin B12 absorption in patients with pernicious anemia. *J. Clin. Invest.* 45, 803–812.
- Jorge, A.D., Sanchez, D., 1973. The effect of azathioprine on gastric mucosal histology and acid secretion in chronic gastritis. *Gut* 14, 104–106.
- Judd, L.M., Gleeson, P.A., Toh, B.H., van Driel, I.R., 1999. Autoimmune gastritis results in disruption of gastric epithelial cell development. *Am. J. Physiol. Gastrointest. Liver Physiol.* 277, G209–G218.
- Kang, W., Rathinavelu, S., Samuelson, L.C., Merchant, J.L., 2005. Interferon gamma induction of gastric mucous neck cell hypertrophy. *Lab. Invest.* 85, 702–715.
- Karlsson, F.A., Burman, P., Loof, L., Mardh, S., 1988. Major parietal cell antigen in autoimmune gastritis with pernicious anaemia is the acid-producing H,K-adenosine triphosphatase of the stomach. *J. Clin. Invest.* 81, 475–479.
- Katakai, T., Mori, K.J., Masuda, T., Shimizu, A., 1998. Differential localization of T(H)1 and T(H)2 cells in autoimmune gastritis. *Int. Immunol.* 10, 1325–1334.
- Kaye, M.D., Whorwell, P.J., Wright, R., 1983. Gastric mucosal lymphocyte subpopulations in pernicious anaemia and in normal stomach. *Clin. Immunol. Immunopathol.* 28, 431–440.
- Kekki, M., Varis, K., Pohjanpalo, H., Isokoski, M., Ihmäki, T., Siurala, M., 1983. Course of antrum and body gastritis in pernicious anemia families. *Dig. Dis. Sci.* 28, 698–704.
- Kojima, A., Prehn, R.T., 1981. Genetic susceptibility to post-thymectomy autoimmune disease. *Immunogenetics* 14, 15–27.
- Kokkola, A., Sjöblom, S.M., Haapiaisen, R., Sipponen, P., Puolakkainen, P., Järvinen, H., 1998. The risk of gastric carcinoma and carcinoid tumours in patients with pernicious anaemia. A prospective follow-up study. *Scand. J. Gastroenterol.* 33, 88–92.

- Lahner, E., Norman, G.L., Severi, C., Encabo, S., Shums, Z., Vannella, L., et al., 2009. Reassessment of intrinsic factor and parietal cell autoantibodies in atrophic gastritis with respect to cobalamin deficiency. *Am. J. Gastroenterol.* 104, 2071–2079.
- Levin, D., Dipaolo, R.J., Brinster, C., Revilleza, M.J., Boyd, L.F., Teyton, L., et al., 2008. Availability of autoantigenic epitopes controls phenotype, severity, and penetrance in TCR Tg autoimmune gastritis. *Eur. J. Immunol.* 38, 3339–3353.
- Lindblom, A., Quadt, N., Marsh, T., Aeschlimann, D., Mörgelin, M., Mann, K., et al., 1999. The intrinsic factor-vitamin B12 receptor, cubilin, is assembled into trimers via a coiled-coil alpha-helix. *J. Biol. Chem.* 274, 6374–6380.
- Ma, J.Y., Borch, K., Mardh, S., 1994. Human gastric H,K-adenosine tri-phosphatase beta-subunit is a major autoantigen in atrophic corpus gastritis—expression of the recombinant human glycoprotein in insect cells. *Scand. J. Gastroenterol.* 29, 790–794.
- MacCuish, A.C., Urbaniak, S.J., Goldstone, A.H., Irvine, W.J., 1974. PHA responsiveness and subpopulations of circulating lymphocytes in pernicious anemia. *Blood* 44, 849–855.
- Mackay, I.R., Burnet, E.M., 1963. Autoimmune Diseases, Pathogenesis, Chemistry and Therapy. Charles Thomas, Springfield, IL.
- Markson, J.L., Moore, J.M., 1962. Thyroid auto-antibodies in pernicious anaemia. *Br. Med. J.* 2, 1352–1355.
- Marshall, A.C.J., Alderuccio, F., Toh, B.-H., 2002. Fas/CD95 is required for gastric mucosal damage in autoimmune gastritis. *Gastroenterology* 123, 780–789.
- Martinelli, T.M., van Driel, I.R., Alderuccio, F., Gleeson, P.A., Toh, B.H., 1996. Analysis of mononuclear cell infiltrate and cytokine production in murine autoimmune gastritis. *Gastroenterology* 110, 1791–1802.
- McHugh, R.S., Shevach, E.M., Margulies, D.H., Natarajan, K., 2001. A T cell receptor transgenic model of severe, spontaneous organ-specific autoimmunity. *Eur. J. Immunol.* 31, 2094–2103.
- McIntyre, P.A., Hahn, R., Conley, C.L., Glass, B., 1959. Genetic factors in predisposition to pernicious anemia. *Bull. Johns Hopkins Hosp.* 104, 309–342.
- Monteiro, J.P., Farache, J., Mercadante, A.C., Mignaco, J.A., Bonamino, M., Bonomo, A., 2008. Pathogenic effector T cell enrichment overcomes regulatory T cell control and generates autoimmune gastritis. *J. Immunol.* 181, 5895–5903.
- Muller, H.K., McGiven, A.R., Nairn, R.C., 1971. Immunofluorescent staining of rat gastric parietal cells by human antibody unrelated to pernicious anaemia. *J. Clin. Pathol.* 24, 13–14.
- Murphy, K., Biondo, M., Toh, B.-H., Alderuccio, F., 2003. Tolerance established in autoimmune disease by mating or bone marrow transplantation that target autoantigen to thymus. *Int. Immunol.* 15, 269–277.
- Nguyen, T.-L.M., Sullivan, N.L., Ebel, M., Teague, R.M., Dipaolo, R.J., 2011. Antigen-specific TGF- $\beta$ -induced regulatory T cells secrete chemokines, regulate T cell trafficking, and suppress ongoing autoimmunity. *J. Immunol.* 187, 1745–1753.
- Pearce, J.M.S., 2008. Subacute combined degeneration of the cord: Putnam-Dana syndrome. *Eur. Neurol.* 60, 53–56.
- Pettitt, J.M., Humphris, D.C., Barrett, S.P., Toh, B.H., van Driel, I.R., Gleeson, P.A., 1995. Fast freeze-fixation/freeze-substitution reveals the secretory membranes of the gastric parietal cell as a network of helically coiled tubule—a new model for parietal cell transformation. *J. Cell Sci.* 108, 1127–1141.
- Prinz, C., Kajimura, M., Scott, D., Helander, H., Shin, J.M., Besancon, M., et al., 1992. Acid secretion and the H,K-ATPase of stomach. *Yale J. Biol. Med.* 65, 577–596.
- Rabon, E.C., Reuben, M.A., 1990. The mechanism and structure of the gastric H,K ATPase. *Annu. Rev. Physiol.* 52, 321–344.
- Read, S., Hogan, T.V., Zwar, T.D., Gleeson, P.A., Van Driel, I.R., 2007. Prevention of autoimmune gastritis in mice requires extra-thymic T-cell deletion and suppression by regulatory T cells. *Gastroenterology* 133, 547–558.
- Renshaw, A., 1930. Treatment of pernicious anaemia with desiccated hog's stomach. *Br. Med. J.* 1, 334–335.
- Rickes, E.L., Brink, N.G., Koniuszky, F.R., Wood, T.R., Folkers, K., 1948. Crystalline vitamin B12. *Science* 107, 396–397.
- Rodbro, P., Dige-Petersen, H., Schwartz, M., Dalgaard, O.Z., 1967. Effect of steroids on gastric mucosal structure and function in pernicious anemia. *Acta Med. Scand.* 181, 445–452.
- Rose, M.S., Doniach, D., Chanarin, I., Brostoff, J., Ardeman, S., 1970. Intrinsic-factor antibodies in absence of pernicious anaemia. 3–7 year follow-up. *Lancet* 2, 9–12.
- Rothenberg, S.P., Kantha, K.R., Ficarra, A., 1971. Autoantibodies to intrinsic factor: their determination and clinical usefulness. *J. Lab. Clin. Med.* 77, 476–484.
- Samloff, I.M., Kleinman, M.S., Turner, M.D., Sobel, M.V., Jeffrie, G.H., 1968. Blocking and binding antibody to intrinsic factor and parietal cell antibody in pernicious anaemia. *Gastroenterology* 55, 575–583.
- Samloff, I.M., Varis, K., Ihamaki, T., Siurala, M., Rotter, J.I., 1982. Relationships among serum pepsinogen I, serum pepsinogen II, and gastric mucosal histology. A study in relatives of patients with pernicious anemia. *Gastroenterology* 83, 204–209.
- Samy, E.T., Wheeler, K.M., Roper, R.J., Teuscher, C., Tung, K.S., 2008. Cutting edge: Autoimmune disease in day 3 thymectomized mice is actively controlled by endogenous disease-specific regulatory T cells. *J. Immunol.* 180, 4366–4370.
- Savage, D.G., Lindenbaum, J., 1995. Neurological complications of acquired cobalamin deficiency: clinical aspects. *Baillieres Clin. Haematol.* 8, 657–678.
- Scarff, K.J., Pettitt, J.M., Driel, I.R.V., Gleeson, P.A., Toh, B.H., 1997. Immunisation with gastric H/K ATPase induces reversible autoimmune gastritis. *Immunology* 92, 91–98.
- Scheinecker, C., McHugh, R., Shevach, E.M., Germain, R.N., 2002. Constitutive presentation of a natural tissue autoantigen exclusively by dendritic cells in the draining lymph node. *J. Exp. Med.* 196, 1079–1090.
- Schwartz, M., 1958. Intrinsic-factor-inhibiting substance in serum of orally treated patients with pernicious anaemia. *Lancet* 2, 61–62.
- Serafini, U., Masala, C., Pala, A.M., 1970. Studies on gastric autoimmunity. *Folia Allergol.* 17, 433–434.
- Sharp, E.A., 1929. An antianaemic factor in desiccated hog stomach. *JAMA* 93, 749–751.
- Silveira, P.A., Baxter, A.G., Cain, W.E., van Driel, I.R., 1999. A major linkage region on distal chromosome 4 confers susceptibility to mouse autoimmune gastritis. *J. Immunol.* 162, 5106–5111.
- Silveira, P.A., Wilson, W.E., Esteban, L.M., Jordan, M.A., Hawke, C.G., van Driel, I.R., et al., 2001. Identification of the Gasa3 and Gasa4 autoimmune gastritis susceptibility genes using congenic mice and partitioned, segregative and interaction analyses. *Immunogenetics* 53, 741–750.

- Smith, E.L., 1948. Purification of anti-pernicious anaemia factors from liver. *Nature* 161, 638–639.
- Song, Y.H., Ma, J.Y., Mardh, S., Liu, T., Sjostrand, S.E., Rask, L., et al., 1994. Localization of a pernicious anaemia autoantibody epitope on the alpha-subunit of human H,K-adenosine triphosphatase. *Scand. J. Gastroenterol.* 29, 122–127.
- Stabler, S.P., 2013. Vitamin B12 deficiency. *N. Engl. J. Med.* 368, 2041–2042.
- Strickland, R.G., 1969. Pernicious anemia and polyendocrine deficiency. *Ann. Intern. Med.* 70, 1001–1005.
- Strickland, R.G., Mackay, I.R., 1973. A reappraisal of the nature and significance of chronic atrophic gastritis. *Am. J. Dig. Dis.* 18, 426–440.
- Stummvoll, G.H., DiPaolo, R.J., Huter, E.N., Davidson, T.S., Glass, D., Ward, J.M., et al., 2008. Th1, Th2, and Th17 effector T cell-induced autoimmune gastritis differs in pathological pattern and in susceptibility to suppression by regulatory T cells. *J. Immunol.* 181, 1908–1916.
- Sturgis, C.C., Isaacs, R., 1929. Desiccated stomach in the treatment of pernicious anaemia. *JAMA* 93, 747–749.
- Suri-Payer, E., Cantor, H., 2001. Differential cytokine requirements for regulation of autoimmune gastritis and colitis by CD4(+)CD25(+) T cells. *J. Autoimmun.* 16, 115–123.
- Suri-Payer, E., Amar, A.Z., McHugh, R., Natarajan, K., Margulies, D.H., Shevach, E.M., 1999. Post-thymectomy autoimmune gastritis: fine specificity and pathogenicity of anti-H/K ATPase-reactive T cells. *Eur. J. Immunol.* 29, 669–677.
- Tai, C., McGuigan, J.E., 1969. Immunologic studies in pernicious anaemia. *Blood* 34, 63–71.
- Taylor, K.B., 1959. Inhibition of intrinsic factor by pernicious anaemia sera. *Lancet* 2, 106–108.
- Taylor, K.B., Morton, J.A., 1959. An antibody to Castle's intrinsic factor. *J. Pathol. Bacteriol.* 77, 117–122.
- Toh, B.H., 2014. Diagnosis and classification of autoimmune gastritis. *Autoimmun. Rev.* 13, 459–462.
- Toh, B.H., 2017. Pathophysiology and laboratory diagnosis of pernicious anaemia. *Immunol. Res.* 65, 326–330.
- Toh, B.H., Gleeson, P.A., Simpson, R.J., Moritz, R.L., Callaghan, J., Goldkorn, I., et al., 1990. The 60–90 kDa parietal cell autoantigen associated with autoimmune gastritis is a b subunit of the gastric H<sub>1</sub>K<sub>1</sub>-ATPase (proton pump). *Proc. Natl. Acad. Sci. U.S.A.* 87, 6418–6422.
- Toh, B.H., van Driel, I.R., Gleeson, P.A., 1997. Mechanisms of disease: pernicious anaemia. *N. Engl. J. Med.* 337, 1441–1448.
- Toh, B.H., Chan, J., Kyaw, T., Alderuccio, F., 2012a. Cutting edge issues in autoimmune gastritis. *Clin. Rev. Allergy. Immunol.* 42, 269–278.
- Toh, B.H., Kyaw, T., Taylor, R., Pollock, W., Schlumberger, W., 2012b. Parietal cell antibody identified by ELISA is superior to immunofluorescence, rises with age and is associated with intrinsic factor antibody. *Autoimmunity* 45, 527–532.
- Tu, E., Ang, D.K.Y., Hogan, T.V., Read, S., Chia, C.P.Z., Gleeson, P.A., et al., 2011. A convenient model of severe, high incidence autoimmune gastritis caused by polyclonal effector T cells and without perturbation of regulatory T cells. *PLoS One* 6, e27153.
- Tudhope, G.R., Wilson, G.M., 1960. Anaemia in hypothyroidism. Incidence, pathogenesis, and response to treatment. *Q. J. Med.* 29, 513–537.
- Twomey, J.J., Jordan, P.H., Jarrold, T., Trubowitz, S., Ritz, N.D., Conn, H.O., 1969. The syndrome of immunoglobulin deficiency and pernicious anaemia. *Am. J. Med.* 47, 340–350.
- Uibo, R., Krohn, K., Villako, K., Tammur, R., Tamm, A., 1984. The relationship of parietal cell, gastrin cell, and thyroid autoantibodies to the state of the gastric mucosa in a population sample. *Scand. J. Gastroenterol.* 19, 1075–1080.
- Ungar, B., Whittingham, S., Francis, C.M., 1967. Pernicious anaemia: incidence and significance of circulating antibodies to intrinsic factor and to parietal cells. *Australas. Ann. Med.* 16, 226–229.
- Ungar, B., Stocks, A.E., Martin, F.I., Whittingham, S., Mackay, I.R., 1968. Intrinsic-factor antibody, parietal-cell antibody, and latent pernicious anaemia in diabetes mellitus. *Lancet* 2, 415–417.
- Ungar, B., Mathews, J.D., Tait, B.D., Cowling, D.C., 1977. HLA patterns in pernicious anaemia. *Br. Med. J.* 1, 798–800.
- Vargas, J.A., Alvarezmon, M., Manzano, L., Albillos, A., Fernandezcorugedo, A., Albaran, F., et al., 1995. Functional defect of T cells in autoimmune gastritis. *Gut* 36, 171–175.
- Varis, K., 1981. Family of behaviour of chronic gastritis. *Ann. Clin. Res.* 13, 123–129.
- Varis, K., Samloff, I.M., Ihämaki, T., Siurala, M., 1979. An appraisal of tests for severe atrophic gastritis in relatives of patients with pernicious anaemia. *Dig. Dis. Sci.* 24, 187–191.
- Wall, A.J., Whittingham, S., Mackay, I.R., Ungar, B., 1968. Prednisolone and gastric atrophy. *Clin. Exp. Immunol.* 3, 359–366.
- Wangel, A.G., Callender, S.T., Spray, G.H., Wright, R., 1968a. A family study of pernicious anaemia. II. Intrinsic factor secretion, vitamin B12 absorption and genetic aspects of gastric autoimmunity. *Br. J. Haematol.* 14, 183–204.
- Wangel, A.G., Callender, S.T., Spray, G.H., Wright, R., 1968b. A family study of pernicious anaemia. I. Autoantibodies, achlorhydria, serum pepsinogen and vitamin B12. *Br. J. Haematol.* 14, 161–181.
- Whittingham, S., Ungar, B., Mackay, I.R., Mathews, J.D., 1969. The genetic factor in pernicious anaemia. A family study in patients with gastritis. *Lancet* 1, 951–954.
- Whittingham, S., Youngchaiyud, U., Mackay, I.R., Buckley, J.D., Morris, P.J., 1975. Thyrogastric autoimmune disease. Studies on the cell-mediated immune system and histocompatibility antigens. *Clin. Exp. Immunol.* 19, 289–299.
- Whittingham, S., Mackay, I.R., Tait, B.D., 1991. The immunogenetics of pernicious anaemia. In: Farid, N.R. (Ed.), *Immunogenetics of Autoimmune Disease*. CRC Press, London, pp. 215–227.
- Wilkinson, J.F., 1949. Megalocytic anaemias. *Lancet* 1, 249–255.
- Wood, I.J., Doig, R.K., Motterham, R., Hughes, A., 1949. Gastric biopsy; report on 55 biopsies using a new flexible gastric biopsy tube. *Lancet* 1, 18–21.
- Zwar, T.D., Read, S., van Driel, I.R., Gleeson, P.A., 2006. CD4<sup>+</sup> CD25<sup>+</sup> regulatory T cells inhibit the antigen-dependent expansion of self-reactive T cells in vivo. *J. Immunol.* 176, 1609–1617.

## Further Reading

- Tu, E., Bourges, D., Gleeson, P.A., Ang, D.K., van Driel, I.R., 2013. Pathogenic T cells persist after reversal of autoimmune disease by immunosuppression with regulatory T cells. *Eur. J. Immunol.* 43, 1286–1296.

# Celiac Disease

Ludvig M. Sollid<sup>1</sup> and Knut E.A. Lundin<sup>2</sup>

<sup>1</sup>Centre for Immune Regulation and KG Jebsen Coeliac Disease Research Centre, Department of Immunology, Oslo University Hospital, University of Oslo, Oslo, Norway <sup>2</sup>KG Jebsen Coeliac Disease Research Centre, Department of Gastroenterology, Oslo University Hospital, University of Oslo, Oslo, Norway

## O U T L I N E

General Introduction	849	Pathogenic Mechanisms	857
Historical Aspects	850	Gluten-Specific CD4 + T Cells	857
Epidemiology	851	Transglutaminase 2	859
Clinical Features and Disease Associations	851	Gluten Antigen Presentation by Disease Associated HLA-DQ Molecules	859
Pathological Features	852	Macrophages and Dendritic Cells	860
Autoimmune Features	852	Plasma Cells	861
Autoantibodies	852	Effector Mechanisms Leading to Mucosal Alterations	861
Autoreactive Intraepithelial Lymphocytes	854	Autoantibodies as Immunologic Markers	861
Genetics	854	Serology	862
HLA Genes	855	Staining of Immune Complexes	862
Non-HLA Genes	855	Treatment and Outcome	862
Environmental Factors	856	Current Treatment	862
Gluten Proteins	856	Novel Treatments	863
Other Environmental Factors	856	Perspectives	863
In Vivo and In Vitro Disease Models	857	Acknowledgments	863
Animal Models	857	References	863
Organ Culture Assays	857		

## GENERAL INTRODUCTION

Celiac disease (also termed gluten-sensitive enteropathy) was early on considered a food hypersensitivity disorder as it precipitates in genetically susceptible individuals by the ingestion of cereal gluten proteins. This perception of the etiology of the disorder has changed as the disease has several autoimmune features of which the highly disease-specific antibodies to the enzyme transglutaminase 2 (TG2) are particularly striking. There is also tissue-specific destruction of enterocytes in the small bowel—not very different from the destruction of islet cells in the pancreas of subjects affected with type 1 diabetes. The combination of the disease driving antigen being identified, the relatively easy access to the target organ by gastroduodenoscopy, and an unusually clear

association to certain human leukocyte antigen (HLA) allotypes has led to a detailed understanding of the pathogenic mechanisms. Hence, among the chronic inflammatory disorders with autoimmune components, celiac disease stands out as a particularly instructive model. Insight into the pathogenesis of this disorder is relevant for the diseases for which the genetic and environmental components are yet poorly characterized.

## HISTORICAL ASPECTS

Celiac disease was probably first described in the 2nd century A.D. by Aretaeus of Cappadocia. However, the important discovery of the Dutch pediatrician Willem Karel Dicke that this is a condition dependent on oral gluten exposure is fairly recent. Apparently, Dicke's suspicion was alerted by a young mother who reported that her child's rash improved by removal of bread from the diet, and he began experimenting with gluten-free diet treatment in the mid-1930s (van Berge-Henegouwen and Mulder, 1993). His first publication on the dietary treatment of the condition was in Dutch in 1941 (Dicke, 1941). Dicke (1950) defended his thesis entitled "Coeliac disease investigations of the harmful effects of certain types of cereal on patients suffering from coeliac disease" at the University of Utrecht. Elimination and provocation diets played a central role in the early diagnostic workup schemes reflecting upon the fact that celiac disease was considered a food intolerance disorder (Meeuwisse, 1970). Now, celiac disease is equally considered to be much an autoimmune condition where the diagnosis in the recent guidelines for pediatric disease is diagnosed essentially on detection of high levels of autoantibodies (Husby et al. 2012). Gluten-free diets are popular today and staying gluten free is adopted by many more people than those affected with celiac disease. Some of the people staying gluten free suffer from nonceliac gluten sensitivity. The diagnostic criteria of this condition are solely based on symptoms and an exclusion of celiac disease, and its etiology, which while still being unknown, may as well not involve immunological mechanisms. In Box 45.1, some key historical findings which have led to our current understanding of the pathogenesis and the clinical handling of celiac disease are listed.

### BOX 45.1

#### IMPORTANT HISTORICAL ACHIEVEMENTS IN THE STUDY OF CELIAC DISEASE.

- First description of a condition resembling celiac disease by Aretaeus of Cappadocia (2nd century A.D.)
- First precise description of the disorder (Gee, 1888)
- Suggestion that banana diet can cure the disease (Haas, 1932)
- Wheat gluten identified as a causative agent (Dicke, 1950)
- Villous atrophy and crypt hyperplasia found to be pathognomonic for the disease (Paulley, 1954)
- Celiac disease of childhood and adult nontropical sprue share the same pathogenesis (Rubin et al., 1960)
- Familial clustering of biopsy proven disease (MacDonald et al., 1965)
- First diagnostic criteria of pediatric celiac disease from ESPGAN (Meeuwisse, 1970)
- Existence of autoantibodies to reticulin detected (Alp and Wright, 1971; Seah et al., 1971)
- Celiac disease found to be an HLA associated disorder (Falchuk et al., 1972; Stokes et al., 1972)
- Cell-mediated immunity to gliadin in celiac disease intestinal biopsy specimens (Ferguson et al., 1975)
- HLA-DQ2 heterodimer ( $DQA1^*05/DQB1^*02$ ) identified as the primary HLA susceptibility factor (Sollid et al., 1989)
- Isolation of gliadin-specific, HLA-DQ2 restricted T cells from intestinal biopsies (Lundin et al., 1993)
- Celiac disease autoantigen identified as transglutaminase 2 (Dieterich et al., 1997)
- Diagnosis in children can be done without a gut biopsy examination (Husby et al., 2012)

## EPIDEMIOLOGY

Celiac disease was previously considered to be a disease primarily of Caucasians but new observations suggest that the disease has an almost worldwide distribution. For instance, a study from China based on serology indicated that the prevalence in the Shandong province in north China, where wheat is a staple in the diet, is 0.76% (Yuan et al., 2017). In Europe and the United States the prevalence is around 1% (Choung et al., 2017; Mustalahti et al., 2010). There are unexplained differences in prevalence between European countries with adult prevalence figures varying from 2.4% in Finland to 0.3% in Germany (Mustalahti et al., 2010). Similarly, there is a striking difference in prevalence between Finnish and Russian Karelia, which should be genetically homogenous, as the region was divided after the Second World War (Kondrashova et al., 2008). The strong regional differences in the prevalence speak of the involvement of environmental factors in the etiology of the disease.

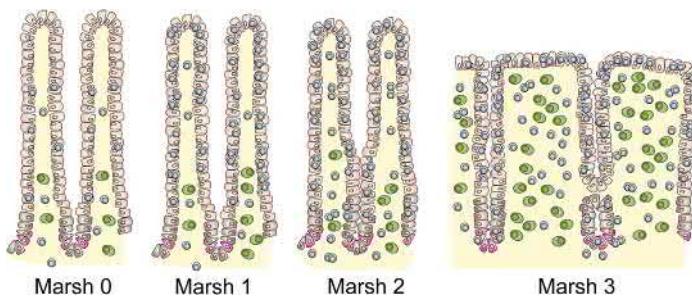
More than half of the cases of celiac disease are now diagnosed in adult life, and some patients are diagnosed after the age of 60 years (Holmes and Muirhead, 2017; Rubio-Tapia et al., 2012). Many patients may have had undetected disease since childhood whereas in others, the disease has started later (Catassi et al., 2010). The natural history and the timing for conversion to seropositive and/or mucosal inflammation are not fully understood. Studies have reported that some children have transient positive serology that normalizes without dietary manipulation (Mäki et al., 2003; Simell et al., 2007). These events suggest that at least certain aspects of celiac disease may be transient.

Between 1985 and 1995, there was an epidemic of celiac disease in Sweden among children below 2 years of age with a threefold increase in incidence (Namatovu et al., 2014). The sharp rise and subsequent abrupt decrease in incidence was investigated and explained by changes in infant feeding habits, including an increase in amount of gluten given, the age at introduction of gluten to the diet, and whether breastfeeding was ongoing or not when gluten was introduced. These observations fueled hope that it should be possible to prevent celiac disease by introducing small amounts of gluten at 4–6 months of age. This approach was tested in a multicenter, randomized, double-blind, placebo-controlled dietary intervention study (Vriezinga et al., 2014). Disappointingly, no preventive effect by this intervention was observed in children at the age of 3 years. Neither did a parallel intervention study that awaited gluten introduction to after 1 year of age demonstrate a preventive effect (Lionetti et al., 2014). Thus prevention of celiac disease by dietary intervention is not possible today.

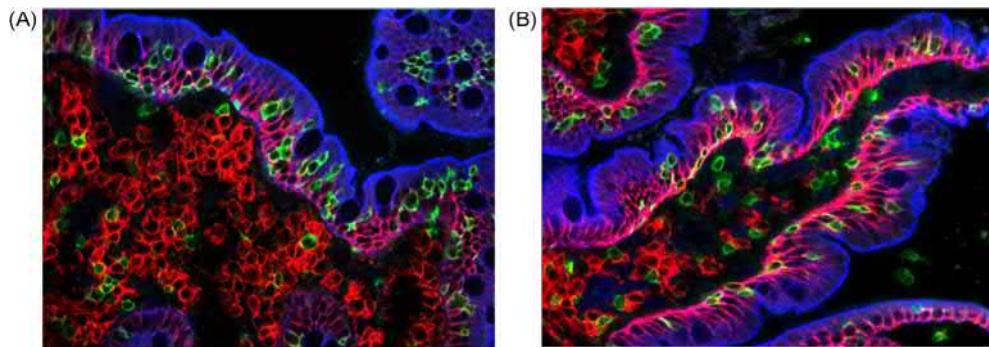
## CLINICAL FEATURES AND DISEASE ASSOCIATIONS

Celiac disease may present in early childhood soon after the introduction of gluten containing food. A dramatic and even fatal clinical picture with diarrhea, anorexia, failure to thrive, abdominal distension, and growth retardation was once regarded as typical. This is now rare, and most patients, both children and adults, present with less and milder symptoms (Lebwohl et al., 2017). Many of the symptoms associated with the milder disease form, such as chronic fatigue, joint pain, and headache do not directly point to a small intestinal disorder. Neither do complications, such as osteoporosis, reduced fertility, peripheral neuropathy, ataxia, epilepsy with cerebral calcifications, or dermatitis herpetiformis (Ciacci et al., 1995; Collin and Reunala, 2003; Hadjivassiliou et al., 2003; Sanders et al., 2003). The coexistence of autoimmune diseases is striking; in particular, there is an overrepresentation of type 1 diabetes (Cronin et al., 1997; Green et al., 1962; Koletzko et al., 1988), Sjögren's syndrome (Collin et al., 1994), autoimmune thyroid disorders (Collin et al., 1994; Counsell et al., 1994), connective tissue disease (Collin et al., 1994), and IgA deficiency (Cataldo et al., 1997; Mawhinney and Tomkin, 1971; Wang et al., 2011). It has been debated whether gluten consumption in undiagnosed celiac disease can induce other autoimmune diseases; however, the available evidence supporting this notion is weak (Lundin and Wijmenga, 2015). Like many autoimmune disorders, pediatric and adult celiac disease show a gender bias with a female to male ratio of approximately 2:1 (Ciacci et al., 1995; Ivarsson et al., 2003b).

Recently, there has been much interest in the clinical entity of nonceliac gluten sensitivity. Such patients may have symptoms resembling celiac disease but the enteropathy, serology, and HLA association typical of celiac disease are lacking as are clear diagnostic criteria (Ludvigsson et al., 2013).



**FIGURE 45.1** Schematic representation of the stages of the intestinal lesion in celiac disease according to Marsh. These stages are dynamically related. The normal state is Marsh type 0. In Marsh type 1, there is increased number of intraepithelial lymphocytes. Marsh type 2 has increased number of intraepithelial lymphocytes and enlarged, hyperplastic crypts. Marsh type 3 has, in addition to increased number of intraepithelial lymphocytes and crypt hyperplasia, partial to complete blunting of the villous structures also. The number of leukocytes in the lamina propria, such as plasma cells and T cells, are increased in the full-blown celiac disease lesion but their presence is not included as a parameter for the Marsh staging. Source: Courtesy of A.-C.R. Beitnes and Jorunn Stamnaes.



**FIGURE 45.2** The celiac lesion is characterized by the increased infiltration of T cells and plasma cells. Immunohistochemical staining of T cells (CD3, green), plasma cells (CD138, red), and epithelial cells (cytokeratin, blue) in the biopsy sections of (A) a patient with untreated celiac disease and (B) a normal subject. Source: Courtesy of A.-C.R. Beitnes.

## PATHOLOGICAL FEATURES

The intestinal lesion in celiac disease can be classified into three dynamically connected stages as suggested by Marsh—the infiltrative, hyperplastic, and destructive lesion (Marsh, 1992) (Fig. 45.1). In the infiltrative lesion (Marsh 1), the mucosal architecture is normal but there is an increased infiltration of intraepithelial lymphocytes (IELs) in the villous epithelium. The hyperplastic lesion (Marsh 2) is similar to the infiltrate lesion but in addition, has enlarged hyperplastic crypts. The last stage is the destructive lesion which can be subgrouped into partial, subtotal, or total villous atrophy (Marsh 3A-C) (Oberhuber et al., 1999). The latter is corresponding to the classic flat lesion, which in addition to the increased IELs, is characterized by swelling and infiltration of plasma cells, CD4 +  $\alpha\beta$  T cells, macrophages/dendritic cells, mast cells, and neutrophils in the lamina propria (Fig. 45.2).

## AUTOIMMUNE FEATURES

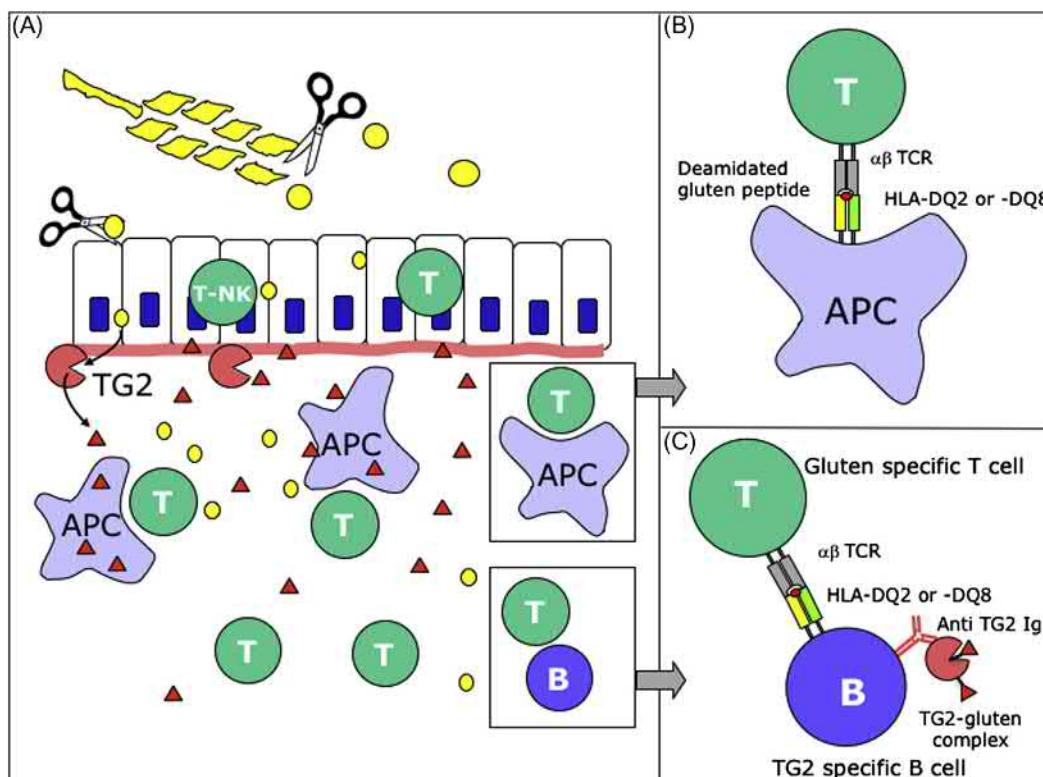
### Autoantibodies

Untreated celiac disease patients (on a regular diet) usually have increased levels of antibodies against gluten, other food antigens, and autoantigens. Initially, autoantibodies in celiac disease were detected as antireticulin antibodies by staining of various rat tissues (Alp and Wright, 1971; Seah et al., 1971). Later IgA antiendomysium antibodies (EMA), either as detected by staining of monkey esophagus (Chorzelski et al., 1984) or human umbilical cord (Ladinser et al., 1994), were described. The enzyme TG2 was identified as the target antigen of

both antireticulin antibodies and EMA (Dieterich et al., 1997), although antibodies to calreticulin and actin are also found in the serum of some patients (Clemente et al., 2000; Sanchez et al., 2000). The antibodies to TG2 are both of the IgA and IgG isotypes but the IgA antibodies are most closely linked with celiac disease and are the best predictor of disease (Rostom et al., 2005).

Anti-TG2 antibodies only occur in individuals who are HLA-DQ2 or HLA-DQ8 (Bjorck et al., 2010). Moreover, the production of the IgA anti-TG2 antibodies is dependent on dietary gluten exposure (Dieterich et al., 1998; Sulkkanen et al., 1998). These two observations indicate that gluten-specific T cells are implicated in the generation of the antibodies. A model where gluten-specific T cells may provide help to TG2-specific B cells by means of haptene-carrier-like gluten-TG2 complexes can explain the gluten and HLA dependence of the anti-TG2 antibody production (Solidi et al., 1997) (Fig. 45.3C). The same T cells should also be able to provide T-cell help to the B cells specific for the deamidated gluten peptides.

The production of anti-TG2 antibodies is vigorous in the active celiac lesion. On an average, 10% of the plasma cells in the lesion produce anti-TG2 antibodies and the IgA isotype dominates the response (Di Niro et al., 2012). The TG2-specific plasma cells have a responsive population dynamics and a wide seeding in the duodenal lamina propria (Di Niro et al., 2016). On commencement of a gluten-free diet, the frequency of the cells in the lesion drops dramatically within 6 months, yet some cells remain. The anti-TG2 antibodies have a unique repertoire with a biased usage of certain heavy chain genes, such as *IGHV5-51* and *IGHV3-48*.



**FIGURE 45.3** The celiac small intestinal lesion. (A) Depiction of the intestinal mucosa with emphasis on the factors taking part in the development and control of celiac disease. The parts of the gluten proteins that are resistant to processing by the luminal and brush border enzymes will survive digestion and can be transported across the epithelial barrier as polypeptides. Gluten peptides are deamidated by TG2, which, in the intestinal mucosa, is mainly located extracellularly in the subepithelial region but is also found in the brush border. TG2 may also be expressed by APC like macrophages and dendritic cells. CD4+ T cells in the lamina propria recognize predominantly deamidated gluten peptides, presented by HLA-DQ2 or HLA-DQ8 molecules on the cell surface of the APC. (B) DQ2 and DQ8 molecules have preference for binding peptides with negatively charged amino acids and thereby bind gluten peptides deamidated by TG2 with increased affinities. (C) Model of how gluten-specific T cells control the formation of anti-TG2 antibodies. Covalent complexes of gluten peptides and TG2 (involving glutamine residues of peptide either by isopeptide bond linkage to TG2's cell surface lysine residues or by thiolester linkage to TG2's active site cysteine) can be taken up by TG2-specific B cells via cell surface immunoglobulin. In endosomes, deamidated gluten peptides can be loaded onto DQ2 or DQ8 molecules. After transport to the cell surface of the HLA molecules with bound peptides, gluten-specific T cells can recognize the deamidated gluten peptides and thereby provide T-cell help to the TG2-specific B cells. *T*, T cell; *T-NK*, T cell with natural killer cell receptors; *B*, B cell; *APC*, antigen presenting cell; *TG2*, transglutaminase 2.

(Di Niro et al., 2012; Marzari et al., 2001) and light chain genes, such as *IGKV1-39p* and *IGKV5-1* (Roy et al., 2017). Further, the antibodies have few mutations despite being part of a chronic inflammatory response (Di Niro et al., 2012; Roy et al., 2017). Albeit few, displacement mutations dominate over silent mutations, thus speaking of the involvement of T cells in the antibody response (Di Niro et al., 2012; Marzari et al., 2001). Further, there is a reduction in the binding affinity of the antibodies by reversion to presumed germline sequence indicating that affinity maturation takes place in the generation of the antibodies (Di Niro et al., 2012). The plasma cells specific for the deamidated gluten peptides are less frequent in the celiac lesion than the TG2-specific plasma cells, but these cells, as the anti-TG2 plasma cells, have restricted the VH/VL usage and limited degree of mutations (Steinsbo et al., 2014). Interesting information on the generation of the celiac disease-relevant antibodies was obtained by the comparative analysis of the immunoglobulin protein sequences of serum and biopsy explants with the gene sequences of the gut plasma cells (Iversen et al., 2017). Serum IgA specific for either TG2 or a deamidated gluten peptide has the V-gene usage that match with those of the gut plasma cells. Further, the CDR-H3 sequences of the gut plasma cells are abundant in serum IgA and also detectable in serum IgG. As the serum IgA antibodies were found to be mostly monomeric and the gut IgA antibodies mostly dimeric, the observations indicate that the same B cell clones give rise to gut plasma cells and contribute to the serum antibody pool, but that there are distinct plasma cell origins of the immunoglobulins.

The high disease sensitivity and specificity of the antibodies to TG2 and the deamidated gluten peptides suggest that the antibodies somehow are involved in the pathogenesis of celiac disease. This could be at the level of the soluble antibodies, but not mutually exclusive, it could also be at the level of the antigen receptor of the B cells (i.e., cell surface immunoglobulin). The B cell receptor mediated capture of either TG2 decorated with deamidated gluten peptides and capture of the deamidated gluten peptides would both lead to the increased presentation of the gluten antigen to T cells, and as such, an amplification of the anti-gluten T cell response. Notably, a higher prevalence of celiac disease among patients with IgA deficiency (Wang et al., 2011) argues against the involvement of the IgA antibodies per se in the pathogenesis.

The autoantibodies may well be implicated in the extraintestinal manifestations of celiac disease. In dermatitis herpetiformis, which could be considered as celiac disease with additional skin lesions, the IgA autoantibodies are prime suspects. These patients, in addition to the anti-TG2 antibodies, have the antibodies that target the transglutaminase 3 (TG3) and these IgA anti-TG3 antibodies are more closely associated with the disease than the IgA anti-TG2 antibodies (Sardy et al., 2002). TG3, being closely related to TG2, can also use the gluten peptides as the substrates (Stamnaes et al., 2010). It is expressed in the epidermis but not in the dermal papillae, where the granular IgA deposits in dermatitis herpetiformis are located.

## Autoreactive Intraepithelial Lymphocytes

Untreated celiac disease is characterized by an increased density of proliferating  $\text{TCR}\alpha\beta + \text{CD8} + \text{CD4} -$  and  $\text{TCR}\gamma\delta + \text{CD8} + \text{CD4} -$  cells within the epithelium (Fig. 45.3A). In contrast to the  $\text{TCR}\alpha\beta + \text{CD8} +$  IELs that return to normal when gluten is removed from the diet, the  $\text{TCR}\gamma\delta +$  IELs remain at an elevated level (Kutlu et al., 1993). This may suggest that these two types of T cells play different roles in the pathogenesis. Many of the IELs co-express the innate (NK cell) receptors recognizing the nonclassical HLA molecules, such as MIC molecules and HLA-E. In celiac disease, there is a decrease in IELs expressing the inhibitory receptor CD94-NKG2A (Meresse et al., 2006) and an increase of the cells expressing the activating receptors NKG2D (Hüe et al., 2004; Meresse et al., 2004) and CD94/NKG2C (Meresse et al., 2006) (Fig. 45.3A). The activated IELs can kill the enterocytes by use of the NK cell receptors without any involvement of the T-cell receptor (Hüe et al., 2004; Meresse et al., 2004).

## GENETICS

A high prevalence (10%) among the first-degree relatives of celiac disease patients indicates that the susceptibility to develop celiac disease is strongly influenced by the inherited factors (Ellis, 1981). The familial clustering is stronger in celiac disease than in most other chronic inflammatory diseases with multifactorial etiology (Risch, 1987). The strong genetic influence in celiac disease is further supported by a high concordance

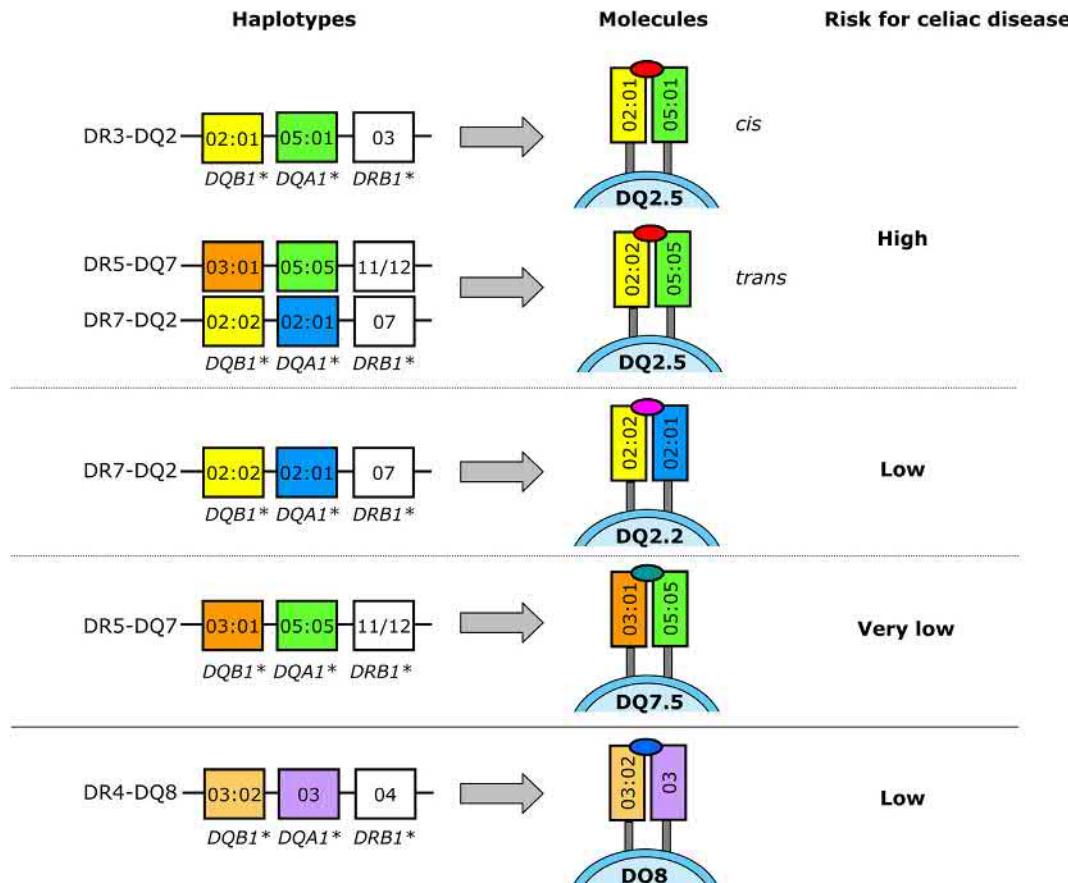
rate (approximately 75%) in monozygotic twins (Nistico et al., 2006). Both HLA and non-HLA genes contribute to the genetic predisposition.

## HLA Genes

The strongest HLA association in celiac disease is with a variant of DQ2 that is often termed DQ2.5 (Fig. 45.4). This HLA molecule is usually carried by 90% or more of celiac disease patients. The DQ2.5 molecule is in individuals who carry the DR3-DQ2 haplotype encoded by *DQA1* and *DQB1* alleles (*DQA1*\*05:01 and *DQB1*\*02:01), which are located on the same chromosome (*cis* position). Alternatively, it can be expressed by the individuals who are DR5DQ7/DR7DQ2 heterozygous and then the alleles (*DQA1*\*05:05 and *DQB1*\*02:02) are located on the opposite chromosomes (*trans* position) (Sollid et al., 1989). About half of the remaining celiac patients carry HLA-DQ8 encoded by the *DQA1*\*03 and *DQB1*\*03:02 alleles. The rest of the patients are either DQ2.2 or DQ7.5 (Karell et al., 2003); thus they carry either the genes for the  $\alpha$ -chain or the  $\beta$ -chain of the DQ2.5 molecule. Only a small fraction of the individuals with celiac disease associated DQ allotypes develop celiac disease. HLA can thus be considered a necessary, but not sufficient, genetic factor for disease development.

## Non-HLA Genes

The genome wide association studies (GWAS) have advanced the knowledge of the non-HLA genes in celiac disease. So far, 42 loci outside of HLA have been identified to harbor one or more genes predisposing to or protecting against celiac disease (Trynka et al., 2011; Withoff et al., 2016). Each of these non-HLA genes



**FIGURE 45.4** HLA association in celiac disease. The majority of celiac disease patients carry the DQ2.5 molecule, which can be encoded in *cis* or in *trans* positions. Most of the remaining patients express DQ8 whereas the rest of celiac disease patients express DQ2.2 or DQ7.5. The polypeptides encoded by *DQA1*\*05:01 and *DQA1*\*05:05 differ by one residue in the leader peptide whereas those encoded by *DQB1*\*02:01 and *DQB1*\*02:02 differ by one residue in the membrane proximal domain. It is unlikely that these substitutions have functional consequences.

contributes little to the genetic risk. It has been estimated that the 42 non-HLA loci account for 15% of the genetic variance whereas HLA in comparison accounts for about 25%–40% (Withoff et al., 2016). Evidence suggests the existence of many additional non-HLA genes with even smaller effect sizes than those identified to date.

Most of the 42 non-HLA loci contain genes of immunological function. Many of these genes are related to the function of the T cells. Thus the pathogenesis model established by the studies of immune cells of celiac disease patients is confirmed by the findings of the GWAS. Most celiac disease associated risk variants in the 42 non-HLA loci are not located in protein coding regions but rather in the regions implicated in gene regulation (Withoff et al., 2016). This suggests that the differences in gene expression play an important role in the pathogenesis of celiac disease. Finally, celiac disease and other immune mediated diseases, in particular type 1 diabetes (Smyth et al., 2008), share the risk gene variants that speak of the sharing of the critical pathways in the development of the various diseases. New therapeutics for the treatment of the autoimmune diseases, including the biologicals, may thus find a rational basis for the testing in celiac disease as well.

## ENVIRONMENTAL FACTORS

### Gluten Proteins

Gluten is the cohesive mass that remains when the dough is washed to remove the starch. Gluten was originally used to denote the wheat proteins only but it is now increasingly used as a term for the proline- and glutamine-rich proteins of wheat, barley, and rye (and oat, see later). In wheat, gluten consists of the gliadin and glutenin subcomponents. The gliadin proteins can be subdivided into the  $\alpha$ -,  $\gamma$ -, and  $\omega$ -gliadins while the glutenin proteins can be subdivided into high molecular weight and low molecular weight subunits (Shewry et al., 2003). The number of unique gluten proteins in common bread wheat is enormous due to a hexaploid genome, multiple encoding loci, and allelic variation. Thus, in a single wheat variety, there are several hundred different gluten proteins, many of which only differ by a few amino acids. The high content of proline residues makes the gluten proteins particularly resistant to gastrointestinal digestion (Shan et al., 2002). This has important implications for their immunogenicity for the T cells.

The gluten proteins of barley are termed hordeins, those of rye secalins and those of oat avenins. The gluten proteins of barley and rye are also harmful to celiac disease patients (Anand et al., 1978) and the T-cell epitopes of barley and rye have been defined (see below). In general, the feeding studies have indicated that oat is safe for celiac disease patients (Hoffenberg et al., 2000; Janatuinen et al., 2002). Occasional oat-intolerant celiac patients exist (Arentz-Hansen et al., 2004; Lundin et al., 2003). The lesions of these rare patients harbor the T cells specific for the oat avenin peptides that closely resemble the gliadin T-cell epitopes, but which are present in low amounts (Arentz-Hansen et al., 2004).

### Other Environmental Factors

Infections may play a role in the development of celiac disease. Perhaps the best epidemiological evidence comes from the studies of Sweden. In the children below the age of 2 years, a positive correlation was found between celiac disease risk and being born during the summer (Ivarsson et al., 2003a). The children born in the summer are first exposed to the dietary gluten during the winter when these infections are more prevalent. Moreover, the same population case control studies indicated that the celiac subjects had experienced three or more infection episodes more frequently than the referents. The evidence for the involvement of rotavirus in the pathogenesis was provided by a prospective multinational study (the TEDDY study), which showed that the rotavirus vaccination reduced the risk of celiac disease in the children vaccinated against rotavirus and introduced to gluten before the age of 6 months (Kempainen et al., 2017). Further, reovirus was proposed as the candidate agent as a certain strain of reovirus in mice was able to trigger an inflammatory response in the gut to the orally administered antigens (Bouziat et al., 2017). It was demonstrated that the virus, via action on the dendritic cells, suppressed peripheral regulatory T cell conversion and promoted the immunity to the dietary antigen dominated by the interferon- $\gamma$  production. The same study found some serological evidence that reovirus is involved in the human celiac disease but failed to find any similar evidence for rotavirus. Clearly, more studies are required to understand which environmental factors can trigger celiac disease.

---

## IN VIVO AND IN VITRO DISEASE MODELS

---

### Animal Models

The attempts to establish good animal models for celiac disease have been troublesome. The gluten-sensitive conditions in macaques (Bethune et al., 2008) and horses (van der Kolk et al., 2012) have been described, but whether these faithfully represent celiac disease is unclear. Until recently, all attempts to establish a mouse model have been unsuccessful. The gluten-sensitive enteropathy could not be induced in mice made transgenic for HLA-DQ2.5 (Chen et al., 2003; Chen et al., 2002) or HLA-DQ8 (Black et al., 2002). Not even HLA-DQ2.5 transgenic mice made transgenic for T-cell receptors specific for the celiac disease-relevant gluten epitopes developed enteropathy (de Kauwe et al., 2009; du Pré et al., 2011). Dermatitis herpetiformis, such as skin lesions occurred in a fraction of the HLA-DQ8 transgenic mice on a NOD background after systemic immunization with gluten but the mice did not have enteropathy (Marietta et al., 2004). A model with overexpression of IL-15 is more promising (Abadie et al., 2017). Mice made triply transgenic, expressing HLA-DQ8 and IL-15 both in the lamina propria and epithelium, have a gluten-dependent phenotype with the gut villous atrophy, intraepithelial lymphocytosis, and antibodies to the deamidated gluten peptides. Importantly, the disease phenotype is dependent on the involvement of the CD4+ T cells.

### Organ Culture Assays

The culturing of the small intestinal biopsies ex vivo has frequently been used to dissect the pathogenic events in celiac disease. This assay for instance has been used to examine the innate effects of gluten (Maiuri et al., 2000) and to show that the gluten challenge induces a stress response with the upregulation of the IL-15, MICA, and heat shock proteins (Hüe et al., 2004). Although the ex vivo culture system has been very important in clarifying the pathogenic mechanisms of celiac disease (Lindfors et al., 2012), this result should be carefully interpreted. One of the problems with the ex vivo organ culture system may relate to the uneven distribution of the inflammatory cells in the mucosa (our unpublished results).

---

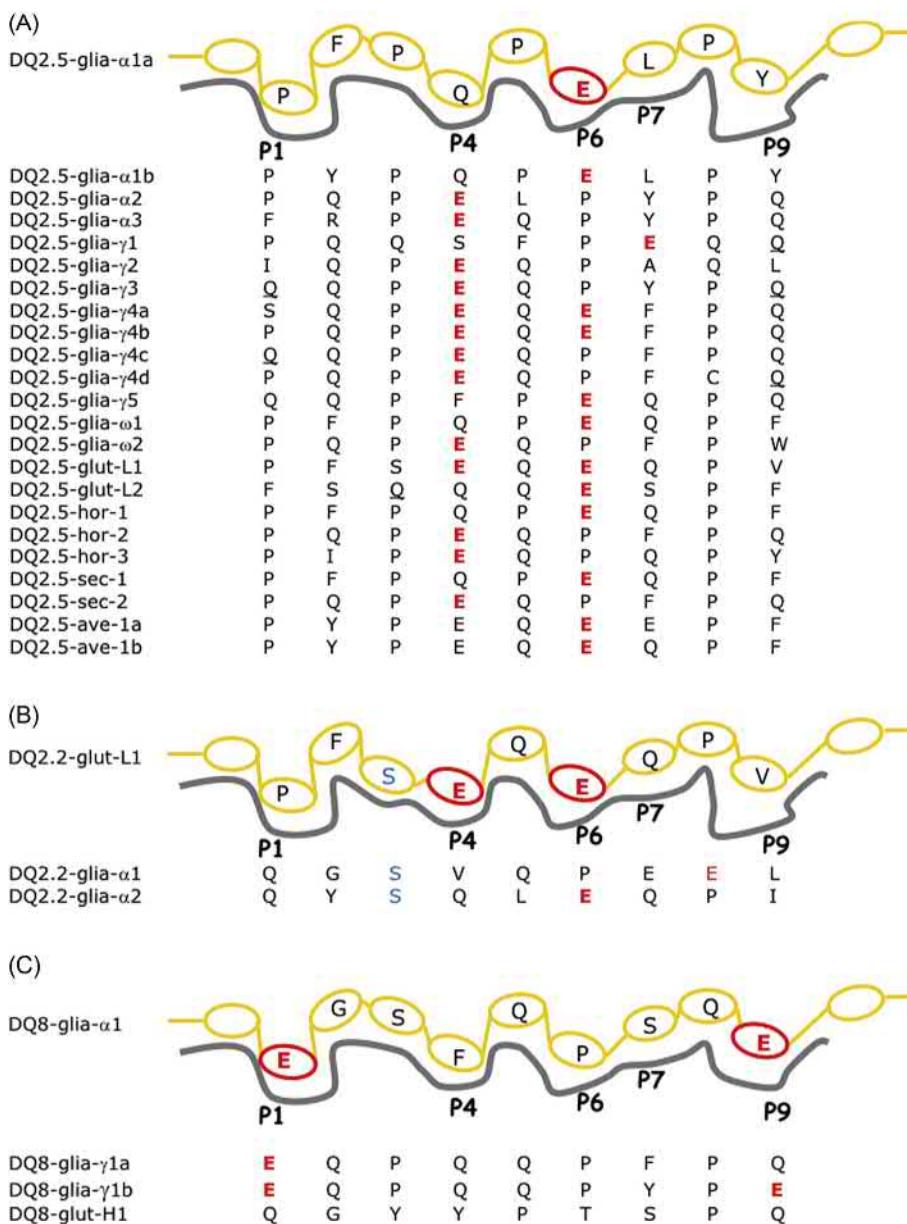
## PATHOGENIC MECHANISMS

---

The strong HLA association in celiac disease and overrepresentation of non-HLA susceptibility genes related to the T-cell function implicate the T cells in the pathogenesis of celiac disease.

### Gluten-Specific CD4+ T Cells

CD4+ T cells specific for the gluten proteins can be isolated from the small intestinal biopsies of the majority of celiac disease patients (Lundin et al., 1993; van de Wal et al., 1998b) but not from the biopsies obtained of disease controls (Molberg et al., 1997). By contrast, both the patients and many control subjects have gluten-specific T cells in their peripheral blood. These T cells use many different HLA molecules for peptide presentation (DR, DQ, and DP) (Gjertsen et al., 1994) and their reactivity is not enhanced by the deamidation of the gluten antigen (Molberg et al., 1998). This implies that the subjects can be immunologically sensitized to gluten without developing any small intestinal pathology. The gluten-specific T cells derived from the intestinal celiac lesions display a remarkable feature in that these invariably recognize the epitopes restricted by the disease associated HLA-DQ molecules, DQ2.5, DQ8, and DQ2.2 (Bodd et al., 2012; Lundin et al., 1994; Lundin et al., 1993) (Fig. 45.3B). Many distinct gluten T-cell epitopes that are derived from a variety of different classes of the gluten proteins exist (Solid et al., 2012). In general, there are unique sets of epitopes presented by the DQ2.5, DQ2.2, and DQ8 molecules reflecting distinct peptide binding motifs of the different HLA molecules (see later) (Fig. 45.5). For each HLA:peptide complex, the patients use a variety of receptors for T-cell recognition. Notwithstanding, there is a selection of the preferred T-cell receptors and this biased usage is observed across the patients, thereby representing the public type of responses. Such public responses are more pronounced for the recognition of certain HLA:peptide complexes. For instance, the pair TRAV26-1/TRBV7-2 is preferred by the T cells recognizing the HLA-DQ2.5:DQ2.5-glia- $\alpha$ 2 complex (Qiao et al., 2014; Qiao et al., 2011). These T cells also use a certain CRD3 $\beta$  motif with a nongermline encoded arginine residue (Qiao et al., 2014) that serves as the lynchpin in the interaction with the HLA:peptide complex (Petersen et al., 2014).



**FIGURE 45.5** The T-cell epitopes of gluten presented by HLA-DQ molecules and recognized by CD4+ T cells of celiac disease patients. Epitopes presented by DQ2.5 are shown in (A), epitopes presented by DQ2.2 are shown in (B), and epitopes presented by DQ8 are shown in (C). The 9-mer core regions of the epitopes are given with one letter amino acid codes in the register with which they bind to the HLA molecules. The T-cell receptors recognizing the epitopes are often sensitive to the residues outside the 9-mer core region. Glutamate residues (E, in red) formed by TG2-mediated deamidation, which are important for recognition by the T cells, are shown. Additional glutamine residues (Q, also targeted by TG2, are underlined. Epitopes presented by DQ2.2 have commonly serine (S, in blue) at position P3 hence complying with the binding motif of this HLA molecule. Of note is the abundance of proline (P) residues within the epitopes. Further information about epitopes and epitope nomenclature can be found elsewhere (Sollid et al., 2012).

The gluten-specific T cells can be detected with the HLA-DQ:gluten tetramers (Quarsten et al., 2001). In these reagents, the biotinylated HLA:DQ molecules are tethered with the gluten epitopes and multimerized onto a fluorescently labeled streptavidin thereby obtaining sufficient avidity of the reagent to stain the antigen-specific T cells. Although around 1% of the CD4+ T cells in the intestinal lamina propria of the untreated patients bind the HLA-DQ:gluten tetramers (Bodd et al., 2013), such cells are rare in blood, ranging 1–100 cells per million CD4+ T cells of the untreated patients and even 5-fold less in the treated patients (Christophersen et al., 2014). Of note, maybe only half of all the celiac disease-relevant and gluten-specific T cells will be captured by the HLA-DQ:gluten tetramers used in these studies. The majority of the HLA-DQ:gluten tetramer staining cells in the celiac patients are the effector memory T cells (Christophersen et al., 2014). Such cells are almost completely absent in the nonceliac DQ2.5 controls and thus HLA-DQ:gluten tetramer staining has a potential as a diagnostic marker. The gluten-specific T cells increase substantially in frequency in blood on day six after the short-term oral gluten challenge either as detected by ELISPOT (Anderson et al., 2000) or by HLA tetramer staining (Brottveit et al., 2011; Ráki et al., 2007). Along with the surge of the gluten-specific CD4+ T cells in blood on day six after the gluten challenge, there is also a surge of the activated CD8+ T cells and  $\gamma\delta$  T cells (Han et al., 2013). The specificities of these cells are unknown.

## Transglutaminase 2

The enzyme TG2 is not only the target of the autoantibodies in celiac disease because it is also involved in the creation of the T-cell epitopes for being recognized by the HLA-DQ restricted CD4+ T cells (Molberg et al., 1998; van de Wal et al., 1998a). This dual role of TG2 is hardly coincidental. TG2 catalyzes a posttranslational transamidation or deamidation of specific glutamine residues within substrate proteins (Folk, 1983; Lorand and Graham, 2003). In the transamidation reaction, the glutamine becomes cross-linked to a protein-bound lysine or polyamine whereas the deamidation reaction results in the conversion of glutamine to the glutamic acid. The enzyme displays regional selectivity for the glutamine residues in the gliadin peptides, and QXP is a particularly good target sequence (Fleckenstein et al., 2002; Piper et al., 2002; Vader et al., 2002). It is the deamidation reaction that is critical for the creation of the T-cell epitopes and there is mounting evidence that the enzyme is centrally involved in the selection of the HLA-DQ restricted T-cell epitopes in celiac disease (see later). Exactly where in the body TG2 is creating the T-cell epitopes is not known, but there is the indication of increased activity in the celiac lesion; assessing enzymatic activity in the lysates of gut biopsies revealed higher activity in the untreated celiac disease patients compared to controls (Bruce et al., 1985).

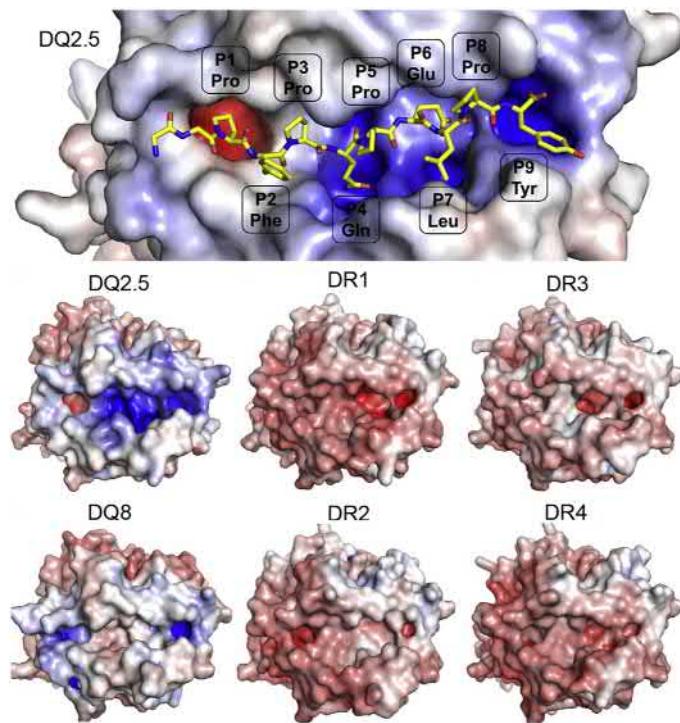
## Gluten Antigen Presentation by Disease Associated HLA-DQ Molecules

The MHC class II molecules bind the antigenic peptides in a groove in their membrane distal part. In this groove, there are pockets that accommodate the side chains of amino acids of the peptide, the so-called anchor residues. In the MHC class II peptides, the anchor residues are usually found at the positions P1, P4, P6, P7, and P9. All DQ2.5, DQ2.2, and DQ8, which are associated with celiac disease, have a fairly unique preference for binding peptides with multiple negatively charged residues. Both DQ2.5 and DQ2.2 have preference for the negatively charged anchor residues at the positions P4, P6, and P7 (Kim et al., 2004; Tollefse et al., 2006) whereas DQ8 has preference for the negatively charged anchor residues at the positions P1 and P9 (Henderson et al., 2007; Tollefse et al., 2006) (Fig. 45.6). The binding motif of DQ2.2 is similar to that of DQ2.5 but in addition, the DQ2.2 molecule has a preference for binding the serine or threonine residues at the P3 position (Bergseng et al., 2015; van de Wal et al., 1997). Overall, the HLA association in celiac disease can be explained by a superior ability of the disease associated HLA-DQ molecules to bind the biased repertoire of the proline-rich gluten peptides that have survived the gastrointestinal digestion and which have been deamidated by TG2.

There is a hierarchy among the gluten T-cell epitopes both in terms of how many T cells of a single patient respond to each, and in terms of the responder frequency among different patients (Marti et al., 2005; Tye-Din et al., 2010). The same epitopes dominate in each of these settings, likely reflecting variation in immunogenicity between the epitopes. In  $\alpha$ -gliadin of wheat, there are two immunodominant epitopes (i.e., DQ2.5-glia- $\alpha$ 1a/b and DQ2.5-glia- $\alpha$ 2) (Arentz-Hansen et al., 2000). In some  $\alpha$ -gliadin proteins, these epitopes are expressed in altogether 6 copies within a 33-mer fragment. This potent antigen is resistant to degradation by the gastric, pancreatic, and intestinal brush border membrane proteases (Shan et al., 2002). Other immunodominant epitopes are the DQ2.5-glia- $\omega$ 2 epitope that derives from  $\omega$ -gliadin of wheat and a shared 9 amino acid sequence-stretch found in the proteins of wheat, rye, and barley (termed DQ2.5-glia- $\omega$ 1, DQ2.5-hor-1, and DQ2-sec-1) (Tye-Din et al., 2010). A similar hierarchy probably exists for the HLA-DQ8 restricted epitopes. Celiac disease patients who express DQ2.2 but not DQ2.5 or DQ8 have T cells that do respond to the common DQ2.5 restricted epitopes but often respond to the immunodominant DQ2.2 restricted epitope (DQ2.2-glut-L1) that has a serine residue at the P3 position (Bodd et al., 2012). The common DQ2.5 restricted epitopes bind stably to DQ2.5 but unstably to DQ2.2 (Fallang et al., 2009) while the immunodominant DQ2.2 epitope binds stably to DQ2.2 but not to DQ2.5 (Bodd et al., 2012).

The stable binding of a gluten peptide to the MHC molecule appears to be important for a successful T-cell priming (Fallang et al., 2009). Notably, deamidation of the gluten peptides decreases the off-rate for binding to DQ2.5 (Xia et al., 2005) and hence, one effect of deamidation can be that more gluten-DQ complexes are generated resulting in better T-cell priming.

Only a few sequences in the huge gluten proteome are recognized by the CD4+ T cells of celiac disease patients. This suggests that there are strong guidance factors in the selection of the T-cell epitopes. Three factors have been identified as being particularly important—protease resistance, MHC binding, and TG2 efficiency. The clustering of epitopes to regions of gluten proteins that have high contents of proline residues and that are proteolytically stable suggests that proteolytic stability is critical (Arentz-Hansen et al., 2002; Shan et al., 2002). Antigen concentration is important for T-cell stimulation and fragments that are resistant to breakdown will survive at higher concentrations. The selection by MHC would relate to the selection of peptides with the correct binding



**FIGURE 45.6** Three-dimensional structures of the binding sites of HLA class II molecules. (A) HLA-DQ2.5 complexed with the deamidated gluten epitope DQ2.5-glia- $\alpha$ 1 (PDB 1S9V). The peptide is shown with a stick representation (carbon = yellow, nitrogen = blue, oxygen = red; amino acid residues in the positions P1–P9 are labeled). (B) The electrostatic potential surface of the HLA molecules at pH7.0 (red, negative; blue, positive). HLA-DQ2.5 (PDB 5KSU), HLA-DR1 (3PDO), HLA-DR3 (PDB 1A6A), HLA-DQ8 (PDB 2NNA), HLA-DR2 (PDB 1H15), and HLA-DR4 (PDB 2SEB) are depicted. Note the unique distribution of positively charged binding pockets in the peptide binding groove of DQ2.5 (P4, P6, P7) and DQ8 (P1, P9). Source: Figure in panel (A) has been prepared with help from Chu-Young Kim and figure in panel (B) is adapted from reference (Nguyen, T.B., Jayaraman, P., Bergseng, E., Madhusudhan, M.S., Kim, C.Y., Sollid, L.M. 2017. Unraveling the structural basis for the unusually rich association of human leukocyte antigen DQ2.5 with class-II-associated invariant chain peptides. *J. Biol. Chem.* 292, 9218–9228; Nguyen et al., 2017).

motif as well as the selection of peptides that form stable MHC complexes. The observation that the DQ2.5 and DQ8 molecules in general select discrete epitopes of the gluten proteome underscores the importance of HLA allotype selection (Tollefson et al., 2006). Finally, TG2 specificity appears to be vital. There is a correlation between how frequently the T-cell epitopes are recognized by celiac disease patients and their propensity to be targeted as substrates for TG2 (Dørum et al., 2009). Moreover, using TG2 to select its best substrates from a proteolytic digest of gluten consisting of several thousand different peptides, the majority of about 30 peptides selected contained celiac disease related T-cell epitopes (Dørum et al., 2010). This suggests that TG2 specificity is a major force in the T-cell epitope selection process.

## Macrophages and Dendritic Cells

Several types of HLA-DQ positive cells can serve as antigen presenting cells (APC) in the small intestinal mucosa (Beitnes et al., 2012; Ráki et al., 2006). There are CD163 + CD11c – macrophages and CD11c + dendritic cells which express either CD163 or CD103 and CD1c. The CD163 + CD11c + dendritic cells likely derive from circulating monocytes as these co-express CD14 and CCR2. The CD11c + dendritic cells expressing CD103 + or CD1c + do not express CD163 or CD14, suggesting the existence of separate functional lineages. In the active celiac disease lesion, there is an increase of CD163 + CD11c + dendritic cells whereas the CD163 + CD11c – macrophages and CD103 + and CD1c + dendritic cells are all decreased (Beitnes et al., 2011). Based on the studies in mice (Schulz et al., 2009), it is tempting to speculate that the CD11c + CD103 + dendritic cells function to transport antigen to the mesenteric lymph nodes for presentation to the naïve or central memory T cells, whereas the CD163 + CD11c – macrophages and CD163 + CD11c + dendritic cells serve to present antigen locally to the effector T cells.

## Plasma Cells

The density of IgA and IgM plasma cells in the lesion of active celiac disease patient increase 2.4 and 4.6 fold, respectively (Baklien et al., 1977). Strikingly, these plasma cells express surface immunoglobulin as a functional B-cell receptor (Di Niro et al., 2010; Pinto et al., 2013) raising the question whether they might sense antigen. The sensing could either only involve the B-cell receptor but conceivably if the cells have sufficient MHC class II molecules, they could also serve as the antigen-presenting cells for T cells. If so, this could have consequences for the function of the plasma cells themselves but also for the T cells in the lamina propria.

## Effector Mechanisms Leading to Mucosal Alterations

Despite the lack of detailed knowledge of effector mechanisms involved in the creation of the celiac lesion, evidence suggests that inflammatory cytokines, particularly interferon- $\gamma$  and IL-15, are involved. The messenger RNA for interferon- $\gamma$  is abundant in the biopsies taken from untreated patients and is rapidly induced when biopsies from the treated patients are challenged ex vivo with gluten (Nilsen et al., 1998). Interferon- $\gamma$  is also produced by the intestinal, gluten-specific CD4+ T cells (Nilsen et al., 1995) and by IELs (Forsberg et al., 2002; Olaussen et al., 2002). The gluten-specific CD4+ T cells also produce IL-21 (Bodd et al., 2010). Importantly, IL-21, together with IL-2 and tumor necrosis factor, induce proliferation and expansion of IELs (Kooy-Winkelaar et al., 2017). The same effect on IELs is mediated by the enterocyte bound IL-15 (Mention et al., 2003). In active celiac disease, there is increased expression of IL-15, both in the lamina propria (Maiuri et al., 2000) and in the epithelium (Mention et al., 2003). The expression of IL-15 increases following in vitro gluten challenge of the celiac biopsies and certain gluten peptides appear to cause innate immune activation and IL-15 production (Maiuri et al., 2003). IL-15 is a key factor for reprogramming IELs in celiac disease, where the cells downregulate inhibitory and upregulate activating NK cell receptors (Meresse et al., 2006) (Fig. 45.3A). The activated IELs kill the stressed enterocytes and this appears to be an important component in creating the celiac lesion (Jabri and Sollid, 2009).

Taken together, currently available data places the activation of CD4+ T cells recognizing gluten peptides presented by DQ2 or DQ8 molecules as the central key gateway for the control of celiac disease development. The activation of naïve T cells probably takes place in the mesenteric lymph nodes and effector cells then seed to the lamina propria in the small intestine where these are reactivated upon challenge with gluten. This model would explain the dominant genetic role of HLA. The products of the other predisposing genes likely feed into the processes that lead to T-cell activation or tissue destruction but as each of the non-HLA susceptibility genes have small effects, this indicates a lack of critical checkpoints or redundancy in pathways.

While regulatory T cells are frequently described in the gut and breakage of oral tolerance to gluten conceivably could be the underlying cause of celiac disease, there is still meager evidence for the existence of such cells (Sollid, 2017). In addition, there is no evidence of a protective effect of HLA allotypes in celiac disease that one could expect if the regulatory T cells were using certain HLA allotypes for recognition of their antigenic peptides.

## AUTOANTIBODIES AS IMMUNOLOGIC MARKERS

In adults, the examination of gut biopsy is still considered mandatory in the diagnostic workup although serology has become an increasingly important diagnostic adjunct. To state definite diagnosis of CD, villous atrophy is required (Bai and Ciacci, 2017; Ludvigsson et al., 2014). In children, gut histology examination is no more deemed necessary in many cases. Updated European guidelines for the pediatric celiac disease recommend that the diagnosis can be made without the assessment of intestinal histology by using a combination of strongly positive serology ( $>10 \times$  upper limit of normal of IgA anti-TG2), positive IgA EMA in independent blood sample, HLA typing, and clinical criteria (Husby et al., 2012). Two large prospective multicenter studies assessing the diligence of the new diagnostic scheme concluded that children can be accurately diagnosed with celiac disease without biopsy analysis resulting in more than half the children with celiac disease avoiding the risks and costs of endoscopy (Werkstetter et al., 2017; Wolf et al., 2017). Detection of autoantibodies is the single most important diagnostic factor of the new scheme. It is striking that celiac disease that previously was diagnosed as a food intolerance based on elimination and provocation diets, is now essentially diagnosed as an autoimmune disease by detection of autoantibodies.

## Serology

More than 95% of the untreated celiac disease patients (at least in selected patient cohorts) have high titers of serum IgA antibodies reactive with TG2 (Dieterich et al., 1998; Sulkkanen et al., 1998). Most tests are ELISA based with human recombinant TG2 as the antigen. In the recent European guidelines for pediatric celiac disease (Husby et al., 2012), the EMA test is included in the diagnostic algorithm because this test was judged to outperform the anti-TG2 ELISA for prediction of celiac disease (Giersiepen et al., 2012). This may relate to the fact that the celiac anti-TG2 antibodies are reactive with conformational epitopes and that the TG2 antigen in some commercial kits is suboptimally refolded. IgG anti-TG2 antibodies can be useful to evaluate in the patients with selective IgA deficiency but should otherwise not be tested for (Murray, 1999).

The patients with untreated celiac disease also have increased levels of IgA and IgG anti-gluten antibodies. Monitoring of such antibodies, in particular anti-gliadin antibodies, was used for many years in the clinical workup of celiac disease. However, the IgA EMA and anti-TG2 tests gradually took over in clinical practice due to better performance. A fraction of the anti-gluten antibodies are directed against deamidated epitopes (Osman et al., 2000) and tests based on reactivity to deamidated gliadin peptides are performed at the level of anti-TG2 tests (Lewis and Scott, 2010).

Some celiac disease patients present with negative serology (Dahele et al., 2001; Kaukinen et al., 2002) and an intestinal biopsy should therefore be examined irrespective of serology when there is high clinical suspicion of celiac disease (Ludvigsson et al., 2014). Notably, false positive anti-TG2 tests do also occur, particularly in patients with inflammatory bowel disease (Carroccio et al., 2002; Dahele et al., 2001).

## Staining of Immune Complexes

In patients with active celiac disease, there are deposits of IgA in the small intestine corresponding to the distribution of fibronectin bound extracellular TG2, typically as a band beneath the epithelium (Korponay-Szabo et al., 2004). Such deposits of specific IgA complexed with TG2 can be found in the intestinal mucosa even in those patients where serum autoantibodies are undetectable (Salmi et al., 2006b). In IgA deficient patients, these deposits are made up of IgM (Borrelli et al., 2010). Case reports have described autoantibody deposits years before any intestinal damage was observed and the production of TG2-specific autoantibodies is therefore considered an early marker for developing celiac disease (Salmi et al., 2006a). Because detection of IgA deposits can only be done with frozen tissue sections, this method has not reached widespread usage.

## TREATMENT AND OUTCOME

### Current Treatment

The current treatment of celiac disease is a lifelong gluten exclusion diet. This disease is largely a benign disorder and particularly so in patients detected by screening (West et al., 2003). Although celiac patients overall have an increased relative risk for nonHodgkin's lymphoma of the gastrointestinal tract, the absolute risk is low and lower than previously anticipated (Askling et al., 2002; Catassi et al., 2002). In an Italian study, it was found that the overall mortality rate in celiac disease was two times greater than in the controls (Corrao et al., 2001). This increased mortality was accounted for by the increased death rates in the first 3 years after diagnosis. The gluten-free diet has been considered to be protective against the development of malignancy but this notion was not supported by a study from the United States (Green et al., 2003). Persistent mucosal inflammation (compatible with either lack of compliance or other autoimmune phenomena) is of particular concern (Ludvigsson et al., 2009).

The most frequent reason for absent or incomplete clinical improvement is poor diet compliance (Ciacci et al., 2002), but it is clear that some patients have refractory disease that do not respond to an adequate diet. Refractory celiac disease is classified into type 1 and type 2 (Rishi et al., 2016). Type 2 patients, in contrast to type 1 patients, have monoclonal expansions of IELs that can progress to become overt enteropathy associated T-cell lymphoma (Cellier et al., 2000). While the clinical management of type 2 is more difficult than that of type 1, the treatment of enteropathy associated T-cell lymphoma is particularly difficult with poor outcome for the patients (Rishi et al., 2016). Recent insight into the mechanism of malignant transformation may give clues for better treatments (Ettersperger et al., 2016). The cells that undergo malignant transformation are IL-15 dependent T cell-like innate IELs and they do so by acquiring gain-of-function mutations in Janus kinase 1 (JAK1) or signal transducer and activator of transcription 3 (STAT3).

## Novel Treatments

Many patients cope with the gluten-free diet easily. Others find that the dietary restrictions are laborious and negatively impacting their quality of life. Suboptimal and even poor compliance is frequent. Better alternatives are thus called for. It is hence promising that the insight into the molecular mechanisms involved in the intestinal T-cell reactivity to gluten has uncovered novel targets for therapy. Several avenues are being pursued. One possibility, which is basically an extension of today's treatment with a gluten-free diet, is to produce cereals with bread-making properties that are devoid of the T-cell epitopes, either by breeding programs or transgenic technology including RNAi or CRISPR/Cas9 technologies (Gil-Humane et al., 2010; Sánchez-León et al., 2018). Another possibility is enzyme supplementation with the aim of either to destroy the T-cell epitopes directly or to facilitate their gastrointestinal proteolysis (Gass et al., 2007; Hausch et al., 2002; Mitea et al., 2008). Prolyl endopeptidases are particularly attractive enzymes as they will target the proline-rich regions of gluten that harbor the T-cell epitopes. In a recent phase 2 trial testing of patients with symptomatic celiac disease and with histologic evidence of duodenal mucosal injury, the protease drug did no better than placebo, which is likely due to a trial effect as the placebo group also showed improvement (Murray et al., 2017). TG2 appears as a target for intervention because of its critical role in generating the gluten T-cell epitopes. A series of TG2 inhibitors have been developed (Keillor and Apperley, 2016) that potentially may be useful in the therapy of celiac disease. A problem with this approach could be unacceptable side effects as TG2 is involved in many different physiological processes (Lorand and Graham, 2003). Another strategy would be to aim directly at the gluten-specific T cells. This can possibly be achieved by peptide vaccination (Goel et al., 2017) or by removal of the antigen-specific T cells by eradication of the activated T cells after the oral gluten challenge. Yet another possible target would be to block the presentation of gluten peptides by DQ2 and DQ8, and thereby the activation of gluten-specific T cells. The approach does not appear to be very efficient in preclinical testing. Whatever new therapeutic modality is introduced in uncomplicated celiac disease, it will have to prove to be as good as or even better than the current gluten-free diet regime with regard to its long-term safety and outcome. For complicated celiac disease, in particular refractory celiac disease type 2 and enteropathy associated T-cell lymphoma, better treatment alternatives are urgently needed. The next years will tell whether targeting of IL-15, JAK1, or STAT3 will be effective treatment modalities.

## PERSPECTIVES

It has become clear that gluten serves as the driver of autoimmune reactions in celiac disease (Sollid and Jabri, 2013). Considerable progress has been made in recent years on the understanding of the molecular basis for celiac disease, but several new questions have emerged. Many of these relate to the autoimmune aspects of the disease. What is the trigger event that transforms the mucosal immune system from gluten-tolerant to gluten-intolerant? To what extent are the autoimmune components of celiac disease involved in the disease development? How relevant are the findings of T-cell recognition of posttranslationally modified peptides and autoantibody formation driven by an exogenous food antigen for other autoimmune disorders? Can it be that the other autoimmune disorders are driven by immune responses to foreign, not yet identified, antigens? Celiac disease, this fascinating condition in the gray zone between food intolerance and autoimmunity, continues to give us food for thought.

## Acknowledgments

Studies in the authors' laboratory are supported by grants from Stiftelsen Kristian Gerhard Jebsen (project number SKGJ-MED-017), the Research Council of Norway through the Centre of Excellence funding scheme (project number 179573/V40), and the South-Eastern Norway Regional Health Authority. We thank Ann-Christin R. Beitnes, Jorunn Stamnaes, and Chu-Young Kim for help in preparation of figures.

## References

- Abadie, V., Kim,S.M., Lejune, T., Ernst, J., Dumaine, A., Ciszewski, C., et al. 2017. From human to mouse and back: advances in the development of a mouse model for celiac disease. In: 17th International Celiac Disease Symposium. Abstract O8, New Delhi.
- Alp, M.H., Wright, R., 1971. Autoantibodies to reticulin in patients with idiopathic steatorrhoea, coeliac disease, and Crohn's disease, and their relation to immunoglobulins and dietary antibodies. Lancet 2, 682–685.
- Anand, B.S., Piris, J., Truelove, S.C., 1978. The role of various cereals in coeliac disease. Q. J. Med. 47, 101–110.
- Anderson, R.P., Degano, P., Godkin, A.J., Jewell, D.P., Hill, A.V., 2000. In vivo antigen challenge in celiac disease identifies a single transglutaminase-modified peptide as the dominant A-gliadin T-cell epitope. Nat. Med. 6, 337–342.

- Arentz-Hansen, H., Körner, R., Molberg, Ø., Quarsten, H., Vader, W., Kooy, Y.M., et al., 2000. The intestinal T cell response to  $\alpha$ -gliadin in adult celiac disease is focused on a single deamidated glutamine targeted by tissue transglutaminase. *J. Exp. Med.* 191, 603–612.
- Arentz-Hansen, H., McAdam, S.N., Molberg, Ø., Fleckenstein, B., Lundin, K.E., Jorgensen, T.J., et al., 2002. Celiac lesion T cells recognize epitopes that cluster in regions of gliadins rich in proline residues. *Gastroenterology* 123, 803–809.
- Arentz-Hansen, H., Fleckenstein, B., Molberg, Ø., Scott, H., Koning, F., Jung, G., et al., 2004. The molecular basis for oat intolerance in patients with celiac disease. *PLoS Med.* 1, e1.
- Asklung, J., Linet, M., Gridley, G., Halstensen, T.S., Ekstrom, K., Ekbom, A., 2002. Cancer incidence in a population-based cohort of individuals hospitalized with celiac disease or dermatitis herpetiformis. *Gastroenterology* 123, 1428–1435.
- Bai, J.C., Ciacci, C., 2017. World Gastroenterology Organisation global guidelines: celiac disease February 2017. *J. Clin. Gastroenterol.* 51, 755–768.
- Baklien, K., Brandtzaeg, P., Fausa, O., 1977. Immunoglobulins in jejunal mucosa and serum from patients with adult coeliac disease. *Scand. J. Gastroenterol.* 12, 149–159.
- Beitnes, A.C., Raki, M., Lundin, K.E., Jahnson, J., Sollid, L.M., Jahnson, F.L., 2011. Density of CD163+ CD11c+ dendritic cells increases and CD103+ dendritic cells decreases in the coeliac lesion. *Scand. J. Immunol.* 74, 186–194.
- Beitnes, A.C., Raki, M., Brottveit, M., Lundin, K.E., Jahnson, F.L., Sollid, L.M., 2012. Rapid accumulation of CD14+ CD11c+ dendritic cells in gut mucosa of celiac disease after in vivo gluten challenge. *PLoS One* 7, e33556.
- Bergseng, E., Dørum, S., Arntzen, M.O., Nielsen, M., Nygård, S., Buus, S., et al., 2015. Different binding motifs of the celiac disease-associated HLA molecules DQ2.5, DQ2.2, and DQ7.5 revealed by relative quantitative proteomics of endogenous peptide repertoires. *Immunogenetics* 67, 73–84.
- Bethune, M.T., Borda, J.T., Ribka, E., Liu, M.X., Phillipi-Falkenstein, K., Jandacek, R.J., et al., 2008. A non-human primate model for gluten sensitivity. *PLoS One* 3, e1614.
- Bjorck, S., Brundin, C., Lorinc, E., Lynch, K.F., Agardh, D., 2010. Screening detects a high proportion of celiac disease in young HLA-genotyped children. *J. Pediatr. Gastroenterol. Nutr.* 50, 49–53.
- Black, K.E., Murray, J.A., David, C.S., 2002. HLA-DQ determines the response to exogenous wheat proteins: a model of gluten sensitivity in transgenic knockout mice. *J. Immunol.* 169, 5595–5600.
- Bodd, M., Ráki, M., Tollesen, S., Fallang, L.E., Bergseng, E., Lundin, K.E., et al., 2010. HLA-DQ2-restricted gluten-reactive T cells produce IL-21 but not IL-17 or IL-22. *Mucosal Immunol.* 3, 594–601.
- Bodd, M., Kim, C.Y., Lundin, K.E., Sollid, L.M., 2012. T-cell response to gluten in patients with HLA-DQ2.2 reveals requirement of peptide-MHC stability in celiac disease. *Gastroenterology* 142, 552–561.
- Bodd, M., Ráki, M., Bergseng, E., Jahnson, J., Lundin, K.E., Sollid, L.M., 2013. Direct cloning and tetramer staining to measure the frequency of intestinal gluten-reactive T cells in celiac disease. *Eur. J. Immunol.* 43, 2605–2612.
- Borrelli, M., Maglio, M., Agnese, M., Paparo, F., Gentile, S., Colicchio, B., et al., 2010. High density of intraepithelial gammadelta lymphocytes and deposits of immunoglobulin (Ig)M anti-tissue transglutaminase antibodies in the jejunum of coeliac patients with IgA deficiency. *Clin. Exp. Immunol.* 160, 199–206.
- Bouziat, R., Hinterleitner, R., Brown, J.J., Stencel-Baerenwald, J.E., Ikitler, M., Mayassi, T., et al., 2017. Reovirus infection triggers inflammatory responses to dietary antigens and development of celiac disease. *Science* 356, 44–50.
- Brottveit, M., Ráki, M., Bergseng, E., Fallang, L.E., Simonsen, B., Lovik, A., et al., 2011. Assessing possible celiac disease by an HLA-DQ2-gliadin tetramer test. *Am. J. Gastroenterol.* 106, 1318–1324.
- Bruce, S.E., Bjarnason, I., Peters, T.J., 1985. Human jejunal transglutaminase: demonstration of activity, enzyme kinetics and substrate specificity with special relation to gliadin and coeliac disease. *Clin. Sci.* 68, 573–579.
- Carroccio, A., Vitale, G., Di Prima, L., Chifari, N., Napoli, S., La Russa, C., et al., 2002. Comparison of anti-transglutaminase ELISAs and an anti-endomysial antibody assay in the diagnosis of celiac disease: a prospective study. *Clin. Chem.* 48, 1546–1550.
- Cataldo, F., Marino, V., Bottaro, G., Greco, P., Ventura, A., 1997. Celiac disease and selective immunoglobulin A deficiency. *J. Pediatr.* 131, 306–308.
- Catassi, C., Fabiani, E., Corrao, G., Barbato, M., De Renzo, A., Carella, A.M., et al., 2002. Risk of non-Hodgkin lymphoma in celiac disease. *JAMA* 287, 1413–1419.
- Catassi, C., Kryszak, D., Bhatti, B., Sturgeon, C., Helzlsouer, K., Clipp, S.L., et al., 2010. Natural history of celiac disease autoimmunity in a USA cohort followed since 1974. *Ann. Med.* 42, 530–538.
- Cellier, C., Delabesse, E., Helmer, C., Patey, N., Matuchansky, C., Jabri, B., et al., 2000. Refractory sprue, coeliac disease, and enteropathy-associated T-cell lymphoma. French Coeliac Disease Study Group. *Lancet* 356, 203–208.
- Chen, Z., Dudek, N., Wijburg, O., Strugnell, R., Brown, L., Deliannis, G., et al., 2002. A 320-kilobase artificial chromosome encoding the human HLA DR3-DQ2 MHC haplotype confers HLA restriction in transgenic mice. *J. Immunol.* 168, 3050–3056.
- Chen, D., Ueda, R., Harding, F., Patil, N., Mao, Y., Kurahara, C., et al., 2003. Characterization of HLA DR3/DQ2 transgenic mice: a potential humanized animal model for autoimmune disease studies. *Eur. J. Immunol.* 33, 172–182.
- Chorzelski, T.P., Beutner, E.H., Sulej, J., Tchorzewska, H., Jablonska, S., Kumar, V., et al., 1984. IgA anti-endomysium antibody. A new immunological marker of dermatitis herpetiformis and coeliac disease. *Br. J. Dermatol.* 111, 395–402.
- Choung, R.S., Larson, S.A., Khaleghi, S., Rubio-Tapia, A., Ovsyannikova, I.G., King, K.S., et al., 2017. Prevalence and morbidity of undiagnosed celiac disease from a community-based study. *Gastroenterology* 152, 830–839.e5.
- Christophersen, A., Ráki, M., Bergseng, E., Lundin, K.E., Jahnson, J., Sollid, L.M., et al., 2014. Tetramer-visualized gluten-specific CD4+ T cells in blood as a potential diagnostic marker for coeliac disease without oral gluten challenge. *United Eur. Gastroenterol. J.* 2, 268–278.
- Ciacci, C., Cirillo, M., Sollazzo, R., Savino, G., Sabbatini, F., Mazzacca, G., 1995. Gender and clinical presentation in adult celiac disease. *Scand. J. Gastroenterol.* 30, 1077–1081.
- Ciacci, C., Cirillo, M., Cavallaro, R., Mazzacca, G., 2002. Long-term follow-up of celiac adults on gluten-free diet: prevalence and correlates of intestinal damage. *Digestion* 66, 178–185.
- Clemente, M.G., Musu, M.P., Frau, F., Brusco, G., Sole, G., Corazza, G.R., et al., 2000. Immune reaction against the cytoskeleton in coeliac disease. *Gut* 47, 520–526.

- Collin, P., Reunala, T., 2003. Recognition and management of the cutaneous manifestations of celiac disease: a guide for dermatologists. *Am. J. Clin. Dermatol.* 4, 13–20.
- Collin, P., Reunala, T., Pukkala, E., Laippala, P., Keyrilainen, O., Pasternack, A., 1994. Coeliac disease—associated disorders and survival. *Gut* 35, 1215–1218.
- Corrao, G., Corazza, G.R., Bagnardi, V., Brusco, G., Ciacci, C., Cottone, M., et al., 2001. Mortality in patients with coeliac disease and their relatives: a cohort study. *Lancet* 358, 356–361.
- Counsell, C.E., Taha, A., Ruddell, W.S., 1994. Coeliac disease and autoimmune thyroid disease. *Gut* 35, 844–846.
- Cronin, C.C., Feighery, A., Ferriss, J.B., Liddy, C., Shanahan, F., Feighery, C., 1997. High prevalence of celiac disease among patients with insulin-dependent (type I) diabetes mellitus. *Am. J. Gastroenterol.* 92, 2210–2212.
- Dahele, A.V., Aldhous, M.C., Humphreys, K., Ghosh, S., 2001. Serum IgA tissue transglutaminase antibodies in coeliac disease and other gastrointestinal diseases. *Q. J. M.* 94, 195–205.
- de Kauwe, A.L., Chen, Z., Anderson, R.P., Keech, C.L., Price, J.D., Wijburg, O., et al., 2009. Resistance to celiac disease in humanized HLA-DR3-DQ2-transgenic mice expressing specific anti-gliadin CD4+ T cells. *J. Immunol.* 182, 7440–7450.
- Di Niro, R., Mesin, L., Raki, M., Zheng, N.Y., Lund-Johansen, F., Lundin, K.E., et al., 2010. Rapid generation of rotavirus-specific human monoclonal antibodies from small-intestinal mucosa. *J. Immunol.* 185, 5377–5383.
- Di Niro, R., Mesin, L., Zheng, N.Y., Stammae, J., Morrissey, M., Lee, J.H., et al., 2012. High abundance of plasma cells secreting transglutaminase 2-specific IgA autoantibodies with limited somatic hypermutation in celiac disease intestinal lesions. *Nat. Med.* 18, 441–445.
- Di Niro, R., Snir, O., Kaukinen, K., Yaari, G., Lundin, K.E., Gupta, N.T., et al., 2016. Responsive population dynamics and wide seeding into the duodenal lamina propria of transglutaminase-2-specific plasma cells in celiac disease. *Mucosal Immunol.* 9, 254–264.
- Dicke, W.K., 1950. Coeliac Disease. Investigations of the Harmful Effects of Certain Types of Cereal on Patients Suffering From Coeliac Disease (Thesis). University of Utrecht.
- Dicke, W.K., 1941. Simple dietary treatment for the syndrome of Gee-Herter. *Ned. Tijdschr. Geneesk.* 85, 1715–1716 (in Dutch).
- Dieterich, W., Ehnis, T., Bauer, M., Donner, P., Volta, U., Riecken, E.O., et al., 1997. Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nat. Med.* 3, 797–801.
- Dieterich, W., Laag, E., Schopper, H., Volta, U., Ferguson, A., Gillett, H., et al., 1998. Autoantibodies to tissue transglutaminase as predictors of celiac disease. *Gastroenterology* 115, 1317–1321.
- Dørum, S., Qiao, S.W., Sollid, L.M., Fleckenstein, B., 2009. A quantitative analysis of transglutaminase 2-mediated deamidation of gluten peptides: implications for the T-cell response in celiac disease. *J. Proteome Res.* 8, 1748–1755.
- Dørum, S., Arntzen, M.O., Qiao, S.W., Holm, A., Koehler, C.J., Thiede, B., et al., 2010. The preferred substrates for transglutaminase 2 in a complex wheat gluten digest are peptide fragments harboring celiac disease T-cell epitopes. *PLoS One* 5, e14056.
- du Pré, M.F., Kozijn, A.E., van Berkel, L.A., ter Borg, M.N., Lindenbergh-Kortleve, D., Jensen, L.T., et al., 2011. Tolerance to ingested deamidated gliadin in mice is maintained by splenic, type 1 regulatory T cells. *Gastroenterology* 141, 610–620.e1–e2.
- Ellis, A., 1981. Coeliac disease: previous family studies. In: McConnell, R.B. (Ed.), *The Genetics of Coeliac Disease*. MTP Press, Lancaster, pp. 197–200.
- Ettersperger, J., Montcuquet, N., Malamut, G., Guegan, N., Lopez-Lastra, S., Gayraud, S., et al., 2016. Interleukin-15-dependent T-cell-like innate intraepithelial lymphocytes develop in the intestine and transform into lymphomas in celiac disease. *Immunity* 45, 610–625.
- Falchuk, Z.M., Rogentine, G.N., Strober, W., 1972. Predominance of histocompatibility antigen HL-A8 in patients with gluten-sensitive enteropathy. *J. Clin. Invest.* 51, 1602–1605.
- Fallang, L.E., Bergseng, E., Hotta, K., Berg-Larsen, A., Kim, C.Y., Sollid, L.M., 2009. Differences in the risk of celiac disease associated with HLA-DQ2.5 or HLA-DQ2.2 are related to sustained gluten antigen presentation. *Nat. Immunol.* 10, 1096–1101.
- Ferguson, A., MacDonald, T.T., McClure, J.P., Holden, R.J., 1975. Cell-mediated immunity to gliadin within the small-intestinal mucosa in coeliac disease. *Lancet* 1, 895–897.
- Fleckenstein, B., Molberg, Ø., Qiao, S.W., Schmid, D.G., Von Der, M.F., Elgstoer, K., et al., 2002. Gliadin T cell epitope selection by tissue transglutaminase in celiac disease. Role of enzyme specificity and pH influence on the transamidation versus deamidation process. *J. Biol. Chem.* 277, 34109–34116.
- Folk, J.E., 1983. Mechanism and basis for specificity of transglutaminase-catalyzed ε-(γ-glutamyl) lysine bond formation. *Adv. Enzymol. Relat. Areas Mol. Biol.* 54, 1–56.
- Forsberg, G., Hernell, O., Melgar, S., Israelsson, A., Hammarstrom, S., Hammarstrom, M.L., 2002. Paradoxical coexpression of proinflammatory and down-regulatory cytokines in intestinal T cells in childhood celiac disease. *Gastroenterology* 123, 667–678.
- Gass, J., Bethune, M.T., Siegel, M., Spencer, A., Khosla, C., 2007. Combination enzyme therapy for gastric digestion of dietary gluten in patients with celiac sprue. *Gastroenterology* 133, 472–480.
- Gee, S.J., 1888. On the coeliac affection. *St. Batholomew's Hosp. Res.* 24, 17–20.
- Giersiepen, K., Lelgemann, M., Stuhldreher, N., Ronfani, L., Husby, S., Koletzko, S., et al., 2012. Accuracy of diagnostic antibody tests for coeliac disease in children: summary of an evidence report. *J. Pediatr. Gastroenterol. Nutr.* 54, 229–241.
- Gil-Humanez, J., Piston, F., Tollesen, S., Sollid, L.M., Barro, F., 2010. Effective shutdown in the expression of celiac disease-related wheat gliadin T-cell epitopes by RNA interference. *Proc. Natl. Acad. Sci. U.S.A.* 107, 17023–17028.
- Gjertsen, H.A., Sollid, L.M., Ek, J., Thorsby, E., Lundin, K.E.A., 1994. T cells from the peripheral blood of coeliac disease patients recognize gluten antigens when presented by HLA-DR, -DQ, or -DP molecules. *Scand. J. Immunol.* 39, 567–574.
- Goel, G., King, T., Daveson, A.J., Andrews, J.M., Krishnarajah, J., Krause, R., et al., 2017. Epitope-specific immunotherapy targeting CD4-positive T cells in coeliac disease: two randomised, double-blind, placebo-controlled phase 1 studies. *Lancet Gastroenterol. Hepatol.* 2, 479–493.
- Green, P.A., Wollaeger, E.E., Sprague, R.G., Brown, A.L., 1962. Diabetes mellitus associated with tropical sprue. *Diabetes* 18, 388–392.
- Green, P.H., Fleischauer, A.T., Bhagat, G., Goyal, R., Jabri, B., Neugut, A.I., 2003. Risk of malignancy in patients with celiac disease. *Am. J. Med.* 115, 191–195.
- Haas, S.V., 1932. Celiac disease, its specific treatment and cure without nutritional relapse. *JAMA* 99, 448–452.
- Hadjivassiliou, M., Grunewald, R., Sharrack, B., Sanders, D., Lobo, A., Williamson, C., et al., 2003. Gluten ataxia in perspective: epidemiology, genetic susceptibility and clinical characteristics. *Brain* 126, 685–691.

- Han, A., Newell, E.W., Glanville, J., Fernandez-Becker, N., Khosla, C., Chien, Y.H., et al., 2013. Dietary gluten triggers concomitant activation of CD4+ and CD8+  $\alpha\beta$  T cells and  $\gamma\delta$  T cells in celiac disease. *Proc. Natl. Acad. Sci. U.S.A.* 110, 13073–13078.
- Hausch, F., Shan, L., Santiago, N.A., Gray, G.M., Khosla, C., 2002. Intestinal digestive resistance of immunodominant gliadin peptides. *Am. J. Physiol. Gastrointest. Liver Physiol.* 283, G996–G1003.
- Henderson, K.N., Tye-Din, J.A., Reid, H.H., Chen, Z., Borg, N.A., Beissbarth, T., et al., 2007. A structural and immunological basis for the role of human leukocyte antigen DQ8 in celiac disease. *Immunity* 27, 23–34.
- Hoffenberg, E.J., Haas, J., Drescher, A., Barnhurst, R., Osberg, I., Bao, F., et al., 2000. A trial of oats in children with newly diagnosed celiac disease. *J. Pediatr.* 137, 361–366.
- Holmes, G.K.T., Muirhead, A., 2017. Epidemiology of coeliac disease in a single centre in Southern Derbyshire 1958–2014. *BMJ Open Gastroenterol.* 4, e000137.
- Hüe, S., Mention, J.J., Monteiro, R.C., Zhang, S., Cellier, C., Schmitz, J., et al., 2004. A direct role for NKG2D/MICA interaction in villous atrophy during celiac disease. *Immunity* 21, 367–377.
- Husby, S., Koletzko, S., Korponay-Szabo, I.R., Mearin, M.L., Phillips, A., Shamir, R., et al., 2012. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. *J. Pediatr. Gastroenterol. Nutr.* 54, 136–160.
- Ivarsson, A., Hernell, O., Nyström, L., Persson, L.A., 2003a. Children born in the summer have increased risk for coeliac disease. *J. Epidemiol. Community Health* 57, 36–39.
- Ivarsson, A., Persson, L.A., Nyström, L., Hernell, O., 2003b. The Swedish coeliac disease epidemic with a prevailing twofold higher risk in girls compared to boys may reflect gender specific risk factors. *Eur. J. Epidemiol.* 18, 677–684.
- Iversen, R., Snir, O., Stensland, M., Kroll, J.E., Steinsbo, O., Korponay-Szabo, I.R., et al., 2017. Strong clonal relatedness between serum and gut IgA despite different plasma cell origins. *Cell Rep.* 20, 2357–2367.
- Jabri, B., Sollid, L.M., 2009. Tissue-mediated control of immunopathology in coeliac disease. *Nat. Rev. Immunol.* 9, 858–870.
- Janatuinen, E.K., Kemppainen, T.A., Julkunen, R.J., Kosma, V.M., Maki, M., Heikkinen, M., et al., 2002. No harm from five year ingestion of oats in coeliac disease. *Gut* 50, 332–335.
- Karell, K., Louka, A.S., Moodie, S.J., Ascher, H., Clot, F., Greco, L., et al., 2003. HLA types in celiac disease patients not carrying the DQA1\*05–DQB1\*02 (DQ2) heterodimer: results from the European Genetics Cluster on Celiac Disease. *Hum. Immunol.* 64, 469–477.
- Kaukinen, K., Sulkanen, S., Maki, M., Collin, P., 2002. IgA-class transglutaminase antibodies in evaluating the efficacy of gluten-free diet in coeliac disease. *Eur. J. Gastroenterol. Hepatol.* 14, 311–315.
- Keillor, J.W., Aupperley, K.Y., 2016. Transglutaminase inhibitors: a patent review. *Expert Opin. Ther. Pat.* 26, 49–63.
- Kemppainen, K.M., Lynch, K.F., Liu, E., Lonnrot, M., Simell, V., Briese, T., et al., 2017. Factors that increase risk of celiac disease autoimmunity after a gastrointestinal infection in early life. *Clin. Gastroenterol. Hepatol.* 15, 694–702.e5.
- Kim, C.Y., Quarsten, H., Bergseng, E., Khosla, C., Sollid, L.M., 2004. Structural basis for HLA-DQ2-mediated presentation of gluten epitopes in celiac disease. *Proc. Natl. Acad. Sci. U.S.A.* 101, 4175–4179.
- Koletzko, S., Burgin-Wolff, A., Koletzko, B., Knapp, M., Burger, W., Gruneklee, D., et al., 1988. Prevalence of coeliac disease in diabetic children and adolescents. A multicentre study. *Eur. J. Pediatr.* 148, 113–117.
- Kondrashova, A., Mustalahti, K., Kaukinen, K., Viskari, H., Volodicheva, V., Haapala, A.M., et al., 2008. Lower economic status and inferior hygienic environment may protect against celiac disease. *Ann. Med.* 40, 223–231.
- Kooy-Winkelhaar, Y.M., Bouwer, D., Janssen, G.M., Thompson, A., Brugman, M.H., Schmitz, F., et al., 2017. CD4 T-cell cytokines synergize to induce proliferation of malignant and nonmalignant innate intraepithelial lymphocytes. *Proc. Natl. Acad. Sci. U.S.A.* 114, E980–E989.
- Korponay-Szabo, I.R., Halttunen, T., Szalai, Z., Laurila, K., Kiraly, R., Kovacs, J.B., et al., 2004. In vivo targeting of intestinal and extraintestinal transglutaminase 2 by coeliac autoantibodies. *Gut* 53, 641–648.
- Kutlu, T., Brousse, N., Rambaud, C., Le Deist, F., Schmitz, J., Cerf-Bensussan, N., 1993. Numbers of T cell receptor (TCR)  $\alpha\beta$  + but not of TcR  $\gamma\delta$  + intraepithelial lymphocytes correlate with the grade of villous atrophy in coeliac patients on a long term normal diet. *Gut* 34, 208–214.
- Ladinser, B., Rossipal, E., Pittschielder, K., 1994. Endomysium antibodies in coeliac disease: an improved method. *Gut* 35, 776–778.
- Lebwohl, B., Sanders, D.S., Green, P.H.R., 2017. Coeliac disease. *Lancet* 391, 70–81.
- Lewis, N.R., Scott, B.B., 2010. Meta-analysis: deamidated gliadin peptide antibody and tissue transglutaminase antibody compared as screening tests for coeliac disease. *Aliment. Pharmacol. Ther.* 31, 73–81.
- Lindfors, K., Rauhavirta, T., Stenman, S., Maki, M., Kaukinen, K., 2012. In vitro models for gluten toxicity: relevance for celiac disease pathogenesis and development of novel treatment options. *Exp. Biol. Med.* 237, 119–125.
- Lionetti, E., Castellaneta, S., Francavilla, R., Pulvirenti, A., Tonutti, E., Amarri, S., et al., 2014. Introduction of gluten, HLA status, and the risk of celiac disease in children. *N. Engl. J. Med.* 371, 1295–1303.
- Lorand, L., Graham, R.M., 2003. Transglutaminases: crosslinking enzymes with pleiotropic functions. *Nat. Rev. Mol. Cell Biol.* 4, 140–156.
- Ludvigsson, J.F., Montgomery, S.M., Ekbom, A., Brandt, L., Granath, F., 2009. Small-intestinal histopathology and mortality risk in celiac disease. *JAMA* 302, 1171–1178.
- Ludvigsson, J.F., Leffler, D.A., Bai, J.C., Biagi, F., Fasano, A., Green, P.H., et al., 2013. The Oslo definitions for coeliac disease and related terms. *Gut* 62, 43–52.
- Ludvigsson, J.F., Bai, J.C., Biagi, F., Card, T.R., Ciacci, C., Ciclitira, P.J., et al., 2014. Diagnosis and management of adult coeliac disease: guidelines from the British Society of Gastroenterology. *Gut* 63, 1210–1228.
- Lundin, K.E., Wijmenga, C., 2015. Coeliac disease and autoimmune disease-genetic overlap and screening. *Nat. Rev. Gastroenterol. Hepatol.* 12, 507–515.
- Lundin, K.E.A., Scott, H., Hansen, T., Paulsen, G., Halstensen, T.S., Fausa, O., et al., 1993. Gliadin-specific, HLA-DQ( $\alpha 1^*0501, \beta 1^*0201$ ) restricted T cells isolated from the small intestinal mucosa of celiac disease patients. *J. Exp. Med.* 178, 187–196.
- Lundin, K.E.A., Scott, H., Fausa, O., Thorsby, E., Sollid, L.M., 1994. T cells from the small intestinal mucosa of a DR4, DQ7/DR4, DQ8 celiac disease patient preferentially recognize gliadin when presented by DQ8. *Hum. Immunol.* 41, 285–291.
- Lundin, K.E.A., Nilsen, E.M., Scott, H.G., Løberg, E.M., Gjøen, A., Bratlie, J., et al., 2003. Oats induced villous atrophy in coeliac disease. *Gut* 52, 1149–1152.

- MacDonald, W.C., Dobbins, I.W.O., Rubin, C.E., 1965. Studies of the familial nature of celiac sprue using biopsy of the small intestine. *N. Engl. J. Med.* 272, 448–456.
- Maiuri, L., Ciacci, C., Auricchio, S., Brown, V., Quarantino, S., Londei, M., 2000. Interleukin 15 mediates epithelial changes in celiac disease. *Gastroenterology* 119, 996–1006.
- Maiuri, L., Ciacci, C., Ricciardelli, I., Vacca, L., Raia, V., Auricchio, S., et al., 2003. Association between innate response to gliadin and activation of pathogenic T cells in coeliac disease. *Lancet* 362, 30–37.
- Mäki, M., Mustalahti, K., Kokkonen, J., Kulmala, P., Haapalahti, M., Karttunen, T., et al., 2003. Prevalence of celiac disease among children in Finland. *N. Engl. J. Med.* 348, 2517–2524.
- Marietta, E., Black, K., Camilleri, M., Krause, P., Rogers, R.S., David, C., et al., 2004. A new model for dermatitis herpetiformis that uses HLA-DQ8 transgenic NOD mice. *J. Clin. Invest.* 114, 1090–1097.
- Marsh, M.N., 1992. Mucosal pathology in gluten sensitivity. In: Marsh, M.N. (Ed.), *Celiac Disease*. Blackwell Scientific Publications, Oxford, pp. 136–191.
- Marti, T., Molberg, Ø., Li, Q., Gray, G.M., Khosla, C., Sollid, L.M., 2005. Prolyl endopeptidase-mediated destruction of T cell epitopes in whole gluten: chemical and immunological characterization. *J. Pharmacol. Exp. Ther.* 312, 19–26.
- Marzari, R., Sblattero, D., Florian, F., Tongiorgi, E., Not, T., Tommasini, A., et al., 2001. Molecular dissection of the tissue transglutaminase autoantibody response in celiac disease. *J. Immunol.* 166, 4170–4176.
- Mawhinney, H., Tomkin, G.H., 1971. Gluten enteropathy associated with selective IgA deficiency. *Lancet* 2, 121–124.
- Meeuwisse, G.W., 1970. European Society for Paediatric Gastroenterology meeting in Interlaken September 18, 1969. *Acta Paediat. Scand.* 59, 461–463.
- Mention, J.J., Ben Ahmed, M., Begue, B., Barbe, U., Verkarre, V., Asnafi, V., et al., 2003. Interleukin 15: a key to disrupted intraepithelial lymphocyte homeostasis and lymphomagenesis in celiac disease. *Gastroenterology* 125, 730–745.
- Meresse, B., Chen, Z., Ciszewski, C., Tretiakova, M., Bhagat, G., Krausz, T.N., et al., 2004. Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease. *Immunity* 21, 357–366.
- Meresse, B., Curran, S.A., Ciszewski, C., Orbelyan, G., Setty, M., Bhagat, G., et al., 2006. Reprogramming of CTLs into natural killer-like cells in celiac disease. *J. Exp. Med.* 203, 1343–1355.
- Mitea, C., Havenaar, R., Drijfhout, J.W., Edens, L., Dekking, L., Koning, F., 2008. Efficient degradation of gluten by a prolyl endoprotease in a gastrointestinal model: implications for coeliac disease. *Gut* 57, 25–32.
- Molberg, K., Kett, H., Scott, E., Thorsby, L.M., Sollid, Lundin, K.E.A., 1997. Gliadin specific, HLA DQ2-restricted T cells are commonly found in small intestinal biopsies from coeliac disease patients, but not from controls. *Scand. J. Immunol.* 46, 103–109.
- Molberg, Ø., McAdam, S.N., Korner, R., Quarsten, H., Kristiansen, C., Madsen, L., et al., 1998. Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease. *Nat. Med.* 4, 713–717.
- Murray, J.A., 1999. The widening spectrum of celiac disease. *Am. J. Clin. Nutr.* 69, 354–365.
- Murray, J.A., Kelly, C.P., Green, P.H.R., Marcantonio, A., Wu, T.T., Maki, M., et al., 2017. No difference between latiglutinase and placebo in reducing villous atrophy or improving symptoms in patients with symptomatic celiac disease. *Gastroenterology* 152, 787–798.e2.
- Mustalahti, K., Catassi, C., Reunanan, A., Fabiani, E., Heier, M., McMillan, S., et al., 2010. The prevalence of celiac disease in Europe: results of a centralized, international mass screening project. *Ann. Med.* 42, 587–595.
- Namatovu, F., Sandstrom, O., Olsson, C., Lindkvist, M., Ivarsson, A., 2014. Celiac disease risk varies between birth cohorts, generating hypotheses about causality: evidence from 36 years of population-based follow-up. *BMC Gastroenterol.* 14, 59.
- Nguyen, T.B., Jayaraman, P., Bergseng, E., Madhusudhan, M.S., Kim, C.Y., Sollid, L.M., 2017. Unraveling the structural basis for the unusually rich association of human leukocyte antigen DQ2.5 with class-II-associated invariant chain peptides. *J. Biol. Chem.* 292, 9218–9228.
- Nilsen, E.M., Lundin, K.E.A., Krajci, P., Scott, H., Sollid, L.M., Brandtzaeg, P., 1995. Gluten specific, HLA-DQ restricted T cells from coeliac mucosa produce cytokines with Th1 or Th0 profile dominated by interferon gamma. *Gut* 37, 766–776.
- Nilsen, E.M., Jahnson, F.L., Lundin, K.E.A., Johansen, F.E., Fausa, O., Sollid, L.M., et al., 1998. Gluten induces an intestinal cytokine response strongly dominated by interferon gamma in patients with celiac disease. *Gastroenterology* 115, 551–563.
- Nistico, L., Fagnani, C., Coto, I., Percopo, S., Cotichini, R., Limongelli, M.G., et al., 2006. Concordance, disease progression, and heritability of coeliac disease in Italian twins. *Gut* 55, 803–808.
- Oberhuber, G., Granditsch, G., Vogelsang, H., 1999. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. *Eur. J. Gastroenterol. Hepatol.* 11, 1185–1194.
- Olaussen, R.W., Johansen, F.E., Lundin, K.E., Jahnson, J., Brandtzaeg, P., Farstad, I.N., 2002. Interferon-gamma-secreting T cells localize to the epithelium in coeliac disease. *Scand. J. Immunol.* 56, 652–664.
- Osman, A.A., Gunnell, T., Dietl, A., Uhlig, H.H., Amin, M., Fleckenstein, B., et al., 2000. B cell epitopes of gliadin. *Clin. Exp. Immunol.* 121, 248–254.
- Paulley, J.W., 1954. Observation on the aetiology of idiopathic steatorrhoea; jejunal and lymph-node biopsies. *Br. Med. J.* 2, 1318–1321.
- Petersen, J., Montserrat, V., Mujico, J.R., Loh, K.L., Beringer, D.X., van Lummel, M., et al., 2014. T-cell receptor recognition of HLA-DQ2-gliadin complexes associated with celiac disease. *Nat. Struct. Mol. Biol.* 21, 480–488.
- Pinto, D., Montani, E., Bolli, M., Garavaglia, G., Sallusto, F., Lanzavecchia, A., et al., 2013. A functional BCR in human IgA and IgM plasma cells. *Blood* 121, 4110–4114.
- Piper, J.L., Gray, G.M., Khosla, C., 2002. High selectivity of human tissue transglutaminase for immunoactive gliadin peptides: implications for celiac sprue. *Biochemistry* 41, 386–393.
- Quarsten, H., McAdam, S.N., Jensen, T., Arentz-Hansen, H., Molberg, Ø., Lundin, K.E., et al., 2001. Staining of celiac disease-relevant T cells by peptide-DQ2 multimers. *J. Immunol.* 167, 4861–4868.
- Qiao, S.W., Ráki, M., Gunnarsen, K.S., Løset, G.A., Lundin, K.E., Sandlie, I., et al., 2011. Posttranslational modification of gluten shapes TCR usage in celiac disease. *J. Immunol.* 187, 3064–3071.
- Qiao, S.W., Christoffersen, A., Lundin, K.E., Sollid, L.M., 2014. Biased usage and preferred pairing of  $\alpha$ - and  $\beta$ -chains of TCRs specific for an immunodominant gluten epitope in coeliac disease. *Int. Immunol.* 26, 13–19.

- Ráki, M., Tollefse, S., Molberg, O., Lundin, K.E., Sollid, L.M., Jähnsen, F.L., 2006. A unique dendritic cell subset accumulates in the celiac lesion and efficiently activates gluten-reactive T cells. *Gastroenterology* 131, 428–438.
- Ráki, M., Fallang, L.E., Brottveit, M., Bergseng, E., Quarsten, H., Lundin, K.E., et al., 2007. Tetramer visualization of gut-homing gluten-specific T cells in the peripheral blood of celiac disease patients. *Proc. Natl. Acad. Sci. U.S.A.* 104, 2831–2836.
- Risch, N., 1987. Assessing the role of HLA-linked and unlinked determinants of disease. *Am. J. Hum. Genet.* 40, 1–14.
- Rishi, A.R., Rubio-Tapia, A., Murray, J.A., 2016. Refractory celiac disease. *Expert Rev. Gastroenterol. Hepatol.* 10, 537–546.
- Rostom, A., Dube, C., Cranney, A., Saloojee, N., Sy, R., Garrity, C., et al., 2005. The diagnostic accuracy of serologic tests for celiac disease: a systematic review. *Gastroenterology* 128, S38–S46.
- Roy, B., Neumann, R.S., Snir, O., Iversen, R., Sandve, G.K., Lundin, K.E.A., et al., 2017. High-throughput single-cell analysis of B cell receptor usage among autoantigen-specific plasma cells in celiac disease. *J. Immunol.* 199, 782–791.
- Rubin, C.E., Brandborg, L.L., Phelps, P.C., Taylor, H.C., 1960. Studies of celiac disease. I. Apparent identical and specific nature of the duodenal and proximal jejunal lesion in celiac disease and idiopathic sprue. *Gastroenterology* 38, 28–49.
- Rubio-Tapia, A., Ludvigsson, J.F., Brantner, T.L., Murray, J.A., Everhart, J.E., 2012. The prevalence of celiac disease in the United States. *Am. J. Gastroenterol.* 107, 1538–1544.
- Salmi, T.T., Collin, P., Jarvinen, O., Haimila, K., Partanen, J., Laurila, K., et al., 2006a. Immunoglobulin A autoantibodies against transglutaminase 2 in the small intestinal mucosa predict forthcoming coeliac disease. *Aliment. Pharmacol. Ther.* 24, 541–552.
- Salmi, T.T., Collin, P., Korponay-Szabo, I.R., Laurila, K., Partanen, J., Huhtala, H., et al., 2006b. Endomysial antibody-negative coeliac disease: clinical characteristics and intestinal autoantibody deposits. *Gut* 55, 1746–1753.
- Sanchez, D., Tuckova, L., Sebo, P., Michalak, M., Whelan, A., Sterzl, I., et al., 2000. Occurrence of IgA and IgG autoantibodies to calreticulin in coeliac disease and various autoimmune diseases. *J. Autoimmun.* 15, 441–449.
- Sánchez-León, S., Gil-Humane, J., Ozuna, C.V., Giménez, M.J., Sousa, C., Voytas, D.F., et al., 2018. Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9. *Plant Biotechnol. J.* 16, 902–910.
- Sanders, D.S., Patel, D., Stephenson, T.J., Ward, A.M., McCloskey, E.V., Hadjivassiliou, M., et al., 2003. A primary care cross-sectional study of undiagnosed adult coeliac disease. *Eur. J. Gastroenterol. Hepatol.* 15, 407–413.
- Sardy, M., Karpati, S., Merkl, B., Paulsson, M., Smyth, N., 2002. Epidermal transglutaminase (TGase 3) is the autoantigen of dermatitis herpetiformis. *J. Exp. Med.* 195, 747–757.
- Schulz, O., Jaensson, E., Persson, E.K., Liu, X., Worbs, T., Agace, W.W., et al., 2009. Intestinal CD103+, but not CX3CR1+, antigen sampling cells migrate in lymph and serve classical dendritic cell functions. *J. Exp. Med.* 206, 3101–3114.
- Seah, P.P., Fry, L., Rossiter, M.A., Hoffbrand, A.V., Holborow, E.J., 1971. Anti-reticulin antibodies in childhood coeliac disease. *Lancet* 2, 681–682.
- Shan, L., Molberg, O., Parrot, I., Hausch, F., Filiz, F., Gray, G.M., et al., 2002. Structural basis for gluten intolerance in celiac sprue. *Science* 297, 2275–2279.
- Shewry, P.R., Halford, N., Lafiandra, D., 2003. Genetics of wheat gluten proteins. *Adv. Genet.* 49, 111–184.
- Simell, S., Hoppu, S., Hekkala, A., Simell, T., Stahlberg, M.R., Viander, M., et al., 2007. Fate of five celiac disease-associated antibodies during normal diet in genetically at-risk children observed from birth in a natural history study. *Am. J. Gastroenterol.* 102, 2026–2035.
- Smyth, D.J., Plagnol, V., Walker, N.M., Cooper, J.D., Downes, K., Yang, J.H., et al., 2008. Shared and distinct genetic variants in type 1 diabetes and celiac disease. *N. Engl. J. Med.* 359, 2767–2777.
- Sollid, L.M., 2017. The roles of MHC class II genes and post-translational modification in celiac disease. *Immunogenetics* 69, 605–616.
- Sollid, L.M., Jabri, B., 2013. Triggers and drivers of autoimmunity: lessons from coeliac disease. *Nat. Rev. Immunol.* 13, 294–302.
- Sollid, L.M., Markussen, G., Ek, J., Gjerde, H., Vartdal, F., Thorsby, E., 1989. Evidence for a primary association of celiac disease to a particular HLA-DQ  $\alpha/\beta$  heterodimer. *J. Exp. Med.* 169, 345–350.
- Sollid, L.M., Molberg, Ø., McAdam, S., Lundin, K.E., 1997. Autoantibodies in coeliac disease: tissue transglutaminase—guilt by association? *Gut* 41, 851–852.
- Sollid, L.M., Qiao, S.W., Anderson, R.P., Gianfrani, C., Koning, F., 2012. Nomenclature and listing of celiac disease relevant gluten T-cell epitopes restricted by HLA-DQ molecules. *Immunogenetics* 64, 455–460.
- Stamnaes, J., Dorum, S., Fleckenstein, B., Aeschlimann, D., Sollid, L.M., 2010. Gluten T cell epitope targeting by TG3 and TG6; implications for dermatitis herpetiformis and gluten ataxia. *Amino Acids* 39, 1183–1191.
- Steinsbo, Ø., Henry Dunand, C.J., Huang, M., Mesin, L., Salgado-Ferrer, M., Lundin, K.E., et al., 2014. Restricted VH/VL usage and limited mutations in gluten-specific IgA of coeliac disease lesion plasma cells. *Nat. Commun.* 5, 4041.
- Stokes, P.L., Asquith, P., Holmes, G.K., Mackintosh, P., Cooke, W.T., 1972. Histocompatibility antigens associated with adult coeliac disease. *Lancet* 2, 162–164.
- Sulkanen, S., Halattunen, T., Laurila, K., Kolho, K.L., Korponay-Szabo, I.R., Sarnesto, A., et al., 1998. Tissue transglutaminase autoantibody enzyme-linked immunosorbent assay in detecting celiac disease. *Gastroenterology* 115, 1322–1328.
- Tollefse, S., Arentz-Hansen, H., Fleckenstein, B., Molberg, Ø., Ráki, M., Kwok, W.W., et al., 2006. HLA-DQ2 and -DQ8 signatures of gluten T cell epitopes in celiac disease. *J. Clin. Invest.* 116, 2226–2236.
- Trynka, G., Hunt, K.A., Bockett, N.A., Romanos, J., Mistry, V., Szperl, A., et al., 2011. Dense genotyping identifies and localizes multiple common and rare variant association signals in celiac disease. *Nat. Genet.* 43, 1193–1201.
- Tye-Din, J.A., Stewart, J.A., Dromey, J.A., Beissbarth, T., van Heel, D.A., Tatham, A., et al., 2010. Comprehensive, quantitative mapping of T cell epitopes in gluten in celiac disease. *Sci. Transl. Med.* 2, 41ra51.
- Vader, L.W., de Ru, A., van Der, W.Y., Kooy, Y.M., Benckhuijsen, W., Mearin, M.L., et al., 2002. Specificity of tissue transglutaminase explains cereal toxicity in celiac disease. *J. Exp. Med.* 195, 643–649.
- van Berge-Henegouwen, G.P., Mulder, C.J., 1993. Pioneer in the gluten free diet: Willem-Karel Dicke 1905–1962, over 50 years of gluten free diet. *Gut* 34, 1473–1475.
- van de Wal, Y., Kooy, Y.C., Drijfhout, J.W., Amons, R., Papadopoulos, G.K., Koning, F., 1997. Unique peptide binding characteristics of the disease-associated DQ( $\alpha 1^*0501, \beta 1^*0201$ ) vs the non-disease-associated DQ( $\alpha 1^*0201, \beta 1^*0202$ ) molecule. *Immunogenetics* 46, 484–492.

- van de Wal, Y., Kooy, Y.M., van Veelen, P.A., Pena, S.A., Mearin, L.M., Molberg, Ø., et al., 1998b. Small intestinal T cells of celiac disease patients recognize a natural pepsin fragment of gliadin. *Proc. Natl. Acad. Sci. U.S.A.* 95, 10050–10054.
- van de Wal, Y., Kooy, Y., van Veelen, P., Pena, S., Mearin, L., Papadopoulos, G., et al., 1998a. Selective deamidation by tissue transglutaminase strongly enhances gliadin-specific T cell reactivity. *J. Immunol.* 161, 1585–1588.
- van der Kolk, J.H., van Putten, L.A., Mulder, C.J., Grinwis, G.C., Reijm, M., Butler, C.M., et al., 2012. Gluten-dependent antibodies in horses with inflammatory small bowel disease (ISBD). *Vet. Q.* 32, 3–11.
- Vriezinga, S.L., Auricchio, R., Bravi, E., Castillejo, G., Chmielewska, A., Crespo Escobar, P., et al., 2014. Randomized feeding intervention in infants at high risk for celiac disease. *N. Engl. J. Med.* 371, 1304–1315.
- Wang, N., Shen, N., Vyse, T.J., Anand, V., Gunnarson, I., Sturfelt, G., et al., 2011. Selective IgA deficiency in autoimmune diseases. *Mol. Med.* 17, 1383–1396.
- Werkstetter, K.J., Korponay-Szabo, I.R., Popp, A., Villanacci, V., Salemmé, M., Heilig, G., et al., 2017. Accuracy in diagnosis of celiac disease without biopsies in clinical practice. *Gastroenterology*.
- West, J., Logan, R.F., Hill, P.G., Lloyd, A., Lewis, S., Hubbard, R., et al., 2003. Seroprevalence, correlates, and characteristics of undetected coeliac disease in England. *Gut* 52, 960–965.
- Withoff, S., Li, Y., Jonkers, I., Wijmenga, C., 2016. Understanding celiac disease by genomics. *Trends Genet.* 32, 295–308.
- Wolf, J., Petroff, D., Richter, T., Auth, M.K.H., Uhlig, H.H., Laass, M.W., et al., 2017. Validation of antibody-based strategies for diagnosis of pediatric celiac disease without biopsy. *Gastroenterology* 153, 410–419.e17.
- Xia, J., Sollid, L.M., Khosla, C., 2005. Equilibrium and kinetic analysis of the unusual binding behavior of a highly immunogenic gluten peptide to HLA-DQ2. *Biochemistry* 44, 4442–4449.
- Yuan, J., Zhou, C., Gao, J., Li, J., Yu, F., Lu, J., et al., 2017. Prevalence of celiac disease autoimmunity among adolescents and young adults in China. *Clin. Gastroenterol. Hepatol.* 15, 1572–1579.e1.

# Inflammatory Bowel Disease

Michael W. Winter and Joel V. Weinstock

Division of Gastroenterology-Hepatology, Department of Internal Medicine, Tufts Medical Center, Boston, MA,  
United States

## OUTLINE

<b>General Introduction</b>	871	<b>Genetics</b>	879
<b>Historical Aspects</b>	872	<b>Animal Models</b>	880
<b>Epidemiology</b>	872	<b>Diagnostic Procedures</b>	881
<b>Clinical Features and Disease Associations</b>	873	Serologic Markers	881
<i>Crohn's Disease</i>	873	Stool Markers	882
<i>Ulcerative Colitis</i>	874	Imaging	882
Extraintestinal Manifestations Common to			
<i>Ulcerative Colitis and Crohn's Disease</i>	875	<b>Treatment</b>	883
Other Diseases With a Link to Inflammatory		Medical	883
Bowel Disease	876	Aminosalicylates	884
Cancer	876	Surgical	888
<b>Pathological Features</b>	876	<b>Future Prospects</b>	889
<b>Etiology Including Autoimmune Features</b>	877	<b>Acknowledgment</b>	889
<i>Microbiota</i>	877	<b>Abbreviations</b>	889
<i>Intestinal Epithelium and Barrier Function</i>	878	<b>References</b>	890
<i>Innate and Adoptive Immunity</i>	878		

## GENERAL INTRODUCTION

Inflammatory bowel disease (IBD) traditionally is divided into two related subgroups called Crohn's disease (CD) and ulcerative colitis (UC) that often differ in location of bowel involved, pathological features, and surgical outcomes. They were thought to be distinct entities. It now appears more likely that both CD and UC are various conditions with somewhat overlapping clinical and pathological features. They are the manifestations of intestinal mucosal immune dysregulation in which the host immunologically overresponds to substances in the natural intestinal stream. IBD presently is not considered a classic autoimmune disease in which host T cells and antibodies inappropriately recognize and attack host tissue. Yet, the microbiota and molecular components of our inner intestinal lumen may be considered a functional "organ" essential for our health. In this sense, IBD could be considered an autoimmune disease in which a poorly regulated mucosal immune response to luminal contents leads to severe collateral damage to the intestinal lining. Advances in genomics have detected gene variants that, to a limited degree, predispose some people to these diseases. However, poorly defined environmental factors were

the major cause for the rapid growth of IBD in industrial societies in the latter half of the 20th century and are the primary drive for the precipitous spread of IBD worldwide in the 21st century. Advances in therapy are coming with increasing speed because of exciting new discoveries providing insight into the immune regulatory pathways that drive and limit intestinal inflammation as well as from the enhanced appreciation of the importance of the intestinal microbiota. This also comes with the sobering realization that there is a continual increase in both the prevalence and severity of these diseases, which will increasingly challenge healthcare systems and the quality of life of people everywhere.

## HISTORICAL ASPECTS

Both UC and CD were recognized in the 19th century. It is likely that the high prevalence of enteric mycobacterial infections, partly due to drinking unpasteurized milk, and other enteric infections masked the recognition of many cases.

With regard to CD, various case reports described patients with chronic inflammatory diseases of the small bowel and colon that did not appear to have enteric infection (Baron, 2000). With refinements in microscopy, small case series emerged documenting patients with inflammation and strictures of the ileocecal region without any evidence of malignancy or tuberculosis.

Doctors Burrill B. Crohn, Leon Ginzburg, and Gordon D. Oppenheimer practicing at Mount Sinai Hospital in New York published the seminal paper in 1932 entitled "Regional Ileitis: A Pathologic and Clinical Entity" (Crohn et al., 1932). The paper described 14 patients requiring surgery, performed by Dr. A. A. Berg, for idiopathic granulomatous inflammation of the terminal ileum. The condition was called regional ileitis because Dr. Crohn believed the disease only involved the terminal ileum (Wells, 1952) despite evidence presented by Ginzburg, Oppenheimer (Ginzburg and Oppenheimer, 1932), and others of cases with inflammation involvement in the colon and small bowel. Crohn's name was attached to the disease because the authors listed their names alphabetically on the paper and the surgeon, Dr. Berg, humbly declined to be included as an author (Baron, 2000).

Samuel Wilks (Wilks, 1859) receives credit for the discovery of UC as a distinct entity, which he called "idiopathic colitis." Hale-White (1888) coined the term "ulcerative colitis." However, his patients may have had irritable bowel syndrome rather than UC (Baron, 2000). The development of the electric sigmoidoscope allowed Sir William Hurst (Hurst, 1909) and Lockhart-Mummery (Mummery, 1907) to help clarify the appearance of the colon in UC.

## EPIDEMIOLOGY

The highest prevalence of IBD is in wealthy industrialized nations of North America and Europe (Molodecky et al., 2012). It is particularly common in the Northern regions of these geographical areas. These diseases were rare in the middle of the 20th century. Population-based, epidemiologic studies substantiated the rise in disease prevalence over time (Cosnes et al., 2011). It is estimated that perhaps 0.3% of the population in these Western regions now has UC or CD (Molodecky et al., 2012; Kaplan and Ng, 2017).

IBD was rare in less developed countries, but this is no longer the case. IBD presently is spreading around the world as nations undergo the process of industrialization (Molodecky et al., 2012). Although the incidence of IBD is lower in Eastern Europe, Africa, and Asia compared to North America and Western Europe, there are well-documented increases in disease prevalence in countries such as South Korea, India, China, Japan, and the nations of the Middle East (Goh and Xiao, 2009). IBD are diseases that usually begin in the second-to-third decade of life. The disease in less developed countries tends to be milder and come on at a later age, a situation similar to that seen in the United States during the early years of the IBD epidemic. Cohort studies suggest that children acquire an increased risk of developing IBD if they were born to immigrants who moved from regions with a low IBD incidence to areas of high incidence (Benchimol et al., 2015).

These observations strongly suggest that environmental factors greatly affect the prevalence of IBD. The "IBD Hygiene Hypothesis," which is now supported by substantial experimental and clinical data, suggests that an important cause is unintentional alterations in the composition of the various organisms residing within the intestines (Elliott et al., 2000). Another important factor could be a shift in the nature and composition of diets (Owczarek et al., 2016). However, data supporting this idea remain sparse.

Smoking affects the frequency of IBD (Rubin and Hanauer, 2000). People who smoke double their risk for CD. Also, the disease is more virulent and resistant to therapy in people who smoke. However, smoking lowers the risk for UC. Tobacco smoke is a complex molecular mix. Not yet identified are the factors in smoke responsible for these observations.

Another substantiated observation is the effect of appendectomy on IBD. Appendectomy, below the age of 20, for the confirmed diagnosis of appendicitis decreases the risk for UC.

Industrialization causes may alter lifestyle and environment that correlate with the increase in IBD incidence, but these are mostly irrelevant. Other proposed risk factors include use of antibiotics, failure to breastfeed, use of oral contraceptives, insufficient vitamin D intake, living in an urban versus a rural environment, and lack of exposure to farm animals to name a few. Evidence supporting causation remains weak for most of these associations.

## CLINICAL FEATURES AND DISEASE ASSOCIATIONS (TABLE 46.1)

**TABLE 46.1** Findings That Help Differentiate Crohn's Disease (CD) From Ulcerative Colitis (UC)

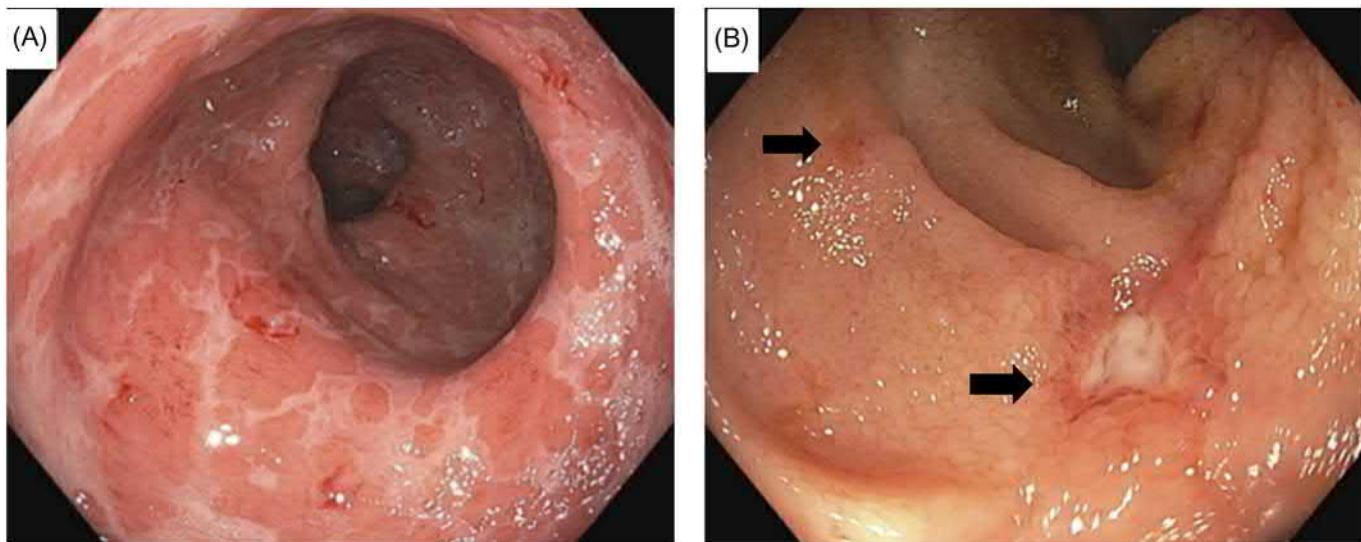
	Crohn's disease	Ulcerative colitis
Symptoms and physical findings	Right lower abdominal pain, crampy periumbilical pain, perianal pain with defecation  Soreness in the right lower abdomen ± palpable mass  Anal fissure or perianal fistula	Tenesmus  Bloody diarrhea
Area and pattern of mucosal involvement	Small bowel involvement  Rectal sparing  "Skip lesions"  Strictures  Fistulas or fissures	Rectal involvement with confluent proximal disease of variable extension
Histologic features	Transmural inflammation  T lymphocyte and macrophage predominant infiltration  Noncaseating granulomas	Inflammation limited to mucosal epithelium  Lymphocytic with variable inclusion of eosinophils and mast cells, and neutrophils near the crypts
Common extraintestinal manifestations	Aphthous ulcers of the mouth	Primary sclerosing cholangitis  Pyoderma gangrenosum

### Crohn's Disease

CD can involve any part of the gastrointestinal tract from mouth to anus. Among all patients, about 50% have disease in the small bowel and colon, 30% have isolated small bowel disease, and 20% have disease just in the colon. The terminal ileum is the most commonly affected segment of bowel. A few patients have involvement of the more proximal small bowel and/or stomach. Rare sites of disease include the mouth, esophagus, and skin in the perineal area. When CD involves the colon, it usually spares the rectum.

The areas of inflamed mucosa can be confluent or discontinuous ("skip lesions"). In a subset of patients, the inflammation stimulates extensive fibrosis that can lead to intestinal strictures and bowel obstruction. Early mucosal lesions of CD are 1–2 mm superficial ulcers with surrounding erythema (aphthous ulcers) that can expand into larger and deeper ulcers (Fig. 46.1). These lesions, in turn, can grow into longitudinally oriented, "linear ulcers."

About 30% of patients develop fistulas (Schwartz et al., 2002). This is the consequence of the transmural nature of the inflammation, which erodes deeply into the intestinal wall. The most common site for fistulas is the



**FIGURE 46.1** (A) Moderately severe ulcerative colitis in the rectum showing diffuse inflammation, edema, and superficial ulceration. (B) Early CD in the terminal ileum showing a 1 cm ulcer and a 1 mm “aphthous” ulcer with surrounding inflammation (arrows). CD, Crohn’s disease.

perianal region followed by the terminal ileum. These abnormal channels extend from the intestines to either the skin or some internal structure conveying stool and pus. Perianal fistulas usually extend to the skin surrounding the anus. Those entering the bladder or vagina are the most difficult to treat. Ileal fistulas have the propensity to cause regional intrabdominal abscesses, enter other nearby segments of bowel creating enteral shunts or extend to the abdominal wall.

Patients often develop anal fissures. These are longitudinal tears in the mucosa of the anal canal. Patients with fissures experience pain with defecation and see fresh blood on the stool or toilet paper. Chronic fissures and fistulas can be the first manifestation of CD.

The clinical presentation depends on the location of mucosal inflammation and the presence or absence of strictures or fistulae. CD most commonly leads to inflammation in the terminal ileum and colon. CD typically presents as diarrhea with or without blood, crampy lower abdominal pain, abdominal soreness particularly in the right lower abdomen and fatigue. The presence of an anal fissure or fistula can result in rectal pain and perhaps fever secondary to perianal abscess. Common associated laboratory findings are iron and vitamin B12 deficiency with associated anemia. Partial small bowel obstruction presents as intermittent, crescendo-decrescendo perumbilical pain often associated with eating high-fiber foods. CD can produce additional symptoms if it involves the mouth, esophagus, stomach, and proximal small bowel.

### Ulcerative Colitis

The chronic inflammation in UC is isolated to the colon with occasional involvement of the distal terminal ileum (“backwash ileitis”). Unlike CD, UC is characterized by confluent inflammation with no skip lesions. It involves the rectum and more proximal colonic mucosa to a variable extent. The disease can present as inflammation localized to the rectum (“proctitis”) or involve the entire colon (“pan-colitis”). About 10% of patients with left-sided colonic disease also have an additional “patch” of inflammation around the appendix, which is on the right side of the colon. Unlike CD, the inflammation in active UC does not extend beyond the inner mucosal and submucosal lining and has a more variable cellular content ranging from lymphocytic to mixed eosinophilic and neutrophilic infiltration. Mild disease may present just as thickening of the mucosa with edema. More serious disease displays superficial ulcerations, areas covered with purulent exudate and a friable mucosa that tends to bleed upon scope contact (“friability”) (Fig. 46.1). The worst disease could present with nearly total loss of the epithelial lining.

Patients typically present with diarrhea (bloody or nonbloody), fecal urgency, nighttime symptoms, tenesmus (repeating urge to defecate with no result), and crampy lower abdominal pain. Fistulas do not develop, and colonic strictures are rare.

In severe pan-colitis, the colon can dilate presenting as “toxic megacolon” and threaten perforation. Associated findings may include tachycardia, diffuse abdominal tenderness, reduced frequency of defecation due to loss of colonic motility, fever, and leukocytosis. This is a dangerous condition requiring immediate intense medical therapy or colectomy if patients do not respond quickly to medical treatment (Autenrieth and Baumgart, 2012).

## Extraintestinal Manifestations Common to Ulcerative Colitis and Crohn's Disease (Brown and Coviello, 2015; Vavricka et al., 2015)

IBD is associated with several extraintestinal manifestations (EIM), one or more of which develop in 50% of patients. About 25% of the patients have EIM before developing the clinical symptoms of IBD. EIM commonly involve the skin, joints, eyes, mouth, and, less frequently, the hepatobiliary tree.

### **Skin**

IBD is associated with several skin diseases. Erythema nodosum (EN) presents as tender, raised erythematous nodules on extensor surfaces and develops in up to 15% of patients. EN appears in many immunologic diseases and some chronic infections (i.e., sarcoidosis, tuberculosis) and is not specific to IBD. Pyoderma gangrenosum (PG) is less common than EN and manifests as quickly developing, nontender purulent necrotic ulcers mostly on the lower extremities but can develop elsewhere. Unlike EN, 50% of patients with PG have IBD. Sweet's syndrome is a rare dermatologic condition characterized by diffusely distributed tender nodules. Sweet's syndrome can develop on its own or associates with underlying malignancy or infection. IBD is one of the more common associated conditions.

### **Joints**

Joint pain is the most common EIM manifestation in IBD, which occurs in 20%–30% of the patients (Bernstein et al., 2005). There are several forms of joint involvement. Patients can develop nondestructive arthropathy of the extremities characterized by morning stiffness and the lack of swelling and erythema in the involved joints. Some patients present with asymmetrical, large joint involvement. Others have bilateral involvement of the proximal small joints of the hands and perhaps feet. Still others get sacroileitis. Patients who are HLA B27 positive are prone to ankylosing spondylitis, and 50% of the patients with ankylosing spondylitis have IBD that can be quite mild.

### **Eyes**

IBD is associated with several ophthalmologic conditions, notably uveitis, episcleritis, and scleritis. Uveitis is most common. It may present with sudden onset of blurry vision, photophobia, and localized eye pain. Most patients have minimal symptoms that are easy to overlook. Patients should have routine eye examinations by an ophthalmologist to avoid glaucoma and the other complications associated with this condition.

### **Mouth**

Aphthous ulcers are lesions that periodically develop in the mouth. These painful ulcers appear most commonly on the side of the tongue or buccal mucosal along the bite zone. They are a common manifestation of IBD, especially CD, which frequently flare during IBD exacerbation. Most people who get aphthous ulcers have no known associated disease. They need to be distinguished from CD of the mouth. Mouth ulcers also can be a sign of other conditions such as lupus and syphilis.

### **Hepatobiliary**

IBD is strongly associated with primary sclerosing cholangitis (PSC), a disease causing chronic fibrosis and biliary duct strictures (Lazaridis and LaRusso, 2016). Damage to the ducts causes cholestasis that gradually progresses to cirrhosis. Ninety percent of patients with PSC have IBD, 80% of which will be UC. Colectomy does not stop disease progression. Unlike regular UC, many patients with PSC have inflammation localized mostly to the right side of the colon. Some have an open, atrophic ileocecal valve with a fish mouth appearance. These patients are at particularly high risk for developing colorectal cancer and require annual surveillance colonoscopy. They also are highly prone to cholangiocarcinoma and gallbladder cancer.

Bile salt malabsorption in CD predisposed to the formation of gallbladder stones, which can cause biliary cholangitis or cholecystitis. Bile salt malabsorption also can induce chronic diarrhea independent of IBD activity.

### Kidney Disease

Frequent dehydration and aberrant absorption of oxalate predispose to calcium oxalate and other types of kidney stones. Aminosalicylates (5-ASA) medications, commonly used in UC, can cause interstitial nephritis.

### Other Diseases With a Link to Inflammatory Bowel Disease

Other immune-mediated diseases have some association with IBD. Patients with IBD are more likely to have psoriasis, and patients with psoriasis are more apt to develop IBD (Takeshita et al., 2017). There also are links to multiple sclerosis and asthma. More frequent in IBD is alopecia areata, an autoimmune disease in which lymphocytes attack the hair follicles causing hair loss.

Nonimmunologic autoimmune disease associations include hidradenitis suppurativa and osteoporosis. The latter association is at least in part due to corticosteroid use.

### Cancer

Patients with IBD have higher cancer risks. Patients with UC and CD are at increased risk for colon cancer depending on the extent, severity, and duration of colitis (Velayos et al., 2017). Screening colonoscopy commences after about 8 years of disease and continues at frequent intervals (1–3 years) for patients with disease involving more than a third of the colon. Patients with just proctitis or CD with limited colon involvement are at low risk for the colon cancer compared to other IBD patients and require colonoscopy perhaps less frequently. CD also predisposes to small bowel cancer for which there currently is no screening test.

Patients with PSC are extremely prone to colon and biliary cancers. They receive screening colonoscopy annually commencing at the time of PSC diagnosis.

The aim of screening colonoscopy is to detect dysplastic lesions that predispose to colon cancer. Using high-definition colonoscopy, chromoendoscopy increases the dysplasia detection rate in some circumstances. Patients with dysplasia may require colectomy or local endoscopic resection (Velayos et al., 2017).

Medications used to treat IBD also can predispose to cancer. For instance, azathioprine increases the risk for lymphoma, some skin cancers, and a select group of other malignancies.

## PATHOLOGICAL FEATURES

Endoscopic biopsies are helpful in the diagnosis of IBD and the differentiation of UC from CD through the recognition of microscopic changes suggestive of UC, CD, or both (Magro et al., 2013). They help differentiate IBD from other conditions associated with intestinal inflammation such as lymphocytic colitis, collagenous colitis, ischemic colitis, radiation colitis, some forms of infectious enteritis (Nostrant et al., 1987), celiac disease, and graft-versus-host disease.

At times, the differentiation of CD from UC is problematic when CD only involves the colon (Feehans, 2014). In active colitis, both CD and UC display an inflammatory cell infiltration of the mucosa and submucosa with associated epithelial crypt abscesses and crypt distortion suggestive of chronic epithelial injury. Crypt abscesses are aggregates of neutrophils that invade degenerating crypt epithelium. There can be Paneth cell metaplasia distal to the ascending colon, the depletion of goblet cell mucin and ulceration. Chronic, focally severe mucosa injury due to inflammation and ulceration can regenerate producing “pseudopolyps,” which are finger-like outgrowths of the mucosal lining. These are benign lesions composed mostly of normal or regenerative epithelial lining.

The most histologically specific feature of CD is the presence of noncaseating granulomas anywhere in the gut (Mathew and Weinstock, 1994). However, these are identified in only about 30%–50% of patients with CD. The granulomas in CD can take on one of three distinct forms. The presence of granulomas is not pathognomonic for CD since some bacterial, chlamydial, fungal, and helminthic infections also induce intestinal granulomas, and they can be found in sarcoidosis, granulomatosis with polyangiitis (formerly called Wegener's granulomatosis), and idiopathic granulomatous gastritis. The distinct morphology of CD granulomas helps distinguish them from granulomas of other causes.

CD may have other distinguishing features. A primarily T-cell and macrophage infiltration is somewhat more supportive of CD. In addition, CD can display transmural inflammation, but this feature is more fully

appreciated in surgical resections than in superficial endoscopic biopsies. Also, supporting the diagnosis of CD is the microscopic evidence of interspersed areas of normal mucosa next to diseased tissue (skip lesions).

Typically, UC displays a neutrophilic infiltrate mostly near the epithelial crypts with a chronic inflammatory infiltrate in the lamina propria—containing T lymphocytes and plasma cells, and variable numbers of eosinophils and mast cells. The inflammatory infiltrate does not extend deeper into the intestinal wall unless UC is severe. Skip lesions are not the histologic features of UC. In UC, areas of normal mucosa often mark the proximal extent of the diseased mucosa. Granulomas are infrequent and small in UC, forming only at the boundary of a degenerating epithelial crypt.

The absence of granulomas, skip lesions, or transmural inflammation does not exclude CD, and clinicians must rely on the entire clinical scenario when diagnosing UC or CD ([Table 46.1](#)). It is not possible to distinguish UC from CD in about 10% of the patients with inflammation suggestive of IBD limited to the colon. This is called “indeterminate” colitis.

## ETIOLOGY INCLUDING AUTOIMMUNE FEATURES

The intestinal lumen contains a huge number of microorganisms, mostly concentrated in the colon and distant small bowel, food antigens, and various mitogenic and toxic substances. These factors constantly interact with the intestinal lining. The intestinal surface is covered with epithelial cells under which lay many inflammatory cells of various types. This normal inflammatory layer in the intestinal lining is evident on microscopic examination of histological sections of the gut wall and is called the “physiological inflammation.” These immunocytes function to corral our intestinal microbiota and gate our immune responses to luminal substances to prevent inappropriate immune reactions to food antigens and other molecules. Yet, the gut immune system is able to respond to maladjusted, invading intraluminal pathogenic organisms and clear them from the body. Studies using animals and some human investigation over the last few years provided remarkable insight into the complex processes of immune regulatory, epithelial biology, and mucosal barrier function that leads to mucosal homeostasis.

It seems increasing likely that IBD is a series of chronic inflammatory disorders with somewhat similar pathological expression. This makes identification of a precise etiology for these diseases most difficult. IBDs presently are not considered autoimmune disorders induced by an autoantibody or cellular immunological response directed to self-antigens. Instead, they are considered inappropriate immune reactions to intraluminal organisms and other substances. This hyperreactive immune response indirectly damages the intestinal lining. A predisposing factor is considered intestinal dysbiosis, which is an alteration in the composition and functional state of our “normal” intraluminal organisms resulting from “modern day” living. Two proposed factors that may contribute to dysbiosis are the use of antibiotics and changes in diet ([Chan et al., 2015](#)). Probably other not yet well defined environmental factors also promote these diseases. Also, some patients carry gene variants that affect susceptibility for IBD. The topics of environment and genetics are discussed elsewhere in this chapter. There are inconclusive and controversial data suggesting that some forms of IBD are caused by “infectious agents.”

The current hypotheses of disease pathogenesis do not adequately explain some of the clinical observations related to these diseases. For unexplained reasons, these diseases usually manifest themselves during the second and third decades of life. The triggers that induce disease are mostly not defined. IBD can damage some regions of the gut, while leaving others alone, often with a clean demarcation between healthy and diseased tissue. Also, biopsies taken throughout the intestines, which clearly disrupt barrier function, do not spread the disease to these physician-induced areas of mucosal injury. IBD does not spread to spouses or healthcare workers in close contact with patients, which suggests IBD is not caused by a classical pathogen–host interaction. Immune suppression does not cause the disease to spread within the body. Patients can spontaneously go into complete remission without apparent explanation.

### Microbiota

There is growing recognition of the importance of the gut microbiome in maintenance of health ([Bellaguarda and Chang, 2015](#)). The human microbiome is quite heterogeneous in composition comprising 500 to several thousand species of bacteria. There also are fungi ([Richard et al., 2015](#)), viruses, and worm-like parasites (helminths); the latter are present much more often in less-developed countries ([Weinstock, 1996](#)). The composition and density of the gut flora/fauna varies in different regions of the intestine as well as to its proximity to the intestinal lining.

The diversity and composition of the intestinal flora form during early childhood and becomes more stable later in life. About 90% of our intestinal bacteria fall into one of two bacterial phyla: Firmicutes and Bacteroidetes.

Witnessed in daily medical practice is evidence of the importance of flora in the disease process. For instance, fecal diversion via construction of an ileostomy can promote healing of CD in distal regions of the bowel, and disease usually relapses with take-down of the ileostomy and reestablishment of the fecal stream.

Loss of diversity has been thoroughly documented in patients with IBD (Putignani et al., 2016). Studies of luminal bacterial composition in patients with IBD revealed a decrease in “beneficial” bacteria such as the bifidobacteria *Lactobacilli* and *Firmicutes* and an increase in putative pathogenic bacteria such as *Bacteroides* and *Escherichia coli*. Yet, human investigation still has not definitively shown if dysbiosis is an important cause or the result of having IBD.

Animal models of colitis support the premise. There are species of bacteria (Lathrop et al., 2011) and helminths (Weinstock and Elliott, 2014) that inhibit murine colitis through suppression of the adaptive immune response that drives the disease. Some of these organisms induce within the gut regulatory T cells, macrophages, and/or dendritic cells, and they promote the production of regulatory cytokines such as IL10 and TGF $\beta$ . Regulatory immune cells and factors inhibit the pathogenic T-cell responses that drive the inflammation. There are other organisms shown to promote, rather than inhibit colitis.

## Intestinal Epithelium and Barrier Function

The intestinal epithelium is the interface between the contents of the intestines and the body. Under normal circumstances, the epithelial lining of the gut turns over every 4–5 days. Intestinal stem cells at the base of the crypts drive this regeneration. The epithelial lining is comprised of many different, highly specialized cells that vary in function. Some display specialized activities depending on the region of intestine in which they reside. There are cells that participate in digestion, absorption, secretion, and host defense. Some produce hormones and other regulatory molecules.

It is proposed that defects in epithelial cell barrier function are risk factors for the development of IBD (Okamoto and Watanabe, 2016). As demonstrated in animal models, the disruption of the tight junctions that hold these cells together can lead to chronic intestinal inflammation (Rudolph et al., 1995; Hornquist et al., 1997). The surface epithelium is covered with a layer of mucus. The disruption of the cells that make this mucus (goblet cells) can result in murine colitis (Van der Sluis et al., 2006). Paneth cells located in the base of the lining secrete defensins and other molecules that help keep bacterial away from the lining. Genetic variants that affect Paneth cell function are mild risk factors for the development of IBD in humans (Welkamp et al., 2005; Kernbauer and Cadwell, 2014). Mice with extensive disruption of Paneth cell function spontaneously develop colitis (Adolph et al., 2013).

## Innate and Adoptive Immunity

Under normal circumstances, it is likely that innate, non-T-cell immune responses are the major guardians of mucosal homeostasis. Mice lacking both functional T and B cells survive without difficulty harboring a normal intestinal flora. Excessive adoptive immunity drives pathology.

The conductor of the mucosal immune response is the dendritic cell. Intestinal dendritic cells sample luminal contents. They may do so via allowing luminal molecules to engage their receptors of innate immunity (e.g., tolls, C-type lectins (CLECs), nucleotide-binding oligomerization domain containing 2 (NOD2))located on the cell surface or present in intracellular locations. The dendritic cells present antigen to nearby T cells and instruct the T cells to activate or go into the inactive state of anergy (Kalekar and Mueller, 2017). It is of intense interest to determine the factors that instruct intestinal dendritic cells to favor an innate, rather than an adoptive immune response.

Dendritic cells produce, or direct other immune cells to produce, regulatory cytokines such as TGF $\beta$  and IL10. They also promote the generation of regulatory T cells that dampen T-cell responses. Particularly in the intestines, TGF $\beta$  is an exceptionally important cytokine for driving T cells to differentiate into their regulatory phenotypes (Sekiya et al., 2016). In transgenic mice, T cells bio-engineered to express TGF $\beta$  receptors that cannot signal upon TGF $\beta$  ligation develop colitis (Ince et al., 2009; Gorelik and Flavell, 2000). This shows the importance of the interaction of TGF $\beta$  with T cells in preventing disease. Regulatory T cells in the gut are an important source of IL10 (Hang et al., 2013; Uhlig et al., 2006; Singh et al., 2001; Rubtsov et al., 2008), which, among other functions, inhibits the production T helper 1 (Th1) cells, IFN $\gamma$ , and perhaps IL17, all of which drive inflammation.

Dendritic cells and other cell types also can be sources of IL12 and IL23 that promote development of proinflammatory Th1 and T helper 17 (Th17) cells, respectively. Also, some of the important intracellular signaling

pathways used to promote gut inflammation include spleen tyrosine kinase (SYK) (Hang et al., 2016), Janus kinase (JAK), and nuclear factor of kappa light polypeptide gene enhancer in B-cells (NF $\kappa$ B).

The inflammation of CD is notable for the infiltration of gut tissue with abundant numbers of inflammatory T lymphocytes. These cells are drawn into the gut through the local production of chemokines and the ability of some T cells, via expression of integrins, to recognize gut-specific adhesion molecules (addressin) present on the gut vascular endothelium. One such T-cell-specific integrin called  $\alpha$ 4/ $\beta$ 7 binds the gut-associated, vascular addressin called MadCAM1.

It is believed that some of the key cytokines driving the inflammatory response in CD are IL12, IL23, IL17, and IFN $\gamma$ . IL12 and IL23 induce the T cells that produce IFN $\gamma$  and IL17, respectively. These factors, in turn, drive cells to produce tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), IL6, IL1 and other cytokines that amplify the inflammatory response.

These observations have pharmaceutical relevance in IBD. Agents that block IL12/IL13, TNF $\alpha$ , the JAK1 signaling pathway, or that promote TGF $\beta$  production (Monteleone et al., 2015) display clinical utility in subsets of patients with CD. Also of clinical importance are molecules that block gut-specific, integrins-like  $\alpha$ 4/ $\beta$ 7 which impede entrance of T cells into the gut. Under development are additional therapies that target other presumed critical inflammatory pathways relevant to IBD.

The inflammatory response in UC appears more complex and variable than that of CD. In addition to lymphocytes, there can be large numbers of neutrophils and eosinophils. The cytokine pattern of UC may contain additional cytokines such as IL13, IL5, and perhaps IL4. These are cytokines associated with Th2 cells and allergic responses. Yet, subsets of patients with UC respond equally well to most of the same medications displaying efficacy in patients with CD.

## GENETICS

Environmental factors influence the risk for IBD (Loftus and Sandborn, 2002). Cigarette smoking increases the likelihood of developing CD (Calkins, 1989). Appendectomy for appendicitis decreases the incidence of UC (Andersson et al., 2001; Derby and Jick, 1998; Russel et al., 1997). Some enteric infections due to exposure to organisms such as the ameba *Entameba histolytica* or cytomegalovirus can induce IBD. Studies have observed geographic variations in IBD frequency. IBD was more common in the northern versus southern regions of the United States and Europe in the latter half of the 20th century (Sonnenberg et al., 1991; Shivananda et al., 1996; Kappelman et al., 2007) and is more frequent in Western versus Eastern Europe. Greater risk for disease is departed to children born to people who relocate from regions of low CD or UC frequency to areas of high IBD prevalence (Carr and Mayberry, 1999; Jayanthi et al., 1992; Probert et al., 1993). IBD prevalence has increased in North America and Western Europe and is approaching about 1 in 250 people in some prosperous countries (Bernstein et al., 1999; Loftus et al., 2002). IBD is a growing problem in many Asian nations. People with blue-collar jobs exposing them to dirt (Sonnenberg, 1990) and US military personnel who served in tropical regions (Delco and Sonnenberg, 1998) are less likely to acquire IBD. Thus, environmental factors may play an equal or greater role in the development of IBD than genetic variation.

While 5%–15% of people with IBD have a first-degree relative afflicted with the disease (Binder, 1998), such associations could still result from shared environmental risk factors. Ashkenazi Jews are at high risk for development of IBD compared to non-Jew European ancestry cohorts. Genetic studies in this population have yet to discover prevalent, distinct disease susceptibility genes (Vacic et al., 2014).

Monozygotic twin studies were the first to provide convincing evidence that IBD had a genetic component (Gordon et al., 2015). This led to genome-wide association studies that have discovered about 200 genetic loci/regions that affect the risk for development of IBD. It now is clear that no one gene is “the cause” of IBD. Some gene variations affect the risk for UC or CD or for both conditions (McGovern et al., 2015). Many of the gene variants fall into one of three categories: regulation of (1) inflammation, (2) autophagy, and (3) microbial sensing to activate autophagy (Uniken Venema et al., 2017). Knowledge of these variants also has provided some insight into processes that may drive IBD. While these genes do depart risk, the vast majority of people with one or more of these risk factors will never develop IBD, and most patients with the condition fail to display known susceptibility genes. IBD develops in all racial and ethnic groups. Many susceptibility genes uniformly depart risk among different populations, although they are expressed at variable frequencies within different groups. Some are unique for a particular population. This latter observation may reflect the complex multigenetic nature of IBD susceptibility or perhaps worldwide differences in disease pathogenesis.

The first identified genetic variants associated with IBD, present mostly in Western societies, were loss-of-function mutations in the NOD2 gene. It predisposes to the development of CD particularly of the terminal ileum. The prevalence of the NOD2 defect ranges from 0% to 28% in CD and 0% to 14% in healthy control populations depending on the population studied. While NOD2 is perhaps the most clinically significant disease susceptibility gene, much less than 5% of people who carry one of the NOD2 variants will ever develop IBD. Carriers of two NOD2 variants are at even greater risk.

NOD2 is an intracellular receptor for muramyl dipeptide derived from bacterial lipopolysaccharides and has a role in driving the innate immune response to such organisms. Cells expressing the NOD2 variant fail to respond to NOD2 ligation with NF $\kappa$ B activation (Li et al., 2004), an important intracellular signaling pathway that drives proinflammatory responses. Within the gut, it is speculated that NOD2 helps protect us through promoting the production of  $\alpha$ -defensins that help destroy some types of microorganisms if they approach the intestinal lining (Wehkamp et al., 2005; Simms et al., 2008). Ligation of NOD2 also downmodulates Toll-like receptors expression in dendritic cells (Watanabe et al., 2006), which remain responsive in the NOD2-deficient state. It is speculated that loss of both of these functions promotes development of CD.

Also, variants of the autophagy-related 16-like 1 (ATG16L1) gene are expressed somewhat more frequently in people of European ancestry with CD compared to the healthy general population (Hampe et al., 2007; Rioux et al., 2007). Autophagy is the natural, regulated cellular mechanism through which cells allow the orderly degradation and recycling of unnecessary or dysfunctional intracellular components including bacteria. ATG16L1 encodes a protein important for the process of autophagy. The T300A variant of ATG16L1 is highly susceptible to cleavage by caspase-3 and -7, which are activated during cellular stress (Murthy et al., 2014). Reduction in the amount of this protein can lead to defective microbial clearance and increased cytokine production (Lassen et al., 2014).

Other gene variants afford protection from IBD. There are gene variants in the locus-containing IL23R that are associated with increased CD and UC susceptibility. However, people with the loss-of-function Arg381Gln variant of the IL23R gene are much less likely to get IBD (Duerr et al., 2006; Zwiers et al., 2012; Pidasheva et al., 2011). The gene is present in about 14% of people of European ancestry and is more protective for CD than UC. Among its functions, IL23, via engagement of IL23R, drives the development of T cells that express IL17 (Gaffen et al., 2014). Interleukin 17 is a proinflammatory cytokine (Burkett et al., 2015). It recruits monocytes and neutrophils to sites of inflammation in response to invasion by pathogens and stimulates the production of many other cytokines. Already in use or under clinical investigation are the inhibitors of IL23 that appears efficacious for some patients with CD (Deepak and Loftus, 2016).

Gene testing presently is of limited value in patients with IBD. IBD is a complex disorder in which gene mutations contribute to various extents to the disease. For instance, having the NOD2 defect at least doubles the risk for developing CD. Other variant genes associated with IBD depart relatively little risk. Most healthy people carrying these same genes will never develop IBD. Thus, genetic testing will not be helpful in predicting disease expression or the diagnosis of disease because of the lack of sensitivity and specificity. It currently is unknown if detection of gene variants will help predict disease prognosis, risk for EIM of disease, or pharmaceutical responsiveness (Gabryel et al., 2016).

## ANIMAL MODELS

It is likely that IBD is a heterogeneous group of conditions caused by expression of various different host and environmental factors what cumulate in chronic intestinal inflammation often with similar phenotypic appearance. Thus, it presently is not possible to develop a single, precise animal model of the human disease when the actual pathophysiology of the human condition remains murky and multifactorial.

However, the animal models of gut inflammation have grown in sophistication revealing various important intestinal mechanisms governing injury and repair, mucosal immune regulation, gut flora/mucosal interactions, epithelial cell biology, and inflammatory-induced carcinogenesis (Westbrook et al., 2016; Valatas et al., 2015) all of which are processes highly relevant to the human disease. Also, some of the animal models have demonstrated utility in early pharmaceutical testing of promising new therapeutic agents. For instance, they predicted the therapeutic safety and efficacy of molecules that block IL12, IL23, homing of T cells to the gut, SMAD7, and the JAK signaling pathway, which are now in use to treat IBD or are in the advanced stages of testing.

There are more than 75 distinct mouse models of intestinal inflammation (Mizoguchi et al., 2016). Due to the inconsistency of disease expression, relevance of the model and/or cost of murine colony maintenance, only a

relatively few of these models are used repeatedly and in many laboratories to study the mechanisms of mucosal homeostasis. Even the best models have their weaknesses. Thus, some models may prove superior to others for addressing particular questions pertaining to enteric biology and IBD.

Some induce short-term inflammation via injury to the intestinal lining, such as 2,4,6-trinitrobenzenesulfonic acid, which is given rectally, or dextran sodium sulfate (DSS) administered orally. The models dependent on DSS-induced injury have proven particularly important for the identification of mechanisms of mucosal injury and repair.

The genetic manipulation of mice allowing over- or underexpression of various molecules has created a large number of inflammatory models. They have revealed an array of complex interactions needed to maintain immune homeostasis in the gut mucosa, which abuts against many potentially harmful food antigens and a multitude of diverse and potentially invasive microorganisms.

Various genetic alterations in humans can increase the risk for developing IBD. Genetic manipulations in mice have provided insight into the function and importance of some of these disease susceptibility genes (Mizoguchi et al., 2016). For instance, some loss of function polymorphisms in NOD2 increases susceptibility to CD (Strober et al., 2014). Mouse models displaying NOD2 defects showed its role in sensing molecular components of some bacterial species and triggering innate immune responses. Defects in a process called autophagy also predisposes to CD disease. Study of autophagy in animal models revealed how it works to degrade invading intracellular microorganisms (Kernbauer and Cadwell, 2014; Baxt and Xavier, 2015).

Several models of IBD depend on the transfer of T-cell subsets or genetically manipulated cells into immune-deficient murine hosts. Most notable among these models have been the reconstitution of T and B cell-deficient mice (e.g., Rag<sup>-/-</sup>) with CD45RBhi or CD25+ T cells or T cells that cannot make IL10 (Blum et al., 2004; Keubler et al., 2015; Powrie et al., 1994; Izcue et al., 2009). The colitis that develops in these mice led to the discovery of the importance of TGFβ, IL10, and regulatory T cells in the control of intestinal inflammation.

Various models of IBD proved useful for studying the role of the host gut microbiota (Khanna et al., 2014) in the disease process. The microbiota comprises the various organisms that live within our intestines, such as bacteria, viruses, and fungi, and also includes the helminthic parasites (Weinstock and Elliott, 2014). Many of the IBD models fail to develop colitis in the absence of the intestinal microbiota, revealing the importance of the gut microflora in the disease process. In several animal models, the presence of some microbial species, such as *Helicobacter* or *Citrobacter*, helps to trigger disease, whereas other microbial species afford protection. However, in mice and humans with the NOD2 defect, *Bacteroides* species promote intestinal inflammation, whereas *Clostridiales* are protective (Ramanan et al., 2016). These studies have provided insight into the importance of the composition and functional state of the various components of the gut microbiome in maintaining mucosal integrity.

## DIAGNOSTIC PROCEDURES

IBD is a syndrome, not a specific disease. The diagnosis involves recognition of symptoms and other clinical observations associated with supportive endoscopic, radiologic, and pathologic findings in the absence of enteric infection. No one clinical observation or test is pathognomonic for IBD. Patients may have an acute colitis from an undiagnosed enteric infection that spontaneously resolves over time. Thus, another important element in diagnosis is documenting disease chronicity or recurrence in the absence of potential inciting factors such as infection, IBD-inducing drugs, vasculitis, or ischemia. Gaining favor is the use of several stool and serologic biomarkers to help predict ongoing intestinal inflammation, which could help decrease the repeated use of invasion and expensive testing in patients with dubious symptoms. They also may prove useful for predicting relapse in patients with IBD. However, all the presently used biomarkers are imperfect tools and should be used with care. In the absence of biomarkers that are strongly predictive for disease activity, clinicians often rely on endoscopy to monitor these patients (Chang et al., 2015). There are no available biomarkers with adequate sensitivity or specificity to directly diagnose IBD, rule out disease expression or that can distinguish hard to differentiate CD from UC.

### Serologic Markers

C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are acute phase reactants that often, but not always, elevate in active IBD. Neither is specific for intestinal inflammation (Chang et al., 2015; Panes et al., 2017). Both CRP and ESR increase in response to many inflammatory and infectious conditions and have limited

clinical utility in most patients with IBD. CRP frequently increases in patients with CD having transmural inflammation. The CRP 717 gene variant carried in 25% of people renders CRP an unreliable marker in many patients (Chang et al., 2015; Jones et al., 2008). Furthermore, there is no demonstrated association between clinical disease activity and elevated CRP in CD (Crohn et al., 1932). In UC, CRP elevation is associated with increased symptomatology and extent of inflammation, but not with severity of inflammation (Solem et al., 2005).

There is great interest in developing a serologic test that can accurately diagnose and/or exclude IBD. In principle, such markers would help decrease reliance on invasive endoscopic procedures in patients with a low pre-test probability of IBD (Iliev et al., 2012). Several marker antibodies have been identified in patients with IBD: perinuclear antineutrophil cytoplasmic antibody, anti-*Saccharomyces cerevisiae* antibody, anti-*E. coli* outer membrane porin (OmpC), anti-*Pseudomonas fluorescens*-associated sequence (I2) and antiflagellin (CBir1). As a panel, these serologic markers are not highly specific for IBD and are not used as routine screening tests in health people or those with nonspecific symptoms (Benor et al., 2010; Zhou et al., 2016). They do tend to predict UC or CD in patients whom the diagnosis of UC and CD already is quite evident. Specific antibodies are associated with particular IBD phenotypes, but they are not particularly useful in guiding management decisions or in differentiating between CD and UC in cases of indeterminate IBD (Zhou et al., 2016). Thus, they have little use in clinical practice.

## Stool Markers

Fecal biomarkers, as opposed to serologic markers, are more specific for luminal inflammation (Wright, 2016). Many stool markers have been explored for use in the diagnosis and surveillance of disease activity in IBD, though none are clinically validated for replacement of endoscopy with biopsy. The best studied and most commercially available are fecal calprotectin and fecal lactoferrin (Wright, 2016; Sipponen and Kolho, 2015; Mendall et al., 2016). Fecal calprotectin and lactoferrin concentrations often increase in the stool of patients with active IBD. They have been used to distinguish IBD from irritable bowel syndrome, which can have similar presentations and symptom overlap. Fecal calprotectin has value as a surrogate for severity of endoscopic- and histologic-documented, disease activity in both UC and CD. There is insufficient data to support the use of either fecal lactoferrin or calprotectin as a surrogate test to document mucosal healing, and neither biomarker distinguishes CD from UC.

## Imaging

Several nonendoscopic imaging modalities help in the diagnosis IBD and can define disease extent, activity, and associated complications such as stricture, abscess, and fistula (Panes et al., 2017; Fletcher et al., 2011). Before the availability of cross-sectional imaging via computed tomography (CT) and magnetic resonance (MR) imaging, small-bowel follow-through (SBFT) X-ray series was used to evaluate the small intestine for the thickening of the bowel wall, strictures, and fistulas. Barium enema X-ray and colonoscopy were used for the colon. SBFT and barium enema are time-consuming studies that require patients to swallow contrast or receive it rectally. Then, the radiologist uses fluoroscopy to visualize the bowel and takes still X-rays to document the findings. CT (CTE) and MR enterography (MRE) have largely replaced SBFT for the luminal assessment of the small intestines since they take less physician time and provide information pertaining to peri-intestinal pathology.

Patients with IBD are prone to receiving many X-ray studies over their lifetime resulting in potentially dangerously high cumulative radiation exposure. Several epidemiological studies suggest that there is an increased risk of all types of cancer with repeated X-ray exposure. Compared to MRE, CTE is a relatively fast imaging modality, but it delivers between 1 and 14 mSv of radiation per study (Guimaraes et al., 2010). MRE does not expose the patient to radiation. Thus, in IBD, it often is the procedure of choice for small bowel examination and for the assessment of pelvic pathology (Sheedy et al., 2017).

Endoscopy (colonoscopy and esophagogastroduodenoscopy) is the best imaging modality to directly assess luminal-side inflammation in the colon, terminal ileum, and upper GI track, and is the only procedure that allows biopsy. It permits visualization of the mucosal surface with reliable characterization of disease activity and remission. It facilitates the diagnosis and treatment of strictures and detection of luminal infections (e.g., infection with mycobacteria or cytomegalovirus and cancer).

Sometimes, video capsule endoscopy (VCE) is used to visualize hard-to-reach regions of the small intestine. The camera, which is the size of a pill, is swallowed providing direct visualization of the entirety of the inner surface of the small bowel. It is necessary to use VCE with extreme caution in CD, since the rigid device can obstruct the intestines at areas of stricture requiring surgery for removal (Al-Bawardi et al., 2015).

## TREATMENT

At the end of the 1980s, there were few useful treatments for patients with IBD. These included glucocorticoids with systemic side effects, aminosalicylates requiring frequent dosing, thiopurines, some antibiotics (e.g., metronidazole), total parenteral, or enteral nutrition to provide bowel rest and surgery. New therapies have emerged due to revelations in disease immune pathogenesis, growing knowledge of the mechanisms driving and controlling mucosal inflammation and the eagerness of the pharmaceutical industry to explore new therapeutic options for these conditions. Most of these medications are useful for management of both UC and CD. These advances come with the sobering recognition that IBD is increasing in frequency in developed countries and is rapidly spreading to less-developed nations around the world. Also, IBD is more virulent than in previous years. Many of these new and expensive therapies can cause significant side effects and, thus, require careful patient monitoring. Also, these agents only work on a limited number of patients whose therapeutic response cannot be predicted in advance of therapy. Not infrequently, responding patients are subject to disease relapse even after many years of successful management. Patient age, disease presentation (including EIM of IBD), concomitant illnesses, family history of various diseases, pregnancy, and more influence drug selection as do the various limitations and peculiarities of each of the medications on hand.

### Medical (Table 46.2)

**TABLE 46.2** Treatments for Inflammatory Bowel Disease

Class	Modes of administration	Disease	Common uses
5-ASA	Oral, rectal	UC, ± CD	Induce remission and maintenance
<b>CORTICOSTEROID</b>			
Systemic	Oral, rectal, IV	UC, CD	Induce remission, bridging therapy
Minimal systemic absorption	Oral, rectal	UC, CD	Induce remission, bridging therapy
Thiopurines	Oral	UC, CD	Steroid sparing, maintenance, block antibody development
Methotrexate	IM	UC, CD	Steroid sparing, maintenance, block antibody development
Cyclosporine	Oral, IV	UC	Urgent rescue therapy, bridging therapy
Anti-TNF	IV or SQ	UC, CD	Induce remission and maintenance
Antiintegrin	IV (oral, SQ in development)	UC greater than CD	Induce remission and maintenance
Anti-IL12/23	SQ	CD	Induce remission and maintenance
Tofacitinib (JAK 1/3 inhibitor)	Oral	UC	Induce remission and maintenance
Other JAK inhibitors (under development)	Oral	Probably UC and CD	Expected use: Induce remission and maintenance
Nutritional support	Oral, IV	CD	Induce remission, bridging therapy
Nutritional support	Oral, IV	CD, UC	Short bowel syndrome (CD), malnutrition, bridge for surgery, serious intraabdominal complications

CD, Crohn's disease; JAK, Janus kinase; UC, ulcerative colitis; 5-ASA, aminosalicylates.

## Aminosalicylates

5-ASA (mesalamine) is the treatment most commonly used for first-line therapy of mild-to-moderate UC ([Hauso et al., 2015](#); [Harris and Lichtenstein, 2011](#)). It has proven efficacious for both induction and maintenance of remission. It is an antiinflammatory medication that works via direct contact with the colonic mucosa. 5-ASA is rapidly absorbed in the small bowel and must be protected to reach the colon. The first 5-ASA derivative was sulfasalazine. This medication was sulfapyridine, an antibiotic, connected to 5-ASA via an azo-bond. Bacteria in the colon enzymatically cleaved the azo-bond releasing the 5-ASA. The drug was not “bioengineered” for the treatment of IBD. Its efficacy was discovered by happenstance. The sulfa moiety tended to cause allergic reactions, nausea, headaches, and other symptoms. Although still available, this medication has fallen mostly out of use due to the development of safer 5-ASA formulations.

Since the benefit of the drug rested mostly with the 5-ASA moiety, developed were oral formulations just containing 5-ASA (or similar medication, e.g., balsalazide) that were targeted for release in various regions of the gut. In some, the agent was enclosed in capsules designed to open near pH 7, which is the typical pH in the distal bowel. Additional targeting strategies led to the development of extended release forms of 5-ASA and formulations that would adhere to the colonic lining promoting longer drug retention. Most of these formulations only require once daily administration to achieve maximal benefit. Although most of the modern-day preparations contain the same 5-ASA (mesalamine), the various forms do not necessarily optimally target the same regions of the bowel. Thus, factors such as disease location and intestinal transit time may influence drug selection.

Also developed were rectally delivered 5-ASA preparations that had the advantage of providing extraordinarily high concentrations of the medication in the distal colon of patients with mostly distal colitis. They also found use in combination with oral 5-ASA to more effectively treat the entire colon. Disadvantages include the difficulty retaining rectally administered 5-ASA for an adequate length of time in the face of active proctitis, and patient emotional resistance to the use of rectal medications.

The use of 5-ASA in CD is controversial. It appears that 5-ASA is minimally effective in CD. Their use in CD is limited mostly to small subsets of patients with minimal disease who may not require or desire medications more adverse side effects. They also may be employed in patient with Crohn's or indeterminate disease limited mostly to the colon.

The drug has many proposed mechanisms of action. For instance, they are stated to inhibit proinflammatory processes such as NF $\kappa$ B activation ([Bantel et al., 2000](#)) and IL1 production ([Mahida et al., 1991](#)). Still others propose that they impede TNF $\alpha$  binding ([Shanahan et al., 1990](#)) and scavenger injurious free radicals ([Ahnfelt-Ronne et al., 1990](#)).

5-ASA has a high safety profile. Toxicity can include interstitial nephritis that develops in perhaps one in 500 patients. This can lead to permanent renal dysfunction. Thus, the chronic use of these medications requires routine monitoring of blood creatinine levels. Other problems may include temporary nausea or hair loss. There are unusual reports of 5-ASA-inducing pulmonary fibrosis, allergic reactions, and paradoxical worsening of disease. They appear safe for use during pregnancy.

## Glucocorticoids

Corticosteroids are commonly employed for the acute management of IBD ([Katz, 2004](#)). However, their use has greatly diminished since the development of new alternative therapies. No other treatment provides such a rapid and major clinical benefit in both patients with UC and CD. They can be life-saving for patients with extreme flares of UC (e.g., toxic megacolon). However, there are many patients with IBD flare who do not respond to corticosteroids or only do so if the medication is given at high dosage. Progressive steroid resistance is common in patients who repeatedly cycle on and off of these medications. Some patients relapse as the dose is diminished and cannot wean off. This is a condition called “corticosteroid dependency.”

Corticosteroids given rectally or orally, depending on the extent and severity of disease, can induce remission in patients with UC. Rectal medications of all types administered in foam formulation are retained the longest. Some patients with relatively mild or moderate UC, often taking maintenance 5-ASA, occasionally flare. Some of these patients can be brought back into prolonged remission with a 1–2 month course of steroid therapy associated with a slow and prolonged steroid taper. Such patients may not need to progress to other medications.

Steroids are less effective at inducing clinical remission in CD, and relapse occurs more readily since this therapy does not usually induce complete healing of intestinal lesions. Because of their speed of action, corticosteroids frequently are used as bridging therapy to allow some other concomitantly administered slower acting

medication to take hold. Corticosteroids are not effective maintenance therapy in either UC or CD. Thus, it is preferable to avoid their long-term use.

A major problem with corticosteroids is that they enter the systemic circulation and cause short and long-term side effects. The chronic use of steroids can induce cataracts and osteoporosis. They can induce aseptic necrosis of bones often requiring joint replacement. Other side effects may include insulin resistance (diabetes), hypertension, muscle weakness (myopathy), increased risk of infection, insomnia, acne, undesirable weight gain, mood changes, increased intraocular pressure (glaucoma), "moon face" and adrenal insufficiency.

Budesonide is an oral glucocorticoid that undergoes extensive first-pass metabolism in the liver (Abdalla and Herfarth, 2016). Perhaps 10% or less of this medication enters the systemic circulation when taken orally. Budesonide has been formulated to reach the terminal ileum and/or colon after oral administration, and it is much less likely to cause systemic side effects than conventional steroids. Budesonide given rectally can induce short-term improvement or clinical remission in UC of the distal colon (Rubin et al., 2015; Sandborn et al., 2015). Also, administered orally, it can induce improvement or remission in UC that extends throughout the colon and in CD. As with other corticosteroids, they quickly lose efficacy with long-term use. Budesonide is less efficacious than other corticosteroids that reach the systemic circulation. It is most commonly used in patients who have a relative contraindication for the use of the more powerful systemic corticosteroids or who cannot tolerate their side effects.

Corticosteroids work via the engagement of a specific cytosolic steroid receptor. The engaged receptors translocate to the nucleus affecting transcription of various genes in a multitude of cell types (Carlstedt-Duke and Gustafsson, 1987). They affect the production of many cytokines, activation of lymphocytes, release of arachidonic acid metabolites, and function of macrophages.

### **Thiopurines**

The thiopurines include azathioprine and 6-mercaptopurine (6-MP). Azathioprine is metabolized in the body to 6-MP, which in turn is broken down to other metabolites.

There is dispute regarding the overall value of thiopurines in the management of UC and CD (Axelrad et al., 2016). Thiopurines are used in UC and CD for the induction and maintenance of clinical remission. Thiopurines are slow-acting agents that may require 2–4 months to take effect. In highly symptomatic patients, their use may require temporary concomitant bridging therapy with corticosteroids-like prednisone or budesonide to suppress symptoms sufficiently to allow time for the thiopurine to work. They also can help close perianal fissures and fistulae.

Also, anti-TNF therapy achieves a higher rate of short- and long-term remission when used in combination with thiopurines. This is due, at least in part, to the ability of thiopurines to inhibit neutralizing antibody responses to anti-TNFs, which are immunogenic molecules. It also slows metabolism of anti-TNFs allowing higher anti-TNF blood level.

Thiopurine therapy has other applications. It is employed to decrease postoperative CD recurrence after bowel resection. They also have "corticosteroid-sparing" properties, allowing reduction or discontinuation of steroids in patients requiring continuous or frequent steroid use. However, recent trials in CD found azathioprine ineffective at achieving sustained corticosteroid-free remission (Cosnes et al., 2013; Panes et al., 2013).

Thiopurines are most effective when used at dosages just below the toxic range. Thus, the use of thiopurines requires close monitoring. Perhaps 20% of patients starting on thiopurines develop, in the first month of use, serious side effects including pancreatitis, myelosuppression, fever, rash, malaise, muscle, and/or joint pain requiring drug discontinuation. Leukopenia, hepatitis, and pancreatitis also can develop latter in therapy. Dose reduction can manage mild hepatitis and leukopenia. Acceptable therapeutic side effects of these medications are moderate lymphopenia and mild anemia with red-cell macrocytosis. Thiopurines predispose patients to infections. This includes cutaneous warts. Thiopurines are carcinogenic agents predisposing patients most notably to basal and squamous cell carcinoma of the skin as well as lymphoma. Azathioprine and 6-MP are similar medications. An adverse drug reaction to one predicts a similar adverse reaction to the other. However, with all these toxicities, present studies have revealed no reason to discontinue thiopurines during pregnancy.

In the body, competing metabolic pathways degrade 6-MP. The enzyme thiopurines methyltransferase (TPMT) converts 6-MP to 6-methylmercaptopurine. Patients with loss- or reduction-of-function genetic variants of TPMT shunt 6-MP into other metabolic products that can induce profound leukopenia. Screening patients for TPMT deficiency can reduce but does not obviate the risk of leukopenia and other toxicities in thiopurine therapy.

The mechanism of action of thiopurines is mediated by its metabolites (Coskun et al., 2016). 6-Thioguanine nucleotides incorporate as false purine analogs into RNA or DNA inducing immunosuppressive and cytotoxic effects. It also is proposed that the metabolite 6-thioguanine triphosphate inhibits T-cell activation and proliferation.

### **Methotrexate**

Methotrexate (MTX) has a place in the management of IBD (Coskun et al., 2016; Herfarth et al., 2016). It is a folate analog that inhibits dihydrofolate reductase, which interferes with DNA synthesis. It has antiinflammatory and proapoptotic activity. MTX is efficacious for induction and maintenance of remission in CD. MTX possibly is beneficial in UC when dosed properly. Due to better drug availability, the benefit of the drug is seen with weekly intramuscular (15–25 mg) but not oral administration. When used in combination with anti-TNF therapy, it suppresses anti-TNF antibody formation. MTX is given with folate supplementation to minimize side effects induced by the effect of the drug on folate metabolism and folate-dependent pathways.

The most common side effect is nausea developing shortly after drug administration. This may disappear with dose reduction or may be manageable with antiemetics. Among others, additional side effects may include diarrhea, hepatitis, and myelotoxicity. MTX is teratogenic making it risky to use in woman in their reproductive years and is contraindicated in lactating women.

### **Cyclosporine**

Cyclosporine is useful as a rescue therapy in patients with life-threatening, corticosteroid-resistant UC. It has significant side effects including nephrotoxicity, hypertension, and seizures, which limits its utility. Anti-TNF medications have better long-term tolerability relegating cyclosporine to second-class status, although they induce clinical remission at comparable rates (Narula et al., 2016). A potential utility is in patients with life-threatening UC with previously proven anti-TNF resistance. In this situation, cyclosporine may prove useful as a therapeutic bridge to some other slower acting alternative therapy. Cyclosporine inhibits calcineurin, which is a cytoplasmic enzyme needed for T-cell activation.

### **Anti-TNF Therapies**

Anti-TNF therapies [e.g., infliximab, CT-P13 (infliximab biosimilar), adalimumab, golimumab, certolizumab] were major advances in the management of IBD. All but certolizumabs are derivatives of IgG1 monoclonal antibodies directed against TNF and are given intravenously (infliximab, CT-P13) or subcutaneously. These bind Fc receptors strongly. Certolizumab is a pegylated anti-TNF(ab)2 fragment lacking the Fc-binding site. Anti-TNFs appear to work through engaging TNF bound to the surface of activated lymphocytes causing them to undergo cell death (apoptosis) (Levin et al., 2016). There also may be some Fc-dependent mechanisms of action as well.

Anti-TNFs can induce and maintain remission in UC and CD. They can close fistulae and may have efficacy in maintaining remission in postoperative patients with CD (Regueiro et al., 2016; Carla-Moreau et al., 2015). Infliximab remains the anti-TNF of choice for patients with steroid-resistant, life-threatening UC because of its perceived speed of action. Anti-TNF therapy was the first treatment to frequently induce total mucosal healing in patients with IBD. Unfortunately, large numbers of patients do not respond to anti-TNFs, and some drug responders eventually develop drug resistance and relapse.

One frequent cause of resistance is the development of antibodies that block the action of anti-TNF and speed drug clearance. Concomitant administration of immunomodulators (e.g., thiopurines or MTX) reduces the incidence of antibody development. Switching to a molecularly dissimilar anti-TNF medication will overcome antibody-induced, anti-TNF resistance. However, it is necessary to switch to other classes of medications that function independent of TNF blockade if a patient is a primary anti-TNF nonresponder or subsequently develops resistance to the medication in the absence of blocking antibodies.

Optimal dosing of anti-TNFs affords the best results (Ding et al., 2016). This requires occasional drug monitoring to check drug levels and to detect the early development of anti-TNF antibodies. However, drug testing is not yet a completely reliable science. Patients with low levels of drug in their blood stream just before the next dose of the medication are more prone to relapse. Another sign of “underdosing” is the development of symptoms prior to the next dose of the medication.

Anti-TNF therapy is not without significant potential side effects. These drugs leave patients more prone to infections. Patients with latent tuberculosis, occult histoplasmosis, or carriers of the hepatitis B virus are at great risk of the activation of their latent infection. Significant abscesses should be drained and treated with antibiotics before initiating anti-TNF therapy. Anti-TNFs can worsen congestive heart failure, exacerbate multiple sclerosis,

and trigger psoriasis. They can induce hepatitis, cause serious allergic reactions, and perhaps predispose to lymphoma. They appear safe to use in pregnancy (Androulakis et al., 2015).

### **Anti-IL12/13 Therapies**

Ustekinumab (Stelara) is a fully human IgG1 monoclonal antibody that binds to the shared p40 subunit of the IL12 and IL23 receptor, blocking IL12/IL23 activity. IL12 and IL23 drive the production of the important proinflammatory cytokines IFN $\gamma$  and IL17, respectively (Catana et al., 2015). One or both of these cytokines likely drive the pathological inflammation in some patients with CD.

In 2016, it was approved for the treatment of moderate-to-severe CD in patients older than 17. For several years, ustekinumab has been used for the treatment of psoriasis at lower doses than that for CD. Eight weeks after initiation of the therapy, two randomized double-blind phase III trials in CD revealed that ustekinumab induced remission in about 15%–20% more patients than that of placebo and proved efficacy. A 44-week extension trial containing patients who responded or went into remission at week-8 showed that about 15% more test subjects were in remission than those receiving placebo. Response rates were somewhat higher, although still only about 15% better than placebo. Patients who failed anti-TNF therapy were less likely to respond. More extensive use of this medication in clinical practice will help determine the actual benefit of ustekinumab in CD (Deepak and Loftus, 2016; Feagan et al., 2016).

Ustekinumab is a reasonable choice for patients refractory or intolerant to TNF antagonists. Its overall efficacy seems similar to that of the antiintegrin vedolizumab. It has the advantage of subcutaneous versus intravenous administration, which is required for vedolizumab. It could be advantageous for some CD patients with concomitant psoriasis or PG, which are conditions unresponsive to vedolizumab. It is not yet established if ustekinumab readily closes fistulae or benefits patients with various extracolonic manifestations of CD.

Ustekinumab appears to have a good safety profile in CD. Serious adverse events using this drug are similar or close to that of placebo. There is extremely limited data regarding the use of this medication during pregnancy. Also, there is a low incidence of antibody development against this biologic.

Under investigation is a selective inhibitor of the p19 subunit of the IL13 receptor that only blocks IL23. Present data suggest that this will prove beneficial in CD.

### **Inhibitors of Leukocyte Infiltration**

Another approach for the treatment of IBD is blocking the infiltration of lymphocytes into the intestinal wall. The first drug in this class was natalizumab, which bound the  $\alpha 4$  component of the two integrins  $\alpha 4\beta 1$  and  $\alpha 4\beta 7$ . While proving efficacious for CD, it also promoted the development of John Cunningham (JC) virus-induced, progressive multifocal leukoencephalopathy. This is a dangerous and neurologically devastating disease. Thus, natalizumab is rarely, if ever, used in the clinical management of IBD.

Vedolizumab, a humanized monoclonal antibody, is a selective inhibitor of the  $\alpha 4\beta 7$  integrin expression on lymphocytes. The integrin  $\alpha 4\beta 7$  binds exclusively to the mostly gut-specific addressin MAdCAM1, an adhesion molecule located primarily on intestinal vascular endothelial cells. Blockade of  $\alpha 4\beta 7$  impedes lymphocyte adhesion to the endothelium. This, in turn, prevents the egress of memory CD4+ T cells into the gut and perhaps mesenteric lymph nodes.

Vedolizumab has proven effective in UC and, to a lesser degree, in CD (Feagan et al., 2013; Sandborn et al., 2013). It is not proven that this drug can close fistula in CD, and it is of questionable value managing extraintestinal problems associated with IBD such as joint pain, ankylosing spondylitis, EN, and uveitis. It could be a desirable therapy in patients with ongoing nonintestinal infections, multiple sclerosis, heart failure, TNF-induce psoriasis, and patients undergoing chemotherapy for cancer.

The incidence of major adverse events to vedolizumab is close to that of placebo (Novak et al., 2017). Unlike natalizumab, the selectivity of vedolizumab only for  $\alpha 4\beta 7$  allows the infiltration of lymphocytes into the CNS, and it does not increase the risk of developing progressive multifocal leukoencephalopathy. Patients can develop antibodies to vedolizumab that block drug action (perhaps 5%), and can have allergic reactions. Over time, patients can develop nonantibody-mediated drug resistance. It may predispose to sinus and upper respiratory infections. This medication requires intravenous administration, which is an additional expense and inconvenience. Other similar agents under development will allow subcutaneous and perhaps oral use. Among these may include medication that directly blocks MAdCAM1.

Ozanimod is an inhibitor of the sphingosine-1-phosphate receptor subtype 1 and 5 (S1P1). It shows promise in UC (Sandborn et al., 2016). S1P1 is located on lymphocytes. Blockade of this molecule prevents the vascular adhesion and egress of lymphocytes out of lymph nodes. The drug is orally administered.

### **Janus Kinase Inhibitors and Some Other Medications on the Horizon**

Tofacitinib, an inhibitor of JAK 1 and 3, and upadacitinib and filgotinib, which are selective blockers of JAK1, appear to have efficacy in IBD. Tofacitinib is used to manage rheumatoid arthritis and psoriasis, and showed benefit in UC (Sandborn et al., 2017). Upadacitinib may prove effective in both UC and CD with fewer side effects. JAKs are important intracellular signaling pathways that drive proinflammatory responses.

Smad7 blockade, using an orally active, gut-directed mRNA inhibitory oligonucleotide, showed promise in CD (Monteleone et al., 2015). Smad7 is a natural inhibitor of T-cell TGF $\beta$  signaling. Blockade of Smad7 allows TGF $\beta$  to more effectively stimulate the development of regulatory T cells and block inflammation. However, advanced phase 3 trials in CD and UC failed to show efficacy.

### **Nutritional Support**

It is appreciated that patients with CD, but not UC, often quickly go into remission and close fistulae if they are nutritionally restricted to enteral or intravenous feeding solutions (called bowel rest). Some data suggest that enteral and parenteral feeding is comparably effective. The disease rapidly returns with the resumption of a regular diet. There is prolonged benefit if the patient maintains the dietary restriction. This form of therapy can prove beneficial as a bridging therapy in patients with advanced CD who need time to respond to some other, slower acting medication and who do not respond to or tolerate rapid acting corticosteroids. Also, it provides life-saving support to CD patients with short bowel syndrome often resulting from repeated small bowel resections and to people suffering from severe nutritional depletion.

The mechanism of action is unknown. It is assumed that patients benefit from the reduction of the bacterial and antigenic load in the distal bowel.

### **Surgical**

The development of new medications to manage IBD has decreased the need for major bowel resections and fistula repairs.

The surgical indications for UC include toxic megacolon, severe disease unresponsive to appropriate therapy, development of colonic dysplasia or cancer, significant side effects to medication, steroid dependency, severe hemorrhage, unremitting disease that severely impairs quality of life, and intractable severe EIM of IBD (Bohl, 2015). Surgery usually involves total colectomy often using minimally invasive surgical approaches.

Most patients, particularly below the age of 60, receive a surgically constructed ileal J-pouch attached to the anal verge to retain fecal continence and to avoid an ileostomy (Bohl, 2015). People with a J-pouch have to defecate about 5.5 times daily and some have seepage. Removal of the colon should in theory cure UC, since the inflammation of UC is limited to the colon with the exception of the occasional patient with "back-wash ileitis." However, patients with J-pouches can get complications, and they not infrequently develop "pouchitis" requiring intermittent medical therapy (Bohl, 2015). In perhaps 10%–20% of such patients, pouchitis morphs into a condition that looks like CD requiring intensive medical therapy and sometimes removal of the pouch.

Ileostomies may be desirable for people having dysfunctional anal sphincters, do not want the two to three surgeries required to create a J-pouch, or who are older and perhaps physically unfit for multiple surgeries. It also is needed for patients with J-pouches that failed due to recurring disease. Ileostomies require the patient to wear a bag to collect daily stool output, which necessitates emptying the bag a few times daily. Ileostomies are subject to various medical problems (Bafford and Irani, 2013). Also, perhaps 5%–10% of patients having an ileostomy for treatment of classic UC eventually develop CD in their terminal ileum.

The surgical indications for CD are similar to UC (Harb, 2015; Toh et al., 2016). After resection of the diseased segment of bowel, unlike UC, CD almost inevitably returns to previously healthy regions of the gut. CD occurs most frequently in the ileocecal area. Thus, the most common surgery is ileocecal resection with subsequent attachment of the residual ileum to the colon. For CD limited to the ileal/cecal region, recurrence rates after resection are about 70%–90% in the subsequent 5–10 years after surgery.

In CD, complications resulting from strictures and fistula formation may require surgery. Patients with previous extensive small bowel resection or with multiple short segments of small bowel narrowing may receive a "stricturoplasty" to widen these areas of narrowing avoiding further bowel resection.

Fistulae develop in about 30% of the CD patients (Schwartz et al., 2002). There are various bowel-sparing medical and surgical approaches for management of perianal fistula in CD.

## FUTURE PROSPECTS

We live in an exciting time in which scientific research has identified many of the molecules and processes that drive and regulate inflammation. Advances in our appreciation of the workings of mucosal immunity and the intestinal microbiome are no less spectacular. Serendipity no longer dictates drug discovery in IBD. The new medications to treat IBD and those under development represent attempts to block or modulate the newly discovered inflammatory pathways that are assumed to drive IBD. This has resulted in a high rate of success leading to increasing availability of exciting new medications to treat these diseases. This process will continue. "Biologic" therapy based on monoclonal antibody technology has its limitations. Newer targeting strategies will allow the oral administration of medications that inhibit the same pathways, which in many cases could render "biologics" obsolete.

We used to consider IBD just two distinct entities, UC and CD. Investigation of the genomics of IBD failed to identify distinct gene variants that underlay the pathology. Rather, IBD probably is a larger collection of disorders with overlapping clinical phenotypes.

This makes therapy much more difficult. Many of the new medications provide major benefit to small subsets of the entire IBD patient population. Each of these new agents requires several months of administration before establishing efficacy. Thus, successful therapeutic intervention could follow many months of trial and failure, a significant clinical problem. Thus, happenstance, rather than logical therapeutic selection, will be the approach to treatment until we learn more about the underlying pathophysiology of each of these diseases.

The therapeutic road in IBD is littered with patients who experienced spectacular successful treatment followed by relapse for unknown reasons. This just illustrates how much more we need to learn about mucosal immune regulation and our immune interactions with the intestinal microbiome.

The present understanding of disease pathogenesis does not explain many clinical observations. For example, in both UC and CD, disease can be highly segmental with razor sharp cutoffs. Extensive mucosal biopsy, which disrupts the mucosal barrier, does not spread the disease. Nutritional support can place nearly all forms of CD into temporary clinical and endoscopic remission but fails to control the raging inflammation of UC. We have a lot to learn.

Not all patients have an aggressive clinical course. Not every patient requires early intervention with expensive and sometimes risky therapies. Presently, we have limited ability to stratify patients according to risk.

Colonoscopy remains the "gold standard" for assessing disease progression and other risks. Symptoms often are unreliable indicators of disease activity. Required are more inexpensive and less invasive tests to predict disease activity. This could permit earlier, appropriate therapeutic intervention to prevent disease progression, permanent intestinal damage, and cancer.

IBD is a growing menace around the world with increasing clinical and economic impact. It was a rare disease in the early 20th century, but now is quite common. It has a huge emotional, financial, and social impact on the patients and their families. The solution to IBD is not just the development of new medications, but the discovery of the environmental factors which allow emergence of these diseases. Poorly defined changes in daily living are the major cause of IBD. Modifications in life style, everyday exposure or avoidance of particular environmental factors, could be the ultimate solution to these diseases.

### Acknowledgment

Supported by NIH grants DK38327, DK058755, Schneider Family, Gilman Family

## ABBREVIATIONS

<b>5-ASA</b>	aminosalicylates
<b>OmpC</b>	anti- <i>Escherichia coli</i> outer membrane porin
<b>ATG16L1</b>	autophagy related 16-like 1
<b>DSS</b>	C-reactive protein (CRP) and dextran sodium sulfate
<b>CT</b>	computed tomography
<b>CTE</b>	computed tomography enterography
<b>CD</b>	Crohn's disease
<b>EN</b>	erythema nodosum
<b>EIM</b>	extra-intestinal manifestations

ESR	erythrocyte sedimentation rate
IBD	inflammatory bowel disease
JAK	Janus kinase
MRE	magnetic resonance enterography
6-MP	6-mercaptopurine
MTX	methotrexate
NOD2	nucleotide-binding oligomerization domain containing 2
PSC	primary sclerosing cholangitis
PG	pyoderma gangrenosum
SBFT	small bowel follow-through X-ray series
Th1	T helper 1
Th17	T helper 17
TPMT	thiopurines methyltransferase
UC	ulcerative colitis
VCE	video capsule endoscopy

## References

- Abdalla, M.I., Herfarth, H., 2016. Budesonide for the treatment of ulcerative colitis. [Review]. *Expert Opin. Pharmacother.* 17, 1549–1559.
- Adolph, T.E., Tomczak, M.F., Niederreiter, L., Ko, H.J., Bock, J., Martinez-Naves, E., et al., 2013. Paneth cells as a site of origin for intestinal inflammation. *Nature* 503, 272–276.
- Ahnfelt-Ronne, I., Nielsen, O.H., Christensen, A., Langholz, E., Binder, V., Riis, P., 1990. Clinical evidence supporting the radical scavenger mechanism of 5-aminosalicylic acid. *Gastroenterology* 98, t-9.
- Al-Bawardi, B., Locke, G., Huprich, J.E., Fletcher, J.G., Fidler, J.L., Barlow, J.M., et al., 2015. Retained capsule endoscopy in a large tertiary care academic practice and radiologic predictors of retention. *Inflamm. Bowel Dis.* 21, 2158–2164.
- Andersson, R.E., Olaison, G., Tysk, C., Ekbom, A., 2001. Appendectomy and protection against ulcerative colitis. *N. Eng. J. Med.* 344, 808–814.
- Androulakis, I., Zavos, C., Christopoulos, P., Mastorakos, G., Gazouli, M., 2015. Safety of anti-tumor necrosis factor therapy during pregnancy in patients with inflammatory bowel disease. [Review]. *World J. Gastroenterol.* 21, 13205–13211.
- Autenrieth, D.M., Baumgart, D.C., 2012. Toxic megacolon. [Review]. *Inflamm. Bowel Dis.* 18, 584–591.
- Axelrad, J.E., Roy, A., Lawlor, G., Korelitz, B., Lichtiger, S., 2016. Thiopurines and inflammatory bowel disease: current evidence and a historical perspective. [Review]. *World J. Gastroenterol.* 22, 10103–10117.
- Bafford, A.C., Irani, J.L., 2013. Management and complications of stomas. [Review]. *Surg. Clin. North Am.* 93, 145–166.
- Bantel, H., Berg, C., Vieth, M., Stolte, M., Kruis, W., Schulze-Osthoff, K., 2000. Mesalazine inhibits activation of transcription factor NF- $\kappa$ B in inflamed mucosa of patients with ulcerative colitis. *Am. J. Gastroenterol.* 95, 3452–3457.
- Baron, J.J., 2000. Inflammatory bowel disease up to 1932. *Mt. Sinai J. Med.* 67, 174–189.
- Baxt, L.A., Xavier, R.J., 2015. Role of autophagy in the maintenance of intestinal homeostasis. [Review]. *Gastroenterology* 149, 553–562.
- Bellaguarda, E., Chang, E.B., 2015. IBD and the gut microbiota—from bench to personalized medicine. [Review]. *Curr. Gastroenterol. Rep.* 17, 15.
- Benchimol, E.I., Manuel, D.G., To, T., Mack, D.R., Nguyen, G.C., Gommerman, J.L., et al., 2015. Asthma, type 1 and type 2 diabetes mellitus, and inflammatory bowel disease amongst South Asian immigrants to Canada and their children: a population-based cohort study. *PLoS One* [Electronic Resource] 10, e0123599.
- Benor, S., Russell, G.H., Silver, M., Israel, E.J., Yuan, Q., Winter, H.S., 2010. Shortcomings of the inflammatory bowel disease Serology 7 panel. *Pediatrics* 125, 1230–1236.
- Bernstein, C.N., Blanchard, J.F., Rawsthorne, P., Wajda, A., 1999. Epidemiology of Crohn's disease and ulcerative colitis in a central Canadian province: a population-based study. *Am. J. Epidemiol.* 149, 916–924.
- Bernstein, C.N., Wajda, A., Blanchard, J.F., 2005. The clustering of other chronic inflammatory diseases in inflammatory bowel disease: a population-based study. *Gastroenterology* 129, 827–836.
- Binder, V., 1998. Genetic epidemiology in inflammatory bowel disease. [Review] [27 refs]. *Dig. Dis.* 16, 351–355.
- Blum, A.M., Metwali, A., Elliott, D.E., Berg, D.J., Weinstock, J.V., 2004. CD4+ T cells from IL-10-deficient mice transfer susceptibility to NSAID-induced Rag colitis. *Am. J. Physiol. – Gastrointest. Liver Physiol.* 287 (2), G320–G325.
- Bohl, J.L., Sobba, K., 2015. Indications and options for surgery in ulcerative colitis. [Review]. *Surg. Clin. North Am.* 95, 1211–1232.
- Brown, S.R., Coviello, L.C., 2015. Extraintestinal manifestations associated with inflammatory bowel disease. [Review]. *Surg. Clin. North Am.* 95, 1245–1259.
- Burkett, P.R., Meyer zu, H.G., Kuchroo, V.K., 2015. Pouring fuel on the fire: Th17 cells, the environment, and autoimmunity. [Review]. *J. Clin. Invest.* 125, 2211–2219.
- Calkins, B.M., 1989. A meta-analysis of the role of smoking in inflammatory bowel disease. *Dig. Dis. Sci.* 34, 1841–1854.
- Carla-Moreau, A., Paul, S., Roblin, X., Genin, C., Peyrin-Biroulet, L., 2015. Prevention and treatment of postoperative Crohn's disease recurrence with anti-TNF therapy: a meta-analysis of controlled trials. [Review]. *Dig. Liver Dis.* 47, 191–196.
- Carlstedt-Duke, J., Gustafsson, J.A., 1987. Structure and function of the glucocorticoid receptor. *J. Steroid Biochem.* 27, 99–104.
- Carr, I., Mayberry, J.F., 1999. The effects of migration on ulcerative colitis: a three-year prospective study among Europeans and first- and second-generation South Asians in Leicester (1991–1994). *Am. J. Gastroenterol.* 94, 2918–2922.
- Catana, C.S., N. Berindan, I., Cozma, V., Magdas, C., Tabaran, F., Dumitrascu, D.L., 2015. Contribution of the IL-17/IL-23 axis to the pathogenesis of inflammatory bowel disease. [Review]. *World J. Gastroenterol.* 21, 5823–5830.

- Chan, D., Kumar, D., Mendall, M., 2015. What is known about the mechanisms of dietary influences in Crohn's disease? [Review] *Nutrition* 31, 1195–1203.
- Chang, S., Malter, L., Hudesman, D., 2015. Disease monitoring in inflammatory bowel disease. [Review]. *World J. Gastroenterol.* 21, 11246–11259.
- Coskun, M., Steenholdt, C., de Boer, N.K., Nielsen, O.H., 2016. Pharmacology and optimization of thiopurines and methotrexate in inflammatory bowel disease. [Review]. *Clin. Pharmacokinet.* 55, 257–274.
- Cosnes, J., Gower-Rousseau, C., Seksik, P., Cortot, A., 2011. Epidemiology and natural history of inflammatory bowel diseases. *Gastroenterology* 140, 1785–1794.
- Cosnes, J., Bourrier, A., Laharie, D., Nahon, S., Bouhnik, Y., Carbonnel, F., et al., 2013. Early administration of azathioprine vs conventional management of Crohn's disease: a randomized controlled trial and Groupe d'Etude Therapeutique des Affections Inflammatoires du Tube Digestif (GETAID) *Gastroenterology* 145, 758–765.
- Crohn, B.B., Ginzburg, L., Oppenheimer, J., 1932. Regional ileitis. *JAMA* 99, 1323–1329.
- Deepak, P., Loftus Jr., E.V., 2016. Ustekinumab in treatment of Crohn's disease: design, development, and potential place in therapy. [Review]. *Drug Des. Dev. Ther.* 10, 3685–3698.
- Delco, F., Sonnenberg, A., 1998. Military history of patients with inflammatory bowel disease: an epidemiological study among U.S. veterans. [see comments]. *Am. J. Gastroenterol.* 93, 1457–1462.
- Derby, L.E., Jick, H., 1998. Appendectomy protects against ulcerative colitis. *Epidemiology* 9, 205–207.
- Ding, N.S., Hart, A., De, C.P., 2016. Systematic review: predicting and optimising response to anti-TNF therapy in Crohn's disease – algorithm for practical management. [Review]. *Aliment. Pharmacol. Ther.* 43, 30–51.
- Duerr, R.H., Taylor, K.D., Brant, S.R., Rioux, J.D., Silverberg, M.S., Daly, M.J., et al., 2006. A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. [see comment]. *Science* 314 (5804), 1461–1463.
- Elliott, D.E., Urban Jr., J.F., Argo, C.K., Weinstock, J.V., 2000. Does the failure to acquire helminthic parasites predispose to Crohn's disease? *FASEB J.* 14, 1848–1855.
- Feagan, B.G., Rutgeerts, P., Sands, B.E., Hanauer, S., Colombel, J.F., Sandborn, W.J., et al., 2013. Vedolizumab as induction and maintenance therapy for ulcerative colitis and Study Group N. *Engl. J. Med.* 369, 699–710.
- Feagan, B.G., Sandborn, W.J., Gasink, C., Jacobstein, D., Lang, Y., Friedman, J.R., et al., 2016. Ustekinumab as induction and maintenance therapy for Crohn's disease and UNITI-IM-UNITI Study Group N. *Engl. J. Med.* 375, 1946–1960.
- Feakins, R.M., 2014. Ulcerative colitis or Crohn's disease? Pitfalls and problems. [Review]. *Histopathology* 64, 317–335.
- Fletcher, J.G., Fidler, J.L., Bruining, D.H., Huprich, J.E., 2011. New concepts in intestinal imaging for inflammatory bowel diseases. [Review]. *Gastroenterology* 140, 1795–1806.
- Gabryel, M., Skrzypczak-Zielinska, M., Kucharski, M.A., Slomski, R., Dobrowolska, A., 2016. The impact of genetic factors on response to glucocorticoids therapy in IBD. [Review]. *Scand. J. Gastroenterol.* 51, 654–665.
- Gaffen, S.L., Jain, R., Garg, A.V., Cua, D.J., 2014. The IL-23-IL-17 immune axis: from mechanisms to therapeutic testing. [Review]. *Nat. Rev. Immunol.* 14, 585–600.
- Ginzburg, L., Oppenheimer, G.D., 1932. Non-specific granulomata of the intestines (inflammatory tumors and strictures of the bowel). *Trans. Am. Gastro-Enterol. Assoc.* 35, 241–283.
- Goh, K., Xiao, S.D., 2009. Inflammatory bowel disease: a survey of the epidemiology in Asia. [Review] [45 refs]. *J. Dig. Dis.* 10, 1–6.
- Gordon, H., Trier, M.F., Andersen, V., Harbord, M., 2015. Heritability in inflammatory bowel disease: from the first twin study to genome-wide association studies. [Review]. *Inflamm. Bowel Dis.* 21, 1428–1434.
- Gorelik, L., Flavell, R.A., 2000. Abrogation of TGF $\beta$  signaling in T cells leads to spontaneous T cell differentiation and autoimmune disease. *Immunity* 12, 171–181.
- Guimaraes, L.S., Fletcher, J.G., Yu, L., Huprich, J.E., Fidler, J.L., Manduca, A., et al., 2010. Feasibility of dose reduction using novel denoising techniques for low kV (80 kV) CT enterography: optimization and validation. *Acad. Radiol.* 17, 1203–1210.
- Hale-White, W., 1888. On Simple Ulcerative Colitis and Other Rare Intestinal Ulcers. 30 ed. pp. 131–163.
- Hampe, J., Franke, A., Rosenstiel, P., Till, A., Teuber, M., Huse, K., et al., 2007. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. *Nat. Genet.* 39, 207–211.
- Hang, L., Blum, A.M., Urban, J., Stoyanoff, K., Weinstock, J.V., 2013. *Heligmosomoides bakeri* infection activates colonic Foxp3+ T cells enhancing their capacity to prevent colitis. *J. Immunol.* 191, 1927–1934.
- Hang, L., Blum, A.M., Kumar, S., Urban, J.F., Mitreva, M., Geary, T.G., et al., 2016. Downregulation of the Syk signaling pathway in intestinal dendritic cells is sufficient to induce dendritic cells that inhibit colitis. *J. Immunol.* 197, 2948–2957.
- Harb, W.J., 2015. Crohn's disease of the colon, rectum, and anus. [Review]. *Surg. Clin. North Am.* 95, 1195–1210.
- Harris, M.S., Lichtenstein, G.R., 2011. Review article: delivery and efficacy of topical 5-aminosalicylic acid (mesalazine) therapy in the treatment of ulcerative colitis. [Review]. *Aliment. Pharmacol. Ther.* 33, 996–1009.
- Hauso, O., Martinsen, T.C., Waldum, H., 2015. 5-Aminosalicylic acid, a specific drug for ulcerative colitis. [Review]. *Scand. J. Gastroenterol.* 50, 933–941.
- Herfarth, H.H., Kappelman, M.D., Long, M.D., Isaacs, K.L., 2016. Use of methotrexate in the treatment of inflammatory bowel diseases. [Review]. *Inflamm. Bowel Dis.* 22, 224–233.
- Hornquist, C.E., Lu, X., Rogers-Fani, P.M., Rudolph, U., Shappell, S., Birnbaumer, L., et al., 1997. G( $\alpha$ )i2-deficient mice with colitis exhibit a local increase in memory CD4+ T cells and proinflammatory Th1-type cytokines. *J. Immunol.* 158, 1068–1077.
- Hurst, A.F., 1909. Ulcerative colitis. *Guy's Hosp. Rep.* 71, 26.
- Iliev, I.D., Funari, V.A., Taylor, K.D., Nguyen, Q., Reyes, C.N., Strom, S.P., et al., 2012. Interactions between commensal fungi and the C-type lectin receptor Dectin-1 influence colitis. *Science* 336, 1314–1317.
- Ince, M.N., Elliott, D.E., Setiawan, T., Metwali, A., Blum, A., Chen, H.L., et al., 2009. Role of T cell TGF- $\beta$  signaling in intestinal cytokine responses and helminthic immune modulation. *Eur. J. Immunol.* 39 (7), 1870–1878.

- Izcue, A., Coombes, J.L., Powrie, F., 2009. Regulatory lymphocytes and intestinal inflammation. [Review] [165 refs]. *Annu. Rev. Immunol.* 27, 313–338.
- Jayanthi, V., Probert, C.S., Pinder, D., Wicks, A.C., Mayberry, J.F., 1992. Epidemiology of Crohn's disease in Indian migrants and the indigenous population in Leicestershire. *Q. J. Med.* 82, 125–138.
- Jones, J., Loftus Jr., E.V., Panaccione, R., Chen, L.S., Peterson, S., McConnell, J., et al., 2008. Relationships between disease activity and serum and fecal biomarkers in patients with Crohn's disease. *Clin. Gastroenterol. Hepatol.* 6, 1218–1224.
- Kalekar, L., Mueller, D., 2017. Relationship between CD4 regulatory T cells and anergy in vivo. *J. Immunol.* 198, 2527–2533.
- Kaplan, G.G., Ng, S.C., 2017. Understanding and preventing the global increase of inflammatory bowel disease. [Review]. *Gastroenterology* 152, 313–321.
- Kappelman, M.D., Rifas-Shiman, S.L., Kleinman, K., Ollendorf, D., Bousvaros, A., Grand, R.J., et al., 2007. The prevalence and geographic distribution of Crohn's disease and ulcerative colitis in the United States.[see comment]. *Clin. Gastroenterol. Hepatol.* 5 (12), 1424–1429.
- Katz, J.A., 2004. Treatment of inflammatory bowel disease with corticosteroids. [Review] [108 refs]. *Gastroenterol. Clin. North Am.* 33, 171–189.
- Kernbauer, E., Cadwell, K., 2014. Autophagy, viruses, and intestinal immunity. [Review]. *Curr. Opin. Gastroenterol.* 30, 539–546.
- Keubler, L.M., Buettner, M., Hager, C., Bleich, A., 2015. A multihit model: colitis lessons from the interleukin-10-deficient mouse. [Review]. *Inflamm. Bowel Dis.* 21, 1967–1975.
- Khanna, P.V., Shih, D.Q., Haritunians, T., McGovern, D.P., Targan, S., 2014. Use of animal models in elucidating disease pathogenesis in IBD. [Review]. *Semin. Immunopathol.* 36, 541–551.
- Lassen, K.G., Kuballa, P., Conway, K.L., Patel, K.K., Becker, C.E., Peloquin, J.M., et al., 2014. Atg16L1 T300A variant decreases selective autophagy resulting in altered cytokine signaling and decreased antibacterial defense. *Proc. Natl. Acad. Sci. U.S.A.* 111, 7741–7746.
- Lathrop, S.K., Bloom, S.M., Rao, S.M., Nutsch, K., Lio, C.W., Santacruz, N., et al., 2011. Peripheral education of the immune system by colonic commensal microbiota. *Nature* 478, 250–254.
- Lazaridis, K.N., LaRusso, N.F., 2016. Primary sclerosing cholangitis. *N. Engl. J. Med.* 375, 2501–2502.
- Levin, A.D., Wildenberg, M.E., van den Brink, G.R., 2016. Mechanism of action of anti-TNF therapy in inflammatory bowel disease. [Review]. *J. Crohn's Colitis* 10, 989–997.
- Li, J., Moran, T., Swanson, E., Julian, C., Harris, J., Bonen, D.K., et al., 2004. Regulation of IL-8 and IL-1beta expression in Crohn's disease associated NOD2/CARD15 mutations. *Hum. Mol. Genet.* 13, 1715–1725.
- Loftus Jr., E.V., Sandborn, W.J., 2002. Epidemiology of inflammatory bowel disease. [Review] [173 refs]. *Gastroenterol. Clin. North Am.* 31, 1–20.
- Loftus Jr., E.V., Schoenfeld, P., Sandborn, W.J., 2002. The epidemiology and natural history of Crohn's disease in population-based patient cohorts from North America: a systematic review. [Review] [25 refs]. *Aliment. Pharmacol. Ther.* 16, 51–60.
- Magro, F., Langner, C., Driessen, A., Ensari, A., Geboes, K., Mantzaris, G.J., et al., 2013. European consensus on the histopathology of inflammatory bowel diseaseEuropean Society of Pathology (ESP), and European Crohn's and Colitis Organisation (ECCO) *J. Crohn's Colitis* 7, 827–851.
- Mahida, Y.R., Lamming, C.E., Gallagher, A., Hawthorne, A.B., Hawkey, C.J., 1991. 5-Aminosalicylic acid is a potent inhibitor of interleukin 1 beta production in organ culture of colonic biopsy specimens from patients with inflammatory bowel disease. *Gut* 32, 50–54.
- Mathew, R.C., Weinstock, J.V., 1994. Granuloma formation. In: Targan, S.R., Shanahan, F. (Eds.), *Inflammatory Bowel Disease: From Bench to Bedside*. Williams & Wilkins, pp. 151–159.
- McGovern, D.P., Kugathasan, S., Cho, J.H., 2015. Genetics of inflammatory bowel diseases. [Review]. *Gastroenterology* 149, 1163–1176.
- Mendall, M.A., Chan, D., Patel, R., Kumar, D., 2016. Faecal calprotectin: factors affecting levels and its potential role as a surrogate marker for risk of development of Crohn's disease. [Review]. *BMC Gastroenterol.* 16, 126.
- Mizoguchi, A., Takeuchi, T., Himuro, H., Okada, T., Mizoguchi, E., 2016. Genetically engineered mouse models for studying inflammatory bowel disease. [Review]. *J. Pathol.* 238, 205–219.
- Molodecky, N.A., Soon, I.S., Rabi, D.M., Ghali, W.A., Ferris, M., Chernoff, G., et al., 2012. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. [Review]. *Gastroenterology* 142, 46–54.
- Monteleone, G., Neurath, M.F., Ardizzone, S., Di, S.A., Fantini, M.C., Castiglione, F., et al., 2015. Mongersen, an oral SMAD7 antisense oligonucleotide, and Crohn's disease. *N. Engl. J. Med.* 372, 1104–1113.
- Mummery, L.P., 1907. The causes of colitis with special reference to its surgical treatment. With an account of 36 cases. *Lancet* 1, 1638–1643.
- Murthy, A., Li, Y., Peng, I., Reichelt, M., Katakam, A.K., Noubaide, R., et al., 2014. A Crohn's disease variant in Atg16l1 enhances its degradation by caspase 3. *Nature* 506, 456–462.
- Narula, N., Marshall, J.K., Colombel, J.F., Leontiadis, G.I., Williams, J.G., Muqtadir, Z., et al., 2016. Systematic review and meta-analysis: infliximab or cyclosporine as rescue therapy in patients with severe ulcerative colitis refractory to steroids. [Review]. *Am. J. Gastroenterol.* 111, 477–491.
- Nostrant, T.T., Kumar, N.B., Appelman, H.D., 1987. Histopathology differentiates acute self-limited colitis from ulcerative colitis. *Gastroenterology* 92, 318–328.
- Novak, G., Hindryckx, P., Khanna, R., Jairath, V., Feagan, B.G., 2017. The safety of vedolizumab for the treatment of ulcerative colitis. [Review]. *Expert Opin. Drug Saf.* 16, 501–507.
- Okamoto, R., Watanabe, M., 2016. Role of epithelial cells in the pathogenesis and treatment of inflammatory bowel disease. [Review]. *J. Gastroenterol.* 51, 11–21.
- Owczarek, D., Rodacki, T., Domagala-Rodacka, R., Cibor, D., Mach, T., 2016. Diet and nutritional factors in inflammatory bowel diseases. [Review]. *World J. Gastroenterol.* 22, 895–905.
- Panes, J., Lopez-Sanroman, A., Bermejo, F., Garcia-Sanchez, V., Esteve, M., Torres, Y., et al., 2013. Early azathioprine therapy is no more effective than placebo for newly diagnosed Crohn's diseaseand AZTEC Study Group *Gastroenterology* 145, 766–774.
- Panes, J., Jairath, V., Levesque, B.G., 2017. Advances in use of endoscopy, radiology, and biomarkers to monitor inflammatory bowel diseases. [Review]. *Gastroenterology* 152, 362–373.

- Pidasheva, S., Trifari, S., Phillips, A., Hackney, J.A., Ma, Y., Smith, A., et al., 2011. Functional studies on the IBD susceptibility gene IL23R implicate reduced receptor function in the protective genetic variant R381Q. *PLoS One [Electronic Resource]*, 6, p. e25038.
- Powrie, F., Leach, M.W., Mauze, S., Menon, S., Caddle, L.B., Coffman, R.L., 1994. Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RBhi CD4+ T cells. *Immunity* 1, 553–562.
- Probert, C.S., Jayanthi, V., Hughes, A.O., Thompson, J.R., Wicks, A.C., Mayberry, J.F., 1993. Prevalence and family risk of ulcerative colitis and Crohn's disease: an epidemiological study among Europeans and south Asians in Leicestershire. *Gut* 34, 1547–1551.
- Putignani, L., Del, C.F., Vernocchi, P., Cicala, M., Cucchiara, S., Dallapiccola, B., 2016. Gut microbiota dysbiosis as risk and premorbid factors of IBD and IBS along the childhood-adulthood transition. [Review] and Dysbiotrack Study Group. *Inflamm. Bowel Dis.* 22, 487–504.
- Ramanan, D., Bowcutt, R., Lee, S.C., Tang, M.S., Kurtz, Z.D., Ding, Y., et al., 2016. Helminth infection promotes colonization resistance via type 2 immunity. *Science* 352, 608–612.
- Regueiro, M., Feagan, B.G., Zou, B., Johanns, J., Blank, M.A., Chevrier, M., et al., 2016. Infliximab reduces endoscopic, but not clinical, recurrence of Crohn's disease after ileocolonic resection and PREVENT Study Group. *Gastroenterology* 150, 1568–1578.
- Richard, M.L., Lamas, B., Liguori, G., Hoffmann, T.W., Sokol, H., 2015. Gut fungal microbiota: the Yin and Yang of inflammatory bowel disease. [Review]. *Inflamm. Bowel Dis.* 21, 656–665.
- Rioux, J.D., Xavier, R.J., Taylor, K.D., Silverberg, M.S., Goyette, P., Huett, A., et al., 2007. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. *Nat. Genet.* 39, 596–604.
- Rubin, D.T., Hanauer, S.B., 2000. Smoking and inflammatory bowel disease. [Review] [73 refs]. *Eur. J. Gastroenterol. Hepatol.* 12, 855–862.
- Rubin, D.T., Sandborn, W.J., Bosworth, B., Zakko, S., Gordon, G.L., Sale, M.E., et al., 2015. Budesonide foam has a favorable safety profile for inducing remission in mild-to-moderate ulcerative proctitis or proctosigmoiditis. *Dig. Dis. Sci.* 60, 3408–3417.
- Rubtsov, Y.P., Rasmussen, J.P., Chi, E.Y., Fontenot, J., Castelli, L., Ye, X., et al., 2008. Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. *Immunity* 28, 546–558.
- Rudolph, U., Finegold, M.J., Rich, S.S., Harriman, G.R., Srinivasan, Y., Brabet, P., et al., 1995. Gi2 alpha protein deficiency: a model of inflammatory bowel disease. *J. Clin. Immunol.* 15, 101S–105S.
- Russel, M.G., Dorant, E., Brummer, R.J., van de Kruyjs, M.A., Muris, J.W., Bergers, J.M., et al., 1997. Appendectomy and the risk of developing ulcerative colitis or Crohn's disease: results of a large case-control study. South Limburg Inflammatory Bowel Disease Study Group. [see comments]. *Gastroenterology* 113, 377–382.
- Sandborn, W.J., Feagan, B.G., Rutgeerts, P., Hanauer, S., Colombel, J.F., Sands, B.E., et al., 2013. Vedolizumab as induction and maintenance therapy for Crohn's disease and Study Group N. *Engl. J. Med.* 369, 711–721.
- Sandborn, W.J., Bosworth, B., Zakko, S., Gordon, G.L., Clemons, D.R., Golden, P.L., et al., 2015. Budesonide foam induces remission in patients with mild to moderate ulcerative proctitis and ulcerative proctosigmoiditis. *Gastroenterology* 148, 740–750.
- Sandborn, W.J., Feagan, B.G., Wolf, D.C., D'Haens, G., Vermeire, S., Hanauer, S.B., et al., 2016. Ozanimod induction and maintenance treatment for ulcerative colitis and TOUCHSTONE Study Group N. *Engl. J. Med.* 374, 1754–1762.
- Sandborn, W.J., Su, C., Sands, B.E., D'Haens, G.R., Vermeire, S., Schreiber, S., et al., 2017. Tofacitinib as induction and maintenance therapy for ulcerative colitis. *N. Engl. J. Med.* 376, 1723–1736.
- Schwartz, D.A., Loftus Jr., E.V., Tremaine, W.J., Panaccione, R., Harmsen, W.S., et al., 2002. The natural history of fistulizing Crohn's disease in Olmsted County, Minnesota. *Gastroenterology* 122, 875–880.
- Sekiya, T., Nakatsukasa, H., Lu, Q., Yoshimura, A., 2016. Roles of transcription factors and epigenetic modifications in differentiation and maintenance of regulatory T cells. [Review]. *Microbes Infect.* 18, 378–386.
- Shanahan, F., Niederlechner, A., Carramanzana, N., Anton, P., 1990. Sulfasalazine inhibits the binding of TNF alpha to its receptor. *Immunopharmacology* 20, 217–224.
- Sheedy, S.P., Bruining, D.H., Dozois, E.J., Faubion, W.A., Fletcher, J.G., 2017. MR imaging of perianal Crohn disease. [Review]. *Radiology* 282, 628–645.
- Shivananda, S., Lennard-Jones, J., Logan, R., Fear, N., Price, A., Carpenter, L., et al., 1996. Incidence of inflammatory bowel disease across Europe: is there a difference between north and south? Results of the European Collaborative Study on inflammatory bowel disease (EC-IBD). *Gut* 39, 690–697.
- Simms, L.A., Doecke, J.D., Walsh, M.D., Huang, N., Fowler, E.V., Radford-Smith, G.L., 2008. Reduced alpha-defensin expression is associated with inflammation and not NOD2 mutation status in ileal Crohn's disease. *Gut* 57, 903–910.
- Singh, B., Read, S., Asseman, C., Malmstrom, V., Mottet, C., Stephens, L.A., et al., 2001. Control of intestinal inflammation by regulatory T cells. [Review] [83 refs]. *Immunol. Rev.* 182, 190–200.
- Sipponen, T., Kolho, K.L., 2015. Fecal calprotectin in diagnosis and clinical assessment of inflammatory bowel disease. [Review]. *Scand. J. Gastroenterol.* 50, 74–80.
- Solem, C.A., Loftus Jr., E.V., Tremaine, W.J., Harmsen, W.S., Zinsmeister, A.R., Sandborn, W.J., 2005. Correlation of C-reactive protein with clinical, endoscopic, histologic, and radiographic activity in inflammatory bowel disease. *Inflamm. Bowel Dis.* 11, 707–712.
- Sonnenberg, A., 1990. Occupational distribution of inflammatory bowel disease among German employees. *Gut* 31, 1037–1040.
- Sonnenberg, A., McCarty, D.J., Jacobsen, S.J., 1991. Geographic variation of inflammatory bowel disease within the United States. [see comments]. *Gastroenterology* 100, 143–149.
- Strober, W., Asano, N., Fuss, I., Kitani, A., Watanabe, T., 2014. Cellular and molecular mechanisms underlying NOD2 risk-associated polymorphisms in Crohn's disease. [Review]. *Immunol. Rev.* 260, 249–260.
- Takeshita, J., Grewal, S., Langan, S.M., Mehta, N.N., Oggie, A., Van Voorhees, A.S., et al., 2017. Psoriasis and comorbid diseases: epidemiology. [Review]. *J. Am. Acad. Dermatol.* 76, 377–390.
- Toh, J.W., Stewart, P., Rickard, M.J., Leong, R., Wang, N., Young, C.J., 2016. Indications and surgical options for small bowel, large bowel and perianal Crohn's disease. [Review]. *World J. Gastroenterol.* 22, 8892–8904.
- Uhlig, H.H., Coombes, J., Mottet, C., Izcue, A., Thompson, C., Fanger, A., et al., 2006. Characterization of Foxp3+ CD4+ CD25+ and IL-10-secreting CD4+ CD25+ T cells during cure of colitis. *J. Immunol.* 177 (9), 5852–5860.

- Uniken Venema, W.T., Voskuil, M.D., Dijkstra, G., Weersma, R.K., Festen, E.A., 2017. The genetic background of inflammatory bowel disease: from correlation to causality. [Review]. *J. Pathol.* 241, 146–158.
- Vacic, V., Ozelius, L.J., Clark, L.N., Bar-Shira, A., Gana-Weisz, M., Gurevich, T., et al., 2014. Genome-wide mapping of IBD segments in an Ashkenazi PD cohort identifies associated haplotypes. *Hum. Mol. Genet.* 23, 4693–4702.
- Valatas, V., Bamias, G., Kolios, G., 2015. Experimental colitis models: insights into the pathogenesis of inflammatory bowel disease and translational issues. [Review]. *Eur. J. Pharmacol.* 759, 253–264.
- Van der Sluis, M., De Koning, B.A., de Bruijn, A.C., Velich, A., Meijerink, J.P., Van Goudoever, J.B., et al., 2006. Muc2-deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection. *Gastroenterology* 131, 117–129.
- Vavricka, S.R., Schoepfer, A., Scharl, M., Lakatos, P.L., Navarini, A., Rogler, G., 2015. Extraintestinal manifestations of inflammatory bowel disease. [Review]. *Inflamm. Bowel Dis.* 21, 1982–1992.
- Velayos, F., Kathpalia, P., Finlayson, E., 2017. Changing paradigms in detection of dysplasia and management of patients with inflammatory bowel disease: is colectomy still necessary? [Review] *Gastroenterology* 152, 440–450.
- Watanabe, T., Kitani, A., Murray, P.J., Wakatsuki, Y., Fuss, I.J., Strober, W., 2006. Nucleotide binding oligomerization domain 2 deficiency leads to dysregulated TLR2 signaling and induction of antigen-specific colitis. *Immunity* 25, 473–485.
- Wehkamp, J., Salzman, N.H., Porter, E., Nuding, S., Weichenthal, M., Petras, R.E., et al., 2005. Reduced Paneth cell alpha-defensins in ileal Crohn's disease. *Proc. Natl. Acad. Sci. U.S.A.* 102, 18129–18134.
- Parasitic Diseases of the Liver and Intestines. 1996. W.B. Saunders Company, Philadelphia, London, Toronto, Montreal, Sydney, Tokyo.
- Weinstock, J.V., Elliott, D.E., 2014. Helminth infections decrease host susceptibility to immune-mediated diseases. *J. Immunol.* 193, 3239–3247.
- Wells, C., 1952. Ulcerative colitis and Crohn's disease. *Ann. R. Coll. Surg. Engl.* 11, 105–120.
- Westbrook, A.M., Szakmary, A., Schiestl, R.H., 2016. Mouse models of intestinal inflammation and cancer. [Review]. *Arch. Toxicol.* 90, 2109–2130.
- Wilks, S., 1859. Lectures on Pathological Anatomy, third ed Longmans, London, UK.
- Wright, E.K., 2016. Calprotectin or lactoferrin: do they help. [Review]. *Dig. Dis.* 34, 98–104.
- Zhou, G., Song, Y., Yang, W., Guo, Y., Fang, L., Chen, Y., et al., 2016. ASCA, ANCA, ALCA and many more: are they useful in the diagnosis of inflammatory bowel disease? [Review]. *Dig. Dis.* 34, 90–97.
- Zwiers, A., Kraal, L., van de Pouw Kraan, T.C., Wurdinger, T., Bouma, G., Kraal, G., 2012. Cutting edge: a variant of the IL-23R gene associated with inflammatory bowel disease induces loss of microRNA regulation and enhanced protein production. *J. Immunol.* 188, 1573–1577.

# Autoimmune Hemolytic Anemia

Mark A. Vickers<sup>1,2</sup> and Robert N. Barker<sup>2</sup>

<sup>1</sup>Scottish National Blood Transfusion Service, Aberdeen, United Kingdom <sup>2</sup>Immunity, Infection and Inflammation, Institute of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom

## OUTLINE

Historical Background	897	Treatment of Autoimmune Hemolytic Anemia	903
Classification of Autoimmune Hemolytic Anemia	898	Etiology of Autoimmune Hemolytic Anemia and Predisposing Factors	903
Animal Models of Autoimmune Hemolytic Anemia	898	Genetic Predisposition	903
Mechanisms of Red Blood Cells Destruction in Autoimmune Hemolytic Anemia	899	Gender and Age	904
Cold Reactive Antibodies	899	Infectious Agents	904
Warm Reactive Antibodies	899	Drugs	904
Pathogenicity of Warm Reactive IgG Antibodies	899	Neoplasia	904
Additional Mechanisms of Hemolysis by Warm Antibodies	901	Immune Mechanisms Underlying Loss of Self Tolerance in Warm Autoimmune Hemolytic Anemia	904
Red Blood Cell Autoantigens	902	B Cells and Tolerance	905
Clinical Signs of Autoimmune Hemolytic Anemia	902	T-Helper Cells and Tolerance	905
Laboratory Diagnosis of Autoimmune Hemolytic Anemia	902	Concluding Remarks	906
		References	906

## HISTORICAL BACKGROUND

The earliest descriptions of autoimmune hemolytic anemia (AIHA) date from the 19th century, and the disease was one of the first shown to have an autoimmune pathology. Pioneering work by [Donath and Landsteiner \(1904\)](#) demonstrated that the destruction of red blood cells (RBC) was dependent on the absorption of hemolysins and complement from serum. Further studies were impeded by the difficulty in distinguishing acquired from congenital hemolytic anemias, but an important advance was made using the antiglobulin, or Coombs', test, which detected RBC coated with autoantibodies by agglutinating them with antiserum to human globulin ([Coombs et al., 1945; Boorman et al., 1946; Loutit and Mollison, 1946](#)). Methods for measuring survival of circulating cells in vivo further demonstrated that RBC from patients with acquired hemolytic anemias were destroyed by a "random hemolytic process," rather than being "intrinsically defective" ([Loutit and Mollison, 1946; Mollison, 1959](#)). Together, these developments identified AIHA as a disease in which autoantibodies bind

**TABLE 47.1** Classification of Autoimmune Hemolytic Anemia (AIHA)

	Cold reactive	Warm reactive
<b>CLASSIFICATION BY AUTOANTIBODY TYPE</b>		
Optimum temperature for binding RBC	4°C	37°C
Predominant autoantibody class	IgM (cold agglutinin syndrome) IgG (paroxysmal cold hematuria)	IgG
Predominant site of hemolysis	Intravascular	Extravascular (spleen, liver)
Predominant mechanism of hemolysis	Complement lysis (membrane attack complex)	Phagocytosis via macrophage IgG Fc and complement receptors
<b>CLASSIFICATION BY UNDERLYING DISEASE</b>		
Primary (idiopathic)		Secondary
No underlying disease		Underlying disease (causal or shared etiology)
		Infection
		Other immune disease neoplasia
		Drug induced

RBC and shorten their lifespan, affecting 1–3 per 100,000 of the population (Sokol et al., 1992; Petz and Garratty, 2004a).

## CLASSIFICATION OF AUTOIMMUNE HEMOLYTIC ANEMIA

AIHA can be classified both by the type of autoantibody and by the presence of underlying disease (Sokol et al., 1992; Petz and Garratty, 2004a) (Table 47.1). Pathogenic autoantibodies are divided into either cold (Petz, 2008) or warm (Packman, 2008) reactive, depending on the optimum temperature at which they bind RBC. Up to 7% of the patients have “mixed” pathogenic autoantibodies of both types (Sokol et al., 1981, 1983, 1992). AIHA can also be described as primary, or idiopathic, in the absence of any associated condition, or as secondary if there is a concurrent disease that may be considered causal or has shared etiology (Packman, 2008; Petz, 2008).

## ANIMAL MODELS OF AUTOIMMUNE HEMOLYTIC ANEMIA

Examples of AIHA in laboratory mice have proved valuable in understanding the pathogenesis of the disease. The New Zealand Black (NZB) mouse (Helyer and Howie, 1963; Barker et al., 1993b) develops AIHA spontaneously, and hemolysis can be recapitulated by transgenic expression of a monoclonal anti-RBC autoantibody derived from this strain (Murakami et al., 1992). AIHA is also one of the autoimmune pathologies arising in the nonobese diabetic mouse (Baxter and Mandel, 1991) and from genetic modification to prevent expression of interleukin-2 (IL-2) (Hoyer et al., 2009). In addition to these spontaneous examples, the disease can be induced in healthy murine strains by repeated immunization with rat RBC (Playfair and Marshall-Clarke, 1973; Naysmith et al., 1981) or by infection of C3HeB/FeJ mice with the docile strain of lymphocytic choriomeningitis virus (LCMV) (Coutelier et al., 1994).

AIHA has also been described as a cause of anemia in the domestic dog (Barker et al., 1991), cat (Switzer and Jain, 1981), rabbit (Fox et al., 1971), horse (Mair et al., 1990), and ox (Dixon et al., 1978).

## MECHANISMS OF RED BLOOD CELLS DESTRUCTION IN AUTOIMMUNE HEMOLYTIC ANEMIA

AIHA is a classic example of type II hypersensitivity, with autoantibody-coated RBC removed from the circulation by phagocytes of the reticuloendothelial system (RES), predominantly splenic macrophages, and/or lysis by complement fixation (Packman, 2008; Petz, 2008). Anemia results if the hemolysis is insufficiently compensated for by increased RBC production.

### Cold Reactive Antibodies

Cold reactive anti-RBC autoantibodies are responsible for 15%–20% of human AIHA cases (Petz, 2008). They bind more strongly at 4°C than at higher temperatures, and the pathogenic effects of these antibodies depend more on their thermal amplitude than their titer. Cold autoagglutinins that bind RBC below 10°C–15°C can be demonstrated in the sera of most healthy individuals (Landsteiner and Levine, 1926). In contrast, cold autoantibodies active up to 30°C are associated with cold agglutinin syndrome (CAS) (Petz, 2008), since temperatures in the peripheral circulation can fall below this level. The antibody, usually immunoglobulin (Ig) M, causes intravascular hemolysis if it activates complement to form membrane attack complexes (MAC) (Engelfriet et al., 1981; Petz and Garratty, 2004b), overcoming protective regulators on the RBC such as CD35 (complement receptor 1 CR1), CD55 (decay-accelerating factor), and CD59 (protectin) (Nicholson-Weller et al., 1982; Krych-Goldberg and Atkinson, 2001; Ruiz-Argüelles and Llorente, 2007). RBC coated with C3b may also be temporarily sequestered by macrophages (Engelfriet et al., 1981). In some patients, cold IgM autoantibodies agglutinate RBC in extremities that become chilled, blocking small blood vessels and causing ischemia (Petz, 2008). CAS can be either transient, most frequently as a complication of mycoplasma infection (Costea et al., 1972), or chronic, typically associated with clonal lymphoproliferative disease (Berentsen et al., 2006).

Pathogenic cold reactive autoantibodies also include the Donath–Landsteiner (DL) hemolysins, which cause paroxysmal cold hemoglobinuria (PCH), a dramatic form of AIHA precipitated by chilling of the patient (Petz, 2008). These antibodies, which are IgG, bind RBC if the temperature falls below 37°C, and then fix complement to trigger MAC formation and fulminant intravascular hemolysis when warmed again. PCH was commonly secondary to syphilis when it was first described in the late 19th century but is now rare and typically follows childhood viral infections (Sokol et al., 1982, 1984, 1999).

### Warm Reactive Antibodies

Warm autoantibodies are the most common cause of AIHA and react as well, or more strongly, with RBC at 37°C than at lower temperatures (Sokol et al., 1992; Packman, 2008). Most are of the IgG class and cause extravascular hemolysis, predominantly by Fc receptor (FcγR)-mediated phagocytosis (Engelfriet et al., 1981; Petz and Garratty, 2004b; Packman, 2008). The complement regulators on the RBC membrane (Ruiz-Argüelles and Llorente, 2007) typically prevent MAC formation, but deposition of C3b and C3d is common and can strongly enhance opsonization (Kurlander et al., 1978) by interacting with specific receptors including CR1 and CR3 (Ross and Medof, 1985). Although blood monocytes and hepatic Kupffer cells also express appropriate sets of receptors, splenic macrophages are the main effectors of RBC destruction (Engelfriet et al., 1981; Petz and Garratty, 2004b; Packman, 2008). Some sensitized RBC may be only partially phagocytosed and released back into the circulation as spherocytes, which have a short half-life (Garratty, 1983).

Between 22% (Dausset and Colombani, 1959) and 81% (Pirofsky, 1976) of warm AIHA cases have been reported to be secondary, varying with different interpretations of this classification. The most common associations are with other immune-based conditions, most notably ulcerative colitis, rheumatoid arthritis, and systemic lupus erythematosus (SLE), with neoplasia, particularly chronic lymphocytic leukemia (CLL), with a growing number of drug treatments (Garratty and Arndt, 2014), and with a variety of infectious diseases (Sokol et al., 1992; Petz and Garratty, 2004a).

### Pathogenicity of Warm Reactive IgG Antibodies

Warm reactive IgG anti-RBC autoantibodies vary in their pathogenicity, exemplified by the finding of a positive direct agglutination test (DAT) in a small proportion (1 in 7–15,000) of healthy blood donors (Hernandez-

Jodra et al., 1990; Win et al., 1997; Petz and Garratty, 2004b). The ability to cause hemolysis has been attributed to multiple factors, including titer, subclass, affinity for autoantigen, patterns of heavy chain glycosylation, and also the activity of phagocytes responsible for clearance (Garratty, 1990; Sokol et al., 1992; Petz and Garratty, 2004b).

The amount of IgG blood group alloantibody-coating RBC determines their rate of clearance in healthy subjects (Mollison and Hughes-Jones, 1967; Kelton et al., 1985), but it is less easy to demonstrate a similar relationship for autoantibodies in human AIHA (Rosse, 1971; Chaplin, 1990; Petz and Garratty, 2004b). Serial measurements from individual patients reveal some correlation of hemolysis with autoantibody titer, but many cross-sectional studies fail to do so, using either the DAT or more sensitive and quantitative flow cytometric or ELISA-based techniques to measure RBC-bound IgG (Van der Meulen et al., 1980; Garratty and Nance, 1990; Sokol et al., 1992; Petz and Garratty, 2004b). Autoantibody titer alone also appears to be an unreliable predictor of the severity of hemolysis in canine (Barker et al., 1992b) and murine AIHA (Naysmith et al., 1981; Shen et al., 2003).

The subclass of RBC autoantibody is a potentially important factor in its ability to cause hemolysis, by determining interactions with different types of Fc $\gamma$ R and fixation of complement (Sokol et al., 1992; Petz and Garratty, 2004b). Comprehensive analyses of IgG subclass switch variants of monoclonal anti-RBC antibodies originally derived from NZB mice have established how differences in these Fc-associated effector functions can critically influence pathogenicity (Baudino et al., 2006), with affinity for autoantigen playing a less important role (Fossati-Jimack et al., 1999). In mice there are three activating Fc $\gamma$ R types (Fc $\gamma$ RI, Fc $\gamma$ RIII, and Fc $\gamma$ RIV), all of which are expressed on macrophages and can bind complexed IgG, while only Fc $\gamma$ RI also has high affinity for monomeric IgG (Nimmerjahn and Ravetch, 2008). IgG2a and IgG2b autoantibody switch variants, each of which interacts efficiently with Fc $\gamma$ RIII and activate complement, and mediate the most severe hemolysis in vivo (Baudino et al., 2006). Fc $\gamma$ RIV makes an additional contribution to uptake by these isotypes, and IgG2a also promotes clearance via Fc $\gamma$ RI if it coats RBC at sufficient density to compete with the free monomer in serum (Baudino et al., 2008). The next most pathogenic subclass is IgG3, which activates complement but does not bind any Fc $\gamma$ R, followed by the IgG1 subclass that interacts only with Fc $\gamma$ RIII and fails to fix complement. Overall, IgG2a and IgG2b autoantibodies are 20-fold more potent in causing hemolysis than IgG1 (Baudino et al., 2006). Complement fixation by the murine autoantibodies does not lead to MAC formation, but CR-mediated erythrophagocytosis can be important if there is an extensive opsonization of RBC by C3 associated with binding of high-affinity IgG2b or IgG3 (da Silveira et al., 2002).

Compared with murine studies, the relationships between human RBC autoantibody subclass and hemolysis are less clear (Petz and Garratty, 2004b). Based on interactions with Fc $\gamma$ R and complement, IgG3 would be predicted to be the most pathogenic subclass, followed by IgG1, with IgG2 and IgG4 relatively benign (Engelfriet et al., 1981; Petz and Garratty, 2004b; Sokol et al., 1992). However, in both patients with AIHA and healthy DAT-positive donors with no evidence of hemolysis, IgG1 autoantibody predominates and is the only isotype detected on RBC using agglutination-based techniques in up to 80% of each group (Garratty, 1989). Furthermore, IgG3 can be detected by DAT not only in patients but also in normal blood donors, although RBC sensitization with IgG4 alone may be restricted to healthy individuals (Garratty, 1989). Sensitive flow cytometric and ELISA methods (Sokol et al., 1990a; Garratty and Nance, 1990) have confirmed that increased levels of RBC-bound IgG3 are not necessarily associated with disease and suggest instead that autoantibodies of multiple IgG subclasses are common in AIHA and that this diversity is important in promoting hemolysis via synergistic effects (Sokol et al., 1990a). The three families of human activating Fc $\gamma$ R (Fc $\gamma$ RI, Fc $\gamma$ RIIa/Fc $\gamma$ RIIc, and Fc $\gamma$ RIIIa/Fc $\gamma$ RIIIb) (Nimmerjahn and Ravetch, 2008) may each play a role in RBC uptake. As in mice, only Fc $\gamma$ RI has a high affinity for monomeric IgG but may mediate erythrophagocytosis under conditions that allow RBC-bound IgG to compete with a monomer in serum, particularly in the hemoconcentrated environment of the spleen (Kelton et al., 1985; Barker et al., 1992b). Although MAC formation rarely contributes to hemolysis in the patients with warm antibodies, complement fixation appears to be an important determinant of disease (Petz and Garratty, 2004b). C3 can be detected by DAT in up to 50% of AIHA patients (Garratty, 1989) and is quantitatively associated with hemolysis (Freedman et al., 1982), reflecting synergy between Fc $\gamma$ R and CR in RBC uptake (Sokol et al., 1992).

Changes in glycosylation of the CH2 domain of the IgG heavy chain may also influence interactions with Fc $\gamma$ R and complement, with loss of either terminal sialic acid or galactose residues suggested to alter the pathogenicity of murine RBC autoantibody (Baudino et al., 2006). There is evidence in rheumatoid arthritis that IgG lacking galactose (G0) can interact with mannose-binding protein, thereby fixing complement and triggering inflammation (Malhotra et al., 1995). However, in AIHA it seems likely that RBC autoantibody may be less hemolytic than G0, since the loss of galactose reduces the affinity of IgG for Fc $\gamma$ RIII (Hadley et al., 1995). Although G0 RBC

autoantibodies have been identified in some AIHA patients and in NZB mice, the levels can vary widely over time in individuals and show no correlation with the severity of the disease (Barker et al., 1999a,b).

The final, important, factor determining the hemolytic potential of warm autoantibodies is the effectiveness of the RES in clearing sensitized RBC (Sokol et al., 1992; Petz and Garratty, 2004b). The ability of macrophages to phagocytose RBC can be enhanced by infections that upregulate Fc $\gamma$ R expression (Atkinson and Frank, 1974; Coutelier et al., 2007) or compromised by saturation with immune complexes in AIHA secondary to SLE (Frank et al., 1979). Drugs such as corticosteroids can also inhibit phagocytosis by downregulating Fc $\gamma$ R expression (Fries et al., 1983; Kelton, 1985). The cytokine milieu is a major influence on macrophage activation state, and the severity of NZB AIHA can be ameliorated by gene therapy to increase levels of circulating IL-4 (Youssef et al., 2005).

## Additional Mechanisms of Hemolysis by Warm Antibodies

Although Fc $\gamma$ R- and CR-mediated erythrophagocytosis of IgG-coated RBC is the major cause of hemolysis in warm AIHA, there is evidence for other pathogenic mechanisms (Sokol et al., 1992; Petz and Garratty, 2004b).

In rare patients with warm AIHA, the main autoantibody class may be IgM or IgA, and not IgG. If warm IgM autoantibodies predominate, they can trigger MAC formation and fulminant intravascular hemolysis (Freedman et al., 1987). IgA autoantibodies may cause hemolysis by Fc-mediated uptake and cytotoxicity (Clark et al., 1984), or hemagglutination in the spleen (Baudino et al., 2007). More sensitive techniques than the DAT detect a higher prevalence of cosensitization of IgG with low levels of IgM and/or IgA, and such coating of RBC with multiple antibody classes is associated with severe hemolysis, suggesting the importance of synergistic effects (Sokol et al., 1990b).

**TABLE 47.2** Red Blood Cells Autoantigens in Human Autoimmune Hemolytic Anemia (AIHA) Caused by Cold Autoantibodies

Form of AIHA	Common autoantibody Specificity	Rare autoantibody Specificity
CAS	I (~90% patients)	i, Pr, A, B
PCH	P (~90% patients)	i, p, HI, I

CAS, Cold agglutinin syndrome; PCH, paroxysmal cold hematuria.

**TABLE 47.3** Red Blood Cells (RBC) Autoantigens in Human and Animal Autoimmune Hemolytic Anemia (AIHA) Caused by Warm Autoantibodies

Species	B-cell autoantigen (dominant antigen in bold)	Th-cell autoantigen identified	Unprimed autoreactive Th cells in health	Regulatory T-cell response
Human AIHA	Rh proteins (~70% patients) Glycophorin A Band 3	Rh proteins	Yes	IL-10 response to Rh protein Epitopes
Canine AIHA	Glycophorins (~50% patients) Band 3	Glycophorins	Yes	Not examined
Murine AIHA				
NZB mouse	<b>Band 3</b> Band 4.1 (pr) Phosphatidylcholine (pr)	Band 3	Not applicable	Weak IL-10 response to Band 3
Induced by rat RBC	<b>Band 3</b> (cr) Glycophorins (cr)	Band 3	Yes	Recovery due to suppression by CD25 <sup>+</sup> cells
Induced by LCMV	<b>Band 3</b>	Not examined	Yes	Not examined

Pr, Polyreactive antibody also binds nuclear antigens, for example, histones; cr, cross reacts with rat RBC antigen.

Modified from Barker, R.N., Vickers, M.A., Ward, F.J., 2007. Controlling autoimmunity—lessons from the study of red blood cells as model antigens. *Immunol. Lett.* 108, 20–26 (Barker et al., 2007).

Another mechanism, which may be of particular relevance in patients with very low levels of RBC-bound IgG, is that splenic macrophages are instead “armed” with the autoantibody bound to Fc $\gamma$ RI, allowing them to capture circulating RBC (Griffiths et al., 1994). Antibody-dependent cell-mediated cytotoxicity may also play a role in hemolysis (Garratty, 1983; Griffiths et al., 1994), mediated not only by macrophages but also potentially by K cells (Urbaniak and Griess, 1980) or activated neutrophils (Engelfriet et al., 1981). In some patients, there is evidence that autoantibodies can interfere with erythropoiesis (Crosby and Rappaport, 1956) or RBC egress from the bone marrow (Conley et al., 1982), as well as causing hemolysis.

## Red Blood Cell Autoantigens

Many of the major RBC autoantigens in AIHA have been identified (Tables 47.2 and 47.3).

Serological studies have established that cold reactive RBC autoantibodies in healthy individuals, and pathogenic species with high thermal amplitude in CAS, are most commonly directed to the Ii blood group system of carbohydrate differentiation antigens (Berentsen and Tjønnfjord, 2012). On adult RBC, the I antigen predominates, while i is expressed at low levels. Antibodies from approximately 90% of CAS patients recognize the I antigen (Jenkins et al., 1960), with anti-i (Marsh and Jenkins, 1960) accounting for most of the remainder. Other, very rare specificities for autoantibodies in CAS include Pr (Dellagi et al., 1981). In PCH, anti-P autoantibodies are detected in at least 90% of the patients (Sokol et al., 1999).

The most common targets in human warm AIHA, recognized in over 70% of cases, are the Rh proteins (Weiner and Voss, 1963; Barker et al., 1992a; Leddy et al., 1993), which also express important blood groups (Avent and Reid, 2000). Autoantibodies reactive against the glycophorins, or against the RBC anion channel protein, Band 3, are produced in some patients (Victoria et al., 1990; Barker et al., 1992a; Leddy et al., 1993). The major canine RBC autoantigens are the glycophorins, with autoantibodies from some cases specific for Band 3 (Barker et al., 1991). In mice, Band 3 is the dominant autoantigen in NZB disease (Barker et al., 1993b; de Sá Oliveira et al., 1996) and in AIHA following LCMV infection (Mazza et al., 1997) and, together with glycophorins, is also a target for autoantibodies induced by rat RBC (Barker et al., 1993a).

In addition to the autoantigens relevant to AIHA pathogenesis, RBC can also express cryptic determinants recognized by naturally occurring IgG autoantibodies. These include spectrin, the major component of the internal RBC cytoskeleton (Lutz and Wipf, 1982; Ballas, 1989; Barker et al., 1991, 1993a) and senescent red cell antigen (Alderman et al., 1981), which is exposed on Band 3 by aged RBC (Kay et al., 1990). The autoantibodies are thought to provide physiological mechanisms for disposing of damaged and effete RBC (Wiener et al., 1986; Pantaleo et al., 2008).

## CLINICAL SIGNS OF AUTOIMMUNE HEMOLYTIC ANEMIA

In both CAS (Petz, 2008) and warm AIHA (Packman, 2008), the predominant clinical features reflect the anemia, which most commonly causes lethargy and dyspnea. Signs include pallor and icterus, and massive hemolysis may precipitate hemoglobinuria. In CAS there may also be cyanosis or even necrosis of the bodily extremities (Petz, 2008). AIHA due to DL antibodies is typified by recurrent bouts of anemia and hemoglobinuria precipitated by exposure to cold (Petz, 2008). In warm AIHA, splenomegaly or hepatomegaly can be associated with extravascular hemolysis (Packman, 2008). Where AIHA is secondary, the signs of the underlying disease may predominate.

## LABORATORY DIAGNOSIS OF AUTOIMMUNE HEMOLYTIC ANEMIA

In addition to anemia, most cases show evidence of erythroid regeneration, with reticulocytosis (Packman, 2008; Petz, 2008). However, there can be a poor erythroid response (Liesveld et al., 1987), due to the physiological lag in increasing RBC production following acute hemolysis, or to autoimmune reactions inhibiting RBC regeneration (Conley et al., 1982), or to an underlying bone marrow disorder (Lefrere et al., 1986). Evidence of hemolysis can also be provided by increased bilirubin, aspartate transaminase, and lactate dehydrogenase levels. RBC autoagglutination or spherocytes may be seen in cold and warm AIHA, respectively.

Detection of RBC autoantibodies confirms the diagnosis of AIHA. The DAT has been the classic tool for measuring RBC-bound autoantibodies and complement (Petz and Garratty, 2004a). However, benign immunoproteins on the RBC surface can cause a positive DAT (Heddle et al., 1988; Huh et al., 1988), and the test also gives false-negative results in 3%–11% of AIHA cases (Sokol et al., 1985, 1988; Petz and Garratty, 2004a). These limitations have led to more sensitive methods to detect RBC-bound immunoglobulins, including radioimmunoassay (Kaplan and Quimby, 1983), flow cytometry (Van der Meulen et al., 1980; Garratty and Nance, 1990), or ELISA (Sokol et al., 1985, 1988).

## TREATMENT OF AUTOIMMUNE HEMOLYTIC ANEMIA

Anemia in both cold and warm AIHA requires supportive care and, if life-threatening, transfusion (Packman, 2008; Petz, 2008).

Patients with pathogenic cold reactive autoantibodies should be protected from unnecessary exposure to low temperatures (Petz, 2008). Cases of secondary disease, for example, CAS or PCH associated with infection, may be transient or resolve with treatment of the underlying condition. Corticosteroids or cytotoxic drugs have been used to treat CAS, but the response is frequently poor (Petz, 2008), and better results have been obtained by targeting B cells with the anti-CD20 monoclonal antibody rituximab (Berentsen et al., 2004), which is now considered the first line of therapy (Zanella and Barcellini, 2014).

Corticosteroids, such as prednisolone, are the most common first-line therapy for warm AIHA and can be highly effective, although many patients relapse after withdrawal of these drugs (Packman, 2008). Corticosteroids can both downregulate macrophage Fc $\gamma$ R to improve the survival of IgG-sensitized RBC (Fries et al., 1983) and reduce autoantibody production (Rosse, 1971; Sokol and Hewitt, 1985), but the rapid response they typically elicit suggests the importance of the former effect (Packman, 2008). Cytotoxic drugs such as cyclophosphamide or azathioprine may be used as second-line therapies to suppress immune responsiveness, or splenectomy can also be considered to remove a major site of extravascular hemolysis (Packman, 2008; Crowther et al., 2011). Ablation of B cells with rituximab has emerged as an effective treatment for AIHA that is refractory to conventional treatments (Zecca et al., 2003; Peñalver et al., 2010; Crowther et al., 2011), is now the preferred second-line therapy after corticosteroids in some centers, and also shows promise as a first-line treatment when combined with steroids (Dierickx et al., 2015).

## ETOLOGY OF AUTOIMMUNE HEMOLYTIC ANEMIA AND PREDISPOSING FACTORS

It is clear that autoimmune diseases result from the interaction of multiple factors (Shoenfeld and Isenberg, 1989; Cho and Gregersen, 2011). Genetic background, gender, age, environmental factors such as infections, drugs, and neoplasia have all been implicated in the etiology of AIHA.

### Genetic Predisposition

The possibility of a genetic predisposition to human AIHA was raised by rare reports of familial disease (Cordova et al., 1966; Pirofsky, 1968; Pollock et al., 1970; Lippman et al., 1982; Olanoff and Fudenberg, 1983). Particular human leucocyte antigen (HLA) haplotypes are the strongest genetic determinants of many autoimmune diseases (Shoenfeld and Schwartz, 1984; Caillat-Zucman, 2009; Cho and Gregersen, 2011; Lessard et al., 2012), and warm AIHA is positively associated with HLA-DR15, with approximately 60% of the patients expressing this allele (Stott et al., 2002). Genome-wide association studies of other human autoimmune diseases reveal that large numbers of non-HLA genes further contribute to susceptibility (Cho and Gregersen, 2011; Lessard et al., 2012), and predisposition of the NZB mouse to AIHA has also been attributed to multiple loci (Chused et al., 1987; Lee et al., 2004; Scatizzi et al., 2012). The molecular bases of most such associations remain unknown. However, in common with other autoimmune-prone strains, the NZB mouse shares a promoter haplotype that is associated with reduced expression and function of the inhibitory Fc $\gamma$ R Fc $\gamma$ RIIb (Pritchard et al., 2000). The effects of the polymorphism on Fc $\gamma$ RIIb expressed by macrophages and B cells are enhanced phagocytosis of IgG-opsonized RBC and increased antibody responsiveness (Pritchard et al., 2000; Kikuchi et al., 2006).

## Gender and Age

Unlike most other human autoimmune diseases (Talal and Ahmed, 1987), the incidence of AIHA is no higher in women than in men (Pirofsky, 1976; Sokol et al., 1981, 1992). AIHA becomes progressively more common with age (Sokol et al., 1981, 1992), perhaps reflecting defects in immune regulation (Tomer and Shoenfeld, 1988; Talor and Rose, 1991; Akbar and Fletcher, 2005).

## Infectious Agents

Infectious agents are commonly implicated in provoking autoimmune disease in susceptible individuals (Shoenfeld and Isenberg, 1989), with almost 10% of human AIHA patients reported to have concurrent bacterial or viral conditions (Sokol et al., 1981, 1992), and RBC autoantibodies induced in mice by LCMV (Coutelier et al., 1994). Cross-reactivity between bacterial lipopolysaccharide and the blood group antigen I has been proposed to explain the high incidence of transient CAS which follows human *Mycoplasma pneumoniae* infection (Costea et al., 1972). The potential for mimicry to induce warm AIHA is exemplified by the disease that develops in mice following repeated injections of RBC expressing cross-reactive antigens from a closely related species, the rat (Playfair and Marshall-Clarke, 1973; Barker et al., 1993a). Studies of NZB mice also suggest that cross-reactivity between a microbe and a self-epitope can focus a predisposition to autoimmunity onto a particular target, even when not a primary or sufficient cause of disease (Hall et al., 2007). A second mechanism linking infection and AIHA is the ability of innate microbial stimuli and the cytokines they induce to activate antigen-presenting cells (APC) and therefore to enhance the immunogenicity of RBC autoantigens (Elson et al., 1995; Coutelier et al., 2007). Changes to the cytokine milieu resulting from infection, particularly IFN- $\gamma$  production, can also enhance RBC phagocytosis by modulating both the subclass of autoantibody and the activation of macrophages (Atkinson and Frank, 1974; Coutelier et al., 2007). Finally, particular infectious agents associated with AIHA, such as Epstein–Barr virus, may directly infect and dysregulate immune cells (Bowman et al., 1974).

## Drugs

Immune-mediated hemolytic anemias are a rare side effect of many drugs, including the penicillins, but in most cases, the antibodies are not strictly autoreactive and only bind RBC in the presence of the drug (Garratty, 2010; Garratty and Arndt, 2014). However, other examples such as  $\alpha$ -methyldopa can induce true RBC autoantibodies that may cause AIHA (Sokol et al., 1981), by perturbing immune regulation (Kirtland et al., 1980) or altering the antigenic structure of RBC (Owens et al., 1982).

## Neoplasia

Up to 22% of the human AIHA cases suffer from some form of concurrent neoplastic disease (Sokol et al., 1981, 1992). Many patients with CAS have a monoclonal RBC autoantibody associated with a clonal lymphoproliferative disorder (Silberstein et al., 1986), most frequently classified as lymphoplasmacytic lymphoma (Berentsen and Tjønnfjord, 2012). Warm AIHA and autoimmune thrombocytopenia are both closely associated with CLL. Over 10% of AIHA cases are also diagnosed with the condition (Sokol et al., 1992), and circulating leukocytes with an abnormal CLL-like phenotype can be detected in further 19% of the patients classified with apparent primary AIHA (Mittal et al., 2008). Conversely, up to 14% of CLL patients have AIHA or elevated levels of RBC-bound autoantibody (Dearden et al., 2008). One model to explain this association is that the large numbers of the malignant CLL cells present in the spleen drive an autoimmune response to circulating cells by acting as aberrant APC (Hall et al., 2005).

## IMMUNE MECHANISMS UNDERLYING LOSS OF SELF TOLERANCE IN WARM AUTOIMMUNE HEMOLYTIC ANEMIA

The study of specific pathogenic responses to RBC autoantigens has enabled mechanisms underlying the loss of self-tolerance in warm AIHA to be characterized.

## B Cells and Tolerance

Although RBC destruction is autoantibody mediated, it is not necessary to invoke a defect in B-cell repertoire selection to explain the loss of tolerance in AIHA. Central tolerance of self-reactive B cells is incomplete in healthy individuals, since anti-RBC autoantibodies with a wide range of specificities can be induced to cause AIHA in murine strains that have no predisposition to spontaneous disease (Day et al., 1989; Barker et al., 1993a). Nevertheless, one model of AIHA, created by transgenic expression of an anti-RBC monoclonal autoantibody from NZB mice, does illustrate the potential for pathology to result from failure to censor autoreactive B cells (Murakami et al., 1992). The B cells producing monoclonal antibody are sequestered in the peritoneal cavity and survive to cause disease in only a proportion of mice, depending on whether they are deleted by contact with RBC (Murakami et al., 1992).

## T-Help Cells and Tolerance

There is a long-standing belief that self-tolerance in the T-cell compartment is less secure than for B cells, and that, in health, antibody-mediated diseases such as AIHA are prevented due to lack of effective help (Naysmith et al., 1981; Elson and Barker, 2000). The vast majority of IgG responses are T dependent (Kelsoe, 1995), and the production of warm autoantibodies in AIHA appears to be no exception (Elson and Barker, 2000). NZB IgG autoantibody production in vivo is retarded by treatment with anti-CD4 monoclonal antibody (Oliveira et al., 1994), or by CD4 gene deletion (Chen et al., 1996), and splenic T-helper (Th) cells from NZB mice but not MHC-matched healthy strains, proliferate in vitro in response to the major murine RBC autoantigen, Band 3 (Perry et al., 1996; Shen et al., 1996). Furthermore, NZB disease is accelerated by immunization with an insoluble peptide bearing the dominant Th-cell epitope from Band 3 and ameliorated by mucosal administration of a soluble analog of this sequence (Shen et al., 2003). Other murine models are also Th dependent, since anti-CD4 mAb-treated mice do not develop AIHA induced by LCMV (Coutelier et al., 1994), and T-cell depletion prevents RBC autoantibody production in response to immunization with cross-reactive rat antigens (Naysmith et al., 1981). Findings in human AIHA are also consistent with the need for help. Rh autoantigen-specific effector Th cells that have been activated in vivo can be demonstrated in the peripheral blood and/or spleen from all patients with anti-Rh autoantibodies (Barker et al., 1997), but from very few healthy donors (Barker and Elson, 1994; Barker et al., 1997).

Warm AIHA in patients and NZB mice is associated with specific helper responses that are dominated by the Th1 subset, and inducing a corresponding Th2 bias can prevent or ameliorate NZB disease (Shen et al., 1996, 2003; Hall et al., 2002). Such a shift may be therapeutically beneficial partly because of the associated switch of the autoantibody to a less pathogenic isotype. Recent studies of human AIHA reveal that disease is also strongly associated with IL-17 responses to RBC, raising the possibility that Th17 cells may also provide help for autoreactive B cells to produce pathogenic IgG subclasses (Hall et al., 2012).

In common with B cells, it appears that potentially autoaggressive T cells can escape central tolerance as part of normal immune development and that failure of peripheral mechanisms to control their activation results in AIHA. Healthy mice (Naysmith et al., 1981; Barker et al., 1993a, 2002), dogs (Corato et al., 1997), and humans (Barker and Elson, 1994) all harbor naïve Th cells that can be stimulated to proliferate in vitro by RBC autoantigens. Comparison with AIHA patients demonstrates that they differ, not in the presence or fine specificity of circulating RBC-specific autoreactive Th cells but in the finding that these lymphocytes are activated in vivo (Barker and Elson, 1994; Barker et al., 1997). One possibility is that the surviving autoreactive Th cells are specific for RBC self-epitopes that are normally inefficiently processed and presented by APC from the intact antigen (Elson et al., 1995) and therefore unavailable to induce tolerance in the thymus. This model is supported by studies of human AIHA, where activated Th cells are specific for epitopes on the Rh protein autoantigens that are “cryptic” or subdominant (Hall et al., 1999). Such epitopes may be more efficiently presented and drive an autoaggressive Th response if APC are activated, for example, by infection (Elson et al., 1995) or by the accumulation of aberrant APC types such as CLL cells (Hall et al., 2005). The importance of antigen presentation is also illustrated by observations that the immunogenicity of RBC can be increased by changes in their innate receptor recognition by dendritic cells (Yi et al., 2015).

It is now recognized that CD4<sup>+</sup> regulatory T (Treg) cells are important mediators of peripheral self-tolerance (Roncarolo et al., 2006; Sakaguchi et al., 2010; Shevach, 2011). AIHA induced by rat RBC immunization of mice provided an early example of such “infectious tolerance,” since the autoimmune response is transient and the mice become refractory to further induction of disease, with splenocytes transferred from recovered animals

providing protection to naïve recipients (Playfair and Marshall-Clarke, 1973; Naysmith et al., 1981). Both the “adaptive” IL-10<sup>+</sup> (Roncarolo et al., 2006) and “natural” CD25<sup>+</sup>FoxP3<sup>+</sup> (Sakaguchi et al., 2010; Shevach, 2011) forms of Treg cell have been implicated in maintaining or restoring tolerance to RBC autoantigens. In murine AIHA induced by rat RBC, recovery is associated with protective CD251 T cells (Mqadmi et al., 2005) and the development of AIHA in gene-deleted mice that lack IL-2 has been attributed to a deficiency of the “natural” Treg population (Hoyer et al., 2009). Treg cells specific for the target Rh autoantigens can be found in the peripheral blood or spleen of patients with AIHA and are capable of inhibiting the Th1 effector responses in vitro by secretion of IL-10 (Hall et al., 2002). The Rh-specific Treg cells have been cloned and shown to mediate inhibitory activity only after stimulation by cognate antigen, and not in response to polyclonal activators, illustrating the importance of specificity in their function (Ward et al., 2008). Although able to secrete the “adaptive” inhibitory cytokine IL-10, these Treg cells also express the “natural” marker FoxP3, and the Th1 transcription factor T-bet, revealing plasticity between the different regulatory forms and effector subsets (Ward et al., 2008).

## CONCLUDING REMARKS

In AIHA, many of the pathogenetic mechanisms by which autoantibodies can cause disease have been defined. The identification of major human and murine RBC autoantigens has also provided unique insights into the control of specific, pathogenic immune responses in both human and experimental animal disease. This work to understand how immunological tolerance is lost and can be restored holds out the prospect of more effective, specific therapies.

## References

- Akbar, A.N., Fletcher, J.M., 2005. Memory T cell homeostasis and senescence during aging. *Curr. Opin. Immunol.* 17, 480–485.
- Alderman, E.M., Fudenberg, H.H., Lovins, R.E., 1981. Isolation and characterization of an age-related antigen present on senescent human red blood cells. *Blood* 58, 341–349.
- Atkinson, J.P., Frank, M.M., 1974. The effect of *Bacillus Calmette-Guérin*-induced macrophage activation on the in vivo clearance of sensitized erythrocytes. *J. Clin. Invest.* 53, 1742–1749.
- Avent, N.D., Reid, M.E., 2000. The Rh blood group system: a review. *Blood* 95, 375–387.
- Ballas, S.K., 1989. Spectrin autoantibodies in normal human serum and in polyclonal blood grouping sera. *Br. J. Haematol.* 71, 137–139.
- Barker, R.N., Elson, C.J., 1994. Multiple self-epitopes on the Rhesus polypeptides stimulate immunologically ignorant human T-cells in vitro. *Eur. J. Immunol.* 2, 1578–1582.
- Barker, R.N., Gruffydd-Jones, T.J., Stokes, C.R., Elson, C.J., 1991. Identification of autoantigens in canine autoimmune haemolytic anaemia. *Clin. Exp. Immunol.* 85, 33–40.
- Barker, R.N., Casswell, K.M., Reid, M.E., Sokol, R.J., Elson, C.J., 1992a. Identification of autoantigens in autoimmune haemolytic anaemia by a non-radioisotope immunoprecipitation method. *Br. J. Haematol.* 8, 126–132.
- Barker, R.N., Gruffydd-Jones, T.J., Stokes, C.R., Elson, C.J., 1992b. Autoimmune haemolysis in the dog: relationship between anaemia and the levels of red blood cell immunoglobulins and complement measured by an enzyme-linked antiglobulin test. *Vet. Immunol. Immunopathol.* 3, 1–20.
- Barker, R.N., Casswell, K.M., Elson, C.J., 1993a. Identification of murine erythrocyte autoantigens and cross-reactive rat antigens. *Immunology* 78, 568–573.
- Barker, R.N., De Sá Oliveira, G.G., Elson, C.J., Lydyard, P.M., 1993b. Pathogenic autoantibodies in the NZB mouse are specific for erythrocyte Band 3 protein. *Eur. J. Immunol.* 23, 1723–1726.
- Barker, R.N., Hall, A.M., Standen, G.R., Jones, J., Elson, C.J., 1997. Identification of T-cell epitopes on the Rhesus polypeptides in autoimmune hemolytic anemia. *Blood* 90, 2701–2715.
- Barker, R.N., Leader, K.A., Elson, C.J., 1999a. Serial changes in the galactosylation of autoantibody and serum IgG in autoimmune haemolytic anaemia. *Autoimmunity* 31, 103–108.
- Barker, R.N., Young, R.D., Leader, K.A., Elson, C.J., 1999b. Galactosylation of serum IgG and autoantibodies in murine models of autoimmune haemolytic anaemia. *Clin. Exp. Immunol.* 117, 449–454.
- Barker, R.N., Shen, C.-R., Elson, C.J., 2002. T-cell specificity in murine autoimmune haemolytic anaemia induced by rat red blood cells. *Clin. Exp. Immunol.* 129, 208–213.
- Barker, R.N., Vickers, M.A., Ward, F.J., 2007. Controlling autoimmunity—lessons from the study of red blood cells as model antigens. *Immunol. Lett.* 108, 20–26.
- Baudino, L., da Silveira, S.A., Nakata, M., Izui, S., 2006. Molecular and cellular basis for pathogenicity of autoantibodies, lessons from murine monoclonal autoantibodies. *Springer Semin. Immunopathol.* 28, 175–184.
- Baudino, L., Fossati-Jimack, L., Chevalley, C., Martinez-Soria, E., Shulman, M.J., Izui, S., 2007. IgM and IgA anti-erythrocyte autoantibodies induce anemia in a mouse model through multivalency-dependent hemagglutination but not through complement activation. *Blood* 109, 5355–5362.

- Baudino, L., Nimmerjahn, F., da Silveira, S.A., Martinez-Soria, E., Saito, T., Carroll, M., et al., 2008. Differential contribution of three activating IgG Fc receptors (Fc $\gamma$ RI, Fc $\gamma$ RIII, and Fc $\gamma$ RIV) to IgG2a- and IgG2b-induced autoimmune hemolytic anemia in mice. *J. Immunol.* 180, 1948–1953.
- Baxter, A.G., Mandel, T.E., 1991. Hemolytic anemia in non-obese diabetic mice. *Eur. J. Immunol.* 21, 2051–2055.
- Berentsen, S., Tjønnfjord, G.E., 2012. Diagnosis and treatment of cold agglutinin mediated autoimmune hemolytic anemia. *Blood. Rev.* 26, 107–115.
- Berentsen, S., Ulvestad, E., Gjertsen, B.T., Hjorth-Hansen, H., Langholm, R., Knutsen, H., et al., 2004. Rituximab for primary chronic cold agglutinin disease, a prospective study of 37 courses of therapy in 27 patients. *Blood* 103, 2925–2928.
- Berentsen, S., Ulvestad, E., Langholm, R., Beiske, K., Hjorth-Hansen, H., Ghanima, W., et al., 2006. Primary chronic cold agglutinin disease, a population based clinical study of 86 patients. *Haematologica* 91, 460–466.
- Boorman, K.E., Dodd, B.E., Loutit, J.F., 1946. Haemolytic icterus (acholuric jaundice), congenital and acquired. *Lancet* 1, 812–814.
- Bowman, H.S., Marsh, W.L., Schumacher, H.R., Oyen, R., Reihart, J., 1974. Auto anti-N immunohemolytic anemia in infectious mononucleosis. *Am. J. Clin. Pathol.* 61, 465–472.
- Caillat-Zucman, S., 2009. Molecular mechanisms of HLA association with autoimmune diseases. *Tissue Antigens* 73, 1–8.
- Chaplin Jr., H., 1990. Red cell-bound immunoglobulin as a predictor of severity of hemolysis in patients with autoimmune hemolytic anemia. *Transfusion* 30, 576–578.
- Chen, S., Takeoka, Y., Ansari, A.A., Boyd, R., Klinman, D.M., Gershwin, M.E., 1996. The natural history of disease expression in CD4 and CD8 gene-deleted New Zealand Black (NZB) mice. *J. Immunol.* 157, 2676–2684.
- Cho, J.H., Gregersen, P.K., 2011. Genomics and the multifactorial nature of human autoimmune disease. *N. Engl. J. Med.* 365, 1612–1623.
- Chused, T.M., McCoy, K.L., Lal, R.B., Brown, E.M., Baker, P.J., 1987. Multigenic basis of autoimmune disease in New Zealand mice. *Concepts Immunopathol.* 4, 129–143.
- Clark, D.A., Dessypris, E.N., Jenkins Jr., D.E., Krantz, S.B., 1984. Acquired immune hemolytic anemia associated with IgA erythrocyte coating, investigation of hemolytic mechanisms. *Blood* 64, 1000–1005.
- Conley, C.L., Lippman, S.M., Ness, P.M., Petz, L.D., Branch, D.R., Gallagher, M.T., 1982. Autoimmune hemolytic anemia with reticulocytopenia and erythroid marrow. *N. Engl. J. Med.* 306, 281–286.
- Coombs, R.R.A., Mourant, A.E., Race, R.R., 1945. A new test for the detection of weak and “incomplete” Rh agglutinins. *Br. J. Exp. Pathol.* 26, 255–266.
- Corato, A., Shen, C.-R., Mazza, G., Barker, R.N., Day, M.J., 1997. Proliferative responses of peripheral blood mononuclear cells from normal dogs and dogs with autoimmune haemolytic anaemia to red blood cell antigens. *Vet. Immunol. Immunopathol.* 59, 191–204.
- Cordova, M.S., Baez-Villasenor, J., Mendez, J.J., Campos, E., 1966. Acquired hemolytic anemia with positive antiglobulin (Coombs') test in mother and daughter. *Arch. Intern. Med.* 117, 692–695.
- Costea, N., Yakulis, V.J., Heller, P., 1972. Inhibition of cold agglutinins (anti-I) by *M. pneumoniae* antigens. *Proc. Soc. Exp. Biol. Med.* 139, 476–479.
- Coutelier, J.-P., Johnston, S.J., El Idrissi, M. el-A., Pfau, C.J., 1994. Involvement of CD4+ cells in lymphocytic choriomeningitis virus-induced autoimmune anemia and hypergammaglobulinemia. *J. Autoimmun* 7, 589–599.
- Coutelier, J.-P., Detalle, L., Musaji, A., Meite, M., Izui, S., 2007. Two-step mechanism of virus-induced autoimmune hemolytic anemia. *Ann. N. Y. Acad. Sci.* 1109, 151–157.
- Crosby, W.H., Rappaport, H., 1956. Reticulocytopenia in autoimmune hemolytic anemia. *Blood* 11, 926–936.
- Crowther, M., Chan, Y.L., Garbett, I.K., Lim, W., Vickers, M.A., Crowther, M.A., 2011. Evidence-based focused review of the treatment of idiopathic warm immune hemolytic anemia in adults. *Blood* 118, 4036–4040.
- da Silveira, S.A., Kikuchi, S., Fossati-Jimack, L., Moll, T., Saito, T., Verbeek, J.S., et al., 2002. Complement activation selectively potentiates the pathogenicity of the IgG2b and IgG3 isotypes of a high affinity anti-erythrocyte autoantibody. *J. Exp. Med.* 195, 665–672.
- Dausset, J., Colombani, J., 1959. The serology and prognosis of 128 cases of autoimmune hemolytic anemia. *Blood* 14, 1280–1301.
- Day, M.J., Russell, J., Kitwood, A.J., Ponsford, M., Elson, C.J., 1989. Expression and regulation of erythrocyte auto-antibodies in mice following immunization with rat erythrocytes. *Eur. J. Immunol.* 19, 795–801.
- de Sá Oliveira, G.G., Izui, S., Ravirajan, C.T., Mageed, R.A.K., Lydyard, P.M., Elson, C.J., et al., 1996. Diverse antigen specificity of erythrocyte-reactive monoclonal autoantibodies from NZB mice. *Clin. Exp. Immunol.* 10, 313–320.
- Dearden, C., Wade, R., Else, M., Richards, S., Milligan, D., Hamblin, T., et al., 2008. The prognostic significance of a positive direct anti-globulin test in chronic lymphocytic leukemia, a beneficial effect of the combination of fludarabine and cyclophosphamide on the incidence of hemolytic anemia. *Blood* 111, 1820–1826.
- Dellagi, K., Brouet, J.C., Schenmetzler, C., Praloran, V., 1981. Chronic hemolytic anemia due to a monoclonal IgG cold agglutinin with anti-Pr specificity. *Blood* 57, 189–191.
- Dierickx, D., Kentos, A., Delannoy, A., 2015. The role of rituximab in adults with warm antibody autoimmune hemolytic anemia. *Blood* 125, 3223–3229.
- Dixon, P.M., Matthews, A.G., Brown, R., Millar, P.M., Ritchie, J.S.D., 1978. Bovine auto-immune haemolytic anaemia. *Vet. Rec.* 103, 155–157.
- Donath, J., Landsteiner, K., 1904. Über paroxysmale Hämoglobinurie. *Munch. Med. Wochenschr.* 51, 1590–1593.
- Elson, C.J., Barker, R.N., 2000. Helper T cells in antibody-mediated, organ specific autoimmunity. *Curr. Opin. Immunol.* 12, 664–669.
- Elson, C.J., Barker, R.N., Thompson, S.J., Williams, N.A., 1995. Immunologically ignorant T-cells, epitope spreading and repertoire limitation. *Immunol. Today* 1, 71–76.
- Engelfriet, C.P., Von dem Borne, A.E.G.Kr, Beckers, D., Van der Meulen, F.W., Fleer, A., Roos, D., et al., 1981. Immune destruction of red cells. Seminar on Immune Mediated Cell Destruction. American Association of Blood Banks, Washington DC, pp. 93–130.
- Fossati-Jimack, L., Reininger, L., Chicheportiche, Y., Clynes, R., Ravetch, J.V., Honjo, T., et al., 1999. High pathogenic potential of low-affinity autoantibodies in experimental autoimmune hemolytic anemia. *J. Exp. Med.* 190, 1689–1696.
- Fox, R.R., Meier, H., Crary, D.D., Norberg, R.F., Myers, D.D., 1971. Hemolytic anemia associated with thymoma in the rabbit. Genetic studies and pathological findings. *Oncology* 25, 372–382.

- Frank, M.M., Hamburger, M.I., Lawley, T.J., Kimberley, R.P., Plotz, P.H., 1979. Defective reticuloendothelial system Fc-receptor function in systemic lupus erythematosus. *N. Eng. J. Med.* 300, 518.
- Freedman, J., Ho, M., Barefoot, C., 1982. Red blood cell-bound C3d in selected hospital patients. *Transfusion* 22, 515–520.
- Freedman, J., Wright, J., Lim, F.C., Garvey, M.B., 1987. Hemolytic warm IgM autoagglutinins in autoimmune hemolytic anemia. *Transfusion* 27, 464–467.
- Fries, L.F., Brickman, C.M., Frank, M.M., 1983. Monocyte receptors for the Fc portion of IgG increase in number in autoimmune hemolytic anemia and other hemolytic states and are decreased by glucocorticoid therapy. *J. Immunol.* 131, 1240–1245.
- Garratty, G., 1983. Mechanisms of immune red cell destruction, and red cell compatibility testing. *Hum. Pathol.* 14, 204–212.
- Garratty, G., 1989. Factors affecting the pathogenicity of red cell autoantibodies and alloantibodies. In: Nance, S.J. (Ed.), *Immune Destruction of Red Blood Cells*. American Association of Blood Banks, Arlington, VA, pp. 109–169.
- Garratty, G., 1990. Predicting the clinical significance of red cell antibodies with in vitro cellular assays. *Transfus. Med. Rev IV*, 297–312.
- Garratty, G., 2010. Immune hemolytic anemia associated with drug therapy. *Blood Rev.* 24, 143–150.
- Garratty, G., Arndt, P.A., 2014. Drugs that have been shown to cause drug-induced immune hemolytic anemia or positive direct antiglobulin tests: some interesting findings since 2007. *Immunohematology* 30, 66–79.
- Garratty, G., Nance, S.J., 1990. Correlation between in vivo hemolysis and the amount of red cell-bound IgG measured by flow cytometry. *Transfusion* 30, 617–621.
- Griffiths, H.L., Kumpel, B.M., Elson, C.J., Hadley, A.G., 1994. The functional activity of human monocytes passively sensitized with monoclonal anti-D suggests a novel role for Fc gamma RI in the immune destruction of blood cells. *Immunology* 83, 370–377.
- Hadley, A.G., Zupanska, B., Kumpel, B.M., Pilkington, C., Griffiths, H.L., Leader, K.A., et al., 1995. The glycosylation of red cell autoantibodies affects their functional activity in vitro. *Br. J. Haematol.* 91, 587–594.
- Hall, A.M., Stott, L.-M., Wilson, D.W.L., Urbaniak, S.J., Barker, R.N., 1999. Different epitopes are targeted by helper T-cells responding to the same human protein as an autoantigen or foreign antigen. *J. Autoimmun.* 27, 80.
- Hall, A.M., Ward, F.J., Vickers, M.A., Stott, L.-M., Urbaniak, S.J., Barker, R.N., 2002. Interleukin-10 mediated regulatory T-cell responses to epitopes on a human red blood cell autoantigen. *Blood* 100, 4529–4536.
- Hall, A.M., Vickers, M.A., McLeod, E., Barker, R.N., 2005. Rh autoantigen presentation to helper T cells in chronic lymphocytic leukemia by malignant B-cells. *Blood* 105, 2007–2015.
- Hall, A.M., Shen, C.-R., Ward, F.J., Rowe, C., Bowie, L., Devine, A., et al., 2007. Deletion of the dominant autoantigen in NZB mice with autoimmune hemolytic anemia: effects on autoantibody and T-helper responses. *Blood* 110, 4511–4517.
- Hall, A.M., Zamzami, O.M., Whibley, N., Hampsey, D.P., Haggart, A.M., Vickers, M.A., et al., 2012. Production of the effector cytokine interleukin-17, rather than interferon- $\gamma$ , is more strongly associated with autoimmune hemolytic anemia. *Hematologica* 97, 1494–1500.
- Heddle, N.M., Kelton, J.G., Turchyn, K.L., Ali, M.A., 1988. Hypergammaglobulinemia can be associated with a positive direct antiglobulin test, a nonreactive eluate, and no evidence of hemolysis. *Transfusion* 28, 29–33.
- Helyer, B.J., Howie, J.B., 1963. Spontaneous auto-immune disease in NZB/B1 mice. *Br. J. Haematol.* 9, 119–131.
- Hernandez-Jodra, M., Hudnall, S.D., Petz, L.D., 1990. Studies of in vitro red cell autoantibody production in normal donors and in patients with autoimmune hemolytic anemia. *Transfusion* 30, 411–417.
- Hoyer, K.K., Kuswanto, W.F., Gallo, E., Abbas, A.K., 2009. Distinct roles of helper T-cell subsets in a systemic autoimmune disease. *Blood* 113, 389–395.
- Huh, Y.O., Liu, F.J., Rogge, K., Chakrabarty, L., Lichtiger, B., 1988. Positive direct antiglobulin test and high serum immunoglobulin G values. *Am. J. Clin. Pathol.* 90, 197–200.
- Jenkins, W.J., Marsh, W.J., Noades, J., Tippett, P., Sanger, R., Race, R.R., 1960. The I antigen and antibody. *Vox Sang.* 5, 97–121.
- Kaplan, A.V., Quimby, F.W., 1983. A radiolabelled staphylococcal protein A assay for detection of anti-erythrocyte IgG in warm agglutinin autoimmune hemolytic anemia in dogs and man. *Vet. Immunol. Immunopathol.* 4, 307–317.
- Kay, M.M., Marchalonis, J.J., Hughes, J., Watanabe, K., Schluter, S.F., 1990. Definition of a physiologic aging autoantigen by using synthetic peptides of membrane protein band 3: localization of the active antigenic sites. *Proc. Natl. Acad. Sci. U.S.A.* 87, 5734–5738.
- Kelsoe, G., 1995. The germinal center reaction. *Immunol. Today* 16, 324–326.
- Kelton, J.G., 1985. Impaired reticuloendothelial function in patients treated with methyldopa. *N. Engl. J. Med.* 313, 596–600.
- Kelton, J.G., Singer, J., Rodger, C., Gauldie, J., Horsewood, P., Dent, P., 1985. The concentration of IgG in the serum is a major determinant of Fc-dependent reticuloendothelial function. *Blood* 66, 490–495.
- Kikuchi, S., Santiago-Raber, M.L., Amano, H., Amano, E., Fossati-Jimack, L., Moll, T., et al., 2006. Contribution of NZB autoimmunity 2 to Y-linked autoimmunity acceleration-induced monocytosis in association with murine systemic lupus. *J. Immunol.* 176, 3240–3247.
- Kirtland, H.H., Mohler, D.N., Horwitz, D.A., 1980. Methyldopa inhibition of suppressor-lymphocyte function. A proposed cause of autoimmune hemolytic anemia. *N. Engl. J. Med.* 302, 825–832.
- Krych-Goldberg, M., Atkinson, J.P., 2001. Structure-function relationships of complement receptor type 1. *Immunol. Rev.* 180, 112–122.
- Kurlander, R.J., Rosse, W.F., Logue, G.L., 1978. Quantitative influence of antibody and complement coating of red cells in monocyte-mediated cell lysis. *J. Clin. Invest.* 61, 1309–1319.
- Landsteiner, K., Levine, P., 1926. On the cold agglutinins in human serum. *J. Immunol.* 12, 441–460.
- Leddy, J.P., Falany, J.L., Kissel, G.E., Passador, S.T., Rosenfeld, S.I., 1993. Erythrocyte membrane proteins reactive with human (warm reacting) anti-red cell autoantibodies. *J. Clin. Invest.* 91, 1672–1680.
- Lee, N.J., Rigby, R.J., Gill, H., Boyle, J.J., Fossati-Jimack, L., Morley, B.J., et al., 2004. Multiple loci are linked with anti-red blood cell antibody production in NZB mice—comparison with other phenotypes implies complex modes of action. *Clin. Exp. Immunol.* 138, 39–46.
- Lefrere, J.-J., Courouce, A.-M., Bertrand, Y., Girot, R., Soulier, J.-P., 1986. Human parvovirus and aplastic crisis in chronic hemolytic anemias: a study of 24 observations. *Am. J. Hematol.* 23, 271–275.
- Lessard, C.J., Ice, J.A., Adrianto, I., Wiley, G.B., Kelly, J.A., Gaffney, P.M., et al., 2012. The genomics of autoimmune disease in the era of genome-wide association studies and beyond. *Autoimmun. Rev.* 11, 267–275.

- Liesveld, J.L., Rowe, J.M., Lichtman, M.A., 1987. Variability of the erythropoietic response in autoimmune hemolytic anemia. Analysis of 109 cases. *Blood* 69, 820–826.
- Lippman, S.M., Arnett, F.C., Conley, C.L., Ness, P.M., Meyers, D.A., Bias, W.B., 1982. Genetic factors predisposing to autoimmune diseases: autoimmune hemolytic anemia, chronic thrombocytopenic purpura and systemic lupus erythematosus. *Am. J. Med.* 73, 827–840.
- Loutit, J.F., Mollison, P.L., 1946. Haemolytic icterus (acholuric jaundice), congenital and acquired. *J. Pathol. Bacteriol.* 58, 711–728.
- Lutz, H.U., Wipf, G., 1982. Naturally occurring autoantibodies to skeletal proteins from human red blood cells. *J. Immunol.* 128, 1695–1699.
- Mair, T.S., Taylor, F.G.R., Hillyer, M.H., 1990. Autoimmune haemolytic anaemia in eight horses. *Vet. Rec.* 126, 51–53.
- Malhotra, R., Wormald, M.R., Rudd, P.M., Fischer, T.B., Dwek, R.A., Sims, R.B., 1995. Alterations in glycosylation of IgG associated with rheumatoid arthritis; activating complement via the mannose binding protein. *Nat. Med.* 1, 237–243.
- Marsh, W.L., Jenkins, W.J., 1960. Anti-i, a new cold antibody. *Nature* 188, 753.
- Mazza, G., el Idrissi, M.E., Coutelier, J.P., Corato, A., Elson, C.J., Pfau, C.J., et al., 1997. Infection of C3HeB/FeJ mice with the docile strain of lymphocytic choriomeningitis virus induces autoantibodies specific for erythrocyte Band 3. *Immunology* 91, 239–245.
- Mittal, S., Blaylock, M., Culligan, D.J., Barker, R.N., Vickers, M.A., 2008. A high rate of "CLL phenotype" lymphocytes in autoimmune hemolytic anemia and immune thrombocytopenic purpura. *Haematologica* 93, 151–152.
- Mollison, P.L., 1959. Measurement of survival and destruction of red cells in haemolytic syndromes. *Br. Med. Bull.* 15, 59–66.
- Mollison, P.L., Hughes-Jones, N.C., 1967. Clearance of Rh-positive red cells by low concentrations of Rh antibody. *Immunology* 12, 63–73.
- Mqadmi, A., Zheng, X., Yazdanbakhsh, K., 2005. CD4+ CD25+ regulatory T cells control induction of autoimmune hemolytic anemia. *Blood* 105, 3746–3748.
- Murakami, M., Tsubata, T., Okamoto, M., Shimizu, A., Kumagai, S., Imura, H., et al., 1992. Antigen-induced apoptotic death of Ly-1 B cells responsible for autoimmune disease in transgenic mice. *Nature* 357, 77–80.
- Naysmith, J.D., Ortega-Pierres, M.G., Elson, C.J., 1981. Rat erythrocyte induced anti-erythrocyte autoantibody production and control in normal mice. *Immunol. Rev.* 55, 55–87.
- Nicholson-Weller, A., Burge, J., Fearon, D.T., Weller, P.F., Austen, K.F., 1982. Isolation of a human erythrocyte membrane glycoprotein with decay accelerating activity for C3 convertases of the human complement system. *J. Immunol.* 129, 184–189.
- Nimmerjahn, F., Ravetch, J.V., 2008. Fc $\gamma$  receptors as regulators of immune responses. *Nat. Rev. Immunol.* 8, 34–47.
- Olanoff, L.S., Fudenberg, H.H., 1983. Familial autoimmunity. Twenty years later. *J. Clin. Lab. Immunol.* 11, 105–111.
- Oliveira, G.G., Hutchings, P.R., Roitt, I.M., Lydyard, P.M., 1994. Production of erythrocyte autoantibodies in NZB mice is inhibited by CD4 antibodies. *Clin. Exp. Immunol.* 96, 297–302.
- Owens, N.A., Hui, H.L., Green, F.A., 1982. Induction of direct Coombs positivity with alpha-methyldopa in chimpanzees. *J. Med.* 13, 473–477.
- Packman, C.H., 2008. Hemolytic anemia due to warm autoantibodies. *Blood Rev.* 22, 17–31.
- Pantaleo, A., Giribaldi, G., Mannu, F., Arese, P., Turrini, F., 2008. Naturally occurring anti-Band 3 antibodies and red blood cell removal under physiological and pathological conditions. *Autoimmun. Rev.* 7, 457–462.
- Peñalver, F.J., Alvarez-Larrán, A., Díez-Martín, J.L., Gallur, L., Jarque, I., Caballero, D., et al., 2010. Multi-institutional retrospective study on the use of rituximab in refractory AIHA. Rituximab is an effective and safe therapeutic alternative in adults with refractory and severe autoimmune hemolytic anemia. *Ann. Hematol.* 89, 1073–1080.
- Perry, F.E., Barker, R.N., Mazza, G., Day, M.J., Wells, A.D., Shen, C.R., et al., 1996. Autoreactive T-cell specificity in autoimmune hemolytic anemia of the NZB mouse. *Eur. J. Immunol.* 2, 136–141.
- Petz, L.D., 2008. Cold antibody autoimmune hemolytic anemias. *Blood Rev.* 22, 1–15.
- Petz, L.D., Garratty, G., 2004a. Classification and characteristics of autoimmune hemolytic anemias. In: Petz, L.D., Garratty, G. (Eds.), *Immune Hemolytic Anemias*, second ed Churchill Livingstone, Philadelphia, PA, pp. 61–131.
- Petz, L.D., Garratty, G., 2004b. Mechanisms of immune hemolysis. In: Petz, L.D., Garratty, G. (Eds.), *Immune Hemolytic Anemias*, second ed Churchill Livingstone, Philadelphia, PA, pp. 133–165.
- Pirofsky, B., 1968. Hereditary aspects of autoimmune hemolytic anemia. A retrospective analysis. *Vox Sang.* 14, 334–347.
- Pirofsky, B., 1976. Clinical aspects of autoimmune hemolytic anemia. *Semin. Hematol.* 13, 251–265.
- Playfair, J.H.L., Marshall-Clarke, S., 1973. Induction of red cell autoantibodies in normal mice. *Nat. New Biol.* 243, 213–214.
- Pollock, J.G., Fenton, E., Barrett, K.E., 1970. Familial autoimmune haemolytic anaemia associated with rheumatoid arthritis and pernicious anaemia. *Br. J. Haematol.* 18, 171–182.
- Pritchard, N.R., Cutler, A.J., Uribe, S., Chadban, S.J., Morley, B.J., Smith, K.G., 2000. Autoimmune-prone mice share a promoter haplotype associated with reduced expression and function of the Fc receptor Fc $\gamma$ RII. *Curr. Biol.* 10, 227–230.
- Roncarolo, M.G., Gregori, S., Battaglia, M., Bacchetta, R., Fleischhauer, K., Levings, M.K., 2006. Interleukin-10-secreting type 1 regulatory T cells in rodents and humans. *Immunol. Rev.* 212, 28–50.
- Ross, G.D., Medof, M.E., 1985. Membrane complement receptors specific for bound fragments of C3. *Adv. Immunol.* 37, 217–267.
- Rosse, W.F., 1971. Quantitative immunology of immune hemolytic anemia. II. The relationship of cell-bound antibody to hemolysis and the effect of treatment. *J. Clin. Invest.* 50, 734–743.
- Ruiz-Argüelles, A., Llorente, L., 2007. The role of complement regulatory proteins (CD55 and CD59) in the pathogenesis of autoimmune hemolytic anemias. *Autoimmun. Rev.* 6, 155–161.
- Sakaguchi, S., Miyara, M., Costantino, C.M., Hafler, D.A., 2010. FOXP3+ regulatory T cells in the human immune system. *Nat. Rev. Immunol.* 10, 490–500.
- Scatizzi, J.C., Haraldsson, M.K., Pollard, K.M., Theofilopoulos, A.N., Kono, D.H., 2012. The Lbw2 locus promotes autoimmune hemolytic anemia. *J. Immunol.* 188, 3307–3314.
- Shen, C.-R., Mazza, G., Perry, F.E., Beech, J.T., Thompson, S.J., Corato, A., et al., 1996. T-helper 1 dominated responses to erythrocyte Band 3 in NZB mice. *Immunology* 8, 195–199.
- Shen, C.-R., Youssef, A.-R., Devine, A., Bowie, L., Hall, A.M., Wraith, D.C., et al., 2003. Peptides containing a dominant T-cell epitope from red cell Band 3 have in vivo immunomodulatory properties in NZB mice with autoimmune hemolytic anemia. *Blood* 102, 3800–3806.

- Shevach, E.M., 2011. Biological functions of regulatory T cells. *Adv. Immunol.* 112, 137–176.
- Shoenfeld, Y., Schwartz, R.S., 1984. Immunologic and genetic factors in autoimmune diseases. *N. Engl. J. Med.* 311, 1019–1029.
- Shoenfeld, Y., Isenberg, D.A., 1989. The mosaic of autoimmunity. *Immunol. Today* 10, 123–126.
- Silberstein, L.E., Robertson, G.A., Harris, A.C., Moreau, L., Besa, E., Nowell, P.C., 1986. Etiologic aspects of cold agglutinin disease, Evidence for cytogenetically defined clones of lymphoid cells and the demonstration that an anti-Pr cold autoantibody is derived from a chromosomally aberrant B cell clone. *Blood* 67, 1705–1709.
- Sokol, R.J., Hewitt, S., 1985. Autoimmune hemolysis. A critical review. *CRC Crit. Rev. Oncol. Hematol.* 4, 125–154.
- Sokol, R.J., Hewitt, S., Stamps, B.K., 1981. Autoimmune haemolysis: an 18-year study of 865 cases referred to a regional transfusion centre. *Br. Med. J.* 282, 2023–2027.
- Sokol, R.J., Hewitt, S., Stamps, B.K., 1982. Autoimmune haemolysis associated with Donath-Landsteiner antibodies. *Acta Haemat.* 68, 268–277.
- Sokol, R.J., Hewitt, S., Stamps, B.K., 1983. Autoimmune haemolysis. Mixed warm and cold antibody type. *Acta Haemat.* 69, 266–274.
- Sokol, R.J., Hewitt, S., Stamps, B.K., Hitchen, P.A., 1984. Autoimmune haemolysis in childhood and adolescence. *Acta Haemat.* 72, 245–257.
- Sokol, R.J., Hewitt, S., Booker, D.J., Stamps, R., 1985. Enzyme linked direct antiglobulin tests in patients with autoimmune haemolysis, *J. Clin. Pathol.* 38, 912–914.
- Sokol, R.J., Hewitt, S., Booker, D.J., Stamps, R., Booth, J.R., 1988. An enzyme-linked direct antiglobulin test for assessing erythrocyte bound immunoglobulins. *J. Immunol. Meth.* 106, 31–35.
- Sokol, R.J., Hewitt, S., Booker, D.J., Bailey, A., 1990a. Erythrocyte autoantibodies, subclasses of IgG and autoimmune haemolysis. *Autoimmunity*, 6, 99–104.
- Sokol, R.J., Hewitt, S., Booker, D.J., Bailey, A., 1990b. Erythrocyte autoantibodies, multiple immunoglobulin classes and autoimmune haemolysis. *Transfusion*, 30, 714–717.
- Sokol, R.J., Booker, D.J., Stamps, R., 1992. The pathology of autoimmune haemolytic anaemia, *J. Clin. Pathol.* 45, 1047–1052.
- Sokol, R.J., Booker, D.J., Stamps, R., 1999. Erythropoiesis: paroxysmal cold haemoglobinuria, a clinico-pathological study of patients with a positive Donath-Landsteiner test. *Hematology*, 4, 137–164.
- Stott, L.-M., Urbaniak, S.J., Barker, R.N., 2002. Specific production of regulatory T-cell cytokines, responsiveness to the RhD blood group, and expression of HLA-DRB1\*15. *Immunology* 107, 6.
- Switzer, J.W., Jain, N.C., 1981. Autoimmune hemolytic anemia in dogs and cats. *Vet. Clin. North Am. Small Anim. Pract.* 11, 405–420.
- Talal, N., Ahmed, S.A., 1987. Immunomodulation by hormones—an area of growing importance. *J. Rheumatol.* 14, 191–193.
- Talor, E., Rose, N.R., 1991. Hypothesis. The aging paradox and autoimmune disease. *Autoimmunity* 8, 245–249.
- Tomer, Y., Shoenfeld, Y., 1988. Ageing and autoantibodies. *Autoimmunity* 1, 141–149.
- Urbaniak, S.J., Griess, M.A., 1980. ADCC (K-cell) lysis of human erythrocytes sensitized with Rhesus alloantibodies. III. Comparison of IgG anti-D agglutinating and lytic (ADCC) activity and the role of IgG subclasses. *Br. J. Haematol.* 46, 447–453.
- Van der Meulen, F.W., De Bruin, H.G., Goosen, P.C.M., Bruynes, E.C.E., Joustra-Maas, C.J., Telkamp, H.G., et al., 1980. Quantitative aspects of the destruction of red cells sensitized with IgG1 autoantibodies. An application of flow cytometry. *Br. J. Haematol.* 46, 47–56.
- Victoria, E.J., Pierce, S.W., Branks, M.J., Masouredis, S.P., 1990. IgG red blood cell autoantibodies in autoimmune hemolytic anemia bind to epitopes on red blood cell membrane band 3 glycoprotein. *J. Lab. Clin. Med.* 115, 74–88.
- Ward, F.J., Hall, A.M., Cairns, L.S., Leggat, A.S., Urbaniak, S.J., Vickers, M.A., et al., 2008. Clonal regulatory T cells specific for a red blood cell autoantigen in human autoimmune hemolytic anemia. *Blood* 111, 680–687.
- Weiner, W., Vos, G.H., 1963. Serology of acquired hemolytic anemias. *Blood* 22, 606–613.
- Wiener, E., Hughes-Jones, N.C., Irish, W.T., Wickramasinghe, S.N., 1986. Elution of antispectrin antibodies from red cells in homozygous β-thalassaemia. *Clin. Exp. Immunol.* 63, 680–686.
- Win, N., Islam, S.I., Peterkin, M.A., Walker, I.D., 1997. Positive direct antiglobulin test due to antiphospholipid antibodies in normal healthy blood donors. *Vox Sang.* 72, 182–184.
- Yi, T., Li, J., Chen, H., Wu, J., An, J., Xu, Y., et al., 2015. Splenic dendritic cells survey red blood cells for missing self-CD47 to trigger adaptive immune responses. *Immunity* 43, 764–775.
- Youssef, A.R., Shen, C.-R., Lin, C.-L., Barker, R.N., Elson, C.J., 2005. IL-4 and IL-10 modulate autoimmune haemolytic anaemia in NZB mice. *Clin. Exp. Immunol.* 139, 84–89.
- Zanella, A., Barcellini, W., 2014. Treatment of autoimmune hemolytic anemias. *Haematologica* 99, 1547–1554.
- Zecca, M., Nobili, B., Ramenghi, U., Perrotta, S., Amendola, G., Rosito, P., et al., 2003. Rituximab for the treatment of refractory autoimmune hemolytic anemia in children. *Blood* 101, 3857–3861.

# Immune Thrombocytopenia: A Complex Autoimmune Disease

Eun-Ju Lee<sup>1</sup> and James B. Bussel<sup>2</sup>

<sup>1</sup>Department of Medicine, New York Presbyterian Hospital, Weill Cornell Medical Center, New York, NY, United States <sup>2</sup>Departments of Pediatrics, Medicine, and Obstetrics and Gynecology, New York Presbyterian Hospital, Weill Cornell Medical Center, New York, NY, United States

## O U T L I N E

Introduction	911	First-Line Therapies	916
Epidemiology	912	Second-Line Therapies	916
Diagnosis	913	Splenectomy	916
Pathogenesis	914	Rituximab	917
Platelet Autoantibodies	914	Thrombopoietin Receptor Agonists	917
T-Cell Involvement	914	Conclusion	918
Megakaryopoiesis	915	References	918
Treatment	916		

## INTRODUCTION

ITP is an acquired autoimmune disorder characterized by a platelet count  $<100 \times 10^9 \text{ L}^{-1}$  due to accelerated platelet destruction and impaired platelet production (McMillan, 1981; Heyns Adu et al., 1986). It is generally believed that the autoimmune response is mediated by both antiplatelet antibodies and T cell-mediated cytotoxicity. Platelets are derived from megakaryocytes (MKs), whose production and maturation in the bone marrow are regulated by thrombopoietin (TPO) (Kaushansky, 1995). Normal platelet values range from 150 to  $450 \times 10^9 \text{ L}^{-1}$ . Bleeding due to impaired primary hemostasis and platelet plug formation is a major clinical consequence of thrombocytopenia.

ITP occurs in both children and adults. The incidence of ITP is estimated at 3.3/100,000 adults per year and between 1.9 and 6.4/100,000 children per year (Terrell et al., 2010). Adults and children have similar platelet counts at diagnosis and bleeding symptoms with severe thrombocytopenia. However, the underlying disease processes are likely distinct as the majority of children with ITP achieve spontaneous remission while most adults face a chronic disorder (Terrell et al., 2010; Lambert and Gernsheimer, 2017; Stasi et al., 1995).

**TABLE 48.1** Causes of Secondary Immune Thrombocytopenia

Infection	HIV, HCV, <i>H. pylori</i> , CMV, Varicella zoster
Autoimmune disorders	SLE, APS, Evans syndrome
Immunodeficiency	CVID, ALPS, mild SCID
Lymphoproliferative disorders	CLL, HD, LGL, NHL
Postvaccination	Especially MMR
Drug-induced	Depakote, quinine, quinidine
Bone marrow transplantation side effect	

*HIV*, Human immunodeficiency virus; *HCV*, hepatitis C virus; *H. pylori*, *Helicobacter pylori*; *CMV*, cytomegalovirus; *SLE*, systemic lupus erythematosus; *APS*, antiphospholipid syndrome; *CVID*, common variable immune deficiency; *ALPS*, autoimmune lymphoproliferative syndrome; *SCID*, severe combined immunodeficiency; *CLL*, chronic lymphocytic leukemia; *HD*, Hodgkin disease; *LGL*, large granular T-lymphocyte leukemia; *NHL*, non-Hodgkin lymphoma; *MMR*, measles-mumps-rubella.

Adapted from Neunert, C., Lim, W., Crowther, M., et al., 2011. The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. *Blood* 117 (16), 4190–4207; Cines, D.B., Bussel, J.B., Liebman, H.A., et al., 2009. The ITP syndrome: pathogenic and clinical diversity. *Blood* 113 (26), 6511–6521.

According to the 2010 International Working Group (IWG) consensus of ITP experts, the acronym ITP represents immune thrombocytopenia, avoiding the terms “idiopathic” and “purpura” that had been included in the past. This reflects the enhanced understanding of the pathophysiology of ITP and lack of purpura in many cases. The IWG defines primary ITP as an isolated thrombocytopenia in the absence of other identified conditions and secondary if occurring in the context of drug exposures or other disorders associated with immune dysregulation (Table 48.1) (Rodeghiero et al., 2009).

This chapter reviews the epidemiology, diagnosis, pathogenesis, and treatment of ITP with specific emphasis on the autoimmune aspects of the disease.

## EPIDEMIOLOGY

Studies indicate a slightly higher prevalence of ITP in males in childhood and in older adulthood with increased incidence in women in the middle (child-bearing) adult years (30–60 years age group) (Segal and Powe, 2006; Moulis et al., 2014). Peak incidence occurs during childhood and in adults greater than 60 years of age (Moulis et al., 2014). Though limited, there are some data suggesting decreased incidence among black populations and seasonal variation with a peak in the winter and spring months (Moulis et al., 2014, 2017).

Severe spontaneous or posttraumatic bleeding, such as gastrointestinal, genitourinary, and gynecologic hemorrhage, skin and mucosal hemorrhage, or intracranial hemorrhage (ICH), may occur with platelet values  $<10 \times 10^9 \text{ L}^{-1}$  and can occur but is less frequent in patients with platelets between  $10 \times 10^9$  and  $20 \times 10^9 \text{ L}^{-1}$  (Cortelazzo et al., 1991; Cines and Bussel, 2005). Factors associated with increased risk of bleeding include lower platelet counts, male gender, older age, and prior hemorrhage (Lambert and Gernsheimer, 2017; Cortelazzo et al., 1991; Neunert et al., 2011, 2015). Adults have a higher overall rate of intracranial bleeding than children, 1.4% versus 0.4% (Neunert et al., 2015) potentially reflecting the presence of medical comorbidities. ICH is especially prevalent in patients over the age of 60 (Cortelazzo et al., 1991).

Although bleeding is a significant concern with thrombocytopenia, recent reports indicate an increased risk of thromboembolism in ITP patients with a relative risk of 1.6 compared to the general population (Doobaree et al., 2016). The reasons behind this remain unclear but could be related to increased platelet activation and to cell-derived microparticles (Bidot et al., 2008; Sewify et al., 2013; Psaila et al., 2011) with possibly some contribution of treatment effect. For example, patients who have undergone splenectomy have a higher rate of thromboembolism than nonsplenectomized ITP patients (Doobaree et al., 2016; Boyle et al., 2013). Patients receiving TPO receptor agonists (TPO-RAs) also have a higher risk of thromboembolic events (6% over several years of treatment) (Wong et al., 2017).

In the US population, there was greater in-hospital mortality for ITP patients compared to non-ITP hospitalizations (Danese et al., 2009). This increased mortality also exists in ITP patients compared with the general population along with higher rates of cardiovascular disease, thromboembolic events, bleeding, and infection (Norgaard et al., 2011; Frederiksen et al., 2014).

## DIAGNOSIS

An isolated platelet count  $<100 \times 10^9 \text{ L}^{-1}$  in the absence of other underlying disorders characterizes ITP. Current guidelines recommend a thorough evaluation of a patient's history, physical examination, complete blood count (CBC), and peripheral blood smear (Neunert et al., 2011; Provan et al., 2010). In taking the history, particular attention should be given to the presence of systemic diseases, infections, chronicity, and severity of bleeding/bruising events, medications, vaccinations, and personal or family history of thrombocytopenia or easy bleeding/bruising. The physical exam should be unremarkable save for manifestations of bleeding or bruising. Careful examination of the liver, the spleen, the lymph nodes, and the radial ray is particularly warranted. Detection of a significantly enlarged spleen should prompt evaluation for disorders other than ITP. The CBC should show intact hemoglobin and white blood cell count. Abundant platelet clumping and signs of hemolysis (schistocytes) should be absent on the peripheral blood smear.

The IWG consensus report from 2010 recommends checking for human immunodeficiency virus (HIV), hepatitis C virus, *Helicobacter pylori*, quantitative immunoglobulin levels, and a direct antiglobulin test (DAT) (Provan et al., 2010). The evaluation of antiphospholipid antibodies, antinuclear antibodies, antiplatelet antibodies, thyroid function testing, and cytomegalovirus could be helpful but are not routinely recommended, unless guided by symptoms or history (Neunert et al., 2011; Provan et al., 2010). Both the IWG consensus report (2010) and the American Society of Hematology (ASH) 2011 practice guidelines advise against routine bone marrow biopsy in the evaluation of children and younger adults (Neunert et al., 2011; Provan et al., 2010; Jubelirer and Harpold, 2002; Mak et al., 2000). The IWG suggests consideration of bone marrow biopsy in certain situations such as patients older than 60 years of age, presence of systemic symptoms, or prior to splenectomy (Provan et al., 2010). Bone marrow aspirate and biopsy are important tools in patients with refractory disease to rule out other causes of thrombocytopenia. Note that both documents may be revised in the near future with the next rendition of the ASH guidelines expected by early 2018.

ITP remains a diagnosis of exclusion. The majority of ITP cases are primary and around 20% are secondary (Table 48.1), the most common causes varying by location. In the United States, the most common causes of secondary ITP are common variable immune deficiency (CVID), systemic lupus erythematosus, hepatitis C infection, and chronic lymphocytic leukemia (Moulis et al., 2014; Cines et al., 2009). Whether ITP is primary or secondary, transient substantial response to intravenous immunoglobulin (IVIG) is supportive of the diagnosis.

The phases of ITP are defined as acute, persistent, and chronic (Table 48.2). Once diagnosed, most adults will go on to have chronic ITP (Cuker et al., 2015). Those with clinically significant bleeding and very low platelet counts requiring initiation of treatment, additional therapies, or increased doses of medications are described as having "severe" ITP (Rodeghiero et al., 2009). Severe ITP can occur during any phase of ITP. "Refractory" ITP describes disease not responsive to splenectomy with severe ITP or significant risk of bleeding to warrant treatment (Rodeghiero et al., 2009).

**TABLE 48.2** Phases of Immune Thrombocytopenia

Acute	Diagnosis to 3 months
Persistent	3–12 months from diagnosis
Chronic	More than 12 months from diagnosis

*Adapted from Rodeghiero, F., Stasi, R., Gernsheimer, T., et al., 2009. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. Blood 113 (11), 2386–2393.*

## PATHOGENESIS

Primary ITP is a complicated acquired autoimmune condition with thrombocytopenia broadly resulting from pathologic antiplatelet autoantibodies (Shulman et al., 1965), T cell–mediated platelet destruction (Olsson et al., 2003), and impaired MK production (Khodadi et al., 2016). It is a very heterogeneous disease whereby immune dysregulation of many causes results in the antiplatelet response described above. It is worth noting that initially in the first stage of B-cell development, 50% or so are autoantibodies which, given the marrow milieu, are directed against blood cells especially platelets. Therefore with preformed antibodies that can be stimulated, ITP can develop as a loss of control (Nemazee et al., 1991).

## PLATELET AUTOANTIBODIES

Autoantibodies, typically IgG, are produced against single or multiple platelet surface antigens particularly components of the glycoprotein (GP) IIb/IIIa and GPIb/IX complexes but also others that may have been underestimated in the past such as anti-GPVI (He et al., 1995; Woods et al., 1984; Audia et al., 2017). IgA and IgM anti-platelet antibodies are seen less often and typically in conjunction with IgG autoantibodies (He et al., 1994); their role is largely unclear.

B lymphocytes may secrete autoreactive antibodies and are present in increased frequency in patients with ITP (Kuwana et al., 2014; Chen et al., 2012). The spleen is the main site of antiplatelet antibody production with the peripheral blood, bone marrow, and probably lymph nodes serving as secondary sources of antibody-producing B and plasma cells (McMillan et al., 1974). Phagocytes with Fc $\gamma$ -receptors recognize antiplatelet antibody bound to platelets, facilitating their phagocytosis and destruction. Given the local production of antibodies and the constant presence of about one-third of the intravascular platelet mass, the spleen is the primary site of platelet destruction with usually a smaller contribution via the reticuloendothelial system of the liver (Ballem et al., 1987; Zufferey et al., 2017; McMillan, 2007). In addition to antibody-mediated destruction via phagocytosis, antibody binding can activate complement-mediated platelet lysis (Tsubakio et al., 1986), though this has been poorly studied and the significance of complement components is not completely clear.

Currently, neither the IWG nor the ASH practice guidelines recommend routine testing for platelet autoantibodies (Neunert et al., 2011; Provan et al., 2010). No detectable antibodies are found in up to 30%–40% of the patients (Zufferey et al., 2017), and this is reflected in the low sensitivity (i.e., percent of patients with ITP with positive antibodies) of 49%–66% of platelet antibody testing in ITP. In addition, a specificity of only 78%–93% leads to a significant number of false-positive tests (Warner et al., 1999; Brighton et al., 1996; McMillan et al., 2003). Possible reasons behind the absence of detectable antibodies in a substantial percent of ITP patients include suppression of antibody levels due to preceding treatment, the presence of antibodies to other platelet antigens that are not being assayed, and a significant contribution of antibody-independent mechanisms of disease such as T cell–mediated platelet destruction and/or suppression of platelet production (Zufferey et al., 2017; McMillan, 2007).

In addition to the production of autoreactive antibodies, other mechanisms of immune dysregulation involving B cells may also participate in the pathogenesis ITP. B cell–activating factor (BAFF or B lymphocyte stimulator) is a cytokine that plays a critical role in regulating B-cell survival, maturation, and stimulation (Schneider et al., 1999). Elevated levels of BAFF in ITP may contribute to disease activity by rescuing autoreactive B and T cells from apoptosis and by promoting survival of long-lived splenic plasma cells (Audia et al., 2017; Zhu et al., 2009; Mahevas et al., 2013). A subset of IL-10 producing B-regulatory cells (Bregs) helps to maintain intact immune tolerance by promoting the differentiation of T-regulatory cells (Tregs), regulating T helper 1 (Th1)/T helper 2 (Th2) balance, and suppressing activation of monocytes, thus playing a vital role in immune suppression (Li et al., 2012; Semple, 2012; Lemoine et al., 2011). Not only are Bregs decreased in patients with ITP but they are also functionally impaired (Li et al., 2012).

## T-CELL INVOLVEMENT

T cells and dysregulation of T-cell populations are involved in the pathogenesis of ITP through a variety of mechanisms. A change in T-helper cell balance occurs with decreased Th2 polarization resulting in an increased Th1/Th2 ratio (Ogawara et al., 2003; Wang et al., 2005) and with an inverse correlation of the Th1/Th2 ratio with

platelet count in ITP (Takahashi et al., 2017; Panitsas et al., 2004). The significance of this altered T-cell balance is not yet known but as Th1 polarization is required for macrophage stimulation, this may result in increased platelet phagocytosis (Audia et al., 2017).

The most discussed mechanism, possibly due to the current lack of clarity, involves the role of cytotoxic T cells. CD8+ cytotoxic T cells can directly lyse platelets (Zhao et al., 2008) and accumulate in the bone marrow inhibiting platelet production (Olsson et al., 2008). T cells can also enhance antibody formation as demonstrated by a population of autoreactive GPIIb/IIIa CD4+ T cells that promote the production of antiplatelet antibodies (Kuwana et al., 1998). The latter may involve the CD40–CD40 ligand (CD154) interaction (Audia et al., 2014).

Tregs suppress self-reactive lymphocytes and preserve immunological self-tolerance (Hori et al., 2003; Sakaguchi et al., 2009). The spontaneous development of severe autoimmune disease in animal models with depleted Treg populations, and in humans with specific deficiency of Tregs, highlights the importance of Tregs in maintaining immune homeostasis (Kim et al., 2007; Yu et al., 2017). As shown in multiple studies, both decreased number and impaired function of Tregs characterize the abnormal immune environment of ITP (Sakakura et al., 2007; Liu et al., 2007).

Why all of these mechanisms result in ITP, rather than perhaps other autoimmune diseases, remains to be clarified. Possibilities include the preexistence of autoantiplatelet antibodies or the resemblance of platelets to lymphocytes with multiple immunologically active molecules. The latter may lead to platelet participation in the immune response resulting in the presentation of platelet GPs to antigen-presenting cells.

Another largely unresolved issue is why ITP resolves in certain cases and not in others. Several approaches have been explored. In children, if the initial inciting infection results in oxidative damage to the platelet membrane, then this increases the chance of developing chronic ITP (Zhang and Zehnder, 2013). In support of this hypothesis is an Egyptian study randomizing addition of antioxidative therapy showing a benefit to this form of treatment (Elalfy et al., 2015). Another study of children with chronic ITP suggested a number of polymorphisms in T-cell pathways contributed to chronic ITP (Zhang et al., 2014). One appeal of these findings is that they strongly support a polygenic instead of monogenic etiology of chronic disease. A corroborative set of findings comes from the study of a B cell–directed treatment (rituximab plus dexamethasone). Nonresponders had a significantly increased frequency of monoclonal and oligoclonal T-cell repertoires compared to responders (Chapin et al., 2016). This suggests that the persistence of abnormal T cells relates to the persistence of ITP. The majority of patients with ITP gradually improve but certain patients worsen over time. One of the reasons behind this appears to include processes such as epitope spreading whereby epitopes distinct from the initial inciting epitope become major targets of the ongoing immune response (Cornaby et al., 2015). But, as with many of the discussed immunologic phenomena, this has been shown to occur but to be of unclear clinical significance.

## MEGAKARYOPOIESIS

Despite the normal or increased number of MKs seen in bone marrow biopsies of ITP patients, the platelet production is actually impaired (Louwes et al., 1999; Dameshek and Miller, 1946). Abnormalities in MK development contribute to insufficient platelet production. Early microscopy studies demonstrated extensive damage to 50%–75% of MKs (Stahl et al., 1986). More recent studies confirm these findings showing features of apoptosis and para-apoptosis in the majority of mature MKs with surrounding neutrophils and macrophages (Houwerzijl et al., 2004). Since MKs express platelet surface antigens (i.e., GPIIb/IIIa, GPIb/IX), these antiplatelet antibodies, and potentially autoreactive T cells, can affect MK maturation/platelet production in the bone marrow (Houwerzijl et al., 2004; McMillan et al., 2004; Chang et al., 2003).

Another reason for the suboptimal platelet production involves TPO, produced by the liver and the main growth factor for MKs (de Sauvage et al., 1996). After TPO binds to its receptor, c-Mpl, on platelets and MKs, it is internalized and eventually destroyed along with the platelet (Kuter and Rosenberg, 1995; Debili et al., 1995). Thus the total cell mass of c-Mpl expressing cells regulates circulating TPO levels; the same is true of granulocyte colony-stimulating factor although not erythropoietin (Corbacioglu et al., 2000). While patients with ITP have low absolute platelet number, TPO levels remain low due to uptake by the increased population of MKs and platelets with a short lifespan (Chang et al., 1999).

An additional pathway leading to TPO production has recently been discovered. Desialylation of membrane proteins occurs as platelets age, leading to their binding by the hepatic Ashwell–Morell receptor which induces platelet phagocytosis and TPO production (Audia et al., 2017; Grozovsky et al., 2015). However, in ITP, the majority of antibody-coated platelets are believed to be destroyed via FcR-mediated phagocytosis and not in this

fashion. These findings of low circulating TPO levels in ITP may partly explain why TPO-RA is an often effective treatment.

Reduced proplatelet formation in patients with undetectable antiplatelet antibodies suggests a nonimmune, inherent defect in late megakaryopoiesis (Riviere et al., 2015). As mentioned briefly above, CD8+ cytotoxic T cells accumulate in the bone marrow (Olsson et al., 2008) where they suppress proper MK apoptosis, a step required for platelet production (Li et al., 2007). In one study, nonresponders to TPO-RAs had increased MKs in the marrow but reduced platelet reticulocytes suggesting that their lack of response was due to impaired ability of proplatelets to release platelets (Barsam et al., 2011). Responders to TPO-RAs had clearly increased platelet reticulocytes compared to those patients who responded to treatments such as IVIG, IV anti-D, and even an anti-FcR III antibody, demonstrating their unique mechanism of effect, that is, stimulation of platelet production (Barsam et al., 2011).

## TREATMENT

Generally, patients with platelets above  $30 \times 10^9 \text{ L}^{-1}$  are not at risk for serious bleeding and may be followed by observation alone. Patients with platelets  $<10 \times 10^9 \text{ L}^{-1}$  are treated, and the decision to treat those with platelets between  $10 \times 10^9$  and  $30 \times 10^9 \text{ L}^{-1}$  depends on factors such as bleeding/bruising symptoms, medical comorbidities, and fatigue (Cooper, 2017); most adults with platelet counts in this range are likely to be treated. The goal of treatment is to achieve a durably improved platelet count without the need for ongoing therapy.

## FIRST-LINE THERAPIES

Historically, first-line treatments include steroids, IVIG, and IV anti-D. The overall aim of these therapies is to decrease autoantibody-mediated platelet destruction, although which of their multiple potential mechanisms of action are most significant is not completely understood (Zufferey et al., 2017). IVIG slows platelet destruction and increases platelet half-life but exactly how this occurs remains controversial. There are various types and doses of steroids used in ITP but no consensus regarding the optimal regimen. Some studies suggest improved response rates using one or multiple rounds of high-dose dexamethasone ( $40 \text{ mg/day} \times 4 \text{ days}$ ) compared to a prolonged prednisone taper (Wei et al., 2016; Mazzucconi et al., 2007). In patients with contraindications to high doses of steroids, IVIG  $1 \text{ g/kg}$  may be used for 1 or 2 days depending on platelet response (Cooper, 2017). The primary common toxicity is headache, which can be severe.

Patients who are RhD antigen positive, are DAT negative, and have intact spleens are candidates for treatment with IV anti-D (Scaradavou et al., 1997; Cooper et al., 2002). IV anti-D has been shown to be particularly efficacious in children and in HIV-related ITP (Scaradavou et al., 1997), though all treated patients must be monitored for intravascular hemolysis. Intravascular hemolysis and fever–chill–nausea–vomiting cytokine-driven reactions to IV anti-D can both be ameliorated substantially by premedication with high dose IV steroids, for example, methylprednisolone  $30 \text{ mg/kg}$  up to  $1 \text{ g}$ .

## SECOND-LINE THERAPIES

If the initial attempt at therapy does not result in lasting improvement and/or clinical remission, multiple agents are available as second-line treatments. The selection among these agents is very difficult and the only clear, unequivocal tenet is *not* to continue steroids.

## SPLENECTOMY

Splenectomy remains a reasonable option with around 60% of the patients achieving a normal platelet count postsurgery and maintaining a sustained response at 5 years (Kojouri et al., 2004; Kumar et al., 2002; Ahmed et al., 2016). However, this potential benefit should be balanced with the surgical risk and long-term increased risk of infection/sepsis and thrombosis especially stroke (Ahmed et al., 2016). The decreased usage of splenectomy (Boyle et al., 2013) reflects the possibility of other treatment options, the long-term response in only 60%,

the inability to predict the response except in very few centers, and the hope that the ITP will improve over 1–3 years such that no ongoing treatment will be necessary. If very good prediction of response were widely available, it would probably be selected more frequently.

## RITUXIMAB

Rituximab is an anti-CD-20 monoclonal antibody that depletes circulating CD-20-positive antibody-producing B cells. The overall response rate is around 50%–60% and about 20%–30% of the patients have a long-term (>5 years) response (Patel et al., 2012; Arnold et al., 2007). Apparently, the population with the best response to the combination of rituximab and three cycles of dexamethasone is women with ITP of <1 year's duration (Chapin et al., 2016). Caution should be taken as B-cell depletion can lead to decreased vaccine response for up to 6 months (Nazi et al., 2013) and repeated courses of rituximab, especially in combination with dexamethasone, can lead to severe hypogammaglobulinemia (Cooper et al., 2009). Those with existing CVID, and patients who are on immunosuppressive medications, may be at greater risk for hypogammaglobulinemia and infection but those with CVID should already be on IVIG and they respond to rituximab particularly well (Gobert et al., 2011). Obtaining baseline immunoglobulin levels is recommended prior to treatment with rituximab (Cooper, 2017; Kado et al., 2016). In addition, patients should be tested for hepatitis B due to the risk of viral reactivation with rituximab. Overall, rituximab is generally well tolerated with toxicities including very occasional severe infusion reaction, serum sickness, prolonged immunosuppression, and rare reports of progressive multifocal leukoencephalopathy, only one or two of which occurred in patients with ITP (Carson et al., 2009; Cuker and Neunert, 2016).

## THROMBOPOIETIN RECEPTOR AGONISTS

TPO-RAs bind to the TPO receptor stimulating megakaryopoiesis and hence platelet production. Currently, there are two TPO-RAs, eltrombopag and romiplostim, approved by the US Food and Drug Administration (FDA) and European Medicines Agency. Both agents lack sequence or structural homology with endogenous TPO avoiding the risk of developing cross-reactive antibodies (Kuter, 2007). Eltrombopag is an orally available, small molecule, nonpeptide TPO-RA that binds the transmembrane domain of the TPO receptor (Kuter, 2007; Rodeghiero and Carli, 2017). It is FDA approved for the treatment of ITP in adults and children  $\geq 1$  year-old refractory to corticosteroids, IVIg, or splenectomy; adults with severe aplastic anemia; and thrombocytopenia due to chronic hepatitis C infection. Romiplostim is a recombinant fusion protein, a so-called peptibody, given weekly via subcutaneous injection that shares the same TPO receptor binding site as endogenous TPO (Rodeghiero and Carli, 2017). Romiplostim is FDA approved for the treatment of chronic ITP in adults refractory corticosteroids, IVIg, or splenectomy.

In a double-blind randomized controlled trial of 63 splenectomized and 62 nonsplenectomized patients with ITP, Romiplostim had an overall response rate (4 of 24 weeks) of 80%–90% with durable platelet response rates (platelet count  $\geq 50 \times 10^9/L$  for at least 6 of the final 8 weeks of the study) of 38% in splenectomized and 61% in nonsplenectomized patients. The majority of patients who had been on concomitant treatment were able to reduce the dosage of or discontinue other ITP medications, and rescue treatments for low counts were reduced as was bleeding (Kuter et al., 2008). Longer term studies of up to 5 years demonstrated ongoing efficacy and safety of Romiplostim (Bussel et al., 2009a; Kuter et al., 2013). Randomized controlled trials of eltrombopag in adults and children show initial platelet response rates of 59%–79%, with a significant proportion of patients able to decrease or discontinue concomitant ITP therapies (Bussel et al., 2009b; Grainger et al., 2015; Cheng et al., 2011).

Potential risks of both TPO-RAs include thrombosis, headaches, and myalgia and bone marrow fibrosis. Eltrombopag may result in cataracts and transaminitis or hyperbilirubinemia (Bussel et al., 2009a; Kuter et al., 2013). Romiplostim has a higher rate of cycling counts in some responders, and there appears to be a 1% incidence of neutralizing antibodies (which do not cross-react with native TPO) (Carpenedo et al., 2016). Should a patient not respond to or not tolerate one of the TPO-RAs switching to the other is a reasonable option (Khellaf et al., 2013). Although not meant as curative therapy, there are reports of patients sustaining adequate platelet counts after TPO-RA discontinuation (Cuker et al., 2015) but how to decide when to taper or stop a TPO-RA remains uncertain. The Sunshine Pharmaceutical Co., Ltd. has a TPO-RA that resembles native TPO which is licensed in China and several Asian countries (Zhao et al., 2004). Two additional TPO-RAs are in clinical trial at present.

Multiple other agents are used as second-line treatments in ITP including mycophenolate mofetil, danazol, azathioprine, dapsone, vincristine, and cyclophosphamide. There are a number of single arm studies with each of these agents albeit with response rates mostly <50% in difficult patients (Taylor et al., 2015; Hou et al., 2003; Maloisel et al., 2004; Provan et al., 2006; Quiquandon et al., 1990; Stirnemann et al., 2016). The latter two agents (vincristine and cyclophosphamide) have been used much less frequently in recent years because of their toxicities.

## CONCLUSION

ITP is a prototypic organ-specific autoimmune disease. Its relative frequency is attributed to the development of immature pre-B and B cells in the marrow where they are exposed to platelets. Studies of tolerance have shown that the most immature B cells have an autoselectivity in approximately 50% of cells that steadily falls to <1%. Rupture of tolerance in one of many areas can thus readily result in ITP.

ITP is a final common pathway disease that can result from many different etiologies. The current inability to dissect out which one is occurring in a given patient has been one of the primary limitations in improving the management of ITP. With the increasing number of acceptable treatment options, it can be very difficult to decide which one to select in a given patient. Studies not only of various polymorphisms or mutations in patients linked to their outcomes of their ITP, for example, not only bleeding and fatigue but also response to different therapies and whether spontaneous improvement will occur are badly needed. If and when this happens, the information should allow a more rational approach to the management of ITP in the future. There is general agreement that curative therapies are more effective in patients very close to diagnosis so that prediction of outcome, for example, chronicity, would be very useful in the decision of minimal treatment, just enough to avoid bleeding, as compared to a rituximab and dexamethasone-based regimen intended to be curative.

## References

- Ahmed, R., Devasia, A.J., Viswabandya, A., et al., 2016. Long-term outcome following splenectomy for chronic and persistent immune thrombocytopenia (ITP) in adults and children: Splenectomy in ITP. *Ann. Hematol.* 95 (9), 1429–1434.
- Arnold, D.M., Dentali, F., Crowther, M.A., et al., 2007. Systematic review: efficacy and safety of rituximab for adults with idiopathic thrombocytopenic purpura. *Ann. Intern. Med.* 146 (1), 25–33.
- Audia, S., Rossato, M., Santegoets, K., et al., 2014. Splenic TFH expansion participates in B-cell differentiation and antiplatelet antibody production during immune thrombocytopenia. *Blood* 124 (18), 2858–2866.
- Audia, S., Mahevas, M., Samson, M., et al., 2017. Pathogenesis of immune thrombocytopenia. *Autoimmun. Rev.* 16 (6), 620–632.
- Ballem, P.J., Segal, G.M., Stratton, J.R., et al., 1987. Mechanisms of thrombocytopenia in chronic autoimmune thrombocytopenic purpura. Evidence of both impaired platelet production and increased platelet clearance. *J. Clin. Invest.* 80 (1), 33–40.
- Barsam, S.J., Psaila, B., Forestier, M., et al., 2011. Platelet production and platelet destruction: assessing mechanisms of treatment effect in immune thrombocytopenia. *Blood* 117 (21), 5723–5732.
- Bidot, L., Jy, W., Bidot Jr, C., et al., 2008. Microparticle-mediated thrombin generation assay: increased activity in patients with recurrent thrombosis. *J. Thromb. Haemost.* 6 (6), 913–919.
- Boyle, S., White, R.H., Brunson, A., et al., 2013. Splenectomy and the incidence of venous thromboembolism and sepsis in patients with immune thrombocytopenia. *Blood* 121 (23), 4782–4790.
- Brighton, T.A., Evans, S., Castaldi, P.A., et al., 1996. Prospective evaluation of the clinical usefulness of an antigen-specific assay (MAIPA) in idiopathic thrombocytopenic purpura and other immune thrombocytopenias. *Blood* 88 (1), 194–201.
- Bussel, J.B., Kuter, D.J., Pullarkat, V., et al., 2009a. Safety and efficacy of long-term treatment with romiplostim in thrombocytopenic patients with chronic ITP. *Blood* 113 (10), 2161–2171.
- Bussel, J.B., Provan, D., Shamsi, T., et al., 2009b. Effect of eltrombopag on platelet counts and bleeding during treatment of chronic idiopathic thrombocytopenic purpura: a randomised, double-blind, placebo-controlled trial. *Lancet* 373 (9664), 641–648.
- Carpenedo, M., Cantoni, S., Coccini, V., et al., 2016. Response loss and development of neutralizing antibodies during long-term treatment with romiplostim in patients with immune thrombocytopenia: a case series. *Eur. J. Haematol.* 97 (1), 101–103.
- Carson, K.R., Evans, A.M., Richey, E.A., et al., 2009. Progressive multifocal leukoencephalopathy after rituximab therapy in HIV-negative patients: a report of 57 cases from the Research on Adverse Drug Events and Reports project. *Blood* 113 (20), 4834–4840.
- Chang, M., Qian, J.X., Lee, S.M., et al., 1999. Tissue uptake of circulating thrombopoietin is increased in immune-mediated compared with irradiated thrombocytopenic mice. *Blood* 93 (8), 2515–2524.
- Chang, M., Nakagawa, P.A., Williams, S.A., et al., 2003. Immune thrombocytopenic purpura (ITP) plasma and purified ITP monoclonal auto-antibodies inhibit megakaryocytopoiesis in vitro. *Blood* 102 (3), 887–895.
- Chapin, J., Lee, C.S., Zhang, H., et al., 2016. Gender and duration of disease differentiate responses to rituximab-dexamethasone therapy in adults with immune thrombocytopenia. *Am. J. Hematol.* 91 (9), 907–911.
- Chen, J.F., Yang, L.H., Chang, L.X., et al., 2012. The clinical significance of circulating B cells secreting anti-glycoprotein IIb/IIIa antibody and platelet glycoprotein IIb/IIIa in patients with primary immune thrombocytopenia. *Hematology* 17 (5), 283–290.

- Cheng, G., Saleh, M.N., Marcher, C., et al., 2011. Eltrombopag for management of chronic immune thrombocytopenia (RAISE): a 6-month, randomised, phase 3 study. *Lancet* 377 (9763), 393–402.
- Cines, D.B., Bussel, J.B., 2005. How I treat idiopathic thrombocytopenic purpura (ITP). *Blood* 106 (7), 2244–2251.
- Cines, D.B., Bussel, J.B., Liebman, H.A., et al., 2009. The ITP syndrome: pathogenic and clinical diversity. *Blood* 113 (26), 6511–6521.
- Cooper, N., 2017. State of the art - how I manage immune thrombocytopenia. *Br. J. Haematol.* 177 (1), 39–54.
- Cooper, N., Woloski, B.M., Fodero, E.M., et al., 2002. Does treatment with intermittent infusions of intravenous anti-D allow a proportion of adults with recently diagnosed immune thrombocytopenic purpura to avoid splenectomy? *Blood* 99 (6), 1922–1927.
- Cooper, N., Davies, E.G., Thrasher, A.J., et al., 2009. Repeated courses of rituximab for autoimmune cytopenias may precipitate profound hypogammaglobulinaemia requiring replacement intravenous immunoglobulin. *Br. J. Haematol.* 146 (1), 120–122.
- Corbacioglu, S., Bux, J., Konig, A., et al., 2000. Serum granulocyte colony-stimulating factor levels are not increased in patients with autoimmune neutropenia of infancy. *J. Pediatr.* 137 (1), 96–99.
- Cornaby, C., Gibbons, L., Mayhew, V., et al., 2015. B cell epitope spreading: mechanisms and contribution to autoimmune diseases. *Immunol. Lett.* 163 (1), 56–68.
- Cortelazzo, S., Finazzi, G., Buelli, M., et al., 1991. High risk of severe bleeding in aged patients with chronic idiopathic thrombocytopenic purpura. *Blood* 77 (1), 31–33.
- Cuker, A., Neunert, C.E., 2016. How I treat refractory immune thrombocytopenia. *Blood* 128 (12), 1547–1554.
- Cuker, A., Prak, E.T., Cines, D.B., 2015. Can immune thrombocytopenia be cured with medical therapy? *Semin. Thromb. Hemost.* 41 (4), 395–404.
- Dameshek, W., Miller, E.B., 1946. The megakaryocytes in idiopathic thrombocytopenic purpura, a form of hypersplenism. *Blood* 1, 27–50.
- Danese, M.D., Lindquist, K., Gleeson, M., et al., 2009. Cost and mortality associated with hospitalizations in patients with immune thrombocytopenic purpura. *Am. J. Hematol.* 84 (10), 631–635.
- Debili, N., Wendling, F., Cosman, D., et al., 1995. The Mpl receptor is expressed in the megakaryocytic lineage from late progenitors to platelets. *Blood* 85 (2), 391–401.
- Doobaree, I.U., Nandigam, R., Bennett, D., et al., 2016. Thromboembolism in adults with primary immune thrombocytopenia: a systematic literature review and meta-analysis. *Eur. J. Haematol.* 97 (4), 321–330.
- Elalfy, M.S., Elhenawy, Y.I., Deifalla, S., et al., 2015. Oxidant/antioxidant status in children and adolescents with immune thrombocytopenia (ITP) and the role of an adjuvant antioxidant therapy. *Pediatr. Blood Cancer* 62 (5), 830–837.
- Frederiksen, H., Maegbaek, M.L., Norgaard, M., 2014. Twenty-year mortality of adult patients with primary immune thrombocytopenia: a Danish population-based cohort study. *Br. J. Haematol.* 166 (2), 260–267.
- Gobert, D., Bussel, J.B., Cunningham-Rundles, C., et al., 2011. Efficacy and safety of rituximab in common variable immunodeficiency-associated immune cytopenias: a retrospective multicentre study on 33 patients. *Br. J. Haematol.* 155 (4), 498–508.
- Grainger, J.D., Locatelli, F., Chotsampancharoen, T., et al., 2015. Eltrombopag for children with chronic immune thrombocytopenia (PETIT2): a randomised, multicentre, placebo-controlled trial. *Lancet* 386 (10004), 1649–1658.
- Grozovsky, R., Begonja, A.J., Liu, K., et al., 2015. The Ashwell-Morell receptor regulates hepatic thrombopoietin production via JAK2-STAT3 signaling. *Nat. Med.* 21 (1), 47–54.
- He, R., Reid, D.M., Jones, C.E., et al., 1994. Spectrum of Ig classes, specificities, and titers of serum antiglycoproteins in chronic idiopathic thrombocytopenic purpura. *Blood* 83 (4), 1024–1032.
- He, R., Reid, D.M., Jones, C.E., et al., 1995. Extracellular epitopes of platelet glycoprotein Ib alpha reactive with serum antibodies from patients with chronic idiopathic thrombocytopenic purpura. *Blood* 86 (10), 3789–3796.
- Heyns Adu, P., Badenhorst, P.N., Lotter, M.G., et al., 1986. Platelet turnover and kinetics in immune thrombocytopenic purpura: results with autologous 111In-labeled platelets and homologous 51Cr-labeled platelets differ. *Blood* 67 (1), 86–92.
- Hori, S., Nomura, T., Sakaguchi, S., 2003. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 299 (5609), 1057–1061.
- Hou, M., Peng, J., Shi, Y., et al., 2003. Mycophenolate mofetil (MMF) for the treatment of steroid-resistant idiopathic thrombocytopenic purpura. *Eur. J. Haematol.* 70 (6), 353–357.
- Houwerzijl, E.J., Blom, N.R., van der Want, J.J., et al., 2004. Ultrastructural study shows morphologic features of apoptosis and para-apoptosis in megakaryocytes from patients with idiopathic thrombocytopenic purpura. *Blood* 103 (2), 500–506.
- Jubelirer, S.J., Harpold, R., 2002. The role of the bone marrow examination in the diagnosis of immune thrombocytopenic purpura: case series and literature review. *Clin. Appl. Thromb. Hemost.* 8 (1), 73–76.
- Kado, R., Sanders, G., McCune, W.J., 2016. Suppression of normal immune responses after treatment with rituximab. *Curr. Opin. Rheumatol.* 28 (3), 251–258.
- Kaushansky, K., 1995. Thrombopoietin: the primary regulator of platelet production. *Blood* 86 (2), 419–431.
- Khellaf, M., Viallard, J.F., Hamidou, M., et al., 2013. A retrospective pilot evaluation of switching thrombopoietic receptor-agonists in immune thrombocytopenia. *Haematologica* 98 (6), 881–887.
- Khodadi, E., Asnafi, A.A., Shahrobi, S., et al., 2016. Bone marrow niche in immune thrombocytopenia: a focus on megakaryopoiesis. *Ann. Hematol.* 95 (11), 1765–1776.
- Kim, J.M., Rasmussen, J.P., Rudensky, A.Y., 2007. Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. *Nat. Immunol.* 8 (2), 191–197.
- Kojouri, K., Vesely, S.K., Terrell, D.R., et al., 2004. Splenectomy for adult patients with idiopathic thrombocytopenic purpura: a systematic review to assess long-term platelet count responses, prediction of response, and surgical complications. *Blood* 104 (9), 2623–2634.
- Kumar, S., Diehn, F.E., Gertz, M.A., et al., 2002. Splenectomy for immune thrombocytopenic purpura: long-term results and treatment of post-splenectomy relapses. *Ann. Hematol.* 81 (6), 312–319.
- Kuter, D.J., 2007. New thrombopoietic growth factors. *Blood* 109 (11), 4607–4616.
- Kuter, D.J., Rosenberg, R.D., 1995. The reciprocal relationship of thrombopoietin (c-Mpl ligand) to changes in the platelet mass during busulfan-induced thrombocytopenia in the rabbit. *Blood* 85 (10), 2720–2730.

- Kuter, D.J., Bussel, J.B., Lyons, R.M., et al., 2008. Efficacy of romiplostim in patients with chronic immune thrombocytopenic purpura: a double-blind randomised controlled trial. *Lancet* 371 (9610), 395–403.
- Kuter, D.J., Bussel, J.B., Newland, A., et al., 2013. Long-term treatment with romiplostim in patients with chronic immune thrombocytopenia: safety and efficacy. *Br. J. Haematol.* 161 (3), 411–423.
- Kuwana, M., Kaburaki, J., Ikeda, Y., 1998. Autoreactive T cells to platelet GPIIb-IIIa in immune thrombocytopenic purpura. Role in production of anti-platelet autoantibody. *J. Clin. Invest.* 102 (7), 1393–1402.
- Kuwana, M., Okazaki, Y., Ikeda, Y., 2014. Detection of circulating B cells producing anti-GPIb autoantibodies in patients with immune thrombocytopenia. *PLoS One* 9 (1), e86943.
- Lambert, M.P., Gernsheimer, T.B., 2017. Clinical updates in adult immune thrombocytopenia. *Blood* 129 (21), 2829–2835.
- Lemoine, S., Morva, A., Youinou, P., et al., 2011. Human T cells induce their own regulation through activation of B cells. *J. Autoimmun.* 36 (3-4), 228–238.
- Li, S., Wang, L., Zhao, C., et al., 2007. CD8+ T cells suppress autologous megakaryocyte apoptosis in idiopathic thrombocytopenic purpura. *Br. J. Haematol.* 139 (4), 605–611.
- Li, X., Zhong, H., Bao, W., et al., 2012. Defective regulatory B-cell compartment in patients with immune thrombocytopenia. *Blood* 120 (16), 3318–3325.
- Liu, B., Zhao, H., Poon, M.C., et al., 2007. Abnormality of CD4(+)CD25(+) regulatory T cells in idiopathic thrombocytopenic purpura. *Eur. J. Haematol.* 78 (2), 139–143.
- Louwes, H., Zeinali Lathori, O.A., Vellenga, E., et al., 1999. Platelet kinetic studies in patients with idiopathic thrombocytopenic purpura. *Am. J. Med.* 106 (4), 430–434.
- Mahevas, M., Patin, P., Huetz, F., et al., 2013. B cell depletion in immune thrombocytopenia reveals splenic long-lived plasma cells. *J. Clin. Invest.* 123 (1), 432–442.
- Mak, Y.K., Yu, P.H., Chan, C.H., et al., 2000. The management of isolated thrombocytopenia in Chinese adults: does bone marrow examination have a role at presentation? *Clin. Lab. Haematol.* 22 (6), 355–358.
- Maloisel, F., Andres, E., Zimmer, J., et al., 2004. Danazol therapy in patients with chronic idiopathic thrombocytopenic purpura: long-term results. *Am. J. Med.* 116 (9), 590–594.
- Mazzucconi, M.G., Fazi, P., Bernasconi, S., et al., 2007. Therapy with high-dose dexamethasone (HD-DXM) in previously untreated patients affected by idiopathic thrombocytopenic purpura: a GIMEMA experience. *Blood* 109 (4), 1401–1407.
- McMillan, R., 1981. Chronic idiopathic thrombocytopenic purpura. *N. Engl. J. Med.* 304 (19), 1135–1147.
- McMillan, R., 2007. The pathogenesis of chronic immune thrombocytopenic purpura. *Semin. Hematol.* 44 (4Suppl 5), S3–S11.
- McMillan, R., Longmire, R.L., Yelenosky, R., et al., 1974. Quantitation of platelet-binding IgG produced in vitro by spleens from patients with idiopathic thrombocytopenic purpura. *N. Engl. J. Med.* 291 (16), 812–817.
- McMillan, R., Wang, L., Tani, P., 2003. Prospective evaluation of the immunobead assay for the diagnosis of adult chronic immune thrombocytopenic purpura (ITP). *J. Thromb. Haemost.* 1 (3), 485–491.
- McMillan, R., Wang, L., Tomer, A., et al., 2004. Suppression of in vitro megakaryocyte production by antiplatelet autoantibodies from adult patients with chronic ITP. *Blood* 103 (4), 1364–1369.
- Moulis, G., Palmaro, A., Montastruc, J.L., et al., 2014. Epidemiology of incident immune thrombocytopenia: a nationwide population-based study in France. *Blood* 124 (22), 3308–3315.
- Moulis, G., Guenin, S., Limal, N., et al., 2017. Seasonal variations of incident primary immune thrombocytopenia in adults: an ecological study. *Eur. J. Intern. Med.* 37, e26–e28.
- Nazi, I., Kelton, J.G., Larche, M., et al., 2013. The effect of rituximab on vaccine responses in patients with immune thrombocytopenia. *Blood* 122 (11), 1946–1953.
- Nemazee, D., Russell, D., Arnold, B., et al., 1991. Clonal deletion of autospesific B lymphocytes. *Immunol. Rev.* 122, 117–132.
- Neunert, C., Lim, W., Crowther, M., et al., 2011. The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. *Blood* 117 (16), 4190–4207.
- Neunert, C., Noroozi, N., Norman, G., et al., 2015. Severe bleeding events in adults and children with primary immune thrombocytopenia: a systematic review. *J. Thromb. Haemost.* 13 (3), 457–464.
- Norgaard, M., Jensen, A.O., Engebjerg, M.C., et al., 2011. Long-term clinical outcomes of patients with primary chronic immune thrombocytopenia: a Danish population-based cohort study. *Blood* 117 (13), 3514–3520.
- Ogawara, H., Handa, H., Morita, K., et al., 2003. High Th1/Th2 ratio in patients with chronic idiopathic thrombocytopenic purpura. *Eur. J. Haematol.* 71 (4), 283–288.
- Olsson, B., Andersson, P.O., Jernas, M., et al., 2003. T-cell-mediated cytotoxicity toward platelets in chronic idiopathic thrombocytopenic purpura. *Nat. Med.* 9 (9), 1123–1124.
- Olsson, B., Ridell, B., Carlsson, L., et al., 2008. Recruitment of T cells into bone marrow of ITP patients possibly due to elevated expression of VLA-4 and CX3CR1. *Blood* 112 (4), 1078–1084.
- Panitsas, F.P., Theodoropoulou, M., Kourakis, A., et al., 2004. Adult chronic idiopathic thrombocytopenic purpura (ITP) is the manifestation of a type-1 polarized immune response. *Blood* 103 (7), 2645–2647.
- Patel, V.L., Mahevas, M., Lee, S.Y., et al., 2012. Outcomes 5 years after response to rituximab therapy in children and adults with immune thrombocytopenia. *Blood* 119 (25), 5989–5995.
- Provan, D., Moss, A.J., Newland, A.C., et al., 2006. Efficacy of mycophenolate mofetil as single-agent therapy for refractory immune thrombocytopenic purpura. *Am. J. Hematol.* 81 (1), 19–25.
- Provan, D., Stasi, R., Newland, A.C., et al., 2010. International consensus report on the investigation and management of primary immune thrombocytopenia. *Blood* 115 (2), 168–186.
- Psaila, B., Bussel, J.B., Frelinger, A.L., et al., 2011. Differences in platelet function in patients with acute myeloid leukemia and myelodysplasia compared to equally thrombocytopenic patients with immune thrombocytopenia. *J. Thromb. Haemost.* 9 (11), 2302–2310.

- Quiquandon, I., Fenaux, P., Caulier, M.T., et al., 1990. Re-evaluation of the role of azathioprine in the treatment of adult chronic idiopathic thrombocytopenic purpura: a report on 53 cases. *Br. J. Haematol.* 74 (2), 223–228.
- Riviere, E., Viallard, J.F., Guy, A., et al., 2015. Intrinsically impaired platelet production in some patients with persistent or chronic immune thrombocytopenia. *Br. J. Haematol.* 170 (3), 408–415.
- Rodeghiero, F., Carli, G., 2017. Beyond immune thrombocytopenia: the evolving role of thrombopoietin receptor agonists. *Ann. Hematol.* 96 (9), 1421–1434.
- Rodeghiero, F., Stasi, R., Gernsheimer, T., et al., 2009. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. *Blood* 113 (11), 2386–2393.
- Sakaguchi, S., Wing, K., Onishi, Y., et al., 2009. Regulatory T cells: how do they suppress immune responses? *Int. Immunol.* 21 (10), 1105–1111.
- Sakakura, M., Wada, H., Tawara, I., et al., 2007. Reduced Cd4+ Cd25+ T cells in patients with idiopathic thrombocytopenic purpura. *Thromb. Res.* 120 (2), 187–193.
- de Sauvage, F.J., Carver-Moore, K., Luoh, S.M., et al., 1996. Physiological regulation of early and late stages of megakaryocytopoiesis by thrombopoietin. *J. Exp. Med.* 183 (2), 651–656.
- Scaradavou, A., Woo, B., Woloski, B.M., et al., 1997. Intravenous anti-D treatment of immune thrombocytopenic purpura: experience in 272 patients. *Blood* 89 (8), 2689–2700.
- Schneider, P., MacKay, F., Steiner, V., et al., 1999. BAFF, a novel ligand of the tumor necrosis factor family, stimulates B cell growth. *J. Exp. Med.* 189 (11), 1747–1756.
- Segal, J.B., Powe, N.R., 2006. Prevalence of immune thrombocytopenia: analyses of administrative data. *J. Thromb. Haemost.* 4 (11), 2377–2383.
- Semple, J.W., 2012. Bregging rights in ITP. *Blood* 120 (16), 3169.
- Sewify, E.M., Sayed, D., Abdel Aal, R.F., et al., 2013. Increased circulating red cell microparticles (RMP) and platelet microparticles (PMP) in immune thrombocytopenic purpura. *Thromb. Res.* 131 (2), e59–e63.
- Shulman, N.R., Marder, V.J., Weinrach, R.S., 1965. Similarities between known antiplatelet antibodies and the factor responsible for thrombocytopenia in idiopathic purpura. Physiologic, serologic and isotopic studies. *Ann. N. Y. Acad. Sci.* 124 (2), 499–542.
- Stahl, C.P., Zucker-Franklin, D., McDonald, T.P., 1986. Incomplete antigenic cross-reactivity between platelets and megakaryocytes: relevance to ITP. *Blood* 67 (2), 421–428.
- Stasi, R., Stipa, E., Masi, M., et al., 1995. Long-term observation of 208 adults with chronic idiopathic thrombocytopenic purpura. *Am. J. Med.* 98 (5), 436–442.
- Stirnemann, J., Kaddouri, N., Khellaf, M., et al., 2016. Vincristine efficacy and safety in treating immune thrombocytopenia: a retrospective study of 35 patients. *Eur. J. Haematol.* 96 (3), 269–275.
- Takahashi, N., Saitoh, T., Gotoh, N., et al., 2017. The cytokine polymorphisms affecting Th1/Th2 increase the susceptibility to, and severity of, chronic ITP. *BMC Immunol.* 18 (1), 26.
- Taylor, A., Neave, L., Solanki, S., et al., 2015. Mycophenolate mofetil therapy for severe immune thrombocytopenia. *Br. J. Haematol.* 171 (4), 625–630.
- Terrell, D.R., Beebe, L.A., Vesely, S.K., et al., 2010. The incidence of immune thrombocytopenic purpura in children and adults: a critical review of published reports. *Am. J. Hematol.* 85 (3), 174–180.
- Tsubakio, T., Tani, P., Curd, J.G., et al., 1986. Complement activation in vitro by antiplatelet antibodies in chronic immune thrombocytopenic purpura. *Br. J. Haematol.* 63 (2), 293–300.
- Wang, T., Zhao, H., Ren, H., et al., 2005. Type 1 and type 2 T-cell profiles in idiopathic thrombocytopenic purpura. *Haematologica* 90 (7), 914–923.
- Warner, M.N., Moore, J.C., Warkentin, T.E., et al., 1999. A prospective study of protein-specific assays used to investigate idiopathic thrombocytopenic purpura. *Br. J. Haematol.* 104 (3), 442–447.
- Wei, Y., Ji, X.B., Wang, Y.W., et al., 2016. High-dose dexamethasone vs prednisone for treatment of adult immune thrombocytopenia: a prospective multicenter randomized trial. *Blood* 127 (3), 296–302. quiz 370.
- Wong, R.S.M., Saleh, M.N., Khelifi, A., et al., 2017. Safety and efficacy of long-term treatment of chronic/persistent ITP with eltrombopag: final results of the EXTEND study. *Blood* 130, 2527–2536.
- Woods Jr, V.L., Oh, E.H., Mason, D., et al., 1984. Autoantibodies against the platelet glycoprotein IIb/IIIa complex in patients with chronic ITP. *Blood* 63 (2), 368–375.
- Yu, H., Paiva, R., Flavell, R.A., 2017. Harnessing the power of regulatory T cells to control autoimmune diabetes: overview and perspective. *Immunology* 153 (2), 161–170.
- Zhang, B., Zehnder, J.L., 2013. Oxidative stress and immune thrombocytopenia. *Semin. Hematol.* 50 (3), e1–e4.
- Zhang, D., Zhang, X., Ge, M., et al., 2014. The polymorphisms of T cell-specific TBX21 gene may contribute to the susceptibility of chronic immune thrombocytopenia in Chinese population. *Hum. Immunol.* 75 (2), 129–133.
- Zhao, Y.Q., Wang, Q.Y., Zhai, M., et al., 2004. [A multi-center clinical trial of recombinant human thrombopoietin in chronic refractory idiopathic thrombocytopenic purpura]. *Zhonghua Nei Ke Za Zhi.* 43 (8), 608–610.
- Zhao, C., Li, X., Zhang, F., et al., 2008. Increased cytotoxic T-lymphocyte-mediated cytotoxicity predominant in patients with idiopathic thrombocytopenic purpura without platelet autoantibodies. *Haematologica* 93 (9), 1428–1430.
- Zhu, X.J., Shi, Y., Peng, J., et al., 2009. The effects of BAFF and BAFF-R-Fc fusion protein in immune thrombocytopenia. *Blood* 114 (26), 5362–5367.
- Zufferey, A., Kapur, R., Semple, J.W., 2017. Pathogenesis and therapeutic mechanisms in immune thrombocytopenia (ITP). *J. Clin. Med.* 6 (2), pii: E16.

# Acquired Aplastic Anemia

*Robert A. Brodsky and Richard J. Jones*

Division of Hematology, Department of Medicine and The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD, United States

## OUTLINE

Historic Background	923	Immunosuppressive Therapy	927
Genetic Features	924	Eltrombopag	928
Clinical, Pathologic, and Epidemiologic Features	924	High-Dose Cyclophosphamide Without Bone Marrow Transplantation	929
Autoimmune Features and Pathogenic Mechanisms	925	Human Leukocyte Antigen Haploididential Bone Marrow Transplant With Posttransplant Cyclophosphamide	929
Environmental Features	926	Aplastic Anemia and Clonality	931
Animal Models	926	Concluding Remarks—Future Prospects	931
Therapy for Aplastic Anemia	926	References	932
Bone Marrow Transplantation	926		
Bone Marrow Transplantation From Unrelated Donors	927		

Aplastic anemia manifests with pancytopenia and a hypocellular bone marrow (Brodsky and Jones, 2005). The disease may be acquired or inherited. Most cases of acquired aplastic anemia result from autoimmune destruction of hematopoietic stem/progenitors and respond to immunosuppressive therapies. The inherited forms of aplastic anemia are less common and usually present within the first decade of life (Tsangaris et al., 2011). The inherited bone marrow failure may be due to a variety of genetic mutations such as DNA repair defects (Fanconi anemia), telomerase defects [dyskeratosis congenita (DKC)], ribosomopathies (Shwachman–Diamond syndrome), or cMPL mutations (amegakaryocytic thrombocytopenia). Immunosuppressive therapy is not helpful for the most inherited forms of bone marrow failure. This chapter will predominantly focus on acquired aplastic anemia; however, it is important to be aware of these less common inherited forms of aplastic anemia since they can sometimes be hard to distinguish from the acquired form of the disease.

## HISTORIC BACKGROUND

The earliest case description of aplastic anemia in 1888 was by Dr. Paul Ehrlich (Ehrlich, 1888). He described a young woman who died following an abrupt illness characterized by severe anemia, bleeding, high fever, and a markedly hypocellular bone marrow. Until the early 1970s, most patients with severe aplastic anemia (SAA) died within a year of diagnosis. The advent of allogeneic (allo) bone marrow transplantation (BMT) and

immunosuppressive therapy markedly improved the outcome for these patients and prompted vigorous clinical and laboratory investigation. These studies have generated an important insight into hematopoietic stem cell biology, immunology, and autoimmunity. Today the majority of patients will survive this potentially fatal autoimmune disorder.

## GENETIC FEATURES

Distinctive genetic abnormalities are more common with congenital bone marrow failure syndromes since most acquired aplastic anemia is autoimmune. Congenital aplastic anemia tends to present in the first decade of life and is often, but not always, associated with other physical anomalies. Fanconi anemia, the most common form of congenital bone marrow failure, predisposes to cancer and is frequently associated with other congenital abnormalities (e.g., short stature, upper limb anomalies, hypogonadism, café-au-lait spots, etc.) (Bagby, 2003). DKA is another congenital bone marrow failure disorder that can be either X-linked recessive, autosomal dominant, or autosomal recessive (Dokal and Vulliamy, 2003). The X-linked recessive form results from mutations in a gene known as *DKC1* whose gene product, dyskerin is important for stabilizing telomerase. The resulting telomerase deficiency leads to short telomeres, bone marrow failure, and premature aging. The autosomal dominant form of DKA results from human telomerase RNA component (*hTERC*) gene mutations, the RNA component of telomerase. This chapter will focus on the acquired form of aplastic anemia. Genetic abnormalities are less well characterized in acquired aplastic anemia; however, there appears to be an underlying genetic predisposition to acquired aplastic anemia, as evidenced by the overrepresentation of human leukocyte antigen (HLA) DR2 subtypes (Nimer et al., 1994).

## CLINICAL, PATHOLOGIC, AND EPIDEMIOLOGIC FEATURES

Aplastic anemia manifests as pancytopenia in conjunction with a hypocellular bone marrow. The disease may present abruptly (over days) or insidiously, over weeks to months. The most common clinical manifestations reflect the low blood counts and include dyspnea on exertion, fatigue, easy bruising, petechia, epistaxis, gingival bleeding, heavy menses, headaches, and fever. A complete blood count, leukocyte differential, reticulocyte count, and a bone marrow aspirate and biopsy are essential for diagnosis. Peripheral blood flow cytometry to detect glycosylphosphatidylinositol (GPI) anchor deficient blood cells (Brodsky et al., 2000; Borowitz et al., 2010) as well as cytogenetics and fluorescent in situ hybridization (FISH) analysis should be performed on the bone marrow aspirate. Up to 70% of patients with acquire aplastic anemia have a detectable paroxysmal nocturnal hemoglobinuria (PNH) clone which essentially rules out inherited forms of aplastic anemia (Dezern et al., 2014). Cytogenetic or FISH abnormalities are suggestive of a hypoplastic form of myelodysplasia. Patients under the age of 40 years old should be screened for Fanconi anemia using the clastogenic agents diepoxybutane or mitomycin C, and telomere lengths should be obtained on patients with a family history of bone marrow failure, premature graying, pulmonary fibrosis, or other stigmata of DKA.

A hypocellular bone marrow is required for the diagnosis of aplastic anemia. However, some patients will have residual pockets of ongoing hematopoiesis; thus an adequate biopsy (1–2 cm in length) is essential for establishing the diagnosis. Dyserythropoiesis is not uncommon in aplastic anemia, especially in cases with coincidental small-to-moderate PNH populations; however, a small percentage of myeloid blasts, or dysplastic features in the myeloid or megakaryocyte lineages, is more typical of hypoplastic myelodysplastic syndromes (MDS). CD34 is expressed on early hematopoietic progenitors and the number of CD34<sup>+</sup> cells has also been used to help discriminate between aplastic anemia and hypoplastic myelodysplastic syndrome (hMDS). In aplastic anemia the percentage of cells expressing CD34 is usually less than 0.1%; in hMDS the CD34 count is either normal (0.5%–1.0%) or elevated (Matsui et al., 2006).

As with other autoimmune diseases, there is a wide spectrum of disease severity in aplastic anemia. The prognosis in aplastic anemia is proportional to degree of peripheral blood cytopenias. Accordingly, aplastic anemia is classified as nonsevere, severe, and very severe based largely upon the degree of neutropenia (Table 49.1). SAA is defined as bone marrow cellularity of less than 25% and markedly decreased values of at least two of three hematopoietic lineages (neutrophil count <500/ $\mu$ L, platelet count <20,000/ $\mu$ L, and absolute reticulocyte count of <60,000/ $\mu$ L). Very SAA satisfies the above criteria except the neutrophil count is <200/ $\mu$ L, while non-SAA is characterized by a hypocellular bone marrow but with cytopenias that do not meet the criteria for severe disease.

**TABLE 49.1** Aplastic Anemia: Diagnosis and Definitions

Peripheral blood counts	Nonsevere aplastic anemia (not meeting criteria for severe disease)	Severe aplastic anemia (any 2 of 3)	Very-severe aplastic anemia (meets criteria for severe disease and absolute neutrophils <200)
absolute neutrophils		<500/ $\mu$ L	<200/ $\mu$ L
platelets		<20,000/ $\mu$ L	
reticulocyte count		<1.0% corrected or <60,000/ $\mu$ L	

Bone marrow cellularity <25%.

The 2-year mortality rate with supportive care alone for patients with SAA exceeds 50% (Camitta et al., 1979), with invasive fungal infections and overwhelming bacterial sepsis being the most frequent causes of death. Non-SAA is seldom life-threatening and in many instances requires no therapy. Although some cases of non-SAA will progress, many will remain stable for years, and some may spontaneously improve.

Aplastic anemia has been associated with drugs, benzene exposure, insecticides, viruses, and other agents. However, over 80% of cases are classified as idiopathic. The disease most commonly affects children and young adults but may occur at any age. Precise estimates of the incidence of aplastic anemia are difficult due to the rarity of the disease and imprecision in establishing the diagnosis. The best estimates of incidence are case-control studies that report an incidence of two cases/million inhabitants in Europe (Kaufman et al., 1991) and Israel (Modan et al., 1975), but the incidence may be two to threefold higher in Southeast Asia (Issaragrisil et al., 1997; Szklo et al., 1985). A population-based case-control study of aplastic anemia in Thailand found that drugs, the most commonly implicated etiology, explain only 5% of newly diagnosed cases (Issaragrisil et al., 1997).

An intriguing association exists between seronegative hepatitis and aplastic anemia. The hepatitis-aplastic anemia syndrome accounts for 3%–5% of newly diagnosed cases of aplastic anemia. The disease predominantly affects young males, with a precipitous onset of severe pancytopenia occurring within 2–3 months after the onset of hepatitis (Brown et al., 1997). Moreover, aplastic anemia has been reported to occur in up to 30% of patients following orthotopic liver transplantation for seronegative hepatitis (Tzakis et al., 1988; Cattral et al., 1994). The aplastic anemia in the hepatitis/aplastic anemia syndrome is also thought to be autoimmune since most cases respond to immunosuppressive therapy (Savage et al., 2007; Locasciulli et al., 2010).

## AUTOIMMUNE FEATURES AND PATHOGENIC MECHANISMS

Aplastic anemia was originally thought to result from a quantitative deficiency of hematopoietic stem cells precipitated by a direct toxic effect on stem cells. However, the attempts to treat aplastic anemia by simple transfusion of bone marrow from an identical twin failed to reconstitute hematopoiesis in most patients. Retransplant of many of these patients following a high-dose cyclophosphamide preparative regimen was successful, suggesting that the pathophysiology of aplastic anemia was more complicated (Champlin et al., 1984; Hinterberger et al., 1997). In the late 1960s Mathe et al. (1970) were among the first to postulate an immune basis for aplastic anemia. They performed BMT in patients with aplastic anemia using partially mismatched donors after administering antilymphocyte globulin as an immunosuppressive conditioning regimen. Although the patients failed to engraft, the investigators witnessed autologous recovery of hematopoiesis in some patients. This suggested that functional hematopoietic stem cells exist in aplastic anemia patients and that the immune system was somehow suppressing the growth and differentiation of hematopoietic stem cells. The response to immunosuppressive therapy was the first clear evidence that aplastic anemia was truly an autoimmune disease.

The first laboratory experiments implicating an autoimmune pathophysiology were coculture experiments showing that T lymphocytes from aplastic anemia patients inhibited hematopoietic colony formation in vitro (Hoffman et al., 1977; Nissen et al., 1980). Since then, it has been shown that the immune destruction of hematopoietic stem cells in aplastic anemia is mediated by cytotoxic T cells and involves inhibitory Th1 cytokines and the Fas-dependent cell death pathway. The cytotoxic T cells are usually more conspicuous in the bone marrow than in the peripheral blood (Zoumbos et al., 1985; Selleri et al., 1994; Melenhorst et al., 1997) and overproduce interferon- $\gamma$  and tumor necrosis factor (TNF) (Nakao, 1997; Nistico and Young, 1994). TNF and interferon- $\gamma$  are

direct inhibitors of hematopoiesis and appear to upregulate Fas expression on CD34<sup>+</sup> cells (Maciejewski et al., 1995). Immortalized CD4<sup>+</sup> and CD8<sup>+</sup> T-cell clones from some aplastic anemia patients have been shown to secrete Th1 cytokines and are capable of lysing autologous CD34 cells (Nakao et al., 1997; Zeng et al., 2001). Evidence for a humoral autoimmune response in aplastic anemia has also been reported (Hirano et al., 2000; Feng et al., 2004). Studies examining T-cell diversity using complementarity-determining region (CDR3) spectratyping have further implicated the role of the immune system in aplastic anemia. Several groups have now found limited heterogeneity of the T-cell receptor β-chain (BV) in aplastic anemia, suggesting that there is oligoclonal or even clonal expansion of T cells in response to a specific antigen (Melenhorst et al., 1997; Zeng et al., 2001; Manz et al., 1997).

## ENVIRONMENTAL FEATURES

The medical literature is replete with reports of environmental exposures, most notably benzene and radiation, causing aplastic anemia. However, rigorous epidemiologic studies supporting an association between environmental toxins and aplastic anemia are lacking. A major confounder is that benzene, radiation, and other toxins also predispose to MDS and leukemia. Older literature was unlikely to have been able to distinguish different types of marrow failure, such as aplastic anemia, MDS, and hypoplastic leukemia, leading to an overestimation of the association between benzene and aplastic anemia. While the magnitude of the risk remains uncertain, benzene is probably not a major risk factor for aplastic anemia in countries with modern standards of industrial hygiene. A large case-control study in Thailand employing modern diagnostic and epidemiologic methods found that individuals of lower economic status and younger age are at greater risk than their counterparts in other countries following exposure to solvents, glues, and hepatitis A (likely a surrogate marker). Grain farmers were also found to have a higher risk of developing aplastic anemia (relative risk = 2.7) regardless of whether they use insecticides (Issaragrisil et al., 1997). These same investigators noted marked differences in the incidence between northern and southern rural regions of Thailand and among Bangkok suburbs implicating potential environmental factors in causing the disease (Issaragrisil et al., 1999).

## ANIMAL MODELS

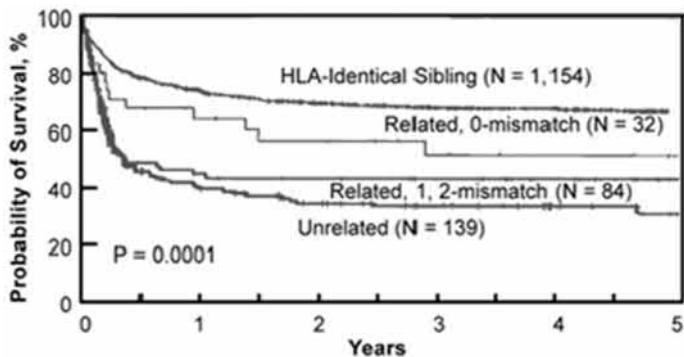
Animal models of bone marrow failure exist, but none of these models fully replicate the human disease acquired aplastic anemia (Chen, 2005). Busulfan, benzene, and irradiation have all been used to establish the models of marrow failure. All three of these agents lead to pancytopenia, and a hypocellular marrow but the marrow failure is due to stem cell injury and damage to the microenvironment rather than autoimmune-mediated suppression of hematopoiesis. More recently, infusion of lymphocytes from congenic mice was used to model immune-mediated marrow failure; this model induces a hypocellular bone marrow and severe pancytopenia but is not truly autoimmune (Chen et al., 2004).

## THERAPY FOR APLASTIC ANEMIA

Definitive therapy for aplastic anemia includes BMT or immunosuppressive therapy. A variety of immunosuppressive agents have been studied, but antithymocyte globulin (ATG) and cyclosporine A (CSA) are the most commonly employed (Bacigalupo, 2017). Supportive care with blood transfusions and antibiotics is commonly required. Administration of hematopoietic growth factors has not been shown to improve survival in aplastic anemia.

### Bone Marrow Transplantation

BMT is the treatment of choice for young patients who have an HLA-matched sibling donor. Cyclophosphamide (50 mg/kg/day × 4 days) with or without ATG is most commonly used for conditioning before BMT. This regimen is nonmyeloablative; however, the immunosuppression is sufficient to allow engraftment in most cases (Storb et al., 1997; Kahl et al., 2005). Alternative regimens using fludarabine,



**FIGURE 49.1** Survival after allogeneic bone marrow transplantation for severe aplastic anemia. Data for HLA-identical siblings and related matched/mismatched transplants is from the IBMTR. Data for unrelated donor transplants is from the EBMT registry, Fred Hutchinson Cancer Research Center, IBMTR, and the IMUST study group. Survival curves are not adjusted for varying patient, disease, and transplant regimen characteristics. HLA, Human leukocyte antigen; EBMT, European Bone Marrow Transplant; IBMTR, International Bone Marrow Transplant Registry; IMUST, International Marrow Unrelated Search and Transplant Study. Source: Reproduced with permission from the National Bone Marrow Donor Program.

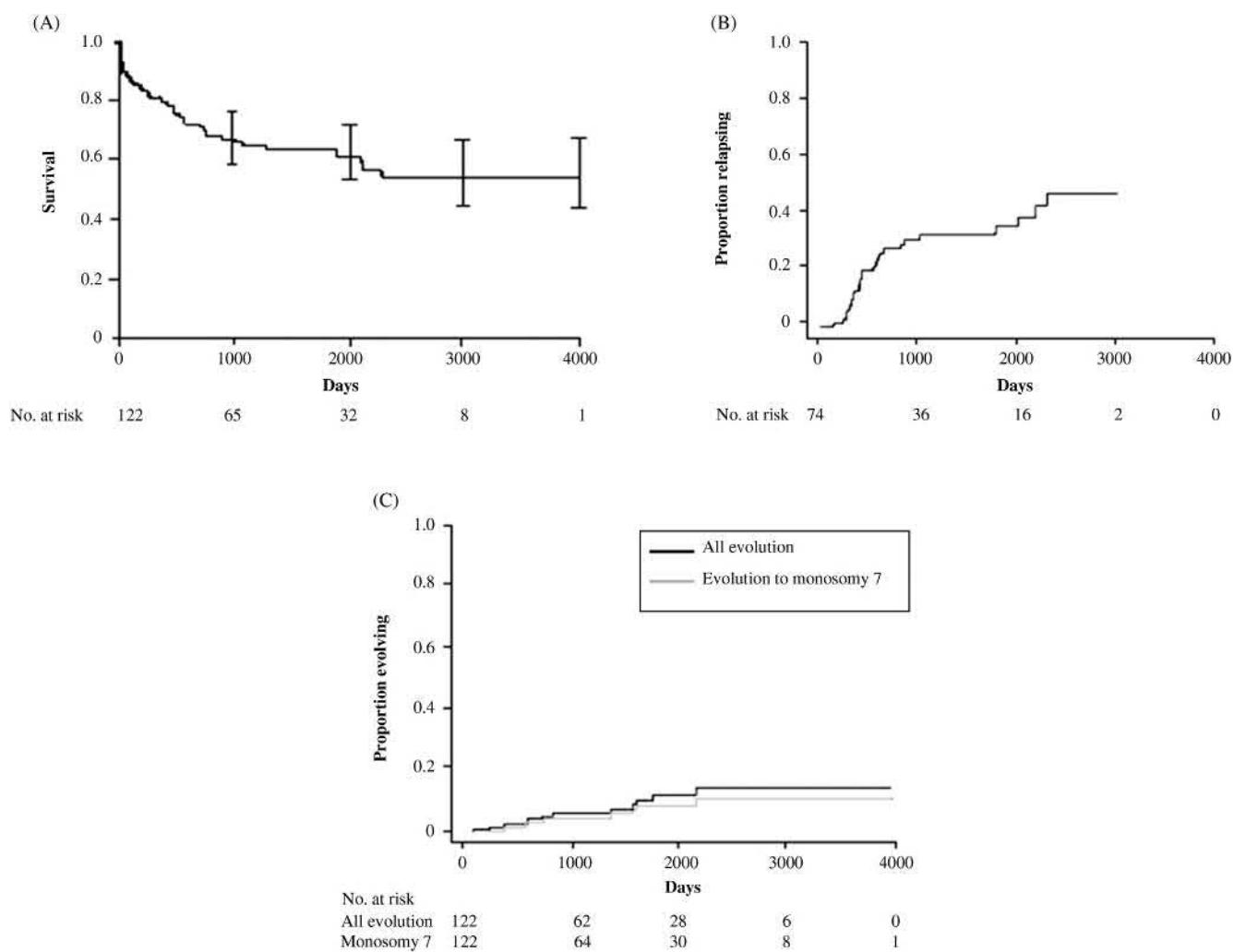
cyclophosphamide, and ATG are increasingly being used (Bacigalupo et al., 2010). Survival rates following matched sibling allo BMT have steadily improved since the 1970s largely because of improved supportive care, improved HLA typing, and better graft-versus-host disease (GVHD) prophylaxis (Bacigalupo, 1999). Late BMT-related complications such as chronic GVHD occur in up to one-third of patients, with many of these patients requiring long-term therapy for their GVHD (Storb et al., 2001). Patient age and the type of allograft (HLA-matched sibling, unrelated, or mismatched donors) are the most important factors influencing outcome. In patients under 30 years of age the cure rate after HLA-matched sibling BMT ranges from 70% to 90% (Ades et al., 2004; Horowitz, 2000). However, the risk of GVHD steadily increases with age, leading to reduced survival.

### Bone Marrow Transplantation From Unrelated Donors

BMT from HLA-matched unrelated donors is usually reserved for patients who fail to respond to one or more courses of immunosuppressive therapy. The risk for transplant-related mortality and GVHD is almost twice that of BMT from matched sibling donors (Bacigalupo, 2017). The European Group for Blood and Marrow Transplantation has been conditioning SAA patients for BMT with fludarabine, cyclophosphamide, and ATG  $\pm$  total body irradiation. The European Society for Blood and Marrow Transplantation (EBMT) has reported survival rates as high as 75% using this conditioning regimen (Bacigalupo et al., 2010). Survival was the best in children and in patients who undergo BMT within 2 years of diagnosis (Fig. 49.1).

## IMMUNOSUPPRESSIVE THERAPY

ATG is produced by immunizing animals (horse or rabbit) against human thymocytes and kills human T cells through its cytolytic activity. Both horse (hATG) and rabbit (rATG) are approved for the use in the United States; hATG appears to be superior as first-line treatment (Scheinberg et al., 2011). Cyclosporine A (CSA) suppresses T-cell function by inhibiting the expression of nuclear regulatory proteins. Both single-agent ATG and single-agent CSA can induce remissions in acquired aplastic anemia; however, the combination ATG/CSA leads to a higher response rate and a greater likelihood of achieving transfusion independence (Frickhofen et al., 1991; Marsh et al., 1999). A randomized controlled demonstrated that hATG/CSA is superior to rATG/CSA (Scheinberg et al., 2011). The combination of ATG/CSA leads to 5-year survival rates comparable to BMT, but most of these patients are not cured of their disease. Response rates to hATG/CSA range between 60% and 80%, but in contrast to BMT, most patients do not acquire normal blood counts (Frickhofen et al., 1991; Rosenfeld et al., 2003). Another limitation of this approach is that many patients relapse, become dependent on cyclosporine, or develop secondary clonal disease such as PNH or MDS (Rosenfeld et al., 2003; Bacigalupo et al., 2000; Scheinberg et al., 2006). These late events often lead to substantial morbidity and mortality. The National Institutes of Health treated 122 patients (median age, 35 years) with the combination of ATG/CSA and methylprednisolone over a period of 8 years (Rosenfeld et al., 2003). The response rate was 58% and actuarial survival at 7 years was 55%; 13% of patients died within 3 months of treatment, most from fungal infections (Fig. 49.2). The relapse rate for responders was 40% and 13 patients developed MDS. In an attempt to improve response rate and survival, and to decrease the relapse rate and secondary MDS that occurs after hATG/CSA, the NIH added mycophenolate (1 g twice daily for 18 months) to the standard hATG/CSA



**FIGURE 49.2** (A) Survival probability for 122 patients with severe aplastic anemia following treatment with antithymocyte globulin and cyclosporine. (B) Probability of relapse in 74 patients with aplastic anemia classified as responders at 3 months after treatment with antithymocyte globulin and cyclosporine. (C) Proportion of patients experiencing clonal evolution. Source: Reproduced from Rosenfeld, S., Follmann, D., Nunez, O., Young, N.S., 2003. Antithymocyte globulin and cyclosporine for severe aplastic anemia: association between hematologic response and long-term outcome. *JAMA* 289 (9), 1130–1135 with permission from JAMA.

regimen. This three drug regimen resulted in a 62% response rate, but 37% of the responders relapsed (most while taking mycophenolate) and 9% progressed to either MDS or leukemia; thus the addition of mycophenolate did not improve response or survival (Scheinberg et al., 2006). Alemtuzumab is a highly immunosuppressive monoclonal antibody that binds to cell surface CD52, which is expressed primarily on B and T cells and monocytes. Alemtuzumab has activity in treating SAA, but response rates in therapy naive patients are less than 30% (Scheinberg et al., 2012).

## ELTROMBOPAG

Eltrombopag is a small molecule agonist of the c-mpl (TpoR) receptor, which is the physiological target of the hormone thrombopoietin, and is the only new drug approved for the treatment of SAA in the past 30 years. Eltrombopag may be used in an attempt to improve the cytopenias in patients at the refractory state (Desmond et al., 2015). Up to 20% of patients with refractory SAA become transfusion-independent within 3 months; however, similar to immunosuppressive therapy, there is a relatively high likelihood of relapse and secondary clonal disease (Desmond et al., 2014; Marsh and Kulasekararaj, 2013; Olnes et al., 2012). Eltrombopag is now

being studied as an adjuvant to ATG/CSA in newly diagnosed patients in the hopes that it will improve response rates and prevent relapse.

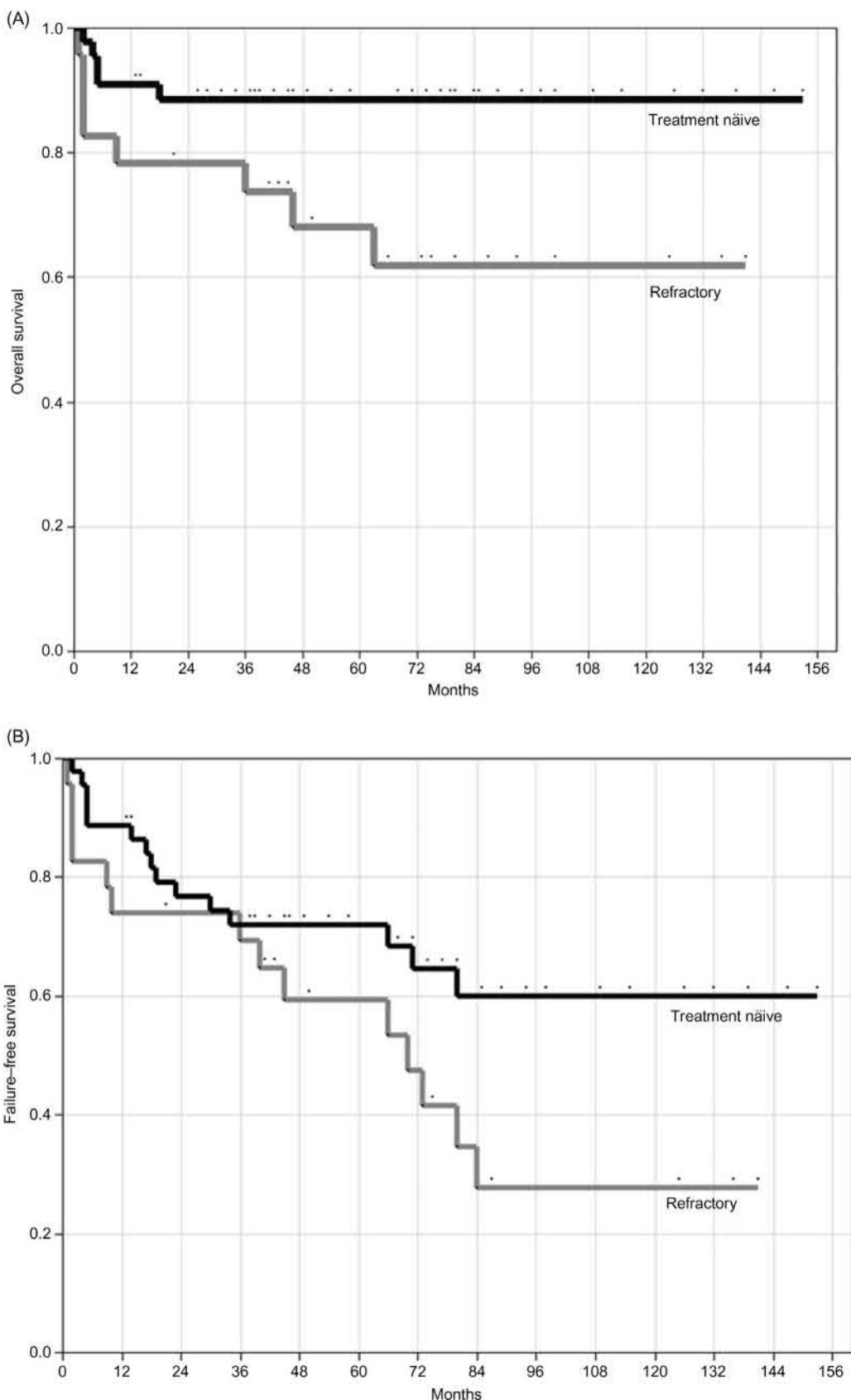
## HIGH-DOSE CYCLOPHOSPHAMIDE WITHOUT BONE MARROW TRANSPLANTATION

The first successful human allo BMT, reported in 1972 by Thomas et al. (1972) in a patient with aplastic anemia, employed high-dose cyclophosphamide, and this remains (often in conjunction with ATG) the most commonly employed conditioning regimen for aplastic anemia (Storb et al., 2001). Complete reconstitution of autologous hematopoiesis occurs in 10%–15% of patients undergoing allo BMT for aplastic anemia (Thomas et al., 1976; Sensenbrenner et al., 1977; Gmur et al., 1979). The EBMT reported that 10% of SAA patients experience autologous reconstitution following BMT using a cyclophosphamide + ATG conditioning regimen. Interestingly, 10-year survival (84%) in patients with autologous recovery was better than in patients who engrafted (74%) (Piccin et al., 2010).

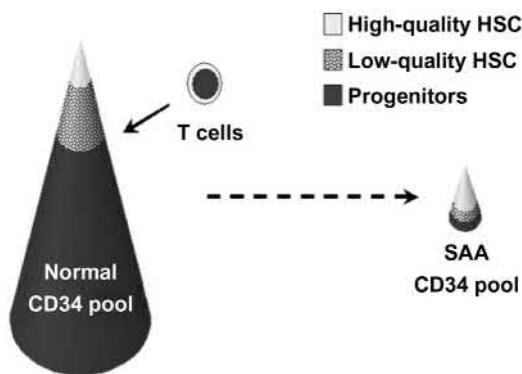
The unique pharmacology of cyclophosphamide explains the autologous hematopoietic recovery (Emadi et al., 2009). Cyclophosphamide is a prodrug that is converted to 4-hydroxycyclophosphamide and its tautomer aldophosphamide in the liver. These compounds diffuse into the cell and are converted to the active compound phosphoramide mustard, or they are inactivated by aldehyde dehydrogenase to form the inert carboxyphosphamide. Lymphocytes have low levels of aldehyde dehydrogenase and are rapidly killed by high doses of cyclophosphamide; hematopoietic stem cells possess high levels of aldehyde dehydrogenase and are resistant to cyclophosphamide (Hilton, 1984; Jones et al., 1995). Thus, high-dose cyclophosphamide is highly immunosuppressive, but not myeloablative, allowing endogenous hematopoietic stem cells to reconstitute hematopoiesis. With this background, high-dose cyclophosphamide without BMT was used successfully in aplastic anemia patients who lacked appropriate donor (Brodsy et al., 1996a; Tisdale et al., 2000; Jaime-Prez et al., 2001; Brodsky et al., 2010). The largest and most mature study with high-dose cyclophosphamide is from Johns Hopkins (Brodsky et al., 2010; Gamper et al., 2016). These investigators treated 67 SAA patients with high-dose cyclophosphamide; 44 patients were treatment-naïve and 23 were refractory to one or more previous immunosuppressive regimens. At 10 years, the overall actuarial survival, response rate, and event-free survival were 88%, 71%, and 58%, respectively, for the 44 treatment-naïve patients. Patients with refractory SAA fared less well; at 10 years, the overall actuarial survival, response, and event-free survival rates were 62%, 48%, and 27%, respectively. For the treatment-naïve patients, the median time to a neutrophil count of  $0.5 \times 10^9/L$  was 60 (range, 28–104) days, and the median time to last platelet and red cell transfusion was 117 and 186 days, respectively. Relapse occurred in just two of the treatment-naïve patients, one of whom was retreated with high-dose cyclophosphamide into a second complete remission. Despite the high response rate and low risk of relapse and secondary clonal disease, the duration of deep aplasia (median 60 days to neutrophil recovery) and risk for invasive fungal infections is a major drawback for this approach (Fig. 49.3).

## HUMAN LEUKOCYTE ANTIGEN HAPLOIDENTICAL BONE MARROW TRANSPLANT WITH POSTTRANSPLANT CYCLOPHOSPHAMIDE

Investigators at Johns Hopkins, in an effort to expand the donor pool for patients in need of a allo bone marrow transplant, have pioneered the use of high-dose cyclophosphamide 3 and 4 days after bone marrow transplant to improve engraftment and decrease the risk of GVHD after HLA-haploidential bone marrow grafts (Luznik et al., 2008). These authors recently reported on a prospective trial of this approach in 16 consecutive patients with refractory SAA (DeZern et al., 2017). Between July 2011 and August 2016, 16 patients underwent allo BMT for refractory SAA from 13 haploidential donors and 3 unrelated donors. All 16 patients engrafted and survived with a median follow-up of 21 (range, 3–64) months at the time of publication. Two patients had grade 1 or 2 skin-only acute GVHD. These same two also had mild chronic GVHD of the skin/mouth requiring systemic steroids. One of these GVHD patients was able to come off all immunosuppressive therapy (IST) by 15 months and the other by 17 months. All other patients stopped IST at 1 year (Fig. 49.4).



**FIGURE 49.3** Survival probability following high-dose cyclophosphamide without BMT in 67 patients with SAA. (A) Overall survival for 44 patients with treatment-naïve SAA (topline) and 23 patients with refractory SAA. (B) Failure-free survival after high-dose cyclophosphamide therapy for 44 patients with treatment-naïve SAA (topline) and 23 patients with refractory SAA. BMT, Bone marrow transplantation; SAA, severe aplastic anemia. Source: Reproduced with permission from *Blood* 2010 (Brodsky, R. A., Chen, A. R., Dorr, D., Fuchs, E. J., Huff, C. A., Luznik, L., et al., (2010). High-dose cyclophosphamide for severe aplastic anemia: long-term follow-up. *Blood*, 115(11), 2136–2141).



**FIGURE 49.4** Model depicting the pathophysiology of bone marrow failure in acquired aplastic anemia. Autoaggressive lymphocytes lyse CD34 + bone marrow progenitor cells but seem to spare more immature CD34 + cells known as high-quality stem cells.

## APLASTIC ANEMIA AND CLONALITY

The survivors of aplastic anemia are at high risk of clonal progression following immunosuppressive therapy (Socie et al., 1993). PNH and MDS are the most common clonal disorders to evolve from aplastic anemia (de Planque et al., 1989; Tichelli et al., 1988). Even before the widespread use of immunosuppressive therapy, 5% of the patients progressed to clonal hematopoiesis. This suggests that the increase in MDS and PNH following immunosuppressive therapy is not caused by the immunosuppression; rather, the increased survival following immunosuppressive therapy may allow time for these underlying clones to expand (Mukhina et al., 2001; Pu et al., 2011).

PNH results from the expansion of an abnormal hematopoietic stem cell that harbors a somatic mutation of the X-linked gene, termed phosphatidylinositol glycan class A (*PIG-A*) (Brodsy, 2014). The product of the *PIG-A* gene is required for GPI anchor biosynthesis; consequently, PNH cells are deficient in GPI-anchored proteins. Several GPI-anchored proteins (CD59 and CD55) protect cells from complement-mediated destruction, and their absence explains the hemolytic anemia associated with PNH. It is unclear how the PNH stem cell and its progeny achieve clonal dominance in the setting of aplastic anemia, despite the fact that PNH cells are more vulnerable to complement-mediated destruction; however, it may relate to relative resistance to the autoimmune attack due to intrinsic mutations (Brodsy et al., 1996b; Inoue et al., 2006). Specifically, it has been suggested that PNH cells may be relatively resistant to an autoimmune attack, because they are deficient in GPI-anchored ULBPs that serve as ligands for the NKG2D receptor found on natural killer cells and T cells (Hanaoka et al., 2006; Savage et al., 2009). Alternatively, it has been proposed that “second hit” mutations may also give the PNH clone a growth advantage (Inoue et al., 2006; Babushok et al., 2017).

MDS also commonly arises in aplastic anemia patients treated with immunosuppressive therapy. In a retrospective review of children with SAA, 11 of 86 patients who received immunosuppressive therapy developed MDS (Ohara et al., 1997). Up to 15% of adult patients with aplastic anemia will also develop MDS following immunosuppressive therapy with monosomy 7 being the most common chromosomal abnormality (Rosenfeld et al., 2003).

## CONCLUDING REMARKS—FUTURE PROSPECTS

Aplastic anemia was originally thought to be due to a defect in hematopoietic stem cells or their microenvironment. It is now clear that most cases of acquired aplastic anemia are caused by autoreactive lymphocytes that target bone marrow stem/progenitor cells. With modern therapies, the 5-year survival rate for SAA exceeds 85%. BMT offers the best chance for cure but is not available to all patients. Immunosuppressive therapy remains the standard of care for patients who are not suitable candidates for BMT. Remissions are achieved in up to 75% of patients, but the high rate of relapse and secondary clonal diseases limits the efficacy of immunosuppressive therapy. Even complete responders may relapse or develop MDS 5–10 years after immunosuppressive therapy.

Currently, the advances in mitigating graft failure and GVHD in the setting of alternative donor BMT appear to be outpacing the development of more effective IST therapies for SAA. In the coming years, there is likely to be great use of unrelated and HLA-mismatched BMT to treat SAA, especially in patients who don't respond or relapse after immunosuppressive therapy. The development of posttransplant cyclophosphamide (CY) to expand the donor pool and mitigate GVHD appears promising (DeZern et al., 2017).

## References

- Ades, L., Mary, J.Y., Robin, M., et al., 2004. Long-term outcome after bone marrow transplantation for severe aplastic anemia. *Blood* 103 (7), 2490–2497.
- Babushok, D.V., Stanley, N., Xie, H.M., Huang, H., Bagg, A., Olson, T.S., et al., 2017. Clonal replacement underlies spontaneous remission in paroxysmal nocturnal haemoglobinuria. *Br. J. Haematol.* 176 (3), 487–490.
- Bacigalupo, A., 1999. Bone marrow transplantation for severe aplastic anemia from HLA identical siblings. *Haematologica* 84 (1), 2–4.
- Bacigalupo, A., 2017. How I treat acquired aplastic anemia. *Blood* 129 (11), 1428–1436.
- Bacigalupo, A., Brand, R., Oneto, R., et al., 2000. Treatment of acquired severe aplastic anemia: bone marrow transplantation compared with immunosuppressive therapy—The European Group for Blood and Marrow Transplantation experience. *Semin. Hematol.* 37 (1), 69–80.
- Bacigalupo, A., Socie', G., Lanino, E., et al., 2010. Fludarabine, cyclophosphamide, antithymocyte globulin, with or without low dose total body irradiation, for alternative donor transplants, in acquired severe aplastic anemia: a retrospective study from the EBMT-SAA working party. *Haematologica* 95 (6), 976–982.
- Bagby Jr., G.C., 2003. Genetic basis of Fanconi anemia. *Curr. Opin. Hematol.* 10 (1), 68–76.
- Borowitz, M.J., Craig, F.E., DiGiuseppe, J.A., et al., 2010. Guidelines for the diagnosis and monitoring of paroxysmal nocturnal hemoglobinuria and related disorders by flow cytometry. *Cytometry B Clin. Cytom.* 78 (4), 211–230.
- Brodsky, R.A., 2014. Paroxysmal nocturnal hemoglobinuria. *Blood* 124 (18), 2804–2811.
- Brodsky, R.A., Jones, R.J., 2005. Aplastic anaemia. *Lancet* 365 (9471), 1647–1656.
- Brodsky, R.A., Sensenbrenner, L.L., Jones, R.J., 1996a. Complete remission in severe aplastic anemia after high-dose cyclophosphamide without bone marrow transplantation. *Blood* 87 (2), 491–494.
- Brodsky, R.A., Vala, M.S., Barber, J.P., Medoff, M.E., Jones, R.J., 1996b. Resistance to apoptosis caused by *PIG-A* gene mutations in paroxysmal nocturnal hemoglobinuria (PNH). *Blood* 88 (10), 140a.
- Brodsky, R.A., Mukhina, G.L., Li, S., et al., 2000. Improved detection and characterization of paroxysmal nocturnal hemoglobinuria using fluorescent aerolysin. *Am. J. Clin. Pathol.* 114 (3), 459–466.
- Brodsky, R.A., Chen, A.R., Dorr, D., Fuchs, E.J., Huff, C.A., Luznik, L., et al., 2010. High-dose cyclophosphamide for severe aplastic anemia: long-term follow-up. *Blood* 115 (11), 2136–2141.
- Brown, K.E., Tisdale, J., Barrett, A.J., Dunbar, C.E., Young, N.S., 1997. Hepatitis-associated aplastic anemia [see comments]. *N. Engl. J. Med.* 336 (15), 1059–1064.
- Camitta, B.M., Thomas, E.D., NATHAN, D.G., et al., 1979. A prospective study of androgens and bone marrow transplantation for treatment of severe aplastic anemia. *Blood* 53, 504–514.
- Catral, M.S., Langnas, A.N., Markin, R.S., et al., 1994. Aplastic anemia after liver transplantation for fulminant liver failure. *Hepatology* 20 (4), 813–818.
- Champlin, R.E., Feig, S.A., Sparkes, R.S., Galen, R.P., 1984. Bone marrow transplantation from identical twins in the treatment of aplastic anemia: implication for the pathogenesis of the disease. *Br. J. Haematol.* 56 (3), 455–463.
- Chen, J., 2005. Animal models for acquired bone marrow failure syndromes. *Clin. Med. Res.* 3 (2), 102–108.
- Chen, J., Lipovsky, K., Ellison, F.M., Calado, R.T., Young, N.S., 2004. Bystander destruction of hematopoietic progenitor and stem cells in a mouse model of infusion-induced bone marrow failure. *Blood* 104 (6), 1671–1678.
- DeZern, A.E., Zahurak, M., Symons, H., Cooke, K., Jones, R.J., Brodsky, R.A., 2017. Alternative donor transplantation with high-dose post-transplantation cyclophosphamide for refractory severe aplastic anemia. *Biol. Blood Marrow Transplant.* 23 (3), 498–504.
- Desmond, R., Townsley, D.M., Dumitriu, B., et al., 2014. Eltrombopag restores trilineage hematopoiesis in refractory severe aplastic anemia that can be sustained on discontinuation of drug. *Blood* 123 (12), 1818–1825.
- Desmond, R., Townsley, D.M., Dunbar, C., Young, N.S., 2015. Eltrombopag in aplastic anemia. *Semin. Hematol.* 52 (1), 31–37.
- Dezern, A.E., Symons, H.J., Resar, L.S., Borowitz, M.J., Armanios, M.Y., Brodsky, R.A., 2014. Detection of paroxysmal nocturnal hemoglobinuria clones to exclude inherited bone marrow failure syndromes. *Eur. J. Haematol.* 92, 467–470.
- Dokal, I., Vulliamy, T., 2003. Dyskeratosis congenita: its link to telomerase and aplastic anaemia. *Blood Rev.* 17 (4), 217–225.
- Ehrlich, P., 1888. Ueber einem Fall von Anämie mit Bemer-kungen über regenerative Veränderungen des Knochenmarks. *Charite-Annalen* 13, 301–309.
- Emadi, A., Jones, R.J., Brodsky, R.A., 2009. Cyclophosphamide and cancer: golden anniversary. *Nat. Rev. Clin. Oncol.* 6 (11), 638–647.
- Feng, X., Chuhjo, T., Sugimori, C., et al., 2004. Diazepam-binding inhibitor-related protein 1: a candidate autoantigen in acquired aplastic anemia patients harboring a minor population of paroxysmal nocturnal hemoglobinuria-type cells. *Blood* 104 (8), 2425–2431.
- Frickhofen, N., Kaltwasser, J.P., Schrezenmeier, H., et al., 1991. Treatment of aplastic anemia with antilymphocyte globulin and methylprednisolone with or without cyclosporine. *N. Engl. J. Med.* 324, 1297–1304.
- Gamper, C.J., Takemoto, C.M., Chen, A.R., et al., 2016. High-dose cyclophosphamide is effective therapy for pediatric severe aplastic anemia. *J. Pediatr. Hematol. Oncol.* 38 (8), 627–635.
- Gmur, J., Von Felten, A., Rhyner, K., Frick, P.G., 1979. Autologous hematologic recovery from aplastic anemia following high dose cyclophosphamide and HLA-matched allogeneic bone marrow transplantation. *Acta Haematol.* 62 (1), 20–24.
- Hanaoka, N., Kawaguchi, T., Horikawa, K., Nagakura, S., Mitsuya, H., Nakakuma, H., 2006. Immunoselection by natural killer cells of PIGA mutant cells missing stress-inducible ULBP. *Blood* 107 (3), 1184–1191.

- Hilton, J., 1984. Role of aldehyde dehydrogenase in cyclophosphamide-resistant L1210 leukemia. *Cancer Res.* 44 (11), 5156–5160.
- Hinterberger, W., Rowlings, P.A., Hinterberger-Fischer, M., et al., 1997. Results of transplanting bone marrow from genetically identical twins into patients with aplastic anemia [see comments]. *Ann. Intern. Med.* 126 (2), 116–122.
- Hirano, N., Kojima, S., von Bergwelt-Baildon, M.S., et al., 2000. Distinct autoantigens in American and Japanese immune-mediated aplastic anemia. *Blood* 96, 8a.
- Hoffman, R., Zanjani, E.D., Lutton, J.D., Zalusky, R., Wasserman, L.R., 1977. Suppression of erythroid-colony formation by lymphocytes from patients with aplastic anemia. *N. Engl. J. Med.* 296 (1), 10–13.
- Horowitz, M.M., 2000. Current status of allogeneic bone marrow transplantation in acquired aplastic anemia. *Semin. Hematol.* 37 (1), 30–42.
- Inoue, N., Izui-Sarumaru, T., Murakami, Y., et al., 2006. Molecular basis of clonal expansion of hematopoiesis in 2 patients with paroxysmal nocturnal hemoglobinuria (PNH). *Blood* 108 (13), 4232–4236.
- Inoue, N., Izui-Sarumaru, T., Murakami, Y., et al., 2006. Molecular basis of clonal expansion of hematopoiesis in two patients with paroxysmal nocturnal hemoglobinuria (PNH). *Blood* 108, 4232–4236.
- Issaragrisil, S., Kaufman, D.W., Anderson, T., et al., 1997. Low drug attributability of aplastic anemia in Thailand. *Blood* 89 (11), 4034–4039.
- Issaragrisil, S., Chansung, K., Kaufman, D.W., Sirijirachai, J., Thamprasit, T., Young, N.S., 1997. Aplastic anemia in rural Thailand: its association with grain farming and agricultural pesticide exposure. *Aplastic Anemia Study Group. Am. J. Public Health* 87 (9), 1551–1554.
- Issaragrisil, S., Leaverton, P.E., Chansung, K., et al., 1999. Regional patterns in the incidence of aplastic anemia in Thailand. *The Aplastic Anemia Study Group. Am. J. Hematol.* 61 (3), 164–168.
- Jaime-Prez, J.C., Gonzlez-Llano, O., Gmez-Almaguer, D., 2001. High-dose cyclophosphamide in the treatment of severe aplastic anemia in children [6]. *Am. J. Hematol.* 66 (1), 71.
- Jones, R.J., Barber, J.P., Vala, M.S., Collector, M.I., Kaufmann, S.H., Ludeman, S.M., et al., 1995. Assessment of aldehyde dehydrogenase in viable cells. *Blood* 85 (10), 2742–2746.
- Kahl, C., Leisenring, W., Deeg, H.J., et al., 2005. Cyclophosphamide and antithymocyte globulin as a conditioning regimen for allogeneic marrow transplantation in patients with aplastic anaemia: a long-term follow-up. *Br. J. Haematol.* 130 (5), 747–751.
- Kaufman, D.W., Kelly, J.P., Levy, M., Shapiro, S., 1991. *The Drug Etiology of Agranulocytosis and Aplastic Anemia* (first ed.). Oxford University Press, Inc, New York.
- Locasciulli, A., Bacigalupo, A., Bruno, B., et al., 2010. Hepatitis-associated aplastic anaemia: epidemiology and treatment results obtained in Europe. A report of The EBMT aplastic anaemia working party. *Br. J. Haematol.* 149 (6), 890–895.
- Luznik, L., O'Donnell, P.V., Symons, H.J., et al., 2008. HLA-haploididentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. *Biol. Blood Marrow Transplant.* 14 (6), 641–650.
- Maciejewski, J.P., Selleri, C., Sato, T., Anderson, S., Young, N.S., 1995. Increased expression of Fas antigen on bone marrow CD34+ cells of patients with aplastic anaemia. *Br. J. Haematol* 91 (1), 245–252.
- Manz, C.Y., Dietrich, P.Y., Schnuriger, V., Nissen, C., Wodnar-Filipowicz, A., 1997. T-cell receptor beta chain variability in bone marrow and peripheral blood in severe acquired aplastic anemia. *Blood Cells Mol. Dis.* 23 (1), 110–122.
- Marsh, J., Schrezenmeier, H., Marin, P., et al., 1999. Prospective randomized multicenter study comparing cyclosporin alone versus the combination of antithymocyte globulin and cyclosporin for treatment of patients with nonsevere aplastic anemia: a report from the European Blood and Marrow Transplant (EBMT) Severe Aplastic Anaemia Working Party. *Blood* 93 (7), 2191–2195.
- Marsh, J.C., Kulasekararaj, A.G., 2013. Management of the refractory aplastic anemia patient: what are the options? *Blood* 122, 3561–3567.
- Mathe, G., Amiel, J.L., Schwarzenberg, L., et al., 1970. Bone marrow graft in man after conditioning by antilymphocytic serum. *Br. Med. J.* 2, 131–136.
- Matsui, W.H., Brodsky, R.A., Smith, B.D., Borowitz, M.J., Jones, R.J., 2006. Quantitative analysis of bone marrow CD34 cells in aplastic anemia and hypoplastic myelodysplastic syndromes. *Leukemia* 20, 458–462.
- Melenhorst, J.J., Fibbe, W.E., Struyk, L., van der Elsen, P.J., Willemze, R., Landegent, J.E., 1997. Analysis of T-cell clonality in bone marrow of patients with acquired aplastic anaemia. *Br. J. Haematol.* 96 (1), 85–91.
- Modan, B., Segal, S., Shani, M., Sheba, C., 1975. Aplastic anemia in Israel: evaluation of the etiological role of chloramphenicol on a community-wide basis. *Am. J. Med. Sci.* 270 (3), 441–445.
- Mukhina, G.L., Buckley, J.T., Barber, J.P., Jones, R.J., Brodsky, R.A., 2001. Multilineage glycosylphosphatidylinositol anchor deficient hematopoiesis in untreated aplastic anemia. *Br. J. Haematol.* 115, 476–482.
- Nakao, S., 1997. Immune mechanism of aplastic anemia. [Review] [26 refs]. *Int. J. Hematol.* 66 (2), 127–134.
- Nakao, S., Takami, A., Takamatsu, H., et al., 1997. Isolation of a T-cell clone showing HLA-DRB1\*0405-restricted cytotoxicity for hematopoietic cells in a patient with aplastic anemia. *Blood* 89 (10), 3691–3699.
- Nimer, S.D., Ireland, P., Meshkinpour, A., Frane, M., 1994. An increased HLA DR2 frequency is seen in aplastic anemia patients. *Blood* 84 (3), 923–927.
- Nissen, C., Cornu, P., Gratwohl, A., Speck, B., 1980. Peripheral blood cells from patients with aplastic anaemia in partial remission suppress growth of their own bone marrow precursors in culture. *Br. J. Haematol.* 45 (2), 233–243.
- Nistico, A., Young, N.S., 1994. gamma-Interferon gene expression in the bone marrow of patients with aplastic anemia. *Ann. Intern. Med.* 120 (6), 463–469.
- Ohara, A., Kojima, S., Hamajima, N., et al., 1997. Myelodysplastic syndrome and acute myelogenous leukemia as a late clonal complication in children with acquired aplastic anemia. *Blood* 90 (3), 1009–1013.
- Olnes, M.J., Scheinberg, P., Calvo, K.R., et al., 2012. Eltrombopag and improved hematopoiesis in refractory aplastic anemia. *N. Engl. J. Med.* 367 (1), 11–19.
- de Planque, M.M., Bacigalupo, A., Wursch, A., et al., 1989. Long-term follow-up of severe aplastic anaemia patients treated with antithymocyte globulin. *Br. J. Haematol.* 73, 121–126.
- Piccin, A., McCann, S., Socié, G., Oneto, R., Bacigalupo, A., Locasciulli, A., et al., 2010. Survival of patients with documented autologous recovery after SCT for severe aplastic anemia: a study by the WPSAA of the EBMT. *Bone Marrow Transplant.* 45 (6), 1008–1013.

- Pu, J.J., Mukhina, G., Wang, H., Savage, W.J., Brodsky, R.A., 2011. Natural history of paroxysmal nocturnal hemoglobinuria clones in patients presenting as aplastic anemia. *Eur. J. Haematol.* 87 (1), 37–45.
- Rosenfeld, S., Follmann, D., Nunez, O., Young, N.S., 2003. Antithymocyte globulin and cyclosporine for severe aplastic anemia: association between hematologic response and long-term outcome. *JAMA* 289 (9), 1130–1135.
- Savage, W.J., DeRusso, P.A., Resar, L.M., et al., 2007. Treatment of hepatitis-associated aplastic anemia with high-dose cyclophosphamide. *Pediatr. Blood Cancer* 49 (7), 947–951.
- Savage, W.J., Barber, J.P., Mukhina, G.L., Hu, R., Chen, G., Matsui, W., et al., 2009. Glycosylphosphatidylinositol-anchored protein deficiency confers resistance to apoptosis in PNH. *Exp. Hematol.* 37 (1), 42–51.e41.
- Scheinberg, P., Nunez, O., Young, N.S., 2006. Retreatment with rabbit anti-thymocyte globulin and ciclosporin for patients with relapsed or refractory severe aplastic anaemia. *Br. J. Haematol.* 133 (6), 622–627.
- Scheinberg, P., Nunez, O., Wu, C., Young, N.S., 2006. Treatment of severe aplastic anaemia with combined immunosuppression: anti-thymocyte globulin, ciclosporin and mycophenolate mofetil. *Br. J. Haematol.* 133 (6), 606–611.
- Scheinberg, P., Nunez, O., Weinstein, B., et al., 2011. Horse versus rabbit antithymocyte globulin in acquired aplastic anemia. *N. Engl. J. Med.* 365 (5), 430–438.
- Scheinberg, P., Nunez, O., Weinstein, B., Scheinberg, P., Wu, C.O., Young, N.S., 2012. Activity of alemtuzumab monotherapy in treatment-naïve, relapsed, and refractory severe acquired aplastic anemia. *Blood* 119 (2), 345–354.
- Selleri, C., Anderson, S., Young, N.S., Maciejewski, J.P., 1994. Interferon-gamma and tumor necrosis factor- $\alpha$  suppress early and late stages of hematopoiesis *in vitro* and induce programmed cell death. *Blood* 84, 215a.
- Sensenbrenner, L.L., Steele, A.A., Santos, G.W., 1977. Recovery of hematologic competence without engraftment following attempted bone marrow transplantation for aplastic anemia: Report of a case with diffusion chamber studies. *Exp. Hematol.* 5 (1), 51–58.
- Socie, G., Henry-Amar, M., Bacigalupo, A., et al., 1993. Malignant tumors occurring after treatment of aplastic anemia. *N. Engl. J. Med.* 329, 1152–1157.
- Storb, R., Leisenring, W., Anasetti, C., et al., 1997. Long-term follow-up of allogeneic marrow transplants in patients with aplastic anemia conditioned by cyclophosphamide combined with antithymocyte globulin. *Blood* 89 (10), 3890–3891.
- Storb, R., Blume, K.G., O'Donnell, M.R., et al., 2001. Cyclophosphamide and antithymocyte globulin to condition patients with aplastic anemia for allogeneic marrow transplantations: the experience in four centers. *Biol. Blood Marrow Transplant.* 7 (1), 39–44.
- Szklo, M., Sensenbrenner, L., Markowitz, J., Weida, S., Warm, S., Linet, M., 1985. Incidence of aplastic anemia in metropolitan Baltimore: a population-based study. *Blood* 66 (1), 115–119.
- Thomas, E.D., Storb, R., Fefer, A., Slichter, S.J., Bryant, J.I., Buckner, C.D., et al., 1972. Aplastic anaemia treated by marrow transplantation. *Lancet* 1 (7745), 284–289.
- Thomas, E.D., Storb, R., Giblett, E.R., Longpre, B., Weiden, P.L., Fefer, A., et al., 1976. Recovery from aplastic anemia following attempted marrow transplantation. *Exp. Hematol.* 4 (2), 97–102.
- Tichelli, A., Gratwohl, A., Wursch, A., Nissen, C., Speck, B., 1988. Late haematological complications in severe aplastic anaemia. *Br. J. Haematol.* 69, 413–418.
- Tisdale, J.F., Dunn, D.E., Geller, N., Plante, M., Nunez, O., Dunbar, C.E., et al., 2000. High-dose cyclophosphamide in severe aplastic anaemia: a randomised trial. *Lancet* 356 (9241), 1554–1559.
- Tsangaris, E., Klaassen, R., Fernandez, C.V., Yanofsky, R., Shereck, E., Champagne, J., et al., 2011. Genetic analysis of inherited bone marrow failure syndromes from one prospective, comprehensive and population-based cohort and identification of novel mutations. *J. Med. Genet.* 48 (9), 618–628.
- Tzakis, A.G., Arditì, M., Whittington, P.F., et al., 1988. Aplastic anemia complicating orthotopic liver transplantation for non-A, non-B hepatitis. *N. Engl. J. Med.* 319 (7), 393–396.
- Zeng, W., Maciejewski, J.P., Chen, G., Young, N.S., 2001. Limited heterogeneity of T cell receptor BV usage in aplastic anemia. *J. Clin. Invest.* 108 (5), 765–773.
- Zoumbos, N.C., Gascón, P., Djeu, J.Y., Trost, S.R., Young, N.S., 1985. Circulating activated suppressor T lymphocytes in aplastic anemia. *N. Engl. J. Med.* 312, 257–265.

# Autoimmune Clotting Dysfunction

*Christoph Königs*

Department of Pediatrics and Adolescent Medicine, Clinical and Molecular Hemostasis, Goethe University,  
Frankfurt am Main, Germany

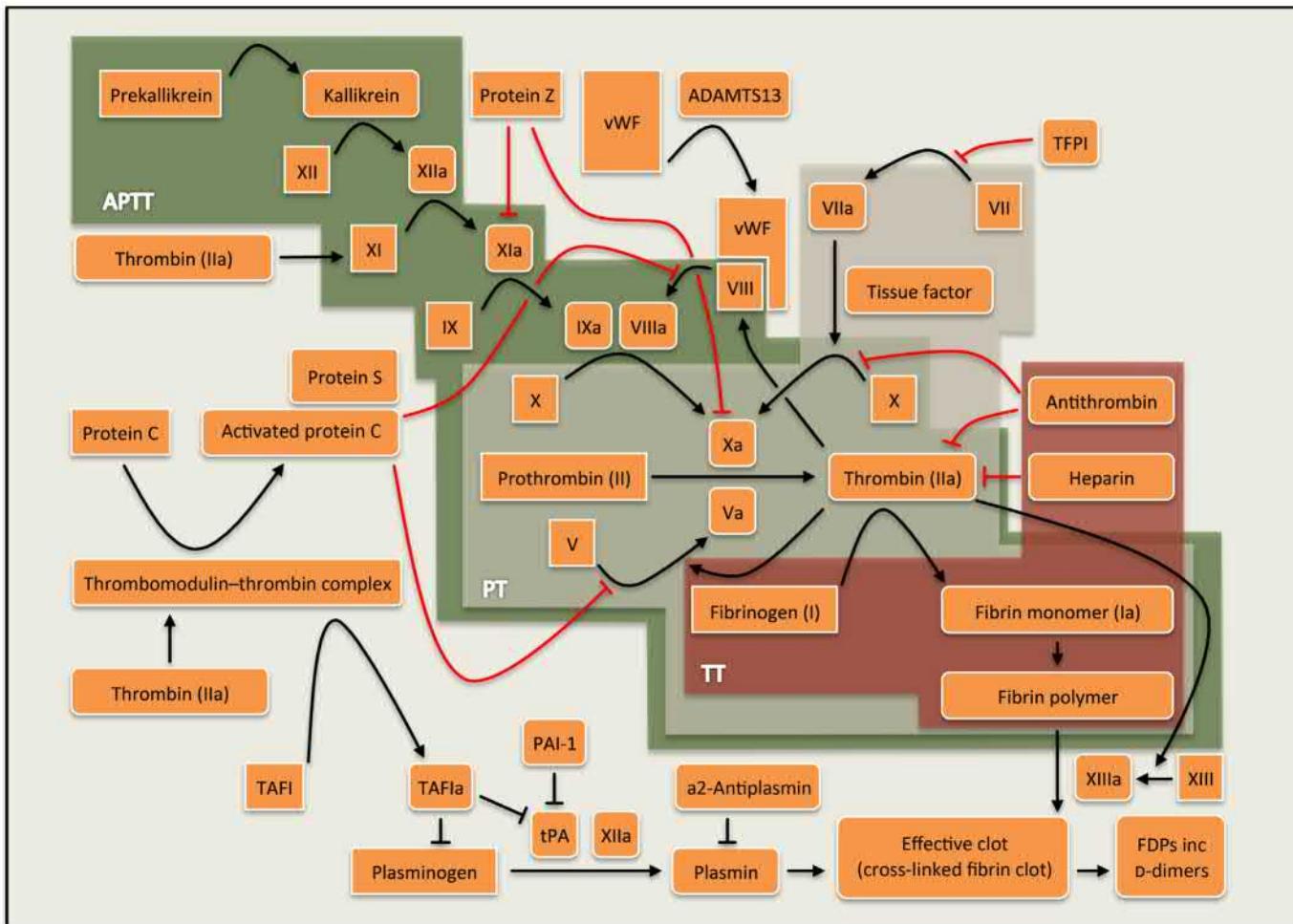
## OUTLINE

Prothrombotic Disorders	937	Autoimmune Inhibitors to Factor X	946
Autoimmune Inhibitors to a Disintegrin and Metalloproteinase With a Thrombospondin Type 1 Motif 13	937	Autoimmune Inhibitors to Factor XI	946
Anticoagulant Disorders	939	Autoimmune Inhibitors to Factor XII	947
Autoimmune Inhibitors to Fibrinogen (Factor I) and Fibrin	939	Autoimmune Inhibitors to Factor XIII	947
Autoimmune Inhibitors to Prothrombin (Factor II) and Thrombin	940	Autoimmune Inhibitors to Von Willebrand Factor	948
Autoimmune Inhibitors to Factor V	941	Autoimmune Inhibitors to Further Proteins	949
Autoimmune Inhibitors to Factor VII	942	Conclusion and Future Aspects	949
Autoimmune Inhibitors to Factor VIII	942	Acknowledgments	949
Autoimmune Inhibitors to Factor IX	945	References	949

Immunologically mediated dysfunction of coagulation is observed as a specific autoimmune response to the molecules of the coagulation system and occurs as specific disease entities or secondary to a number of different autoimmune conditions. The immune response directly or indirectly leads to an imbalance of hemostasis inducing hyper- or hypocoagulability.

Autoimmunity is found in primary and secondary—also called plasmatic—hemostasis. Primary hemostasis describes the initial reaction after injury, including the contraction of the blood vessel and the activation of platelets after exposure to subendothelial structures and tissue factor (TF). These steps lead to the initial closure of the injury. In parallel secondary hemostasis is initiated transforming the initial platelet clot into a stable fibrin clot, which is then cross-linked and further stabilized by FXIII and controlled by fibrinolysis. The classical schematic presentation of the plasmatic coagulation cascade and the interplay of activation and inactivation are shown in Fig. 50.1 to illustrate its complexity of regulation without showing the cellular aspects of coagulation.

This chapter aims to provide a short but comprehensive overview of autoimmunity to various proteins involved in secondary hemostasis, thus summarizing completely different and independent autoimmune entities (Chang and Chiang, 2014; Cugno et al., 2014). The autoimmune response to coagulation factors needs to be distinguished from alloantibodies in patients with congenital bleeding disorders after substitution of plasma or



**FIGURE 50.1** The coagulation cascade. The complexity of coagulation and the interplay of the different factors in regards of inhibition and activation are illustrated in the diagram. The classical scheme has been used to demonstrate the complexity of molecules and interaction. Although extremely rare, autoimmune inhibitors have been described against most coagulation factors. The factors influencing the different global tests aPTT, PT, and TT are shaded green, gray, and red, respectively. *aPTT*, Activated partial thromboplastin time; *PT*, prothrombin time; *TT*, thrombin time.

plasma proteins (antidrug antibodies), which are not covered in this chapter. Autoantibodies to platelets interfering with primary hemostasis are described in Chapter 47, Autoimmune Hemolytic Anemia.

In general, autoantibodies interfering with hemostasis are rare phenomena, for some proteins only individual cases have been described in literature. Nevertheless, the understanding of the autoimmune response and potential therapeutic options has increased in recent years for some autoimmune coagulation disorders. In general, such conditions remain extremely rare and most information on therapeutic interventions provided in this chapter reflects only data from individual reports or expert opinion based on cohort studies or case series.

## PROTHROMBOTIC DISORDERS

Several autoimmune entities are known to interfere with the homeostasis of the coagulation system leading to a procoagulant state. These include the antiphospholipid syndrome (APS), which interferes with several components of the coagulation cascade and is discussed in Chapter 34, Rheumatoid Arthritis. Except for APS and antibodies to a disintegrin and metalloproteinase with a thrombospondin type 1 motif 13 (ADAMTS13) (see next), only single reports on autoantibodies to other anticoagulant proteins of the coagulation system have been published.

Lupus anticoagulants are known to interfere with the protein C pathway and possibly block the inactivation of activated factor VIII (FVIII) (FVIIIa) ([Urbanus and de Laat, 2010; Saenz et al., 2011](#)). Antibodies to protein C and protein S itself have been described, whereas antibodies to protein S appear to be more clinically relevant. Both autoantibodies were found in higher frequencies in individuals with clinical relevant thrombosis compared to healthy controls. Antibodies to protein C have been suggested as a potential biomarker for the severity of thromboembolic events in patients with lupus anticoagulants ([Arachchillage et al., 2014](#)). The presence of specific IgM but not IgG as determined by enzyme linked immunosorbent assay (ELISA) correlated with the clinical phenotype ([Rossetto et al., 2009](#)). Also both isotypes have been associated with fetal growth restrictions and preeclampsia in pregnancy ([Torricelli et al., 2009](#)). Antibodies to protein S have been detected for example in Beçet disease associated with thrombosis in a few cases ([Guermazi et al., 1997; Lechner et al., 2011](#)). Further they were prevalent in a number of patients with acquired protein S deficiency and in patients with activated protein C resistance in the absence of the factor V (FV) Leiden mutation with or without underlying systemic lupus erythematosus (SLE) ([Sorice et al., 1996; Nojima et al., 2002a, 2009](#)). Antibodies to protein S and clinically relevant thrombosis have also been detected in children after infections including varicella or human herpes virus 6 ([Larakeb et al., 2009; Levin et al., 1995; Boccaro et al., 2009](#)).

IgG and IgM autoantibodies to protein Z, which inactivates FXa and FXIa, have been detected in women with recurrent spontaneous miscarriages but not in healthy controls and were also associated with pregnancy complications such as preeclampsia and fetal growth retardation in individual cases ([Sater et al., 2011; Erez et al., 2009; Gris et al., 2003](#)). In a case–control study, low protein Z levels and autoantibodies to protein Z were also found in patients with arterial or venous thrombosis ([Pardos-Gea et al., 2008](#)). The role of autoantibodies present in the APS on the protein Z–mediated inactivation of FXa and on the thrombosis is not clear ([Sailer et al., 2008; Forastiero et al., 2003](#)). Thrombotic complication also occurs due to inhibitors to prothrombin or ADAMTS13 (see next).

## AUTOIMMUNE INHIBITORS TO A DISINTEGRIN AND METALLOPROTEINASE WITH A THROMBOSPONDIN TYPE 1 MOTIF 13

Autoimmunity to ADAMTS13 causes the clinical picture of immune-mediated thrombotic–thrombocytopenic purpura (iTTP). Thrombotic events in terminal capillaries and arterioles are seen in iTTP. ADAMTS13 is a zinc protease mainly synthesized by hepatic stellate cells, which cleaves ultralarge von Willebrand multimers ([Tsai, 1996; Furlan et al., 1997; Zheng et al., 2001; Fujikawa et al., 2001; Dong et al., 2002; Zhou et al., 2005](#)). Prior to the discovery of the enzyme, the presence of ultralarge multimers has already been demonstrated in TTP patients ([Moake et al., 1982](#)). If not processed by ADAMTS13, the multimers rapidly form a hyaline thrombus together with thrombocytes mainly in brain, kidney, adrenal gland, heart, pancreas, and spleen.

The incidence of iTTP is estimated 2.17/million per year with women being affected 2.5–3.5 times as often as men. There is also a higher incidence and a more severe course seen in the individuals of African descent ([Torok et al., 1995; Miller et al., 2004; Terrell et al., 2005; Cataland et al., 2009](#)). iTTP is mostly seen in adults with a

median age of 42 years (range: 2–78), but also pediatric cases have been described (McDonald et al., 2010; Scully et al., 2008). In some patients iTPP is seen in combination with underlying conditions including malignancies, drugs, infections, autoimmune diseases, primary immunodeficiency, after bone marrow transplantation, and pregnancy (Murrin and Murray, 2006; Yamada et al., 2011; Scully et al., 2008; Kawasaki et al., 2013). A clinical pentad combining thrombocytopenia, hemolytic anemia, renal impairment, fever, and neurological symptoms was seen initially in almost 90% of the patients diagnosed with iTPP. Today the full classical pentad is present in less than 5% of the affected patients. Severe neurological symptoms are usually multifocal and often recurrent (Murrin and Murray, 2006). A characteristic presentation is the combination of thrombosis and thrombocytopenia accompanied by petechial bleedings. The full clinical picture depends on organs affected by microthromboses. This includes affection of the heart with pathological ECG and cardiac enzymes.

The differential diagnosis includes but is not limited to congenital TTP, hemolytic uremic syndrome, disseminated intravascular coagulation and other microangiopathies, or combinations of the above. The diagnosis may be difficult as ADAMTS13 is reduced in a number of conditions.

In suspected iTPP a blood cell count, von Willebrand factor (vWF) parameters including multimers, ADAMTS13 activity, antigen, and antibodies need to be determined. Different assays to determine ADAMTS13 antigen or activity are available. A residual activity of ADAMTS13 <10% appears to predict the clinical outcome of the first episode and potential relapses. Antibodies can be detected by ELISA using immobilized ADAMTS13. Inhibitory antibodies are detected by mixing studies. Von Willebrand antigen and activity appear higher in TTP. In the acute phase, the large vWF multimers are missing in the plasma of affected patients but reappear in remission. The extent of missing ultralarge multimers correlates with the severity of hemolysis and thrombocytopenia. Fragmented erythrocytes, an elevated lactate dehydrogenase and retention parameters depending on kidney affection, are observed (Lotta et al., 2011; Wu et al., 2006; Whitelock et al., 2004; Peyvandi et al., 2010; Kremer Hovinga et al., 2010, 2017, 2018). Mainly antibodies of the isotypes IgG, but also IgA and IgM are found in almost all patients with iTPP. Most antibodies are inhibitory, only in 10%–15% of the patients with ADAMTS13 deficiency noninhibitory antibodies are found, suggesting an increased clearance of the protease. Also antinuclear antibodies have been detected in most iTPP patients at first diagnosis (Tsai et al., 2006; Ferrari et al., 2007; Shelat et al., 2006; Scheiflinger et al., 2003; Pos et al., 2011; Kremer Hovinga et al., 2018). Antibodies isolated from peripheral B cells and switched memory B cells show somatic hypermutations suggestive for a role of T cells (Luken et al., 2005). IgG to ADAMTS13 is also seen in healthy controls but to a much lesser extent compared to iTPP patients (Tsai et al., 2006). Specific antibodies mainly belong to the IgG isotype, including the IgG4 subclass, but also IgG1, IgG2 and 3, IgA, and IgM have been identified (Ferrari et al., 2009). High levels of autoantibodies and in particular high levels of IgG4 and the combination of IgG1 and IgA have been associated with an unfavorable outcome. Antibody levels decrease during successful treatment (Coppo et al., 2006; Ferrari et al., 2007; Scully et al., 2007). In other study levels of IgA, IgG1 and IgG3 correlated with disease severity in the acute phase. In almost all patients antibodies against the cysteine-rich and the spacer region were identified, followed by antibodies against the metalloprotease. The cysteine-rich region is required for the cleavage of vWF. Antibodies against all other domains have been detected at lower frequencies. Longitudinal analyses including relapses showed antibody maturation and epitope spreading (Klaus et al., 2004; Luken et al., 2005; Zheng et al., 2010; Soejima et al., 2003; Yamaguchi et al., 2011; Thomas et al., 2015).

There are indications for a genetic predisposition for the development of anti-ADAMTS13 autoantibodies: in different cohorts the MHC class II alleles DRB1\*11, DQB1\*0301, DQB1\*02:02, and DRB3\* are found more frequently in patients with iTPP, while the DRB1\*04 and DRB4 alleles appear to be protective. Also heterozygous mutations in the ADAMTS13 gene have been associated with a potential higher risk of iTPP (Coppo et al., 2010; Scully et al., 2010; Pos et al., 2011; John et al., 2012; Kremer Hovinga et al., 2017).

Timely diagnosis and adequate treatment is crucial. In untreated iTPP, the mortality is around 90%. Thus treatment is often based on a presumptive diagnosis. Treatment is successful in most patients, but 30%–60% relapse over the following 10 years (Shumak et al., 1995; Tsai, 2006; Kremer Hovinga et al., 2010). The benefit of plasmapheresis and plasma transfusions compared to transfusions alone was shown in a randomized trial (Rock et al., 1991). With the combination of plasma exchange and steroids remission of 50% to >90% was achieved (Ferrari et al., 2007; Ferrari et al., 2014). Immunosuppression was mainly based on corticosteroids alone. Combinations with cyclosporine have also been successfully used (Cataland et al., 2007). Treatment with rituximab was successful and associated with a faster time to remission and a reduced number of relapses (Ojeda-Uribe et al., 2010; Scully, 2012; Ling et al., 2009; Foley et al., 2009; Tun and Villani, 2012; Westwood et al., 2017). A successful treatment with bortezomib has also been reported in a patient refractory to immunosuppression including rituximab (Shortt et al., 2013). The benefit of ivIg is doubtful. Single reports discuss the benefit of defibrotide

(Pogliani et al., 2000). In patients with refractory iTPP, splenectomy has been performed successfully (Kremer Hovinga et al., 2004). Thrombocyte transfusions are contraindicated and associated with a higher mortality. Many patients suffer from sequelae and a reduced life-expectancy even after successful treatment of the initial clinical presentation. A potential chronic affection even after the successful treatment of the acute episode is supported clinical presentations, the correlation of persistent antibodies with relapses and the detection of immunocomplexes (Ferrari et al., 2014; Bettoni et al., 2012).

Novel therapies are currently being evaluated including gain of function mutants resistant to autoantibodies and recombinant ADAMTS13, which restored the vWF-cleaving activity at higher doses in vitro despite the presence of autoantibodies to ADAMTS13. The clinical benefit remains to be determined. A novel antibody fragment (nanobody) targeting vWF is being evaluated. It reduces the clinical impact of the acute episode but does not promote immune tolerance induction (Jian et al., 2012; Plaimauer et al., 2011; Scully et al., 2017; Peyvandi et al., 2016).

## ANTICOAGULANT DISORDERS

Autoimmune inhibitors to proteins of the coagulation cascade potentially cause a severe bleeding phenotype. A rapid diagnosis of these rare conditions and appropriate treatment are often crucial for the prognosis of the patients. Inhibitors should be suspected in patients with hemorrhages without a personal or family history of bleeding, without trauma or anticoagulants. The provided algorithm describes a summary of a potential and rational approach to investigate patients with unexpected and unexplained bleeding events or abnormal coagulation tests and suspected inhibitors (Fig. 50.2).

As only limited data are available for each individual condition epidemiological, clinical, and immunological aspects of inhibitors are described next.

## AUTOIMMUNE INHIBITORS TO FIBRINOGEN (FACTOR I) AND FIBRIN

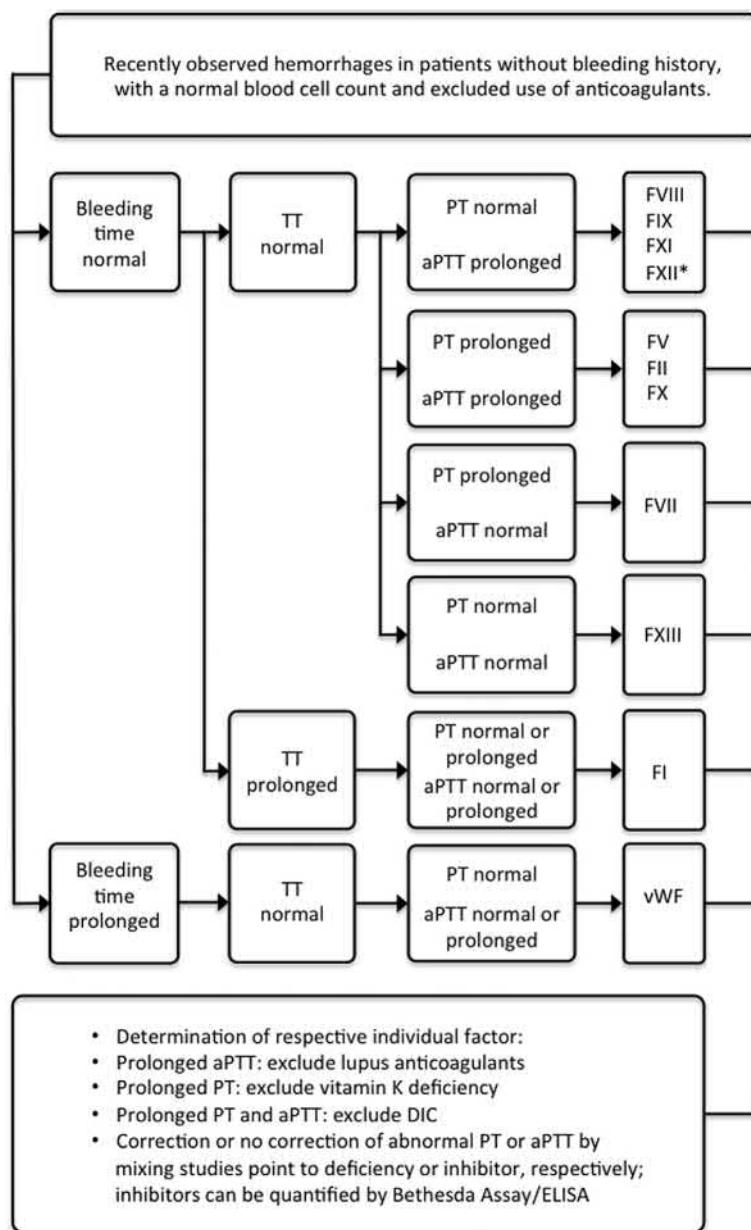
The fibrin clot is the final product of the coagulation cascade. Fibrin is generated from fibrinogen after thrombin cleavage. The glycoprotein fibrinogen is a heterohexamer composed of two  $\alpha$ -,  $\beta$ -, and  $\gamma$ -chains. Autoantibodies to fibrin or fibrinogen are rare. They have been described in combination with different underlying conditions, in pregnancy or as idiopathic. These conditions include other autoimmune disorders such as SLE or monoclonal gammopathies (Galanakis et al., 1978; Cohen et al., 1970; Coleman et al., 1972; Panzer and Thaler, 1993; Ruiz-Arguelles, 1988). The clinical phenotype ranges from mild-to-severe bleeding symptoms. Antibodies observed in pregnant women and newborns were not associated with a bleeding phenotype (Kondera-Anasz, 1998).

More frequently cross-reactive antibodies occurred after exposure to bovine thrombin glue [see next (Chouhan et al., 1997)].

Laboratory analysis reveals a prolonged thrombin time (TT) or reptiles time (RT), with both either normal or prolonged prothrombin time (PT) and activated partial thromboplastin time (aPTT). Fibrinogen levels may be normal or decreased. The TT is not corrected in mixing studies.

Antibodies against fibrinogen or fibrin usually belong to the IgG isotype. In a single report, the subclasses IgG1 and IgG3 were detected (Galanakis et al., 1978). Most antibodies described interfere with the monomer formation and the release of fibrinopeptide A (Marciniak and Greenwood, 1979; Gris et al., 1992) or with the monomer polymerization (Ghosh et al., 1983). Two further cases have been described with antibodies directed against the fibrinopeptide B also interfering with its release. These patients had no bleeding history, a prolonged TT normal RT (Llobet et al., 2007; Nawarawong et al., 1991). The correlation of the clinical phenotype and the epitope location might reflect the different kinetics of thrombin cleavage for the two different fibrinopeptides and their distinct role in the three-dimensional formation of the fibrin clot (Pechik et al., 2006).

The few published cases showed a spontaneous remission after the detection of an antiidiotypic antibody (Ruiz-Arguelles, 1988) or report a normalization of clotting assays after treatment of the underlying disease (Panzer and Thaler, 1993). There are no treatment algorithms; an immunosuppressive or modulative therapy with corticosteroids or ivIg may be necessary in case of severe symptoms.



**FIGURE 50.2** Possible guideline for the interpretation of coagulation tests for the diagnosis of factor inhibitors. \*, Factor XII deficiency/inhibitors are not associated with a bleeding phenotype; aPTT, Activated partial thromboplastin time; PT, prothrombin time; TT, thrombin time; vWF, von Willebrand factor. Source: Based on, extended, and modified from Kershaw, G., Favaloro, E.J., 2012. Laboratory identification of factor inhibitors: an update. *Pathology* 44, 293–302.

## AUTOIMMUNE INHIBITORS TO PROTHROMBIN (FACTOR II) AND THROMBIN

Thrombin plays a central role in the coagulation, activating fibrinogen and also to some extent other proteins of the coagulation cascade. This leads to a thrombin burst by a positive feedback amplification. Thrombin also binds to thrombomodulin, which then activates protein C to control coagulation in a negative feedback loop. Thrombin is generated from prothrombin by activated factor X (FX) and its cofactor activated FV. Prothrombin is a glycoprotein structurally including the gamma-carbylglytamic acid (Gla) domain, two kringle domains, and a C-terminal catalytic domain.

Antibodies to thrombin and prothrombin are rare and associated with a mild-to-severe bleeding phenotype. Such antibodies to prothrombin may also lead to a prothrombotic state (Bertolaccini et al., 1998). This is especially seen in patients with lupus anticoagulants (see Nojima et al., 2002b; Roubey, 1998; Knobe et al., 2012, also see Chapter 34, Rheumatoid Arthritis). The occurrence of such antibodies has been described as idiopathic (La Spada et al., 1995; Knobe et al., 2012) or in association with gammopathies, liver cirrhosis, SLE, or infections including epstein-barr-virus (Colwell et al., 1997; Barthels and Heimbürger, 1985; Atsumi et al., 2000; Bertolaccini et al., 1998).

More often antibodies to thrombin have been described after topical exposure to bovine thrombin during surgery with the use of fibrin glues (Chouhan et al., 1997; Berruyer et al., 1993; Fastenau and McIntyre, 2000). In some patients there is no bleeding tendency and the prolonged TT is an in vitro phenomenon and due to the use of bovine thrombin in the laboratory assay: when bovine thrombin is replaced by human thrombin, the TT is normalized due to lacking cross-reactivity of the antibodies with human thrombin (Stricker et al., 1988; Flaherty et al., 1989; Lawson et al., 1990; Berruyer et al., 1993). In contrast some patients develop antibodies with cross-reactivity to human thrombin: TT does not normalize if bovine thrombin is replaced by the human molecule in vitro (Lawson et al., 1990). In a prospective trial ( $n = 150$ ) the outcome for patients with exposure to bovine glue was less favorable (Ortel et al., 2001). The use of human recombinant thrombin has been shown to be less immunogenic in clinical trials. Antibodies developed in <1% of the patients without showing cross-reactivity with endogenous thrombin (Ballard et al., 2010; Singla et al., 2012).

Coagulation test show a markedly prolonged TT and a prolonged aPTT and PT without any correction in mixing studies. RT is normal. Thrombin levels and activity are reduced (Gabriel et al., 1987; Scully et al., 1982). Antibodies can also be detected by ELISA.

Antibodies to thrombin or prothrombin may be neutralizing or nonneutralizing increasing the clearance of prothrombin (Bajaj et al., 1985). Isotypes IgG or IgM have been identified (Bertolaccini et al., 1998). Antibodies directed to the catalytic center of thrombin (Sie et al., 1991) or the exosites of thrombin block the corresponding functions including the cleavage of fibrinogen (Mollica et al., 2006; Arnaud et al., 1994; La Spada et al., 1995). In an individual patient, antibodies have been described that form a complex with prothrombin leading to an interaction with antithrombin III and a direct inactivation without the conversion to thrombin (Madoiwa et al., 2001). The affinity of anticoagulant prothrombin antibodies appeared to be lower compared to the affinity of lupus anticoagulants (Bajaj et al., 1983; Field et al., 2001).

Establishment of a normal hemostasis in the presence of inhibitors to prothrombin or thrombin can be challenging. Fatal cases have been described (La Spada et al., 1995). The bypassing agents activated factor VII (FVII) (FVIIa) or activated prothrombin complex concentrate (aPCC) have been used (Giovannini et al., 2004). Also plasmapheresis, the use of fresh frozen plasma (FFP), immunosuppressive therapy including corticosteroids and ivIg has been applied (Scully et al., 1982). During therapy, a hypercoagulant situation may occur with the need for anticoagulation. A timely diagnosis and treatment reduces morbidity and mortality.

## AUTOIMMUNE INHIBITORS TO FACTOR V

Activated FV represents an essential cofactor of the complex converting prothrombin to thrombin. FV is structurally related to FVIII and composed of the A1, A2, A3, B, C1, and C2 domains. After activation by thrombin, the B domain is released and an A1-A2/A3-C1-C2 heterodimer is formed (Lollar, 2005).

Most FV antibodies have been described after surgical procedures especially after exposure to bovine thrombin glue, which contains traces of bovine FV (Chouhan et al., 1997; Berruyer et al., 1993; Spero, 1993; Zehnder and Leung, 1990; Muntean et al., 1994; Wang et al., 2017). These alloantibodies cross react with human FV. In addition autoantibodies to FV have been described as idiopathic (23%), related to the use of antibiotics (33%) (Knobl and Lechner, 1998), to surgery (even without the use of thrombin glue, 26%), malignancies (17%), infections (17%), or autoimmune diseases (11%), including SLE, hashimoto thyroiditis, primary biliary cirrhosis, or rheumatoid arthritis (Ang et al., 2009; Shastri et al., 1999; Takahashi et al., 2003; Franchini et al., 2012; Franchini and Lippi, 2011; Wang et al., 2017). In an analysis of FV inhibitors not associated with fibrin glue, 126 cases have been reported between 1955 and 2016. The estimated incidence was 0.09 (Singapore) to 0.23 (Australia) cases per million person years. About two-third of the patients with autoantibodies show a bleeding phenotype with hemorrhages mainly at mucosal membranes including hematuria, gastrointestinal, and gingival bleedings but also intracranial or retroperitoneal hemorrhages including fatal bleeds. Hemorrhages seem to correlate with the FV levels and with the aPTT (Ang et al., 2009; Favaloro et al., 2004; Olson et al., 2017).

Coagulation assays show a prolonged PT and aPTT, while the TT is normal. In contrast to FV deficiency or DIC mixing experiments fail to correct the test results. FV antigen and activity is reduced. FV inhibitors are also quantified by the Bethesda Assay.

Inhibitors belong to the isotypes IgG and IgA (Lane et al., 1978; Ortel et al., 1992; Takahashi et al., 2003). In a single publication an FV-specific IgG4 was described (Suehisa et al., 1995). The antibodies bind to the light chain of FV (A3-C1-C2) (Ortel et al., 1992). Interestingly, antibodies from patients showing a bleeding phenotype bind to the N-terminal part of C2 and inhibit the binding of FV to phospholipids and, therefore, interfering with the

formation of the prothrombinase complex (Ortel et al., 1998; Izumi et al., 2001). Platelet bound FV seems to be protected against most inhibitors, but severe bleedings have been described in a patient with inhibitors also against platelet associated FV (Ajzner et al., 2009; Nesheim et al., 1986). There are also noninhibitory antibodies that increase FV clearance. In addition to the anticoagulant nature of most FV inhibitors a single patient with an FV inhibitor was identified whose antibodies rendered FVa resistant to inactivation by activated protein C and therefore led to thrombophilic state (Kalafatis et al., 2002).

The initial therapy focuses on controlling the acute bleed. The use of platelets, FFP, PCC, aPCC, and FVIIa has been described. FFP was often given prior to diagnosis and needs critical assessment due to the low content of FV. The use of aPCC and FVIIa seems more effective (Ang et al., 2009; Franchini et al., 2012).

The need for eradication of FV inhibitors is discussed controversially. Inhibitors also disappear spontaneously within several weeks (Knobl and Lechner, 1998). There was no statistical difference in inhibitor elimination whether the patients were treated or not (Ang et al., 2009). Nevertheless patients with bleeding symptoms might benefit from inhibitor eradication. Several immunosuppressant approaches have been described including the use of corticosteroids, cyclophosphamide, or rituximab. ivlg, immunoabsorption, or plasmapheresis have also been used (de Raucourt et al., 2003; Jansen et al., 2001; Wang et al., 2017).

Prevention seems feasible as at least half of the published FV inhibitors occurred after the use of bovine thrombin or fibrin glue and therefore appear to be preventable with using alternative preparations (Streiff and Ness, 2002; Ballard et al., 2010).

## AUTOIMMUNE INHIBITORS TO FACTOR VII

Factor VII (FVII) is a serine protease composed of a Gla-domain, two epidermal growth factor (EGF) domains, and a protease domain. FVII binds to TF and is activated by thrombin, FXIa, factor XII (FXII), and FXa. FVIIa is a heterodimer of light chain (Gla and EGF domains) and the heavy chain (protease domain). The complex of TF and FVIIa activates FX and factor IX (FIX) (Vadivel and Bajaj, 2012).

Acquired FVII deficiency is mainly due to vitamin K deficiency and rarely an autoimmune pathology. Antibody mediated FVII deficiency has been described in less than ten reports so far, partly only with indirect evidence for autoantibodies. These inhibitors have been described as idiopathic, in combination with malignancies, autoimmune disorders, HIV-infection or after the administration of penicillin (Delmer et al., 1989; Aguilar et al., 2003; Campbell et al., 1980; de Raucourt et al., 1994; Ndimbie et al., 1989; Mehta et al., 1992; Weisdorf et al., 1989; Okajima and Ishii, 1999; Brunod et al., 1998). The intensity of hemorrhages varied from mild phenotypes to severe life-threatening bleedings and include hematomas, ecchymosis, gastrointestinal bleedings, hematuria, and intracranial hemorrhages (Delmer et al., 1989; Okajima and Ishii, 1999).

Coagulation assays show an isolated prolonged PT with normal aPTT, TT and bleeding time. FVII antigen and activity are decreased. Mixing studies do not correct the PT and inhibitors can be determined by the Bethesda Assay.

FVII inhibition is mediated by an IgG autoantibody belonging to the IgG1 subclass (Campbell et al., 1980; Weisdorf et al., 1989; Brunod et al., 1998). A direct inhibition of FVIIa interaction with TF or phospholipids has been proposed: the autoantibody bound to the light chain with an epitope on or near the Gla-domain in a Ca<sup>2+</sup>-dependent manner. In addition antibodies that increased the clearance of FVII without showing inhibition in vitro have been described (Weisdorf et al., 1989; Kamikubo et al., 2000).

The initial hemorrhage is controlled by administration of FFP, FVII, or FVIIa concentrates (Delmer et al., 1989; Mullighan et al., 2004). Tranexamic acid has also been used (Aguilar et al., 2003). The use of FVII concentrate has been associated with thrombotic events in patients with FVII inhibitors and underlying disease (Brunod et al., 1998). Inhibitor eradication has been achieved by immunosuppression and modulation with corticosteroids with or without azathioprine, ivlg or plasma exchange (Delmer et al., 1989).

## AUTOIMMUNE INHIBITORS TO FACTOR VIII

The FVIII protein includes the domains A1, A2, A3, B, C1, C2, and acidic spacers (Toole et al., 1984; Wood et al., 1984). The domain structure is shared between FV and FVIII. FVIII is mainly produced in endothelial cells; the main source of the protein is liver sinusoidal endothelial cells. During processing FVIII is cleaved into the heavy and the light chain, which include the domains A1-A2-B and A3-C1-C2, respectively. Heavy and



**FIGURE 50.3** Severe soft-tissue hemorrhage in a patient with acquired hemophilia A.

light chains form a noncovalent heterodimer, which is complexed to vWF. Upon activation by thrombin, the B domain and the acidic a3 region are cleaved off and vWF dissociates. FVIIIa interacts with the phospholipid membrane, FIXa and the substrate FX, which form the tenase complex and activate FX (Lenting et al., 1998; Saenko et al., 2002).

Despite rare, acquired hemophilia A (AHA) is by far the most frequent anticoagulant autoimmune condition (Fig. 50.3). In addition to numerous case reports, a small number of studies including one recent prospective trial and metaanalyses with 175–501 patients have been published (Green and Lechner, 1981; Collins et al., 2007; Delgado et al., 2003; Tiede et al., 2015). The reported incidence varies between 1.2 and 1.48/million/year (Collins et al., 2004; Tay et al., 2009). Patients presenting with AHA show a biphasic age peak with postpartum women dominating an early peak between 20 and 40 years (mean: 33.9 years) and men dominating the age peak above 60 years. Overall age groups men (58%) appear to be slightly more affected. The mean age at diagnosis is 73.9–78 years depending on the cohort (Collins et al., 2007; Green and Lechner, 1981; Knoebel et al., 2012; Tiede et al., 2015). A number of AHA cases in children have also been described (Moraca and Ragni, 2002; Brodeur et al., 1980).

AHA should be suspected patients presenting with spontaneously or traumatic bleeds without a prior bleeding history and a prolonged aPTT. The majority of patients show a severe bleeding phenotype. In contrast to congenital hemophilia A (CHA) most observed bleedings are large subcutaneous hematomas, ecchymosis and other soft-tissue bleeds. Many different clinical presentations have been reported including hematuria, gastrointestinal bleedings, intracranial hemorrhage and hemothoraces. Around 6% of the patients do not present with bleeding symptoms at time of diagnosis. Diagnosis is often prolonged, which is associated with increased bleeding but not with overall outcome (Knoebel et al., 2012; Fukushima et al., 2012; Micic et al., 2011; Rezaieyazdi et al., 2012; Webert, 2012). Similar to autoimmunity to other coagulation factors, AHA is associated with underlying conditions in about half of the cases. Malignancies (11.8%) including solid tumors, hematological neoplasias and autoimmune disorders (11.6%) including rheumatoid arthritis, SLE and others have been reported. Postpartum inhibitors comprised 7%–15% of the cohorts (Collins et al., 2007; Knoebel et al., 2012; Delgado et al., 2003; Green and Lechner, 1981). Others include exposure to certain drugs, surgery, infections, primary immunodeficiency and dermatological conditions. The remaining half with no concomitant condition identified is termed idiopathic AHA (Kim et al., 2008; Ozgur et al., 2007; Knoebel et al., 2012; Shetty et al., 2011; Reitter et al., 2011). A significant number of pregnancy associated FVIII autoantibodies are present antepartum. Hemorrhages during birth have been observed. The diagnostic evaluation of the newborn is crucial due to placental transfer of IgG (Tengborn et al., 2012).

Mortality is between 7.9% and 31%, whereas early mortality is related to bleeding and a much higher percentage of the patients experience later mortality due to complications of the immunosuppression or due to the underlying condition (Green and Lechner, 1981; Collins et al., 2007; Lottenberg et al., 1987; Morrison et al., 1993;

Hay et al., 1997; Bossi et al., 1998). In the largest cohort study at the end of follow-up survival of the patients with postpartum inhibitors was 100%, with concomitant autoimmune disease or malignancy 71% or 32%, respectively, and 58% for idiopathic AHA (Collins et al., 2012). In the recent prospective trial, 32% of the patients died during the first year after diagnosis (Tiede et al., 2015).

Laboratory results reveal a prolonged aPTT, low FVIII and normal PT or TT. Mixing studies do not correct the aPTT. Antibodies are measured by ELISA and by the Bethesda assay (Nijmegen modification). Bethesda units or residual FVIII activity do not correlate with the bleeding phenotype (Lindgren et al., 2002; Toschi and Baudo, 2010). Interestingly in a recent prospective trial FVIII activity <1 IU/dL and higher concentrations of FVIII specific IgG but not Bethesda units were associated with a lower rate of remission and a higher rate of death (Tiede et al., 2015; Werwitzke et al., 2016). Type I and type II kinetics of FVIII inactivation are observed with the latter being the dominant kinetic not inactivating FVIII completely. There are indications that type I inhibitors block the activation of FVIII by thrombin, whereas type II inhibitors block the binding to phospholipids resulting in different clinical phenotypes (Matsumoto et al., 2012; Nogami et al., 2001). Affinity for FVIII might also influence the type of kinetics observed. Isotypes IgG, mainly the subclasses IgG4 and IgG1, but also IgA and IgM have been detected at low titers (Whelan et al., 2013). The IgG population is polyclonal. In an analysis of 115 patients antibody epitopes were mainly found on the C1 (78%) and C2 (68%) domains followed by the A2 domain (23%) indicating a different epitope signature than found in the alloimmune response in patients with CHA and inhibitors (Kahle et al., 2017). The FVIII specific IgG subclasses and the recognized epitopes differ in different clinical conditions: while a general dominance of IgG4 is described, in postpartum AHA FVIII specific IgG2 and 3 were more frequent than in patients with other concomitant conditions. In addition, postpartum inhibitors recognized more often the heavy chain especially the A1a1 domain (Lapalud, 2012). The presence of FVIII specific IgA was associated with a higher risk of not achieving complete remission, relapse and death (Tiede et al., 2016). Antibodies block the interaction of FVIII with different molecules of the coagulation cascade including vWF, phospholipids, thrombin, FIX and FX by competition or steric hindrance. Proteolytic FVIII antibodies have also been described (Mahendra et al., 2012; Wootla et al., 2008). In addition, there are indications that inhibitors with type II kinetics also inhibit the proteolysis of FVIIa by activated protein C and establish circulating immune complexes (Nogami et al., 2001). Recently, the presence of hydrolyzing and activating FIX antibodies in AHA patients leading to thrombin generation in about a third of the patients was described (Wootla et al., 2011). The clinical relevance remains to be determined.

Some T-cell epitopes in the C2 domain (amino acids 2291–2330) are shared between AHA, patients with CHA with inhibitors and healthy individuals with additional disease group specific epitopes including the region 2241–2290 for AHA patients. In addition, distinct T-cell epitopes on the A3 domain have been identified for patients with AHA that are not shared by patients with CHA (Reding et al., 2003; Reding et al., 2004).

Polymorphisms in immune response genes as well as HLA genotypes have been described to be associated with AHA: a higher frequency of the CTLA4 49A/G polymorphism has been observed in patients with AHA compared to healthy controls. Interestingly the G allele was more frequent in patients with no idiopathic AHA or with an underlying autoimmune disease. HLA class I haplotypes were not associated with AHA, but for HLA class II haplotypes a higher frequency of DRB1\*16 and DQB1\*0502 and a lower frequency of DRB1\*15 and DQB1\*0602 were detected in affected patients. These results do not correspond to HLA haplotypes seen in CHA patients with inhibitors. The influence of polymorphisms in the FVIII gene remains to be investigated (Pavlova et al., 2008, 2010; Tiede et al., 2010).

The treatment of patients with AHA focuses initially on reestablishing hemostasis followed by the induction of tolerance and treatment of the underlying disease (if applicable). In a recent prospective trial, an immunosuppressive regime based on corticosteroids with the addition of cyclophosphamide and rituximab in a step-wise approach was evaluated in 102 patients. Hemostatic control without the necessity of further hemostatic treatment was achieved in 83% of the patients after a median of 31 days (range: 7–362 days) (Tiede et al., 2015). In current AHA treatment, in about 70% of the patients bypassing agents including FVIIa and aPCC are used to control acute bleeds. Around one-third of the patients did not require any hemostatic treatment. In the largest cohort FVIIa was used in more than half of bleeds and aPCC in 20%. Both agents were equally effective overall (91.8% and 93.3%) but treatment success for each agent differs in the individual patient. The remaining number of patients was treated with FVIII products, D-amino D-arginine vasopressin (DDAVP) and with porcine FVIII. Treatment with bypassing agents was more effective than treatment with human FVIII or DDAVP (69.9%) (Collins et al., 2007; Baudo et al., 2012; Morrison et al., 1993; Kessler and Ludlam, 1993). In about 4% of the patients thromboembolic events occurred during treatment with bypassing agents

(Knoebel et al., 2012; Sumner et al., 2007). Recently recombinant porcine (rp) FVIII was licensed for the treatment of AHA. In the initial treatment rpFVIII provides an alternative to classical bypassing agents with the opportunity to monitor the treatment (Kruse-Jarres et al., 2015). Current guidelines recommend the use of rpFVIII, rFVIIa and aPCC for the control of acute bleeds. The use of human FVIII might also be possible to control bleeds in patients with low titer inhibitors; further immune adsorption high-dose of FVIII treatment is successfully used in the European centers (Kruse-Jarres et al., 2017; Collins et al., 2013).

Immunosuppressive therapy is necessary to induce tolerance in most AHA patients. Various regimens based on corticosteroids, cytotoxic agents, rituximab, ivIg, plasmapheresis, immune adsorption, and protocols for the induction of immune tolerance from CHA with inhibitors using high-dose FVIII have been applied (Stasi et al., 2004; Nemes and Pitlik, 2000; Zanon et al., 2013; Barillari and Pasca, 2013; Tiede et al., 2009; Collins et al., 2007, 2012; Muzaffar et al., 2012; Wiestner et al., 2002; Freedman et al., 2003). Inhibitor eradication (complete remission) was achieved in about two-thirds of the patients. In the largest cohort to date (EACH2,  $n = 501$ ) most patients were treated with corticosteroids alone (43%), in combination with cyclophosphamide (25%) or with rituximab-based regimens (15%) achieving complete remission in 58%, 80%, or 61%, respectively. Successfully treated patients who received a combination of corticosteroids and cyclophosphamide experienced more adverse events (41%), mainly infections compared to the group treated with steroids alone (25%). The final outcome “alive and inhibitor free at last follow up” was 67% of the patients treated with steroids alone compared to 62% of the patients treated with corticosteroids and cyclophosphamide (Collins et al., 2012). In the prospective GTH trial complete remission was achieved in 61% of the patients after a median of 79 days (range: 26–856 days) (Tiede et al., 2015). The modified Bonn–Malmö protocol combining immune adsorption, immunosuppression, FVIII administration and FVIIa to control acute bleeds achieved remission rates of >90% for nonmalignancy associated AHA (Zeitler et al., 2012, 2013). Current guidelines recommend the use of corticosteroids alone, corticosteroids in combination with cyclophosphamide or—as second line—corticosteroids in combination with rituximab are recommended for different clinical situations (Kruse-Jarres et al., 2017; Collins et al., 2013). Treatment success appears to be associated with the underlying condition, younger age and the FVIII specific antibody signature (see above). Spontaneous remissions have also been described, especially in postpartum inhibitors. These groups also showed the highest rates of complete remission when treated with corticosteroids (Baudo and de Cataldo, 2003). When complete remission was achieved life-expectancy appears equal to nonaffected individuals of the same age (Collins et al., 2007).

## AUTOIMMUNE INHIBITORS TO FACTOR IX

FIX is a serine protease structurally related to FVII and FX including a Gla-domain, two EGF-like domains and a protease domain. FIX is activated by FXIa and FVIIa. FIXa forms with FVIIIa the tenase complex, which activates FX (see above).

Autoimmune inhibitors of FIX have been only described in single case reports (Largo et al., 1974; Roberts, 1970; Ozsoylu and Ozer, 1973; Reisner et al., 1977). The incidence is unknown. They mainly occurred in elderly people, but few cases in children have also been described (Jedidi et al., 2011; Mazzucconi et al., 1999; Miller et al., 1978). The condition occurred idiopathic or with underlying diseases including hepatitis C (under therapy), autoimmune liver disease, Sjögren syndrome, immune thrombocytopenia and arthritis, and SLE (Campos-de-Magalhaes et al., 2011; Jedidi et al., 2011; Krishnamurthy et al., 2011; Carmassi et al., 2007; Castro et al., 1972; Torres et al., 1980). Three cases have been reported with both, FVIII and FIX inhibitors (Carmassi et al., 2007) and one patient with an inhibitor to FIX, FX, and prothrombin (Rochanda et al., 2012).

Laboratory assessment reveals a prolonged aPTT while PT, TT and bleeding time are normal. In mixing studies the aPTT does not correct. FIX inhibitors are detected and quantified by the Bethesda Assay.

Due to the few cases, data are scarce on the nature of the autoantibodies to FIX. Alloantibodies described in hemophilia B patients are mainly IgG, belonging to the subclasses IgG1 and IgG4. The antibodies are mainly directed against the protease and the GLA-domain, but also the EGF-like domain (Torres et al., 1980). In one patient antibodies that bound to FIX, FX, and prothrombin belonged to the IgG4 subclass and bound to a common motif on the Gla-domain (Rochanda et al., 2012).

In the few cases described, the acute bleeding situation was treated with FVIIa (Abshire and Kenet, 2008) accompanied by immunosuppression with prednisolone alone (Krishnamurthy et al., 2011), in combination with ivIg (Mazzucconi et al., 1999) or with addition of azathioprine (Carmassi et al., 2007).

## AUTOIMMUNE INHIBITORS TO FACTOR X

FX also is a zymogen of a serine protease in its activated form and related to FVII and FIX with the identical domain structure including the Gla-, EGF-, and protease domains. Upon activation by FVIIa and TF or the tenase complex composed of FVIIIa and FIXa, FXa cleaves prothrombin in the presence of FVa (prothrombinase complex) and phospholipids which generates active thrombin.

Acquired FX deficiency has been described in the context of amyloidosis due to the adsorption of FX to fibrils (Mumford et al., 2000; Gollard et al., 2013; Chan and Ogunsile, 2017) and of malignancies including solid tumors and leukemias. Acquired FX deficiency unrelated to amyloidosis is extremely rare and has been reported in 36 patients with only 11 case reports with evidence for autoantibodies. Acquired inhibitors to FX have been associated with respiratory tract infections (Mulhare et al., 1991; Bayer et al., 1969; Hosker and Jewell, 1983; Currie et al., 1984; Broze, 2014; Chan and Ogunsile, 2017; Lee et al., 2012). One case has been described in a child after extensive burns (Matsunaga and Shafer, 1996). Antibodies as inhibitors were only identified in recent cases. The clinical picture includes hematoma, ecchymosis, mucosal bleeds, hematuria, and also intracranial hemorrhages (Lankiewicz and Bell, 1992; Rochanda et al., 2012; Smith et al., 1998; Edgin et al., 1980).

In laboratory findings PT and aPTT are prolonged while TT and bleeding time are normal. Inhibitors are quantified by the Bethesda assay.

Anti-FX inhibitors belong to the IgG isotype. Several antibodies that were analyzed bound to the light chain near or on the Gla-domain. The antibody inhibited the activation of FX by the TF–FVIIa complex and by the tenase complex (Rao et al., 1994; Lankiewicz and Bell, 1992; Smith et al., 1998; Matsunaga and Shafer, 1996). Another report described the recognition of a common epitope for FII, FIX, and FX also on the Gla-domain (Rochanda et al., 2012). This antibody population belonged to the IgG4 subclass.

Treatment of acute bleeds due to FX inhibitors is challenging due to the central role of FX in the coagulation cascade. The successful use of aPCC has been described but has also been complicated by thromboembolic complications and cerebral infarctions (Smith et al., 1998; Henson et al., 1989; Mulhare et al., 1991). Further PCC, vitamin K, and transfusions of FFP have been described with different success rates to control bleeds. Autoimmune inhibitors of FX are mainly transient, thus inhibitor eradication is not a focus of the primary treatment but corticosteroids or ivIg have been used in individual cases (Matsunaga and Shafer, 1996; Edgin et al., 1980).

## AUTOIMMUNE INHIBITORS TO FACTOR XI

Factor XI (FXI) is a zymogen and after activation by FXIIa, thrombin or autocatalytically it is a serine protease. It is composed of four so-called apple domains in the heavy chain and a protease domain and circulates as a homodimer. FXIa then activates FIX (Gailani and Smith, 2009).

Autoimmune inhibitors to FXI are also extremely rare. The incidence is unknown. Most reports are on elderly people, but also a few children have been described. FXI inhibitors have been found with underlying conditions such as SLE, leukemias, autoimmune gastrointestinal diseases, psoriasis, or membranoproliferative glomerulonephritis (Vercellotti and Mosher, 1982; Goodrick et al., 1992; Kyriakou et al., 2002; McManus et al., 2012; Bortoli et al., 2009; Rustgi et al., 1982; Vazzana et al., 2014). Different bleeding phenotypes due to FXI inhibitors occurred including mostly mild or no bleeding but also life threatening bleedings have been described (Bortoli et al., 2009; Reece et al., 1984).

In coagulation assays, FXI inhibitors present with a prolongation of aPTT with a normal PT, TT, and bleeding time. Inhibitors are quantified by the Bethesda assay.

The isotype of FXI inhibitors is IgG or IgM (Krieger et al., 1975). Antibodies in patients with congenital FXI deficiency are of the IgG1 or three subclasses and are directed against the heavy chain of the molecule (De La Cadena et al., 1988). The mode of FXI inhibition has been identified as increased clearance of FXI or blocking of the activation of FXI (McManus et al., 2012; Krieger et al., 1975; Poon et al., 1984). No such data are available on acquired inhibitors to FXI.

For the treatment of hemorrhages in the presence of FXI inhibitors, the use of FXI-concentrate, FFP, PCC, and rFVIIa have been described. Inhibitor eradication has been achieved by immunosuppression with corticosteroids or by immunosuppression as part of the therapy of the underlying condition (Vercellotti and Mosher, 1982; Billon et al., 2001; Bern et al., 2005; Goodrick et al., 1992).

## AUTOIMMUNE INHIBITORS TO FACTOR XII

FXII is a monomeric zymogen of the serine protease FXIIa. FXII consists of a heavy chain including two fibronectin-type domains, two EGF-like domains, a kringle domain, and a proline-rich domain plus a light chain including the catalytic center. Polyphosphates on the surface of procoagulant thrombocytes (contact phase) preactivate FXII, which is then activated by kallikrein. FXIIa then activates FXI and plasminogen. In addition to initiating the coagulation cascade, FXIIa starts the kallikrein–kinin system.

FXII inhibitors have been described in combination with autoimmune diseases such as SLE, APS, autoimmune hepatitis, malignancies including lymphoma and gastric carcinoma or infections such as hepatitis B (Bertolaccini et al., 2007; Jones et al., 2000; Davidson et al., 2005; Chalkiadakis et al., 1999). The pathology of FXII inhibitors is still being discussed; they are not associated with bleeding symptoms but possibly with a prothrombotic state (Aberg and Nilsson, 1972). In addition FXII inhibitors have been described in context with fetal loss (Jones et al., 2001; D'Uva et al., 2005).

Laboratory results show a very prolonged aPTT with a normal PT, TT, and bleeding time typically without any bleeding symptoms. Antibodies can be detected by ELISA.

For FXII inhibitors, isotype IgG or IgM has been identified (Jones et al., 2000; Davidson et al., 2005). In women with fetal loss most antibodies recognized the N-terminal portion of the heavy chain, which interacts with thrombocytes, whereas in patients with APS epitopes were identified in the catalytic domain and in the EGF-like domain (Inomo et al., 2008; Harris et al., 2005).

Treatment is not required, but anticoagulation may be considered particularly in context of pregnancy and miscarriages.

## AUTOIMMUNE INHIBITORS TO FACTOR XIII

FXIII is a tetrameric molecule composed of two A and two B domains, which is activated by thrombin and stabilizes the fibrin clot by cross-linking fibrin  $\gamma$ -chains (Ariens et al., 2002). Autoantibodies to FXIII occur very rarely but are likely to be under diagnosed. In recent years reported cases rose close to 100 due to a national registry (Ichinose and Japanese Collaborative Research Group on AH13, 2017). FXIII inhibitors are also associated with underlying conditions, including a monoclonal gammopathy (Luo et al., 2010) or other autoimmune diseases including SLE (Lorand et al., 2002; Ahmad et al., 1996; Ajzner et al., 2009; Luo and Zhang, 2011). Antibodies to FXIII have also been described after exposure to drugs such as isoniazid (Otis et al., 1974; Shires et al., 1979; Krumdieck et al., 1991). Almost half of cases have been termed idiopathic without any apparent underlying condition (Nijenhuis et al., 2004; Ichinose and Japanese Collaborative Research Group on AH13, 2017; Franchini et al., 2013).

Patients present clinically with recurrent hemorrhages of different intensity including mucosal, intramuscular, or large soft-tissue hematomas but also very severe to life-threatening events such as intracranial or retroperitoneal hemorrhages in 11% and 19% of the patients, respectively (Lorand et al., 2002; Daly et al., 1991; Ichinose and Japanese Collaborative Research Group on AH13, 2017). Mortality rate due to hemorrhages has been reported as high as 18%. An additional 18% of a subset of patients receiving immunosuppressive therapy died due to infections. The mean age at diagnosis was 65.8 years and ranged from 10 to 87 years with a median age between 60 and 70 years. Slightly more women than men were affected (Luo and Zhang, 2011; Ichinose and Japanese Collaborative Research Group on AH13, 2017). Diagnosis is based on a clinical score in combination with laboratory findings (Ichinose et al., 2016).

The laboratory findings typically reveal normal global coagulation tests including PTT, PT, bleeding time, and platelet counts. FXIII activity or levels are reduced and can be determined by chromogenic assays, ELISA for the FXIIIA or B subunits. The thromboelastogram reflects a reduced clot formation and increased fibrinolysis. Alternatively, a dissolved clot after treatment with 5 M urea indicates a FXIII deficiency. Inhibitors to FXIII can be measured by mixing studies and by ELISA to immobilized FXIII subunits. In addition immunocomplexes have been detected that may interfere with laboratory diagnosis. The residual FXIII activity correlates with the severity of bleeds (Ichinose and Japanese Collaborative Research Group on AH13, 2017).

Antibodies are mainly directed only against the enzymatically active A subunit of FXIII (80%) and rarely against the B subunit (7%) or against both (13%). Depending on the binding site, antibodies block the activation by thrombin, catalysis, and the binding to fibrin or other substrates of FXIII. Noninhibitory antibodies increase

the degradation of FXIII (Ahmad et al., 1996; Luo and Zhang, 2011; Ajzner et al., 2009; Lorand et al., 2002; Nakamura et al., 1988; Fukue et al., 1992).

The treatment of patients was based on replacement therapy and immunosuppression. Despite the presence of antibodies, treatment of acute bleeds with mainly FXIII concentrate or FFP (if concentrate was not available) has been successful. In addition antifibrinolytics, FVIIa and platelets have been used. Hemostatic therapy is mainly combined with immunosuppression, including corticosteroids and/or cyclophosphamide (Hayashi et al., 2012; Tosetto et al., 1995; Luo and Zhang, 2011; Ishida et al., 2010). The use of rituximab, ivIg, plasma exchange or immune adsorption, azathioprine, or cyclosporine has also been described. Despite initial successful treatment, recent data suggest an ongoing autoimmune response in around 10% of the patients (Ajzner et al., 2009; Gregory and Cooper, 2006; Ichinose and Japanese Collaborative Research Group on AH13, 2017).

## AUTOIMMUNE INHIBITORS TO VON WILLEBRAND FACTOR

Although vWF is not a classical coagulation factor, it plays a crucial role in primary as well as secondary hemostasis. The protein binds to FVIII prolonging its half-life prior to activation, interacts with subendothelial layers after vascular injury and promotes thrombocyte adhesion and aggregation. The glycoprotein includes the domains D', D1-4, A1-3, B, and C1-2. The vWF monomers assemble to multimers of different sizes up to 20,000 kDa. Ultralarge multimers are cleaved by ADAMTS 13 (see above, Section, Prothrombotic Disorders) (Schneppenheim and Budde, 2008).

Acquired von Willebrand disease (avWD) is also a rare condition. A few hundred patients have been described in literature, one report stated an incidence of 10% in a small, preselected cohort of patients with bleeding disorders (Mohri et al., 1998). Among patients with avWD, 10%–30% have a detectable autoantibody against vWF; their overall incidence has been estimated to be 0.04% (Kumar et al., 2003). The pathophysiology of avWD in most patients is not based on autoimmune phenomena but rather on a reduced production of vWF as seen in hypothyroidism, an increased clearance following attachment to tumor cells or paraneoplastic products, an increased mechanical clearance due to mechanical cardiac devices or due to certain drugs (Veyradier et al., 2000). Thus most cases of avWD are associated with underlying conditions such as hypothyroidism, lympho- or myeloproliferative disorders, solid tumors including Wilms tumor, infections, and acquired or congenital heart defects. Drugs that may cause avWD include certain antibiotics, hydroethyl starch, and valproic acid. Autoimmune avWD has been described as idiopathic or in combination with underlying autoimmune diseases, including SLE or monoclonal gammopathies (Will, 2006; Federici et al., 2000; Michiels et al., 2001). Patients present with a spectrum of no-to-severe bleeding symptoms including mainly ecchymoses but also epistaxis, gastrointestinal, and mucosal bleeds (Collins et al., 2008; Mohri et al., 1998). Patients with detectable inhibitors show a higher bleeding tendency than patients without inhibitors. Autoantibodies bind to the large or intermediate size multimers. They have been identified as mainly IgG isotypes, but also IgA and IgM have been described depending on the underlying condition. IgG antibodies belong to the subclasses IgG1 and IgG4. Epitopes have been mapped to the A1 and the A3 domain inhibiting collagen binding and binding to GP Ib and GP IIb/IIIa (Mohri et al., 1998; van Genderen et al., 1994).

Laboratory diagnosis of avWD and differentiation to congenital vWD is often difficult. The analysis reveals a prolonged bleeding time, with normal PT and normal or prolonged aPTT. The vWF antigen, ristocetin cofactor, or collagen-binding activity is reduced. The FVIII activity is often reduced as well. Large vWF multimers are often missing. Anti-vWF antibodies can be detected. In a Bethesda-like assay several approaches have to be considered due to the large size of vWF and different epitope-related effect of the antibodies. A pharmacokinetic analysis might be useful as noninhibitory antibodies that enhance clearance are not detected by the Bethesda assay (Siaka et al., 2003; Mohri et al., 1998; Tiede et al., 2008; Luboshitz et al., 2001; Federici et al., 2013).

The treatment of avWD is based on the underlying condition and pathomechanism. The use of desmopressin (DDAVP) to release vWF, the substitution of a vWF-containing FVIII concentrate or FFP, has been used successfully in nonautoimmune avWD and to control acute bleedings. The response to FVIII/vWF concentrates or DDAVP is usually very poor when an inhibitor is present. In severe uncontrollable bleedings FVIIa has also been used successfully. Autoimmune avWD has successfully been treated with immunomodulation, including the administration of immunoglobulins and immunosuppression with corticosteroids, rituximab, plasma exchange, or immune absorption (Collins et al., 2008; Sucker et al., 2009; Tiede et al., 2011; Gavva et al., 2017).

## AUTOIMMUNE INHIBITORS TO FURTHER PROTEINS

In addition to autoimmune responses to proteins described above, a case of an autoantibody to prekallikrein has been identified to date. An IgG1 and 4 polyclonal autoantibody population was detected in a patient who presented with a prolonged aPTT without any symptoms of bleeding or thrombosis (Page et al., 1994).

## CONCLUSION AND FUTURE ASPECTS

Autoimmune inhibitors to coagulation factors are rare phenomena and poorly understood entities of autoimmune diseases despite progress some entities. In case of bleeding in individuals without bleeding history, inhibitors to coagulation factors need to be assessed. The timely diagnosis and treatment of affected patients remains challenging. Further efforts are needed to understand the underlying pathology of autoimmunity leading to hemorrhages or thrombosis and to improve treatment outcome for affected individuals.

### Acknowledgments

The author would like to thank Stephan Schultze-Strasser, PhD, and Manuela Krause, MD, for assistance with or provision of figure, Christine Heller, MD, for critical comments on the manuscript and also his family, and the editors for support and patience.

### References

- Aberg, H., Nilsson, I.M., 1972. Recurrent thrombosis in a young woman with a circulating anticoagulant directed against factors XI and XII. *Acta Med. Scand.* 192, 419–425.
- Abshire, T., Kenet, G., 2008. Safety update on the use of recombinant factor VIIa and the treatment of congenital and acquired deficiency of factor VIII or IX with inhibitors. *Haemophilia* 14, 898–902.
- Aguilar, C., Lucia, J.F., Hernandez, P., 2003. A case of an inhibitor autoantibody to coagulation factor VII. *Haemophilia* 9, 119–120.
- Ahmad, F., Solymoss, S., Poon, M.C., Berube, C., Sullivan, A.K., 1996. Characterization of an acquired IgG inhibitor of coagulation factor XIII in a patient with systemic lupus erythematosus. *Br. J. Haematol.* 93, 700–703.
- Ajzner, E., Schlammadinger, A., Kerenyi, A., Bereczky, Z., Katona, E., Haramura, G., et al., 2009. Severe bleeding complications caused by an autoantibody against the B subunit of plasma factor XIII: a novel form of acquired factor XIII deficiency. *Blood* 113, 723–725.
- Ang, A.L., Kuperan, P., Ng, C.H., Ng, H.J., 2009. Acquired factor V inhibitor. A problem-based systematic review. *Thromb. Haemost.* 101, 852–859.
- Arachchilage, D.R., Efthymiou, M., Mackie, I.J., Lawrie, A.S., Machin, S.J., Cohen, H., 2014. Anti-protein C antibodies are associated with resistance to endogenous protein C activation and a severe thrombotic phenotype in antiphospholipid syndrome. *J. Thromb. Haemost.* 12, 1801–1809.
- Ariens, R.A., Lai, T.S., Weisel, J.W., Greenberg, C.S., Grant, P.J., 2002. Role of factor XIII in fibrin clot formation and effects of genetic polymorphisms. *Blood* 100, 743–754.
- Arnaud, E., Lafay, M., Gaussem, P., Picard, V., Jandrot-Perrus, M., Aiach, M., et al., 1994. An autoantibody directed against human thrombin anion-binding exosite in a patient with arterial thrombosis: effects on platelets, endothelial cells, and protein C activation. *Blood* 84, 1843–1850.
- Atsumi, T., Ieko, M., Bertolaccini, M.L., Ichikawa, K., Tsutsumi, A., Matsuura, E., et al., 2000. Association of autoantibodies against the phosphatidylserine-prothrombin complex with manifestations of the antiphospholipid syndrome and with the presence of lupus anticoagulant. *Arthritis Rheum.* 43, 1982–1993.
- Bajaj, S.P., Rapaport, S.I., Fierer, D.S., Herbst, K.D., Schwartz, D.B., 1983. A mechanism for the hypoprothrombinemia of the acquired hypoprothrombinemia-lupus anticoagulant syndrome. *Blood* 61, 684–692.
- Bajaj, S.P., Rapaport, S.I., Barclay, S., Herbst, K.D., 1985. Acquired hypoprothrombinemia due to non-neutralizing antibodies to prothrombin: mechanism and management. *Blood* 65, 1538–1543.
- Ballard, J.L., Weaver, F.A., Singla, N.K., Chapman, W.C., Alexander, W.A., 2010. Safety and immunogenicity observations pooled from eight clinical trials of recombinant human thrombin. *J. Am. Coll. Surg.* 210, 199–204.
- Barillari, G., Pasca, S., 2013. pdFVIII/VWF may be an alternative treatment for old medical patient with acquired haemophilia A and systemic vascular disease? *Transfus. Apher. Sci.* 48, 59–62.
- Barthels, M., Heimburger, N., 1985. Acquired thrombin inhibitor in a patient with liver cirrhosis. *Haemostasis* 15, 395–401.
- Baudo, F., de Cataldo, F., 2003. Acquired factor VIII inhibitors in pregnancy: data from the Italian Haemophilia Register relevant to clinical practice. *Br. J. Obstet. Gynaecol.* 110, 311–314.
- Baudo, F., Collins, P., Huth-Kuhne, A., Levesque, H., Marco, P., Nemes, L., et al., 2012. Management of bleeding in acquired hemophilia A: results from the European Acquired Haemophilia (EACH2) Registry. *Blood* 120, 39–46.
- Bayer, W.L., Curiel, D., Szeto, I.L., Lewis, J.H., 1969. Acquired factor X deficiency in a Negro boy. *Pediatrics* 44, 1007–1009.
- Bern, M.M., Sahud, M., Zhukov, O., Qu, K., Mitchell JR., W., 2005. Treatment of factor XI inhibitor using recombinant activated factor VIIa. *Haemophilia* 11, 20–25.

- Berruyer, M., Amiral, J., Ffrench, P., Belleville, J., Bastien, O., Clerc, J., et al., 1993. Immunization by bovine thrombin used with fibrin glue during cardiovascular operations. Development of thrombin and factor V inhibitors. *J. Thorac. Cardiovasc. Surg.* 105, 892–897.
- Bertolaccini, M.L., Atsumi, T., Khamashta, M.A., Amengual, O., Hughes, G.R., 1998. Autoantibodies to human prothrombin and clinical manifestations in 207 patients with systemic lupus erythematosus. *J. Rheumatol.* 25, 1104–1108.
- Bertolaccini, M.L., Mepani, K., Sanna, G., Hughes, G.R., Khamashta, M.A., 2007. Factor XII autoantibodies as a novel marker for thrombosis and adverse obstetric history in patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* 66, 533–536.
- Bettoni, G., Palla, R., Valsecchi, C., Consonni, D., Lotta, L.A., Trisolini, S.M., et al., 2012. ADAMTS-13 activity and autoantibodies classes and subclasses as prognostic predictors in acquired thrombotic thrombocytopenic purpura. *J. Thromb. Haemost.* 10, 1556–1565.
- Billon, S., Niger, L.E., Escoffre-Barbe, C., Vicariot, M., Abgrall, J.F., 2001. The use of recombinant factor VIIa (NovoSeven) in a patient with a factor XI deficiency and a circulating anticoagulant. *Blood Coagul. Fibrinolysis* 12, 551–553.
- Boccara, O., Lesage, F., Regnault, V., Lasne, D., Dupic, L., Bourdon-Lanoy, E., et al., 2009. Nonbacterial purpura fulminans and severe autoimmune acquired protein S deficiency associated with human herpesvirus-6 active replication. *Br. J. Dermatol.* 161, 181–183.
- Bortoli, R., Monticielo, O.A., Chakr, R.M., Palominos, P.E., Rohsig, L.M., Kohem, C.L., et al., 2009. Acquired factor XI inhibitor in systemic lupus erythematosus—case report and literature review. *Semin. Arthritis Rheum.* 39, 61–65.
- Bossi, P., Cabane, J., Ninet, J., Dhote, R., Hanslik, T., Chosidow, O., et al., 1998. Acquired hemophilia due to factor VIII inhibitors in 34 patients. *Am. J. Med.* 105, 400–408.
- Brodeur, G.M., O'Neill, P.J., Willimas, J.A., 1980. Acquired inhibitors of coagulation in nonhemophiliac children. *J. Pediatr.* 96, 439–441.
- Broze JR, G.J., 2014. An acquired, calcium-dependent, factor X inhibitor. *Blood Cells Mol. Dis.* 52, 116–120.
- Brunod, M., Chatot-Henry, C., Mehdaoui, H., Richer, C., Fonteau, C., 1998. Acquired anti-factor VII (proconvertin) inhibitor: hemorrhage and thrombosis. *Thromb. Haemost.* 79, 1065–1066.
- Campbell, E., Sanal, S., Mattson, J., Walker, L., Estry, S., Mueller, L., et al., 1980. Factor VII inhibitor. *Am. J. Med.* 68, 962–964.
- Campos-de-Magalhaes, M., Eduardo Brandao-Mello, C., Lucia Elias Pires, M., Cecilia Da Fonseca Salgado, M., Barcelo De Brito, S., Jose De Almeida, A., 2011. Factor VIII and IX deficiencies related to acquired inhibitors in a patient with chronic hepatitis C virus infection receiving treatment with pegylated interferon plus ribavirin. *Hematology* 16, 80–85.
- Carmassi, F., Giannarelli, C., De Giorgi, A., De Negri, F., 2007. Combined factor VIII and IX inhibitors in a non-haemophilic patient: successful treatment with immunosuppressive drugs. *Haemophilia* 13, 106–107.
- Castro, O., Farber, L.R., Clyne, L.P., 1972. Circulating anticoagulants against factors IX and XI in systemic lupus erythematosus. *Ann. Intern. Med.* 77, 543–548.
- Cataland, S.R., Jin, M., Ferketich, A.K., Kennedy, M.S., Kraut, E.H., George, J.N., et al., 2007. An evaluation of cyclosporin and corticosteroids individually as adjuncts to plasma exchange in the treatment of thrombotic thrombocytopenic purpura. *Br. J. Haematol.* 136, 146–149.
- Cataland, S.R., Yang, S.B., Witkoff, L., Kraut, E.H., Lin, S., George, J.N., et al., 2009. Demographic and ADAMTS13 biomarker data as predictors of early recurrences of idiopathic thrombotic thrombocytopenic purpura. *Eur. J. Haematol.* 83, 559–564.
- Chalkiadakis, G., Kyriakou, D., Oekonomaki, E., Tsiaoussis, J., Alexandrakis, M., Vasilakis, S., et al., 1999. Acquired inhibitors to the coagulation factor XII associated with liver disease. *Am. J. Gastroenterol.* 94, 2551–2553.
- Chan, I.S., Ogunsile, F.J., 2017. An acquired factor X inhibitor: the importance of understanding coagulation. *Am. J. Med.* 130, e307–e308.
- Chang, H.H., Chiang, B.L., 2014. The diagnosis and classification of autoimmune coagulopathy: an updated review. *Autoimmun. Rev.* 13, 587–590.
- Chouhan, V.D., De La Cadena, R.A., Nagaswami, C., Weisel, J.W., Kajani, M., Rao, A.K., 1997. Simultaneous occurrence of human antibodies directed against fibrinogen, thrombin, and factor V following exposure to bovine thrombin: effects on blood coagulation, protein C activation and platelet function. *Thromb. Haemost.* 77, 343–349.
- Cohen, I., Amir, J., Ben-Shaul, Y., Pick, A., De Vries, A., 1970. Plasma cell myeloma associated with an unusual myeloma protein causing impairment of fibrin aggregation and platelet function in a patient with multiple malignancy. *Am. J. Med.* 48, 766–776.
- Coleman, M., Vigliano, E.M., Weksler, M.E., Nachman, R.L., 1972. Inhibition of fibrin monomer polymerization by lambda myeloma globulins. *Blood* 39, 210–223.
- Collins, P., Macartney, N., Davies, R., Lees, S., Giddings, J., Majer, R., 2004. A population based, unselected, consecutive cohort of patients with acquired haemophilia A. *Br. J. Haematol.* 124, 86–90.
- Collins, P.W., Hirsch, S., Baglin, T.P., Dolan, G., Hanley, J., Makris, M., et al., 2007. Acquired hemophilia A in the United Kingdom: a 2-year national surveillance study by the United Kingdom Haemophilia Centre Doctors' Organisation. *Blood* 109, 1870–1877.
- Collins, P., Budde, U., Rand, J.H., Federici, A.B., Kessler, C.M., 2008. Epidemiology and general guidelines of the management of acquired haemophilia and von Willebrand syndrome. *Haemophilia* 14 (Suppl 3), 49–55.
- Collins, P., Baudo, F., Knoebl, P., Levesque, H., Nemes, L., Pellegrini, F., et al., 2012. Immunosuppression for acquired hemophilia A: results from the European Acquired Haemophilia Registry (EACH2). *Blood* 120, 47–55.
- Collins, P.W., Chalmers, E., Hart, D., Jennings, I., Liesner, R., Rangarajan, S., et al., 2013. Diagnosis and management of acquired coagulation inhibitors: a guideline from UKHCDO. *Br. J. Haematol.* 162, 758–773.
- Colwell, N.S., Tollefson, D.M., Blinder, M.A., 1997. Identification of a monoclonal thrombin inhibitor associated with multiple myeloma and a severe bleeding disorder. *Br. J. Haematol.* 97, 219–226.
- Coppo, P., Wolf, M., Veyradier, A., Bussel, A., Malot, S., Millot, G.A., et al., 2006. Prognostic value of inhibitory anti-ADAMTS13 antibodies in adult-acquired thrombotic thrombocytopenic purpura. *Br. J. Haematol.* 132, 66–74.
- Coppo, P., Busson, M., Veyradier, A., Wynckel, A., Poullin, P., Azoulay, E., et al., 2010. HLA-DRB1\*11: a strong risk factor for acquired severe ADAMTS13 deficiency-related idiopathic thrombotic thrombocytopenic purpura in Caucasians. *J. Thromb. Haemost.* 8, 856–859.
- Cugno, M., Gualtierotti, R., Tedeschi, A., Meroni, P.L., 2014. Autoantibodies to coagulation factors: from pathophysiology to diagnosis and therapy. *Autoimmun. Rev.* 13, 40–48.
- Currie, M.S., Stein, A.M., Rustagi, P.K., Behrens, A.N., Logue, G.L., 1984. Transient acquired factor X deficiency associated with pneumonia. *N.Y. State J. Med.* 84, 572–573.

- Daly, H.M., Carson, P.J., Smith, J.K., 1991. Intracerebral haemorrhage due to acquired factor XIII inhibitor—successful response to factor XIII concentrate. *Blood Coagul. Fibrinolysis* 2, 507–514.
- Davidson, S.J., Burman, J.F., Nicholson, A.G., Jones, D.W., Dusmet, M.E., 2005. Factor XII auto-antibodies present in a patient with a B-cell lymphoma. *Blood Coagul. Fibrinolysis* 16, 365–367.
- De La Cadena, R.A., Baglia, F.A., Johnson, C.A., Wenk, R.E., Amernick, R., Walsh, P.N., et al., 1988. Naturally occurring human antibodies against two distinct functional domains in the heavy chain of FXI/FXIIa. *Blood* 72, 1748–1754.
- Delgado, J., Jimenez-Yuste, V., Hernandez-Navarro, F., Villar, A., 2003. Acquired haemophilia: review and meta-analysis focused on therapy and prognostic factors. *Br. J. Haematol.* 121, 21–35.
- Delmer, A., Horellou, M.H., Andreu, G., Lecompte, T., Rossi, F., Kazatchkine, M.D., et al., 1989. Life-threatening intracranial bleeding associated with the presence of an antifactor VII autoantibody. *Blood* 74, 229–232.
- de Raucourt, E., Barbier, C., Sinda, P., Dib, M., Peltier, J.Y., Ternisien, C., 2003. High-dose intravenous immunoglobulin treatment in two patients with acquired factor V inhibitors. *Am. J. Hematol.* 74, 187–190.
- De Raucourt, E., Dumont, M.D., Tourani, J.M., Hubesch, J.P., Riquet, M., Fischer, A.M., 1994. Acquired factor VII deficiency associated with pleural liposarcoma. *Blood Coagul. Fibrinolysis* 5, 833–836.
- Dong, J.F., Moake, J.L., Nolasco, L., Bernardo, A., Arceneaux, W., Shrimpton, C.N., et al., 2002. ADAMTS-13 rapidly cleaves newly secreted ultralarge von Willebrand factor multimers on the endothelial surface under flowing conditions. *Blood* 100, 4033–4039.
- D'Uva, M., Strina, I., Mollo, A., Ranieri, A., De Placido, G., Di Micco, P., 2005. Acquired factor XII deficiency in a woman with recurrent pregnancy loss: working on a differential diagnosis in a single case. *J. Transl. Med.* 3, 43.
- Edgin, R.A., Metz, E.N., Fromkes, J.J., Beman, F.M., 1980. Acquired factor X deficiency with associated defects in platelet aggregation. A response to corticosteroid therapy. *Am. J. Med.* 69, 137–139.
- Erez, O., Romero, R., Vaisbuch, E., Mazaki-Tovi, S., Kusanovic, J.P., Chaiworapongsa, T., et al., 2009. Maternal anti-protein Z antibodies in pregnancies complicated by pre-eclampsia, SGA and fetal death. *J. Matern. Fetal Neonatal Med.* 22, 662–671.
- Fastenau, D.R., McIntyre, J.A., 2000. Immunochemical analysis of polyspecific antibodies in patients exposed to bovine fibrin sealant. *Ann. Thorac. Surg.* 69, 1867–1872.
- Favaloro, E.J., Posen, J., Ramakrishna, R., Soltani, S., Mcrae, S., Just, S., et al., 2004. Factor V inhibitors: rare or not so uncommon? A multi-laboratory investigation. *Blood Coagul. Fibrinolysis* 15, 637–647.
- Federici, A.B., Rand, J.H., Buccarelli, P., Budde, U., Van Genderen, P.J., Mohri, H., et al., 2000. Acquired von Willebrand syndrome: data from an international registry. *Thromb. Haemost.* 84, 345–349.
- Federici, A.B., Budde, U., Castaman, G., Rand, J.H., Tiede, A., 2013. Current diagnostic and therapeutic approaches to patients with acquired von Willebrand syndrome: a 2013 update. *Semin. Thromb. Hemost.* 39, 191–201.
- Ferrari, S., Scheiflinger, F., Rieger, M., Mudde, G., Wolf, M., Coppo, P., et al., 2007. Prognostic value of anti-ADAMTS 13 antibody features (Ig isotype, titer, and inhibitory effect) in a cohort of 35 adult French patients undergoing a first episode of thrombotic microangiopathy with undetectable ADAMTS 13 activity. *Blood* 109, 2815–2822.
- Ferrari, S., Mudde, G.C., Rieger, M., Veyradier, A., Kremer Hovinga, J.A., Scheiflinger, F., 2009. IgG subclass distribution of anti-ADAMTS13 antibodies in patients with acquired thrombotic thrombocytopenic purpura. *J. Thromb. Haemost.* 7, 1703–1710.
- Ferrari, S., Palavra, K., Gruber, B., Kremer Hovinga, J.A., Knobl, P., Caron, C., et al., 2014. Persistence of circulating ADAMTS13-specific immune complexes in patients with acquired thrombotic thrombocytopenic purpura. *Haematologica* 99, 779–787.
- Field, S.L., Chesterman, C.N., Dai, Y.P., Hogg, P.J., 2001. Lupus antibody bivalence is required to enhance prothrombin binding to phospholipid. *J. Immunol.* 166, 6118–6125.
- Flaherty, M.J., Henderson, R., Wener, M.H., 1989. Iatrogenic immunization with bovine thrombin: a mechanism for prolonged thrombin times after surgery. *Ann. Intern. Med.* 111, 631–634.
- Foley, S.R., Webert, K., Arnold, D.M., Rock, G.A., Clark, W.F., Barth, D., et al., 2009. A Canadian phase II study evaluating the efficacy of rituximab in the management of patients with relapsed/refractory thrombotic thrombocytopenic purpura. *Kidney Int. Suppl.* S55–S58. Available from: <https://doi.org/10.1038/ki.2008.629>.
- Forastiero, R.R., Martinuzzo, M.E., Lu, L., Broze, G.J., 2003. Autoimmune antiphospholipid antibodies impair the inhibition of activated factor X by protein Z/protein Z-dependent protease inhibitor. *J. Thromb. Haemost.* 1, 1764–1770.
- Franchini, M., Lippi, G., 2011. Acquired factor V inhibitors: a systematic review. *J. Thromb. Thrombolysis* 31, 449–457.
- Franchini, M., Lippi, G., Favaloro, E.J., 2012. Acquired inhibitors of coagulation factors: part II. *Semin. Thromb. Hemost.* 38, 447–453.
- Franchini, M., Frattini, F., Crestani, S., Bonfanti, C., 2013. Acquired FXIII inhibitors: a systematic review. *J. Thromb. Thrombolysis* 36, 109–114.
- Freedman, J., Rand, M.L., Russell, O., Davis, C., Cheatley, P.L., Blanchette, V., et al., 2003. Immunoabsorption may provide a cost-effective approach to management of patients with inhibitors to FVIII. *Transfusion* 43, 1508–1513.
- Fujikawa, K., Suzuki, H., McMullen, B., Chung, D., 2001. Purification of human von Willebrand factor-cleaving protease and its identification as a new member of the metalloproteinase family. *Blood* 98, 1662–1666.
- Fukue, H., Anderson, K., McPhedran, P., Clyne, L., McDonagh, J., 1992. A unique factor XIII inhibitor to a fibrin-binding site on factor XIIIa. *Blood* 79, 65–74.
- Fukushima, T., Mikane, T., Ono, D., Oku, S., Kobayashi, H., Watanabe, Y., et al., 2012. A case of acquired hemophilia A with massive hemothorax. *J. Anesth.* 26, 262–264.
- Furlan, M., Robles, R., Solenthaler, M., Wassmer, M., Sandoz, P., Lammle, B., 1997. Deficient activity of von Willebrand factor-cleaving protease in chronic relapsing thrombotic thrombocytopenic purpura. *Blood* 89, 3097–3103.
- Gabriel, D.A., Carr, M.E., Cook, L., Roberts, H.R., 1987. Spontaneous antithrombin in a patient with benign paraprotein. *Am. J. Hematol.* 25, 85–93.
- Gailani, D., Smith, S.B., 2009. Structural and functional features of factor XI. *J. Thromb. Haemost.* 7 (Suppl 1), 75–78.
- Galankis, D.K., Ginzler, E.M., Fikrig, S.M., 1978. Monoclonal IgG anticoagulants delaying fibrin aggregation in two patients with systemic lupus erythematosus (SLE). *Blood* 52, 1037–1046.
- Gavva, C., Patel, P., Shen, Y.M., Frenkel, E., Sarode, R., 2017. A case of autoimmune severe acquired von Willebrand syndrome (type 3-like). *Transfus. Apher. Sci.* 56, 431–433.

- Ghosh, S., McEvoy, P., McVerry, B.A., 1983. Idiopathic autoantibody that inhibits fibrin monomer polymerization. *Br. J. Haematol.* 53, 65–72.
- Giovannini, L., Appert, A., Monpoux, F., Fischer, F., Boutte, P., Sirvent, N., 2004. Successful use of recombinant factor VIIa for management of severe menorrhagia in an adolescent with an acquired inhibitor of human thrombin. *Acta Paediatr.* 93, 841–843.
- Gollard, R., Rahman, S., Ratnasabapathy, R., 2013. Factor X inhibitor: a fulminant presentation and fatal course of a rare syndrome in a 59-year-old male. *Acta Haematol.* 129, 40–44.
- Goodrick, M.J., Prentice, A.G., Copplestone, J.A., Pamphilon, D.H., Boon, R.J., 1992. Acquired factor XI inhibitor in chronic lymphocytic leukaemia. *J. Clin. Pathol.* 45, 352–353.
- Green, D., Lechner, K., 1981. A survey of 215 non-hemophilic patients with inhibitors to factor VIII. *Thromb. Haemost.* 45, 200–203.
- Gregory, T.F., Cooper, B., 2006. Case report of an acquired factor XIII inhibitor: diagnosis and management. *Proc. (Baylor Univ. Med. Cent.)* 19, 221–223.
- Gris, J.C., Schved, J.F., Branger, B., Aguilar-Martinez, P., Vecina, F., Oules, R., et al., 1992. Autoantibody to plasma fibrinopeptide A in a patient with a severe acquired haemorrhagic syndrome. *Blood Coagul. Fibrinolysis* 3, 519–529.
- Gris, J.C., Amadio, C., Mercier, E., Lavigne-Lissalde, G., Dechaud, H., Hoffet, M., et al., 2003. Anti-protein Z antibodies in women with pathologic pregnancies. *Blood* 101, 4850–4852.
- Guermazi, S., Hamza, M., Dellagi, K., 1997. Protein S deficiency and antibodies to protein S in patients with Behcet's disease. *Thromb. Res.* 86, 197–204.
- Harris, S.L., Jones, D.W., Gallimore, M.J., Nicholls, P.J., Winter, M., 2005. The antigenic binding site(s) of antibodies to factor XII associated with the antiphospholipid syndrome. *J. Thromb. Haemost.* 3, 969–975.
- Hay, C.R., Negrier, C., Ludlam, C.A., 1997. The treatment of bleeding in acquired haemophilia with recombinant factor VIIa: a multicentre study. *Thromb. Haemost.* 78, 1463–1467.
- Hayashi, T., Kadohira, Y., Morishita, E., Asakura, H., Souris, M., Ichinose, A., 2012. A case of acquired FXIII deficiency with severe bleeding symptoms. *Haemophilia* 18, 618–620.
- Henson, K., Files, J.C., Morrison, F.S., 1989. Transient acquired factor X deficiency: report of the use of activated clotting concentrate to control a life-threatening hemorrhage. *Am. J. Med.* 87, 583–585.
- Hosker, J.P., Jewell, D.P., 1983. Transient, selective factor X deficiency and acute liver failure following chest infection treated with erythromycin BP. *Postgrad. Med. J.* 59, 514–515.
- Ichinose, A., Japanese Collaborative Research Group on AH13, 2017. Autoimmune acquired factor XIII deficiency due to anti-factor XIII/13 antibodies: a summary of 93 patients. *Blood Rev.* 31, 37–45.
- Ichinose, A., Kohler, H.P., Philippou, H., Factor XIII and Fibrinogen SSC Subcommittee of the ISTH, 2016. Recommendation for ISTH/SSC Criterion 2015 for autoimmune acquired factor XIII/13 deficiency. *Thromb. Haemost.* 116, 772–774.
- Inomo, A., Sugi, T., Fujita, Y., Matsubayashi, H., Izumi, S., Mikami, M., 2008. The antigenic binding sites of autoantibodies to factor XII in patients with recurrent pregnancy losses. *Thromb. Haemost.* 99, 316–323.
- Ishida, F., Okubo, K., Ito, T., Okumura, N., Souris, M., Ichinose, A., 2010. Spontaneous regression of the inhibitor against the coagulation factor XIII A subunit in acquired factor XIII deficiency. *Thromb. Haemost.* 104, 1284–1285.
- Izumi, T., Kim, S.W., Greist, A., Macedo-Ribeiro, S., Fuentes-Prior, P., Bode, W., et al., 2001. Fine mapping of inhibitory anti-factor V antibodies using factor V C2 domain mutants. Identification of two antigenic epitopes involved in phospholipid binding. *Thromb. Haemost.* 85, 1048–1054.
- Jansen, M., Schmaldienst, S., Banyai, S., Quehenberger, P., Pabinger, I., Derfler, K., et al., 2001. Treatment of coagulation inhibitors with extracorporeal immunoabsorption (Ig-Therasorb). *Br. J. Haematol.* 112, 91–97.
- Jedidi, I., Hdjii, S., Ajmi, N., Makni, F., Masmoudi, S., Elloumi, M., et al., 2011. [Acquired haemophilia B: a case report and literature review]. *Ann. Biol. Clin. (Paris)* 69, 685–688.
- Jian, C., Xiao, J., Gong, L., Skipwith, C.G., Jin, S.Y., Kwaan, H.C., et al., 2012. Gain-of-function ADAMTS13 variants that are resistant to autoantibodies against ADAMTS13 in patients with acquired thrombotic thrombocytopenic purpura. *Blood* 119, 3836–3843.
- John, M.L., Hitzler, W., Scharrer, I., 2012. The role of human leukocyte antigens as predisposing and/or protective factors in patients with idiopathic thrombotic thrombocytopenic purpura. *Ann. Hematol.* 91, 507–510.
- Jones, D.W., Gallimore, M.J., Mackie, I.J., Harris, S.L., Winter, M., 2000. Reduced factor XII levels in patients with the antiphospholipid syndrome are associated with antibodies to factor XII. *Br. J. Haematol.* 110, 721–726.
- Jones, D.W., Mackie, I.J., Gallimore, M.J., Winter, M., 2001. Antibodies to factor XII and recurrent fetal loss in patients with the anti-phospholipid syndrome. *Br. J. Haematol.* 113, 550–552.
- Kahle, J., Orlowski, A., Stichel, D., Healey, J.F., Parker, E.T., Jacquemin, M., et al., 2017. Frequency and epitope specificity of anti-factor VIII C1 domain antibodies in acquired and congenital hemophilia A. *Blood* 130, 808–816.
- Kalafatis, M., Simioni, P., Tormene, D., Beck, D.O., Luni, S., Girolami, A., 2002. Isolation and characterization of an antifactor V antibody causing activated protein C resistance from a patient with severe thrombotic manifestations. *Blood* 99, 3985–3992.
- Kamikubo, Y., Miyamoto, S., Iwasa, A., Ishii, M., Okajima, K., 2000. Purification and characterization of factor VII inhibitor found in a patient with life threatening bleeding. *Thromb. Haemost.* 83, 60–64.
- Kawasaki, Y., Toyoda, H., Otsuki, S., Iwasa, T., Iwamoto, S., Azuma, E., et al., 2013. A novel Wiskott-Aldrich syndrome protein mutation in an infant with thrombotic thrombocytopenic purpura. *Eur. J. Haematol.* 90, 164–168.
- Kershaw, G., Favalaro, E.J., 2012. Laboratory identification of factor inhibitors: an update. *Pathology* 44, 293–302.
- Kessler, C.M., Ludlam, C.A., 1993. The treatment of acquired factor VIII inhibitors: worldwide experience with porcine factor VIII concentrate. International Acquired Hemophilia Study Group. *Semin. Hematol.* 30, 22–27.
- Kim, M.S., Kilgore, P.E., Kang, J.S., Kim, S.Y., Lee, D.Y., Kim, J.S., et al., 2008. Transient acquired hemophilia associated with *Mycoplasma pneumoniae* pneumonia. *J. Korean Med. Sci.* 23, 138–141.
- Klaus, C., Plaimauer, B., Studt, J.D., Dorner, F., Lammle, B., Mannucci, P.M., et al., 2004. Epitope mapping of ADAMTS13 autoantibodies in acquired thrombotic thrombocytopenic purpura. *Blood* 103, 4514–4519.

- Knobe, K., Tedgard, U., Ek, T., Sandstrom, P.E., Hillarp, A., 2012. Lupus anticoagulants in two children—bleeding due to nonphospholipid-dependent antiprothrombin antibodies. *Eur. J. Pediatr.* 171, 1383–1387.
- Knobl, P., Lechner, K., 1998. Acquired factor V inhibitors. *Baillieres Clin. Haematol.* 11, 305–318.
- Knoebl, P., Marco, P., Baudo, F., Collins, P., Huth-Kuhne, A., Nemes, L., et al., 2012. Demographic and clinical data in acquired hemophilia A: results from the European Acquired Haemophilia Registry (EACH2). *J. Thromb. Haemost.* 10, 622–631.
- Kondera-Anasz, Z., 1998. Antibodies against fibrinogen in pregnant women, in post delivery women and in the newborns. *Thromb. Haemost.* 79, 963–968.
- Kremer Hovinga, J.A., Studt, J.D., Demarmels Biasiutti, F., Solenthaler, M., Alberio, L., Zwicky, C., et al., 2004. Splenectomy in relapsing and plasma-refractory acquired thrombotic thrombocytopenic purpura. *Haematologica* 89, 320–324.
- Kremer Hovinga, J.A., Vesely, S.K., Terrell, D.R., Lammle, B., George, J.N., 2010. Survival and relapse in patients with thrombotic thrombocytopenic purpura. *Blood* 115, 1500–1511. quiz 1662.
- Kremer Hovinga, J.A., Coppo, P., Lammle, B., Moake, J.L., Miyata, T., Vanhoorelbeke, K., 2017. Thrombotic thrombocytopenic purpura. *Nat. Rev. Dis. Primers* 3, 17020.
- Kremer Hovinga, J.A., Heeb, S.R., Skowronksa, M., Schaller, M., 2018. Pathophysiology of thrombotic thrombocytopenic purpura and hemolytic uremic syndrome. *J. Thromb. Haemost.* 16, 618–629.
- Krieger, H., Leddy, J.P., Breckenridge, R.T., 1975. Studies on a circulating anticoagulant in systemic lupus erythematosus: evidence for inhibition of the function of activated plasma thromboplastin antecedent (factor XIa). *Blood* 46, 189–197.
- Krishnamurthy, P., Hawche, C., Evans, G., Winter, M., 2011. A rare case of an acquired inhibitor to factor IX. *Haemophilia* 17, 712–713.
- Krumdieck, R., Shaw, D.R., Huang, S.T., Poon, M.C., Rustagi, P.K., 1991. Hemorrhagic disorder due to an isoniazid-associated acquired factor XIII inhibitor in a patient with Waldenstrom's macroglobulinemia. *Am. J. Med.* 90, 639–645.
- Kruse-Jarres, R., St-Louis, J., Greist, A., Shapiro, A., Smith, H., Chowdary, P., et al., 2015. Efficacy and safety of OBI-1, an antihaemophilic factor VIII (recombinant), porcine sequence, in subjects with acquired haemophilia A. *Haemophilia* 21, 162–170.
- Kruse-Jarres, R., Kempton, C.L., Baudo, F., Collins, P.W., Knoebl, P., Leissinger, C.A., et al., 2017. Acquired hemophilia A: updated review of evidence and treatment guidance. *Am. J. Hematol.* 92, 695–705.
- Kumar, S., Pruthi, R.K., Nichols, W.L., 2003. Acquired von Willebrand's syndrome: a single institution experience. *Am. J. Hematol.* 72, 243–247.
- Kyriakou, D.S., Alexandrakis, M.G., Passam, F.H., Foundouli, K., Matalliotakis, E., Koutroubakis, I.E., et al., 2002. Acquired inhibitors to coagulation factors in patients with gastrointestinal diseases. *Eur. J. Gastroenterol. Hepatol.* 14, 1383–1387.
- Lane, T.A., Shapiro, S.S., Burka, E.R., 1978. Factor V antibody and disseminated intravascular coagulation. *Ann. Intern. Med.* 89, 182–185.
- Lankiewicz, M.W., Bell, W.R., 1992. A unique circulating inhibitor with specificity for coagulation factor X. *Am. J. Med.* 93, 343–346.
- Lapalud, P., et al., 2012. The IgG autoimmune response in postpartum acquired hemophilia A targets mainly the A1a1 domain of FVIII. *J. Thromb. Haemost.* 10, 1814–1822.
- Larakeb, A.S., Evrard, S., Louillet, F., Kwon, T., Djaffar, H., Llanas, B., et al., 2009. Acute renal cortical necrosis due to acquired antiprotein S antibodies. *Pediatr. Nephrol.* 24, 207–209.
- Largo, R., Sigg, P., Von Felten, A., Straub, P.W., 1974. Acquired factor-IX inhibitor in a nonhaemophilic patient with autoimmune disease. *Br. J. Haematol.* 26, 129–140.
- Lawson, J.H., Pennell, B.J., Olson, J.D., Mann, K.G., 1990. Isolation and characterization of an acquired antithrombin antibody. *Blood* 76, 2249–2257.
- Lechner, K., Simonitsch, I., Haselböck, J., Jager, U., Pabinger, I., 2011. Acquired immune-mediated thrombophilia in lymphoproliferative disorders. *Leuk. Lymphoma* 52, 1836–1843.
- Lee, G., Duan-Porter, W., Metjian, A.D., 2012. Acquired, non-amyloid related factor X deficiency: review of the literature. *Haemophilia* 18, 655–663.
- Lenting, P.J., Van Mourik, J.A., Mertens, K., 1998. The life cycle of coagulation factor VIII in view of its structure and function. *Blood* 92, 3983–3996.
- Levin, M., Eley, B.S., Louis, J., Cohen, H., Young, L., Heyderman, R.S., 1995. Postinfectious purpura fulminans caused by an autoantibody directed against protein S. *J. Pediatr.* 127, 355–363.
- Lindgren, A., Wadenvik, H., Tengborn, L., 2002. Characterization of inhibitors to FVIII with an ELISA in congenital and acquired haemophilia A. *Haemophilia* 8, 644–648.
- Ling, H.T., Field, J.J., Blinder, M.A., 2009. Sustained response with rituximab in patients with thrombotic thrombocytopenic purpura: a report of 13 cases and review of the literature. *Am. J. Hematol.* 84, 418–421.
- Llobet, D., Borrell, M., Vila, L., Vallve, C., Felices, R., Fontcuberta, J., 2007. An acquired inhibitor that produced a delay of fibrinopeptide B release in an asymptomatic patient. *Haematologica* 92, e17–e19.
- Lollar, P., 2005. Pathogenic antibodies to coagulation factors. Part II. Fibrinogen, prothrombin, thrombin, factor V, factor XI, factor XII, factor XIII, the protein C system and von Willebrand factor. *J. Thromb. Haemost.* 3, 1385–1391.
- Lorand, L., Velasco, P.T., Hill, J.M., Hoffmeister, K.J., Kaye, F.J., 2002. Intracranial hemorrhage in systemic lupus erythematosus associated with an autoantibody against factor XIII. *Thromb. Haemost.* 88, 919–923.
- Lotta, L.A., Lombardi, R., Mariani, M., Lancillotti, S., De Cristofaro, R., Hollestelle, M.J., et al., 2011. Platelet reactive conformation and multimeric pattern of von Willebrand factor in acquired thrombotic thrombocytopenic purpura during acute disease and remission. *J. Thromb. Haemost.* 9, 1744–1751.
- Lottenberg, R., Kentro, T.B., Kitchens, C.S., 1987. Acquired hemophilia. A natural history study of 16 patients with factor VIII inhibitors receiving little or no therapy. *Arch. Intern. Med.* 147, 1077–1081.
- Luboshitz, J., Lubetsky, A., Schliamser, L., Kotler, A., Tamarin, I., Inbal, A., 2001. Pharmacokinetic studies with FVIII/von Willebrand factor concentrate can be a diagnostic tool to distinguish between subgroups of patients with acquired von Willebrand syndrome. *Thromb. Haemost.* 85, 806–809.
- Luken, B.M., Turenhout, E.A., Hulstein, J.J., Van Mourik, J.A., Fijnheer, R., Voorberg, J., 2005. The spacer domain of ADAMTS13 contains a major binding site for antibodies in patients with thrombotic thrombocytopenic purpura. *Thromb. Haemost.* 93, 267–274.

- Luo, Y.Y., Zhang, G.S., 2011. Acquired factor XIII inhibitor: clinical features, treatment, fibrin structure and epitope determination. *Haemophilia* 17, 393–398.
- Luo, Y., Zhang, G., Zuo, W., Zheng, W., Dai, C., 2010. Acquired factor XIII inhibitor in monoclonal gammopathy of undetermined significance: characterization and cross-linked fibrin ultrastructure. *Ann. Hematol.* 89, 833–834.
- Madoiwa, S., Nakamura, Y., Mimuro, J., Furusawa, S., Koyama, T., Sugo, T., et al., 2001. Autoantibody against prothrombin aberrantly alters the proenzyme to facilitate formation of a complex with its physiological inhibitor antithrombin III without thrombin conversion. *Blood* 97, 3783–3789.
- Mahendra, A., Padiolleau-Lefevre, S., Kaveri, S.V., Lacroix-Desmazes, S., 2012. Do proteolytic antibodies complete the panoply of the autoimmune response in acquired haemophilia A? *Br. J. Haematol.* 156, 3–12.
- Marciniak, E., Greenwood, M.F., 1979. Acquired coagulation inhibitor delaying fibrinopeptide release. *Blood* 53, 81–92.
- Matsumoto, T., Nogami, K., Ogiwara, K., Shima, M., 2012. A putative inhibitory mechanism in the tenase complex responsible for loss of coagulation function in acquired haemophilia A patients with anti-C2 autoantibodies. *Thromb. Haemost.* 107, 288–301.
- Matsunaga, A.T., Shafer, F.E., 1996. An acquired inhibitor to factor X in a pediatric patient with extensive burns. *J. Pediatr. Hematol. Oncol.* 18, 223–226.
- Mazzucconi, M.G., Peraino, M., Bizzoni, L., Bernasconi, S., Luciani, M., Rossi, G.D., 1999. Acquired inhibitor against factor IX in a child: successful treatment with high-dose immunoglobulin and dexamethasone. *Haemophilia* 5, 132–134.
- McDonald, V., Liesner, R., Grainger, J., Gattens, M., Machin, S.J., Scully, M., 2010. Acquired, noncongenital thrombotic thrombocytopenic purpura in children and adolescents: clinical management and the use of ADAMTS 13 assays. *Blood Coagul. Fibrinolysis* 21, 245–250.
- McManus, M.P., Frantz, C., Gailani, D., 2012. Acquired factor XI deficiency in a child with membranoproliferative glomerulonephritis. *Pediatr. Blood Cancer* 59, 173–175.
- Mehta, J., Singhal, S., Mehta, B.C., 1992. Factor VII inhibitor. *J. Assoc. Phys. India* 40, 44.
- Michiels, J.J., Budde, U., Van Der Planken, M., Van Vliet, H.H., Schroyens, W., Berneman, Z., 2001. Acquired von Willebrand syndromes: clinical features, aetiology, pathophysiology, classification and management. *Best Pract. Res. Clin. Haematol.* 14, 401–436.
- Micic, D., Williams, E.C., Medow, J.E., 2011. Cerebellar hemorrhage as a first presentation of acquired hemophilia A. *Neurocrit. Care* 15, 170–174.
- Miller, K., Neely, J.E., Kravit, W., Edson, J.R., 1978. Spontaneously acquired factor IX inhibitor in a nonhemophiliac child. *J. Pediatr.* 93, 232–234.
- Miller, D.P., Kaye, J.A., Shea, K., Ziyadeh, N., Cali, C., Black, C., et al., 2004. Incidence of thrombotic thrombocytopenic purpura/hemolytic uremic syndrome. *Epidemiology* 15, 208–215.
- Moake, J.L., Rudy, C.K., Troll, J.H., Weinstein, M.J., Colannino, N.M., Azocar, J., et al., 1982. Unusually large plasma factor VIII: von Willebrand factor multimers in chronic relapsing thrombotic thrombocytopenic purpura. *N. Engl. J. Med.* 307, 1432–1435.
- Mohri, H., Motomura, S., Kanamori, H., Matsuzaki, M., Watanabe, S., Maruta, A., et al., 1998. Clinical significance of inhibitors in acquired von Willebrand syndrome. *Blood* 91, 3623–3629.
- Mollica, L., Preston, R.J., Chion, A.C., Lees, S.J., Collins, P., Lewis, S., et al., 2006. Autoantibodies to thrombin directed against both of its cryptic exosites. *Br. J. Haematol.* 132, 487–493.
- Moraca, R.J., Ragni, M.V., 2002. Acquired anti-FVIII inhibitors in children. *Haemophilia* 8, 28–32.
- Morrison, A.E., Ludlam, C.A., Kessler, C., 1993. Use of porcine factor VIII in the treatment of patients with acquired hemophilia. *Blood* 81, 1513–1520.
- Mulhare, P.E., Tracy, P.B., Golden, E.A., Branda, R.F., Bovill, E.G., 1991. A case of acquired factor X deficiency with in vivo and in vitro evidence of inhibitor activity directed against factor X. *Am. J. Clin. Pathol.* 96, 196–200.
- Mullighan, C.G., Rischbieth, A., Duncan, E.M., Lloyd, J.V., 2004. Acquired isolated factor VII deficiency associated with severe bleeding and successful treatment with recombinant FVIIa (NovoSeven). *Blood Coagul. Fibrinolysis* 15, 347–351.
- Mumford, A.D., O'donnell, J., Gillmore, J.D., Manning, R.A., Hawkins, P.N., Laffan, M., 2000. Bleeding symptoms and coagulation abnormalities in 337 patients with AL-amyloidosis. *Br. J. Haematol.* 110, 454–460.
- Muntean, W., Zenz, W., Finding, K., Zobel, G., Beitzke, A., 1994. Inhibitor to factor V after exposure to fibrin sealant during cardiac surgery in a two-year-old child. *Acta Paediatr.* 83, 84–87.
- Murkin, R.J., Murray, J.A., 2006. Thrombotic thrombocytopenic purpura: aetiology, pathophysiology and treatment. *Blood Rev.* 20, 51–60.
- Muzaffar, J., Katragadda, L., Haider, S., Javed, A., Anaissie, E., Usmani, S., 2012. Rituximab and intravenous immunoglobulin (IVIG) for the management of acquired factor VIII inhibitor in multiple myeloma: case report and review of literature. *Int. J. Hematol.* 95, 102–106.
- Nakamura, S., Kato, A., Sakata, Y., Aoki, N., 1988. Bleeding tendency caused by IgG inhibitor to factor XIII, treated successfully by cyclophosphamide. *Br. J. Haematol.* 68, 313–319.
- Nawarawong, W., Wyshock, E., Meloni, F.J., Weitz, J., Schmaier, A.H., 1991. The rate of fibrinopeptide B release modulates the rate of clot formation: a study with an acquired inhibitor to fibrinopeptide B release. *Br. J. Haematol.* 79, 296–301.
- Ndimbie, O.K., Raman, B.K., Saeed, S.M., 1989. Lupus anticoagulant associated with specific inhibition of factor VII in a patient with AIDS. *Am. J. Clin. Pathol.* 91, 491–493.
- Nemes, L., Pitlik, E., 2000. New protocol for immune tolerance induction in acquired hemophilia. *Haematologica* 85, 64–68.
- Nesheim, M.E., Nichols, W.L., Cole, T.L., Houston, J.G., Schenk, R.B., Mann, K.G., et al., 1986. Isolation and study of an acquired inhibitor of human coagulation factor V. *J. Clin. Invest.* 77, 405–415.
- Nijenhuis, A.V., Van Bergeijk, L., Huijgens, P.C., Zweegman, S., 2004. Acquired factor XIII deficiency due to an inhibitor: a case report and review of the literature. *Haematologica* 89, ECR14.
- Nogami, K., Shima, M., Giddings, J.C., Hosokawa, K., Nagata, M., Kamisue, S., et al., 2001. Circulating factor VIII immune complexes in patients with type 2 acquired hemophilia A and protection from activated protein C-mediated proteolysis. *Blood* 97, 669–677.
- Nojima, J., Kuratsune, H., Suehisa, E., Kawasaki, T., Machii, T., Kitani, T., et al., 2002a. Acquired activated protein C resistance associated with anti-protein S antibody as a strong risk factor for DVT in non-SLE patients. *Thromb. Haemost.* 88, 716–722.

- Nojima, J., Kuratsune, H., Suehisa, E., Kawasaki, T., Machii, T., Kitani, T., et al., 2002b. Acquired activated protein C resistance is associated with the co-existence of anti-prothrombin antibodies and lupus anticoagulant activity in patients with systemic lupus erythematosus. *Br. J. Haematol.* 118, 577–583.
- Nojima, J., Iwatani, Y., Ichihara, K., Tsuneoka, H., Ishikawa, T., Yanagihara, M., et al., 2009. Acquired activated protein C resistance is associated with IgG antibodies to protein S in patients with systemic lupus erythematosus. *Thromb. Res.* 124, 127–131.
- Ojeda-Uribe, M., Federici, L., Wolf, M., Coppo, P., Veyradier, A., 2010. Successful long-term rituximab maintenance for a relapsing patient with idiopathic thrombotic thrombocytopenic purpura. *Transfusion* 50, 733–735.
- Okajima, K., Ishii, M., 1999. Life-threatening bleeding in a case of autoantibody-induced factor VII deficiency. *Int. J. Hematol.* 69, 129–132.
- Olson, N.J., Robert, D., Hedayat, A.A., Liu, X., Ornstein, D.L., 2017. Fatal hemorrhage due to a spontaneous factor V inhibitor with lupus anti-coagulant properties. *Blood Coagul. Fibrinolysis* 28, 407–410.
- Ortel, T.L., Quinn-Allen, M.A., Charles, L.A., Devore-Carter, D., Kane, W.H., 1992. Characterization of an acquired inhibitor to coagulation factor V. Antibody binding to the second C-type domain of factor V inhibits the binding of factor V to phosphatidylserine and neutralizes procoagulant activity. *J. Clin. Invest.* 90, 2340–2347.
- Ortel, T.L., Moore, K.D., Quinn-Allen, M.A., Okamura, T., Sinclair, A.J., Lazarchick, J., et al., 1998. Inhibitory anti-factor V antibodies bind to the factor V C2 domain and are associated with hemorrhagic manifestations. *Blood* 91, 4188–4196.
- Ortel, T.L., Mercer, M.C., Thames, E.H., Moore, K.D., Lawson, J.H., 2001. Immunologic impact and clinical outcomes after surgical exposure to bovine thrombin. *Ann. Surg.* 233, 88–96.
- Otis, P.T., Feinstein, D.I., Rapaport, S.I., Patch, M.J., 1974. An acquired inhibitor of fibrin stabilization associated with isoniazid therapy: clinical and biochemical observations. *Blood* 44, 771–781.
- Ozgur, T.T., Asal, G.T., Gurgey, A., Tezcan, I., Ersoy, F., Sanal, O., 2007. Acquired factor VIII deficiency associated with a novel primary immunodeficiency suggestive of autosomal recessive hyper IgE syndrome. *J. Pediatr. Hematol. Oncol.* 29, 327–329.
- Ozsoylu, S., Ozer, F.L., 1973. Acquired factor IX deficiency. A report of two cases. *Acta Haematol.* 50, 305–314.
- Page, J.D., Dela Cadena, R.A., Humphries, J.E., Colman, R.W., 1994. An autoantibody to human plasma prekallikrein blocks activation of the contact system. *Br. J. Haematol.* 87, 81–86.
- Panzer, S., Thaler, E., 1993. An acquired cryoglobulinemia which inhibits fibrin polymerization in a patient with IgG kappa myeloma. *Haemostasis* 23, 69–76.
- Pardos-Gea, J., Ordi-Ros, J., Serrano, S., Balada, E., Nicolau, I., Vilardell, M., 2008. Protein Z levels and anti-protein Z antibodies in patients with arterial and venous thrombosis. *Thromb. Res.* 121, 727–734.
- Pavlova, A., Diaz-Lacava, A., Zeitler, H., Satoguina, J., Niemann, B., Krause, M., et al., 2008. Increased frequency of the CTLA-4 49 A/G polymorphism in patients with acquired haemophilia A compared to healthy controls. *Haemophilia* 14, 355–360.
- Pavlova, A., Zeitler, H., Scharrer, I., Brackmann, H.H., Oldenburg, J., 2010. HLA genotype in patients with acquired haemophilia A. *Haemophilia* 16, 107–112.
- Pechik, I., Yakovlev, S., Mosesson, M.W., Gilliland, G.L., Medved, L., 2006. Structural basis for sequential cleavage of fibrinopeptides upon fibrin assembly. *Biochemistry* 45, 3588–3597.
- Peyvandi, F., Palla, R., Lotta, L.A., Mackie, I., Scully, M.A., Machin, S.J., 2010. ADAMTS-13 assays in thrombotic thrombocytopenic purpura. *J. Thromb. Haemost.* 8, 631–640.
- Peyvandi, F., Scully, M., Kremer Hovinga, J.A., Cataland, S., Knobl, P., Wu, H., et al., 2016. Caplacizumab for acquired thrombotic thrombocytopenic purpura. *N. Engl. J. Med.* 374, 511–522.
- Plaimauer, B., Kremer Hovinga, J.A., Juno, C., Wolfsegger, M.J., Skalicky, S., Schmidt, M., et al., 2011. Recombinant ADAMTS13 normalizes von Willebrand factor-cleaving activity in plasma of acquired TTP patients by overriding inhibitory antibodies. *J. Thromb. Haemost.* 9, 936–944.
- Pogliani, E.M., Perseghin, P., Parma, M., Pioltelli, P., Corneo, G., 2000. Defibrotide in recurrent thrombotic thrombocytopenic purpura. *Clin. Appl. Thromb. Hemost.* 6, 69–70.
- Poon, M.C., Saito, H., Koopman, W.J., 1984. A unique precipitating autoantibody against plasma thromboplastin antecedent associated with multiple apparent plasma clotting factor deficiencies in a patient with systemic lupus erythematosus. *Blood* 63, 1309–1317.
- Pos, W., Luken, B.M., Sorvillo, N., Kremer Hovinga, J.A., Voorberg, J., 2011. Humoral immune response to ADAMTS13 in acquired thrombotic thrombocytopenic purpura. *J. Thromb. Haemost.* 9, 1285–1291.
- Rao, L.V., Zivelin, A., Iturbe, I., Rapaport, S.I., 1994. Antibody-induced acute factor X deficiency: clinical manifestations and properties of the antibody. *Thromb. Haemost.* 72, 363–371.
- Reding, M.T., Okita, D.K., Diethylm-Okita, B.M., Anderson, T.A., Conti-Fine, B.M., 2003. Human CD4+ T-cell epitope repertoire on the C2 domain of coagulation factor VIII. *J. Thromb. Haemost.* 1, 1777–1784.
- Reding, M.T., Okita, D.K., Diethylm-Okita, B.M., Anderson, T.A., Conti-Fine, B.M., 2004. Epitope repertoire of human CD4(+) T cells on the A3 domain of coagulation factor VIII. *J. Thromb. Haemost.* 2, 1385–1394.
- Reece, E.A., Clyne, L.P., Romero, R., Hobbins, J.C., 1984. Spontaneous factor XI inhibitors. Seven additional cases and a review of the literature. *Arch. Intern. Med.* 144, 525–529.
- Reisner, H.M., Roberts, H.R., Krumholz, S., Yount, W.J., 1977. Immunochemical characterization of a polyclonal human antibody to factor IX. *Blood* 50, 11–19.
- Reitter, S., Knoebl, P., Pabinger, I., Lechner, K., 2011. Postoperative paraneoplastic factor VIII auto-antibodies in patients with solid tumours. *Haemophilia* 17, e889–e894.
- Rezaieyazdi, Z., Sharifi-Doloui, D., Hashemzadeh, K., Shirdel, A., Mansouritorghabeh, H., 2012. Acquired haemophilia A in a woman with autoimmune hepatitis and systemic lupus erythematosus; review of literature. *Blood Coagul. Fibrinolysis* 23, 71–74.
- Roberts, H.R., 1970. Acquired inhibitors to factor IX. *N. Engl. J. Med.* 283, 543–544.
- Rochanda, L., Del Zoppo, G.J., Feinstein, D.I., Liebman, H.A., 2012. Approach to the treatment, characterization and diagnosis of an acquired auto-antibody directed against factors prothrombin, factor X and factor IX: a case report and review of the literature. *Haemophilia* 18, 102–107.

- Rock, G.A., Shumak, K.H., Buskard, N.A., Blanchette, V.S., Kelton, J.G., Nair, R.C., et al., 1991. Comparison of plasma exchange with plasma infusion in the treatment of thrombotic thrombocytopenic purpura. Canadian Apheresis Study Group. *N. Engl. J. Med.* 325, 393–397.
- Rossetto, V., Spiezia, L., Franz, F., Salmaso, L., Pozza, L.V., Gavasso, S., et al., 2009. The role of antiphospholipid antibodies toward the protein C/protein S system in venous thromboembolic disease. *Am. J. Hematol.* 84, 594–596.
- Roubey, R.A., 1998. Mechanisms of autoantibody-mediated thrombosis. *Lupus* 7 (Suppl 2), S114–S119.
- Ruiz-Arguelles, A., 1988. Spontaneous reversal of acquired autoimmune dysfibrinogenemia probably due to an antiidiotypic antibody directed to an interspecies cross-reactive idiotype expressed on antifibrinogen antibodies. *J. Clin. Invest.* 82, 958–963.
- Rustgi, R.N., Laduca, F.M., Tourbaf, K.D., 1982. Circulating anticoagulant against factor XI in psoriasis. *J. Med.* 13, 289–301.
- Saenko, E.L., Ananyeva, N.M., Tuddenham, E.G., Kemball-Cook, G., 2002. Factor VIII—novel insights into form and function. *Br. J. Haematol.* 119, 323–331.
- Saenz, A.J., Johnson, N.V., Van Cott, E.M., 2011. Acquired activated protein C resistance caused by lupus anticoagulants. *Am. J. Clin. Pathol.* 136, 344–349.
- Sailer, T., Vormittag, R., Koder, S., Quehenberger, P., Kaider, A., Pabinger, I., 2008. Clinical significance of anti-protein Z antibodies in patients with lupus anticoagulant. *Thromb. Res.* 122, 153–160.
- Sater, M.S., Finan, R.R., Al-Hammad, S.A., Mohammed, F.A., Issa, A.A., Almawi, W.Y., 2011. High frequency of anti-protein Z IgM and IgG autoantibodies in women with idiopathic recurrent spontaneous miscarriage. *Am. J. Reprod. Immunol.* 65, 526–531.
- Scheiflinger, F., Knobl, P., Trattner, B., Plainauer, B., Mohr, G., Dockal, M., et al., 2003. Nonneutralizing IgM and IgG antibodies to von Willebrand factor-cleaving protease (ADAMTS-13) in a patient with thrombotic thrombocytopenic purpura. *Blood* 102, 3241–3243.
- Schneppenheim, R., Budde, U., 2008. [Inborn and acquired von Willebrand disease]. *Hamostaseologie* 28, 312–319.
- Scully, M., 2012. Rituximab in the treatment of TTP. *Hematology* 17 (Suppl 1), S22–S24.
- Scully, M.F., Ellis, V., Kakkar, V.V., Savidge, G.F., Williams, Y.F., Sterndale, H., 1982. An acquired coagulation inhibitor to factor II. *Br. J. Haematol.* 50, 655–664.
- Scully, M., Cohen, H., Cavenagh, J., Benjamin, S., Starke, R., Killick, S., et al., 2007. Remission in acute refractory and relapsing thrombotic thrombocytopenic purpura following rituximab is associated with a reduction in IgG antibodies to ADAMTS-13. *Br. J. Haematol.* 136, 451–461.
- Scully, M., Yarranton, H., Liesner, R., Cavenagh, J., Hunt, B., Benjamin, S., et al., 2008. Regional UK TTP registry: correlation with laboratory ADAMTS 13 analysis and clinical features. *Br. J. Haematol.* 142, 819–826.
- Scully, M., Brown, J., Patel, R., McDonald, V., Brown, C.J., Machin, S., 2010. Human leukocyte antigen association in idiopathic thrombotic thrombocytopenic purpura: evidence for an immunogenetic link. *J. Thromb. Haemost.* 8, 257–262.
- Scully, M., Knobl, P., Kentouche, K., Rice, L., Windyga, J., Schneppenheim, R., et al., 2017. Recombinant ADAMTS-13: first-in-human pharmacokinetics and safety in congenital thrombotic thrombocytopenic purpura. *Blood* 130, 2055–2063.
- Shastri, K.A., Ho, C., Logue, G., 1999. An acquired factor V inhibitor: clinical and laboratory features. *J. Med.* 30, 357–366.
- Shelat, S.G., Smith, P., Ai, J., Zheng, X.L., 2006. Inhibitory autoantibodies against ADAMTS-13 in patients with thrombotic thrombocytopenic purpura bind ADAMTS-13 protease and may accelerate its clearance in vivo. *J. Thromb. Haemost.* 4, 1707–1717.
- Shetty, S., Bhave, M., Ghosh, K., 2011. Acquired hemophilia a: diagnosis, aetiology, clinical spectrum and treatment options. *Autoimmun. Rev.* 10, 311–316.
- Shires, L., Gomperts, E.D., Bradlow, B.A., 1979. An acquired inhibitor to factor XIII A case report. *S. Afr. Med. J.* 56, 70–72.
- Shortt, J., Oh, D.H., Opat, S.S., 2013. ADAMTS13 antibody depletion by bortezomib in thrombotic thrombocytopenic purpura. *N. Engl. J. Med.* 368, 90–92.
- Shumak, K.H., Rock, G.A., Nair, R.C., 1995. Late relapses in patients successfully treated for thrombotic thrombocytopenic purpura. Canadian Apheresis Group. *Ann. Intern. Med.* 122, 569–572.
- Siaka, C., Rugeri, L., Caron, C., Goudemand, J., 2003. A new ELISA assay for diagnosis of acquired von Willebrand syndrome. *Haemophilia* 9, 303–308.
- Sie, P., Bezeaud, A., Dupouy, D., Archipoff, G., Freyssinet, J.M., Dugoujon, J.M., et al., 1991. An acquired antithrombin autoantibody directed toward the catalytic center of the enzyme. *J. Clin. Invest.* 88, 290–296.
- Singla, N.K., Foster, K.N., Alexander, W.A., Pribble, J.P., 2012. Safety and immunogenicity of recombinant human thrombin: a pooled analysis of results from 10 clinical trials. *Pharmacotherapy* 32, 998–1005.
- Smith, S.V., Liles, D.K., White II, G.C., Brecher, M.E., 1998. Successful treatment of transient acquired factor X deficiency by plasmapheresis with concomitant intravenous immunoglobulin and steroid therapy. *Am. J. Hematol.* 57, 245–252.
- Soejima, K., Matsumoto, M., Kokame, K., Yagi, H., Ishizashi, H., Maeda, H., et al., 2003. ADAMTS-13 cysteine-rich/spacer domains are functionally essential for von Willebrand factor cleavage. *Blood* 102, 3232–3237.
- Sorice, M., Arcieri, P., Griggi, T., Circella, A., Misasi, R., Lenti, L., et al., 1996. Inhibition of protein S by autoantibodies in patients with acquired protein S deficiency. *Thromb. Haemost.* 75, 555–559.
- La Spada, A.R., Skalhegg, B.S., Henderson, R., Schmer, G., Pierce, R., Chandler, W., 1995. Brief report: fatal hemorrhage in a patient with an acquired inhibitor of human thrombin. *N. Engl. J. Med.* 333, 494–497.
- Spero, J.A., 1993. Bovine thrombin-induced inhibitor of factor V and bleeding risk in postoperative neurosurgical patients. Report of three cases. *J. Neurosurg.* 78, 817–820.
- Stasi, R., Brunetti, M., Stipa, E., Amadori, S., 2004. Selective B-cell depletion with rituximab for the treatment of patients with acquired hemophilia. *Blood* 103, 4424–4428.
- Streiff, M.B., Ness, P.M., 2002. Acquired FV inhibitors: a needless iatrogenic complication of bovine thrombin exposure. *Transfusion* 42, 18–26.
- Stricker, R.B., Lane, P.K., Leffert, J.D., Rodgers, G.M., Shuman, M.A., Corash, L., 1988. Development of antithrombin antibodies following surgery in patients with prosthetic cardiac valves. *Blood* 72, 1375–1380.
- Sucker, C., Scharf, R.E., Zottz, R.B., 2009. Use of recombinant factor VIIa in inherited and acquired von Willebrand disease. *Clin. Appl. Thromb. Hemost.* 15, 27–31.

- Suehisa, E., Toku, M., Akita, N., Fushimi, R., Takano, T., Tada, H., et al., 1995. Study on an antibody against F1F2 fragment of human factor V in a patient with Hashimoto's disease and bullous pemphigoid. *Thromb. Res.* 77, 63–68.
- Sumner, M.J., Geldziler, B.D., Pedersen, M., Seremetis, S., 2007. Treatment of acquired haemophilia with recombinant activated FVII: a critical appraisal. *Haemophilia* 13, 451–461.
- Takahashi, H., Fuse, I., Abe, T., Yoshino, N., Aizawa, Y., 2003. Acquired factor V inhibitor complicated by Hashimoto's thyroditis, primary biliary cirrhosis and membranous nephropathy. *Blood Coagul. Fibrinolysis* 14, 87–93.
- Tay, L., Duncan, E., Singhal, D., Al-Qunfoidi, R., Coghlan, D., Jaksic, W., et al., 2009. Twelve years of experience of acquired hemophilia A: trials and tribulations in South Australia. *Semin. Thromb. Hemost.* 35, 769–777.
- Tengborn, L., Baudo, F., Huth-Kuhne, A., Knoebl, P., Levesque, H., Marco, P., et al., 2012. Pregnancy-associated acquired haemophilia A: results from the European Acquired Haemophilia (EACH2) registry. *Br. J. Obstet. Gynaecol.* 119, 1529–1537.
- Terrell, D.R., Williams, L.A., Vesely, S.K., Lammle, B., Hovinga, J.A., George, J.N., 2005. The incidence of thrombotic thrombocytopenic purpura-hemolytic uremic syndrome: all patients, idiopathic patients, and patients with severe ADAMTS-13 deficiency. *J. Thromb. Haemost.* 3, 1432–1436.
- Thomas, M.R., De Groot, R., Scully, M.A., Crawley, J.T., 2015. Pathogenicity of anti-ADAMTS13 autoantibodies in acquired thrombotic thrombocytopenic purpura. *EBioMedicine* 2, 942–952.
- Tiede, A., Priesack, J., Werwitzke, S., Bohlmann, K., Oortwijn, B., Lenting, P., et al., 2008. Diagnostic workup of patients with acquired von Willebrand syndrome: a retrospective single-centre cohort study. *J. Thromb. Haemost.* 6, 569–576.
- Tiede, A., Huth-Kuhne, A., Oldenburg, J., Grossmann, R., Geisen, U., Krause, M., et al., 2009. Immunosuppressive treatment for acquired haemophilia: current practice and future directions in Germany, Austria and Switzerland. *Ann. Hematol.* 88, 365–370.
- Tiede, A., Eisert, R., Czwalinna, A., Miesbach, W., Scharrer, I., Ganser, A., 2010. Acquired haemophilia caused by non-haemophilic factor VIII gene variants. *Ann. Hematol.* 89, 607–612.
- Tiede, A., Rand, J.H., Budde, U., Ganser, A., Federici, A.B., 2011. How I treat the acquired von Willebrand syndrome. *Blood* 117, 6777–6785.
- Tiede, A., Klamroth, R., Scharf, R.E., Trappe, R.U., Holstein, K., Huth-Kuhne, A., et al., 2015. Prognostic factors for remission of and survival in acquired hemophilia A (AHA): results from the GTH-AH 01/2010 study. *Blood* 125, 1091–1097.
- Tiede, A., Hofbauer, C.J., Werwitzke, S., Knobl, P., Gottstein, S., Scharf, R.E., et al., 2016. Anti-factor VIII IgA as a potential marker of poor prognosis in acquired hemophilia A: results from the GTH-AH 01/2010 study. *Blood* 127, 2289–2297.
- Toole, J.J., Knopf, J.L., Wozney, J.M., Sultzman, L.A., Buecker, J.L., Pittman, D.D., et al., 1984. Molecular cloning of a cDNA encoding human antihaemophilic factor. *Nature* 312, 342–347.
- Torok, T.J., Holman, R.C., Chorba, T.L., 1995. Increasing mortality from thrombotic thrombocytopenic purpura in the United States—analysis of national mortality data, 1968–1991. *Am. J. Hematol.* 50, 84–90.
- Torres, A., Lucia, J.F., Oliveros, A., Vazquez, C., Torres, M., 1980. Anti-factor IX circulating anticoagulant and immune thrombocytopenia in a case of Takayasu's arteritis. *Acta Haematol.* 64, 338–340.
- Torricelli, M., Sabatini, L., Florio, P., Scaccia, V., Voltolini, C., Biliotti, G., et al., 2009. Levels of antibodies against protein C and protein S in pregnancy and in preeclampsia. *J. Matern. Fetal Neonatal Med.* 22, 993–999.
- Toschi, V., Baudo, F., 2010. Diagnosis, laboratory aspects and management of acquired hemophilia A. *Intern. Emerg. Med.* 5, 325–333.
- Tosetto, A., Rodeghiero, F., Gatto, E., Manotti, C., Poli, T., 1995. An acquired hemorrhagic disorder of fibrin crosslinking due to IgG antibodies to FXIII, successfully treated with FXIII replacement and cyclophosphamide. *Am. J. Hematol.* 48, 34–39.
- Tsai, H.M., 1996. Physiologic cleavage of von Willebrand factor by a plasma protease is dependent on its conformation and requires calcium ion. *Blood* 87, 4235–4244.
- Tsai, H.M., 2006. Current concepts in thrombotic thrombocytopenic purpura. *Annu. Rev. Med.* 57, 419–436.
- Tsai, H.M., Raoufi, M., Zhou, W., Guinto, E., Grafos, N., Ranzurmal, S., et al., 2006. ADAMTS13-binding IgG are present in patients with thrombotic thrombocytopenic purpura. *Thromb. Haemost.* 95, 886–892.
- Tun, N.M., Villani, G.M., 2012. Efficacy of rituximab in acute refractory or chronic relapsing non-familial idiopathic thrombotic thrombocytopenic purpura: a systematic review with pooled data analysis. *J. Thromb. Thrombolysis* 34, 347–359.
- Urbanus, R.T., de Laat, B., 2010. Antiphospholipid antibodies and the protein C pathway. *Lupus* 19, 394–399.
- Vadivel, K., Bajaj, S.P., 2012. Structural biology of factor VIIa/tissue factor initiated coagulation. *Front. Biosci.* 17, 2476–2494.
- van Genderen, P.J., Vink, T., Michiels, J.J., Van 'T Veer, M.B., Sixma, J.J., Van Vliet, H.H., 1994. Acquired von Willebrand disease caused by an autoantibody selectively inhibiting the binding of von Willebrand factor to collagen. *Blood* 84, 3378–3384.
- Vazzana, N., Scarti, L., Beltrame, C., Picchi, A., Taccetti, G., Fortini, A., 2014. Acquired factor XI inhibitor presenting as spontaneous bilateral subdural hematoma in an elderly patient. *Case Rep. Hematol.* 2014, 626831.
- Vercellotti, G.M., Mosher, D.F., 1982. Acquired factor XI deficiency in systemic lupus erythematosus. *Thromb. Haemost.* 48, 250–252.
- Veyradier, A., Jenkins, C.S., Fressinaud, E., Meyer, D., 2000. Acquired von Willebrand syndrome: from pathophysiology to management. *Thromb. Haemost.* 84, 175–182.
- Wang, X., Qin, X., Yu, Y., Wang, R., Liu, X., Ji, M., et al., 2017. Acquired factor V deficiency in a patient with a urinary tract infection presenting with haematuria followed by multiple haemorrhages with an extremely low level of factor V inhibitor: a case report and review of the literature. *Blood Coagul. Fibrinolysis* 28, 334–341.
- Webert, K.E., 2012. Acquired hemophilia A. *Semin. Thromb. Hemost.* 38, 735–741.
- Weisdorf, D., Hasegawa, D., Fair, D.S., 1989. Acquired factor VII deficiency associated with aplastic anaemia: correction with bone marrow transplantation. *Br. J. Haematol.* 71, 409–413.
- Werwitzke, S., Geisen, U., Nowak-Gottl, U., Eichler, H., Stephan, B., Scholz, U., et al., 2016. Diagnostic and prognostic value of factor VIII binding antibodies in acquired hemophilia A: data from the GTH-AH 01/2010 study. *J. Thromb. Haemost.* 14, 940–947.
- Westwood, J.P., Thomas, M., Alwan, F., McDonald, V., Benjamin, S., Lester, W.A., et al., 2017. Rituximab prophylaxis to prevent thrombotic thrombocytopenic purpura relapse: outcome and evaluation of dosing regimens. *Blood Adv.* 1, 1159–1166.
- Whelan, S.F., Hofbauer, C.J., Horling, F.M., Allacher, P., Wolfsegger, M.J., Oldenburg, J., et al., 2013. Distinct characteristics of antibody responses against factor VIII in healthy individuals and in different cohorts of hemophilia A patients. *Blood* 121, 1039–1048.

- Whitelock, J.L., Nolasco, L., Bernardo, A., Moake, J., Dong, J.F., Cruz, M.A., 2004. ADAMTS-13 activity in plasma is rapidly measured by a new ELISA method that uses recombinant VWF-A2 domain as substrate. *J. Thromb. Haemost.* 2, 485–491.
- Wiestner, A., Cho, H.J., Asch, A.S., Michelis, M.A., Zeller, J.A., Peerschke, E.I., et al., 2002. Rituximab in the treatment of acquired factor VIII inhibitors. *Blood* 100, 3426–3428.
- Will, A., 2006. Paediatric acquired von Willebrand syndrome. *Haemophilia* 12, 287–288.
- Wood, W.I., Capon, D.J., Simonsen, C.C., Eaton, D.L., Gitschier, J., Keyt, B., et al., 1984. Expression of active human factor VIII from recombinant DNA clones. *Nature* 312, 330–337.
- Wootla, B., Dasgupta, S., Dimitrov, J.D., Bayry, J., Levesque, H., Borg, J.Y., et al., 2008. Factor VIII hydrolysis mediated by anti-factor VIII auto-antibodies in acquired hemophilia. *J. Immunol.* 180, 7714–7720.
- Wootla, B., Christophe, O.D., Mahendra, A., Dimitrov, J.D., Repesse, Y., Ollivier, V., et al., 2011. Proteolytic antibodies activate factor IX in patients with acquired hemophilia. *Blood* 117, 2257–2264.
- Wu, J.J., Fujikawa, K., Lian, E.C., McMullen, B.A., Kulman, J.D., Chung, D.W., 2006. A rapid enzyme-linked assay for ADAMTS-13. *J. Thromb. Haemost.* 4, 129–136.
- Yamada, R., Nozawa, K., Yoshimine, T., Takasaki, Y., Ogawa, H., Takamori, K., et al., 2011. A case of thrombotic thrombocytopenia purpura associated with systemic lupus erythematosus: diagnostic utility of ADAMTS-13 activity. *Autoimmune Dis.* 2011, 483642.
- Yamaguchi, Y., Moriki, T., Igari, A., Nakagawa, T., Wada, H., Matsumoto, M., et al., 2011. Epitope analysis of autoantibodies to ADAMTS13 in patients with acquired thrombotic thrombocytopenic purpura. *Thromb. Res.* 128, 169–173.
- Zanon, E., Milan, M., Brandolin, B., Barbar, S., Spiezia, L., Saggiorato, G., et al., 2013. High dose of human plasma-derived FVIII-VWF as first-line therapy in patients affected by acquired haemophilia A and concomitant cardiovascular disease: four case reports and a literature review. *Haemophilia* 19, e50–e53.
- Zehnder, J.L., Leung, L.L., 1990. Development of antibodies to thrombin and factor V with recurrent bleeding in a patient exposed to topical bovine thrombin. *Blood* 76, 2011–2016.
- Zeitler, H., Ulrich-Merzenich, G., Panek, D., Goldmann, G., Vidovic, N., Brackmann, H.H., et al., 2012. Extracorporeal treatment for the acute and long-term outcome of patients with life-threatening acquired hemophilia. *Transfus. Med. Hemother.* 39, 264–270.
- Zeitler, H., Goldmann, G., Marquardt, N., Ulrich-Merzenich, G., 2013. Long term outcome of patients with acquired haemophilia—a monocentre interim analysis of 82 patients. *Atheroscler. Suppl.* 14, 223–228.
- Zheng, X., Chung, D., Takayama, T.K., Majerus, E.M., Sadler, J.E., Fujikawa, K., 2001. Structure of von Willebrand factor-cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic purpura. *J. Biol. Chem.* 276, 41059–41063.
- Zheng, X.L., Wu, H.M., Shang, D., Falls, E., Skipwith, C.G., Cataland, S.R., et al., 2010. Multiple domains of ADAMTS13 are targeted by auto-antibodies against ADAMTS13 in patients with acquired idiopathic thrombotic thrombocytopenic purpura. *Haematologica* 95, 1555–1562.
- Zhou, W., Inada, M., Lee, T.P., Benten, D., Lyubsky, S., Bouhassira, E.E., et al., 2005. ADAMTS13 is expressed in hepatic stellate cells. *Lab. Invest.* 85, 780–788.

# Multiple Sclerosis

Sarah Wesley and David A. Hafler

Departments of Neurology and Immunobiology, Yale School of Medicine, New Haven, CT, United States

## OUTLINE

<b>Historical Background</b>	<b>961</b>	<b>Immune Pathogenesis</b>	<b>969</b>
<b>Clinical Features</b>	<b>962</b>	<i>T-Cell Pathogenesis</i>	970
<i>Radiologically Isolated Syndrome</i>	962	<i>Immune Dysregulation</i>	971
<i>Clinically Isolated Syndrome</i>	963	<i>Autoantigens</i>	973
<i>Relapsing-Remitting Multiple Sclerosis</i>	963	<i>The Role of B Cells</i>	973
<i>Progressive Multiple Sclerosis</i>	963	<b>Treatment</b>	<b>974</b>
<i>Infusion-Based Therapies</i>			974
<i>Oral Therapies</i>			976
<i>Injection-Based Therapies</i>			977
<b>Diagnostic Criteria</b>	<b>964</b>	<b>Concluding Remarks</b>	<b>978</b>
<b>Imaging</b>	<b>964</b>	<b>References</b>	<b>979</b>
<b>Immunological Markers in Diagnosis</b>	<b>965</b>	<b>Further Reading</b>	<b>986</b>
<b>Pathology</b>	<b>966</b>		
<b>Epidemiology of MS</b>	<b>967</b>		
<i>Genetic Factors</i>	967		
<i>Environmental Factors</i>	968		

## HISTORICAL BACKGROUND

It is widely held that the earliest known reference of multiple sclerosis (MS) can be attributed to Scottish pathologist Robert Carswell (reviewed by [Murray, 2009](#)). In his atlas, *Pathological Anatomy: Illustrations of the Elementary Forms of Disease* (1838), Carswell described two patients affected by paralysis, both with lesions along the spinal cord and lower brainstem accompanied by atrophy. Carswell believed that the extensive paralysis the patients suffered was directly related to the impressive pathology he encountered ([Behan, 1982](#)). However, compelling Carswell's account was that it was not until 30 years later that MS was named by a French neurologist, Jean Martin Charcot. Charcot first described a comprehensive account of the features of MS in 1868 by correlating the clinical and pathological features of the illness in patients he examined both while they were alive and at autopsy. While aspects of his findings would go on to be revised, he noted the accumulation of inflammatory cells in a perivascular distribution, demyelination, and axonal sparing within the lesions or "plaques" in the brain and spinal cord white matter of patients with intermittent episodes of neurologic dysfunction ([Charcot, 1868a,b, 1877](#)). This led to the term "sclérose en plaques disseminées," or MS.

Over the last 100 years, there have been many important historical milestones that have led to the fundamental understanding that MS is a multifocal inflammatory disease primarily affecting central nervous system (CNS) white matter resulting in progressive neurodegeneration in genetically susceptible hosts ([Nylander and Hafler, 2012](#)).

The hypothesis that MS is an autoimmune disease can be attributed to observations by Thomas Rivers at the then Rockefeller Institute. In 1933 Rivers demonstrated that injection of rabbit brain and spinal cord into primates resulted in a demyelinating disease in mammals (Rivers et al., 1933). This disease, known as experimental autoimmune encephalomyelitis (EAE), is the result of immunization of CNS myelin and has served as an important animal model for MS.

Schumacker et al. (1965) defined clinical diagnostic criteria based on the notion that MS is a disease disseminated in time and space throughout the CNS. Such criteria continue to be utilized and revised into the present day. Since then, the advent of magnetic resonance imaging (MRI) and the Food and Drug Administration (FDA) approval of disease-modifying immunotherapies, beginning with interferon (IFN)- $\beta$ 1b in 1993, have revolutionized how we examine and treat patients with MS (Young et al., 1981; Arnason, 1993). During the past 20 years, MS has evolved from a disease with no therapy to one with 15 approved therapies in the United States to date. These major advances have established MS as a treatable neurological illness. Nonetheless, the development of more effective and safer treatments that can be used at the time of diagnosis for this potentially disabling illness is paramount, and it is predicated on a more thorough understanding of the underlying immunopathology. Advances in immunology and neurology have provided clinicians with powerful tools to understand better the underlying causes of MS, leading to new therapeutic advances. The future calls for extending the original observations of Carswell and Charcot by continuing to define the molecular pathology of MS in relation to growing knowledge surrounding immune-related pathology and DNA haplotype structure, in addition to CNS and peripheral mRNA and protein expression, leading to the generation of a new series of disease-related hypotheses.

## CLINICAL FEATURES

The signs and symptoms of MS are variable as the disease can affect anywhere within the CNS. Demyelinating lesions may develop at any site along myelinated CNS white matter tracts, and symptoms of MS therefore depend on the functions subserved by the pathways involved. Although the primary insult involves demyelination, there is edema, inflammation, gliosis, and axonal loss which all contribute to the symptomatology of a lesion. The most common symptoms and signs involve alteration or loss of sensation due to involvement of spinothalamic or posterior column fibers, visual loss from optic neuritis, limb weakness and spasticity related to disruption of corticospinal tracts, tremors and incoordination of gait or limbs largely related to cerebellar or spinocerebellar fiber involvement, and abnormalities of cranial nerve function (such as double vision due to disturbance in conjugate eye movement) secondary to brainstem lesions (Noseworthy et al., 2000). Bowel, bladder, and sexual dysfunction occur in over two-thirds of the patients at some time during the course of their illness (Betts et al., 1993; Mattson et al., 1995), largely due to disruption in spinal cord pathways. Fatigue, depression, and cognitive changes are common symptoms of unclear etiology that can significantly interfere with daily functioning and are now being recognized as significant contributors to disability (Whitlock and Siskind, 1980; Freal et al., 1984; Sadovnick et al., 1996). A correlation with progressive brain atrophy, cognitive decline, and impairment on MRI has implicated axonal loss as the pathologic substrate of the cognitive deterioration in MS (Rao et al., 1989; Hohol et al., 1997; Amato et al., 2004).

While MS might have variability in clinical presentation and course of the illness, it can follow a number of rather predictable courses. MS can be divided into disease subtypes: radiologically isolated syndromes (RISs), clinically isolated syndromes (CISs), relapsing-remitting MS (RRMS), secondary progressive MS (SPMS), and primary progressive MS (PPMS).

### Radiologically Isolated Syndrome

RIS is often identified incidentally on MRI of the brain in the absence of signs or symptoms. However, in the presence of a high lesion burden and evidence of CNS inflammation with positive oligoclonal bands (OCBs) or increased IgG index in the cerebrospinal fluid (CSF), there is a significant risk of developing the clinical symptoms of MS. Patients with RIS are often followed with close serial examinations and MRIs over time. In some cases where new lesions occur in the absence of clinical symptomatology, immunotherapy should be considered.

## Clinically Isolated Syndrome

CIS generally occurs in young adults and is defined by the presentation of a first episode of demyelination, typically in the form of an optic neuritis, cerebellar, or brainstem syndrome. During an episode, neurological symptoms develop over hours to several days, persist for days to several weeks, and gradually dissipate (Miller et al., 2012). In order for such episodes of acute inflammatory CNS demyelinating events to be considered CIS, they must last for at least 24 hours with no more than 30 days between attacks (Polman et al., 2005). The resolution of symptoms appears to be due to the reduction of inflammation and edema at the site of the responsible lesion rather than to the reversal of demyelination, which may persist even in the absence of symptoms (McDonald et al., 2001).

A number of prospective studies have helped to risk-stratify patients with CIS. Those patients who have concurrent but clinically silent lesions on MRI are at a higher risk for developing MS, but those without MRI lesions in other parts of the CNS have a lower probability of developing MS (Achiron and Barak, 2000; Fisniku et al., 2008). It is prudent to consider that while the patient might not meet strict McDonalds Criteria, the presence of other inactive or clinically silent lesions on MRI likely indicates that the patient is hosting underlying pathology consistent with MS. We at times call CIS “singular sclerosis” thus explaining to the patients that we know what is going on and that there is a high likelihood of developing MS (Rovira et al., 2009). There should be a low threshold to treat such patients, particularly if the first clinical attack occurred in the presence of added risk factors for long-term physical disability, such as incomplete recovery from first attack and bladder or bowel symptoms at onset (Langer-Gould et al., 2006).

## Relapsing-Remitting Multiple Sclerosis

If a patient with a CIS develops further clinical episodes followed by relapses, then the patient meets criteria for a diagnosis of RRMS. Early in the course of RRMS, patients often make complete recovery from relapses. As the disease progresses, the recovery from a relapse diminishes, which causes a patient to accrue disability. A relapsing-remitting onset is observed in 85%–90% of the patients, with relapses often lasting an average of 4 weeks in duration. The outcome in patients with RRMS is variable; untreated, previous reports suggested that approximately 50% of all MS patients require the use of a walking aid by 10 years after clinical onset (Weinshenker, 1994). The consequences on prognosis of newer treatment regimens are not well delineated; however, many studies indicate improvement of disability status following continued treatment regimens (Bates, 2011). Increased attack frequency and poor recovery from attacks in the first years of clinical disease predict a more rapid deterioration (Confavreux and Vukusic, 2006). New treatment paradigms will be discussed later in this chapter.

## Progressive Multiple Sclerosis

Ultimately, approximately 40%–50% of the untreated RRMS patients stop having attacks and develop what may be a neurodegenerative progressive disease secondary to the chronic CNS inflammation, known as SPMS (Confavreux et al., 2000). The evolution to this secondary progressive form of the disease is associated with significantly fewer gadolinium-enhancing lesions and a decrease in brain parenchymal volume (Khoury et al., 1994; Filippi et al., 1995; Weiner et al., 2000). Similarly, while earlier RRMS is sensitive to immunotherapy (Hohol et al., 1999), as times goes on, responsiveness to treatment decreases and ultimately disappears in secondary progressive disease (Rieckmann et al., 2004).

Lastly, PPMS is a form of disease that occurs in close to 15% of the patients and unlike RRMS is more frequent in males. It is characterized from the onset by the absence of acute attacks and instead involves a gradual clinical decline traditionally in the form of a progressive myelopathy (Miller and Leary, 2007). Patients might also present with a progressive cerebellar syndrome in the form of ataxia. Clinically, this form of the disease is associated with a lack of significant response to immunotherapy, although anti-CD20 monoclonal antibody (ocrelizumab) has a modest effect on disease progression and has been FDA approved for patients with this form of disease. Given some challenges in making the diagnosis of PPMS, there are specific proposed diagnostic criteria that will be discussed later in the chapter.

## DIAGNOSTIC CRITERIA

In the absence of a specific immune-based assay, the diagnosis of RRMS continues to be predicated on the clinical history and neurological exam demonstrating multiple lesions disseminated in time and space within the CNS (McDonald et al., 2001). Using McDonald's criteria, the diagnosis of RRMS can be made solely on the basis of history of two relapses and objective findings on exam of two lesions disseminated in the CNS (periventricular, juxtacortical, infratentorial, or spinal cord). However, MRI of the neuroaxis is often sought to confirm the diagnosis or to rule out other mimics of the illness. The use of the MRI and other imaging modalities has had a major impact on early diagnosis. Moreover, in practice, MRI helps to monitor disease course and response to therapy. T1-weighted scans generally provide appreciable contrast between gray and white matter with water appearing darker and fat brighter. In T2-weighted scans, fat is also differentiated from water; however, fat appears darker and water lighter in the image, making T2-weighted scans well suited for imaging edema since CSF appears lighter (Filippi and Agosta, 2009). As part of revised 2010 McDonald's criteria for RRMS diagnosis, new T1-weighted lesions or T2-weighted gadolinium enhancing-lesions on follow-up MRI scans may serve as criteria for dissemination in space (DIS) or dissemination in time, thus allowing for a diagnosis fulfilling the criteria for MS. For diagnosis of PPMS under McDonald's Criteria, there must be 1 year of disease progression plus two of the following three elements: (1) evidence for DIS in the brain based on having  $\geq 1$  T2 lesions in MS characteristic regions; (2) evidence for DIS in the spinal cord based on  $\geq 2$  T2 lesions in the cord; and (3) CSF with presence of OCBs and/or elevated IgG index (Polman et al., 2011). In addition to meeting criteria for DIS and dissemination in time, a thorough evaluation should be done to rule out alternative diagnoses. The extent of the workup is often dependent on the appearance of the lesions, the patient's clinical course, and the patient's other medical risk factors. Differential diagnosis includes but is not limited to rheumatologic conditions, metabolic derangements, infections of the CNS, vitamin deficiencies, and malignancies of the CNS.

## IMAGING

MRI has gained a principal role in the assessment of MS because it allows clinicians to readily obtain an understanding of the pathophysiology of the lesions, CNS involvement, and ultimately the overall illness without invasive procedures. Currently, common MRI measures of disease burden include the quantification of brain lesions using T1- and T2-weighted images, gadolinium contrast, proton density, and fluid attenuated inversion recovery sequences. Each of these markers represents many possible histopathological correlates, including demyelination, edema, axonal loss, matrix destruction, and inflammation (Filippi and Rocca, 2011). The lesions on MRI are often ovoid in shape, ranging from a few mm to more than 1 cm in size. Their location is crucial, considering that MS lesions have a high propensity to locate in the periventricular white matter, brainstem, and cerebellum. In addition, MRI and pathological data suggest that the evolution of MS lesions depends on whether they occur during early versus chronic phases of the disease course (Filippi et al., 2012).

T2/FLAIR is the best sequence to date for visualizing the white matter lesions from MS, although it has some limitations in the posterior fossa, in which case a regular T2-weighted image might help to differentiate artifact from true hyperintensity. Typical MS protocol for MRI should include the following: precontrast axial, coronal, and sagittal T2/FLAIR sequences, axial T1- and T2-weighted images, and axial, coronal, and sagittal postcontrast T1-weighted images.

Lesions on T2-weighted images are often clinically silent and correlate weakly with a patient's disability despite correlating well with the location on plaques in the CNS of postmortem MS patients (Newcombe et al., 1991). Hypointense lesions on T1-weighted images may be persistent or nonpersistent (Rovaris et al., 1999). Persistent hypointense T1-weighted lesions represent areas indicative of axonal loss and severe tissue destruction and correlate better than T2 lesion load with clinical severity of the disease (Van Waesberghe et al., 1999). Multiple T2-weighted and/or gadolinium-enhancing lesions on initial MRI scans indicative of diffuse cortical lesions and atrophy also predict a more severe subsequent course related not only to physical disability but also to diminished cognitive outcome (Calabrese et al., 2009; Deloire et al., 2011). Nonpersistent hypointense lesions on T1-weighted images represent reversible edema due to abatement of inflammation. Postcontrast gadolinium enhancement of lesions on T1-weighted images represents acute disruption of the blood–brain barrier (BBB) from inflammation. On average, disruption can last 3 weeks but can range anywhere from 2 to 6 weeks and is

dependent on gadolinium dose, the characteristics and delay of image acquisition, and steroid treatment of acute attacks (Filippi, 2000).

Intermittent MRI imaging might underestimate severity of disease burden since weekly MRI scanning suggests that a significant proportion of MS lesions have very short-lived enhancement (Cotton et al., 2003). Brain and spinal cord atrophy may occur in MS and can represent loss of myelin, oligodendrocytes, and axons, in addition to contraction of astrocyte volume.

Quantitative susceptibility mapping (QSM) is a novel sequence derivative of susceptibility-weighted imaging that provides a direct measure of tissue susceptibility and can indicate the presence of iron deposition, a known component of activated microglia in chronically activated lesions. Therefore it is potentially a measure of chronically active lesions after gadolinium enhancement has subsided as well as a possible marker for lesions that will go on to cause atrophy and progressive features. Continued work in improving the use of various imaging modalities to more accurately characterize active disease is imperative in moving forward.

## IMMUNOLOGICAL MARKERS IN DIAGNOSIS

CSF immunologic markers can function as adjuncts to clinical findings when considering the diagnosis of MS. The CSF of patients with MS typically shows normal glucose, 0–5 lymphocytes (predominantly T cells), normal to mildly elevated total protein, increase in IgG CSF synthesis (IgG index), and OCBs. OCBs are uncovered when CSF from MS patients is electrophoresed. The cathode region reveals a number of discrete bands that represent excess antibody production by one or more clones of B cells. Such bands are not evident when CSF from healthy controls is electrophoresed. Often absent early in the disease, OCBs can eventually be detected in over 90% of the patients with MS (Cruz et al., 1987; Mclean et al., 1990).

CSF OCBs have also been described in conditions where there is CNS inflammation such as subacute sclerosing panencephalomyelitis (Mattson et al., 1980), neurosyphilis (Pedersen et al., 1982), Varicella zoster virus infection (Vartdal et al., 1982), HIV infection (Skotzek et al., 1988), and in multisystem autoimmune diseases. They are more rarely seen in cerebrovascular accidents, and up to 5% of the normal individuals. Of note, OCBs are continuously present in MS regardless of disease activity, whereas they are transient in other conditions due to infection clearance (Link and Huang, 2006). Furthermore, OCBs have been reported to correlate with disease course and disability progression (Link and Huang, 2006; Mandrioli et al., 2008) and with the conversion to MS in patients with CIS and a negative MRI or an MRI with few lesions (Tintore et al., 2008). In diseases such as the viral encephalitides, OCBs commonly bind virus determinants, in contrast to MS, where there is continued evidence to suggest that OCB antibodies are heterogeneous in their structures and roles without one particular antibody target identified to date (Olek, 2000; Brändle et al., 2016).

Due to intrathecal synthesis from plasma cells, CSF immunoglobulin levels are also elevated in patients with MS. The immunoglobulins are mainly composed of IgG, with lesser amounts being IgM and immunoglobulin A (IgA). Specifically, studies have delineated differences between IgG and IgM levels with the latter correlating more strongly with MS disease course and IgG reflecting local B-cell responses accompanying CNS inflammation. Nearly 90% of the patients demonstrate elevated levels of CSF IgG production when the IgG index formula (spinal fluid IgG/spinal fluid albumin)/(serum IgG/serum albumin) is calculated. A spinal fluid IgG index greater than 0.58 implies that IgG is being synthesized in the CNS.

Apart from OCBs, numerous CSF markers have demonstrated specificity for the MS disease process. Several nonspecific proteins may function as markers of disease process, including tau protein and myelin basic protein (MBP), in addition to light and heavy neurofilament chains appearing during relapse and correlating with long-term functional outcome and the likelihood of conversion from CIS to RRMS (Graber and Dhib-Jalbut, 2011). While measuring neurofilaments may have some future clinical utility, there is no usefulness in measuring MBP in the era of MRI imaging. This remains an example of a “legacy test” discovered over 35 years ago that is of no clear utility.

There are reports of serum and CSF anti-MBP, anti-myelin oligodendrocyte glycoprotein (MOG), and anti-proteolipid protein (PLP) autoantibodies (Warren and Catz, 1994; Warren et al., 1994; Berger et al., 2003) in patients with MS, and it has become clear that these findings represent nonspecific binding of low-affinity antibodies to denatured proteins on ELISA plates. There are observations that high-affinity antibodies against MOG epitopes are present in a small proportion of the patients (Menge et al., 2011). However, using sensitive solution phase assays, high-affinity autoantibodies to MOG can be detected in the serum and CSF of patients with acute disseminated encephalomyelitis and pediatric MS (O'Connor et al., 2003, 2005, 2007; Zhou et al., 2006). A recent

study aiming to characterize intrathecal MOG antibodies in MS indicated that the rMOG index, a marker of intrathecal MOG antibody production, may provide complementary information to routine CSF testing in the diagnosis of MS (Klawiter et al., 2010). Moreover, anti-MOG autoantibodies can be eluted from the brain tissue of a subset of patients with MS (O'Connor et al., 2005). Such markers support the ongoing hypothesis of MS as a disease linked to immune dysfunction, ongoing inflammation, and tissue damage and repair. A serum autoantibody, neuromyelitis optica immunoglobulin (NMO-IgG), was identified in patients with neuromyelitis optica, an inflammatory demyelinating disease similar to, and considered by some, to be a variant of MS affecting the optic nerves and spinal cord, thereby highlighting the importance of the differential diagnosis between MS, NMO, and NMO spectrum disorders. NMO and the associated autoantibody (NMO-IgG/anti-aquaporin 4) are discussed further in Chapter 57, Hepatitis.

## PATHOLOGY

Gross examination of MS brain tissue has largely been limited to autopsy specimens of individuals with long-standing disease. Such pathological examination reveals multiple sharply demarcated gray colored plaques in the CNS white matter with a predilection for the optic nerves and white matter tracts of the periventricular regions, brainstem, and spinal cord. The gray matter contains less myelin, and thus lesions in the gray matter are less conspicuous on gross examination (Geurts et al., 2005). When examining the histological features of an MS lesion, there exist three major components: inflammation, gliosis, and demyelination. The inflammation in lesions is composed of lymphocytes, monocytes, and macrophages whose proportions depend on the activity and age of the lesion (Frohman et al., 2006). The second component of a lesion occurs when reactive astrocytes and fibrillary gliosis are present in the lesion.

Demyelination is an important feature of the MS lesion. Although MS is described as a disease causing a loss of myelin, the notion of axonal loss has been suggested as the major cause of irreversible disability in patients with MS. Trapp et al. (1998) first reported that substantial axonal injuries with axonal transections are also abundant throughout active MS lesions, even in patients in the early stages of the disease process. Axonal reduction and acute damage have previously been correlated with demyelination and meningeal inflammation (Ferguson et al., 1997). Interestingly, one study suggested that differences in axonal loss exist among subsets of MS, with the least axonal loss being demonstrated in PPMS and the most pronounced in SPMS (Bitsch et al., 2000). Notably, there have been case reports of early RRMS presenting with inflammatory cortical demyelination prior to the appearance of white matter lesions, indicating possible inflammation being initiated at the subpial layer (Popescu et al., 2011). Recent work aimed at elucidating the pathogenesis of axonal damage and loss has implicated several mechanisms including inflammatory secretions, Wallerian degeneration, disruption of axonal ion concentrations, loss of myelin-derived support, damage from nitric oxide and reactive oxygen species, energy failure from mitochondrial dysfunction, and  $\text{Ca}^{2+}$  accumulation (Smith and Lassmann, 2002; Dutta and Trapp, 2007; Dziedzic et al., 2010).

MS lesions can also be classified from a pathogenesis point of view into three types based on the age of the lesion: active, chronic active, and chronic inactive (Lassmann, 1998). This classification system has recently been corroborated by interval MRIs of lesions in live individuals as well as MRIs of tissue specimens using QSM imaging modalities which show the evolution of acute lesions into either remyelinated areas, chronically active lesions, or chronically inactive lesions. Macrophages are most prominent in the center of the active plaques and are seen to contain myelin debris, while oligodendrocyte counts are reduced and generally present in lesions demonstrating signs of remyelination. Hypertrophic astrocytes and mild astroglial scarring are also characteristic of active lesions (Frohman et al., 2006). Lymphocytes may be found in normal appearing white matter beyond the margin of active demyelination (Prineas, 1975; Booss et al., 1983). The inflammatory cell profile of active lesions is characterized by perivascular infiltration of oligoclonal T cells (Wucherpfennig et al., 1992b) consisting predominantly of clonally expanded CD8<sup>+</sup> T cells in the plaque margins and perivascular cuffs, and to a lesser extent of CD4<sup>+</sup> cells invading the normal appearing white matter around the lesion (Traugott et al., 1983; Hauser et al., 1986; Babbe et al., 2000). The inflammatory infiltrate may also include monocytes, occasional B cells,  $\gamma\delta$  T cells, and rare plasma cells (Booss et al., 1983; Wucherpfennig et al., 1992a; Babbe et al., 2000). Remarkably, demyelination in acute lesions may be related to an antimyelin antibody-mediated mechanism in which normal myelin is coated with anti-MOG immunoglobulin and phagocytosed in the presence of complement by local macrophages (Genain et al., 1999). Chronic-active lesions are sharply demarcated with perivascular cuffs

of infiltrating cells, lipid- and myelin-laden macrophages, activated microglia, and hypertrophic astrocytes. These cells disappear from the center core suggesting the presence of ongoing inflammatory activity along the lesion edge. Within the hypocellular core, there are naked axons embedded within a matrix of fibrous astrocytes, lipid-laden macrophages, a few infiltrating leukocytes, and no oligodendrocytes. The chronic-inactive plaque does not have macrophages at the border or center of the plaque. There is vast hypocellularity and no ongoing demyelination with histology demonstrating demyelinated axons with fibrillary gliosis (Raine, 1991).

## EPIDEMIOLOGY OF MS

The prevalence rates of MS vary based on latitude and patient populations in North America. According to the Center for Disease Control (CDC), the prevalence in one part of Texas was 47.2 per 100,000 while it was 109.5 per 100,000 in Ohio, which is more than 1 in 1000 people (Noonan et al., 2010). It is possible that prevalence data do not reflect the true number of cases, with some populations being undiagnosed or not included in these statistics. The median age of onset of symptoms is 23–24 years, with a peak age of onset for women in the early twenties, and for men in the late twenties (Schumacker et al., 1965; Paty et al., 1994). As in most diseases classified as autoimmune, there is a clear female predominance in MS cases, with a 3:2 female-to-male ratio (Olek, 2000). Studies of MS incidence rates in migrants (Dean et al., 1976) and apparent epidemics of MS at geographical locations (Kurtzke et al., 1982), also discussed below, indicate a clear role for environmental factors.

### Genetic Factors

Studies in twins (Mackay and Myrianthopoulos, 1966; Williams et al., 1980; Heltberg and Holm, 1982; Ebers et al., 1986; Kinnunen et al., 1987; [No authors listed], 1992; Mumford et al., 1992) demonstrate shared genetic risk factors for MS. While the risk of developing MS in the general population is 1/750, our early understanding of the genetic factors linked to MS epidemiological studies and disease concordance within family members or twins. Such studies have demonstrated that approximately 15%–20% of the patients have a family history of MS and when both parents are affected with MS, 9% of the children develop the disease (Hogancamp et al., 1997; Sadovnick et al., 1997; Sadovnick, 2006). Twin studies have indicated that the monozygotic concordance rate is 30% versus the dizygotic rate of 5% (Holmes et al., 1967; Sadovnick et al., 1993). Nevertheless, large extended pedigrees are relatively uncommon.

Most recently, work involving genome-wide association studies (GWAS) using single nucleotide polymorphisms from the haplotype map (HapMap) project has provided insights into the genetic architecture of autoimmune diseases (Tishkoff and Verrelli, 2003; Frazer et al., 2007). Specifically, the sequencing of the human genome and the generation of the HapMap has finally allowed the identification of the genetic architecture underlying risk for developing autoimmune diseases by GWAS (Tishkoff and Verrelli, 2003). GWAS have afforded an unbiased and widespread approach in scanning the whole genome and identifying haplotypes associated with risk of developing human diseases. They provide an alternative approach to classic linkage analysis and have greater statistical power to detect variants conferring a modest disease risk (Risch and Merikangas, 1996; Yang et al., 2005).

In 2007, we performed among the first GWAS in patients with autoimmune diseases in subjects with MS and identified several gene regions associated with disease risk (Hafler et al., 2009). Over the past decade, we have performed a series of deeper investigations of MS genetic risk (Wellcome Trust Case Control Consortium et al., 2007; Aulchenko et al., 2008; Comabella et al., 2008; Australia and New Zealand Multiple Sclerosis Genetics Consortium, 2009; Baranzini et al., 2009; De Jager et al., 2009b; Jakkula et al., 2010; Nischwitz et al., 2010; Sanna et al., 2010; International Multiple Sclerosis Genetics Consortium et al., 2009).

In our most recent study, we assembled and analyzed genetic data of 47,351 MS patients and 68,284 control subjects. These data established a reference map of the genetic architecture of MS that includes 233 autosomal susceptibility variants outside the major histocompatibility complex (MHC), 1 chromosome X variant, and 32 independent associations within the extended MHC. We have used a number of different methods to map MS susceptibility genes onto cell types. These studies implicate multiple innate and adaptive pathways distributed across the cellular components of the immune system, particularly CD4 and B cells (Farh et al., 2015). Using expression profiles from purified human microglia, we found enrichment for MS genes in these brain-resident immune cells. Thus while MS is most likely initially triggered by perturbation of peripheral immune responses,

the functional responses of microglia and other brain cells are also altered and may have a role in targeting an autoimmune process to the CNS.

One of the additional aims of GWAS has been to refine our understanding of the genetic risk associated with the MHC through looking more closely at HLA types at six loci (A, B, C, DQA1, DQB1, and DRB1) (Sawcer et al., 2011). Prior to GWAS, our understanding of the association and linkage of MS with respect to MHC was limited to alleles and haplotypes on chromosome 6p21. Recently, studies have successfully established HLA DRB\*1501 as being the allele variant involved in MS as opposed to HLA DQA1 or DQB1 (Brynedal et al., 2007; Lincoln et al., 2009). Further work has implicated HLA-A2 as being negatively associated with MS, thereby potentially serving as a protective allele with consistent effects across cohorts.

Many of the alleles identified in MS are shared not only among other autoimmune diseases but also are strongly associated with immune pathways (Farh et al., 2015). Given these genetic commonalities, it is therefore not surprising that MS and other autoimmune diseases share related defects in immune function and regulation. Recent work aimed at uncovering the intricacies of the relationships between autoimmune diseases has confirmed commonality across seven autoimmune diseases through identifying shared genes among some but not all of the diseases (Cotsapas et al., 2011). A model addressing the overarching interconnectivity of various autoimmune disease mechanisms is likely to be elucidated in the future. As MS is a complex disease, understanding which combinations of genes within the population confer the greatest risk of developing autoimmune disease is a central goal of present genetic research efforts. Nevertheless, these studies have provided convincing evidence that MS fits into the autoimmune disease category and is caused by common allelic variants each with only subtle but important variations on immune function.

## Environmental Factors

Global maps of MS prevalence rates, constructed based on multiple descriptive epidemiological studies, reveal a nonrandom geographical distribution of the disease. A diminishing north to south gradient of MS prevalence was described in the Northern Hemisphere (Beebe et al., 1967; Kurtzke, 1977; Kurtzke et al., 1979; Hammond et al., 1987), with an opposite trend identified in the Southern Hemisphere (Hammond et al., 1988; Hogancamp et al., 1997; Kurtzke, 2000). Notably, there is a marked absence of MS cases directly near the equator. When taking continental differences into consideration, there is increased prevalence of MS with large dispersion in Western Europe and North America, both regions being highly populated by Caucasians, whereas areas in Central and Eastern Europe, Australia, and New Zealand have lower prevalence with the lowest prevalence occurring in Asia, the Middle East, and Africa. Such variation calls into question the possibility that ethnicity might be linked to the continental differences seen (Koch-Henriksen and Sorensen, 2010).

Previously, the study of latitudinal differences demonstrated only modest effect in Europe and North America (Lauer, 1995; Weinshenker, 1996), with the only clear trend of increased incidence attributed to the Southern Hemisphere where the majority of studies have come from New Zealand and Australia (Skegg et al., 1987; Mcleod et al., 1994). Descendants living New Zealand and Australia have a lower risk of MS than those descendants living in the United Kingdom. This general distribution likely reflects a combination of genetic and environmental influences particularly with respect to exposure to sunlight (Ebers, 2008). However, the aforementioned more recent study of populations in North America by the CDC did demonstrate a clear latitudinal gradient with higher prevalence of MS cases occurring farther from the equator, even when controlled for ethnic differences (Noonan et al., 2010).

Relatedly, environmental deficiencies have long been associated with MS, as indicated by the inverse correlation of the world prevalence of MS and environmental supply of vitamin D. Vitamin D may be supplied from the environment via sunlight exposure or via dietary intake of vitamin D3 (Vanamerongen et al., 2004; Ascherio et al., 2010). At higher latitudes, the exposure to sunlight is insufficient to produce vitamin D given the lower exposure to the sun in winter months. Vitamin D and its immunomodulatory effect have been established both in response to sunlight and in dietary vitamin D(3) intake (Smolders et al., 2008; Kragt et al., 2009). Subtle defects in vitamin D metabolism have suggested a possible genetic origin. Identification of a highly conserved vitamin D responsive element in the promoter region of the HLA-DRB\*1501 haplotype has sparked ongoing debate as to whether HLA-DRB\*1501 might be involved in the response to vitamin D particularly since studies suggest that the beneficial effect of vitamin D on MS may in fact be attenuated in those with the risk allele (Ramagopalan et al., 2009; Simon et al., 2011). Nevertheless, few genetic determinants have been identified.

Epstein–Barr virus (EBV) infection has been linked to MS risk since exposure to EBV is associated with 1.5 times greater risk of developing MS. Over 99% of the individuals with MS have evidence of prior infection with EBV in comparison to 90% in humans overall (Bagert, 2009). However, the role of EBV in the development of MS is not known. EBV generally infects resting B lymphocytes transforming them into memory cells that survive long term largely undetected by the immune system (Thorley-Lawson and Gross, 2004). Interestingly, serostatus in individuals with MS has demonstrated elevated titers to EBV prior to the development of any neurologic sequelae (Thacker et al., 2006).

Diet has been investigated as a potential environmental factor contributing to the development of MS. In particular, the marked increase in salt intake in the Western diet has been studied with interesting results. Based on evidence that development of EAE appears to be linked to pathogenic IL-23-dependent T<sub>H</sub>17 cells, a recent study demonstrated that elevated in vivo sodium chloride concentrations might increase the expression of pathogenic T<sub>H</sub>17 in murine and human cells, leading to increased production of proinflammatory cytokines. Researchers also showed that mice with high-salt diets developed greater expression of IL-17a-producing CD4<sup>+</sup> cells and more severe forms of EAE (Kleinewietfeld et al., 2013). Based on these results, another study looked at salt intake in the human population, demonstrating a 2.75-fold increase in exacerbation rate in RRMS patients with moderate-to-high salt diets when controlled for a number of factors including treatment, age, and disease duration (Farez et al., 2015). Of note, there was one study that found possible evidence to the contrary. Using estimates of 24-hour urine sodium extrapolated from spot urine samples over 5 years in a subanalyzed group of patients enrolled in the BENEFIT trial, researchers did not find an association between urinary sodium as a marker of dietary intake and progression clinically or radiographically of CIS or MS (Fitzgerald et al., 2017).

There has also been speculation about the role of childhood obesity and the risk of developing MS. A recent study looking at pediatric MS found an adjusted odds ratio of 3.76 for development of MS in extremely obese girls compared to healthy controls, but researchers found no effect in boys with obesity (Langer-Gould et al., 2013).

Finally, a major area of research for environmental factors is emerging from investigation of the role of the gut microbiome as a factor in mediating host immune responses and gene expression. A 2016 study used rRNA sequencing to analyze the microbiome in healthy, untreated, and treated MS cohorts. It characterized populations of the microbiome and found differences between healthy and MS populations at the phylum level. These results also suggested that certain microbiome populations might be considered proinflammatory, and treatment of MS might shift the population to a more normalized profile. In a more recent study, mice with EAE tended to have more severe disease when exposed to the microbiome from MS patients. Specific populations of gut bacteria were identified as either proinflammatory or antiinflammatory, specifically regulating expression of IL-10-positive T-cells and regulatory T cell (Treg) populations. Not surprisingly, the latter bacteria populations were decreased in the MS population, compared to healthy controls, and the former were increased (Cekanaviciute et al., 2017).

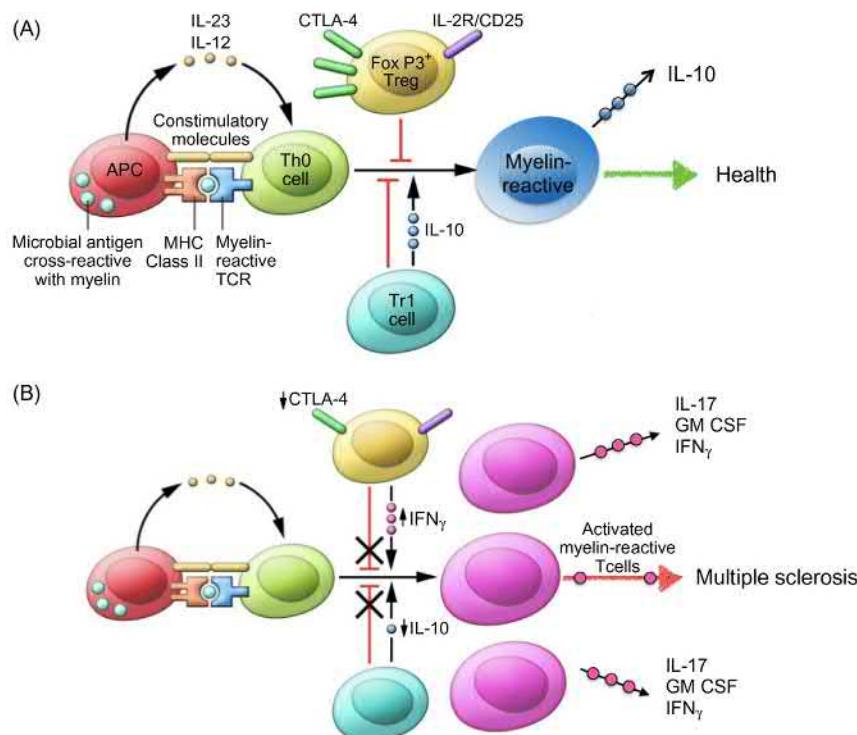
## IMMUNE PATHOGENESIS

Until recently, there were discussions as to whether MS is a primary degenerative disease with secondary inflammation or an autoimmune disease with immune-mediated destruction of the CNS. The sharing of almost half of the allelic variants between MS and other autoimmune disorders from the GWAS has, to a large extent, provided convincing evidence for an autoimmune pathophysiology for the disease (Hafler, 2012). Our current understanding of MS strongly implicates involvement of the immune system and autoreactive proinflammatory T cells that are critical to the propagation of CNS tissue injury. Elucidating the mechanism of disease initiation and how it contributes to the transition from physiologic immune-surveillance to pathologic cascade continues to be an area of ongoing investigation. Our foundation is based on the understanding that peripherally activated cross-reactive T cells migrate into the CNS of genetically susceptible hosts and mount proinflammatory responses to myelin epitopes. Myelin-reactive T cells appear to be both increased in frequency and activation state in individuals with MS (Raddassi et al., 2011; Cao et al., 2015), suggesting a peripheral breach of tolerance to CNS antigen. However, the presence of autoreactive cells in the periphery is an insufficient explanation for the development of autoimmune disease given that myelin-reactive T cells can be found in the peripheral blood of both healthy individuals and patients with MS.

## T-Cell Pathogenesis

Upon interaction of the T cell with the antigen-presenting cell (APC), the antigen-specific T cells proliferate and divide into subsets (see Fig. 51.1). Of specific interest to MS are the Th1, Th2, Th17, and Treg subsets. T cells have been classified according to the cytokine profiles that they produce upon activation (Abbas et al., 1996; O'Garra, 1998; O'Shea and Paul, 2010). MHC class II restricted CD4 T cells, producing IFN- $\gamma$ , GM-CSF, IL-2, and TNF- $\alpha$ , have been defined as Th1 (inflammatory) cells and have demonstrated pathogenicity in EAE models (Leonard et al., 1995; Segal et al., 1998; Severson and Hafler, 2010). The Th1 cytokines activate macrophages for cellular immunity with the assistance of IgG<sub>1</sub> secreted by B cells. In contrast, CD4 T cells producing IL-4, IL-5, IL-10, or IL-13 have been termed Th2 (antiinflammatory) cells and have demonstrated a protective role in EAE (Khoury et al., 1992; Owens et al., 1994; Begolka et al., 1998; Antel and Owens, 1999). Th2 cytokines promote humoral immune responses alongside IgG<sub>4</sub>. Th17 cells are thought to be proinflammatory in nature and secrete the cytokines IL-17A, IL-17F, IL-21, and IL-22, which have been strongly implicated in the pathogenesis of autoimmune diseases (Harrington et al., 2005). Tregs are a subset of T cells that are involved in the regulation of the immune system, maintenance of tolerance to self-antigens, and surveillance of autoimmune disease. They can secrete a variety of cytokines including transforming growth factor- $\beta$  (TGF- $\beta$ ), IL-10, and IFN- $\gamma$  in addition to utilizing FoxP3 as a transcription factor (Fontenot et al., 2003; Hori et al., 2003). Current work involving Tregs has linked this subset with immune dysregulation, a concept which will be discussed later.

Several lines of evidence support the hypothesis that Th1 cells may be pathogenic in MS. Th1 and Th2 cells express distinct profiles of chemokine receptors, including CXCR3/CCR5 and CCR3/CCR4, respectively



**FIGURE 51.1** Pathophysiology of MS. Defects in peripheral immune regulation lower the activation barrier for autoreactive T cells. (A) In normal homeostasis, APCs digest microbial antigens or self proteins and present them to naïve T cells in the context of costimulatory molecules. An appropriate cytokine milieu can drive differentiation of these naïve autoreactive T cells to a Th1 or Th17 cell phenotype; however, these potentially pathogenic T cells are not activated due to the actions of peripheral regulatory immune cell populations, such as FoxP3 Tregs and Tr1 cells. Via the actions of coinhibitory molecules and cytokines such as IL-10 and TGF- $\beta$ , autoreactive T cells become anergic and autoimmune disease is prevented. Other mechanisms, such as thymic deletion and lack of costimulatory molecules on APCs, are also involved in controlling autoreactive T cells. (B) MS patients have defects in peripheral immune regulation, including higher expression of costimulatory molecules on APCs, lower CTLA-4 levels, and lower IL-10 production. In addition, MS patients have an increased frequency of IFN- $\gamma$ -secreting Tregs relative to healthy controls. Thus the barrier for activation of autoreactive T cells is lowered for MS patients. Activated myelin-reactive T cells can then adhere to and extravasate across the choroid plexus and BBB, where they can initiate an inflammatory milieu that gives license to further waves of inflammation and eventual epitope spreading. APCs, Antigen-presenting cell; BBB, blood–brain barrier; MS, multiple sclerosis; TGF- $\beta$ , transforming growth factor- $\beta$ .

(Bonecchi et al., 2009). An increased proportion of T cells from MS patients was shown to express the characteristic Th1 chemokine receptor pattern and MS plaques were found to express increased levels of the corresponding chemokine (Siveke and Hamann, 1998; Balashov et al., 1999; Sorensen et al., 1999). The analysis of cytokine mRNA in CSF from MS patients showed a bias toward Th1 cytokines (Blain et al., 1994). Immunohistochemical studies of MS plaques *in situ* have demonstrated the presence of the proinflammatory cytokines TNF- $\alpha$  and IL-12 (Hofman et al., 1989; Selmaj et al., 1991; Windhagen et al., 1995). Markedly, MBP-reactive T cells derived from patients with MS secrete cytokines that are more consistent with Th1-mediated response, whereas MBP-reactive T cells from healthy individuals are more likely to produce cytokines that characterize a Th2-mediated response (Crawford et al., 2004). Interventions that shift or deviate the cytokine responses away from a Th1 and toward a Th2 profile have been deemed favorable.

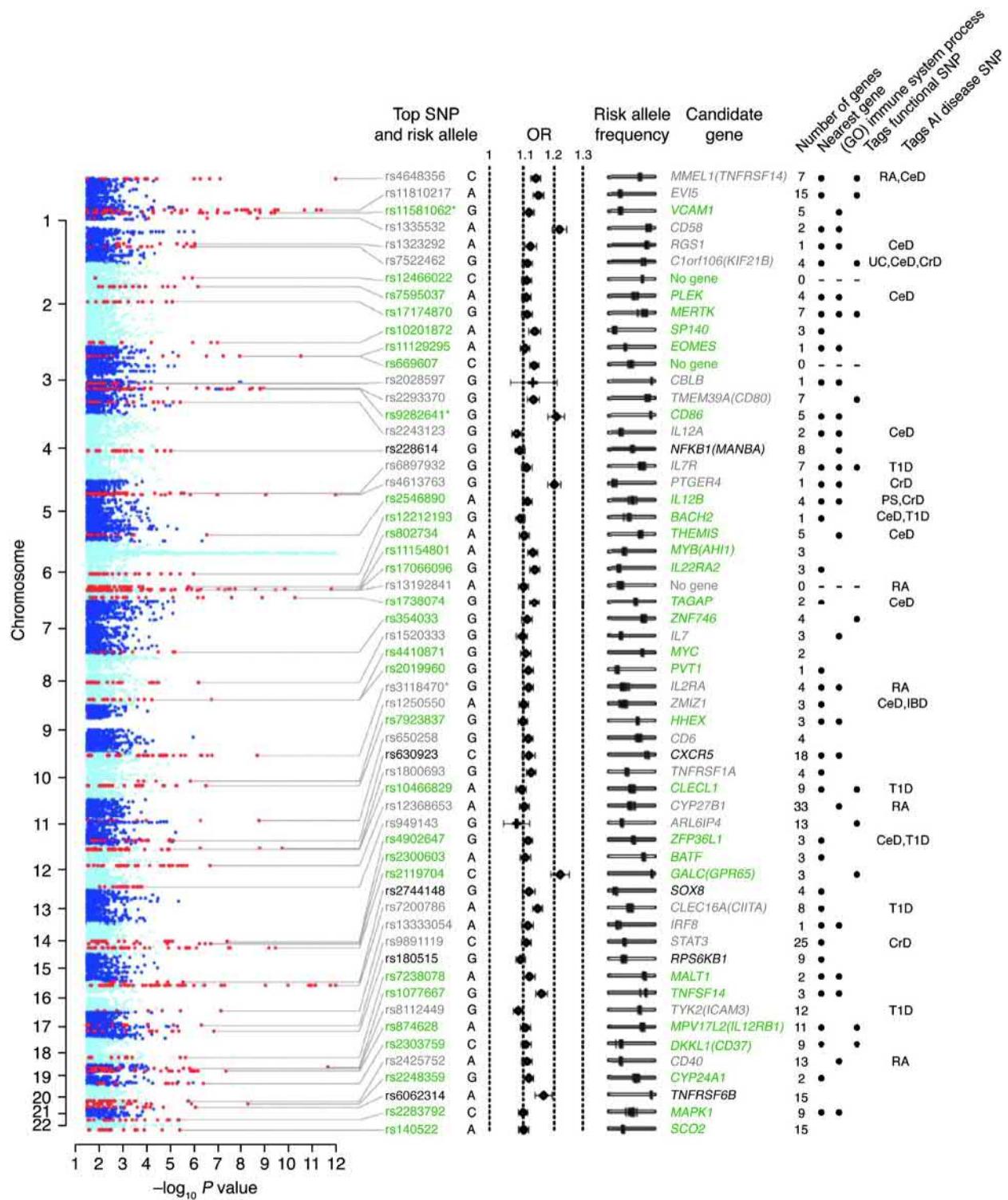
Despite the prior suggestion that Th1 cells are unique in driving the inflammatory response, recent work has characterized the putative role of proinflammatory Th17 cells in establishing the MS phenotype. Studies implicate pathogenic Th17 cells in gaining early access to the CNS (Steinman, 2007; Rebaldi et al., 2009). Entry to the CNS is mediated via the choroid plexus, which, in addition to producing CSF, spans the blood–CSF barrier and facilitates immune-surveillance. The Th17 mechanism implicates the CCR6/CCL20 axis in disease initiation as defined in the EAE model, with Th17 cells expressing CCR6 and choroid plexus endothelial cells expressing CCL20.

While myelin-specific autoreactive T cells are known to target myelin antigens, there was previously no characterization of the functional or transcriptional differences between these cells in healthy and MS populations. We recently compared responses of naïve CD4 $^{+}$  T cells, CCR6 $^{-}$  memory T cells, and CCR6 $^{+}$  memory T cells to various myelin peptides, including MBP, PLP, and MOG. It was found that naïve T cells and CCR6 $^{-}$  memory cells exhibited no response to the myelin epitopes. However, CCR6 $^{+}$  memory T cells produced GM-CSF, IFN- $\gamma$ , and IL-17 along with increasing their proliferation. Of note, in the absence of a myelin antigen, there was no difference in response amongst the populations. Moreover, this study found that CCR6 $^{+}$  memory cells were increased in number in MS patients (Cao et al., 2015). Finally, it was found that myelin-reactive T cells from healthy subjects secreted predominantly IL-10, explaining perhaps why healthy individuals have autoreactive T cells that we speculate may be involved in mediating responses to tissue damage.

In applying this information to human disease, it is likely that peripherally activated Th17 cells are able to bind to adhesion molecules and chemokine receptors expressed on the choroid plexus thereby migrating across the blood–CSF barrier and gaining access to circulating CSF. Dysregulated Th17 cells are subsequently able to access perivascular tissue, initiating a cascade of proinflammatory events. Th17 cells secrete IL-23, which, in EAE models, alongside the transcription factor ROR $\gamma$ t, promotes production of GM-CSF. This induces a positive feedback loop where further secretion of IL-23 occurs and is implicated in the encephalogenicity and incidence of axoglial damage (Codarri et al., 2011; El-Behi et al., 2011). In relating Th17 cells to the formation of perivascular infiltrates, it is possible that their secretion of IL-17 and IL-22 may increase BBB permeability and thus facilitate the influx of immune cells, such as autoreactive Th17 cells, Th1 (IFN- $\gamma$  secreting),  $\gamma\delta$  T cells, cytotoxic CD8 $^{+}$  cells, B cells, and immunoglobulin-secreting plasma cells (Kebir et al., 2007). This leads to the possibility of a two-step process for initiation of MS, one in which Th17 cells prime the entrance of other dysregulated immune cells and therefore creating the appropriate inflammatory environment containing infiltrates of cells that result in downstream damage of the CNS (Nylander and Hafler, 2012).

## Immune Dysregulation

In elaborating on the concept of immune dysregulation, GWAS have shed light on the likely involvement of allelic variants in diseases states (see Fig. 51.2) (Sawcer et al., 2011). A number of these variants involved genes for cytokine receptors and costimulatory molecules and have been associated with defects in Treg homeostasis. Costimulatory molecules have recently been found to function as negative regulators of the immune system. TIM-3 has been implicated in modulating Th1 and Th17 cytokine secretion and loss of TIM-3 functional T-cell regulation has been established in MS patients (Koguchi et al., 2006; Yang et al., 2008; Hastings et al., 2009). GWAS studies have linked CD226 to MS risk (Hafler et al., 2009), and recent work has implicated the CD226/TIGIT axis in regulating human T-cell function via a mechanism similar to that employed by CD28/CTLA4, in which binding to B7 induces coexpression of inhibitory and excitatory signals that modulate immune responses (Joller et al., 2011; Lozano et al., 2012). Costimulation between CD58/CD2 appears to be implicated in T-cell receptor signaling, including activation of Tregs, and has been suggested to provide a protective effect for MS (De Jager et al., 2009a).



**FIGURE 51.2** Regions of genome showing association to MS. Genome regions showing association with MS. Evidence for association from linear mixed model analysis of the discovery data (threshold at a  $2 \log_{10} P$  value of 12) is shown at left. Non-MHC regions containing associated SNPs are indicated in red and labeled with the rs number (green text for newly identified loci, black text for loci with strong evidence of association, and gray text for previously reported loci) and risk allele of the most significant SNP. Asterisks indicate that the locus contains a secondary SNP signal. ORs (diamonds) and 95% confidence intervals (whiskers) are estimated from a metaanalysis of discovery and replication data (1 indicates estimates for previously known loci from discovery data only). Risk allele frequency estimates in the control populations are indicated by vertical bars (scale of 0–1, left to right). A candidate gene and the number of genes are reported for each region of association. Black dots indicate that the candidate gene is physically the nearest gene included in the GO immune system process term. “Tags functional SNP” indicates whether the most significant SNP tags a SNP predicted to affect the function of the candidate gene. Where such an SNP exists, the gene is selected as the candidate gene; otherwise, the nearest gene is selected unless there are strong biological reasons for a different choice. The final column indicates whether SNPs are correlated ( $r^2 > 0.1$ ) with SNPs associated with other autoimmune diseases. CeD, Celiac disease; CrD, Crohn’s disease; PS, psoriasis; RA, rheumatoid arthritis; T1D, type 1 diabetes; UC, ulcerative colitis; OR, odds ratios; MHC, major histocompatibility complex; SNPs, single nucleotide polymorphisms; MS, multiple sclerosis. Source: Reproduced with permission from Sawcer, S., Hellenthal, G., Pirinen, M., Spencer, C.C., Patsopoulos, N.A., Moutsianas, L., et al., 2011. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature* 476, 214–219.

Despite normal frequency of Tregs in MS patients, their suppressive ability is compromised and substantially decreased in response to autoreactive T cells in comparison to healthy individuals (Viglietta et al., 2004; Haas et al., 2005). Notably, Tregs have demonstrated great functional plasticity. Their ability to be reprogrammed suggests that Tregs may be able to function as a biomarker in MS (Nylander and Hafler, 2012). In the presence of IL-1 $\beta$  and IL-6, Tregs are able to produce the inflammatory cytokine IL-17, further implicating this subset in autoimmune disease (Koenen et al., 2008; Ayyoub et al., 2009; Beriou et al., 2009). Moreover, the notion of Treg reprogramming has arisen due to the observation that patients with RRMS have the ability to produce IFN- $\gamma$ -secreting Tregs, thereby characterizing a Th1-type Treg effector phenotype. This finding was observed in vitro under IL-12 stimulation of Tregs and recapitulated in ex vivo Tregs derived from patients with untreated RRMS. This role of Th1/Treg effector cells was suggested by the use of IFN- $\beta$ , a first-line therapy for RRMS, which has been shown to decrease IL-12 levels and normalize the ratio of IFN- $\gamma$ Foxp3 $^{+}$  Tregs to that of healthy controls (Dominguez-Villar et al., 2011). Costimulatory molecules and Treg plasticity in MS undoubtedly provide clues into disease pathogenesis.

## Autoantigens

Autoantigen-specific T cells have been identified both in healthy individuals and in patients with MS. Their entrance into the inflamed CNS environment is likely mediated by the immune mechanisms posited above, whereupon entering, the autoreactive T cells are able to subsequently contribute to myelin destruction and axonal damage in addition to secondary inflammation following activation by local APCs. Attention has been directed toward uncovering frequencies of autoantigen-specific T-cell binding to myelin proteins including MOG, MBP, and proteolipid in addition to heat shock protein  $\alpha$ B-crystallin and oligodendroglia-specific enzyme transaldolase in CD4 cells isolated from MS patients (Banki et al., 1994; Greer and Pender, 2008). Anti-MOG has previously been isolated from postmortem CNS tissue in MS patients (O'Connor et al., 2005). In an assay based on self-assembly of radiolabeled MHC tetramers, it was demonstrated that such tetramers were more sensitive for MOG autoantibody detection. Nevertheless, MOG-specific autoantibodies were found more so in patients with acute disseminated encephalomyelitis than in adults with MS (O'Connor et al., 2007). Likewise, the ability to detect and clone autoantigen-specific T cells from blood has allowed further quantification of MOG frequencies, work which demonstrated an increase in MOG-specific T cells in MS patients in comparison to healthy individuals (Raddassi et al., 2011).

Despite the inability to identify a putative role for environmental triggers, infectious factors, or microbial antigens in establishing MS risk, studies have suggested potential cross-reactivity between epitopes with microbial antigens. Finally, regardless of what antigen event initiates the self-reactive cascade, epitope spreading is likely to contribute to an array of activated immune cells that can respond to multiple antigens. Nevertheless, no single antigen has been clearly implicated in the pathogenesis of MS.

## The Role of B Cells

Given the marked presence of OCBs and increased intrathecal synthesis of IgG within the CSF of individuals with MS, in addition to the ongoing debate about the increased incidence of EBV and its ability to prime the immune system in individuals with MS, the notion of B-cell involvement has received great attention in playing a crucial role in MS pathogenesis. There appears to be great connectivity between the cell population in the CNS and CSF since clonally expanded B cells and plasmablast clones have been associated with the observed intrathecal immunoglobulin production (Lovato et al., 2011; Obermeier et al., 2011). There is evidence that B-cell clones populate the meninges, leading to the hypothesis that these aggregate structures can be related to the B-cell infiltrates found in MS lesions (Lovato et al., 2011). Likewise, ectopic lymphoid follicles, strongly resembling germinal centers, have been identified in the meninges of SPMS patients (Serafini et al., 2004). These follicles contain proliferating B cells, plasma cells, T cells, and dendritic cells with the corresponding diffuse meningeal inflammation being suggested to play a role in the manifestation of cerebral cortical gray matter pathology in MS. Follicles have been located more frequently in the deep sulci of the temporal cingulate, insula, and frontal cortex (Howell et al., 2011).

Moreover, the success of B-cell depleting agents in treating rapidly reducing inflammation and inducing remission (Hauser et al., 2008) suggests that B cells might be serving as APCs via MHC class II to autoreactive T cells and thus mediating the cascade of proinflammatory cytokine production within the CNS (Cross et al., 2006).

Furthermore, there is evidence from EAE mice that B cells might directly produce proinflammatory cytokines, such as IL-6 (Barr et al., 2012). Apart from being associated with cortical pathology, studies have implicated that meningeal B-cell follicles can be associated with early onset of disease in patients with SPMS (Maglizzi et al., 2007). Overall, such humoral activation within ectopic lymph tissue and CSF has been postulated to play an imperative role in disease progression secondary to the ongoing persistence of antigens driving a constitutive inflammatory and humoral response.

## TREATMENT

Therapeutic approaches in MS may be broadly divided into treatments that are symptomatic and/or supportive in nature and treatments that are directed at the underlying pathophysiology of the disorder. There are currently 15 agents approved by the United States FDA (see Table 51.1 for comparison of FDA-approved therapies) as disease-modifying therapies. They are expensive, with 10-year disease-related costs averaging US \$467,712 for patients on a single disease-modifying therapy (DMT) (Noyes et al., 2011). Immunotherapies as of 2017 are available in a number of administration forms: infusion-based, oral, and self-injection medications.

With only IFN- $\beta$  and glatiramer acetate, it was not uncommon for neurologists to take a “stepwise” approach to initiation of therapy, starting with the lower efficacy drugs first and then escalating care in conjunction with breakthrough disease to more immunosuppressive drugs such as cyclophosphamide. Now with the availability of more effective medications with similar risk profiles, it is no longer appropriate to allow patients to fail multiple agents before using an effective drug. Immunotherapies have shown the most efficacy in the RRMS, CIS, and early progressive forms of MS wherein there exists an inflammatory component. There is only one agent approved for PPMS; however, to date, there is no immunotherapy that has shown significant response in progressive forms of MS, such as SPMS and PPMS.

### Infusion-Based Therapies

The most effective FDA-approved immunotherapies for MS to date are the infusion-based medications, ocrelizumab (Ocrevus), alemtuzumab (Lemtrada), and natalizumab (Tysabri). Of note, mitoxantrone (Novantrone) is also an FDA-approved infusion which will be discussed, but given severe adverse effects, it is no longer used in modern practice.

#### **Natalizumab (Tysabri)**

Natalizumab is a monoclonal antibody to the very late activation antigen-4 (VLA-4), the  $\alpha 4\beta 7$  integrin expressed on activated T cells and monocytes, and is the ligand for vascular cell adhesion molecule (VCAM) expressed on CNS endothelial cells. Natalizumab, by preventing adhesion of activated T cells to endothelial cells, has been able to decrease influx of potentially autoreactive T cells into perivascular tissue. Natalizumab-treated patients have developed cases of progressive multifocal leukoencephalopathy, a rare and fatal disease caused by JC virus and characterized by progressive inflammatory damage of CNS white matter. This prompted both its manufacturer and the FDA to review its utility in treating MS. Patients must be screened for the JC virus in blood prior to initiating therapy. If negative, natalizumab can be safely started, and patients/prescribers are required to enroll in a REMS program (risk evaluation and mitigation strategy). JC virus is checked every 6 months, and if a patient converts to being JC virus positive, then the patient must transition to a new DMT. The timing of the transition is dependent on a number of factors. More recently, with the advent of JC virus index value testing using a two-step ELISA, the index value can risk-stratify patients for progressive multifocal leucoencephalopathy (PML).

Regardless, its clinical benefits have been deemed to outweigh the risks involved. Its use has been restricted to individuals with highly active disease. Two trials involving RRMS patients have demonstrated interesting clinical results. The AFFIRM study compared natalizumab alone to placebo and the SENTINEL study looked at adding natalizumab to ongoing IFN- $\beta 1a$  therapy. The AFFIRM trial demonstrated a 42% reduced risk of sustained progression of disability, a 68% reduction in rate of clinical relapse at 1 year, and an 83% reduction in accumulation of new or enlarging hyperintense lesions over 2 years (Polman et al., 2006). Results of the SENTINEL trial were similar to those of AFFIRM with the exception of a 64% decreased risk of sustained disability progression with natalizumab (Calabresi et al., 2007; Hutchinson et al., 2009).

**TABLE 51.1** Food and Drug Administration Approved Therapies for Multiple Sclerosis (MS) as of 2018

Brand name	Indications	Results	Mechanism of action
<b>T-CELL CYTOKINE DEVIATION</b>			
IFN- $\beta$ 1a (IM weekly)	Avonex	Treatment of RRMS	<p>Reduction of relapses and new MRI T2 lesions by about one-third and the volume of enlarging T2 lesions; reduction in the number and volume of Gd-enhancing lesions</p> <p>Slowing of brain atrophy</p> <ul style="list-style-type: none"> <li>• Acts on blood–brain barrier by interfering with T-cell adhesion to the endothelium by binding VLA-4 on T cells or by inhibiting the T-cell expression of MMP reduction in T-cell activation by interfering with HLA class II and costimulatory molecules B7/CD28 and CD40: CD40L</li> <li>• Immune deviation of Th2 over Th1 cytokine profile</li> <li>• Normalizing ratio of IFN-<math>\gamma</math> Foxp3 Tregs</li> </ul>
IFN- $\beta$ 1a (SC three times weekly)	Rebif	Treatment of RRMS	Same as IFN- $\beta$ 1a
Peginterferon- $\beta$ 1a (SC every 14 days)	Plegridy	Treatment of RRMS	Same as IFN- $\beta$ 1a
IFN- $\beta$ 1b (SC every other day)	Betaseron, Extavia	Treatment of RRMS	Same as IFN- $\beta$ 1a
<b>T-CELL RECEPTOR-MEDIATED CYTOKINE DEVIATION</b>			
Glatiramer acetate (SC daily)	Copaxone	Treatment of RRMS	<p>Reduction of relapses by one-third</p> <p>Reduction of 57% in the number and volume of Gd-enhancing lesions (Johnson et al., 1995)</p> <ul style="list-style-type: none"> <li>• Induces cytokine shift from one that is proinflammatory to one that is antiinflammatory and regulatory in nature (Duda et al., 2000)</li> </ul>
<b>BLOCK T-CELL TRAFFICKING</b>			
Fingolimod (given orally once a day)	Gilenya	Treatment of RRMS	<p>Reduction of 54% in risk of relapse</p> <p>Lower risk of disability progression by 30% (Cohen et al., 2010)</p> <ul style="list-style-type: none"> <li>• S1P agonist, causing internalization of S1P1 receptors on lymph-node T cells</li> <li>• Subsequent decrease in migration of activation lymphocytes into circulation</li> </ul>
Natalizumab (IV monthly infusion)	Tysabri	Treatment of RRMS	<p>Reduced rate of relapse up to 68% and the development of new MRI lesions by 83% (Polman et al., 2006)</p> <p>Monoclonal antibody that blocks alpha 4-integrin on surface T cells preventing them from crossing the blood–brain barrier</p> <p>Nature Steinman Yevodnick</p>
<b>METABOLIC INHIBITOR OF PYRIMIDINE SYNTHESIS</b>			
Teriflunomide (given orally once a day)	Aubagio	Treatment of RRMS	<p>Reduction of 36% in risk of relapse</p> <p>Lower risk of disability progression by 31% (O'Connor et al., 2011)</p> <ul style="list-style-type: none"> <li>• Inhibits de novo nucleotide synthesis</li> <li>• Decreases T-cell and B-cell proliferation</li> <li>• Interrupts T-cell and APC interactions</li> <li>• Subsequently possesses antiinflammatory properties</li> </ul>
<b>MODULATION OF NRF2 PATHWAY/INHIBITION OF AEROBIC GLYCOLYSIS</b>			
BG-12, dimethyl fumarate (given orally twice a day)	Tecfidera	Treatment of RRMS	<p>Reduction of 49% in risk of relapse</p> <p>Lower risk of disability progression by 38% (Fox et al., 2012; Gold et al., 2012)</p> <ul style="list-style-type: none"> <li>• Inhibits immune cells and molecules</li> <li>• Decreases myelin damage in the CNS</li> <li>• Exhibits antioxidant properties that may be protective against damage to the brain and spinal cord</li> </ul>
<b>LYMPHOCYTE DEPLETION</b>			
Alemtuzumab	Lemtrada	Treatment of RRMS	<p>78% relapse free at 2 years compared to 59% IFN-<math>\beta</math>1a; ~1/3 develop autoimmune thyroid disease, ~2% develop idiopathic thrombocytopenia (Cohen et al., 2012; Coles et al., 2012)</p> <p>Humanized CD52-directed monoclonal antibody that causes depletion and repopulation of B lymphocytes and T lymphocytes</p>
Ocrelizumab	Ocrevus	Treatment of RRMS and PPMS	<p>RRMS: 47% ARR reduction compared to Rebif; 95% decrease in enhancing T1 lesions</p> <p>PPMS: 24% reduction in progression of EDSS compared to placebo at 12 weeks; 25% reduction in sustained 24-week disability (Hauser et al., 2017; Montalban et al., 2017)</p> <p>Fully humanized monoclonal antibody targeting CD20-positive B cells</p>

RRMS, Relapsing-remitting MS; Tregs, regulatory T cell; PPMS, primary progressive MS; CNS, central nervous system; APC, antigen-presenting cell; IFN, interferon; VLA-4, very late activation antigen-4; EDSS, Extended disability status scale; MMP, metalloproteinase.

### Alemtuzumab (Lemtrada)

Alemtuzumab is a humanized CD52-directed monoclonal antibody that causes depletion and repopulation of B lymphocytes and T lymphocytes. It was approved by the FDA in 2014 for use in RRMS, with a requirement that patients be enrolled in a REMS program due to its significant side-effect profile. It is given via infusion on 5 consecutive days at month 0 and then 3 consecutive days at month 12. In the CARE-MS I&II trials, it showed that 78% of the patients were relapse free at 2 years compared to 59% of the patients in an IFN- $\beta$ 1a group. About one-third of the patients develop autoimmune thyroid disease, with around 2% developing idiopathic thrombocytopenia, as well as other autoimmune conditions in lesser numbers (Cohen et al., 2012; Coles et al., 2012).

### Ocrelizumab (Ocrevus)

Ocrelizumab (Ocrevus) is fully humanized monoclonal antibody targeting CD20-positive B cells that was approved for both RRMS and PPMS in 2017. It was developed based on the success of the chimeric CD20 monoclonal antibody rituximab that has been used off-label for MS for several years. The OPERA I&II trials for RRMS showed a 47% ARR reduction compared to Rebif along with a 95% decrease in enhancing T1 lesions. These data suggest that at this time, Ocrelizumab should be considered as a first-line therapy for new onset RRMS.

The ORATORIO trial for PPMS showed a 24% reduction in progression of extended disability status scale (EDSS) compared to placebo at 12 weeks with a 25% reduction in sustained 24-week disability; however, the success in PPMS was strongly correlated with patients who were younger and still had evidence of inflammatory disease activity (Hauser et al., 2017; Montalban et al., 2017).

### Mitoxantrone (Novantrone)

Mitoxantrone, a small molecule chemotherapeutic agent able to cross the BBB, functions as a type II topoisomerase inhibitor, which disrupts DNA synthesis and DNA repair, both events which function in an immunosuppressant capacity thereby inhibiting T-cell, B-cell, and macrophage proliferation. Overall, this leads to enhanced T-cell suppressor function, inhibition of B-cell function and antibody production, decreased secretion of proinflammatory cytokines, and inhibition of macrophage-mediated myelin degradation (Fox, 2004). Mitoxantrone, administered intravenously, has been approved by the FDA as treatment for patients with worsening forms of MS including SPMS, worsening RRMS, and PRMS. The MIMS trial indicated a 44% reduction in time to first relapse; a 24% decrease in the expanded disability status scale, however, did not demonstrate a positive impact on MRI disease burden (Hartung et al., 2002; Krapf et al., 2005). Due to concerns related to increased incidence of systolic dysfunction and therapy-related acute leukemia, it is no longer used in modern MS treatment (Marriott et al., 2010).

## Oral Therapies

There are currently three FDA-approved oral medications for MS, starting with fingolimod (Gilenya) which was the first to be approved in 2010, followed by teriflunomide (Aubagio) and dimethyl fumarate, BG-12 (Tecfidera).

### Fingolimod (Gilenya)

Fingolimod's pharmacologic activity is targeted toward lymphocyte migration out of lymph nodes. This action is highly dependent on the engagement of a G-protein-coupled receptor, S1P<sub>1</sub>, present on the surface of the lymphocytes. Fingolimod is structurally similar to S1P and can function as an agonist by engaging four of the five known S1P receptors (S1P<sub>1</sub>, S1P<sub>3</sub>, S1P<sub>4</sub>, S1P<sub>5</sub>). This leads to a reduction in activated T cells that are able to exit the lymph node and subsequently cross the BBB to exert their potential pathogenic effects on perivascular tissue (Schwab and Cyster, 2007; Pham et al., 2008). Studies have indicated the potential for S1P receptors to be present on other cells, including neurons, microglial cells, oligodendrocytes, and astrocytes, suggesting a putative role for fingolimod in influencing myelin repair, modulating survival of oligodendrocyte progenitor cells, and directing astrocyte migration and proliferation (Yamagata et al., 2003; Miron et al., 2008, 2010).

In September 2010 fingolimod became the first FDA-approved first-line oral agent for the treatment of MS. The FREEDOMS trial demonstrated, after 2 years, an overall 54% decreased risk of relapse in the group treated with fingolimod versus those taking placebo. The risk of disability progression was 30% lower in patients receiving the lower dose (0.5 mg) as opposed to placebo. With regard to MRI disease burden, those taking fingolimod

presented with fewer new lesions and less brain tissue atrophy. The TRANSFORMS trial aimed at characterizing the efficacy of fingolimod versus intramuscular IFN- $\beta$ 1a (Avonex). The group taking fingolimod had a 52% lower risk of having a relapse than those taking IFN- $\beta$ 1a, with 82.5% of the fingolimod group and 70% of the IFN- $\beta$ 1a presenting with no relapses during the 1-year study period. Furthermore, the group taking fingolimod had fewer signs of MRI disease burden. There was no difference among the groups in the risk of disease progression (Cohen et al., 2010).

### **Teriflunomide (Aubagio)**

Teriflunomide is the active metabolite of leflunomide, an approved therapy for rheumatoid arthritis. It inhibits de novo pyrimidine nucleotide synthesis and therefore potently decreases T-cell and B-cell proliferation (Hartung et al., 2010). Reports also indicate that teriflunomide may interrupt T cell and APC interactions in addition to possessing antiinflammatory properties (Zeyda et al., 2005; Gold and Wolinsky, 2011). The TEMSO trial demonstrated a 31% reduction in annual relapse rate, a 21% reduction in disability progression, and a 76% reduction in number of new or enlarging T2 lesions on MRI in the higher dose cohort (O'Connor et al., 2011). Although FDA pregnancy categories A–X have been rendered obsolete, it was initially designated as Category X due to severe teratogenicity concerns. Main side effects include alopecia, transaminitis, and reactivation of viral infections including varicella zoster virus (VZV). Teriflunomide was approved by the FDA in September 2012 for patients with RRMS.

### **Dimethyl Fumarate, BG-12 (Tecfidera)**

BG-12 is an oral formulation of fumaric acid, which is metabolized to monomethyl fumarate. It was approved to be a second-line agent in 2013. Other oral formulations of fumaric acid have previously been used to treat psoriasis. Both dimethyl fumarate and the active metabolite induce activation of the nuclear factor E2-related factor-2 pathway, which exerts neuroprotective effects and decreases myelin damage in the CNS (Kappos et al., 2008; Fontoura and Garren, 2010; Linker et al., 2011). Antiinflammatory mechanisms have also been attributed to dimethyl fumarate (Gold, 2011). The DEFINE trial was completed in 2011. As a randomized, double-blind, placebo-controlled phase III study, patients with RRMS were assigned to receive either oral BG-12 at a dose of 240 mg twice or thrice daily, or placebo. The primary end point was the proportion of patients who had a relapse 2 years later in addition to disability progression and MRI disease burden. The study demonstrated positive results with a significant reduction in relapse rate (27% with BG-12 twice daily and 26% with BG-12 thrice daily versus 46% with placebo), in the rate of disability progression, in the number of new or enlarging T2 lesions, and in new gadolinium-enhancing lesions (Gold et al., 2012). The CONFIRM trial was also completed in 2011. In a phase III, randomized study, it aimed to ascertain the efficacy and safety of BG-12 at a dose of 240 twice or thrice daily in comparison to both placebo and glatiramer acetate in patients with RRMS. Similar to the DEFINE trial, both BG-12 and glatiramer acetate significantly reduced relapse rates and MRI disease burden in relation to the placebo group (Fox et al., 2012). In early 2013, BG-12 was approved as a first-line therapy for adults with RRMS.

## **Injection-Based Therapies**

The injection-based therapies are the oldest FDA-approved treatments for MS, with the exception of daclizumab (Zinbryta) which was recently approved in 2016 for both RRMS and early SPMS. This group includes the interferons, glatiramer acetate, and daclizumab.

### **Interferons**

Compared to placebo-treated RRMS controls, treatment with alternate-day subcutaneous injections of 8 million units of IFN- $\beta$ 1b (Betaseron) was shown to decrease the primary efficacy outcome measure of frequency of relapses by 34% after 2 years ([No authors listed], 1993). A significant decrease in the accumulation of MRI lesions was observed with treatment (Paty and Li, 1993) and 5-year follow-up data reported that disease progression in the IFN- $\beta$ 1b-treated group was 35%, compared with 46% progression in the placebo group ([No authors listed], 1995). A 30% decrease in the annual exacerbation rate in the treated group was maintained. IFN- $\beta$ 1a (Avonex, weekly IM injections), a glycosylated recombinant beta-interferon, was evaluated in a 2-year study of weekly intramuscular injections of six million units (30  $\mu$ g). The proportion of patients progressing by the end of the trial was 21.9% in the treated group compared to 34.9% in the placebo group. The annual exacerbation rate

was decreased by 32% in the treated group versus the placebo group. Treatment was also associated with a 40% reduction in mean MRI lesion load (Jacobs et al., 1995, 1996). Clinical and MRI benefits of IFN- $\beta$ 1a (Rebif, subcutaneous, three times weekly) doses for up to 4 years were demonstrated in PRISMS-4, thereby suggesting that early treatment with IFN- $\beta$ 1a was of increased benefit to patients with RRMS as compared with those treated later in disease course (PRISMS, 2001).

$\beta$ -Interferon therapy utilizes recombinant forms of naturally occurring cytokines intrinsically possessing a wide range of properties. The mechanism of action involving  $\beta$ -interferons appears to be quite complex with the DMT exerting its effects on the pathophysiology of MS at several sites. Studies have suggested that  $\beta$ -interferons inhibit the migration of activated inflammatory cells across the BBB and into the CNS parenchyma through decreasing the function of the VCAM-1/very late activation antigen-4 cell adhesion axis (see natalizumab). IFN- $\beta$  may potentially intercept inflammatory cell adhesion and subsequent migration across the BBB (Calabresi et al., 1997; Gruber et al., 2005). The rapid effect of  $\beta$ -interferons on the gadolinium-enhancing lesions of MS represents a biological marker of treatment response and establishes the BBB as an important site of action of  $\beta$ -interferon therapy (Stone et al., 1997).

Studies have also suggested that the  $\beta$ -interferons may exert their pharmacologic effects by shifting cytokines and immune cell profiles in MS patients toward one that is antiinflammatory and protective (Brod et al., 1996). IFN- $\beta$  has also demonstrated ability to upregulate expression of costimulatory molecules needed for antigen presentation (CD80, CD86, and CD40) on monocytes, thus decreasing the generation of autoreactive T cells and limiting T-cell activation. As addressed earlier, IFN- $\beta$  has been implicated in normalizing the ratio of IFN- $\gamma$ <sup>1</sup>Foxp3<sup>1</sup> Tregs to that of healthy controls and in decreasing IL-12 levels, thereby potentially restoring the ability of Tregs to regulate immune cells (Dominguez-Villar et al., 2011).

### **Glatiramer Acetate (Copaxone)**

Treatment with glatiramer acetate/copolymer 1 (Copaxone, GA, subcutaneous, daily) was associated with a 2-year relapse rate reduction of 29% compared to placebo control (Johnson et al., 1995). The clinical benefit of GA for relapse rate in comparison to placebo was sustained following an 11-month extension period utilizing the same study design (Johnson et al., 1998). Subsequent studies revealed a beneficial effect of GA in MRI with a 35% reduction in total gadolinium-enhancing lesions and 57% reduction in new gadolinium-enhancing lesions, with an overall decrease in T2 disease burden (Mancardi et al., 1998; Comi et al., 2001).

GA is a random sequence polypeptide of the four amino acids alanine (A), lysine (K), glutamate (E), and tyrosine (Y). As a sequence of amino acids, GA has been proposed to function as a T-cell receptor antagonist to MBP/MHC at MBP-specific T-cell receptors and operate as an altered peptide ligand to the MBP (Aharoni et al., 1999; Duda et al., 2000). Thus research suggests that GA elicits its effect on the immune system by inducing deviation of cytokine production in response to MBP from Th1 cytokines to Th2 cytokines, a change that is characterized by increased secretion of antiinflammatory cytokines, with prolonged GA treatment leading to a Th2 bias in MS patients (Miller et al., 1998; Duda et al., 2000; Gran et al., 2000; Neuhaus et al., 2000; Valenzuela et al., 2007). In addition, GA has been implicated in altering the cytokine profile to one that is more consistent with a regulatory population (Hong et al., 2005). Furthermore, increases in FoxP3<sup>1</sup> expression in Tregs have been demonstrated following GA, also supporting theories that GA facilitates a regulatory population (Hong et al., 2005).

## **CONCLUDING REMARKS**

Over the past decade, major strides have been made in our understanding of the immunopathogenesis underlying the development and course of MS, an autoimmune disease that predominantly impacts the CNS with white matter damage and demyelination of neurons thought to be primarily driven by T-cell dysregulation, inflammation, and immune dysfunction. Nevertheless, our ever-growing fund of knowledge continues to inspire future directions. Novel insights are providing hints for future prospects related to early cortical demyelination, gray matter pathology, imaging, and B-cell involvement in MS further establishing this disease as a multifocal entity. Overall, our increasing knowledge pertaining to MS continues to be multifactorial. The ability for clinicians and researchers to utilize our understanding of the immunopathogenesis in relation to known pharmacologic mechanisms, clinical findings, and imaging modalities will be paramount in taking the necessary steps toward eradicating MS.

## References

- Abbas, A.K., Murphy, K.M., Sher, A., 1996. Functional diversity of helper T lymphocytes. *Nature* 383, 787–793.
- Achiron, A., Barak, Y., 2000. Multiple sclerosis—from probable to definite diagnosis: a 7-year prospective study. *Arch. Neurol.* 57, 974–979.
- Aharoni, R., Teitelbaum, D., Arnon, R., Sela, M., 1999. Copolymer 1 acts against the immunodominant epitope 82-100 of myelin basic protein by T cell receptor antagonism in addition to major histocompatibility complex blocking. *Proc. Natl. Acad. Sci. U.S.A.* 96, 634–639.
- Amato, M.P., Bartolozzi, M.L., Zipoli, V., Portaccio, E., Mortilla, M., Guidi, L., et al., 2004. Neocortical volume decrease in relapsing-remitting MS patients with mild cognitive impairment. *Neurology* 63, 89–93.
- Antel, J.P., Owens, T., 1999. Immune regulation and CNS autoimmune disease. *J. Neuroimmunol.* 100, 181–189.
- Arnason, B.G., 1993. Interferon beta in multiple sclerosis. *Neurology* 43, 641–643.
- Ascherio, A., Munger, K.L., Simon, K.C., 2010. Vitamin D and multiple sclerosis. *Lancet Neurol.* 9, 599–612.
- Aulchenko, Y.S., Hoppenbrouwers, I.A., Ramagopalan, S.V., Broer, L., Jafari, N., Hillert, J., et al., 2008. Genetic variation in the KIF1B locus influences susceptibility to multiple sclerosis. *Nat. Genet.* 40, 1402–1403.
- Australia and New Zealand Multiple Sclerosis Genetics Consortium, 2009. Genome-wide association study identifies new multiple sclerosis susceptibility loci on chromosomes 12 and 20. *Nat. Genet.* 41, 824–828.
- Ayyoub, M., Deknuydt, F., Raimbaud, I., Dousset, C., Leveque, L., Bioley, G., et al., 2009. Human memory FOXP3<sup>+</sup> Tregs secrete IL-17 ex vivo and constitutively express the T(H)17 lineage-specific transcription factor ROR $\gamma$ t. *Proc. Natl. Acad. Sci. U.S.A.* 106, 8635–8640.
- Babbe, H., Roers, A., Waisman, A., Lassmann, H., Goebels, N., Hohlfeld, R., et al., 2000. Clonal expansions of CD8(+) T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. *J. Exp. Med.* 192, 393–404.
- Bagert, B.A., 2009. Epstein-Barr virus in multiple sclerosis. *Curr. Neurol. Neurosci. Rep.* 9, 405–410.
- Balashov, K.E., Rottman, J.B., Weiner, H.L., Hancock, W.W., 1999. CCR5(+) and CXCR3(+) T cells are increased in multiple sclerosis and their ligands MIP-1alpha and IP-10 are expressed in demyelinating brain lesions. *Proc. Natl. Acad. Sci. U.S.A.* 96, 6873–6878.
- Banki, K., Colombo, E., Sia, F., Halladay, D., Mattson, D.H., Tatum, A.H., et al., 1994. Oligodendrocyte-specific expression and autoantigenicity of transaldolase in multiple sclerosis. *J. Exp. Med.* 180, 1649–1663.
- Baranzini, S.E., Wang, J., Gibson, R.A., Galwey, N., Naegelin, Y., Barkhof, F., et al., 2009. Genome-wide association analysis of susceptibility and clinical phenotype in multiple sclerosis. *Hum. Mol. Genet.* 18, 767–778.
- Barr, T.A., Shen, P., Brown, S., Lampropoulou, V., Roch, T., Lawrie, S., et al., 2012. B cell depletion therapy ameliorates autoimmune disease through ablation of IL-6-producing B cells. *J. Exp. Med.* 209 (5), 1001–1010.
- Bates, D., 2011. Treatment effects of immunomodulatory therapies at different stages of multiple sclerosis in short-term trials. *Neurology* 76, S14–S25.
- Beebe, G.W., Kurtzke, J.F., Kurland, L.T., Auth, T.L., Nagler, B., 1967. Studies on the natural history of multiple sclerosis. 3. Epidemiologic analysis of the army experience in World War II. *Neurology* 17, 1–17.
- Begolka, W.S., Vanderlugt, C.L., Rahbe, S.M., Miller, S.D., 1998. Differential expression of inflammatory cytokines parallels progression of central nervous system pathology in two clinically distinct models of multiple sclerosis. *J. Immunol.* 161, 4437–4446.
- Behan, P.B., 1982. Sir Robert Carswell: Scotland's Pioneer Pathologist. Raven Press, New York.
- Berger, T., Rubner, P., Schautzer, F., Egg, R., Ulmer, H., Mayringer, I., et al., 2003. Antimyelin antibodies as a predictor of clinically definite multiple sclerosis after a first demyelinating event. *N. Engl. J. Med.* 349, 139–145.
- Beriou, G., Costantino, C.M., Ashley, C.W., Yang, L., Kuchroo, V.K., Baecher-Allan, C., et al., 2009. IL-17-producing human peripheral regulatory T cells retain suppressive function. *Blood* 113, 4240–4249.
- Betts, C.D., D'Mellow, M.T., Fowler, C.J., 1993. Urinary symptoms and the neurological features of bladder dysfunction in multiple sclerosis. *J. Neurol. Neurosurg. Psychiatry* 56, 245–250.
- Bitsch, A., Churchyard, J., Bukowski, S., Ullmann, T., Brock, W., 2000. Acute axonal injury in multiple sclerosis. Correlation with demyelination and inflammation. *Brain* 123 (Pt 6), 1174–1183.
- Blain, M., Alkanol, J., Antel, J., 1994. Interferon-gamma mRNA expression in immediately ex-vivo CSF T cells. *J. Neuroimmunol.* 54, 149 (abstract).
- Bonecchi, R., Galleria, E., Boronia, E.M., Corse, M.M., Locate, M., Mantuan, A., 2009. Chemokines and chemokine receptors: an overview. *Front. Biosci.* 14, 540–551.
- Booss, J., Siri, M.M., Tartelette, W.W., Mason, D.Y., 1983. Immunohistological analysis of T lymphocyte subsets in the central nervous system in chronic progressive multiple sclerosis. *J. Neurol. Sci.* 62, 219–232.
- Brändle, S.M., Obermeier, B., Senel, M., Bruder, J., Mentele, R., Khademi, M., et al., 2016. Distinct oligoclonal band antibodies in multiple sclerosis recognize ubiquitous self-proteins. *Proc. Natl. Acad. Sci. U.S.A.* 113 (28), 7864–7869. Available from: <https://doi.org/10.1073/pnas.1522730113>.
- Brod, S.A., Marshall Jr., G.D., Henninger, E.M., Sriram, S., Khan, M., et al., 1996. Interferon-beta 1b treatment decreases tumor necrosis factor-alpha and increases interleukin-6 production in multiple sclerosis. *Neurology* 46, 1633–1638.
- Brynedal, B., Duvefelt, K., Jonasdottir, G., Roos, I.M., Akesson, E., Palmgren, J., et al., 2007. HLA-A confers an HLA-DRB1 independent influence on the risk of multiple sclerosis. *PLoS One* 2, e664.
- Wellcome Trust Case Control Consortium, Burton, P.R., Clayton, D.G., Cardon, L.R., Craddock, N., Deloukas, P., et al., 2007. Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. *Nat. Genet.* 39, 1329–1337.
- Calabrese, M., Agosta, F., Rinaldi, F., Mattisi, I., Grossi, P., Favaretto, A., et al., 2009. Cortical lesions and atrophy associated with cognitive impairment in relapsing-remitting multiple sclerosis. *Arch. Neurol.* 66, 1144–1150.
- Calabresi, P.A., Pelfrey, C.M., Tranquill, L.R., Maloni, H., McFarland, H.F., 1997. VLA-4 expression on peripheral blood lymphocytes is downregulated after treatment of multiple sclerosis with interferon beta. *Neurology* 49, 1111–1116.
- Calabresi, P.A., Giovanni, G., Confavreux, C., Aletta, S.L., Haviv, E., Hutchinson, M., et al., 2007. The incidence and significance of anti-natalizumab antibodies: results from AFFIRM and SENTINEL. *Neurology* 69, 1391–1403.

- Cao, Y., Goods, B.A., Raddassi, K., Nepom, G.T., Kwok, W.W., Love, J.C., et al., 2015. Distinct inflammatory profiles of myelin-reactive T cells from patients with multiple sclerosis. *Sci. Transl. Med.* 7 (287), 287ra74. Available from: <https://doi.org/10.1126/scitranslmed.aaa8038>.
- Cekanaviciute, E., Yoo, B.B., Runia, T.F., Debelius, J.W., Singh, S., Nelson, C.A., et al., 2017. Gut bacteria from multiple sclerosis patients modulate human T cells and exacerbate symptoms in mouse models. *Proc. Natl. Acad. Sci. U.S.A.* Available from: <https://doi.org/10.1073/pnas.1711235114>.
- Charcot, J., 1868a. RE: Comptes rendus des séances et mémoires lus à la Société de Biologie. Mars 14.
- Charcot, J., 1868b. *Histologic de la sclérose en plaque. Gazette Hopital* 41, 554–566.
- Charcot, J., 1877. Lectures on the Diseases of the Nervous System. The New Sydenham Society, London.
- Codarri, L., Gyulveszi, G., Tosevski, V., Hesske, L., Fontana, A., Magnenat, L., et al., 2011. RORγmat drives production of the cytokine GM-CSF in helper T cells, which is essential for the effector phase of autoimmune neuroinflammation. *Nat. Immunol.* 12, 560–567.
- Cohen, J.A., Barkhof, F., Comi, G., Hartung, H.P., Khatri, B.O., Montalban, X., et al., 2010. Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis. *N. Engl. J. Med.* 362, 402–415.
- Cohen, J.A., Coles, A.L., Arnold, D.L., Confavreux, C., Fox, E.J., Hartung, H.P., et al., for the CARE-MS I Investigators 2012. Alemtuzumab versus interferon beta 1a as first-line treatment for patients with relapsing-remitting multiple sclerosis: a randomised controlled phase 3 trial. *Lancet* 380, 1819–1828. Available from: [https://doi.org/10.1016/S0140-6736\(12\)61769-3](https://doi.org/10.1016/S0140-6736(12)61769-3).
- Coles, A.J., Twyman, C.L., Arnold, D.L., Cohen, J.A., Confavreux, C., Fox, E.J., et al., for the CARE-MS II Investigators 2012. Alemtuzumab for patients with relapsing multiple sclerosis after disease-modifying therapy: a randomised controlled phase 3 trial. *Lancet* 380, 1829–1839. Available from: [https://doi.org/10.1016/S0140-6736\(12\)61768-1](https://doi.org/10.1016/S0140-6736(12)61768-1).
- Comabella, M., Craig, D.W., Camina-Tato, M., Morcillo, C., Lopez, C., Navarro, A., et al., 2008. Identification of a novel risk locus for multiple sclerosis at 13q31.3 by a pooled genome-wide scan of 500,000 single nucleotide polymorphisms. *PLoS One* 3, e3490.
- Comi, G., Filippi, M., Wolinsky, J.S., 2001. European/Canadian multi-center, double-blind, randomized, placebo-controlled study of the effects of glatiramer acetate on magnetic resonance imaging—measured disease activity and burden in patients with relapsing multiple sclerosis. European/Canadian Glatiramer Acetate Study Group. *Ann. Neurol.* 49, 290–297.
- Confavreux, C., Vukusic, S., 2006. Natural history of multiple sclerosis: a unifying concept. *Brain* 129, 606–616.
- Confavreux, C., Vukusic, S., Moreau, T., Adeleine, P., 2000. Relapses and progression of disability in multiple sclerosis. *N. Engl. J. Med.* 343, 1430–1438.
- Cotsapas, C., Voight, B.F., Rossin, E., Lage, K., Neale, B.M., Wallace, C., et al., 2011. Pervasive sharing of genetic effects in autoimmune disease. *PLoS Genet.* 7, e1002254.
- Cotton, F., Weiner, H.L., Jolesz, F.A., Guttmann, C.R., 2003. MRI contrast uptake in new lesions in relapsing-remitting MS followed at weekly intervals. *Neurology* 60, 640–646.
- Crawford, M.P., Yan, S.X., Ortega, S.B., Mehta, R.S., Hewitt, R.E., Price, D.A., et al., 2004. High prevalence of autoreactive, neuroantigen-specific CD8<sup>+</sup> T cells in multiple sclerosis revealed by novel flow cytometric assay. *Blood* 103, 4222–4231.
- Cross, A.H., Stark, J.L., Lauber, J., Ramsbottom, M.J., Lyons, J.A., 2006. Rituximab reduces B cells and T cells in cerebrospinal fluid of multiple sclerosis patients. *J. Neuroimmunol.* 180, 63–70.
- Cruz, M., Olsson, T., Ernerudh, J., Hojeberg, B., Link, H., 1987. Immunoblot detection of oligoclonal anti-myelin basic protein IgG antibodies in cerebrospinal fluid in multiple sclerosis. *Neurology* 37, 1515–1519.
- De Jager, P.L., Baecher-Allan, C., Maier, L.M., Arthur, A.T., Ottoboni, L., Barcellos, L., et al., 2009a. The role of the CD58 locus in multiple sclerosis. *Proc. Natl. Acad. Sci. U.S.A.* 106, 5264–5269.
- De Jager, P.L., Jia, X., Wang, J., De Bakker, P.I., Ottoboni, L., Aggarwal, N.T., et al., 2009b. Meta-analysis of genome scans and replication identify CD6, IRF8 and TNFRSF1A as new multiple sclerosis susceptibility loci. *Nat. Genet.* 41, 776–782.
- Dean, G., McLoughlin, H., Brady, R., Adelstein, A.M., Tallett-Williams, J., 1976. Multiple sclerosis among immigrants in Greater London. *Br. Med. J.* 1, 861–864.
- Deloire, M.S., Ruet, A., Hamel, D., Bonnet, M., Dousset, V., Brochet, B., 2011. MRI predictors of cognitive outcome in early multiple sclerosis. *Neurology* 76, 1161–1167.
- Dominguez-Villar, M., Baecher-Allan, C.M., Hafler, D.A., 2011. Identification of T helper type 1-like, Foxp3<sup>+</sup> regulatory T cells in human autoimmune disease. *Nat. Med.* 17, 673–675.
- Duda, P.W., Schmied, M.C., Cook, S.L., Krieger, J.I., Hafler, D.A., 2000. Glatiramer acetate (Copaxone) induces degenerate, Th2-polarized immune responses in patients with multiple sclerosis. *J. Clin. Invest.* 105, 967–976.
- Dutta, R., Trapp, B.D., 2007. Pathogenesis of axonal and neuronal damage in multiple sclerosis. *Neurology* 68, S22–S31, discussion S43–S54.
- Dziedzic, T., Metz, I., Dallenga, T., Konig, F.B., Muller, S., Stadelmann, C., et al., 2010. Wallerian degeneration: a major component of early axonal pathology in multiple sclerosis. *Brain Pathol.* 20, 976–985.
- Ebers, G.C., 2008. Environmental factors and multiple sclerosis. *Lancet Neurol.* 7, 268–277.
- Ebers, G.C., Bulman, D.E., Sadovnick, A.D., Paty, D.W., Warren, S., Hader, W., et al., 1986. A population-based study of multiple sclerosis in twins. *N. Engl. J. Med.* 315, 1638–1642.
- El-Behi, M., Ceric, B., Dai, H., Yan, Y., Cullimore, M., Safavi, F., et al., 2011. The encephalitogenicity of T(H)17 cells is dependent on IL-1- and IL-23-induced production of the cytokine GM-CSF. *Nat. Immunol.* 12, 568–575.
- Farez, M.F., Fiol, M.P., Gaitán, M.I., Quintana, F.J., Correale, J., 2015. Sodium intake is associated with increased disease activity in multiple sclerosis. *J. Neurol. Neurosurg. Psychiatry* 86 (1), 26–31.
- Farh, K.K.-H., Marson, A., Zhu, J., Kleinewietfeld, M., Housley, W.J., Beik, S., et al., 2015. Genetic and epigenetic fine mapping of causal autoimmune disease variants. *Nature* 518 (7539), 337–343.
- Ferguson, B., Matyszak, M.K., Esiri, M.M., Perry, V.H., 1997. Axonal damage in acute multiple sclerosis lesions. *Brain* 120 (Pt 3), 393–399.
- Filippi, M., 2000. Enhanced magnetic resonance imaging in multiple sclerosis. *Mult. Scler.* 6, 320–326.
- Filippi, M., Agosta, F., 2009. Magnetic resonance techniques to quantify tissue damage, tissue repair, and functional cortical reorganization in multiple sclerosis. *Prog. Brain Res.* 175, 465–482.
- Filippi, M., Rocca, M.A., 2011. MR imaging of multiple sclerosis. *Radiology* 259, 659–681.

- Filippi, M., Paty, D.W., Kappos, L., Barkhof, F., Compston, D.A., Thompson, A.J., et al., 1995. Correlations between changes in disability and T2-weighted brain MRI activity in multiple sclerosis: a follow-up study. *Neurology* 45, 255–260.
- Filippi, M., Rocca, M.A., Barkhof, F., Bruck, W., Chen, J.T., Comi, G., et al., 2012. Association between pathological and MRI findings in multiple sclerosis. *Lancet Neurol.* 11, 349–360.
- Fisniku, L.K., Brex, P.A., Altmann, D.R., Miszkiel, K.A., Benton, C.E., Lanyon, R., et al., 2008. Disability and T2 MRI lesions: a 20-year follow-up of patients with relapse onset of multiple sclerosis. *Brain* 131, 808–817.
- Fitzgerald, K.C., Munger, K.L., Hartung, H.-P., Freedman, M.S., Montalbán, X., Edan, G., et al., 2017. Sodium intake and multiple sclerosis activity and progression in BENEFIT. *Ann. Neurol.* 82 (1), 20–29.
- Fontenot, J.D., Gavin, M.A., Rudensky, A.Y., 2003. Foxp3 programs the development and function of CD4 1 CD25 1 regulatory T cells. *Nat. Immunol.* 4, 330–336.
- Fontoura, P., Garren, H., 2010. Multiple sclerosis therapies: molecular mechanisms and future. *Results Probl. Cell Differ.* 51, 259–285.
- Fox, E.J., 2004. Mechanism of action of mitoxantrone. *Neurology* 63, S15–S18.
- Fox, R.J., Miller, D.H., Phillips, J.T., Hutchinson, M., Havrdova, E., Kita, M., et al., 2012. Placebo-controlled phase 3 study of oral BG-12 or glatiramer in multiple sclerosis. *N. Engl. J. Med.* 367, 1087–1097.
- Frazer, K.A., Ballinger, D.G., Cox, D.R., Hinds, D.A., Stuve, L.L., Gibbs, R.A., et al., 2007. A second generation human haplotype map of over 3.1 million SNPs. *Nature* 449, 851–861.
- Freal, J.E., Kraft, G.H., Coryell, J.K., 1984. Symptomatic fatigue in multiple sclerosis. *Arch. Phys. Med. Rehabil.* 65, 135–138.
- [No authors listed], 1992. MS in 54 twinships: concordance rate is independent of zygosity. French Research Group on Multiple Sclerosis. *Ann. Neurol.* 32, 724–727.
- Frohman, E.M., Racke, M.K., Raine, C.S., 2006. Multiple sclerosis—the plaque and its pathogenesis. *N. Engl. J. Med.* 354, 942–955.
- Genain, C.P., Cannella, B., Hauser, S.L., Raine, C.S., 1999. Identification of autoantibodies associated with myelin damage in multiple sclerosis. *Nat. Med.* 5, 170–175.
- Geurts, J.J., Bo, L., Pouwels, P.J., Castelijns, J.A., Polman, C.H., Barkhof, F., 2005. Cortical lesions in multiple sclerosis: combined postmortem MR imaging and histopathology. *AJNR Am. J. Neuroradiol.* 26, 572–577.
- Gold, R., 2011. Oral therapies for multiple sclerosis: a review of agents in phase III development or recently approved. *CNS Drugs* 25, 37–52.
- Gold, R., Wolinsky, J.S., 2011. Pathophysiology of multiple sclerosis and the place of teriflunomide. *Acta Neurol. Scand.* 124, 75–84.
- Gold, R., Kappos, L., Arnold, D.L., Bar-Or, A., Giovannoni, G., Selma, K., et al., 2012. Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis. *N. Engl. J. Med.* 367, 1098–1107.
- Graber, J., Zhan, M., Ford, D., Kursch, F., Francis, G., Bever, C., et al., 2005. Interferon-beta-1a induces increases in vascular cell adhesion molecule: implications for its mode of action in multiple sclerosis. *J. Neuroimmunol.* 161, 169–176.
- Graber, J.J., Dhib-Jalbut, S., 2011. Biomarkers of disease activity in multiple sclerosis. *J. Neurol. Sci.* 305, 1–10.
- Gran, B., Tranquill, L.R., Chen, M., Bielekova, B., Zhou, W., Dhib-Jalbut, S., et al., 2000. Mechanisms of immunomodulation by glatiramer acetate. *Neurology* 55, 1704–1714.
- Greer, J.M., Pender, M.P., 2008. Myelin proteolipid protein: an effective autoantigen and target of autoimmunity in multiple sclerosis. *J. Autoimmun.* 31, 281–287.
- Haas, J., Hug, A., Viehoever, A., Fritzsching, B., Falk, C.S., Filser, A., et al., 2005. Reduced suppressive effect of CD41CD25 high regulatory T cells on the T cell immune response against myelin oligodendrocyte glycoprotein in patients with multiple sclerosis. *Eur. J. Immunol.* 35, 3343–3352.
- Hafler, D.A., 2012. Perspective: deconstructing a disease. *Nature* 484, S6.
- Hafler, J.P., Maier, L.M., Cooper, J.D., Plagnol, V., Hinks, A., Simmonds, M.J., et al., 2009. CD226 Gly307Ser association with multiple autoimmune diseases. *Genes Immun.* 10, 5–10.
- Hammond, S.R., De Wytt, C., Maxwell, I.C., Landy, P.J., English, D., Mcleod, J.G., et al., 1987. The epidemiology of multiple sclerosis in Queensland, Australia. *J. Neurol. Sci.* 80, 185–204.
- Hammond, S.R., Mcleod, J.G., Millingen, K.S., Stewart-Wynne, E.G., English, D., Holland, J.T., et al., 1988. The epidemiology of multiple sclerosis in three Australian cities: Perth, Newcastle and Hobart. *Brain* 111 (Pt 1), 1–25.
- Harrington, L.E., Hatton, R.D., Mangan, P.R., Turner, H., Murphy, T.L., Murphy, K.M., et al., 2005. Interleukin 17-producing CD4 1 effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat. Immunol.* 6, 1123–1132.
- Hartung, H.P., Gonsette, R., Konig, N., Kwiecinski, H., Guseo, A., Morrissey, S.P., et al., 2002. Mitoxantrone in progressive multiple sclerosis: a placebo-controlled, double-blind, randomised, multicentre trial. *Lancet* 360, 2018–2025.
- Hartung, H.P., Aktas, O., Kieseier, B., Giancarlo Comi, G.C., 2010. Development of oral cladribine for the treatment of multiple sclerosis. *J. Neurol.* 257, 163–170.
- Hastings, W.D., Anderson, D.E., Kassam, N., Koguchi, K., Greenfield, E.A., Kent, S.C., et al., 2009. TIM-3 is expressed on activated human CD4 1 T cells and regulates Th1 and Th17 cytokines. *Eur. J. Immunol.* 39, 2492–24501.
- Hauser, S.L., Bar-Or, A., Comi, G., Giovannoni, G., Hartung, H.P., Hemmer, B., et al., for the OPERA I and OPERA II Clinical Investigators 2017. Ocrelizumab versus Interferon Beta-1a in Relapsing Multiple Sclerosis. *N. Engl. J. Med.* 376, 221–234. Available from: <https://doi.org/10.1056/NEJMoa1601277>.
- Hauser, S.L., Bhan, A.K., Gilles, F., Kemp, M., Kerr, C., Weiner, H.L., 1986. Immunohistochemical analysis of the cellular infiltrate in multiple sclerosis lesions. *Ann. Neurol.* 19, 578–587.
- Hauser, S.L., Waubant, E., Arnold, D.L., Vollmer, T., Antel, J., Fox, R.J., et al., 2008. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. *N. Engl. J. Med.* 358 (7), 676–688. Available from: <https://doi.org/10.1056/NEJMoa0706383>.
- Heltberg, A., Holm, N., 1982. Concordance in twins and recurrence in sibships in MS. *Lancet* 1, 1068.
- Hofman, F.M., Hinton, D.R., Johnson, K., Merrill, J.E., 1989. Tumor necrosis factor identified in multiple sclerosis brain. *J. Exp. Med.* 170, 607–612.
- Hogancamp, W.E., Rodriguez, M., Weinshenker, B.G., 1997. The epidemiology of multiple sclerosis. *Mayo Clin. Proc.* 72, 871–878.

- Hohol, M.J., Guttmann, C.R., Orav, J., Mackin, G.A., Kikinis, R., Khouri, S.J., et al., 1997. Serial neuropsychological assessment and magnetic resonance imaging analysis in multiple sclerosis. *Arch. Neurol.* 54, 1018–1025.
- Hohol, M.J., Olek, M.J., Orav, E.J., Stazzone, L., Hafler, D.A., Khouri, S.J., et al., 1999. Treatment of progressive multiple sclerosis with pulse cyclophosphamide/methylprednisolone: response to therapy is linked to the duration of progressive disease. *Mult. Scler.* 5, 403–409.
- Holmes, F.F., Stubbs, D.W., Larsen, W.E., 1967. Systemic lupus erythematosus and multiple sclerosis in identical twins. *Arch. Intern. Med.* 119, 302–304.
- Hong, J., Li, N., Zhang, X., Zheng, B., Zhang, J.Z., 2005. Induction of CD4 1 CD25 1 regulatory T cells by copolymer-I through activation of transcription factor Foxp3. *Proc. Natl. Acad. Sci. U.S.A.* 102, 6449–6454.
- Hori, S., Nomura, T., Sakaguchi, S., 2003. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 299, 1057–1061.
- Howell, O.W., Reeves, C.A., Nicholas, R., Carassiti, D., Radotra, B., Gentleman, S.M., et al., 2011. Meningeal inflammation is widespread and linked to cortical pathology in multiple sclerosis. *Brain* 134, 2755–2771.
- Hutchinson, M., Kappos, L., Calabresi, P.A., Confavreux, C., Giovannoni, G., Galetta, S.L., et al., 2009. The efficacy of natalizumab in patients with relapsing multiple sclerosis: subgroup analyses of AFFIRM and SENTINEL. *J. Neurol.* 256, 405–415.
- [No authors listed], 1993. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. I. Clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. The IFNB Multiple Sclerosis Study Group. *Neurology* 43, 655–661.
- [No authors listed], 1995. Interferon beta-1b in the treatment of multiple sclerosis: final outcome of the randomized controlled trial. The IFNB Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group. *Neurology* 45, 1277–1285.
- International Multiple Sclerosis Genetics Consortium, Hafler, D.A., Compston, A., Sawcer, S., Lander, E.S., Daly, M.J., De Jager, P.L., et al., 2007. Risk alleles for multiple sclerosis identified by a genomewide study. *N. Engl. J. Med.* 357 (9), 851–862. Epub 2007 Jul 29.
- International Multiple Sclerosis Genetics Consortium, 2009. The expanding genetic overlap between multiple sclerosis and type I diabetes. *Genes Immun.* 11, 11–14.
- Jacobs, L.D., Cookfair, D.L., Rudick, R.A., Herndon, R.M., Richert, J.R., Salazar, A.M., et al., 1995. A phase III trial of intramuscular recombinant interferon beta as treatment for exacerbating-remitting multiple sclerosis: design and conduct of study and baseline characteristics of patients. Multiple Sclerosis Collaborative Research Group (MSCRG). *Mult. Scler.* 1, 118–135.
- Jacobs, L.D., Cookfair, D.L., Rudick, R.A., Herndon, R.M., Richert, J.R., Salazar, A.M., et al., 1996. Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. The Multiple Sclerosis Collaborative Research Group (MSCRG). *Ann. Neurol.* 39, 285–294.
- Jakkula, E., Leppa, V., Sulonen, A.M., Varilo, T., Kallio, S., Kemppainen, A., et al., 2010. Genome-wide association study in a high-risk isolate for multiple sclerosis reveals associated variants in STAT3 gene. *Am. J. Hum. Genet.* 86, 285–291.
- Johnson, K.P., Brooks, B.R., Cohen, J.A., Ford, C.C., Goldstein, J., Lisak, R.P., et al., 1995. Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis: results of a phase III multicenter, double-blind placebo-controlled trial. The Copolymer 1 Multiple Sclerosis Study Group. *Neurology* 45, 1268–1276.
- Johnson, K.P., Brooks, B.R., Cohen, J.A., Ford, C.C., Goldstein, J., Lisak, R.P., et al., 1998. Extended use of glatiramer acetate (Copaxone) is well tolerated and maintains its clinical effect on multiple sclerosis relapse rate and degree of disability. Copolymer 1 Multiple Sclerosis Study Group. *Neurology* 50, 701–708.
- Joller, N., Hafler, J.P., Brynedal, B., Kassam, N., Spoerl, S., Levin, S.D., et al., 2011. Cutting edge: TIGIT has T cell-intrinsic inhibitory functions. *J. Immunol.* 186, 1338–1342.
- Kappos, L., Gold, R., Miller, D.H., Macmanus, D.G., Havrdova, E., Limmroth, V., et al., 2008. Efficacy and safety of oral fumarate in patients with relapsing-remitting multiple sclerosis: a multicentre, randomised, double-blind, placebo-controlled phase IIb study. *Lancet* 372, 1463–1472.
- Kebir, H., Kreymborg, K., Ifergan, I., Dodelet-Devillers, A., Cayrol, R., Bernard, M., et al., 2007. Human TH17 lymphocytes promote blood–brain barrier disruption and central nervous system inflammation. *Nat. Med.* 13, 1173–1175.
- Khouri, S.J., Hancock, W.W., Weiner, H.L., 1992. Oral tolerance to myelin basic protein and natural recovery from experimental autoimmune encephalomyelitis are associated with downregulation of inflammatory cytokines and differential upregulation of transforming growth factor beta, interleukin 4, and prostaglandin E expression in the brain. *J. Exp. Med.* 176, 1355–1364.
- Khouri, S.J., Guttmann, C.R., Orav, E.J., Hohol, M.J., Ahn, S.S., Hsu, L., et al., 1994. Longitudinal MRI imaging in multiple sclerosis: correlation between disability and lesion burden. *Neurology* 44, 2120–2124.
- Kinnunen, E., Koskenvuo, M., Kaprio, J., Aho, K., 1987. Multiple sclerosis in a nationwide series of twins. *Neurology* 37, 1627–1629.
- Klawiter, E.C., Piccio, L., Lyons, J.A., Mikesell, R., O'Connor, K.C., Cross, A.H., 2010. Elevated intrathecal myelin oligodendrocyte glycoprotein antibodies in multiple sclerosis. *Arch. Neurol.* 67, 1102–1108.
- Kleinewietfeld, M., Manzel, A., Titze, J., Kvakan, H., Yosef, N., Linker, R.A., et al., 2013. Sodium chloride drives autoimmune disease by the induction of pathogenic TH17 cells. *Nature* 496 (7446), 518–522.
- Koch-Henriksen, N., Sorensen, P.S., 2010. The changing demographic pattern of multiple sclerosis epidemiology. *Lancet Neurol.* 9, 520–532.
- Koenen, H.J., Smeets, R.L., Vink, P.M., Van Rijssen, E., Boots, A.M., Joosten, I., 2008. Human CD25highFoxp3pos regulatory T cells differentiate into IL-17-producing cells. *Blood* 112, 2340–2352.
- Koguchi, K., Anderson, D.E., Yang, L., O'Connor, K.C., Kuchroo, V.K., Hafler, D.A., 2006. Dysregulated T cell expression of TIM3 in multiple sclerosis. *J. Exp. Med.* 203, 1413–1418.
- Kragt, J., Van Amerongen, B., Killestein, J., Dijkstra, C., Uitdehaag, B., Polman, C., et al., 2009. Higher levels of 25-hydroxyvitamin D are associated with a lower incidence of multiple sclerosis only in women. *Mult. Scler.* 15, 9–15.
- Krapf, H., Morrissey, S.P., Zenker, O., Zwingers, T., Gonsette, R., Hartung, H.P., 2005. Effect of mitoxantrone on MRI in progressive MS: results of the MIMS trial. *Neurology* 65, 690–695.
- Kurtzke, J.F., 1977. Geography in multiple sclerosis. *J. Neurol.* 215, 1–26.
- Kurtzke, J.F., 2000. Epidemiology of multiple sclerosis. Does this really point toward an etiology? *Lectio Doctoralis. Neurol. Sci.* 21, 383–403.
- Kurtzke, J.F., Beebe, G.W., Norman Jr., J.E., 1979. Epidemiology of multiple sclerosis in U.S. veterans: 1. Race, sex, and geographic distribution. *Neurology* 29, 1228–1235.

- Kurtzke, J.F., Gudmundsson, K.R., Bergmann, S., 1982. MS in Iceland: 1. Evidence of a post-war epidemic. *Neurology* 32, 143–150.
- Langer-Gould, A., Popat, R.A., Huang, S.M., Cobb, K., Fontoura, P., Gould, M.K., et al., 2006. Clinical and demographic predictors of long-term disability in patients with relapsing-remitting multiple sclerosis a systematic review. *Arch. Neurol.* 63 (12), 1686–1691. Available from: <https://doi.org/10.1001/archneur.63.12.1686>.
- Langer-Gould, A., Brara, S.M., Beaber, B.E., Koebnick, C., 2013. Childhood obesity and risk of pediatric multiple sclerosis and clinically isolated syndrome. *Neurology* 80 (6), 548–552.
- Lassmann, H., 1998. Neuropathology in multiple sclerosis: new concepts. *Mult. Scler.* 4, 93–98.
- Lauer, K., 1995. Environmental associations with the risk of multiple sclerosis: the contribution of ecological studies. *Acta Neurol. Scand.* Suppl. 161, 77–88.
- Leonard, J.P., Waldburger, K.E., Goldman, S.J., 1995. Prevention of experimental autoimmune encephalomyelitis by antibodies against interleukin 12. *J. Exp. Med.* 181, 381–386.
- Lincoln, M.R., Ramagopalan, S.V., Chao, M.J., Herrera, B.M., Deluca, G.C., Orton, S.M., et al., 2009. Epistasis among HLA-DRB1, HLA-DQA1, and HLA-DQB1 loci determines multiple sclerosis susceptibility. *Proc. Natl. Acad. Sci. U.S.A.* 106, 7542–7547.
- Link, H., Huang, Y.M., 2006. Oligoclonal bands in multiple sclerosis cerebrospinal fluid: an update on methodology and clinical usefulness. *J. Neuroimmunol.* 180, 17–28.
- Linker, R.A., Lee, D.H., Ryan, S., Van Dam, A.M., Conrad, R., Bista, P., et al., 2011. Fumaric acid esters exert neuroprotective effects in neuroinflammation via activation of the Nrf2 antioxidant pathway. *Brain* 134, 678–692.
- Lovato, L., Willis, S.N., Rodig, S.J., Caron, T., Almendlinger, S.E., Howell, O.W., et al., 2011. Related B cell clones populate the meninges and parenchyma of patients with multiple sclerosis. *Brain* 134, 534–541.
- Lozano, E., Dominguez-Villar, M., Kuchroo, V., Hafler, D.A., 2012. The TIGIT/CD226 axis regulates human T cell function. *J. Immunol.* 188, 3869–3875.
- Mackay, R.P., Myrianthopoulos, N.C., 1966. Multiple sclerosis in twins and their relatives. *Arch. Neurol.* 15, 449–462.
- Maglizzi, R., Howell, O., Vora, A., Serafini, B., Nicholas, R., Puopolo, M., et al., 2007. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. *Brain* 130, 1089–1104.
- Mancardi, G.L., Sardanelli, F., Parodi, R.C., Melani, E., Capello, E., Inglese, M., et al., 1998. Effect of copolymer-1 on serial gadolinium-enhanced MRI in relapsing remitting multiple sclerosis. *Neurology* 50, 1127–1133.
- Mandrioli, J., Sola, P., Bedin, R., Gambini, M., Merelli, E., 2008. A multifactorial prognostic index in multiple sclerosis. *Cerebrospinal fluid IgM oligoclonal bands and clinical features to predict the evolution of the disease.* *J. Neurol.* 255, 1023–1031.
- Marriott, J.J., Miyasaki, J.M., Gronseth, G., O'Connor, P.W., 2010. Evidence report: the efficacy and safety of mitoxantrone (Novantrone) in the treatment of multiple sclerosis: Report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. *Neurology* 74, 1463–1470.
- Mattson, D., Petrie, M., Srivastava, D.K., Mcdermott, M., 1995. Multiple sclerosis. Sexual dysfunction and its response to medications. *Arch. Neurol.* 52, 862–868.
- Mattson, D.H., Roos, R.P., Arnason, B.G., 1980. Isoelectric focusing of IgG eluted from multiple sclerosis and subacute sclerosing panencephalitis brains. *Nature* 287, 335–337.
- McDonald, W.I., Compston, A., Edan, G., Goodkin, D., Hartung, H.P., Lublin, F.D., et al., 2001. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann. Neurol.* 50, 121–127.
- McLean, B.N., Luxton, R.W., Thompson, E.J., 1990. A study of immunoglobulin G in the cerebrospinal fluid of 1007 patients with suspected neurological disease using isoelectric focusing and the Log IgG-Index. A comparison and diagnostic applications. *Brain* 113 (Pt 5), 1269–1289.
- McLeod, J.G., Hammond, S.R., Hallpike, J.F., 1994. Epidemiology of multiple sclerosis in Australia. With NSW and SA survey results. *Med. J. Aust.* 160, 117–122.
- Menge, T., Lalive, P.H., Von Boldingen, H.C., Genain, C.P., 2011. Conformational epitopes of myelin oligodendrocyte glycoprotein are targets of potentially pathogenic antibody responses in multiple sclerosis. *J. Neuroinflamm.* 8, 161.
- Miller, A., Shapiro, S., Gershtein, R., Kinarty, A., Rawashdeh, H., Honigman, S., et al., 1998. Treatment of multiple sclerosis with copolymer-1 (Copaxone): implicating mechanisms of Th1 to Th2/ Th3 immune-deviation. *J. Neuroimmunol.* 92, 113–121.
- Miller, D.H., Leary, S.M., 2007. Primary-progressive multiple sclerosis. *Lancet Neurol.* 6, 903–912.
- Miller, D.H., Chard, D.T., Ciccarelli, O., 2012. Clinically isolated syndromes. *Lancet Neurol.* 11, 157–169.
- Miron, V.E., Jung, C.G., Kim, H.J., Kennedy, T.E., Soliven, B., Antel, J.P., 2008. FTY720 modulates human oligodendrocyte progenitor process extension and survival. *Ann. Neurol.* 63, 61–71.
- Miron, V.E., Ludwin, S.K., Darlington, P.J., Jarjour, A.A., Soliven, B., Kennedy, T.E., et al., 2010. Fingolimod (FTY720) enhances remyelination following demyelination of organotypic cerebellar slices. *Am. J. Pathol.* 176, 2682–2694.
- Montalban, X., Hauser, S.L., Kappos, L., Arnold, D.L., Bar-Or, A., Comi, G., et al., for the ORATORIO Clinical Investigators 2017. Ocrelizumab versus placebo in primary progressive multiple sclerosis. *N. Engl. J. Med.* 376, 209–220. Available from: <https://doi.org/10.1056/NEJMoa1606468>.
- Mumford, C., Wood, N., Kellar-Wood, H., et al., 1992. The UK study of MS in twins. *J. Neurol.* 239, 62.
- Murray, T.J., 2009. Robert Carswell: the first illustrator of MS. *Int. MS J.* 16, 98–101.
- Neuhaus, O., Farina, C., Yassouridis, A., Wiendl, H., Then Bergh, F., Dose, T., et al., 2000. Multiple sclerosis: comparison of copolymer-1-reactive T cell lines from treated and untreated subjects reveals cytokine shift from T helper 1 to T helper 2 cells. *Proc. Natl. Acad. Sci. U.S.A.* 97, 7452–7457.
- Newcombe, J., Hawkins, C.P., Henderson, C.L., Patel, H.A., Woodroffe, M.N., Hayes, G.M., et al., 1991. Histopathology of multiple sclerosis lesions detected by magnetic resonance imaging in unfixed post-mortem central nervous system tissue. *Brain* 114 (Pt 2), 1013–1023.
- Nischwitz, S., Cepok, S., Kröner, A., Wolf, C., Knop, M., Müller-Sarnowski, F., et al., 2010. Evidence for VAV2 and ZNF433 as susceptibility genes for multiple sclerosis. *J. Neuroimmunol.* 227, 162–166.

- Noonan, C.W., Williamson, D.M., Henry, J.P., Indian, R., Lynch, S.G., Neuberger, J.S., et al., 2010. The prevalence of multiple sclerosis in 3 US communities. *Prev Chronic Dis* 7 (1), A12.
- Noseworthy, J.H., Lucchinetti, C., Rodriguez, M., Weinshenker, B.G., 2000. Multiple sclerosis. *N. Engl. J. Med.* 343, 938–952.
- Noyes, K., Bajorska, A., Chappel, A., Schwid, S.R., Mehta, L.R., Weinstock-Guttman, B., et al., 2011. Cost-effectiveness of disease-modifying therapy for multiple sclerosis: a population-based study. *Neurology* 77, 355–363.
- Nylander, A., Hafler, D.A., 2012. Multiple sclerosis. *J. Clin. Invest.* 122, 1180–1188.
- Obermeier, B., Lovato, L., Mentele, R., Bruck, W., Forne, I., Imhof, A., et al., 2011. Related B cell clones that populate the CSF and CNS of patients with multiple sclerosis produce CSF immunoglobulin. *J. Neuroimmunol.* 233, 245–248.
- Olek, M.D., 2000. Multiple sclerosis and other inflammatory demyelinating diseases of the central nervous system. In: Bradley, W.D., Fenichel, G., Marsden, C.D. (Eds.), *Neurology in Clinical Practice*, third ed Butterworth-Heinemann, Woburn.
- Owens, T., Renno, T., Taupin, V., Krakowski, M., 1994. Inflammatory cytokines in the brain: does the CNS shape immune responses? *Immunol. Today*. 15, 566–571.
- O'Connor, K.C., Chitnis, T., Griffin, D.E., Piyasirisilp, S., Bar-Or, A., Khouri, S., et al., 2003. Myelin basic protein-reactive autoantibodies in the serum and cerebrospinal fluid of multiple sclerosis patients are characterized by low-affinity interactions. *J. Neuroimmunol.* 136, 140–148.
- O'Connor, K.C., Appel, H., Bregoli, L., Call, M.E., Catz, I., Chan, J.A., et al., 2005. Antibodies from inflamed central nervous system tissue recognize myelin oligodendrocyte glycoprotein. *J. Immunol.* 175, 1974–1982.
- O'Connor, K.C., McLaughlin, K.A., De Jager, P.L., Chitnis, T., Bettelli, E., Xu, C., et al., 2007. Self-antigen tetramers discriminate between myelin autoantibodies to native or denatured protein. *Nat. Med.* 13, 211–217.
- O'Connor, P., Wolinsky, J.S., Confavreux, C., Comi, G., Kappos, L., Olsson, T.P., et al., 2011. Randomized trial of oral teriflunomide for relapsing multiple sclerosis. *N. Engl. J. Med.* 365, 1293–1303.
- O'Garra, A., 1998. Cytokines induce the development of functionally heterogeneous T helper cell subsets. *Immunity*. 8, 275–283.
- O'Shea, J.J., Paul, W.E., 2010. Mechanisms underlying lineage commitment and plasticity of helper CD41 T cells. *Science* 327, 1098–1102.
- PRISMS, 2001. PRISMS-4: long-term efficacy of interferon-beta-1a in relapsing MS. *Neurology* 56, 1628–1636.
- Paty, D., Studney, D., Redekop, K., Lublin, F., 1994. MS COSTAR: a computerized patient record adapted for clinical research purposes. *Ann. Neurol.* 36 (Suppl), S134–S135.
- Paty, D.W., Li, D.K., 1993. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. II. MRI analysis results of a multicenter, randomized, double-blind, placebo-controlled trial. UBC MS/ MRI Study Group and the IFNB Multiple Sclerosis Study Group. *Neurology* 43, 662–667.
- Pedersen, N.S., Kam-Hansen, S., Link, H., Mavra, M., 1982. Specificity of immunoglobulins synthesized within the central nervous system in neurosyphilis. *Acta Pathol. Microbiol. Immunol. Scand. C*. 90, 97–104.
- Pham, T.H., Okada, T., Matloubian, M., Lo, C.G., Cyster, J.G., 2008. S1P1 receptor signaling overrides retention mediated by G alpha i-coupled receptors to promote T cell egress. *Immunity*. 28, 122–133.
- Polman, C.H., Reingold, S.C., Edan, G., Filippi, M., Hartung, H.P., Kappos, L., et al., 2005. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". *Ann. Neurol.* 58, 840–846.
- Polman, C.H., O'Connor, P.W., Havrdova, E., Hutchinson, M., Kappos, L., Miller, D.H., et al., 2006. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. *N. Engl. J. Med.* 354, 899–910.
- Polman, C.H., Reingold, S.C., Banwell, B., Clanet, M., Cohen, J.A., Filippi, M., et al., 2011. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann. Neurol.* 69, 292–302.
- Popescu, B.F., Bunyan, R.F., Parisi, J.E., Ransohoff, R.M., Lucchinetti, C.F., 2011. A case of multiple sclerosis presenting with inflammatory cortical demyelination. *Neurology* 76, 1705–1710.
- Prineas, J., 1975. Pathology of the early lesion in multiple sclerosis. *Hum. Pathol.* 6, 531–554.
- Raddassi, K., Kent, S.C., Yang, J., Bourcier, K., Bradshaw, E.M., Seyfert-Margolis, V., et al., 2011. Increased frequencies of myelin oligodendrocyte glycoprotein/MHC class II-binding CD4 cells in patients with multiple sclerosis. *J. Immunol.* 187, 1039–1046.
- Raine, C., 1991. Demyelinating diseases. In: Davis, R.L., Robertson, D.M. (Eds.), *Textbook of Neuropathology*, second ed Williams and Wilkins, Baltimore.
- Ramagopalan, S.V., Maugeri, N.J., Handunnetthi, L., Lincoln, M.R., Orton, S.M., Dyment, D.A., et al., 2009. Expression of the multiple sclerosis-associated MHC class II Allele HLA-DRB1\*1501 is regulated by vitamin D. *PLoS Genet.* 5, e1000369.
- Rao, S.M., Leo, G.J., Haughton, V.M., Aubin-Faubert St, P., Bernardin, L., 1989. Correlation of magnetic resonance imaging with neuropsychological testing in multiple sclerosis. *Neurology* 39, 161–166.
- Reboldi, A., Coisne, C., Baumjohann, D., Benvenuto, F., Bottinelli, D., Lira, S., et al., 2009. C-C chemokine receptor 6-regulated entry of TH-17 cells into the CNS through the choroid plexus is required for the initiation of EAE. *Nat. Immunol.* 10, 514–523.
- Rieckmann, P., Toyka, K.V., Bassetti, C., Beer, K., Beer, S., Buettner, U., et al., 2004. Escalating immunotherapy of multiple sclerosis—new aspects and practical application. *J. Neurol.* 251, 1329–1339.
- Risch, N., Merikangas, K., 1996. The future of genetic studies of complex human diseases. *Science* 273, 1516–1517.
- Rivers, T.M., Sprunt, D.H., Berry, G.P., 1933. Observations on attempts to produce acute disseminated encephalomyelitis in monkeys. *J. Exp. Med.* 58, 39–53.
- Rovaris, M., Bozzali, M., Rodegher, M., Tortorella, C., Comi, G., Filippi, M., 1999. Brain MRI correlates of magnetization transfer imaging metrics in patients with multiple sclerosis. *J. Neurol. Sci.* 166, 58–63.
- Rovira, A., Swanton, J., Tintore, M., Huerga, E., Barkhof, F., Filippi, M., et al., 2009. A single, early magnetic resonance imaging study in the diagnosis of multiple sclerosis. *Arch. Neurol.* 66, 587–592.
- Sadovnick, A.D., 2006. The genetics and genetic epidemiology of multiple sclerosis: the "hard facts. *Adv. Neurol.* 98, 17–25.
- Sadovnick, A.D., Armstrong, H., Rice, G.P., Bulman, D., Hashimoto, L., Paty, D.W., et al., 1993. A population-based study of multiple sclerosis in twins: update. *Ann. Neurol.* 33, 281–285.
- Sadovnick, A.D., Remick, R.A., Allen, J., Swartz, E., Yee, I.M., Eisen, K., et al., 1996. Depression and multiple sclerosis. *Neurology* 46, 628–632.
- Sadovnick, A.D., Dymant, D., Ebers, G.C., 1997. Genetic epidemiology of multiple sclerosis. *Epidemiol. Rev.* 19, 99–106.

- Sanna, S., Pitzalis, M., Zoledziewska, M., Zara, I., Sidore, C., Murru, R., et al., 2010. Variants within the immunoregulatory CBLB gene are associated with multiple sclerosis. *Nat. Genet.* 42, 495–497.
- Sawcer, S., Hellenthal, G., Pirinen, M., Spencer, C.C., Patsopoulos, N.A., Moutsianas, L., et al., 2011. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature* 476, 214–219.
- Schumacker, G.A., Beebe, G., Kibler, R.F., Kurland, L.T., Kurtzke, J.F., McDowell, F., et al., 1965. Problems of Experimental trials of therapy in multiple sclerosis: report by the panel on the evaluation of experimental trials of therapy in multiple sclerosis. *Ann. N. Y. Acad. Sci.* 122, 552–568.
- Schwab, S.R., Cyster, J.G., 2007. Finding a way out: lymphocyte egress from lymphoid organs. *Nat. Immunol.* 8, 1295–1301.
- Segal, B.M., Dwyer, B.K., Shevach, E.M., 1998. An interleukin (IL)-10/IL-12 immunoregulatory circuit controls susceptibility to autoimmune disease. *J. Exp. Med.* 187, 537–546.
- Selmaj, K., Raine, C.S., Cannella, B., Brosnan, C.F., 1991. Identification of lymphotoxin and tumor necrosis factor in multiple sclerosis lesions. *J. Clin. Invest.* 87, 949–954.
- Serafini, B., Rosicarelli, B., Maglione, R., Stigliano, E., Aloisi, F., 2004. Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. *Brain Pathol.* 14, 164–174.
- Severson, C., Hafler, D.A., 2010. T-cells in multiple sclerosis. *Results Probl. Cell Differ.* 51, 75–98.
- Simon, K.C., Munger, K.L., Kraft, P., Hunter, D.J., De Jager, P.L., Ascherio, A., 2011. Genetic predictors of 25-hydroxyvitamin D levels and risk of multiple sclerosis. *J. Neurol.* 258, 1676–1682.
- Siveke, J.T., Hamann, A., 1998. T helper 1 and T helper 2 cells respond differentially to chemokines. *J. Immunol.* 160, 550–554.
- Skegg, D.C., Corwin, P.A., Craven, R.S., Malloch, J.A., Pollock, M., 1987. Occurrence of multiple sclerosis in the north and south of New Zealand. *J. Neurol. Neurosurg. Psychiatry* 50, 134–139.
- Skotzek, B., Sander, T., Zimmermann, J., Kolmel, H.W., 1988. Oligoclonal bands in serum and cerebrospinal fluid of patients with HIV infection. *J. Neuroimmunol.* 20, 151–152.
- Smith, K.J., Lassmann, H., 2002. The role of nitric oxide in multiple sclerosis. *Lancet Neurol.* 1, 232–241.
- Smolders, J., Damoiseaux, J., Menheere, P., Hupperts, R., 2008. Vitamin D as an immune modulator in multiple sclerosis, a review. *J. Neuroimmunol.* 194, 7–17.
- Sorensen, T.L., Tani, M., Jensen, J., Pierce, V., Lucchinetti, C., Folcik, V.A., et al., 1999. Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients. *J. Clin. Invest.* 103, 807–815.
- Steinman, L., 2007. A brief history of T(H)17, the first major revision in the T(H)1/T(H)2 hypothesis of T cell-mediated tissue damage. *Nat. Med.* 13, 139–145.
- Stone, L.A., Frank, J.A., Albert, P.S., Bash, C.N., Calabresi, P.A., Maloni, H., et al., 1997. Characterization of MRI response to treatment with interferon beta-1b: contrast-enhancing MRI lesion frequency as a primary outcome measure. *Neurology* 49, 862–869.
- Thacker, E.L., Mirzaei, F., Ascherio, A., 2006. Infectious mononucleosis and risk for multiple sclerosis: a meta-analysis. *Ann. Neurol.* 59, 499–503.
- Thorley-Lawson, D.A., Gross, A., 2004. Persistence of the Epstein-Barr virus and the origins of associated lymphomas. *N. Engl. J. Med.* 350, 1328–1337.
- Tintore, M., Rovira, A., Rio, J., Tur, C., Pelayo, R., Nos, C., et al., 2008. Do oligoclonal bands add information to MRI in first attacks of multiple sclerosis? *Neurology* 70, 1079–1083.
- Tishkoff, S.A., Verrelli, B.C., 2003. Role of evolutionary history on haplotype block structure in the human genome: implications for disease mapping. *Curr. Opin. Genet. Dev.* 13, 569–575.
- Trapp, B.D., Peterson, J., Ransohoff, R.M., Rudick, R., Mork, S., Bo, L., 1998. Axonal transection in the lesions of multiple sclerosis. *N. Engl. J. Med.* 338, 278–285.
- Traugott, U., Reinherz, E.L., Raine, C.S., 1983. Multiple sclerosis: distribution of T cell subsets within active chronic lesions. *Science* 219, 308–310.
- Valenzuela, R.M., Costello, K., Chen, M., Said, A., Johnson, K.P., Dhib-Jalbut, S., 2007. Clinical response to glatiramer acetate correlates with modulation of IFN-gamma and IL-4 expression in multiple sclerosis. *Mult. Scler.* 13, 754–762.
- Van Waesberghe, J.H., Kamphorst, W., De Groot, C.J., Van Walderveen, M.A., Castelijns, J.A., Ravid, R., et al., 1999. Axonal loss in multiple sclerosis lesions: magnetic resonance imaging insights into substrates of disability. *Ann. Neurol.* 46, 747–754.
- Vanamerongen, B.M., Dijkstra, C.D., Lips, P., Polman, C.H., 2004. Multiple sclerosis and vitamin D: an update. *Eur. J. Clin. Nutr.* 58, 1095–1109.
- Vartdal, F., Vandvik, B., Norrby, E., 1982. Intrathecal synthesis of virus-specific oligoclonal IgG, IgA and IgM antibodies in a case of varicella-zoster meningoencephalitis. *J. Neurol. Sci.* 57, 121–132.
- Viglietta, V., Baecher-Allan, C., Weiner, H.L., Hafler, D.A., 2004. Loss of functional suppression by CD41CD251 regulatory T cells in patients with multiple sclerosis. *J. Exp. Med.* 199, 971–979.
- Warren, K.G., Catz, I., 1994. Relative frequency of autoantibodies to myelin basic protein and proteolipid protein in optic neuritis and multiple sclerosis cerebrospinal fluid. *J. Neurol. Sci.* 121, 66–73.
- Warren, K.G., Catz, I., Johnson, E., Mielke, B., 1994. Anti-myelin basic protein and anti-proteolipid protein specific forms of multiple sclerosis. *Ann. Neurol.* 35, 280–289.
- Weiner, H.L., Guttman, C.R., Khouri, S.J., Orav, E.J., Hohol, M.J., Kikinis, R., et al., 2000. Serial magnetic resonance imaging in multiple sclerosis: correlation with attacks, disability, and disease stage. *J. Neuroimmunol.* 104, 164–173.
- Weinshenker, B.G., 1994. Natural history of multiple sclerosis. *Ann. Neurol.* 36 (Suppl), S6–S11.
- Weinshenker, B.G., 1996. Epidemiology of multiple sclerosis. *Neurol. Clin.* 14, 291–308.
- Whitlock, F.A., Siskind, M.M., 1980. Depression as a major symptom of multiple sclerosis. *J. Neurol. Neurosurg. Psychiatry* 43, 861–865.
- Williams, A., Eldridge, R., McFarland, H., Houff, S., Krebs, H., McFarlin, D., 1980. Multiple sclerosis in twins. *Neurology* 30, 1139–1147.
- Windhagen, A., Scholz, C., Hollsberg, P., Fukaura, H., Sette, A., Hafler, D.A., 1995. Modulation of cytokine patterns of human autoreactive T cell clones by a single amino acid substitution of their peptide ligand. *Immunity*. 2, 373–380.

- Wucherpfennig, K.W., Newcombe, J., Li, H., Keddy, C., Cuzner, M.L., Hafler, D.A., 1992a. Gamma delta T-cell receptor repertoire in acute multiple sclerosis lesions. *Proc. Natl. Acad. Sci. U.S.A.* 89, 4588–4592.
- Wucherpfennig, K.W., Newcombe, J., Li, H., Keddy, C., Cuzner, M.L., Hafler, D.A., 1992b. T cell receptor V alpha-V beta repertoire and cytokine gene expression in active multiple sclerosis lesions. *J. Exp. Med.* 175, 993–1002.
- Yamagata, K., Tagami, M., Torii, Y., Takenaga, F., Tsumagari, S., Itoh, S., et al., 2003. Sphingosine 1-phosphate induces the production of glial cell line-derived neurotrophic factor and cellular proliferation in astrocytes. *Glia.* 41, 199–206.
- Yang, L., Anderson, D.E., Kuchroo, J., Hafler, D.A., 2008. Lack of TIM-3 immunoregulation in multiple sclerosis. *J. Immunol.* 180, 4409–4414.
- Yang, Q., Khouri, M.J., Friedman, J., Little, J., Flanders, W.D., 2005. How many genes underlie the occurrence of common complex diseases in the population? *Int. J. Epidemiol.* 34, 1129–1137.
- Young, I.R., Hall, A.S., Pallis, C.A., Legg, N.J., Bydder, G.M., Steiner, R.E., 1981. Nuclear magnetic resonance imaging of the brain in multiple sclerosis. *Lancet* 2, 1063–1066.
- Zeyda, M., Poglitsch, M., Geyeregger, R., Smolen, J.S., Zlabinger, G.J., Horl, W.H., et al., 2005. Disruption of the interaction of T cells with antigen-presenting cells by the active leflunomide metabolite teriflunomide: involvement of impaired integrin activation and immunologic synapse formation. *Arthritis Rheum.* 52, 2730–2739.
- Zhou, D., Srivastava, R., Nessler, S., Grummel, V., Sommer, N., Bruck, W., et al., 2006. Identification of a pathogenic antibody response to native myelin oligodendrocyte glycoprotein in multiple sclerosis. *Proc. Natl. Acad. Sci. U.S.A.* 103, 19057–19062.

## Further Reading

- Absinta, M., Sati, P., Schindler, M., Leibovitch, E.C., Ohayon, J., Wu, T., et al., 2016. Persistent 7-tesla phase rim predicts poor outcome in new multiple sclerosis patient lesions. *J Clin Invest.* 126 (7), 2597–2609. Available from: <https://doi.org/10.1172/JCI86198>.
- Chen, W., Gauthier, S.A., Gupta, A., Comunale, J., Liu, T., Wang, S., et al., 2014. Quantitative susceptibility Mapping of Multiple sclerosis lesions at Various ages. *Radiology* 271 (1), 183–192. Available from: <https://doi.org/10.1148/radiol.13130353>. Apr.
- Jangi, S., Gandhi, R., Cox, L.M., Li, N., von Glehn, F., Yan, R., et al., 2016. Alterations of the human gut microbiome in multiple sclerosis. *Nat. Commun.* 7, 12015.
- Lassmann, H., Niedobitek, G., Aloisi, F., Middeldorp, J.M., 2011. Epstein-Barr virus in the multiple sclerosis brain: a controversial issue—report on a focused workshop held in the Centre for Brain Research of the Medical University of Vienna, Austria. *Brain* 134, 2772–2786.
- Plavina, T., Subramanyam, M., Bloomgren, G., Ticho, B., et al., 2014. Anti-JC virus antibody levels in serum or plasma further define risk of natalizumab-associated progressive multifocal leukoencephalopathy. *Ann. Neurol.* 76 (6), 802–812. Available from: <https://doi.org/10.1002/ana.24286>.
- Torkamani, A., Topol, E.J., Schork, N.J., 2008. Pathway analysis of seven common diseases assessed by genome-wide association. *Genomics* 92, 265–272.
- Xavier, R.J., Rioux, J.D., 2008. Genome-wide association studies: a new window into immune-mediated diseases. *Nat. Rev. Immunol.* 8, 631–643.
- Zhernakova, A., Van Diemen, C.C., Wijmenga, C., 2009. Detecting shared pathogenesis from the shared genetics of immune-related diseases. *Nat. Rev. Genet.* 10, 43–55.

## Peripheral Neuropathies

Michael P.T. Lunn<sup>1</sup>, Helmar C. Lehmann<sup>2</sup> and Kazim A. Sheikh<sup>3</sup>

<sup>1</sup>National Hospital for Neurology and Neurosurgery, London, United Kingdom <sup>2</sup>Department of Neurology, University Hospital of Cologne, Cologne, Germany <sup>3</sup>University of Texas Medical School at Houston, Houston, TX, United States

### OUTLINE

<b>Introduction</b>	987	<b>Chronic Neuropathies: Chronic Inflammatory Demyelinating Polyradiculoneuropathy</b>	<b>998</b>
<b>Acute Neuropathies: The Guillain–Barré Syndrome</b>		<i>History</i>	998
<i>Historical Background</i>	988	<i>Epidemiology and Clinical Features</i>	998
<i>Epidemiology</i>	988	<i>Autoimmune Features</i>	999
<i>Clinical Features and Subtypes of Guillain–Barré Syndrome</i>	988	<i>Immunogenetic Features</i>	1000
<i>Autoimmune Features</i>	989	<i>Environmental Influences</i>	1001
<i>Environmental Effects</i>	993	<i>Animal Models</i>	1001
<i>Animal Models of Disease</i>	993	<i>Pathogenic Mechanisms</i>	1002
<i>Cellular Mechanisms</i>	995	<i>Treatment and Outcome</i>	1002
<i>Cellular and Humoral Immune Elements Are Synergistic</i>	997	<i>Concluding Remarks and Future Prospects</i>	1002
<i>Genetic Aspects of Guillain–Barré Syndrome</i>	997	<i>Acknowledgments</i>	1002
<i>Treatment and Outcomes</i>	997	<i>References</i>	1003
		<i>Further Reading</i>	1009

### INTRODUCTION

Autoimmunity is implicated in a small but important group of peripheral nerve diseases. These include the acute inflammatory neuropathies eponymously referred to as the Guillain–Barré syndrome (GBS) and Fisher syndrome (FS), and the chronic inflammatory demyelinating polyradiculoneuropathy (CIDP), both idiopathic and associated with a serum paraprotein. Substantial evidence exists for an autoimmune pathogenesis in GBS and its subtypes. The evidence is still gathering to support similar processes in the chronic inflammatory neuropathies including the demyelinating neuropathy associated with antibodies to myelin-associated glycoprotein (MAG). Although the most current evidence supports an antibody-driven pathogenesis triggered by infection for GBS and FS, T-cell mechanisms predominate in CIDP and other cellular immune components are crucial effectors of disease. Experimental allergic neuritis (EAN), an inflammatory model of neuropathy, has been instructive in the detailed study of the pathogenesis of immune-mediated neuropathies and will be discussed.

## ACUTE NEUROPATHIES: THE GUILAIN–BARRÉ SYNDROME

### Historical Background

Guillain, Barré, and Strohl described a rapidly evolving flaccid paralysis with areflexia and albuminocytological dissociation in the cerebrospinal fluid (CSF) in 1916 (Guillain et al., 1916). Early autopsies demonstrated both T-cell inflammation and demyelination in peripheral nerves (Asbury et al., 1969; Haymaker and Kernohan, 1949) leading to the notion of GBS being a single pathophysiological entity synonymous with acute inflammatory demyelinating polyradiculoneuropathy (AIDP). AIDP is by far the most common variant of GBS in the developed world. Variants such as the FS (Fisher, 1956) and the axonal variants (1986) (Feasby et al., 1986), acute motor axonal neuropathy (AMAN), and acute motor and sensory axonal neuropathy (AMSAN) (Yuki et al., 1990; McKhann et al., 1991, 1993) are now part of a spectrum of disease with variable worldwide occurrence (see later and Box 52.1).

### Epidemiology

Since the near eradication of poliomyelitis, GBS has become the commonest cause of acute flaccid neuromuscular paralysis in the world. The incidence of GBS is 0.81–1.89 per 100,000 (Hughes and Rees, 1997; Sejvar et al., 2011). The incidence increases steadily with advancing age (0.62 per 100,000 in 0–9 year olds and 2.99 per 100,000 in 80–89 year olds) and males are affected more than females by 1.25:1 (Hadden and Gregson, 2001; Sejvar et al., 2011). Case-control studies implicate infections as precipitating events (see later).

Severity may vary from mild with full recovery in 10% of the patients only, to bedbound in 40%, to complete paralysis with ventilatory dependence in 20%. Death occurs in 3.5%–12% of the patients (Guillain-Barre Syndrome Study Group, 1985; Rees et al., 1998; Cornblath, 2005).

### Clinical Features and Subtypes of Guillain–Barré Syndrome

#### **Acute Inflammatory Demyelinating Polyradiculoneuropathy**

The diagnosis of GBS remains clinical. Electrophysiological studies help to subtype patients into diagnostic categories. AIDP accounts for over 95% of the patients with GBS in Europe and North America. Patients present with a rapidly evolving neuropathic (sensory-)motor paralysis, usually ascending, in two or more limbs over less than 4 weeks. The illness is monophasic. Most patients have numbness, tingling, or pain and many complain of bladder disturbance, facial weakness, or swallowing difficulty (Hughes, 1990). The autonomic disturbance is common with arrhythmia and fluctuating blood pressures. Tendon reflexes are absent or reduced. The CSF contains less than 50 leukocytes per  $\mu\text{L}$  (Asbury and Cornblath, 1990) and CSF protein is raised in 80% of the cases. Antiganglioside antibodies may be detected in the serum (see below) by enzyme-linked immunosorbent assay (ELISA), often with thin-layer chromatography confirmation, but novel solid-phase multiplex and combinatorial ganglioside assays have emerged and are being increasingly used (Rinaldi et al., 2009). Electrophysiological studies typically show slowed motor conduction velocities, delayed F-waves, and preserved compound muscle action potential (CMAP) amplitudes consistent with demyelination, and a “spared” sural nerve but conduction failure and axonal degeneration may complicate the picture.

Multifocal perivascular T-cell infiltration with demyelination, typically patchy with the involvement of proximal and terminal nerve segments, characterizes the pathology of AIDP (Asbury et al., 1969; Hall et al., 1992).

#### BOX 52.1

#### THE DIVERSITY OF GUILAIN–BARRÉ VARIANTS

- Acute inflammatory demyelinating polyradiculoneuropathy (AIDP)
- Regional variants, e.g., pharyngo-cervical-brachial
- Acute motor axonal neuropathy (AMAN)
- Acute motor and sensory axonal neuropathy (AMSAN)
- (Miller) Fisher syndrome (ataxia, ophthalmoplegia, and areflexia)
- Acute panautonomic neuropathy
- Acute pure sensory neuropathy
- Acute motor conduction block neuropathy

Indications of blood–nerve barrier (BNB) breakdown and deposition of activated complement components can be seen in some but not all cases of AIDP (Hafer-Macko et al., 1996b). These observations raise the possibility that T cell- or antibody-mediated immune injury can predominate in an individual case, although the evidence is increasingly in favor of an antibody-driven process in humans.

### **Acute Motor Axonal Neuropathy**

AMAN is a pure motor variant of GBS seen most commonly in China, Japan, and Mexico (McKhann et al., 1991, 1993; Ogawara et al., 2000). Here, it accounts for almost half of the cases, but in Europe and North America, it accounts for only 5%–20% (Rees et al., 1995b; Visser et al., 1995), clinically probably nearer 5%. In China, AMAN occurs in seasonal epidemics, affects more children (McKhann et al., 1991), and is strongly associated with *Campylobacter jejuni* infection. Sensory impairment is minimal and autonomic involvement less common. Electrophysiological studies are characterized by reduced CMAP amplitudes, absent F-waves with normal distal motor latencies, and conduction velocity (Kuwabara et al., 2000) but many feel criteria of this sort misclassify AMAN as AIDP and the prevalence is higher (Uncini and Kuwabara, 2012). Sensory involvement is absent.

The pathology of axonal GBS has largely been described from AMAN cases in northern China. The pathological changes indicate an antibody-mediated immune attack directed preferentially against the motor axons causing primary axonal degeneration in the absence of prominent T-cell inflammation (Griffin et al., 1995, 1996a; Hafer-Macko et al., 1996a). Macrophages may be found in the periaxonal space suggesting that the antigen of interest is on the axolemma (Fig. 52.1). Animal models of AMAN have disrupted nodal sodium channel clusters and detachment of paranodal myelin terminal loops. This would significantly reduce the safety factor for impulse transmission and might be responsible for the rapidly reversible conduction block frequently present in human AMAN (Yuki and Kuwabara, 2007). However, since in some patients little pathology is found (Griffin et al., 1996b) and in others recovery is too rapid for nerve fiber degeneration and regeneration (Ho et al., 1997; Kuwabara et al., 1998), axonal conduction failure and distal neuromuscular terminal failure must also make a prominent contribution to clinical weakness in AMAN.

### **Acute Motor and Sensory Neuropathy**

AMSAN is a more severe form of AMAN with a more severe course, sensory involvement, and delayed recovery (Feasby et al., 1986; Griffin et al., 1995). Sensory as well as motor nerve roots are involved. The pathology is similar to that of AMAN.

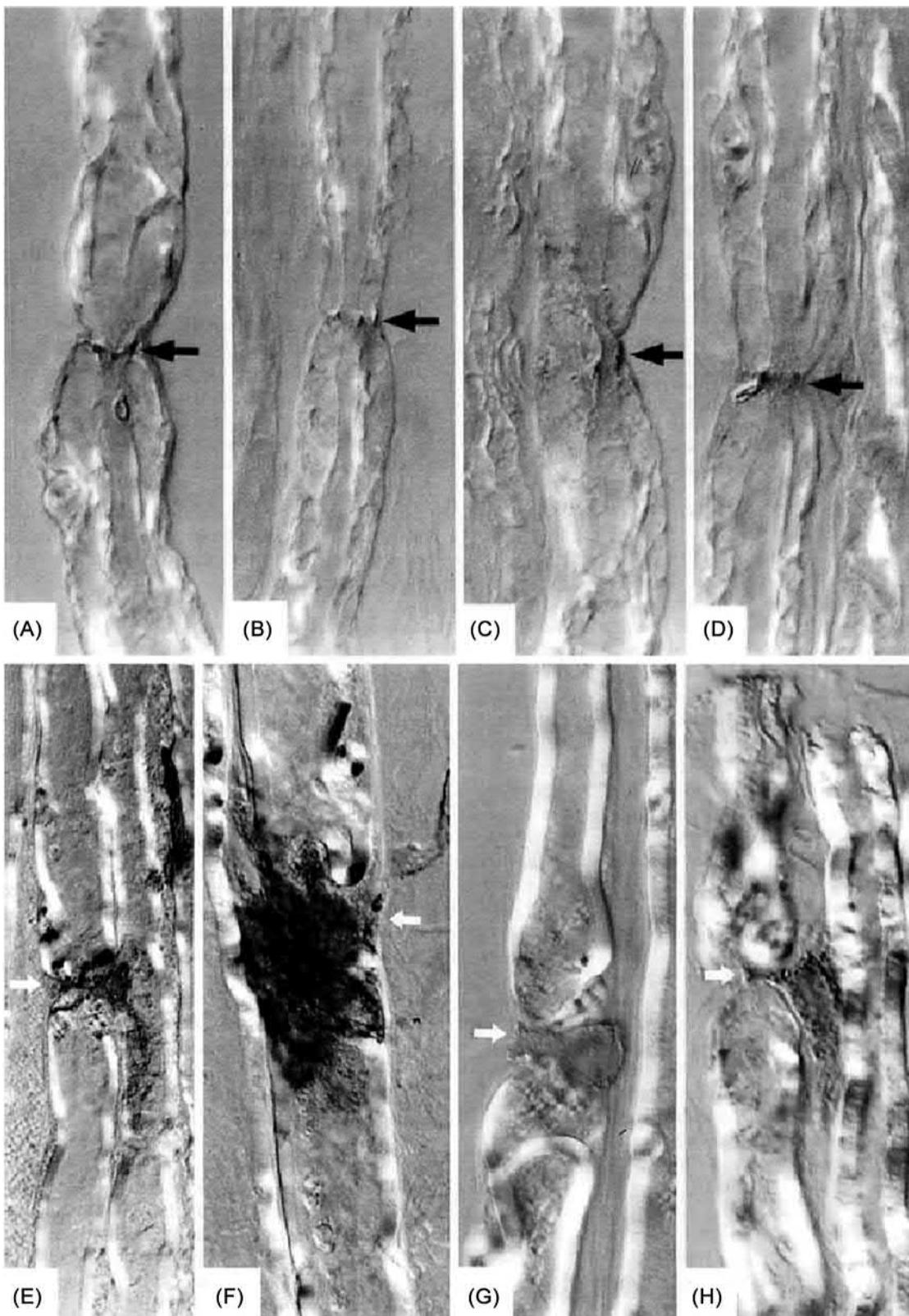
### **Miller Fisher Syndrome**

The Miller Fisher or FS (Fisher, 1956) accounts for approximately 5% of GBS cases and comprises ophthalmoplegia, ataxia, and areflexia without limb weakness. In common usage, facial and bulbar weakness have been included as part of the syndrome. Overlapping forms with AIDP are not uncommon. This syndrome is strongly associated with preceding *C. jejuni* infection, and more than 90% of the patients have antibodies to the ganglioside GQ1b that are almost certainly pathogenic (see below) (Mizoguchi, 1998; O'Hanlon et al., 2001). Anti-GQ1b antibodies are also found in GBS patients with ophthalmoplegia. The pathophysiological and structural basis of the clinical manifestations in FS are still not completely resolved, although complement has been shown to be important in models of disease (Willison et al., 2008).

## **Autoimmune Features**

### **Molecular Mimicry**

Molecular mimicry has been invoked as a mechanism in a variety of autoimmune diseases. AMAN and FS provide some of the best available evidence to support the hypothesis of molecular mimicry as a pathogenic mechanism underlying postinfectious autoimmune disorders. AIDP may be very similar and as more evidence of complex ganglioside antigen associations emerges, AIDP may be resolved into the same pathogenic category as AMAN and FS. AMAN and FS fulfill some of the Koch–Witebsky postulates supporting autoantibody as pathogenic (Witebsky et al., 1957; Rose and Bona, 1993). Antiganglioside antibodies can be demonstrated in patient serum. They have cognate antigens (gangliosides) enriched in peripheral nerve. It is possible to immunize animals (see below) with ganglioside antigens either purified or as whole bacteria to produce autoantibody and disease albeit with difficulty. The transfer of antibody from patient/model animal to a normal subject can sometimes transfer disease. Furthermore, mechanisms of antibody action are now starting to be understood.



**FIGURE 52.1** Immunostained teased ventral root fibers from a case of AMAN 4 days after the onset of neurologic symptoms. (A–D) The complement activation product C3d was localized discretely at nodes of Ranvier (black arrows) of large myelinated motor fibers. The golden-brown immunoreaction product is at the nodes. (E–H) Many motor fibers had macrophages overlying, and extending processes into, the nodes of Ranvier (white arrows designate nodes of Ranvier). [(E) and (F) were immunostained with the macrophage marker HAM-56; (G) and (H) for HLA-DR (major histocompatibility locus class II)]. AMAN, Acute motor axonal neuropathy. Reprinted with permission of John Wiley & Sons Inc. from Figure 1, p. 639 of Hafer-Macko, C., Hsieh, S.T., Li, C.Y., Ho, T.W., Sheikh, K., Cornblath, D.R., et al., 1996a. Acute motor axonal neuropathy: an antibody-mediated attack on axolemma. *Ann. Neurol.* 40, 635–644.

### **Antiganglioside Antibodies in Guillain–Barré Syndrome Variants**

Antibodies to ganglioside species are found in the serum of patients with GBS (both AMAN and AIDP). They are polyclonal, predominantly IgG, and generally complement-fixing IgG1 and IgG3 (Willison and Veitch, 1994; Ogino et al., 1995; Yuki et al., 1995; Ho et al., 1999). This implies class switching usually with T-cell help, both atypical of human anticerbohydrate responses (see below). Antibodies to single gangliosides GM1, GM1(NeuGc), GM1b, GalNAc-GM1b, GD1a, GalNAc-GD1a, GD1b, 9-O-acetyl GD1b, GD3, GT1b, GQ1b, GQ1b $\alpha$ , LM1, galactocerebroside, and sulfated glucuronyl paragloboside (SGPG) have been reported in more than 200 papers on inflammatory neuropathies (Willison and Yuki, 2002). Clinico-serological correlations between GBS subtypes and serum antibodies to putative ganglioside antigens have been drawn (Ho et al., 1999; Ogawara et al., 2000; Rees et al., 1995a). Antibodies to gangliosides GM1 and GD1a, implicated as the major target antigens in AMAN (Hadden et al., 1998; Ho et al., 1999), can be detected in 50%–60% of AMAN patients in the Far East (Ho et al., 1999; Ogawara et al., 2000). Antibodies to GalNAc-GD1a and GM1b are found in motor predominant GBS in about 10%–15% of the cases (Ang et al., 1999; Yuki et al., 2000). Anti-GQ1b antibodies, frequently cross-reactive with structurally related gangliosides, are present in 80%–90% of the patients with FS (Willison et al., 1993; Yuki et al., 1993; Carpo et al., 1998). This correlation provides the strongest association between antibodies to a specific ganglioside and a clinical phenotype.

More recently, the serendipitous identification of antibodies to complex gangliosides has led to a resurgence of interest in antibodies (Kusunoki's first reference) (Greenshields et al., 2009; Kusunoki and Kaida, 2011). Antibody activity to a ganglioside species can be enhanced or entirely abrogated by the close association of a second species of ganglioside presumably making a complex epitope. Antibodies to ganglioside complexes may explain the lack of antibodies found in sera when only single ganglioside activities were sought, the difficulties of consistent identification of clinic-serological phenotypes, and the apparent inconsistency in the spatial distribution of single gangliosides and antibody binding.

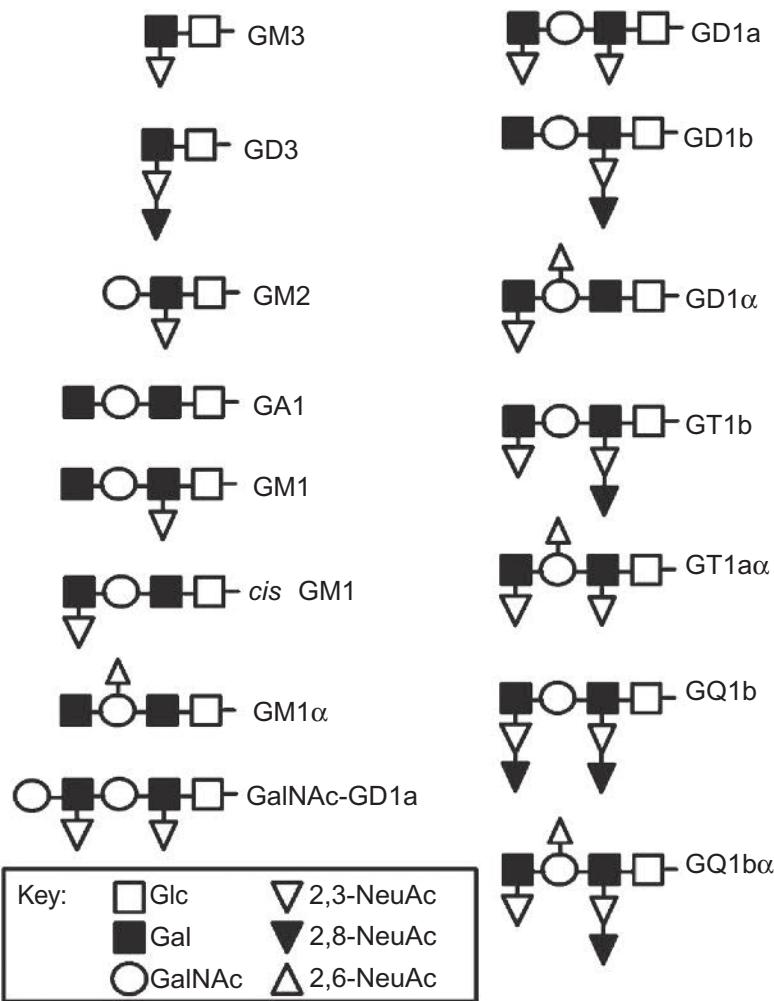
The serological studies have also identified associations of specific antiganglioside or ganglioside complex antibodies with poor recovery (reviewed in Lopez et al., 2010; Sheikh and Zhang, 2010). These association studies imply that specific antiganglioside antibodies can not only injure intact nerve fibers to induce neuropathy but can also adversely affect recovery by either inducing more severe neuropathic disease or interfering with the nerve repair process required for recovery. Experimental studies with antiganglioside antibodies support the latter hypothesis (Lehmann et al., 2007; Lopez et al., 2010).

### **Gangliosides in Peripheral Nerve**

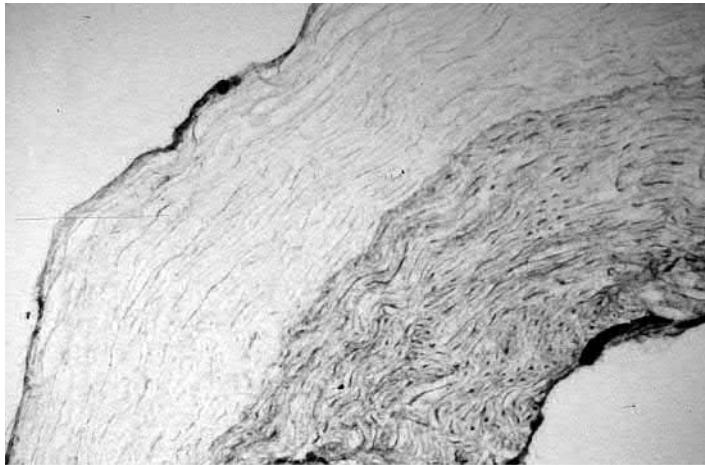
Gangliosides are sialic acid containing glycolipids (see Fig. 52.2), widely distributed in mammalian tissues, but enriched in the nervous system. GM1, GD1b, GD1a, and GT1b are most abundant. A simple hypothesis to explain the differences between GBS variants is based on the premise that target gangliosides have differential distribution in the peripheral nervous system (PNS), but this hypothesis is being modified in response to the discovery of complex ganglioside epitopes. In relation to FS, GQ1b is relatively enriched in the oculomotor cranial nerves (Chiba et al., 1993, 1997) although anti-GQ1b antibodies may bind elsewhere (Goodyear et al., 1999). Although ganglioside immunolocalization studies are technically difficult, the AMAN-associated gangliosides GM1 and GD1a are localized at the nodes of Ranvier and in motor nerve terminals (MNTs) (Sheikh et al., 1999; Gong et al., 2002). Furthermore, preferential staining of motor nerve fibers has been demonstrated with monoclonal anti-GD1a antibodies in rats (Fig. 52.3) and also with human anti-GD1a antibodies from a patient with AMAN (De Angelis et al., 2001). The distribution of ganglioside complexes in vivo will be even more difficult. Other factors such as variations in permeability of the BNB, density and accessibility of target gangliosides, and relationship to functional components of the axolemma, such as ion channels, are likely to be relevant.

### **Functional Effects of Antibodies**

Antibodies bind to nerves at nodes of Ranvier where gangliosides (especially GM1) and channels are enriched. Early studies indicated that anti-GM1 antibodies possibly blocked or altered channel function, although the exact mechanism was unclear (Sheikh et al., 1999). Intraneuronal injection of GBS patient serum, purified immunoglobulin, or specific antiganglioside antibodies has produced mixed results (Saida et al., 1979; Winer et al., 1988; Sumner et al., 1992). Powerful models developed over the last 10 years have demonstrated clear binding of antibodies and complement to nodal structures, dissolution of the axonal cytoskeletal architecture, and disruption of nodal and paranodal channels (Susuki et al., 2007). The electrical effect of this is to destabilize the membrane resulting in trains of uncontrolled miniature end-plate potentials identifiable in models.



**FIGURE 52.2** Some of the ganglioside species in peripheral nerve, potentially targets for neuropathy-associated antibodies. Note the similarity in structures between species, which allows for some cross-reactivity. *Glc*, Glucose; *Gal*, galactose; *GalNAc*, 5 *N*-acetylgalactosamine; *NeuAc*, 5 neuraminic (sialic) acid.



**FIGURE 52.3** Unfixed fresh-frozen rat motor-sensory nerve root stained with a monospecific murine IgG anti-GD1a antibody. There is preferential strong staining of the motor axons of the ventral root (below) compared to the sensory axons of the adjacent dorsal root (above). Magnification  $\times 320$ .

An alternative site of the attack is at the roots and MNTs where the BNB is relatively deficient. MNTs degenerate in AMAN (Ho et al., 1997) and are disturbed electrophysiologically in FS (Uncini and Lugaresi, 1999). In an ex vivo phrenic nerve-diaphragm preparation, Willison et al. showed that anti-GQ1b antibodies bind to nerve terminals, cause complement-dependent quantal acetylcholine release resulting in neuromuscular blockade and a calcium-dependent disruption of the terminal bouton (Goodear et al., 1999; Plomp et al., 1999; O'Hanlon et al., 2001). The same effects were shown with anti-GD1a antibodies dependent upon GD1a antigen density (Goodfellow et al., 2005).

In a parallel series of patch-clamp experiments, IgG GQ1b, GD1a, GD1b, and GM1 antibodies have been shown to cause reversible complement independent pre- and postsynaptic blockade depending upon the antibody used (Buchwald et al., 2002).

The clinical, pathological, and electrical effects are almost entirely abrogated by the application of an inhibitor of the C5 component of complement (eculizumab) which results in a failure of formation of the membrane attack complex (Halstead et al., 2008). This pathophysiological understanding and subsequent proof of concept in an animal model have led to the design of human trials.

Antiganglioside antibodies can inhibit neurite growth and growth cone extension in primary neuronal cultures consistent with the notion that these antibodies can adversely affect axon and nerve regeneration (Zhang et al., 2011b). Small GTPase RhoA is part of the downstream inhibitory intracellular signaling that mediates antiganglioside antibody-induced inhibition of axon growth (Zhang et al., 2011b). At the cellular level, antiganglioside antibody-mediated inhibition of axon regeneration requires the engagement of activating Fc-gamma receptors on macrophage lineage cells.

A recent study has shown that pleiotropic cytokine erythropoietin (EPO) with neurotrophic properties reverses the inhibitory effects of antiganglioside antibodies via EPO receptors and the Janus kinase 2/Signal transducer and activator of transcription 5 pathway (Zhang et al., 2011a).

## Environmental Effects

*C. jejuni* is a Gram-negative, nonspore-forming enteropathogen and is one of the most common causes of bacterial gastroenteritis worldwide (Friedman et al., 2000; Oberhelman and Taylor, 2000). Infection with *C. jejuni* is found in 13%–72% of the patients with AMAN or GBS (Hughes and Rees, 1997; Hadden and Gregson, 2001) with an overall prevalence estimated around 30% (Moran et al., 2002). Only 1 in 1000 cases of *C. jejuni* infection is complicated by GBS. The exact characteristics of *C. jejuni* that determine whether GBS follows infection are still unclear. However, a relationship of GBS with a number of Penner serotypes is recognized (Prendergast and Moran, 2000). Penner serotyping distinguishes *C. jejuni* strains on the basis of strain-specific, heat-stable extractable capsular lipopolysaccharide (LPS) and lipooligosaccharide (LOS). Penner strains HS:19 (Rees et al., 1995b; Sheikh et al., 1998) and HS:41 (Goddard et al., 1997) are particularly overrepresented in GBS and uncommon in patients with uncomplicated gastroenteritis. The LPS and LOS of *C. jejuni* carry ganglioside-like moieties. Several studies have characterized these in GBS- and diarrhea-associated *C. jejuni* strains. GM1-, GD1a-, GalNAc-GD1a-, GM1b-, GT1a-, GD2-, GD3-, and GM2-like structures have all been identified (Aspinall et al., 1993; Yuki et al., 1994; Nachamkin et al., 2002). Although no GQ1b-like structure exists, antibody-binding assays have shown the presence of GQ1b- and GT1a-cross-reactive moieties in *C. jejuni* LPS/LOSs (Yuki et al., 1994; Jacobs et al., 1995, 1997). It is these structures that are likely to provide the initial stimulus to autoimmune activation.

Upper respiratory tract infection or other febrile episodes caused by cytomegalovirus (5%–22%), Epstein–Barr virus (2%–10%), *Mycoplasma pneumoniae* (5%), and *Haemophilus influenzae* have all been identified as potentially causative (Hadden and Gregson, 2001). *H. influenzae* carries ganglioside-like moieties. Swine flu and rabies vaccinations have also been causatively implicated, but recent surveillance for an association with swine flu vaccination failed to demonstrate any conclusive link (Crawford et al., 2012).

More recently, Zika virus, a mosquito-borne RNA flavivirus, has been identified as a potential trigger for GBS. During a Zika virus outbreak in 2013–14 in French Polynesia, an increase of GBS cases was observed. Notably, these post-Zika virus infection GBS cases were mostly AMAN but negative for antiganglioside antibodies typically associated with this variant of GBS. However, 31% of the patients had antiglycolipid antibodies detected by ELISA and 46% detected by glycoarray antibodies. A temporal association between increased rates of GBS cases and Zika virus infection was also observed during the major Zika virus outbreak in 2015–16 in South America. A detailed description of a cohort of GBS cases in Colombia during Zika virus outbreak revealed that in contrast to the classic clinical course of GBS, many patients (20%) showed a parainfectious, rapid disease onset.

## Animal Models of Disease

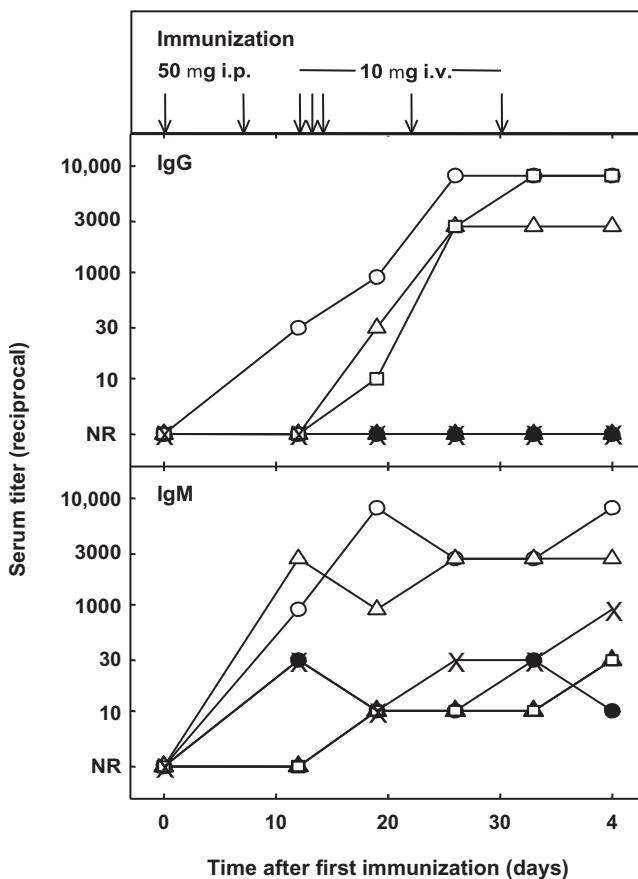
Attempts to generate either IgG antiganglioside antibodies or neuropathy in animals by immunization with *C. jejuni* were for a long while either unforthcoming or not reproducible. Immunization of mice with *C. jejuni* LPSs/LOSs generates mainly low-titer IgM antibodies (Wirguin et al., 1997; Goodey et al., 1999) reflecting a

high level of tolerance to self-gangliosides (Bowes et al., 2002). Tolerance to self-gangliosides can be overcome by immunization with gangliosides or *C. jejuni* LPSs conjugated to adjuvant in transgenic animals lacking complex gangliosides (Lunn et al., 2000) (see Fig. 52.4). This model illustrates that where tolerance is circumvented, potentially pathogenic antibodies can be generated.

A sensory ataxic neuropathy with pathological changes in the nerve and the cord has been induced in rabbits by GD1b immunization and passive antibody transfer (Kusunoki et al., 1999). Immunization with mixed bovine brain gangliosides or GM1 alone produced an acute flaccid paralysis in rabbits reminiscent of human disease (Susuki et al., 2003). The implantation of a murine IgG anti-GD1a secreting hybridoma into mice but not passive transfer of the same antibody led to an axonal neuropathy (Sheikh et al., 2004). Additional cytokine factors may be required to initiate the neuropathy.

The combined research in GBS models has clearly demonstrated that antibodies bind to ganglioside targets on the nerve, fix complement, destroy the nodal, and cytoskeletal architecture through complement and calcium-dependent mechanisms and result in electrophysiological instability and axonal dissolution (Willison et al., 2008).

A passive transfer animal model has been established to examine the effects of antiganglioside antibodies on peripheral nerve repair/axon regeneration (Lehmann et al., 2007). These passive transfer studies show that patient and experimental antibodies inhibit axon regeneration in a nerve crush model mimicking the regenerative response of degenerating/injured axons in GBS (Lehmann et al., 2007; Lopez et al., 2010). The impaired regenerative responses and ultrastructure of injured peripheral axons mimicked dystrophic and stalled the growth cones



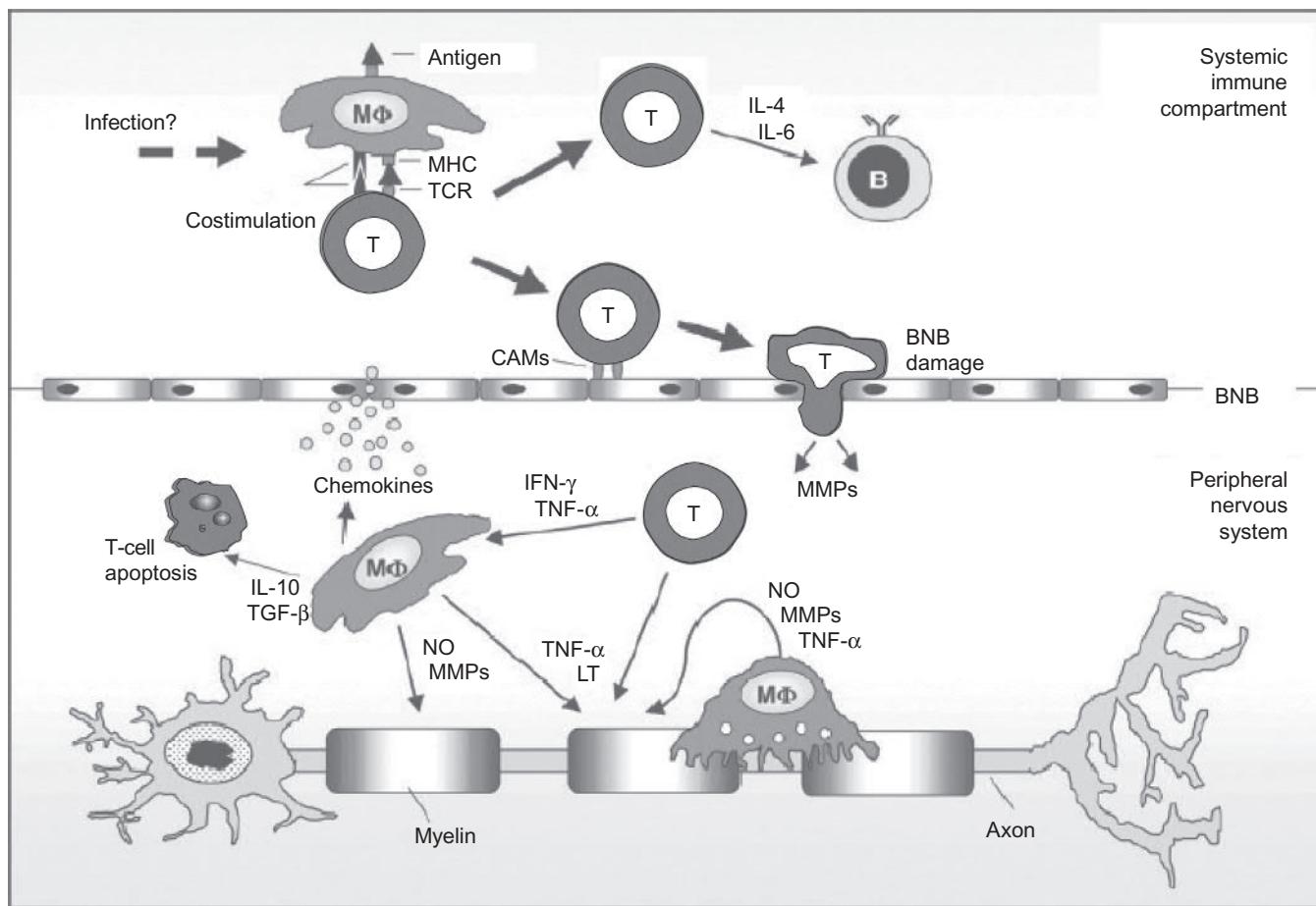
**FIGURE 52.4** Mice lacking complex ganglioside species (*GalNAcT*) are able to class switch to complement-fixing IgG production when immunized with KLH-conjugated ganglioside. The figure shows serological responses of *GalNAcT*<sup>-/-</sup> and wild-type mice immunized with GD1a-KLH or KLH alone. Sera collected at the indicated times were tested for IgG and IgM anti-GD1a antibody titers by ELISA. Open symbols, individual *GalNAcT* mice immunized with GD1a-KLH; filled symbols, individual wild-type mice immunized with GD1a-KLH; X, *GalNAcT*<sup>-/-</sup> mouse immunized with unconjugated KLH. Titer values are presented as the greatest dilution resulting in a signal which exceeded 3 SD above the control mean. *i.p.*, Intraperitoneal; *i.v.*, intravenous; *KLH*, keyhole limpet hemocyanin. Reprinted with permission of Blackwell Publishing Ltd. from Figure 2 of Lunn, M.P., Johnson, L.A., Fromholt, S.E., Itonori, S., Huang, J., Vyas, A.A., et al., 2000. High-affinity anti-ganglioside IgG antibodies raised in complex ganglioside knockout mice: reexamination of GD1a immunolocalization. *J. Neurochem.* 75, 404–412. ©2000 Blackwell Publishing Ltd.

typically seen after central nervous system (CNS) injury. Such dystrophic/stalled growth cones can also be seen in nerve biopsies of GBS patients with poor recovery (Sheikh and Zhang, 2010). These observations support the notion that inhibition of axon regeneration is one mechanism of poor recovery in GBS patients with antiganglioside antibodies. Further, this antibody-induced nerve injury model was used to show beneficial proregenerative effects of EPO (Zhang et al., 2011a).

## Cellular Mechanisms

Comprehensive evidence of T-cell involvement in GBS has not been so forthcoming despite the central role of T cells in the pathogenesis of EAN. Studies of animal models and predominantly human AIDP have generated a complex multistep pathogenesis for cellular involvement in the autoimmune neuropathies (see Fig. 52.5).

Multifocal lymphocytic infiltration was established as the hallmark of the pathology of GBS in early postmortem studies (Asbury et al., 1969) but it is not always seen (Cornblath et al., 1990; Honavar et al., 1991).



**FIGURE 52.5** Schematic illustration of the immune responses in the inflamed peripheral nervous system. Basic principles of the cellular immune responses: autoreactive T cells (T) recognize a specific autoantigen presented by MHC class II molecules and the simultaneous delivery of costimulatory signals on the cell surface of antigen-presenting cells, such as macrophages (MΦ), in the systemic immune compartment. Activated T lymphocytes can cross the BNB in order to enter the PNS. Within the PNS, T cells activate macrophages that enhance phagocytic activity, production of cytokines, and the release of toxic mediators, such as NO, MMPs, and proinflammatory cytokines, propagating demyelination and axonal loss. The termination of the inflammatory response is mediated, in part, by macrophages by the induction of T-cell apoptosis and the release of antiinflammatory Th2/Th3 cytokines, such as IL-10 and TGF- $\beta$ . BNB, Blood–nerve barrier; IL-10, interleukin-10; TGF- $\beta$ , transforming growth factor- $\beta$ ; MMPs, matrix metalloproteinases; MHC, major histocompatibility complex; PNS, peripheral nervous system; NO, nitric oxide. Reprinted with permission from John Wiley & Sons Inc. from Figure 1, p. 136 of Kieseier, B.C., Kiefer, R., Gold, R., Hemmer, B., Willison, H.J., Hartung, H.P., 2004. Advances in understanding and treatment of immune-mediated disorders of the peripheral nervous system. *Muscle Nerve* 30, 131–156. ©2004 John Wiley & Sons Inc.

Circulating-activated T cells are found early in the course of GBS (Taylor and Hughes, 1989; Hartung and Toyka, 1990).  $\alpha\beta$ T cells with CD4 and CD8 ratios in similar proportions to peripheral blood (Cornblath et al., 1990) are the predominant cells found in nerve. The restricted usage of  $V\beta$  genes, especially  $V\beta 15$ , suggests activation by a common antigen or superantigen (Khalili-Shirazi et al., 1997). Furthermore,  $\gamma\delta$ T cells have also been found in, and isolated from, GBS-affected nerves (Khalili-Shirazi et al., 1998; Winer et al., 2002).  $\gamma\delta$ T cells are capable of recognizing nonprotein antigen and are thus candidates for responding to putative carbohydrate and ganglioside antigens (see above) (Bukowski et al., 1998). They proliferate in vitro in response to *C. jejuni* sonicates but require either  $\alpha\beta$ T cells or interleukin (IL)-2/IL-15 to do so. The predominant use of  $V\gamma 8\delta 1$  suggests the activation of epithelial (possibly gut) resident  $\gamma\delta$ T cells (Cooper et al., 2000).  $\alpha\beta$  and  $\gamma\delta$ T cells also probably provide the necessary help to orchestrate class switching of antiganglioside antibodies to IgG1 and IgG3.

Autoantigen in the systemic compartment is processed and presented by antigen-presenting cells. EAN can be initiated with neuritogenic epitopes of peripheral nerve proteins P0, P2, and PMP22 (Hughes et al., 1999) or by adoptive transfer of sensitized T cells. Disease severity depends upon the cell or antigen dosage (Hartung et al., 1996). Disease is dependent on the presence of T cells, their normal function (Holmdahl et al., 1985; Hartung et al., 1987; Jung et al., 1992), and normal function of the costimulatory partners B7.1/CD80 or B7.2/CD86 and CTLA-4/CD28 (Kiefer et al., 2000; Zhu et al., 2001; Zehntner et al., 2003).

Lymphocyte activation is revealed by greater numbers of circulating T cells—bearing activation markers and increased concentrations of Th1 cytokines such as IFN- $\gamma$ , IL-2, IL-2 receptor, and tumor necrosis factor (TNF)- $\alpha$  (Taylor and Hughes, 1989; Hartung et al., 1991; Exley et al., 1994; Creange et al., 1996). Levels of transforming growth factor (TGF)- $\beta 1$  are depressed (Creange et al., 1998). In EAN, levels of IFN- $\gamma$ , IL-1 $\beta$ , IL-6, TNF- $\alpha$ , TNF- $\beta$ , and IL-12 are raised during the development of disease (Zhu et al., 1997).

Homing and migration of activated T cells to the peripheral nerve are modulated by E-selectin and mucins binding L-selectin and sialyl Lewis antigens (Hartung et al., 2002) and then VCAM-1 and ICAM-1, both upregulated early in GBS and EAN progression (Enders et al., 1998; Creange et al., 2001). Blockade of VCAM-1 or its ligand VLA-4/ $\alpha 4\beta 1$  integrin ameliorates EAN (Enders et al., 1998). Selectin and integrin released into the circulation may downregulate inflammation (Hartung et al., 1988). Chemokines assist in leukocyte recruitment localization and trafficking (Bagnolini, 1998; Campbell et al., 1998). The chemokine receptors CCR1, CCR2, CCR4, CCR5, and CXCR3 have been characterized in AIDP-affected nerves, differentially upregulated by infiltrating cell populations (Kieseier et al., 2002).

Diapedesis through the vascular endothelium and the basal lamina is facilitated by the matrix metalloproteinases (MMPs). MMP-2, MMP-3, MMP-7, and especially MMP-9 have been implicated in the pathogenesis of EAN and GBS (Kieseier et al., 1998; Creange et al., 1999) and correlate with GBS severity.

Macrophages, both resident and recruited from the circulation (Hartung et al., 2002), remain the key component in perpetuating endoneurial inflammatory damage through the release of specific immune mediators. Under inflammatory conditions, they continue to express antigen and induce Schwann cells to do so also (Gold et al., 1995). Depletion of macrophages abrogates the development of EAN, indicating their central role in the final common pathway of nerve damage. A recent study used a function-neutralizing monoclonal antibody against CD11b to ameliorate disease onset and severity in murine EAN to reduce leukocyte trafficking in the inflamed nerve. Activated macrophages target normal looking nerves and Schwann cells in EAN and AIDP (Hartung et al., 1996; Hughes et al., 1999) probably by antibody-targeted cellular cytotoxicity and complement-dependent mechanisms (Hafer-Macko et al., 1996a,b). Macrophage processes insinuate themselves between myelin lamellae and strip the myelin in AIDP (Hughes et al., 1999) or directly attack the axon in AMAN (see Fig. 52.1) (Hafer-Macko et al., 1996a). In the endoneurium, macrophages secrete a host of inflammatory mediators including MMPs, TNF- $\alpha$ , nitric oxide, eicosanoids, neutral proteases, lipases, and phospholipases, all contributing to nerve damage (Gregson and Hall, 1973; Redford et al., 1997; Smith et al., 1999).

Macrophages may also influence recovery from GBS. They direct T-cell apoptosis reducing the ongoing response. During recovery, the T-cell response shifts toward Th2 with rises in IL-4 in patients (Dahle et al., 1997) and upregulation of TGF- $\beta 1$ , IL-10, and catolysin in models of disease (Kiefer et al., 1996). TGF- $\beta 1$  favors recovery (Vriesendorp et al., 1996; Zhu et al., 1997) and levels are correlated to the severity of GBS (Creange et al., 1998).

A recent study (Mausberg et al., 2011) showed that EPO reduced disease severity and also shortened the recovery phase of EAN. The clinical improvement in this model correlated with decreased T-cell inflammation within the peripheral nerve and produced less nerve fiber injury. In contrast, EPO increased the number of macrophages in the recovery phase of EAN. The beneficial effects of macrophages and the modulation of the

immune system toward antiinflammatory responses in the PNS were associated with the upregulation of anti-inflammatory cytokine TGF-beta.

## Cellular and Humoral Immune Elements Are Synergistic

Understanding of the pathomechanisms of the acute inflammatory neuropathies is incomplete. Neither antibodies nor T cells generate disease in isolation and immunization with any antigen which induces responses in both cellular and humoral arms of the immune system. The apparent absence of T cells in some biopsies, and hence their possible nonnecessity, is discussed above. Arguments against the early and significant involvement of antibodies include the absence of detectable antiganglioside antibodies, using the current methodology, in a significant proportion of GBS (usually AIDP) cases, and the onset of adoptive transfer-EAN (AT-EAN) 4 days after transfer, before antibodies could be synthesized. However, recent advances have reduced the strength of these arguments. First, disease severity in AT-EAN is usually enhanced by cotransferring antibodies recognizing myelin or oligodendrocytes/Schwann cell epitopes ([Spies et al., 1995](#)), although this was not confirmed when pretreatment AIDP GBS sera were coadministered to rats with mild EAN ([Hadden et al., 2001](#)). Gangliosides are not necessarily targeted by antibodies as single entities explaining why many sera were apparently negative for antiganglioside antibodies. Combinatorial epitopes made from adjacent but differing molecules that enhance or reduce antibody affinity in vitro and probably in vivo are recognized as targets ([Kusunoki and Kaida, 2011](#)).

## Genetic Aspects of Guillain–Barré Syndrome

The role of host genetics in susceptibility to GBS is still in its infancy. No strong correlations have been established between disease susceptibility or GBS subtypes and host major histocompatibility complex (MHC) class I or class II haplotypes in several studies ([Magira et al., 2003](#); [Gelejns et al., 2005a](#)). The study of single nucleotide polymorphisms in genes for various components of the immune response has not identified any significant contributors ([Gelejns et al., 2005a,b](#)). One Dutch study identified that GBS patients homozygous for the Fc $\gamma$  receptor IIa-H131 had a higher chance of developing severe disease than patients with other genotypes ([van der Pol et al., 2000](#)). Polymorphisms in CD1 molecules which present ganglioside to T cells were associated with GBS in two studies but the largest study to date failed to confirm this ([Kuijf et al., 2008](#)). Metaanalysis focusing on TNF- $\alpha$ , CD1, and Fc $\gamma$ R suggests that the TNF- $\alpha$  308A allele may be a moderate risk factor for GBS ([Wu et al., 2012](#)), but this awaits the confirmation in directed larger studies.

## Treatment and Outcomes

The effectiveness of other immunotherapies for GBS is supported by good evidence ([Hughes et al., 2003](#)). Two to five sessions of plasma exchange (PEx) hasten recovery in nonambulant patients preferably started within 2 weeks of disease onset ([Chevret et al., 2017](#)). Intravenous immunoglobulin (ivIg) is as effective as PEx and is still probably the intervention of choice ([Hughes et al., 2014](#)). The concern about possible contamination of ivIg with prions means that the written informed consent is essential before administration, although advances in ivIg purification technology and prion detection may reduce this concern. The drive to search for new, more effective, and safer therapies is stronger than ever.

A large randomized controlled trial, previous smaller trials, and a Cochrane metaanalysis have shown steroids to be at best ineffective and in some cases harmful. There is no indication for their use in GBS.

Complement inhibitors such as eculizumab are effective in other complement-mediated conditions such as paroxysmal nocturnal hemoglobinuria and possibly effective in multifocal motor neuropathy. Two phase-II trials explored the safety of eculizumab in GBS. In the ICA-GBS trial, five patients with GBS received eculizumab in addition to standard ivIg treatment. The combination treatment was not associated with an increased frequency of severe adverse events. Similarly, in the Japanese Eculizumab Trial, GBS patients safely received eculizumab in conjunction with ivIg and although the primary outcome was not reached, secondary outcomes were positive, and further, larger studies are awaited. Immunoabsorption of pathogenic antiganglioside antibodies has been infrequently used ([Willison et al., 2004](#)). Preclinical studies in antibody and T cell–induced models of GBS suggest that EPO is a viable candidate drug to develop further for neuroprotection and enhancing nerve repair in patients with GBS ([Zhang et al., 2011a](#)).

## CHRONIC NEUROPATHIES: CHRONIC INFLAMMATORY DEMYELINATING POLYRADICULONEUROPATHY

CIDP is an acquired PNS disease characterized by progressive or relapsing proximal and distal weakness with or without sensory loss. There is good evidence to indicate that CIDP is autoimmune.

### History

Osler recognized a chronic relapsing or progressive form of "multiple neuritis" in 1892. Austin (1958) described a slowly progressive or recurrent "steroid responsive polyneuropathy" with histology indistinguishable from that seen in GBS. The term "chronic inflammatory demyelinating polyradiculoneuropathy" was coined in 1975 (Dyck et al., 1975).

### Epidemiology and Clinical Features

The prevalence of CIDP is 1.25–7 per 100,000 (Lunn et al., 1998; Mygland and Monstad, 2001). Symmetrical sensory and motor deficits both proximal and distal reach their nadir over more than 8 weeks. The clinical course is commonly chronic and progressive particularly in untreated patients but some patients have a relapsing-remitting pattern of disease. Some case series have shown that up to 15% of the patients with CIDP can start acutely with GBS-like onset (Dionne et al., 2010). Cranial nerve and the diaphragm are infrequently involved. CSF examination demonstrates albuminocytological dissociation with raised protein in more than 90% of the cases (Bouchard et al., 1999), and the cell count is <10 mm<sup>3</sup> unless complicated by human immunodeficiency virus. Electrophysiology typically demonstrates multifocal motor conduction slowing, temporal dispersion, and block with delayed or absent F-waves. Half of the patients require treatment at any one time and 13% require long-term aid to walk (Lunn et al., 1999). In a single study, 6 of 21 patients were shown to have serum antibodies to the peripheral myelin protein P0 (Yan et al., 2001) but this remains unconfirmed. Morphological examination demonstrates macrophage-associated demyelination in nerve roots, plexuses, and nerve trunks with T cells in the endoneurium. The edema, Schwann cell proliferation, and inflammatory infiltrates testify to the ongoing inflammation. Axonal degeneration occurs in severe or late cases or in distal sensory nerve biopsies (Hadden and Hughes, 2003).

As with GBS, CIDP is heterogeneous, and a number of subtypes or related conditions have emerged.

### Multifocal Motor Neuropathy With Conduction Block

Multifocal motor neuropathy with conduction block (MMNCB) (Pestronk et al., 1988) is a rare but treatable condition sometimes misdiagnosed as motor neuron disease. Wasting and weakness begin asymmetrically in the distribution of a motor nerve usually more distal than proximal, most commonly in the upper limb. Fasciculations and cramps occur. Males account for 70% of the cases. Sensory symptoms are reported in 20% of the patients. Depending upon assay methods, between 30% and 80% of the patients have anti-GM1 antibodies in the serum (Sander and Latov, 2003), some as a paraprotein. Rarely, anti-GD1a antibodies are described (Carpo et al., 1996). Electrophysiological studies demonstrate multifocal conduction blocks in two or more nerves with normal sensory conduction. Many studies demonstrate the evidence of more widespread slowing or axonal degeneration.

The etiology of MMNCB remains unresolved, as it is seldom fatal, and neuropathological material is seldom sought. Perivascular CIDP-like inflammation has been described in a motor nerve biopsy (Kaji et al., 1993), and there are minimal findings in sensory nerves (Corse et al., 1996). Inflammatory cellular infiltration and immunoglobulin deposition were described in the motor roots of an autopsy case (Oh et al., 1995). The therapeutic response to immunomodulation supports an autoimmune pathogenesis (Umapathi et al., 2005).

### Multifocal-Acquired Demyelinating Sensory and Motor Neuropathy

Lewis and Sumner described the entity now referred to as multifocal-acquired demyelinating sensory and motor neuropathy in 1982 (Lewis et al., 1982). Electrophysiological examination demonstrates multifocal sensory and motor involvement with conduction block.

### Multifocal-Acquired Sensory and Motor Neuropathy

This group of patients has only been recently described (Alaedini et al., 2003). Antiganglioside antibodies are found in the serum of 48%, and there is a beneficial response to immunomodulation. The nerve conduction

studies do not suggest demyelination and are more consistent with axonal degeneration. These findings require confirmation.

### **Paraproteinemic Demyelinating Peripheral Neuropathy**

Ten percent of the patients with a peripheral neuropathy will have a paraprotein in the serum. Although this should initiate a search for a malignant source, such as multiple or solitary myeloma, most are monoclonal gammopathies of undetermined significance (MGUS). A number of MGUS-neuropathy syndromes are described.

The commonest is a progressive sensory ataxic neuropathy with unsteadiness and tremor associated with an IgM<sub>k</sub> paraprotein that reacts with the HNK-1 epitope of the peripheral nerve antigen MAG. Up to 80% of the patients with an IgM paraprotein and a demyelinating neuropathy have anti-MAG antibodies. Nerve conduction studies reveal motor conduction slowing, particularly distally (Cocito et al., 2001). Electron microscopic examination of myelin in sural nerve biopsies reveals the characteristic widening of the intraperiod line not seen in other conditions (see Fig. 52.6).

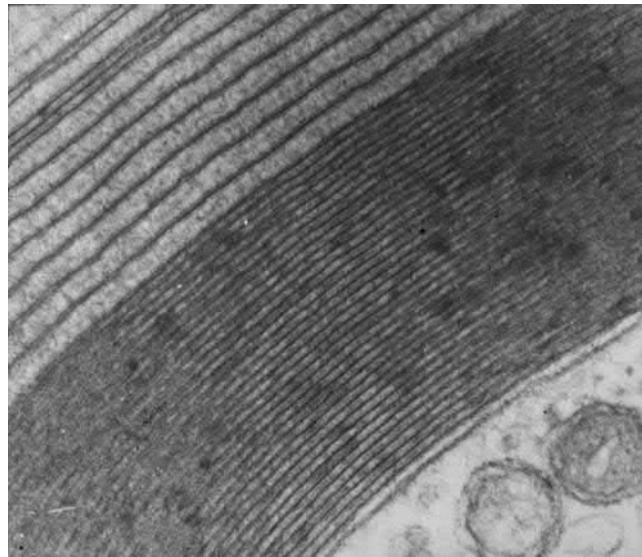
Neuropathies associated with IgG or IgA paraproteins are found in a diverse group of patients with axonal and demyelinating forms of the disease. No consistent pathogenesis has yet emerged. The demyelinating cases have a similar therapeutic response to CIDP (Stork et al., 2015).

Chronic ataxic neuropathy with ophthalmoplegia, M-protein, and antidisialosyl antibodies (CANOMAD) is a rare paraproteinemic syndrome rather like a chronic FS. Its pathogenesis is not yet resolved.

The POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes) is a rare condition associated with both osteosclerotic myeloma and Castleman's disease. The paraprotein required to make the diagnosis does not seem to participate in the pathogenesis as it is often still found after successful treatment. Furthermore, cytokines (especially IL-6 and IL-1 $\beta$ ) and vascular growth endothelial factor are implicated in causation (Lagueny et al., 2004; Dispenzieri, 2012).

### **Autoimmune Features**

Many of the features of autoimmunity displayed in the acute neuropathies are also seen in CIDP. Cellular and humoral responses are both important to the pathogenesis. Raised circulating levels of IL-2 and TNF- $\alpha$  correlate with disease activity (Misawa et al., 2001). A TH-17 response is active with raised levels of IL-17 detectable (Chi et al., 2010), and reduced levels of CD25/CD4 and FoxP3 cells indicate reduced regulatory T cell (Treg) capacity (Chi et al., 2008). Cytokines that affect B-cell responses such as B cell-activating factor are also elevated in sera



**FIGURE 52.6 Widely spaced myelin.** The normal myelin lamellae are tightly compacted and have a periodicity in electron microscope preparations of 12–15 nm. In the demyelinating neuropathy associated with IgM paraprotein that has activity against MAG (anti-MAG PDPN), the intraperiod line becomes split giving an overall periodicity of 30–40 nm. Note the suggestion of material within the widened spaces, possibly immunoglobulin M. Electron micrograph  $\times 100,000$ . MAG, Myelin-associated glycoprotein; MAG PDPN, myelin-associated glycoprotein paraproteinemic demyelinating peripheral neuropathy.

of CIDP patients. T-lymphocyte migration into the endoneurium is facilitated by the increased levels of chemokines, cell adhesion molecules, and MMPs (Previtali et al., 2001; Kieseier et al., 2004). The expression of the BNB constituents claudin-5 and ZO-1 is altered in CIDP perhaps contributing to increased BNB permeability (Kanda et al., 2004). Within the endoneurium, T cells secrete IFN- $\gamma$ , IL-2, and TNF- $\alpha$  (Mathey et al., 1999) and probably others, contributing to inflammatory upregulation of effectors such as macrophages, continued recruitment, and direct nerve damage. The expression of downregulatory cytokines IL-4, IL-10, and TGF- $\beta$  is also found, and in CIDP, expression of nerve growth factor (NGF), glial cell derived neurotrophic factor (GDNF), Leukemia inhibitory factor (LIF), and their receptors may contribute to nerve regeneration (Yamamoto et al., 2002). Clear evidence of Fas deficiency gives support to an inability to halt the ongoing response as effectively as in a monophasic disease (Comi et al., 2009).

Macrophage and Schwann cells in the endoneurium express the costimulatory molecules CD80 (B7-1) and CD86 (B7-2) and their cognate partners CTLA-4 and CD28 are found on endoneurial T cells (Hu et al., 2007).

Antibodies to the glycopeptides P0 and P2, and glycolipids and gangliosides LM1 and GD1b, and LM1 ganglioside complexes have been described but remain unconfirmed, or difficult to confirm, in other studies (Yan et al., 2001; Sanvito et al., 2009; Nobile-Orazio et al., 2010).

More recently, several groups independently reported the occurrence of autoantibodies against nodal-complex proteins in CIDP. These autoantibodies are infrequent and found in 3%–18% of CIDP cases and directed against the neurofascins (predominantly NF155), contactin-1, and/or contactin-associated protein-1 (Caspr). They belong predominantly to the IgG4 subtype and are associated with distinct clinical phenotypes. Patients with anti-NF155 antibodies tend to be younger and have prominent tremor, sensory ataxia, and distal demyelinating symmetric features. CIDP patients with contactin-1 antibodies are older and show a predominant motor involvement and axonal damage. In common, they show a poor response to ivIg treatment and may respond to anti-CD20 treatments.

Pathological studies in nerve samples from these patients demonstrated segmental demyelination with detachment of terminal myelin loops from the axolemma in the paranodal region of the affected nerve fibers.

Unlike GBS or CIDP, in the anti-MAG paraproteinemic demyelinating peripheral neuropathy (anti-MAG PDPN), there is evidence of only antibody-mediated immunity. There is no evidence of macrophage-associated demyelination, T-cell infiltration into the endoneurium, or upregulation of T-cell costimulatory molecules. There is quite strong evidence that anti-MAG antibodies may fulfill the Koch–Witebsky postulates. Anti-MAG antibodies can be found in the serum of patients with characteristic clinical presentation. MAG is expressed at the Schmidt-Lanterman incisures, the paranodes, and on the periaxonal myelin (Gabriel et al., 1998). It displays the HNK-1 carbohydrate epitope shared by several other peripheral nerve antigens. Histological studies demonstrate IgM anti-MAG deposits on Schwann cells that colocalize with MAG (Takatsu et al., 1985). Electron-dense material seen between widened lamellae is consistent with anti-MAG IgM insinuated between the layers (Mendell et al., 1985) (see Fig. 52.6). Activated complement components have been described by some researchers (Monaco et al., 1990). The disruption of MAG functions and complement-mediated damage may result in alterations in the axonal neurofilament cytoskeleton (Lunn et al., 2002), and these, in turn, may lead to slowing of nerve conduction and axonal degeneration.

## Immunogenetic Features

Restricted usage of T-cell receptor (TCR) genes has been demonstrated in CIDP. The predominant TCR in CIDP is  $\alpha\beta$  but  $\gamma\delta$  T cells are found in the majority of nerve biopsy specimens (Winer et al., 2002), again implying a possible nonprotein immune response. No clonality has been demonstrated in isolated T cells. However, significant numbers of highly Th1 inflammatory natural killer T cells identified by expression of V $\alpha$ 24J $\alpha$ Q invariant TCR chain are found (Illes et al., 2000).

A number of putative immunogenetic modifiers have been proposed. Polymorphisms in TAG-1 are not associated with treatment response or outcome (Pang et al., 2012), and no association has been found with CD1 in small studies (Uncini et al., 2011). Recently, studies to suggest that homozygous genotype for a low number of GA repeats in SH2D2A (Uncini et al., 2011) and defective AIRE-mediated central tolerance to P0 (Su et al., 2012a) may be implicated in disease have been presented.

## Environmental Influences

A preceding illness, infection, or vaccination has been identified in patients with CIDP in 32% in the 6 months and 16% in the 6 weeks preceding their illness. Others have found no convincing evidence of vaccination being a trigger (Pritchard et al., 2002). No specific provoking environmental events have so far been recognized for CIDP or the paraproteinemic peripheral neuropathies.

## Animal Models

The pathogenesis of CIDP is not completely defined. Widely accepted animal models that recapitulate all the clinical aspects of CIDP are not available. However, recently developed spontaneous autoimmune polyneuropathy (SAP) models have provided useful insights into pathogenic mechanisms involved in inflammatory demyelinating nerve injury relevant to CIDP. SAP can be seen in transgenic mice expressing the MHC class II  $\text{I}\alpha^b$  molecule. This MHC presents a single peptide, E $\alpha$ 52-68, in mice overexpressing IL-10 under the control of human VMD2 promoter, and in nonobese diabetic (NOD) mice alongside modulations of the costimulatory molecule B7-2 (CD86) and the autoimmune regulator Aire. The subsequent pathogenic mechanisms in NOD mice are fairly well characterized. Elimination of B7-2 in NOD mice induces protection against diabetes mellitus in these animals but triggers the onset of a CIDP-like illness at 6–7 months of age that is almost 100% penetrant in female mice (Salomon et al., 2001; Ubogu et al., 2012). These mice develop a chronic progressive neuropathic disorder that has overlapping clinical, electrodiagnostic, and morphological features with CIDP (Ubogu et al., 2012). B7-2 knockout NOD mice point to the pathogenic role of costimulatory pathways such as B7-1/B7-2:CD28/CTLA-4 molecules in the development of SAP due to disruption of the balance between pathogenic and Tregs maintained by this pathway. Nerve injury in these animals is mediated by Th1 cells that are reactive against myelin P0, the most prominent PNS myelin protein (Bour-Jordan et al., 2005; Louvet et al., 2009). Sera from these animals also contain antibodies to P0, which may contribute to peripheral nerve injury (Kim et al., 2008; Su et al., 2012a).

The importance of the immunogenetic repertoire in the development of SAP is suggested by the absence of neuropathic disorder in B7-2 knockout mice on C57BL/6 or 129/Sv nonautoimmune mouse background (Salomon et al., 2001). How the immunogenetic repertoire contributes to the development of immune neuropathies was exemplified by the development of SAP in transgenic NOD mice with hypomorphic Aire gene function (NOD. Aire $^{GW/+}$  mice) (Su et al., 2012a). Aire is critical to central tolerance. Aire functions by upregulating the ectopic expression of a variety of tissue-specific self-Ags in medullary thymic epithelial cells (mTECs) (Anderson et al., 2002) and increasing the probability of negative selection of antigen-specific high affinity (developing) thymocytes (Anderson et al., 2005). Interestingly, NOD. Aire $^{GW/+}$  mice have increased immune cell and autoantibody reactivity toward P0 in the peripheral immune compartment (Su et al., 2012a). These observations support P0 being an Aire-regulated Ag in mTECs and that decreased P0 expression in mTECs is linked to the development of antigen-specific autoimmunity. Further, CD4 $^+$  T cells from NOD. Aire $^{GW/+}$  mice are sufficient to transfer neuropathy, and Th1 effector cells are dominant in the peripheral nerves in this model (Su et al., 2012a). The clinical relevance of these experimental findings was supported by the following observation. The patients with autoimmune polyendocrinopathy syndrome type 1 (APS-1) have genetic mutations in Aire and develop multior gan autoimmunity. Recently, a CIDP-like neuropathy was recognized as a potential novel component of APS-1 in two unrelated children in whom there were confirmed Aire mutations (Valenzise et al., 2009). These patients have evidence of defective tolerance to P0 and harbor anti-P0 antibodies in their sera (Su et al., 2012a). Overall, these studies show defective tolerance to P0 and antigen-specific dysregulated cellular and humoral immune responses in both Aire-deficient mice and APS-1 patients with CIDP-like neuropathy.

Findings of autoantibodies against nodal proteins in CIDP have prompted animal studies demonstrating pathogenicity of these antibodies. Passive transfer studies with antibodies against neurofascin-155 and Caspr suggest that these antibodies bind corresponding paranodal protein and destroy the nodal architecture in motor nerve fibers, resulting in conduction deficits. These effects were not observed in neurofascin-deficient mice which indicate that specific binding to the target paranodal proteins is required for the pathological effects. In contrast, the relevance of myelin P0 as a potential antigenic target in CIDP remains contentious; however, Yan et al. demonstrated that 6/21 sera (28%) from patients with CIDP responsive to PEx contained IgG anti-P0 antibodies. Four of these sera containing IgG anti-P0 antibodies produced conduction block and demyelination by passive transfer when the BNB was breached, either by direct injection or by preceding passive T-cell transfer (Yan et al., 2000).

Animal models of MMNCB are lacking, and animal studies that explored passive transfer of IgM GM1 antibodies failed to demonstrate pathogenicity. However, *in vitro* studies showed that anti-GM1 IgM antibodies in sera from multifocal motor neuropathy (MMN) patient activate complement.

In anti-MAG PDPN, passive transfer of antibodies results in complement-mediated demyelination in cats and rabbits (Hays et al., 1987; Willison et al., 1988) although the pathological features were not similar to those seen in human disease. Widened myelin lamellae, almost pathognomonic of the condition, were demonstrated after passive transfer to chicks (Tatum, 1993). Induction of high-titer IgM antibodies cross-reactive with human MAG by immunization with the peripheral nerve glycolipid SGPG has been achieved in rats, rabbits, and cats. However, no convincing neuropathy, as a result, has been demonstrated.

## Pathogenic Mechanisms

The final common path of macrophage-mediated demyelination in CIDP is thought to be similar to that seen in GBS (see above).

## Treatment and Outcome

Steroids, ivIg, PEx, and immunosuppressive drugs have all been used in the treatment of CIDP. PEx and ivIg are both very potent (Mehndiratta et al., 2015; Eftimov et al., 2013). There was no statistically significant difference between steroids and ivIg in an early comparative trial (Hughes et al., 2001, 2017), and even though steroid is cheaper in the short term, ivIg was the treatment of choice. More recent evidence suggests that steroids may have a disease-modifying effect, driving patients into remission more frequently and effectively than ivIg (Nobile-Orazio et al., 2012). Interferon- $\beta$ 1a was shown to be ineffective in otherwise treatment-resistant patients (Hadden et al., 1999). Campath-1H (alemtuzumab) and fingolimod, both designed to severely reduce circulating numbers of pathogenic T cells, are currently under trial. There is inadequate evidence to properly assess anecdotally beneficial drugs such as azathioprine, cyclosporin, and mycophenolate mofetil.

In MMNCB, a dramatic therapeutic response is sometimes seen to ivIg demonstrated in controlled trials, and cyclophosphamide may have a place in primary treatment (Umapathi et al., 2015). Many other agents including interferon- $\beta$ 1a, rituximab, mycophenolate, azathioprine, and cyclosporin have been used with evidence for no effect for mycophenolate and only anecdotal evidence of partial benefit for the others (Umapathi et al., 2015). PEx has no effect, and interestingly steroids often worsen the condition by an unknown mechanism. Predictors of favorable outcome are anti-GM1 antibodies, younger age, the presence of conduction block, a normal creatine kinase (CK), and less severe disease at outset (Hadden and Hughes, 2003). Despite the treatment, conduction blocks are dynamic, and weakness probably progresses slowly overtime (Taylor et al., 2000).

In anti-MAG PDPN, patients only need treatment for their neuropathy if it becomes severe and disabling. Randomized controlled evidence of benefit is available for ivIg in the short term (Lunn and Nobile-Orazio, 2016; Su et al., 2012a). Rituximab (an anti-CD20 monoclonal) was promising in case series but had disappointing benefit in individual randomized control trials (RCTs). However, a metaanalysis of the trial outcomes demonstrates meaningful benefits in a number of outcome domains (Lunn and Nobile-Orazio, 2016).

## CONCLUDING REMARKS AND FUTURE PROSPECTS

The last decade has witnessed huge advances in the understanding of the pathogenesis of both acute and chronic inflammatory peripheral neuropathies. GBS and CIDP are heterogeneous clinical conditions in which humoral and cellular mechanisms conspire to cause disabling diseases. Although there are still innumerable advances to be made, our greater understanding of pathomechanisms is inspiring trials of novel agents for the treatment which will transform treatment in the next decade. Our understanding of disease with the emergence of further exciting discoveries will reduce significantly death and disability from GBS and CIDP.

## Acknowledgments

MPTL is supported by the Biomedical Research Centre at the National Hospital for Neurology, Queen Square, London. KAS is supported by NIH grant numbers NS42888, NS054962, and NS070888.

## References

- Alaedini, A., Sander, H.W., Hays, A.P., Latov, N., 2003. Antiganglioside antibodies in multifocal acquired sensory and motor neuropathy. *Arch. Neurol.* 60, 42–46.
- Anderson, M.S., Venanzi, E.S., Klein, L., Chen, Z., Berzins, S.P., Turley, S.J., et al., 2002. Projection of an immunological self shadow within the thymus by the aire protein. *Science* 298, 1395–1401.
- Anderson, M.S., Venanzi, E.S., Chen, Z., Berzins, S.P., Benoist, C., Mathis, D., 2005. The cellular mechanism of Aire control of T cell tolerance. *Immunity* 23, 227–239.
- Ang, C.W., Yuki, N., Jacobs, B.C., Koga, M., van Doorn, P.A., Schmitz, P.I., et al., 1999. Rapidly progressive, predominantly motor Guillain-Barre syndrome with anti-GalNAc-GD1a antibodies. *Neurology* 53, 2122–2127.
- Asbury, A.K., Cornblath, D.R., 1990. Current diagnostic criteria for Guillain-Barre syndrome. *Ann. Neurol.* 27, S22–S24.
- Asbury, A.K., Arnason, B.G., Adams, R.D., 1969. The inflammatory lesion in idiopathic polyneuritis. *Medicine (Baltimore)* 48, 173–215.
- Aspinall, G.O., McDonald, A.G., Raju, T.S., Pang, H., Moran, A.P., et al., 1993. Chemical structures of the core regions of *Campylobacter jejuni* serotypes O:1, O:4, O:23, and O:36 lipopolysaccharides. *Eur. J. Biochem.* 213, 1017–1027.
- Austin, J.H., 1958. Recurrent polyneuropathies and their corticosteroid treatment. *Brain* 81, 157–192.
- Bagliolini, M., 1998. Chemokines and leukocyte traffic. *Nature* 392, 565–568.
- Bouchard, C., Lacroix, C., Plante, V., Adams, D., Chedru, F., Guglielmi, J.M., et al., 1999. Clinicopathologic findings and prognosis of chronic inflammatory demyelinating polyneuropathy. *Neurology* 52, 498–503.
- Bour-Jordan, H., Thompson, H.L., Bluestone, J.A., 2005. Distinct effector mechanisms in the development of autoimmune neuropathy versus diabetes in nonobese diabetic mice. *J. Immunol.* 175, 5649–5655.
- Bowes, T., Wagner, E.R., Boffey, J., Nicholl, D., Cochrane, L., Benboubetra, M., et al., 2002. Tolerance to self gangliosides is the major factor restricting the antibody response to lipopolysaccharide core oligosaccharides in *Campylobacter jejuni* strains associated with Guillain-Barre syndrome. *Infect. Immun.* 70, 5008–5018.
- Buchwald, B., Ahangari, R., Toyka, K.V., 2002. Differential blocking effects of the monoclonal anti-GQ1b IgM antibody and alpha-latrotoxin in the absence of complement at the mouse neuromuscular junction. *Neurosci. Lett.* 334, 25–28.
- Bukowski, J.F., Morita, C.T., Band, H., Brenner, M.B., 1998. Crucial role of TCR gamma chain junctional region in prenyl pyrophosphate antigen recognition by gamma delta T cells. *J. Immunol.* 161, 286–293.
- Campbell, J.J., Hedrick, J., Zlotnik, A., Siani, M.A., Thompson, D.A., Butcher, E.C., 1998. Chemokines and the arrest of lymphocytes rolling under flow conditions. *Science* 279, 381–384.
- Carpo, M., Nobile-Orazio, E., Meucci, N., Gamba, M., Barbieri, S., Allaria, S., et al., 1996. Anti-GD1a ganglioside antibodies in peripheral motor syndromes. *Ann. Neurol.* 39, 539–543.
- Carpo, M., Pedotti, R., Lolli, F., Pitrola, A., Allaria, S., Scarlato, G., et al., 1998. Clinical correlate and fine specificity of anti-GQ1b antibodies in peripheral neuropathy. *J. Neurol. Sci.* 155, 186–191.
- Chevret, S., Hughes, R.A., Annane, D., 2017. Plasma exchange for Guillain-Barré syndrome. *Cochrane Database Syst Rev* 2, CD001798.
- Chi, L.J., Wang, H.B., Wang, W.Z., 2008. Impairment of circulating CD4 1 CD25 1 regulatory T cells in patients with chronic inflammatory demyelinating polyradiculoneuropathy. *J. Peripher. Nerv. Syst.* 13, 54–63.
- Chi, L.J., Xu, W.H., Zhang, Z.W., Huang, H.T., Zhang, L.M., Zhou, J., 2010. Distribution of Th17 cells and Th1 cells in peripheral blood and cerebrospinal fluid in chronic inflammatory demyelinating poly-radiculoneuropathy. *J. Peripher. Nerv. Syst.* 15, 345–356.
- Chiba, A., Kusunoki, S., Obata, H., Machinami, R., Kanazawa, I., 1993. Serum anti-GQ1b IgG antibody is associated with ophthalmoplegia in Miller Fisher syndrome and Guillain-Barre syndrome: clinical and immunohistochemical studies. *Neurology* 43, 1911–1917.
- Chiba, A., Kusunoki, S., Obata, H., Machinami, R., Kanazawa, I., 1997. Ganglioside composition of the human cranial nerves, with special reference to pathophysiology of Miller Fisher syndrome. *Brain Res.* 745, 32–36.
- Cocito, D., Isoardo, G., Ciaramitaro, P., Migliaretti, G., Pipieri, A., Barbero, P., et al., 2001. Terminal latency index in polyneuropathy with IgM paraproteinemia and anti-MAG antibody. *Muscle Nerve* 24, 1278–1282.
- Comi, C., Osio, M., Ferretti, M., Mesturini, R., Cappellano, G., Chiocchetti, A., et al., 2009. Defective Fas-mediated T-cell apoptosis predicts acute onset CIDP. *J. Peripher. Nerv. Syst.* 14, 101–106.
- Cooper, J.C., Ben Smith, A., Savage, C.O., Winer, J.B., 2000. Unusual T cell receptor phenotype V gene usage of gamma delta T cells in a line derived from the peripheral nerve of a patient with Guillain-Barre syndrome. *J. Neurol. Neurosurg. Psychiatry* 69, 522–524.
- Cornblath, D.R., 2005. Guillain-Barre syndrome. *Lancet* 366, 1653–1666.
- Cornblath, D.R., Griffin, D.E., Welch, D., Griffin, J.W., McArthur, J.C., 1990. Quantitative analysis of endoneurial T-cells in human sural nerve biopsies. *J. Neuroimmunol.* 26, 113–118.
- Corse, A.M., Chaudhry, V., Crawford, T.O., Cornblath, D.R., Kuncl, R.W., Griffin, J.W., 1996. Sensory nerve pathology in multifocal motor neuropathy. *Ann. Neurol.* 39, 319–325.
- Crawford, N.W., Cheng, A., Andrews, N., Charles, P.G., Clothier, H.J., Day, B., et al., 2012. Guillain-Barré syndrome following pandemic (H1N1) 2009 influenza A immunisation in Victoria: a self-controlled case series. *Med. J. Aust.* 19, 574–578.
- Creange, A., Belec, L., Clair, B., Raphael, J.C., Gherardi, R.K., 1996. Circulating tumor necrosis factor (TNF)-alpha and soluble TNF-alpha receptors in patients with Guillain-Barre syndrome. *J. Neuroimmunol.* 68, 95–99.
- Creange, A., Belec, L., Clair, B., Degos, J.D., Raphael, J.C., Gherardi, R.K., 1998. Circulating transforming growth factor beta 1 (TGF-beta1) in Guillain-Barre syndrome: decreased concentrations in the early course and increase with motor function. *J. Neurol. Neurosurg. Psychiatry* 64, 162–165.
- Creange, A., Sharshar, T., Planchenault, T., Christov, C., Poron, F., Raphael, J.C., et al., 1999. Matrix metalloproteinase-9 is increased and correlates with severity in Guillain-Barre syndrome. *Neurology* 53 (8), 1683–1691.
- Creange, A., Chazaud, B., Sharshar, T., Plonquet, A., Poron, F., Eliezer, M.C., et al., 2001. Inhibition of the adhesion step of leukodapedesis: a critical event in the recovery of Guillain-Barre syndrome associated with accumulation of proteolytically active lymphocytes in blood. *J. Neuroimmunol.* 114, 188–196.

- Dahle, C., Ekerfelt, C., Vrethem, M., Samuelsson, M., Ernerudh, J., 1997. T helper type 2 like cytokine responses to peptides from P0 and P2 myelin proteins during the recovery phase of Guillain-Barre syndrome. *J. Neurol. Sci.* 153, 54–60.
- De Angelis, M.V., Di Muzio, A., Lupo, S., Gambi, D., Uncini, A., Lugaresi, A., 2001. Anti-GD1a antibodies from an acute motor axonal neuropathy patient selectively bind to motor nerve fiber nodes of Ranvier. *J. Neuroimmunol.* 121, 79–82.
- Dionne, A., Nicolle, M.W., Hahn, A.F., 2010. Clinical and electrophysiological parameters distinguishing acute-onset chronic inflammatory demyelinating polyneuropathy from acute inflammatory demyelinating polyneuropathy. *Muscle Nerve* 41, 202–207.
- Dispenzieri, A., 2012. How I treat POEMS syndrome. *Blood* 119, 5650–5658.
- Dyck, P.J., Lais, A.C., Ohta, M., Bastron, J.A., Okazaki, H., Groover, R.V., 1975. Chronic inflammatory polyradiculoneuropathy. *Mayo Clin. Proc.* 50, 621–637.
- Eftimov, F., Winer, J.B., Vermeulen, M., de Haan, R., van Schaik, I.N., 2013. Intravenous immunoglobulin for chronic inflammatory demyelinating polyradiculoneuropathy. *Cochrane Database Syst Rev* (12), CD001797.
- Enders, U., Lobb, R., Pepinsky, R.B., Hartung, H.P., Toyka, K.V., Gold, R., 1998. The role of the very late antigen-4 and its counterligand vascular cell adhesion molecule-1 in the pathogenesis of experimental autoimmune neuritis of the Lewis rat. *Brain* 121, 1257–1266.
- Exley, A.R., Smith, N., Winer, J.B., 1994. Tumour necrosis factor-alpha and other cytokines in Guillain-Barre syndrome. *J. Neurol. Neurosurg. Psychiatry* 57, 1118–1120.
- Feasby, T.E., Gilbert, J.J., Brown, W.F., Bolton, C.F., Hahn, A.F., Koopman, W.F., et al., 1986. An acute axonal form of Guillain-Barre polyneuropathy. *Brain* 109, 1115–1126.
- Fisher, M., 1956. Syndrome of ophthalmoplegia, ataxia and areflexia. *N. Engl. J. Med.* 255, 57–65.
- Friedman, C.R., Neumann, J., Wegener, H.C., Tauxe, R.V., 2000. Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. In: Nachamkin, I., Blaser, M.J. (Eds.), *Campylobacter*. American Society for Microbiology, Washington, DC, pp. 121–138.
- Gabriel, J.M., Erne, B., Bernasconi, L., Tosi, C., Probst, A., Landmann, L., et al., 1998. Confocal microscopic localization of anti-myelin-associated glycoprotein autoantibodies in a patient with peripheral neuropathy initially lacking a detectable IgM gammopathy. *Acta Neuropathol.* 95, 540–546.
- Geleijns, K., Laman, J.D., van Rijssen, W., Tio-Gillen, A.P., Hintzen, R.Q., van Doorn, P.A., et al., 2005a. Fas polymorphisms are associated with the presence of anti-ganglioside antibodies in Guillain-Barre syndrome. *J. Neuroimmunol.* 161, 183–189.
- Geleijns, K., Schreuder, G.M., Jacobs, B.C., Sint Nicolaas, K., van Koningsveld, R., Meulstee, J., et al., 2005b. HLA class II alleles are not a general susceptibility factor in Guillain-Barre syndrome. *Neurology* 64, 44–49.
- Goddard, E.A., Lastovica, A.J., Argent, A.C., 1997. *Campylobacter* 0:41 isolation in Guillain-Barre syndrome. *Arch. Dis. Child.* 76, 526–528.
- Gold, R., Toyka, K.V., Hartung, H.P., 1995. Synergistic effect of IFN-gamma and TNF-alpha on expression of immune molecules and antigen presentation by Schwann cells. *Cell. Immunol.* 165, 65–70.
- Gong, Y., Tagawa, Y., Lunn, M.P., Laroy, W., Heffer-Lauk, M., Li, C.Y., et al., 2002. Localization of major gangliosides in the PNS: implications for immune neuropathies. *Brain* 125, 2491–2506.
- Goodfellow, J.A., Bowes, T., Sheikh, K., Odaka, M., Halstead, S.K., Humphreys, P.D., et al., 2005. Overexpression of GD1a ganglioside sensitizes motor nerve terminals to anti-GD1a antibody-mediated injury in a model of acute motor axonal neuropathy. *J. Neurosci.* 25, 1620–1628.
- Goodear, C.S., O'Hanlon, G.M., Plomp, J.J., Wagner, E.R., Morrison, R., Veitch, J., et al., 1999. Monoclonal antibodies raised against Guillain-Barre syndrome-associated *Campylobacter jejuni* lipopoly-saccharides react with neuronal gangliosides and paralyze muscle-nerve preparations. *J. Clin. Invest.* 104, 697–708.
- Greenshields, K.N., Halstead, S.K., Zitman, F.M., Rinaldi, S., Brennan, K.M., O'Leary, C., et al., 2009. The neuropathic potential of anti-GM1 autoantibodies is regulated by the local glycolipid environment in mice. *J. Clin. Invest.* 119, 595–610.
- Gregson, N.A., Hall, S.M., 1973. A quantitative analysis of the effects of the intraneurral injection of lysophosphatidyl choline. *J. Cell Sci.* 13, 257–277.
- Griffin, J.W., Li, C.Y., Ho, T.W., Xue, P., Macko, C., Gao, C.Y., et al., 1995. Guillain-Barre syndrome in northern China. The spectrum of neuro-pathological changes in clinically defined cases. *Brain* 118, 577–595.
- Griffin, J.W., Li, C.Y., Ho, T.W., Tian, M., Gao, C.Y., Xue, P., et al., 1996a. Pathology of the motor-sensory axonal Guillain-Barre syndrome. *Ann. Neurol.* 39, 17–28.
- Griffin, J.W., Li, C.Y., Macko, C., Ho, T.W., Hsieh, S.T., Xue, P., et al., 1996b. Early nodal changes in the acute motor axonal neuropathy pattern of the Guillain-Barre syndrome. *J. Neurocytol.* 25, 33–51.
- Guillain, G., Barré, J.A., Strohl, A., 1916. Sur un syndrome de radiculonévrite avec hyperalbuminose du liquide céphalorachidien sans réaction cellulaire. Remarques sur les caractères cliniques et graphiques des réflexes tendineux. *Bull. Soc. Méd. Hôp. Paris* 40, 1462–1470.
- Guillain-Barre Syndrome Study Group, 1985. Plasmapheresis and acute Guillain-Barre syndrome. *Neurology* 35, 1096–1104.
- Hadden, R.D., Gregson, N.A., 2001. Guillain-Barre syndrome and *Campylobacter jejuni* infection. *Symp. Ser. Soc. Appl. Microbiol.* 30, 145S–154S.
- Hadden, R.D., Hughes, R.A., 2003. Management of inflammatory neuropathies. *J. Neurol. Neurosurg. Psychiatry* 74, ii9–ii14.
- Hadden, R.D., Cornblath, D.R., Hughes, R.A., Zielasek, J., Hartung, H.P., Toyka, K.V., et al., 1998. Electrophysiological classification of Guillain-Barre syndrome: clinical associations and outcome. *Plasma Exchange/Sandoglobulin Guillain-Barre Syndrome Trial Group. Ann. Neurol.* 44, 780–788.
- Hadden, R.D., Sharrack, B., Bensa, S., Soudain, S.E., Hughes, R.A., 1999. Randomized trial of interferon beta-1a in chronic inflammatory demyelinating polyradiculoneuropathy. *Neurology* 53, 57–61.
- Hadden, R.D., Gregson, N.A., Gold, R., Willison, H.J., Hughes, R.A., 2001. Guillain-Barre syndrome serum and anti-*Campylobacter* antibody do not exacerbate experimental autoimmune neuritis. *J. Neuroimmunol.* 119, 306–316.
- Hafer-Macko, C., Hsieh, S.T., Li, C.Y., Ho, T.W., Sheikh, K., Cornblath, D.R., et al., 1996a. Acute motor axonal neuropathy: an antibody-mediated attack on axolemma. *Ann. Neurol.* 40, 635–644.
- Hafer-Macko, C.E., Sheikh, K.A., Li, C.Y., Ho, T.W., Cornblath, D.R., McKhann, G.M., et al., 1996b. Immune attack on the Schwann cell surface in acute inflammatory demyelinating polyneuropathy. *Ann. Neurol.* 39 (5), 625–635.

- Hall, S.M., Hughes, R.A., Atkinson, P.F., McColl, I., Gale, A., 1992. Motor nerve biopsy in severe Guillain-Barre syndrome. *Ann. Neurol.* 31, 441–444.
- Halstead, S.K., Zitman, F.M., Humphreys, P.D., Greenshields, K., Verschuuren, J.J., Jacobs, B.C., et al., 2008. Eculizumab prevents anti-ganglioside antibody-mediated neuropathy in a murine model. *Brain* 131, 1197–1208.
- Hartung, H.P., Toyka, K.V., 1990. T-cell and macrophage activation in experimental autoimmune neuritis and Guillain-Barre syndrome. *Ann. Neurol.* 27, S57–S63.
- Hartung, H.P., Willison, H.J., Kieseier, B.C., 2002. Acute immunoinflammatory neuropathy: update on Guillain-Barre syndrome. *Curr. Opin. Neurol.* 15, 571–577.
- Hartung, H.P., Schafer, B., Fierz, W., Heininger, K., Toyka, K.V., 1987. Ciclosporin A prevents P2 T cell line-mediated experimental autoimmune neuritis (AT-EAN) in rat. *Neurosci. Lett.* 83, 195–200.
- Hartung, H.-P., Heininger, K., Schafer, B., Fierz, W., Toyka, K.V., 1988. Immune mechanisms in inflammatory neuropathy. *Adv. Neuroimmunol.* 540, 122–161.
- Hartung, H.P., Reiners, K., Schmidt, B., Stoll, G., Toyka, K.V., 1991. Serum interleukin-2 concentrations in Guillain-Barre syndrome and chronic idiopathic demyelinating polyradiculoneuropathy: comparison with other neurological diseases of presumed immunopathogenesis. *Ann. Neurol.* 30, 48–53.
- Hartung, H.-P., Willison, H.J., Jung, S., Pette, M., Toyka, K.V., Giegerich, G., 1996. Autoimmune responses in peripheral nerve. *Springer Semin. Immunopathol.* 18, 97–123.
- Haymaker, W., Kernohan, J.W., 1949. The Landry-Guillain-Barré syndrome: a clinicopathologic report of fifty fatal cases and a critique of the literature. *Medicine (Baltimore)* 28, 59–141.
- Hays, A.P., Latov, N., Takatsu, M., Sherman, W.H., 1987. Experimental demyelination of nerve induced by serum of patients with neuropathy and an anti-MAG IgM M-protein. *Neurology* 37, 242–256.
- Ho, T.W., Hsieh, S.T., Nachamkin, I., Willison, H.J., Sheikh, K., Kiehlbauch, J., et al., 1997. Motor nerve terminal degeneration provides a potential mechanism for rapid recovery in acute motor axonal neuropathy after *Campylobacter* infection. *Neurology* 48, 717–724.
- Ho, T.W., Willison, H.J., Nachamkin, I., Li, C.Y., Veitch, J., et al., 1999. Anti-GD1a antibody is associated with axonal but not demyelinating forms of Guillain-Barre syndrome. *Ann. Neurol.* 45, 168–173.
- Holmdahl, R., Olsson, T., Moran, T., Klareskog, L., 1985. In vivo treatment of rats with monoclonal anti-T-cell antibodies. Immunohistochemical and functional analysis in normal rats and in experimental allergic neuritis. *Scand. J. Immunol.* 22, 157–169.
- Honavar, M., Tharakan, J.K., Hughes, R.A., Leibowitz, S., Winer, J.B., 1991. A clinicopathological study of the Guillain-Barre syndrome. Nine cases and literature review. *Brain* 114, 1245–1269.
- Hu, W., Janke, A., Ortler, S., Hartung, H.P., Leder, C., Kieseier, B.C., et al., 2007. Expression of CD28-related costimulatory molecule and its ligand in inflammatory neuropathies. *Neurology* 68, 277–282.
- Hughes, R.A., 1990. Guillain-Barré Syndrome. Springer-Verlag, London. Hughes, R.A.
- Hughes, R.A., Rees, J.H., 1997. Clinical and epidemiologic features of Guillain-Barre syndrome. *J. Infect. Dis.* 176, S92–S98.
- Hughes, R.A., Swan, A.V., van Doorn, P.A., 2014. Intravenous immunoglobulin for Guillain-Barré syndrome. *Cochrane Database Syst Rev* (9), CD002063.
- Hughes, R.A., Mehdiratta, M.M., Rajabally, Y.A., 2017. Corticosteroids for chronic inflammatory demyelinating polyradiculoneuropathy. *Cochrane Database Syst Rev* 11.
- Hughes, R.A., Hadden, R.D., Gregson, N.A., Smith, K.J., 1999. Pathogenesis of Guillain-Barre syndrome. *J. Neuroimmunol.* 100, 74–97.
- Hughes, R., Bensa, S., Willison, H., Van den, B.P., Comi, G., Illa, I., et al., 2001. Randomized controlled trial of intravenous immunoglobulin versus oral prednisolone in chronic inflammatory demyelinating polyradiculoneuropathy. *Ann. Neurol.* 50, 195–201.
- Hughes, R.A., Wijdicks, E.F., Barohn, R., Benson, E., Cornblath, D.R., Hahn, A.F., et al., 2003. Practice parameter: immunotherapy for Guillain-Barre syndrome: report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* 61, 736–740.
- Illes, Z., Kondo, T., Newcombe, J., Oka, N., Tabira, T., Yamamura, T., 2000. Differential expression of NK T cell V alpha 24J alpha Q invariant TCR chain in the lesions of multiple sclerosis and chronic inflammatory demyelinating polyneuropathy. *J. Immunol.* 164, 4375–4381.
- Jacobs, B.C., Endtz, H., van der Meche, F.G., Hazenberg, M.P., Achtereekte, H.A., van Doorn, P.A., 1995. Serum anti-GQ1b IgG antibodies recognize surface epitopes on *Campylobacter jejuni* from patients with Miller Fisher syndrome. *Ann. Neurol.* 37, 260–264.
- Jacobs, B.C., Endtz, H.P., van der Meche, F.G., Hazenberg, M.P., De Klerk, M.A., van Doorn, P.A., 1997. Humoral immune response against *Campylobacter jejuni* lipopolysaccharides in Guillain-Barre and Miller Fisher syndrome. *J. Neuroimmunol.* 79, 62–68.
- Jung, S., Kramer, S., Schluesener, H.J., Hunig, T., Toyka, K., Hartung, H.P., 1992. Prevention and therapy of experimental autoimmune neuritis by an antibody against T cell receptors-alpha/beta. *J. Immunol.* 148, 3768–3775.
- Kaji, R., Oka, N., Tsuji, T., Mezaki, T., Nishio, T., Akiguchi, I., et al., 1993. Pathological findings at the site of conduction block in multi-focal motor neuropathy. *Ann. Neurol.* 33, 152–158.
- Kanda, T., Numata, Y., Mizusawa, H., 2004. Chronic inflammatory demyelinating polyneuropathy: decreased claudin-5 and relocated ZO-1. *J. Neurol. Neurosurg. Psychiatry* 75, 765–769.
- Khalili-Shirazi, A., Gregson, N.A., Hall, M.A., Hughes, R.A., Lanchbury, J.S., 1997. T cell receptor V beta gene usage in Guillain-Barre syndrome. *J. Neurol. Sci.* 145, 169–176.
- Khalili-Shirazi, A., Gregson, N.A., Londef, M., Summers, L., Hughes, R.A., 1998. The distribution of CD1 molecules in inflammatory neuropathy. *J. Neurol. Sci.* 158, 154–163.
- Kiefer, R., Funa, K., Schweitzer, T., Jung, S., Bourde, O., Toyka, K.V., et al., 1996. Transforming growth factor-beta 1 in experimental autoimmune neuritis. Cellular localization and time course. *Am. J. Pathol.* 148, 211–223.
- Kiefer, R., Dangond, F., Mueller, M., Toyka, K.V., Hafler, D.A., Hartung, H.P., 2000. Enhanced B7 costimulatory molecule expression in inflammatory human sural nerve biopsies. *J. Neurol. Neurosurg. Psychiatry* 69, 362–368.
- Kieseier, B.C., Kiefer, R., Clements, J.M., Miller, K., Wells, G.M., Schweitzer, T., et al., 1998. Matrix metalloproteinase-9 and -7 are regulated in experimental autoimmune encephalomyelitis. *Brain* 121, 159–166.

- Kieseier, B.C., Dalakas, M.C., Hartung, H.P., 2002. Immune mechanisms in chronic inflammatory demyelinating neuropathy. *Neurology* 59, S7–S12.
- Kieseier, B.C., Kiefer, R., Gold, R., Hemmer, B., Willison, H.J., Hartung, H.P., 2004. Advances in understanding and treatment of immune-mediated disorders of the peripheral nervous system. *Muscle Nerve* 30, 131–156.
- Kim, H.J., Jung, C.G., Jensen, M.A., Dukala, D., Soliven, B., 2008. Targeting of myelin protein zero in a spontaneous autoimmune polyneuropathy. *J. Immunol* 181, 8753–8760.
- Kuij, M.L., Geleijns, K., Ennaji, N., van Doorn, P.A., Jacobs, B.C., 2008. Susceptibility to Guillain-Barre syndrome is not associated with CD1A and CD1E gene polymorphisms. *J. Neuroimmunol.* 205, 110–112.
- Kusunoki, S., Kaida, K., 2011. Antibodies against ganglioside complexes in Guillain-Barre syndrome and related disorders. *J. Neurochem.* 116, 828–832.
- Kusunoki, S., Hitoshi, S., Kaida, K., Murayama, S., Kanazawa, I., 1999. Degeneration of rabbit sensory neurons induced by passive transfer of anti-GD1b antiserum. *Neurosci. Lett.* 273, 33–36.
- Kuwabara, S., Yuki, N., Koga, M., Hattori, T., Matsuura, D., Miyake, M., et al., 1998. IgG anti-GM1 antibody is associated with reversible conduction failure and axonal degeneration in Guillain-Barre syndrome. *Ann. Neurol.* 44, 202–208.
- Kuwabara, S., Ogawara, K., Mizobuchi, K., Koga, M., Mori, M., Hattori, T., et al., 2000. Isolated absence of F waves and proximal axonal dysfunction in Guillain-Barre syndrome with antiganglioside antibodies. *J. Neurol. Neurosurg. Psychiatry* 68, 191–195.
- Lagueny, A., Bouillot, S., Vital, C., Ferrer, X., Larrieu, J.M., Vital, A., 2004. [POEMS syndrome (or Crow-Fukase syndrome)]. *Rev. Neurol. (Paris)* 160, 285–295.
- Lehmann, H.C., Lopez, P.H., Zhang, G., Ngyuen, T., Zhang, J., Kieseier, B.C., et al., 2007. Passive immunization with antiganglioside antibodies directly inhibits axon regeneration in an animal model. *J. Neurosci.* 27, 27–34.
- Lewis, R.A., Sumner, A.J., Brown, M.J., Asbury, A.K., 1982. Multifocal demyelinating neuropathy with persistent conduction block. *Neurology* 32, 958–964.
- Lopez, P.H., Zhang, G., Zhang, J., Lehmann, H.C., Griffin, J.W., Schnaar, R.L., et al., 2010. Passive transfer of IgG anti-GM1 antibodies impairs peripheral nerve repair. *J. Neurosci.* 30, 9533–9541.
- Louvet, C., Kabre, B.G., Davini, D.W., Martinier, N., Su, M.A., DeVoss, J.J., et al., 2009. A novel myelin P0-specific T cell receptor transgenic mouse develops a fulminant autoimmune peripheral neuropathy. *J. Exp. Med.* 206, 507–514.
- Lunn, M.P., Nobile-Orazio, E., 2016. Immunotherapy for IgM anti-myelin-associated glycoprotein paraprotein-associated peripheral neuropathies. *Cochrane Database Syst Rev* 10, CD002827.
- Lunn, M.P.T., Manji, H., Choudhary, P.P., Hughes, R.A.C., Thomas, P.K., 1998. Chronic inflammatory demyelinating polyradiculoneuropathy: a prevalence study in South East England. *J. Neurol. Neurosurg. Psychiatry* 66, 677–680.
- Lunn, M.P., Manji, H., Choudhary, P.P., Hughes, R.A., Thomas, P.K., 1999. Chronic inflammatory demyelinating polyradiculoneuropathy: a prevalence study in south east England. *J. Neurol. Neurosurg. Psychiatry* 66, 677–680.
- Lunn, M.P., Johnson, L.A., Fromholt, S.E., Itonori, S., Huang, J., Vyas, A.A., et al., 2000. High-affinity anti-ganglioside IgG antibodies raised in complex ganglioside knockout mice: reexamination of GD1a immunolocalization. *J. Neurochem.* 75, 404–412.
- Lunn, M.P., Crawford, T.O., Hughes, R.A., Griffin, J.W., Sheikh, K.A., 2002. Anti-myelin-associated glycoprotein antibodies alter neurofilament spacing. *Brain* 125, 904–911.
- Magira, E.E., Papaoakim, M., Nachamkin, I., Asbury, A.K., Li, C.Y., Ho, T.W., et al., 2003. Differential distribution of HLA-DQ beta/DR beta epitopes in the two forms of Guillain-Barre syndrome, acute motor axonal neuropathy and acute inflammatory demyelinating polyneuropathy (AIDP): identification of DQ beta epitopes associated with susceptibility to and protection from AIDP. *J. Immunol.* 170, 3074–3080.
- Mathey, E.K., Pollard, J.D., Armati, P.J., 1999. TNF alpha, IFN gamma and IL-2 mRNA expression in CIDP sural nerve biopsies. *J. Neurol. Sci.* 163, 47–52.
- Mausberg, A.K., Meyer zu, H.G., Dehmel, T., Stettner, M., Lehmann, H.C., Sheikh, K.A., et al., 2011. Erythropoietin ameliorates rat experimental autoimmune neuritis by inducing transforming growth factor-beta in macrophages. *PLoS One* 6, e26280.
- McKhann, G.M., Cornblath, D.R., Ho, T., Li, C.Y., Bai, A.Y., Wu, H.S., et al., 1991. Clinical and electrophysiological aspects of acute paralytic disease of children and young adults in northern China. *Lancet* 338, 593–597.
- McKhann, G.M., Cornblath, D.R., Griffin, J.W., Ho, T.W., Li, C.Y., Jiang, Z., et al., 1993. Acute motor axonal neuropathy: a frequent cause of acute flaccid paralysis in China. *Ann. Neurol.* 33, 333–342.
- Mehndiratta, M.M., Hughes, R.A., Pritchard, J., 2015. Plasma exchange for chronic inflammatory demyelinating polyradiculoneuropathy. *Cochrane Database Syst Rev* (8), CD003906.
- Mendell, J.R., Schenk, Z., Whittaker, J.N., Trapp, B.D., Yates, A.J., Griggs, R.C., et al., 1985. Polyneuropathy and IgM monoclonal gammopathy: studies on the pathogenic role of the myelin-associated glycoprotein antibody. *Ann. Neurol.* 17, 243–254.
- Misawa, S., Kuwabara, S., Mori, M., Kawaguchi, N., Yoshiyama, Y., Hattori, T., 2001. Serum levels of tumor necrosis factor-alpha in chronic inflammatory demyelinating polyneuropathy. *Neurology* 56, 666–669.
- Mizoguchi, K., 1998. Anti-GQ1b IgG antibody activities related to the severity of Miller Fisher syndrome. *Neurol. Res.* 20, 617–624.
- Monaco, S., Bonetti, B., Ferrari, S., Moretto, G., Nardelli, E., Tedesco, F., et al., 1990. Complement-mediated demyelination in patients with IgM monoclonal gammopathy and polyneuropathy. *N. Engl. J. Med.* 322, 649–652.
- Moran, A.P., Prendergast, M.M., Hogan, E.L., 2002. Sialosyl-galactose: a common denominator of Guillain-Barre and related disorders? *J. Neurol. Sci.* 196, 1–7.
- Myglan, A., Monstad, P., 2001. Chronic polyneuropathies in Vest-Agder, Norway. *Eur. J. Neurol.* 8, 157–165.
- Nachamkin, I., Liu, J., Li, M., Ung, H., Moran, A.P., Prendergast, M.M., et al., 2002. *Campylobacter jejuni* from patients with Guillain-Barre syndrome preferentially expresses a GD1a-like epitope. *Infect. Immun.* 70, 5299–5303.
- Nobile-Orazio, E., Giannotta, C., Briani, C., 2010. Anti-ganglioside complex IgM antibodies in multifocal motor neuropathy and chronic immune-mediated neuropathies. *J. Neuroimmunol.* 219, 119–122.

- Nobile-Orazio, E., Cocito, D., Jann, S., Uncini, A., Beghi, E., Messina, P., et al., 2012. Intravenous immunoglobulin versus intravenous methylprednisolone for chronic inflammatory demyelinating polyradiculoneuropathy: a randomised controlled trial. *Lancet Neurol.* 11, 493–502.
- Oberhelman, R.A., Taylor, D.N., 2000. *Campylobacter* infections in developing countries. In: Nachamkin, I., Blaser, M.J. (Eds.), *Campylobacter*. American Society for Microbiology, Washington DC, pp. 139–153.
- Ogawara, K., Kuwabara, S., Mori, M., Hattori, T., Koga, M., Yuki, N., 2000. Axonal Guillain-Barre syndrome: relation to anti-ganglioside antibodies and *Campylobacter jejuni* infection in Japan. *Ann. Neurol.* 48, 624–631.
- Ogino, M., Orazio, N., Latov, N., 1995. IgG anti-GM1 antibodies from patients with acute motor neuropathy are predominantly of the IgG1 and IgG3 subclasses. *J. Neuroimmunol.* 58, 77–80.
- Oh, S.J., Claussen, G.C., Odabasi, Z., Palmer, C.P., 1995. Multifocal demyelinating motor neuropathy: pathologic evidence of “inflammatory demyelinating polyradiculoneuropathy”. *Neurology* 45, 1828–1832.
- O'Hanlon, G.M., Plomp, J.J., Chakrabarti, M., Morrison, I., Wagner, E.R., Goodyear, C.S., et al., 2001. Anti-GQ1b ganglioside antibodies mediate complement-dependent destruction of the motor nerve terminal. *Brain* 124, 893–906.
- Pang, S.Y., Chan, K.H., Mak, W.W., Kung, M.H., Lee, C.N., Tsoi, T.H., et al., 2012. Single-nucleotide polymorphism of transient axonal glycoprotein-1 and its correlation with clinical features and prognosis in chronic inflammatory demyelinating polyneuropathy. *J. Peripher. Nerv. Syst.* 17, 72–75.
- Pestronk, A., Cornblath, D.R., Ilyas, A.A., Baba, H., Quarles, R.H., Griffin, J.W., et al., 1988. A treatable multifocal motor neuropathy with antibodies to GM1 ganglioside. *Ann. Neurol.* 24, 73–78.
- Plomp, J.J., Molenaar, P.C., O'Hanlon, G.M., Jacobs, B.C., Veitch, J., Daha, M.R., et al., 1999. Miller Fisher anti-GQ1b antibodies: alpha-latrotoxin-like effects on motor end plates. *Ann. Neurol.* 45, 189–199.
- van der Pol, W.L., Van den Berg, L.H., Scheepers, R.H., van der Bom, J.G., van Doorn, P.A., van Koningsveld, R., et al., 2000. IgG receptor IIa alleles determine susceptibility and severity of Guillain-Barre syndrome. *Neurology* 54, 1661–1665.
- Prendergast, M.M., Moran, A.P., 2000. Lipopolysaccharides in the development of the Guillain-Barre syndrome and Miller Fisher syndrome forms of acute inflammatory peripheral neuropathies. *J. Endotoxin Res.* 6, 341–359.
- Previtali, S.C., Feltri, M.L., Archelos, J.J., Quattrini, A., Wrabetz, L., Hartung, H., 2001. Role of integrins in the peripheral nervous system. *Prog. Neurobiol.* 64, 35–49.
- Pritchard, J., Mukherjee, R., Hughes, R.A., 2002. Risk of relapse of Guillain-Barre syndrome or chronic inflammatory demyelinating polyradiculoneuropathy following immunization. *J. Neurol. Neurosurg. Psychiatry* 73, 348–349.
- Redford, E.J., Smith, K.J., Gregson, N.A., Davies, M., Hughes, P., Gearing, A.J., et al., 1997. A combined inhibitor of matrix metalloproteinase activity and tumour necrosis factor-alpha processing attenuates experimental autoimmune neuritis. *Brain* 120, 1895–1905.
- Rees, J.H., Gregson, N.A., Hughes, R.A., 1995a. Anti-ganglioside GM1 antibodies in Guillain-Barre syndrome and their relationship to *Campylobacter jejuni* infection. *Ann. Neurol.* 38, 809–816.
- Rees, J.H., Soudain, S.E., Gregson, N.A., Hughes, R.A., 1995b. *Campylobacter jejuni* infection and Guillain-Barre syndrome. *N. Engl. J. Med.* 333, 1374–1379.
- Rees, J.H., Thompson, R.D., Smeeton, N.C., Hughes, R.A., 1998. Epidemiological study of Guillain-Barre syndrome in south east England. *J. Neurol. Neurosurg. Psychiatry* 64, 74–77.
- Rinaldi, S., Brennan, K.M., Goodyear, C.S., O'Leary, C., Schiavo, G., Crocker, P.R., et al., 2009. Analysis of lectin binding to glycolipid complexes using combinatorial glycoarrays. *Glycobiology* 19, 789–796.
- Rose, N.R., Bona, C., 1993. Defining criteria for autoimmune diseases (Witebsky's postulates revisited). *Immunol. Today* 14, 426–430.
- Saida, K., Saida, T., Brown, M.J., Silberberg, D.H., 1979. In vivo demyelination induced by intraneuronal injection of anti-galactocerebroside serum: a morphologic study. *Am. J. Pathol.* 95, 99–116.
- Salomon, B., Rhee, L., Bour-Jordan, H., Hsin, H., Montag, A., Soliven, B., et al., 2001. Development of spontaneous autoimmune peripheral polyneuropathy in B7-2-deficient NOD mice. *J. Exp. Med.* 194, 677–684.
- Sander, H.W., Latov, N., 2003. Research criteria for defining patients with CIDP. *Neurology* 60, S8–S15.
- Sanvito, L., Makowska, A., Mahdi-Rogers, M., Hadden, R.D., Peakman, M., Gregson, N., et al., 2009. Humoral and cellular immune responses to myelin protein peptides in chronic inflammatory demyelinating polyradiculoneuropathy. *J. Neurol. Neurosurg. Psychiatry* 80, 333–338.
- Sejvar, J.J., Baughman, A.L., Wise, M., Morgan, O.W., 2011. Population incidence of Guillain-Barre syndrome: a systematic review and meta-analysis. *Neuroepidemiology* 36, 123–133.
- Sheikh, K.A., Zhang, G., 2010. An update on pathobiologic roles of anti-glycan antibodies in Guillain-Barre syndrome. *F1000 Biol. Rep* 2, 21.
- Sheikh, K.A., Nachamkin, I., Ho, T.W., Willison, H.J., Veitch, J., Ung, H., et al., 1998. *Campylobacter jejuni* lipopolysaccharides in Guillain-Barre syndrome: molecular mimicry and host susceptibility. *Neurology* 51, 371–378.
- Sheikh, K.A., Deerinck, T.J., Ellisman, M.H., Griffin, J.W., 1999. The distribution of ganglioside-like moieties in peripheral nerves. *Brain* 122, 449–460.
- Sheikh, K.A., Zhang, G., Gong, Y., Schnaar, R.L., Griffin, J.W., 2004. An anti-ganglioside antibody-secreting hybridoma induces neuropathy in mice. *Ann. Neurol.* 56, 228–239.
- Smith, K.J., Kapoor, R., Felts, P.A., 1999. Demyelination: the role of reactive oxygen and nitrogen species. *Brain Pathol.* 9, 69–92.
- Spies, J.M., Pollard, J.D., Bonner, J.G., Westland, K.W., McLeod, J.G., 1995. Synergy between antibody and P2-reactive T cells in experimental allergic neuritis. *J. Neuroimmunol.* 57, 77–84.
- Stork, A.C., Lunn, M.P., Nobile-Orazio, E., Notermans, N.C., 2015. Treatment for IgG and IgA paraproteinaemic neuropathy. *Cochrane Database Syst Rev* (3), CD005376.
- Su, M.A., Davini, D., Cheng, P., Giang, K., Fan, U., DeVoss, J.J., et al., 2012a. Defective autoimmune regulator-dependent central tolerance to myelin protein zero is linked to autoimmune peripheral neuropathy. *J. Immunol.* 188, 4906–4912.
- Sumner, A.J., Said, G., Idy, I., Metral, S., 1992. Demyelinative conduction block produced by intraneuronal injection of human Guillain-Barré syndrome serum into rat sciatic nerve. *Neurology* 32, A106.

- Susuki, K., Nishimoto, Y., Yamada, M., Baba, M., Ueda, S., Hirata, K., et al., 2003. Acute motor axonal neuropathy rabbit model: immune attack on nerve root axons. *Ann. Neurol.* 54, 383–388.
- Susuki, K., Rasband, M.N., Tohyama, K., Koibuchi, K., Okamoto, S., Funakoshi, K., et al., 2007. Anti-GM1 antibodies cause complement-mediated disruption of sodium channel clusters in peripheral motor nerve fibers. *J. Neurosci.* 27, 3956–3967.
- Takatsu, M., Hays, A.P., Latov, N., Abrams, G.M., Nemni, R., Sherman, W.H., et al., 1985. Immunofluorescence study of patients with neuropathy and IgM M proteins. *Ann. Neurol.* 18, 173–181.
- Tatum, A.H., 1993. Experimental paraprotein neuropathy, demyelination by passive transfer of human IgM anti-myelin-associated glycoprotein. *Ann. Neurol.* 33, 502–506.
- Taylor, W.A., Hughes, R.A., 1989. T lymphocyte activation antigens in Guillain-Barre syndrome and chronic idiopathic demyelinating polyradiculoneuropathy. *J. Neuroimmunol.* 24, 33–39.
- Taylor, B.V., Wright, R.A., Harper, C.M., Dyck, P.J., 2000. Natural history of 46 patients with multifocal motor neuropathy with conduction block. *Muscle Nerve* 23, 900–908.
- Ubogu, E.E., Yosef, N., Xia, R.H., Sheikh, K.A., 2012. Behavioral, electrophysiological, and histopathological characterization of a severe murine chronic demyelinating polyneuritis model. *J. Peripher. Nerv. Syst.* 17, 53–61.
- Umapathi, T., Hughes, R.A., Nobile-Orazio, E., Léger, J.M., 2015. Immunosuppressant and immunomodulatory treatments for multifocal motor neuropathy. *Cochrane Database Syst Rev* (3), CD003217.
- Umapathi, T., Hughes, R.A., Nobile-Orazio, E., Léger, J.M., 2005. Immunosuppressant and immunomodulatory treatments for multifocal motor neuropathy. *Cochrane Database Syst. Rev.* (3), CD003217.
- Uncini, A., Lugaresi, A., 1999. Fisher syndrome with tetraparesis and antibody to GQ1b: evidence for motor nerve terminal block. *Muscle Nerve* 22, 640–644.
- Uncini, A., Kuwabara, S., 2012. Electrodiagnostic criteria for Guillain-Barre syndrome: a critical revision and the need for an update. *Clin. Neurophysiol.* 123, 1487–1495.
- Uncini, A., Notturno, F., Pace, M., Caporale, C.M., 2011. Polymorphism of CD1 and SH2D2A genes in inflammatory neuropathies. *J. Peripher. Nerv. Syst.* 16, 48–51.
- Valenzise, M., Meloni, A., Betterle, C., Giometto, B., Autunno, M., Mazzeo, A., et al., 2009. Chronic inflammatory demyelinating polyneuropathy as a possible novel component of autoimmune poly-endocrine-candidiasis-ectodermal dystrophy. *Eur. J. Pediatr.* 168, 237–240.
- Visser, L.H., van der Meche, F.G., van Doorn, P.A., Meulstee, J., Jacobs, B.C., Oomes, P.G., et al., 1995. Guillain-Barre syndrome without sensory loss (acute motor neuropathy). A subgroup with specific clinical, electrodiagnostic and laboratory features. Dutch Guillain-Barre Study Group. *Brain* 118, 841–847.
- Vriesendorp, F.J., Flynn, R.E., Khan, M., Pappolla, M.A., Brod, S.A., 1996. Oral administration of type I interferon modulates the course of experimental allergic neuritis. *Autoimmunity* 24, 157–165.
- Willison, H.J., Veitch, J., 1994. Immunoglobulin subclass distribution and binding characteristics of anti-GQ1b antibodies in Miller Fisher syndrome. *J. Neuroimmunol.* 50, 159–165.
- Willison, H.J., Yuki, N., 2002. Peripheral neuropathies and anti-glycolipid antibodies. *Brain* 125, 2591–2625.
- Willison, H.J., Trapp, B.D., Bacher, J.D., Dalakas, M.C., Griffin, J.W., Quarles, R.H., 1988. Demyelination induced by intraneuronal injection of human antimyelin-associated glycoprotein antibodies. *Muscle Nerve* 11, 1169–1176.
- Willison, H.J., Veitch, J., Paterson, G., Kennedy, P.G., 1993. Miller Fisher syndrome is associated with serum antibodies to GQ1b ganglioside. *J. Neurol. Neurosurg. Psychiatry* 56, 204–206.
- Willison, H.J., Townson, K., Veitch, J., Boffey, J., Isaacs, N., Andersen, S.M., et al., 2004. Synthetic disialylgalactose immunoabsorbents deplete anti-GQ1b antibodies from autoimmune neuropathy sera. *Brain* 127, 680–691.
- Willison, H.J., Halstead, S.K., Beveridge, E., Zitman, F.M., Greenshields, K.N., Morgan, B.P., et al., 2008. The role of complement and complement regulators in mediating motor nerve terminal injury in murine models of Guillain-Barre syndrome. *J. Neuroimmunol.* 201–202, 172–182.
- Winer, J.B., Gray, I.A., Gregson, N.A., Hughes, R.A., Leibowitz, S., et al., 1988. A prospective study of acute idiopathic neuropathy. III. Immunological studies. *J. Neurol. Neurosurg. Psychiatry* 51, 619–625.
- Winer, J., Hughes, S., Cooper, J., Ben Smith, A., Savage, C., 2002. Gamma delta T cells infiltrating sensory nerve biopsies from patients with inflammatory neuropathy. *J. Neurol.* 249, 616–621.
- Wirguin, I., Briani, C., Suturkova-Milosevic, L., Fisher, T., Della-Latta, P., Chalif, P., et al., 1997. Induction of GM1 ganglioside antibodies by *Campylobacter jejuni* lipopolysaccharides. *J. Neuroimmunol.* 78, 138–142.
- Witebsky, E., Rose, N.R., Terplan, K., Paine, J.R., Egan, R.W., 1957. Chronic thyroiditis and autoimmunization. *J. Am. Med. Assoc.* 164, 1439–1447.
- Wu, L.Y., Zhou, Y., Qin, C., Hu, B.L., 2012. The effect of TNF-alpha, FcgammaR and CD1 polymorphisms on Guillain-Barre syndrome risk: evidences from a meta-analysis. *J. Neuroimmunol.* 243, 18–24.
- Yamamoto, M., Ito, Y., Mitsuma, N., Li, M., Hattori, N., Sobue, G., 2002. Parallel expression of neurotrophic factors and their receptors in chronic inflammatory demyelinating polyneuropathy. *Muscle Nerve* 25, 601–604.
- Yan, W.X., Taylor, J., Andrias-Kauba, S., Pollard, J.D., 2000. Passive transfer of demyelination by serum or IgG from chronic inflammatory demyelinating polyneuropathy patients. *Ann. Neurol.* 47, 765–775.
- Yan, W.X., Archelos, J.J., Hartung, H.P., Pollard, J.D., 2001. P0 protein is a target antigen in chronic inflammatory demyelinating polyradiculoneuropathy. *Ann. Neurol.* 50, 286–292.
- Yuki, N., Kuwabara, S., 2007. Axonal Guillain-Barre syndrome: carbohydrate mimicry and pathophysiology. *J. Peripher. Nerv. Syst.* 12, 238–249.
- Yuki, N., Yoshino, H., Sato, S., Miyatake, T., 1990. Acute axonal poly-neuropathy associated with anti-GM1 antibodies following *Campylobacter enteritis*. *Neurology* 40, 1900–1902.
- Yuki, N., Sato, S., Tsuji, S., Ohsawa, T., Miyatake, T., 1993. Frequent presence of anti-GQ1b antibody in Fisher's syndrome. *Neurology* 43, 414–417.

- Yuki, N., Taki, T., Takahashi, M., Saito, K., Yoshino, H., Tai, T., et al., 1994. Molecular mimicry between GQ1b ganglioside and lipopolysaccharides of *Campylobacter jejuni* isolated from patients with Fisher's syndrome. Ann. Neurol. 36, 791–793.
- Yuki, N., Ichihashi, Y., Taki, T., 1995. Subclass of IgG antibody to GM1 epitope-bearing lipopolysaccharide of *Campylobacter jejuni* in patients with Guillain-Barre syndrome. J. Neuroimmunol. 60, 161–164.
- Yuki, N., Ang, C.W., Koga, M., Jacobs, B.C., van Doorn, P.A., Hirata, K., et al., 2000. Clinical features and response to treatment in Guillain-Barre syndrome associated with antibodies to GM1b ganglioside. Ann. Neurol. 47, 314–321.
- Zehntner, S.P., Brisebois, M., Tran, E., Owens, T., Fournier, S., 2003. Constitutive expression of a costimulatory ligand on antigen-presenting cells in the nervous system drives demyelinating disease. FASEB J. 17, 1910–1912.
- Zhang, G., Lehmann, H.C., Bogdanova, N., Gao, T., Zhang, J., Sheikh, K.A., 2011a. Erythropoietin enhances nerve repair in anti-ganglioside antibody-mediated models of immune neuropathy. PLoS One 6, e27067.
- Zhang, G., Lehmann, H.C., Manoharan, S., Hashmi, M., Shim, S., Ming, G.L., et al., 2011b. Anti-ganglioside antibody-mediated activation of RhoA induces inhibition of neurite outgrowth. J. Neurosci. 31, 1664–1675.
- Zhu, J., Bai, X.F., Mix, E., Link, H., 1997. Experimental allergic neuritis: cytolsin mRNA expression is upregulated in lymph node cells during convalescence. J. Neuroimmunol. 78, 108–116.
- Zhu, J., Zou, L., Zhu, S., Mix, E., Shi, F., Wang, H., et al., 2001. Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) blockade enhances incidence and severity of experimental auto-immune neuritis in resistant mice. J. Neuroimmunol. 115, 111–117.

## Further Reading

- Hughes, R.A., Swan, A.V., van Doorn, P.A., 2012. Intravenous immunoglobulin for Guillain-Barre syndrome. Cochrane Database Syst. Rev. (7), CD002063.
- Mehndiratta, M.M., Hughes, R.A., 2012. Plasma exchange for chronic inflammatory demyelinating polyradiculoneuropathy. Cochrane Database Syst. Rev. (9), CD003906.

# Myasthenia Gravis and Related Disorders

Valentina Damato<sup>1,2</sup>, Stuart Viegas<sup>3</sup> and Angela Vincent<sup>1,4</sup>

<sup>1</sup>Nuffield Department of Clinical Neurosciences, Oxford University, Oxford, United Kingdom <sup>2</sup>Department of Neuroscience, Institute of Neurology, Catholic University, Rome, Italy <sup>3</sup>Department of Neurology, Charing Cross Hospital, Imperial College NHS Trust, London, United Kingdom <sup>4</sup>Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, United Kingdom

## O U T L I N E

<b>Introduction</b>			
The Neuromuscular Junction	1012	The Role of Muscle-Specific Kinase in Neuromuscular Junction Development and Maintenance	1019
Neuromuscular Transmission	1013	LRP4 Antibodies	1020
Acetylcholine Receptor and Muscle-Specific Kinase, the Main Antigenic Targets	1014	Novel Targets	1021
<b>Myasthenia Gravis</b>			
Epidemiology	1014	<b>The Thymus and Cellular Immunity in Myasthenia Gravis</b>	1021
Etiology of Myasthenia	1015	Role of T Lymphocytes in Myasthenia Gravis	1021
General Clinical Aspects	1015	Advances in the Cellular Immunology of Acetylcholine Receptor Myasthenia Gravis	1022
<b>Clinical Heterogeneity of Myasthenia</b>			
Different Forms Related to Antibodies and Thymic Pathology	1015	The Thymus in Myasthenia Gravis	1023
Early-Onset Acetylcholine Receptor-Antibody Positive Myasthenia Gravis	1015	Thymoma	1024
Late-Onset Acetylcholine Receptor-Antibody Myasthenia Gravis	1016	<b>Treatments in Myasthenia Gravis</b>	1024
Thymoma Associated Myasthenia Gravis	1016	General Approach	1024
Muscle-Specific Kinase Antibody Positive Myasthenia Gravis	1017	Biologics	1024
Neonatal Myasthenia Gravis	1017	<b>Lambert–Eaton Myasthenic Syndrome</b>	1025
<b>Antibodies in Myasthenia</b>			
Evidence for Pathogenicity of Acetylcholine Receptor and Muscle-Specific Kinase Antibodies	1018	Introduction	1025
Acetylcholine Receptor Antibodies	1018	Epidemiology and Etiology	1025
Characteristics and Mechanisms	1019	Clinical Features	1025
Muscle-Specific Kinase Antibodies	1019	Investigation and Treatment	1026
		Pathophysiology	1026
		<b>Conclusions and Future Prospects</b>	1026
		<b>References</b>	1027

## INTRODUCTION

In recent years, the recognition and ability to detect antibodies directed against receptors, ion channels, and relevant proteins within both the peripheral and central nervous system has continued to evolve. Nevertheless, myasthenia gravis (MG) and related autoimmune disorders of the neuromuscular junction remain the paradigm diseases, serving to highlight those features which help define an antibody mediated disorder. The history of the discoveries in this archetypal autoimmune disease is summarized in [Table 53.1](#).

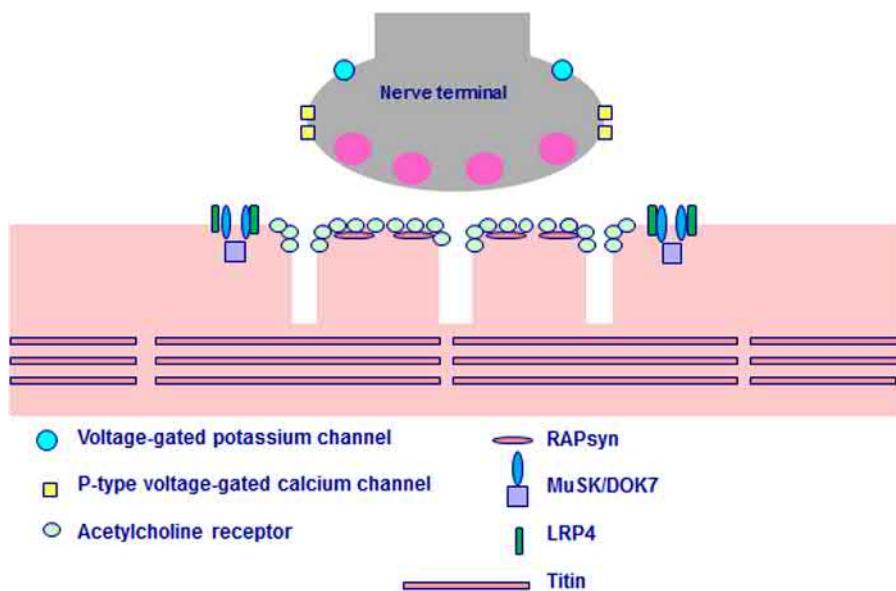
### The Neuromuscular Junction

The neuromuscular junction (NMJ) consists of the presynaptic motor nerve terminal and the postsynaptic motor “endplate.” At the NMJ, the distal motor axon loses its myelin sheath and expands to form the boutons of the presynaptic nerve terminal. These contain mitochondria and the synaptic vesicles that store the acetylcholine (ACh). The vesicles are organized within specialized active zones alongside voltage-gated calcium channels

**TABLE 53.1** A History of Myasthenia Gravis Research

Date	Key observation	Reference
1672	Thomas Willis publishes what is arguably the first clinical description of MG	Willis
1895	Jolly shows that defect is at the neuromuscular junction	Jolly
1913	Thymectomy appears to produce clinical improvement in patients with thymoma or nonthymomatous MG	Sauerbruch
1939		Blalock
1934	Mary Walker demonstrates the effectiveness of cholinesterase inhibitors as treatment	<a href="#">Walker (1934)</a>
1960	Simpson proposes that MG is caused by antibodies to an “endplate” protein	<a href="#">Simpson(1960)</a>
1962	The snake toxin, $\alpha$ -Bungarotoxin, can be used as a label for AChRs at the neuromuscular junction	<a href="#">Chang and Lee (1963)</a>
1964	Elmqvist and colleagues show that the miniature endplate potentials are reduced in MG	<a href="#">Elmqvist et al. (1964)</a>
1971	Several groups begin to purify AChRs from electric organs of electric rays using affinity chromatography on neurotoxin columns	
1973	Immunization against purified electric ray AChR leads to an EAMG in rabbits	<a href="#">Patrick and Lindstrom (1973)</a>
1973	AChRs are reduced in number at neuromuscular junctions, as determined by $^{125}\text{I}$ - $\alpha$ -Bungarotoxin binding	<a href="#">Fambrough et al. (1973)</a>
1975	MG can be passively transferred to mice by injection of patients’ IgG	<a href="#">Toyka et al. (1975)</a>
1976	MG patients have AChR antibodies as shown by radioimmunoprecipitation of $^{125}\text{I}$ - $\alpha$ -Bungarotoxin-tagged AChRs	<a href="#">Lindstrom et al. (1976)</a>
1976–78	Plasma exchange produces striking clinical improvement in MG, which correlates inversely with AChR antibody levels	<a href="#">Newsom-Davis et al. (1978)</a>
1977	IgG and complement are present at the neuromuscular junctions in MG patients and in mice with EAMG	<a href="#">Engel et al. (1977)</a>
1980	MG can present with different HLA, thymic pathology, age at onset, and muscle antibodies	<a href="#">Compston et al. (1980)</a>
1981	The MG thymus contains plasma cells making AChR antibody	<a href="#">Scadding et al. (1981)</a>
1977–Present	Experimental autoimmune model used to determine pathogenic and immunological mechanisms	on-going
1984–Present	Study of T cells from MG patients and their responses to the AChR epitopes	on-going
1995–Present	Study of immune mechanisms in the MG thymus	on-going
2001–Present	Discovery of MuSK antibodies and their distinctive mechanisms	on-going
2010–present	Development of new biologics and benefits of Rituximab and Eculizumab demonstrated in MG	on-going

MG, Myasthenia gravis; AChR, acetylcholine receptor; MuSK, muscle-specific kinase; EAMG, experimental autoimmune myasthenia gravis.



**FIGURE 53.1** Ion channel targets for autoantibodies at the neuromuscular junction.

Neuromuscular transmission depends on the calcium-dependent release of vesicles of ACh. ACh binds to the AChRs on the postsynaptic membrane resulting in a depolarization which, if it reaches a critical threshold, initiates an action potential in the muscle leading to contraction. ACh is immediately destroyed by acetylcholinesterase. AChRs, VGCCs, and VGKCs are all targets for the antibody mediated neurological diseases. The receptor tyrosine kinase MuSK and the protein that activates it, LRP4, have been found to be targets for antibodies in a proportion of patients with MG without AChR antibodies.

(VGCCs). In addition, voltage-gated potassium channels (VGKCs) are also present on the presynaptic nerve terminal (Fig. 53.1).

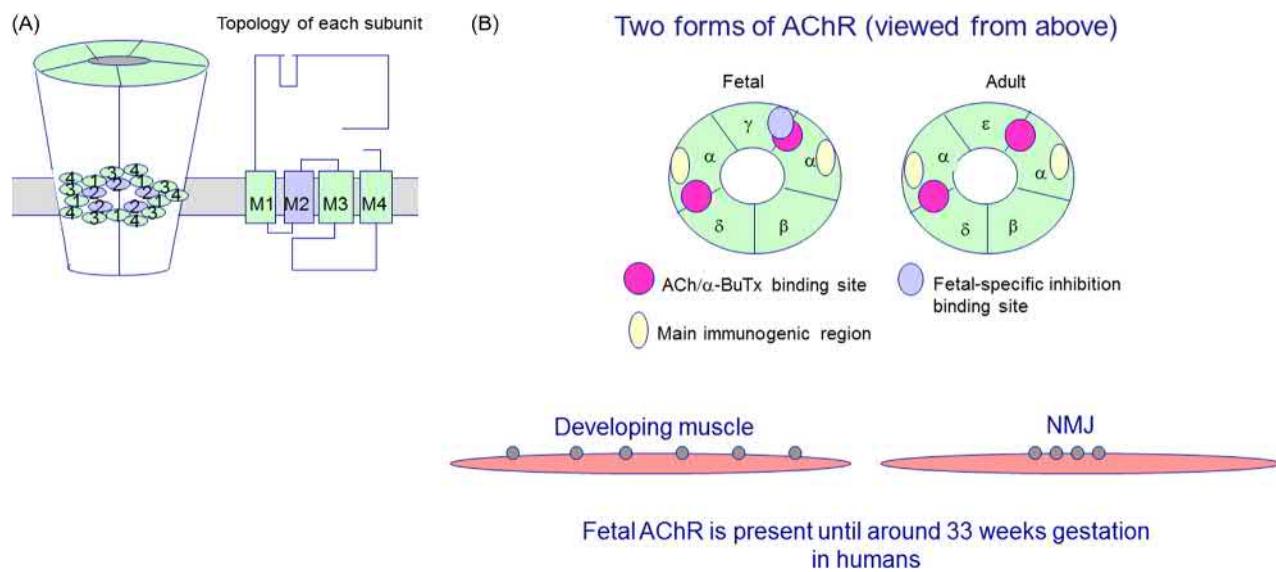
The postsynaptic membrane is deeply infolded to create “junctional” folds. The crests of these, lying in close alignment to the presynaptic active zones, contain the highest density of acetylcholine receptors (AChR). At the depths of these folds, there are relatively few AChRs, but an abundance of voltage-gated sodium channels (VGSC). The synaptic cleft between the presynaptic and postsynaptic membranes contains the basal lamina composed of collagen IV, heparan sulfate and laminin, and many other proteins. The enzyme acetylcholinesterase is anchored to the basal lamina through its collagen-like tail (ColQ).

The clustering of the AChRs is critical for efficient neurotransmission. As will be discussed below, the development and maintenance of the NMJ structure are dependent on a number of key proteins: agrin, low-density lipoprotein receptor related protein 4 (LRP4), muscle-specific kinase (MuSK), docking protein 7 (DOK7), and rapsyn (Singhal and Martin, 2011) (see Fig. 53.1) in addition to other intracellular proteins that are less well characterized.

## Neuromuscular Transmission

Neuromuscular transmission in mature muscle begins with propagation of the action potential into the motor nerve terminal. This depolarization causes opening of the VGCCs and the resulting calcium influx results in the fusion of the ACh containing vesicles and release of ACh. This is terminated by the closing of VGCCs and the opening of the VGKCs with subsequent repolarization of the nerve terminal. ACh binding to the AChR results in the opening of the AChR central ion pore and a localized depolarization of the motor endplate. If sufficient, this will cause the opening of the VGSCs and propagation of the action potential along the muscle fiber to initiate contraction.

The amount of ACh released by a single vesicle is termed a quantum. The spontaneous release of a single quantum is responsible for the generation of a local depolarization, termed a miniature endplate potential (MEPP); a nerve impulse releases multiple vesicles (around 25–30 in humans) leading to a greater depolarization, termed the endplate potential (EPP). It is possible to calculate the number of quanta released, the quantal content, from these parameters (Wood and Slater, 2001). An inherent safety factor exists in normal muscles, whereby more ACh is released than is required to reach the activation threshold for the opening of the VGSCs.



**FIGURE 53.2** The AChR (A,B). The AChR is a transmembrane protein with  $(\alpha)_2$ ,  $\beta$ ,  $\gamma$ , and  $\delta$  subunits in the fetal form and  $(\alpha)_2$ ,  $\beta$ ,  $\delta$ , and  $\epsilon$  subunits in the adult form. A high proportion of antibodies in MG bind to the main immunogenic regions that are on both the  $\alpha$  subunits. In addition, many patients' antibodies bind to the fetal-specific  $\gamma$  subunit. In some cases, antibodies that inhibit the function of the fetal form, selectively, cross the placenta causing fetal muscle paralysis with severe and often fatal deformities. MG, Myasthenia gravis; AChR, acetylcholine receptor; MuSK, muscle-specific kinase; VGCC, voltage-gated calcium channel; VGKC, voltage-gated potassium channel; Ach, acetylcholine.

At the neuromuscular junction, as described above, the AChRs are clustered by the agrin/LRP4/Musk/DOK7 pathway but this clustering process is balanced by binding of ACh that stimulates AChR dispersal. Any defect in the clustering process, as occurs in patients with MuSK antibodies, will be followed by dispersal of the AChRs.

### Acetylcholine Receptor and Muscle-Specific Kinase, the Main Antigenic Targets

The nicotinic AChR remains the major antigenic target in MG. The AChR is a pentameric ligand-gated ion channel that exists in adult and fetal isoforms. The adult form consists of two  $\alpha$ -subunits and one each of the  $\beta$ -,  $\delta$ -, and  $\epsilon$ -subunits, with each subunit being composed of a large extracellular domain, glycosylation sites, and four transmembrane domains. During development, the fetal-specific  $\gamma$  subunit is replaced by the  $\epsilon$ -subunit to form the adult isoform. If the muscle is denervated by nerve injury, the fetal isoform expressing the  $\gamma$  subunit is expressed throughout the muscle until reinnervation occurs.

The AChR subunits are organized around a central ion channel (Fig. 53.2). The two ACh binding sites are between the  $\alpha$ - and  $\epsilon$ - or  $\gamma$ -subunits and between the  $\alpha$ - and the  $\delta$ -subunits, respectively. Both sites need to be occupied for the ion channel to be in the open state. The main immunogenic region is a conformation dependent region on the extracellular component of each of the  $\alpha$ -subunits (Lindstrom, 2000).

MuSK is a receptor tyrosine kinase. The extracellular portion consists of three immunoglobulin-like domains and a cysteine-rich domain. The intracellular portion consists of a juxtamembrane domain, followed by a tyrosine kinase catalytic domain. MuSK is critical both for the development (DeChiara et al., 1996) and ongoing maintenance (Kong et al., 2004) of the NMJ. The role of MuSK and its coreceptor LRP4 is described in detail below (see MuSK antibodies).

## MYASTHENIA GRAVIS

### Epidemiology

Myasthenia affects all races and can occur at any age from the first year of life to the age of ninety. In the Western countries, MG with AChR antibodies (AChR-MG) typically shows two age peaks, in the third decade in

females and in the sixth and seventh decades in males. While the frequency of the early-onset disease has remained unchanged, an increased incidence in the elderly has been consistently reported in the last two decades. The explanation could be the increased longevity of the population and the improvement in diagnosis but these factors only do not appear to be responsible. A metaanalysis of 55 published studies calculated a pooled incidence rate of 5.3 per million person-years (CI 4.4–6.1) and a pooled prevalence rate of 77.7 per million (CI 64–93) (Carr et al., 2010), but a prevalence approaching 150 per million was suggested in a recent study (Andersen et al., 2014).

Interestingly, in Asian populations, particularly the Chinese, there is a high prevalence of limited forms of the disease with childhood onset (Zhang et al., 2007). The incidence and prevalence is difficult to evaluate because of different healthcare systems but it is possible that there is a specific genetic predisposition to childhood MG in these populations or that there is an environmental contribution.

The incidence of MuSK and LRP4 antibody forms of MG is much lower and difficult to assess. For MuSK-MG, it appears to vary with latitude with the highest rates in Italy, Spain, and Turkey—all Mediterranean countries—compared with much lower rates in northern Europe. It is not uncommon in Japan but relatively infrequent in the Chinese (Yeh et al., 2004).

## Etiology of Myasthenia

In the majority of cases, no single cause is identifiable. There is genetic predisposition, which most likely reflects the contribution of polymorphic MHC class I and II loci. Other possible genetic susceptibility markers include the AChR alpha subunit (Garchon et al., 1994; Giraud et al., 2007), Immunoglobulin G (IgG) heavy and light chains (Dondi et al., 1994), Fc gamma receptor IIa (Amdahl et al., 2007), TAP (Hjelmstrom et al., 1997), CTLA4 (Wang et al., 2008), and PTPN22 (Provenzano et al., 2012). Molecular mimicry between AChR subunits and microbial proteins has been proposed as a possible initiating process with autosensitization against muscle AChR occurring as a result of determinant spreading, but there is little robust evidence. Certain drugs, notably penicillamine, may also trigger the development of a reversible form of MG in genetically susceptible individuals (Drosos et al., 1993) but changes in clinical practice means that these cases are seldom seen now.

## General Clinical Aspects

MG is an autoimmune disorder characterized by fatigable muscle weakness. It often involves the extraocular muscles at onset causing diplopia and/or ptosis. If it remains confined to these muscles, it is termed ocular MG. If other muscle groups are involved, often facial, axial, limb, bulbar, and respiratory muscles, it is termed generalized MG. Bulbar and respiratory involvement can be life-threatening.

The diagnosis rests on a compatible clinical presentation supported by serological confirmation (see below) and/or electromyographic evidence (with repetitive nerve stimulation and/or single fiber electromyography) of a defect in neurotransmission. MG is commonly associated with thymic abnormalities, notably thymic hyperplasia and thymoma, and appropriate imaging of the thymus is therefore recommended at presentation.

Symptomatic treatment includes the use of cholinesterase inhibitors but the majority of cases will also require immunosuppressive agents including the corticosteroids and steroid sparing agents (azathioprine, mycophenolate mofetil, methotrexate, and cyclosporine). Thymectomy is a therapeutic option in younger patients with detectable AChR antibodies and a recent study has shown its beneficial effect (Wolfe et al., 2016). Intravenous immunoglobulin and plasma exchange may be employed in severe, life-threatening, or refractory disease. Newer biological agents, such as the monoclonal anti-CD20 agent, Rituximab (RTX) have shown promise in refractory cases (Maddison et al., 2011). Most of these aspects are discussed in more detail below. The treatment modalities available for MG are more fully reviewed elsewhere (Sanders and Evoli, 2010).

## CLINICAL HETEROGENEITY OF MYASTHENIA

### Different Forms Related to Antibodies and Thymic Pathology

MG is not a single disease entity but can be classified into different groups. Defined serologically, it is possible to delineate five main subgroups of MG (see Table 53.2) that differ also by means of age of onset, HLA association, thymic pathology (Compston et al., 1980), and presence of antibodies directed against non-AChR

**TABLE 53.2** MG (Myasthenia Gravis) Patients Divided on the Basis of Antibody Status, Age at Onset, Thymic Pathology and HLA Association

Subtype of MG	Age at onset	Sex M:F	Typical thymic pathology	HLA association	Associated autoantibodies
Early-onset	<51 years	1:3	Thymitis or hyperplasia	B8, DR3	AChR. May have other tissue antibodies, e.g., thyroid
Thymoma associated	Mainly 40–60 years	1:1	Epithelial tumor containing many lymphocytes	No clear association	AChR. Titin and ryanodine receptor antibodies very common. Also cytokine antibodies
Late-onset	>50 years	1.5:1	Normal or atrophied	B7, DR2 in males	AChR. Titin and ryanodine receptor antibodies common, particularly after age 60 years
AChR antibody negative MuSK antibody positive	2–70 years	1:3	Normal or atrophied in most	Not known	MuSK. Other antibodies very uncommon
AChR antibody negative MuSK antibody negative	1–80 years	2:3	Mild thymitis/hyperplasia in some	DR14.DQ5	Cell-based assays can demonstrate antibodies to clustered AChR, MuSK, and/or LRP4 in a proportion

These subgroups are not appropriate in patients with purely ocular MG and in other ethnic populations. *AChR*, Acetylcholine receptor; *MuSK*, muscle-specific kinase.

proteins. The latter group includes MuSK, LRP4 and the striational muscle proteins, titin and ryanodine receptor (RyR).

### Early-Onset Acetylcholine Receptor-Antibody Positive Myasthenia Gravis

These patients are usually defined as presenting before the age of the 50 years. There is a marked female predominance and an incidence that has remained relatively stable for many years. There is an association with HLA A1, B8, DR3, DR2, and DR52 amongst Northern Europeans (Compston et al., 1980; Janer et al., 1999; Hill et al., 1999) and HLA DPB1, DQB1, and DR9 amongst the Japanese (Horiki et al., 1994). Childhood onset AChR-Ab MG is relatively rare in North Europeans but more prevalent amongst Oriental populations as mentioned above (Vincent et al., 2001).

Clinically, the MG often involves extraocular muscles at onset before generalizing, although a proportion will remain purely ocular. The early response to cholinesterase inhibitors is usually good but the majority of patients will still require some form of immunosuppressive therapy. The thymus is typically hyperplastic and thymectomy is a therapeutic option in early-onset generalized AChR-Ab MG, with current available evidence suggestive of benefit in over half of cases (see below). Antibodies (Abs) against titin and RyR are rarely found in the early-onset cases and should raise concerns regarding a thymic tumor.

### Late-Onset Acetylcholine Receptor-Antibody Myasthenia Gravis

By conventional definition, these patients present after 50 years of age and males exceed females by 3:2 ratio. Employing a registry to identify all individuals with positive AChR antibody levels, it has been found that the age specific incidence rises between 45 and 75 years before rapidly falling (Vincent et al., 2003). There is a weak association with HLA B7, DR2 (Compston et al., 1980) and DR4, DQw8 (Carlsson et al., 1990).

Clinically, these patients have a similar phenotype to the early-onset form, although ocular MG may be more common (Zivkovic et al., 2012). The overall response to immunosuppressive treatment is similar to early-onset disease but a greater proportion of patients will encounter side effects, presumably due to comorbid disease (Sanders and Evoli, 2010). However, the thymus is typically atrophic unlike the hyperplastic early-onset thymus. The response to thymectomy is poorer and it is not routinely offered to patients over 60 years of age.

Over half of these late-onset cases have detectable Abs against titin and RyR (Buckley et al., 2001) whilst 25% have Abs against the cytokines, interferon- $\alpha$ , or interleukin-12 (Meager et al., 2003). Titin and RyR Abs are more prevalent in thymoma cases and some authors have speculated that their presence in late-onset MG may represent an immune response against occult thymomas that are subsequently destroyed (Marx et al., 2010).

## Thymoma Associated Myasthenia Gravis

Thymomas are tumors derived from thymic epithelial cells, thereby distinguishing them from lymphoma, neuroendocrine, and germ cell tumors. These are conventionally classified by means of the WHO classification (A, AB, B, and C). A coexisting thymoma is identified in 10% of the MG patients. It can occur at any age but is most common amongst the 40–60 age group. There is no gender difference or consistent HLA association.

Clinically, MG is generalized with detectable AChR antibodies, although ocular and seronegative cases have been reported (Maggi et al., 2008). Myasthenia may be more refractory to treatment than other forms of MG (Sanders and Evoli, 2010). Following thymectomy, AChR Ab levels do not necessarily fall without additional treatments but in contrast to early-onset disease, myasthenia rarely improves without further immunotherapies (Somnier, 1994).

Serum Abs against striated muscle were recognized first in the 1960s. Their major targets are two intracellular proteins, titin and the RyR, both of which are expressed in thymoma (Skeie et al., 1997; Mygland et al., 1995). These antibodies are typically observed in >90% of the thymoma associated MG cases but there are no convincing data supporting their pathogenic role and Abs against the AChR are invariably identified. Neutralizing Abs against interferon- $\alpha$  and interleukin-12 are observed in approximately 70% and 50% of the cases, respectively (Buckley et al., 2001; Meager et al., 2003). These are useful markers for identification of recurrence which occurs in about 10% of the thymomas.

## Muscle-Specific Kinase Antibody Positive Myasthenia Gravis

These patients can present at any age, peaking in the 30s with a female predominance. There is significant worldwide variation with a correlation with geographical latitude, suggesting potential environmental influences (Vincent et al., 2008). On the other hand, despite the small numbers in individual studies, a significant association with HLA DR14 and DQ5 in a Dutch cohort (Niks et al., 2006) and DRB16 and DQB5 in an Italian cohort (Bartoccioni et al., 2009) have been identified suggesting genetic susceptibility. It is not yet clear whether environmental or genetic factors or both predispose to this form of MG.

Clinically, the phenotype is often different from AChR-MG with prominent ocular, bulbar, neck, and respiratory weakness (Evoli et al., 2003; Sanders et al., 2003). Muscle wasting and atrophy of the tongue and facial muscles may be evident both clinically and radiologically (Farrugia et al., 2006). The response to treatment can also differ with a comparatively poorer response (and frequent intolerance) to cholinesterase inhibitors (Evoli et al., 2003; Pasnoor et al., 2010). A proportion can be refractory to conventional immunosuppressive treatment (Evoli et al., 2003). In such cases, plasma exchange may be more effective than intravenous immunoglobulin (Pasnoor et al., 2010) but interestingly, rituximab (RTX) may be more effective in MuSK patients than in those with AChR antibodies (Maddison et al., 2011; Diaz-Manera et al., 2012). The thymus is typically normal or atrophic (Evoli et al., 2008; Leite et al., 2005) in direct comparison to that seen in AChR-Ab MG, and most centers do not perform thymectomy. Thymoma is very rare in MuSK-MG (Saka et al., 2005).

## Neonatal Myasthenia Gravis

This is caused by passive transfer of maternal antibodies across the placenta. It may occur in up to 10% of the female patients with AChR antibodies (Vincent et al., 2001). The affected newborn babies exhibit transient symptomatic weakness, requiring the use of cholinesterase inhibitors for a few weeks. Rarely, it can occur in women who are symptom free but have AChR antibodies.

Arthrogryposis multiplex congenita is a condition where the newborn have multiple joint contractures as a consequence of absent fetal movement in the uterus. It can occur if there are high levels of maternal antibodies directed against the fetal isoform (see Fig. 53.2) of the AChR (Barnes et al., 1995). These antibodies can block the ion channel function of the fetal isoform leading to paralysis during development. The adult isoform takes over during the third trimester and thus, although the condition is not usually reversible, it does not progress after birth. The pathogenic mechanisms were examined in a mouse model of maternal-to-fetal transfer (Jacobson et al., 1999b). There are also rare case reports of neonatal MG occurring in MuSK MG with both transient (Niks et al., 2008) and more persistent disease (Behin et al., 2008) described.

## ANTIBODIES IN MYASTHENIA

### Evidence for Pathogenicity of Acetylcholine Receptor and Muscle-Specific Kinase Antibodies

Both AChR and MuSK antibodies are pathogenic, satisfying the strict criteria required for establishing causation in autoimmune disease (Rose and Bona, 1993). Both are directed against autoantigens that are highly relevant to a disorder of neurotransmission and are highly specific for MG.

The main criteria in these diseases are the passive transfer of MG from man to animal (usually mice or rats) and the response to treatments that reduce Ab levels. Passive transfer models involve injection of IgG from MG patients into animals and lead either to objective weakness or at least to neurophysiological evidence of impaired neurotransmission. Plasma exchange dramatically reduces antibody levels within a few days and leads to striking clinical improvement even in patients with long-standing disease. In addition, maternal–fetal transfer of the disease has been reported in both serological forms of MG.

Further *in vivo* evidence comes from replication of the human disease in animals that have been immunized with the relevant antigen, termed experimental autoimmune MG (EAMG). This active immunization model has been used extensively to study the immune biology of MG, as will be mentioned below.

### Acetylcholine Receptor Antibodies

AChR Abs were first detected by means of a radioimmunoprecipitation assay (RIA) employing  $^{125}\text{I}$   $\alpha$ -bungarotoxin that binds strongly to AChRs and labels AChRs in detergent extracts of human muscle (Lindstrom et al., 1976). Modern RIAs employ AChR extracted from muscle cell lines expressing mixtures of fetal and adult AChRs (Beeson et al., 1996). Directly radiolabeled recombinant MuSK are used for detection of MuSK antibodies (Matthews et al., 2004). Enzyme linked immunosorbent assays are not found to be as sensitive or as specific in our hands. AChR antibodies belong to variable IgG subclasses, although IgG1 and IgG3 predominate (Rodgaard et al., 1987; Vincent et al., 1987). A significant proportion of Abs are directed against the MIR on  $\alpha$ -subunits, although other sites (Whiting et al., 1986) and other AChR subunits (Jacobson et al., 1999a) can also be targets. These Abs have a high affinity (around 100 pM) and are highly specific for the intact receptor with limited binding to recombinant polypeptides or denatured AChR subunits; the Abs bind predominantly to the extracellular portion of the receptor.

The diagnostic sensitivity of the RIA is high in adult-onset generalized MG (80%–85%) but quite low in ocular MG (around 50%) (Wong et al., 2014) and in prepubertal-onset disease (50%–70%) (Finnis and Jayawant, 2011). Between patients, there is variation in the Ab specificity, isoelectric heterogeneity, and avidity for the AChR and no clear correlation with disease activity is observed. Although some reports indicate a poor correlation between Ab levels and clinical severity in MG, the Ab titer can be useful in monitoring disease activity in individual patients (Vincent and Newsom Davis, 1980). This association has been reported after thymectomy, plasma exchange, or immunotherapies (Heldal et al., 2014). Moreover, in a recent study including 223 patients with ocular presentation, AChR antibodies were detected in 71% of the cases and an increased antibody titer predicted the risk for MG generalization (Peeler et al., 2015).

In generalized MG, 85% have AChR Abs and 0%–10% have MuSK Abs; a rather variable but usually low percentage may have LRP4 Abs, although there are few systematic studies at present and assays are not well standardized. There are very rare case reports of patients with both AChR and MuSK Abs (Saulat et al., 2007; Rajakulendran et al., 2012) and LRP4 Abs may coexist with much higher levels of MuSK Abs (Higuchi et al., 2011) raising questions about their importance.

Cell-based assays (CBAs) have now been developed that use human embryonic kidney (HEK) cells transfected with DNA for the antigen of interest that is then expressed on the cell surface. Indirect immunofluorescence can be used to detect the binding of patients' Abs. This method is sensitive and, importantly, measures potentially pathogenic Abs that only bind to extracellular determinants of the antigen (Leite et al., 2010). This was demonstrated in previously seronegative cases using cells transfected with AChR subunits which were clustered with the scaffold protein, rapsyn (Leite et al., 2008). CBAs have also been developed to detect MuSK or LRP4 Abs (Higuchi et al., 2011) as mentioned below.

The "clustered AChR" Abs were found in 38% of the RIA-seronegative MG patients and were useful in confirming the MG diagnosis especially in childhood, ocular, and mild disease forms (Rodriguez Cruz et al., 2015). More recently, a French study reported the presence of clustered AChR Abs in 16% of the adult patients with generalized MG with a phenotype resembling those positive on standard RIA but with a milder disease course

as shown previously. Interestingly, thymectomy was performed in a proportion of patients with evidence of thymoma in one case (Devic et al., 2014). In future, these results will need confirmation by larger, multicenter studies. Of relevance, experimental studies of passive transfer with purified patients' IgG into mice resulted in the reduction of MEPP amplitudes indicating the loss of postsynaptic AChR that mirrors that found in typical MG patients (Jacob et al., 2012) confirming the pathogenicity of the clustered AChR Abs (Vincent and Newsom Davis, 1980).

## Characteristics and Mechanisms

AChR Abs cause loss of AChR through three principal mechanisms. These include complement mediated destruction, crosslinking and accelerated degradation, and functional blockade. AChR Abs belong predominantly to the complement fixing IgG1 and IgG3 subclasses (Rodgaard et al., 1987) that are divalent for the AChR and can therefore cause neuromuscular transmission failure by internalizing the AChRs or activating complement (Rodgaard et al., 1987; Toyka et al., 1975). Internalization or antigenic modulation is an increase in the normal rate of turnover of the AChRs that is observed in both MG patients (Drachman et al., 1978) and EAMG (Lindstrom and Einarson, 1979). It is most prominent when Abs are directed against the MIR (Tzartos et al., 1991) and lead to reduced AChR expression on the postsynaptic membrane without membrane damage. The activation of complement results in the generation of the membrane activation complex that is responsible for lysis and destruction of the postsynaptic membrane, loss of postsynaptic folding, and ultimately loss of AChR and related proteins. IgG colocalizing with activated complement was shown both in EAMG (Sahashi et al., 1978) and MG (Engel et al., 1977) muscle biopsies. The importance of complement in the pathogenesis of this form of MG came from several lines of evidence. Depletion (Lennon et al., 1978), inhibition (Biesecker and Gomez, 1989), or blockade (Piddlesden et al., 1996) of complement all resulted in resistance to developing the disease in EAMG. Further evidence was provided by the study of transgenic mice lacking components of the classical complement cascade (Tuzun et al., 2003) and complement (Morgan et al., 2006) and recently anticomplement therapy has been found effective in severe cases (see Biologics below). By contrast, direct block of ACh binding to the AChR or of the ion channel itself appears to be uncommon in majority of the patients.

Whatever the mechanisms, failure of neuromuscular transmission results from the loss of AChRs, which leads to reduced MEPP and EPP amplitudes. The reduced EPP amplitude falls below the required threshold to initiate an action potential, leading to blocking of neurotransmission. There is partial compensation for this through an increase in AChR synthesis by the muscle and in the number of quanta of ACh released, which appears to be a compensatory mechanism in both MG and EAMG (Plomp et al., 1995).

## Muscle-Specific Kinase Antibodies

MuSK is a receptor tyrosine kinase critical for the formation and maintenance of the neuromuscular junction. The extracellular portion consists of three immunoglobulin-like domains and a cysteine-rich domain. MuSK-Abs are detected by RIA in 40% of the AChR-negative patients with variable frequency across populations.

CBA techniques can be applied to the detection of MuSK-Abs and may improve the diagnosis in RIA-negative patients. A multicenter study investigating seronegative sera from 13 countries searching for MuSK-Abs by CBA achieved positive results in 13% of the samples (Tsonis et al., 2015) but many of the Abs detected were of the IgM type and their specificity is still uncertain. Recently, a study from the Oxford group found that 8% of the RIA-negative MG samples bound MuSK antigen expressed on HEK cells. Their pathogenic role on AChR clustering was confirmed in vitro in C2C12 myotubes (Huda et al., 2017). Interestingly, in this study, the patients with MuSK-Abs only on CBA had a milder phenotype than those positive on standard RIA.

## The Role of Muscle-Specific Kinase in Neuromuscular Junction Development and Maintenance

To understand the effects of antibodies in MuSK-MG, it is necessary to appreciate the complex mechanism of neuromuscular junction formation and maintenance, for which MuSK has a critical role (Kong et al., 2004). During development, a large soluble protein N-agrin is released from the motor nerve and binds to the MuSK coreceptor, LRP4; this binds to and activates MuSK phosphorylation. Phosphorylated MuSK recruits the intracellular protein DOK7 that is also phosphorylated and together they initiate the complex pathway that results in the clustering of rapsyn and AChRs on top of the postsynaptic folds under the nerve terminals. This process can be

studied to a large extent in cultured mouse C2C12 myotubes. The C2C12 myoblasts can be induced by serum deprivation to fuse and become myotubes with high AChR expression throughout the membrane. When agrin is added to the myotubes, the LRP4/MuSK/DOK7 pathway is activated and AChRs form high density clusters ( $>3\text{ }\mu\text{m}$ ) on the myotube surface. These clusters can be observed and quantified using fluorescently labeled alpha-bungarotoxin, the snake toxin that is used extensively to measure muscle AChRs and for the RIA. If DOK7 is virally transduced into the C2C12 cells, the AChR clusters occur without the requirement of agrin, LRP4, or MuSK, showing that DOK7 is the ultimate initiator of the clustering process. These phenomena can be used to study the effects of MuSK and LRP4 Abs.

MuSK Abs belong predominantly to the noncomplement fixing IgG4 subclass (McConville et al., 2004). These Abs are able to undergo Fab arm exchange to produce bispecific Abs that do not cross-link identical antigens (van der Zee et al., 1986), and which therefore function monovalently (Schuurman et al., 1999). This suggests that the effector mechanisms are unlikely to be the same as those in AChR mediated disease. MuSK Abs have been shown to be monovalent for MuSK but they can interfere with protein–protein interactions (Koneczny et al., 2017). MuSK-Abs bind to an epitope within the first Ig-like MuSK domain, preventing its interaction with LRP4, which results in the impairment of the AChR clustering pathway (Huijbers et al., 2013; Koneczny et al., 2013). A recent study showed that the level of IgG4 directed against the first Ig-like MuSK domain correlates better with disease severity than the total MuSK Ab titer (Huijbers et al., 2016) while no correlation was found with Abs directed to the other MuSK epitopes (e.g., Frizzled-like domain). Some studies have also found that MuSK interacts with ColQ, a collagen-like protein that anchors AChE to the basal lamina in the synaptic cleft.

MuSK Abs inhibit the binding of LRP4 to MuSK and thus interfere with the agrin induced clustering pathway. The divalent IgG1-3 Abs do not block LRP4 binding to MuSK. Nevertheless, both IgG4 and IgG1-3 MuSK Abs inhibit agrin induced AChR clustering in the C2C12 myotubes, suggesting that the IgG1-3 antibodies alter MuSK function in an LRP4-independent manner (Koneczny et al., 2013). Since the IgG1-3 antibodies are less frequent, their functional roles, which could include complement mediated damage, have not been explored adequately in animal models.

There are very little data from human muscle, and the only published pathological study has demonstrated normal motor endplates, normal AChR numbers, and little evidence of complement deposition (Shiraishi et al., 2005). By contrast, both active immunization against MuSK (Shigemoto et al., 2006; Viegas et al., 2012) and passive transfer of human MuSK Ab IgG (Cole et al., 2008) models have demonstrated AChR loss in clinically affected animals, although complement deposition has not been observed and complement deficient mice remain susceptible to the disease (Mori et al., 2012).

Of particular interest, combined pre- and postsynaptic morphological changes have been observed in animal models (Cole et al., 2008; Richman et al., 2012) and may explain the severe phenotype that is observed in the mice and in patients. There is also electrophysiological evidence of both pre- and postsynaptic defects (Viegas et al., 2012; Klooster et al., 2012) with the failure of the presynaptic compensatory mechanism (Plomp et al., 1995) further impacting on underlying neurotransmission.

## LRP4 Antibodies

The proportion of LRP4-positive patients among the AChR/MuSK seronegative cases varies widely from 0% to 50% in different reports, probably due to the different techniques applied, such as luciferase reporter immunoprecipitation (Higuchi et al., 2011), ELISA (Zhang et al., 2012), and CBA (Pevzner et al., 2012; Marino et al., 2015). In a recent European multicenter study, LRP4 Abs were detected in 18.7% of the 635 seronegative patients, with a difference in the frequency among different countries, suggesting a possible role of ethnicity in disease susceptibility (Zisimopoulou et al., 2014). A recent Chinese study reported a frequency of LRP4 antibodies of only 4%, associated with AChR and MuSK-Abs in a proportion of cases (Li Y, Muscle Nerve, 2017).

LRP4 Abs were predominantly of the IgG1 subclass and their potential pathogenicity has been demonstrated in active immunization models by disrupting agrin–LRP4 binding to MuSK and by complement–activation (Shen et al., 2013). However, there have been no reports on passive transfer models from human LRP4 Abs. Moreover, somewhat worryingly, LRP4 Abs have been reported in a significant proportion of other neurological diseases including neuromyelitis optica (NMO) (Zhang et al., 2012) and amyotrophic lateral sclerosis (Tzartos et al., 2014). Future studies are needed in order to standardize the assays and clarify the pathogenicity potential of LRP4.

Most of the positive patients have a mild generalized disease. In some of the patients, a double association with MuSK (predominantly) or AChR Abs were considered responsible for a more severe disease course

(Higuchi et al., 2011; Zhang et al., 2012; Pevzner et al., 2012; Marino et al., 2015). There is a female and early-onset preponderance (only 16% were >50 years). Thymic hyperplasia can occur but there are no reports of thymoma.

## Novel Targets

While the search for novel antigens in MG is keenly pursued, there are still some patients without detectable Abs. The frequency of the so-called triple-negative cases is variable. Abs to agrin, ColQ, and cortactin have recently been described in MG patients (Zhang et al., 2014; Zoltowska Katarzyna et al., 2015; Gallardo et al., 2014; Cortes-Vicente et al., 2016) but their role in the disease pathogenesis has not been proved and they are not currently used in routine diagnostic assays. Antibodies against rapsyn have also been described (Agius et al., 1998), but as the antigen is intracellular, the Abs are unlikely to be pathogenic.

One of the problems with many studies on triple negative cases is that the sera are seldom taken at the onset of symptoms and most patients have been treated with immunotherapies. In addition, it is possible that some of the antibodies that are found are secondary to the disease process and not the primary pathogenic entity.

## THE THYMUS AND CELLULAR IMMUNITY IN MYASTHENIA GRAVIS

### Role of T Lymphocytes in Myasthenia Gravis

High affinity AChR Abs are thought to be dependent on CD4+ T lymphocytes but as these are rarely observed in myasthenic muscle and almost never found at the neuromuscular junction; they are not thought to be effector cells in MG. Nevertheless, their critical role in the autoimmune pathogenesis is demonstrated through several lines of evidence in both MG and EAMG. EAMG was first described following the immunization of rabbits with AChR purified from the electric organ of *Torpedo californica* (Patrick and Lindstrom, 1973) and later reproduced in a number of other species. In the murine model, there are both disease sensitive and resistant strains, related to their different H-2 alleles (Berman and Patrick, 1980).

AChR-specific CD4+ T lymphocytes occur in both the peripheral blood and thymuses of MG patients. They may also be observed in the healthy controls but the clinical improvement observed following their removal with anti-CD4 monoclonal Abs (Ahlberg et al., 1994) and in HIV (Nath et al., 1990) supports a pathogenic role for these lymphocytes. These isolated CD4+ T lymphocytes may respond to stimulation with the intact AChR, recombinant subunits or AChR peptides (Conti-Fine et al., 1998; Wang et al., 1998), and T cell lines or clones propagated from MG patients will respond more vigorously to stimulation in vitro than those derived from the healthy controls. The epitopes are most commonly found on the  $\alpha$ -subunit of the AChR (e.g., Ong et al., 1991). In EAMG, a dominant epitope within the  $\alpha$ 146-162 activates MHC class II restricted CD4+ T lymphocytes, leading to pathogenic antibody production (Christadoss et al., 2000). No clearly immunodominant epitope has been identified reproducibly in a high proportion of MG patients, although an epsilon subunit epitope was identified in some (Hill et al., 1999). The TCR V $\beta$  families show preferential expansion of the V $\beta$  4 and 6 amongst MG patients (Navaneetham et al., 1998) whilst mice lacking V $\beta$  6 respond poorly to immunization with AChR (Krcio et al., 1991; Ahlberg et al., 1994).

Mice genetically deficient in functioning CD4+ T lymphocytes don't develop EAMG (Kaul et al., 1994) whilst severe combined immunodeficient mice will only produce AChR Abs and develop myasthenic symptoms if the human grafted cells contain CD4+ T lymphocytes (Wang et al., 1999).

CD4+ T lymphocytes and the cytokines they secrete will influence the type of autoimmune response generated in both MG and EAMG. Analysis of blood from MG patients has confirmed the presence of T helper 1 (Th1), Th2, Th17, and T regulatory (Treg; Foxp $^+$ ) cells (Li et al., 2008) but the role of these T lymphocyte subsets in the development of the disease is best examined using transgenic mice. Interleukin-12 (IL-12) is essential for promoting development of Th1 cells and IL-12 - / - mice are resistant to the development of EAMG despite a significant Ab response (Karachunski et al., 2000). The role of IFN- $\gamma$  remains unclear with conflicting reports of EAMG susceptibility (Balasa et al., 1997; Wang et al., 2007). IL-4 appears to be either neutral (Balasa et al., 1998) or confer a protective effect (Karachunski et al., 1999). There is an apparent increase in Th17 and decrease in Tregs during development of EAMG in rats (Mu et al., 2009) and administration of Tregs to myasthenic rats inhibited progression of EAMG (Aricha et al., 2008). Initial studies identified no change in Treg numbers in MG subjects compared with the healthy controls (Huang et al., 2004), although a specific functional impairment in those Foxp3+ Tregs (Balandina et al., 2005) was subsequently identified.

T cell activation requires the interaction of TCR/MHC peptide in addition to the interaction between CD28/CTLA4 on the T lymphocytes and CD80 (B7) on antigen presenting cells (the CD28–CD80 interaction). It also requires the crosslinking of the CD40 ligand (CD40–CD40L interaction). Using transgenic mice, it has been demonstrated that both interactions are essential for the primary immune response (Shi et al., 1998), although their contribution to the secondary response is thought to be less prominent. Here, the CD278 (inducible T-cell costimulator) is thought to be important for the secondary response (Scott et al., 2004).

### Advances in the Cellular Immunology of Acetylcholine Receptor Myasthenia Gravis

The breakdown of central and peripheral tolerance checkpoints is an important aspect involved in the pathogenesis of MG. A recent study showed that the frequency of autoreactive B cell receptors was higher in patients with AChR and MuSK MG than in the healthy controls (Lee et al., 2016). In addition, studies on regulatory B and T cells provide new insights into the involvement of defective immune regulatory pathways in the immunopathogenic mechanisms likely responsible for MG. The understanding of the mechanisms that account for B-cell shift from a proinflammatory to a regulatory phenotype and defective B cell tolerance has important therapeutic implications in terms of developing durable B and T cell–targeted therapies for MG.

The B-cell population is functionally heterogeneous and B cells can be divided into different subsets according to the cytokine that they produce. One functional B cell subset, regulatory B cells (Bregs), has recently been shown to downregulate the immune response through the production of IL-10, IL-35, and TGF- $\beta$  (Fillatreau et al., 2008; Shen et al., 2014). In particular, IL-10 appears to inhibit dendritic cell production of IL-12 and to suppress Th1 and Th17 cell responses in several models of autoimmune diseases (Li et al., 2012; Quan et al., 2013).

The most accepted cell markers to identify Bregs are considered CD1d + CD5 + and CD24 + CD38 + B cells, which are phenotypically indistinguishable from transitional immature B cells and which have been shown to produce the highest percentage of IL-10. The involvement of CD19 + CD24 + CD38 + immature Bregs in autoimmunity was first described in studies on RTX-treated Systemic lupus erythematosus patients, in which the disease remission was associated with a reconstituted B cell population predominantly characterized by immature Bregs (Blair et al., 2010). This led to the hypothesis that after the B cell depletion therapy, the newly repopulated B cells are constituted by competent Bregs that can efficiently suppress the immune response and restore the immune balance in favor of tolerance (Palanichamy et al., 2009).

Both frequency and function of Bregs were reduced in MG patients compared with the healthy controls and were associated with disease activity (Sheng et al., 2016; Karim et al., 2017; Yi et al., 2017) and response to RTX (Sun et al., 2014). These findings were confirmed in a recent study showing a lower percentage of Bregs in patients with MuSK MG (Guptill et al., 2015). From these findings, Bregs may serve as a marker for disease activity in MG patients and as a promising therapeutic target.

Early studies searching for AChR-specific T cells were technically demanding and have not proven to be particularly helpful in understanding the disease etiology or providing new targets for therapies. More recently the emphasis has been on the regulatory T cells. Thymic T CD4 + lymphocytes include Tregs involved in self-tolerance mechanisms and follicular T helper cells (ThF) that play a critical role in B lymphocyte differentiation and affinity maturation. Treg lymphocytes play important immunosuppressive functions (Sakaguchi et al., 2010; Campbell and Koch, 2011) and both quantitative and functional alterations of CD4 + CD25 + FoxP3 + Tregs have been described in several autoimmune pathologies (Long and Buckner, 2011; Noack and Miossec, 2014) but with contradictory results. In MG, where it is well known that thymus pathology is implicated in the pathogenesis, Treg should be even more relevant. Reduced Tregs were found in a recent study on AChR-MG patients, in which different subpopulations of Treg were significantly reduced after the thymectomy and immunosuppression and were, in general, lower than in healthy subjects (Kohler et al., 2017). Therapies focused on ameliorating Treg function through enhancing (Long and Buckner, 2011) their suppressive activity or their migration appeared to be promising in preclinical models of MG (Sheng et al., 2006) because of the adoptive transfer of ex vivo generated Treg (Aricha et al., 2008).

A subpopulation of Treg, called follicular regulatory T (Tfr) cells, are thought to control the function of ThF that promote B cell maturation and high affinity Ab production in germinal centers. An abnormal production of thymus derived ThF might be involved in the development of several autoimmune diseases including MG. A recent study showed an increased frequency of ThF and a decreased rate of Tfr in the peripheral blood of MG patients compared to the healthy control that inversely correlated with the disease severity (Wen et al., 2016). These results were confirmed in the patients with generalized MG in a study which showed a correlation between ThF, plasma cell frequency, and AChR-Ab titer (Zhang et al., 2016).

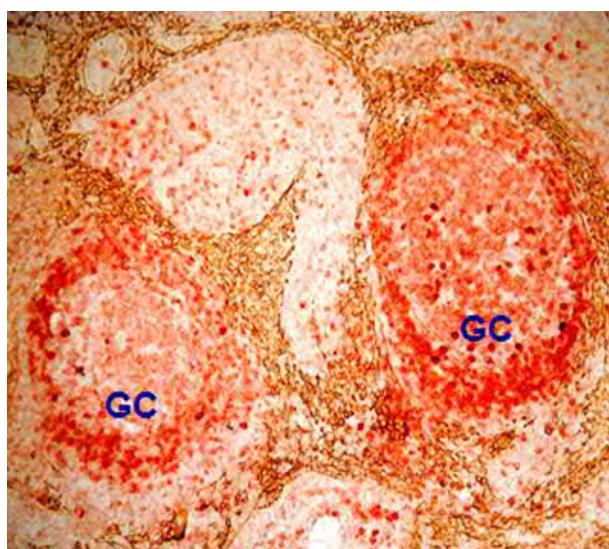
## The Thymus in Myasthenia Gravis

Thymus is involved in the maintenance of self-tolerance. While MuSK-MG is rarely associated with thymus alterations, in AChR-MG thymus changes are common, especially in the early-onset subtype. In case of thymoma, thymectomy is always mandatory while the rationale for thymectomy in nonthymomatous patients is related to the role of the thymus follicular hyperplasia (TFH) in the pathogenesis of the disease. Several studies showed that the TFH includes infiltrates of lymphocytes- and AChR-specific germinal centers that can be targeted by complement factors (Leite et al., 2007).

The thymus is an epithelial organ that can be morphologically divided into a distinct cortex, medulla and corticomedullary zone. The cortex contains densely packed immature lymphocytes alongside a sparse population of epithelial cells and bone marrow derived macrophages. The medulla is less cellular containing more mature T lymphocytes, more prominent epithelial cells, dendritic cells, B lymphocytes, and rare myoid cells (Pearse, 2006). The thymus has a critical role in self-tolerance with a fine balance between the generation of protective T lymphocytes and deletion of autoreactive T lymphocytes required. Relevant autoantigens including the  $\alpha$ -subunit of the AChR are expressed on medullary thymic epithelial cells (mTECs) under the control of the autoimmune regulator gene (AIRE) (Giraud et al., 2007). Central T cell tolerance relies on the close interaction between these mTECs and the nearby dendritic cells and their effect on the T lymphocyte development and subsequent differentiation.

The thymus is thought to have a critical role in the development of early-onset AChR antibody-positive MG. The cortex is typically normal but the medulla contains lymphocytic infiltrates and germinal centers with distinct areas of B lymphocyte proliferation, differentiation, somatic hyper-mutation, and immunoglobulin class switching. A typical pathological section of an MG thymus is shown in Fig. 53.3. These B lymphocytes, when cultured in vitro, are capable of secreting AChR Abs spontaneously (Vincent et al., 1978; Scadding et al., 1981). It is, therefore, to be expected that antibody levels fall post thymectomy (Vincent et al., 1983) but they seldom disappear.

Whether early-onset AChR antibody-positive MG begins in the thymus, or whether these changes are a reflection of a systemic process, remains unresolved. Individual AChR subunits are expressed on mTECs (Salmon et al., 1998), presumably as part of a self-tolerance mechanism, and these are targeted by both autoantibodies (Safar et al., 1991) and complement (Leite et al., 2007). Native AChR is also expressed by the muscle-like myoid cells, which whilst comparatively rare, are more abundant in the hyperplastic thymus (Kirchner et al., 1986). The germinal centers appear to be focused around these myoid cells. Given that they lack MHC class II or costimulatory molecules, they rely on the antigen presenting dendritic cells to prime the CD4<sup>+</sup> T lymphocytes. One proposed multistep hypothesis is that the mTECs first present epitopes from isolated AChR subunits to CD4<sup>+</sup> T lymphocytes, evoking the production of early antibodies capable of attacking the thymic myoid cells



**FIGURE 53.3** Hyperplastic thymus with the presence of GC in an early-onset MG patient with AChR antibodies. Double CD79a (red)/CK19 (brown) immunostaining. Intense CD79a<sup>+</sup> staining marks the periphery of germinal centers, CK19 is specific for the thymic epithelial tissue. Original magnifications,  $\times 250$ . MG, Myasthenia gravis; AChR, acetylcholine receptor; GC, germinal centers. Source: Courtesy of Professor L. Lauriola, Institute of Pathology, Catholic University, Roma.

that express the intact AChR. As the immune response continues to proliferate, germinal centers are formed that allow these antibodies to diversify allowing recognition of native AChRs (Willcox et al., 2008).

The thymus is typically involuted and atrophic in older patients. The aging thymus is gradually replaced with fat, although residual foci of mTECs may persist and myoid cells are only rarely encountered. The histological analysis of the thymus tissue from the late-onset MG cases previously suggested no differences from normal controls (Myking et al., 1998). Nevertheless, a more recent study looking at young and late-onset MG cases identified residual lymphocyte accumulation amongst the older cohort and no qualitative differences between the two groups (Ishii et al., 2007).

## Thymoma

Thymomas are heterogenous neoplasms of thymic epithelial cells (TEC) with mixed cortical and medullary markers. They may develop from either early TEC progenitors or from more mature cortical or medullary TECs (Hasserjian et al., 2005). They are responsible for the generation of numerous maturing polyclonal T lymphocytes (thymocytes) capable of maturing into CD4 + or CD8 + T lymphocytes. The degree of thymopoiesis is known to vary according to the thymoma subtype (Nenninger et al., 1998). The B2 subtype is the most common type associated with MG (accounting for ~50% of the cases) and contains an abundance of mature CD4 + T lymphocytes ready for export (Strobel et al., 2004). It should be noted that corticosteroids can deplete the immature T lymphocytes and thereby modify the histological subtype.

There are certain features in thymomas that are likely to promote inefficient self-tolerance including defective AIRE and HLA class II expression, an absence of myoid cells, failure to generate Foxp3 + Treg cells as well as defective T lymphocyte signaling (Marx et al., 2010). Other antigenic targets are also recognized which is unsurprising given the wide range of systemic, hematological, endocrine, cutaneous, gastrointestinal, and renal disorders associated with thymoma (Marx et al., 2010). A lower production of Treg was observed in thymoma and confirmed by immunohistochemistry (Scarpino et al., 2007).

Genetic aberrations and polymorphisms may also be identified in thymoma. These include HLA genes (notably loci at 6p21) which may affect MHC class II expression and non-HLA genes (including CTLA4 and PTPN22) which influence T-cell receptor signaling.

## TREATMENTS IN MYASTHENIA GRAVIS

### General Approach

The current treatment for MG consists of symptomatic therapy with acetylcholinesterase inhibitors and generalized immunosuppression with corticosteroids, azathioprine, mycophenolate, and immunomodulation with intravenous immunoglobulin and plasmapheresis. Thymectomy is common in early-onset MG. In response to the need for more effective or more rapid treatments in some patients, biologics have been applied to MG management as described below.

The efficacy of thymectomy in early AChR-MG was suggested in several small case series but results from the thymectomy trial (MGTX) have been recently published (Wolfe et al., 2016). This multicenter (40 centers included) trial was conducted between 2006 and 2012 and recruited 126 nonthymomatous AChR-MG patients comparing prednisone alone versus prednisone with transsternal thymectomy. Patients who underwent thymectomy had a lower time-weighted average Quantitative myasthenia gravis (QMG) score over a 3-year period and required lower average alternate-day prednisone than those who received prednisone alone. Moreover, immunosuppressive treatment, need for hospitalization, and treatment side effects were less in the thymectomy group. The MGTX trial represents a milestone in the management of MG patients even if it leaves some open questions, such as the efficacy of thymectomy in late-onset AChR-MG, where the thymus is normally atrophic or in other MG types and the benefit from less invasive surgical approaches (Keijzers et al., 2015).

### Biologics

Several recent studies have demonstrated the benefits of Rituximab (RTX)-mediated B cell depletion in refractory MG, especially in MuSK MG (Iorio et al., 2015). RTX, a monoclonal antibody targeting CD20 on B cells, led

to a sustained clinical improvement in parallel to a reduction or discontinuation of corticosteroid and plasma exchange treatments.

The expression of the CD20 antigen is restricted to the late pre-B-cell stage and is maintained until their differentiation to plasma cells when expression is usually lost. Interestingly RTX targeting the precursor of plasma cells depletes the niche of short-lived plasma cells while it has no effect on the long-lived plasma cell subset (Winter et al., 2012).

It has been hypothesized that MuSK IgG4 Abs are produced almost exclusively by short-lived plasma cells (Diaz-Manera et al., 2012), which may explain why RTX is particularly effective in MuSK-MG and why MuSK antibody titer markedly decreases after RTX treatment. On the other hand, this finding might be related to a modification of the B-cell repertoire induced by RTX with the disappearance of the initially expanded populations and the reconstitution of a diverse B-cell repertoire that might account for its long-lasting effect.

RTX appeared to be safe and effective even in patients with refractory AChR-MG, who were observed to have a long-lasting response after treatment and a significant decrease of the Ab titers that correlated with the clinical improvement (Robeson et al., 2017). The ongoing trial in AChR-MG will provide more definite conclusions on the efficacy and safety of this treatment but one of the main concerns regards the number of reinfusions needed to prevent a relapse. Based on evidences from NMO studies, the reemerging of CD27+ memory B cells to 0.05% of the peripheral blood cells has been recommended as target value to repeat RTX in MG (Lebrun et al., 2016).

Eculizumab, a humanized monoclonal antibody, inhibits the formation of terminal complement complex by preventing the enzymatic cleavage of complement 5 (C5). It was tried for the first time in MG in a small trial involving 14 refractory AChR-MG patients with promising results (Howard et al., 2013). Currently, a phase III study is assessing the efficacy of eculizumab in refractory AChR-MG.

Belimumab, a human monoclonal antibody that prevents B-cell activating factor (BAFF) from binding to B cells provoking the inhibition of B cells proliferation and survival, has not been shown to be effective in a phase II trial in patients with AChR-MG and MuSK-MG (clinicaltrialgov.com: NCT01480596).

## LAMBERT-EATON MYASTHENIC SYNDROME

### Introduction

The Lambert–Eaton Myasthenic Syndrome (Wirtz et al.) is clinically and electrophysiologically distinct from MG. Approximately 50% of the LEMS cases are paraneoplastic, typically associated with small cell lung carcinoma (SCLC). There are certain features that make it possible to distinguish paraneoplastic and nonparaneoplastic forms.

### Epidemiology and Etiology

LEMS is less common than MG with an annual incidence of 0.48 per million in a Dutch study (Wirtz et al., 2003). The median age of onset is 60 years in both the paraneoplastic and nonparaneoplastic forms (O'Neill et al., 1988) although there is another smaller peak at 35 years in the nonparaneoplastic forms (Titulaer et al., 2011a). There is a male predominance in the paraneoplastic form and a slight female predominance in the nonparaneoplastic form. In the latter, similar to early-onset MG, there is an association with HLA B8 and DR3.

### Clinical Features

The cardinal clinical features of LEMS include muscle weakness, autonomic dysfunction, and areflexia. Typical findings include proximal muscle weakness that is more marked in the lower limbs. Ocular and bulbar symptoms may occur later in the disease course. The speed of progression is often more rapid in the paraneoplastic form (Titulaer et al., 2008). Autonomic involvement is seen in over 80% of the cases. Commonly encountered symptoms include dry mouth, erectile dysfunction, and constipation. Micturition difficulties and orthostatic syncope are less common. In contrast to MG, the muscle strength in LEMS will improve after a period of maximal voluntary contraction. A recent update on the clinical features (Titulaer et al., 2011a) and a clinical method predicting the presence of a small cell cancer can be found elsewhere (Titulaer et al., 2011b).

## Investigation and Treatment

Electromyography will confirm a disorder of neurotransmission although a significant increase in the compound muscle action potential following a period of exercise or high frequency stimulation allows electrophysiological differentiation from MG. Serological confirmation involves detection of antibodies against the P/Q type voltage-gated calcium channels which are found in approximately 90% of the LEMS cases and are invariably present in paraneoplastic SCLC cases (see below).

To detect the antibodies, the P/Q type VGCCs are extracted from mammalian brain, labeled with  $^{125}\text{I}$   $\omega$ -conotoxin which binds specifically to these VGCCs and used in an RIA. In addition, antibodies against N type (30%–40%) and L type (25%) VGCCs may be present (Motomura et al., 1997; Johnston et al., 1994). Other antibodies which have been identified include those against Synaptotagmin (Takamori et al., 1995) and more recently against SOX-1 in 65% of the paraneoplastic cases and around 5% of the nonparaneoplastic cases (Sabater et al., 2008; Titulaer et al., 2009).

Symptomatic treatment requires 3,4-diaminopyridine (3-4-DAP). If additional treatment is required, the corticosteroids and other immunosuppressive agents are used. Intravenous immunoglobulin, plasma exchange, and RTX may be used in severe and refractory cases. Any underlying SCLC should be treated in appropriate manner. Treatment options are reviewed in more detail (Titulaer et al., 2011a).

## Pathophysiology

VGCC contains several subunits but the  $\alpha_1$ -subunit is primarily responsible for the biochemical and electrophysiological functions of the protein. Similar to the MIR on the  $\alpha$ -subunit of the AChR, there may be particularly immunogenic sequences; 50% of the LEMS patients have antibodies against linker domains on the  $\alpha_1$ -subunit (Takamori et al., 1997).

Freeze fracture electron microscope studies of the presynaptic motor nerve terminal have demonstrated an ordered array of intramembranous particles. These are located close to the site of transmitter exocytosis. There is some evidence that VGCC constitutes at least some of the intramembranous particles in the active zone (Robitaille et al., 1990). LEMS patients have both a reduction in the total number of active zone particles and the number of particles per active zone (Nagel et al., 1988).

The evidence for the pathogenic nature of VGCC comes from both clinical and experimental studies. Patients respond to plasma exchange (Newsom-Davis et al., 1982) and cases of maternal-to-fetal transfer of the disease have also been reported (Lecky, 2006). Passive transfer with LEMS plasma or IgG produces the same neurophysiological abnormalities in mice (Lang et al., 1983; Fukunaga et al., 1983), although no weakness was observed. Further studies confirmed the localization of IgG close to the presynaptic active zones (Fukuoka et al., 1987). In addition, active immunization of rats with peptides from the  $\alpha_1$  subunit led to mild weakness and compatible neurophysiological changes (Komai et al., 1999). Finally, mice with mutations in the P/Q type VGCC (CACNA1a) share some of the electrophysiological characteristics of LEMS (Kaja et al., 2007).

## CONCLUSIONS AND FUTURE PROSPECTS

Historically, the classification of MG has been based on AChR Ab status, age of onset, and thymic pathology. MuSK antibodies were first recognized 17 years ago and whilst there was some initial skepticism about their relevance, it is now well established that they are pathogenic, satisfying all the necessary criteria for causation in autoimmune diseases. Defined serologically, this particular form of MG is typically more severe and more refractory to conventional immunosuppressive therapy. From an immunological perspective, the predominance of non-complement fixing IgG4 Abs in a subclass of MG patients contrasts with the predominantly IgG1 AChR Abs in the classical form and raises interesting questions regarding the mechanisms involved in initiation of these two, otherwise similar, forms of autoimmune disease, and the different treatments that might be optimal.

Over recent years further advances in serological assays for MG have been made. Alongside established RIA for detecting AChR and MuSK Abs, cell-based assays have been developed that helped to identify clustered AChR Abs and then more recently LRP4 Abs. Given that LRP4 Abs are predominantly of the IgG1 subclass, it is likely that the role of complement may be more akin to that observed in AChR Ab mediated MG, but LRP4 Abs are usually low titer and most patients have relatively mild disease.

There have been numerous studies on the thymus histology and immunology that is so clearly different from the thymus in healthy individuals. However, it is still unclear to what extent the observed differences are primary to the disease or secondary features associated with the lymphocytic infiltrations and germinal centers that are hall marks of the early-onset disease. Moreover, very few studies have been done on the late-onset MG patients who now represent the greatest and increasing number of patients. The underlying immunological mechanisms may be similar but they don't appear to be present in the thymus.

Conventional treatment for MG relies on symptomatic treatment, oral immunosuppressive agents, and immunomodulatory treatment with intravenous immunoglobulin and plasma exchange. Newer biological agents, such as RTX have shown promise and may benefit those with refractory disease, particularly in patients with MuSK-MG. Thymectomy has been shown to be enhance the beneficial effects of steroids and recent trials inhibiting complement inhibition (Eculizumab) have shown promise for severe cases. In the future, our greater understanding of the immunology of MG may allow the development of other targeted immunotherapies. Potential drugs could include those directed against B lymphocyte proliferation, relevant cytokines and their receptors, lymphocyte adhesion, and migration pathways.

## References

- Agius, M.A., Zhu, S., Kirvan, C.A., Schafer, A.L., Lin, M.Y., Fairclough, R.H., et al., 1998. Rapsyn antibodies in myasthenia gravis. *Ann N. Y. Acad. Sci.* 841, 516–521.
- Ahlberg, R., Yi, Q., Pirskaanen, R., Matell, G., Swerup, C., Rieber, E.P., et al., 1994. Treatment of myasthenia gravis with anti-CD4 antibody: improvement correlates to decreased T-cell autoreactivity. *Neurology* 44, 1732–1737.
- Amdahl, C., Alseth, E.H., Gilhus, N.E., Nakkestad, H.L., Skeie, G.O., 2007. Polygenic disease associations in thymomatous myasthenia gravis. *Arch. Neurol.* 64, 1729–1733.
- Andersen, J.B., Heldal, A.T., Engeland, A., Gilhus, N.E., 2014. Myasthenia gravis epidemiology in a national cohort; combining multiple disease registries. *Acta Neurol. Scand. Suppl.* 26–31.
- Aricha, R., Feferman, T., Fuchs, S., Souroujon, M.C., 2008. Ex vivo generated regulatory T cells modulate experimental autoimmune myasthenia gravis. *J. Immunol.* 180, 2132–2139.
- Balandina, A., Lecart, S., Darteville, P., Saoudi, A., Berrih-Aknin, S., 2005. Functional defect of regulatory CD4(+)CD25+ T cells in the thymus of patients with autoimmune myasthenia gravis. *Blood* 105, 735–741.
- Balasa, B., Deng, C., Lee, J., Bradley, L.M., Dalton, D.K., Christadoss, P., et al., 1997. Interferon gamma (IFN-gamma) is necessary for the genesis of acetylcholine receptor-induced clinical experimental autoimmune myasthenia gravis in mice. *J. Exp. Med.* 186, 385–391.
- Balasa, B., Deng, C., Lee, J., Christadoss, P., Sarvetnick, N., 1998. The Th2 cytokine IL-4 is not required for the progression of antibody-dependent autoimmune myasthenia gravis. *J. Immunol.* 161, 2856–2862.
- Barnes, P.R., Kanabar, D.J., Brueton, L., Newsom-Davis, J., Huson, S.M., Mann, N.P., et al., 1995. Recurrent congenital arthrogryposis leading to a diagnosis of myasthenia gravis in an initially asymptomatic mother. *Neuromuscul. Disord.* 5, 59–65.
- Bartoccioni, E., Scuderi, F., Augugliaro, A., Chiatamone Ranieri, S., Sauchelli, D., Alboino, P., et al., 2009. HLA class II allele analysis in MuSK-positive myasthenia gravis suggests a role for DQ5. *Neurology* 72, 195–197.
- Beeson, D., Jacobson, L., Newsom-Davis, J., Vincent, A., 1996. A transfected human muscle cell line expressing the adult subtype of the human muscle acetylcholine receptor for diagnostic assays in myasthenia gravis. *Neurology* 47, 1552–1555.
- Behin, A., Mayer, M., Kassis-Makhoul, B., Jugie, M., Espil-Taris, C., Ferrer, X., et al., 2008. Severe neonatal myasthenia due to maternal anti-MuSK antibodies. *Neuromuscul. Disord.* 18, 443–446.
- Berman, P.W., Patrick, J., 1980. Linkage between the frequency of muscular weakness and loci that regulate immune responsiveness in murine experimental myasthenia gravis. *J. Exp. Med.* 152, 507–520.
- Biesecker, G., Gomez, C.M., 1989. Inhibition of acute passive transfer experimental autoimmune myasthenia gravis with Fab antibody to complement C6. *J. Immunol.* 142, 2654–2659.
- Blair, P.A., Norena, L.Y., Flores-Borja, F., Rawlings, D.J., Isenberg, D.A., Ehrenstein, M.R., et al., 2010. CD19(+)CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic lupus erythematosus patients. *Immunity* 32, 129–140.
- Buckley, C., Newsom-Davis, J., Willcox, N., Vincent, A., 2001. Do titin and cytokine antibodies in MG patients predict thymoma or thymoma recurrence? *Neurology* 57, 1579–1582.
- Campbell, D.J., Koch, M.A., 2011. Phenotypical and functional specialization of FOXP3+ regulatory T cells. *Nat. Rev. Immunol.* 11, 119–130.
- Carlsson, B., Wallin, J., Pirskaanen, R., Matell, G., Smith, C.I., 1990. Different HLA DR-DQ associations in subgroups of idiopathic myasthenia gravis. *Immunogenetics* 31, 285–290.
- Carr, A.S., Cardwell, C.R., McCarron, P.O., McConville, J., 2010. A systematic review of population based epidemiological studies in myasthenia gravis. *BMC Neurol.* 10, 46.
- Chang, C., Lee, C., 1963. Isolation of neurotoxin from the venom of *Bungarus multicinctus* and their modes of neuro muscular blocking action. *Arch. Int. Pharmacodyn. Ther.* 144, 241–257.
- Christadoss, P., Poussin, M., Deng, C., 2000. Animal models of myasthenia gravis. *Clin. Immunol.* 94, 75–87.
- Cole, R.N., Reddel, S.W., Gervasio, O.L., Phillips, W.D., 2008. Anti-MuSK patient antibodies disrupt the mouse neuromuscular junction. *Ann. Neurol.* 63, 782–789.
- Compston, D.A., Vincent, A., Newsom-Davis, J., Batchelor, J.R., 1980. Clinical, pathological, HLA antigen and immunological evidence for disease heterogeneity in myasthenia gravis. *Brain* 103, 579–601.

- Conti-Fine, B.M., Navaneetham, D., Karachunski, P.I., Raju, R., Diethylm-Okita, B., Okita, D., et al., 1998. T cell recognition of the acetylcholine receptor in myasthenia gravis. *Ann. N.Y. Acad. Sci.* 841, 283–308.
- Cortes-Vicente, E., Gallardo, E., Martinez, M.A., Diaz-Manera, J., Querol, L., Rojas-Garcia, R., et al., 2016. Clinical characteristics of patients with double-seronegative myasthenia gravis and antibodies to cortactin. *JAMA Neurol.* 73, 1099–1104.
- DeChiara, T.M., Bowen, D.C., Valenzuela, D.M., Simmons, M.V., Poueymirou, W.T., Thomas, S., et al., 1996. The receptor tyrosine kinase MuSK is required for neuromuscular junction formation in vivo. *Cell* 85, 501–512.
- Devic, P., Petiot, P., Simonet, T., Stojkovic, T., Delmont, E., Franques, J., et al., 2014. Antibodies to clustered acetylcholine receptor: expanding the phenotype. *Eur. J. Neurol.* 21, 130–134.
- Diaz-Manera, J., Martinez-Hernandez, E., Querol, L., Klooster, R., Rojas-Garcia, R., Suarez-Calvet, X., et al., 2012. Long-lasting treatment effect of rituximab in MuSK myasthenia. *Neurology* 78, 189–193.
- Dondi, E., Gajdos, P., Bach, J.F., Garchon, H.J., 1994. Association of Km3 allotype with increased serum levels of autoantibodies against muscle acetylcholine receptor in myasthenia gravis. *J. Neuroimmunol.* 51, 221–224.
- Drachman, D.B., Angus, C.W., Adams, R.N., Michelson, J.D., Hoffman, G.J., 1978. Myasthenic antibodies cross-link acetylcholine receptors to accelerate degradation. *N. Engl. J. Med.* 298, 1116–1122.
- Drosos, A.A., Christou, L., Galanopoulou, V., Tzioufas, A.G., Tsakou, E.K., 1993. D-Penicillamine induced myasthenia gravis: clinical, serological and genetic findings. *Clin. Exp. Rheumatol.* 11, 387–391.
- Elmqvist, D., Hofmann, W.W., Kugelberg, J., Quastel, D.M., 1964. An electrophysiological investigation of neuromuscular transmission in myasthenia gravis. *J. Physiol.* 174, 417–434.
- Engel, A.G., Lambert, E.H., Howard, F.M., 1977. Immune complexes (IgG and C3) at the motor end-plate in myasthenia gravis: ultrastructural and light microscopic localization and electrophysiologic correlations. *Mayo Clin. Proc.* 52, 267–280.
- Evoli, A., Tonali, P.A., Padua, L., Monaco, M.L., Scuderi, F., Batocchi, A.P., et al., 2003. Clinical correlates with anti-MuSK antibodies in generalized seronegative myasthenia gravis. *Brain* 126, 2304–2311.
- Evoli, A., Bianchi, M.R., Riso, R., Minicuci, G.M., Batocchi, A.P., Servidei, S., et al., 2008. Response to therapy in myasthenia gravis with anti-MuSK antibodies. *Ann. N.Y. Acad. Sci.* 1132, 76–83.
- Fambrough, D.M., Drachman, D.B., Satyamurti, S., 1973. Neuromuscular junction in myasthenia gravis: decreased acetylcholine receptors. *Science* 182, 293–295.
- Farrugia, M.E., Robson, M.D., Clover, L., Anslow, P., Newsom-Davis, J., Kennett, R., et al., 2006. MRI and clinical studies of facial and bulbar muscle involvement in MuSK antibody-associated myasthenia gravis. *Brain* 129, 1481–1492.
- Fillatreau, S., Gray, D., Anderton, S.M., 2008. Not always the bad guys: B cells as regulators of autoimmune pathology. *Nat. Rev. Immunol.* 8, 391–397.
- Finnis, M.F., Jayawant, S., 2011. Juvenile myasthenia gravis: a paediatric perspective. *Autoimmune Dis.* 2011, 404101.
- Fukunaga, H., Engel, A.G., Lang, B., Newsom-Davis, J., Vincent, A., 1983. Passive transfer of Lambert-Eaton myasthenic syndrome with IgG from man to mouse depletes the presynaptic membrane active zones. *Proc. Natl. Acad. Sci. U. S. A.* 80, 7636–7640.
- Fukuoka, T., Engel, A.G., Lang, B., Newsom-Davis, J., Vincent, A., 1987. Lambert-Eaton myasthenic syndrome: II. Immunoelectron microscopy localization of IgG at the mouse motor end-plate. *Ann. Neurol.* 22, 200–211.
- Gallardo, E., Martinez-Hernandez, E., Titulaer, M.J., Huijbers, M.G., Martinez, M.A., Ramos, A., et al., 2014. Cortactin autoantibodies in myasthenia gravis. *Autoimmun. Rev.* 13, 1003–1007.
- Garchon, H.J., Djabiri, F., Viard, J.P., Gajdos, P., Bach, J.F., 1994. Involvement of human muscle acetylcholine receptor alpha-subunit gene (CHRNA) in susceptibility to myasthenia gravis. *Proc. Natl. Acad. Sci. U. S. A.* 91, 4668–4672.
- Giraud, M., Taubert, R., Vandiedonck, C., Ke, X., Levi-Strauss, M., Pagani, F., et al., 2007. An IRF8-binding promoter variant and AIRE control CHRNA1 promiscuous expression in thymus. *Nature* 448, 934–937.
- Guptill, J.T., Yi, J.S., Sanders, D.B., Guidon, A.C., Juel, V.C., Massey, J.M., et al., 2015. Characterization of B cells in muscle-specific kinase antibody myasthenia gravis. *Neurol. Neuroimmunol. Neuroinflamm.* 2, e77.
- Hassnerian, R.P., Strobel, P., Marx, A., 2005. Pathology of thymic tumors. *Semin. Thorac. Cardiovasc. Surg.* 17, 2–11.
- Heldal, A.T., Eide, G.E., Romi, F., Owe, J.F., Gilhus, N.E., 2014. Repeated acetylcholine receptor antibody-concentrations and association to clinical myasthenia gravis development. *PLoS One* 9, e114060.
- Higuchi, O., Hamuro, J., Motomura, M., Yamanashi, Y., 2011. Autoantibodies to low-density lipoprotein receptor-related protein 4 in myasthenia gravis. *Ann. Neurol.* 69, 418–422.
- Hill, M., Beeson, D., Moss, P., Jacobson, L., Bond, A., Corlett, L., et al., 1999. Early-onset myasthenia gravis: a recurring T-cell epitope in the adult-specific acetylcholine receptor epsilon subunit presented by the susceptibility allele HLA-DR52a. *Ann. Neurol.* 45, 224–231.
- Hjelmstrom, P., Giscombe, R., Lefvert, A.K., Pirskanen, R., Kockum, I., Landin-Olsson, M., et al., 1997. TAP polymorphisms in Swedish myasthenia gravis patients. *Tissue Antigens* 49, 176–179.
- Horiki, T., Inoko, H., Moriuchi, J., Ichikawa, Y., Arimori, S., 1994. Combinations of HLA-DPB1 and HLA-DQB1 alleles determine susceptibility to early-onset myasthenia gravis in Japan. *Autoimmunity* 19, 49–54.
- Howard Jr., J.F., Barohn, R.J., Cutter, G.R., Freimer, M., Juel, V.C., Mozaffar, T., et al., 2013. A randomized, double-blind, placebo-controlled phase II study of eculizumab in patients with refractory generalized myasthenia gravis. *Muscle Nerve* 48, 76–84.
- Huang, Y.M., Pirskanen, R., Giscombe, R., Link, H., Lefvert, A.K., 2004. Circulating CD4 + CD25 + and CD4 + CD25 + T cells in myasthenia gravis and in relation to thymectomy. *Scand. J. Immunol.* 59, 408–414.
- Huda, S., Waters, P., Woodhall, M., Leite, M.I., Jacobson, L., De Rosa, A., et al., 2017. IgG-specific cell-based assay detects potentially pathogenic MuSK-Abs in seronegative MG. *Neurol. Neuroimmunol. Neuroinflamm.* 4, e357.
- Huijbers, M.G., Zhang, W., Klooster, R., Niks, E.H., Friese, M.B., Straasheim, K.R., et al., 2013. MuSK IgG4 autoantibodies cause myasthenia gravis by inhibiting binding between MuSK and Lrp4. *Proc. Natl. Acad. Sci. U. S. A.* 110, 20783–20788.
- Huijbers, M.G., Vink, A.F., Niks, E.H., Westhuis, R.H., van Zwet, E.W., de Meel, R.H., et al., 2016. Longitudinal epitope mapping in MuSK myasthenia gravis: implications for disease severity. *J. Neuroimmunol.* 291, 82–88.

- Iorio, R., Damato, V., Alboini, P.E., Evoli, A., 2015. Efficacy and safety of rituximab for myasthenia gravis: a systematic review and meta-analysis. *J. Neurol.* 262, 1115–1119.
- Ishii, W., Matsuda, M., Hanyuda, M., Momose, M., Nakayama, J., Ehara, T., et al., 2007. Comparison of the histological and immunohistochemical features of the thymus in young- and elderly-onset myasthenia gravis without thymoma. *J. Clin. Neurosci.* 14, 110–115.
- Jacob, S., Viegas, S., Leite, M.I., Webster, R., Cossins, J., Kennett, R., et al., 2012. Presence and pathogenic relevance of antibodies to clustered acetylcholine receptor in ocular and generalized myasthenia gravis. *Arch. Neurol.* 69, 994–1001.
- Jacobson, L., Beeson, D., Tzartos, S., Vincent, A., 1999a. Monoclonal antibodies raised against human acetylcholine receptor bind to all five subunits of the fetal isoform. *J. Neuroimmunol.* 98, 112–120.
- Jacobson, L., Polizzi, A., Morrissey-Kay, G., Vincent, A., 1999b. Plasma from human mothers of fetuses with severe arthrogryposis multiplex congenita causes deformities in mice. *J. Clin. Invest.* 103, 1031–1038.
- Janer, M., Cowland, A., Picard, J., Campbell, D., Pontarotti, P., Newsom-Davis, J., et al., 1999. A susceptibility region for myasthenia gravis extending into the HLA-class I sector telomeric to HLA-C. *Hum. Immunol.* 60, 909–917.
- Johnston, I., Lang, B., Leys, K., Newsom-Davis, J., 1994. Heterogeneity of calcium channel autoantibodies detected using a small-cell lung cancer line derived from a Lambert-Eaton myasthenic syndrome patient. *Neurology* 44, 334–338.
- Kaja, S., van de Ven, R.C., van Dijk, J.G., Verschueren, J.J., Arahata, K., Frants, R.R., et al., 2007. Severely impaired neuromuscular synaptic transmission causes muscle weakness in the Cacna1a-mutant mouse rolling Nagoya. *Eur. J. Neurosci.* 25, 2009–2020.
- Karachunski, P.I., Ostlie, N.S., Okita, D.K., Conti-Fine, B.M., 1999. Interleukin-4 deficiency facilitates development of experimental myasthenia gravis and precludes its prevention by nasal administration of CD4+ epitope sequences of the acetylcholine receptor. *J. Neuroimmunol.* 95, 73–84.
- Karachunski, P.I., Ostlie, N.S., Monfardini, C., Conti-Fine, B.M., 2000. Absence of IFN-gamma or IL-12 has different effects on experimental myasthenia gravis in C57BL/6 mice. *J. Immunol.* 164, 5236–5244.
- Karim, M.R., Zhang, H.Y., Yuan, J., Sun, Q., Wang, Y.F., 2017. Regulatory B cells in seropositive myasthenia gravis versus healthy controls. *Front. Neurol.* 8, 43.
- Kaul, R., Shenoy, M., Goluszko, E., Christadoss, P., 1994. Major histocompatibility complex class II gene disruption prevents experimental autoimmune myasthenia gravis. *J. Immunol.* 152, 3152–3157.
- Keijzers, M., de Baets, M., Hochstenbag, M., Abdul-Hamid, M., Zur Hausen, A., van der Linden, M., et al., 2015. Robotic thymectomy in patients with myasthenia gravis: neurological and surgical outcomes. *Eur. J. Cardiothorac. Surg.* 48, 40–45.
- Kirchner, T., Schalke, B., Melms, A., von Kugelgen, T., Muller-Hermelink, H.K., 1986. Immunohistological patterns of non-neoplastic changes in the thymus in myasthenia gravis. *Virchows Arch. B Cell Pathol. Incl. Mol. Pathol.* 52, 237–257.
- Klooster, R., Plomp, J.J., Huijbers, M.G., Niks, E.H., Straasheijm, K.R., Detmers, F.J., et al., 2012. Muscle-specific kinase myasthenia gravis IgG4 autoantibodies cause severe neuromuscular junction dysfunction in mice. *Brain* 135, 1081–1101.
- Kohler, S., Keil, T.O.P., Hoffmann, S., Swierzy, M., Ismail, M., Ruckert, J.C., et al., 2017. CD4(+) FoxP3(+) T regulatory cell subsets in myasthenia gravis patients. *Clin. Immunol.* 179, 40–46.
- Komai, K., Iwasa, K., Takamori, M., 1999. Calcium channel peptide can cause an autoimmune-mediated model of Lambert-Eaton myasthenic syndrome in rats. *J. Neurol. Sci.* 166, 126–130.
- Koneczny, I., Cossins, J., Waters, P., Beeson, D., Vincent, A., 2013. MuSK myasthenia gravis IgG4 disrupts the interaction of LRP4 with MuSK but both IgG4 and IgG1-3 can disperse preformed agrin-independent AChR clusters. *PLoS One* 8, e80695.
- Koneczny, I., Stevens, J.A., De Rosa, A., Huda, S., Huijbers, M.G., Saxena, A., et al., 2017. IgG4 autoantibodies against muscle-specific kinase undergo Fab-arm exchange in myasthenia gravis patients. *J. Autoimmun.* 77, 104–115.
- Kong, X.C., Barzaghi, P., Ruegg, M.A., 2004. Inhibition of synapse assembly in mammalian muscle in vivo by RNA interference. *EMBO Rep.* 5, 183–188.
- Krco, C.J., David, C.S., Lennon, V.A., 1991. Mouse T lymphocyte response to acetylcholine receptor determined by T cell receptor for antigen V beta gene products recognizing Mls-1a. *J. Immunol.* 147, 3303–3305.
- Lang, B., Newsom-Davis, J., Prior, C., Wray, D., 1983. Antibodies to motor nerve terminals: an electrophysiological study of a human myasthenic syndrome transferred to mouse. *J. Physiol.* 344, 335–345.
- Lebrun, C., Bourg, V., Bresch, S., Cohen, M., Rosenthal-Allieri, M.A., Desnuelle, C., et al., 2016. Therapeutic target of memory B cells depletion helps to tailor administration frequency of rituximab in myasthenia gravis. *J. Neuroimmunol.* 298, 79–81.
- Lecky, B.R., 2006. Transient neonatal Lambert-Eaton syndrome. *J. Neurol. Neurosurg. Psychiatry* 77, 1094.
- Lee, J.Y., Stathopoulos, P., Gupta, S., Bannock, J.M., Barohn, R.J., Cotzomi, E., et al., 2016. Compromised fidelity of B-cell tolerance checkpoints in AChR and MuSK myasthenia gravis. *Ann. Clin. Transl. Neurol.* 3, 443–454.
- Leite, M.I., Strobel, P., Jones, M., Micklem, K., Moritz, R., Gold, R., et al., 2005. Fewer thymic changes in MuSK antibody-positive than in MuSK antibody-negative MG. *Ann. Neurol.* 57, 444–448.
- Leite, M.I., Jones, M., Strobel, P., Marx, A., Gold, R., Niks, E., et al., 2007. Myasthenia gravis thymus: complement vulnerability of epithelial and myoid cells, complement attack on them, and correlations with autoantibody status. *Am. J. Pathol.* 171, 893–905.
- Leite, M.I., Jacob, S., Viegas, S., Cossins, J., Clover, L., Morgan, B.P., et al., 2008. IgG1 antibodies to acetylcholine receptors in 'seronegative' myasthenia gravis. *Brain* 131, 1940–1952.
- Leite, M.I., Waters, P., Vincent, A., 2010. Diagnostic use of autoantibodies in myasthenia gravis. *Autoimmunity* 43, 371–379.
- Lennon, V.A., Seybold, M.E., Lindstrom, J.M., Cochrane, C., Ulevitch, R., 1978. Role of complement in the pathogenesis of experimental autoimmune myasthenia gravis. *J. Exp. Med.* 147, 973–983.
- Li, X., Xiao, B.G., Xi, J.Y., Lu, C.Z., Lu, J.H., 2008. Decrease of CD4(+)CD25(high)Foxp3(+) regulatory T cells and elevation of CD19(+)BAFF-R (+) B cells and soluble ICAM-1 in myasthenia gravis. *Clin. Immunol.* 126, 180–188.
- Li, X., Zhong, H., Bao, W., Boulad, N., Evangelista, J., Haider, M.A., et al., 2012. Defective regulatory B-cell compartment in patients with immune thrombocytopenia. *Blood* 120, 3318–3325.
- Lindstrom, J.M., 2000. Acetylcholine receptors and myasthenia. *Muscle Nerve* 23, 453–477.

- Lindstrom, J., Einarson, B., 1979. Antigenic modulation and receptor loss in experimental autoimmune myasthenia gravis. *Muscle Nerve* 2, 173–179.
- Lindstrom, J.M., Seybold, M.E., Lennon, V.A., Whittingham, S., Duane, D.D., 1976. Antibody to acetylcholine receptor in myasthenia gravis. Prevalence, clinical correlates, and diagnostic value. *Neurology* 26, 1054–1059.
- Long, S.A., Buckner, J.H., 2011. CD4 + FOXP3 + T regulatory cells in human autoimmunity: more than a numbers game. *J. Immunol.* 187, 2061–2066.
- Maddison, P., McConville, J., Farrugia, M.E., Davies, N., Rose, M., Norwood, F., et al., 2011. The use of rituximab in myasthenia gravis and Lambert-Eaton myasthenic syndrome. *J. Neurol. Neurosurg. Psychiatry* 82, 671–673.
- Maggi, L., Andreetta, F., Antozzi, C., Confalonieri, P., Cornelio, F., Scaioli, V., et al., 2008. Two cases of thymoma-associated myasthenia gravis without antibodies to the acetylcholine receptor. *Neuromuscul. Disord.* 18, 678–680.
- Marino, M., Scuderi, F., Samengo, D., Saltelli, G., Maiuri, M.T., Shen, C., et al., 2015. Flow cytofluorimetric analysis of anti-LRP4 (LDL receptor-related protein 4) autoantibodies in Italian patients with myasthenia gravis. *PLoS One* 10, e0135378.
- Marx, A., Willcox, N., Leite, M.I., Chuang, W.Y., Schalke, B., Nix, W., et al., 2010. Thymoma and paraneoplastic myasthenia gravis. *Autoimmunity* 43, 413–427.
- Matthews, I., Chen, S., Hewer, R., McGrath, V., Furmaniak, J., Rees Smith, B., 2004. Muscle-specific receptor tyrosine kinase autoantibodies—a new immunoprecipitation assay. *Clin. Chim. Acta* 348, 95–99.
- McConville, J., Farrugia, M.E., Beeson, D., Kishore, U., Metcalfe, R., Newsom-Davis, J., et al., 2004. Detection and characterization of MuSK antibodies in seronegative myasthenia gravis. *Ann. Neurol.* 55, 580–584.
- Meager, A., Wadhwa, M., Dilger, P., Bird, C., Thorpe, R., Newsom-Davis, J., et al., 2003. Anti-cytokine autoantibodies in autoimmunity: preponderance of neutralizing autoantibodies against interferon-alpha, interferon-omega and interleukin-12 in patients with thymoma and/or myasthenia gravis. *Clin. Exp. Immunol.* 132, 128–136.
- Morgan, B.P., Chamberlain-Banoub, J., Neal, J.W., Song, W., Mizuno, M., Harris, C.L., 2006. The membrane attack pathway of complement drives pathology in passively induced experimental autoimmune myasthenia gravis in mice. *Clin. Exp. Immunol.* 146, 294–302.
- Mori, S., Kubo, S., Akiyoshi, T., Yamada, S., Miyazaki, T., Hotta, H., et al., 2012. Antibodies against muscle-specific kinase impair both presynaptic and postsynaptic functions in a murine model of myasthenia gravis. *Am. J. Pathol.* 180, 798–810.
- Motomura, M., Lang, B., Johnston, I., Palace, J., Vincent, A., Newsom-Davis, J., 1997. Incidence of serum anti-P/Q-type and anti-N-type calcium channel autoantibodies in the Lambert-Eaton myasthenic syndrome. *J. Neurol. Sci.* 147, 35–42.
- Mu, L., Sun, B., Kong, Q., Wang, J., Wang, G., Zhang, S., et al., 2009. Disequilibrium of T helper type 1, 2 and 17 cells and regulatory T cells during the development of experimental autoimmune myasthenia gravis. *Immunology* 128, e826–e836.
- Mygland, A., Kuwajima, G., Mikoshiba, K., Tysnes, O.B., Aarli, J.A., Gilhus, N.E., 1995. Thymomas express epitopes shared by the ryanodine receptor. *J. Neuroimmunol.* 62, 79–83.
- Myking, A.O., Skeie, G.O., Varburg, J.E., Andersen, K.S., Gilhus, N.E., Aarli, J.A., 1998. The histomorphology of the thymus in late onset, non-thymoma myasthenia gravis. *Eur. J. Neurol.* 5, 401–405.
- Nagel, A., Engel, A.G., Lang, B., Newsom-Davis, J., Fukuoka, T., 1988. Lambert-Eaton myasthenic syndrome IgG depletes presynaptic membrane active zone particles by antigenic modulation. *Ann. Neurol.* 24, 552–558.
- Nath, A., Kerman, R.H., Novak, I.S., Wolinsky, J.S., 1990. Immune studies in human immunodeficiency virus infection with myasthenia gravis: a case report. *Neurology* 40, 581–583.
- Navaneetham, D., Penn, A.S., Howard Jr., J.F., Conti-Fine, B.M., 1998. TCR-Vbeta usage in the thymus and blood of myasthenia gravis patients. *J. Autoimmun.* 11, 621–633.
- Nenninger, R., Schultz, A., Hoffacker, V., Helmreich, M., Wilisch, A., Vandekerckhove, B., et al., 1998. Abnormal thymocyte development and generation of autoreactive T cells in mixed and cortical thymomas. *Lab. Invest.* 78, 743–753.
- Newsom-Davis, J., Vincent, A., Wilson, S.G., Ward, C.D., Pinching, A.J., Hawkey, C., 1978. Plasmapheresis for myasthenia gravis. *N. Engl. J. Med.* 298, 456–457.
- Newsom-Davis, J., Murray, N., Wray, D., Lang, B., Prior, C., Gwilt, M., et al., 1982. Lambert-Eaton myasthenic syndrome: electrophysiological evidence for a humoral factor. *Muscle Nerve* 5, S17–S20.
- Niks, E.H., Kuks, J.B., Roep, B.O., Haasnoot, G.W., Verduijn, W., Ballieux, B.E., et al., 2006. Strong association of MuSK antibody-positive myasthenia gravis and HLA-DR14-DQ5. *Neurology* 66, 1772–1774.
- Niks, E.H., Verrips, A., Semmekrot, B.A., Prick, M.J., Vincent, A., van Tol, M.J., et al., 2008. A transient neonatal myasthenic syndrome with anti-musk antibodies. *Neurology* 70, 1215–1216.
- Noack, M., Miossec, P., 2014. Th17 and regulatory T cell balance in autoimmune and inflammatory diseases. *Autoimmun. Rev.* 13, 668–677.
- O'Neill, J.H., Murray, N.M., Newsom-Davis, J., 1988. The Lambert-Eaton myasthenic syndrome. A review of 50 cases. *Brain* 111 (Pt 3), 577–596.
- Ong, B., Willcox, N., Wordsworth, P., Beeson, D., Vincent, A., Altmann, D., et al., 1991. Critical role for the Val/Gly86 HLA-DR beta dimorphism in autoantigen presentation to human T cells. Critical role for the Val%2FGly86 HLA-DR beta dimorphism in autoantigen presentation to human T cells. *Proceedings of the National Academy of Sciences of the United States of America*. PNAS 88, 7343–7347.
- Palanichamy, A., Barnard, J., Zheng, B., Owen, T., Quach, T., Wei, C., et al., 2009. Novel human transitional B cell populations revealed by B cell depletion therapy. *J. Immunol.* 182, 5982–5993.
- Pasnoor, M., Wolfe, G.I., Nations, S., Trivedi, J., Barohn, R.J., Herbelin, L., et al., 2010. Clinical findings in MuSK-antibody positive myasthenia gravis: a U.S. experience. *Muscle Nerve* 41, 370–374.
- Patrick, J., Lindstrom, J., 1973. Autoimmune response to acetylcholine receptor. *Science* 180, 871–872.
- Pearse, G., 2006. Normal structure, function and histology of the thymus. *Toxicol. Pathol.* 34, 504–514.
- Peeler, C.E., De Lott, L.B., Nagia, L., Lemos, J., Eggenberger, E.R., Cornblath, W.T., 2015. Clinical utility of acetylcholine receptor antibody testing in ocular myasthenia gravis. *JAMA Neurol.* 72, 1170–1174.

- Pevzner, A., Schoser, B., Peters, K., Cosma, N.C., Karakatsani, A., Schalke, B., et al., 2012. Anti-LRP4 autoantibodies in AChR- and MuSK- antibody-negative myasthenia gravis. *J. Neurol.* 259, 427–435.
- Piddlesden, S.J., Jiang, S., Levin, J.L., Vincent, A., Morgan, B.P., 1996. Soluble complement receptor 1 (sCR1) protects against experimental autoimmune myasthenia gravis. *J. Neuroimmunol.* 71, 173–177.
- Plomp, J.J., Van Kempen, G.T., De Baets, M.B., Graus, Y.M., Kuks, J.B., Molenaar, P.C., 1995. Acetylcholine release in myasthenia gravis: regulation at single end-plate level. *Ann. Neurol.* 37, 627–636.
- Provenzano, C., Ricciardi, R., Scuderi, F., Maiuri, M.T., Maestri, M., La Carpia, F., et al., 2012. PTPN22 and myasthenia gravis: replication in an Italian population and meta-analysis of literature data. *Neuromuscul. Disord.* 22, 131–138.
- Quan, C., Yu, H., Qiao, J., Xiao, B., Zhao, G., Wu, Z., et al., 2013. Impaired regulatory function and enhanced intrathecal activation of B cells in neuromyelitis optica: distinct from multiple sclerosis. *Mult. Scler.* 19, 289–298.
- Rajakulendran, S., Viegas, S., Spillane, J., Howard, R.S., 2012. Clinically biphasic myasthenia gravis with both AChR and MuSK antibodies. *J. Neurol.* 259, 2736–2739.
- Richman, D.P., Nishi, K., Morell, S.W., Chang, J.M., Ferns, M.J., Wollmann, R.L., et al., 2012. Acute severe animal model of anti-muscle-specific kinase myasthenia: combined postsynaptic and presynaptic changes. *Arch. Neurol.* 69, 453–460.
- Robeson, K.R., Kumar, A., Keung, B., DiCapua, D.B., Grodinsky, E., Patwa, H.S., et al., 2017. Durability of the rituximab response in acetylcholine receptor autoantibody-positive myasthenia gravis. *JAMA Neurol.* 74, 60–66.
- Robitaille, R., Adler, E.M., Charlton, M.P., 1990. Strategic location of calcium channels at transmitter release sites of frog neuromuscular synapses. *Neuron* 5, 773–779.
- Rodgaard, A., Nielsen, F.C., Djurup, R., Somnier, F., Gammeltoft, S., 1987. Acetylcholine receptor antibody in myasthenia gravis: predominance of IgG subclasses 1 and 3. *Clin. Exp. Immunol.* 67, 82–88.
- Rodriguez Cruz, P.M., Al-Hajjar, M., Huda, S., Jacobson, L., Woodhall, M., Jayawant, S., et al., 2015. Clinical features and diagnostic usefulness of antibodies to clustered acetylcholine receptors in the diagnosis of seronegative myasthenia gravis. *JAMA Neurol.* 72, 642–649.
- Rose, N.R., Bona, C., 1993. Defining criteria for autoimmune diseases (Witebsky's postulates revisited). *Immunol. Today* 14, 426–430.
- Sabater, L., Titulaer, M., Saiz, A., Verschueren, J., Gure, A.O., Graus, F., 2008. SOX1 antibodies are markers of paraneoplastic Lambert-Eaton myasthenic syndrome. *Neurology* 70, 924–928.
- Safar, D., Aime, C., Cohen-Kaminsky, S., Berrih-Aknin, S., 1991. Antibodies to thymic epithelial cells in myasthenia gravis. *J. Neuroimmunol.* 35, 101–110.
- Sahashi, K., Engel, A.G., Linstrom, J.M., Lambert, E.H., Lennon, V.A., 1978. Ultrastructural localization of immune complexes (IgG and C3) at the end-plate in experimental autoimmune myasthenia gravis. *J. Neuropathol. Exp. Neurol.* 37, 212–223.
- Saka, E., Topcuoglu, M.A., Akkaya, B., Galati, A., Onal, M.Z., Vincent, A., 2005. Thymus changes in anti-MuSK-positive and -negative myasthenia gravis. *Neurology* 65, 782–783. author reply 82–83.
- Sakaguchi, S., Miyara, M., Costantino, C.M., Hafler, D.A., 2010. FOXP3+ regulatory T cells in the human immune system. *Nat. Rev. Immunol.* 10, 490–500.
- Salmon, A.M., Bruand, C., Cardona, A., Changeux, J.P., Berrih-Aknin, S., 1998. An acetylcholine receptor alpha subunit promoter confers intrathymic expression in transgenic mice. Implications for tolerance of a transgenic self-antigen and for autoreactivity in myasthenia gravis. *J. Clin. Invest.* 101, 2340–2350.
- Sanders, D.B., Evoli, A., 2010. Immunosuppressive therapies in myasthenia gravis. *Autoimmunity* 43, 428–435.
- Sanders, D.B., El-Salem, K., Massey, J.M., McConville, J., Vincent, A., 2003. Clinical aspects of MuSK antibody positive seronegative MG. *Neurology* 60, 1978–1980.
- Saulat, B., Maertens, P., Hamilton, W.J., Bassam, B.A., 2007. Anti-musk antibody after thymectomy in a previously seropositive myasthenic child. *Neurology* 69, 803–804.
- Scadding, G.K., Vincent, A., Newsom-Davis, J., Henry, K., 1981. Acetylcholine receptor antibody synthesis by thymic lymphocytes: correlation with thymic histology. *Neurology* 31, 935–943.
- Scarpino, S., Di Napoli, A., Stoppacciaro, A., Antonelli, M., Pilozzi, E., Chiarle, R., et al., 2007. Expression of autoimmune regulator gene (AIRE) and T regulatory cells in human thymomas. *Clin. Exp. Immunol.* 149, 504–512.
- Schuurman, J., Van Ree, R., Perdok, G.J., Van Doorn, H.R., Tan, K.Y., Aalberse, R.C., 1999. Normal human immunoglobulin G4 is bispecific: it has two different antigen-combining sites. *Immunology* 97, 693–698.
- Scott, B.G., Yang, H., Tuzun, E., Dong, C., Flavell, R.A., Christadoss, P., 2004. ICOS is essential for the development of experimental autoimmune myasthenia gravis. *J. Neuroimmunol.* 153, 16–25.
- Shen, C., Lu, Y., Zhang, B., Figueiredo, D., Bean, J., Jung, J., et al., 2013. Antibodies against low-density lipoprotein receptor-related protein 4 induce myasthenia gravis. *J. Clin. Invest.* 123, 5190–5202.
- Shen, P., Roch, T., Lampropoulou, V., O'Connor, R.A., Stervbo, U., Hilgenberg, E., et al., 2014. IL-35-producing B cells are critical regulators of immunity during autoimmune and infectious diseases. *Nature* 507, 366–370.
- Sheng, J.R., Li, L., Ganesh, B.B., Vasu, C., Prabhakar, B.S., Meriggioli, M.N., 2006. Suppression of experimental autoimmune myasthenia gravis by granulocyte-macrophage colony-stimulating factor is associated with an expansion of FoxP3+ regulatory T cells. *J. Immunol.* 177, 5296–5306.
- Sheng, J.R., Rezania, K., Soliven, B., 2016. Impaired regulatory B cells in myasthenia gravis. *J. Neuroimmunol.* 297, 38–45.
- Shi, F.D., He, B., Li, H., Matusevicius, D., Link, H., Ljunggren, H.G., 1998. Differential requirements for CD28 and CD40 ligand in the induction of experimental autoimmune myasthenia gravis. *Eur. J. Immunol.* 28, 3587–3593.
- Shigemoto, K., Kubo, S., Maruyama, N., Hato, N., Yamada, H., Jie, C., et al., 2006. Induction of myasthenia by immunization against muscle-specific kinase. *J. Clin. Invest.* 116, 1016–1024.
- Shiraishi, H., Motomura, M., Yoshimura, T., Fukudome, T., Fukuda, T., Nakao, Y., et al., 2005. Acetylcholine receptors loss and postsynaptic damage in MuSK antibody-positive myasthenia gravis. *Ann. Neurol.* 57, 289–293.
- Simpson, J.A., 1960. Myasthenia gravis: a new hypothesis. *Scot. Med. J.* 5, 419–436.

- Singhal, N., Martin, P.T., 2011. Role of extracellular matrix proteins and their receptors in the development of the vertebrate neuromuscular junction. *Dev. Neurobiol.* 71, 982–1005.
- Skeie, G.O., Freiburg, A., Kolmerer, B., Labeit, S., Aarli, J.A., Appiah-Boadu, S., et al., 1997. Titin transcripts in thymomas. *J. Autoimmun.* 10, 551–557.
- Sommier, F.E., 1994. Exacerbation of myasthenia gravis after removal of thymomas. *Acta Neurol. Scand.* 90, 56–66.
- Strobel, P., Rosenwald, A., Beyersdorf, N., Kerkau, T., Elert, O., Murumagi, A., et al., 2004. Selective loss of regulatory T cells in thymomas. *Ann. Neurol.* 56, 901–904.
- Sun, F., Ladha, S.S., Yang, L., Liu, Q., Shi, S.X., Su, N., et al., 2014. Interleukin-10 producing-B cells and their association with responsiveness to rituximab in myasthenia gravis. *Muscle Nerve* 49, 487–494.
- Takamori, M., Takahashi, M., Yasukawa, Y., Iwasa, K., Nemoto, Y., Suenaga, A., et al., 1995. Antibodies to recombinant synaptotagmin and calcium channel subtypes in Lambert-Eaton myasthenic syndrome. *J. Neurol. Sci.* 133, 95–101.
- Takamori, M., Iwasa, K., Komai, K., 1997. Antibodies to synthetic peptides of the alpha1A subunit of the voltage-gated calcium channel in Lambert-Eaton myasthenic syndrome. *Neurology* 48, 1261–1265.
- Titulaer, M.J., Wirtz, P.W., Kuks, J.B., Schelhaas, H.J., van der Kooi, A.J., Faber, C.G., et al., 2008. The Lambert-Eaton myasthenic syndrome 1988–2008: a clinical picture in 97 patients. *J. Neuroimmunol.* 201–202, 153–158.
- Titulaer, M.J., Klooster, R., Potman, M., Sabater, L., Graus, F., Hegeman, I.M., et al., 2009. SOX antibodies in small-cell lung cancer and Lambert-Eaton myasthenic syndrome: frequency and relation with survival. *J. Clin. Oncol.* 27, 4260–4267.
- Titulaer, M.J., Lang, B., Verschueren, J.J., 2011a. Lambert-Eaton myasthenic syndrome: from clinical characteristics to therapeutic strategies. *Lancet Neurol.* 10, 1098–1107.
- Titulaer, M.J., Maddison, P., Sont, J.K., Wirtz, P.W., Hilton-Jones, D., Klooster, R., et al., 2011b. Clinical Dutch-English Lambert-Eaton Myasthenic syndrome (LEMS) tumor association prediction score accurately predicts small-cell lung cancer in the LEMS. *J. Clin. Oncol.* 29, 902–908.
- Toyka, K.V., Brachman, D.B., Pestronk, A., Kao, I., 1975. Myasthenia gravis: passive transfer from man to mouse. *Science* 190, 397–399.
- Tsonis, A.I., Zisimopoulou, P., Lazaridis, K., Tzartos, J., Matsigkou, E., Zouvelou, V., et al., 2015. MuSK autoantibodies in myasthenia gravis detected by cell based assay—a multinational study. *J. Neuroimmunol.* 284, 10–17.
- Tuzun, E., Scott, B.G., Goluszko, E., Higgs, S., Christadoss, P., 2003. Genetic evidence for involvement of classical complement pathway in induction of experimental autoimmune myasthenia gravis. *J. Immunol.* 171, 3847–3854.
- Tzartos, S.J., Cung, M.T., Demange, P., Loutrari, H., Mamalaki, A., Marraud, M., et al., 1991. The main immunogenic region (MIR) of the nicotinic acetylcholine receptor and the anti-MIR antibodies. *Mol. Neurobiol.* 5, 1–29.
- Tzartos, J.S., Zisimopoulou, P., Rentzos, M., Karandreas, N., Zouvelou, V., Evangelakou, P., et al., 2014. LRP4 antibodies in serum and CSF from amyotrophic lateral sclerosis patients. *Ann. Clin. Transl. Neurol.* 1, 80–87.
- van der Zee, J.S., van Swieten, P., Aalberse, R.C., 1986. Inhibition of complement activation by IgG4 antibodies. *Clin. Exp. Immunol.* 64, 415–422.
- Viegas, S., Jacobson, L., Waters, P., Cossins, J., Jacob, S., Leite, M.I., et al., 2012. Passive and active immunization models of MuSK-Ab positive myasthenia: electrophysiological evidence for pre and postsynaptic defects. *Exp. Neurol.* 234, 506–512.
- Vincent, A., Newsom Davis, J., 1980. Anti-acetylcholine receptor antibodies. *J. Neurol. Neurosurg. Psychiatry* 43, 590–600.
- Vincent, A., Scadding, G.K., Thomas, H.C., Newsom-Davis, J., 1978. In-vitro synthesis of anti-acetylcholine-receptor antibody by thymic lymphocytes in myasthenia gravis. *Lancet* 1, 305–307.
- Vincent, A., Newsom-Davis, J., Newton, P., Beck, N., 1983. Acetylcholine receptor antibody and clinical response to thymectomy in myasthenia gravis. *Neurology* 33, 1276–1282.
- Vincent, A., Whiting, P.J., Schluep, M., Heidenreich, F., Lang, B., Roberts, A., et al., 1987. Antibody heterogeneity and specificity in myasthenia gravis. *Ann. N.Y. Acad. Sci.* 505, 106–120.
- Vincent, A., Palace, J., Hilton-Jones, D., 2001. Myasthenia gravis. *Lancet* 357, 2122–2128.
- Vincent, A., Clover, L., Buckley, C., Grimley Evans, J., Rothwell, P.M., 2003. Evidence of underdiagnosis of myasthenia gravis in older people. *J. Neurol. Neurosurg. Psychiatry* 74, 1105–1108.
- Vincent, A., Leite, M.I., Farrugia, M.E., Jacob, S., Viegas, S., Shiraishi, H., et al., 2008. Myasthenia gravis seronegative for acetylcholine receptor antibodies. *Ann. N.Y. Acad. Sci.* 1132, 84–92.
- Walker, M.B., 1934. Treatment of myasthenia gravis with physostigmine. *Lancet* 1, 1200–1201.
- Wang, Z.Y., Okita, D.K., Howard Jr., J.F., Conti-Fine, B.M., 1998. CD4+ epitope spreading and differential T cell recognition of muscle acetylcholine receptor subunits in myasthenia gravis. *Ann. N.Y. Acad. Sci.* 841, 334–337.
- Wang, Z.Y., Karachunksi, P.I., Howard Jr., J.F., Conti-Fine, B.M., 1999. Myasthenia in SCID mice grafted with myasthenic patient lymphocytes: role of CD4+ and CD8+ cells. *Neurology* 52, 484–497.
- Wang, W., Milani, M., Ostlie, N., Okita, D., Agarwal, R.K., Caspi, R.R., et al., 2007. C57BL/6 mice genetically deficient in IL-12/IL-23 and IFN-gamma are susceptible to experimental autoimmune myasthenia gravis, suggesting a pathogenic role of non-Th1 cells. *J. Immunol.* 178, 7072–7080.
- Wang, X.B., Pirskanen, R., Giscombe, R., Lefvert, A.K., 2008. Two SNPs in the promoter region of the CTLA-4 gene affect binding of transcription factors and are associated with human myasthenia gravis. *J. Intern. Med.* 263, 61–69.
- Wen, Y., Yang, B., Lu, J., Zhang, J., Yang, H., Li, J., 2016. Imbalance of circulating CD4(+)CXCR5(+)FOXP3(+) Tfr-like cells and CD4(+)CXCR5(+)FOXP3(-) Tfh-like cells in myasthenia gravis. *Neurosci Lett* 630, 176–182.
- Whiting, P.J., Vincent, A., Newsom-Davis, J., 1986. Myasthenia gravis: monoclonal antihuman acetylcholine receptor antibodies used to analyze antibody specificities and responses to treatment. *Neurology* 36, 612–617.
- Willcox, N., Leite, M.I., Kadota, Y., Jones, M., Meager, A., Subrahmanyam, P., et al., 2008. Autoimmunizing mechanisms in thymoma and thymus. *Ann. N.Y. Acad. Sci.* 1132, 163–173.
- Winter, O., Dame, C., Jundt, F., Hiepe, F., 2012. Pathogenic long-lived plasma cells and their survival niches in autoimmunity, malignancy, and allergy. *J. Immunol.* 189, 5105–5111.

- Wirtz, P.W., Nijhuis, M.G., Sotodeh, M., Willems, L.N., Brahim, J.J., Putter, H., et al., 2003. The epidemiology of myasthenia gravis, Lambert-Eaton myasthenic syndrome and their associated tumours in the northern part of the province of South Holland. *J. Neurol.* 250, 698–701.
- Wolfe, G.I., Kaminski, H.J., Aban, I.B., Minisman, G., Kuo, H.C., Marx, A., et al., 2016. Randomized trial of thymectomy in myasthenia gravis. *N. Engl. J. Med.* 375, 511–522.
- Wong, S.H., Huda, S., Vincent, A., Plant, G.T., 2014. Ocular myasthenia gravis: controversies and updates. *Curr. Neurol. Neurosci. Rep.* 14, 421.
- Wood, S.J., Slater, C.R., 2001. Safety factor at the neuromuscular junction. *Prog. Neurobiol.* 64, 393–429.
- Yeh, J.H., Chen, W.H., Chiu, H.C., Vincent, A., 2004. Low frequency of MuSK antibody in generalized seronegative myasthenia gravis among Chinese. *Neurology* 62, 2131–2132.
- Yi, J.S., Russo, M.A., Massey, J.M., Juel, V., Hobson-Webb, L.D., Gable, K., et al., 2017. B10 cell frequencies and suppressive capacity in myasthenia gravis are associated with disease severity. *Front. Neurol.* 8, 34.
- Zhang, X., Yang, M., Xu, J., Zhang, M., Lang, B., Wang, W., et al., 2007. Clinical and serological study of myasthenia gravis in HuBei Province, China. *J. Neurol. Neurosurg. Psychiatry* 78, 386–390.
- Zhang, B., Tzartos, J.S., Belimezi, M., Ragheb, S., Bealmeir, B., Lewis, R.A., et al., 2012. Autoantibodies to lipoprotein-related protein 4 in patients with double-seronegative myasthenia gravis. *Arch. Neurol.* 69, 445–451.
- Zhang, B., Shen, C., Bealmeir, B., Ragheb, S., Xiong, W.C., Lewis, R.A., et al., 2014. Autoantibodies to agrin in myasthenia gravis patients. *PLoS One* 9, e91816.
- Zhang, C.J., Gong, Y., Zhu, W., Qi, Y., Yang, C.S., Fu, Y., et al., 2016. Augmentation of circulating follicular helper T cells and their impact on autoreactive B cells in myasthenia gravis. *J. Immunol.* 197, 2610–2617.
- Zisimopoulou, P., Evangelakou, P., Tzartos, J., Lazaridis, K., Zouvelou, V., Mantegazza, R., et al., 2014. A comprehensive analysis of the epidemiology and clinical characteristics of anti-LRP4 in myasthenia gravis. *J. Autoimmun.* 52, 139–145.
- Zivkovic, S.A., Clemens, P.R., Lacomis, D., 2012. Characteristics of late-onset myasthenia gravis. *J. Neurol.* 259, 2167–2171.
- Zoltowska Katarzyna, M., Belyaeva, K., Leite, M., Patrick, W., Vincent, A., Beeson, D., 2015. Collagen Q—a potential target for autoantibodies in myasthenia gravis. *J. Neurol. Sci.* 348, 241–244.

## Ocular Disease

Sapna Gangaputra<sup>1,\*</sup>, Benjamin Chaigne-Delalande<sup>2,\*</sup>,  
Igal Gery<sup>3</sup> and H. Nida Sen<sup>4,5</sup>

<sup>1</sup>Vanderbilt eye Institute, Nashville, TN, United States <sup>2</sup>National Eye Institute, National Institutes of Health, Bethesda, MD, United States <sup>3</sup>Scientist Emeritus Laboratory of Immunology, National Eye Institute, NIH, Bethesda, MD, United States <sup>4</sup>Unit on Clinical and Translational Studies, Uveitis and Ocular Immunology Fellowship Program, National Eye Institute, National Institutes of Health, Bethesda, MD, United States <sup>5</sup>Department of Ophthalmology, The George Washington University, Washington, DC, United States

### O U T L I N E

Historical Background	1035	Autoimmune Features	1042
Clinical Features	1036	Pathogenic Mechanisms	1043
Pathologic Features	1039	Immunological Markers	1044
Epidemiologic Features	1040	Treatment and Outcomes	1044
Genetic Factors	1040	Concluding Remarks and Future Projects	1045
The Unique Immune System of the Eye	1042	References	1046
Animal Models	1042		

### HISTORICAL BACKGROUND

The concept that the eye harbors autoimmune-inducing or uveitogenic materials has been suggested by many since the beginning of the last century. It was the demonstration by Uhlenhuth (1903) of autoantibody production to the lens that pioneered investigation in this area. Several investigators used homogenates from the eye, which appeared to be capable of inducing an intraocular inflammatory response when injected at locations distant from the animal eyes. Noteworthy of mention are Wacker and Lipton (1968) and Faure (1980).

The presence of uveitogenic antigens in the human eye that are capable of inducing disease is a well-established concept, proposed by Elschnig (1910). Since then several antigens capable of inducing ocular inflammatory disease similar to that seen in humans have been isolated. Retinal S-antigen, or arrestin, one of the most potent uveitogenic antigens defined to date, was isolated and its immunologic properties partially characterized by Wacker et al. (1977). It causes an immune-mediated, bilateral inflammatory response in the eye, named "experimental autoimmune uveitis" (EAU) when injected in microgram quantities at a site far from the globe (Pfister et al., 1986). S-antigen and its clinical relevance in uveitis were widely studied in the 1980s by many investigators (Nussenblatt et al., 1980a,b; Gregerson et al., 1981; Forrester and Borthwick, 1983;

\*Co-first authors.

Gregerson and Abrahams 1983; Nussenblatt et al., 1983). Several other uveitogenic antigens have since been identified, including interphotoreceptor retinoid-binding protein (IRBP) (Gery et al., 1986; Hirose et al., 1986), recoverin (Gery et al., 1994), bovine melanin protein (Chan et al., 1994), rhodopsin (Schalken et al., 1989), phosducin (Lee et al., 1990), RPE65 (Ham et al., 2002), and tyrosinase proteins (Yamaki et al., 2000).

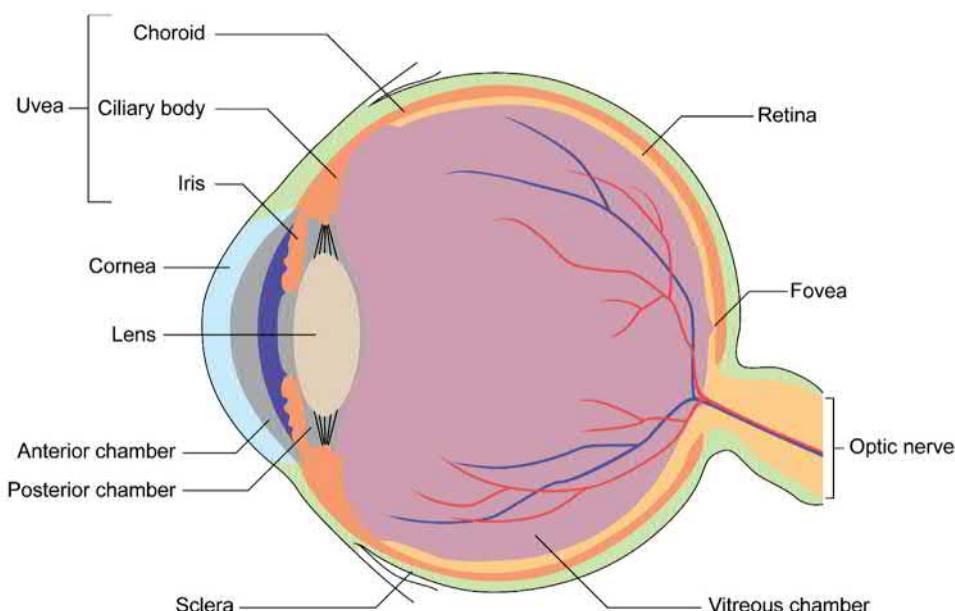
At least some of the mentioned ocular-specific antigens are currently assumed to be the targets for pathogenic processes in patients with autoimmune uveitic conditions. The notion concerning the involvement of autoimmunity in the pathogenesis of certain inflammatory eye diseases received early strong support from data concerning sympathetic ophthalmia. In this rare disease, penetrating injury to one eye is followed by inflammation in the opposite uninjured eye. It is of note that sympathetic ophthalmia had historically provided crucial evidence for the notion concerning pathogenic autoimmunity. This notion was later supported by the findings of cellular and humoral responses to ocular antigens in uveitic patients (Nussenblatt et al., 1980a,b) and by experimental data showing that animals immunized with ocular-specific antigens develop ocular diseases that resemble the human/diseases. These data are discussed in detail below.

## CLINICAL FEATURES

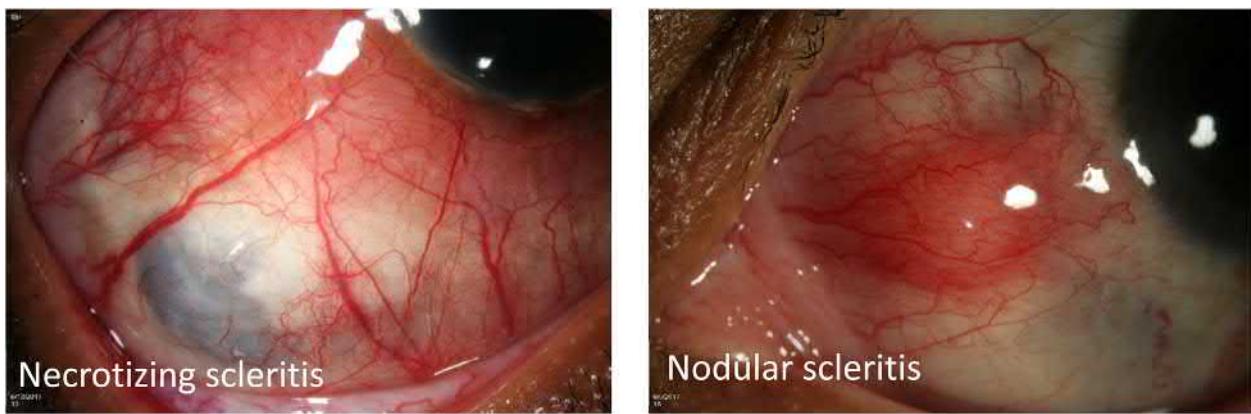
The uveal tract can be divided anatomically into the iris, ciliary body, and choroid (Fig. 54.1). Uveitis is usually defined as any inflammation of the uveal tract. This inflammation may be an antigen-specific, immune-mediated response, or a nonspecific response, which can be elicited by infection, trauma, or surgery. In clinical practice, any intraocular inflammatory reaction involving the structures of the eye (see Fig. 54.1) is collectively referred to as uveitis.

Episcleritis is an inflammatory disease involving the tissue that lies superficial to the sclera. Scleritis is a painful and potentially sight-threatening inflammatory disease involving the sclera. Episcleritis is typically more acute and less severe than scleritis and it is often idiopathic; however, many of the diseases associated with scleritis have also been associated with episcleritis. Clinical examination evaluates for the presence of erythema and edema of the episclera or sclera as well as injection of the deep episcleral vessels (Fig. 54.2). The inflamed sclera is usually characterized by a violaceous hue which can be graded in a standardized fashion (Sen et al., 2011). Many inflammatory systemic disorders are associated with scleritis, the most common being rheumatoid arthritis (RA). Others include juvenile idiopathic arthritis (JIA), reactive arthritis, ankylosing spondylitis, inflammatory bowel disease, polymyositis, polyarteritis nodosa, systemic lupus erythematosus (SLE), granulomatous polyangiitis, sarcoidosis, Lyme disease, and Cogan syndrome. In up to 40% of scleritis, no associated disease can be identified (Sainz de la Maza et al., 2012); however, almost 60% will require oral corticosteroid or immunosuppressive drugs to control the inflammation (Jabs et al., 2000).

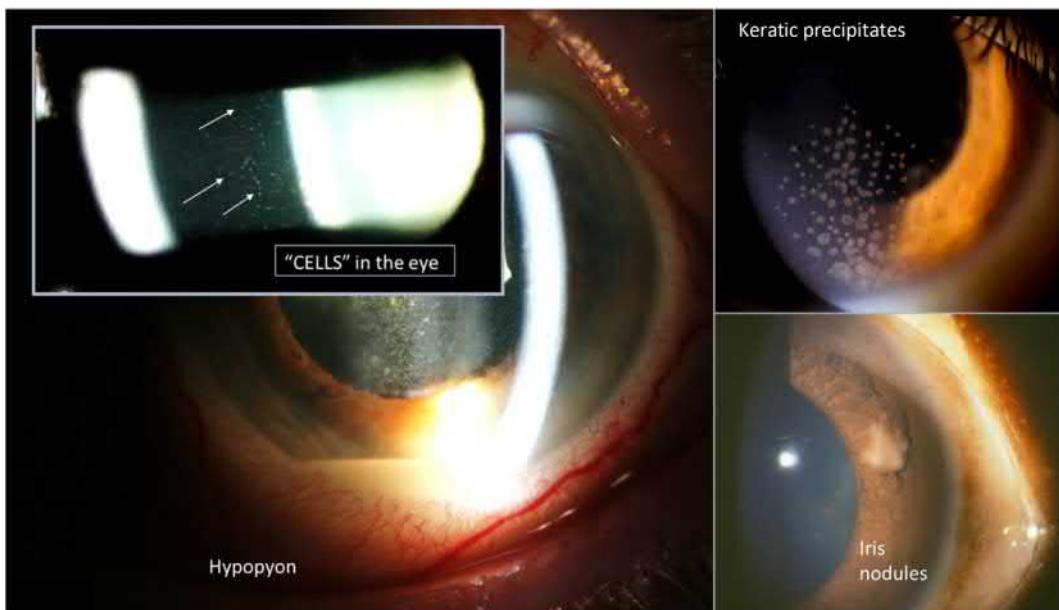
According to the standardization of uveitis nomenclature working group criteria (Jabs et al., 2005), uveitis can be classified based on anatomic location: anterior, intermediate, posterior, and panuveitis. *Anterior uveitis*



**FIGURE 54.1** Key anatomic features of the eye.



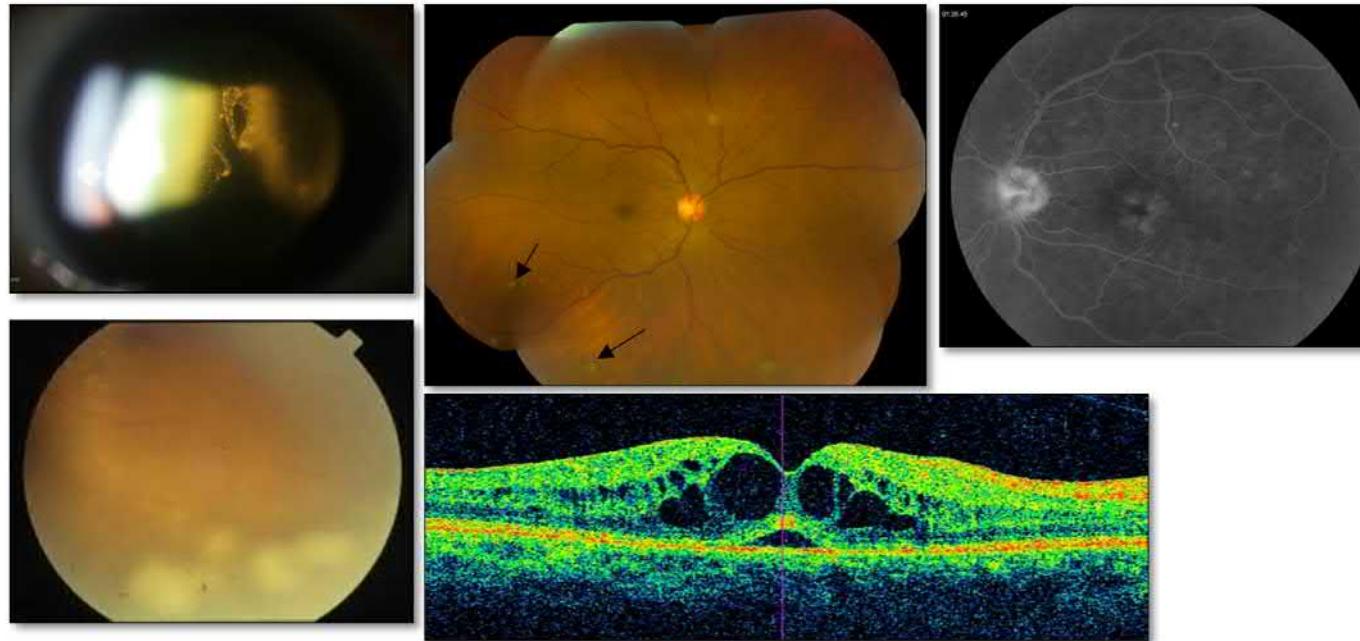
**FIGURE 54.2** Left panel shows necrotizing scleritis with severe thinning of the sclera and visibility of the underlying bluish choroid. Right panel shows a single large nodule on the inner surface of the eye.



**FIGURE 54.3** Various manifestations of inflammation in the anterior chamber of the eye (from top left clockwise)—cells in anterior chamber, “mutton fat” keratic precipitates, iris nodules, and hypopyon.

describes a disease predominantly limited to the anterior segment. There are many inflammatory systemic diseases associated with anterior uveitis, including JIA, human leukocyte antigen (HLA)-B27-associated diseases, Behçet’s disease, and sarcoidosis. The corneal examination may reveal keratic precipitates (Fig. 54.3), small aggregates of inflammatory cells which can be granulomatous or nongranulomatous in nature, which accumulate on the endothelial surface of the cornea. Examination of the anterior chamber via biomicroscopy will reveal the presence of inflammatory cells (Fig. 54.3) and increased protein (flare), resulting from spillover of inflammation from the iris and the ciliary body, graded in a standardized fashion (Nussenblatt and Whitcup, 2004; Jabs et al., 2005). An accumulation of leukocytes associated with fibrin, layered in the lower angle of the anterior chamber, is called a hypopyon (Fig. 54.3) and is commonly associated with Behçet’s disease and the HLA-B27-associated uveitides. Inflammation of the iris may cause synechiae (adhesions) between the iris and the lens capsule or the cornea. It may also develop accumulations of inflammatory cells called nodules on the papillary margin, referred to as Koeppe nodules, or on the iris surface, known as Busacca nodules, commonly seen with sarcoidosis and other granulomatous diseases (Fig. 54.3).

*Intermediate uveitis* refers to uveitis where the vitreous is the major site of inflammation. Inflammation in the vitreous is characterized by increased cells (Fig. 54.4) and protein, vitreous haze (Nussenblatt et al., 1985;



**FIGURE 54.4** Various manifestations of inflammation in the vitreous chamber of the eye (clockwise from top left image)—vitreous cells, collections of cells into “snowballs” in the vitreous cavity, fluorescein angiogram showing dye in a petaloid pattern, cross-sectional optical coherence tomography (OCT) image showing cystic fluid in the fovea “cystoid macular edema,” and hazy view of retina due to large inflammatory snowballs.



**FIGURE 54.5** Various manifestations of inflammation in the retina and choroid (clockwise from top left image)—ocular toxoplasmosis with a new lesion and overlying vitreous inflammation causing “headlight in the fog” sign, Behçet’s disease with inflammation of the retinal blood vessels evidenced by yellow sheathing of blood vessels and hemorrhages; birdshot chorioretinopathy, with yellow choroidal lesions radiating from the optic nerve; viral retinitis, with exudates and hemorrhage and necrosis of the retina due to cytomegalovirus (CMV); serpiginous chorioretinopathy with serpentine lesions spreading outward from the optic disc.

Jabs et al., 2005), or pars plana exudates. Retinal inflammation may cause cystoid macular edema (Fig. 54.4) and retinal vasculitis. There are specific systemic autoimmune diseases associated with this type of uveitis: multiple sclerosis, sarcoidosis, and Lyme disease. A common form of intermediate uveitis is referred to as *pars planitis*, which is by definition idiopathic. Syphilis and tuberculosis are common infectious etiologies of intermediate uveitis.

*Posterior uveitis* refers to inflammation affecting the posterior segment, particularly the retina and the choroid. There are numerous autoimmune diseases that are associated with posterior uveitis: Behçet’s disease (Fig. 54.5), SLE, polyarteritis nodosa, Wegener’s granulomatosis, sarcoidosis, syphilis, and Vogt–Koyanagi–Harada (VKH) syndrome. There are also many infectious etiologies: ocular histoplasmosis, cytomegalovirus retinitis (Fig. 54.5), acute retinal necrosis (varicella zoster virus, herpes simplex virus), toxoplasmosis (Fig. 54.5), and immune-mediated local ocular disorders: sympathetic ophthalmia, birdshot retinochoroidopathy (BCR) (Fig. 54.5), and the “white-dot syndromes,” which can cause posterior uveitis. In addition, primary intraocular lymphoma can masquerade as an intermediate or posterior uveitis.

Finally, *panuveitis* is a term reserved for inflammation involving all segments of the eye in which there is no predominant site of inflammation (Nussenblatt and Whitcup, 2004; Jabs et al., 2005). Typical systemic diseases associated with this form of uveitis are Behçet’s disease, sarcoidosis, VKH syndrome, and syphilis.

## PATHOLOGIC FEATURES

Uveitis can be classified as granulomatous or nongranulomatous. This is a clinical definition, based on the type of inflammatory cells infiltrating the ocular tissues, as seen by biomicroscopy. It is not based on histopathologic analysis. Biomicroscopy will reveal inflammatory precipitates throughout the eye. The most common type of corneal (keratic) precipitates is nongranulomatous, characterized by fine, white-colored lymphocytes, plasma cells, and pigment. Many etiologic factors may be responsible for this type of inflammation. Granulomatous inflammation forms large, greasy-appearing collections of lymphocytes, plasma cells, and giant cells, also called “mutton-fat” keratic precipitates (Fig. 54.3). It can also cause iris nodules, vitreous inflammatory cells, called “snowballs,” (Fig. 54.4) and retinal vascular inflammation, called “candle wax drippings.” This clinical classification is an important diagnostic clue, because the etiologic agents associated with granulomatous uveitis form a fairly short list including sarcoidosis, VKH syndrome, syphilis, tuberculosis, and toxoplasmosis.

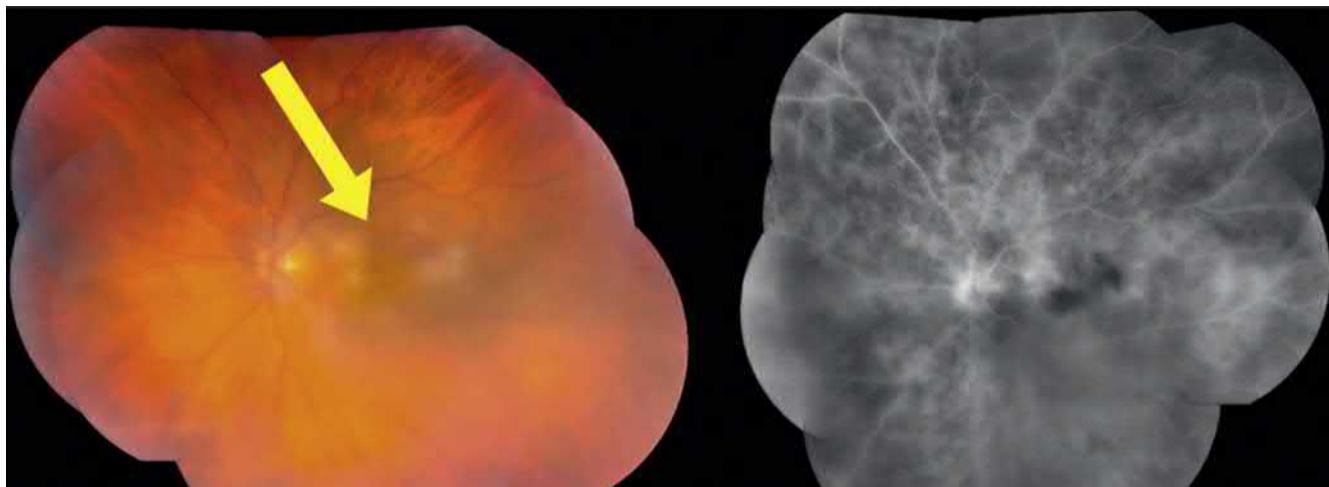
The histopathological changes in eyes of patients with uveitic conditions are described in published reviews (e.g., [Chan and Li, 1998](#); [Boyd et al., 2001](#)). The pathogenic processes responsible for these changes are generally assumed to be similar to those identified in experimental animals, described below.

## EPIDEMIOLOGIC FEATURES

According to a study by [Gritz and Wong \(2004\)](#), the incidence of uveitis was 52/100,000 person-years, and the period prevalence was 115/100,000 persons. It has been found that women have a higher prevalence than men, and the highest incidence and prevalence of disease are in those 65 years and older ([Gritz and Wong 2004](#)), although the age-group with the highest prevalence is still under debate. An insurance claims based dataset estimated that the prevalence of noninfectious uveitis among adults was 121 cases/100,000 persons (95% CI—117.5–124.3), and pediatric uveitis was 29 cases/100,000 among 4 million eligible participants in 2012 ([Thorne et al., 2016](#)). [Rodriguez et al. \(1996\)](#) analyzed their university/referral-based population and found 83% of cases were noninfectious in etiology. There are many demographic features that offer important clues when evaluating a patient with uveitis such as age, sex, race, ethnic heritage, and geographic residence. Specific examples include JIA associated with chronic anterior uveitis seen in children under age of 16. One half of the JIA patients in one study reportedly had chronic uveitis by the age of 6 ([Key and Kimura 1975](#)). It is mostly seen in young females with pauciarticular arthritis and a positive antinuclear antibody test ([Cassidy et al., 1986](#)). The uveitis associated with ankylosing spondylitis is typically a recurrent anterior uveitis seen in young men between the ages of 20 and 30 years ([Brewerton et al., 1973](#)). Sarcoidosis affects young adults aged 20–50 years and has a slightly increased prevalence in women. It can affect all races, but African-Americans are 10 times more likely to be affected compared to white persons, and with chronic sarcoidosis they are more likely to develop ocular manifestations than are white persons ([Jabs and Johns, 1986](#)). VKH syndrome is a multisystem disorder, with ocular, central nervous system, cutaneous, and vestibular-auditory manifestations; the ocular manifestation is usually a severe, bilateral, and granulomatous panuveitis. It is common in Japan and certain parts of Latin America. We have also noted a fairly high Native American ancestry among our patients. Finally, Behcet's disease is a multisystem disorder with ocular involvement; it is especially common in the Far East and the Mediterranean basin ([Ohno and Matsuda, 1986](#)). The ocular disease may be an anterior and/or posterior uveitis, associated with retinal vasculitis ([Fig. 54.6](#)).

## GENETIC FACTORS

Genetic factors play a significant role in the development of endogenous uveitis. Most researchers agree that mechanisms of the autoimmune disease of the eye are multifactorial, so several factors must be present to cause the disease, otherwise disease expression would be far more common. Family studies in patients with uveitis



**FIGURE 54.6** Fundus photograph with corresponding fluorescein angiogram of a patient with Behcet's disease and retinal vasculitis. Arrow points to areas of macular retinitis.

show that the susceptibility to particular types of idiopathic uveitis is possibly due to genetic background (Kimura and Hogan, 1963; Hogan et al., 1965; Giles and Tanton, 1980; Augsburger et al., 1981; Culbertson et al., 1983; DOFT, 1983; Wetzig et al., 1988; Duinkerke-Eerola et al., 1990; Tejada et al., 1994; Lee, 1995). The ability to respond to a specific immune stimulant is genetically determined. Genes for HLA coding for major histocompatibility complex (MHC) proteins are clustered on the short arm of chromosome 6. They have been linked to various uveitic syndromes (Table 54.1). MHC plays an important role in determining disease susceptibility. Genetic predisposition may determine the severity of the disease. A permissive MHC in a nonpermissive background will result in a mild disease or no disease at all (Caspi et al., 1992).

Brewerton et al. (1973) were among the first to observe that a high percentage of white patients with ankylosing spondylitis showed *HLA-B27* positivity. Khan et al. (1977) demonstrated that *HLA-B7* was associated with ankylosing spondylitis in African-Americans to a greater degree than *HLA-B27*. It is clear that HLA associations may be different for various ethnic groups. It is possible that different genes initiate responses that finally lead to a common pathway resulting in a particular disease. The reasons for association between HLA and diseases are unclear.

Among systemic diseases that have associated anterior uveitis, gene association studies show that *HLA-B27*, interleukin (*IL*)-23 receptor, and endoplasmic reticulum aminopeptidase 1 predispose to anterior uveitis and

**TABLE 54.1** HLA Class Association with Autoimmune Diseases That Affect the Eye

HLA	Autoimmune disease
Class I HLA	HLA-A11 Sympathetic ophthalmia
	HLA-A24 Tubulointerstitial nephritis uveitis syndrome
	HLA-A29 Birdshot retinochoroiditis
	HLA-B5 Adamantiades–Behçet’s disease
	HLA-B7 Presumed ocular histoplasmosis Serpiginous chorioretinopathy Acute posterior multifocal placoid epitheliopathy Ankylosing spondylitis
	HLA-B8 Acute anterior uveitis Sarcoidosis
	HLA-B12 Ocular cicatricial pemphigoid
	HLA-B27 Acute anterior uveitis Reiter syndrome
	HLA-B51 Adamantiades–Behçet’s disease
Class II HLA	HLA-DQw3 VKH syndrome
	HLA-DR2 Presumed ocular histoplasmosis
	HLA-DR4 Rheumatoid arthritis VKH syndrome Relapsing polychondritis
	HLA-DR6 Tubulointerstitial nephritis uveitis syndrome
	HLA-DR15 Intermediate uveitis Multiple sclerosis
	HLA-DRB1 Sarcoidosis
	HLA-DRB1*0405 Sympathetic ophthalmia
	HLA-DRw53 VKH syndrome

HLA, Human leukocyte antigens; VKH, Vogt–Koyanagi–Harada.

ankylosing spondylitis (Rosenbaum, 2017). Birdshot retinochoroiditis has a very strong HLA association with HLA-A29, seen on over 95% of cases with odds ratio (OR) 157.5 for the disease when the HLA-A29.02 allele is present (Papadia and Herbort, 2015; Herbort et al., 2017). Behcet's disease has shown a strong association with HLA-B51 with 40%–80% of cases having the allele as compared to 10%–30% controls (Horai et al., 2017), Tubulointerstitial nephritis and uveitis is associated with HLA-DQA1\*01, HLA-DQB1\*05, and HLA-DRB1\*01 (Levinson et al., 2003), and HLA-DRB1\*04/HLA-DR4 carriers have an increased risk of VKH with OR 8.42. The risk increases with ethnicity, with highest risk in Eastern Asians. HLA-DRB1\*0401 has been shown to be protective for VKH (Shi et al., 2014).

## THE UNIQUE IMMUNE SYSTEM OF THE EYE

To defend tissues and cell populations of the eye that are highly susceptible to the pathogenic effects of inflammatory processes, the eye is equipped with multiple layers of defense against these processes. The defense layers provide the eye with a status of "immune privileged organ" and include (1) an efficient blood–retina barrier, as well as relatively poor lymphatic drainage of the intraocular tissues; (2) an immunoinhibitory environment composed of several soluble molecules [e.g., transforming growth factor (TGF)- $\beta$ ,  $\alpha$ -melanocyte stimulating hormone, and thrombospondin], as well as cell-attached molecules (e.g., Fas ligand, PD-1), that inhibit immune cells and processes; (3) an active process named anterior chamber associated immune deviation in which foreign antigens reaching the anterior chamber of the eye initiate a complex immunosuppressive process [for details, see review by Taylor (2016)].

Despite these inhibitory mechanisms, pathogenic autoimmune processes do develop in the eye when T lymphocytes specific to ocular antigens are activated and acquire pathogenic capacity. These processes have been mostly investigated in rodents. T cells are generated in the thymus and cells specific against self-antigens are eliminated in this organ when exposed to their target molecules that are expressed in this organ (Derbinski and Kyewski, 2010). Ocular-specific antigens were found to be expressed in the thymus (Zhang et al., 2003; Takase et al., 2005) and are assumed to eliminate the vast majority of T cell specific to these antigens. Small numbers of these cells do escape the elimination process, however, and enter the lymphocyte pool, in their naïve nonpathogenic state. When stimulated, these cells acquire the capacity to cross the blood–retina barrier and initiate inflammatory processes in the eye (Shi et al., 2013).

## ANIMAL MODELS

Animal models for ocular autoimmune diseases have been critical to collect information on the pathogenic processes involved in uveitic diseases, as well as approaches for suppression of these processes. The major animal model, EAU, induced by immunization with uveitogenic antigens, has been established in several species of mammals including guinea pigs (Collins, 1949; Kalsow and Wacker, 1973), rabbits (Wacker and Lipton, 1968), rats (Faure, 1980; de Kozak et al., 1981; Gery et al., 1986), mice (Caspi et al., 1988), and primates (Nussenblatt et al., 1981). Currently, mice and rats are the two most commonly used animals for EAU studies. Lewis rats provide high susceptibility to EAU induction, whereas mice offer the availability of inbred and other genetically modified strains, such as gene knockout or transgenic animals. Typically, uveitogenic antigens are emulsified with complete Freund's adjuvant and injected subcutaneously into the animals ("active immunization"). Coinjection of pertussis toxin is essential for the EAU induction in most strains of mice. EAU can also be induced by adoptively transferring T cells from animals with active EAU (Mochizuki et al., 1985), or uveitogenic cell lines (Cox et al., 2008; Luger et al., 2008), to syngeneic normal recipients.

## AUTOIMMUNE FEATURES

The two known mechanisms that activate naïve T cells specific to autoantigens are initiated by (1) necrotic tissue and by (2) microbial components. Necrotic tissue triggers immune responses (Kono et al., 2014), and such a process is assumed to play a major role in the pathogenesis of sympathetic ophthalmia, mentioned above. It is assumed that the necrotic tissue activates antigen-presenting cells that present the now exposed ocular-specific antigens to naïve T cells specific to these antigens. The activated T cells are capable of invading the opposite eye and initiating pathogenic process typical to this condition. The notion concerning pathogenic autoimmunity as

the mechanism for sympathetic ophthalmia was further supported by the finding that pathological changes similar to those of the human condition are induced in animals immunized with retinal-specific antigens (Gery et al., 1986; Caspi, 2010). The animal disease, EAU, is dealt with below.

The other mechanism assumed to be responsible for activation of naïve T cells specific to ocular antigens is provided by microbial components that include amino acid sequences which “mimic” the uveitogenic epitopes of ocular-specific antigens. This hypothesis is supported by studies in animals: immunization with such mimicking peptides induces EAU in rats (Singh et al., 1990). A recent study by Horai et al. (2015) has provided new data showing the involvement of gut microbiota in initiation of autoimmune ocular disease. In this study, elimination of the gut microbiota prevented the development of spontaneous EAU in mice with T cells transgenically expressing a T-cell receptor (TCR) against a component of IRBP. Further, naïve CD4<sup>+</sup> T cells specific to the IRBP peptide were stimulated by fecal extracts of these mice (Horai et al., 2015). In addition to providing mimicking peptides, the microbial components and products play an important role in initiation of the autoimmune pathogenic process by their capacity to interact with Toll-like receptors expressed on immune cells that stimulate these cells to initiate disease. Thus naïve T CD4<sup>+</sup> cells, with specificity to an ocular antigen, induce uveitic changes in recipient mice when microbial components, such as lipopolysaccharides, are injected as well (Fujimoto et al., 2006; Shi et al., 2013).

Finally, the notion of microbial involvement in ocular autoimmunity in humans has been supported by anecdotal observations of relationship between uveitic conditions and certain microbial infections (Kijlstra et al., 1986; Kaneko et al., 2008).

## PATHOGENIC MECHANISMS

The use of the EAU mouse model has greatly facilitated the understanding of the pathogenic mechanisms of uveitis. The cellular events were dissected mainly in systems in which the disease is induced by adoptively transferred purified populations of T<sub>H</sub> cells, transgenically expressing TCR specific to the uveitogenic antigen, IRBP (Luger et al., 2008), or to a neo-self-antigen (Cox et al., 2008). Two cell subsets are playing major roles in initiating the pathogenic process of EAU, namely, T<sub>H</sub>1 and T<sub>H</sub>17 cells that express interferon (IFN)- $\gamma$  or IL-17A, respectively. Purified T<sub>H</sub>1 or T<sub>H</sub>17 cells induce EAU when adoptively transferred to naïve recipients (Cox et al., 2008; Luger et al., 2008). Furthermore, both cell subsets are present among the inflammatory cells in eyes with actively induced EAU (Shi et al., 2013). Importantly, similar to experimental autoimmune encephalitis, a model for multiple sclerosis (Panitch and Ciccone, 1981; Ben-Nun et al., 1981), the adoptively transferred T<sub>H</sub> cells induce EAU only following activation in vitro (Vistica, Gery et al., unpublished data). Recent studies showed that in addition to the activation in vitro, the T<sub>H</sub> cells acquire the capacity to initiate EAU only after undergoing a second activation phase (“licensing”) in lymphoid organs of the recipient animals (Tan et al., 2017). These authors also identified cellular molecules apparently involved in changes in the mobility of the pathogenic T<sub>H</sub> cells (Tan et al., 2017). T<sub>H</sub> cells, that initiate EAU, secrete cytokines that recruit into the affected eye other inflammatory cells, including macrophages and polymorphonuclear (PMN) cells (Shi et al., 2013). A study by Foxman et al. (2002) identified a large number of transcripts of cytokines, chemokines, and chemokine receptors whose expression was increased in eyes that developed EAU. The major proinflammatory cytokines identified in EAU are IFN- $\gamma$ , IL-1 $\beta$ , IL-6, IL-12, IL-17, IL-23, and tumor necrosis factor (TNF)- $\alpha$ , as well as the chemokine ligands (CCL) CCL2 (MCP-1), CCL3 (MIP-1 $\alpha$ ), CCL4 (MIP-1 $\beta$ ), CCL5 (RANTES), and CCL20 (MIP-3 $\alpha$ ). It is noteworthy that certain cytokines show dual effect. Thus in addition to their pathogenic activity, IFN- $\gamma$  and IL-27 have immunosuppressive capacity (Tarrant et al., 1999; Aparicio-Siegmund and Garbers, 2015).

In addition to the inflammation-inducing cells, the lymphoid cell populations in the inflamed eye with EAU include T regulatory cells (Tregs) that are capable of inhibiting the pathogenic process of this experimental disease (Lee and Taylor, 2015; Silver et al., 2015). There are several subpopulations of Tregs, with their majority expressing the transcription factor FoxP3, a molecule that is immunoinhibitory and serves as a marker for these cells (Morikawa and Sakaguchi, 2014; Tao et al., 2017). The inhibitory activity of Tregs is multifaceted and includes cell–cell contact with the target cells and secretion of immunosuppressive agents, including IL-10, IL-27, IL-35, and TGF- $\beta$  (Sakaguchi et al., 2008; Noval Rivas and Chatila, 2016). The attractive notion of using Tregs to suppress uveitis in humans has not been materialized yet, mainly because the target antigens in individual patients are not known.

Besides T cells, other immune cells have also been investigated for their roles in the pathogenesis of EAU. Recently, studies unraveled new roles for immunosuppressive B lymphocytes, namely, B regulatory cells (Bregs) in

EAU (Wang et al., 2014; Egwuagu and Yu, 2015). Bregs exert their activity similarly to Tregs (Rincon-Arevalo et al., 2016). In addition, natural killer (NK) cells and NKT cells have also been implied in the pathogenesis of EAU. One report suggested that NK cells might play a detrimental role in EAU (Kitaichi et al., 2002), while another study showed increased NK and NKT cells in association with IFN- $\beta$  amelioration of EAU (Suzuki et al., 2002).

Dendritic cells and macrophages are also important in EAU induction and tissue destruction (Jiang et al., 1999). Damage to the ocular tissues of eyes affected by uveitis is mainly induced by macrophages (type "M1") and PMNs recruited to the affected eye by the T<sub>H</sub> cells that initiate the inflammatory process (see above). The damage to the inflamed eye is mainly the result of phagocytic activity and products of the inflammatory cells, including enzymes, such as proteinases, and oxidizing molecules such as nitric oxide (Hoey et al., 1997).

The sera of patients with uveitis often contain antibodies specific to retinal antigens (Heckenlively and Ferreyra, 2008; Adamus, 2009). It is generally assumed that the antibodies are produced against ocular-specific antigens, released as a result of the tissue destruction, but it is conceivable they exert pathogenic effect that may further aggravate the primary damage.

## IMMUNOLOGICAL MARKERS

Recent advances have demonstrated that cytokines are important in the pathogenesis of noninfectious uveitis. T<sub>H</sub>1 cytokines, such as IFN- $\gamma$ , IL-2, TNF- $\alpha$ , and IL-12, are an essential factor in the pathogenesis of uveitis. IFN- $\gamma$  and IL-2 were found to be elevated in ocular tissues with concomitant infiltrating T cells in uveitic patients (Hooks et al., 1988). IL-6 has been shown to be elevated in the aqueous humor of patients with noninfectious uveitis (Murray et al., 1990; Franks et al., 1992). Several studies showed correlation between elevated cytokines levels and disease activity in various uveitic diseases, however, a consensus is still missing. For example, in acute anterior uveitis (AAU), one study found elevated serum levels of IL-1 $\beta$  in patients compared to healthy controls (Zhao et al., 2015), whereas another study found IL-6 elevated but not IL-1 $\beta$  (Chen et al., 2015). In Behcet's disease, patients with active disease show elevated serum level of IL-8 compared to healthy controls or inactive patients (Akkurt et al., 2015). Higher levels of TNF- $\alpha$  and IL-1 have also been demonstrated in both peripheral sera and aqueous humor samples (Palexas et al., 1992; Sakaguchi et al., 1998). Cytokine profile analysis of peripheral T cells from uveitic patients suggested that the intracellular level of IFN- $\gamma$  was increased (Frassanito et al., 2003), but similar analysis of T cells derived from aqueous humor samples showed decreased IFN- $\gamma$  compared to those from peripheral blood (Hill et al., 2005).

Recent interest has surrounded the role of T<sub>H</sub>17/IL-17, which is believed to play a role in chronic inflammation, whereas T<sub>H</sub>1 drives the early pathogenic response (Hoyer et al., 2009). The elevated levels of T<sub>H</sub>17-associated cytokines (IL-22, IL-23, and IL-17A) have been correlated with disease or disease activity in several uveitic diseases, such as BCR, AAU, VKH, sarcoidosis, and idiopathic uveitis (Jawad et al., 2013; Molins et al., 2016). Moreover, IL-17A levels were even higher in active compared to inactive disease (Jawad et al., 2013).

Data derived from animal EAU studies, however, have provided more consistent insights into the molecular mechanisms of autoimmune uveitis. In EAU, TNF- $\alpha$  is associated with the recruitment of CD11b macrophages and CD4 $^{+}$  T cells into the eye (Nakamura et al., 1994; Dick et al., 1996; Robertson et al., 2003), while IL-10 and IL-12 were suggested to be protective to the development of disease (Rizzo et al., 1998; Tarrant et al., 1999). Consistent with findings in humans, IFN- $\gamma$  was also found in the ocular tissues of EAU animals and correlated with the disease course (Charteris and Lightman, 1992). However, the treatment with anti-IFN- $\gamma$  antibody exacerbated EAU, while the treatment with recombinant IFN- $\gamma$  ameliorated the diseases in a murine EAU model (Caspi et al., 1994), leading investigators to conclude that endogenous IFN- $\gamma$  was protective for EAU. The same investigators also showed that IFN- $\gamma$  gene deficient mice developed EAU similar to their wild-type littermates, suggesting that IFN- $\gamma$  is not necessary for the development of EAU (Jones et al., 1997). To further complicate the issue, an independent study using IFN- $\gamma$  transgenic rats showed that overexpression of IFN- $\gamma$  resulted in more severe EAU (Egwuagu et al., 1999). These studies underscore the complexity of cytokine networks in the pathogenesis of EAU.

## TREATMENT AND OUTCOMES

Corticosteroids have been the mainstay of therapy for ocular inflammatory disease since the early 1950s. Both systemic (oral, intravenous) and local (topical, periocular, and intravitreal) formulations are available. In addition, steroid implants [fluocinolone acetonide (Retisert, Iluvien) and dexamethasone (Ozurdex)] have recently become

popular choices as both adjuvant and monotherapy. They offer the benefit of sustained corticosteroid delivery to the eye while avoiding systemic complications (Larson et al., 2011; Multicenter Uveitis Steroid Treatment Trial Research et al., 2015; Writing Committee for the Multicenter Uveitis Steroid Treatment, Follow-up Study Research et al., 2017).

Some diseases are steroid resistant and for others, long-term steroid therapy carries the risk of developing unacceptable systemic side effects. For these patients, other immunomodulatory agents need to be added as a steroid-sparing agent. Steroid-sparing agents include alkylating agents (cyclophosphamide, chlorambucil), anti-metabolites (azathioprine, methotrexate, mycophenolate mofetil, cyclosporine, tacrolimus, rapamycin), antibodies, and monoclonal antibodies (daclizumab, etanercept, infliximab, adalimumab, rituximab).

Antibodies and monoclonal antibodies directed against various parts of the immune cascade represent the new era of immunomodulation through their ability to interfere with specific molecules or pathways based on our understanding of the inflammatory process (Heiligenhaus et al., 2010). Daclizumab is a humanized anti-CD25 (Tac) monoclonal recombinant antibody. CD25 is a subunit of the IL-2R high-affinity complex. Caspi et al. (1986) demonstrated the presence of high-affinity IL-2R in animal models of uveitis. It was used safely and effectively in the treatment of intermediate and posterior uveitis in adults and children, as well as active JIA-associated anterior uveitis (Yeh et al., 2008; Sen et al., 2009). However, it was pulled from the market in 2009 due to insufficient market demand (Smith et al., 2012).

TNF- $\alpha$  is found during the acute phase of experimental autoimmune uveoretinitis (Kim et al., 2001). Etanercept is a TNF fusion protein that binds to and inactivates TNF, while infliximab is a chimeric monoclonal antibody directed against TNF- $\alpha$ , and adalimumab is fully humanized monoclonal IgG1 antibody against TNF- $\alpha$ . Etanercept was found to be effective in some systemic diseases; however, a review of multiple case series showed new onset of uveitis in previously unaffected rheumatologic patients (Smith et al., 2012). As a result, etanercept is not used in uveitis. Infliximab, demonstrated to be effective in several clinical studies in reducing ocular inflammation, reported successful control of inflammation within 10 weeks of initiating treatment in approximately 75% of patients with refractory uveitides including idiopathic, sarcoidosis, Behcet's disease-associated uveitis, and BCR (Suhler et al., 2009). Adalimumab has also been effective for the treatment of several autoimmune diseases including RA, ankylosing spondylitis, psoriatic arthritis, JIA, and Crohn's disease and is now FDA approved for chronic uveitis in adults and pediatric age-groups (Smith et al., 2012; Jaffe et al., 2016; Nguyen et al., 2016; Ramanan et al., 2017). Rituximab is a chimeric anti-CD20 monoclonal antibody which targets CD20, a surface antigen expressed on pre-B and mature B cells. It has demonstrated success in RA, SLE, and Wegener's-associated scleritis (Smith et al., 2012).

In our treatment approach, cytotoxic agents are often used last, after corticosteroids, cyclosporine, and monoclonal antibodies, for sight-threatening intraocular inflammation. Most uveitis practices follow a stepladder approach, customized to the patient's response to treatment with the goal of maintaining quiescence of inflammation (Foster et al., 2016). Other less commonly used therapeutic modalities include intravenous immunoglobulin therapy, oral tolerance, plasmapheresis, and IFN- $\alpha$ . In addition, nonsteroidal antiinflammatory agents have been used for specific diseases; however, they have not been shown to be effective as a sole agent in the treatment of noninfectious uveitis.

In general, the following therapeutic guidelines can be used:

- Topical corticosteroids for anterior segment disease.
- Periorcular corticosteroids for unilateral disease, the presence of intermediate or posterior complications, and in select uveitic entities to avoid systemic complications of corticosteroids.
- Systemic immunosuppressive agents for bilateral, sight-threatening intermediate uveitis, posterior uveitis, or panuveitis. Biological immunomodulatory therapy for those who fail (or are intolerant of) standard immunosuppressive therapy. Some patients may need more than one immunosuppressive agent at a time.
- Consideration of corticosteroid implants for unilateral disease, contraindication or intolerance to standard agents, or multiple systemic comorbidities which limit the use of systemic immunosuppressive therapy.
- Reserving cytotoxic agents for therapeutic failures.
- For uveitis associated with infectious etiologies, specific agents are indicated, including antivirals and antibacterial or antiparasitic therapy.

## Concluding Remarks and Future Projects

We have learned a great deal since the advent of steroids for uveitis in the early 1950s. Most therapies during the 20th century tended to be monotherapy, and combination therapy only began to be used more frequently

during the 1990s. Concerns about the side effects of steroids, that is, osteoporosis, came to the attention of treating physicians relatively early, as did the goals for therapy, including long-term approaches that reduced secondary effects. Because of this, the use of local therapy, including intraocular therapy, whether by injection or a time-released device, has become more popular. So where will we be going in the 21st century? Some of the possibilities include pharmacogenetics study of variability in drug responses attributed to hereditary factors in different populations; pharmacogenomics determination and analysis of the genome and its products (RNA and proteins) as they relate to drug response; evaluation of patient genomes to identify which patients are the best candidates for a specific therapy; and finally, using cytokines or other biomarkers as a marker for inflammation, as potential therapeutic targets, and to determine the true level of immunological quiescence in chronic uveitis.

## References

- Adamus, G., 2009. Autoantibody targets and their cancer relationship in the pathogenicity of paraneoplastic retinopathy. *Autoimmun. Rev.* 8 (5), 410–414.
- Akkurt, Z.M., Bozkurt, M., Ucmak, D., Yuksei, H., Ucak, H., Sula, B., et al., 2015. Serum cytokine levels in Behcet's disease. *J. Clin. Lab. Anal.* 29 (4), 317–320.
- Aparicio-Siegmund, S., Garbers, C., 2015. The biology of interleukin-27 reveals unique pro- and anti-inflammatory functions in immunity. *Cytokine Growth Factor Rev.* 26 (5), 579–586.
- Augsburger, J.J., Annesley Jr., W.H., Sergott, R.C., Felberg, N.T., Bowman, J.H., Raymond, L.A., 1981. Familial pars planitis. *Ann. Ophthalmol.* 13 (5), 553–557.
- Ben-Nun, A., Wekerle, H., Cohen, I.R., 1981. The rapid isolation of clonal antigen-specific T lymphocyte lines capable of mediating autoimmune encephalomyelitis. *Europ. J. Immunol.* 11 (3), 195–199.
- Boyd, S.R., Young, S., Lightman, S., 2001. Immunopathology of the noninfectious posterior and intermediate uveitides. *Surv. Ophthalmol.* 46 (3), 209–233.
- Brewerton, D., Hart, F., Nicholls, A., et al., 1973. Ankylosing spondylitis and HL-A27. *Lancet* 1, 904.
- Caspi, R.R., 2010. A look at autoimmunity and inflammation in the eye. *J. Clin. Invest.* 120 (9), 3073–3083.
- Caspi, R., Roberge, F., McAllister, C., et al., 1986. T-cell lines mediating experimental autoimmune uveoretinitis (EAU) in the rat. *J. Immunol.* 136, 928–933.
- Caspi, R.R., Roberge, F.G., Chan, C.C., Wiggert, B., Chader, G.J., Rozenszajn, L.A., et al., 1988. A new model of autoimmune disease. Experimental autoimmune uveoretinitis induced in mice with two different retinal antigens. *J. Immunol.* 140 (5), 1490–1495.
- Caspi, R., Grubbs, B., Chan, C., Chader, G., Wiggert, B., 1992. Genetic control of susceptibility to experimental autoimmune uveoretinitis in the mouse model: concomitant regulation by MHC and non-MHC genes. *J. Immunol.* 148, 2384–2389.
- Caspi, R.R., Chan, C.C., Grubbs, B.G., Silver, P.B., Wiggert, B., Parsa, C.F., et al., 1994. Endogenous systemic IFN-gamma has a protective role against ocular autoimmunity in mice. *J. Immunol.* 152 (2), 890–899.
- Cassidy, J.T., Levinson, J.E., Bass, J.C., Baum, J., Brewer Jr., E.J., Fink, C.W., et al., 1986. A study of classification criteria for a diagnosis of juvenile rheumatoid arthritis. *Arthritis Rheum.* 29 (2), 274–281.
- Chan, C.C., Li, Q., 1998. Immunopathology of uveitis. *Br. J. Ophthalmol.* 82 (1), 91–96.
- Chan, C., Hikita, N., Dastgheib, K., Whitcup, S., Gery, I., Nussenblatt, R., 1994. Experimental melanin-protein-induced uveitis in the Lewis rat. Immunopathologic processes. *Ophthalmology* 101, 1275–1280.
- Charteris, D.G., Lightman, S.L., 1992. Interferon-gamma (IFN-gamma) production in vivo in experimental autoimmune uveoretinitis. *Immunology* 75 (3), 463–467.
- Chen, W., Zhao, B., Jiang, R., Zhang, R., Wang, Y., Wu, H., et al., 2015. Cytokine expression profile in aqueous humor and sera of patients with acute anterior uveitis. *Curr. Mol. Med.* 15 (6), 543–549.
- Collins, R.C., 1949. Experimental studies on sympathetic ophthalmia. *Am. J. Ophthalmol.* 32 (12), 1687–1699. illust.
- Cox, C.A., Shi, G., Yin, H., Vistica, B.P., Wawrousek, E.F., Chan, C.C., et al., 2008. Both Th1 and Th17 are immunopathogenic but differ in other key biological activities. *J. Immunol.* 180 (11), 7414–7422.
- Culbertson, W.W., Giles, C.L., West, C., Stafford, T., 1983. Familial pars planitis. *Retina* 3 (3), 179–181.
- de Kozak, Y., Sakai, J., Thillaye, B., Faure, J.P., 1981. S antigen-induced experimental autoimmune uveo-retinitis in rats. *Curr. Eye Res.* 1 (6), 327–337.
- Derbinski, J., Kyewski, B., 2010. How thymic antigen presenting cells sample the body's self-antigens. *Curr. Opin. Immunol.* 22 (5), 592–600.
- Dick, A.D., McMenamin, P.G., Korner, H., Scallon, B.J., Ghrayeb, J., Forrester, J.V., et al., 1996. Inhibition of tumor necrosis factor activity minimizes target organ damage in experimental autoimmune uveoretinitis despite quantitatively normal activated T cell traffic to the retina. *Eur. J. Immunol.* 26 (5), 1018–1025.
- Doft, B.H., 1983. Pars planitis in identical twins. *Retina* 3 (1), 32–33.
- Duinkerke-Eerola, K.U., Pinckers, A., Cruysberg, J.R., 1990. Pars planitis in father and son. *Ophthalmic Paediatr. Genet.* 11 (4), 305–308.
- Egwuagu, C.E., Yu, C.R., 2015. Interleukin 35-producing B cells (i35-Breg): a new mediator of regulatory B-cell functions in CNS autoimmune diseases. *Crit. Rev. Immunol.* 35 (1), 49–57.
- Egwuagu, C.E., Sztein, J., Mahdi, R.M., Li, W., Chao-Chan, C., Smith, J.A., et al., 1999. IFN-gamma increases the severity and accelerates the onset of experimental autoimmune uveitis in transgenic rats. *J. Immunol.* 162 (1), 510–517.
- Elschnig, A., 1910. Studien zur sympathischen ophthalmis. Die antigen wirkung des augenpigmentes. *Albrecht von Graefes Arch. Ophthalmol.* 76, 509–546.
- Faure, J., 1980. Autoimmunity and the retina. *Curr. Top. Eye Res.* (2), 215–302.

- Forrester, J.V., Borthwick, G.M., 1983. Clinical relevance of S-antigen induced experimental uveoretinitis. *Trans. Ophthalmol. Soc. U. K.* 103 (Pt 5), 497–502.
- Foster, C.S., Kothari, S., Anesi, S.D., Vitale, A.T., Chu, D., Metzinger, J.L., et al., 2016. The Ocular Immunology and Uveitis Foundation preferred practice patterns of uveitis management. *Surv. Ophthalmol.* 61 (1), 1–17.
- Foxman, E.F., Zhang, M., Hurst, S.D., Muchamuel, T., Shen, D., Wawrousek, E.F., et al., 2002. Inflammatory mediators in uveitis: differential induction of cytokines and chemokines in Th1- versus Th2-mediated ocular inflammation. *J. Immunol.* 168, 2483–2492.
- Franks, W.A., Limb, G.A., Stanford, M.R., Ogilvie, J., Wolstencroft, R.A., Chignell, A.H., et al., 1992. Cytokines in human intraocular inflammation. *Curr. Eye Res.* 11, 187–191.
- Frassanito, M.A., Dammacco, R., Fusaro, T., Cusmai, A., Guerriero, S., Sborgia, C., 2003. Combined cyclosporin-A/prednisone therapy of patients with active uveitis suppresses IFN-gamma production and the function of dendritic cells. *Clin. Exp. Immunol.* 133 (2), 233–239.
- Fujimoto, C., Yu, C.R., Shi, G., Vistica, B.P., Wawrousek, E.F., Klinman, D.M., et al., 2006. Pertussis toxin is superior to TLR ligands in enhancing pathogenic autoimmunity, targeted at a neo-self antigen, by triggering robust expansion of Th1 cells and their cytokine production. *J. Immunol.* 177 (10), 6896–6903.
- Gery, I., Mochizuki, M., Nussenblatt, R., 1986. Retinal specific antigens and immunopathogenic processes they provoke. In: Osborne, N., Chader, J. (Eds.), *Progress in Retinal Research*, 5. Pergamon Press, Oxford, pp. 75–109.
- Gery, I., Chanaud 3rd, N.P., Anglade, E., 1994. Recoverin is highly uveitogenic in Lewis rats. *Invest. Ophthalmol. Vis. Sci.* 35 (8), 3342–3345.
- Giles, C.L., Tanton, J.H., 1980. Peripheral uveitis in three children of one family. *J. Pediatr. Ophthalmol. Strabismus* 17 (5), 297–299.
- Gregerson, D.S., Abrahams, I.W., 1983. Immunologic and biochemical properties of several retinal proteins bound by antibodies in sera from animals with experimental autoimmune uveitis and uveitis patients. *J. Immunol.* 131 (1), 259–264.
- Gregerson, D.S., Abrahams, I.W., Thirkill, C.E., 1981. Serum antibody levels of uveitis patients to bovine retinal antigens. *Invest. Ophthalmol. Vis. Sci.* 21 (5), 669–680.
- Gritz, D.C., Wong, I.G., 2004. Incidence and prevalence of uveitis in Northern California; the Northern California Epidemiology of Uveitis Study. *Ophthalmology* 111 (3), 491–500. discussion 500.
- Ham, D.I., Gentleman, S., Chan, C.C., McDowell, J.H., Redmond, T.M., Gery, I., 2002. RPE65 is highly uveitogenic in rats. *Invest. Ophthalmol. Vis. Sci.* 43, 2258–2263.
- Heckenlively, J.R., Ferreyra, H.A., 2008. Autoimmune retinopathy: a review and summary. *Semin. Immunopathol.* 30 (2), 127–134.
- Heiligenhaus, A., Thurau, S., Hennig, M., Grajewski, R.S., Wildner, G., 2010. Anti-inflammatory treatment of uveitis with biologicals: new treatment options that reflect pathogenetic knowledge of the disease. *Graefes Arch. Clin. Exp. Ophthalmol.* 248 (11), 1531–1551.
- Herbort Jr., C.P., Pavesio, C., LeHoang, P., Bodaghi, B., Fardeau, C., Kestelyn, P., et al., 2017. Why birdshot retinochoroiditis should rather be called 'HLA-A29 uveitis'? *Br. J. Ophthalmol.* 101 (7), 851–858.
- Hill, T., Galatowicz, G., Akerele, T., Lau, C.H., Calder, V., Lightman, S., 2005. Intracellular T lymphocyte cytokine profiles in the aqueous humor of patients with uveitis and correlation with clinical phenotype. *Clin. Exp. Immunol.* 139 (1), 132–137.
- Hirose, S., Kuwabara, T., Nussenblatt, R.B., Wiggert, B., Redmond, T.M., Gery, I., 1986. Uveitis induced in primates by interphotoreceptor retinoid-binding protein. *Arch. Ophthalmol.* 104 (11), 1698–1702.
- Hoey, S., Grabowski, P.S., Ralston, S.H., Forrester, J.V., Liversidge, J., 1997. Nitric oxide accelerates the onset and increases the severity of experimental autoimmune uveoretinitis through an IFN-gamma-dependent mechanism. *J. Immunol.* 159 (10), 5132–5142.
- Hogan, M.J., Kimura, S.J., O'Connor, G.R., 1965. Peripheral retinitis and chronic cyclitis in children. *Trans. Ophthalmol. Soc. U. K.* 85, 39–52.
- Hooks, J.J., Chan, C.C., Detrick, B., 1988. Identification of the lymphokines, interferon-gamma and interleukin-2, in inflammatory eye diseases. *Invest. Ophthalmol. Vis. Sci.* 29 (9), 1444–1451.
- Horai, R., Zarate-Blades, C.R., Dillenburg-Pilla, P., Chen, J., Kielczewski, J.L., Silver, P.B., et al., 2015. Microbiota-dependent activation of an autoreactive T cell receptor provokes autoimmunity in an immunologically privileged site. *Immunity* 43 (2), 343–353.
- Horai, R., Sen, H.N., Caspi, R.R., 2017. Commensal microbiota as a potential trigger of autoimmune uveitis. *Expert Rev. Clin. Immunol.* 13 (4), 291–293.
- Hoyer, K.K., Kuswanto, W.F., Gallo, E., Abbas, A.K., 2009. Distinct roles of helper T-cell subsets in a systemic autoimmune disease. *Blood* 113 (2), 389–395.
- Jabs, D.A., Johns, C.J., 1986. Ocular involvement in chronic sarcoidosis. *Am. J. Ophthalmol.* 102 (3), 297–301.
- Jabs, D.A., Mudun, A., Dunn, J.P., Marsh, M.J., 2000. Episcleritis and scleritis: clinical features and treatment results. *Am. J. Ophthalmol.* 130 (4), 469–476.
- Jabs, D.A., Nussenblatt, R.B., Rosenbaum, J.T., G. Standardization of Uveitis Nomenclature Working, 2005. Standardization of uveitis nomenclature for reporting clinical data. Results of the First International Workshop. *Am. J. Ophthalmol.* 140 (3), 509–516.
- Jaffe, G.J., Dick, A.D., Brezin, A.P., Nguyen, Q.D., Thorne, J.E., Kestelyn, P., et al., 2016. Adalimumab in patients with active noninfectious uveitis. *N. Engl. J. Med.* 375 (10), 932–943.
- Jawad, S., Liu, B., Agron, E., Nussenblatt, R.B., Sen, H.N., 2013. Elevated serum levels of interleukin-17A in uveitis patients. *Ocul. Immunol. Inflamm.* 21 (6), 434–439.
- Jiang, H.R., Lumsden, L., Forrester, J.V., 1999. Macrophages and dendritic cells in IRBP-induced experimental autoimmune uveoretinitis in B10RIII mice. *Invest. Ophthalmol. Vis. Sci.* 40 (13), 3177–3185.
- Jones, L.S., Rizzo, L.V., Agarwal, R.K., Tarrant, T.K., Chan, C.C., Wiggert, B., et al., 1997. IFN-gamma-deficient mice develop experimental autoimmune uveitis in the context of a deviant effector response. *J. Immunol.* 158 (12), 5997–6005.
- Kalsow, C.M., Wacker, W.B., 1973. Localization of a uveitogenic soluble retinal antigen in the normal guinea pig eye by an indirect fluorescent antibody technique. *Int. Arch. Allergy Appl. Immunol.* 44 (1), 11–20.
- Kaneko, F., Oyama, N., Yanagihori, H., Isogai, E., Yokota, K., Oguma, K., 2008. The role of streptococcal hypersensitivity in the pathogenesis of Behcet's Disease. *Eur. J. Dermatol.* 18 (5), 489–498.
- Key 3rd, S.N., Kimura, S.J., 1975. Iridocyclitis associated with juvenile rheumatoid arthritis. *Am. J. Ophthalmol.* 80 (3 Pt 1), 425–429.
- Khan, M.A., Kushner, I., Braun, W.E., Schacter, B.Z., Steinberg, A.G., 1977. HLA-B7 and ankylosing spondylitis in American blacks. *N. Engl. J. Med.* 297 (9), 513.

- Kijlstra, A., Luyendijk, L., van der Gaag, R., van Kregten, E., Linssen, A., Willers, J.M., 1986. IgG and IgA immune response against Klebsiella in HLA-B27-associated anterior uveitis. *Br. J. Ophthalmol.* 70 (2), 85–88.
- Kim, H.S., Yoon, S.K., Joo, C.K., 2001. The expression of multiple cytokines and inducible nitric oxide synthase in experimental melanin-protein-induced uveitis. *Ophthalmic Res.* 33 (6), 329–335.
- Kimura, S.J., Hogan, M.J., 1963. Chronic cyclitis. *Trans. Am. Ophthalmol. Soc.* 61, 397–417.
- Kitaichi, N., Kotake, S., Morohashi, T., Onoe, K., Ohno, S., Taylor, A.W., 2002. Diminution of experimental autoimmune uveoretinitis (EAU) in mice depleted of NK cells. *J. Leukoc. Biol.* 72 (6), 1117–1121.
- Kono, H., Onda, A., Yanagida, T., 2014. Molecular determinants of sterile inflammation. *Curr. Opin. Immunol.* 26, 147–156.
- Larson, T., Nussenblatt, R.B., Sen, H.N., 2011. Emerging drugs for uveitis. *Expert Opin. Emerg. Drugs* 16 (2), 309–322.
- Lee, A.G., 1995. Familial pars planitis. *Ophthalmic Genet.* 16 (1), 17–19.
- Lee, D.J., Taylor, A.W., 2015. Recovery from experimental autoimmune uveitis promotes induction of antiuveitic inducible Tregs. *J. Leukoc. Biol.* 97 (6), 1101–1109.
- Lee, R., Fowler, A., McGinnis, J., Lolley, R., Craft, C., 1990. Amino acid and cDNA sequence of bovine phosducin, a soluble phosphoprotein from photoreceptor cells. *J. Biol. Chem.* 265, 15867–15873.
- Levinson, R.D., Park, M.S., Rikkens, S.M., Reed, E.F., Smith, J.R., Martin, T.M., et al., 2003. Strong associations between specific HLA-DQ and HLA-DR alleles and the tubulointerstitial nephritis and uveitis syndrome. *Invest. Ophthalmol. Vis. Sci.* 44 (2), 653–657.
- Luger, D., Silver, P.B., Tang, J., Cua, D., Chen, Z., Iwakura, Y., et al., 2008. Either a Th17 or a Th1 effector response can drive autoimmunity: conditions of disease induction affect dominant effector category. *J. Exp. Med.* 205 (4), 799–810.
- Mochizuki, M., Kuwabara, T., McAllister, C., Nussenblatt, R.B., Gery, I., 1985. Adoptive transfer of experimental autoimmune uveoretinitis in rats. Immunopathogenic mechanisms and histologic features. *Invest. Ophthalmol. Vis. Sci.* 26 (1), 1–9.
- Molins, B., Mesquida, M., Llorente, V., Sainz de la Maza, M., Adan, A., 2016. Elevated serum immune mediators and subclinical inflammation in HLA-A29-associated birdshot chorioretinopathy. *Ocul. Immunol. Inflamm.* 24 (6), 647–652.
- Morikawa, H., Sakaguchi, S., 2014. Genetic and epigenetic basis of Treg cell development and function: from a FoxP3-centered view to an epigenome-defined view of natural Treg cells. *Immunol. Rev.* 259 (1), 192–205.
- Multicenter Uveitis Steroid Treatment Trial Research, G., Kempen, J.H., Altawee, M.M., Drye, L.T., Holbrook, J.T., Jabs, D.A., et al., 2015. Benefits of systemic anti-inflammatory therapy versus fluocinolone acetonide intraocular implant for intermediate uveitis, posterior uveitis, and panuveitis: fifty-four-month results of the multicenter uveitis steroid treatment (MUST) trial and follow-up study. *Ophthalmology* 122 (10), 1967–1975.
- Murray, P.I., Hoekzema, R., Luyendijk, L., Konings, S., Kijlstra, A., 1990. Analysis of aqueous humor immunoglobulin G in uveitis by enzyme-linked immunosorbent assay, isoelectric focusing, and immunoblotting. *Invest. Ophthalmol. Vis. Sci.* 31 (10), 2129–2135.
- Nakamura, S., Yamakawa, T., Sugita, M., Kijima, M., Ishioka, M., Tanaka, S., et al., 1994. The role of tumor necrosis factor-alpha in the induction of experimental autoimmune uveoretinitis in mice. *Invest. Ophthalmol. Vis. Sci.* 35 (11), 3884–3889.
- Nguyen, Q.D., Merrill, P.T., Jaffe, G.J., Dick, A.D., Kurup, S.K., Sheppard, J., et al., 2016. Adalimumab for prevention of uveitic flare in patients with inactive non-infectious uveitis controlled by corticosteroids (VISUAL II): a multicentre, double-masked, randomised, placebo-controlled phase 3 trial. *Lancet* 388 (10050), 1183–1192.
- Noval Rivas, M., Chatila, T.A., 2016. Regulatory T cells in allergic diseases. *J. Allergy Clin. Immunol.* 138 (3), 639–652.
- Nussenblatt, R.B., Whitcup, S.M., 2004. Uveitis: Fundamentals and Clinical Practice. Mosby, Philadelphia, PA.
- Nussenblatt, R.B., Gery, I., Ballantine, E., et al., 1980a. Cellular immune responsiveness of uveitis patients to retinal S-antigen. *Am. J. Ophthalmol.* 89, 173–179.
- Nussenblatt, R.B., Gery, I., Wacker, W.B., 1980b. Experimental autoimmune uveitis: cellular immune responsiveness. *Invest. Ophthalmol. Vis. Sci.* 19 (6), 686–690.
- Nussenblatt, R.B., Kuwabara, T., de Monasterio, F.M., Wacker, W.B., 1981. S-antigen uveitis in primates. A new model for human disease. *Arch. Ophthalmol.* 99 (6), 1090–1092.
- Nussenblatt, R.B., Salinas-Carmona, M., Leake, W., Scher, I., 1983. T lymphocyte subsets in uveitis. *Am. J. Ophthalmol.* 95 (5), 614–621.
- Nussenblatt, R.B., Palestine, A.G., Chan, C.C., Roberge, F., 1985. Standardization of vitreal inflammatory activity in intermediate and posterior uveitis. *Ophthalmology* 92 (4), 467–471.
- Ohno, S., Matsuda, H., 1986. In: Lehner, T., Barnes, C. (Eds.), Studies of HLA Antigens in Behcet's Disease in Japan. Recent Advances in Behcet's Disease. Royal Society of Medicine Press, London, pp. 11–16.
- Palexas, G.N., Sussman, G., Welsh, N.H., 1992. Ocular and systemic determination of IL-1 beta and tumour necrosis factor in a patient with ocular inflammation. *Scand. J. Immunol. Suppl.* 11, 173–175.
- Panitch, H.S., Ciccone, C., 1981. Adoptive transfer of experimental allergic encephalomyelitis: spleen requirement for macrophages in activation of spleen cells in vitro by concanavalin A, or myelin basic protein. *Cell. Immunol.* 60 (1), 24–33.
- Papadia, M., Herbort Jr., C.P., 2015. New concepts in the appraisal and management of birdshot retinochoroiditis, a global perspective. *Int. Ophthalmol.* 35 (2), 287–301.
- Pfister, C., Chabre, M., Plouet, J., Van Tuyen, V., de Kozak, Y., Faure, J., et al., 1986. Retinal S antigen identified as the 48k protein regulating light-dependent phosphodiesterase in rods. *Science* 228, 891–893.
- Ramanan, A.V., Dick, A.D., Jones, A.P., McKay, A., Williamson, P.R., Compeyrot-Lacassagne, S., et al., 2017. Adalimumab plus methotrexate for uveitis in juvenile idiopathic arthritis. *N. Engl. J. Med.* 376 (17), 1637–1646.
- Rincon-Arevalo, H., Sanchez-Parra, C.C., Castano, D., Yassin, L., Vasquez, G., 2016. Regulatory B cells and mechanisms. *Int. Rev. Immunol.* 35 (2), 156–176.
- Rizzo, L.V., Xu, H., Chan, C.C., Wiggert, B., Caspi, R.R., 1998. IL-10 has a protective role in experimental autoimmune uveoretinitis. *Int. Immunol.* 10 (6), 807–814.
- Robertson, M., Liversidge, J., Forrester, J.V., Dick, A.D., 2003. Neutralizing tumor necrosis factor-alpha activity suppresses activation of infiltrating macrophages in experimental autoimmune uveoretinitis. *Invest. Ophthalmol. Vis. Sci.* 44 (7), 3034–3041.

- Rodriguez, A., Calonge, M., Pedroza-Seres, M., Akova, Y.A., Messmer, E.M., D'Amico, D.J., et al., 1996. Referral patterns of uveitis in a tertiary eye care center. *Arch. Ophthalmol.* 114 (5), 593–599.
- Rosenbaum, J.T., 2017. New developments in uveitis associated with HLA B27. *Curr. Opin. Rheumatol.* 29 (4), 298–303.
- Sainz de la Maza, M., Molina, N., Gonzalez-Gonzalez, L.A., Doctor, P.P., Tauber, J., Foster, C.S., 2012. Clinical characteristics of a large cohort of patients with scleritis and episcleritis. *Ophthalmology* 119 (1), 43–50.
- Sakaguchi, M., Sugita, S., Sagawa, K., Itoh, K., Mochizuki, M., 1998. Cytokine production by T cells infiltrating in the eye of uveitis patients. *Jpn. J. Ophthalmol.* 42 (4), 262–268.
- Sakaguchi, S., Yamaguchi, T., Nomura, T., Ono, M., 2008. Regulatory T cells and immune tolerance. *Cell* 133 (5), 775–787.
- Schalken, J., Winkens, H., van Vugt, A., De Grip, W., Broekhuysse, R., 1989. Rhodopsin induced experimental autoimmune uveoretinitis in monkeys. *Br. J. Ophthalmol.* 73, 68–172.
- Sen, H.N., Levy-Clarke, G., Faia, L.J., Li, Z., Yeh, S., Barron, K.S., et al., 2009. High-dose daclizumab for the treatment of juvenile idiopathic arthritis-associated active anterior uveitis. *Am. J. Ophthalmol.* 148 (5), 696–703.e691.
- Sen, H.N., Sangave, A.A., Goldstein, D.A., Suhler, E.B., Cunningham, D., Vitale, S., et al., 2011. A standardized grading system for scleritis. *Ophthalmology* 118 (4), 768–771.
- Shi, G., Vistica, B.P., Nugent, L.F., Tan, C., Wawrousek, E.F., Klinman, D.M., et al., 2013. Differential involvement of Th1 and Th17 in pathogenic autoimmune processes triggered by different TLR ligands. *J. Immunol.* 191 (1), 415–423.
- Shi, T., Lv, W., Zhang, L., Chen, J., Chen, H., 2014. Association of HLA-DR4/HLA-DRB1\*04 with Vogt-Koyanagi-Harada disease: a systematic review and meta-analysis. *Sci. Rep.* 4, 6887.
- Silver, P.B., Horai, R., Chen, J., Jittayasothorn, Y., Chan, C.C., Villasmil, R., et al., 2015. Retina-specific T regulatory cells bring about resolution and maintain remission of autoimmune uveitis. *J. Immunol.* 194 (7), 3011–3019.
- Singh, V.K., Kalra, H.K., Yamaki, K., Abe, T., Donoso, L.A., Shinohara, T., 1990. Molecular mimicry between a uveitopathogenic site of S-antigen and viral peptides. Induction of experimental autoimmune uveitis in Lewis rats. *J. Immunol.* 144 (4), 1282–1287.
- Smith, W.M., Sen, H.N., Nussenblatt, R.B., 2012. Noncorticosteroid immune therapy for ocular inflammation. In: Tasman, W., Jaeger, E.A. (Eds.), *Duane's Ophthalmology*. Lippincott Williams & Wilkins, Philadelphia, PA.
- Suhler, E.B., Smith, J.R., Giles, T.R., Lauer, A.K., Wertheim, M.S., Kurz, D.E., et al., 2009. Infliximab therapy for refractory uveitis: 2-year results of a prospective trial. *Arch. Ophthalmol.* 127 (6), 819–822.
- Suzuki, J., Sakai, J., Okada, A.A., Takada, E., Usui, M., Mizuguchi, J., 2002. Oral administration of interferon-beta suppresses experimental autoimmune uveoretinitis. *Graefes Arch. Clin. Exp. Ophthalmol.* 240 (4), 314–321.
- Takase, H., Yu, C.R., Mahdi, R.M., Douek, D.C., Dirusso, G.B., Midgley, F.M., et al., 2005. Thymic expression of peripheral tissue antigens in humans: a remarkable variability among individuals. *Int. Immunopharmacol.* 17 (8), 1131–1140.
- Tan, C., Wandu, W.S., Lee, R.S., Hinshaw, S.H., Klinman, D.M., Wawrousek, E., et al., 2017. Shedding new light on the process of "Licensing" for pathogenicity by Th lymphocytes. *J. Immunol.* 198 (2), 681–690.
- Tao, J.H., Cheng, M., Tang, J.P., Liu, Q., Pan, F., Li, X.P., 2017. Foxp3, regulatory T cell, and autoimmune diseases. *Inflammation* 40 (1), 328–339.
- Tarrant, T.K., Silver, P.B., Wahlsten, J.L., Rizzo, L.V., Chan, C.C., Wiggert, B., et al., 1999. Interleukin 12 protects from a T helper type 1-mediated autoimmune disease, experimental autoimmune uveitis, through a mechanism involving interferon gamma, nitric oxide, and apoptosis. *J. Exp. Med.* 189 (2), 219–230.
- Taylor, A.W., 2016. Ocular immune privilege and transplantation. *Front. Immunol.* 7, 37.
- Tejada, P., Sanz, A., Criado, D., 1994. Pars planitis in a family. *Int. Ophthalmol.* 18 (2), 111–113.
- Thorne, J.E., Suhler, E., Skup, M., Tari, S., Macaulay, D., Chao, J., et al., 2016. Prevalence of noninfectious uveitis in the United States: a claims-based analysis. *JAMA Ophthalmol.* 134 (11), 1237–1245.
- Uhlenhuth, P., 1903. Zur lehre von der unterscheidung verschiedener eiweissarten mit hilfe spezifischer sera. *Festschrift zum 60 geburstag von Robert Koch*. Fischer, Jena, pp. 49–74.
- Wacker, W.B., Lipton, M.M., 1968. Experimental allergic uveitis. II. Serologic and hypersensitive responses of the guinea pig following immunization with homologous retina. *J. Immunol.* 101 (1), 157–165.
- Wacker, W., Donoso, L., Kalsow, C., 1977. Experimental allergic uveitis. Isolation, characterization, and localization of a soluble uveitopathogenic antigen from bovine retina. *J. Immunol.* 119, 1949–1958.
- Wang, R.X., Yu, C.R., Dambuza, I.M., Mahdi, R.M., Dolinska, M.B., Sergeev, Y.V., et al., 2014. Interleukin-35 induces regulatory B cells that suppress autoimmune disease. *Nat. Med.* 20 (6), 633–641.
- Wetzig, R.P., Chan, C.C., Nussenblatt, R.B., Palestine, A.G., Mazur, D.O., Mittal, K.K., 1988. Clinical and immunopathological studies of pars planitis in a family. *Br. J. Ophthalmol.* 72 (1), 5–10.
- Writing Committee for the Multicenter Uveitis Steroid Treatment, T., G. Follow-up Study Research, Kempen, J.H., Altawee, M.M., Holbrook, J.T., Sugar, E.A., Thorne, J.E., et al., 2017. Association between long-lasting intravitreous fluocinolone acetonide implant vs systemic anti-inflammatory therapy and visual acuity at 7 years among patients with intermediate, posterior, or panuveitis. *JAMA* 317 (19), 1993–2005.
- Yamaki, K., Kondo, I., Nakamura, H., Miyano, M., Konno, S., Sakuragi, S., 2000. Ocular and extraocular inflammation induced by immunization of tyrosinase related protein 1 and 2 in Lewis rats. *Exp. Eye Res.* 71 (4), 361–369.
- Yeh, S., Wroblewski, K., Buggage, R., Li, Z., Kurup, S.K., Sen, H.N., et al., 2008. High-dose humanized anti-IL-2 receptor alpha antibody (daclizumab) for the treatment of active, non-infectious uveitis. *J. Autoimmun.* 31 (2), 91–97.
- Zhang, M., Vacchio, M.S., Vistica, B.P., Lesage, S., Egwuagu, C.E., Yu, C.R., et al., 2003. T cell tolerance to a neo-self antigen expressed by thymic epithelial cells: the soluble form is more effective than the membrane-bound form. *J. Immunol.* 170 (8), 3954–3962.
- Zhao, B., Chen, W., Jiang, R., Wang, L., Gordon, L., Chen, L., 2015. Expression profile of IL-1 family cytokines in aqueous humor and sera of patients with HLA-B27 associated anterior uveitis and idiopathic anterior uveitis. *Exp. Eye Res.* 138, 80–86.

## Immune-Mediated Inner Ear Disease

Claudio Lunardi<sup>1</sup>, Elisa Tinazzi<sup>1</sup>, Lorenzo Delfino<sup>1</sup> and Antonio Puccetti<sup>2</sup>

<sup>1</sup>Clinical Immunology, Section of Internal Medicine, Department of Medicine, University Hospital, Verona, Italy

<sup>2</sup>Department of Experimental Medicine, University of Genova, Genova, Italy

### OUTLINE

Introduction	1051	Animal Models	1057
Clinical Features	1052	Treatment	1058
<i>Immune-Mediated Inner Ear Disease Associated With Systemic Autoimmune Diseases</i>	1053	Concluding Remarks and Future Perspectives	1059
<i>Immune-Mediated Inner Ear Disease Associated With Primary Vasculitides</i>	1053	Acknowledgment	1060
Evidence of Autoimmunity	1056	References	1060
Genetic Susceptibility	1056	Further Reading	1065

### INTRODUCTION

The inner ear has been considered for a long time an immune-privileged site, spared from organ-specific autoimmunity and rarely involved in systemic autoimmune diseases thanks to the blood–labyrinthine barrier (Matsuoka and Harris, 2013).

The hypothesis that sudden or rapidly progressive sensorineural hearing loss (SNHL) often accompanied by vestibular symptoms might result in an autoimmune process was first hypothesized by Lehnhardt (1958) and then proposed by McCabe (1979), who based his findings on the clinical features, presence of abnormal immunological tests, and a positive response to immunosuppressive therapy. Since then, a number of syndromes characterized by SNHL with overlapping clinical features have been described and termed in different ways: autoimmune SNHL, immune-mediated inner ear disease (IMIED), idiopathic progressive bilateral SNHL, sudden SNHL, idiopathic SNHL, bilateral immune-mediated Menière disease, autoimmune vestibulo-cochlear disorders (Rahman et al., 2001a), generating a great confusion in the identification of patients and in the evaluation of different studies. SNHL in adult patients remains idiopathic in the vast majority of the cases (71%); known causes are viral infections (12.8%), inner ear abnormality (4.7%), trauma (4.2%), vascular or hematologic (2.8%), neoplastic (2.3%), central nervous system (CNS) abnormality (Chau et al., 2010), and genetics (Koide et al., 2018). Recently, a case of temporary or permanent SNHL after vaccination with tetanus–diphtheria, meningococcal vaccine, and with influenza vaccine has been reported (De Marco et al., 2018; Kolarov et al., 2019).

In a series of children with idiopathic SNHL, Berti et al. (2013) confirmed an autoimmune origin in the majority of the cases included in the study. It is still debated whether we can define autoimmune in the majority of the cases of SNHL without any apparent cause (Greco et al., 2011a). Although most of the authors in the field refer

to these cases with the term “autoimmune inner ear disease” or “autoimmune SNHL” (Solares et al., 2003; Mathews and Kumar, 2003; Ciorba et al., 2018), we and others (Stone and Francis, 2000; García-Berrocal et al., 2003; Peneda et al., 2018) still prefer the definition of IMIED, since an autoimmune process cannot always be identified. IMIED may be a process confined to the inner ear (primary ear disease) and antibodies against a vast array of different molecular weights inner ear antigens may be found in a percentage of these patients, that is, the inner ear is the direct target of the immune response. Moreover, the inner ear can be damaged by a deposition of circulating immune complexes; in these cases the process can be identified as an organ-specific autoimmune disease. In other cases, IMIED is a feature of a systemic disorder, such as primary vasculitides or of systemic autoimmune diseases (secondary ear disease) (Ciorba et al., 2018; Ralli et al., 2018). Indeed, a systemic autoimmune disorder can be present in one-third of the patients with IMIED (Rossini et al., 2017).

Several studies have focused on the humoral and cellular response of the adaptive immune system. Little was known on the innate immune system of the inner ear. Recently, O’Malley et al. (2016) have identified cells with staining characteristics and the morphology of macrophages/microglia in the human cochlea. The authors suggested that these cells may have an important role in inner ear pathology due to the increased level of proinflammatory cytokines and reactive oxygen species induced by microglia.

## CLINICAL FEATURES

IMIED is characterized by the presence of rapidly progressive (usually between 1 and 90 days), often bilateral SNHL, and in about 50% of the patients by vertigo, tinnitus, and a sense of aural fullness, sometimes indistinguishable from Menière’s disease at the beginning. IMIED usually leads to irreversible damage within hours or days from the onset and involves both ears often asynchronously. Sometimes, the disease shows an initial fluctuating course of remissions and relapses typical of an autoimmune disease. Since the devastating sequelae of IMIED may be avoided with the early institution of aggressive immunosuppression, a prompt diagnosis represents a major goal for the clinician. Indeed, the clinical manifestations are shared with the entities of different etiologies, such as vascular, toxic, metabolic, neoplastic, genetic, traumatic, and infective (Massimo et al., 2012; Abdelfatah et al., 2013; Yariz et al., 2012; Gao et al., 2013; Beyea et al., 2012; Lin et al., 2013; Koide et al., 2018). Therefore all the possible nonimmune-mediated causes of SNHL need to be excluded. Overall, the incidence of SNHL has been estimated to range from 5 to 20 per 100,000 subjects per year (Chau et al., 2010), whereas the estimated yearly incidence of an autoimmune origin is <5 cases per 100,000, and the estimated prevalence is about 15/100,000 (Ciorba et al., 2018).

Menière’s syndrome, characterized by hearing loss and episodes of vertigo, tinnitus, and aural fullness, may accompany several causes of inner ear inflammation, including IMIED. In the absence of an identifiable cause, usually viral, this syndrome is termed Menière’s disease, considered of an autoimmune origin at least in 30%–40% of the cases although the immunological mechanisms involved are not clear (Riente et al., 2004; Greco et al., 2012, 2013; Kim et al., 2014; Kangasniemi and Hietikko, 2018). Recently, some researchers have identified alterations in the TWEAK/Fn14 pathway, which is involved in the modulation of inflammation by increasing the translation of NF- $\kappa$ B, in genetically susceptible individuals (Frejo et al., 2017). Time course is the most important criterion to differentiate IMIED from Menière’s disease where hearing loss occurs over a long period of time, sometimes for several years. Moreover, Menière’s disease is usually limited to one ear in the majority of the cases.

Due to the many different etiologies that can lead to SNHL and to the absence of specific diagnostic markers, García-Berrocal and Ramirez-Camacho (2002) proposed the following criteria to correctly assess IMIED as a distinct entity:

1. Major criteria: Bilateral involvement, the presence of a systemic autoimmune disease, positive antinuclear antibodies (ANAs), reduced number of naïve T cells (CD4RA), and recovery of hearing >80%.
2. Minor criteria: Unilateral involvement, young/middle-aged patient, often female, the presence of antibodies against heat shock protein 70 (HSP70), and good response to steroid therapy.

The suspect of IMIED would be supported by the presence of three major criteria or two major and more than two minor criteria. García-Berrocal et al. (2003) used these criteria to characterize and evaluate the response to therapy of 69 patients with a recent onset of SNHL of different origin, such as viral, vascular, immune mediated, and idiopathic. Patients with IMIED had the best and the earliest recovery rate of hearing after therapy, but also a higher rate of recurrence, typical of an autoimmune disorder. However, profound hearing loss (>90 dB)

presents a low percentage of recoveries, regardless of the etiology. The criteria proposed by Garcia-Berrocal et al. still need to be validated by different groups in a greater number of patients. In particular, we think that the presence of not only ANA but also of other autoantibodies, such as anticardiolipin antibodies, antithyroid antibodies, rheumatoid factor, anticitrullinated peptide antibodies, or myeloperoxidase–antineutrophil cytoplasmic antibodies, anti-CD148 and anticochlin, anti- $\beta$ -tubulin, and anti- $\beta$ -actin antibodies (Bachor et al., 2005; Toubi et al., 2004; Takagi et al., 2004; Lunardi et al., 2002; Berti et al., 2013; Ciorba et al., 2018), may suggest the presence of an autoimmune aggression of the inner ear. Moreover, we believe that a positive therapeutic response to corticosteroid administration should be considered a major criteria for the diagnosis of IMIED (Ruckenstein, 2004; Gallo et al., 2013; Yu et al., 2018; Witsell et al., 2018). More recently, Ciorba et al. (2018) have proposed a possible flowchart to use for the diagnosis and therapy of autoimmune inner ear disease.

Due to the lack of a reliable diagnostic tests (Bovo et al., 2009; Ciorba et al., 2018), MRI/3 Tesla-MRI, positron emission tomography (PET) of the inner ear in association with anti-HSP70 antibody determination, had been proposed as a useful technique for assessing IMIED (Mazlumzadeh et al., 2003). More recently, intratympanic gadolinium MRI (Lobo et al., 2018) and F-FDG PET/TC (Muentes Rasilla et al., 2018) have been proposed as a useful tool in the evaluation of IMIED.

The utility of the determination of antibodies anti-HSP70 is still unclear, and they are not more frequent in Menière's disease compared to healthy controls; moreover, the presence of such autoantibodies was not correlated with bilateral disease, activity or stage of the disease (Hornbrook et al., 2011). According to other authors, these antibodies could correlate to specific subgroups of IMIED (Bonauguri et al., 2014).

## Immune-Mediated Inner Ear Disease Associated With Systemic Autoimmune Diseases

Hearing loss, both sensorineural and conductive, has been reported in patients with rheumatoid arthritis (RA) (Raut et al., 2001; Oczan et al., 2002; Salvinelli et al., 2004), psoriatic arthritis (Giani et al., 2006), primary Sjögren's syndrome (Boki et al., 2001; Ralli et al., 2018), and with inflammatory bowel disease (IBD) (Vavricka et al., 2015). Anticardiolipin antibodies have been found associated with the presence of SNHL in patients with Sjögren's syndrome (Tumiati et al., 1997; Tucci et al., 2005) and with systemic lupus erythematosus (SLE) (Naarendorp and Spiera, 1998; Green and Miller, 2001; Kastanioudakis et al., 2002; Batueca-Caletrio et al., 2013). Moreover, SNHL can be present in patients with primary antiphospholipid syndrome (Vyse et al., 1994; Chapman et al., 2003; Galicia-López et al., 2016). The histopathologic features of temporal bone described in subjects affected by SLE showed the involvement of both cochlear and vestibular structures with various degrees of type I vestibular hair cell and inner hair cell loss, atrophy of the organ of Corti, marked cochlear inflammation with a polymorphonuclear infiltration and vasculitis or the formation of fibrous tissue and new bone throughout the cochlea, degeneration of the spiral ligament and hydrops, findings very similar to the known features of IMIED (Sone et al., 1999; Di Stadio and Ralli, 2017).

Similarly, the histopathology in four patients with Sjögren's syndrome showed severe loss of intermediate cells of stria vascularis and immunoglobulin G (IgG) deposition on the basement membrane of stria vascularis blood vessels. These pathologic changes are very similar to the inner ear histology found in a mouse model of Sjögren's syndrome (Calzada et al., 2012a).

SNHL has also been reported in progressive systemic sclerosis with a prevalence between 20% and 27% of the patients (Kastanioudakis et al., 2001; Mancini et al., 2018) and in ulcerative colitis (Kumar et al., 2000). Granulomatous inner ear disease has been described in Crohn's disease (Dettmer et al., 2011).

Finally, Meniere's disease displays an elevated prevalence of systemic autoimmune diseases, such as RA, SLE, and ankylosing spondylitis (Gazquez et al., 2011).

In conclusion the presence of a systemic autoimmune disease needs to be always rule out in SNHL (Mijovic et al., 2013; Mancini et al., 2018).

## Immune-Mediated Inner Ear Disease Associated With Primary Vasculitides

SNHL is often an early symptom of primary vasculitides, which usually affect both the middle and the inner ear (Ralli et al., 2018). The best example is Wegener's granulomatosis characterized by chronic otitis media leading to conductive hearing loss; SNHL is often associated and is related to vasculitis of the inner ear, and otological involvement can be the first sign of the disease (Takagi et al., 2002; Safavi Naini et al., 2017). Similar findings may be present in patients with relapsing polychondritis (Malard et al., 2002) characterized by vasculitis

of the labyrinthine artery and inflammation of the cartilage within the inner ear. SNHL has been described in polyarteritis nodosa (Tsunoda et al., 2001), microscopic polyangiitis (Koseki et al., 1997), Behcet's disease (Adler et al., 2002), and Kawasaki disease (Silva et al., 2002).

### Cogan's Syndrome

It was first described in 1934 by Morgan and Baumgartner as a nonsyphilitic interstitial keratitis but was defined as a clinical entity in 1945 by the ophthalmologist David Cogan. Cogan's syndrome is a rare chronic inflammatory disease characterized by three main clinical features: vestibulo-auditory dysfunction, interstitial keratitis, and vasculitis (St. Clair and McCallum, 1999; Greco et al., 2012, 2013). It occurs primarily in children and young adults and was first described by Cogan in 1945. Systemic manifestations occurs in approximately half of the cases (Van Doornum et al., 2001); fever and weight loss are associated with active vasculitis that can involve the aorta, aortic arch vessels, or medium vessels (Vollersten, 1990; Weissen-Plenz et al., 2010; Branislava et al., 2011). Central and peripheral nervous system abnormalities may be present in up to 50% of the subjects (Bicknell and Holland, 1978; Albayram et al., 2001). Morbidity in Cogan's syndrome results from permanent hearing loss and cardiovascular disease. In 1980 Haynes and other authors proposed a subdivision of Cogan's syndrome into "typical" and "atypical" forms. The "typical" form is characterized by the ocular involvement, such as nonsyphilitic interstitial keratitis, and by audiovestibular involvement similar to Meniere's disease, progressive loss of hearing to the point of deafness within 1–2 months. The interval between the onset of ocular and audiovestibular manifestations is less than 2 years. Cogan's syndrome is considered "atypical" when is present another type of ocular involvement (such as scleritis, episcleritis, retinal artery occlusion, choroiditis, retinal hemorrhages, papilloedema, and exophthalmos) associated with audiovestibular symptoms that do not resemble Meniere's disease or arise more than 2 years before or after ocular symptoms. Systemic manifestations are often found in the "atypical" Cogan's syndrome. It is important to underline that timing and association between symptoms may be extremely variable and not always is possible to make a clear distinction between the two forms (Iliescu et al., 2015; D'Aguanno et al., 2017).

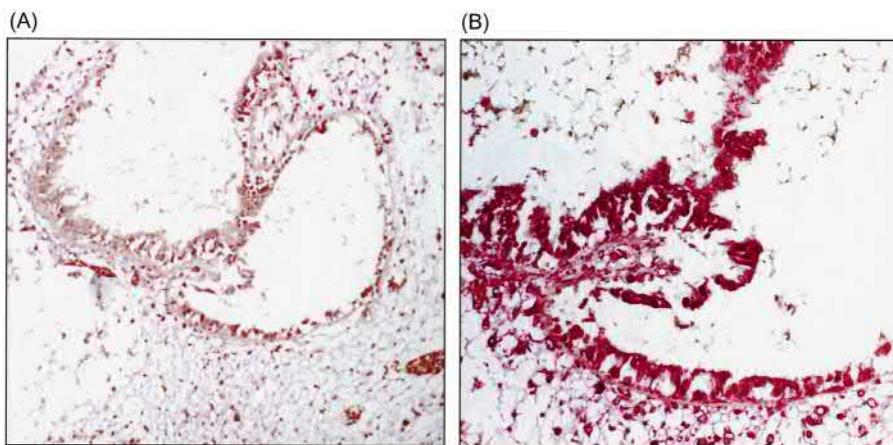
Inner ear pathology reveals endolymphatic hydrops, infiltration of the spiral ligament with lymphocytes and plasma cells, degeneration of the sensory receptors and supporting structures of the cochlea and vestibular apparatus, and demyelination and atrophy of the vestibular and cochlear branches of the eighth cranial nerve. In some cases, extensive new bone formation can be observed (Jung et al., 2016).

The cause of the disease is unknown; upper respiratory tract infections may precede the onset of the disease, suggesting an infectious origin (Vollertsen et al., 1986). Autoimmunity has been implicated because of the presence of serum antibodies to a mixture of corneal antigens and inner ear extracts (Helmchen et al., 1999; Disher et al., 1997). In a few cases, antineutrophil cytoplasmic autoantibodies have been reported.

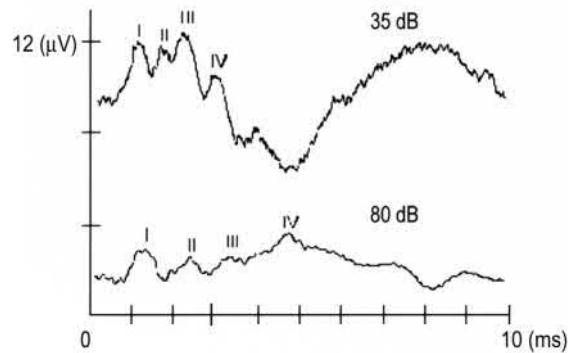
We have demonstrated that Cogan's syndrome is an autoimmune disease and that DEP1/CD148 is the pathogenetically relevant autoantigen (Lunardi et al., 2002). Using the peptide library approach (Puccetti and Lunardi, 2010), already applied to the study of systemic sclerosis (Lunardi et al., 2000), we identified a peptide recognized by the sera of all the patients. The peptide shares homology with autoantigens and with the major core protein lambda 1 (Bartlett and Joklik, 1988) of reovirus type III that causes mild rhinitis and pharyngitis. Peptide-specific IgG antibodies isolated from patients' sera recognized the viral protein, suggesting a viral involvement in the pathogenesis of the disease, possibly through a molecular mimicry mechanism (Zhao et al., 1998).

The peptide showed homology with the high cell density-enhanced protein tyrosine phosphatase-1 (DEP-1/CD148) that is highly expressed on both endothelial cells (Takahashi et al., 1999) and supporting cells of the inner ear (Kruger et al., 1999) and with connexin-26, a gap junction protein expressed in the inner ear (Kikuchi et al., 2000). Affinity-purified antibodies against the peptide obtained from the patients recognized CD148 and connexin-26 in human cochlear extracts. DEP1/CD148 is a widespread cell-surface antigen, and its distribution explains the clinical spectrum of Cogan's syndrome. Connexin-26 represents a major system of intercellular communication, and its loss results in local intoxication of the organ of Corti, leading to hearing loss; moreover, mutations at the connexin-26 gene are responsible for the majority of congenital deafness. Connexin-26 shows homology with connexin-43 and connexin-50, gap junction proteins present in corneal fibroblasts and epithelium, and this homology may explain the eye involvement in the disease. Peptide-specific antibodies bound human cochlea by immunohistochemistry (Fig. 55.1A and B).

To prove that these autoantibodies are pathogenic and that the identified autoantigen is relevant to Cogan's syndrome we induced the clinical features of the disease in animals (Balb/c mice and rabbits New Zealand white (NZW)) following either passive transfer of peptide-specific autoantibodies or active immunization with autoantigen peptides. The animal developed hearing loss as assessed by the auditory brainstem responses evaluation (Fig. 55.2).



**FIGURE 55.1** Antibodies against the Cogan peptide bind human cochlea. Human cochlea immunostained with antibodies purified against an irrelevant peptide (negative control, A) and with antibodies purified against the Cogan peptide (higher magnitude, B). Source: Reprinted with permission from Elsevier, Lunardi, C., Bason, C., Leandri, M., Navone, R., Lestani, M., Millo, E., et al., 2002. Autoantibodies to inner ear and endothelial antigens in Cogan's syndrome. *Lancet* 360, 915–921.



**FIGURE 55.2** Grand average of Auditory Brainstem Responses obtained from the same 6 mice before (upper trace) and one week after (lower trace) the third injection of purified antibodies directed against the Cogan peptide. A higher stimulus intensity (80 vs 35 dB) was needed to obtain much smaller and delayed responses (lower trace), consistent with hearing loss. The traces shown were obtained with above threshold stimuli in order to clearly identify the single components. The delay of waves II, III and IV suggests an impaired transmission along the auditory pathway from the acoustic nerve to the midbrain. Data represent amplitude of the response in microvolt ( $\mu$ V) (vertical axis); time elapsed from stimulus in milliseconds (horizontal axis). The time of stimulus delivery (click) is coincident with the 0 of the horizontal axis; no pre-stimulus baseline is shown. Source: Reprinted with permission from Elsevier, Lunardi, C., Bason, C., Leandri, M., Navone, R., Lestani, M., Millo, E., et al., 2002. Autoantibodies to inner ear and endothelial antigens in Cogan's syndrome. *Lancet* 360, 915–921.

We have so far tested many patients with Cogan's syndrome and with idiopathic SNHL for the presence of antibodies directed against the Cogan peptide, DEP-1/CD148, reovirus, and connexin-26 peptides in order to define the specificity and the sensitivity of the test. We consider positive those patients who have at least two antibodies directed against the four peptides, and one of the two positivity needs to be either anti-Cogan peptide or anti-DEP1/CD148 antibodies. Interestingly, antibodies against Cogan peptide and DEP1/CD148 have been found also in patients diagnosed as affected by an autoimmune SNHL. Interestingly, these autoantibodies can cross-react with an epitope of SSA/Ro60 protein. As well known, anti-SSA/Ro antibodies can cause immune-mediated damage of the cardiac conduction system in fetuses during the pregnancy; we have recently shown that these antiinner ear antibodies can only recognize a part of the whole protein Ro60, so they are not able to cause a congenital heart block in fetus (Bason et al., 2017).

Recently, the role of anti-HSP70 antibodies has been investigated. A group of researchers showed a significant relationship with IMIED and particularly with Cogan's syndrome. According to these authors, anti-HSP70 antibodies has an extremely high sensitivity for the "typical" form of Cogan's syndrome and, in the absence of immunosuppressive therapy, a negative test for these antibodies can rule out the diagnosis of "typical" Cogan's syndrome (Bonauguri et al., 2014). However, the role of these antibodies in the pathogenesis and in the diagnostic evaluation (see next) is debated and further data are needed.

## EVIDENCE OF AUTOIMMUNITY

Autoantibodies and autoreactive T cells have been implicated in the etiopathogenesis of idiopathic SNHL (Yehudai et al., 2006). Different antibodies directed either against inner ear specific or widely distributed autoantigens have been described in patients with SNHL of unknown origin. Antibodies against type II and type IX collagens as well as against other autoantigens in patients with Menière disease and with IMIED have been reported (Yoo et al., 2002). The Kresge Hearing Research Institute-3 (KHRI-3) antibody binds to guinea pig inner ear supporting cell antigen that has been found to be homologous to the human choline transporter-like protein 2 (CTL2), expressed in the inner ear, making this protein a possible target for an autoimmune aggression in IMIED (Nair et al., 2004). Antiendothelial cell autoantibodies have been reported in some patients with SNHL and may represent a marker of vasculitis or vascular damage of the inner ear, which leads to leucocyte infiltration and local immunoglobulin production (Cadoni et al., 2002; Mathews and Kumar, 2003). The detection of antibodies against the myelin protein P0 (30 kDa) has given conflicting results (Passali et al., 2004; Pham et al., 2007). Besides antibodies against the 30 kDa protein, Boulassel et al. (2001a) reported also the presence of antibodies against a 42 kDa protein identified as  $\beta$ -actin and against a 68 kDa protein, identified as HSP70 by some authors (Billings et al., 1998; Bloch et al., 1999) but not by others (Yeom et al., 2003). HSP70 is considered a marker of early cell damage of inner ear, but it is considered by some authors as aspecific and not useful in the diagnosis of IMIED (Lobo et al., 2014). The Western blot test for HSP70 has a very low sensitivity (García-Berrocal et al., 2002); however, its positivity seems to correlate with steroid responsiveness in subjects with IMIED (Hirose et al., 1999). The 58 kDa inner ear protein, recognized by the sera of some patients with SNHL, has been identified as cochlin, a molecule highly expressed in the cochlea (Boulassel et al., 2001b). Mutation at the "coagulation factor C homology gene" (COCH gene) encoding for cochlin causes progressive DFNA9 hearing loss and vestibular disorder (Roberston et al., 2006). Increased expression of cochlin and decreased expression of its associated basement membrane proteins have also been found in Meniere's disease (Calzada et al., 2012b). Evidence has been reported for the role of this protein in the pathogenesis of autoimmune inner ear disease (Baruah, 2014).

Another protein, highly expressed in the inner ear tissues, such as hair cells, supporting cells, spiral ligament of stria vascularis, is  $\beta$ -tubulin. Antibodies against the 55 kDa protein  $\beta$ -tubulin have been identified in 59% of the patients with an autoimmune SNHL and Ménière's disease (Zhou et al., 2011a).

Cochlin,  $\beta$ -tectorin, and  $\beta$ -tubulin have been used to induce SNHL in animal models (see next). A 68–72 kDa inner ear membrane glycoprotein called CTL2 has been identified as a target of autoantibodies in patients with an autoimmune hearing loss. Moreover, the presence of these antibodies seems to be correlated with response to corticosteroids (Kommareddi et al., 2009). Table 55.1 summarizes the putative autoantigens believed or proved to be involved in the pathogenesis of IMIED. The search for immune epitopes of the inner ear has not led to the identification of novel autoantigen targets so far (Platt et al., 2014).

We can conclude that the sera of patients with SNHL recognizes a large array of proteins of variable molecular weight only a few of which have been identified so far. However, there is not direct proof that any of the antibodies directed against such autoantigens may be immunopathogenic and cochleopathic in IMIED. Indeed, the majority of them could not induce SNHL in immunized animals (see next).

The first evidence for an implication of autoreactive T cells was reported by Mc Cabe and McCormick (1984) who observed leukocyte migration inhibition by T cells exposed to inner ear extracts. Lorenz et al. (2002) reported an increased number of inner ear-specific interferon gamma (IFN- $\gamma$ )-producing T cells (Th1 immune response) in the peripheral blood of patients with SNHL believed to be of an autoimmune origin. These findings indicated that proinflammatory T cells specific for as yet unknown inner ear antigens may play a role in the development and progression of inner ear autoimmunity. Moreover, abnormalities in the cytokine milieu in patients with IMIED have been reported (Goodall and Siddiq, 2015).

## GENETIC SUSCEPTIBILITY

Since IMIED and Meniere's disease can be of an autoimmune origin, interactions between genetic factors and environmental factors play a pivotal role in the pathogenesis of the disease.

Variability in acute immune response genes could determine susceptibility and prognosis of SNHL.

**TABLE 55.1** Putative Autoantigen Targets in IMIED (A) and Cogan's Syndrome (B)

IMIED	Molecular weight (kDa)	Reference	Induces hearing loss in animals	Reference
Collagen type II		Yoo et al. (2002)	No	Harris et al. (1986)
Collage type IX		Yoo et al. (2002)	?	—
Myelin protein P0	30	Boulassel et al. (2001a)	No	Boulassel et al. (2001a)
Beta actin	42	Boulassel et al. (2001a)	?	—
HSP-70	68	Billings et al. (1995) Bloch et al. (1999)	No	Billings et al. (1998)
Cochlin	58	Boulassel et al. (2001b)	Yes	Solares et al. (2004)
Beta tectorin	43	Boulassel et al. (2001b)	Yes	Solares et al. (2004)
Beta tubulin	55	Zhou et al. (2011a)	Yes	Zhou et al. (2011a)
CTL2	68–72	Kommareddi et al. (2009)	?	—
<b>COGAN'S SYNDROME</b>				
DEP1/CD148	200	Lunardi et al. (2002)	Yes	Lunardi et al. (2002)
Connexin 26	26	Lunardi et al. (2002)	Yes	Lunardi et al. (2002)

Functional allelic variants of genes encoding for the proinflammatory cytokines, including tumor necrosis factor alpha (TNF-alpha), IFN- $\gamma$ , and macrophage migration inhibitory factor, have not been found associated with disease susceptibility or hearing loss progression in patients with Menière's disease (Gásquez et al., 2012).

Since increased permeability of blood vessels, as shown by MRI, suggests inflammation of the inner ear, different genes involved in inflammatory pathways have been analyzed; polymorphisms of the gene encoding for IL-6 (Hiramatsu et al., 2012) and for IL-1 $\beta$  (Um et al., 2013) have been found associated with the risk of sudden SNHL. Moreover, patients nonresponder to steroid therapy show an overexpression and a dysregulation of IL-1 $\beta$  (Pathak et al., 2011); consensually, they have higher MMP-9 levels than TIMP-1 levels (Eisner et al., 2017). Finally, SNHL has been observed in autoinflammatory syndromes, such as Muckle–Wells syndrome whose hallmark is IL-1 $\beta$  dysregulation (Kuemmerle-Deschmer et al., 2013). Therefore IL-1 $\beta$  blockade may be an alternative method to restore hearing in patients who do not respond to steroid therapy.

Oxidative stress seems to be related to the pathology of inner ear; however, polymorphisms of genes involved in oxidative stress have not been found associated with the risk of SNHL or Menière's disease (Teranishi et al., 2012).

Aquaporins are water channel proteins, which play a pivotal role in the regulation of perilymph and endolymph volume; two of the eight aquaporin subtypes seems to be involved in impaired fluid regulation present in Menière's disease and in Sjögren's syndrome (Eckhard et al., 2012).

Finally, the study of coagulation has identified risk factors, such as hyperhomocysteinemia and polymorphism of the MTHFR gene associated with SNHL (Massimo et al., 2012), whereas decrease in plasminogen activator inhibitor-1 levels due to gene polymorphism may be associated with the reduced risk of sudden SNHL in Italian population (Cho et al., 2012). Recently, a polymorphism of the uncoupling protein 2, a mitochondrial transporter abundant in the inner ear, has been reported to be associated with SNHL (Koide et al., 2018). Finally, polymorphism in transmembrane channel 1 can cause both autosomal dominant and recessive hearing loss (Wang et al., 2018).

## ANIMAL MODELS

A major barrier in understanding the pathophysiology of IMIED derives from the paucity of inner ear tissue available. This problem underlines the importance of animal models of this disorder, which may provide insights into the pathogenesis, diagnosis, and treatment of IMIED.

A recent animal model of an autoimmune inner ear disease has been obtained in Sprague-Dawley rats by combination with the high dose of pertussis toxin; cellular infiltration, missing hair cells, degeneration of the spiral ganglion cells, endolymphatic hydrops, and autoantibodies directed to inner ear-specific antigens were all noted after immunization (Kong et al., 2011).

Immunization with the better characterized putative autoantigens, such as collagen type II, HSP70, myelin P0, have failed to elicit hearing loss (Harris et al., 1986; Billings et al., 1998; Boulassel et al., 2001c). Attempts to develop animal models of IMIED have been made by immunizing guinea pigs with either isologous or bovine inner ear homogenates (Harris, 1987; Gong et al., 2002); this model was hampered by the vast array of immunologically active components involved, making impossible to identify the specific self-antigens involved in disease initiation and progression. The use of fractions of inner ear proteins has partially addressed this problem (Tomiyama, 2002) that will be solved by the availability of recombinant antigens (Billings, 2004). Gloddek et al. (1999) induced SNHL in naïve Lewis rats following a passive transfer of activated T cells specific for bovine inner ear extracts, demonstrating the role of T cells in the initiation and pathogenesis of SNHL. A confirmation of the importance of T cells was recently provided by Solares et al. (2004) who showed that SWXJ mice immunized with peptides derived from two proteins of the inner ear, cochlin and  $\beta$ -tectorin, had significant hearing loss. Two selected peptides elicited a CD4 $+$  T cell response of the Th1-like phenotype. Moreover, the passive transfer of peptide-activated CD4 $+$  T cells into naïve SWXJ recipient induced the leukocytic infiltration of inner ear and hearing loss. However, it is not clear whether cochlin and  $\beta$ -tectorin are implicated in IMIED in humans and how accurately this model reflects events occurring in the spontaneous disease (Billings, 2004). Zhou et al. (2011a) have shown that immunization of Balb/c mice with  $\beta$ -tubulin is able to cause lesions in the cochlear hair cells and cochlear damage of the spiral ganglion, mediated by CD4 $+$  T cells producing IFN- $\gamma$ . Hearing loss was induced by the passive transfer of CD4 $+$  T cells specific for  $\beta$ -tubulin to naïve mice and that this is accompanied by a decreased frequency and impaired suppressive function of regulatory T cells (Tregs). In this murine model of autoimmune hearing loss, systemic infusion of adipose tissue-derived mesenchymal stem cells significantly improved hearing function and protected hair cells by decreasing proliferation of antigen-specific Th1/Th17 cells and by inducing generation of antigen-specific Tregs (Zhou et al., 2011b). Moreover, the severity of  $\beta$ -tubulin-induced experimental autoimmune hearing loss is exacerbated by IL-10 deficiency (Zhou et al., 2012).

Interestingly, oral administration of  $\beta$ -tubulin in female C57BL/6 mice induced decreased hearing loss and inner ear damage through the induction of oral tolerance by increasing Th2 type cytokines (Cai et al., 2009).

The administration of monoclonal antibodies directed either against the guinea pig inner ear supporting cell antigen (KHRI-3 antibody) (Nair et al., 1995) or type II collagen fragment CB11 peptide-induced SNHL with the loss of hair cells, inflammatory cell migration, and endolymphatic hydrops (Matsuoka et al., 2002), suggesting that antibodies also play a critical role in autoimmunity of the inner ear.

IL-1 $\beta$  has been observed as highly expressed in an animal model of autoimmune inner ear disease; in this model, lipopolysaccharide (LPS) was required in addition to Ag reexposure to initiate cochlear IL-1 $\beta$  expression, leukocyte ingress into the cochlea, and hearing loss (Pathak et al., 2011).

## TREATMENT

Since IMIED may result in severe deafness and vestibular dysfunction, patients must be treated aggressively and immediately after the onset of the symptoms. The mainstay of therapy is high dose of corticosteroids (1 mg/kg/day) (Chen et al., 2003) continued for at least 2 weeks and then for other 2 weeks in case of improvement. The steroid is then tapered in a period variable between 2 and 3 months and in some cases maintained at low dosage for a long period (e.g., methylprednisolone 4 mg/day). Different schemes of steroids administration, including intratympanic dexamethasone and 6-methylprednisolone injections, have been used with good results (Garcia-Berrocal et al., 2006; Rauch et al., 2009; Alexander et al., 2009; Gallo et al., 2013; Lim et al., 2012; Witsell et al., 2018; Tang et al., 2018; Yu et al., 2018). In the case of deterioration of symptoms or of a not significant improvement during the first 2 weeks of treatment, other immunosuppressive agents (Buniel et al., 2009) are added, such as cyclophosphamide (CYP), 1–2 mg/kg/die, or methotrexate (MTX), 7,5–20 mg/wk. Because of the well-known side effects of CYP, clinicians prefer the use of MTX; however, despite the preliminary favorable responses (Kilpatrick et al., 2000; Matteson et al., 2001; Rahman et al., 2001a; Matteson et al., 2003), the unique controlled trial published so far does not support the efficacy of long-term MTX in maintaining the improvement achieved with glucocorticoid therapy (Harris et al., 2003). Since MTX is slow acting, its effect may start too late in a disease that rapidly leads to hearing loss. Mc Cabe (1989) reported the promising results obtained with the

CYP–prednisolone combination therapy. We have used oral methylprednisolone and CYP pulse therapy in two particularly rapid and severe cases; this therapy blocked and reverted the hearing loss. Encouraging results derive from the use of azathioprine that would be able to reduce the risk of relapse and to maintain the hearing threshold, modifying the course of the disease (Meta-Castro et al., 2018).

The results obtained with steroids and aggressive immunosuppression are variable (Broughton et al., 2004; Loveman et al., 2004; Ruckenstein, 2004) depending on the characteristics of the patients included, on the severity of the hearing loss at the beginning of the treatment and on how early the therapy is started. Also, proinflammatory cytokines, cytokines receptors polymorphism may affect the steroid responsiveness (Vambutas et al., 2009).

Biological agents can play a role in the management of patients with IMIED (reviewed by Lobo et al., 2012), most studies achieved a hearing improvement or stabilization in more than 70% of the patients treated. The biological agents used include anti-TNF-alpha inhibitors (etanercept, infliximab, and adalimumab), anti-IL-1 antagonist (anakinra) and anti-CD20 surface antigen (rituximab). Infliximab has been also delivered locally through transtympanic administration (Van Wijk et al., 2006) and has been used in the treatment of Cogan's syndrome (Beccastrini et al., 2010). Controversial data are reported on the efficacy of rituximab, and further studies are needed (Orsoni et al., 2010; Bunker and Kerr, 2016).

There are reports on the utility of plasmapheresis (Luetje and Berliner, 1997), therapeutic apheresis of low density lipoprotein (LDL) (Bosch, 2003) and fibrinogen (Suzuki et al., 2003). Suckfull (2002) reported the beneficial effects of a single fibrinogen/LDL apheresis compared to conventional infusion treatment and prednisolone for 10 days. In controlled studies the use of antiviral therapy with valacyclovir (Tucci et al., 2002) and acyclovir (Uri et al., 2003; Westerlaken et al., 2003) in addition to steroids was not more beneficial than steroids alone. There are reports on the utility of low-molecular-weight heparins (Yue et al., 2003; Mora et al., 2004) and antioxidants (Joachims et al., 2003), such as N-acetylcysteine (Pathac et al., 2015) in addition to the usual therapy, and on the efficacy of intravenous infusion of tissue plasminogen activator (Mora et al., 2003) alone.

Emerging therapies appear to target cell death pathways, influence oxidant stressors, and favor the regeneration of hair cells (Crowson et al., 2017).

Hyperbaric oxygen therapy seems to be helpful in increasing the effect of pharmacotherapy (Krajcovicova et al., 2018).

In the case of permanent severe bilateral hearing loss the auditory function may be partially replaced with a cochlear implant, an electrical prosthesis with electrodes inserted into the cochlea through mastoidectomy (Cohen et al., 1993). Cochlear implantation has been successfully used in patients with SNHL (Gaylor et al., 2013), with IMIED and with Cogan's syndrome (Malik et al., 2012; Aftab et al., 2010; Wang et al., 2010). Of the 21 patients with Cogan's syndrome we are following at the moment, 11 (age 7–30) have undergone multichannel cochlear implant, resulting in a great improvement of their quality of life, without any complication. Cochlear implantation has been applied with benefit in children of 12 months of age or younger (Holman et al., 2013). Bilateral cochlear implantation seems to be more beneficial than unilateral implantation (Van Schoonhoven et al., 2013), and the use of implants with thin electronics capsules seems to simplify the simultaneous bilateral cochlear implantation (Perenyi et al., 2018).

Hematopoietic stem cell transplantation led to improvement in sensorineural hearing in patients with mucopolysaccharidosis (Da Costa et al., 2012). The recent emergence of stem cell technology has the potential to open new approaches for the regeneration of hair cells (Okano and Kelley, 2012; Crowson et al., 2017).

## CONCLUDING REMARKS AND FUTURE PERSPECTIVES

It is now evident that the inner ear is not an "immunologically privileged" site and may mount a cross-reactive immune response against both foreign (i.e., viral) and self-antigens, through a mechanism of molecular mimicry. The association of IMIED with systemic autoimmune diseases provides evidence that autoimmunity can damage the inner ear, but it does not address organ-specific disease (Mijovic et al., 2013). Antibodies directed against different inner ear antigens have been identified in some patients; however, they are neither diagnostic nor correlate with disease state. In the future the major goals for research in this field will be (1) the identification of pathogenetically relevant autoantigen(s), (2) the development of highly specific diagnostic test, and (3) a better knowledge of the immunopathological mechanisms in an organ as inaccessible as the inner ear and define the best timing and treatment for the disease. Indeed, despite recent researches, there is nonconsensus on diagnosis and optimal treatment (Peneda et al., 2018).

## Acknowledgment

We are grateful to Dr. C. Bason.

## References

- Abdelfatah, N., McComiskey, D.A., Doucette, L., Griffin, A., Mooere, S.J., Negrijn, C., et al., 2013. Identification of a novel in-frame deletion in KCNQ4 (DFNA2A) and evidence of multiple phenocopies of unknown origin in a family with ADSNHL. *Eur. J. Hum. Genet.* 21, 1112–1119 [Epub ahead of print].
- Adler, Y.D., Jovanovic, S., Jivanjee, A., Krause, L., Zouboulis, C.C., 2002. Adamantiades-Behcet's disease with inner ear involvement. *Clin. Exp. Rheumatol.* 20, S40–S42.
- Aftab, S., Semaan, M.T., Murray, G.S., Megerian, C.A., 2010. Cochlear implantation outcomes in patients with autoimmune and immune-mediated inner ear disease. *Otol. Neurotol.* 31, 1337–1342.
- Albayram, M.S., Wityk, R., Yousem, D.M., Zinreich, S.J., 2001. The cerebral angiographic findings in Cogan syndrome. *Am. J. Neuroradiol.* 22, 751–754.
- Alexander, T.H., Weisman, M.H., Derebery, J.M., Espeland, M.A., Gantz, B.J., Gulya, A.J., et al., 2009. Safety of high-dose corticosteroids for the treatment of autoimmune inner ear disease. *Otol. Neurotol.* 30, 443–448.
- Bachor, E., Kremmer, S., Kreuzfelder, E., Jahnke, K., Seidahmadi, S., 2005. Antiphospholipid antibodies in patients with sensorineural hearing loss. *Eur. Arch. Otorhinolaryngol.* 262, 622–626 [Epub ahead of print].
- Bartlett, J.A., Joklik, W.K., 1988. The sequence of the reovirus serotype 3 L3 genome segment which encodes the major core protein lambda 1. *Virology* 167, 31–37.
- Baruah, P., 2014. Cochlin in autoimmune inner ear disease: is the search for an inner ear autoantigen over? *Auris Nasus Larynx* 41, 499–501.
- Bason, C., Pagnini, I., Brucato, A., Maestroni, S., Puccetti, A., Lunardi, C., et al., 2017. Congenital heart block and immune mediated sensorineural hearing loss: possible cross reactivity of immune response. *Lupus* 26, 835–840.
- Batueca-Caletro, A., Del Pin-Montes, J., Cordero-Civantos, C., Calle-Cabanillas, M., Lopez-Escamez, J., 2013. Hearing and vestibular disorders in patients with systemic lupus erythematosus. *Lupus* 22, 437–442 [Epub ahead of print].
- Beccastrini, E., Emmi, G., Squarrito, D., Vannucchi, P., Emmi, L., 2010. Infliximab and Cogan's syndrome. *Clin. Otolaryngol.* 35, 439–450.
- Berti, E., Vannucci, G., Lunardi, C., Bianchi, B., Bason, C., Puccetti, A., et al., 2013. Identification of autoantibodies against inner ear antigens in a cohort of children with idiopathic sensorineural hearing loss. *Autoimmunity* 46, 525–530.
- Beyea, J.A., Agrawal, S.K., Parnes, L.S., 2012. Recent advances in viral inner ear disorders. *Curr. Opin. Otolaryngol. Head Neck Surg.* 20, 404–408.
- Bicknell, J.M., Holland, J.V., 1978. Neurologic manifestations of Cogan's syndrome. *Neurology* 28, 278–288.
- Billings, P., 2004. Experimental autoimmune hearing loss. *J. Clin. Invest.* 113, 1114–1117.
- Billings, P.B., Keithley, E.M., Harris, J.P., 1995. Evidence linking the 68 kilodalton antigen identified in progressive sensorineural hearing loss patient sera with heat shock protein 70. *Ann Otol Rhinol Laryngol* 104, 181–188.
- Billings, P., Shin, S.O., Harris, J.P., 1998. Assessing the role of anti-hsp70 in cochlear function. *Hear. Res.* 126, 210–212.
- Bloch, D.B., Gutierrez, J.A., Guerriero Jr., V., Rauch, S.D., Bloch, K.J., 1999. Recognition of a dominant epitope in bovine heat-shock protein 70 in inner ear disease. *Laryngoscope* 109, 621–625.
- Boki, K.A., Ioannidis, J.P., Segas, J.V., Maragkoudakis, P.V., Petrou, D., Adamopoulos, G.K., et al., 2001. How significant is sensorineural hearing loss in primary Sjogren's syndrome? An individually matched case-control study. *J. Rheumatol.* 28, 798–801.
- Bonauguri, C., Orsoni, J., Russo, A., Rubino, P., Bacci, S., Lippi, G., et al., 2014. Cogan's syndrome: anti-Hsp70 antibodies are a serological marker in the typical form. *IMAJ* 16, 285–288.
- Bosch, T., 2003. Recent advances in therapeutic apheresis. *J. Artif. Organs.* 6, 1–8.
- Boulassel, M.R., Deggouj, N., Tomasi, J.P., Gersdorff, M., 2001a. Inner ear autoantibodies and their targets in patients with autoimmune inner ear diseases. *Acta Otolaryngol.* 121, 28–34.
- Boulassel, M.R., Tomasi, J.P., Deggouj, N., Gersdorff, M., 2001b. COCH5B2 is a target antigen of anti-inner ear antibodies in autoimmune inner ear diseases. *Otol. Neurotol.* 22, 614–618.
- Boulassel, M.R., Guerit, J.M., Denison, S., de Tourchaninoff, M., Wenderickx, L., Boterman, N., et al., 2001c. No evidence of auditory dysfunction in guinea pigs immunized with myelin P0 protein. *Hear. Res.* 152, 10–16.
- Bovo, R., Ciorba, A., Martini, A., 2009. The diagnosis of autoimmune inner ear disease: evidence and critical pitfalls. *Eur. Arch. Otorhinolaryngol.* 266, 37–40.
- Branislava, I., Marijana, T., Nemanja, D., Dragan, S., Maja, Z., 2011. Atypical Cogan's syndrome associated with coronary disease. *Chin. Med. J.* 124, 3192–3194.
- Broughton, S.S., Meyerhoff, W.E., Cohen, S.B., 2004. Immune-mediated inner ear disease: 10-year experience. *Semin. Arthritis Rheum.* 34, 544–548.
- Buniel, M.C., Geelan-Hansen, K., Weber, P.C., Tuohy, V.K., 2009. Immunosuppressive therapy for autoimmune inner ear disease. *Immunotherapy* 1, 425–434.
- Bunker, D.R., Kerr, L.D., 2016. Rituximab not effective for hearing loss in Cogan's syndrome. *Case Rep. Rheumatol.* 2016, 8352893.
- Cadoni, G., Fetoni, A.R., Agostino, S., DeSantis, A., Manna, R., Ottaviani, F., et al., 2002. Autoimmunity in sudden sensorineural hearing loss: possible role of anti-endothelial cell autoantibodies. *Acta Otolaryngol. Suppl.* 548, 30–33.
- Cai, Q., Du, X., Zhou, B., Cai, C., Kermany, M.H., Yoo, T., 2009. Induction of tolerance by oral administration of beta-tubulin in an animal model of autoimmune inner ear disease. *ORL* 71, 135–141.
- Calzada, A.P., Balaker, A.E., Ishiyama, G., Lopez, I.A., Ishiyama, A., 2012a. Temporal bone histopathology and immunoglobulin deposition in Sjögren's syndrome. *Otol. Neurotol.* 33, 258–266.

- Calzada, A.P., Lopez, I.A., Parrazal, L.B., Ishiyama, A., Ishiyama, G., 2012b. Cochlin expression in vestibular endorgans obtained from patients with Meniere's disease. *Cell Tissue Res.* 350, 373–384.
- Chapman, J., Rand, J.H., Brey, R.L., Levine, S.R., Blatt, I., Khamashta, M.A., et al., 2003. Non-stroke neurological syndromes associated with antiphospholipid antibodies: evaluation of clinical and experimental studies. *Lupus* 12, 514–517.
- Chau, J.K., Lin, J.R.J., Atashband, S., Irvine, R.A., Westerberg, B.D., 2010. Systematic review of the evidence for the etiology of adult sudden sensorineural hearing loss. *Laryngoscope* 120, 1011–1021.
- Chen, C.Y., Halpin, C., Rauch, S.D., 2003. Oral steroid treatment of sudden sensorineural hearing loss: a ten year retrospective analysis. *Otol. Neurotol.* 24, 728–733.
- Cho, S.H., Chen, H., Kim, I.S., Yokose, C., Kang, J., Cho, D., et al., 2012. Association of the 4g/5g polymorphism of plasminogen activator inhibitor-1 gene with sudden sensorineural hearing loss. A case control study. *BMC Ear Nose Throat Disord.* 6, 12–15.
- Ciorba, A., Corazzi, V., Bianchini, C., Aimoni, C., Pelucchi, S., Skarzynski, P.H., et al., 2018. Autoimmune inner ear disease (AIED): a diagnostic challenge. *Int. J. Immunopathol. Pharmacol.* 32, 1–5.
- Clair St., E.W., McCallum, R.M., 1999. Cogan's syndrome. *Curr. Opin. Rheumatol.* 11, 47–52.
- Cogan, D., 1945. Syndrome of nonsyphilitic interstitial keratitis and vestibuloauditory symptoms. *Arch Ophthalmol.* 33, 144–149.
- Cohen, N.L., Waltzman, S.B., Fisher, S.G., 1993. A prospective, randomized study of cochlear implants and the Department of Veterans Affairs Cochlear Implant Study Group N. *Engl. J. Med.* 328, 233–237.
- Crowson, M.G., Hertzano, R., Tucci, D.L., 2017. Emerging therapies for sensorineural hearing loss. *Otol Neurotol.* 38, 792–803.
- Da Costa, V., O'Grady, G., Jackson, L., Kaylie, D., Raynor, E., 2012. Improvements in sensorineural hearing loss after cord blood transplant in patients with mucopolysaccharidosis. *Arch. Otolaryngol. Head Neck Surg.* 138, 1071–1076.
- D'Aguanno, V., Ralli, M., de Vincentiis, M., Greco, A., 2017. Optimal management of Cogan's syndrome: a multidisciplinary approach. *J Multidiscip. Health* 11, 1–11.
- De Marco, F., De Cesare, D.P., Di Folco, F., Massoni, F., Tomei, G., Di Luca, N.M., et al., 2018. Post vaccinal temporary sensorineural hearing loss. *Int. J. Environ. Res. Public Health* 19, 15. Available from: <https://doi.org/10.3390/ijerph15081780>. pii: E1780.
- Dettmer, M., Hegemann, I., Hegemann, S.C., 2011. Extraintestinal Crohn's disease mimicking autoimmune inner ear disease: a histopathological approach. *Audiol. Neurotol.* 16, 36–40.
- Di Stadio, A., Ralli, M., 2017. Systemic lupus erythematosus and hearing disorders: Literature review and meta-analysis of clinical and temporal bone findings. *J. Int. Med. Res.* 45, 1470–1480.
- Disher, M.J., Ramakrishnan, A., Nair, T.S., Miller, J.M., Telian, S.A., Arts, H.A., et al., 1997. Human autoantibodies and monoclonal antibody KHRI-3 bind to a phylogenetically conserved inner-ear supporting cell antigen. *Ann. N.Y. Acad. Sci.* 830, 253–267.
- Eckhard, A., Gleiser, C., Arnold, H., Rask-Andersen, H., Kumagami, H., Müller, M., et al., 2012. Water channel proteins in the inner ear and their link to hearing impairment and deafness. *Mol. Aspects Med.* 33, 612–637.
- Eisner, L., Vambutas, A., Pathak, S., 2017. The balance of tissue inhibitor of metalloproteinase-1 and matrix metalloproteinase-9 in the autoimmune inner ear disease patients. *J. Interferon Cytokine Res.* 37, 354–361.
- Frejo, L., Requena, T., Okawa, S., Gallego-Martinez, A., Martinez-Bueno, M., Arani, I., et al., 2017. Regulation of Fn14 receptor and NF- $\kappa$ B underlies inflammation in Meniere's disease. *Front. Immunol.* 8, 1739.
- Galicia-López, A., Anda-Garay, J.C., García de la Peña, M., 2016. Bilateral sudden sensorineural hearing loss in a patient with microangiopathic antiphospholipid syndrome. *Reumatol. Clin.* 12, 175–177.
- Gallo, E., Khojasteh, E., Gloor, M., Hegemann, S.C., 2013. Effectiveness of systemic high-dose dexamethasone therapy for idiopathic sudden sensorineural hearing loss. *Audiol. Neurotol.* 18, 161–170.
- Gao, Y., Yechikov, S., Vazquez, A.E., Chen, D., Nie, L., 2013. Distinct roles of molecular chaperones HSP90 $\alpha$  and HSP90 $\beta$  in the biogenesis of KCNQ4 channels. *PLoS One* 8, e57282.
- García-Berrocal, J.R., Ramírez-Camacho, R., 2002. Sudden sensorineural hearing loss: supporting the immunologic theory. *Ann. Otol. Rhinol. Laryngol.* 111, 989–997.
- García-Berrocal, J.R., Ramírez-Camacho, R., Arellano, B., Vargas, J.A., 2002. Validity of the Western blot immunoassay for heat shock protein-70 in associated and isolated immunorelated inner ear disease. *Laryngoscope* 112, 304–309.
- García-Berrocal, J.R., Ramírez-Camacho, R., Millà, I., Gorri, C., Trinidad, A., Arellano, B., et al., 2003. Sudden presentation of immune-mediated inner ear disease: characterization and acceptance of a cochleovestibular dysfunction. *J. Laringol. Otol.* 117, 775–779.
- Garcia-Berrocal, J.R., Ibañez, A., Rodríguez, A., González-García, J.A., Verdaguera, J.M., Trinidad, A., et al., 2006. Alternatives to systemic steroid therapy for refractory immune-mediated inner ear disease: a physiopathologic approach. *Eur. Arch. Otorhinolaryngol.* 263, 977–982.
- Gásquez, I., Moreno, A., Requena, T., Ohmen, J., Santos-Perez, S., Aran, I., et al., 2012. Functional variants of MIF, INF $\gamma$  and TFNA genes are not associated with disease susceptibility or hearing loss progression in patients with Ménière's disease. *Eur. Arch. Otorhinolaryngol.* 270, 1521–1529 [Epub ahead of print].
- Gaylor, J.M., Raman, G., Chung, M., Lee, J., Rao, M., Lau, J., et al., 2013. Cochlear implantation in adults: a systematic review and meta-analysis. *JAMA Otolaryngol. Head Neck Surg.* 21, 1–8.
- Gazquez, I., Soto-Varela, A., Aran, I., Santos, S., Batuecas, A., Trinidad, G., et al., 2011. High prevalence of systemic autoimmune diseases in patients with Meniere's disease. *PLoS One* 6, 1–7.
- Giani, T., Simonini, G., Lunardi, C., Puccetti, A., De Martino, M., Falcini, F., 2006. Juvenile psoriatic arthritis and acquired sensorineural hearing loss in a teenager: is there an association? *Clin. Exp. Rheumatol.* 24, 344–346.
- Gloddek, B., Gloddek, J., Arnold, W., 1999. A rat T cell line that mediates autoimmune disease of the inner ear in the Lewis rat. *ORL J. Otorhinolaryngol. Relat. Spec.* 61, 181–187.
- Gong, S.S., Yu, D.Z., Wang, J.B., 2002. Relationship between three inner ear antigens with different molecular weights and autoimmune inner ear disease. *Acta Otolaryngol.* 122, 5–9.
- Goodall, A.F., Siddiq, M.A., 2015. Current understanding of the pathogenesis of autoimmune inner ear disease: a review. *Clin Otolaryngol.* 40, 412–419.
- Greco, A., Fusconi, M., Gallo, A., Marinelli, C., Macri, G.F., De Vincentiis, M., 2011a. Sudden sensorineural hearing loss: an autoimmune disease? *Autoimmun. Rev.* 10, 756–761.

- Greco, A., Gallo, A., Fusconi, M., Marinelli, C., Macri, G.F., De Vincentiis, M., 2012. Meniere's disease might be an autoimmune condition? *Autoimmun. Rev.* 11, 731–738.
- Greco, A., Gallo, A., Fusconi, M., Magliulo, G., Turchetta, R., Marinelli, C., et al., 2013. Cogan's syndrome: an autoimmune inner ear disease. *Autoimmun. Rev.* 12 (3), 396–400.
- Green, L., Miller, E.B., 2001. Sudden sensorineural hearing loss as a first manifestation of systemic lupus erythematosus: association with anti-*cardiolipin* antibodies. *Clin. Rheumatol.* 20, 220–222.
- Harris, J.P., 1987. Experimental autoimmune sensorineural hearing loss. *Laryngoscope* 97, 63–76.
- Harris, J.P., Woolf, N.K., Ryan, A.F., 1986. A re-examination of experimental type II collagen autoimmunity: middle and inner ear morphology and function. *Ann. Otol. Rhinol. Laryngol.* 95, 176–180.
- Harris, J.P., Weisman, M.H., Derebery, J.M., Espeland, M.A., Gantz, B.J., Gulya, A.J., et al., 2003. Treatment of corticosteroid-responsive autoimmune inner ear disease with methotrexate: a randomized controlled trial. *JAMA* 290, 1875–1883.
- Helmchen, C., Arbusow, V., Jager, L., Strupp, M., Stocker, W., Schulz, P., 1999. Cogan's syndrome: clinical significance of antibodies against inner ear and cornea. *Acta Otorhinolaryngol.* 119, 528–536.
- Hiramatsu, M., Teranishi, M., Uchida, Y., Nishio, N., Suzuki, H., Kato, K., et al., 2012. Polymorphisms in genes involved in inflammatory pathways in patients with sudden sensorineural hearing loss. *J. Neurogenet.* 26, 387–396.
- Hirose, K., Wener, M.H., Duckert, L.G., 1999. Utility of laboratory testing in autoimmune inner ear disease. *Laryngoscope* 109, 1749–1754.
- Holman, M.A., Carlson, M.L., Driscoll, C.L., Grim, K.J., Petersson, R.S., Sladen, D.P., et al., 2013. Cochlear implantation in children 12 months of age and younger. *Otol. Neurotol.* 34, 251–258.
- Hornibrook, J., George, P., Spellerberg, M., Gourley, J., 2011. HSP70 antibodies in 80 patients with "clinically certain" Meniere's disease. *Ann. Otol. Rhinol. Laryngol.* 120, 651–655.
- Iliescu, D.A., Timaru, C.M., Batras, M., De Simone, A., Stefan, C., 2015. Cogan's syndrome. *Rom. J. Ophthalmol.* 59, 6–13.
- Joachims, H.Z., Segal, J., Golz, A., Netzer, A., Goldenberg, D., 2003. Antioxidants in treatment of idiopathic sudden hearing loss. *Otol. Neurotol.* 24, 572–575.
- Jung, D.H., Nadol Jr., J.B., Folkerth, R.D., Merola, J.-F., 2016. Histopathology of the inner ear in a case with recent onset of Cogan's syndrome: evidence for vasculitis. *Ann Otol Rhinol Laryngol.* 125, 20–24.
- Kangasniemi, E., Hietikko, E., 2018. The theory of autoimmunity in Meniere's disease is lacking evidence. *Auris Nasus Larynx* 45, 399–406.
- Kastanioudakis, I., Ziavra, N., Politis, E.N., Exarchakos, G., Drosos, A.A., Skevas, A., 2001. Hearing loss in progressive systemic sclerosis patients: a comparative study. *Otolaryngol. Head Neck Surg.* 124, 522–525.
- Kastanioudakis, I., Ziavra, N., Voulgari, P.V., Exarchakos, G., Skevas, A., Drosos, A.A., 2002. Ear involvement in systemic lupus erythematosus patients: a comparative study. *J. Laryngol. Otol.* 116, 103–107.
- Kikuchi, T., Kimura, R.S., Paul, D.L., Takasaka, T., Adams, J.C., 2000. Gap junction systems in the mammalian cochlea. *Brain Res. Rev.* 32, 163–166.
- Kilpatrick, J.K., Sismanis, A., Spencer, R.F., Wise, C.M., 2000. Low dose oral methotrexate management of patients with bilateral Meniere's disease. *Ear Nose Throat J.* 79, 82–83, 86–88, 91–92.
- Kim, S.H., Kim, J.Y., Lee, H.J., Gi, M., Kim, B.G., Choi, J.Y., 2014. Autoimmunity as a candidate for the etiopathogenesis of Meniere's disease: detection of autoimmune reactions and diagnostic biomarker candidate. *PLoS One* 9, e111039.
- Koide, Y., Teranishi, M., Sugiura, S., Uchida, Y., Nishio, N., Kato, K., et al., 2018. Association between uncoupling protein 2 gene Ala55val polymorphism and sudden sensorineural hearing loss. *J. Int. Adv. Otol.* 14, 166–169.
- Kolarov, C., Löbermann, M., Fritzsche, C., Hemmer, C., Mlynki, R., Reisinger, E.C., 2019. Bilateral deafness two days following influenza vaccination: a case report. *Hum. Vaccin. Immunother.* 15, 107–108.
- Kommareddi, P.K., Nair, T.S., Vallurupalli, M., Telian, S.A., Arts, H.A., El-Kshlan, H.K., et al., 2009. Autoantibodies to recombinant human CTL2 in autoimmune hearing loss. *Laryngoscope* 119, 924–932.
- Kong, W.J., Wang, D.Y., Huang, X., Ding, G.F., 2011. High dose combination pertussis toxin induces autoimmune inner ear disease in Sprague-Dawley rats. *Acta Otolaryngol.* 131, 692–700.
- Koseki, Y., Suwa, A., Nojima, T., Ishijama, K., Nakajima, A., Tanabe, M., et al., 1997. A case of microscopic polyangiitis accompanied by hearing loss as the initial sign of the disease. *Ryumachi* 37, 804–809.
- Krajcovicova, Z., Melus, V., Zigo, R., Matisakova, I., Vecera, J., Kaslikova, K., 2018. Efficacy of hyperbaric oxygen therapy as a supplementary therapy of sudden sensorineural hearing loss in the Slovak Republic. *Undersea Hyperb. Med.* 45, 363–370.
- Kruger, R.P., Goodyear, R.J., Legan, P.K., Warchol, M.E., Raphael, Y., Cotanche, D.A., et al., 1999. The supporting-cell antigen: a receptor-like protein tyrosine phosphatase expressed in the sensory epithelia of the avian inner ear. *J. Neurosci.* 19, 4815–4827.
- Kuemmerle-Deschner, J.B., Koitschev, A., Ummenhofer, K., Hansmsnn, S., Plontke, S.K., Koitschev, C., et al., 2013. Hearing loss in Muckle-Wells syndrome. *Arthritis Rheum.* 65, 824–831.
- Kumar, B.N., Smith, M.S.H., Walsh, R.M., 2000. Sensorineural hearing loss in ulcerative colitis. *Clin. Otolaryngol.* 25, 143–145.
- Lehnhardt, E., 1958. Sudden hearing disorders occurring simultaneously or successively on both sides. *Z. Laryngol. Rhinol. Otol.* 37, 1–16.
- Lim, H.J., Kim, Y.T., Choi, S.J., Lee, J.B., Park, K., Choung, Y.H., 2012. Efficacy of 3 different steroid treatments for sudden sensorineural hearing loss: a prospective, randomized trial. *Otolaryngol. Head Neck Surg.* 148, 121–127 [Epub ahead of print].
- Lin, C., Lin, S.W., Weng, S.F., Lin, Y.S., 2013. Increased risk of sudden sensorineural hearing loss in patients with human immunodeficiency virus aged 18 to 35 years: a population-based cohort study. *JAMA Otolaryngol. Head Neck Surg.* 21, 1–5 [Epub ahead of print].
- Lobo, D., Garcia-Berrocal, J.R., Trinidad, A., Verdaguera, J.M., Ramirez-Camacho, R., 2012. Review of the biologic agents used for immune-mediated inner ear disease. *Acta Otorrinolaringol. Esp.* 64, 223–229 [Epub ahead of print].
- Lobo, D., Tunon, M., Villarreal, I., Brea, B., Garcia-Berrocal, J.R., 2018. Intratympanic gadolinium magnetic resonance imaging supports the role of endolymphatic hydrops in the pathogenesis of immune-mediated inner-ear disease. *J. Laryngol. Otol.* 132, 554–559.
- Lobo, D.R., García-Berrocal, J.R., Ramirez-Camacho, R., 2014. New prospects in the diagnosis and treatment of immune-mediated inner ear disease. *World J. Methodol.* 4, 91–98.

- Lorenz, R.R., Solares, C.A., Williams, P., Sikora, J., Pelfrey, C.M., Hughes, G.B., et al., 2002. Interferon-gamma production to inner ear antigens by T cells from patients with autoimmune sensorineural hearing loss. *J. Neuroimmunol.* 130, 173–178.
- Loveman, D.M., de Comarmond, C., Cepero, R., Baldwin, D.M., 2004. Autoimmune sensorineural hearing loss: clinical course and treatment outcome. *Semin. Arthritis Rheum.* 34, 538–543.
- Luetje, C.M., Berliner, K.I., 1997. Plasmapheresis in autoimmune inner ear disease: long-term follow-up. *Am. J. Otol.* 18, 572–576.
- Lunardi, C., Bason, C., Navone, R., Millo, E., Da monte, G., Corrocher, R., et al., 2000. Systemic sclerosis immunoglobulin G autoantibodies bind the human cytomegalovirus late protein UL94 and induce apoptosis in human endothelial cells. *Nat. Med.* 6, 1183–1186.
- Lunardi, C., Bason, C., Leandri, M., Navone, R., Lestani, M., Millo, E., et al., 2002. Autoantibodies to inner ear and endothelial antigens in Cogan's syndrome. *Lancet* 360, 915–921.
- Malard, O., Hamidou, M., Toquet, C., Bailleuil, S., Bordure, P., Beauvillain De Montreuil, C., 2002. Relapsing polychondritis revealed by ENT symptoms: clinical characteristics in three patients. *Ann. Otolaryngol. Chir. Cervicofac.* 119, 202–208.
- Malik, M.U., Pandian, V., Masood, H., Diaz, D.A., Varela, V., Dávalos-Balderas, A.J., et al., 2012. Spectrum of immune-mediated inner ear disease and cochlear implant results. *Laryngoscope* 122, 2557–2562.
- Mancini, P., Atturo, F., Di Mario, A., Portanova, G., Ralli, M., De Virgilio, A., et al., 2018. Hearing loss in autoimmune disorders: prevalence and therapeutic options. *Autoimmunity Rev.* 17, 644–652.
- Massimo, F., Antonio, C., Armand de V., Antonio, G., Fulvio, M., Rosaria, T., et al., 2012. Sudden sensorineural hearing loss: a vascular cause? Analysis of prothrombotic risk factors in head and neck. *Int. J. Audiol.* 51, 800–805.
- Mathews, J., Kumar, B.N., 2003. Autoimmune sensorineural hearing loss. *Clin. Otolaryngol.* 28, 479–488.
- Matsuoka, A.J., Harris, J.P., 2013. Autoimmune inner ear disease: a retrospective review of forty-seven patients. *Audiol. Neurotol.* 18, 228–239.
- Matsuoka, H., Kwon, S.S., Yazawa, Y., Barbieri, M., Yoo, T.J., 2002. Induction of endolymphatic hydrops by directly infused monoclonal antibody against type II collagen CB11 peptide. *Ann. Otol. Rhinol. Laryngol.* 111, 587–592.
- Matteson, E.L., Fabry, D.A., Facer, G.W., Beatty, W., Driscoll, C.L., Strome, S.E., et al., 2001. Open trial of methotrexate as treatment for autoimmune hearing loss. *Arthritis Rheum.* 45, 146–150.
- Matteson, E.L., Fabry, D.A., Strome, S.E., Driscoll, C.L., Beatty, C.W., McDonald, T.J., 2003. Autoimmune inner ear disease: diagnostic and therapeutic approaches in a multidisciplinary setting. *J. Am. Acad. Audiol.* 14, 225–230.
- Mazlumzadeh, M., Lowe, V.J., Mullan, B.P., Fabry, D.A., Mc Donald, T.J., Matteson, E.L., 2003. The utility of positron emission tomography in the evaluation of autoimmune hearing loss. *Otol. Neurotol.* 24, 201–204.
- Mc Cabe, B.F., 1979. Autoimmune sensorineural hearing loss. *Ann. Otol. Rhinol. Laringol.* 88, 585–589.
- Mc Cabe, B.F., 1989. Autoimmune inner ear disease: therapy. *Am. J. Otol.* 10, 196–197.
- Mc Cabe, B.F., McCormick, K.J., 1984. Tests for autoimmune disease in otology. *Am. J. Otol.* 5, 447–449.
- Meta-Castro, N., Gavilanes-Plasencia, J., Ramirez-Camacho, R., Garcia-Fernandez, A., Garcia-Berrocal, J.R., 2018. Azathioprine reduces the risk of audiometric relapse in immune-mediated hearing loss. *Acta Otorrinolaringol. Esp.* 69, 260–267.
- Mijovic, T., Zeitouni, A., Colmegna, I., 2013. Autoimmune sensorineural hearing loss: the otology-rheumatology interface. *Rheumatology* 52, 780–789 (Epub ahead of print).
- Mora, R., Barbieri, M., Mora, F., Mora, M., Yoo, T.J., 2003. Intravenous infusion of recombinant tissue plasmonogen activator for treatment of patients with sudden and/or chronic hearing loss. *Ann. Otol. Rhinol. Laryngol.* 112, 665–670.
- Mora, R., Mora, F., Passali, F.M., Cordone, M.P., Crippa, B., Barbieri, M., 2004. Restoration of immune-mediated sensorineural hearing loss with sodium enoxaparin: a case report. *Acta Otolaryngol. Suppl.* 552, 25–28.
- Muñecas Rasilla, J., Ortiz Evan, L., Villarreal, I., Garcia-Berrocal, J.R., 2018. Can positron emission tomography support the characterization of immune-mediated inner ear disease? *Rev. Esp. Med. Nucl. Imagen. Mol.* 37 (5), 290–295.
- Naarendorp, M., Spiera, H., 1998. Sudden sensorineural hearing loss in patients with systemic lupus erythematosus or lupus-like syndromes and antiphospholipid antibodies. *J. Rheumatol.* 25, 589–592.
- Nair, T.S., Raphael, Y., Dolan, D.F., Parrett, T.J., Perlman, L.S., Brahmbhatt, V.R., et al., 1995. Monoclonal antibody induced hearing loss. *Hear. Res.* 83, 101–113.
- Nair, T.S., Kozma, K.E., Hoefling, N.L., Kommareddi, P.K., Ueda, Y., Gong, T.W., et al., 2004. Identification and characterization of choline transporter-like protein 2, an inner ear glycoprotein of 68 and 72 kDa that is the target of antibody-induced hearing loss. *J. Neurosci.* 24, 1772–1779.
- O'Malley, J.T., Nadol Jr, J.B., McKenna, M.J., 2016. Anti CD163 +, Iba1 +, and CD68 + cells in the adult human Inner ear: Normal distribution of an unappreciated class of macrophages/microglia and implications for inflammatory otopathology in humans. *Otol. Neurotol.* 37, 99–108.
- Oczan, M., Karakus, M.F., Gunduz, O.H., Tuncel, U., Sahin, H., 2002. Hearing loss and middle ear involvement in rheumatoid arthritis. *Rheumatol. Int.* 22, 16–19.
- Okano, T., Kelley, M.W., 2012. Stem cell therapy for the inner ear: recent advances and future directions. *Trends Amplif.* 16, 4–18.
- Orsoni, J.G., Laganà, B., Rubino, P., Zavota, L., Bacciu, S., Mora, P., 2010. Rituximab ameliorated severe hearing loss in Cogan's syndrome: a case report. *Orphanet. J. Rare Dis.* 5, 18.
- Passali, D., Damiani, V., Mora, R., Passali, F.M., Pssali, G.C., Bellussi, L., 2004. P0 antigen detection in sudden hearing loss and Meniere's disease: a new diagnostic marker? *Acta Otolaryngol.* 124, 1145–1148.
- Pathac, S., Stern, C., Vambutas, A., 2015. N-Acetylcisteine attenuates tumor necrosis factor alpha levels in autoimmune inner ear disease patients. *Immunol. Res.* 63, 236–245.
- Pathak, S., Elliot, G., Vivas, E.X., Bonagura, V.R., Vambutas, A., 2011. IL-1 $\beta$  is overexpressed and aberrantly regulated in corticosteroid nonresponders with autoimmune inner ear disease. *J. Immunol.* 186, 1870–1879.
- Penéda, J.F., Lima, N.B., Monteiro, F., Silva, J.V., Gama, R., Condé, A., 2018. Immune-mediated inner ear disease: diagnostic and therapeutic approaches. *Acta Otorrinolaringol. Esp.* Available from: <https://doi.org/10.1016/j.otorri.2017.08.008>.

- Perenyi, A., Toth, F., Nagy, A.A., Skrivan, J., Boucek, J., Gheorghe, D.C., et al., 2018. Early experience on a modern, thin cochlear implant family. A retrospective, international multicenter study. *J. Med. Life* 11, 146–152.
- Pham, B.-N., Rudic, M., Bouccara, D., Sterkers, O., Belmatoug, N., Bébéar, J.-P., et al., 2007. Antibodies to myelin protein zero (P0) protein as markers of auto-immune inner ear diseases. *Autoimmunity* 40, 202–207.
- Platt, M., Dilwali, S., Elackattu, A., Parikh, J.R., Stankovic, K.M., 2014. *Otolaryngol. Head Neck Surg.* 150, 460–463.
- Puccetti, A., Lunardi, C., 2010. The role of peptide libraries in the identification of novel autoantigen targets in autoimmune diseases. *Discov. Med.* 9, 224–228.
- Rahman, M.U., Poe, D.S., Choi, H.K., 2001a. Autoimmune vestibulo-cochlear disorders. *Curr. Opin. Rheumatol.* 13, 184–189.
- Ralli, M., D'Aguanno, V., Di Studio, A., De virgilio, A., Croce, A., Longo, L., et al., 2018. Audiovestibular symptoms in systemic autoimmune diseases. *J. Immunol. Res.* 2018, 5798103.
- Raut, V.V., Cullen, J., Cathers, G., 2001. Hearing loss in rheumatoid arthritis. *J Otolaryngol.* 30, 289–294.
- Riente, L., Bongiorni, F., Nacci, A., Migliorini, P., Segnini, G., Delle Sedie, A., et al., 2004. Antibodies to inner ear antigens in Meniere's disease. *Clin. Exp. Immunol.* 135, 159–163.
- Roberston, N.G., Cremers, C.W.R.J., Huygen, P.L.M., Ikezono, T., Krastins, B., Kremer, H., et al., 2006. Cochlin immunostaining of inner ear pathologic deposits and proteomic analysis in DFNA9 deafness and vestibular dysfunction. *Hum. Mol. Genet.* 15, 1071–1085.
- Rossini, B.A.A., Penido, N.O., Munhoz, M.S.L., Bogaz, E.A., Curi, R.S., 2017. Sudden sensorineural hearing loss and autoimmune systemic diseases. *Int. Arch. Otorhinolaryngol.* 21, 213–223.
- Ruckenstein, M.J., 2004. Autoimmune inner ear disease. *Curr. Opin. Otolaryngol. Head Neck Surg.* 12, 426–430.
- Safavi Naini, A., Ghorbani, J., Montazer Lotte Elahi, S., Beigomi, M., 2017. Otologic manifestations and progression in patients with Wegener's granulomatosis: a survey in 55 patients. *Iran. J. Otorhinolaryngol.* 29, 327–331.
- Salvinelli, F., Cancilleri, F., Casale, M., Luccarelli, V., Di Poco, V., D'Ascanio, L., et al., 2004. Hearing thresholds in patients affected by rheumatoid arthritis. *Clin. Otolaryngol.* 29, 75–79.
- Silva, C.H., Roscoe, I.C., Fernandes, K.P., Novaes, R.M., Lazari, C.S., 2002. Sensorineural hearing loss associated to Kawasaki disease. *J. Pediatr.* 78, 71–74.
- Solares, C.A., Hughes, G.B., Tuohy, V.K., 2003. Autoimmune sensorineural hearing loss: an immunologic perspective. *J. Neuroimmunol.* 138, 1–7.
- Solares, C.A., Edling, A.E., Johnson, J.M., Baek, M., Hirose, K., Hughes, G.B., et al., 2004. Murine autoimmune hearing loss mediated by CD4+ T cells specific for inner ear peptides. *J. Clin. Invest.* 113, 1210–1217.
- Sone, M., Schachern, P.A., Paparella, M.M., Morizono, N., 1999. Study of systemic lupus erythematosus in temporal bones. *Ann. Otol. Rhinol. Laryngol.* 108, 338–344.
- Stone, J.H., Francis, H.W., 2000. Immune-mediated inner ear disease. *Curr. Opin. Rheumatol.* 12, 32–40.
- Suckfull, M., 2002. Fibrinogen and LDL apheresis in treatment of sudden hearing loss: a randomized multicentre trial and the Hearing Loss Study Group Lancet 360, 1811–1817.
- Suzuki, H., Furukawa, M., Kumagai, M., Takahashi, E., Matsuura, K., Katori, Y., et al., 2003. Defibrinogenation therapy for idiopathic sudden sensorineural hearing loss in comparison with high-dose steroid therapy. *Acta Otolaryngol.* 123, 46–50.
- Takagi, D., Nakamaru, Y., Maguchi, S., Furuta, Y., Fukuda, S., 2002. Otologic manifestations of Wegener's granulomatosis. *Laryngoscope* 112, 1684–1690.
- Takagi, D., Nakamaru, Y., Maguchi, S., Furuta, Y., Fukuda, S., 2004. Clinical features of bilateral progressive hearing loss associated with myeloperoxidase-antineutrophil cytoplasmic antibody. *Ann. Otol. Rhinol. Laryngol.* 113, 388–393.
- Takahashi, T., Takahashi, K., Mernaugh, R., Drozdoff, V., Sipe, C., Schoecklmann, H., et al., 1999. Endothelial localization of receptor tyrosine phosphatase, ECRTP/DEP1, in developing and mature renal vasculature. *J. Am. Soc. Nephrol.* 10, 2135–2145.
- Tang, B., Jia, Y., Shi, Z., Shen, Y., Li, D., Huang, H., et al., 2018. Intratympanic injection of dexamethasone after failure of intravenous prednisolone in simultaneous bilateral sudden sensorineural hearing loss. *Am. J. Otolaryngol.* 39, 676–678.
- Teranishi, M., Uchida, Y., Nishio, N., Kato, K., Otake, H., Yoshida, T., et al., 2012. Polymorphisms in genes involved in oxidative stress response in patients with sudden sensorineural hearing loss and Ménière's disease in a Japanese population. *DNA Cell Biol.* 31, 1555–1562.
- Tomiyama, S., 2002. Experimental autoimmune labyrinthitis: assessment of molecular size of autoantigens in fractions of inner ear proteins eluted on the Mini Whole Gel Eluter. *Acta Otolaryngol.* 122, 692–697.
- Toubi, E., Ben-David, J., Kessel, A., Hals, K., Sabo, E., Luntz, M., 2004. Immune-mediated disorders associated with idiopathic sudden sensorineural hearing loss. *Ann. Otol. Rhinol. Laryngol.* 113, 445–449.
- Tsunoda, K., Akaogi, J., Ohya, N., Murofushi, T., 2001. Sensorineural hearing loss as the initial manifestation of polyarteritis nodosa. *J. Laryngol. Otol.* 115, 311–312.
- Tucci, D.L., Farmer Jr, J.C., Kitch, R.D., Witsell, D.L., 2002. Treatment of sudden sensorineural hearing loss with systemic steroids and valacyclovir. *Otol. Neurotol.* 23, 301–308.
- Tucci, M., Quatraro, C., Silvestris, F., 2005. Sjögren's syndrome: an autoimmune disorder with otolaryngological involvement. *Acta Otorhinolaryngol. Ital.* 25, 139–144.
- Tumati, B., Casoli, P., Parmeggiani, A., 1997. Hearing loss in Sjogren's syndrome. *Ann. Inter. Med.* 126, 450–453.
- Um, J.Y., Jang, C.H., Kim, H.L., Cho, Y.B., Park, J., Lee, S.J., et al., 2013. Proinflammatory cytokine IL-1 $\beta$  polymorphisms in sudden sensorineural hearing loss. *Immunopharmacol. Immunotoxicol.* 35, 52–56.
- Uri, N., Doweck, I., Cohen-Kerem, R., Greenberg, E., 2003. Acyclovir in the treatment of idiopathic sudden sensorineural hearing loss. *Otolaryngol. Head Neck Surg.* 128, 544–549.
- Vambutas, A., DeVoti, J., Goldofsky, E., Gordon, M., Lesser, M., Bonagura, V., 2009. Alternate splicing of Interleukin-1 receptor type II (IL1R2) in vitro correlates with clinical glucocorticoid responsiveness in patients with AIED. *PLoS One* 4, 1–9.
- Van Doornum, S., McColl, G., Walter, M., Jennens, I., Bhathal, P., Wicks, I.P., 2001. Prolonged prodrome, systemic vasculitis, and deafness in Cogan's syndrome. *Ann. Rheum. Dis.* 60, 69–71.

- Van Schoonhoven, J., Sparreboom, M., van Zanten, B.G., Scholten, R.J., Mylanus, E.A., Dreschler, W.A., et al., 2013. The effectiveness of bilateral cochlear implants for severe-to-profound deafness in adults: a systemic review. *Otol. Neurotol.* 34, 190–198.
- Van Wijk, F., Staecker, H., Keithley, E., Lefebvre, P.P., 2006. Local perfusion of the tumor necrosis factor alpha blocker infliximab to the inner ear improves autoimmune neurosensory hearing loss. *Audiol. Neurotol.* 11, 357–365.
- Vavricka, S.R., Greuter, T., Scharl, M., Mantzaris, G., Shitrit, A.B., Filip, R., et al., 2015. Cogan's syndrome in patients with inflammatory bowel disease—a case seriesECCO CONFER investigators *J. Crohns Colitis.* 9, 886–890.
- Vollersten, R., 1990. Vasculitis and Cogan's syndrome. *Rheum. Dis. Clin. North Am.* 16, 433–438.
- Vollertsen, R.S., McDonald, T.J., Younge, B., et al., 1986. Cogan's syndrome: 18 cases and a review of the literature. *Mayo Clin. Proc.* 61, 344–361.
- Vyse, T., Luxon, L.M., Walport, M.J., 1994. Audiovestibular manifestations of the antiphospholipid syndrome. *J. Laryngol. Otol.* 108, 57–59.
- Wang, H., Wu, K., Guan, J., Yang, J., Xie, L., Xiong, F., et al., 2018. Identification of four TMC1 variations in different Chinese families with hereditary hearing loss. *Mol. Genet. Genomic Med.* Available from: <https://doi.org/10.1002/mgg3.394>.
- Wang, J.R., Yuen, H.W., Shipp, D.B., Stewart, S., Lin, V.Y.W., Chen, J.M., et al., 2010. Cochlear implantation in patients with autoimmune inner ear disease including Cogan syndrome: a comparison with age- and sex-matched controls. *Laryngoscope* 120, 2478–2483.
- Weissen-Plenz, G., Sezer, Ö., Vahlhaus, C., Robenek, H., Hoffmeier, A., Tjan, T.D.T., et al., 2010. Aortic dissection associated with Cogans's syndrome: deleterious loss of vascular structural integrity is associated with GM-CSF overstimulation in macrophages and smooth muscle cells. *J. Cardiothorac. Surg.* 5, 66–70.
- Westerlaken, B.O., Stokroos, T.J., Dhooge, I.J., Wit, H.P., Albers, F.W., 2003. Treatment of idiopathic sudden sensorineural hearing loss with antiviral therapy: a prospective, randomised, double-blind clinical trial. *Ann. Otol. Rhinol. Laryngol.* 112, 993–1000.
- Witsell, D.L., Mulder, H., Rauch, S., Schulz, K.A., Tucci, D.L., 2018. Steroid Use for Sudden Sensorineural Hearing Loss: a CHEER Network study. *Otolaryngol. Head Neck Surg.* 159, 895–899.
- Yariz, K.O., Duman, D., Seco, C.Z., Dallman, J., Huang, M., Peters, T.A., et al., 2012. Mutations in OTOGL, encoding the inner ear protein otogelin-like, cause moderate sensorineural hearing loss. *Am. J. Hum. Genet.* 91, 872–882.
- Yehudai, D., Shoenfeld, Y., Toubi, E., 2006. The autoimmune characteristics of progressive or sudden sensorineural hearing loss. *Autoimmunity* 39, 153–158.
- Yeom, K., Gray, J., Nair, T.S., Arts, H.A., Telian, S.A., Disher, M.J., et al., 2003. Antibodies to HSP-70 in normal donors and autoimmune hearing loss patients. *Laryngoscope* 113, 1770–1776.
- Yoo, T.J., Du, X., Known, S.S., 2002. Molecular mechanism of autoimmune hearing loss. *Acta Otolaringol. Suppl.* 548, 3–9.
- Yu, G.H., Choi, Y.J., Jung, H.J., Lim, Y.S., Park, S.W., Cho, C.G., et al., 2018. A comparison of single-dose and multiple divided daily-dose oral steroids for sudden sensorineural hearing loss. *Braz. J. Otorhinolaryngol.* Available from: [https://doi.org/10.1016/j.bjorl.2018.06.001pii:S1808-8694\(18\)30425-7](https://doi.org/10.1016/j.bjorl.2018.06.001pii:S1808-8694(18)30425-7).
- Yue, W.L., Li, P.Y., Qi, P.Y., Li, H.J., Zhou, H., 2003. Role for low-molecular-weight heparins in the treatment of sudden hearing loss. *Am. J. Otolaryngol.* 24, 328–333.
- Zhao, Z.S., Granucci, F., Yeh, L., Schaffer, P.A., Cantor, H., 1998. Molecular mimicry by herpes simplex virus-type 1: autoimmune disease after viral infection. *Science* 279, 1344–1347.
- Zhou, B., Kermany, M.H., Glickstein, J., Cai, Q., Cai, C., Zhou, Y., et al., 2011a. Murine autoimmune hearing loss mediated by CD4 + T cells specific for β-tubulin. *Clin. Immunol.* 138, 222–230.
- Zhou, B., Kermany, M.H., Cai, C., Zhou, Y., Nair, U., Liu, W., et al., 2012. Experimental autoimmune hearing loss is exacerbated in IL-10-deficient mice and reversed by IL-10 gene transfer. *Gene Ther.* 19, 228–235.
- Zhou, Y., Yuan, J., Zhou, B., Lee, A.J., Lee, A.J., Ghawji, M. Jr, et al., 2011b. The therapeutic efficacy of human adipose tissue-derived mesenchymal stem cells on experimental autoimmune hearing loss in mice. *Immunology* 133, 133–140.

## Further Reading

- Harris, J.P., Sharp, P., 1990. Inner ear autoantibodies in patients with rapidly progressive sensorineural hearing loss. *Laryngoscope* 100, 516–524.
- Kempf, H.G., 1989. Ear involvement in Wegener's granulomatosis. *Clin. Otorhinolaryngol.* 14, 451–456.

# Autoimmune and Autoantibody-Associated Encephalomyopathies

Ralf Gold<sup>1</sup>, Ilya Ayzenberg<sup>1,2</sup> and Kalliopi Pitarokoili<sup>1</sup>

<sup>1</sup>Department of Neurology, Ruhr University, St. Josef-Hospital, Bochum, Germany <sup>2</sup>Department of Neurology, Sechenov First Moscow State Medical University, Moscow, Russia

## O U T L I N E

Introduction	1067	Behçet's Disease	1076
Systemic Immunopathic Disorders With Encephalitis and Myelitis		Sarcoidosis	1077
Systemic Vasculitides	1068	Antibody-Associated Diseases of the Central Nervous System	1078
Large Vessel Vasculitides	1068	General Considerations	1078
Medium Vessel Vasculitides	1069	Antibody-Associated Clinical Syndromes	1083
Small Vessel—Antineutrophil Antibodies Positive Vasculitides	1070	Target Antigens: Main Functions and Role in the Central Nervous System Autoimmunity	1093
Small Vessel—Antineutrophil Antibodies Negative Vasculitides	1071		
	1073	References	1099

## INTRODUCTION

Autoimmune central nervous system (CNS) affection comprises an expanding group of potentially treatable disorders that should be included in the differential diagnosis of any type of encephalitis or myelitis.

The extent of CNS involvement in systemic immunopathic disorders such as lupus erythematosus, rheumatoid arthritis (RA), or sarcoidosis has been recognized since long. However, the identification of underlying pathogenic mechanisms has led to the development of revolutionary antibody (Ab)-based therapies improving the prognosis of this group of patients.

Further advances in autoimmune involvement of the nervous system have led to the identification of new clinical syndromes, associated with antineuronal Abs that have transformed the diagnostic and therapeutic approach to these disorders. Starting with auto-Abs to the acetylcholine receptor for myasthenia gravis and against intracellular antineuronal nuclear Ab 1 (ANNA-1) (Hu) antigen, there is still a continuous expansion of the number of cell surface, synaptic, and intracellular molecules, which expose antigenic epitopes for autoimmune neurological disorders. Numerous of these Abs were associated with an extraneuronal malignancy, causing “paraneoplastic neurological syndromes,” while others occur as primary autoimmune diseases. Furthermore, identification of several Abs targeting glial antigens, such as aquaporin-4 (AQP4), has enabled the classification of these disorders as distinct clinical entities.

The particular focus of this chapter is on autoimmune disorders associated with encephalitis or myelitis, subdivided into two groups: (1) systemic diseases with CNS manifestations and (2) Ab-associated disorders of the CNS.

## SYSTEMIC IMMUNOPATHIC DISORDERS WITH ENCEPHALITIS AND MYELITIS

### Systemic Vasculitides

#### **Definition and Classification**

Vasculitides constitute a heterogeneous group of immune-mediated vascular disorders characterized by inflammation and necrosis of the blood vessel wall. Their occurrence can be primary or secondary to a broad variety of systemic infectious, malignant, or connective tissue diseases, whereas they are responsible for marked morbidity and societal burden. Their pathogenic mechanisms have not been fully understood; the initial inflammation can be mediated by circulating auto-Abs, immune complexes, and cell-mediated mechanisms.

An overview of the different vasculitides taking into account the size of affected vessels, histological characteristics, suspected pathogenetic mechanisms with reference to CNS involvement is presented in [Table 56.1](#) (Jennette and Falk, 2007).

#### **Neurological Manifestation**

The spectrum of cerebral vasculitic involvement includes self-limiting benign variants, as well as severe, relapsing, or chronic progressive forms leading to high morbidity and mortality. The involvement of the peripheral nervous system (PNS), often characterized by a painful mononeuritis multiplex, normally precedes the cerebral manifestation. The clinical vasculitic picture differs from atherosclerotic vascular diseases mainly due to the presence of headache in combination with encephalopathic and focal neurological symptoms and a frequently earlier manifestation age.

#### **Central Nervous System Imaging**

In cases of suspected vasculitis as well as for treatment monitoring, a cerebral magnetic resonance (MR) imaging (MRI) with contrast medium including MR-angiography (MRA) should be performed. MRI should include diffusion-weighted images for detection of recent ischemia and T2\*-weighted gradient echo sequences for detection of previous microhemorrhages (Pomper et al., 1999).

Various lesion patterns are associated with cerebral vasculitis: multiple subcortical lesions, cortical lesions, and lesions in the basal ganglia. Individual parenchymal lesions and the meninges may show a contrast enhancement. In the case of a large-vessel involvement, a contrast enhancement of the vessel wall can occasionally be detected as a correlate of the inflammatory vessel wall thickening (Pfefferkorn et al., 2010; Moore and Richardson, 1998).

**TABLE 56.1** Neurological Manifestation of Systemic Vasculitides

Frequency of neurological symptoms	Large vessel		Medium vessel cPAN	Small-vessel (ANCA positive)				Small-vessel (immune complex mediated)				
	GCA	Takayashu arteritis		WG	MPA	EGPA	Cryoglobulinemia	SLE	RA	Sjögren syndrome	Behcet syndrome	
PNS (%)	5–15	0	50–75	15	<1	50–78	10		13	20	20	5
CNS (%)	10	10–36	24–40	23–50	<1	<5	10		10	<5	<5	10–40
Acute encephalopathy	(+)	(+)	+	(+)	0	0	(+)		(+)	(+)	(+)	+
Memory loss	+	(+)	+	+	0	0	0		(+)	(+)	0	(+)
Behavioral disorders	(+)	(+)	(+)	0	0	0	0		(+)	(+)	0	+
Epilepsy	0	+	+	+	0	(+)	(+)		(+)	(+)	(+)	(+)
Other focal signs	(+)	+	+	+	0	(+)	(+)		(+)	(+)	(+)	+
Cranial nerves involvement	+	+	(+)	+	0	(+)	0		(+)	(+)	(+)	+
Spinal symptoms	0	(+)	(+)	(+)	0	0	0		(+)	(+)	(+)	(+)
Meningeal symptoms	0	0	(+)	+	(+)	0	(+)		(+)	(+)	(+)	+
Cerebral hemorrhages	0	(+)	(+)	(+)	0	(+)	(+)		(+)	(+)	0	(+)

ANCA, Antineutrophil antibodies; GCA, giant cell arteritis; cPAN, classical panarteritis nodosa; WG, Wegener's granulomatosis; MPA, microscopic polyangiitis; EGPA, eosinophilic granulomatosis with polyangiitis (formerly Churg–Strauss syndrome); SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; PNS, peripheral nervous system; CNS, central nervous system; +, often, (+), not so often; 0, rare or not reported.

Digital subtraction angiography can detect vessels up to 0.1 mm in diameter and is superior to the MRA in the representation of caliber irregularities (Demaerel et al., 2004). Vascular irregularities, stenoses, vasocompressions, or aneurysmal dilatations are not specific for vasculitis and are also present in noninflammatory vascular processes (e.g., in reversible vasoconstriction syndrome). The specificity of the DSA is, therefore, low (14%–65%, Kadkhodayan et al., 2004). In the absence of extracranial atherosclerotic lesions, intracerebral changes imply a probable vasculitis. The sensitivity of angiography varies between 76% and 94% (Duna and Calabrese, 1995; Chu et al., 1998; Demaerel et al., 2004; Kobayashi et al., 2005).

### **Biopsy**

Due to the severe side-effects of the immunosuppressive treatment of cerebral vasculitis with cyclophosphamide and steroids, the greatest possible diagnostic certainty is required. In patients without bioptic evidence, immunosuppressive treatment does not provide any prognostic advantage (Alreshaid and Powers, 2003).

For systemic vasculitides with CNS involvement, the biopsy should be taken from a clinically affected organ. "Blind biopsies" (e.g., of skin, muscle, sural nerve) do not offer any advantage. Histological confirmation is not necessary if the diagnostic criteria are fulfilled and clinical and radiological involvement of the CNS is present.

An additional advantage of CNS biopsy is the exclusion of other treatable causes such as chronic, indolent inflammation of the basal meninges (tuberculosis, fungi, sarcoidosis), neoplasia, or other noninflammatory angiopathies (e.g., amyloid angiopathy). The brain biopsy has a sensitivity of about 75% and a specificity of 80%. The risk of side effects is estimated to be 1%–5%, so that the indication for biopsy should be restricted to patients with suspected vasculitis after passing through the noninvasive diagnosis. The complication risk is certainly lower than the morbidity risk of long-term cyclophosphamide therapy (Duna and Calabrese, 1995).

## **Large Vessel Vasculitides**

### **Giant Cell Arteritis**

*Definition and epidemiology:* Giant cell arteritis (GCA, temporal arteritis, Horton's disease) is an inflammatory vasculopathy of medium and large arteries and the most common primary systemic vasculitis (Nordborg and Bengtsson, 1989, 1990). Predilection sites are external branches of the carotid artery (Arteria temporalis, rarely occipital artery), the A. ophthalmica and Arteriae ciliates posterior (30%) and the aortic arch and its branches (distal subclavian, axillary arteries) (15%–30%). Only rarely (<1%) the intracranial vessels (mostly posterior circulation) or other organ systems (Salvarani et al., 2012) are afflicted. Approximately 50% of the patients with giant-cell arteritis may also present with polymyalgia rheumatica before, at the time of, or after the diagnosis of vasculitis (Kermani et al., 2013; González-Gay et al., 2017; Dejaco et al., 2017).

*Immune mechanisms:* Histologically, there is a granulomatous panarteritis of medium-sized and large arteries with giant cells, lymphomononuclear infiltration, and narrowing of the lumen by intimal proliferation. Two major immune response networks have been identified: the interleukin-12-type 1 helper T-cell (Th1)–interferon- $\gamma$  axis and the interleukin-6-type 17 helper T-cell (Th17)–interleukin-17 or interleukin-21 axis; only the latter is effectively suppressed with glucocorticoid treatment (Kermani and Warrington, 2013). A genetic predisposition (association with HLA-DR4 or DRB1 \* 04 alleles) has been also reported. Various infectious agents (varicella zoster virus (VZV), *Mycoplasma pneumoniae*, parvoviruses, chlamydiae) have been discussed as putative environmental triggers (Weyand and Goronyz, 2013).

*Neurological manifestations:* The most common neurological symptom (70%–85%) is a new-onset severe headache of throbbing or piercing quality, often unilateral, usually frontotemporal, with a prominent and tender temporal artery (30%–60%). Sometimes jaw muscle claudication is present (30%–40%). Headache often precedes further symptoms such as inappetence, weight loss, and general malaise.

Involvement of the ciliary arteries occurs in 30% of the cases and can lead to sudden unilateral or bilateral vision loss or diplopia (15%–45%). The (often irreversible) blindness caused by anterior ischemic optic neuropathy can follow transient visual disturbances mimicking amaurosis fugax. An involvement of the ocular muscles can lead to pain during eye movement, double vision, and ptosis. Significantly less frequent is the involvement of other brain nerves (Nesher, 2014). Cerebral ischemia, transient ischemic attacks, and other neuropsychiatric manifestations are present in cases of involvement of intracranial arteries mostly of the posterior cerebral circulation (<15%). Vestibulo-auditory manifestations include hearing loss, tinnitus, vertigo, and are present in 5%–25% of the cases. The PNS (14%) can be affected in the form of a mononeuritis multiplex (<10%) (Caselli et al., 1988).

**CNS imaging:** The high-resolution ( $\geq 10$  MHz) color-coded duplex sonography can be used to visualize superficial arteries, with the halo sign (vessel wall edema) distinguishing vasculitis from arteriosclerotic changes (median sensitivity 88% and specificity 97% in 13 studies (Schmidt and Blockmans, 2005). MRA or computed tomographic angiography (CTA) shows wall thickening and increased intramural hematoma, which may not be reversible with treatment and should not be used to assess the inflammatory burden or disease activity. Intramural leaky microvessels give rise to delayed enhancement of the arterial wall, which is consistent with but not specific for inflammatory activity. Traditional angiography is now reserved for planning revascularization procedures, when required. 18F-fluorodeoxyglucose (FDG) PET can detect the increased metabolic activity in the inflamed vessel wall with higher sensitivity than MRI in patients with corresponding symptoms of large vessel vasculitis, but negative temporal biopsy and excludes an occult malignancy (Bley et al., 2005a,b).

**Biopsy:** In cases of suspected giant-cell arteritis, histologic verification of vasculitis should be sought by means of a temporal artery biopsy with assessment of a clinically and sonographically affected vascular segment that is 1.5–2.0 cm in length. A negative biopsy finding does not rule out giant-cell arteritis as it identifies 85%–95% of the cases (Kermani et al., 2013). Treatment prior to biopsy is unlikely to affect the test result, but the biopsy should not be delayed beyond 1–2 weeks of glucocorticoid therapy begin (Achkar et al., 1994; Narváez et al., 2007).

**Treatment:** Prednisone remains the cornerstone of treatment for GCA. No glucocorticoid-sparing agents have been approved for the treatment of giant-cell arteritis; however, a broad spectrum of secondary agents is used in patients with giant-cell arteritis (methotrexate, cyclophosphamide, azathioprine, and antimalarial agents) (Kötter et al., 2012). Randomized controlled trials of anti-TNF- $\alpha$  therapy in GCA have showed disappointing results (Martinez-Taboada et al., 2008; Seror et al., 2014; Roberts and Clifford, 2017). The clinical effectiveness of a treatment with methotrexate can be assessed after approximately 6 months of treatment (Dasgupta et al., 2010; Mukhtyar et al., 2009; Bienvenu et al., 2016).

Elevated levels of IL-6 have been found in both the inflamed arteries and peripheral circulation of patients with GCA (Garcia-Martinez et al., 2010) and data of GiACTA clinical study, showed that subcutaneous tocilizumab (humanized monoclonal Ab against IL-6-receptor, 162 mg weekly or every other week) was superior to prednisolone monotherapy, although serious infections of the respiratory tract and skin/soft tissue have occasionally been described. Other promising options for future consideration include abatacept (CD80, CD86-signal blocker) and ustekinumab (anti-IL-12, anti-IL-23 p40 unit Ab) (Villiger et al., 2016; Stone et al., 2016, 2017; Roberts and Clifford, 2017).

## Medium Vessel Vasculitides

### **Classical Polyarteritis Nodosa**

**Definition and epidemiology:** Classical polyarteritis nodosa (PAN) (cPAN) is a severe, systemic, necrotizing inflammation of the medium-sized arteries without the involvement of smaller vessels. The microscopic form of polyarteritis associated strongly with *p*-antineutrophil Abs (*p*-ANCA)/myeloperoxidase (MPO) should be separated from the classical disease (Jennette et al., 2013). Neurological symptoms occur in both forms, yet more frequently in the classical form. The diagnosis of cPAN should be based on the American College of Rheumatology criteria after exclusion of other small-vessel vasculitides (Watts et al., 2007). More than 50% of the patients have an HBsAg carrier status, so that an infection-associated pathogenesis mediated by immune complexes is suspected. PAN with and without HBV association differ in aspects of clinical course, outcome, and response to treatment; the presence of Hepatitis virus goes along with a high incidence of mononeuritis multiplex and a worse prognosis (Özen et al., 2006).

**Immune mechanisms:** The exact pathogenesis of PAN largely remains unknown (Guillevin et al., 2005). It is probably a type III or immune complex reaction (Trepo et al., 1974; Zuckerman, 1976). The presence of large masses of HBs antigen immune complexes in the recent vascular lesions, lesser amount of these complexes in regressing lesions, and their absence in healed lesions points toward the primary role played by the immune complexes. The interaction of immunocomplexes, Fc $\gamma$  receptors and adhesion molecules probably lead to disturbances in transmigration and activation of polymorphonuclear neutrophils, with consequent vessel damage (Sindrilaru et al., 2007; Scott et al., 1982).

**Neurologic manifestations:** They are among the main clinical characteristics of PAN patients (79%) but since most of them are restricted to the PNS (painful mononeuritis multiplex and distal symmetric sensorimotor polyneuropathy, 50%–75%), 4%–20% of the patients show CNS involvement (Pagnoux et al., 2010; Sharma and Sharma, 2013). This usually occurs only in the later course of the disease and includes a variable

combination of headache (34%), retinopathy (32%), and encephalopathy (23%). Focal symptoms such as hemiparesis due to ischemic lesions or cerebral hemorrhage, epilepsy, and cranial nerve involvement are less frequent (10%). In MRA/DSA multiple-vessel stenoses of medium-sized cerebral and early signs of cerebral ischemia have been described (Provenzale and Allen, 1996). Occasionally there is a spinal cord affliction. The prognosis clearly depends on the organ manifestations (five-factor score, FFS). The 5-year survival probability decreases from 90% with an FFS from 0% to 65% with an FFS of  $\geq 2$  (Guillemin et al., 1995; Gayraud et al., 2001). A combined biopsy of muscle and nerve demonstrates the necrotizing granulomatous inflammation (Khellaf et al., 2007).

*Treatment and prognosis:* In severe cases relapses with neurologic, renal, or cardiac manifestations are frequent. In PAN with negative hepatitis serology induction treatment is started with prednisone and cyclophosphamide. In cases of rapid clinical progression plasmapheresis may be tried. In the case of positive hepatitis serology prednisone is combined with modern virustatics such as lamivudine (in hepatitis B) or interferone alpha and ribavirine (in hepatitis C). Alternatively, rituximab or intravenous immunoglobulins (IVIGs) may be used, the former NOT in the presence of florid hepatitis. The 5-year survival period of the PAN was previously without immuno-suppression around 13%. The combination with cyclophosphamide significantly increased the 5-year survival time to 75%–85% (Gayraud et al., 2001).

## Small Vessel—Antineutrophil Antibodies Positive Vasculitides

ANCA-associated vasculitis is a heterogeneous group of rare diseases potentially involving all organs and systems. During the last years, it has become increasingly evident that it is the specificity of ANCA, rather than clinical diagnosis of granulomatosis with polyangiitis (GPA) or microscopic polyangiitis (MPA) that influences the disease phenotype.

### **Granulomatosis With Polyangiitis (Formerly Wegener's Granulomatosis)**

*Definition and epidemiology:* This small vessel arteritis is characterized by a necrotizing granulomatous inflammation of the blood vessels of the respiratory tract and later in the disease course by glomerulonephritis (Elefante et al., 2017). In Caucasians it is associated with the detection of cytoplasmic ANCA (c-ANCA) against proteinase 3 (c-ANCA-PR3), whereas in Asians dominating Abs against MPO are present. C-ANCA/PR3 are present only in 50% of the patients with limited disease and in >90% of the systemic GPA cases. Interestingly, Rahmattulla et al. (2016) showed specific genetic associations of GPA patients with their ANCA phenotype: c-ANCA-PR3 showed a significant association with HLA-DPB1 and HLA-DPA1, whereas p-ANCA-MPO with HLA-DQA2 and HLA-DQB1 variants. Fibrosing interstitial disease and polyneuropathy are associated with a higher mortality (Miloslavsky et al., 2016).

*Immune mechanisms:* According to the widely recognized pathogenetic model of GPA, ANCA react with PR3 and MPO on the surface of neutrophils and increase their adhesion to the endothelium, which results to the production of reactive oxygen species, proteolytic enzymes, and subsequently to vessel damage (Yang et al., 2016).

A very important aspect relating to the role of neutrophils in GPA pathogenesis is the increased release of neutrophil extracellular traps (NETs) through neutrophil activation. NETs contain various proinflammatory mediators, such as histones, HMGB1, PR3, MPO, and neutrophil elastase that concur to vessel inflammation by damaging endothelial cells and by increasing complement activation. The initial events, which lead to the ANCA production, still need to be elucidated. Lepse et al. (2016) reported that endogenous and exogenous factors including TLR9 agonists, together with BAFF (B-cell activating factor) and IL-21, can promote PR3-ANCA production.

*Neurological symptoms:* Neurologic involvement in GPA has been described in 22%–50% of the GPA patients. When excluding cranial nerve palsies from CNS manifestations, CNS involvement is however rare, representing 7%–11% of the GPA patients (de Groot et al., 2001). Three different pathological CNS-involvement patterns are known:

1. Contiguous granulomatous CNS invasion from extracranial sites: In the limited stage of GPA, necrotizing granulomas of the nose and the paranasal sinuses may lead to compression of neighborhood structures with cranial nerve lesions (II, VI, VII, VIII), cerebral venous sinus thrombosis, diabetes insipidus, or exophthalmus. These patients have often a saddle nose deformity and an episcleritis.
2. Intracerebral, meningeal (pachymeningitis) or cerebral granulomatous lesions (Seror, 2006).

3. A manifestation of generalized GPA, with vasculitis of small-sized cerebral or spinal cord vessels: CNS parenchyma vasculitic involvement presents with ischemic stroke, intracerebral hemorrhages, and encephalopathic symptoms with or without seizures (10%) mostly at late stages of the disease in combination with rapid progressive glomerulonephritis.

The most typical and frequent PNS complication is mononeuritis multiplex (10%–22% or 30%–50%). Symmetrical sensorimotor polyneuropathy with relatively rapid progression, though rare, has also been reported (de Groot et al., 2001).

*CNS imaging:* Aseptic meningitis with enhancement of the basal meninges especially of the tentorium in MRI and the development of an occlusive or communicating hydrocephalus are possible findings. Conventional angiography is inconspicuous for small vessels. In MRI, however, T2-intense parenchyma lesions, hemorrhages, and strongly thickened and contrast agent-containing meninges (pachymeningitis) can be detected (Nishino et al., 1993).

*Biopsy:* The diagnosis is made by the histological examination of a biopsy specimen from the affected area; up to three biopsies may be necessary in the nasal mucosa area as the first biopsy is only diagnostic in 30% of the cases. In addition, biopsies can also be performed from muscle, skin, sural nerve, and especially the kidney.

### **Microscopic Polyangiitis**

MPA is a necrotizing nongranulomatous angiitis of small vessels without immune complex deposits and often associated with necrotizing glomerulonephritis and pulmonary capillaritis (pulmorenal syndrome). Usually pANCA/MPO-ANCA is found in over 90% of the patients. A case of neurological involvement in the form of a hypertrophic pachymeningitis has been reported (Furukawa et al., 2004). The clinical characteristics of small vessels vasculitis in the MPA are similar to those of the GPA.

*Treatment and prognosis for GPA/MPA:* Standard treatment of GPA/MPA in the generalized disease stage with CNS involvement is the combined administration of cyclophosphamide and prednisolone (response rate over 90%, 5-year survival rate 85%, de Groot et al., 2001). However, the side effects include severe infections, the risk of secondary tumors (bladder cancer, lymphoma), and ovarian failure. Based on the results of CYCAZAREM study, treatment deescalation to azathioprine is performed after reaching a remission with cyclophosphamide during the first 12–24 months of treatment (Walsh et al., 2014). Methotrexate and azathioprine were equally effective (Pagnoux et al., 2008). Mycophenolate mofetil is classified as substance of second choice (Hiemstra et al., 2010; Unizony et al., 2016).

Results from two studies published in 2010 (RAVE: Stone et al., 2010; RITUXVAS: Jones et al., 2010) demonstrated a similar efficacy of cyclophosphamide and rituximab (375 mg/m<sup>2</sup> per week for 4 weeks). The secondary malignancy risk was lower in rituximab-treated patients than in those treated with cyclophosphamide and similar to that of the general population (van Daalen et al., 2017; Yates et al., 2016; McGeoch et al., 2016). Further studies have shown a therapeutic potential for ofatumumab, a fully humanized mAb directed against a distinct extracellular epitope of CD20 (McAdoo et al., 2016) and of an orally administered inhibitor of C5a complement receptor (CCX168, CLEAR-Study). IVIG has exerted a beneficial effect as adjunctive therapy (Crickx et al., 2016), whereas a recently published case report of a patient with refractory MPA introduced a potential role for bortezomib, a proteasome inhibitor approved for the treatment of multiple myeloma and that has already showed promising results in systemic lupus erythematosus (SLE) (Novikov et al., 2016a,b).

### **Eosinophilic Granulomatosis With Polyangiitis (Formerly Churg–Strauss Syndrome)**

*Definition and epidemiology:* Eosinophilic GPA (EGPA) is an entity widely considered at a cross-road of ANCA positive vasculitis and hypereosinophilic-associated conditions (Harrold et al., 2005).

EGPA typically develops into three sequential phases: the allergic phase, distinguished by the occurrence of asthma, allergic rhinitis, and sinusitis; the eosinophilic phase, in which the main pathological finding is the eosinophilic organ infiltrations (e.g., lungs, heart, and gastrointestinal system); and the vasculitic phase, characterized by purpura, peripheral neuropathy, and constitutional symptoms. p-ANCA/MPO are present in 40%–60% of the patients (Sablé-Fourtassou et al., 2005). These show an increased frequency of vasculitic renal, lung, and CNS involvement. Patients without ANCA present more frequently with an eosinophilic form of cardiac disease and pulmonary infiltrates (Grau, 2008).

*Immune mechanisms:* The neurological findings in allergic granulomatosis are caused by systemic necrotizing vasculitis, with the eosinophilic infiltrate affecting small vessels. The disease is probably the result of a complex interaction in which genetically and environmental factors lead to an inflammatory response whose principal

players are eosinophils, T, and B lymphocytes. Among the acquired pathogenetic factors, the exposure to different allergens, infections, vaccinations, drugs, and silica exposure have been involved (Gomez-Puerta et al., 2013). An association with HLA-DRB1\*04 and \*07 (Wieczorek et al., 2008a) and with HLA-DRB4 (Vaglio et al., 2007) has been proven. EGPA ANCA-negative subset has been associated with the interleucin-10.2 haplotype of the IL-10 promoter gene, a condition, which leads to an increased production of IL-10 (Wieczorek et al., 2008b). This is apparently in line with EGPA pathogenesis, which is characterized by an increased Th-2 response and IgG4 levels, both of which seem to be mediated by IL-10.

*Neurological manifestations:* CNS events are rare and resemble those of PAN (Puechal et al., 2008; Jennette et al., 2013; Wechsler et al., 2009). CNS involvement may include paralysis of seventh cranial nerve and phrenic nerve palsy, cerebral, or subarachnoid hemorrhage or cerebral infarction, seizures, and coma (Sehgal et al., 1995; Sablé-Fourtassou et al., 2005).

On the other hand, PNS involvement is noted in about 50%–78% of the patients, mainly in the form of painful mononeuritis multiplex and rarely as symmetric mild sensory axonal neuropathy. The neuropathy is caused mainly by nerve ischemia due to occlusion of vasa nervorum; with pANCA probably playing a key pathogenetic role. Symmetrical polyneuropathies possibly caused by eosinophil infiltrates are seen in the ANCA negative group. Nerve biopsy is characteristic and useful to confirm diagnosis (Chao et al., 2007).

*Treatment/prognosis:* Cyclophosphamide (6–12 pulses) in combination with steroids is recommended in case of CNS participation (Cohen et al., 2007). The remission rate is 80%–90%. Patient survival varies between 60% and 97% at 5 years (Mukhtyar et al., 2009; Ribi et al., 2008). Azathioprine, methotrexate, or interferon- $\alpha$  is used after reaching remission. Various case series reported the efficacy of rituximab; in long-term observations studies a decline in serum immunoglobulin levels on long-term rituximab treatment has been reported (Mohammad et al., 2016; Novikov et al., 2016a,b; Thiel et al., 2017). Results of a phase 3 study in patients with treatment refractory EGPA show an efficacy of the monoclonal anti-IL-5 Abs (high-affinity receptor binding site on IgE) mepolizumab (Wechsler et al., 2017).

## Small Vessel—Antineutrophil Antibodies Negative Vasculitides

### Rheumatoid Arthritis

*Definition:* RA is the most common chronic disease of the connective tissue, mainly affecting the joints. Extraarticular features are observed in up to 40% of the patients and are associated with increased morbimortality. Vasculitis is a known complication of RA, often occurring several years after the initial onset of disease (Scott et al., 2010).

*Immune mechanisms:* In RA and SLE there is evidence of a diminished relative percentage of CD4 $^{+}$ CD25 $^{+}$  regulatory T cells and for auto-Abs production such as antiendothelial cell Abs. These as well as circulating immune complexes are thought to activate endothelial cells, leading to upregulation of adhesion molecules and other major histocompatibility class II molecules. Of importance, endothelial cells play a crucial role in inflammation and coagulation, as their activation leads to a procoagulatory/prothrombotic status. Recent theories on the pathogenesis of RA suggest that the synovial cells of these patients chronically express an antigen that triggers the production of rheumatoid factor and polymorphonuclear leukocyte infiltration (Fernández et al., 2004).

## NEUROLOGICAL MANIFESTATIONS

The following mechanisms lead to nervous system involvement:

### 1. Systemic inflammatory processes, resulting to pachymeningitis or vasculopathy/vasculitis.

The exact incidence of cerebral involvement in RA is low compared with spinal and peripheral nerve involvement. The patients may present with stroke-like episodes. Inflammation of the meninges may present with acute or progressive focal deficits, seizures, headache, and cranial nerve affection (Starosta and Brandwein, 2007). Definitive diagnosis requires a meningeal biopsy. Interestingly, these manifestations can occur even in the absence of systemic disease activity (Kurne et al., 2009; Aguilar-Amat et al., 2011).

MRI findings can demonstrate meningeal enhancement after contrast administration (pachymeningitis). White matter lesions with hypointense signal on T1-weighted sequence and hyperintense signal on T2-weighted sequence can suggest diffuse vasculitis. Cerebrospinal fluid (CSF) analysis may present with elevated white blood cells and normal to slightly elevated protein concentration (Joaquim and Appenzeller, 2015).

The main causes of peripheral neuropathy in RA can be divided in two groups: (1) compressive neuropathies (most frequently carpal tunnel syndrome secondary to tendosynovitis of the flexor tendons of the fingers) and (2) noncompressive neuropathies (20%, asymptomatic 85%) mostly painful mononeuritis multiplex but also axonal-length dependent sensorimotor neuropathy probably secondary to vasculopathy/vasculitis (Bayrak et al., 2010; Sim et al., 2014). Finally, an autonomic neuropathy characterized by auto-Abs specific for nicotinic acetylcholine receptors in the autonomic ganglia was described, whereas a decrease of Abs levels correlates with clinical improvement (Vernino et al., 2000).

2. Joint and bone destruction leading to neural compression (e.g., cervical myelopathy symptoms caused by destruction of the atlanto-axial joints) (Richioud et al., 2012; Blom et al., 2013).
3. Potential side effects of the medication used for RA treatment, such as corticosteroids, disease-modifying drugs, and biological agents.

Treatment with anti-TNF drugs can lead to cerebral or spinal demyelinating events (including optic neuritis) mostly from 1 week to 12 months after starting the agents, but delayed presentations are also described (Faillace et al., 2013; Kaltsonoudis et al., 2014). Demyelinating polyradiculoneuropathy has also been observed in RA patients on anti-TNF- $\alpha$  (Alshekhlee et al., 2010).

4. Accelerated atherosclerosis associated with systemic inflammation and auto-Abs.

Observational studies have shown that compared with healthy normal individuals, patients with RA are at double the risk for developing coronary artery disease and the assessment and treatment of risk factors, such as hypertension, diabetes, and obesity is mandatory (Solomon et al., 2003; Maradit-Kremers et al., 2005).

**Treatment—Prognosis:** Currently, corticosteroids and immunosuppressants such as cyclophosphamide are the mainstay of treatment for vasculitis (Krishnan et al., 2004; Hurlmann et al., 2002). The availability of biological immunomodulatory therapies, such as rituximab, tocilizumab, anti-TNF- $\alpha$  inhibitors (infliximab, certolizumab, etanercept) IL-1 blockers such as anakinra and T-cell costimulatory blocking agent (CTLA-4) abatacept, has potentially increased therapeutic options in systemic vasculitis. It has been reported that the combination of rituximab with methotrexate or anti-TNF- $\alpha$  inhibitors and methotrexate are more effective in improving symptoms compared to methotrexate alone, with etanercept appearing to be the safest TNF- $\alpha$  blocker (Aaltonen et al., 2012).

### **Systemic Lupus Erythematosus**

**Definition and epidemiology:** SLE is characterized by multisystem organ involvement, heterogeneity of clinical features, and varied severity levels, and similarities with other autoimmune diseases (Yu et al., 2014).

**Immune mechanisms:** The etiology of the loss of immunological tolerance for nuclear autoantigens in SLE is multifactorial, and includes environmental factors and genetic susceptibility.

Auto-Abs play the main role since most of lupus patients (approximately 95%) present high titers of autoreactive Abs mainly raised against nuclear antigens [such as double-stranded DNA (dsDNA) and ribonucleoproteins (RNPs)], which form circulating immune complexes. Immune complex deposition in target organs initiates and maintains the auto-inflammatory response (Tsokos et al., 2016).

**Autoantibodies:** Disease activity is known to correlate with auto-Ab titers, especially antidouble-stranded dsDNA IgG (Liu and Davidson, 2012). A secondary antiphospholipid syndrome (APS) is present in approximately 25% of all SLE cases. Most antiphospholipid Abs, in particular, anticardiolipin (aCL) and lupus anticoagulant (LAC), have been widely investigated in SLE with neuropsychiatric manifestations but were also brought in association with neurovascular manifestations, migraine headaches, and diffuse neurological manifestations including cognitive impairment (Sanna et al., 2003; Mikdashi and Handwerger, 2004; Govoni et al., 2012). In adult SLE patients, aCL has been found to be associated with neuropsychiatric involvement, whereas LAC was most strongly associated with stroke and in some studies with seizures (Zirkzee et al., 2012). Data from the SLICC study provide evidence that anti-RNP, which are present in 46% of the subjects is significantly associated with psychosis attributed to SLE (Eber et al., 2005; Isenberg and Ramsey-Goldman, 2006; Hanly et al., 2011).

The N-methyl-D-aspartate (NMDA) receptors (NMDAR), NR2a and NR2b, that bind the neurotransmitter glutamate are present on neuronal cells throughout the brain and play a role in many neurological functions including memory and learning (Zandman-Goddard et al., 2007). Studies in animal models of SLE have shown that a subset of anti-ds-DNA Abs cross-react with the extracellular, ligand-binding domain of NR2 receptors, thereby suggesting a plausible biological role for these auto-Abs in the pathogenesis of neuropsychiatric syndromes. Further studies revealed that anti-NR2 Abs can occur in up to 30% of the patients with SLE. Nevertheless, they are infrequent in the absence of detectable anti-dsDNA Abs and their presence in the circulation is not associated with any of the neuropsychiatric syndromes associated with SLE (Gono et al., 2011).

**Neurological manifestations:** The American College of Rheumatology proposed criteria for 19 distinct neuropsychiatric symptoms associated with SLE with 12 of them involving the CNS.

Approximately 13% of the patients with SLE present with the following CNS disorders: headache (39%–61%), mood and anxiety disorders (69%–74%), cognitive disorders (delirium, dementia, mild cognitive impairment, 75%–80%), cerebrovascular disease (stroke, transient ischemic attacks, and cerebral venous sinus thrombosis, 2%–8%), seizures (8%–18%), psychosis (3%–5%), cranial neuropathy (1.5%–2.1%), movement disorders (chorea or ataxia, 1%), aseptic meningitis, demyelinating syndromes; their mortality rate was 18.8% ([Chiewthanakul et al., 2012](#)). In patients with neuropsychiatric features attributed to SLE, these were the presenting signs in some 40% ([Padovan et al., 2012](#)).

Although risk factors for atherosclerosis in SLE patients are similar to those in patients with RA, there are additional SLE-related risk factors due to auto-Abs to endothelium, immunocomplexes, high-density lipoprotein, and phospholipids, as well as dyslipidemia, which contribute to the greatly increased cardiovascular risk.

Transverse myelitis with typical sensory, motor, and urinary dysfunction is described in 1%–2% of the SLE patients ([Schulz et al., 2012](#)). Birnbaum et al. distinguished two subgroups with myelitis: those with primarily long-tract signs (spasticity, hyperreflexia) and those with flaccid paralysis (indicating gray matter damage). The former group was more likely to meet criteria for neuromyelitis optica, and the second group had a worse outcome ([Birnbaum et al., 2009](#)). Episodes of transverse myelitis are accompanied by other signs of active SLE in only half of the cases, and in 25% of the patients with SLE-associated myelitis, there were no prior systemic signs of SLE ([Espinosa et al., 2010](#)).

The complications of the PNS in SLE are rare, and arise in approximately 10% of the patients, due to vasculitis. They include trigeminal neuropathy or distal symmetric sensory or sensorimotor polyneuropathy. The involvement of the neuromuscular synapse in coexisting myasthenia is rare (10%).

**MRI manifestation:** MRI shows T2 hyperintense lesions of the cervical or mid-lower thoracic spinal cord in some patients with less extensive disease and some of them with lesions involving more than four cord segments ([Kovacs et al., 2000](#); [Espinosa et al., 2010](#)). The inflammatory vasopathy is not detectable by MRA—a cerebral DSA is not helpful for diagnosis of a neuro-SLE. Although in individual studies a higher accuracy of PET and SPECT regarding the detection of inflammatory activity in SLE, MRI in combination with CSF diagnostics is usually sufficient for the clarification of a patients with neuropsychiatric manifestation ([Govoni et al., 2004](#)). Recent MR techniques such as MR spectroscopy, diffusion, and perfusion measurements may be used for the early detection of vascular lesions.

**Treatment and prognosis:** Cyclophosphamide is the most effective substance for the treatment of neuropsychiatric manifestations ([Ortmann and Klippel, 2000](#)). Mostly azathioprine or alternative mycophenolate mofetil are used after induction of remission ([Mok et al., 2002](#)). Antiplatelet/anticoagulation therapy is indicated when manifestations related to antiphospholipid Abs and particularly thrombotic cerebrovascular disease are present ([Ruiz-Irastorza et al., 2001](#)). Furthermore, medication side effects have to be considered in the differential diagnosis as high-dose corticosteroids can lead to psychopathological abnormalities.

The initial promising results from the last decade for the use of rituximab in SLE are in clear contrast with the poor results of the completed EXPLORER and LUNAR randomized controlled trials. In contrast to EXPLORER and LUNAR results controlled trials for belimumab (a humanized IgG1 monoclonal Ab that antagonizes BLyS, a B lymphocyte stimulator) showed positive results and subsequently, belimumab was the first drug approved for the treatment of SLE patients ([Hahn, 2013](#)). Further studies with anti-CD22 Abs (epratuzumab), anti-BLyS Abs (blisibimod, tabalumab), anti-IL-6 receptors (tocilizumab), anti-IL-1 receptor antagonists (anankira), antiinterferon-alpha (sifalimumab) showed positive preliminary results ([Cuadrado et al., 2013](#); [Bakshi et al., 2017](#)).

### Sjögren's Syndrome

**Definition:** Sjögren's syndrome (SS) is characterized by CD4<sup>+</sup> T-cell infiltration and destruction of salivary and lacrimal glands leading to loss of tears (keratoconjunctivitis sicca) and saliva (xerostomia). Primary SS is associated with enhanced risk for B-cell lymphoma, in which the presence of purpura or vasculitis is clinical risk indicators for future non-Hodgkin's lymphoma development ([Soliotis et al., 2004](#)).

**Immune mechanisms:** The vascular injury may be related to the presence of antineuronal Abs. SS-A/Ro Abs against extractable nuclear antigens occurs in patients with SS (up to 90% of the cases), however also in SLE (40%–60% of the cases) and RA. SS-B/La Abs are found primarily in patients with SS or SLE (60% or 15%, respectively). SS-B/La Abs occur only infrequently in the absence of SS-A/Ro Abs. A direct role of these Abs in damaging tissues is postulated ([Both et al., 2017](#)).

**Neurological manifestations:** Approximately 25% of the patients have features of PNS or CNS involvement and may precede the sicca symptoms in 40%–93% of the cases, most typically a sensory neuropathy or mononeuritis multiplex (Sène et al., 2011). Very characteristic is the ataxic sensory neuronopathy (ganglionitis) with pseudoathetosis, gait ataxia, and dysesthesia and is associated with antiganglionic acetylcholine receptor Ab (Soliotis and Moutsopoulos, 2004). It was recently observed that Abs against the type-3 muscarinic receptor may eventually explain part of the broader autonomic dysfunction found in patients with SS. There is often the involvement of the cerebral nerves, in particular of the trigeminal, facial, and vestibulocochlear nerve.

The true percentage of CNS affected patients is controversial, in part due to the wide diversity of manifestations such as encephalitis, cerebellitis, optic neuritis, aseptic meningitis, brainstem syndromes, spinal cord involvement, migraine, seizures and psychiatric symptoms (Soliotis et al., 2004). Alexander et al. (Alexander, 1993; Alexander et al., 1986) demonstrated intrathecal Ab synthesis in patients with encephalopathic SS, suggested vasculopathy as a pathogenic mechanism, and reported an association between the presence in serum of anti-Ro (SS-A) and more severe CNS disease. Estiasari et al. (2012) found that one-third of patients with SS, particularly those with spinal cord lesions or optic neuritis, had Abs to AQP4. The pathophysiology of anti-AQP4-negative patients with SS and CNS symptoms is unclear (Carvalho et al., 2014). The MRI changes of the SS may resemble those of multiple sclerosis. Since also mild lymphomonocytic pleocytosis, protein elevation, and positive oligoclonal bands can be detectable in the cerebrospinal fluid, the differential diagnosis is difficult in individual cases. However, in contrast to multiple sclerosis, oligoclonal bands are not only present in the IgG region, but also in the IgA and above all IgM band (Reske et al., 2005).

**Treatment and prognosis:** In general, intravenous corticosteroids are first-line therapy for patients with primary SS associated neuropathy. Methotrexate is also used in the treatment of arthritis in SS patients. Further options are mycophenolate mofetil, cyclosporine, azathioprine. Cyclophosphamide or IVIGs can be considered in patients who do not improve with corticosteroids (Skopouli et al., 1996).

Several studies have demonstrated a favorable effect of rituximab in approximately 60% of the patients after 6 months. As a consequence of rituximab treatment, serum BAFF levels are increasing in order to stimulate B-cell maturation, which can be countered by anti-BAFF treatment (belimumab) to achieve a longer B-cell depletion and associated longer treatment effect (Ramos-Casals et al., 2010; Pollard et al., 2013; Quartuccio et al., 2016). Currently, new potential anti-B-cell therapies are being evaluated in preclinical trials including anti-CD40 (decreases antigen presentation by B cells), anti-BAFF receptor (inhibits the effects of BAFF), antiinducible costimulatory ligand (ICOSL, decreases activation of T cells), and phosphoinositide 3-kinase delta inhibitor (PI3Kδ, inhibition of B-cell development and activation) (Sharma et al., 2016; Belkhir et al., 2014; Nakamura et al., 2013; Le et al., 2016).

## Behçet's Disease

**Definition and epidemiology:** Behçet's disease is a multisystem disease of vasculitic genesis which affects skin/mucous membranes (oral ulcers), eyes (relapsing uveitis), the joints (mono- or oligoarthritis), the gastrointestinal tract (mucous ulcers in the ileum or cecum), the pulmonary artery (pulmonary arteritis), and the aortic extremity vessels (thrombophlebitis, arteritis with the development of pseudoaneurysms) (Akman-Demir et al., 1999).

**Immune mechanisms:** Although the cause and pathogenesis are still unclear, possible factors contributing to the disease are infectious triggers, autoimmune-mediated processes, prothrombotic anomalies of the coagulation system, and a genetic predisposition. There is an association with circulating immune complexes and the tissue antigen HLA-B5 (Siva and Saip, 2009). There is evidence that natural killer T cells and distinct  $\gamma\delta$  T cells may also play a role. Pathological studies have revealed perivascular lymphocytic or neutrophilic infiltrates, especially in the basal ganglia, midbrain, and thalamus.

**Neurological manifestations:** Nervous system involvement occurs in about 30% of the patients after an average of 5 years, almost always involving the CNS (Borhani Haghghi et al., 2005). Of these, 80% present parenchymal disease (Mirsattari et al., 2004). Only 3% of the patients develop neurologic symptoms without mucocutaneous lesions or ocular symptoms (Akman-Demir et al., 1999).

The following parenchymal syndromes have been described:

- Brainstem (most frequently): Ophthalmoparesis, cranial nerves affection, symptoms of cerebellar, or pyramidal dysfunction.
- Multifocal (diffuse): Variable combination of brainstem signs and symptoms, cerebral or spinal cord involvement

- Myelopathy
- Cerebral: Sensory and motor symptoms and signs suggestive of cerebral hemispheric involvement as well as encephalopathic symptoms, optic neuropathy, seizures, cognitive impairment, and psychosis

The following nonparenchymal syndromes are described:

- Cerebral venous thrombosis ([Seyahi et al., 2012](#))
- Intracranial hypertension syndrome (pseudotumor cerebri, 20%)
- Acute meningeal syndromes

In the cerebrospinal fluid, 50% of the patients show pleocytosis and protein increase. Usually there is a lymphocytic, more rarely a mixed cell or predominantly granulocytic pleocytosis (0–485, median 30/ $\mu$ L) or isolated protein elevation. Whilst in 70% of the cases there is a pathological IgG index, oligoclonal bands are often only temporarily positive, whereas an increase of IL-6 in CSF has been reported ([Hatemi et al., 2008](#)).

*Cerebral imaging:* Interestingly, brain perfusion MRI showed decreased relative cerebral blood flow in patients with Behcet's disease with and without neurological symptoms ([Alkan et al., 2012](#)), suggesting that subclinical CNS involvement may be common. MRI typically shows contrast-absorbing extensive T2 lesions, preferably in the basal ganglia or in the brain stem. These lesions are not restricted in one vessel territory; no clear predisposition for periventricular regions can be seen; in 10%–20% of the cases the spinal cord is also affected ([Banna and el-Ramah, 1991](#)).

The vascular neuro-Behcet (20% of the total group) shows intracranial hypertension as a guiding symptom. Sinus vein thromboses are presented in MRA ([Akman-Demir et al., 1999](#)). A vasculitic inflammatory affection of the arteries is rare. In the vascular Behcet syndrome, cerebrospinal fluid is usually normal except for an increased opening pressure.

*Treatment and prognosis:* There is no evidence-based standard therapy for Behcet's syndrome with neurological manifestations. Combinations of high doses of corticosteroids and immunosuppressive drugs are recommended. Cyclophosphamide, methotrexate, interferon-alpha, or azathioprine (up to 3 mg/kg) may be used as immunosuppressive agents in parenchymal Behcet. Because of its potential neurotoxicity, cyclosporine A should not be used in the treatment of NB patients. Chlorambucil should be avoided because of its myelotoxicity and increased risk of malignancies ([Hatemi et al., 2008, 2009](#)). In treatment of refractory patients infliximab or etanercept may be tried ([Sfikakis et al., 2004](#)). Interferon-alpha was found to induce long-lasting remissions. Small observational studies with non-TNF biologics such as ustekinumab, anakinra, and canakinumab report beneficial results, which await confirmation ([Hatemi et al., 2017](#)).

## Sarcoidosis

*Definition:* Sarcoidosis is not formally characterized as an autoimmune disease, although this possibility has been raised from time to time. Sarcoidosis is defined as a multisystemic granulomatosis of unknown etiology affecting mainly young adults. The characteristic histology exhibits nonacidifying epithelioid-cell granulomas. Main manifestations are located in the lymph nodes of the lung hilus, the lung parenchyma, the skin, and the eyes.

*Neurological manifestations:* Neural tissues are clinically affected in 5%–13% of the patients, including peripheral nerves, cranial nerves, meninges, brain (including hypothalamus), and/or spinal cord ([Lacomis, 2011](#)) and in autopsy in up to 27% of the cases. This is the case in the early course of a systemic sarcoidosis and rather in younger patients (30–40 years) as in about half of the cases, the neurological symptoms are included in the initial presentation ([Joseph and Scolding, 2009](#)).

Ischemia of the nervous system is the first manifestation of sarcoidosis in about 50% and in the case of cerebral infarction even 85%. In over 90% of the patients with neurosarcoidosis, there are also other system manifestations mostly hilus lymphomas (80%) and eye disease (uveitis, keratoconjunctivitis) (50%).

Cranial nerve involvement of the optic, facial, or multiple cranial nerves is common (50%–70%) ([Joseph and Scolding, 2009; Pawate et al., 2009](#)).

The second most common manifestation is an aseptic meningitis (18%–26%), especially of the basal meninges with typical meningeal signs ([Gullapalli and Phillips, 2002](#)). The third most frequent manifestation is a hydrocephalus (9%–17%), either obstructive with granulomas in the region of the III. or IV. ventricle or communicating with an inflammatory occlusion of pachyionic granulations. Hypothalamus and pituitary gland (15%–26%) are particularly frequently infected presenting with diabetes insipidus, bulimic behavior, and hypersomnia. Cerebral, subdural, and meningeal (en plaque) mass lesions can also lead to epileptic seizures (about 20%). A myelopathy

due to compressive extramedullary or intramedullary granulomas is observed in 6%–10%, peripheral neuropathy (mainly type mononeuritis multiplex) in 4%–14%, and myopathy in 7%–12% (Stern, 2004). The clinical course is unpredictable in individual cases; in two-thirds of cases it is monophasic, recurring in one-third (Zajicek et al., 1999).

**Cerebral imaging:** The typical MRI finding is a knotted or also flat thickening of the meninges, especially on the cranial base with strong contrast enhancement (Smith et al., 2004). T2 lesions can also be found without a typical distribution, therefore depending on the MRI findings; the differential diagnosis is very broad (lymphoma, glioma, meningioma, tuberculosis, multiple sclerosis in predominantly periventricular lesions, leukemic or carcinomatous infiltrations, etc.). Therefore CSF analysis including bacteriology and cytology (to rule out infectious or neoplastic differential diagnoses) is necessary. In the case of isolated CNS affliction meningeal and/or cerebral biopsies depending on the MRI findings are necessary.

**Treatment and prognosis:** Patients refractory to initial therapy with corticosteroids should receive other immunosuppressive medications, including azathioprine, cyclophosphamide, and methotrexate (Lower et al., 1997; Scott et al., 2007; Joseph and Scolding, 2009; Lacomis, 2011), or even cranial irradiation (Chapelon et al., 1990; Agbogu et al., 1995). The actual immunological nature of the sarcoid process remains obscure. In view of the recognized morbidity and mortality of the neurosarcoidosis, most authors recommend early and aggressive therapy. However, there are currently no controlled studies for therapeutic guidelines (Hoitsma et al., 2004) Single cases were also successfully treated with mycophenolate mofetil, hydroxychloroquine, TNF- $\alpha$  blockers (infliximab), and monoclonal anti-CD20 Abs (rituximab) (Scott et al., 2007).

## ANTIBODY-ASSOCIATED DISEASES OF THE CENTRAL NERVOUS SYSTEM

### General Considerations

#### **Antibodies Targeting Intracellular and Cell Surface Antigens**

Neurological diseases associated with antineuronal Abs are mostly caused by a primary autoimmune- or tumor-induced (paraneoplastic) disturbance of immune tolerance to neuronal antigens. The intracellular or surface location of the target antigen is probably the most important factor, determining pathophysiological mechanisms as well as clinical course, treatment response, and prognosis of these diseases (Table 56.2).

The Abs directed against intracellular proteins are mostly associated with tumors (so-called obligatory paraneoplastic or onconeural) with an exception of glutamic acid decarboxylase (GAD)-Abs, which are paraneoplastic in the minority of cases only. Effective antitumor immune response can partly suppress tumor growth, however results in a breakthrough of the immunological tolerance to self-antigens. Accordingly, those tumors, expressing neuronal proteins, are of the highest relevance. Tumor size often remains relatively small, so that paraneoplastic syndromes (PNS) develop in two-thirds of the cases prior to cancer diagnosis (Giometto et al., 2010). The Abs against intracellular proteins have probably no direct pathogenetic role and serve, first of all, as an important diagnostic marker. Cell-mediated immune response has a leading pathogenetic role, resulting in progressive and often irreversible neurological deficits within a few weeks or months. Neuropathological studies reveal infiltration with CD8+ cytotoxic lymphocytes, inducing neuronal apoptosis by releasing perforin and granzyme B (Bauer and Bien, 2016). Compartmentalization of cellular immune response in the nervous system and irreversibility of neuronal cell loss limit therapy efficacy and determine generally unfavorable prognosis in these syndromes. Being nonpathogenic, Abs directed against the same intracellular antigen can be associated with a number of clinically distinct neurological syndromes as well as with different tumors (Pittock et al., 2004).

Detection of onconeural Abs confirms paraneoplastic nature of the disease; however, approximately 18% of the cases remain seronegative (Giometto et al., 2010). Some common clinical features support the suspicion of PNS in these cases:

- Subacute initial manifestation, followed by progression of symptoms over weeks or months;
- Multilocular clinical presentation (several structures of the central and PNS can be affected simultaneously);
- Age >45 years (few exceptions, e.g., anti-NMDAR encephalitis associated with teratoma);
- Symmetrical lesions on the cranial MRI, for example, in the cerebellum or temporal lobe, with initial contrast enhancement and atrophy in later stages (however, often MRI is unremarkable, especially in the early stage);
- Inflammatory changes in the CSF: isolated intrathecal immunoglobulin synthesis or identical oligoclonal bands in CSF and serum, moderate lymphocytic pleocytosis (<100/ $\mu$ L), blood–brain barrier disruption.

**TABLE 56.2** Antineuronal and Antiglial Antibodies: Associated Neurological Syndromes and Tumors

Target antigen	Clinical presentation <sup>a</sup>	Association with tumor
<b>OBLIGATE PARANEOPLASTIC ABS (DIRECTED AGAINST INTRACELLULAR ANTIGENS; TUMOR ASSOCIATION &gt;95%)</b>		
Hu (ANNA-1)	Polyneuropathy (mostly sensory), encephalomyelitis, limbic encephalitis, opsoclonus–myoclonus syndrome	SCLC, rarely thymoma, prostate cancer, neuroblastoma in children
Ri (ANNA-2)	Cerebellar degeneration, opsoclonus-myoclonus syndrome	Breast cancer or ovarian cancer, SCLC, NSCLC
ANNA-3	Polyneuropathy, cerebellar degeneration, limbic encephalitis	SCLC
Yo (PCA-1)	Cerebellar degeneration	Ovarian cancer or breast cancer
MAP1B (PCA-2)	Encephalitis, LEMS, polyneuropathy	SCLC > NSCLC
CRMP5 (CV2)	Encephalomyelitis, sensory neuropathy, optic neuritis	SCLC, thymoma
PNMA1/2	Limbic encephalitis, brainstem encephalitis, potentially with hypothalamic involvement	Men <45 years: mostly germ-cell tumor; women, men >45 years: NSCLC, breast cancer
Amphiphysin	Stiff-person syndrome, encephalopathy, myopathy, cerebellar degeneration, opsoclonus–myoclonus syndrome	Breast cancer, SCLC
DNER (Tr) <sup>b</sup>	Cerebellar degeneration	Lymphoma
<b>FACULTATIVE PARANEOPLASTIC ABS (TUMOR ASSOCIATION, %)</b>		
1. Abs to synaptic receptors		
NMDAR (20%–50%)	Anti-NMDAR encephalitis	Ovarian teratoma (age: 12–45 years), carcinomas (rare, mostly >45 years)
AMPA <sub>R</sub> (65%)	Limbic encephalitis	SCLC, thymoma, breast cancer
GABA <sub>A</sub> R (50%)	Limbic encephalitis	SCLC
GABA <sub>A</sub> R (5%)	Encephalitis	Thymoma
mGluR5 (70%)	Encephalitis	Hodgkin's lymphoma
mGluR1 (10%)	Cerebellar degeneration	Hodgkin's lymphoma
α-GlyR (20%)	PERM, stiff-person syndrome	Thymoma, lymphoma, breast cancer
2. Abs to ion channels and other surface proteins		
LGI1 (5%–10%)	Limbic encephalitis	Thymoma
Caspr2 (20%)	Limbic encephalitis, Morvan's syndrome	Thymoma
DPPX (<10%)	Encephalitis	Lymphoma
VGCC (50%–70%)	LEMS, cerebellar ataxia	SCLC >> prostate cancer, thymoma, lymphoma
AQP4 (rare)	NMOSD	Breast and lung cancer, lymphoma, cervical carcinoma, and others
MOG (n.a.)	ADEM, recurrent optic neuritis and myelitis, NMOSD	n.d.
IgLON5 (rare)	Brainstem encephalitis, sleep disturbances	Breast cancer, prostate cancer, non-Hodgkin lymphoma
3. Abs to intracellular antigens		
GAD (<10%)	Stiff-person syndrome, limbic encephalitis, cerebellar degeneration	NET, breast cancer, thymoma
GFAP (22%)	Meningoencephalomyelitis	Ovarian teratoma, adenocarcinomas, others

<sup>a</sup>Rare or not fully characterized associations between Abs and clinical syndromes are not listed.<sup>b</sup>Transmembrane protein.

*Abs*, Antibodies; *ADEM*, acute disseminated encephalomyelitis; *AMPA<sub>R</sub>*, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; *ANNA*, antineuronal nuclear antibody; *AQP4*, aquaporin-4; *Caspr2*, contactin-associated protein-related 2; *CRMP5*, collapsin-response mediator protein 5; *DNER*, Delta/Notch-like epidermal growth factor-related receptor; *DPPX*, dipeptidyl-peptidase-like protein-6; *GABA<sub>A</sub>R*, gamma-aminobutyric acid receptor; *GAD65*, glutamate decarboxylase (65 kDa); *α-GlyR*, glycine receptor alpha 1; *GFAP*, glial fibrillary acidic protein; *LGI1*, leucine-rich glioma inactivated 1; *MAP1B*, microtubule-associated protein 1B; *mGluR*, metabotropic glutamate receptor; *NET*, neuroendocrine tumors; *NMDA*, N-methyl-D-aspartate receptor; *NMOSD*, neuromyelitis optica spectrum disorder; (*N*)*SCLC*, (non)small cell lung cancer; *PCA*, Purkinje cell cytoplasmic antibody; *PNMA*, paraneoplastic Ma antigen; *VGCC*, voltage-gated calcium channel; *NMDAR*, N-methyl-D-aspartate receptors.

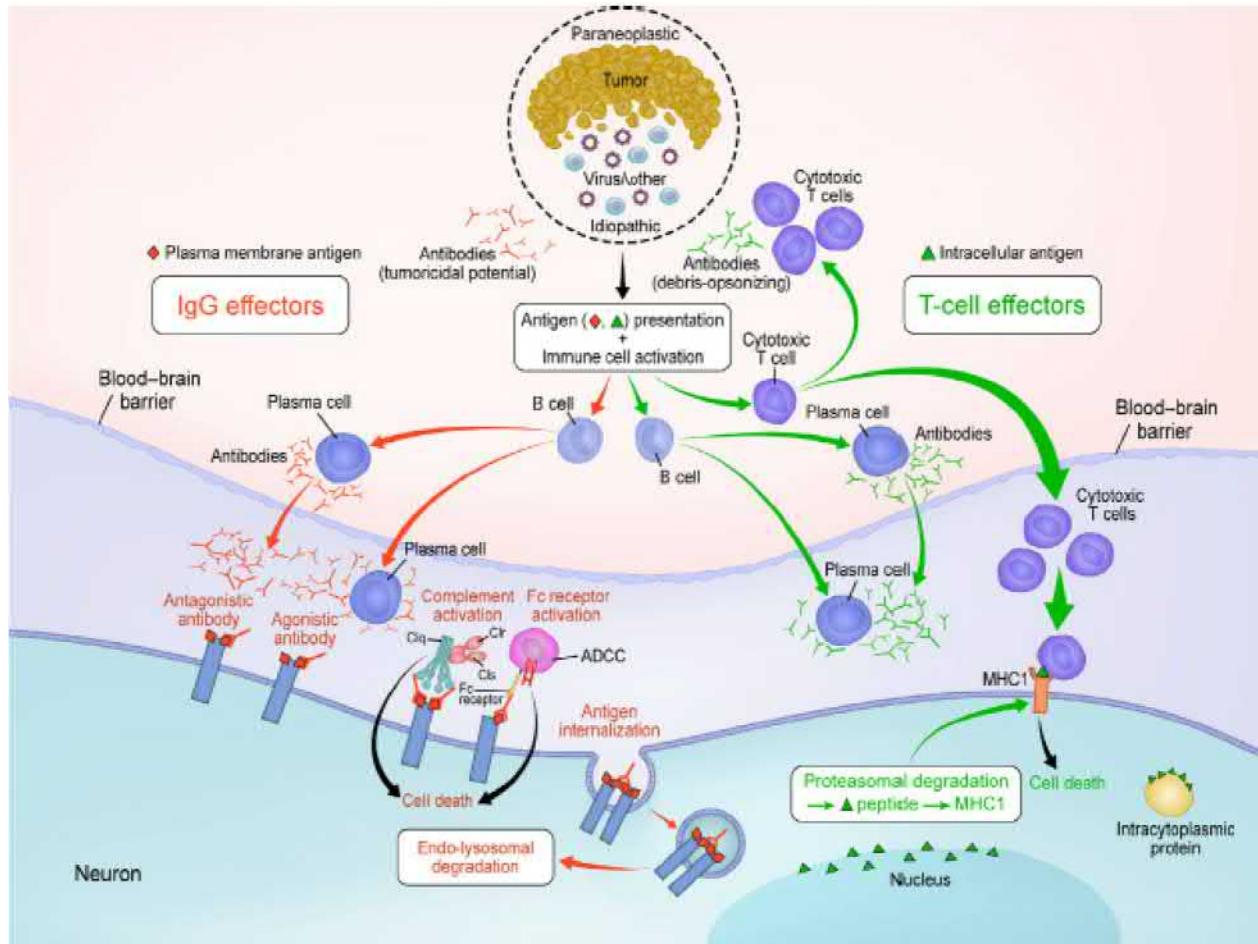
Adapted from Ayzenberg, I., Gold, R., Kleiter, I., 2017. Management of immune-mediated paraneoplastic neurological disorders. *Neuro. Inter. Open* 01 (04), E264–E274. doi: 10.1055/s-0043-112730.

In contrast, *Abs directed against surface antigens* are usually only facultative paraneoplastic and occur, especially in young patients, often as a primary autoimmune disorder. Targeting functionally important surface molecules, for example, synaptic receptors, ion channels, or associated membrane proteins, these Abs are of a direct pathogenetic significance.

Following pathogenetic mechanisms have been supposed (see Fig. 56.1) (Pittock and Vincent, 2016):

- Receptor cross-linking and internalization, leading to a decreased receptor density on the cell membrane;
- Receptor agonistic or antagonistic effects;
- Activation of the complement cascade or Ab-dependent cell-mediated cytotoxicity.

Despite, being usually of IgG1 subclass, several Abs surprisingly demonstrate *predominantly IgG4 reactivity* [leucine-rich glioma inactivated-1 (LGI1)-, contactin-associated protein-like-2 (CASPR2)-, IgLON5-Abs, etc.]. IgG4 are known to be functionally bispecific and monovalent. They are not able to induce cross-linking, complement activation, or cell-mediated cytotoxicity and have been traditionally considered as noninflammatory.



**FIGURE 56.1** Pathogenetic mechanisms of the antibodies, targeting intracellular, and cell surface antigens. Loss of immune tolerability to neural antigens can be initiated as a part of tumor- or infectious-induced immune response or be primary autoimmune (idiopathic). Intracellular antigens (green diamond) are not accessible for antibodies. Accordingly, these antibodies have no pathogenetic role and are diagnostically relevant only. However, peptides derived from these proteins can be displayed on upregulated MHC class I molecules, being accessible to specific cytotoxic T cells. In line with this, denatured proteins can be used for the testing of Abs, targeting nuclear, or cytoplasmic proteins (e.g., Line Blot).

In contrast, Abs-targeting membrane proteins (e.g., receptors, ion channels, or associated molecules, red diamond) are pathogenetic and can cause cellular dysfunction directly. Several injury mechanisms, including agonist or antagonist effects, receptor cross-linking and internalization or its dislocation from the synapse, activation of the complement, or antibody-dependent cell-mediated cytotoxicity have been proposed. Diagnostic tests, using preserved three-dimensional structure of target proteins (e.g., cell-based assays), should be used for their identification. MHC, Major histocompatibility complex. Adapted from Pittock, S.J., Vincent, A. Autoimmune Neurology. Elsevier, Chapter 1, permission requested.

Abs-induced mechanical interference of the ligand-receptor interaction has been supposed as a common pathogenetic mechanism in IgG4-related neurologic diseases (Huijbers et al., 2015).

Dysfunction of the target protein determines characteristic clinical presentation of the syndromes associated with surface antigens [e.g., short faciobrachial dystonic seizures (FBDS) in patients with LGI1-Abs]. Abs-mediated dysfunction of the targeted cell surface proteins is potentially reversible and neuronal or synaptic loss are less severe compared to classical PNS with Abs against intracellular antigens. Accordingly, Abs-depleting immunotherapies, for example, plasma exchange or immunoabsorption in the acute stage, often result in a significant clinical improvement. Patients with Abs to surface targets have a considerably better prognosis compared to those with Abs to intracellular antigens.

### **Antibodies Nomenclature**

Nomenclature of auto-Abs has varied over time and so can be confusing due to a dual terminology. Initially Abs were named after the first two letters of the last name of the index patient (e.g., Hu, Ri) or investigator (e.g., Tr-Abs described by Trotter et al., 1976), later after the antigen localization (ANNA-1) or after clinical syndrome [paraneoplastic cerebellar ataxia 1, Purkinje cell antigen (PCA)-1]. Once target protein identified, the name changed to that of the antigen (e.g., Delta/Notch-like epidermal growth factor-related receptor, DNER).

### **Diagnostical Considerations: Detection of Antibodies**

Antibodies testing can be conducted not only in serum, but also in CSF (Ig level is approx. 300–500 times lower, if no intrathecal production occurs), saliva (100 times lower), or tears (200 times lower) (Burns et al., 1982; Waters et al., 2016). Most of the studies have been performed in serum and CSF. Sensitivity of the Abs-testing may either be better in serum for one disease [e.g., AQP4-Abs bei neuromyelitis optica spectrum disease (NMOSD)] or in CSF for another (e.g., NMDAR-encephalitis). Accordingly, by justified suspicion, both serum and CSF should be tested.

There are several methods for the identification of antineuronal and antiglia Abs.

Indirect immunohistochemistry (IHC) on animal brain tissue is an excellent screening method, detecting Abs against both intracellular and cell surface proteins. Abs binding to intracellular (cytosolic or nuclear) antigens recognize linear, denatured epitopes and so can be further identified with a Western Blot. In practice, commercially available line blots (LBs), allowing testing of multiple targets with high antigen specificity in an individual strip, are the method of choice. In contrast, Abs targeting membrane antigens recognize three-dimensional structure of proteins. Several methods, including cell-based assays (CBAs), primary neuron cultures (in research laboratories), radioactive or fluorescence-based immunoprecipitation assays, or enzyme-linked immunosorbent assay (ELISA), can be used for their identification. Due to several technical issue, ELISA and radioactive or fluorescence-based methods seem to be less specific in some cases (Pitcock et al., 2014; Waters et al., 2012). CBA with (transfected) target antigen expressed in mammalian cells is increasingly being used in the clinical practice.

Combination of IHC, as a screening tool, with an antigen-specific detection assay (LB for intracellular or CBA for surface antigens) in both serum and CSF is diagnostically most useful (Probst et al., 2014). Inconsistent results of several tests and identification of the low titer Abs may occur. Positive reactivity in the brain tissue as well as in CBAs has higher clinical relevance compared to sera positive in CBAs only. Presence of the intrathecal Abs production is of a high significance for some diseases and can even correlate with its course, at least intra-individually (e.g., NMDAR encephalitis) (Dalmau et al., 2017). Presence of low titer Abs in serum without appropriate clinical syndrome have usually no direct significance and can be seen in healthy people as well as in patients with other neurologic disorders as a secondary immune phenomenon. However, in case of positive onconeural Abs, a tumor screening should be performed at diagnosis and up to 4 years later even if there is no corresponding neurological manifestation. Generally, it is unclear if healthy people with positive antineuronal Abs in serum are of an increased risk to become ill in the future. Concerning the Ig-class, the Abs of IgG class (mostly IgG1 or IgG4) are clinically relevant, while those of IgA and IgM class seems to have no pathogenetic role in the Abs-associated CNS diseases (Dahm et al., 2014; Dalmau et al., 2017). Abs testing under treatment with an IVIG or natalizumab (due to  $\alpha$ 4 chain integrin expression on HEK (human embryonic kidney) cells) must be interpreted with caution, as false positive (and probably also false negative under IVIG) findings may occur (Sánchez Gomar et al., 2014).

### **Principles of Treatment**

Given that the majority of the Abs-associated disorders are rare, treatment recommendations are usually based on case series and expert opinions. Considering potentially irreversible damage (especially in case of

intracellular target antigen), treatment should be started as early as possible. There are three main principles of management:

1. Immunotherapy should be started in both primary autoimmune and paraneoplastic cases as early as possible, preferably within few weeks after disease onset (Keime-Guibert et al., 2000; Vernino et al., 2004; Widdess-Walsh et al., 2003). If the suspicion of PNS is clearly supported by clinical evidence, immunotherapy may be started even before definite tumor diagnosis clarified (exception: steroid in case of suspected lymphoma!). Adequate immunosuppression seems not to affect the tumor outcome (Keime-Guibert et al., 1999).
2. In paraneoplastic cases prompt and aggressive tumor therapy must be initiated to remove the source of peripheral antigen stimulation. Adequate tumor treatment alone can result in the stabilization or even improvement of the neurological deficits (Candler et al., 2004; Graus et al., 2001; Shams'ili et al., 2003).
3. Symptomatic treatment can provide effective control in several syndromes.

#### TUMOR DETECTION AND MANAGEMENT

In about two-thirds of the paraneoplastic cases, the tumor remains unknown at the time of onset of neurological symptoms (Giometto et al., 2010). Since the autoimmune response slows down malignant growth, the tumors may be initially relatively small and difficult to diagnose. Following tumors are most frequently associated with PNS: lung cancer (small cell >> non-small cell lung cancer), carcinoma and teratoma of the ovaries, breast cancer, Hodgkin's disease and non-Hodgkin lymphoma, thymoma, and prostate cancer (Darnell and Posner, 2006; Giometto et al., 2010). Testicular tumors in young men and neuroblastoma in small children should be also considered. Multiple anti-neuronal Abs are not rare and help to focus the diagnostic workup on specific tumors (Horta et al., 2014; Pittock et al., 2004). Based on suspected tumor, a targeted stepwise diagnostic approach is recommended (Table 56.3, Titulaer et al., 2011). If standard workup is negative, a whole-body FDG PET/CT should be performed (Hadjivassiliou et al., 2009). Potentially false-negative findings of the FDG PET/CT in tumors with low proliferation rates (e.g., differentiated teratomas) or nonmetastatic skin cancers should be considered. If no tumor is detected, the diagnostic workup should be repeated in 3 months and then every 6 months for a period of at least 4 years (Titulaer et al., 2011).

#### PRINCIPLES OF IMMUNOTHERAPY

Immunosuppression at the acute stage usually includes either corticosteroid pulse therapy (e.g., 5 × 1000 mg methylprednisolone IV, if needed followed by oral tapering) or IVIG G (IVIG, e.g., 0.4 g/kg bodyweight for 5 days). Should the patient not improve, treatment escalation to an apheresis therapy (plasma exchange or immunoabsorption) can be undertaken. IVIG treatment and apheresis therapies are more effective in patients with Abs directed against surface antigens compared to intracellular antigens (Graus et al., 1992; Leyboldt and Wandinger, 2014; Uchuya et al., 1996; Vernino et al., 2004). If no improvement occurs, an early (up to 2 weeks after the primary treatment) escalation to cyclophosphamide (as a short-term high-dose treatment with 750–1000 mg/m<sup>2</sup> IV) or rituximab (e.g., 500 mg IV on days 1 and 15) can be undertaken (Shams'ili et al., 2006; Stark et al., 1995; Vernino et al., 2004).

**TABLE 56.3** Recommendations for Stepwise Cancer Screening in Patients With Paraneoplastic Syndromes (Giometto et al., 2010; Titulaer et al., 2011)

Tumor, prevalence <sup>a</sup>	Stepwise diagnosis (sensitivity of test method)
Lung cancer SCLC 38.4%, NSCLC 7.9%	Chest CT scan (80%–85%) → FDG PET(CT) → bronchoscopy/EB-US with biopsy
Ovarian cancer 10.5%	Transvaginal US (69%–90%), CA125 (62%) → pelvic/abdominal CT scan → FDG PET (CT)
Ovarian teratoma <sup>a</sup>	Transvaginal US (58%–94%) → pelvic/abdominal CT/MRI (93%–96%) → → chest CT scan, if required (extrapelvic teratomas)
Breast cancer 9.7%	Mammography, US → breast MRI (71%–100%), FDG PET (CT), if required
Hodgkin's disease 3.0%, non-Hodgkin's lymphoma 3.4%	Chest/abdomen CT scan, US → FDG PET (CT)
Thymoma 2.7%	Chest CT/MRI (75%–90%) → FDG PET (CT)
Testicular tumors 1.7%	US (72%), AFP + β-HCG → pelvic CT/MRI*, biopsy in the case of microcalcifications

<sup>a</sup>Prevalence among all PNSs, based on Euronetwork Database; no data on ovarian teratoma available.

AFP, alpha fetoprotein; β-HCG, beta human chorionic gonadotropin; CA125, cancer antigen 125; EB-US, endobronchial ultrasound; (N)SCLC, (non)small cell lung cancer; US, ultrasound; PNS, peripheral nervous system.

Adapted from Ayzenberg, I., 2017. *Neurol. Int. Open*, Thieme permission requested.

Long-term treatment usually includes oral immunosuppressants (e.g., azathioprine 2–3 mg/kg/d, mycophenolate mofetil 250–1000 mg b.i.d., cyclophosphamide 1–2 mg/kg/d) as a monotherapy or in combination with oral steroids or rituximab (e.g., every 6 months). IVIG at regular intervals (usually every 4–8 weeks) are effective in some conditions (e.g., stiff-person syndrome).

## Antibody-Associated Clinical Syndromes

Immune response can be directed against proteins expressed in central or PNS, neuromuscular synapses, or muscles. In many cases patients develop a combination of several syndromes (multilocular manifestation) and may have several auto-Abs simultaneously. Clinically, following group of diseases can be differentiated ([Gozzard and Maddison, 2010](#); [Leypoldt and Wandinger, 2014](#)):

- In the central nervous system:
  - Encephalomyelitis
  - Limbic encephalitis (LE)
  - Subacute cerebellar degeneration (SCD)
  - Brainstem encephalitis
  - Opsoclonus–myoclonus syndrome (OMS)
  - Stiff person syndrome
  - Isolated optic neuritis and/or retinopathy
  - Isolated myopathy/myelitis
  - Neuromyelitis optica and further disorders associated with antigliial Abs
- In the PNS, neuromuscular junction and the muscle:
  - Paraneoplastic polyneuropathies (include subacute sensory neuronopathy, distal symmetric sensorimotor neuropathy, polyradiculoneuropathy)
  - Pandysautonomia (include chronic intestinal pseudo-obstruction)
  - Neuromyotonia
  - Myasthenia gravis
  - Lambert–Eaton syndrome
  - Myositis

In this chapter we will focus on the clinical and pathophysiological features of the Abs-associated CNS disorders. With regard to myasthenia gravis, Lambert–Eaton syndrome, polyneuropathies, and myositis reader should refer to appropriate chapters.

Disorders associated with surface antigens often have a characteristic clinical presentation (e.g., NMDA receptor encephalitis) and are described for every well-characterized antigen separately. If no clear association between a distinct syndrome and a specific auto-Ab exists (e.g., SCD), typical clinical features and most common serological findings are discussed. Finally, a short characterization of both intracellular and surface antigens is given.

### **Autoimmune Encephalomyelitis and Limbic Encephalitis**

Clinical spectrum of the Ab-associated encephalomyelitis can be quite heterogeneous, depending on the target antigen and disease severity. The full-blown autoimmune encephalomyelitis (AE) has multilocular manifestation, often involving limbic systems, brainstem, spinal cord as well as PNS ([Giometto et al., 2010](#)). Combination of a psychiatric symptom (from depression and irritability to hallucinations and bizarre behavior), cognitive changes (e.g., short-time memory loss), and seizures is characteristic and classified as LE. This term is diagnostically helpful, as LE is usually autoimmune, however anatomically inaccurate as other areas beside the limbic systems are often involved. Historically, so-called classical variants of AE and LE have been thought to be obligatory paraneoplastic and mostly associated with Abs against intracellular antigens [e.g., Hu, Ma, GAD, CV2/collapsing response mediator protein 5 (CRMP5)]. Over the last decade, this disease spectrum has been substantially expanded, due to the identification of Abs to functionally active surface molecules [NMDAR, voltage-gated potassium channels (VGKC), dipeptidyl-peptidase-like protein 6 (DPPX),  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor (AMPAR),  $\gamma$ -aminobutyric acid (GABA) type-b receptors (GABAbRs), GABA type-a receptors (GABAaR), IgLON5, metabotropic glutamate receptor (mGluR) 5, dopamine receptor 2, glycine receptor (GlyR)].

By "classical" forms the prognosis is usually poor with the exception of Ma2-Ab-associated encephalomyelitis, in which approx. 30% of the patients experience improvement after adequate tumor treatment and immunotherapy (Dalmau et al., 2004). In contrast, patients with Abs to surface antigens have a much better outcome, except for cases with additional onconeural Abs (Höftberger et al., 2013, 2015).

#### ANTI-N-METHYL-D-ASPARTATE RECEPTOR ENCEPHALITIS

**EPIDEMIOLOGY AND CLINICAL CHARACTERISTICS** NMDAR encephalitis is the most common type of Ab-mediated encephalitis and accounts for approx. 4% of all encephalitis (Granerod et al., 2010). About 80% patients are female (with peak incidence between 13 and 30 years), the majority of whom have ovarian teratomas (Dalmau et al., 2008). The occurrence of teratomas increases among adolescents and is at 15% in female patients <14 years, at 30% <18 years and at 60% >18 years (Dalmau et al., 2011). In patients >45 years NMDAR encephalitis can be also associated with carcinomas (Titulaer et al., 2013b). In children <12 years and in men paraneoplastic etiology is rare. Prompt and targeted tumor screening, including abdominal MRI and a thorough gynecological examination, is crucial in NMDAR encephalitis. FDG PET-CT is not sensitive enough to detect well-differentiated teratomas.

Autoimmune encephalitis may also occur as a secondary autoimmune phenomenon after herpes simplex encephalitis (Armangue et al., 2014a). About 20% of these patients develop NMDAR-Abs, resulting in behavioral changes and choreoathetosis in children and cognitive and psychiatric abnormalities in adults (Armangue et al., 2015). Other cases seem to be primary autoimmune or of unknown etiology.

Clinically, NMDAR-encephalitis can manifest with a prodromal phase, resembling a viral infection, and further rapid deterioration. Patients develop behavioral and psychiatric changes (including an acute psychosis, hallucinations, delusions, and agitation), memory loss, and seizures. Afterwards a catatonic or comatose state with autonomic instability develops and patients frequently undergo intensive care unit treatment. The acute stage of the disease can last for several months and mortality, mostly due to secondary complications, is about 7% (Titulaer et al., 2013a). In case of adequate treatment patients slowly recover (up to 1–2 years), often exhibiting previous disease stages in a reverse order. In some patients mild cognitive deficits may persist (Finke et al., 2012).

Positive NMDAR-Abs testing is crucial for the diagnosis. Testing in CSF is more sensitive than in serum and is obligatory if anti-NMDAR-encephalitis is suspected (Gresa-Arribas et al., 2014). NMDAR-Abs of IgM and IgA class have probably no clinical relevance (Dahm et al., 2014; Dalmau et al., 2017). Isolated positive NMDAR-IgG of a low titer in serum only can be also seen in healthy controls. Brain MRI is abnormal in less than a half of patients and usually demonstrates nonspecific transient T2/FLAIR signal abnormalities in medial temporal lobe and hippocampus as well as other cortex areas, basal ganglia, cerebellum, and white matter (Titulaer et al., 2013a). CSF lymphocytosis is common during first weeks and oligoclonal bands appear later (Irani et al., 2010b). Electroencephalographic abnormalities can be seen in the majority of patients, including epileptiform activity, generalized slowing and characteristic "extreme delta brush" pattern in ca. 30% (Schmitt et al., 2012).

**PATHOPHYSIOLOGY** Expression of NMDAR in the neuronal component of teratomas or other tumors (or release by viral encephalitis) triggers an initial peripheral autoimmune response. Further restimulation of memory B-lymphocytes in CNS results in antigen-driven affinity maturation, clonal expansion and differentiation into long-lived plasma cells, producing NMDAR-Abs intrathecally (Dalmau, 2016). Antibodies target NR1 subunit of NMDAR and are directly pathogenic (Hughes et al., 2010; Planagumà et al., 2015). Despite being predominantly of IgG1 subclass, NMDAR-Abs do not cause relevant neuronal loss yet impair their function. Binding of Abs results in cross-linking and internalization of NMDA receptors from the cell surface (Hughes et al., 2010; Moscato et al., 2014). This mechanism explains the good outcome, with good or even complete recovery in about 75% after adequate therapy (Dalmau et al., 2011).

**TREATMENT** Treatment efficacy data of four larger retrospective studies are available (Dalmau et al., 2008; Irani et al., 2010b; Titulaer et al., 2013a; Viaccoz et al., 2014). Any tumor detected should be immediately removed. Up to 80% of the patients improve with tumor resection in combination with a first-line immunotherapy: steroid pulse therapy, IVIG, or apheresis therapy (Dalmau et al., 2011). Early immunotherapy (<40 days after initial manifestation) is associated with a significantly better prognosis (Irani et al., 2010b). Early plasma exchange therapy or immunoabsorption are probably preferable (Dogan Onugoren et al., 2016; Heine et al., 2016; Pham et al., 2011). In nonparaneoplastic cases, first-line immunotherapy is effective in approximately half of the patients only (Dalmau et al., 2011; Titulaer et al., 2013a). If no improvement occurs, second-line therapy with rituximab or cyclophosphamide should be considered (Gastaldi et al., 2016). Cyclophosphamide crosses the

blood–brain barrier but is associated with toxic effects and has a negative impact on fertility which is highly relevant in young women. For this reason, rituximab continues to be increasingly used in clinical practice. In approx. 75% of the cases, second-line therapy results in further significant improvement of the symptoms (Dalmau et al., 2008, 2011; Pham et al., 2011; Titulaer et al., 2013a). Successful administration of other immunosuppressants, including azathioprine, methotrexate, and mycophenolate mofetil, has also been reported (Gastaldi et al., 2016). Of a special interest is an escalation therapy with the proteasome inhibitor bortezomib. Bortezomib results in a targeted reduction of plasma cells and consequently Abs production. In two case series significant clinical improvement and a rapid decrease in serum NMDAR-Ab-titers have been reported (Behrendt et al., 2016; Scheibe et al., 2017).

**RELAPSES** Relapses can occur several months to years after the first episode. The relapse rate lies in the range of 12%–25%; it is higher in patients with nonparaneoplastic disease compared with patients after teratoma resection (Dalmau et al., 2008; Titulaer et al., 2013a). In these cases, second-line immunotherapy for a limited period of time (e.g., 2 years) is recommended. The reason why some patients relapse remains unclear; however, an intraindividual correlation between Ab-titer changes (especially in the CSF) and development of a recurrence has been reported (Gresa-Arribas et al., 2014). In contrast rapid decline in anti-NMDAR-Ab-titers in serum and CSF is characteristic for the monophasic course (Gresa-Arribas et al., 2014; Irani et al., 2010b).

#### ANTIVOLTAGE-GATED POTASSIUM CHANNELS ANTIBODY ENCEPHALITIS (VOLTAGE-GATED POTASSIUM CHANNELS ENCEPHALITIS)

VGKC are expressed both in the CNS and juxtaparanodally on peripheral motor nerves. Depending on the precise target antigen patients may develop both central and PNS disorders. Historically, VGKC-Abs were first described in Isaacs–Mertens syndrome, an autoimmune peripheral nerve hyperexcitability, that manifests with spontaneous muscle contractions and stiffness (Hart et al., 1997). Interestingly, clinical features of this condition resemble symptoms of poisoning with the VGKC (Kv1) toxin alpha-dendrotoxin. However primary antigens in this disease are not the Kv channel subunits, but three VGKC-associated proteins: LGI1, CASPR2, and contactin-2 (Irani et al., 2010a; Lai et al., 2010; Lancaster et al., 2011). While LGI1- and CASPR2-Abs are associated with well-defined clinical syndromes, the pathogenetic role of contactin-2 Abs remains unclear as it never occurs isolated. The significance of other anti-VGKC-Abs (positive in screening test against VGKC, but negative in specific CBA for LGI1-, CASPR2-, and contactin-2-Abs) also remains questionable (van Sonderen et al., 2017). About 38% of these Abs target an intracellular epitope of the Kv1 subunit and are presumably of no clinical significance (Lang et al., 2017).

**LEUCINE-RICH GLIOMA INACTIVATED-1 ENCEPHALITIS** LGI1-Abs are the most prevalent type of VGKC-Abs. In contrast to NMDAR encephalitis, the majority of patients with LGI1-encephalitis (60%–80%) are men, mainly in the second half of life. Anti-LGI1 encephalitis mostly develops as a primary autoimmune disorder. Paraneoplastic cases are rare (5%–10%) and usually associated with thymoma or lung cancer (Irani et al., 2010a; Lai et al., 2010).

Typical clinical manifestation includes LE with a subacute disturbance of memory, behavior changes and loss of spatial orientation, hyponatremia (in approx. 60%), and characteristic FBDS (in approx. 50%). FBDS last only few seconds (<3 seconds), involve unilateral arm and face (or leg) and occur up to 100 times a day (Irani et al., 2011). They usually develop prior to cognitive impairment and prompt start of immunotherapy may probably prevent disease progression (Irani et al., 2013). However, due to atypical presentation FBDS often remain unrecognized by physicians. At a later stage of the disease, patients develop generalized seizures. MRI reveals T2 hyperintensities of the medial temporal lobe in two-thirds of cases (Irani et al., 2010a). Approximately 75% of the patients respond to immunotherapy and have favorable prognosis (van Sonderen et al., 2016a,b). Yet recovery is often incomplete and cognitive deficits including memory impairment may persist (Malter et al., 2014). In line with this, neuropathological findings show immunoglobulin deposits, partially with complement activation and moderate lymphocytic infiltration, as well as neuronal loss in the hippocampus and amygdala (Bauer and Bien, 2016). This finding is unexpected, as LGI1-Abs predominantly belong to IgG4 subclass and do not activate complement (Irani et al., 2012). Correlation of the LGI1-Abs titer and clinical syndrome confirms the pathogenetic role of these Abs. LGI1-Abs have been shown to inhibit interaction between LGI1 and ADAM22/23 proteins, reducing synaptic AMPA receptor clusters and provoking epileptogenic activity (Ohkawa et al., 2013).

**CONTACTIN-ASSOCIATED PROTEIN-LIKE-2 ENCEPHALITIS** Caspr2 encephalitis is a rare variant of LE. It occurs almost exclusively in men (80%–90%) with a manifestation age around 60 years. The reason for the male predominance remains unclear as most of autoimmune diseases occur more frequently in women. Being a transmembrane protein with a large extracellular domain, Caspr2 is expressed in both central and PNS and serves as a good accessible antigen target. Accordingly Caspr2-Abs are associated with a wide range of central and peripheral neurologic syndromes, including autoimmune encephalitis, cerebellar symptoms, neuromyotonia (Isaac–Mertens' syndrome), neuropathic pain, dysautonomia, and weight loss (Joubert et al., 2016). Combination of at least three of these core symptoms can be found in 77% of the patients (van Sonderen et al., 2016a). Full-blown variant, including encephalitis signs and neuromyotonia accompanied by autonomous involvement and insomnia is known as Morvan's syndrome. Tumor incidence lies by approximately 15%–20% (Joubert et al., 2016; van Sonderen et al., 2016a). Thymomas, being most common tumor type, can be also associated with myasthenia gravis in some patients. Signs of neuromyotonia are almost always present in paraneoplastic cases. In contrast tumors are rare in patients with isolated LE. In nonparaneoplastic cases immunotherapy is usually beneficial and the prognosis favorable. CASPR2-Abs have been identified in several cases of Creutzfeldt–Jakob disease, probably as a secondary immune phenomenon. This finding can be diagnostically misleading due to partly similar clinical manifestation of both conditions (Zuhorn et al., 2014).

Precise pathogenesis of Caspr2-Abs associated disorders remains unclear. Similar to LGI1-Abs, Caspr2-Abs of IgG4 subtype are present in all patients, in 63% in combination with IgG1. As known, IgG4 are able to exchange half-molecule (so-called Fab-arm exchange) and so are functionally monovalent (Aalberse et al., 2009). Accordingly, IgG4 are unable to induce cross-linking and internalization of the target antigen. These Abs have only low affinity for the Fc $\gamma$  receptor and do not induce cellular as well as complement-dependent cytotoxicity. Direct binding and alteration of the Caspr2 function (e.g., downregulation of Caspr2/Kv1.1/1.2 complexes on the peripheral nerve) has been supposed as a possible mechanism; however, further investigations are needed (Irani et al., 2010a; van Sonderen et al., 2016c).

**TREATMENT OF LEUCINE-RICH GLIOMA INACTIVATED-1- AND CONTACTIN-ASSOCIATED PROTEIN-LIKE-2-ENCEPHALITIS** Data are scarce and partly reported before identification of LGI1- and CASPR2-Abs. There are no prospective comparative studies available. In most cases, symptomatic treatment with anticonvulsants alone is insufficient. In paraneoplastic cases, treatment of the underlying tumor is crucial. Regarding initial immunotherapy, either steroids, IVIG, or apheresis therapy may be used. Some findings indicate that combined and earlier (within the first 2 months after manifestation) treatment is associated with a better prognosis (Gastaldi et al., 2016; Thieben et al., 2004). Combination of IVIG with steroids was superior to steroid monotherapy in a small retrospective study (Shin et al., 2013). In an open prospective study all patients ( $n = 9$ ) improved when treated with the combination of plasma exchange, IVIG, and steroid pulse therapy, followed by oral steroid treatment (Wong et al., 2010). In most cases first a reduction in seizure frequency is noted, while cognitive deficits tend to respond much later (Vincent et al., 2004). Data regarding long-term therapy are limited. Azathioprine, tacrolimus, mycophenolate mofetil, rituximab, or tocilizumab may be tried in refractory cases (Gastaldi et al., 2016; Krogias et al., 2013). Patients with both LGI1- and CASPR2-encephalitis may experience relapses (van Sonderen et al., 2016a,b). In LGI1-encephalitis a correlation with an increase in Ab-titers has been observed (Irani et al., 2013).

### **Encephalitis Associated With Antibodies to Other Neuronal Surface Proteins**

#### **DIPEPTIDYL-PEPTIDASE-LIKE PROTEIN 6 ENCEPHALITIS**

DPPX encephalitis is a rare and mostly primary autoimmune condition. Target protein is a regulatory subunit of neuronal Kv4.2 potassium channels, highly expressed in hippocampus, cerebellum, and plexus mesentericus (Nadal et al., 2003). Typical clinical manifestation includes LE with symptoms of central hyperexcitability (agitation, myoclonus, tremor, sleep disturbance, and seizures), brainstem pathology (eye movements abnormalities, ataxia, dysarthria, or dysphagia) often preceded or overlapped with intense diarrhea and weight loss (Boronat et al., 2013; Tobin et al., 2014). Few patients with DPPX-Abs and PERM (progressive encephalomyelitis with rigidity and myoclonus) syndrome were also reported (Balint et al., 2014). CSF analysis usually demonstrates inflammatory changes, including pleocytosis and increased IgG index or positive oligoclonal bands. DPPX encephalitis was rarely associated with lymphoma or chronic lymphocytic leukemia. Being often insidious in onset, encephalitis can be severe on the later stages. In a retrospective case series, early immunotherapy (corticosteroids, IVIG, PLEX (plasma exchange) alone or combined with rituximab or cyclophosphamide) resulted in

improvements in about two-thirds of the patients (Tobin et al., 2014). Long-term immunotherapy is usually needed and its decrease or cessation may cause relapses (Boronat et al., 2013).

#### **α-AMINO-3-HYDROXY-5-METHYL-4-ISOXAZOLEPROPIONIC ACID RECEPTOR ENCEPHALITIS**

AMPAR encephalitis mostly occurs in females with a manifestation age between 30 and 70 years. This form of encephalitis is tumor-associated in about two-thirds of cases (mostly thymoma, lung, or breast cancer) and accordingly often characterized by an aggressive course (Lai et al., 2009). Almost all patients develop symptoms of LE such as short-term memory loss, confusion, and abnormal behavior (Höftberger et al., 2015). Additionally, seizures, prominent psychiatric symptoms, ataxia, or abnormal movements may occur. In some cases disease manifests as a pure psychosis (Graus et al., 2010). MRI is usually pathologic and demonstrates abnormalities predominantly located in the temporal lobe. Involvement of other regions, corresponding diffuse encephalitis, is also not rare. AMPAR-Abs testing in CSF seems to be more sensitive than in serum (Höftberger et al., 2015). Auto-Abs target GluA1 or GluA2 subunits of the AMPA receptors (Lai et al., 2009). Similar to NMDAR-encephalitis, binding of the Abs cause selective internalization and degradation of AMPAR clusters as well as decrease of AMPAR-mediated excitatory postsynaptic currents (Peng et al., 2015).

Tumor resection in combination with early immunotherapy (steroids or IVIG) can lead to clinical improvement. However, relapses may occur and are unrelated to tumor recurrence. In most cases, relapses can be prevented by treatment with rituximab and cyclophosphamide (Höftberger et al., 2015). Presence of classical onconeural Abs (e.g., CRMP5, amphiphysin, SOX1) is associated with a poor outcome.

#### **GABA-B RECEPTOR ENCEPHALITIS**

GABA<sub>B</sub>R is a metabotropic inhibitory receptor, mainly expressed in hippocampus, amygdala, cerebellum, and thalamus. Dysfunction of these receptors results in excessive neuronal activity and synchronization, leading to seizures and memory deficits (Emson, 2007; Enna and Bowery, 2004). In line with this, patients with GABA<sub>B</sub>R encephalitis usually develop LE, including early and often refractory seizures, memory loss, and variety of neuropsychiatric symptoms (Lancaster et al., 2010). Rare atypical presentations include cerebellar ataxia and OMS (Höftberger et al., 2013). GABA<sub>B</sub>R-encephalitis is usually monophasic and in 50% paraneoplastic [mostly small-cell lung cancer (SCLC) in patients >50–60 years old] (Höftberger et al., 2013; Lancaster et al., 2010). Both genders are almost equally affected. MRI is pathologic in about half of patients and often demonstrates abnormalities of the mesial temporal lobe. Out of the total number of patients, two-thirds have pleocytosis in CSF.

GABA<sub>B</sub>R-Abs are directed against extracellular B1 subunit of the receptor and are mainly of IgG1 class. It is supposed, that these Abs have a direct pathogenetic role (Höftberger et al., 2013; Lancaster et al., 2010). Few available histopathological findings demonstrated infiltration of plasma cells as well as granzyme B-producing CD8+ lymphocytes, assuming that probably both humoral and cellular (especially in paraneoplastic cases) mechanisms can be involved in the pathogenesis of this condition (Golombeck et al., 2016; Höftberger et al., 2013).

Consistent with surface location of the target protein, immunotherapy (if necessary in combination with tumor resection and chemotherapy) is effective in most cases and may even result in complete recovery (Höftberger et al., 2013). Unfavorable tumor prognosis and presence of the classical onconeural Abs (e.g., Ri, amphiphysin, SOX1) are associated with a poor outcome.

#### **γ-AMINOBUTYRIC ACID TYPE-A RECEPTOR ENCEPHALITIS**

This form of encephalitis seems to be very rare and until now three case series have been reported only (Petit-Pedrol et al., 2014; Pettingill et al., 2015; Spatola et al., 2017). It may occur in children as well as in adults. Paraneoplastic cases of the disease are not rare in adults and usually associated with thymoma (Spatola et al., 2017). Few small children developed GABA<sub>A</sub>R encephalitis few weeks after viral encephalitis (both in combination with NMDAR Abs). Refractory status epilepticus is one of the main clinical features of the disease. Further core symptoms include cognitive impairment, behavior changes, decreased levels of consciousness, altered behavior, and movement disorders (more often in children). In contrast to other LE, MRI reveals extensive multi-locular subcortical and cortical abnormalities predominantly in temporal and frontal lobes in 80% (Spatola et al., 2017). MRI lesions may occur asynchronous. Epileptiform activity can be seen in 76% of the patients. Immunotherapy can be effective and patient recover at least partly, if survive the acute phase of the illness. Usually no relapses occur. The GABA<sub>A</sub>R-Abs targeting α1, β3, or γ2 subunits of the receptor have been reported. The last one is probably not pathogenic, as it has been always detected in association with Abs against α1- or β3-subunits. Patient's sera induce specific loss of synaptic (but not dendritic) GABA<sub>A</sub>R in the cell culture (Petit-Pedrol et al., 2014). High titers of GABA<sub>A</sub>R Abs are associated with severe clinical presentations.

### OPHELIA SYNDROME OR METABOTROPIC GLUTAMATE RECEPTOR 5 ENCEPHALITIS

This rare form of autoimmune encephalitis was firstly described 1982 in the setting of Hodgkin lymphoma. Due to some similarity with a character of Shakespeare's play Hamlet, this clinical entity was termed the Ophelia syndrome (Carr, 1982). Typical clinical features include confusion, progressive loss of memory with bizarre behavior, depression, and hallucinations. The course of this disease is milder comparing to NMDAR encephalitis. Usually patients do not have seizures and autonomic instability. Patients improve dramatically with treatment of the lymphoma. Antibodies to mGluR5 have been identified in this condition and are supposed to be pathogenic (Lancaster et al., 2011). Antibodies targeting another receptor of this group—mGluR1 are linked with cerebellar degeneration (Lopez-Chiriboga et al., 2016). Only minority of these patients have tumor. Consistent with expression of the mGluR1 in the limbic system seizures, memory loss, and psychiatric symptoms may be infrequently present; however, ataxia remains the core symptom. Experimental data support pathogenicity of mGluR1 as well and immunotherapy is usually effective. Despite being 85% homologous, the Abs targeting mGluR1 and mGluR5 do not cross-react and the clinical syndromes are distinct.

### IGLON5 ENCEPHALITIS

Encephalitis associated with IgLON5-Abs is probably a most mysterious Abs-associated neurologic disease with features of both autoimmune and neurodegenerative disorder. It occurs in older patients (median age about 60) (Gaig et al., 2017). Clinical manifestations are multilocular, usually insidious at onset and gradually progressive over years. Complex sleep disturbances (with vocalizations during REM (rapid eye movement) and non-REM phase, sleep apnea, insomnia, and excessive daytime sleepiness) are most characteristic. Further typical presentations include gait instability and brainstem signs such as stridor, dysphagia, and central hypoventilation. Movement disorders are sometimes present and may include Parkinsonism, dystonia, or chorea as well as myoclonus. Sudden death due to laryngospasm or respiratory failure has been reported. Paraneoplastic cases are rare.

IgLON5-Abs are predominantly of IgG4 subclass; however, IgG1 Abs are also present in all patients. In vitro IgLON5 Abs (of IgG1 subclass only) recognize the immunoglobulin-like domain 2 and cross-linked receptors, resulting in an irreversible internalization of IgLON5 clusters from the surface of the hippocampal neurons (Sabater et al., 2016). It remains unclear if Abs of IgG4 subclass have a direct pathogenetic effects due to other mechanisms. Strong association with HLA-DRB1\*1001 and HLA-DQB1\*0501 haplotypes was demonstrated in several studies (Gaig et al., 2017; Sabater et al., 2014). All these findings support an underlying immune pathogenesis. In contrast, neuropathological findings demonstrate lack of inflammatory infiltrates. Characteristic are neuronal loss and accumulation of hyperphosphorylated tau protein, involving the midbrain tegmentum and the hypothalamus (Gelpi et al., 2016). The neuropathological changes cannot be classified within known taupathies such as progressive supranuclear palsy or corticobasal degeneration. Accordingly, it remains unclear if the IgLON5 autoimmunity is primary and pathogenetically relevant or just a secondary immunologic phenomenon.

Despite surface localization of the target antigen, data on immunotherapy remain controversial, with moderate improvement in selected reports (Bonello et al., 2017; Gaig et al., 2017; Honorat et al., 2017; Sabater et al., 2014). It may be speculated that neuronal-specific tau accumulation and further neurodegeneration is Ab-mediated and, thus, a secondary feature. In this case an early immunotherapy could be promising.

### DOPAMINE-2 RECEPTOR (BASAL GANGLIA) ENCEPHALITIS

Dopamine-2 receptor (D2R) Abs have been identified in some children with basal ganglia encephalitis, presenting with different movement disorders: Parkinsonism, chorea, or dystonia. Furthermore these Abs were present in a few patients with Sydenham chorea and Tourette syndrome (Dale et al., 2012). Psychiatric disturbances such as emotional lability, attention deficit, or psychosis are typical in these patients. Half of the patients demonstrated MR-signal abnormalities in basal ganglia. Onset is often associated with previous infections and an early immunosuppression is partly effective. D2R-Abs recognize extracellular N-terminal domain of the receptor and are able to decrease its surface expression on the transfected cells (Sinmaz et al., 2016). Further studies are needed to confirm this finding and better characterize an associated clinical syndrome.

### Subacute Cerebellar Degeneration

SCD is one of the most common Ab-associated diseases of the CNS. It often develops in context of tumors, representing about a half of all paraneoplastic syndromes (Giometto et al., 2010). After acute or subacute onset symptoms of ataxia worsen progressively and reach a plateau within the first weeks to months with 50%–70% of the patients becoming wheelchair-bound (Jones et al., 2015). MRI is usually normal at onset or rarely

demonstrates inflammatory lesions with contrast-enhancement, symmetrical in some cases. In the following course, cerebellar atrophy becomes more apparent. CSF examination demonstrates inflammatory changes with a mild pleocytosis (at initial stage) and intrathecal Ig-production or oligoclonal bands. Interestingly, in some cases antineuronal Abs can be produced intrathecally, making CSF screening diagnostically relevant (Bernal et al., 2003; Mitoma et al., 2016).

In the majority of patients with PCD, Abs to intracellular antigens (mostly Yo, Hu, and GAD; less frequently Ri, Ma2, CV2/CRMP5, amphiphysin, Zic4, ANNA-3, PCA-2/MAP-1B and sporadically, Homer3, CARPVIII, PKC $\gamma$ , Ca/ARHGAP26) can be detected (Jarius and Wildemann, 2015). Abs targeting surface antigens Tr/DNER and very rarely mGluR1 (facultative paraneoplastic) can be found in the setting of Hodgkin's lymphoma and those against voltage-gated calcium channel (VGCC) in patients with lung cancer (with or without Lambert–Eaton myasthenic syndrome) (Dalmau et al., 2017).

Irreversible loss of Purkinje cells is the main underlying cause of subacute cerebellar ataxia. Neuropathological findings usually reveal CD8+ T cells infiltrates and no relevant IgG or complement products. Cell-mediated cytotoxic immune response seems to be pathogenetically most relevant in SCD with Abs targeting intracellular antigens; however, several studies demonstrated possible direct role of Abs as well (Mitoma et al., 2015). Auto-Abs can be internalized by Purkinje cells and at least those targeting Yo were demonstrated to increase neuronal death ex vivo (Greenlee et al., 2015).

The prognosis is generally unfavorable, especially if Hu-, Yo-, or CV2/CRMP5-Abs is present (Shams'ili et al., 2003). In some patients, stabilization can be achieved with early tumor resection, followed by chemotherapy (Vernino et al., 2004). While immunotherapies, including corticosteroids, IVIG, plasma exchange, cyclophosphamide, and tacrolimus, are generally disappointing, some improvement in individual cases has been reported (Peterson et al., 1992; Phuphanich and Brock, 2007; Rojas et al., 2000; Vernino et al., 2004). Patients with Tr/DNER-Abs after successful lymphoma treatment and patients with Ri-Abs under immunotherapy have a more favorable prognosis (Briani et al., 2011; Greenlee, 2013). Prompt immunotherapy can be beneficial in GAD-Abs positive SCD; however, overall efficacy remains much lower comparing to stiff-person syndrome (Ariño et al., 2014; Mitoma et al., 2016). In patients with VGCC-Abs, cerebellar symptoms stabilize in up to 40% of the patients after tumor therapy and immunotherapy (Graus et al., 2002). Rare variant of SCD with mGluR1-Abs (initially described in context of lymphoma) is often nonparaneoplastic and respond well to immunotherapy (Lopez-Chiriboga et al., 2016). In all cases additional symptomatic treatment with propranolol, primidone, topiramate, or clonazepam can be attempted (Deuschl et al., 2012).

### **Stiff-Person Spectrum Disorder**

These syndromes are associated with a storage of inhibitory synaptic transmission mediated by GABA and glycine in the brainstem and spinal cord. Antibodies targeting an intracellular enzyme GAD65 (approx. 40%–80% of the cases) and cell surface-associated GlyR  $\alpha$ 1 subunit (GlyR, approx. 10%–20% of the cases) are by far most frequently identified in stiff-person spectrum disorder (SPSD) (Martinez-Hernandez et al., 2016; McKeon et al., 2012). These Abs can infrequently occur in a paraneoplastic context (see Table 56.2); however, most of the cases are idiopathic. More rarely Abs to synaptic vesicle-associated protein amphiphysin can be identified. These Abs are often associated with breast cancer (typically in women around 60 years) and small-cell lung carcinoma (Pittock et al., 2005). Three rarely reported antigens include DPPX, GABAaR, and gephyrin (Martinez-Hernandez et al., 2016).

Typical symptoms of SPSD reflect hyperexcitability of the CNS and include generalized muscle rigidity with painful muscle spasms, an exaggerated startle response and a characteristic gait abnormality with freezing of gait and hyperlordosis. In case of a limited variant, symptoms can affect one limb only (stiff-limb syndrome). Apart from classical manifestation, GlyR-Abs may induce more aggressive and generalized progressive PERM, as a so-called plus variant of the disease (Carvajal-González et al., 2014). These patients can additionally develop myoclonus, seizures, cognitive decline, brainstem signs and autonomic dysregulation.

In contrast to other Abs-associated disorders, patients with SPSD may experience a significant improvement with symptomatic treatment alone. First-line therapy includes GABAa receptor agonists (e.g., diazepam or clonazepam) and GABAb receptor agonist baclofen. In many cases these medications effectively reduce muscle spasm and rigor; however, tolerance may develop later (McKeon et al., 2012). In extremely severe cases, intrathecal baclofen treatment can be used (Stayer et al., 1997). In paraneoplastic SPSD cancer treatment, including chemotherapy, has the highest priority. With regard to immunotherapy, both corticosteroids and immunosuppressants (e.g., cyclophosphamide) can be effective (Faissner et al., 2016; Murinson and Guarnaccia, 2008; Schmierer et al., 1998). Long-term therapy with oral steroids is usually limited due to often comorbid GAD-Abs associated diabetes, necessitating administration of steroid-saving medications, such as mycophenolate

mofetil, azathioprine, or cyclophosphamide. In SPSD patients with GAD65-Abs, IVIG (2 g/kg per cycle every 4 weeks) is recommended as a first-line therapy (Dalakas et al., 2001; McKeon et al., 2012). Treatment escalation to apheresis therapy can be effective, regardless of the type of Ab (Pagano et al., 2014). Rituximab was effective in individual cases; however, failed in a smaller blinded study (Baker et al., 2005; McKeon et al., 2012). As a general rule, treatment should be started as early as possible. Relevant improvement of symptoms can be achieved during the first year after initial manifestation only (McKeon et al., 2012).

In case of a GlyR-Abs-associated SPSD substantial benefit of immunotherapy and better prognosis has been reported (Carvajal-González et al., 2014). Relapses may occur in approx. 10%.

### ***Opsoclonus–Myoclonus Syndrome***

Abs-associated brainstem syndromes are often quite heterogeneous and can be present as a part of AE. In contrast, OMS is a characteristic-distinct clinical entity that frequently occurs isolated. This syndrome is characterized by the combination of opsoclonus (arrhythmic multidirectional conjugate saccades without an intersaccadic interval) with or without myoclonus and ataxia. The onset is usually acute or subacute with vertigo, vomiting, and gait instability, so that initial diagnosis of vestibular vertigo is common (Armangué et al., 2016). Brain MRI is usually normal, while mild pleocytosis and protein elevation can be seen in CSF.

OMS occurs in small children (usually <5 years, also known as Kinsbourne syndrome) and adults. Children are mostly seronegative for auto-Abs (except for a few cases with anti-Hu-Abs) and in a half of the cases have neuroblastoma (Gorman, 2010). Adult cases are in 40% of the paraneoplastic and in 60% of the idiopathic origin (Armangué et al., 2016). In young adults with paraneoplastic OMS (20–30 years), ovarian teratoma and in older (>45 years) lung or breast cancer (last one in association with Ri-Abs) are most frequently detected. Antibodies to other intracellular antigens (Hu, Ma2, amphiphysin, CV2/CRMP5) have been also less frequently reported. Antibodies targeting surface antigens (GABA<sub>B</sub>R, NMDAR, DPPX, and α-GlyR, last one probably unspecific) have been described in a few patients only (Armangué et al., 2016). In many cases OMS remains seronegative; however, CSF flow cytometry demonstrates B-cell expansion as a possible biomarker of disease activity (Pranzatelli et al., 2010). Parainfectious cases of OMS are mostly associated with HIV and rarely several other infections (Klaas et al., 2012).

Standard treatment (tumor therapy, corticosteroids alone or combined with IVIG, PLEX) is often insufficient in children and associated with residual deficits (Vedeler et al., 2006). More aggressive therapies, such as rituximab and cyclophosphamide, can improve long-term prognosis, including cognitive and behavioral deficits (Mitchell et al., 2015; Pranzatelli et al., 2010). In adults some clinical improvement can be achieved in case of adequate tumor management (Bataller et al., 2001). A subgroup of young patients with teratoma and OMS as a part of an NMDAR-Abs negative encephalitis (mostly with brainstem involvement) have often a very good outcome. After tumor resection and immunotherapy (methylprednisolone, in some cases combined with IVIG and plasma exchange), 8 of 10 patients became symptom-free (Armangue et al., 2014b). Likewise, patients with parainfectious OMS respond well to immunotherapy (e.g., IVIG) and have better prognosis (Klaas et al., 2012). Symptomatic treatment with clonazepam, propranolol, or topiramate can be tried.

### ***Isolated Myopathies and Visual Loss, Associated With Antineuronal Antibodies***

Auto-Abs found in patients with isolated myopathy and/or isolated optic neuritis mostly target glial (see the “Neuromyelitis optica and further disorders associated with antiglial antibodies” section) and only rarely neuronal antigens.

In myopathies most commonly onconeural amphiphysin-, CV2/CRMP5- and more rarely Ri-, ANNA-3-, Hu-, Yo-, Ma1/2-, GAD-, and PCA-2-Abs can be detected (Flanagan et al., 2011; Flanagan, 2016). Paraneoplastic myopathies are mostly associated with breast and lung cancer. In contrast to patients with primary immune-mediated myopathies, they are usually older, with a first manifestation of around 60 years. Cytotoxic (CD8+) cell-mediated immune response is involved and accordingly immunotherapies, targeting T cells (e.g., cyclophosphamide) in parallel to tumor treatment can be tried.

Visual loss due to antineuronal Abs is also rare. Most reported cases describe CRMP/CV2-Abs associated optic neuritis or recoverin-Abs associated retinopathy (Cross et al., 2003). A number of further Abs have been identified in so-called cancer-associated retinopathy (usually in lung or gynecological cancer) and melanoma-associated retinopathy; however, further studies are required for their precise characterization (Braithwaite et al., 2014). Treatment is usually ineffective and prognosis depends on the underlying tumor.

### ***Neuromyelitis Optica and Further Disorders Associated With Antiglial Antibodies***

There are four glial autoantigens with definite or probable clinical relevance: membrane proteins AQP4 and myelin-oligodendrocyte glycoprotein (MOG), cytoplasmatic glial fibrillary acidic protein (GFAP), and nuclear sex determining region Y box protein (SOX1, earlier described as an antiglial nuclear Ab). Antibodies to AQP4 are strongly associated to mostly primary autoimmune neuromyelitis optica spectrum disorder. Direct pathogenetic role of AQP4-Abs and MOG-Abd has been demonstrated in animal models (Saadoun et al., 2012, 2014). Concerning recently described GFAP-Abs further studies are needed in order to evaluate its clinical relevance. Despite its intracellular localization, associated disease seems to be responsive to immunotherapies. SOX1-Abs are first of all diagnostically relevant, as a paraneoplastic marker (mostly by small-cell lung cancer) (Sabater et al., 2008). GFAP- and rarely AQP4-Abs (but not MOG-Abs) can be of paraneoplastic origin also.

#### **NEUROMYELITIS OPTICAL SPECTRUM DISEASE**

NMOSD is one of the best characterized Ab-mediated autoimmune disorders and serves as one of the model diseases of this group. Historically it has been long seen as a rare atypical form of multiple sclerosis (also called Devic's disease). Despite clinical similarity of both diseases the pathophysiology, immunology and treatment of neuromyelitis optica clearly differ. A crucial role in the pathogenesis of the disease plays Abs, targeting astrocytic water protein AQP4, classifying neuromyelitis optica as an autoimmune astrocytopathy (Lennon et al., 2004). AQP4-Abs can be found in approximately 80% of the patients. Being almost always a primary autoimmune disorder of unknown origin, NMOSD can rarely occur in a paraneoplastic context in elderly patients (Ontaneda and Fox, 2014).

**EPIDEMIOLOGY AND CLINICAL CHARACTERISTICS** NMOSD is a rare disease with an overall prevalence of 0.3–4.4 per 100,000 (higher in Asian, South American, and African population) and clear female predominance (f:m 9:1) (Pittock and Lucchinetti, 2016). Diagnostic criteria of NMOSD have been recently reviewed (Wingerchuk et al., 2015). Characteristic clinical features include recurring and often severe attacks of uni- or bilateral optic neuritis and myelitis. The last one classically involves equal to or more than 3 myelon segments (longitudinal extensive transfer myelitis, LETM); however, shorter lesions don't exclude the diagnosis (Flanagan et al., 2015). Further typical yet less prevalent manifestations include area postrema syndrome (presenting as a triad of hiccup, nausea, and vomiting), diencephalic (narcolepsy), or brainstem syndrome. Beside usually centrally located LETM, typical MRI changes can be found in the dorsal medulla and at periependymal surfaces, surrounding ventricular system (e.g., along aqueduct and third ventricle) (Kim et al., 2015). In contrast to MS (multiple sclerosis) periependymal lesions are not ovoid and perpendicular to ventricle, but usually follow ependymal lining. Sometimes tumefactive ( $>3$  cm) and even confluent lesions may occur. Being partly due to a vasogenic edema, they can be largely reversible. CSF analysis often reveals pleocytosis ( $50–1000 \times 10^6$ ) with a predominance of granulocytes and eosinophils, especially during attacks (Wingerchuk et al., 2007). Oligoclonal Abs can be detected in 15%–30% only and are transitory in some cases. Intrathecal synthesis of AQP4-Abs is also rare, reported in 1 of 23 patients only (Jarius et al., 2010).

Approximately 20%–25% of the patients remain seronegative. Interestingly, Abs targeting full length MOG have been detected in a part of NMOSD patients without AQP4-Abs (see the "Neurological syndromes associated with myelin-oligodendrocyte glycoprotein-antibodies" section). Despite overall clinical similarity, another pathogenetic mechanism with leading oligodendrocytopathy underlies this disease (Reindl et al., 2013).

**PATOPHYSIOLOGY** The pathology of NMOSD-lesions reflects an Ab-mediated autoimmune astrocytopathy, with an extensive astrocytic degradation, demyelination, and damage of axons and neurons (Pittock and Lucchinetti, 2016). AQP4-Abs are polyclonal and bind to multiple epitopes on both M1 and M23 isoforms of AQP4, which have identical extracellular domain. AQP4 is colocalized and functionally coupled with an excitatory amino acid transporter (EAAT2), responsible for 90% of the glutamate reuptake. Binding of the Abs results in a cross-linking and internalization of M1-isoform together with EAAT2 and following storage of water transport and glutamate uptake (Hinson et al., 2008). Increased glutamate excitotoxicity is able to cause oligodendrocyte damage with subsequent demyelination. The M23-isoform clusters cannot be internalized due to its size. Abs binding results in its further aggregation on the cell membrane and astrocytic degradation due to a complement-mediated cellular cytotoxicity (Hinson et al., 2012). Accordingly, regions with higher expression of M23 are supposed to undergo more destructive damage, while isolated loss of AQP4 and EAAT2 has been demonstrated in nondestructive lesions (Lucchinetti et al., 2014).

In parallel AQP-Abs induce reactive activation of astrocytes and increased production of proinflammatory cytokines, chemokines, and c1q compliment factor, stimulating recruitment of inflammatory cells (including granulocytes and eosinophiles), blood–brain barrier disruption, and compliment activation. Among cytokines, particular IL-6 seems to play an important role. A CD19intCD27highCD38highCD180– population of plasma-blasts was demonstrated to produce AQP4-Abs under IL-6-stimulation (Chihara et al., 2011). Increased levels of IL-6 in CSF have been demonstrated in NMOSD patients and anti-IL-6 receptor therapy seems to be effective even in refractory NMOSD cases (Ayzenberg et al., 2013; Uzawa et al., 2009).

**THERAPY** Similar to MS, acute attacks can be treated with a high-dose steroid pulse or apheresis therapies. Due to often aggressive course and incomplete recovery, it should be started as soon as possible. In severe attacks, plasma exchange or immunoabsorption can be tried also as a first-line therapy (Bonnan et al., 2017; Kleiter et al., 2015).

Many preventive long-term immunotherapies, being effective in MS, have in contrast no influence on NMOSD (e.g., glatiramer acetate) or can even worsen the course of disease: interferon-beta, fingolimod, natalizumab, alemtuzumab (Ayzenberg et al., 2016; Trebst et al., 2014). Rituximab, azathioprine, or mycophenolate mofetil are used as a first-line therapies, whereby rituximab seems to be more effective than both oral medications but is still off-label (Jeong et al., 2016). Methotrexate or combination therapies can be also used as a second-line therapy. Efficacy of an IL-6-receptor antagonist tocilizumab and recently proteasome-inhibitor bortezomib has been demonstrated in refractory cases (Ayzenberg et al., 2013; Zhang et al., 2017). Other potentially high-effective medications, including complement system inhibitor eculizumab, IL-6-receptor inhibitor SA-237 and anti-CD19 monoclonal Ab inebilizumab, are currently under investigations.

#### NEUROLOGICAL SYNDROMES ASSOCIATED WITH MYELIN-OLIGODENDROCYTE GLYCOPROTEIN-ANTIBODIES

MOG-Abs can be found in the number of pediatric and adult inflammatory CNS diseases, including acute disseminated encephalomyelitis (in 36.4%, especially often in children), AQP4-Abs negative NMOSD (in 26.9% of the cases), recurrent optic neuritis and myelitis, multiple sclerosis (especially with spinal and brainstem involvement), and NMDAR-encephalitis (Hyun et al., 2017; Peschl et al., 2017; Reindl et al., 2013). Monophasic course is usually associated with a transient presence of MOG-Abs and better prognosis. Antibody persistence is more characteristic for recurrent diseases. Modern CBA with the full length, conformationally intact, and correct glycosylated MOG have better sensitivity comparing to older tests. Earlier methods, such as peptide-based ELISA or Western blot, demonstrated partly controversial findings and are considered obsolete (Berger and Reindl, 2015).

Although clinical presentation in adults most commonly resembles those of classical NMOSD, there are several points differentiating these two pathogenetically separate entities (Jarius et al., 2016b; Peschl et al., 2017). MOG-Abs occur more often in males (1:2.8 comparing to 1:9 in NMOSD), these patients are usually younger and have more often simultaneous optic neuritis and myelitis. Beside characteristic findings such as optic neuritis and longitudinally myelitis, MRI frequently demonstrates brain involvement: supratentorial lesions in 47% at least once during the course of the disease and brainstem—in 29%. Periventricular and callosal lesions can be also found in 26% and 17% accordingly. Presence of less than four cerebral lesions and their fluffy pattern as well as absence of MS-typical Dawson’s fingers, ovoid periventricular lesions, and hypointense T1-lesions helps to differentiate it from MS (Jurynczyk et al., 2017). Minority of patients only (13%) has oligoclonal bands (Jarius et al., 2016b). MOG-Abs can be detected in CSF in 67%; however, there is usually no specific intrathecal synthesis (Jarius et al., 2016c).

MOG-Abs belong to complement-activating IgG1 subclass. Available histopathological findings demonstrated MS pattern II lesions, with prominent loss of myelin, macrophages, containing myelin degradation products, perivascular and parenchymal T- and fewer B cells as well as deposition of terminal complement complex C9neo (Jarius et al., 2016a; Spadaro et al., 2015). MOG-immunoreactivity was diminished and the remaining oligodendrocytes were MOG-negative, likely representing oligodendrocyte progenitor cells. In contrast to AQP4-Abs positive NMOSD astrocytes remain largely intact, and there were no neutrophilic or eosinophilic infiltrates (Wang et al., 2016). While GFAP levels in CSF are significantly increased in AQP4-Abs positive cases, elevated myelin basic protein has been reported in MOG-Abs positive variant of the disease (Ikeda et al., 2015). Taking altogether primary occurring autoimmune oligodendrocytes injury indicates that it is pathogenetically a distinct disease.

Relapses can be severe; however, remarkable recovery both clinically and paraclinically (even with normalization of evoked potentials and resolution of MRI lesions) can be generally achieved (Kitley et al., 2014). High-dose steroids or apheresis therapies are usually effective. Flare-up of symptoms after temporary improvement under

high-dose steroids is rather common (44%) and oral tapering should be considered (Jarius et al., 2016c). Due to the lack of studies an optimal long-term immunotherapy remains speculative. Some efficacy has been reported for azathioprine, methotrexate, and anti-CD20 monoclonal Abs.

#### NEUROLOGICAL SYNDROMES ASSOCIATED WITH GLIAL FIBRILLARY ACIDIC PROTEIN-ANTIBODIES

A new GFAP-Abs associated meningoencephalomyelitis has been recently reported in two large case series (Fang et al., 2016; Flanagan et al., 2017). Clinical presentation is not always specific and may include encephalopathy (memory loss, confusion, seizures), myelitic, and meningeal symptoms, optic disc edema with blurred vision and tremor. In 22% this disease occurs in a paraneoplastic context (most common ovarian teratoma) and in 21% in association with other autoimmune diseases (diabetes mellitus type 1, RA, myasthenia gravis). Diagnostically the highest sensitivity and specificity of Abs-testing was demonstrated in CSF. The cell count and protein in CSF can be also markedly elevated, and 54% have CSF-exclusive oligoclonal bands. MRI reveals at least in a half of patients characteristic radial linear and sometimes dotted perivascular contrast enhancement, extending from ventricles and frequently resolving under corticosteroid therapy (Flanagan et al., 2017). Spinal lesions are often extensive and longitudinal along the course of the central canal, however are described as more thin and subtle, comparing to those in AQP4-positive NMOSD.

Being directed toward an intracellular antigen GFAP-Abs have probably no direct pathogenetic role and T cell-mediated mechanisms must be supposed. However, this syndrome seems to be responsive to immunotherapy. The presence of GFAP-Abs is probably not always clinically relevant or can be secondary in a number of cases. In two cases GFAP-Abs have been found in patients with gliomas (astrocytoma and choroid plexus glioma) as a primary CNS disease. These Abs have been also found in serum of 0.5%–1.5% of the healthy controls. In 40% of the patients GFAP-Abs coexisted with other auto-Abs, most often directed against two membrane proteins: NMDAR (22%) and AQP4 (10%). Although no animal model has been yet established, a kind of natural model of the disease, necrotizing meningoencephalitis with GFAP-Abs in CSF, has been previously described in dogs (Uchida et al., 2016).

### Target Antigens: Main Functions and Role in the Central Nervous System Autoimmunity

#### *Neuronal Cell Surface Antigens*

##### EXCITATORY RECEPTORS

**N-METHYL-D-ASPARTATE RECEPTOR** The NMDAR is one of the excitatory ionotropic glutamate receptors (similar to AMPA and kainite receptors) (Kalia et al., 2008). NMDARs are tetramers, composed of polypeptide subunits, forming the ion channel, permeable to K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>++</sup>. Three groups of subunits (NR1, 2, 3) have been identified. Opening of the ion-channel requires binding of glutamate (on the NR2 subunit) and glycine (on the NR1 subunit) as well as simultaneous depolarization (Paoletti and Neyton, 2007). NMDAR plays an important role in calcium-dependent synaptic plasticity. However, increased concentrations of glutamate result in neuronal injury due to excessive NMDAR activity and Ca<sup>++</sup> inflow (glutamate excitotoxicity), being relevant in a number of neurological diseases (Waxman and Lynch, 2005). In case of encephalitis, NMDAR-Abs bind the NR1 subunit of the receptor, resulting in its internalization from the cell surface and reduction of NMDAR-mediated currents (Hughes et al., 2010). For clinical features of NMDAR-encephalitis, see the “Anti-N-methyl-D-aspartate receptor encephalitis” section.

**$\alpha$ -AMINO-3-HYDROXY-5-METHYL-4-ISOXAZOLEPROPIONIC ACID RECEPTOR** The AMPAR is an ionotropic transmembrane glutamate receptor that mediates most of the rapid excitatory synaptic transmission in the CNS (Shepherd and Huganir, 2007). Structurally AMPARs are heterotetramers, assembled from the four subunits (GluA1-4), differently expressed depending on the brain region. Amongst them the GluA2 subunit seems to be the most preferred in the assembly process and functionally important, due to control of Ca<sup>2+</sup> permeability. Regulation of the AMPAR (mostly through subunits phosphorylation and receptor trafficking) is a fundamental mechanism of the postsynaptic LTP and LTD. Activity of the AMPAR can be also regulated by its interaction with LGI1 and metalloproteinase (ADAM22) proteins (Yokoi et al., 2012). Being actively involved in the synaptic plasticity AMPAR (along with NMDAR) is essential for learning and memory (Sprengel, 2006). High expression levels of GluA1/2 and GluA2/3 heterodimers have been demonstrated in the structures of limbic system, especially at CA3-CA1 areas of the hippocampus. In case of encephalitis, AMPAR-Abs target GluA1 and/or GluA2 subunits and induce internalization and degradation of receptors, resulting in typical clinical picture of LE, as

described in detail previously (see the “ $\alpha$ -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor encephalitis” section).

**METABOTROPIC GLUTAMATE RECEPTORS 1 AND 5** Both mGluR1 and mGluR5 are located postsynaptically and belong to the first group of mGluRs (Niswender and Conn, 2010). The mGluR1 receptors are strongly expressed in Purkinje cells of cerebellum and mGluR5 in cerebral cortex, hippocampus, striatum, and dorsal horn, consistent with the clinical presentation of corresponding syndromes (see the “Ophelia syndrome or metabotropic glutamate receptor 5 encephalitis” section). These Gq-protein-coupled receptors are able to activate phospholipase C to influence calcium/inositol triphosphate cascade. Functionally, they increase neuronal excitability and are involved in the regulation of synaptic plasticity, including long-term potentiation and depression. The mGluR5 receptors are also involved in the pathogenesis of fragile X syndrome and could be potential therapeutic target in this disease (Scharf et al., 2015).

There are five isoforms of mGluR1 (a–e), having identical extracellular domains and accordingly being a potential target for mGluR1-Abs. A direct pathogenetic role of mGluR1-Abs has been demonstrated experimentally both on the cerebellar slices ex vivo and in vivo (Coesmans et al., 2003). Postmortem analysis showed extensive damage with a loss of two-third of Purkinje cells in a patient with mGluR1-Abs positive cerebellitis. Interestingly, Abs targeting mGluR1 associated protein Homer3 have been also reported in cerebellar ataxia (Jarius and Wildemann, 2015).

## INHIBITORY RECEPTORS

**GLYCINE RECEPTOR** GlyR is a major ionotropic inhibitory receptor actively involved into regulation of motor neuron activity (Carvajal-González et al., 2014). It is a heteromeric ligand-gated Cl-permeable channel, formed by the combination of one of the four a-subunits and a single b-subunit. Activation of the GlyR in mature neurons leads to influx of Cl<sup>-</sup>, fast hyperpolarization, and postsynaptic inhibition. GlyRs are actively expressed in the spinal cord and brainstem as well as in granular cell layer of the cerebellum and hippocampus. Many spinal and brainstem interneurons express both inhibitory receptors: GlyR and GABAaR (Todd and Sullivan, 1990). On the spinal cord level, they coordinate reciprocal inhibition in muscle stretch reflexes, allowing contraction of agonists and relaxation of antagonist muscles and in brainstem keep motor atonia during rapid-eye movement sleep.

Abs-mediated dysfunction of GlyR results in neuronal hyperexcitability, including encephalopathy, seizures, myoclonus, and signs of stiff-person syndrome (so-called PERM syndrome, see the “Stiff-person spectrum disorder” section). Postsynaptic clustering of GlyRs depends on the interactions with gephyrin, another rare autoantigen reported in stiff-person syndrome (Martinez-Hernandez et al., 2016; Tyagarajan and Fritschy, 2014).

**GAMMA-AMINOBUTYRIC ACID TYPE-A RECEPTORS** The GABAaRs are heteropentameric ligand-gated inhibitory chloride channels. Receptor pentamers may contain  $\alpha$ ,  $\beta$ , and either  $\gamma$  or  $\delta$  subunits. Synaptic GABAaR (benzodiazepine-sensitive) usually consist of  $\alpha$ ,  $\beta$ , and  $\gamma$  subunits and mediate fast inhibitory signal transmission (Jacob et al., 2008). In contrast  $\delta$  subunits (instead of  $\gamma$  subunits) are characteristic for receptors at extrasynaptic sites, mediating tonic inhibitory neurotransmission. Similar to GlyR, gephyrin is one of the central proteins involved in the regulation and clustering of GABAaRs at inhibitory synapses. Impairment of GABAaR function causes increased neuronal excitability and epileptic activity (Hirose, 2014). Genetic alteration of GABAaR was demonstrated in inherited epileptic disorders resembling the main symptom of GABAaR-encephalitis and indirectly confirming a direct pathogenetic role of GABAaR-Abs.

**GAMMA-AMINOBUTYRIC ACID TYPE B RECEPTORS** The GABAbR is a metabotropic inhibitory receptor, mainly expressed in hippocampus, amygdala, cerebellum, and thalamus. This receptor is composed of two subunits, B1 (necessary for GABA binding and also target antigen in GABAbR encephalitis) and B2 (determines localization of the receptor and G-protein coupling) (Emson, 2007). GABAbR are located both pre- and postsynaptically and are involved in pre- and postsynaptical inhibition. GABAbR suppress excessive synchronization in neuronal network, associated with epileptic activity. Accordingly, dysfunction of GABAbR results in an overactivation and generation of epileptic activity. Both genetic and pharmacologically induced alteration of GABAbR function cause seizures as well learning and memory deficits, resembling features of GABAbR encephalitis (Enna and Bowery, 2004; Lancaster et al., 2010; Schuler et al., 2001). GABAbR polymorphism is also associated with temporal lobe epilepsy (Gambardella et al., 2003).

## OTHER NEURONAL CELL SURFACE ANTIGENS

**LEUCINE-RICH GLIOMA INACTIVATED-1** LGI1 is an extracellularly secreted neuronal protein, actively expressed in temporal cortex and hippocampus. In contrast to CASPR2, LGI1 expression in peripheral nerves is weak (Irani et al., 2010a). Binding to presynaptic ADAM23 (a disintegrin and metalloproteinase 23) and postsynaptic ADAM22, LGI1 organizes a transsynaptic complex essential for inhibitory signal transmission from presynaptic VGKC to postsynaptic AMPA receptors. Mutations of LGI1 cause autosomal dominant lateral temporal lobe epilepsy (Kalachikov et al., 2002). LGI1 knock-out mice develop lethal seizures (Fukata et al., 2010). LGI1-Abs induced loss of the interaction between LGI1 and ADAM22 reduces the AMPA receptor-mediated synaptic transmission, resulting in epileptic seizures (Ohkawa et al., 2013).

**CONTACTIN-ASSOCIATED PROTEIN LIKE 2** CASPR2 is a transmembrane protein with a large extracellular part, expressed in the central and PNS. At the neural juxtaparanodes Caspr2 is colocalizes with Kv1.1 and 1.2, being essential for VGKC clustering (Horresh et al., 2008). In the CNS Caspr2 is more prominently expressed in hippocampus and cerebellum. Mutations in Caspr2 gen cause epilepsy and cognitive impairment—signs resembling those of Caspr2-encephalitis (Friedman et al., 2008). It has been supposed that Caspr2-Abs cause down-regulation of Caspr2/Kv complexes on the membrane of peripheral nerves. Similar, dispersed Kv1 have been demonstrated in Caspr2-knockout mice as well as patients with mutations of the Caspr2 encoding gen (Strauss et al., 2006).

**Dipeptidyl-peptidase-like protein 6** DPPX is a cell surface regulatory subunit of the voltage-gated A-type Kv4.2 potassium channels, responsible for transient inhibitory currents and regulating repetitive firing rates and back-propagation of action potentials. Kv4.2 truncation mutation has been identified in temporal lobe epilepsy (Singh et al., 2006). Enhanced neuronal excitability has been also demonstrated in DPPX knockouts as well as experimentally after application of sera containing DPPX-Abs to guinea pig and human enteric neurons (Piepgras et al., 2015; Sun et al., 2011). Taking altogether, both genetic and immunological alterations of the DPPX-Kv4.2 complex seem to result in neuronal hyperexcitability, confirming direct pathogenetic role of DPPX-Abs.

**DOPAMINE-2 RECEPTOR** D2R is one of the five related G-protein-coupled receptors. There are two splice variants of this receptor, short D2Rs, that are predominantly expressed presynaptically and involved in autoreceptor functions, and long D2R, expressed postsynaptically (Beaulieu and Gainetdinov, 2011). These receptors are highly expressed in nucleus accumbens, striatum, substantia nigra, olfactory tubercle, hippocampus, and cortex. D2R are involved in the regulation of motor control, behavior as well as learning, memory, and prolactin secretion. Dopaminergic dysregulation is involved in the pathogenesis of many neurological and psychiatric diseases, such as schizophrenia, depression, bipolar disorder, Parkinsonism, and number of further extrapyramidal disorders.

**IgLON5 RECEPTORS** IgLON5 is a surface adhesion protein, broadly present in the CNS with highest expression level in thalamus and brainstem (Vanaveski et al., 2017). It is highly glycosylated and contains three extracellular immunoglobulin-like domains. In case of IgLON5 encephalitis (for clinical features, see the “IgLON5 encephalitis” section), auto-Abs target the immunoglobulin-like domain 2, independently on its glucosylation, resulting in internalization of IgLON5. Functions of this protein remain incompletely understood, but it is involved in a regulation of neurite outgrowth, neuronal pathfinding, and synaptogenesis during brain development (Sanz et al., 2015). In line with this, proteins of IgLON family are implicated in the pathogenesis of autism spectrum disorders (Minhas et al., 2013).

**AMPHIPHYSIN** Amphiphysin belongs to N-BAR proteins family, involved in the cell membrane remodeling, and is highly expressed in the CNS. It is important for several steps of clathrin-mediated endocytosis, required for the rapid recycling of presynaptic vesicles (Arkhipov et al., 2009). Dysfunction of endocytosis is especially critical for tonic GABAergic inhibitory neurons, characterized by a high vesicles turnover. Amphiphysin-Abs were demonstrated to bind the target antigen in spinal cord presynapses during endocytosis, resulting in fast replenishment of the presynaptic vesicle pool (Geis et al., 2010; Werner et al., 2016). Reduction of GABAergic neurotransmission explains major symptoms of stiff-person syndrome disorder (SPSD)—most characteristic clinical presentation in patients with Amphiphysin-Abs. In contrast to GAD65-Abs positive SPSD, these patients are older and have more prominent cervical stiffness (Murinson and Guarnaccia, 2008). Most of the patients have

breast or lung cancer and further onconeural Abs (Pittock et al., 2005). Beside SPSD, patients may develop neuropathy (including dysautonomia), myelitis, limbic, and brainstem encephalitis or cerebellar ataxia. Spinal cord lesions are often longitudinal, resembling those by NMOSD. Despite an experimentally demonstrated direct pathogenetic role of the Amphiphysin-Abs, CD8+ T-cell infiltrates have been shown histopathologically. Immunotherapy can be effective, especially in nonparaneoplastic and partly in paraneoplastic cases (Moon et al., 2014; Murinson and Guarnaccia, 2008; Pittock et al., 2005).

**DELTA/NOTCH-LIKE EPIDERMAL GROWTH FACTOR-RELATED RECEPTOR (TR)** A punctate staining of the Purkinje cells cytoplasm in a combination with diffuse staining of molecular layer of the cerebellum is characteristic for Tr-Abs (Graus et al., 1997a, 1998). In line with this pattern these Abs are strongly associated with paraneoplastic cerebellar ataxia, mostly in the setting of Hodgkin or more rarely non-Hodgkin lymphoma (Bernal et al., 2003). A transmembrane DNER has been identified as a target autoantigen (de Graaff et al., 2012). DNER is expressed by Purkinje cells and Bergmann glia and is essential for cerebellar development (Saito and Takeshima, 2006). Mice lacking DNER demonstrate impaired cerebellar function, confirming its possible role in the disease (Tohgo et al., 2006). The Abs react with a surface DNER epitope, so that their direct pathogenetic role can be supposed. Improvement of ataxia in some cases under successful tumor treatment or immunotherapy alone (prior to chemotherapy) indirectly confirms it. However, pathological studies demonstrated permanent neuronal loss in others (Bernal et al., 2003; Greene et al., 2014). High sensitivity and specificity of a diagnostic CBA for Tr-Abs has been recently reported (Probst et al., 2015).

### **Intracellular Neuronal Antigens**

#### **ANTINEURONAL NUCLEAR ANTIBODIES**

**HU (ANTINEURONAL NUCLEAR ANTIBODIES 1)** Hu-Abs react with the nuclear and to a lesser extent cytoplasmic antigen, expressed in the central and peripheral (including dorsal root ganglia and enteric plexus) nervous system. Three highly homologous embryonic lethal abnormal visual (ELAV) such as neuron-specific RNA binding proteins 2, 3, and 4 (other names HuB, HuC, and HuD, respectively) have been identified as target antigens (Pignolet et al., 2013). These proteins are highly conserved across species and homologous to the *Drosophila melanogaster* nuclear protein ELAV. ELAV proteins seem to play an important role in the neuronal development (Okano and Darnell, 1997). High titer of Hu-Abs is most frequently associated with paraneoplastic LE and/or paraneoplastic sensory neuronopathy in patients with SCLC. Recovery of neurological deficits is usually poor. Low titers of Hu-Abs may be frequently seen in patients with SCLC without neurologic manifestation and are associated with a better tumor outcome (Graus et al., 1997b). Being rarely found in children, Hu-Abs are associated with neuroblastoma and OMS or LE in tumor-free patients (Honnarat et al., 2013).

**RI (ANTINEURONAL NUCLEAR ANTIBODIES 2)** Unlike Hu-Abs, Ri-Abs react with the neuronal nuclei in CNS only. These Abs can be found in paraneoplastic OMS or cerebellar (often truncal) ataxia mostly in patients with lung, breast, or ovarian cancer. Ri-Abs also target two RNA-binding proteins Nova-1 and 2 (neurooncological ventral antigen), regulating alternative splicing of transcripts encoding synaptic proteins (Yang et al., 1998). Nova-1 expression is mostly restricted to brainstem and spinal cord, being responsible for motor and oculomotor deficits. Nova-2 is more widely expressed in CNS, including cortex and explaining cognitive deficits, occurring in some patients. Prognosis is better, comparing to Hu-Abs. Some patients can improve under tumor and immune therapy (Pittock et al., 2010).

**ANTINEURONAL NUCLEAR ANTIBODIES 3** Target protein of ANNA-3-Abs remains unknown. It is a 170 kDa protein, predominantly expressed in the nuclei of Purkinje cells, granular neurons, enteric neurons, and surprisingly renal glomerular podocytes. Neurological manifestation is usually multifocal and may include polyneuropathies, cerebellar ataxia, myelitis as well as brainstem and LE (Chan et al., 2001). Lung cancer is the most frequently detected tumor in these patients.

**MA (PARANEOPLASTIC MA ANTIGENS)** The Ma-Abs react predominantly with nuclei and nucleoli of all neurons of the central and PNS as well as testicular germinal cells. Three highly homologous proteins paraneoplastic Ma antigens (PNMA)-1, 2, and 3 are targets of the immune response in these patients (Rosenfeld et al., 2001). The precise functions of the PNMA proteins family remain poorly understood. PNMA-2 (expressed exclusively in the brain and number of tumors) seems to be the major antigen, recognized by the serum of almost all patients.

Antibodies to PNMA-1 or -3 almost always occur in combination with PNMA-2. PNMA-1 and -3 proteins can be found in the brain and germ cells of testis. PNMA-1 is known to have proapoptotic function and to be involved in regulated neuronal death in developing brain (Chen and D'Mello, 2010). PNMA-2 seems to play role by the tumorigenesis and to increase chemoresistance of cancer cells, probably inhibiting proapoptotic effects of PNMA-1 (Lee et al., 2016).

LE, often with additional diencephalic or brainstem symptoms, is a most typical clinical presentation; however, cerebellar ataxia, polyneuropathies, and parkinsonism have been also reported (Dalmau et al., 2004; Rosenfeld et al., 2001). Antibodies, targeting Ma2, are usually associated with a testicular cancer (sometimes microscopic tumors!) in men younger than 50 years. Remarkably, neurologic deficits improve in approx. 40% in this subgroup of patients in case of complete response to tumor treatment (Rosenfeld et al., 2001). In contrast, patients with Ma2-Abs in a combination with Ma1 or Ma3 develop more often cerebellar symptoms, have worse prognosis and in 80% are associated with tumors other than germ cells cancer (often lung cancer).

#### CYTOPLASMATIC ANTIGENS

**YO (PURKINJE CELL ANTIGEN-1)** Seres of patients with Yo-Abs react with the cytoplasm of Purkinje cells and some neurons of the deep cerebellar nuclei. Two target antigens have been identified: major (of 62 kDa) and minor (of 34 kDa). The major target protein CDR2 (cerebellar degeneration related-2) is actively expressed in cerebellar and brainstem neurons as well as testes, normal ovary, and ovarian tumors. CDR2 is involved in the cell-cycle control and required for the proper execution of mitosis (O'Donovan et al., 2010). It was also shown to downregulate c-Myc and prevent apoptosis (Okano et al., 1999). Interestingly, Yo-Abs (those targeting CDR2) can be taken up by the neurons and cause dysregulation of calcium homeostasis and neuronal death in the slice culture (Greenlee et al., 2015; Schubert et al., 2014). The precise function of the minor antigen (CDR1) remains unknown.

Yo-Abs are typically associated with an SCD in women with breast or gynecologic cancer (Peterson et al., 1992). Despite effective tumor therapy, neurological deficits usually do not improve, resulting in a significant disability (Rojas et al., 2000).

**MICROTUBULE-ASSOCIATED PROTEIN 1B (PURKINJE CELL ANTIGEN-2)** Initially described in patients with small-cell lung carcinoma and cerebellar ataxia this Ab has been named PCA-2 in order to differentiate it from PCA-1(Yo), associated with ovarian and breast cancer (Vernino and Lennon, 2000). Recently, microtubule-associated protein 1B (MAP1B) has been identified as a target antigen of PCA-2 Abs (Gadoth et al., 2017). As an important component of neural cytoskeleton, MAP1B binds and stabilizes microtubules and is essential for maintenance of the cell shape and polarity, synapse formation, and axonal transport (Tortosa et al., 2011). Mice with genetically deleted MAP1B demonstrate delayed and abnormal nervous system development with lack of corpus callosum and often perinatal death (Takei et al., 1997).

Clinical signs are often heterogeneous. Patients with PCA-Abs may develop brainstem and LE, cerebellar ataxia, and polyneuropathy. Such a mixed presentation can be partly explained by the frequent presence of further antineuronal Abs, such as CV2/CRMP5 (in 26%) or Hu-Abs (in 13%) (Gadoth et al., 2017). PCA-1 Abs are highly predictive for the lung cancer, detected in two-thirds of the cases. Histopathological findings demonstrate infiltration with a cytotoxic CD8 + T-lymphocytes and treatment outcome is usually poor.

**COLLAPSING RESPONSE MEDIATOR PROTEIN 5 (CV2)** Target antigen of the Ab, initially named CV2, is the CRMP5 (Yu et al., 2001). CRMP5 belongs to a family of five cytosolic proteins that are actively expressed during brain development and control neural stem-cell differentiation, neurite outgrowth, and axonal pathfinding. This protein is predominantly located in the dendrites and has negative regulatory effect on dendrite extension, mediated by tubulin binding (Brot et al., 2010). In postnatal brain CRMP5 is actively expressed in the zones of adult neurogenesis (dentate gyrus, olfactory bulb), being involved in the proliferation and survival of newly generated neuroblasts (Veyrac et al., 2011). This protein also promotes proliferation of glioblastoma cells, being an indicator of poor outcome in these patients (Moutal et al., 2015). Interestingly, oligodendrocytes actively express CRMP5 as well and clinical presentation of CRMP5-autoimmunity can mimic symptoms of classical demyelinating disorders (Bretin et al., 2005). Optic neuritis with or without uveitis, loss of olfaction and taste, myelopathy, movement disorders (chorea, Parkinsonism, hemiballism), cerebellar ataxia, behavioral changes as well as involvement of PNS have been described in patients with CRMP5-Abs (Cross et al., 2003; Vernino et al., 2002; Yu et al., 2001). Most of the cases are paraneoplastic, being associated with lung cancer (small cell > nonsmall cell) and sometimes thymoma or other tumors.

**GLUTAMIC ACID DECARBOXYLASE 65** GAD is an intracellular enzyme, converting L-glutamate to GABA by decarboxylation. Both existing enzyme isoforms (65 and 67 kDa) are expressed in the islet cells of pancreas and CNS. In neurons GAD67 is predominantly localized in the cytoplasm and produce a basal level of GABA. GAD65 is concentrated presynaptically, anchored at the cytoplasm-facing side of synaptic vesicles, and is responsible for supplementary GABA-synthesis during sustained neuronal activity. GAD65 is the main clinically relevant autoantigen, while GAD67-Abs often coexist and are rarely autoantigenic in isolation (Fenalti and Buckle, 2010).

Despite being an intracellular protein, GAD65 autoimmunity is relatively rarely associated with tumors (usually thymoma, neuroendocrinial tumors, or breast cancer). A total of 70% neurological manifestation is associated with other autoimmune diseases (mostly type 1 diabetes mellitus, autoimmune thyroid disease, and pernicious anemia). Levels of GAD65-Abs in patients with isolated diabetes are usually much lower (<20 nmol/L) comparing to those with neurological manifestation (often >100 nmol/L) (Pittock et al., 2006). Low values can be also found in up to 8% of the healthy controls (Walikonis and Lennon, 1998). Neurological presentations can be quite heterogeneous, including cerebellar ataxia, stiff-person syndrome, LE (often with seizures), and rarely myelopathy. Intrathecal production of GAD65-Abs can be often detected (Pittock et al., 2006). Target epitopes of T and B cells differ in diabetes patients and those with stiff-person syndrome (Ali et al., 2011; Lohmann et al., 2003). Further antineuronal Abs targeting surface antigens may coexist with GAD65-Abs (Chang et al., 2013). Despite GAD65-Abs were demonstrated to inhibit GABA synthesis and affect transmission ex vivo, it is unlikely to be a central pathogenic mechanism in vivo (McKeon and Tracy, 2017). Pathological findings confirm a T cell-mediated injury in GAD65-Abs-associated autoimmunity (Holmøy et al., 2009). Efficacy of the immunotherapy varies. In patients with LE and seizures improvement occurred in one-third of cases only (McKeon and Tracy, 2017). In another study half of the patients with ataxia improved under immunotherapy (Jones et al., 2015). As in other T cell-mediated diseases an early start immunotherapy is critical.

### **Glial antigens**

#### **AQUAPORIN-4**

AQP4 is the main water channel of the CNS. Its main functions include regulation of the water balance in CNS and extracellular glutamate concentrations. Deletion of AQP4 impairs removal of excess brain water, resulting in increased vasogenic edema and intracranial pressure in experiment (Papadopoulos et al., 2004). AQP4 is especially highly expressed on perivascular endfeet of astrocytes as well as in subependymal and subpial regions, corresponding to the typical localization of NMOSD lesions (see the “Neuromyelitis optica spectrum disease” section). Beside CNS it is expressed in skeletal muscles, placenta, lacrimal and salivary glands, distal renal collecting duct cells, retinal Muller cells, olfactory epithelial cells, and gastric parietal cells. These tissues are generally spared in NMOSD, probably due to coexpression of AQP4 with membrane complement regulators (CD46, CD55, CD59), that are absent on astrocytic endfeets (Saadoun and Papadopoulos, 2015). There are two predominant isoforms of AQP4: M1 and M23. The M23 isoform is able to build tetramers that further cluster to supramolecular assemblies. It has been proposed that due to its size such an assemble cannot be internalized after Abs binding, resulting in the complement-dependent destruction of astrocytes (Hinson et al., 2012).

### **MYELIN OLIGODENDROCYTE GLYCOPROTEIN**

MOG is a CNS-specific minor myelin component. Being involved in myelination, MOG serves as a differentiation marker for oligodendrocytes maturation (Linington et al., 1988). MOG belongs to the Ig superfamily and have 15 alternatively spliced isoforms; however, its function is still not fully understood (Delarasse et al., 2006). It has been supposed to serve as a ligand for dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin on antigen-presenting cells and to keep them in tolerogenic state and as a receptor for nerve growth factor (García-Vallejo et al., 2014; von Büdingen et al., 2015). However, MOG knockout mice display normal myelin and no clinical abnormalities (Delarasse et al., 2003). MOG is one of the best-studied antigens in experimental AE. It is expressed at an outer surface of myelin sheaths and oligodendrocyte processes in CNS, being accessible extracellular target for the auto-Abs. Secreted form of MOG can also probably trigger an autoimmunity (Delarasse et al., 2006). Interestingly, being homologous to butyrophilin proteins of mammary glands, MOG can potentially cause autoimmunity by molecular mimicry (Gardinier et al., 1992; Guggenmos et al., 2004). Earlier results of MOG-Abs testing demonstrated controversial findings (Berger and Reindl, 2015). Modern diagnostic tests, using the full-length, conformationally intact, and glycosylated MOG, have better sensitivity comparing to older one with denatured MOG.

## GLIAL FIBRILLARY ACIDIC PROTEIN

GFAP is the main intermediate filament protein, expressed by astrocytes, ependymal cells, and neural progenitor cells. It is involved in several important astrocytic functions in CNS, including synaptic plasticity, regeneration, and reactive gliosis (Middeldorp and Hol, 2011). Being a cytoplasmic protein, it belongs to intracellular antigens. Among several isoforms of GFAP, mature a-isoform seems to be most relevant as an antigen in GFAP-Abs positive meningoencephalomyelitis (Flanagan et al., 2017). Mutations of GFAP gene result in mental and physical retardation (Alexander disease).

## References

- Aalberse, R.C., Stapel, S.O., Schuurman, J., Rispens, T., 2009. Immunoglobulin G4: an odd antibody. *Clin. Exp. Allergy.* 39, 469–477. Available from: <https://doi.org/10.1111/j.1365-2222.2009.03207.x>.
- Aaltonen, K.J., Virkki, L.M., Malmivaara, A., Konttinen, Y.T., Nordström, D.C., Blom, M., 2012. Systematic review and meta-analysis of the efficacy and safety of existing TNF blocking agents in treatment of rheumatoid arthritis. *PLoS One* 7 (1), e30275.
- Ayzenberg, I., Gold, R., Kleiter, I., 2017. Management of immune-mediated paraneoplastic neurological disorders. *Neuro. Inter. Open* 01 (04), E264–E274. <https://doi.org/10.1055/s-0043-112730>.
- Achkar, A.A., Lie, J.T., Hunder, G.G., O'Fallon, W.M., Gabriel, S.E., 1994. How does previous corticosteroid treatment affect the biopsy findings in giant cell (temporal) arteritis? *Ann. Intern. Med.* 120 (12), 987–992. PubMed PMID: 8185147.
- Agbogu, B.N., Stern, B.J., Sewell, C., Yang, G., 1995. Therapeutic considerations in patients with refractory neurosarcoidosis. *Arch. Neurol.* 52 (9), 875–879. PubMed PMID: 7661724.
- Aguilar-Amat, M.J., Abenza-Abildúa, M.J., Vivancos, F., Rodríguez de Rivera, F.J., Morales-Bastos, C., Gandía-Gonzalez, M.L., et al., 2011. Rheumatoid meningitis mimicking progressive supranuclear palsy. *Neurologist* 17, 136–140.
- Akman-Demir, G., Serdaroglu, P., Tasçi, B., 1999. Clinical patterns of neurological involvement in Behcet's disease: evaluation of 200 patients. The Neuro-Behcet Study Group. *Brain* 122 (Pt 11), 2171–2182. PubMed PMID: 10545401.
- Alexander, E.L., 1993. Neurologic disease in Sjögren's syndrome: mononuclear inflammatory vasculopathy affecting central/peripheral nervous system and muscle. A clinical review and update of immunopathogenesis. *Rheum. Dis. Clin. N. Am.* 19 (4), 869–908. Review. PubMed PMID: 8265827.
- Alexander, E.L., Malinow, K., Lejewski, J.E., Jerdan, M.S., Provost, T.T., Alexander, G.E., 1986. Primary Sjögren's syndrome with central nervous system mimicking multiple sclerosis. *Ann. Intern. Med.* 104 (3), 323–330. PubMed PMID: 3946977.
- Ali, F., Rowley, M., Jayakrishnan, B., Teuber, S., Gershwin, M.E., Mackay, I.R., 2011. Stiff-person syndrome (SPS) and anti-GAD-related CNS degenerations: protean additions to the autoimmune central neuropathies. *J. Autoimmun.* 37, 79–87. Available from: <https://doi.org/10.1016/j.jaut.2011.05.005>.
- Alkan, A., Goktan, A., Karincaoglu, Y., Kamisli, S., Dogan, M., Oztanir, N., et al., 2012. Brain perfusion MRI findings in patients with Behcet's disease. *Sci. World J.* Available from: <https://doi.org/10.1100/2012/261502>. Epub 2012 Apr 30. 2012:261502 PubMed PMID: 22654579; PubMed Central PMCID: PMC3361152.
- Alreshaid, A.A., Powers, W.J., 2003. Prognosis of patients with suspected primary CNS angiitis and negative brain biopsy. *Neurology* 61 (6), 831–833. Review. PubMed PMID: 14504332.
- Alshekhee, A., Basiri, K., Miles, J.D., Ahmad, S.A., Katirji, B., 2010. Chronic inflammatory demyelinating polyneuropathy associated with tumor necrosis factor-alpha antagonists. *Muscle Nerve* 41, 723–727.
- Ariño, H., Gresa-Arribas, N., Blanco, Y., Martínez-Hernández, E., Sabater, L., Petit-Pedrol, M., et al., 2014. Cerebellar ataxia and glutamic acid decarboxylase antibodies: immunologic profile and long-term effect of immunotherapy. *JAMA Neurol.* 71, 1009–1016. Available from: <https://doi.org/10.1001/jamaneurol.2014.1011>.
- Arkhipov, A., Yin, Y., Schulten, K., 2009. Membrane-bending mechanism of amphiphysin N-BAR domains. *Biophys. J.* 97, 2727–2735. Available from: <https://doi.org/10.1016/j.bpj.2009.08.051>.
- Armangue, T., Leypoldt, F., Málaga, I., Raspall-Chaure, M., Martí, I., Nichter, C., et al., 2014a. Herpes simplex virus encephalitis is a trigger of brain autoimmunity. *Ann. Neurol.* 75, 317–323. Available from: <https://doi.org/10.1002/ana.24083>.
- Armangue, T., Titulaer, M.J., Sabater, L., Pardo-Moreno, J., Gresa-Arribas, N., Barbero-Bordallo, N., et al., 2014b. A novel treatment-responsive encephalitis with frequent opsoclonus and teratoma. *Ann. Neurol.* 75, 435–441. Available from: <https://doi.org/10.1002/ana.23917>.
- Armangue, T., Moris, G., Cantarín-Extremera, V., Conde, C.E., Rostasy, K., Erro, M.E., et al., 2015. Autoimmune post-herpes simplex encephalitis of adults and teenagers. *Neurology* 85, 1736–1743. Available from: <https://doi.org/10.1212/WNL.0000000000002125>.
- Armangué, T., Sabater, L., Torres-Vega, E., Martínez-Hernández, E., Ariño, H., Petit-Pedrol, M., et al., 2016. Clinical and immunological features of opsoclonus-myoclonus syndrome in the era of neuronal cell surface antibodies. *JAMA Neurol.* 73, 417–424. Available from: <https://doi.org/10.1001/jamaneurol.2015.4607>.
- Ayzenberg, I., Kleiter, I., Schröder, A., Hellwig, K., Chan, A., Yamamura, T., et al., 2013. Interleukin 6 receptor blockade in patients with neuromyelitis optica nonresponsive to anti-CD20 therapy. *JAMA Neurol.* 70, 394–397. Available from: <https://doi.org/10.1001/jamaneurol.2013.1246>.
- Ayzenberg, I., Schöllhammer, J., Hoepner, R., Hellwig, K., Ringelstein, M., Aktas, O., et al., 2016. Efficacy of glatiramer acetate in neuromyelitis optica spectrum disorder: a multicenter retrospective study. *J. Neurol.* 263, 575–582. Available from: <https://doi.org/10.1007/s00415-015-7991-1>.
- Baker, M., Das, M., Isaacs, J., Fawcett, P., Bates, D., 2005. Treatment of stiff person syndrome with rituximab. *J. Neurol. Neurosurg. Psychiatry* 76, 999–1001. Available from: <https://doi.org/10.1136/jnnp.2004.051144>.

- Bakshi, J., Segura, B.T., Wincup, C., Rahman, A., 2017. Unmet needs in the pathogenesis and treatment of systemic lupus erythematosus. *Clin. Rev. Allergy Immunol.* Available from: <https://doi.org/10.1007/s12016-017-8640-5> [Epub ahead of print] Review. PubMed PMID: 28853005.
- Balint, B., Jarius, S., Ni, S., Haberkorn, U., Probst, C., Blöcker, I.M., et al., 2014. Progressive encephalomyelitis with rigidity and myoclonus: a new variant with DPPX antibodies. *Neurology* 82, 1521–1528. Available from: <https://doi.org/10.1212/WNL.0000000000000372>.
- Banna, M., el-Ramahi, K., 1991. Neurologic involvement in Behcet disease: imaging findings in 16 patients. *AJNR Am. J. Neuroradiol.* 12 (4), 791–796. PubMed PMID: 1882769.
- Bataller, L., Graus, F., Saiz, A., Vilchez, J.J., Spanish Opsclonus-Myoclonus Study Group, 2001. Clinical outcome in adult onset idiopathic or paraneoplastic opsclonus-myoclonus. *Brain J. Neurol.* 124, 437–443.
- Bauer, J., Bien, C.G., 2016. Neuropathology of autoimmune encephalitides. *Handb. Clin. Neurol.* 133, 107–120. Available from: <https://doi.org/10.1016/B978-0-444-63432-0.00007-4>.
- Bayrak, A.O., Durmus, D., Durmaz, Y., Demir, I., Canturk, F., Onar, M.K., 2010. Electrophysiological assessment of polyneuropathic involvement in rheumatoid arthritis: relationships among demographic, clinical and laboratory findings. *Neurol. Res.* 32, 711–714.
- Beaulieu, J.-M., Gainetdinov, R.R., 2011. The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol. Rev.* 63, 182–217. Available from: <https://doi.org/10.1124/pr.110.002642>.
- Behrendt, V., Krogias, C., Reinacher-Schick, A., Gold, R., Kleiter, I., 2016. Bortezomib treatment for patients with anti-N-methyl-D-aspartate receptor encephalitis. *JAMA Neurol.* 73, 1251–1253. Available from: <https://doi.org/10.1001/jamaneurol.2016.2588>.
- Belkhir, R., Gestermann, N., Koutero, M., Seror, R., Tost, J., Mariette, X., et al., 2014. Upregulation of membrane-bound CD40L on CD4+ T cells in women with primary Sjögren's syndrome. *Scand. J. Immunol.* 79, 37–42.
- Berger, T., Reindl, M., 2015. Antibody biomarkers in CNS demyelinating diseases—a long and winding road. *Eur. J. Neurol.* 22, 1162–1168. Available from: <https://doi.org/10.1111/ene.12759>.
- Bernal, F., Shams'ili, S., Rojas, I., Sanchez-Valle, R., Saiz, A., Dalmau, J., et al., 2003. Anti-Tr antibodies as markers of paraneoplastic cerebellar degeneration and Hodgkin's disease. *Neurology* 60, 230–234.
- Bienvenu, B., Ly, K., Lambert, M., et al., 2016. Management of giant cell arteritis: recommendations of the French Study Group for Large Vessel Vasculitis (GEFA). *Rev. Med. Interne* 37, 154–165.
- Birnbaum, J., Petri, M., Thompson, R., Izbudak, I., Kerr, D., 2009. Distinct subtypes of myelitis in systemic lupus erythematosus. *Arthritis Rheum.* 60 (11), 3378–3387. Available from: <https://doi.org/10.1002/art.24937>. PubMed PMID: 19877037.
- Bley, T.A., Weiben, O., Uhl, M., Vaith, P., Schmidt, D., Warnatz, K., et al., 2005a. Assessment of the cranial involvement pattern of giant cell arteritis with 3T magnetic resonance imaging. *Arthritis Rheum.* 52 (8), 2470–2477. PubMed PMID: 16052572.
- Bley, T.A., Wieben, O., Leupold, J., Uhl, M., 2005b. Images in cardiovascular medicine. Magnetic resonance imaging findings in temporal arteritis. *Circulation* 111 (16), e260. PubMed PMID: 15851605.
- Blom, M., Creemers, M.C., Kievit, W., Lemmens, J.A., van Riel, P.L., 2013. Long-term follow-up of the cervical spine with conventional radiographs in patients with rheumatoid arthritis. *Scand. J. Rheumatol.* 42, 281–288.
- Bonello, M., Jacob, A., Ellul, M.A., Barker, E., Parker, R., Jefferson, S., et al., 2017. IgLON5 disease responsive to immunotherapy. *Neurol. Neuroimmunol. Neuroinflamm.* 4, e383. Available from: <https://doi.org/10.1212/NXI.0000000000000383>.
- Bonnan, M., Valentino, R., Debeugny, S., Merle, H., Fergé, J.-L., Mehdaoui, H., et al., 2017. Short delay to initiate plasma exchange is the strongest predictor of outcome in severe attacks of NMO spectrum disorders. *J. Neurol. Neurosurg. Psychiatry*. Available from: <https://doi.org/10.1136/jnnp-2017-316286>.
- Borhani Haghighi, A., Pourmand, R., Nikseresht, A.R., 2005. Neuro-Behcet disease. A review. *Neurologist* 11 (2), 80–89. Review. PubMed PMID: 15733330.
- Boronat, A., Gelfand, J.M., Gresa-Arribas, N., Jeong, H.-Y., Walsh, M., Roberts, K., et al., 2013. Encephalitis and antibodies to DPPX, a subunit of Kv4.2 potassium channels. *Ann. Neurol.* 73, 120–128. Available from: <https://doi.org/10.1002/ana.23756>.
- Both, T., Dalm, V.A., van Hagen, P.M., van Daele, P.L., 2017. Reviewing primary Sjögren's syndrome: beyond the dryness—from pathophysiology to diagnosis and treatment. *Int. J. Med. Sci.* 14 (3), 191–200. Available from: <https://doi.org/10.7150/ijms.17718>. eCollection 2017. Review. PubMed PMID: 28367079; PubMed Central PMCID: PMC5370281.
- Braithwaite, T., Holder, G.E., Lee, R.W.J., Plant, G.T., Tufail, A., 2014. Diagnostic features of the autoimmune retinopathies. *Autoimmun. Rev.* 13, 534–538. Available from: <https://doi.org/10.1016/j.autrev.2014.01.039>.
- Bretin, S., Reibel, S., Charrier, E., Maus-Moatti, M., Auvergnon, N., Thevenoux, A., et al., 2005. Differential expression of CRMP1, CRMP2A, CRMP2B, and CRMP5 in axons or dendrites of distinct neurons in the mouse brain. *J. Comp. Neurol.* 486, 1–17. Available from: <https://doi.org/10.1002/cne.20465>.
- Briani, C., Vitaliani, R., Grisold, W., Honnorat, J., Graus, F., Antoine, J.C., et al., 2011. Spectrum of paraneoplastic disease associated with lymphoma. *Neurology* 76, 705–710. Available from: <https://doi.org/10.1212/WNL.0b013e31820d62eb>.
- Brot, S., Rogemond, V., Perrot, V., Chouinlamountri, N., Auger, C., Honnorat, J., et al., 2010. CRMP5 interacts with tubulin to inhibit neurite outgrowth, thereby modulating the function of CRMP2. *J. Neurosci.* 30, 10639–10654. Available from: <https://doi.org/10.1523/JNEUROSCI.0059-10.2010>.
- Burns, C.A., Ebersole, J.L., Allansmith, M.R., 1982. Immunoglobulin A antibody levels in human tears, saliva, and serum. *Infect. Immun.* 36, 1019–1022.
- Candler, P.M., Hart, P.E., Barnett, M., Weil, R., Rees, J.H., 2004. A follow up study of patients with paraneoplastic neurological disease in the United Kingdom. *J. Neurol. Neurosurg. Psychiatry* 75, 1411–1415. Available from: <https://doi.org/10.1136/jnnp.2003.025171>.
- Carr, I., 1982. The Ophelia syndrome: memory loss in Hodgkin's disease. *Lancet Lond. Engl.* 1, 844–845.
- Carvajal-González, A., Leite, M.I., Waters, P., Woodhall, M., Coutinho, E., Balint, B., et al., 2014. Glycine receptor antibodies in PERM and related syndromes: characteristics, clinical features and outcomes. *Brain* 137, 2178–2192. Available from: <https://doi.org/10.1093/brain/awu142>.
- Carvalho, D.C., Tironi, T.S., Freitas, D.S., Kleinpaul, R., Talim, N.C., Lana-Peixoto, M.A., 2014. Sjögren syndrome and neuromyelitis optica spectrum disorder co-exist in a common autoimmune milieu. *Arq. Neuropsiquiatr.* 72 (8), 619–624. Review. PubMed PMID: 25098478.

- Caselli, R.J., Daube, J.R., Hunder, G.G., Whisnant, J.P., 1988. Peripheral neuropathic syndromes in giant cell (temporal) arteritis. *Neurology* 38 (5), 685–689. PubMed PMID: 2834668.
- Chan, K.H., Vernino, S., Lennon, V.A., 2001. ANNA-3 anti-neuronal nuclear antibody: marker of lung cancer-related autoimmunity. *Ann. Neurol.* 50, 301–311.
- Chang, T., Alexopoulos, H., McMenamin, M., Carvajal-González, A., Alexander, S.K., Deacon, R., et al., 2013. Neuronal surface and glutamic acid decarboxylase autoantibodies in Nonparaneoplastic stiff person syndrome. *JAMA Neurol.* 70, 1140–1149. Available from: <https://doi.org/10.1001/jamaneurol.2013.3499>.
- Chao, C.C., Hsieh, S.T., Shun, C.T., Hsieh, S.C., 2007. Skin denervation and cutaneous vasculitis in eosinophilia-associated neuropathy. *Arch. Neurol.* 64 (7), 959–965. PubMed PMID: 17620485.
- Chapelon, C., Ziza, J.M., Piette, J.C., Levy, Y., Raguin, G., Wechsler, B., et al., 1990. Neurosarcoidosis: signs, course and treatment in 35 confirmed cases. *Medicine (Baltimore)* 69 (5), 261–276. Review. PubMed PMID: 2205782.
- Chen, H.-L., D'Mello, S.R., 2010. Induction of neuronal cell death by paraneoplastic Ma1 antigen. *J. Neurosci. Res.* 88, 3508–3519. Available from: <https://doi.org/10.1002/jnr.22506>.
- Chiewthanakul, P., Sawanyawisuth, K., Focharoen, C., Tiamkao, S., 2012. Clinical features and predictive factors in neuropsychiatric lupus. *Asian Pac. J. Allergy Immunol.* 30 (1), 55–60. Erratum in: *Asian Pac J Allergy Immunol.* 2012 Sep;30(3):246. PubMed PMID: 22523908.
- Chihara, N., Aranami, T., Sato, W., Miyazaki, Y., Miyake, S., Okamoto, T., et al., 2011. Interleukin 6 signaling promotes anti-aquaporin 4 auto-antibody production from plasmablasts in neuromyelitis optica. *Proc. Natl. Acad. Sci. U.S.A.* 108, 3701–3706. Available from: <https://doi.org/10.1073/pnas.1017385108>.
- Chu, C.T., Gray, L., Goldstein, L.B., Hulette, C.M., 1998. Diagnosis of intracranial vasculitis: a multi-disciplinary approach. *J. Neuropathol. Exp. Neurol.* 57 (1), 30–38. PubMed PMID: 9600195.
- Coesmans, M., Smitt, P.A.S., Linden, D.J., Shigemoto, R., Hirano, T., Yamakawa, Y., et al., 2003. Mechanisms underlying cerebellar motor deficits due to mGluR1-autoantibodies. *Ann. Neurol.* 53, 325–336. Available from: <https://doi.org/10.1002/ana.10451>.
- Cohen, P., Pagnoux, C., Mahr, A., Arène, J.P., Mouthon, L., Le Guern, V., et al., 2007. Churg-Strauss syndrome with poor-prognosis factors: a prospective multicenter trial comparing glucocorticoids and six or twelve cyclophosphamide pulses in forty-eight patients. *Arthritis Rheum.* 57 (4), 686–693. PubMed PMID: 17471546.
- Crickx, E., Machelart, I., Lazaro, E., Kahn, J.E., Cohen-Aubart, F., Martin, T., et al., 2016. Intravenous immunoglobulin as an immunomodulating agent in antineutrophil cytoplasmic antibody-associated vasculitides: a French Nationwide Study of ninety-two patients. *Arthritis Rheumatol.* 68 (3), 702–712. Available from: <https://doi.org/10.1002/art.39472>. PubMed PMID: 26473632.
- Cross, S.A., Salomao, D.R., Parisi, J.E., Kryzer, T.J., Bradley, E.A., Mines, J.A., et al., 2003. Paraneoplastic autoimmune optic neuritis with retinitis defined by CRMP-5-IgG. *Ann. Neurol.* 54, 38–50. Available from: <https://doi.org/10.1002/ana.10587>.
- Cuadrado, M.J., Sciascia, S., Bosch, X., Khamashta, M.A., Ramos-Casals, M., 2013. Is it time for biosimilars in autoimmune diseases? *Autoimmun. Rev.* 12, 954–957.
- Dahm, L., Ott, C., Steiner, J., Stepienak, B., Teegen, B., Saschenbrecker, S., et al., 2014. Seroprevalence of autoantibodies against brain antigens in health and disease. *Ann. Neurol.* 76, 82–94. Available from: <https://doi.org/10.1002/ana.24189>.
- Dalakas, M.C., Fujii, M., Li, M., Lutfi, B., Kyhos, J., McElroy, B., 2001. High-dose intravenous immune globulin for stiff-person syndrome. *N. Engl. J. Med.* 345, 1870–1876. Available from: <https://doi.org/10.1056/NEJMoa01167>.
- Dale, R.C., Merheb, V., Pillai, S., Wang, D., Cantrill, L., Murphy, T.K., et al., 2012. Antibodies to surface dopamine-2 receptor in autoimmune movement and psychiatric disorders. *Brain J. Neurol.* 135, 3453–3468. Available from: <https://doi.org/10.1093/brain/aws256>.
- Dalmau, J., 2016. NMDA receptor encephalitis and other antibody-mediated disorders of the synapse: the 2016 Cotzias Lecture. *Neurology* 87, 2471–2482. Available from: <https://doi.org/10.1212/WNL.0000000000003414>.
- Dalmau, J., Graus, F., Villarejo, A., Posner, J.B., Blumenthal, D., Thiessen, B., et al., 2004. Clinical analysis of anti-Ma2-associated encephalitis. *Brain J. Neurol.* 127, 1831–1844. Available from: <https://doi.org/10.1093/brain/awh203>.
- Dalmau, J., Gleichman, A.J., Hughes, E.G., Rossi, J.E., Peng, X., Lai, M., et al., 2008. Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. *Lancet Neurol.* 7, 1091–1098. Available from: [https://doi.org/10.1016/S1474-4422\(08\)70224-2](https://doi.org/10.1016/S1474-4422(08)70224-2).
- Dalmau, J., Lancaster, E., Martinez-Hernandez, E., Rosenfeld, M.R., Balice-Gordon, R., 2011. Clinical experience and laboratory investigations in patients with anti-NMDAR encephalitis. *Lancet Neurol.* 10, 63–74. Available from: [https://doi.org/10.1016/S1474-4422\(10\)70253-2](https://doi.org/10.1016/S1474-4422(10)70253-2).
- Dalmau, J., Geis, C., Graus, F., 2017. Autoantibodies to synaptic receptors and neuronal cell surface proteins in autoimmune diseases of the central nervous system. *Physiol. Rev.* 97, 839–887. Available from: <https://doi.org/10.1152/physrev.00010.2016>.
- Darnell, R.B., Posner, J.B., 2006. Paraneoplastic syndromes affecting the nervous system. *Semin. Oncol.* 33, 270–298. Available from: <https://doi.org/10.1053/j.seminoncol.2006.03.008>.
- Dasgupta, B., Borg, F., Hassan, N., et al., 2010. BSR and BHPR guidelines for the management of giant cell arteritis. *Rheumatology (Oxford)* 49, 1594–1597.
- de Graaff, E., Maat, P., Hulsenboom, E., van den Berg, R., van den Bent, M., Demmers, J., et al., 2012. Identification of delta/notch-like epidermal growth factor-related receptor as the Tr antigen in paraneoplastic cerebellar degeneration. *Ann. Neurol.* 71, 815–824. Available from: <https://doi.org/10.1002/ana.23550>.
- de Groot, K., Adu, D., Savage, C., 2001. The value of pulse cyclophosphamide in ANCA-associated vasculitis: meta-analysis and critical review. *Nephrol. Dial. Transplant.* 16, 2018–2027.
- Dejaco, C., Brouwer, E., Mason, J.C., Buttigereit, F., Matteson, E.L., Dasgupta, B., 2017. Giant cell arteritis and polymyalgia rheumatica: current challenges and opportunities. *Nat. Rev. Rheumatol.* 13 (10), 578–592. Available from: <https://doi.org/10.1038/nrrheum.2017.142>. Epub 2017 Sep 14. Review. PubMed PMID: 28905861.
- Delarasse, C., Daubas, P., Mars, L.T., Vizler, C., Litzenburger, T., Iglesias, A., et al., 2003. Myelin/oligodendrocyte glycoprotein-deficient (MOG-deficient) mice reveal lack of immune tolerance to MOG in wild-type mice. *J. Clin. Invest.* 112, 544–553. Available from: <https://doi.org/10.1172/JCI15861>.
- Delarasse, C., Della Gaspera, B., Lu, C.W., Lachapelle, F., Gelot, A., Rodriguez, D., et al., 2006. Complex alternative splicing of the myelin oligodendrocyte glycoprotein gene is unique to human and non-human primates. *J. Neurochem.* 98, 1707–1717. Available from: <https://doi.org/10.1111/j.1471-4159.2006.04053.x>.

- Demaerel, P., De Ruyter, N., Maes, F., Velghe, B., Wilms, G., 2004. Magnetic resonance angiography in suspected cerebral vasculitis. *Eur. Radiol.* 14 (6), 1005–1012. Epub 2004 Feb 10. PubMed PMID: 14872278.
- Deuschl, G., Kessler, K., Poewe, W., Schulz, J.B., Schnitzler, A., Schwingenschuh, P., et al., 2012. Tremor, Leitlinien der Deutschen Gesellschaft für Neurologie. <https://www.dgn.org/leitlinien/2391-ll-13-2012-tremor> (accessed 01.03.2019)
- Dogan Onugoren, M., Golombeck, K.S., Bien, C., Abu-Tair, M., Brand, M., Bulla-Hellwig, M., et al., 2016. Immunoabsorption therapy in autoimmune encephalitides. *Neurol. Neuroimmunol. Neuroinflamm.* 3, e207. Available from: <https://doi.org/10.1212/NXI.0000000000000207>.
- Duna, G.F., Calabrese, L.H., 1995. Limitations of invasive modalities in the diagnosis of primary angiitis of the central nervous system. *J. Rheumatol.* 22 (4), 662–667. PubMed PMID: 7791160.
- Eber, T., Chapman, J., Shoenfeld, Y., 2005. Anti-ribosomal P-protein and its role in psychiatric manifestations of systemic lupus erythematosus: myth or reality? *Lupus* 14, 571–575.
- Elefante, E., Monti, S., Bond, M., Lepri, G., Quartuccio, L., Talarico, R., et al., 2017. One year in review 2017: systemic vasculitis. *Clin. Exp. Rheumatol.* 35 (Suppl 103(1)), 5–26. Epub 2017 Mar 29. Review. PubMed PMID: 28375840.
- Emson, P.C., 2007. GABA(B) receptors: structure and function. *Progr. Brain Res.* 160, 43–57. Available from: [https://doi.org/10.1016/S0079-6123\(06\)60004-6](https://doi.org/10.1016/S0079-6123(06)60004-6).
- Enna, S.J., Bowery, N.G., 2004. GABA(B) receptor alterations as indicators of physiological and pharmacological function. *Biochem. Pharmacol.* 68, 1541–1548. Available from: <https://doi.org/10.1016/j.bcp.2004.06.037>.
- Espinosa, G., Mendizábal, A., Minguez, S., Ramo-Tello, C., Capellades, J., Olivé, A., et al., 2010. Transverse myelitis affecting more than 4 spinal segments associated with systemic lupus erythematosus: clinical, immunological, and radiological characteristics of 22 patients. *Semin. Arthritis Rheum.* 39 (4), 246–256. Available from: <https://doi.org/10.1016/j.semarthrit.2008.09.002>. Epub 2008 Nov 20. PubMed PMID: 19022478.
- Estiasari, R., Matsushita, T., Masaki, K., Akiyama, T., Yonekawa, T., Isobe, N., et al., 2012. Comparison of clinical, immunological and neuro-imaging features between anti-aquaporin-4 antibody-positive and antibody-negative Sjögren's syndrome patients with central nervous system manifestations. *Mult. Scler.* 18 (6), 807–816. Available from: <https://doi.org/10.1177/1352458511431727>. Epub 2012 Jan 30. PubMed PMID: 22291033.
- Faillace, C., de Almeida, J.R., de Carvalho, J.F., 2013. Optic neuritis after infliximab therapy. *Rheumatol. Int.* 33, 1101–1103.
- Faissner, S., Lukas, C., Reinacher-Schick, A., Tannapfel, A., Gold, R., Kleiter, I., 2016. Amphiphysin-positive paraneoplastic myelitis and stiff-person syndrome. *Neurol. Neuroimmunol. Neuroinflamm.* 3, e285. Available from: <https://doi.org/10.1212/NXI.0000000000000285>.
- Fang, B., McKeon, A., Hinson, S.R., Kryzer, T.J., Pittock, S.J., Aksamit, A.J., et al., 2016. Autoimmune glial fibrillary acidic protein astrocytopathy: a novel meningoencephalomyelitis. *JAMA Neurol.* 73, 1297–1307. Available from: <https://doi.org/10.1001/jamaneurol.2016.2549>.
- Fenalti, G., Buckle, A.M., 2010. Structural biology of the GAD autoantigen. *Autoimmun. Rev.* 9, 148–152. Available from: <https://doi.org/10.1016/j.autrev.2009.05.003>.
- Fernández, N., Jancar, S., Sánchez Crespo, M., 2004. Blood and endothelium in immune complex-mediated tissue injury. *Trends Pharmcol. Sci.* 25, 512–517.
- Finke, C., Kopp, U.A., Prüss, H., Dalmau, J., Wandinger, K.-P., Ploner, C.J., 2012. Cognitive deficits following anti-NMDA receptor encephalitis. *J. Neurol. Neurosurg. Psychiatry* 83, 195–198. Available from: <https://doi.org/10.1136/jnnp-2011-300411>.
- Flanagan, E.P., 2016. Autoimmune myelopathies. *Handb. Clin. Neurol.* 133, 327–351. Available from: <https://doi.org/10.1016/B978-0-444-63432-0.00019-0>.
- Flanagan, E.P., McKeon, A., Lennon, V.A., Kearns, J., Weinshenker, B.G., Krecke, K.N., et al., 2011. Paraneoplastic isolated myelopathy: clinical course and neuroimaging clues. *Neurology* 76, 2089–2095. Available from: <https://doi.org/10.1212/WNL.0b013e31821f468f>.
- Flanagan, E.P., Weinshenker, B.G., Krecke, K.N., Lennon, V.A., Lucchinetti, C.F., McKeon, A., et al., 2015. Short myelitis lesions in aquaporin-4-IgG-positive neuromyelitis optica spectrum disorders. *JAMA Neurol.* 72, 81–87. Available from: <https://doi.org/10.1001/jamaneurol.2014.2137>.
- Flanagan, E.P., Hinson, S.R., Lennon, V.A., Fang, B., Aksamit, A.J., Morris, P.P., et al., 2017. Glial fibrillary acidic protein immunoglobulin G as biomarker of autoimmune astrocytopathy: analysis of 102 patients. *Ann. Neurol.* 81, 298–309. Available from: <https://doi.org/10.1002/ana.24881>.
- Friedman, J.I., Vrijenhoek, T., Markx, S., Janssen, I.M., van der Vliet, W.A., Faas, B.H.W., et al., 2008. CNTNAP2 gene dosage variation is associated with schizophrenia and epilepsy. *Mol. Psychiatry* 13, 261–266. Available from: <https://doi.org/10.1038/sj.mp.4002049>.
- Fukata, Y., Lovero, K.L., Iwanaga, T., Watanabe, A., Yokoi, N., Tabuchi, K., et al., 2010. Disruption of LGI1-linked synaptic complex causes abnormal synaptic transmission and epilepsy. *Proc. Natl. Acad. Sci. U.S.A.* 107, 3799–3804. Available from: <https://doi.org/10.1073/pnas.0914537107>.
- Furukawa, Y., Matsumoto, Y., Yamada, M., 2004. Hypertrophic pachymeningitis as an initial and cardinal manifestation of microscopic polyangiitis. *Neurology* 63 (9), 1722–1724. PubMed PMID: 15534267.
- Gadoth, A., Kryzer, T.J., Fryer, J., McKeon, A., Lennon, V.A., Pittock, S.J., 2017. Microtubule-associated protein 1B: novel paraneoplastic biomarker. *Ann. Neurol.* 81, 266–277. Available from: <https://doi.org/10.1002/ana.24872>.
- Gaig, C., Graus, F., Compta, Y., Högl, B., Bataller, L., Brüggemann, N., et al., 2017. Clinical manifestations of the anti-IgLON5 disease. *Neurology* 88, 1736–1743. Available from: <https://doi.org/10.1212/WNL.000000000003887>.
- Gambardella, A., Manna, I., Labate, A., Chifari, R., La Russa, A., Serra, P., et al., 2003. GABA(B) receptor 1 polymorphism (G1465A) is associated with temporal lobe epilepsy. *Neurology* 60, 560–563.
- Garcia-Martinez, A., Hernandez-Rodriguez, J., Espigol-Frigole, G., et al., 2010. Clinical relevance of persistently elevated circulating cytokines (tumor necrosis factor  $\alpha$  and interleukin-6) in the long-term follow up of patients with giant cell arteritis. *Arthritis Care Res (Hoboken)* 62, 835–841.
- García-Vallejo, J.J., Ilarregui, J.M., Kalay, H., Chamorro, S., Koning, N., Unger, W.W., et al., 2014. CNS myelin induces regulatory functions of DC-SIGN-expressing, antigen-presenting cells via cognate interaction with MOG. *J. Exp. Med.* 211, 1465–1483. Available from: <https://doi.org/10.1084/jem.20122192>.

- Gardinier, M.V., Amiguet, P., Linington, C., Matthieu, J.M., 1992. Myelin/oligodendrocyte glycoprotein is a unique member of the immunoglobulin superfamily. *J. Neurosci. Res.* 33, 177–187. Available from: <https://doi.org/10.1002/jnr.490330123>.
- Gastaldi, M., Thouin, A., Vincent, A., 2016. Antibody-mediated autoimmune encephalopathies and immunotherapies. *Neurother. J. Am. Soc. Exp. Neurother.* 13, 147–162. Available from: <https://doi.org/10.1007/s13311-015-0410-6>.
- Gayraud, M., Guillemin, L., le Toumelin, P., Cohen, P., Lhote, F., Casassus, P., et al., 2001. Long-term followup of polyarteritis nodosa, microscopic polyangiitis, and Churg-Strauss syndrome: analysis of four prospective trials including 278 patients. *Arthritis Rheum.* 44 (3), 666–675. PubMed PMID: 11263782.
- Geis, C., Weishaupt, A., Hallermann, S., Grünewald, B., Wessig, C., Wultsch, T., et al., 2010. Stiff person syndrome-associated autoantibodies to amphiphysin mediate reduced GABAergic inhibition. *Brain J. Neurol.* 133, 3166–3180. Available from: <https://doi.org/10.1093/brain/awq253>.
- Gelpi, E., Höftberger, R., Graus, F., Ling, H., Holton, J.L., Dawson, T., et al., 2016. Neuropathological criteria of anti-IgLON5-related tauopathy. *Acta Neuropathol. (Berl.)* 132, 531–543. Available from: <https://doi.org/10.1007/s00401-016-1591-8>.
- Giometto, B., Grisold, W., Vitaliani, R., Graus, F., Honnorat, J., Bertolini, G., et al., 2010. Paraneoplastic neurologic syndrome in the PNS Euronetwork database: a European study from 20 centers. *Arch. Neurol.* 67, 330–335. Available from: <https://doi.org/10.1001/archneurol.2009.341>.
- Golombeck, K.S., Börte, K., Mönig, C., van Loo, K.M., Hartwig, M., Schwindt, W., et al., 2016. Evidence of a pathogenic role for CD8(+) T cells in anti-GABAB receptor limbic encephalitis. *Neurol. Neuroimmunol. Neuroinflamm.* 3, e232. Available from: <https://doi.org/10.1212/NXI.0000000000000232>.
- Gomez-Puerta, J.A., Gedmintas, L., Costenbader, K.H., 2013. The association between silica exposure and development of ANCA-associated vasculitis: systematic review and meta-analysis. *Autoimmun. Rev.* 12 (12), 1129–1135. Available from: <https://doi.org/10.1016/j.autrev.2013.06.016>.
- Gono, T., Kawaguchi, Y., Kaneko, H., et al., 2011. Anti-NR2A antibody as a predictor for neuropsychiatric systemic lupus erythematosus. *Rheumatology (Oxford)* 50, 1578–1585.
- González-Gay, M.A., Matteson, E.L., Castañeda, S., 2017. Polymyalgia rheumatica. *Lancet*. Available from: [https://doi.org/10.1016/S0140-6736\(17\)31825-1](https://doi.org/10.1016/S0140-6736(17)31825-1) pii: S0140-6736(17)31825-1. [Epub ahead of print] Review. PubMed PMID: 28774422.
- Gorman, M.P., 2010. Update on diagnosis, treatment, and prognosis in opsoclonus-myoclonus-ataxia syndrome. *Curr. Opin. Pediatr.* 22, 745–750. Available from: <https://doi.org/10.1097/MOP.0b013e32833fde3f>.
- Govoni, M., Castellino, G., Padovan, M., Borrelli, M., Trotta, F., 2004. Recent advances and future perspective in neuroimaging in neuropsychiatric systemic lupus erythematosus. *Lupus* 13 (3), 149–158. Review. PubMed PMID: 15119542.
- Govoni, M., Bombardieri, S., Bortoluzzi, A., Caniatti, L., Casu, C., Conti, F., et al., 2012. Factors and comorbidities associated with first neuropsychiatric event in systemic lupus erythematosus: does a risk profile exist? A large multicentre retrospective cross-sectional study on 959 Italian patients. *Rheumatology (Oxford)* 51 (1), 157–168. Available from: <https://doi.org/10.1093/rheumatology/ker310>. Epub 2011 Nov 10. PubMed PMID: 22075066.
- Gozzard, P., Maddison, P., 2010. Which antibody and which cancer in which paraneoplastic syndromes? *Pract. Neurol.* 10, 260–270. Available from: <https://doi.org/10.1136/jnnp.2010.224105>.
- Granerod, J., Ambrose, H.E., Davies, N.W., Clewley, J.P., Walsh, A.L., Morgan, D., et al., 2010. Causes of encephalitis and differences in their clinical presentations in England: a multicentre, population-based prospective study. *Lancet Infect. Dis.* 10, 835–844. Available from: [https://doi.org/10.1016/S1473-3099\(10\)70222-X](https://doi.org/10.1016/S1473-3099(10)70222-X).
- Grau, R.G., 2008. Churg-Strauss syndrome: 2005–2008 update. *Curr. Rheumatol. Rep.* 10 (6), 453–458. Review. PubMed PMID: 19007535.
- Graus, F., Vega, F., Delattre, J.Y., Bonaventura, I., Reñé, R., Arbaiza, D., et al., 1992. Plasmapheresis and antineoplastic treatment in CNS paraneoplastic syndromes with antineuronal autoantibodies. *Neurology* 42, 536–540.
- Graus, F., Dalmau, J., Valldeoriola, F., Ferrer, I., Reñé, R., Marin, C., et al., 1997a. Immunological characterization of a neuronal antibody (anti-Tr) associated with paraneoplastic cerebellar degeneration and Hodgkin's disease. *J. Neuroimmunol.* 74, 55–61.
- Graus, F., Dalmau, J., Reñé, R., Tora, M., Malats, N., Verschueren, J.J., et al., 1997b. Anti-Hu antibodies in patients with small-cell lung cancer: association with complete response to therapy and improved survival. *J. Clin. Oncol.* 15, 2866–2872. Available from: <https://doi.org/10.1200/JCO.1997.15.8.2866>.
- Graus, F., Gultekin, S.H., Ferrer, I., Reiriz, J., Alberch, J., Dalmau, J., 1998. Localization of the neuronal antigen recognized by anti-Tr antibodies from patients with paraneoplastic cerebellar degeneration and Hodgkin's disease in the rat nervous system. *Acta Neuropathol. (Berl.)* 96, 1–7.
- Graus, F., Keime-Guibert, F., Reñé, R., Benyahia, B., Ribalta, T., Ascaso, C., et al., 2001. Anti-Hu-associated paraneoplastic encephalomyelitis: analysis of 200 patients. *Brain J. Neurol.* 124, 1138–1148.
- Graus, F., Lang, B., Pozo-Rosich, P., Saiz, A., Casamitjana, R., Vincent, A., 2002. P/Q type calcium-channel antibodies in paraneoplastic cerebellar degeneration with lung cancer. *Neurology* 59, 764–766.
- Graus, F., Boronat, A., Xifró, X., Boix, M., Svilgelj, V., García, A., et al., 2010. The expanding clinical profile of anti-AMPA receptor encephalitis. *Neurology* 74, 857–859. Available from: <https://doi.org/10.1212/WNL.0b013e3181d3e404>.
- Greene, M., Lai, Y., Baella, N., Dalmau, J., Lancaster, E., 2014. Antibodies to delta/notch-like epidermal growth factor-related receptor in patients with anti-Tr, paraneoplastic cerebellar degeneration, and Hodgkin lymphoma. *JAMA Neurol.* 71, 1003–1008. Available from: <https://doi.org/10.1001/jamaneurol.2014.999>.
- Greenlee, J.E., 2013. Treatment of paraneoplastic cerebellar degeneration. *Curr. Treat. Options Neurol.* 15, 185–200. Available from: <https://doi.org/10.1007/s11940-012-0215-4>.
- Greenlee, J.E., Clawson, S.A., Hill, K.E., Wood, B., Clardy, S.L., Tsunoda, I., et al., 2015. Anti-Yo antibody uptake and interaction with its intracellular target antigen causes Purkinje cell death in rat cerebellar slice cultures: a possible mechanism for paraneoplastic cerebellar degeneration in humans with gynecological or breast cancers. *PLoS One* 10, e0123446. Available from: <https://doi.org/10.1371/journal.pone.0123446>.
- Gresa-Arribas, N., Titulaer, M.J., Torrents, A., Aguilar, E., McCracken, L., Leyboldt, F., et al., 2014. Antibody titres at diagnosis and during follow-up of anti-NMDA receptor encephalitis: a retrospective study. *Lancet Neurol.* 13, 167–177. Available from: [https://doi.org/10.1016/S1474-4422\(13\)70282-5](https://doi.org/10.1016/S1474-4422(13)70282-5).

- Guggenmos, J., Schubart, A.S., Ogg, S., Andersson, M., Olsson, T., Mather, I.H., et al., 2004. Antibody cross-reactivity between myelin oligodendrocyte glycoprotein and the milk protein butyrophilin in multiple sclerosis. *J. Immunol. Baltim. Md* 1950 (172), 661–668.
- Guillevin, L., Lhote, F., Cohen, P., et al., 1995. Polyarteritis nodosa related to hepatitis B virus. A prospective study with long-term observation of 41 patients. *Medicine (Baltimore)* 74, 238–253.
- Guillevin, L., Mahr, A., Callard, P., et al., 2005. Hepatitis B virus-associated polyarteritis nodosa. Clinical characteristics, outcome, and impact of treatment in 115 patients. *Medicine* 84, 313–322.
- Gullapalli, D., Phillips II, L.H., 2002. Neurologic manifestations of sarcoidosis. *Neurol. Clin.* 20 (1), 59–83. vi. Review. PubMed PMID: 11754302.
- Hadjivassiliou, M., Alder, S.J., Van Beek, E.J.R., Hanney, M.B., Lorenz, E., Rao, D.G., et al., 2009. PET scan in clinically suspected paraneoplastic neurological syndromes: a 6-year prospective study in a regional neuroscience unit. *Acta Neurol. Scand.* 119, 186–193. Available from: <https://doi.org/10.1111/j.1600-0404.2008.01089.x>.
- Hahn, B.H., 2013. Belimumab for systemic lupus erythematosus. *N. Engl. J. Med.* 368, 1528–1535.
- Hanly, J.G., Urowitz, M.B., Su, L., et al., 2011. Autoantibodies as biomarkers for the prediction of neuropsychiatric events in systemic lupus erythematosus. *Ann. Rheum. Dis.* 70, 1726–1732.
- Harrold, L.R., Andrade, S.E., Go, A.S., Buist, A.S., Eisner, M., Vollmer, W.M., et al., 2005. Incidence of Churg-Strauss syndrome in asthma drug users: a population-based perspective. *J. Rheumatol.* 32 (6), 1076–1080. PubMed PMID: 15940771.
- Hart, I.K., Waters, C., Vincent, A., Newland, C., Beeson, D., Pongs, O., et al., 1997. Autoantibodies detected to expressed K<sup>+</sup> channels are implicated in neuromyotonia. *Ann. Neurol.* 41, 238–246. Available from: <https://doi.org/10.1002/ana.410410215>.
- Hatemi, G., Silman, A., Bang, D., Bodaghi, B., Chamberlain, A.M., Gul, A., et al., 2008. EULAR recommendations for the management of Behcet disease. *Ann. Rheum. Dis.* 67 (12), 1656–1662. Available from: <https://doi.org/10.1136/ard.2007.080432>. Epub 2008 Jan 31. PubMed PMID: 18245110.
- Hatemi, G., Silman, A., Bang, D., Bodaghi, B., Chamberlain, A.M., Gul, A., et al., 2009. Management of Behcet disease: a systematic literature review for the European League Against Rheumatism evidence-based recommendations for the management of Behcet disease. *Ann. Rheum. Dis.* 68 (10), 1528–1534. Available from: <https://doi.org/10.1136/ard.2008.087957>. Epub 2008 Apr 17. Review. PubMed PMID: 18420940.
- Hatemi, G., Seyahi, E., Fresko, I., Talarico, R., Hamuryudan, V., 2017. One year in review 2017: Behcet's syndrome. *Clin. Exp. Rheumatol.* [Epub ahead of print] Review. PubMed PMID: 28980900.
- Heine, J., Ly, L.-T., Lieker, I., Slowinski, T., Finke, C., Prüss, H., et al., 2016. Immunoabsorption or plasma exchange in the treatment of autoimmune encephalitis: a pilot study. *J. Neurol.* 263, 2395–2402. Available from: <https://doi.org/10.1007/s00415-016-8277-y>.
- Hiemstra, T.F., Walsh, M., Mahr, A., Savage, C.O., de Groot, K., Harper, L., et al., 2010. Mycophenolate mofetil vs azathioprine for remission maintenance in antineutrophil cytoplasmic antibody-associated vasculitis: a randomized controlled trial. *JAMA* 304 (21), 2381–2388. Available from: <https://doi.org/10.1001/jama.2010.1658>. Epub 2010 Nov 8. PubMed PMID: 21060104.
- Hinson, S.R., Roemer, S.F., Lucchinetti, C.F., Fryer, J.P., Kryzer, T.J., Chamberlain, J.L., et al., 2008. Aquaporin-4-binding autoantibodies in patients with neuromyelitis optica impair glutamate transport by down-regulating EAAT2. *J. Exp. Med.* 205, 2473–2481. Available from: <https://doi.org/10.1084/jem.20081241>.
- Hinson, S.R., Romero, M.F., Popescu, B.F.G., Lucchinetti, C.F., Fryer, J.P., Wolburg, H., et al., 2012. Molecular outcomes of neuromyelitis optica (NMO)-IgG binding to aquaporin-4 in astrocytes. *Proc. Natl. Acad. Sci. U.S.A.* 109, 1245–1250. Available from: <https://doi.org/10.1073/pnas.1109980108>.
- Hirose, S., 2014. Mutant GABA(A) receptor subunits in genetic (idiopathic) epilepsy. *Progr. Brain Res.* 213, 55–85. Available from: <https://doi.org/10.1016/B978-0-444-63326-2.00003-X>.
- Höftberger, R., Titulaer, M.J., Sabater, L., Dome, B., Rózsás, A., Hegedus, B., et al., 2013. Encephalitis and GABAB receptor antibodies: novel findings in a new case series of 20 patients. *Neurology* 81, 1500–1506. Available from: <https://doi.org/10.1212/WNL.0b013e3182a9585f>.
- Höftberger, R., van Sonderen, A., Leyboldt, F., Houghton, D., Geschwind, M., Gelfand, J., et al., 2015. Encephalitis and AMPA receptor antibodies: novel findings in a case series of 22 patients. *Neurology* 84, 2403–2412. Available from: <https://doi.org/10.1212/WNL.0000000000001682>.
- Hoitsma, E., Faber, C.G., Drent, M., Sharma, O.P., 2004. Neurosarcoidosis: a clinical dilemma. *Lancet Neurol.* 3 (7), 397–407. Review. PubMed PMID: 15207796.
- Holmøy, T., Skorstad, G., Røste, L.S., Scheie, D., Alvik, K., 2009. Stiff person syndrome associated with lower motor neuron disease and infiltration of cytotoxic T cells in the spinal cord. *Clin. Neurol. Neurosurg.* 111, 708–712. Available from: <https://doi.org/10.1016/j.clineuro.2009.06.005>.
- Honorat, J., Didelot, A., Karantoni, E., Ville, D., Ducray, F., Lambert, L., et al., 2013. Autoimmune limbic encephalopathy and anti-Hu antibodies in children without cancer. *Neurology* 80, 2226–2232. Available from: <https://doi.org/10.1212/WNL.0b013e318296e9c3>.
- Honorat, J.A., Komorowski, L., Josephs, K.A., Fechner, K., St Louis, E.K., Hinson, S.R., et al., 2017. IgLON5 antibody: neurological accompaniments and outcomes in 20 patients. *Neurol. Neuroimmunol. Neuroinflamm.* 4, e385. Available from: <https://doi.org/10.1212/NXI.0000000000000385>.
- Horresh, I., Poliak, S., Grant, S., Bredt, D., Rasband, M.N., Peles, E., 2008. Multiple molecular interactions determine the clustering of Caspr2 and Kv1 channels in myelinated axons. *J. Neurosci.* 28, 14213–14222. Available from: <https://doi.org/10.1523/JNEUROSCI.3398-08.2008>.
- Horta, E.S., Lennon, V.A., Lachance, D.H., Jenkins, S.M., Smith, C.Y., McKeon, A., et al., 2014. Neural autoantibody clusters aid diagnosis of cancer. *Clin. Cancer Res.* 20, 3862–3869. Available from: <https://doi.org/10.1158/1078-0432.CCR-14-0652>.
- Hughes, E.G., Peng, X., Gleichman, A.J., Lai, M., Zhou, L., Tsou, R., et al., 2010. Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. *J. Neurosci.* 30, 5866–5875. Available from: <https://doi.org/10.1523/JNEUROSCI.0167-10.2010>.
- Huibers, M.G., Querol, L.A., Niks, E.H., Plomp, J.J., van der Maarel, S.M., Graus, F., et al., 2015. The expanding field of IgG4-mediated neurological autoimmune disorders. *Eur. J. Neurol.* 22, 1151–1161. Available from: <https://doi.org/10.1111/ene.12758>.
- Hurlmann, D., Forster, A., Noll, G., Enseleit, F., Chenevard, R., Distler, O., et al., 2002. Anti-tumor necrosis factor-alpha treatment improves endothelial function in patients with rheumatoid arthritis. *Circulation* 106, 2184–2187.

- Hyun, J.-W., Woodhall, M.R., Kim, S.-H., Jeong, I.H., Kong, B., Kim, G., et al., 2017. Longitudinal analysis of myelin oligodendrocyte glycoprotein antibodies in CNS inflammatory diseases. *J. Neurol. Neurosurg. Psychiatry* 88, 811–817. Available from: <https://doi.org/10.1136/jnnp-2017-315998>.
- Ikeda, K., Kiyota, N., Kuroda, H., Sato, D.K., Nishiyama, S., Takahashi, T., et al., 2015. Severe demyelination but no astrocytopathy in clinically definite neuromyelitis optica with anti-myelin-oligodendrocyte glycoprotein antibody. *Mult. Scler.* Hounds Mills Basingstoke Engl. 21, 656–659. Available from: <https://doi.org/10.1177/1352458514551455>.
- Irani, S.R., Alexander, S., Waters, P., Kleopa, K.A., Pettingill, P., Zuliani, L., et al., 2010a. Antibodies to Kv1 potassium channel-complex proteins leucine-rich, glioma inactivated 1 protein and contactin-associated protein-2 in limbic encephalitis, Morvan's syndrome and acquired neuromyotonia. *Brain J. Neurol.* 133, 2734–2748. Available from: <https://doi.org/10.1093/brain/awq213>.
- Irani, S.R., Bera, K., Waters, P., Zuliani, L., Maxwell, S., Zandi, M.S., et al., 2010b. N-Methyl-D-aspartate antibody encephalitis: temporal progression of clinical and paraclinical observations in a predominantly non-paraneoplastic disorder of both sexes. *Brain J. Neurol.* 133, 1655–1667. Available from: <https://doi.org/10.1093/brain/awq113>.
- Irani, S.R., Michell, A.W., Lang, B., Pettingill, P., Waters, P., Johnson, M.R., et al., 2011. Faciobrachial dystonic seizures precede Lgi1 antibody limbic encephalitis. *Ann. Neurol.* 69, 892–900. Available from: <https://doi.org/10.1002/ana.22307>.
- Irani, S.R., Pettingill, P., Kleopa, K.A., Schiza, N., Waters, P., Mazia, C., et al., 2012. Morvan syndrome: clinical and serological observations in 29 cases. *Ann. Neurol.* 72, 241–255. Available from: <https://doi.org/10.1002/ana.23577>.
- Irani, S.R., Stagg, C.J., Schott, J.M., Rosenthal, C.R., Schneider, S.A., Pettingill, P., et al., 2013. Faciobrachial dystonic seizures: the influence of immunotherapy on seizure control and prevention of cognitive impairment in a broadening phenotype. *Brain J. Neurol.* 136, 3151–3162. Available from: <https://doi.org/10.1093/brain/awt212>.
- Isenberg, D., Ramsey-Goldman, R., 2006. Systemic Lupus International Collaborating Group—onwards and upwards? *Lupus* 15, 606–607.
- Jacob, T.C., Moss, S.J., Jurd, R., 2008. GABA<sub>A</sub> receptor trafficking and its role in the dynamic modulation of neuronal inhibition. *Nat. Rev. Neurosci.* 9, 331–343. Available from: <https://doi.org/10.1038/nrn2370>.
- Jarius, S., Wildemann, B., 2015. 'Medusa-head ataxia': the expanding spectrum of Purkinje cell antibodies in autoimmune cerebellar ataxia. Part 1: Anti-mGluR1, anti-Homer-3, anti-Sj<sub>1</sub>/ITPR1 and anti-CARP VIII. *J. Neuroinflamm.* 12. Available from: <https://doi.org/10.1186/s12974-015-0356-y>.
- Jarius, S., Franciotta, D., Paul, F., Ruprecht, K., Bergamaschi, R., Rommer, P.S., et al., 2010. Cerebrospinal fluid antibodies to aquaporin-4 in neuromyelitis optica and related disorders: frequency, origin, and diagnostic relevance. *J. Neuroinflamm.* 7, 52. Available from: <https://doi.org/10.1186/1742-2094-7-52>.
- Jarius, S., Metz, I., König, F.B., Ruprecht, K., Reindl, M., Paul, F., et al., 2016a. Screening for MOG-IgG and 27 other anti-glial and anti-neuronal autoantibodies in "pattern II multiple sclerosis" and brain biopsy findings in a MOG-IgG-positive case. *Mult. Scler.* Hounds Mills Basingstoke Engl. 22, 1541–1549. Available from: <https://doi.org/10.1177/1352458515622986>.
- Jarius, S., Ruprecht, K., Kleiter, I., Borisow, N., Asgari, N., Pitarokoili, K., et al., 2016b. MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 2: Epidemiology, clinical presentation, radiological and laboratory features, treatment responses, and long-term outcome. *J. Neuroinflamm.* 13, 280. Available from: <https://doi.org/10.1186/s12974-016-0718-0>.
- Jarius, S., Ruprecht, K., Kleiter, I., Borisow, N., Asgari, N., Pitarokoili, K., et al., 2016c. MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 1: Frequency, syndrome specificity, influence of disease activity, long-term course, association with AQP4-IgG, and origin. *J. Neuroinflamm.* 13, 279. Available from: <https://doi.org/10.1186/s12974-016-0717-1>.
- Jennette, J.C., Falk, R.J., 2007. Nosology of primary vasculitis. *Curr. Opin. Rheumatol.* 19 (1), 10–16. Review. PubMed PMID: 17143090.
- Jennette, J.C., Falk, R.J., Bacon, P.A., Basu, N., Cid, M.C., Ferrario, F., et al., 2013. 2012 revised International Chapel Hill Consensus Conference nomenclature of vasculitides. *Arthritis Rheum.* 65 (1), 1–11.
- Jeong, I.H., Park, B., Kim, S.-H., Hyun, J.-W., Joo, J., Kim, H.J., 2016. Comparative analysis of treatment outcomes in patients with neuromyelitis optica spectrum disorder using multifaceted endpoints. *Mult. Scler.* Hounds Mills Basingstoke Engl. 22, 329–339. Available from: <https://doi.org/10.1177/1352458515587752>.
- Joaquim, A.F., Appenzeller, S., 2015. Neuropsychiatric manifestations in rheumatoid arthritis. *Autoimmun. Rev.* 14 (12), 1116–1122. Available from: <https://doi.org/10.1016/j.autrev.2015.07.015>. Epub 2015 Jul 31. Review. PubMed PMID: 26238502.
- Jones, A.L., Flanagan, E.P., Pittock, S.J., Mandrekar, J.N., Eggers, S.D., Ahlskog, J.E., et al., 2015. Responses to and outcomes of treatment of autoimmune cerebellar ataxia in adults. *JAMA Neurol.* 72, 1304–1312. Available from: <https://doi.org/10.1001/jamaneurol.2015.2378>.
- Joseph, F.G., Scolding, N.J., 2009. Neurosarcoidosis: a study of 30 new cases. *J. Neurol. Neurosurg. Psychiatry* 80 (3), 297–304. Available from: <https://doi.org/10.1136/jnnp.2008.151977>. Epub 2008 Oct 31. PubMed PMID: 18977817.
- Joubert, B., Saint-Martin, M., Noraz, N., Picard, G., Rogemond, V., Ducray, F., et al., 2016. Characterization of a subtype of autoimmune encephalitis with anti-contactin-associated protein-like 2 antibodies in the cerebrospinal fluid, prominent limbic symptoms, and seizures. *JAMA Neurol.* 73, 1115–1124. Available from: <https://doi.org/10.1001/jamaneurol.2016.1585>.
- Juryneczyk, M., Gerald, R., Probert, F., Woodhall, M.R., Waters, P., Tackley, G., et al., 2017. Distinct brain imaging characteristics of autoantibody-mediated CNS conditions and multiple sclerosis. *Brain J. Neurol.* 140, 617–627. Available from: <https://doi.org/10.1093/brain/aww350>.
- Kadkhodayan, Y., Alreshaid, A., Moran, C.J., Cross III, D.T., Powers, W.J., Derdeyn, C.P., 2004. Primary angiitis of the central nervous system at conventional angiography. *Radiology* 233 (3), 878–882. Epub 2004 Oct 21. PubMed PMID: 15498898.
- Kalachikov, S., Evgrafov, O., Ross, B., Winawer, M., Barker-Cummings, C., Martinelli Boneschi, F., et al., 2002. Mutations in LGI1 cause autosomal-dominant partial epilepsy with auditory features. *Nat. Genet.* 30, 335–341. Available from: <https://doi.org/10.1038/ng832>.
- Kalia, L.V., Kalia, S.K., Salter, M.W., 2008. NMDA receptors in clinical neurology: excitatory times ahead. *Lancet Neurol.* 7, 742–755. Available from: [https://doi.org/10.1016/S1474-4422\(08\)70165-0](https://doi.org/10.1016/S1474-4422(08)70165-0).
- Kaltonoudis, E., Voulgari, P.V., Konitsiotis, S., Drosos, A.A., 2014. Demyelination and other neurological adverse events after anti-TNF therapy. *Autoimmun. Rev.* 13, 54–58.
- Keime-Guibert, F., Graus, F., Broët, P., Reñé, R., Molinuevo, J.L., Ascaso, C., et al., 1999. Clinical outcome of patients with anti-Hu-associated encephalomyelitis after treatment of the tumor. *Neurology* 53, 1719–1723.

- Keime-Guibert, F., Graus, F., Fleury, A., René, R., Honnorat, J., Broet, P., et al., 2000. Treatment of paraneoplastic neurological syndromes with antineuronal antibodies (anti-Hu, anti-Yo) with a combination of immunoglobulins, cyclophosphamide, and methylprednisolone. *J. Neurol. Neurosurg. Psychiatry* 68, 479–482.
- Kermani, T.A., Warrington, K.J., 2013. Polymyalgia rheumatica. *Lancet* 381, 63–72. Erratum, *Lancet* 2013;381:28. PubMed: 23051717.
- Kermani, T.A., Warrington, K.J., Crowson, C.S., et al., 2013. Large-vessel involvement in giant cell arteritis: a population-based cohort study of the incidence-trends and prognosis. *Ann. Rheum. Dis.* 72, 1989–1994. PubMed: 23253927.
- Khellaf, M., Hamidou, M., Pagnoux, C., Michel, M., Brisseau, J.M., Chevallier, X., et al., 2007. Vasculitis restricted to the lower limbs: a clinical and histopathological study. *Ann. Rheum. Dis.* 66 (4), 554–556. Epub 2006 Oct 26. PubMed PMID: 17068062; PubMed Central PMCID: PMC1856040.
- Kim, H.J., Paul, F., Lana-Peixoto, M.A., Tenembaum, S., Asgari, N., Palace, J., et al., 2015. MRI characteristics of neuromyelitis optica spectrum disorder: an international update. *Neurology* 84, 1165–1173. Available from: <https://doi.org/10.1212/WNL.0000000000001367>.
- Kitley, J., Waters, P., Woodhall, M., Leite, M.I., Murchison, A., George, J., et al., 2014. Neuromyelitis optica spectrum disorders with aquaporin-4 and myelin-oligodendrocyte glycoprotein antibodies: a comparative study. *JAMA Neurol.* 71, 276–283. Available from: <https://doi.org/10.1001/jamaneurol.2013.5857>.
- Klaas, J.P., Ahlskog, J.E., Pittock, S.J., Matsumoto, J.Y., Aksamit, A.J., Bartleson, J.D., et al., 2012. Adult-onset opsoclonus-myoclonus syndrome. *Arch. Neurol.* 69, 1598–1607. Available from: <https://doi.org/10.1001/archneurol.2012.1173>.
- Kleiter, I., Gahlen, A., Borisow, N., Fischer, K., Wernecke, K.-D., Wegner, B., et al., 2015. Neuromyelitis optica: Evaluation of 871 attacks and 1153 treatment courses. *Ann. Neurol.* Available from: <https://doi.org/10.1002/ana.24554>.
- Kobayashi, Y., Ishii, K., Oda, K., Nariai, T., Tanaka, Y., Ishiwata, K., et al., 2005. Aortic wall inflammation due to Takayasu arteritis imaged with 18F-FDG PET coregistered with enhanced CT. *J. Nucl. Med.* 46 (6), 917–922. PubMed PMID: 15937300.
- Kötter, I., Henes, J.C., Wagner, A.D., Loock, J., Gross, W.L., 2012. Does glucocorticosteroid-resistant large-vessel vasculitis (giant cell arteritis and Takayasu arteritis) exist and how can remission be achieved? A critical review of the literature. *Clin. Exp. Rheumatol.* 30 (Suppl 70), S114–S129. PubMed: 22640655.
- Kovacs, B., Lafferty, T.L., Brent, L.H., DeHoratius, R.J., 2000. Transverse myelopathy in systemic lupus erythematosus: an analysis of 14 cases and review of the literature. *Ann. Rheum. Dis.* 59 (2), 120–124. Review. PubMed PMID: 10666167; PubMed Central PMCID: PMC1753077.
- Krishnan, E., Lingala, V.B., Singh, G., 2004. Declines in mortality from acute myocardial infarction in successive incidence and birth cohorts of patients with rheumatoid arthritis. *Circulation* 110, 1774–1779.
- Krogias, C., Hoepner, R., Müller, A., Schneider-Gold, C., Schröder, A., Gold, R., 2013. Successful treatment of anti-Caspr2 syndrome by interleukin 6 receptor blockade through tocilizumab. *JAMA Neurol.* 70, 1056–1059. Available from: <https://doi.org/10.1001/jamaneurol.2013.143>.
- Kurne, A., Karabudak, R., Karadag, O., Yalcin-Cakmakli, G., Karli-Oguz, K., Yavuz, K., et al., 2009. An unusual central nervous system involvement in rheumatoid arthritis: combination of pachymeningitis and cerebral vasculitis. *Rheumatol. Int.* 29, 1349–1353.
- Lacomis, D., 2011. Neurosarcoidosis. *Curr. Neuropharmacol.* 9 (3), 429–436. Available from: <https://doi.org/10.2174/157015911796557975>. PubMed PMID: 22379457; PubMed Central PMCID: PMC3151597.
- Lai, M., Hughes, E.G., Peng, X., Zhou, L., Gleichman, A.J., Shu, H., et al., 2009. AMPA receptor antibodies in limbic encephalitis alter synaptic receptor location. *Ann. Neurol.* 65, 424–434. Available from: <https://doi.org/10.1002/ana.21589>.
- Lai, M., Huijbers, M.G.M., Lancaster, E., Graus, F., Batailler, L., Balice-Gordon, R., et al., 2010. Investigation of LGI1 as the antigen in limbic encephalitis previously attributed to potassium channels: a case series. *Lancet Neurol.* 9, 776–785. Available from: [https://doi.org/10.1016/S1474-4422\(10\)70137-X](https://doi.org/10.1016/S1474-4422(10)70137-X).
- Lancaster, E., Lai, M., Peng, X., Hughes, E., Constantinescu, R., Raizer, J., et al., 2010. Antibodies to the GABA(B) receptor in limbic encephalitis with seizures: case series and characterisation of the antigen. *Lancet Neurol.* 9, 67–76. Available from: [https://doi.org/10.1016/S1474-4422\(09\)70324-2](https://doi.org/10.1016/S1474-4422(09)70324-2).
- Lancaster, E., Huijbers, M.G.M., Bar, V., Boronat, A., Wong, A., Martinez-Hernandez, E., et al., 2011. Investigations of caspr2, an autoantigen of encephalitis and neuromyotonia. *Ann. Neurol.* 69, 303–311. Available from: <https://doi.org/10.1002/ana.22297>.
- Lang, B., Makuch, M., Moloney, T., Dettmann, I., Mindorf, S., Probst, C., et al., 2017. Intracellular and non-neuronal targets of voltage-gated potassium channel complex antibodies. *J. Neurol. Neurosurg. Psychiatry*. Available from: <https://doi.org/10.1136/jnnp-2016-314758>.
- Le, K.S., Thibault, M.L., Just-Landi, S., Pastor, S., Gondois-Rey, F., Granjeaud, S., et al., 2016. Follicular B Lymphomas Generate Regulatory T Cells via the ICOS/ICOSL Pathway and Are Susceptible to Treatment by Anti-ICOS/ICOSL Therapy. *Cancer Res.* 76 (16), 4648–4660. Available from: <https://doi.org/10.1158/0008-5472.CAN-15-0589>.
- Lee, Y.H., Pang, S.W., Tan, K.O., 2016. PNMA2 mediates heterodimeric interactions and antagonizes chemo-sensitizing activities mediated by members of PNMA family. *Biochem. Biophys. Res. Commun.* 473, 224–229. Available from: <https://doi.org/10.1016/j.bbrc.2016.03.083>.
- Lennon, V.A., Wingerchuk, D.M., Kryzer, T.J., Pittock, S.J., Lucchinetti, C.F., Fujihara, K., et al., 2004. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet* 364, 2106–2112. Available from: [https://doi.org/10.1016/S0140-6736\(04\)17551-X](https://doi.org/10.1016/S0140-6736(04)17551-X).
- Lepse, N., Land, J., Rutgers, A., Kallenberg, C.G., Stegeman, C.A., Abdulahad, W.H., et al., 2016. Toll-like receptor 9 activation enhances B cell activating factor and interleukin-21 induced anti-proteinase 3 autoantibody production in vitro. *Rheumatology (Oxford)* 55 (1), 162–172. Available from: <https://doi.org/10.1093/rheumatology/kev293>. Epub 2015 Aug 28. PubMed PMID: 26320128.
- Leypoldt, F., Wandinger, K.-P., 2014. Paraneoplastic neurological syndromes. *Clin. Exp. Immunol.* 175, 336–348. Available from: <https://doi.org/10.1111/cei.12185>.
- Linington, C., Bradl, M., Lassmann, H., Brunner, C., Vass, K., 1988. Augmentation of demyelination in rat acute allergic encephalomyelitis by circulating mouse monoclonal antibodies directed against a myelin/oligodendrocyte glycoprotein. *Am. J. Pathol.* 130, 443–454.
- Liu, Z., Davidson, A., 2012. Taming lupus—a new understanding of pathogenesis is leading to clinical advances. *Nat. Med.* 18 (6), 871–882. Available from: <https://doi.org/10.1038/nm.2752>. Review. PubMed PMID: 22674006; PubMed Central PMCID: PMC3607103.
- Lohmann, T., Londei, M., Hawa, M., Leslie, R.D.G., 2003. Humoral and cellular autoimmune responses in stiff person syndrome. *Ann. N.Y. Acad. Sci.* 998, 215–222.
- Lopez-Chiriboga, A.S., Komorowski, L., Kümpfel, T., Probst, C., Hinson, S.R., Pittock, S.J., et al., 2016. Metabotropic glutamate receptor type 1 autoimmunity. *Neurology* 86, 1009–1013. Available from: <https://doi.org/10.1212/WNL.0000000000002476>.

- Lower, E.E., Broderick, J.P., Brott, T.G., Baughman, R.P., 1997. Diagnosis and management of neurological sarcoidosis. *Arch. Intern. Med.* 157 (16), 1864–1868. PubMed PMID: 9290546.
- Lucchinetti, C.F., Guo, Y., Popescu, B.F.G., Fujihara, K., Itoyama, Y., Misu, T., 2014. The pathology of an autoimmune astrocytopathy: lessons learned from neuromyelitis optica. *Brain Pathol. Zurich Switz.* 24, 83–97. Available from: <https://doi.org/10.1111/bpa.12099>.
- Malter, M.P., Frisch, C., Schoene-Bake, J.C., Helmstaedter, C., Wandinger, K.P., Stoecker, W., et al., 2014. Outcome of limbic encephalitis with VGKC-complex antibodies: relation to antigenic specificity. *J. Neurol.* 261, 1695–1705. Available from: <https://doi.org/10.1007/s00415-014-7408-6>.
- Maradit-Kremers, H., Crowson, C.S., Nicola, P.J., Ballman, K.V., Roger, V.L., Jacobsen, S.J., et al., 2005. Increased unrecognized coronary heart disease and sudden deaths in rheumatoid arthritis: a population-based cohort study. *Arthritis Rheum.* 52, 402–411.
- Martinez-Hernandez, E., Ariño, H., McKeon, A., Iizuka, T., Titulaer, M.J., Simabukuro, M.M., et al., 2016. Clinical and immunologic investigations in patients with stiff-person spectrum disorder. *JAMA Neurol.* 73, 714–720. Available from: <https://doi.org/10.1001/jamaneurol.2016.0133>.
- Martinez-Taboada, V., Rodriguez-Valverde, V., Carreno, L., et al., 2008. A double-blind placebo controlled trial of etanercept in patients with giant cell arteritis and corticosteroid side effects. *Ann. Rheum. Dis.* 67, 625–630.
- McAdoo, S.P., Bedi, R., Tarzi, R., Griffith, M., Pusey, C.D., Cairns, T.D., 2016. Ofatumumab for B cell depletion therapy in ANCA-associated vasculitis: a single-centre case series. *Rheumatology (Oxford)* 55 (8), 1437–1442. Available from: <https://doi.org/10.1093/rheumatology/kew199>. Epub 2016 Apr 19. PubMed PMID: 27094598; PubMed Central PMCID: PMC4957674.
- McGeoch, L., Twilt, M., Famorca, L., Bakowsky, V., Barra, L., Benseler, S.M., et al., 2016. CanVasc Recommendations for the management of antineutrophil cytoplasm antibody-associated vasculitides. *J. Rheumatol.* 43 (1), 97–120. Available from: <https://doi.org/10.3899/jrheum.150376>. Epub 2015 Nov 1. Review. PubMed PMID: 26523024.
- McKeon, A., Tracy, J.A., 2017. GAD65 neurological autoimmunity. *Muscle Nerve* 56, 15–27. Available from: <https://doi.org/10.1002/mus.25565>.
- McKeon, A., Robinson, M.T., McEvoy, K.M., Matsumoto, J.Y., Lennon, V.A., Ahlskog, J.E., et al., 2012. Stiff-man syndrome and variants: clinical course, treatments, and outcomes. *Arch. Neurol.* 69, 230–238. Available from: <https://doi.org/10.1001/archneuro.2011.991>.
- Middeldorp, J., Hol, E.M., 2011. GFAP in health and disease. *Progr. Neurobiol.* 93, 421–443. Available from: <https://doi.org/10.1016/j.pneurobio.2011.01.005>.
- Mikdashi, J., Handwerger, B., 2004. Predictors of neuropsychiatric damage in systemic lupus erythematosus: data from the Maryland lupus cohort. *Rheumatology (Oxford)* 43 (12), 1555–1560. Epub 2004 Sep 1. PubMed PMID: 15342927.
- Miloslavsky, E.M., Lu, N., Unizony, S., Choi, H.K., Merkel, P.A., Seo, P., et al., 2016. Myeloperoxidase-antineutrophil cytoplasmic antibody (ANCA)-positive and ANCA-negative patients with granulomatosis with polyangiitis (Wegener's): distinct patient subsets. *Arthritis Rheumatol.* 68 (12), 2945–2952. Available from: <https://doi.org/10.1002/art.39812>. PubMed PMID: 27428559; PubMed Central PMCID: PMC5541999.
- Minhas, H.M., Pescosolido, M.F., Schwede, M., Piasecka, J., Gaitanis, J., Tantravahi, U., et al., 2013. An unbalanced translocation involving loss of 10q26.2 and gain of 11q25 in a pedigree with autism spectrum disorder and cerebellar juvenile pilocytic astrocytoma. *Am. J. Med. Genet. A.* 161A, 787–791. Available from: <https://doi.org/10.1002/ajmg.a.35841>.
- Mirsattari, S.M., McGinn, G.J., Halliday, W.C., 2004. Neuro-Behcet disease with predominant involvement of the brainstem. *Neurology* 63 (2), 382–384. PubMed PMID: 15277646.
- Mitchell, W.G., Wooten, A.A., O'Neil, S.H., Rodriguez, J.G., Cruz, R.E., Wittern, R., 2015. Effect of increased immunosuppression on developmental outcome of opsoclonus myoclonus syndrome (OMS). *J. Child Neurol.* 30, 976–982. Available from: <https://doi.org/10.1177/0883073814549581>.
- Mitoma, H., Hadjivassiliou, M., Honnorat, J., 2015. Guidelines for treatment of immune-mediated cerebellar ataxias. *Cerebellum Ataxias* 2. Available from: <https://doi.org/10.1186/s40673-015-0034-y>.
- Mitoma, H., Adhikari, K., Aeschlimann, D., Chattpadhyay, P., Hadjivassiliou, M., Hampe, C.S., et al., 2016. consensus paper: neuroimmune mechanisms of cerebellar ataxias. *Cerebellum Lond. Engl.* 15, 213–232. Available from: <https://doi.org/10.1007/s12311-015-0664-x>.
- Mohammad, A.J., Hot, A., Arndt, F., et al., 2016. Rituximab for the treatment of eosinophilic granulomatosis with polyangiitis (Churg-Strauss). *Ann. Rheum. Dis.* 75, 396–401.
- Mok, C.C., Ho, C.T., Chan, K.W., Lau, C.S., Wong, R.W., 2002. Outcome and prognostic indicators of diffuse proliferative lupus glomerulonephritis treated with sequential oral cyclophosphamide and azathioprine. *Arthritis Rheum.* 46 (4), 1003–1013. PubMed PMID: 11953978.
- Moon, J., Lee, S.-T., Shin, J.-W., Byun, J.-I., Lim, J.-A., Shin, Y.-W., et al., 2014. Non-stiff anti-amphiphysin syndrome: clinical manifestations and outcome after immunotherapy. *J. Neuroimmunol.* 274, 209–214. Available from: <https://doi.org/10.1016/j.jneuroim.2014.07.011>.
- Moore, P.M., Richardson, B., 1998. *Neurology of the vasculitides and connective tissue diseases*. *J. Neurol. Neurosurg. Psychiatry* 65 (1), 10–22. Review. PubMed PMID: 9667555; PubMed Central PMCID: PMC2170162.
- Moscato, E.H., Peng, X., Jain, A., Parsons, T.D., Dalmau, J., Balice-Gordon, R.J., 2014. Acute mechanisms underlying antibody effects in anti-N-methyl-D-aspartate receptor encephalitis. *Ann. Neurol.* 76, 108–119. Available from: <https://doi.org/10.1002/ana.24195>.
- Moutal, A., Honnorat, J., Massoma, P., Désormeaux, P., Bertrand, C., Malleval, C., et al., 2015. CRMP5 controls glioblastoma cell proliferation and survival through notch-dependent signaling. *Cancer Res.* 75, 3519–3528. Available from: <https://doi.org/10.1158/0008-5472.CAN-14-0631>.
- Mukhtyar, C., Guillevin, L., Cid, M.C., et al., 2009. EULAR recommendations for the management of large vessel vasculitis. *Ann. Rheum. Dis.* 68, 318–323. PubMed PMID: 18413441.
- Murinson, B.B., Guarnaccia, J.B., 2008. Stiff-person syndrome with amphiphysin antibodies. *Neurology* 71, 1955–1958. Available from: <https://doi.org/10.1212/01.wnl.0000327342.58936.e0>.
- Nadal, M.S., Ozaita, A., Amarillo, Y., Vega-Saenz de Miera, E., Ma, Y., Mo, W., et al., 2003. The CD26-related dipeptidyl aminopeptidase-like protein DPPX is a critical component of neuronal A-type K<sup>+</sup> channels. *Neuron* 37, 449–461.
- Nakamura, H., Horai, Y., Suzuki, T., Okada, A., Ichinose, K., Yamasaki, S., et al., 2013. TLR3-mediated apoptosis and activation of phosphorylated Akt in the salivary gland epithelial cells of primary Sjögren's syndrome patients. *Rheumatol. Int.* 33, 441–450.

- Narváez, J., Bernad, B., Roig-Vilaseca, D., García-Gómez, C., Gómez-Vaquero, C., Juanola, X., et al., 2007. Influence of previous corticosteroid therapy on temporal artery biopsy yield in giant cell arteritis. *Semin. Arthritis Rheum.* 37 (1), 13–19. Epub 2007 Mar 23. PubMed PMID: 17360027.
- Nesher, G., 2014. The diagnosis and classification of giant cell arteritis. *J. Autoimmun.* 48–49, 73–75. Available from: <https://doi.org/10.1016/j.jaut.2014.01.017>. Epub 2014 Jan 21. Review. PubMed PMID: 24461386.
- Nishino, H., Rubino, F.A., DeRemee, R.A., Swanson, J.W., Parisi, J.E., 1993. Neurological involvement in Wegener's granulomatosis: an analysis of 324 consecutive patients at the Mayo Clinic. *Ann. Neurol.* 33 (1), 4–9. PubMed PMID: 8388187.
- Niswender, C.M., Conn, P.J., 2010. Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Annu. Rev. Pharmacol. Toxicol.* 50, 295–322. Available from: <https://doi.org/10.1146/annurev.pharmtox.011008.145533>.
- Nordborg, E., Bengtsson, B.A., 1989. Death rates and causes of death in 284 consecutive patients with giant cell arteritis confirmed by biopsy. *BMJ* 299 (6698), 549–550. PubMed PMID: 2507065; PubMed Central PMCID: PMC1837376.
- Nordborg, E., Bengtsson, B.A., 1990. Epidemiology of biopsy-proven giant cell arteritis (GCA). *J. Intern. Med.* 227 (4), 233–236. PubMed PMID: 2324677.
- Novikov, P., Moiseev, S., Bulanov, N., Shchegoleva, E., 2016a. Bortezomib in refractory ANCA-associated vasculitis: a new option? *Ann. Rheum. Dis.* 75 (1), e9. Available from: <https://doi.org/10.1136/annrheumdis-2015-207947>. Jan Epub 2015 Jul 21. PubMed PMID: 26199397.
- Novikov, P., Moisev, S., Smitienko, I., Zagvozdina, E., 2016b. Rituximab as induction therapy in relapsing eosinophilic granulomatosis with polyangiitis: a report of 6 cases. *Joint Bone Spine* 83, 81–84.
- O'Donovan, K.J., Diedler, J., Couture, G.C., Fak, J.J., Darnell, R.B., 2010. The onconeural antigen cdr2 is a novel APC/C target that acts in mitosis to regulate C-Myc target genes in mammalian tumor cells. *PLoS One* 5. Available from: <https://doi.org/10.1371/journal.pone.0010045>.
- Ohkawa, T., Fukata, Y., Yamasaki, M., Miyazaki, T., Yokoi, N., Takashima, H., et al., 2013. Autoantibodies to epilepsy-related LGI1 in limbic encephalitis neutralize LGI1-ADAM22 interaction and reduce synaptic AMPA receptors. *J. Neurosci.* 33, 18161–18174. Available from: <https://doi.org/10.1523/JNEUROSCI.3506-13.2013>.
- Okano, H.J., Darnell, R.B., 1997. A hierarchy of Hu RNA binding proteins in developing and adult neurons. *J. Neurosci.* 17, 3024–3037.
- Okano, H.J., Park, W.-Y., Corradi, J.P., Darnell, R.B., 1999. The cytoplasmic Purkinje onconeural antigen cdr2 down-regulates c-Myc function: implications for neuronal and tumor cell survival. *Genes Dev.* 13, 2087–2097.
- Ontaneda, D., Fox, R.J., 2014. Is neuromyelitis optica with advanced age of onset a paraneoplastic disorder? *Int. J. Neurosci.* 124, 509–511. Available from: <https://doi.org/10.3109/00207454.2013.854208>.
- Ortmann, R.A., Klippel, J.H., 2000. Update on cyclophosphamide for systemic lupus erythematosus. *Rheum. Dis. Clin. North. Am.* 26 (2), 363–375. vii. Review. PubMed PMID: 10768217.
- Özen, S., Ruperto, N., Dillon, M.J., Bagga, A., Barron, K., Davin, J.C., et al., 2006. EULAR/PRoS endorsed consensus criteria for the classification of childhood vasculitides. *Ann. Rheum. Dis.* 65 (7), 936–941. Epub 2005 Dec 1. PubMed PMID: 16322081; PubMed Central PMCID: PMC1798210.
- Padovan, M., Castellino, G., Bortoluzzi, A., Caniatti, L., Trotta, F., Govoni, M., 2012. Factors and comorbidities associated with central nervous system involvement in systemic lupus erythematosus: a retrospective cross-sectional case-control study from a single center. *Rheumatol. Int.* 32 (1), 129–135. Available from: <https://doi.org/10.1007/s00296-010-1565-4>. Epub 2010 Jul 31. PubMed PMID: 20676648.
- Pagano, M.B., Murinson, B.B., Tobian, A.A.R., King, K.E., 2014. Efficacy of therapeutic plasma exchange for treatment of stiff-person syndrome. *Transfusion (Paris)* 54, 1851–1856. Available from: <https://doi.org/10.1111/trf.12573>.
- Pagnoux, C., Mahr, A., Hamidou, M.A., Boffa, J.J., Ruivard, M., Ducroix, J.P., et al., 2008. Azathioprine or methotrexate maintenance for ANCA-associated vasculitis. *N. Engl. J. Med.* 359 (26), 2790–2803. Available from: <https://doi.org/10.1056/NEJMoa0802311>. PubMed PMID: 19109574.
- Pagnoux, C., Seror, R., Henegar, C., Mahr, A., Cohen, P., Le Guern, V., et al., 2010. Clinical features and outcomes in 348 patients with polyarteritis nodosa: a systematic retrospective study of patients diagnosed between 1963 and 2005 and entered into the French Vasculitis Study Group Database. *Arthritis Rheum.* 62 (2), 616–626. Available from: <https://doi.org/10.1002/art.27240>. PubMed PMID: 20112401.
- Paoletti, P., Neyton, J., 2007. NMDA receptor subunits: function and pharmacology. *Curr. Opin. Pharmacol.* 7, 39–47. Available from: <https://doi.org/10.1016/j.coph.2006.08.011>.
- Papadopoulos, M.C., Manley, G.T., Krishna, S., Verkman, A.S., 2004. Aquaporin-4 facilitates reabsorption of excess fluid in vasogenic brain edema. *FASEB J.* 18, 1291–1293. Available from: <https://doi.org/10.1096/fj.04-1723fje>.
- Pawate, S., Moses, H., Sriram, S., 2009. Presentations and outcomes of neurosarcoïdosis: a study of 54 cases. *QJM* 102 (7), 449–460. Available from: <https://doi.org/10.1093/qjmed/hcp042>. Epub 2009 Apr 20. PubMed PMID: 19383611.
- Peng, X., Hughes, E.G., Moscato, E.H., Parsons, T.D., Dalmau, J., Balice-Gordon, R.J., 2015. Cellular plasticity induced by anti- $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor encephalitis antibodies. *Ann. Neurol.* 77, 381–398. Available from: <https://doi.org/10.1002/ana.24293>.
- Peschl, P., Bradl, M., Höftberger, R., Berger, T., Reindl, M., 2017. Myelin oligodendrocyte glycoprotein: deciphering a target in inflammatory demyelinating diseases. *Front. Immunol.* 8, 529. Available from: <https://doi.org/10.3389/fimmu.2017.00529>.
- Peterson, K., Rosenblum, M.K., Kotanides, H., Posner, J.B., 1992. Paraneoplastic cerebellar degeneration. I. A clinical analysis of 55 anti-Yo antibody-positive patients. *Neurology* 42, 1931–1937.
- Petit-Pedrol, M., Armangue, T., Peng, X., Bataller, L., Cellucci, T., Davis, R., et al., 2014. Encephalitis with refractory seizures, status epilepticus, and antibodies to the GABA<sub>A</sub> receptor: a case series, characterisation of the antigen, and analysis of the effects of antibodies. *Lancet Neurol.* 13, 276–286. Available from: [https://doi.org/10.1016/S1474-4422\(13\)70299-0](https://doi.org/10.1016/S1474-4422(13)70299-0).
- Pettingill, P., Kramer, H.B., Coebergh, J.A., Pettingill, R., Maxwell, S., Nibber, A., et al., 2015. Antibodies to GABA<sub>A</sub> receptor  $\alpha$ 1 and  $\gamma$ 2 subunits: clinical and serologic characterization. *Neurology* 84, 1233–1241. Available from: <https://doi.org/10.1212/WNL.0000000000001326>.
- Pfefferkorn, T., Schüller, U., Cyran, C., Hüfner, K., Fesl, G., Seelos, K., et al., 2010. Giant cell arteritis of the Basal cerebral arteries: correlation of MRI, dSA, and histopathology. *Neurology* 74 (20), 1651–1653. Available from: <https://doi.org/10.1212/WNL.0b013e3181df0a09>. PubMed PMID: 20479365.

- Pham, H.P., Daniel-Johnson, J.A., Stotler, B.A., Stephens, H., Schwartz, J., 2011. Therapeutic plasma exchange for the treatment of anti-NMDA receptor encephalitis. *J. Clin. Apheresis* 26, 320–325. Available from: <https://doi.org/10.1002/jca.20311>.
- Phuphanich, S., Brock, C., 2007. Neurologic improvement after high-dose intravenous immunoglobulin therapy in patients with paraneoplastic cerebellar degeneration associated with anti-Purkinje cell antibody. *J. Neurooncol.* 81, 67–69. Available from: <https://doi.org/10.1007/s11060-006-9198-x>.
- Piepgras, J., Höltje, M., Michel, K., Li, Q., Otto, C., Drenckhahn, C., et al., 2015. Anti-DPPX encephalitis: pathogenic effects of antibodies on gut and brain neurons. *Neurology* 85, 890–897. Available from: <https://doi.org/10.1212/WNL.0000000000001907>.
- Pignolet, B.S., Gebauer, C.M., Liblau, R.S., 2013. Immunopathogenesis of paraneoplastic neurological syndromes associated with anti-Hu antibodies: a beneficial antitumor immune response going awry. *Oncoimmunology* 2, e27384. Available from: <https://doi.org/10.4161/onci.27384>.
- Pittock, S.J., Lucchinetti, C.F., 2016. Neuromyelitis optica and the evolving spectrum of autoimmune aquaporin-4 channelopathies: a decade later. *Ann. N.Y. Acad. Sci.* 1366, 20–39. Available from: <https://doi.org/10.1111/nyas.12794>.
- Pittock, S.J., Vincent, A., 2016. Introduction to autoimmune neurology. *Handb. Clin. Neurol.* 133, 3–14. Available from: <https://doi.org/10.1016/B978-0-444-63432-0.00001-3>.
- Pittock, S.J., Kryzer, T.J., Lennon, V.A., 2004. Paraneoplastic antibodies coexist and predict cancer, not neurological syndrome. *Ann. Neurol.* 56, 715–719. Available from: <https://doi.org/10.1002/ana.20269>.
- Pittock, S.J., Lucchinetti, C.F., Parisi, J.E., Benarroch, E.E., Mokri, B., Stephan, C.L., et al., 2005. Amphiphysin autoimmunity: paraneoplastic accompaniments. *Ann. Neurol.* 58, 96–107. Available from: <https://doi.org/10.1002/ana.20529>.
- Pittock, S.J., Yoshikawa, H., Ahlskog, J.E., Tisch, S.H., Benarroch, E.E., Kryzer, T.J., et al., 2006. Glutamic acid decarboxylase autoimmunity with brainstem, extrapyramidal, and spinal cord dysfunction. *Mayo Clin. Proc.* 81, 1207–1214. Available from: <https://doi.org/10.4065/81.9.1207>.
- Pittock, S.J., Parisi, J.E., McKeon, A., Roemer, S.F., Lucchinetti, C.F., Tan, K.M., et al., 2010. Paraneoplastic jaw dystonia and laryngospasm with antineuronal nuclear antibody type 2 (anti-Ri). *Arch. Neurol.* 67, 1109–1115. Available from: <https://doi.org/10.1001/archneurol.2010.209>.
- Pittock, S.J., Lennon, V.A., Bakshi, N., Shen, L., McKeon, A., Quach, H., et al., 2014. Seroprevalence of aquaporin-4-IgG in a northern California population representative cohort of multiple sclerosis. *JAMA Neurol.* 71, 1433–1436. Available from: <https://doi.org/10.1001/jamaneurol.2014.1581>.
- Planagumà, J., Leypoldt, F., Mannara, F., Gutiérrez-Cuesta, J., Martín-García, E., Aguilar, E., et al., 2015. Human N-methyl D-aspartate receptor antibodies alter memory and behaviour in mice. *Brain J. Neurol.* 138, 94–109. Available from: <https://doi.org/10.1093/brain/awu310>.
- Pollard, R.P., Abdulahad, W.H., Vissink, A., Hamza, N., Burgerhof, J.G., Meijer, J.M., et al., 2013. Serum levels of BAFF, but not APRIL, are increased after rituximab treatment in patients with primary Sjögren's syndrome: data from a placebo-controlled clinical trial. *Ann. Rheum. Dis.* 72, 146–148.
- Pomper, M.G., Miller, T.J., Stone, J.H., Tidmore, W.C., Hellmann, D.B., 1999. CNS vasculitis in autoimmune disease: MR imaging findings and correlation with angiography. *AJNR Am. J. Neuroradiol.* 20 (1), 75–85. PubMed PMID: 9974060.
- Pranzatelli, M.R., Tate, E.D., Swan, J.A., Travelstead, A.L., Colliver, J.A., Verhulst, S.J., et al., 2010. B cell depletion therapy for new-onset opsoclonus-myoclonus. *Mov. Disord.* 25, 238–242. Available from: <https://doi.org/10.1002/mds.22941>.
- Probst, C., Saschenbrecker, S., Stoecker, W., Komorowski, L., 2014. Anti-neuronal autoantibodies: current diagnostic challenges. *Mult. Scler. Relat. Disord.* 3, 303–320. Available from: <https://doi.org/10.1016/j.msard.2013.12.001>.
- Probst, C., Komorowski, L., de Graaff, E., van Coevorden-Hameete, M., Rogemond, V., Honnorat, J., et al., 2015. Standardized test for anti-Tr/DNER in patients with paraneoplastic cerebellar degeneration. *Neurol. Neuroimmunol. Neuroinflamm.* 2, e68. Available from: <https://doi.org/10.1212/NXI.0000000000000068>.
- Provenzale, J.M., Allen, N.B., 1996. Neuroradiologic findings in polyarteritis nodosa. *AJNR Am. J. Neuroradiol.* 17 (6), 1119–1126. PubMed PMID: 8791926.
- Puechal, X., Rivereau, P., Vinchon, F., 2008. Churg-Strauss syndrome associated with omalizumab. *Eur. J. Intern. Med.* 19 (5), 364–366. 10.1016/j.ejim.2007.09.001.
- Quartuccio, L., Salvin, S., Corazza, L., Gandolfo, S., Fabris, M., De Vita, S., 2016. Efficacy of belimumab and targeting of rheumatoid factor-positive B-cell expansion in Sjögren's syndrome: follow-up after the end of the phase II open-label BELISS study. *Clin. Exp. Rheumatol.* 34, 311–314.
- Rahmattulla, C., Mooyaart, A.L., van Hooven, D., Schoones, J.W., Bruijn, J.A., Dekkers, O.M., et al., 2016. Genetic variants in ANCA-associated vasculitis: a meta-analysis. *Ann. Rheum. Dis.* 75 (9), 1687–1692. Available from: <https://doi.org/10.1136/annrheumdis-2015-207601>. Epub 2015 Oct 6. PubMed PMID: 26443607.
- Ramos-Casals, M., Garcia-Hernandez, F.J., de Ramon, E., Callejas, J.L., Martinez-Berriotxo, A., Pallares, L., et al., 2010. Off-label use of rituximab in 196 patients with severe, refractory systemic autoimmune diseases. *Clin. Exp. Rheumatol.* 28, 468–476.
- Reindl, M., Di Pauli, F., Rostásy, K., Berger, T., 2013. The spectrum of MOG autoantibody-associated demyelinating diseases. *Nat. Rev. Neurol.* 9, 455–461. Available from: <https://doi.org/10.1038/nrneurol.2013.118>.
- Reske, D., Petereit, H.F., Heiss, W.D., 2005. Difficulties in the differentiation of chronic inflammatory diseases of the central nervous system—value of cerebrospinal fluid analysis and immunological abnormalities in the diagnosis. *Acta Neurol. Scand.* 112 (4), 207–213. Review. PubMed PMID: 16146488.
- Ribi, C., Cohen, P., Pagnoux, C., Mahr, A., Arène, J.P., Lauque, D., et al., 2008. Treatment of Churg-Strauss syndrome without poor-prognosis factors: a multicenter, prospective, randomized, open-label study of seventy-two patients. *Arthritis Rheum.* 58 (2), 586–594. Available from: <https://doi.org/10.1002/art.23198>. PubMed PMID: 18240234.
- Richioud, B., Béjot, Y., Ornetti, P., Ricolfi, F., Sautreux, J.L., Ben Salem, D., 2012. Rheumatoid arthritis and meningeal nodules. *Rev. Neurol. (Paris)* 168, 350–356.
- Roberts, J., Clifford, A., 2017. Update on the management of giant cell arteritis. *Ther. Adv. Chronic Dis.* 8 (4–5), 69–79. Available from: <https://doi.org/10.1177/2040622317700089>. Epub 2017 Mar 28. Review. PubMed PMID: 28491267; PubMed Central PMCID: PMC5406009.

- Rojas, I., Graus, F., Keime-Guibert, F., René, R., Delattre, J.Y., Ramón, J.M., et al., 2000. Long-term clinical outcome of paraneoplastic cerebellar degeneration and anti-Yo antibodies. *Neurology* 55, 713–715.
- Rosenfeld, M.R., Eichen, J.G., Wade, D.F., Posner, J.B., Dalmau, J., 2001. Molecular and clinical diversity in paraneoplastic immunity to Ma proteins. *Ann. Neurol.* 50, 339–348.
- Ruiz-Irastorza, G., Khamashta, M.A., Castellino, G., Hughes, G.R., 2001. Systemic lupus erythematosus. *Lancet* 357 (9261), 1027–1032. Review. PubMed PMID: 11293608.
- Saadoun, S., Papadopoulos, M.C., 2015. Role of membrane complement regulators in neuromyelitis optica. *Mult. Scler. Hounds Mills Basingstoke Engl.* 21, 1644–1654. Available from: <https://doi.org/10.1177/1352458515571446>.
- Saadoun, S., Waters, P., MacDonald, C., Bell, B.A., Vincent, A., Verkman, A.S., et al., 2012. Neutrophil protease inhibition reduces neuromyelitis optica-immunoglobulin G-induced damage in mouse brain. *Ann. Neurol.* 71, 323–333. Available from: <https://doi.org/10.1002/ana.22686>.
- Saadoun, S., Waters, P., Owens, G.P., Bennett, J.L., Vincent, A., Papadopoulos, M.C., 2014. Neuromyelitis optica MOG-IgG causes reversible lesions in mouse brain. *Acta Neuropathol. Commun.* 2, 35. Available from: <https://doi.org/10.1186/2051-5960-2-35>.
- Sabater, L., Titulaer, M., Saiz, A., Verschueren, J., Güre, A.O., Graus, F., 2008. SOX1 antibodies are markers of paraneoplastic Lambert-Eaton myasthenic syndrome. *Neurology* 70, 924–928. Available from: <https://doi.org/10.1212/01.wnl.0000281663.81079.24>.
- Sabater, L., Gaig, C., Gelpí, E., Bataller, L., Lewerenz, J., Torres-Vega, E., et al., 2014. A novel non-rapid-eye movement and rapid-eye-movement parasomnia with sleep breathing disorder associated with antibodies to IgLON5: a case series, characterisation of the antigen, and post-mortem study. *Lancet Neurol.* 13, 575–586. Available from: [https://doi.org/10.1016/S1474-4422\(14\)70051-1](https://doi.org/10.1016/S1474-4422(14)70051-1).
- Sabater, L., Planagumà, J., Dalmau, J., Graus, F., 2016. Cellular investigations with human antibodies associated with the anti-IgLON5 syndrome. *J. Neuroinflamm.* 13, 226. Available from: <https://doi.org/10.1186/s12974-016-0689-1>.
- Sablé-Fourtassou, R., Cohen, P., Mahr, A., Pagnoux, C., Mouthon, L., Jayne, D., et al., 2005. Antineutrophil cytoplasmic antibodies and the Churg-Strauss syndrome. *Ann. Intern. Med.* 143 (9), 632–638. PubMed PMID: 16263885.
- Saito, S.-Y., Takeshima, H., 2006. DNER as key molecule for cerebellar maturation. *Cerebellum Lond. Engl.* 5, 227–231. Available from: <https://doi.org/10.1080/14734220600632564>.
- Salvarani, C., Pipitone, N., Versari, A., Hunder, G.G., 2012. Clinical features of polymyalgia rheumatica and giant cell arteritis. *Nat. Rev. Rheumatol.* 8, 509–521. PubMed: 22825731.
- Sánchez Gomar, I., Díaz Sánchez, M., Uclés Sánchez, A.J., Casado Chocán, J.L., Ramírez-Lorca, R., Serna, A., et al., 2014. An immunoassay that distinguishes real neuromyelitis optica signals from a labeling detected in patients receiving natalizumab. *BMC Neurol.* 14, 139. Available from: <https://doi.org/10.1186/1471-2377-14-139>.
- Sanna, G., Bertolaccini, M.L., Cuadrado, M.J., Laing, H., Khamashta, M.A., Mathieu, A., et al., 2003. Neuropsychiatric manifestations in systemic lupus erythematosus: prevalence and association with antiphospholipid antibodies. *J. Rheumatol.* 30 (5), 985–992. PubMed PMID: 12734893.
- Sanz, R., Ferraro, G.B., Fournier, A.E., 2015. IgLON cell adhesion molecules are shed from the cell surface of cortical neurons to promote neuronal growth. *J. Biol. Chem.* 290, 4330–4342. Available from: <https://doi.org/10.1074/jbc.M114.628438>.
- Scharf, S.H., Jaeschke, G., Wettstein, J.G., Lindemann, L., 2015. Metabotropic glutamate receptor 5 as drug target for Fragile X syndrome. *Curr. Opin. Pharmacol.* 20, 124–134. Available from: <https://doi.org/10.1016/j.coph.2014.11.004>.
- Scheibe, F., Prüss, H., Mengel, A.M., Kohler, S., Nümann, A., Köhnlein, M., et al., 2017. Bortezomib for treatment of therapy-refractory anti-NMDA receptor encephalitis. *Neurology* 88, 366–370. Available from: <https://doi.org/10.1212/WNL.0000000000003536>.
- Schmidt, W.A., Blockmans, D., 2005. Use of ultrasonography and positron emission tomography in the diagnosis and assessment of large-vessel vasculitis. *Curr. Opin. Rheumatol.* 17 (1), 9–15. Review. PubMed PMID: 15604899.
- Schmierer, K., Valdueza, J.M., Bender, A., DeCamilli, P., David, C., Solimena, M., et al., 1998. Atypical stiff-person syndrome with spinal MRI findings, amphiphysin autoantibodies, and immunosuppression. *Neurology* 51, 250–252.
- Schmitt, S.E., Pargeon, K., Frechette, E.S., Hirsch, L.J., Dalmau, J., Friedman, D., 2012. Extreme delta brush. *Neurology* 79, 1094–1100. Available from: <https://doi.org/10.1212/WNL.0b013e3182698cd8>.
- Schubert, M., Panja, D., Haugen, M., Bramham, C.R., Vedeler, C.A., 2014. Paraneoplastic CDR2 and CDR2L antibodies affect Purkinje cell calcium homeostasis. *Acta Neuropathol. (Berl.)* 128, 835–852. Available from: <https://doi.org/10.1007/s00401-014-1351-6>.
- Schuler, V., Lüscher, C., Blanchet, C., Klix, N., Sansig, G., Klebs, K., et al., 2001. Epilepsy, hyperalgesia, impaired memory, and loss of pre- and postsynaptic GABA(B) responses in mice lacking GABA(B(1)). *Neuron* 31, 47–58.
- Schulz, S.W., Shenin, M., Mehta, A., Kebede, A., Fluerant, M., Derk, C.T., 2012. Initial presentation of acute transverse myelitis in systemic lupus erythematosus: demographics, diagnosis, management and comparison to idiopathic cases. *Rheumatol. Int.* 32 (9), 2623–2627. Available from: <https://doi.org/10.1007/s00296-011-2053-1>. Epub 2011 Jul 22. PubMed PMID: 21833518.
- Scott, D.G., Bacon, P.A., Elliott, P.J., Tribe, C.R., Wallington, T.B., 1982. Systemic vasculitis in a district general hospital 1972–1980: clinical and laboratory features, classification and prognosis of 80 cases. *QJM* 51, 292–311.
- Scott, T.F., Yandora, K., Valeri, A., Chieffe, C., Schramke, C., 2007. Aggressive therapy for neurosarcoidosis: long-term follow-up of 48 treated patients. *Arch. Neurol.* 64 (5), 691–696. PubMed PMID: 17502468.
- Scott, D.L., Wolfe, F., Huizinga, T.W., 2010. Rheumatoid arthritis. *Lancet* 376, 1094–1108.
- Sehgal, M., Swanson, J.W., DeRemee, R.A., Colby, T.V., 1995. Neurologic manifestations of Churg-Strauss syndrome. *Mayo Clin. Proc.* 70 (4), 337–341. PubMed PMID: 7898138.
- Sène, D., Jallouli, M., Lefacheur, J.P., Saadoun, D., Costedoat-Chalumeau, N., Maisonobe, T., et al., 2011. Peripheral neuropathies associated with primary Sjögren syndrome: nonataxic sensory neuropathy and sensorimotor neuropathy. *Medicine (Baltimore)* 90 (2), 133–138. Available from: <https://doi.org/10.1097/MD.0b013e31820fd2d1>. PubMed PMID: 21358442.
- Seror, A., 2006. Central nervous system involvement in Wegener's granulomatosis. *Medicine* 85, 54–65.
- Seror, R., Baron, G., Hachulla, E., et al., 2014. Adalimumab for steroid sparing in patients with giant cell arteritis: results of a multicenter randomized controlled trial. *Ann. Rheum. Dis.* 73, 2074–2081.

- Seyahi, E., Melikoglu, M., Akman, C., Hamuryudan, V., Ozer, H., Hatemi, G., et al., 2012. Pulmonary artery involvement and associated lung disease in Behcet disease: a series of 47 patients. *Medicine (Baltimore)* 91 (1), 35–48. Available from: <https://doi.org/10.1097/MD.0b013e318242ff37>. PubMed PMID: 22210555.
- Sfikakis, P.P., Kaklamanis, P.H., Elezoglu, A., Katsilambros, N., Theodossiadis, P.G., Papaefthimiou, S., et al., 2004. Infliximab for recurrent, sight-threatening ocular inflammation in Adamantiades-Behcet disease. *Ann. Intern. Med.* 140 (5), 404–406. PubMed PMID: 14996689.
- Shams'ili, S., Grefkens, J., de Leeuw, B., van den Bent, M., Hooijkaas, H., van der Holt, B., et al., 2003. Paraneoplastic cerebellar degeneration associated with antineuronal antibodies: analysis of 50 patients. *Brain J. Neurol* 126, 1409–1418.
- Shams'ili, S., de Beukelaar, J., Gratama, J.W., Hooijkaas, H., van den Bent, M., van't Veer, M., et al., 2006. An uncontrolled trial of rituximab for antibody associated paraneoplastic neurological syndromes. *J. Neurol.* 253, 16–20. Available from: <https://doi.org/10.1007/s00415-005-0882-0>.
- Sharma, A., Kiripolsky, J., Klimatcheva, E., Howell, A., Fereidouni, F., Levenson, R., et al., 2016. Early BAFF receptor blockade mitigates murine Sjögren's syndrome: concomitant targeting of CXCL13 and the BAFF receptor prevents salivary hypofunction. *Clin. Immunol.* 164, 85–94.
- Sharma, A., Sharma, K., 2013. Hepatotropic viral infection associated systemic vasculitides-hepatitis B virus associated polyarteritis nodosa and hepatitis C virus associated cryoglobulinemic vasculitis. *J. Clin. Exp. Hepatol.* 3 (3), 204–212. Available from: <https://doi.org/10.1016/j.jceh.2013.06.001>. Epub 2013 Jul 8. Review. PubMed PMID: 25755502; PubMed Central PMCID: PMC4216827.
- Shepherd, J.D., Huganir, R.L., 2007. The cell biology of synaptic plasticity: AMPA receptor trafficking. *Annu. Rev. Cell. Dev. Biol.* 23, 613–643. Available from: <https://doi.org/10.1146/annurev.cellbio.23.090506.123516>.
- Shin, Y.-W., Lee, S.-T., Shin, J.-W., Moon, J., Lim, J.-A., Byun, J.-I., et al., 2013. VGKC-complex/LGI1-antibody encephalitis: clinical manifestations and response to immunotherapy. *J. Neuroimmunol.* 265, 75–81. Available from: <https://doi.org/10.1016/j.jneuroim.2013.10.005>.
- Sim, M.K., Kim, D.Y., Yoon, J., Park, D.H., Kim, Y.G., 2014. Assessment of peripheral neuropathy in patients with rheumatoid arthritis who complain of neurologic symptoms. *Ann. Rehabil. Med.* 38, 249–255.
- Sindrilaru, A., Seeliger, S., Ehrchen, J.M., Peters, T., Roth, J., Scharffetter-Kochanek, K., et al., 2007. Site of blood vessel damage and relevance of CD18 in a murine model of immune complex-mediated vasculitis. *J. Invest. Dermatol.* 127, 447–454.
- Singh, B., Ogiwara, I., Kaneda, M., Tokonami, N., Mazaki, E., Baba, K., et al., 2006. A Kv4.2 truncation mutation in a patient with temporal lobe epilepsy. *Neurobiol. Dis.* 24, 245–253. Available from: <https://doi.org/10.1016/j.nbd.2006.07.001>.
- Sinmaz, N., Tea, F., Pilli, D., Zou, A., Amatoury, M., Nguyen, T., et al., 2016. Dopamine-2 receptor extracellular N-terminus regulates receptor surface availability and is the target of human pathogenic antibodies from children with movement and psychiatric disorders. *Acta Neuropathol. Commun.* 4. Available from: <https://doi.org/10.1186/s40478-016-0397-1>.
- Siva, A., Saip, S., 2009. The spectrum of nervous system involvement in Behcet's syndrome and its differential diagnosis. *J. Neurol.* 256 (4), 513–529. Available from: <https://doi.org/10.1007/s00415-009-0145-6>. Epub 2009 Apr 27. Review. PubMed PMID: 19444529.
- Skopouli, F.N., Jagiello, P., Tsifetaki, N., Moutsopoulos, H.M., 1996. Methotrexate in primary Sjögren's syndrome. *Clin. Exp. Rheumatol.* 14, 555–558.
- Smith, J.K., Matheus, M.G., Castillo, M., 2004. Imaging manifestations of neurosarcoidosis. *AJR Am. J. Roentgenol.* 182 (2), 289–295. Review. PubMed PMID: 14736648.
- Soliotis, F.C., Moutsopoulos, H.M., 2004. Sjögren's syndrome. *Autoimmunity* 37 (4), 305–307. Review. PubMed PMID: 15518047.
- Soliotis, F.C., Mavragani, C.P., Moutsopoulos, H.M., 2004. Central nervous system involvement in Sjögren's syndrome. *Ann. Rheum. Dis.* 63 (6), 616–620. PubMed PMID: 15140765; PubMed Central PMCID: PMC1755013.
- Solomon, D.H., Karlson, E.W., Rimm, E.B., Cannuscio, C.C., Mandl, L.A., Manson, J.E., et al., 2003. Cardiovascular morbidity and mortality in women diagnosed with rheumatoid arthritis. *Circulation* 107, 1303–1307.
- Spadaro, M., Gerdés, L.A., Mayer, M.C., Ertl-Wagner, B., Laurent, S., Krumbholz, M., et al., 2015. Histopathology and clinical course of MOG antibody-associated encephalomyelitis. *Ann. Clin. Transl. Neurol.* 2, 295–301. Available from: <https://doi.org/10.1002/acn3.164>.
- Spatola, M., Petit-Pedrol, M., Simabukuro, M.M., Aramangue, T., Castro, F.J., Barcelo Artigues, M.I., et al., 2017. Investigations in GABA<sub>A</sub> receptor antibody-associated encephalitis. *Neurology* 88, 1012–1020. Available from: <https://doi.org/10.1212/WNL.0000000000003713>.
- Sprengel, R., 2006. Role of AMPA receptors in synaptic plasticity. *Cell Tissue Res.* 326, 447–455. Available from: <https://doi.org/10.1007/s00441-006-0275-4>.
- Stark, E., Wurster, U., Patzold, U., Sailer, M., Haas, J., 1995. Immunological and clinical response to immunosuppressive treatment in paraneoplastic cerebellar degeneration. *Arch. Neurol.* 52, 814–818.
- Starosta, M.A., Brandwein, S.R., 2007. Clinical manifestations and treatment of rheumatoid pachymeningitis. *Neurology* 68, 1079–1080.
- Stayer, C., Tronnier, V., Dressnandt, J., Mauch, E., Marquardt, G., Rieke, K., et al., 1997. Intrathecal baclofen therapy for stiff-man syndrome and progressive encephalomyopathy with rigidity and myoclonus. *Neurology* 49, 1591–1597.
- Stern, B.J., 2004. Neurological complications of sarcoidosis. *Curr. Opin. Neurol.* 17 (3), 311–316. Review. PubMed PMID: 15167067.
- Stone, J.H., Merkel, P.A., Spiera, R., Seo, P., Langford, C.A., Hoffman, G.S., et al., 2010. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. *N. Engl. J. Med.* 363 (3), 221–232. Available from: <https://doi.org/10.1056/NEJMoa0909905>. PubMed PMID: 20647199; PubMed Central PMCID: PMC3137658.
- Stone, J.H., Tuckwell, K., Dimonaco, S., et al., 2016. Efficacy and safety of tocilizumab in patients with giant cell arteritis: primary and secondary outcomes from a phase 3, randomized, double-blind, placebo-controlled trial [abstract]. *Arthritis Rheumatol* 68 (Suppl. 10).
- Stone, J.H., Tuckwell, K., Dimonaco, S., Klearman, M., Aringer, M., Blockmans, D., et al., 2017. Trial of tocilizumab in giant-cell arteritis. *N. Engl. J. Med.* 377 (4), 317–328. Available from: <https://doi.org/10.1056/NEJMoa1613849>. PubMed PMID: 28745999.
- Strauss, K.A., Puffenberger, E.G., Huettelman, M.J., Gottlieb, S., Dobrin, S.E., Parod, J.M., et al., 2006. Recessive symptomatic focal epilepsy and mutant contactin-associated protein-like 2. *N. Engl. J. Med.* 354, 1370–1377. Available from: <https://doi.org/10.1056/NEJMoa052773>.
- Sun, W., Maffie, J.K., Lin, L., Petralia, R.S., Rudy, B., Hoffman, D.A., 2011. DPP6 establishes the A-type K(+) current gradient critical for the regulation of dendritic excitability in CA1 hippocampal neurons. *Neuron* 71, 1102–1115. Available from: <https://doi.org/10.1016/j.neuron.2011.08.008>.
- Takei, Y., Kondo, S., Harada, A., Inomata, S., Noda, T., Hirokawa, N., 1997. Delayed development of nervous system in mice homozygous for disrupted microtubule-associated protein 1B (MAP1B) gene. *J. Cell. Biol.* 137, 1615–1626.

- Thieben, M.J., Lennon, V.A., Boeve, B.F., Aksamit, A.J., Keegan, M., Vernino, S., 2004. Potentially reversible autoimmune limbic encephalitis with neuronal potassium channel antibody. *Neurology* 62, 1177–1182.
- Thiel, J., Troilo, A., Salzer, U., Schleyer, T., Halmschlag, K., Rizzi, M., et al., 2017. Rituximab as induction therapy in eosinophilic granulomatosis with polyangiitis refractory to conventional immunosuppressive treatment: a 36-month follow-up analysis. *J. Allergy Clin. Immunol. Pract.* Available from: <https://doi.org/10.1016/j.jaip.2017.07.027> pii: S2213-2198(17)30552-4 [Epub ahead of print] PubMed PMID: 28916432.
- Titulaer, M.J., Soffietti, R., Dalmau, J., Gilhus, N.E., Giometto, B., Graus, F., et al., 2011. Screening for tumours in paraneoplastic syndromes: report of an EFNS Task Force. *Eur. J. Neurol.* 18, 19–e3. Available from: <https://doi.org/10.1111/j.1468-1331.2010.03220.x>.
- Titulaer, M.J., McCracken, L., Gabilondo, I., Armangué, T., Glaser, C., Iizuka, T., et al., 2013a. Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study. *Lancet Neurol.* 12, 157–165. Available from: [https://doi.org/10.1016/S1474-4422\(12\)70310-1](https://doi.org/10.1016/S1474-4422(12)70310-1).
- Titulaer, M.J., McCracken, L., Gabilondo, I., Iizuka, T., Kawachi, I., Bataller, L., et al., 2013b. Late-onset anti-NMDA receptor encephalitis. *Neurology* 81, 1058–1063. Available from: <https://doi.org/10.1212/WNL.0b013e3182a4a49c>.
- Tobin, W.O., Lennon, V.A., Komorowski, L., Probst, C., Clardy, S.L., Aksamit, A.J., et al., 2014. DPPX potassium channel antibody. *Neurology* 83, 1797–1803. Available from: <https://doi.org/10.1212/WNL.0000000000000991>.
- Todd, A.J., Sullivan, A.C., 1990. Light microscope study of the coexistence of GABA-like and glycine-like immunoreactivities in the spinal cord of the rat. *J. Comp. Neurol.* 296, 496–505. Available from: <https://doi.org/10.1002/cne.902960312>.
- Tohgo, A., Eiraku, M., Miyazaki, T., Miura, E., Kawaguchi, S.-Y., Nishi, M., et al., 2006. Impaired cerebellar functions in mutant mice lacking DNER. *Mol. Cell. Neurosci.* 31, 326–333. Available from: <https://doi.org/10.1016/j.mcn.2005.10.003>.
- Tortosa, E., Montenegro-Venegas, C., Benoist, M., Härtel, S., González-Billault, C., Esteban, J.A., et al., 2011. Microtubule-associated protein 1B (MAP1B) is required for dendritic spine development and synaptic maturation. *J. Biol. Chem.* 286, 40638–40648. Available from: <https://doi.org/10.1074/jbc.M111.271320>.
- Trebst, C., Jarius, S., Berthele, A., Paul, F., Schippling, S., Wildemann, B., et al., 2014. Update on the diagnosis and treatment of neuromyelitis optica: recommendations of the Neuromyelitis Optica Study Group (NEMOS). *J. Neurol.* 261, 1–16. Available from: <https://doi.org/10.1007/s00415-013-7169-7>.
- Trepö, C., Zuckerman, A., Bird, R., Prince, A., 1974. The role of circulating hepatitis B antigen/antibody immune complexes in the pathogenesis of vascular and hepatic manifestations in polyarteritis nodosa. *J. Clin. Pathol.* 27, 863–868.
- Trotter, J.L., Henden, B.A., Osterland, C.K., 1976. Cerebellar degeneration with Hodgkin disease. An immunological study. *Arch. Neurol.* 33, 660–661.
- Tsokos, G.C., Lo, M.S., Costa Reis, P., Sullivan, K.E., 2016. New insights into the immunopathogenesis of systemic lupus erythematosus. *Nat. Rev. Rheumatol.* 12 (12), 716–730. Available from: <https://doi.org/10.1038/nrrheum.2016.186>. Review. PubMed PMID: 27872476.
- Tyagarajan, S.K., Fritschy, J.-M., 2014. Gephyrin: a master regulator of neuronal function? *Nat. Rev. Neurosci.* 15, 141–156. Available from: <https://doi.org/10.1038/nrn3670>.
- Uchida, K., Park, E., Tsuboi, M., Chambers, J.K., Nakayama, H., 2016. Pathological and immunological features of canine necrotising meningoencephalitis and granulomatous meningoencephalitis. *Vet. J. Lond. Engl.* 213, 72–77. Available from: <https://doi.org/10.1016/j.tvjl.2016.05.002>.
- Uchuya, M., Graus, F., Vega, F., Reñé, R., Delattre, J.Y., 1996. Intravenous immunoglobulin treatment in paraneoplastic neurological syndromes with antineuronal autoantibodies. *J. Neurol. Neurosurg. Psychiatry* 60, 388–392.
- Unizony, S., Villarreal, M., Miloslavsky, E.M., Lu, N., Merkel, P.A., Spiera, R., et al., 2016. Clinical outcomes of treatment of anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis based on ANCA type. *Ann. Rheum. Dis* 75 (6), 1166–1169. Available from: <https://doi.org/10.1136/annrheumdis-2015-208073>. Epub 2015 Nov 30. PubMed PMID: 26621483; PubMed Central PMCID: PMC4908815.
- Uzawa, A., Mori, M., Ito, M., Uchida, T., Hayakawa, S., Masuda, S., et al., 2009. Markedly increased CSF interleukin-6 levels in neuromyelitis optica, but not in multiple sclerosis. *J. Neurol.* 256, 2082–2084. Available from: <https://doi.org/10.1007/s00415-009-5274-4>.
- Vaglio, A., Martorana, D., Maggiore, U., Grasselli, C., Zanetti, A., Pesci, A., et al., 2007. HLA-DRB4 as a genetic risk factor for Churg-Strauss syndrome. *Arthritis Rheum.* 56 (9), 3159–3166. Available from: <https://doi.org/10.1002/art.22834>.
- van Daalen, E.E., Rizzo, R., Kronbichler, A., Wolterbeek, R., Bruijn, J.A., Jayne, D.R., et al., 2017. Effect of rituximab on malignancy risk in patients with ANCA-associated vasculitis. *Ann. Rheum. Dis.* 76 (6), 1064–1069. Available from: <https://doi.org/10.1136/annrheumdis-2016-209925>. Epub 2016 Nov 29. PubMed PMID: 27899372.
- van Sonderen, A., Ariño, H., Petit-Pedrol, M., Leypoldt, F., Körtvélyessy, P., Wandinger, K.-P., et al., 2016a. The clinical spectrum of Caspr2 antibody-associated disease. *Neurology* 87, 521–528. Available from: <https://doi.org/10.1212/WNL.0000000000002917>.
- van Sonderen, A., Thijss, R.D., Coenders, E.C., Jiskoot, L.C., Sanchez, E., de Brujin, M.A.A.M., et al., 2016b. Anti-LGI1 encephalitis: clinical syndrome and long-term follow-up. *Neurology* 87, 1449–1456. Available from: <https://doi.org/10.1212/WNL.0000000000003173>.
- van Sonderen, A., Schreurs, M.W.J., Wirtz, P.W., Silleveld Smitt, P.A.E., Titulaer, M.J., 2016c. From VGKC to LGI1 and Caspr2 encephalitis: the evolution of a disease entity over time. *Autoimmun. Rev.* 15, 970–974. Available from: <https://doi.org/10.1016/j.autrev.2016.07.018>.
- van Sonderen, A., Petit-Pedrol, M., Dalmau, J., Titulaer, M.J., 2017. The value of LGI1, Caspr2 and voltage-gated potassium channel antibodies in encephalitis. *Nat. Rev. Neurol.* 13, 290–301. Available from: <https://doi.org/10.1038/nrneurol.2017.43>.
- Vanaveski, T., Singh, K., Narvik, J., Eskla, K.-L., Visnapuu, T., Heinla, I., et al., 2017. Promoter-specific expression and genomic structure of IgLON family genes in mouse. *Front. Neurosci.* 11, 38. Available from: <https://doi.org/10.3389/fnins.2017.00038>.
- Vedeler, C.A., Antoine, J.C., Giometto, B., Graus, F., Grisold, W., Hart, I.K., et al., 2006. Management of paraneoplastic neurological syndromes: report of an EFNS Task Force. *Eur. J. Neurol.* 13, 682–690. Available from: <https://doi.org/10.1111/j.1468-1331.2006.01266.x>.
- Vernino, S., Lennon, V.A., 2000. New Purkinje cell antibody (PCA-2): marker of lung cancer-related neurological autoimmunity. *Ann. Neurol.* 47, 297–305.
- Vernino, S., Low, P.A., Fealey, R.D., Stewart, J.D., Farrugia, G., Lennon, V.A., 2000. Autoantibodies to ganglionic acetylcholine receptors in autoimmune autonomic neuropathies. *N. Engl. J. Med.* 343, 847–855.
- Vernino, S., Tuite, P., Adler, C.H., Meschia, J.F., Boeve, B.F., Boasberg, P., et al., 2002. Paraneoplastic chorea associated with CRMP-5 neuronal antibody and lung carcinoma. *Ann. Neurol.* 51, 625–630. Available from: <https://doi.org/10.1002/ana.10178>.

- Vernino, S., O'Neill, B.P., Marks, R.S., O'Fallon, J.R., Kimmel, D.W., 2004. Immunomodulatory treatment trial for paraneoplastic neurological disorders. *Neuro Oncol.* 6, 55–62. Available from: <https://doi.org/10.1215/S1152851703000395>.
- Veyrac, A., Reibel, S., Sacquet, J., Mutin, M., Camdessanche, J.-P., Kolattukudy, P., et al., 2011. CRMP5 regulates generation and survival of newborn neurons in olfactory and hippocampal neurogenic areas of the adult mouse brain. *PLoS One* 6, e23721. Available from: <https://doi.org/10.1371/journal.pone.0023721>.
- Viaccoz, A., Desestret, V., Ducray, F., Picard, G., Cavillon, G., Rogemond, V., et al., 2014. Clinical specificities of adult male patients with NMDA receptor antibodies encephalitis. *Neurology* 82, 556–563. Available from: <https://doi.org/10.1212/WNL.0000000000000126>.
- Villiger, P., Adler, S., Kuchen, S., et al., 2016. Tocilizumab for induction and maintenance of remission in giant cell arteritis: a phase 2, randomized, double-blind, placebo-controlled trial. *Lancet* 387, 1921–1927.
- Vincent, A., Buckley, C., Schott, J.M., Baker, I., Dewar, B.-K., Detert, N., et al., 2004. Potassium channel antibody-associated encephalopathy: a potentially immunotherapy-responsive form of limbic encephalitis. *Brain J. Neurol.* 127, 701–712. Available from: <https://doi.org/10.1093/brain/awh077>.
- von Büdingen, H.-C., Mei, F., Greenfield, A., Jahn, S., Shen, Y.-A.A., Reid, H.H., et al., 2015. The myelin oligodendrocyte glycoprotein directly binds nerve growth factor to modulate central axon circuitry. *J. Cell. Biol.* 210, 891–898. Available from: <https://doi.org/10.1083/jcb.201504106>.
- Walikonis, J.E., Lennon, V.A., 1998. Radioimmunoassay for glutamic acid decarboxylase (GAD65) autoantibodies as a diagnostic aid for stiff-man syndrome and a correlate of susceptibility to type 1 diabetes mellitus. *Mayo Clin. Proc.* 73, 1161–1166. Available from: <https://doi.org/10.4065/73.12.1161>.
- Walsh, M., Faurschou, M., Berden, A., Flossmann, O., Bajema, I., Hoglund, P., et al., 2014. Long-term follow-up of cyclophosphamide compared with azathioprine for initial maintenance therapy in ANCA-associated vasculitis. *Clin. J. Am. Soc. Nephrol.* 9 (9), 1571–1576. Available from: <https://doi.org/10.2215/CJN.00100114>. Epub 2014 Jun 26. PubMed PMID: 24970876; PubMed Central PMCID: PMC4152799.
- Wang, J.J., Jaunmuktane, Z., Mummery, C., Brandner, S., Leary, S., Trip, S.A., 2016. Inflammatory demyelination without astrocyte loss in MOG antibody-positive NMOSD. *Neurology* 87, 229–231. Available from: <https://doi.org/10.1212/WNL.0000000000002844>.
- Waters, P.J., McKeon, A., Leite, M.I., Rajasekharan, S., Lennon, V.A., Villalobos, A., et al., 2012. Serologic diagnosis of NMO: a multicenter comparison of aquaporin-4-IgG assays. *Neurology* 78, 665–671. Available from: <https://doi.org/10.1212/WNL.0b013e318248dec1>. discussion 669.
- Waters, P., Pettingill, P., Lang, B., 2016. Detection methods for neural autoantibodies. *Handb. Clin. Neurol.* 133, 147–163. Available from: <https://doi.org/10.1016/B978-0-444-63432-0.00009-8>.
- Watts, R., Lane, S., Hanslik, T., Hauser, T., Hellmich, B., Koldingsnes, W., et al., 2007. Development and validation of a consensus methodology for the classification of the ANCA-associated vasculitides and polyarteritis nodosa for epidemiological studies. *Ann. Rheum. Dis.* 66 (2), 222–227. Epub 2006 Aug 10. PubMed PMID: 16901958; PubMed Central PMCID: PMC1798520.
- Waxman, E.A., Lynch, D.R., 2005. N-Methyl-D-aspartate receptor subtypes: multiple roles in excitotoxicity and neurological disease. *Neuroscientist* 11, 37–49. Available from: <https://doi.org/10.1177/1073858404269012>.
- Wechsler, M.E., Wong, D.A., Miller, M.K., Lawrence-Miyasaki, L., 2009. Churg-Strauss syndrome in patients treated with omalizumab. *Chest* 136 (2), 507–518. Available from: <https://doi.org/10.1378/chest.08-2990>.
- Wechsler, M.E., Akuthota, P., Jayne, D., Khoury, P., Klion, A., Langford, C.A., et al., 2017. Mepolizumab or placebo for eosinophilic granulomatosis with polyangiitis. *N. Engl. J. Med.* 376 (20), 1921–1932. Available from: <https://doi.org/10.1056/NEJMoa1702079>. PubMed PMID: 28514601; PubMed Central PMCID: PMC5548295.
- Werner, C., Pauli, M., Doose, S., Weishaupt, A., Haselmann, H., Grünewald, B., et al., 2016. Human autoantibodies to amphiphysin induce defective presynaptic vesicle dynamics and composition. *Brain J. Neurol.* 139, 365–379. Available from: <https://doi.org/10.1093/brain/awv324>.
- Weyand, C.M., Goronzy, J.J., 2013. Immune mechanisms in medium and large-vessel vasculitis. *Nat. Rev. Rheumatol.* 9, 731–740. PubMed: 24189842.
- Widess-Walsh, P., Tavee, J.O., Schuele, S., Stevens, G.H., 2003. Response to intravenous immunoglobulin in anti-Yo associated paraneoplastic cerebellar degeneration: case report and review of the literature. *J. Neurooncol.* 63, 187–190.
- Wieczorek, S., Hellmich, B., Arning, L., Moosig, F., Lamprecht, P., Gross, W.L., et al., 2008a. Functionally relevant variations of the interleukin-10 gene associated with antineutrophil cytoplasmic antibody-negative Churg-Strauss syndrome, but not with Wegener's granulomatosis. *Arthritis Rheum.* 58 (6), 1839–1848. Available from: <https://doi.org/10.1002/art.23496>.
- Wieczorek, S., Hellmich, B., Gross, W.L., Epplen, J.T., 2008b. Associations of Churg-Strauss syndrome with the HLA-DRB1 locus, and relationship to the genetics of antineutrophil cytoplasmic antibody-associated vasculitides: comment on the article by Vaglio et al. *Arthritis Rheum.* 58 (1), 329–330. Available from: <https://doi.org/10.1002/art.23209>. 6.
- Wingerchuk, D.M., Lennon, V.A., Lucchinetti, C.F., Pittock, S.J., Weinshenker, B.G., 2007. The spectrum of neuromyelitis optica. *Lancet Neurol.* 6, 805–815. Available from: [https://doi.org/10.1016/S1474-4422\(07\)70216-8](https://doi.org/10.1016/S1474-4422(07)70216-8).
- Wingerchuk, D.M., Banwell, B., Bennett, J.L., Cabre, P., Carroll, W., Chitnis, T., et al., 2015. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. *Neurology* . Available from: <https://doi.org/10.1212/WNL.0000000000001729>.
- Wong, S.H., Saunders, M.D., Larner, A.J., Das, K., Hart, I.K., 2010. An effective immunotherapy regimen for VGKC antibody-positive limbic encephalitis. *J. Neurol. Neurosurg. Psychiatry* 81, 1167–1169. Available from: <https://doi.org/10.1136/jnnp.2009.178293>.
- Yang, Y.Y., Yin, G.L., Darnell, R.B., 1998. The neuronal RNA-binding protein Nova-2 is implicated as the autoantigen targeted in POMA patients with dementia. *Proc. Natl. Acad. Sci. U.S.A.* 95, 13254–13259.
- Yang, J., Ge, H., Poultney, C.J., Hogan, S.L., Hu, Y., Jones, B.E., et al., 2016. Histone modification signature at myeloperoxidase and proteinase 3 in patients with anti-neutrophil cytoplasmic autoantibody-associated vasculitis. *Clin Epigenetics* 8, 85. Available from: <https://doi.org/10.1186/s13148-016-0251-0>. eCollection 2016. PubMed PMID: 27752292; PubMed Central PMCID: PMC5057507.
- Yates, M., Watts, R.A., Bajema, I.M., Cid, M.C., Crestani, B., Hauser, T., et al., 2016. EULAR/ERA-EDTA recommendations for the management of ANCA-associated vasculitis. *Ann. Rheum. Dis.* 75 (9), 1583–1594. Available from: <https://doi.org/10.1136/annrheumdis-2016-209133>. Epub 2016 Jun 23. PubMed PMID: 27338776.

- Yokoi, N., Fukata, M., Fukata, Y., 2012. Synaptic plasticity regulated by protein-protein interactions and posttranslational modifications. *Int. Rev. Cell. Mol. Biol.* 297, 1–43. Available from: <https://doi.org/10.1016/B978-0-12-394308-8.00001-7>.
- Yu, Z., Kryzer, T.J., Griesmann, G.E., Kim, K., Benaroch, E.E., Lennon, V.A., 2001. CRMP-5 neuronal autoantibody: marker of lung cancer and thymoma-related autoimmunity. *Ann. Neurol.* 49, 146–154.
- Yu, C., Gershwin, M.E., Chang, C., 2014. Diagnostic criteria for systemic lupus erythematosus: a critical review. *J. Autoimmun.* 48-49, 10–13. Available from: <https://doi.org/10.1016/j.jaut.2014.01.004>. Epub 2014 Jan 21. Review. PubMed PMID: 24461385.
- Zajicek, J.P., Scolding, N.J., Foster, O., Rovaris, M., Evanson, J., Moseley, I.F., et al., 1999. Central nervous system sarcoidosis—diagnosis and management. *QJM* 92 (2), 103–117. PubMed PMID: 10209662.
- Zandman-Goddard, G., Chapman, J., Shoenfeld, Y., 2007. Autoantibodies involved in neuropsychiatric SLE and antiphospholipid syndrome. *Semin. Arthritis Rheum.* 36, 297–315.
- Zhang, C., Tian, D.-C., Yang, C.-S., Han, B., Wang, J., Yang, L., et al., 2017. Safety and efficacy of bortezomib in patients with highly relapsing neuromyelitis optica spectrum disorder. *JAMA Neurol.* 74, 1010–1012. Available from: <https://doi.org/10.1001/jamaneurol.2017.1336>.
- Zirkzee, E.J., Steup-Beekman, G.M., van der Mast, R.C., et al., 2012. Prospective study of clinical phenotypes in neuropsychiatric systemic lupus erythematosus; multidisciplinary approach to diagnosis and therapy. *J. Rheumatol.* 39, 2118–2126.
- Zuckerman, A.J., 1976. Proceedings: hepatitis B, immune complexes, and the pathogenesis of polyarteritis nodosa. *J. Clin. Pathol.* 29, 84–85.
- Zuhorn, F., Hübenthal, A., Rogalewski, A., Dogan Onugoren, M., Glatzel, M., Bien, C.G., et al., 2014. Creutzfeldt-Jakob disease mimicking autoimmune encephalitis with CASPR2 antibodies. *BMC. Neurol.* 14. Available from: <https://doi.org/10.1186/s12883-014-0227-7>.

# Hepatitis

Diego Vergani<sup>1</sup>, Ian R. Mackay<sup>2</sup> and Giorgina Mieli-Vergani<sup>1</sup>

<sup>1</sup>King's College London Faculty of Life Sciences & Medicine, GI & Nutrition Centre, Institute of Liver Studies and Paediatric Liver, GI and Nutrition Centre, MowatLabs King's College Hospital, London, United Kingdom

<sup>2</sup>Department of Biochemistry and Molecular Biology, Monash University, Clayton, VIC, Australia

## OUTLINE

General Introduction	1117	Animal Models	1131
Historical Aspects	1117	Treatment	1132
Epidemiology	1118	Standard Treatment	1132
Clinical Features, Diagnostic Procedures, and Disease Associations	1119	Alternative Treatments	1134
Pathological Features	1123	Duration of Treatment	1135
Autoimmune Features	1124	Liver Transplantation	1136
Genetics	1127	Future Treatment Approaches	1136
Pathogenic Mechanisms	1128	Perspectives	1137
		References	1137

## GENERAL INTRODUCTION

Autoimmune hepatitis (AIH) is an inflammatory liver disease, affecting mainly females, characterized by elevated transaminase and immunoglobulin G (IgG) levels, interface hepatitis on histology, and positive autoantibodies, whose profile allows its distinction into two types. The etiology of AIH is said to be unknown but, as for complex diseases in general, it results from interplay of multiple predisposing causes, genetic and environmental. The outcome is immune reactivity against host liver antigens. AIH responds to immunosuppressive treatment, which should be instituted as soon as the diagnosis is made, as untreated disease progresses to liver failure.

## HISTORICAL ASPECTS

AIH has gone from obscurity to center stage over a period of 70 years. The disease was unrecognized in the 1940s, though scanty reports described some of its features (Amberg, 1942; Wood et al., 1948). The earliest complete description of the clinical characteristics of AIH was made by the Swedish physician Jan Gösta Waldenström during the first meeting after the war of the German society for Digestive and Metabolic Disorders at Bad Kissingen in September 1950 (Waldenstrom, 1950). Waldenström described six patients, five females, with polyclonal hypergammaglobulinemia, hyperproteinemia, and low serum albumin. He suggested that this

hepatitis "sui generis" could derive from viral persistence after an acute infection and interpreted the hypergammaglobulinemia (mainly due to IgG) to antibody formation against viral antigens. Administration of adrenocorticotropic hormone (ACTH) to investigate the nature of amenorrhea resulted in a striking subjective improvement and a dramatic fall in the erythrocyte sedimentation rate, inducing Waldeström to conclude that these "patients may benefit from ACTH treatment." Waldeström's observations were confirmed soon afterward by Kunkel's group (Kunkel et al., 1951; Bearn et al., 1956), who added to the clinical features fever, arthralgia, and arthritis, suggesting a link with systemic disorders such as rheumatoid arthritis and systemic lupus erythematosus (SLE). Joske and King (1955) described lupus erythematosus (LE) cells in the blood of two patients with hypergammaglobulinemic active chronic hepatitis. In a landmark article, Mackay et al. (1956) reported five additional hypergammaglobulinemic chronic hepatitis patients recruited in the same unit and, in view of the presence of LE cells, proposed the term "lupoid hepatitis" to define this condition.

The first to suggest that this type of chronic hepatitis could be due to autoimmunity was the group of Zimmerman (Zimmerman et al., 1951) who, describing a young man with chronic inflammatory liver disease and hypergammaglobulinemia, suggested that an initial liver injury, possibly due to a virus, could have induced alterations of liver proteins, which might have led to the production of antibodies to self-antigens, perpetuating liver damage. Support to the concept of an autoimmune mechanism in the pathogenesis of liver damage in certain acute and chronic liver diseases came from studies performed in Melbourne by Gajdusek and Mackay in 1958 (Gajdusek, 1958; Mackay and Gajdusek, 1958), who described autoimmune reactions against tissue antigens using a complement fixation test. From these initial findings, the idea of investigating liver-specific targets in chronic inflammatory liver diseases was born, leading to a large wealth of information, starting from the pioneering work in Meyer zum Büschenfelde's laboratory in the 1960s (Meyer zum Büschenfelde and Schrank, 1966; Meyer zum Büschenfelde, 1972; Meyer zum and Miescher, 1972; von Meyer zum Büschenfelde et al., 1974; Hopf et al., 1976) to the present time.

The name "lupoid hepatitis" persisted until it became clear that liver disease is rare in typical SLE and that LE cells reflect the presence of antinuclear antibody (ANA), leading Mackay to suggest the alternative name of "autoimmune hepatitis" (Mackay et al., 1965), though already in the mid-1950s, AIH was a well-recognized nosological entity, so much so that at a New York Academy of Sciences meeting on autoimmunity, Mackay proposed the title "Autoimmune Hepatitis" (Mackay, personal communication).

The name "autoimmune hepatitis," however, became universally accepted only in the 1990s, after several different labels (mostly "chronic active hepatitis") had been given to this condition over the years. In addition to seropositivity for ANAs, reports from the early 1960s showed that anti-smooth-muscle antibodies (SMA) are also markers of what is known today as AIH type 1 (zum Büschenfelde, 2003). Thirty years after the first description of "lupoid hepatitis," another milestone in the history of AIH is the discovery that the presence of anti-liver kidney microsomal type 1 and anti-liver cytosol type 1 antibodies (Homberg et al., 1987; Martini et al., 1988) defines a second type of AIH (AIH type 2), typical of children and adolescents.

Awareness over the years that the clinical and pathological picture of chronic hepatitis may be due to causes other than AIH, such as persistent infection with the hepatotropic viruses B and C, alcohol abuse, and nonalcoholic steatohepatitis, prompted the establishment in the 1990s of an expert panel with the brief of defining criteria for the diagnosis of AIH (International Autoimmune Hepatitis Group, IAIHG) (Johnson and McFarlane, 1993), which were later revised (Alvarez et al., 1999) and then simplified for clinical use (Hennes et al., 2008).

## EPIDEMIOLOGY

AIH occurs worldwide. Initial information on prevalence has been obtained for AIH type 1 before the introduction of the IAIHG diagnostic scoring system (Johnson and McFarlane, 1993) (Alvarez et al., 1999), therefore without standard criteria for patient inclusion. Prevalences reported in early papers range from 1.9 cases/100,000 in Norway (Boberg et al., 1998) and 1/200,000 in the US general population (Manns et al., 1998) to 20/100,000 in females over 14 years of age referred to a tertiary center in Spain (Primo et al., 2004). A study from a UK secondary referral center reported an AIH annual incidence of 3.5/100,000 (Whalley et al., 2007). Two studies using standardized criteria for the diagnosis of AIH published in 2002 and 2010 report a point prevalence of 24.5/100,000 in New Zealand (Ngu et al., 2010) and of 34.5/100,000 in Alaskan natives (Hurlburt et al., 2002). In Asia AIH was widely considered less frequent than in Western countries, with a reported incidence in Japan ranging between 0.08 and 0.15 cases/100,000/year (Nishioka and McFarlane, 1998). A better awareness of its clinical characteristics has led to an increased frequency in the diagnosis of AIH in China, where this condition was considered very

rare (Qiu et al., 2011). Also in Japan the incidence and prevalence of AIH may be higher than previously thought (Yoshizawa et al., 2016). AIH prevalence and incidence are, however, reported to be lower in the Asia-Pacific area than in Europe and America (Yang et al., 2015). Studies on large patient cohorts were conducted in Northern Europe. A population-based investigation in Denmark on 1721 patients reports an incidence rate of 1.68/100,000 population/year, which doubled during the 1994–2012 period of observation. Of note, in the first year after diagnosis, patients with AIH had sixfold higher mortality than the general population, while later their mortality remained twofold higher. In a Swedish cohort of 634 AIH patients, AIH point prevalence was reported as 17.3/100,000 inhabitants in 2009, with a yearly incidence of 1.2/100,000 inhabitants between 1990 and 2009 (Danielsson Borsen et al., 2017). In contrast to the Danish study, however, mortality for the first 4 years post diagnosis was similar to that of the normal population, worsening only afterward (Danielsson Borsen et al., 2017). A Dutch study on 1313 patients shows an AIH prevalence of 18.3/100,000, with an annual incidence of 1.1 in adults, the peak incidence being in middle-aged women (van Gerven et al., 2014).

All these epidemiological figures are likely to be underestimates, since AIH may remain undiagnosed for several years and present eventually with decompensated liver disease attributed to “cryptogenic” cirrhosis.

The prevalence of AIH type 2, which affects mainly children and young adults, is unknown, also because the diagnosis is often overlooked. Intriguingly, AIH type 2 has been reported more frequently in Europe than in the United States (Czaja and Freese, 2002), possibly because of the undertesting for anti-LKM1 antibodies in the latter, due to the unsubstantiated belief that AIH type 2 is rare in Northern America and therefore that testing for anti-LKM1 antibodies is not cost-effective (Duchini et al., 2000). In a study in Canada including 159 children/adolescents with AIH, the annual incidence was 0.23/100000 children, type 1 AIH being diagnosed 5.5 times more frequently than type 2 AIH (Jimenez-Rivera et al., 2015).

At the King's College Hospital tertiary pediatric hepatology referral center, there has been a sevenfold increase in incidence of AIH over the last decade, perhaps a result in part of increased awareness of the disease. AIH represents approximately 10% of some 500 new referrals per year, two-third of the cases being AIH type 1 and one-third AIH type 2.

## CLINICAL FEATURES, DIAGNOSTIC PROCEDURES, AND DISEASE ASSOCIATIONS

The diagnosis of AIH is based on a combination of clinical, biochemical, immunological, and histological features and the exclusion of other known causes of liver disease. Liver biopsy is needed to confirm the diagnosis and to evaluate the severity of liver damage (Krawitt, 2006; Manns et al., 2010; European Association for the Study of the Liver, 2015) as transaminase and IgG levels do not reflect the degree of tissue inflammatory changes nor indicate the presence or absence of cirrhosis. Conditions that may share serological and histological features with AIH, such as viral hepatitis B, C, and E, Wilson disease, nonalcoholic steatohepatitis, and drug-induced liver disease, must be excluded by accurate clinical history and appropriate investigations.

Three quarters of patients with AIH are female. AIH type 1 affects all ages with two peaks, one in childhood/adolescence and the other in adulthood around the age of 40 years. Only 20% of the patients are diagnosed after the age of 60 years (Krawitt, 2006; Manns et al., 2010; European Association for the Study of the Liver, 2015). AIH type 2 affects mainly children/adolescents and young adults, being rare, though not absent, in older individuals. In pediatrics, AIH type 2 represents one-third of all cases and has a clinical course similar to AIH type 1, though anti-LKM1-positive children present at a younger age, more often with an acute onset, including fulminant hepatitis, and have associated IgA deficiency (Gregorio et al., 1997; Oettinger et al., 2005).

A percentage of 40–60 of adult patients with AIH type 1 have a chronic disease course with nonspecific symptoms such as fatigue, nausea, abdominal pain, and arthralgia (Al-Chalabi et al., 2008; Czaja et al., 1983). AIH may be diagnosed after the incidental finding of abnormal liver function tests during routine investigations.

About one-third of adult patients present acutely with jaundice, arthralgia, anorexia, and fatigue, symptoms indistinguishable from those of an acute hepatitis due to other causes (Crapper et al., 1986; Amontree et al., 1989). Acute hepatic episodes alternating with spontaneous clinical and biochemical improvement are not uncommon, a relapsing pattern that often leads to a dangerous delay in diagnosis and treatment. The importance of prompt investigation of abnormal liver function is reinforced by a report showing that half of the patients with AIH presenting with a hepatic picture were already cirrhotic and had a high liver-related mortality.

Some of these fatal outcomes could have been prevented by earlier diagnosis, as 49% of the patients had a history of abnormal liver tests, which had not been investigated (Panayi et al., 2014). Occasionally, the first symptoms of AIH are complications of portal hypertension, for example, gastrointestinal bleeding or hypersplenism,

without previous knowledge of liver disease. The acute presentation is more commonly observed in children and young adults than in older patients. At times AIH, particularly AIH type 2, presents as fulminant hepatic failure (Gregorio et al., 1997; Floreani et al., 2013). Because of the variability of its presenting features, AIH should be suspected and excluded in all patients with symptoms and signs of prolonged, relapsing, or severe liver disease so that the appropriate treatment can be instituted promptly.

At least one-third of patients with AIH already have cirrhosis at the time of diagnosis, irrespective of the mode of presentation (Manns et al., 2010; European Association for the Study of the Liver, 2015), indicating that the disease process is longstanding. Also patients presenting acutely have often advanced fibrosis or cirrhosis on liver biopsy (Dohmen et al., 2017). Patients with asymptomatic or symptomatic AIH have been reported to have similar courses of disease progression and responses to immunosuppressive treatment (Muratori et al., 2016; Dohmen et al., 2017).

About 40% of the patients with AIH have a family history of autoimmune disorders, and associated autoimmune features (e.g., thyroiditis, inflammatory bowel disease, type 1 diabetes, arthritis, hemocytopenias, vitiligo) are present at diagnosis or develop during follow-up in some 20% of both adult (Wong et al., 2017; Wong and Heneghan, 2015) and pediatric patients (Gregorio et al., 1997; Mieli-Vergani and Vergani, 2011).

In the absence of a single diagnostic test for AIH, the IAIHG has devised a diagnostic system for comparative and research purposes, which includes several positive and negative scores, the sum of which gives a value indicative of probable or definite AIH (Johnson and McFarlane, 1993). The scoring system was revised in 1999 (Alvarez et al., 1999) (Table 57.1). A simplified IAIHG scoring system published more recently is easier for clinical application (Hennes et al., 2008) (Table 57.2), though the revised scoring system (Alvarez et al., 1999) has been reported to have a superior performance in the diagnosis of patients with AIH compared with the simplified scoring system (43) (Li et al., 2014). In view of ethnic differences in AIH phenotype, modified scoring systems have been proposed for Japanese patients (Ohira et al., 2015; Onji et al., 2014) and specific guidelines have been established for Chinese patients (Chinese Society of Hepatology et al., 2017).

**TABLE 57.1** International Autoimmune Hepatitis Group Revised Diagnostic Scoring System

Parameter	Feature	Score
Sex	Female	+ 2
ALP: AST (or ALT) ratio	> 3	- 2
	1.5–3	0
	< 1.5	+ 2
Serum globulins or IgG (times above normal)	> 2.0	+ 3
	1.5–2.0	+ 2
	1.0–1.5	+ 1
	< 1.0	0
ANA, SMA, or anti-LKM1 titers	> 1:80	+ 3
	1:80	+ 2
	1:40	+ 1
	< 1:40	0
AMA	Positive	- 4
Viral markers of active infection	Positive	- 3
	Negative	+ 3
Hepatotoxic drug history	Yes	- 4
	No	+ 2
Average alcohol	< 25 g/day	+ 2
	> 60 g/day	- 2

(Continued)

**TABLE 57.1** (Continued)

Parameter	Feature	Score
Histological features	Interface hepatitis	+ 3
	Plasma cells	+ 1
	Rosettes	+ 1
	None of the above	- 5
	Biliary changes <sup>a</sup>	- 3
	Atypical changes <sup>b</sup>	- 3
Immune diseases	Thyroiditis, colitis, other	+ 2
HLA	DR3 or DR4	+ 1
Seropositivity for other autoantibodies	Anti-SLA/LP, actin, ASGPR, pANNA	+ 2
Response to therapy	Remission	+ 2
	Relapse	+ 3

<sup>a</sup>Including granulomatous cholangitis, concentric periductal fibrosis, ductopenia, marginal bile duct proliferation, and cholangiolitis.

<sup>b</sup>Any other prominent feature suggesting a different etiology.

Pretreatment score >15: definite AIH; 10–15: probable AIH. Posttreatment score >17: definite AIH; 12–17: probable AIH. ALP, Alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; IgG, immunoglobulin G; ANA, antinuclear antibody; SMA, anti-smooth-muscle antibody; anti-LKM1, anti-liver kidney microsomal type 1 antibodies; AMA, anti-mitochondrial antibodies; SLA/LP, soluble liver antigen/liver pancreas; ASGPR, asialoglycoprotein receptor; pANNA, peripheral antinuclear neutrophil antibody; HLA, human leukocyte antigen.

Adapted from Alvarez, F, et al., 1999a. International Autoimmune Hepatitis Group report: review of criteria for diagnosis of autoimmune hepatitis. *J. Hepatol.* 31, 929–938.

**TABLE 57.2** Simplified Criteria for the Diagnosis of Autoimmune Hepatitis

Variable	Cutoff	Points
ANA or SMA	≥ 1:40	1
ANA or SMA	≥ 1:80	2 <sup>a</sup>
or anti-LKM1	≥ 1:40	
or SLA	Positive	
IgG	> Upper limit of normal	1
	> 1.10 times upper limit of normal	2
Liver histology	Compatible with AIH	1
	Typical of AIH	2
Absence of viral hepatitis	Yes	2

<sup>a</sup>Additional points for all autoantibodies cannot exceed a maximum of 2.

Score ≥ 6: probable AIH; ≥ 7: definite AIH. ANA, Antinuclear antibody; SMA, anti-smooth-muscle antibody; anti-LKM1, anti-liver kidney microsomal antibody type 1; SLA, soluble liver antigen; IgG, immunoglobulin G; AIH, autoimmune hepatitis.

Adapted from Hennes, E.M., et al., 2008b. Simplified criteria for the diagnosis of autoimmune hepatitis. *Hepatology* 48, 169–176.

None of the published scoring systems is perfect, particularly for the juvenile form of the disease, in which diagnostically relevant autoantibodies often have titers lower than the cutoff value considered positive in adults, and none of them can distinguish between AIH and AIH/sclerosing cholangitis (SC) overlap syndrome. A scoring system for the diagnosis of juvenile autoimmune liver disease has been recently proposed by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition and awaits validation (Mieli-Vergani et al., 2018). In addition, the scoring systems give negative weight to the presence of hepatitis B or C viral markers, but concomitant AIH and chronic viral hepatitis have been reported (Rigopoulou et al., 2013).

As acute hepatitis E may present with histological and biochemical features of AIH, infection should be ruled out before the diagnosis of AIH is made by testing for specific IgM antibodies and hepatitis E virus RNA (Calisti et al., 2017; Patel et al., 2016). Intriguingly, evidence of exposure to hepatitis E virus has been reported more frequently among AIH patients than in the normal population (van Gerven et al., 2016; Pischke et al., 2014). Even more intriguingly, living in close contact with a pet, especially a cat, has been reported to be a risk factor for autoimmune liver disease, perhaps indicating exposure to an as yet unidentified agent (Tenca et al., 2016).

A careful enquiry about exposure to drugs is also mandatory before the diagnosis of AIH can be made, as drug-induced liver injury (DILI) occasionally mimics AIH (Andrade et al., 2009; Czaja, 2011). While a drug can unmask or induce classical AIH in a predisposed individual, who will usually have already fibrotic changes on liver biopsy and who will need chronic treatment with immunosuppressants, a drug can also cause a clinical picture indistinguishable from AIH but without fibrosis and without long-term steroid dependence. The IAIHG criteria for the diagnosis of definite or probable AIH are fulfilled both in classical AIH and in drug-induced autoimmune-like hepatitis (DILI-AIH), only the successful withdrawal of immunosuppression distinguishing the two conditions. The drugs most commonly associated with DILI-AIH are nitrofurantoin and minocycline, followed by methyldopa and hydralazine (de Boer et al., 2017). Other drugs associated with DILI-AIH are anti-tumor necrosis factor alpha (TNF- $\alpha$ ) monoclonal antibodies (Rodrigues et al., 2015; Bjornsson et al., 2017) and herbal remedies, especially in the Far East (Czaja, 2011). Interestingly, antibiotic treatment has been reported to be an independent risk factor for the development of AIH (Ngu et al., 2013).

AIH has been associated with a variety of liver and extrahepatic autoimmune diseases. Overlap between AIH and other autoimmune disorders affecting the liver is the focus of intense discussion (Vierling, 2015; Czaja, 2013). Some patients with AIH may also have clinical, histological, and serological features of primary biliary cholangitis (PBC) or SC. These overlap syndromes are probably clinical descriptions rather than distinct pathological entities, the dominant component of the disease determining its designation and therapy. In the presence of AIH features, immunosuppression is of benefit also in PBC and SC (Vierling, 2015; Czaja, 2013; Ozaslan et al., 2014; Yoshioka et al., 2014).

In children and young adults, a form of SC, known as autoimmune SC (ASC), and characterized by ANA and SMA positivity, high levels of IgG, and interface hepatitis, is increasingly recognized (Gregorio et al., 2001; Rodrigues et al., 2016; Deneau et al., 2013) (Gregorio et al., 2001). Rarely, anti-LKM1-positive patients with ASC have been reported (Gregorio et al., 2001; Pratico et al., 2013; Gargouri et al., 2013). Alkaline phosphatase and gamma glutamyl transpeptidase levels, which are usually elevated in cholestatic disease, are often normal or only mildly increased in the early disease stages of ASC and a cholangiography is needed to make the diagnosis. In the absence of bile duct imaging, these patients are diagnosed and treated as AIH. The presence of SC may be discovered during follow-up, after the appearance of an overt cholestatic biochemical profile. In childhood, ASC affects equally males and females (Gregorio et al., 2001). If treatment is started early, the parenchymal liver damage in ASC responds well to the same immunosuppressive treatment used for AIH, with good medium-to-long-term survival. However, the bile duct disease progresses in about 50% of the patients despite treatment (Gregorio et al., 2001). Also in an adult series, patients with overlapping features between SC and AIH, treated with immunosuppression, appear to have a better prognosis than those with classical primary SC, suggesting that immunosuppression might have a positive effect on disease progression (Zenouzi and Lohse, 2014).

A subtype of AIH characterized by infiltration of IgG4-expressing plasma cells and particularly responsive to corticosteroids has been described within the IgG4-related disease (Chung et al., 2010; Yada et al., 2013). AIH has been reported in association with celiac disease (CD), some 5% of the patients with AIH also having CD and over 10% of the patients with CD having AIH (Najafi et al., 2014). In a large Dutch study, CD was found in 3.5% of the AIH patients compared with 0.35% in the general Dutch population, reinforcing the notion that CD should be considered in all AIH patients (van Gerven et al., 2014) also in view of the possible benefit that gluten withdrawal may have on the course of AIH in CD patients (Iqbal et al., 2017). Occasional patients fulfill diagnostic criteria of both AIH and SLE, the liver disease responding satisfactorily to standard AIH immunosuppressive treatment (Beisel et al., 2014). AIH has also been rarely described in patients with systemic sclerosis (Assandri et al., 2016) or multiple sclerosis (Sayin et al., 2016).

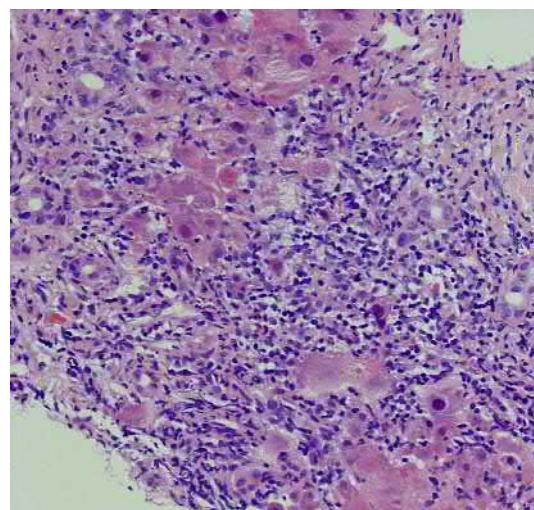
An emerging problem is the association between nonalcoholic fatty liver disease and AIH, resulting from a worldwide increase in metabolic syndrome (Weiler-Normann and Lohse, 2016). Patients with concomitant AIH and nonalcoholic steatohepatitis have been reported to present with cirrhosis and to develop adverse clinical events more frequently than patients with classical AIH, with consequent decreased survival (De Luca-Johnson et al., 2016).

As AIH affects primarily women of childbearing age, most of whom require immunosuppression lifelong, a number of studies have assessed the outcome of pregnancy and postpartum in this condition. Overall, pregnancy and childbirth appear to be safe for both child and mother, even in women with compensated liver cirrhosis, without the need to withdraw azathioprine (Heneghan et al., 2001; Candia et al., 2005; Terrabuio et al., 2009; Braga et al., 2016; Danielsson Borssen et al., 2016). One large series from Sweden reports an increased risk of gestational diabetes, preterm birth, and low-birth-weight infants compared with the general population (Stokkeland et al., 2016). Clinical improvement as well as disease exacerbation have been observed in relation to pregnancy, the latter particularly in the postpartum period (Braga et al., 2016), indicating that high-quality antenatal and postnatal care is essential for women with AIH and their infants.

## PATHOLOGICAL FEATURES

The typical histological feature of AIH is interface hepatitis, which is however not exclusive to this condition (Czaja and Carpenter, 1997). Interface hepatitis is characterized by a dense inflammatory infiltrate composed of lymphocytes and plasma cells, which crosses the limiting plate and invades the surrounding parenchyma (Fig. 57.1). Hepatocytes surrounded by inflammatory cells become swollen and undergo pyknotic changes, representing apoptosis (Searle et al., 1987; Bai and Odin, 2003). We emphasize this feature because not only the liver was the site wherein apoptosis was first recognized nearly a century ago but also, in some other autoimmune diseases, disordered apoptosis can yield potently immunogenic particles—apoptopes (Lleo et al., 2009). Though plasma cells are characteristically abundant at the interface and within the lobule, their presence in low number does not exclude the diagnosis of AIH. When AIH presents acutely, and during episodes of relapse, a common histological finding is panlobular hepatitis with centrilobular and bridging necrosis and, if the disease takes a fulminant course, massive necrosis and multilobular collapse (Tiniakos et al., 2015; Nguyen Canh et al., 2017).

Though sampling variation may occur in needle biopsy specimens, particularly in cirrhotic livers, the severity of the histological appearance is usually of prognostic value (Puustinen et al., 2017). Inflammatory changes surrounding the bile ducts are not uncommon in AIH (Verdonk et al., 2016), but when conspicuous they suggest an overlap with SC or PBC. Other nonspecific features that may point to the diagnosis of AIH are emperipoleisis and hepatocyte rosetting (Miao et al., 2015), which in a recent study have been suggested to be stronger indicators of AIH than interface hepatitis or plasma-cell-rich infiltrate (de Boer et al., 2015). A paper in a pediatric AIH cohort suggests that the finding of hyaline droplets in Kupffer cells is a useful diagnostic marker to distinguish AIH from other forms of chronic hepatitis (Tucker et al., 2015).



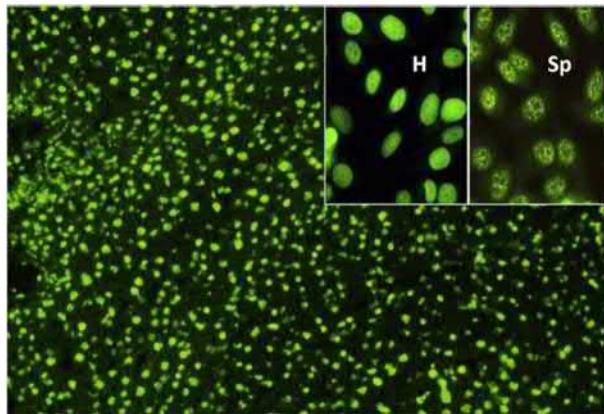
**FIGURE 57.1** Interface hepatitis. Interface hepatitis is the typical histological feature of AIH and is characterized by a dense portal and periportal lymphocyte and plasma cell infiltrate that disrupts the parenchymal limiting plate. Hematoxylin and eosin staining. Original magnification  $\times 40$ . AIH, Autoimmune hepatitis. Source: Courtesy of Dr Alberto Quaglia.

As persisting histological activity despite biochemical remission is frequent in patients with treated AIH and is associated with lower rates of fibrosis regression and reduced long-term survival, repeat biopsy is important in assessing ongoing disease activity, particularly when considering drug withdrawal (Putra et al., 2016; Dhaliwal et al., 2015).

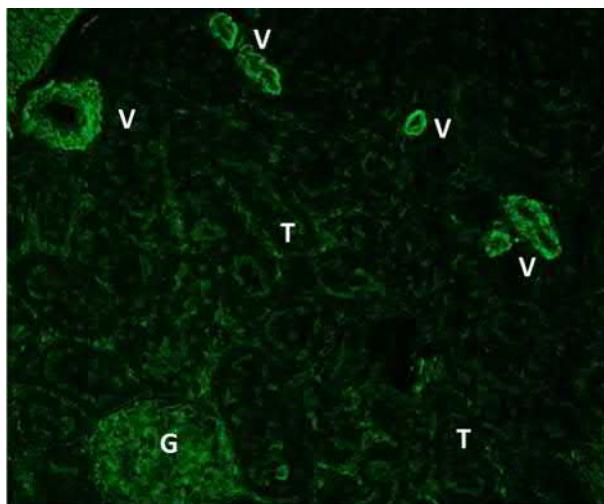
## AUTOIMMUNE FEATURES

AIH has several characteristics of an organ-specific autoimmune disease: it affects mainly females, it is accompanied by serological features of autoimmunity (organ and nonorgan-specific autoantibodies; high levels of IgG), it is associated to human leukocyte antigen (HLA) allotypes predisposing to autoimmunity, it has a strong connectivity with other autoimmune disorders, and it responds satisfactorily to immunosuppressive treatment.

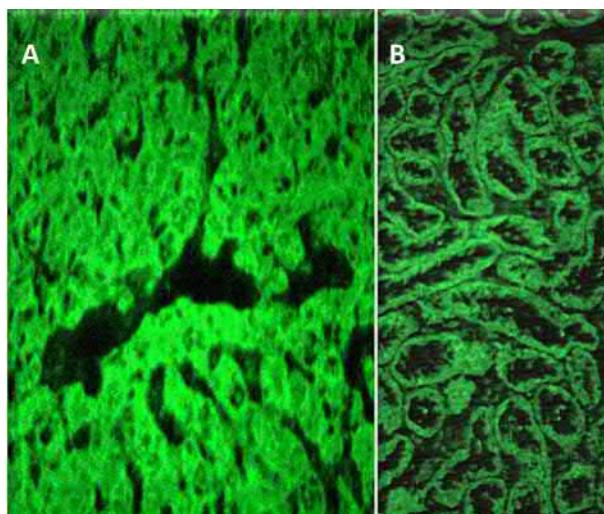
Key to the diagnosis of AIH is positivity for circulating autoantibodies (Johnson and McFarlane, 1993; Alvarez et al., 1999; Hennes et al., 2008; Vergani et al., 2004; Bogdanos et al., 2009; Liberal et al., 2013). Their detection by indirect immunofluorescence (Figs. 57.2–57.5) on a rodent substrate not only assists in the diagnosis but also allows differentiation into two forms of AIH. ANA and SMA characterize AIH type 1, while anti-LKM1 and anti-LC1 define AIH type 2. The two autoantibody profiles may occasionally coexist (Vergani et al., 2004). As



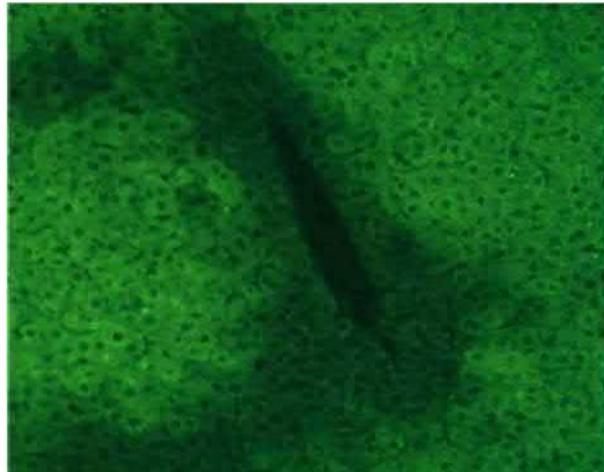
**FIGURE 57.2** Antinuclear antibodies. Immunofluorescence pattern of antinuclear autoantibodies on rodent liver (main image) and HEp2 cells (inset), which, because of their large nuclei, allow pattern recognition. The homogeneous pattern (inset left) is the most common in autoimmune hepatitis; the speckled pattern (inset right) is much rarer. HEp2 cells should not be used for sample screening because of a high frequency of false-positive results.



**FIGURE 57.3** Anti-smooth-muscle antibodies. Immunofluorescence pattern of SMA on rodent kidney. SMA stains the smooth muscle of arterial vessels (V), glomeruli (G), and tubules (T). SMA, Smooth-muscle autoantibodies. Source: Courtesy of Dr Luigi Muratori.



**FIGURE 57.4** Anti–liver kidney microsomal type 1 antibodies. Immunofluorescence pattern of anti-LKM1 autoantibodies on liver (left) and kidney (right) rodent sections; anti-LKM1 stains the cytoplasm of hepatocytes and proximal renal tubules. *LKM1*, Liver kidney microsomal type 1.



**FIGURE 57.5** Anti–liver cytosol type 1 antibodies. Immunofluorescence pattern of anti-LC1 antibodies on a rodent liver section: they stain the cytoplasm of hepatocytes with a weakening of the staining around the central vein. *Anti-LC1*, Anti–liver cytosol type 1.

interpretation of the immunofluorescence patterns can be difficult, guidelines have been provided by the IAIHG regarding methodology and interpretation of liver autoimmune serology (Vergani et al., 2004). A major advantage of testing for autoantibodies by indirect immunofluorescence on a freshly prepared rodent substrate that includes kidney, liver, and stomach is that it allows the concurrent detection of several autoreactivities relevant to AIH. These include ANA, SMA, anti-LKM1, and anti-LC1, as well as anti–mitochondrial antibody (AMA), the serological hallmark of PBC, the presence of which weighs against the diagnosis of AIH (Johnson and McFarlane, 1993; Alvarez et al., 1999; Hennes et al., 2008; Vergani et al., 2004). Autoantibodies are considered positive when present at a dilution of 1:40 or more in adults, while in children, who are rarely positive for autoantibodies in health, positivity at a dilution  $\geq 1:20$  for ANA and SMA or  $\geq 1:10$  for anti-LKM1 is clinically significant (Mieli-Vergani et al., 2018). Both in adults and children, autoantibodies may be present at a low titer or even be negative at disease onset, particularly with acute presentation (Yilmaz et al., 2016; Fujiwara et al., 2015), to become detectable during follow-up (Gregorio et al., 2001). If AIH is suspected, it is advisable to repeat autoantibody testing and to ask the laboratory to report any level of positivity.

ANA is detectable on all rodent tissues and in AIH usually has a homogeneous pattern (Fig. 57.2). For a clearer and easier definition of the pattern, HEp2 cells that have prominent nuclei are used (Fig. 57.2), though

these cells, derived from a laryngeal carcinoma, should be used with caution for screening purposes, because of a high proportion of low titer positivity within the normal population (Tan et al., 1997). There are no ANA molecular targets specific for AIH. A varied profile of ANA reactivities reminiscent of that found in SLE (e.g., to nuclear chromatin, histones, centromere, double-stranded DNA and single-stranded DNA, and ribonucleoproteins (Peakman et al., 1989; Bogdanos et al., 2009; Czaja et al., 1994; Strassburg et al., 1996; Bogdanos et al., 2008)) has been reported in AIH, but at least a third of AIH patients positive for ANA do not react with known nuclear targets (Bogdanos et al., 2009; Bogdanos et al., 2008). Immunofluorescence remains therefore the gold standard for ANA testing, as surmised by the American College of Rheumatology ANA Task Force (Meroni and Schur, 2010), but is not universally practiced. There is an impression, albeit poorly documented, that higher level of autoantibody and/or multiple autoantibodies are a pointer to a diagnosis of AIH, as pertains in Type 1 diabetes.

The immunofluorescent staining of SMA is detected in the arterial walls of rodent kidney, liver, and stomach. In the kidney, SMA can have three patterns: V (vessels), G (glomeruli), and T (tubules) (Bottazzo et al., 1976) (Fig. 57.3). The V pattern is present also in nonautoimmune inflammatory liver disease, in autoimmune diseases not affecting the liver, and in viral infections, but the VG and VGT patterns are indicative of AIH. Interestingly, the accidental finding of SMA with GT pattern in association with any increase of transaminase levels appears to predict the development of AIH (Healey et al., 2016). The VGT pattern corresponds to the "F actin" or microfilament (MF) pattern observed using cultured fibroblasts as substrate. Neither the VGT nor the anti-MF patterns are, however, entirely specific for the diagnosis of AIH type 1. The molecular target of the MF reactivity observed in AIH type 1 remains to be identified. Though "antiactin" reactivity is strongly associated with AIH type 1, some 20% of the SMA-positive AIH type 1 patients do not have the F-actin/VGT pattern. The absence, therefore, of antiactin SMA does not exclude the diagnosis of AIH (Muratori et al., 2002).

The anti-LKM1 pattern is characterized by bright staining of the hepatocyte cytoplasm and of the P3 portion of the renal tubules (Fig. 57.4). Anti-LKM1 is frequently confused with AMA, as both autoantibodies stain liver and kidney, though AMA, in contrast to anti-LKM1, also stains gastric parietal cells. The identification of the molecular targets of anti-LKM1, cytochrome P4502D6 (CYP2D6), and of AMA, enzymes of the 2-oxo-acid dehydrogenase complexes, has allowed the establishment of immunoassays using recombinant or purified antigens (Vergani et al., 2004; Bogdanos et al., 2009), which can be used to resolve doubtful cases. In the context of AIH, there can be positivity for AMA in a small subset of patients (3%–5%), who respond to immunosuppressive treatment such as classical AIH, some of whom having overlapping features with PBC (O'Brien et al., 2008; Montano-Loza et al., 2008; Muratori et al., 2017). Additional LKM reactivities have been described. Anti-LKM2 antibodies, which target cytochrome P4502C9, are of historical interest only because of their association with ticrynafen-induced hepatitis, a uricosuric diuretic withdrawn from clinical use in 1980 because of its severe hepatotoxicity. Anti-LKM3 antibodies are specific for members of the uridine glucuronosyltransferase family 1 and give an immunofluorescence pattern similar to anti-LKM1. Although anti-LKM3 are most commonly detected in patients with hepatitis delta, they have also been reported in approximately 10% of the patients with AIH type 2 (Strassburg et al., 1996).

Anti-LC1 (Fig. 57.5), which is an additional marker for AIH type 2, can be present on its own but frequently occurs in association with anti-LKM1. In addition to indirect immunofluorescence, it can be detected by line-blot (Villalta et al., 2016). Anti-LC1 targets formimino-transferase cyclodeaminase (FTCD) (Lapierre et al., 1999). Anti-FTCD antibody can be detected by commercial enzyme-linked immunosorbent assay (ELISA) (Vergani et al., 2004).

Other autoantibodies less commonly tested, but of diagnostic importance, include anti-soluble liver antigen (anti-SLA) and anti-perinuclear neutrophil cytoplasm antibodies (pANCA).

Anti-SLA is highly specific for the diagnosis of AIH, usually type 1 (Baeres et al., 2002). Its presence identifies patients with more severe disease and worse outcome (Ma et al., 2002; Czaja et al., 2002; Chen et al., 2015). Anti-SLA was thought to identify a third type of AIH in which tests for conventional autoantibodies were negative (Manns et al., 1987). However, early reports predated the publication of the IAIHG recommendations and used a cutoff point for conventional autoantibody levels higher than that currently used for the diagnosis of AIH. Several patients considered to have AIH type 3 were positive for conventional autoantibodies. At variance with standard diagnostic autoantibodies, anti-SLA is not detectable by immunofluorescence. The molecular target of anti-SLA is Sep (O-phosphoserine) tRNA:Sec (selenocysteine) tRNA synthase (SEPSECS) (Wies et al., 2000; Costa et al., 2000; Paloura et al., 2009). Cloning of this reactant has led to the availability of molecularly based diagnostic assays for anti-SLA, but their full evaluation is still under way.

In AIH type 1, akin to primary SC and inflammatory bowel disease, pANCA are frequently detected, but they are atypical, since they react with peripheral nuclear membrane components (perinuclear antinuclear neutrophil

antibodies, pANNA) (Bogdanos et al., 2009). In curious contrast to AIH type 1, pANNA are virtually absent in AIH type 2 (Vergani et al., 2004).

Anti–asialoglycoprotein receptor (ASGPR) antibodies were identified in 1984 during an attempt to detect putative autoantigenic targets located on the hepatocyte membrane. ASGPR is the main constituent of the crude liver cell extract known as liver-specific protein (LSP) and is the only liver-specific autoantigen discovered so far (Bogdanos et al., 2009). Some 50%–90% of the patients with AIH type 1 or 2 are anti-ASGPR/anti-LSP seropositive (Gregorio et al., 2001); anti-ASGPR is found in combination with ANA, SMA, and anti-LKM1 and its level correlates with disease activity. However, the detection of anti-ASGPR requires either purified or recombinant antigen, and the development of reliable molecular assays has been difficult, therefore their applicability to clinical practice is limited. Moreover, since these autoantibodies have also been detected in patients with viral hepatitis, drug-induced hepatitis, and PBC, they are not disease specific (Bogdanos et al., 2009; Rigopoulou et al., 2012).

A number of antibodies of potential diagnostic relevance for AIH have been described more recently. Anti–programmed cell death-1 (PD-1) antibody has been reported to be a serological marker for AIH type 1 in Japan and its presence may distinguish between AIH and DILI (Miyake et al., 2014; Matsumoto et al., 2014), both findings awaiting confirmation. Anti–ribosomal P protein antibody has been reported to define a group of AIH patients with severe prognosis (Calich et al., 2013), though this has been questioned (Muratori et al., 2014) and requires further analysis. Antibodies to self HLA class II alleles, similar to those detected after transplantation, were detected in nontransplanted patients with AIH more frequently than in patients with PBC and healthy subjects (Yamagiwa et al., 2014).

To detect yet unidentified autoantibodies, protein and peptide libraries are scanned with antibodies generated through the isolation of B cells and plasma cells from the peripheral blood or the liver of patients with AIH. Using this approach, novel specificities have been identified in AIH and PBC, whose relevance remains to be evaluated in large series (Mazzara et al., 2015; Norman et al., 2015; Tanaka et al., 2017).

## GENETICS

AIH is a “complex trait” disease, that is, a condition not inherited in a Mendelian autosomal dominant, autosomal recessive, or sex-linked fashion (Donaldson, 2002, 2004). The mode of inheritance of a complex trait disorder involves one or more genes, operating alone or in concert, to increase or reduce the risk of the trait, and interacting with environmental factors.

Several genes have been reported to confer susceptibility to AIH-1 and influence clinical manifestations, response to treatment and prognosis. Most are located within the HLA region [the human major histocompatibility complex (MHC)], the gene products of which are involved in the presentation of antigenic peptides to T cells and the initiation of adaptive immune responses. The strongest associations lie within the HLA-DRB1 locus. Alleles encoding the HLA-DR3 (*DRB1\*0301*) and DR4 (*DRB1\*0401*) molecules confer susceptibility to AIH type 1 in European and North American populations (Donaldson, 2002, 2004; van Gerven et al., 2015), while DR4 to Oriental populations (Furumoto et al., 2015). These associations are sufficiently strong to score positively for the diagnosis of AIH according to the revised diagnostic IAIHG system (Alvarez et al., 1999). The prominent predisposing role of genes encoded in the HLA region has been confirmed in the largest genome-wide association study performed to date in AIH (de Boer et al., 2014). Links have been reported between possession of these predisposing HLA alleles and clinical manifestations, response to treatment and prognosis. Thus among white Northern Europeans, *DRB1\*0301* is more common in patients who deteriorate despite corticosteroid treatment (Donaldson, 2002, 2004). The residues within the cleft of the HLA class II molecules specifically linked to the pathogenesis of AIH reported from various countries differ, suggesting that the antigenic peptides recognized by T cell-mediated immune responses in AIH may derive from different exogenous triggers and that they are embraced by geographically/ethnically distinct HLA molecules (Donaldson, 2002, 2004). These HLA associations may be the molecular footprints of the prevailing triggers that precipitate AIH in different environments. In this context, it is of interest that in South America possession of the HLA *DRB1\*1301* allele, which predisposes to pediatric AIH type 1 in that population, is also associated with persistent infection with the endemic hepatitis A virus (Fainboim et al., 2001). The combination of HLA *DRB1\*1301* and a specific functional form of the killer cell immunoglobulin-like receptor (KIR2DS4-FL) imparts a strong predisposition to pediatric type 1 AIH in South America (Podhorzer et al., 2016).

Susceptibility to and severity of AIH type 2 has been linked to alleles encoding the DRB1\*0301 and DRB1\*0701 molecules in the United Kingdom and Brazil. Allelic variation within HLA-DRB1 has been linked to differences in the autoantibody seropositivity profiles of AIH type 2 patients (Djilali-Saiah et al., 2006).

There are also reports of susceptibility to AIH linked to polymorphisms in genes located outside the MHC; the cytotoxic T lymphocyte antigen-4 (CTLA-4) (Agarwal et al., 2000), the TNF- $\alpha$  gene promoter (Cookson et al., 1999), and Fas (Agarwal et al., 2007) are notable examples.

A form of AIH serologically resembling AIH type 2 affects some 20% of the patients with autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED) (see Chapter 43). APECED is a monogenic autosomal recessive disorder caused by homozygous mutations in the *AIRE1* gene and characterized by a variety of organ-specific autoimmune diseases, the most common of which are hypoparathyroidism and primary adrenocortical failure, accompanied by chronic mucocutaneous candidiasis (Simmonds and Gough, 2004; Liston et al., 2005). Interestingly, there are neutralizing autoantibodies to Type 1 interferons, perhaps accounting for the associated immune deficiencies (see Chapter 76). APECED has a high level of variability in symptoms, especially between populations. Carriers of a single *AIRE1* mutation (heterozygotes) do not develop APECED. However, although the inheritance pattern of APECED indicates a strictly recessive disorder, there are anecdotal data of mutations in a single copy of *AIRE1* being associated with human autoimmunity of a less severe form than classically defined APECED (Simmonds and Gough, 2004; Liston et al., 2005).

The role of the *AIRE1* heterozygote state in the development of type 2 AIH remains to be established, though heterozygous *AIRE1* mutations have been reported in three children with severe AIH type 2 and extrahepatic autoimmune manifestations (Lankisch et al., 2005).

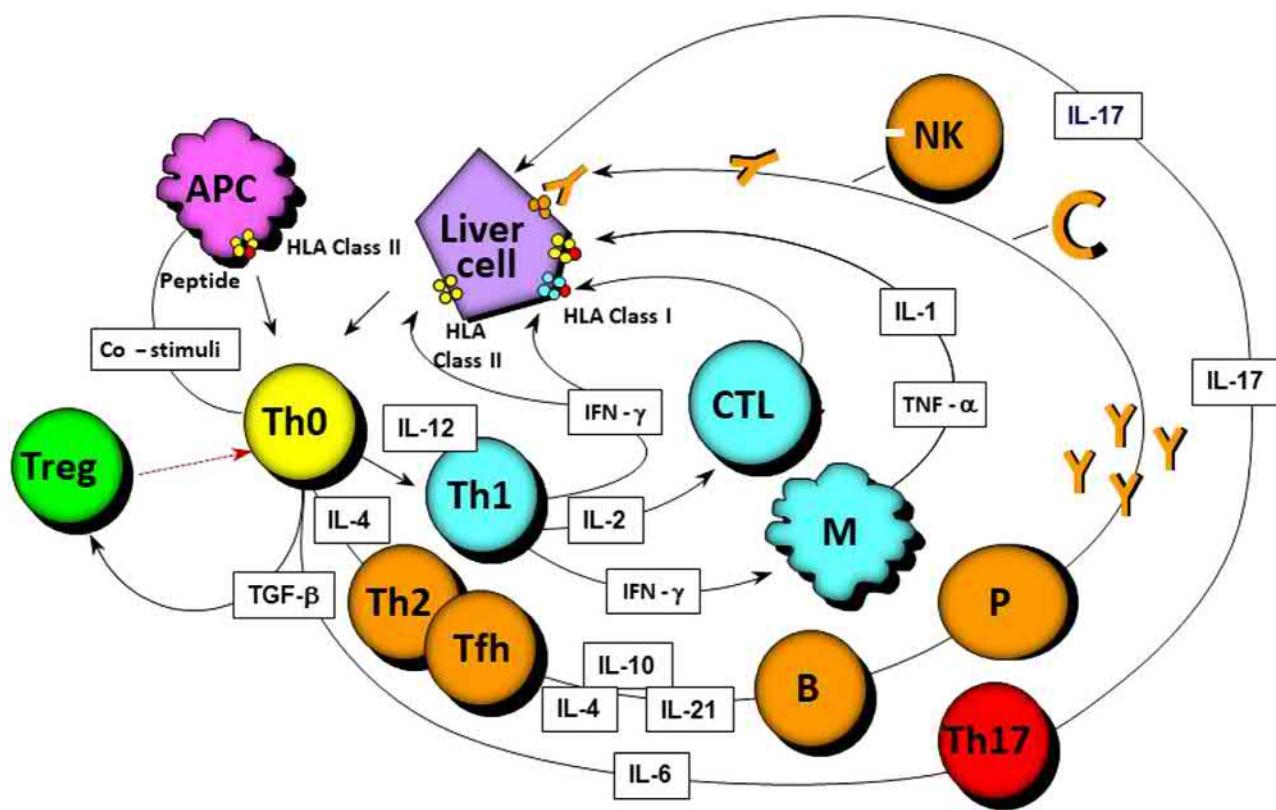
## PATHOGENIC MECHANISMS

In patients with increased genetic susceptibility to AIH, immune responses to liver autoantigens could be triggered by molecular mimicry. Cross-reactivity is an inherent property of the cells of the adaptive immune system (Vergani et al., 2002), derived from the need of T- and B-lymphocytes to recognize a potentially infinite number of non-self-antigens without any prior information as to their structure. This implies that these cells, rather than responding to single antigen specificities, are able to cross-reactively respond to a number of antigens, thus expanding the antigenic specificities of the immune system to a level that reflects the antigenic diversity of the external environment.

This inherent potential for cross-reactivity, whilst allowing efficient responses to a vast array of pathogens, also provides the immune system with the potential to cross-react with self, leading to autoimmunity. This concept has been termed “molecular mimicry,” where immune responses to external pathogens become directed toward structurally similar self components.

The strongest support to this model is in the context of AIH type 2, where T lymphocytes that target a key epitope of CYP2D6 also react with self-mimicking exogenous sequences present on the hepatitis C virus and members of the herpes virus family (cytomegalovirus, Epstein–Barr virus, and herpes simplex virus) (Vergani et al., 2002). It is conceivable that, in genetically predisposed individuals, T cells targeting the self-epitope may be primed and expanded through exposure to the self-mimicking exogenous sequences with consequent initiation and perpetuation of liver autoimmunity. This potential scenario is supported by a case-report describing a 10-year-old girl who acquired hepatitis C virus (HCV) infection following a liver transplant for end-stage liver disease caused by alpha1-antitrypsin deficiency. Two weeks after HCV infection, IgM anti-LKM1 autoantibodies appeared, followed by IgG anti-LKM1 antibodies, suggestive of HCV as the initiator of a primary anti-LKM1/anti-CYP2D6 autoimmune response (Mackie et al., 1994); 10 years later, the patient developed florid AIH type 2, which responded satisfactorily to immunosuppressive treatment, but by this time there was no trace of the previous HCV infection (Bogdanos et al., 2004).

Putative mechanisms of autoimmune liver damage are depicted in Fig. 57.6. The immune response in AIH is believed to be initiated by the presentation of self-antigenic peptides (as yet unknown) to the T-cell receptor of uncommitted naive CD4+ T-helper (Th0) lymphocytes. Self-antigenic peptides are processed and presented by professional antigen-presenting cells (APCs), including dendritic cells (DCs), macrophages, and B lymphocytes. The liver is home to several specialized APC populations, including liver sinusoidal endothelial cells, Kupffer cells, and DCs, so that antigen presentation to both CD4 and CD8 effector T cells can occur in situ, averting the need for trafficking to the regional lymphoid tissues (Crispe, 2011; Ebrahimkhani et al., 2011).



**FIGURE 57.6 Autoimmune attack to the hepatocyte.** An autoantigen is presented to uncommitted T helper (Th0) lymphocytes within the HLA class II molecule of an APC either in the regional lymph nodes or within the liver itself. Activated Th0 cells differentiate into Th1 or Th2 cells in the presence of IL-12 or IL-4, respectively, and according to the nature of the antigen. This triggers a series of immune reactions determined by the cytokines they produce. Th1 cells secrete IL-2 and IFN- $\gamma$ , which are cytokines that stimulate CTL, enhance expression of class I HLA molecules, induce expression of class II HLA molecules on the liver cells, and activate macrophages. Macrophages (M) release IL-1 and TNF. Th2 cells secrete mainly IL-4, IL-10, and IL-13 and stimulate autoantibody production by B lymphocytes. Tregs are derived from Th0 in the presence of TGF- $\beta$ . In the presence of defective Tregs, hepatocyte destruction ensues from the engagement of damaging effector mechanisms, including CTL, cytokines released by Th1 and by activated macrophages, complement activation, or adhesion of NK cells to autoantibody-coated hepatocytes through their Fc receptors. Th17 cells produce the inflammatory cytokine IL-17 and derive from Th0 cells in the presence of TGF- $\beta$  and IL-6. They are the focus of ongoing investigations. Tfh cells, specialized CD4+ T cells that induce activation and differentiation of B cells into immunoglobulin secreting cells through expression of CD40L and production of IL-21, may also have a role in the pathogenesis of AIH, Thf secreted IL-21 being elevated in AIH, its levels correlating with disease activity. APC, Antigen-presenting cell; IL, interleukin; IFN, interferon; CTL, cytotoxic T lymphocytes; HLA, human leukocyte antigen; TNF, tumor necrosis factor; Tregs, regulatory T cells; TGF, transforming growth factor; NK, natural killer; Tfh, T follicular helper.

During antigen presentation, in the presence of appropriate costimulatory signals, CD4+ Th0 cells become activated and undergo differentiation into distinct T-helper cell subsets, depending on the cytokine milieu to which they are exposed. In the presence of IL-12 or IL-4, Th0 lymphocytes differentiate into Th1 or Th2 cells, respectively, while predominance of IL-1beta and IL-6 favors differentiation into Th17 cells. Differentiation into Th1 cells leads to the production of IL-2 and IFN-gamma and the concomitant activation of CD8 T lymphocytes that produce IFN-gamma and TNF- $\alpha$  and exert cytotoxicity upon recognition of an antigen/MHC class I complex (Ichiki et al., 2005). Exposure of hepatocytes to IFN-gamma results in the upregulation of MHC class I and in the aberrant expression of MHC class II molecules, which leads to further T-cell activation and to the perpetuation of liver damage (Lobo-Yeo et al., 1990; Senaldi et al., 1991). IFN-gamma also favors monocyte differentiation, promotes macrophage and immature DC activation (Delneste et al., 2003), and contributes to enhanced natural killer cell killing (Schroder et al., 2004).

Differentiation of Th0 into Th2 cells leads to the secretion of IL-10, IL-4, and IL-13, cytokines essential for B-cell maturation into plasma cells and consequently to the production of autoantibodies, which can participate in mechanisms of damage such as antibody-mediated cellular cytotoxicity and complement activation (Longhi et al., 2010; Liberal et al., 2016). Of note, the titers of several autoantibodies, including anti-liver-specific-protein

and its components, correlate with indices of disease severity (Jensen et al., 1978; McFarlane et al., 1986). Moreover, in AIH type 2, the target of the disease-defining antibody, anti-LKM1, CYP2D6, is present not only in the endoplasmic reticulum but also is expressed on the membrane of hepatocytes and therefore readily accessible to the immune attack (Muratori et al., 2000).

Th17 cells contribute to autoimmunity by producing the proinflammatory cytokines IL-17, IL-22, and TNF- $\alpha$ , and inducing hepatocytes to secrete IL-6 (Zhao et al., 2011), which further enhances Th17 activation. The role of Th17 cells, which has been documented in PBC (Harada et al., 2009), is under investigation also in AIH, where an elevated level of Th17 cells has been reported in both blood and liver (Zhao et al., 2011; Thomas-Dupont et al., 2016). A possible role for T follicular helper (Tfh) cells in the pathogenesis of autoimmune diseases is increasingly been reported (Ma and Deenick, 2014). Tfh cells are specialized CD4+ T cells that induce the activation and differentiation of B cells into immunoglobulin secreting cells. This helper function is provided in the form of expression of molecules such as CD40L and cytokines such as IL-21. Tfh overactivation may result in autoimmunity. Tfh cells are located in secondary lymphoid tissues, but their counterparts can be found also in the circulation. Tfh secreted IL-21 cytokine has been reported to be elevated in AIH, its levels correlating with disease activity (Abe et al., 2016; Ma et al., 2014; Thomas-Dupont et al., 2016; Kimura et al., 2017).

Gamma/Delta T cells, which account for a low proportion of circulating lymphocytes, are relatively abundant in the liver (Wen et al., 1992) and might contribute to the pathogenesis of AIH, as they are elevated during active disease phases. Moreover, in AIH, gamma/delta T cells produce high levels of granzyme B and IFN-gamma, the expression of which correlates with biochemical indices of liver damage, suggesting a direct involvement of this cell population in hepatic injury (Ferri et al., 2010). The involvement of macrophages in AIH liver damage is supported by the finding that soluble CD163, a product of macrophage activation, is markedly elevated during the acute phase of the disease and in poor responders, normalizing with successful treatment (Gronbaek et al., 2016).

A deficiency of immunoregulation, enabling the autoimmune response to develop, has been repeatedly reported in AIH. Thus earlier, patients with AIH were reported to have low levels of circulating CD8+ T cells and impaired T-cell suppressor function which segregated with disease-predisposing HLA alleles B8/DR3 and was correctable by therapeutic doses of corticosteroids (Nouri-Aria et al., 1982). Furthermore, there was a defect in a subpopulation of T cells controlling the immune response to liver-specific membrane antigens (Vento et al., 1984). More recent evidence based on the now “genuine” regulatory T cells (Tregs) confirms an impairment of immunoregulatory function in AIH. Amongst T-cell subsets with potential immunosuppressive function, CD4 cells constitutively expressing the IL-2 receptor alpha chain (CD25) (Tregs) have emerged as the dominant subset. These cells, representing 5%–10% of all peripheral CD4 cells in health, control innate and adaptive immune responses by limiting the proliferation and effector function of autoreactive T cells (Sakaguchi, 2000). Their mechanism of action involves mainly a direct contact with the target cells, and to a lesser extent the release of immunoregulatory cytokines, such as IL-10 and tissue growth factor beta 1. In addition to CD25, which is also present on T cells undergoing activation, Tregs express a further markers such as the glucocorticoid-induced tumor necrosis factor receptor, CD62L, CTLA4, and the forkhead/winged helix transcription factor FOXP3, whose expression has been associated with the acquisition of regulatory properties, while they express little or no CD127—the IL-7 receptor. Treg impairment is linked with various human autoimmune diseases including AIH (Sakaguchi et al., 2010). In patients with AIH type 1 and type 2, Tregs are defective in number compared to normal controls and this reduction is disease stage related, being more evident at diagnosis and during relapses than during drug-induced remission (Longhi et al., 2005; Longhi et al., 2010; Ferri et al., 2010; Liberal et al., 2015). The percentage of Tregs inversely correlated with markers of disease severity, such as anti-SLA and anti-LKM1 autoantibody titers, suggesting that reductions in Tregs favor manifestations of autoimmune liver disease. Moreover, Tregs from AIH patients at diagnosis are impaired in their ability to control the proliferation of CD4 and CD8 effector cells compared to Tregs isolated from AIH patients at remission or from healthy subjects (Longhi et al., 2005; Ferri et al., 2010). Though most published literature indicates a numerical and functional Treg defect in AIH, these findings were disputed in a paper which, however, used a different methodological approach (Peiseler et al., 2012; Longhi et al., 2012).

The immunoregulatory defects described above have been complemented by findings that effector CD4 T cells in AIH are less susceptible to restraints exerted by Tregs. This defect is linked to reduced expression of the receptor molecule Tim-3 (inhibitory receptor T-cell-immunoglobulin-and-mucin-domain-containing-molecule-3), which upon ligation of galectin-9 expressed by Tregs induces effector cell death (Liberal et al., 2012). In AIH, CD39 $^{+}$  Tregs are decreased in number, fail to adequately hydrolyze proinflammatory nucleotides, and do not suppress efficiently IL-17 production by effector CD4 T cells. CD39 $^{+}$  Tregs show plasticity and are unstable upon proinflammatory challenge, suggesting that defective immunoregulation in AIH might result not only from reduced

Treg number and function but also from increased conversion of Tregs into effector cells (Grant et al., 2014). In AIH it has also been reported that low Treg responsiveness to IL-2 results in defective IL-10 production, contributing to Treg functional impairment (Liberal et al., 2015).

An increase of FOXP3-positive cells in the liver of patients with AIH, particularly during active disease, has been reported and interpreted as an enrichment of Tregs in the target tissue (Behairy et al., 2016; Diestelhorst et al., 2017; Taubert et al., 2014). However, these studies rely only on the expression of FOXP3 in tissue lymphocytes, this molecule being associated with activation of CD4 cells—including effector cells (Allan et al., 2007), without functional demonstration of regulatory properties.

If loss of immunoregulation were central to the pathogenesis of autoimmune liver disease, treatment should concentrate on restoring the ability of Tregs to expand, with consequent increase in their number and function. However, we must be mindful that further confirmatory data are needed. Moreover, it is essential to devise strategies preventing Tregs to become effectors of damage within an inflammatory milieu (Liberal et al., 2017; Holder et al., 2014).

Intestinal microbiome may also be involved in the pathogenesis of AIH. Alterations in the composition of the intestinal microbiota (dysbiosis) have been found in experimental AIH (Yuksel et al., 2015). Compared to healthy volunteers, the structural proteins binding intestinal epithelial cells are reduced in patients with AIH; plasma lipopolysaccharide levels are increased; and the number of intestinal anaerobes is decreased (Lin et al., 2015). Also in humans, AIH appears therefore to be associated with dysbiosis, increased gut permeability, and translocation of intestinal microbial products into the systemic circulation.

## ANIMAL MODELS

Research on the pathogenesis of AIH has been hampered by the lack of animal models reproducing faithfully the human condition. Findings in animal models of AIH have been reviewed by Hardtke-Wolenski et al. (2012) and by Christen and Hintermann (2016). Most animal models of AIH, though informative regarding single steps leading to liver inflammation and damage, do not mimic the chronic relapsing course of the human disease. In fact, they demonstrate the difficulty in breaking tolerance toward liver antigens, and the involvement of regulatory mechanisms in maintaining it.

A widely studied model of experimental hepatitis is that induced by concanavalin A (Tiegs et al., 1992). Though this model does not reflect accurately the pathological entity of AIH in humans, it has provided evidence that liver damage mainly occurs within a Th1 scenario, with the involvement of activated CD4 T cells and release of the proinflammatory cytokines interferon gamma and tumor necrosis factor alpha against a specific genetic background. Interleukin-4, a cytokine with mainly regulatory activity, is also required for the establishment of concanavalin A-induced hepatitis. This finding and those of Takeda et al. (2000), who have shown that natural killer T (NKT) cells, which secrete both IL-4 and interferon gamma, are critical to the development of concanavalin A-induced hepatitis in C57/B6 mice, suggest that both adaptive and innate immunity are involved. A distinct member of the interleukin 17 family, IL-17C, produced by hepatocytes, has been shown to have a key role in the liver damage in concanavalin A-induced hepatitis through the binding with its specific receptor IL-17RE present on liver resident T cells (Huang et al., 2017).

The ideal model for AIH should have a well-defined initiating event followed by chronic inflammation leading to fibrosis.

Researchers have been focusing on animal models of AIH type 2, since in this condition the autoantigens are well defined. The model produced by the group of Alvarez (Lapierre et al., 2004) is based on immunizing every 2 weeks for three times C57BL/6 female mice with a plasmid containing the antigenic region of human CYP2D6, the target of anti-LKM1, and FTCD, the target of anti-LC1, together with the murine end terminal region of CTLA-4. The latter was added to facilitate antigen uptake by APCs. In a parallel set of experiments, a plasmid containing the DNA encoding IL-12, a Th1 skewing proinflammatory cytokine, was also used. When autoantigens and IL-12 were used to break tolerance, antigen-specific autoantibodies were produced, a relatively modest elevation of transaminase levels at 4 and 7 months was observed, and a portal and periportal inflammatory infiltrate composed of CD4 and CD8 T cells and, to a lesser extent, B cells was demonstrated 8–10 months after the third immunization. When the same immunization protocol was used in different mouse strains, either a mild hepatitis or no inflammatory changes were observed indicating the importance of a specific genetic background. These authors have also shown, in the same animal model, that adoptive transfer of ex vivo expanded Tregs expressing the chemokine receptor CXCR3 targets efficiently the inflamed liver, restores peripheral tolerance to

FTCD, and induces disease remission (Lapierre et al., 2013). More recently, the same group has shown that liver damage can be controlled by the use of low-dose anti-CD3 (Marceau et al., 2015) or anti-CD20 (Beland et al., 2015) monoclonal antibodies, demonstrating the pathogenic involvement of both T and B cells in liver damage. Using FTDC as immunogen in an Adenovirus shuttle vector, Hardtke-Wolenski et al. (2013) describe a model of AIH evolving to portal and lobular fibrosis, in which the genetic predisposition afforded by the NOD background was the key to the development of the disease. That the development of the disease is genetically controlled was confirmed in a later paper by the same group, in which, however, tolerance breakdown was shown not to be dependent on the genetic background (Hardtke-Wolenski et al., 2017).

Recently, Yuksel et al. (2015) developed a model based on the HLA-DR3 transgenic mouse on the nonobese diabetic background by immunization with a DNA plasmid coding for human CYP2D6/FTCD fusion protein. Immunization leads to increased transaminase levels, development of autoantibodies, interface hepatitis, and fibrosis. Milder liver injury was observed by the same group using HLA-DR4 transgenic mice in the same animal model (Yuksel et al., 2016).

Another model of AIH type 2 uses CYP2D6 transgenic mice and aims at breaking tolerance with an Adenovirus-CYP2D6 vector (Holdener et al., 2008). While focal hepatocyte necrosis was seen in both mice treated with the Adenovirus-CYP2D6 vector and control mice treated with Adenovirus alone, only the former developed chronic histological changes, including fibrosis, reminiscent of AIH. The hepatic lesion was associated to a specific immune response to an immunodominant region of CYP2D6 and a cytotoxic T-cell response to Adenovirus-CYP2D6 vector-infected target cells. Recent data from the same group suggest that preexisting nonalcoholic fatty liver disease potentiates AIH in this mouse model (Muller et al., 2016).

A complex and somewhat artificial strategy, involving neonatal thymectomy to prevent Treg development and egress from the thymus, was used in PD-1-deficient mice to produce a fulminant hepatitis characterized by spontaneous and severe CD4 and CD8 T-cell liver infiltration, lobular necrosis, and elevated titers of ANA. Also in this model, adoptive transfer of Tregs was able to reverse progression to fatal hepatitis, providing support for a protective role of this cell population in AIH (Kido et al., 2008).

In view of the presence of AIH type 2 in some 20% of the patients with APECED, a monogenic autosomal recessive disorder caused by homozygous mutations in the *AIRE1* gene, Hardtke-Wolenski et al. (2015) generated a BALB/c mouse model, in which *AIRE* is truncated at exon 2. Twenty four percent of these mice developed liver damage similar to AIH, disease manifestations being dependent on specific *Aire* mutations and the genetic background of the mice.

Though these experimental approaches provide useful information on some of the possible pathogenic mechanisms leading to AIH type 2, a model closely representative of type 1 AIH in humans is still missing. In this context, of particular interest is the recently developed murine model reminiscent of AIH type 1 created by Bonito et al. (2013). The model is characterized by deletion of medullary thymic epithelial cells. These cells regulate T-cell tolerance by ectopically expressing self-antigens and eliminating autoreactive T cells in the thymus. Unexpectedly, these animals do not suffer from multiorgan autoimmune disease but develop a condition closely resembling human AIH type 1, with interface hepatitis, production of ANA, anti-SLA, and antibodies directed to liver-specific antigens.

## TREATMENT

The aim of treatment in AIH is to attain an early complete remission to prevent disease progression and to maintain it long term using the lowest possible dose of medications. In all types of presentation apart from a fulminant onset with encephalopathy, AIH responds well to immunosuppressive treatment whatever the degree of liver impairment with a reported remission rate of ~80% (Krawitt, 2006; Liberal et al., 2016).

### Standard Treatment

A combination of predniso(lo)ne and azathioprine has remained from the late 1960s, the basis of treatment for AIH (Mackay, 1968; Cook et al., 1971; Soloway et al., 1972; Murray-Lyon et al., 1973). The earlier unfounded suggestion of waiting for 6 months before starting immunosuppression has long been abandoned, since it is now clear that treatment should be started as soon as possible to avoid disease progression (Manns et al., 2010; European Association for the Study of the Liver, 2015).

The American Association for the Study of Liver Diseases (AASLD) practice guidelines, published in 2010, recommend for adult patients either an initial dose of 30 mg prednisolone combined with 1–2 mg/kg of azathioprine daily, or monotherapy with prednisolone at a starting dose of 40–60 mg daily (Manns et al., 2010). To avoid azathioprine hepatotoxicity, particularly in cirrhotic and jaundiced patients (Lohse and Mieli-Vergani, 2011; European Association for the Study of the Liver, 2015), the more recent guidelines by the European Association for the Study of the Liver (EASL) recommend to add azathioprine after 2 weeks of steroid monotherapy [prednisolone 1 mg/kg/day in adults], when partial disease control has been achieved. For children, a dose of 1–2 mg/kg prednisolone up to a daily dose of 60 mg is recommended in combination with azathioprine (1–2 mg/kg) or its parent drug 6-mercaptopurine (6MP) (1.5 mg/kg) (Mieli-Vergani et al., 2018). As children present frequently acutely with jaundice, they should be treated with high-dose prednisolone first and azathioprine should be added later when partial disease control is achieved and jaundice has subsided, to avoid potential hepatotoxicity. The most common side effect after prolonged steroid administration is Cushingoid changes, and recurrent cutaneous warts are also frequent. Less common but severe side effects include osteoporosis, vertebral collapse, diabetes, cataract, hypertension, and psychosis. However, only ~13% of the treated patients develop side effects that necessitate dose reduction or premature drug withdrawal, this being usually for cosmetic changes or obesity, osteopenia with vertebral collapse, and brittle diabetes (Czaja and Freese, 2002). Adverse effects of azathioprine (cholestatic hepatitis, veno-occlusive disease, pancreatitis, nausea and vomiting, rash, bone marrow suppression) affect less than 10% of the patients and usually subside upon drug withdrawal (Manns et al., 2010). Determination of erythrocyte concentrations of thiopurine methyltransferase (TPMT) activity may be advisable before institution of azathioprine therapy but does not invariably predict response to the drug or toxicity (Langley et al., 2002; Heneghan et al., 2006). TPMT genotyping predicts azathioprine hematological toxicity in those rare individuals with variant homozygosity with near-zero erythrocyte concentrations of the enzyme, while heterozygotes do not experience more toxicity than wild-type patients (Newman et al., 2011). Though immunosuppression is tolerated relatively well in AIH, some side effects, particularly cosmetic, are often a great concern for the patients and may lead to poor quality of life (Schramm et al., 2014) and nonadherence, with dangerous consequences for their disease control.

Criteria for complete remission are the disappearance of clinical symptoms, normalization of transaminase and IgG levels in adults and children, and, in addition, abrogation or reduction to a very low titer of the autoantibodies in children (Mieli-Vergani et al., 2018; Liberal et al., 2016). Histological resolution of inflammation lags well behind biochemical improvement (Manns et al., 2010; Sogo et al., 2006; Ustundag et al., 2008).

Response to immunosuppressive treatment in AIH is usually so swift that a lack of response should prompt investigation of other causes of liver disease.

Relapse is characterized by an increased level in serum transaminase enzymes and is common, occurring in 40%–80% of the patients usually during attempts to withdraw treatment or because of nonadherence, and requires a temporary increase in the steroid dose (European Association for the Study of the Liver, 2015; Krawitt, 2006; Manns et al., 2010; Gregorio et al., 1997; Czaja and Freese, 2002). Nonadherence is particularly common in young adults and adolescents (Kerkar et al., 2006) and should not be mistaken for inefficacy of the drugs. Most patients, including those with cirrhosis (Manns et al., 2010; European Association for the Study of the Liver, 2015), attain complete remission on the above treatment schedules.

In the presence of severe steroid side effects, remission can be maintained with azathioprine alone at a dose of up to 2 mg/kg daily (Johnson et al., 1995; Czaja, 2008). Treatment with both steroids and azathioprine can be safely continued during pregnancy (Heneghan et al., 2001; Candia et al., 2005; Terrabuio et al., 2009). Though azathioprine is classified as a category D drug by the Food and Drug Administration, it has no reported teratogenic effects in humans. If concerns remain about its use, women can be temporarily switched to steroid monotherapy.

Early identification of patients who do not respond satisfactorily to conventional treatment would allow tailored monitoring and early intervention with salvage therapy. Montano-Loza et al. (2007) reported that onset at an early age, acute presentation, hyperbilirubinemia, and presence of HLA DRB1\*03 characterize patients with poor response to corticosteroid treatment and that the model for end-stage liver disease (MELD) score (based on serum creatinine and total bilirubin levels, and international normalized ratio value) at presentation may help identifying these patients. In a large cohort presenting with icteric AIH, Yeoman et al. (2011) show that approximately 20% of the patients fail corticosteroid therapy and that failure is best predicted by changes in the MELD score and the United Kingdom end-stage liver disease (UKELD) score (which incorporates serum sodium and reduces the weighting of the creatinine level) at day 7 of treatment. Factors reported to be associated with poor short- and long-term outcome are young age at diagnosis (because of a higher risk of relapse), histological cirrhosis at first diagnosis, and presence of anti-SLA antibodies, while DRB1\*04:01 positivity is associated with a

favorable clinical outcome (Kirstein et al., 2015; Muratori et al., 2016). Interestingly, a recent paper reports alterations of the glucocorticoid receptor signaling pathway in patients who fail to respond to standard therapy (Eriksen et al., 2017).

For adult patients who are asymptomatic, paucisymptomatic or are identified incidentally, the benefit of therapy should be weighed against the adverse effects of corticosteroids, particularly in postmenopausal women or the elderly patients, with the histological severity of inflammation and liver damage being the best guide. In contrast, children, despite being rarely symptomatic, should start treatment promptly, even if the diagnosis is made incidentally, as they have a more aggressive and rapidly progressive disease (Floreani et al., 2013; Czaja et al., 2005).

Long-term immunosuppressive treatment could be associated with the development of malignancies since extrahepatic cancers, including non-Hodgkin lymphoma and skin cancer, are reported to be more frequent in patients with AIH than in age- and sex-matched normal populations (Wang et al., 1989; Werner et al., 2009; Danielsson Borssen et al., 2015; Arinaga-Hino et al., 2017). The risk of developing primary hepatocellular carcinoma (HCC) in AIH is associated with the presence of cirrhosis, akin to other chronic liver diseases (Yeoman et al., 2008; Wong et al., 2011; Werner et al., 2009; Danielsson Borssen et al., 2015; Tansel et al., 2017) though HCC has been anecdotally described also in the absence of cirrhosis (Maeda et al., 2010). Both the AASLD and EASL Autoimmune Hepatitis Guidelines recommend active surveillance for HCC (Manns et al., 2010; European Association for the Study of the Liver, 2015).

The management of AIH with severe acute or fulminant presentation is particularly delicate. In adults, corticosteroid therapy has been reported to be of little benefit and to favor septic complications (Ichai et al., 2007; De Martin et al., 2015), but recent papers show that steroid treatment in severe acute AIH does not jeopardize survival and can improve outcome, suggesting that these patients should be considered for a short trial of corticosteroids, while undergoing assessment for sepsis, and for liver transplant in case of clinical deterioration or development of encephalopathy (Yeoman et al., 2014; Zhu et al., 2014; Sonthalia et al., 2017; Mendizabal et al., 2015). In a pediatric cohort, prednisone treatment was successful in four of nine children with AIH presenting as fulminant hepatic failure, avoiding liver transplant (Di Giorgio A, et al., 2015). Similarly, good results with steroid therapy are reported in a paper from India, where 10 out of 13 children with severe acute presentation of AIH, including encephalopathy in 6, were rescued by prednisone treatment (Ramachandran et al., 2014). Appropriate diagnostic criteria for AIH presenting as severe acute hepatitis or fulminant hepatic failure are not available, though the IAIHG-modified score (Alvarez et al., 1999) performs better than the IAIHG-simplified score (Hennes et al., 2008) as autoantibodies and high IgG levels may not be detectable at presentation, to become detectable or rise during follow-up (Yilmaz et al., 2016; Fujiwara et al., 2015).

## Alternative Treatments

Cyclosporine and tacrolimus, calcineurin inhibitors, have been used as steroid-sparing agents in an attempt to induce remission whilst avoiding high-dose steroid adverse effects (Alvarez et al., 1999; Cuarterolo et al., 2006; Debray et al., 1999; Van Thiel et al., 1995; Zizzo et al., 2017; Marlaka et al., 2012), but whether the use of these toxic and expensive drugs confers any advantage over standard treatment remains to be evaluated in controlled studies.

A large European trial investigating the effect of a combination of budesonide and azathioprine in AIH reported remission in a higher proportion of noncirrhotic patients with less adverse effects than medium-dose standard prednisone and azathioprine (Manns et al., 2010). This study compared the effect of budesonide at a dose of 3 mg three times daily, decreased upon response, with prednisone 40 mg once daily reduced per protocol, irrespective of response. Six months after starting treatment, remission was observed in 60% of the budesonide group, but in only 39% of the prednisone group. When pediatric patients were considered separately, no difference in response was observed between the budesonide and prednisone groups at 6 months (16% vs 15%) and 12 months (50% vs 42%) (Woynarowski et al., 2013). Of note, the remission rate in the prednisone arm of this study is considerably less than that reported both in adults and children (~80%) when a higher starting dose of prednisone is used and tapered according to biochemical response (Gregorio et al., 1997; Kanzler et al., 2001). A controlled trial in treatment-naïve pediatric AIH patients, using drug schedules appropriate for the juvenile disease, is needed to establish whether budesonide has a role in the treatment of this condition (Mieli-Vergani and Vergani, 2013). A limitation of budesonide is that it is ineffective in patients with cirrhosis, who represent at least one-third of all AIH cases (Gregorio et al., 1997; Roberts et al., 1996; Manns et al., 2010; European Association for the Study of the Liver, 2015). Despite its limitations, budesonide could be a valid alternative in patients at selected

risk of adverse effects from prednisolone. Budesonide may also be effective in maintaining remission in patients who have achieved it with prednisolone but has been reported to be ineffective in those resistant to prednisolone, perhaps not surprisingly as prednisone and budesonide target the same receptor (Peiseler et al., 2017).

In patients with azathioprine intolerance, but not in poor responders, 6MP (Hubener et al., 2016) or 6-thioguanine (Legue et al., 2017) appear to be effective and relatively well-tolerated alternatives. As allopurinol, a xanthine oxidase inhibitor, shifts the metabolism of azathioprine from 6-methylmercaptopurine, hepatotoxic, toward 6-thioguanine, not hepatotoxic, the combination of allopurinol and a reduced dose of azathioprine might provide an alternative to more expensive and toxic second line-therapy to induce remission (de Boer et al., 2013; Deswal and Srivastava, 2017). A recent paper reports a beneficial effect of 6-thioguanine treatment (Legue et al., 2017).

Difficult-to-treat cases are reported to respond to mycophenolate mofetil at a dose of 20 mg/kg twice daily in association with prednisone (Richardson et al., 2000; Aw et al., 2009; Devlin et al., 2004; Jothimani et al., 2014; Zizzo et al., 2017; Efe et al., 2017), although this experience is not unanimous (Hennes et al., 2008). In adults, mycophenolate mofetil has been reported to be effective in patients intolerant of but not in those unresponsive to azathioprine (Hennes et al., 2008), though a recent paper from Australia does show a similar remission rate irrespective of intolerance or unresponsiveness to azathioprine (Gazzola et al., 2016).

Mycophenolate mofetil in association with prednisolone has been also used in AIH as first-line treatment and reported to be effective in inducing remission and possibly in achieving a higher rate of successful suspension of treatment than the conventional prednisolone/azathioprine combination (Zachou et al., 2016). However, it is unclear whether mycophenolate mofetil offers a real advantage over azathioprine, as a head-to-head comparison between the two drugs was not performed. Mycophenolate mofetil, moreover, has the major disadvantages of being much more expensive than azathioprine, and, most importantly, of being teratogenic, a highly relevant adverse effect, as AIH affects mainly young females (Janmohamed and Hirschfield, 2016).

For patients who do not respond to, or are intolerant to mycophenolate mofetil (headache, diarrhea, nausea, dizziness, hair loss, and neutropenia), a calcineurin inhibitor in combination with prednisone is suggested (Manns et al., 2010; European Association for the Study of the Liver, 2015; Zizzo et al., 2017; Efe et al., 2017). In patients particularly difficult to treat, the use of biologics has been reported. Rituximab, an anti-CD20 agent targeting B lymphocytes, has shown promising results in a small number of difficult-to-treat AIH (Barth and Clawson, 2010; Burak et al., 2013; D'Agostino et al., 2013), but its safety profile needs to be evaluated carefully, as the drug may have severe long-term side effects, including B-cell depletion (Pavanello et al., 2017). Infliximab, an anti-TNF- $\alpha$  agent (Weiler-Normann et al., 2009, 2013; Bovensiepen et al., 2017), has given promising results in treatment-resistant patients, but it is important to note that anti-TNF- $\alpha$  agents can induce hepatotoxicity resembling AIH (Rodrigues et al., 2015; Bjornsson et al., 2017), as well as other immune-mediated conditions, such as SLE. Moreover, an important risk of these biologic treatments is the occurrence of severe infections (Weiler-Normann et al., 2013).

A single recent paper reports a satisfactory response to methotrexate in 6 of 11 patients refractory or intolerant to first-line therapy. However, two patients developed methotrexate DILI, and the benefit of such treatment remains to be tested (Haridy et al., 2017).

Anecdotally, the m-TOR inhibitors sirolimus and everolimus have been used in difficult-to-treat AIH patients mostly with disappointing results (Chatrath et al., 2014; Kurowski et al., 2014; Ytting and Larsen, 2015).

All these still experimental second-line drugs should be reserved for very difficult to manage AIH cases in specialized centers.

## Duration of Treatment

No optimal length of treatment has been established. Most authors recommend at least 3 years of continuous therapy (Manns et al., 2010; European Association for the Study of the Liver, 2015) before considering withdrawal. Treatment withdrawal should be attempted only in patients with stably normal liver function tests and IgG levels over a period of 1–2 years (Mieli-Vergani et al., 2018; Deneau et al., 2014; Hartl et al., 2015) and when a follow-up liver biopsy shows resolution of inflammation (Manns et al., 2010; European Association for the Study of the Liver, 2015; Mieli-Vergani et al., 2018). Cessation should proceed with caution during or immediately before puberty, when relapses are more frequent, possibly because of poor adherence to treatment during adolescence (Kerkar et al., 2006).

During withdrawal, close monitoring is needed as relapse may be severe and even fatal. While a single relapse may not impact on disease progression, frequent relapses are associated with poor outcome (Bouma and van

Nieuwkerk, 2015). Successful stopping of immunosuppression should be followed up long term, as relapses can occur even several years later (Manns et al., 2010). Some 20% of the patients with juvenile type 1 AIH can stop treatment successfully and permanently, but treatment cessation is achieved only rarely for patients with type 2 AIH (Gregorio et al., 1997; Deneau et al., 2014). Levels of autoantibody and IgG (Luth et al., 2008; Gregorio et al., 2002) are important markers of disease activity and are useful to monitor response to treatment.

Most AIH patients who respond to immunosuppressive treatment have an excellent outcome and lead a normal life on low-dose medication.

## Liver Transplantation

Liver transplantation is the treatment of choice for patients who present with fulminant hepatic failure (grades II–IV encephalopathy) unresponsive to steroids, or who progress to end-stage liver disease despite immunosuppression (~10%–20% of patients) (Liberal et al., 2013). Recurrence of AIH, characterized by high transaminase levels, positive autoantibodies, interface hepatitis, and/or steroid dependence, occurs in ~20% of transplanted patients (Milkiewicz et al., 1999; Duclos-Vallee et al., 2003; Liberal et al., 2016) and can happen even years after transplantation. Prednisolone treatment long term and at a dose higher than that generally used after liver transplantation for other conditions is recommended to avoid recurrence. Besides recurrent AIH, there is a form of graft dysfunction characterized by chronic liver damage with interface hepatitis, high transaminase and IgG levels, and positive tests for autoantibodies that occurs in ~6%–10% of the patients transplanted for nonautoimmune disorders. It has been named “de novo AIH” (Kerkar et al., 1998; Liberal et al., 2013). This condition affects mainly young patients (Kerkar and Yanni, 2016). Early diagnosis is needed, as de novo AIH does not respond satisfactorily to antirejection regimens, but only to the addition of standard treatment for AIH (Liberal et al., 2013). However, despite the good response to AIH treatment, which avoids graft loss, the condition can herald progression to chronic liver disease (Ekong et al., 2017). In resistant cases, rapamycin has been used with success (Kerkar et al., 2005).

## Future Treatment Approaches

As loss of immunoregulation appears central to the pathogenesis of AIH, so treatment should concentrate on restoring the ability of Tregs to expand in number, with consequent increase in their function. The immunotherapeutic use of autologous CD4<sup>+</sup>CD25<sup>+</sup> Tregs derived from patients, however, is hindered by their limited ability to proliferate and by their propensity to apoptosis (Akbar et al., 2003), which usually precludes cell numbers adequate for treatment. The partial Treg restoration in patients during remission (Longhi et al., 2004; Longhi et al., 2005) indicates that Tregs in AIH, although impaired, can expand and regain function. Following in vitro exposure to polyclonal T-cell stimulation, Tregs can be expanded not only in healthy individuals but also in patients with AIH (Longhi et al., 2008). While maintaining the phenotypic features of original CD4<sup>+</sup>CD25<sup>+</sup>CD127<sup>-</sup> T cells, expanded Tregs express increased FOXP3 and display augmented suppressor function (Liberal et al., 2015).

Studies in mice show that Tregs with autoantigen specificity suppress immune effectors more efficiently than do their nonantigen-specific counterparts (Tarbell et al., 2004; Albert et al., 2005). In this regard, type 2 AIH is an excellent model for specific reconstitution of self-tolerance since not only known is the key autoantigen, CYP2D6, but also the specific autoepitope regions (CYP2D6<sub>217–260</sub> and CYP2D6<sub>305–348</sub>), targeted by B cells, CD4 cells, and CD8 T cells (Ma et al., 2006; Longhi et al., 2007; Kerkar et al., 2003). Antigen-specific Tregs have been obtained from patients with type 2 AIH and exert a more powerful immunosuppressive effect than polyclonally expanded Tregs (Longhi et al., 2011). The most efficient suppression of autoreactive T cells was achieved after Tregs were exposed to CYP2D6 peptides cocultured with semimature DCs loaded with the same peptide. Thus adoptive transfer of autologous CYP2D6-specific Tregs could become an effective and even curative therapy for AIH type 2, as suggested also in an animal model of this condition (Lapierre et al., 2013). In type 1 AIH, immune intervention based on SLA could be a possibility, since at least 50% of the patients have autoantibodies to this autoantigen. An even higher proportion of patients with type 1 AIH have cellular immune reactivity to HLA DRB1\*0301 (Meda et al., 2007) or HLA DRB1\*0401 (Zhao et al., 2011) restricted SLA epitopes. SLA-specific Tregs could, therefore, be considered for antigen-specific immune intervention in type 1 AIH as well.

Another possible therapeutic approach is exploitation of Treg response to low-dose IL2, since Saadoun et al. (2011) report encouraging results in the treatment of hepatitis C virus-associated vasculitis that was refractory to

conventional antiviral therapy and anti-CD20 monoclonal antibody, using repeated courses of low-dose IL2, which led to an increase in Treg number and also clinical improvement.

Recent observations on intestinal dysbiosis in AIH may influence future management strategies.

## PERSPECTIVES

AIH requires consideration in the differential diagnosis of any instance of increase in liver enzyme levels. Several pathogenic aspects of AIH have been elucidated, including predisposing genetic factors and (to a degree) disease-specific humoral and cellular immune responses. Prompt immunosuppressive treatment provides a good outcome with a mostly symptom-free long-term survival.

Even so, type 1 (but not type 2) AIH is one of those autoimmune diseases for which clear knowledge on initiation, immunopathogenic mechanisms, and effector processes remain quite lacking. The necessary better understanding of each of these aspects (Sebode et al., 2017) could facilitate our tasks for the future which include establishment of novel treatments aimed specifically at arresting liver autoaggression or, ideally, at reinstating failed tolerance to liver autoantigens, thereby abrogating our hitherto long reliance on nonspecific immunosuppression with all of its discomforts and hazards.

## References

- Abe, K., Takahashi, A., Imaizumi, H., et al., 2016. Interleukin-21 plays a critical role in the pathogenesis and severity of type I autoimmune hepatitis. SpringerPlus 5, 777. Available from: <https://doi.org/10.1186/s40064-016-2512-y>.
- Agarwal, K., Czaja, A.J., Jones, D.E., et al., 2000. Cytotoxic T lymphocyte antigen-4 (CTLA-4) gene polymorphisms and susceptibility to type 1 autoimmune hepatitis. *Hepatology* 31, 49–53.
- Agarwal, K., Czaja, A.J., Donaldson, P.T., 2007. A functional Fas promoter polymorphism is associated with a severe phenotype in type 1 autoimmune hepatitis characterized by early development of cirrhosis. *Tissue Antigens* 69, 227–235. Available from: <https://doi.org/10.1111/j.1399-0039.2006.00794.x>. TAN794 [pii].
- Akbar, A.N., Taams, L.S., Salmon, M., et al., 2003. The peripheral generation of CD4+ CD25+ regulatory T cells. *Immunology* 109, 319–325. Available from: <https://doi.org/10.1046/j.1365-2567.2003.01678.x> [pii].
- Al-Chalabi, T., Underhill, J.A., Portmann, B.C., et al., 2008. Impact of gender on the long-term outcome and survival of patients with autoimmune hepatitis. *J. Hepatol.* 48, 140–147. Available from: <https://doi.org/10.1016/j.jhep.2007.08.013>.
- Albert, M.H., Liu, Y., Anasetti, C., et al., 2005. Antigen-dependent suppression of alloresponses by Foxp3-induced regulatory T cells in transplantation. *Eur. J. Immunol.* 35, 2598–2607. Available from: <https://doi.org/10.1002/eji.200526077>.
- Allan, S.E., Crome, S.Q., Crellin, N.K., et al., 2007. Activation-induced FOXP3 in human T effector cells does not suppress proliferation or cytokine production. *Int. Immunopharmacol.* 19, 345–354. Available from: <https://doi.org/10.1093/intimm/dxm014>.
- Alvarez, F., Ciocca, M., Canero-Velasco, C., et al., 1999. Short-term cyclosporine induces a remission of autoimmune hepatitis in children. *J. Hepatol.* 30, 222–227.
- Alvarez, F., Berg, P.A., Bianchi, F.B., et al., 1999. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. *J. Hepatol.* 31, 929–938.
- Alvarez, F., Berg, P.A., Bianchi, F.B., et al., 1999. International Autoimmune Hepatitis Group report: review of criteria for diagnosis of autoimmune hepatitis. *J. Hepatol.* 31, 929–938.
- Amberg, S., 1942. Hyperproteinaemia associated with severe liver damage. *Proc. Staff Meet. Mayo Clin.* 17, 360–362.
- Amontree, J.S., Stuart, T.D., Bredfeldt, J.E., 1989. Autoimmune chronic active hepatitis masquerading as acute hepatitis. *J. Clin. Gastroenterol.* 11, 303–307.
- Andrade, R.J., Robles, M., Lucena, M.I., 2009. Rechallenge in drug-induced liver injury: the attractive hazard. *Expert Opin. Drug Saf.* 8, 709–714. Available from: <https://doi.org/10.1517/14740330903397378>.
- Arinaga-Hino, T., Ide, T., Miyajima, I., et al., 2018. Risk of malignancies in autoimmune hepatitis type 1 patients with a long-term follow-up in Japan. *Hepatol. Res.* 48, E222–E231. Available from: <https://doi.org/10.1111/hepr.12973>.
- Assandri, R., Monari, M., Montanelli, A., 2016. Development of systemic sclerosis in patients with autoimmune hepatitis: an emerging overlap syndrome. *Gastroenterol. Hepatol. Bed Bench* 9, 211–219.
- Aw, M.M., Dhawan, A., Samyn, M., et al., 2009. Mycophenolate mofetil as rescue treatment for autoimmune liver disease in children: a 5-year follow-up. *J. Hepatol.* 51, 156–160. Available from: <https://doi.org/10.1016/j.jhep.2009.02.024>.
- Baeres, M., Herkel, J., Czaja, A.J., et al., 2002. Establishment of standardised SLA/LP immunoassays: specificity for autoimmune hepatitis, worldwide occurrence, and clinical characteristics. *Gut* 51, 259–264.
- Bai, J., Odin, J.A., 2003. Apoptosis and the liver: relation to autoimmunity and related conditions. *Autoimmun. Rev.* 2, 36–42.
- Barth, E., Clawson, J., 2010. A case of autoimmune hepatitis treated with rituximab. *Case Rep. Gastroenterol.* 4, 502–509. Available from: <https://doi.org/10.1159/000322693>. 322693 [pii].
- Bearn, A.G., Kunkel, H.G., Slater, R.J., 1956. The problem of chronic liver disease in young women. *Am. J. Med.* 21, 3–15.
- Behairy, B.E., El-Araby, H.A., Abd El Kader, H.H., et al., 2016. Assessment of intrahepatic regulatory T cells in children with autoimmune hepatitis. *Ann. Hepatol.* 15, 682–690. Available from: <https://doi.org/10.5604/16652681.1212319>.

- Beisel, C., Weiler-Normann, C., Teufel, A., et al., 2014. Association of autoimmune hepatitis and systemic lupus erythematoses: a case series and review of the literature. *World J. Gastroenterol.* 20, 12662–12667. Available from: <https://doi.org/10.3748/wjg.v20.i35.12662>.
- Beland, K., Marceau, G., Labardy, A., et al., 2015. Depletion of B cells induces remission of autoimmune hepatitis in mice through reduced antigen presentation and help to T cells. *Hepatology* 62, 1511–1523. Available from: <https://doi.org/10.1002/hep.27991>.
- Bjornsson, E.S., Bergmann, O., Jonasson, J.G., et al., 2017. Drug-induced autoimmune hepatitis: response to corticosteroids and lack of relapse after cessation of steroids. *Clin. Gastroenterol. Hepatol.* 15, 1635–1636. Available from: <https://doi.org/10.1016/j.cgh.2017.05.027>.
- Boberg, K.M., Aadland, E., Jahnsen, J., et al., 1998. Incidence and prevalence of primary biliary cirrhosis, primary sclerosing cholangitis, and autoimmune hepatitis in a Norwegian population. *Scand. J. Gastroenterol.* 33, 99–103.
- de Boer, Y.S., van Gerven, N.M., de Boer, N.K., et al., 2013. Allopurinol safely and effectively optimises thiopurine metabolites in patients with autoimmune hepatitis. *Aliment. Pharmacol. Ther.* 37, 640–646. Available from: <https://doi.org/10.1111/apt.12223>.
- de Boer, Y.S., van Gerven, N.M., Zwiers, A., et al., 2014. Genome-wide association study identifies variants associated with autoimmune hepatitis type 1. *Gastroenterology* 147, 443–452.e5. Available from: <https://doi.org/10.1053/j.gastro.2014.04.022>.
- de Boer, Y.S., van Nieuwkerk, C.M., Witte, B.I., et al., 2015. Assessment of the histopathological key features in autoimmune hepatitis. *Histopathology* 66, 351–362. Available from: <https://doi.org/10.1111/his.12558>.
- de Boer, Y.S., Kosinski, A.S., Urban, T.J., et al., 2017. Features of autoimmune hepatitis in patients with drug-induced liver injury. *Clin. Gastroenterol. Hepatol.* 15, 103–112.e2. Available from: <https://doi.org/10.1016/j.cgh.2016.05.043>.
- Bogdanos, D., Ma, Y., Hadzic, N., et al., 2004. Virus-self crossreactivity inducing de novo autoimmune hepatitis eight-years after liver transplantation. *J. Pediatr. Gastroenterol. Nutr.* 39, S169.
- Bogdanos, D.P., Invernizzi, P., Mackay, I.R., et al., 2008. Autoimmune liver serology: current diagnostic and clinical challenges. *World J. Gastroenterol.* 14, 3374–3387.
- Bogdanos, D.P., Mielo-Vergani, G., Vergani, D., 2009. Autoantibodies and their antigens in autoimmune hepatitis. *Semin. Liver Dis.* 29, 241–253. Available from: <https://doi.org/10.1055/s-0029-1233533>.
- Bonito, A.J., Aloman, C., Fiel, M.I., et al., 2013. Medullary thymic epithelial cell depletion leads to autoimmune hepatitis. *J. Clin. Invest.* 123, 3510–3524. Available from: <https://doi.org/10.1172/JCI65414>.
- Bottazzo, G.F., Florin-Christensen, A., Fairfax, A., et al., 1976. Classification of smooth muscle autoantibodies detected by immunofluorescence. *J. Clin. Pathol.* 29, 403–410.
- Bouma, G., van Nieuwkerk, C.M., 2015. Treatment withdrawal in autoimmune hepatitis. *Dig. Dis.* 33 (Suppl. 2), 88–93. Available from: <https://doi.org/10.1159/000440756>.
- Bovensiepen, C.S., Shakat, M., Sebode, M., et al., 2017. TNF $\alpha$  as therapeutic target in autoimmune hepatitis. *J. Hepatol.* 66, S359.
- Braga, A.C., Vasconcelos, C., Braga, J., 2016. Pregnancy with autoimmune hepatitis. *Gastroenterol. Hepatol. Bed Bench* 9, 220–224.
- Burak, K.W., Swain, M.G., Santodomingo-Garzon, T., et al., 2013. Rituximab for the treatment of patients with autoimmune hepatitis who are refractory or intolerant to standard therapy. *Can. J. Gastroenterol.* 27, 273–280.
- zum Büschenfelde, K.H., 2003. Autoimmune hepatitis: "hepatitis sui generis". *J. Hepatol.* 38, 130–135.
- Calich, A.L., Viana, V.S., Cancado, E., et al., 2013. Anti-ribosomal P protein: a novel antibody in autoimmune hepatitis. *Liver Int.* 33, 909–913. Available from: <https://doi.org/10.1111/liv.12155>.
- Calisti, G., Irish, D.N., Ijaz, S., et al., 2017. Acute hepatitis E mimicking a flare of disease in a patient with chronic autoimmune hepatitis. *Ann. Hepatol.* 16, 160–163. Available from: <https://doi.org/10.5604/16652681.1226952>.
- Candia, L., Marquez, J., Espinoza, L.R., 2005. Autoimmune hepatitis and pregnancy: a rheumatologist's dilemma. *Semin. Arthritis Rheum.* 35, 49–56. Available from: <https://doi.org/10.1016/j.semarthrit.2005.03.002>.
- Chatrath, H., Allen, L., Boyer, T.D., 2014. Use of sirolimus in the treatment of refractory autoimmune hepatitis. *Am. J. Med.* 127, 1128–1131. Available from: <https://doi.org/10.1016/j.amjmed.2014.06.016>.
- Chen, Z.X., Shao, J.G., Shen, Y., et al., 2015. Prognostic implications of antibodies to soluble liver antigen in autoimmune hepatitis: a PRISMA-compliant meta-analysis. *Medicine* 94, e953. Available from: <https://doi.org/10.1097/MD.0000000000000953>.
- Chinese Society of Hepatology, Chinese Society of Gastroenterology and Chinese Society of Infectious Diseases, 2017. Chinese consensus on the diagnosis and management of autoimmune hepatitis (2015). *J. Dig. Dis.* 18, 247–264. Available from: <https://doi.org/10.1111/1751-2980.12479>.
- Christen, U., Hintermann, E., 2016. Immunopathogenic mechanisms of autoimmune hepatitis: how much do we know from animal models? *Int. J. Mol. Sci.* 17, 2007. Available from: <https://doi.org/10.3390/ijms17122007>.
- Chung, H., Watanabe, T., Kudo, M., et al., 2010. Identification and characterization of IgG4-associated autoimmune hepatitis. *Liver Int.* 30, 222–231. Available from: <https://doi.org/10.1111/j.1478-3231.2009.02092.x>.
- Cook, G.C., Mulligan, R., Sherlock, S., 1971. Controlled prospective trial of corticosteroid therapy in active chronic hepatitis. *Q. J. Med.* 40, 159–185.
- Cookson, S., Constantini, P.K., Clare, M., et al., 1999. Frequency and nature of cytokine gene polymorphisms in type 1 autoimmune hepatitis. *Hepatology* 30, 851–856.
- Costa, M., Rodriguez-Sanchez, J.L., Czaja, A.J., et al., 2000. Isolation and characterization of cDNA encoding the antigenic protein of the human tRNP(Ser)Sec complex recognized by autoantibodies from patients with type-1 autoimmune hepatitis. *Clin. Exp. Immunol.* 121, 364–374.
- Crapper, R.M., Bhathal, P.S., Mackay, I.R., et al., 1986. 'Acute' autoimmune hepatitis. *Digestion* 34, 216–225.
- Crispe, I.N., 2011. Liver antigen-presenting cells. *J. Hepatol.* 54, 357–365. Available from: <https://doi.org/10.1016/j.jhep.2010.10.005>.
- Quarterolo, M., Ciocca, M., Velasco, C.C., et al., 2006. Follow-up of children with autoimmune hepatitis treated with cyclosporine. *J. Pediatr. Gastroenterol. Nutr.* 43, 635–639.
- Czaja, A.J., 2008. Safety issues in the management of autoimmune hepatitis. *Expert Opin. Drug Saf.* 7, 319–333. Available from: <https://doi.org/10.1517/14740338.7.3.319>.
- Czaja, A.J., 2011. Drug-induced autoimmune-like hepatitis. *Dig. Dis. Sci.* 56, 958–976. Available from: <https://doi.org/10.1007/s10620-011-1611-4>.

- Czaja, A.J., 2013. Diagnosis and management of the overlap syndromes of autoimmune hepatitis. *Can. J. Gastroenterol.* 27, 417–423.
- Czaja, A.J., Carpenter, H.A., 1997. Histological findings in chronic hepatitis C with autoimmune features. *Hepatology* 26, 459–466.
- Czaja, A.J., Freese, D.K., 2002. Diagnosis and treatment of autoimmune hepatitis. *Hepatology* 36, 479–497.
- Czaja, A.J., Davis, G.L., Ludwig, J., et al., 1983. Autoimmune features as determinants of prognosis in steroid-treated chronic active hepatitis of uncertain etiology. *Gastroenterology* 85, 713–717.
- Czaja, A.J., Nishioka, M., Morshed, S.A., et al., 1994. Patterns of nuclear immunofluorescence and reactivities to recombinant nuclear antigens in autoimmune hepatitis. *Gastroenterology* 107, 200–207.
- Czaja, A.J., Donaldson, P.T., Lohse, A.W., 2002. Antibodies to soluble liver antigen/liver pancreas and HLA risk factors for type 1 autoimmune hepatitis. *Am. J. Gastroenterol.* 97, 413–419.
- Czaja, A.J., Bianchi, F.B., Carpenter, H.A., et al., 2005. Treatment challenges and investigational opportunities in autoimmune hepatitis. *Hepatology* 41, 207–215. Available from: <https://doi.org/10.1002/hep.20539>.
- D'Agostino, D., Costaguta, A., Alvarez, F., 2013. Successful treatment of refractory autoimmune hepatitis with rituximab. *Pediatrics* 132, e526–e530. Available from: <https://doi.org/10.1542/peds.2011-1900>.
- Danielsson Borssen, A., Almer, S., Prytz, H., et al., 2015. Hepatocellular and extrahepatic cancer in patients with autoimmune hepatitis—a long-term follow-up study in 634 Swedish patients. *Scand. J. Gastroenterol.* 50, 217–223. Available from: <https://doi.org/10.3109/00365521.2014.983154>.
- Danielsson Borssen, A., Wallerstedt, S., Nyhlin, N., et al., 2016. Pregnancy and childbirth in women with autoimmune hepatitis is safe, even in compensated cirrhosis. *Scand. J. Gastroenterol.* 51, 479–485. Available from: <https://doi.org/10.3109/00365521.2015.1115893>.
- Danielsson Borssen, A., Marschall, H.U., Bergquist, A., et al., 2017. Epidemiology and causes of death in a Swedish cohort of patients with autoimmune hepatitis. *Scand. J. Gastroenterol.* 52, 1022–1028. Available from: <https://doi.org/10.1080/00365521.2017.1335772>.
- De Luca-Johnson, J., Wangensteen, K.J., Hanson, J., et al., 2016. Natural history of patients presenting with autoimmune hepatitis and coincident nonalcoholic fatty liver disease. *Dig. Dis. Sci.* 61, 2710–2720. Available from: <https://doi.org/10.1007/s10620-016-4213-3>.
- De Martin, E., Coilly, A., Ichai, P., et al., 2015. The role of corticosteroids in acute-severe autoimmune hepatitis is still highly debatable. *J. Hepatol.* 63, 1041–1042. Available from: <https://doi.org/10.1016/j.jhep.2015.04.032>.
- Debray, D., Maggiore, G., Giradet, J.P., et al., 1999. Efficacy of cyclosporin A in children with type 2 autoimmune hepatitis. *J. Pediatr.* 135, 111–114.
- Delneste, Y., Charbonnier, P., Herbault, N., et al., 2003. Interferon-gamma switches monocyte differentiation from dendritic cells to macrophages. *Blood* 101, 143–150. Available from: <https://doi.org/10.1182/blood-2002-04-1164>.
- Deneau, M., Jensen, M.K., Holmen, J., et al., 2013. Primary sclerosing cholangitis, autoimmune hepatitis, and overlap in Utah children: epidemiology and natural history. *Hepatology* 58, 1392–1400. Available from: <https://doi.org/10.1002/hep.26454>.
- Deneau, M., Book, L.S., Guthery, S.L., et al., 2014. Outcome after discontinuation of immunosuppression in children with autoimmune hepatitis: a population-based study. *J. Pediatr.* 164, 714–719.e2. Available from: <https://doi.org/10.1016/j.jpeds.2013.12.008>.
- Deswal, S., Srivastava, A., 2017. Role of allopurinol in optimizing thiopurine therapy in patients with autoimmune hepatitis: a review. *J. Clin. Exp. Hepatol.* 7, 55–62. Available from: <https://doi.org/10.1016/j.jceh.2017.01.115>.
- Devlin, S.M., Swain, M.G., Urbanski, S.J., et al., 2004. Mycophenolate mofetil for the treatment of autoimmune hepatitis in patients refractory to standard therapy. *Can. J. Gastroenterol.* 18, 321–326.
- Dhaliwal, H.K., Hoeroldt, B.S., Dube, A.K., et al., 2015. Long-term prognostic significance of persisting histological activity despite biochemical remission in autoimmune hepatitis. *Am. J. Gastroenterol.* 110, 993–999. Available from: <https://doi.org/10.1038/ajg.2015.139>.
- Diestelhorst, J., Junge, N., Schlue, J., et al., 2017. Pediatric autoimmune hepatitis shows a disproportionate decline of regulatory T cells in the liver and of IL-2 in the blood of patients undergoing therapy. *PLoS One* 12, e0181107. Available from: <https://doi.org/10.1371/journal.pone.0181107>.
- Di Giorgio, A., Bravi, M., Bonanomi, E., Alessio, G., Sonzogni, A., Zen, Y., et al., 2015. Fulminant hepatic failure of autoimmune aetiology in children. *J. Pediatr. Gastroenterol. Nutr.* 60, 159–164. Available from: <https://doi.org/10.1097/MPG.0000000000000593>.
- Djilali-Saiah, I., Fakhfakh, A., Louafi, H., et al., 2006. HLA class II influences humoral autoimmunity in patients with type 2 autoimmune hepatitis. *J. Hepatol.* 45, 844–850. Available from: <https://doi.org/10.1016/j.jhep.2006.07.034>.
- Dohmen, K., Tanaka, H., Haruno, M., et al., 2017. Immunoserological and histological differences between autoimmune hepatitis with acute presentation and chronic autoimmune hepatitis. *Hepatol. Res.* 47, 1375–1382. Available from: <https://doi.org/10.1111/hepr.12875>.
- Donaldson, P., 2002. Genetics in autoimmune hepatitis. *Semin. Liver Dis.* 22, 353–364.
- Donaldson, P.T., 2004. Genetics of liver disease: immunogenetics and disease pathogenesis. *Gut* 53, 599–608.
- Duchini, A., McHutchison, J.G., Pockros, P.J., 2000. LKM-positive autoimmune hepatitis in the western United States: a case series. *Am. J. Gastroenterol.* 95, 3238–3241.
- Duclos-Vallee, J.C., Sebagh, M., Rifai, K., et al., 2003. A 10 year follow up study of patients transplanted for autoimmune hepatitis: histological recurrence precedes clinical and biochemical recurrence. *Gut* 52, 893–897.
- Ebrahimkhani, M.R., Mohar, I., Crispe, I.N., 2011. Cross-presentation of antigen by diverse subsets of murine liver cells. *Hepatology* 54, 1379–1387. Available from: <https://doi.org/10.1002/hep.24508>.
- Efe, C., Hagstrom, H., Ytting, H., et al., 2017. Efficacy and safety of mycophenolate mofetil and tacrolimus as second-line therapy for patients with autoimmune hepatitis. *Clin. Gastroenterol. Hepatol.* 15, 1950–1956.e1. Available from: <https://doi.org/10.1016/j.cgh.2017.06.001>.
- Ekong, U.D., McKiernan, P., Martinez, M., et al., 2017. Long-term outcomes of de novo autoimmune hepatitis in pediatric liver transplant recipients. *Pediatr. Transplant.* 21. Available from: <https://doi.org/10.1111/petr.12945>.
- Eriksen, P.L., Kreutzfeldt, M., Gronbaek, H., et al., 2017. Enrichment of genetic variants in the glucocorticoid receptor signalling pathway in autoimmune hepatitis with failure of standard treatment. *Basic Clin. Pharmacol. Toxicol.* 121, 189–194. Available from: <https://doi.org/10.1111/bcpt.12788>.
- European Association for the Study of the Liver, 2015. EASL clinical practice guidelines: autoimmune hepatitis. *J. Hepatol.* 63, 971–1004. Available from: <https://doi.org/10.1016/j.jhep.2015.06.030>.

- Fainboim, L., Canero Velasco, M.C., Marcos, C.Y., et al., 2001. Protracted, but not acute, hepatitis A virus infection is strongly associated with HLA-DRB\*1301, a marker for pediatric autoimmune hepatitis. *Hepatology* 33, 1512–1517.
- Ferri, S., Longhi, M.S., De Molo, C., et al., 2010. A multifaceted imbalance of T cells with regulatory function characterizes type 1 autoimmune hepatitis. *Hepatology* 52, 999–1007. Available from: <https://doi.org/10.1002/hep.23792>.
- Floreani, A., Liberal, R., Vergani, D., et al., 2013. Autoimmune hepatitis: contrasts and comparisons in children and adults—a comprehensive review. *J. Autoimmun.* 46, 7–16. Available from: <https://doi.org/10.1016/j.jaut.2013.08.004>.
- Fujiwara, K., Yasui, S., Yokosuka, O., 2015. Appropriate diagnostic criteria for fulminant autoimmune hepatitis. *Eur. J. Gastroenterol. Hepatol.* 27, 1230–1231. Available from: <https://doi.org/10.1097/MEG.0000000000000441>.
- Furumoto, Y., Asano, T., Sugita, T., et al., 2015. Evaluation of the role of HLA-DR antigens in Japanese type 1 autoimmune hepatitis. *BMC Gastroenterol.* 15, 144. Available from: <https://doi.org/10.1186/s12876-015-0360-9>.
- Gajdusek, D.C., 1958. An autoimmune reaction against human tissue antigens in certain acute and chronic diseases. I. Serological investigations. *AMA Arch. Intern. Med.* 101, 9–29.
- Gargouri, L., Mnif, L., Safi, F., et al., 2013. Type 2 autoimmune hepatitis overlapping with primary sclerosing cholangitis in a 10-year-old boy. *Arch. Pediatr.* 20, 1325–1328. Available from: <https://doi.org/10.1016/j.arcped.2013.09.020>.
- Gazzola, A., Lim, R., Strasser, S.I., et al., 2016. Mycophenolate mofetil in autoimmune hepatitis patients not responsive or intolerant to standard therapy: the Australian TAPESTRY study. *Hepatology* 64, 817A.
- van Gerven, N.M., Verwer, B.J., Witte, B.I., et al., 2014. Epidemiology and clinical characteristics of autoimmune hepatitis in the Netherlands. *Scand. J. Gastroenterol.* 49, 1245–1254. Available from: <https://doi.org/10.3109/00365521.2014.946083>.
- van Gerven, N.M., Bakker, S.F., de Boer, Y.S., et al., 2014. Seroprevalence of celiac disease in patients with autoimmune hepatitis. *Eur. J. Gastroenterol. Hepatol.* 26, 1104–1107. Available from: <https://doi.org/10.1097/MEG.0000000000000172>.
- van Gerven, N.M., de Boer, Y.S., Zwiers, A., et al., 2015. HLA-DRB1\*03:01 and HLA-DRB1\*04:01 modify the presentation and outcome in autoimmune hepatitis type-1. *Genes Immun.* 16, 247–252. Available from: <https://doi.org/10.1038/gene.2014.82>.
- van Gerven, N.M., van der Eijk, A.A., Pas, S.D., et al., 2016. Seroprevalence of hepatitis E virus in autoimmune hepatitis patients in the Netherlands. *J. Gastrointestin. Liver Dis.* 25, 9–13. Available from: <https://doi.org/10.15403/jgld.2014.1121.251.hpe>.
- Grant, C.R., Liberal, R., Holder, B.S., et al., 2014. Dysfunctional CD39(POS) regulatory T cells and aberrant control of T-helper type 17 cells in autoimmune hepatitis. *Hepatology* 59, 1007–1015. Available from: <https://doi.org/10.1002/hep.26583>.
- Gregorio, G.V., Portman, B., Reid, F., et al., 1997. Autoimmune hepatitis in childhood: a 20-year experience. *Hepatology* 25, 541–547.
- Gregorio, G.V., Portmann, B., Karani, J., et al., 2001. Autoimmune hepatitis/sclerosing cholangitis overlap syndrome in childhood: a 16-year prospective study. *Hepatology* 33, 544–553.
- Gregorio, G.V., McFarlane, B., Bracken, P., et al., 2002. Organ and non-organ specific autoantibody titres and IgG levels as markers of disease activity: a longitudinal study in childhood autoimmune liver disease. *Autoimmunity* 35, 515–519.
- Gronbaek, H., Kreutzfeldt, M., Kazankov, K., et al., 2016. Single-centre experience of the macrophage activation marker soluble (s)CD163—associations with disease activity and treatment response in patients with autoimmune hepatitis. *Aliment. Pharmacol. Ther.* 44, 1062–1070. Available from: <https://doi.org/10.1111/apt.13801>.
- Longhi, M.S., Hussain, M.J., Bogdanos, D.P., Quaglia, A., Mieli-Vergani, G., Ma, Y., et al., 2007. Cytochrome P450IID6-specific CD8 T cell immune responses mirror disease activity in autoimmune hepatitis type 2. *Hepatology* 46, 472–484.
- Harada, K., Shimoda, S., Sato, Y., Isse, K., Ikeda, H., Nakanuma, Y., 2009. Periductal interleukin-17 production in association with biliary innate immunity contributes to the pathogenesis of cholangiopathy in primary biliary cirrhosis. *Clin. Exp. Immunol.* 157, 261–270.
- Hardtke-Wolenski, M., Taubert, R., Jaeckel, E., 2012. Animal models for autoimmune liver disease—what is relevant for immune-mediated liver disease. *Dig. Dis.* S1–S20.
- Hardtke-Wolenski, M., Fischer, K., Noyan, F., Schlue, J., Falk, C.S., Stahlhut, M., et al., 2013. Genetic predisposition and environmental danger signals initiate chronic autoimmune hepatitis driven by CD4+ T cells. *Hepatology* 58 (2), 718–728. Available from: <https://doi.org/10.1002/hep.26380>.
- Hardtke-Wolenski, M., Taubert, R., Noyan, F., et al., 2015. Autoimmune hepatitis in a murine autoimmune polyendocrine syndrome type 1 model is directed against multiple autoantigens. *Hepatology* 61, 1295–1305. Available from: <https://doi.org/10.1002/hep.27639>.
- Hardtke-Wolenski, M., Dywicki, J., Fischer, K., et al., 2017. The influence of genetic predisposition and autoimmune hepatitis inducing antigens in disease development. *J. Autoimmun.* 78, 39–45. Available from: <https://doi.org/10.1016/j.jaut.2016.12.001>.
- Haridy, J., Nicoll, A., Sood, S., 2017. Methotrexate therapy for autoimmune hepatitis. *Clin. Gastroenterol. Hepatol.* Available from: <https://doi.org/10.1016/j.cgh.2017.07.003>.
- Hartl, J., Ehlken, H., Weiler-Normann, C., et al., 2015. Patient selection based on treatment duration and liver biochemistry increases success rates after treatment withdrawal in autoimmune hepatitis. *J. Hepatol.* 62, 642–646. Available from: <https://doi.org/10.1016/j.jhep.2014.10.018>.
- Healey, R., Corless, L., Gordins, P., et al., 2016. Do anti-smooth muscle antibodies predict development of autoimmune hepatitis in patients with normal liver function?—a retrospective cohort review. *Autoimmun. Rev.* 15, 668–672. Available from: <https://doi.org/10.1016/j.autrev.2016.03.001>.
- Heneghan, M.A., Norris, S.M., O'Grady, J.G., et al., 2001. Management and outcome of pregnancy in autoimmune hepatitis. *Gut* 48, 97–102.
- Heneghan, M.A., Allan, M.L., Bornstein, J.D., et al., 2006. Utility of thiopurine methyltransferase genotyping and phenotyping, and measurement of azathioprine metabolites in the management of patients with autoimmune hepatitis. *J. Hepatol.* 45, 584–591. Available from: <https://doi.org/10.1016/j.hep.2006.05.011>.
- Hennes, E.M., Zeniya, M., Czaja, A.J., et al., 2008. Simplified criteria for the diagnosis of autoimmune hepatitis. *Hepatology* 48, 169–176. Available from: <https://doi.org/10.1002/hep.22322>.
- Hennes, E.M., Oo, Y.H., Schramm, C., et al., 2008. Mycophenolate mofetil as second line therapy in autoimmune hepatitis? *Am. J. Gastroenterol.* 103, 3063–3070. Available from: <https://doi.org/10.1111/j.1572-0241.2008.02180.x>. DOI: AJG2180 [pii].
- Holdener, M., Hintermann, E., Bayer, M., et al., 2008. Breaking tolerance to the natural human liver autoantigen cytochrome P450 2D6 by virus infection. *J. Exp. Med.* 205, 1409–1422.

- Holder, B.S., Grant, C.R., Liberal, R., et al., 2014. Retinoic acid stabilizes antigen-specific regulatory T-cell function in autoimmune hepatitis type 2. *J. Autoimmun.* 53, 26–32. Available from: <https://doi.org/10.1016/j.aut.2014.02.001>.
- Homberg, J.C., Abuaf, N., Bernard, O., et al., 1987. Chronic active hepatitis associated with antiliver/kidney microsome antibody type 1: a second type of "autoimmune" hepatitis. *Hepatology* 7, 1333–1339.
- Hopf, U., Meyer zum Büschchenfelde, K.H., Arnold, W., 1976. Detection of a liver-membrane autoantibody in HBsAg-negative chronic active hepatitis. *N. Engl. J. Med.* 294, 578–582. Available from: <https://doi.org/10.1056/NEJM19760312941103>.
- Huang, J., Yuan, Q., Zhu, H., et al., 2017. IL-17C/IL-17RE augments t cell function in autoimmune hepatitis. *J. Immunol.* 198, 669–680. Available from: <https://doi.org/10.4049/jimmunol.1600977>.
- Hubener, S., Oo, Y.H., Than, N.N., et al., 2016. Efficacy of 6-mercaptopurine as second-line treatment for patients with autoimmune hepatitis and azathioprine intolerance. *Clin. Gastroenterol. Hepatol.* 14, 445–453. Available from: <https://doi.org/10.1016/j.cgh.2015.09.037>.
- Hurlburt, K.J., McMahon, B.J., Deubner, H., et al., 2002. Prevalence of autoimmune liver disease in Alaska Natives. *Am. J. Gastroenterol.* 97, 2402–2407. Available from: <https://doi.org/10.1111/j.1572-0241.2002.06019.x>.
- Ichai, P., Duclos-Vallee, J.C., Guettier, C., et al., 2007. Usefulness of corticosteroids for the treatment of severe and fulminant forms of autoimmune hepatitis. *Liver Transpl.* 13, 996–1003. Available from: <https://doi.org/10.1002/lt.21036>.
- Ichiki, Y., Aoki, C.A., Bowlus, C.L., et al., 2005. T cell immunity in autoimmune hepatitis. *Autoimmun. Rev.* 4, 315–321. Available from: <https://doi.org/10.1016/j.autrev.2005.01.005>.
- Iqbal, U., Chaudhary, A., Karim, M.A., et al., 2017. Association of autoimmune hepatitis and celiac disease: role of gluten-free diet in reversing liver dysfunction. *J. Invest. Med. High Impact Case Rep.* 5, Available from: <https://doi.org/10.1177/2324709617705679>.
- Janmohamed, A., Hirschfield, G.M., 2016. Editorial: autoimmune hepatitis—identifying options for treatment. *Aliment. Pharmacol. Ther.* 43, 1236–1237. Available from: <https://doi.org/10.1111/apt.13607>.
- Jensen, D.M., McFarlane, I.G., Portmann, B.S., et al., 1978. Detection of antibodies directed against a liver-specific membrane lipoprotein in patients with acute and chronic active hepatitis. *N. Engl. J. Med.* 299, 1–7.
- Jimenez-Rivera, C., Ling, S.C., Ahmed, N., et al., 2015. Incidence and characteristics of autoimmune hepatitis. *Pediatrics* 136, e1237–e1248. Available from: <https://doi.org/10.1542/peds.2015-0578>.
- Johnson, P.J., McFarlane, I.G., 1993. Meeting report: International Autoimmune Hepatitis Group. *Hepatology* 18, 998–1005.
- Johnson, P.J., McFarlane, I.G., Williams, R., 1995. Azathioprine for long-term maintenance of remission in autoimmune hepatitis. *N. Engl. J. Med.* 333, 958–963. Available from: <https://doi.org/10.1056/NEJM199510123331502>.
- Joske, R.A., King, W.E., 1955. The L.E.-cell phenomenon in active chronic viral hepatitis. *Lancet* 269, 477–480.
- Jothimani, D., Cramp, M.E., Cross, T.J., 2014. Role of mycophenolate mofetil for the treatment of autoimmune hepatitis—an observational study. *J. Clin. Exp. Hepatol.* 4, 221–225. Available from: <https://doi.org/10.1016/j.jceh.2014.05.003>.
- Kanzler, S., Lohr, H., Gerken, G., et al., 2001. Long-term management and prognosis of autoimmune hepatitis (AIH): a single center experience. *Z. Gastroenterol.* 39, 339–341. Available from: <https://doi.org/10.1055/s-2001-13708>.
- Kerkar, N., Yanni, G., 2016. 'De novo' and 'recurrent' autoimmune hepatitis after liver transplantation: a comprehensive review. *J. Autoimmun.* 66, 17–24. Available from: <https://doi.org/10.1016/j.aut.2015.08.017>.
- Kerkar, N., Hadzic, N., Davies, E.T., et al., 1998. De-novo autoimmune hepatitis after liver transplantation. *Lancet* 351, 409–413. Available from: [https://doi.org/10.1016/S0140-6736\(97\)06478-7](https://doi.org/10.1016/S0140-6736(97)06478-7).
- Kerkar, N., Choudhuri, K., Ma, Y., et al., 2003. Cytochrome P4502D6(193-212): a new immunodominant epitope and target of virus/self cross-reactivity in liver kidney microsomal autoantibody type 1-positive liver disease. *J. Immunol.* 170, 1481–1489.
- Kerkar, N., Dugan, C., Rumbo, C., et al., 2005. Rapamycin successfully treats post-transplant autoimmune hepatitis. *Am. J. Transplant.* 5, 1085–1089. Available from: <https://doi.org/10.1111/j.1600-6143.2005.00801.x>.
- Kerkar, N., Annunziato, R.A., Foley, L., et al., 2006. Prospective analysis of nonadherence in autoimmune hepatitis: a common problem. *J. Pediatr. Gastroenterol. Nutr.* 43, 629–634.
- Kido, M., Watanabe, N., Okazaki, T., et al., 2008. Fatal autoimmune hepatitis induced by concurrent loss of naturally arising regulatory T cells and PD-1-mediated signaling. *Gastroenterology* 135, 1333–1343. Available from: <https://doi.org/10.1053/j.gastro.2008.06.042>. DOI: S0016-5085(08)01096-2 [pii].
- Kimura, N., Yamagawa, S., Sugano, T., et al., 2017. Possible involvement of CCR7- PD-1+ follicular helper T cell subset in the pathogenesis of autoimmune hepatitis. *J. Gastroenterol. Hepatol.* Available from: <https://doi.org/10.1111/jgh.13844>.
- Kirstein, M.M., Metzler, F., Geiger, E., et al., 2015. Prediction of short- and long-term outcome in patients with autoimmune hepatitis. *Hepatology* 62, 1524–1535. Available from: <https://doi.org/10.1002/hep.27983>.
- Krawitt, E., 2006. Autoimmune hepatitis. *N. Engl. J. Med.* 354, 54–66.
- Kunkel, H.G., Ahrens, J.R., Eisenmenger, W.J., et al., 1951. Extreme hypergammaglobulinemia in young women with liver disease of unknown etiology. *J. Clin. Invest.* 30, 654.
- Kurowski, J., Melin-Aldana, H., Bass, L., et al., 2014. Sirolimus as rescue therapy in pediatric autoimmune hepatitis. *J. Pediatr. Gastroenterol. Nutr.* 58, e4–e6. Available from: <https://doi.org/10.1097/MPG.0b013e318291fea>.
- Langley, P.G., Underhill, J., Tredger, J.M., et al., 2002. Thiopurine methyltransferase phenotype and genotype in relation to azathioprine therapy in autoimmune hepatitis. *J. Hepatol.* 37, 441–447.
- Lankisch, T.O., Strassburg, C.P., Debray, D., et al., 2005. Detection of autoimmune regulator gene mutations in children with type 2 autoimmune hepatitis and extrahepatic immune-mediated diseases. *J. Pediatr.* 146, 839–842. Available from: <https://doi.org/10.1016/j.jpeds.2005.01.050>.
- Lapierre, P., Hajoui, O., Homberg, J.C., et al., 1999. Formiminotransferase cyclodeaminase is an organ-specific autoantigen recognized by sera of patients with autoimmune hepatitis. *Gastroenterology* 116, 643–649.
- Lapierre, P., Djilali-Saïah, I., Vitozzi, S., et al., 2004. A murine model of type 2 autoimmune hepatitis: xenoimmunization with human antigens. *Hepatology* 39, 1066–1074.
- Lapierre, P., Beland, K., Yang, R., et al., 2013. Adoptive transfer of ex vivo expanded regulatory T cells in an autoimmune hepatitis murine model restores peripheral tolerance. *Hepatology* 57, 217–227. Available from: <https://doi.org/10.1002/hep.26023>.

- Legue, C., Legros, L., Kammerer-Jacquet, S., et al., 2017. Safety and efficacy of 6-thioguanine as a second-line treatment for autoimmune hepatitis. *Clin. Gastroenterol. Hepatol.* Available from: <https://doi.org/10.1016/j.cgh.2017.07.032>.
- Li, Y., Peng, M., Gong, G., 2014. Evaluation of the revised versus the simplified scoring system in patients with autoimmune hepatitis. *Exp. Ther. Med.* 7, 131–136. Available from: <https://doi.org/10.3892/etm.2013.1366>.
- Liberal, R., Grant, C.R., Holder, B.S., et al., 2012. The impaired immune regulation of autoimmune hepatitis is linked to a defective galectin-9/tim-3 pathway. *Hepatology* 56, 677–686. Available from: <https://doi.org/10.1002/hep.25682>.
- Liberal, R., Zen, Y., Mieli-Vergani, G., et al., 2013. Liver transplantation and autoimmune liver diseases. *Liver Transpl.* 19, 1065–1077. Available from: <https://doi.org/10.1002/lt.23704>.
- Liberal, R., Mieli-Vergani, G., Vergani, D., 2013. Clinical significance of autoantibodies in autoimmune hepatitis. *J. Autoimmun.* 46, 17–24. Available from: <https://doi.org/10.1016/j.jaut.2013.08.001>.
- Liberal, R., Grant, C.R., Holder, B.S., et al., 2015. In autoimmune hepatitis type 1 or the autoimmune hepatitis-sclerosing cholangitis variant defective regulatory T-cell responsiveness to IL-2 results in low IL-10 production and impaired suppression. *Hepatology* 62, 863–875. Available from: <https://doi.org/10.1002/hep.27884>.
- Liberal, R., Krawitt, E.L., Vierling, J.M., et al., 2016. Cutting edge issues in autoimmune hepatitis. *J. Autoimmun.* 75, 6–19. Available from: <https://doi.org/10.1016/j.jaut.2016.07.005>.
- Liberal, R., Vergani, D., Mieli-Vergani, G., 2016. Recurrence of autoimmune liver disease and inflammatory bowel disease after pediatric liver transplantation. *Liver Transpl.* 22, 1275–1283. Available from: <https://doi.org/10.1002/lt.24490>.
- Liberal, R., Grant, C.R., Yuksel, M., et al., 2017. Treg conditioning endows activated Teff with suppressor function in autoimmune hepatitis/ autoimmune sclerosing cholangitis. *Hepatology*. Available from: <https://doi.org/10.1002/hep.29307>.
- Lin, R., Zhou, L., Zhang, J., et al., 2015. Abnormal intestinal permeability and microbiota in patients with autoimmune hepatitis. *Int. J. Clin. Exp. Pathol.* 8, 5153–5160.
- Liston, A., Lesage, S., Gray, D.H., et al., 2005. Genetic lesions in T-cell tolerance and thresholds for autoimmunity. *Immunol. Rev.* 204, 87–101. Available from: <https://doi.org/10.1111/j.0105-2896.2005.00253.x>.
- Lleo, A., Selmi, C., Invernizzi, P., et al., 2009. Apoptoses and the biliary specificity of primary biliary cirrhosis. *Hepatology* 49, 871–879. Available from: <https://doi.org/10.1002/hep.22736>.
- Lobo-Yeo, A., Senaldi, G., Portmann, B., et al., 1990. Class I and class II major histocompatibility complex antigen expression on hepatocytes: a study in children with liver disease. *Hepatology* 12, 224–232.
- Lohse, A.W., Mieli-Vergani, G., 2011. Autoimmune hepatitis. *J. Hepatol.* 55, 171–182. Available from: <https://doi.org/10.1016/j.jhep.2010.12.012>.
- Longhi, M.S., Ma, Y., Bogdanos, D.P., et al., 2004. Impairment of CD4(+)CD25(+) regulatory T-cells in autoimmune liver disease. *J. Hepatol.* 41, 31–37.
- Longhi, M.S., Ma, Y., Mitry, R.R., et al., 2005. Effect of CD4 + CD25 + regulatory T-cells on CD8 T-cell function in patients with autoimmune hepatitis. *J. Autoimmun.* 25, 63–71.
- Longhi, M.S., Meda, F., Wang, P., et al., 2008. Expansion and de novo generation of potentially therapeutic regulatory T cells in patients with autoimmune hepatitis. *Hepatology* 47, 581–591.
- Longhi, M.S., Ma, Y., Mieli-Vergani G., et al., 2010. Aetiopathogenesis of autoimmune hepatitis. *J. Autoimmun.* 34, 7–14. doi:10.1016/j.jaut.2009.08.010.
- Longhi, M.S., Hussain, M.J., Kwok, W.W., et al., 2011. Autoantigen-specific regulatory Tcells, a potential tool for immune-tolerance reconstitution in type-2 autoimmune hepatitis. *Hepatology* 53, 536–547. Available from: <https://doi.org/10.1002/hep.24039>. Research Support, Non-U.S. Gov't.
- Longhi, M.S., Ma, Y., Mieli-Vergani, G., et al., 2012. Regulatory T cells in autoimmune hepatitis. *J. Hepatol.* 57, 932–933. Available from: <https://doi.org/10.1016/j.jhep.2012.05.022>. author reply 933–934. doi: S0168-8278(12)00424-2 [pii].
- Luth, S., Herkel, J., Kanzler, S., et al., 2008. Serologic markers compared with liver biopsy for monitoring disease activity in autoimmune hepatitis. *J. Clin. Gastroenterol.* 42, 926–930.
- Ma, C.S., Deenick, E.K., 2014. Human T follicular helper (Tfh) cells and disease. *Immunol. Cell Biol.* 92, 64–71. Available from: <https://doi.org/10.1038/icb.2013.55>.
- Ma, L., Qin, J., Ji, H., et al., 2014. Tfh and plasma cells are correlated with hypergammaglobulinaemia in patients with autoimmune hepatitis. *Liver Int.* 34, 405–415. Available from: <https://doi.org/10.1111/liv.12245>.
- Ma, Y., Okamoto, M., Thomas, M.G., et al., 2002. Antibodies to conformational epitopes of soluble liver antigen define a severe form of autoimmune liver disease. *Hepatology* 35, 658–664. Available from: <https://doi.org/10.1053/jhep.2002.32092>.
- Ma, Y., Bogdanos, D.P., Hussain, M.J., et al., 2006. Polyclonal T-cell responses to cytochrome P450IID6 are associated with disease activity in autoimmune hepatitis type 2. *Gastroenterology* 130, 868–882. Available from: <https://doi.org/10.1053/j.gastro.2005.12.020>.
- Mackay, I.R., 1968. Chronic hepatitis: effect of prolonged suppressive treatment and comparison of azathioprine with prednisolone. *Q. J. Med.* 37, 379–392.
- Mackay, I.R., Gajdusek, D.C., 1958. An autoimmune reaction against human tissue antigens in certain acute and chronic diseases. II. Clinical correlations. *AMA Arch. Intern. Med.* 101, 30–46.
- Mackay, I.R., Taft, L.I., Cowling, D.C., 1956. Lupoid hepatitis. *Lancet* 1323–1326. ii.
- Mackay, I.R., Weiden, S., Hasker, J., 1965. Autoimmune hepatitis. *Ann. N.Y. Acad. Sci.* 124, 767–780.
- Mackie, F.D., Peakman, M., Yun, M., et al., 1994. Primary and secondary liver/kidney microsomal autoantibody response following infection with hepatitis C virus. *Gastroenterology* 106, 1672–1675.
- Maeda, C., Tamano, M., Murohisa, T., et al., 2010. Hepatocellular carcinoma associated with noncirrhotic autoimmune hepatitis. *Clin. J. Gastroenterol.* 3, 111–115. Available from: <https://doi.org/10.1007/s12328-010-0137-1>.
- Manns, M., Gerken, G., Kyriatsoulis, A., et al., 1987. Characterisation of a new subgroup of autoimmune chronic active hepatitis by autoantibodies against a soluble liver antigen. *Lancet* 1, 292–294.

- Manns, M.P., Luttig, B., Obermayer-Straub, P., 1998. Autoimmune hepatitis. In: Rose, N.R., Mackay, I.R. (Eds.), *The Autoimmune Diseases*, third ed. Academic Press, pp. 511–525.
- Manns, M.P., Woynarowski, M., Kreisel, W., et al., 2010. Budesonide induces remission more effectively than prednisone in a controlled trial of patients with autoimmune hepatitis. *Gastroenterology* 139, 1198–1206. Available from: <https://doi.org/10.1053/j.gastro.2010.06.046>.
- Manns, M.P., Czaja, A.J., Gorham, J.D., et al., 2010. Diagnosis and management of autoimmune hepatitis. *Hepatology* 51, 2193–2213. Available from: <https://doi.org/10.1002/hep.23584>.
- Marceau, G., Yang, R., Lapierre, P., et al., 2015. Low-dose anti-CD3 antibody induces remission of active autoimmune hepatitis in xenoimmunized mice. *Liver Int.* 35, 275–284. Available from: <https://doi.org/10.1111/liv.12498>.
- Marlaka, J.R., Papadogiannakis, N., Fischler, B., et al., 2012. Tacrolimus without or with the addition of conventional immunosuppressive treatment in juvenile autoimmune hepatitis. *Acta Paediatr.* 101, 993–999. Available from: <https://doi.org/10.1111/j.1651-2227.2012.02745.x>.
- Martini, E., Abuaf, N., Cavalli, F., et al., 1988. Antibody to liver cytosol (anti-LC1) in patients with autoimmune chronic active hepatitis type 2. *Hepatology* 8, 1662–1666.
- Matsumoto, K., Miyake, Y., Matsushita, H., et al., 2014. Anti-programmed cell death-1 antibody as a new serological marker for type 1 autoimmune hepatitis. *J. Gastroenterol. Hepatol.* 29, 110–115. Available from: <https://doi.org/10.1111/jgh.12340>.
- Mazzara, S., Sinisi, A., Cardaci, A., et al., 2015. Two of them do it better: novel serum biomarkers improve autoimmune hepatitis diagnosis. *PLoS One* 10, e0137927. Available from: <https://doi.org/10.1371/journal.pone.0137927>.
- McFarlane, B.M., McSorley, C.G., Vergani, D., et al., 1986. Serum autoantibodies reacting with the hepatic asialoglycoprotein receptor protein (hepatocyte lectin) in acute and chronic liver disorders. *J. Hepatol.* 3, 196–205.
- Meda, F., Wang, P., Longhi, M.S., et al., 2007. Identification of HLA-DR3 restricted CD4 T-cell epitopes on soluble liver antigen in autoimmune hepatitis type 1. *J. Hepatol.* 46, S13.
- Mendizabal, M., Marciano, S., Videla, M.G., et al., 2015. Fulminant presentation of autoimmune hepatitis: clinical features and early predictors of corticosteroid treatment failure. *Eur. J. Gastroenterol. Hepatol.* 27, 644–648. Available from: <https://doi.org/10.1097/MEG.0000000000000353>.
- Meroni, P.L., Schur, P.H., 2010. ANA screening: an old test with new recommendations. *Ann. Rheum. Dis.* 69, 1420–1422. doi: 10.1136/ard.2009.127100 [pii]
- Meyer zum, B., Miescher, P.A., 1972. Liver specific antigens. Purification and characterization. *Clin. Exp. Immunol.* 10, 89–102.
- Meyer zum Büschenthalde, K.H., 1972. Immunopathogenese chronisch entzündlicher Lebererkrankungen. *Ergebnisse Innere Med u Kinderheilkunde* 32, 31–81.
- Meyer zum Büschenthalde, K.H., Schrank, C.H., 1966. Untersuchungen zur Frage organspezifischer Antigene der Leber. *Klin. Wochenschr.* 44, 654–656.
- von Meyer zum Büschenthalde, K.H., Knolle, J., Berger, J., 1974. [Cellular immune reactions towards homologous liver-specific antigens (HLP) in chronic inflammatory liver diseases (author's transl)]. *Klin. Wochenschr.* 52, 246–248.
- Miao, Q., Bian, Z., Tang, R., et al., 2015. Emperipoleisis mediated by CD8 T cells is a characteristic histopathologic feature of autoimmune hepatitis. *Clin. Rev. Allergy Immunol.* 48, 226–235. Available from: <https://doi.org/10.1007/s12016-014-8432-0>.
- Mieli-Vergani, G., Vergani, D., 2011. Autoimmune hepatitis. *Nat. Rev. Gastroenterol. Hepatol.* 8, 320–329. Available from: <https://doi.org/10.1038/nrgastro.2011.69>.
- Mieli-Vergani, G., Vergani, D., 2013. Budesonide for juvenile autoimmune hepatitis? Not yet. *J. Pediatr.* 163, 1246–1248. Available from: <https://doi.org/10.1016/j.jpeds.2013.06.064>.
- Mieli-Vergani, G., Heller, S., Jara, P., et al., 2009. Autoimmune hepatitis. *J. Pediatr. Gastroenterol. Nutr.* 49, 158–164. Available from: <https://doi.org/10.1097/MPG.0b013e3181a1c265>.
- Mieli-Vergani, G., Vergani, D., Baumann, U., et al., 2018. Diagnosis and management of pediatric autoimmune liver disease: ESPGHAN hepatology committee position statement. *J. Pediatr. Gastroenterol. Nutr.* 66, 345–360. Available from: <https://doi.org/10.1097/MPG.0000000000001801>.
- Milkiewicz, P., Hubscher, S.G., Skiba, G., et al., 1999. Recurrence of autoimmune hepatitis after liver transplantation. *Transplantation* 68, 253–256.
- Miyake, Y., Yamamoto, K., Matsushita, H., et al., 2014. Multicenter validation study of anti-programmed cell death-1 antibody as a serological marker for type 1 autoimmune hepatitis. *Hepatol. Res.* 44, 1299–1307. Available from: <https://doi.org/10.1111/hepr.12305>.
- Montano-Loza, A.J., Carpenter, H.A., Czaja, A.J., 2007. Features associated with treatment failure in type 1 autoimmune hepatitis and predictive value of the model of end-stage liver disease. *Hepatology* 46, 1138–1145. Available from: <https://doi.org/10.1002/hep.21787>.
- Montano-Loza, A.J., Carpenter, H.A., Czaja, A.J., 2008. Frequency, behavior, and prognostic implications of antimitochondrial antibodies in type 1 autoimmune hepatitis. *J. Clin. Gastroenterol.* 42, 1047–1053. Available from: <https://doi.org/10.1097/MCG.0b013e3181587d18>.
- Muller, P., Messmer, M., Bayer, M., et al., 2016. Non-alcoholic fatty liver disease (NAFLD) potentiates autoimmune hepatitis in the CYP2D6 mouse model. *J. Autoimmun.* 69, 51–58. Available from: <https://doi.org/10.1016/j.jaut.2016.02.007>.
- Muratori, L., Parola, M., Ripalti, A., et al., 2000. Liver/kidney microsomal antibody type 1 targets CYP2D6 on hepatocyte plasma membrane. *Gut* 46, 553–561.
- Muratori, L., Masi, C., Muratori, P., 2014. Anti-ribosomal P protein antibody: an autoreactivity devoid of prognostic value in patients with autoimmune hepatitis. *Liver Int.* 34, 1446. Available from: <https://doi.org/10.1111/liv.12585>.
- Muratori, P., Muratori, L., Agostinelli, D., et al., 2002. Smooth muscle antibodies and type 1 autoimmune hepatitis. *Autoimmunity* 35, 497–500.
- Muratori, P., Lalanne, C., Bianchi, G., et al., 2016. Predictive factors of poor response to therapy in autoimmune hepatitis. *Dig. Liver Dis.* 48, 1078–1081. Available from: <https://doi.org/10.1016/j.dld.2016.06.018>.
- Muratori, P., Lalanne, C., Barbato, E., et al., 2016. Features and progression of asymptomatic autoimmune hepatitis in Italy. *Clin. Gastroenterol. Hepatol.* 14, 139–146. Available from: <https://doi.org/10.1016/j.cgh.2015.07.017>.
- Muratori, P., Efe, C., Muratori, L., et al., 2017. Clinical implications of antimitochondrial antibody seropositivity in autoimmune hepatitis: a multicentre study. *Eur. J. Gastroenterol. Hepatol.* 29, 777–780. Available from: <https://doi.org/10.1097/MEG.0000000000000870>.

- Murray-Lyon, I.M., Stern, R.B., Williams, R., 1973. Controlled trial of prednisone and azathioprine in active chronic hepatitis. *Lancet* 1, 735–737.
- Najafi, M., Sadjadei, N., Eftekhari, K., et al., 2014. Prevalence of celiac disease in children with autoimmune hepatitis and vice versa. *Iran. J. Pediatr.* 24, 723–728.
- Newman, W.G., Payne, K., Tricker, K., et al., 2011. A pragmatic randomized controlled trial of thiopurine methyltransferase genotyping prior to azathioprine treatment: the TARGET study. *Pharmacogenomics* 12, 815–826. Available from: <https://doi.org/10.2217/pgs.11.32>.
- Ngu, J.H., Bechly, K., Chapman, B.A., et al., 2010. Population-based epidemiology study of autoimmune hepatitis: a disease of older women? *J. Gastroenterol. Hepatol.* 25, 1681–1686. Available from: <https://doi.org/10.1111/j.1440-1746.2010.06384.x>.
- Ngu, J.H., Gearry, R.B., Frampton, C.M., et al., 2013. Autoimmune hepatitis: the role of environmental risk factors: a population-based study. *Hepatol. Int.* 7, 869–875. Available from: <https://doi.org/10.1007/s12072-013-9448-x>.
- Nguyen Canh, H., Harada, K., Ouchi, H., et al., 2017. Acute presentation of autoimmune hepatitis: a multicentre study with detailed histological evaluation in a large cohort of patients. *J. Clin. Pathol.* Available from: <https://doi.org/10.1136/jclinpath-2016-204271>.
- Nishioka, M., McFarlane, I.G., 1998. Geographical variation in the frequency and characteristics of autoimmune liver diseases. In: Krawitt, E. L., Nishioka, M. (Eds.), *Autoimmune Liver Diseases*. Elsevier, Amsterdam, pp. 413–428.
- Norman, G.L., Yang, C.Y., Ostendorff, H.P., et al., 2015. Anti-Kelch-like 12 and anti-hexokinase 1: novel autoantibodies in primary biliary cirrhosis. *Liver Int.* 35, 642–651. Available from: <https://doi.org/10.1111/liv.12690>.
- Nouri-Aria, K.T., Hegarty, J.E., Alexander, G.J., et al., 1982. Effect of corticosteroids on suppressor-cell activity in “autoimmune” and viral chronic active hepatitis. *N. Engl. J. Med.* 307, 1301–1304.
- O'Brien, C., Joshi, S., Feld, J.J., et al., 2008. Long-term follow-up of antimitochondrial antibody-positive autoimmune hepatitis. *Hepatology* 48, 550–556. Available from: <https://doi.org/10.1002/hep.22380>.
- Oettinger, R., Brunnberg, A., Gerner, P., et al., 2005. Clinical features and biochemical data of Caucasian children at diagnosis of autoimmune hepatitis. *J. Autoimmun.* 24, 79–84. Available from: <https://doi.org/10.1016/j.jaut.2004.11.009>.
- Ohira, H., Abe, K., Takahashi, A., et al., 2015. Autoimmune hepatitis: recent advances in the pathogenesis and new diagnostic guidelines in Japan. *Intern. Med.* 54, 1323–1328. Available from: <https://doi.org/10.2169/internalmedicine.54.4125>.
- Onji, M., Zeniya, M., Yamamoto, K., et al., 2014. Autoimmune hepatitis: diagnosis and treatment guide in Japan, 2013. *Hepatol. Res.* 44, 368–370. Available from: <https://doi.org/10.1111/hepr.12300>.
- Ozaslan, E., Efe, C., Heurgue-Berlot, A., et al., 2014. Factors associated with response to therapy and outcome of patients with primary biliary cirrhosis with features of autoimmune hepatitis. *Clin. Gastroenterol. Hepatol.* 12, 863–869. Available from: <https://doi.org/10.1016/j.cgh.2013.09.021>.
- Paliora, S., Sherr, R.L., Steitz, T.A., et al., 2009. The human SepSecS-tRNASEc complex reveals the mechanism of selenocysteine formation. *Science* 325, 321–325. Available from: <https://doi.org/10.1126/science.1173755>.
- Panayi, V., Froud, O.J., Vine, L., et al., 2014. The natural history of autoimmune hepatitis presenting with jaundice. *Eur. J. Gastroenterol. Hepatol.* 26, 640–645. Available from: <https://doi.org/10.1097/MEG.0000000000000085>.
- Patel, I., Ching Companioni, R., Bansal, R., et al., 2016. Acute hepatitis E presenting with clinical feature of autoimmune hepatitis. *J. Community Hosp. Intern. Med. Perspect.* 6, 33342. Available from: <https://doi.org/10.3402/jchimp.v6.33342>.
- Pavanello, F., Zucca, E., Ghielmini, M., 2017. Rituximab: 13 open questions after 20 years of clinical use. *Cancer Treat. Rev.* 53, 38–46. Available from: <https://doi.org/10.1016/j.ctrv.2016.11.015>.
- Peakman, M., Bevis, L., Mieli-Vergani, G., et al., 1989. Double stranded DNA binding in autoimmune chronic active hepatitis and primary sclerosing cholangitis starting in childhood. *Autoimmunity* 3, 271–280.
- Peiseler, M., Sebode, M., Franke, B., et al., 2012. FOXP3+ regulatory T cells in autoimmune hepatitis are fully functional and not reduced in frequency. *J. Hepatol.* 57, 125–132. doi:10.1016/j.jhep.2012.02.029.
- Peiseler, M., Liebscher, T., Sebode, M., et al., 2017. Efficacy and limitations of budesonide as a second-line treatment for patients with autoimmune hepatitis. *Clin. Gastroenterol. Hepatol.* Available from: <https://doi.org/10.1016/j.cgh.2016.12.040>.
- Pischke, S., Gisa, A., Suneetha, P.V., et al., 2014. Increased HEV seroprevalence in patients with autoimmune hepatitis. *PLoS One* 9, e85330. Available from: <https://doi.org/10.1371/journal.pone.0085330>.
- Podhorzer, A., Paladino, N., Cuarterolo, M.L., et al., 2016. The early onset of type 1 autoimmune hepatitis has a strong genetic influence: role of HLA and KIR genes. *Genes Immun.* 17, 187–192. Available from: <https://doi.org/10.1038/gene.2016.7>.
- Pratico, A.D., Salafia, S., Barone, P., et al., 2013. Type II autoimmune hepatitis and small duct sclerosing cholangitis in a seven years old child: an overlap syndrome? *Hepatitis Mon.* 13, e14452. Available from: <https://doi.org/10.5812/hepatmon.14452>.
- Primo, J., Merino, C., Fernandez, J., et al., 2004. [Incidence and prevalence of autoimmune hepatitis in the area of the Hospital de Sagunto (Spain)]. *Gastroenterol. Hepatol.* 27, 239–243.
- Putra, J., Toor, A., Suriawinata, A.A., 2016. The utility of repeat liver biopsy in autoimmune hepatitis: a series of 20 consecutive cases. *Pathology* 48, 449–453. Available from: <https://doi.org/10.1016/j.jpathol.2016.05.001>.
- Puustinen, L., Boyd, S., Mustonen, H., et al., 2017. Prognostic value of clinical variables and liver histology for development of fibrosis and cirrhosis in autoimmune hepatitis. *Scand. J. Gastroenterol.* 52, 321–327. Available from: <https://doi.org/10.1080/00365521.2016.1253768>.
- Qiu, D., Wang, Q., Wang, H., et al., 2011. Validation of the simplified criteria for diagnosis of autoimmune hepatitis in Chinese patients. *J. Hepatol.* 54, 340–347. Available from: <https://doi.org/10.1016/j.jhep.2010.06.032>. doi: S0168-8278(10)00780-4.[pii].
- Ramachandran, J., Sajith, K.G., Pal, S., et al., 2014. Clinicopathological profile and management of severe autoimmune hepatitis. *Trop. Gastroenterol.* 35, 25–31.
- Richardson, P.D., James, P.D., Ryder, S.D., 2000. Mycophenolate mofetil for maintenance of remission in autoimmune hepatitis in patients resistant to or intolerant of azathioprine. *J. Hepatol.* 33, 371–375.
- Rigopoulou, E.I., Roggenbuck, D., Smyk, D.S., et al., 2012. Asialoglycoprotein receptor (ASGPR) as target autoantigen in liver autoimmunity: lost and found. *Autoimmun. Rev.* 12, 260–269. Available from: <https://doi.org/10.1016/j.autrev.2012.04.005>.
- Rigopoulou, E.I., Zachou, K., Gatselis, N., et al., 2013. Autoimmune hepatitis in patients with chronic HBV and HCV infections: patterns of clinical characteristics, disease progression and outcome. *Ann. Hepatol.* 13, 127–135.

- Roberts, S.K., Therneau, T.M., Czaja, A.J., 1996. Prognosis of histological cirrhosis in type 1 autoimmune hepatitis. *Gastroenterology* 110, 848–857.
- Rodrigues, A.T., Liu, P.M., Fagundes, E.D., et al., 2016. Clinical characteristics and prognosis in children and adolescents with autoimmune hepatitis and overlap syndrome. *J. Pediatr. Gastroenterol. Nutr.* 63, 76–81. Available from: <https://doi.org/10.1097/MPG.0000000000001125>.
- Rodrigues, S., Lopes, S., Magro, F., et al., 2015. Autoimmune hepatitis and anti-tumor necrosis factor alpha therapy: A single center report of 8 cases. *World J. Gastroenterol.* 21, 7584–7588. Available from: <https://doi.org/10.3748/wjg.v21.i24.7584>.
- Saadoun, D., Rosenzwajg, M., Joly, F., et al., 2011. Regulatory T-cell responses to low-dose interleukin-2 in HCV-induced vasculitis. *N. Engl. J. Med.* 365, 2067–2077. Available from: <https://doi.org/10.1056/NEJMoa1105143>.
- Sakaguchi, S., 2000. Regulatory T cells: key controllers of immunologic self-tolerance. *Cell* 101, 455–458.
- Sakaguchi, S., Miyara, M., Costantino, C.M., et al., 2010. FOXP3+ regulatory T cells in the human immune system. *Nat. Rev. Immunol.* 10, 490–500. doi:10.1038/nri2785 nri2785 [pii].
- Sayin, R., Gokgul, A., Ebinc, S., et al., 2016. Clinical overlap of multiple sclerosis and autoimmune hepatitis: three cases. *J. Coll. Phys. Surg., Pak.* JCPSP 26, S45–S47.
- Schramm, C., Wahl, I., Weiler-Normann, C., et al., 2014. Health-related quality of life, depression, and anxiety in patients with autoimmune hepatitis. *J. Hepatol.* 60, 618–624. Available from: <https://doi.org/10.1016/j.jhep.2013.10.035>.
- Schroder, K., Hertzog, P.J., Ravasi, T., et al., 2004. Interferon-gamma: an overview of signals, mechanisms and functions. *J. Leukoc. Biol.* 75, 163–189. Available from: <https://doi.org/10.1189/jlb.0603252>.
- Searle, J., Harmon, B.V., Bishop, C.J., et al., 1987. The significance of cell death by apoptosis in hepatobiliary disease. *J. Gastroenterol. Hepatol.* 2, 77–96.
- Sebode, M., Hartl, J., Vergani, D., et al., 2017. Autoimmune hepatitis: from current knowledge and clinical practice to future research agenda. *Liver Int.* Available from: <https://doi.org/10.1111/liv.13458>.
- Senaldi, G., Lobo-Yeo, A., Mowat, A.P., et al., 1991. Class I and class II major histocompatibility complex antigens on hepatocytes: importance of the method of detection and expression in histologically normal and diseased livers. *J. Clin. Pathol.* 44, 107–114.
- Simmonds, M.J., Gough, S.C., 2004. Genetic insights into disease mechanisms of autoimmunity. *Br. Med. Bull.* 71, 93–113. Available from: <https://doi.org/10.1093/bmb/ldh032>.
- Sogo, T., Fujisawa, T., Inui, A., et al., 2006. Intravenous methylprednisolone pulse therapy for children with autoimmune hepatitis. *Hepatol. Res.* 34, 187–192. Available from: <https://doi.org/10.1016/j.hepres.2005.12.002>.
- Soloway, R.D., Summerskill, W.H., Bagenstoss, A.H., et al., 1972. Clinical, biochemical, and histological remission of severe chronic active liver disease: a controlled study of treatments and early prognosis. *Gastroenterology* 63, 820–833.
- Sonthalia, N., Rathi, P.M., Jain, S.S., et al., 2017. Natural history and treatment outcomes of severe autoimmune hepatitis. *J. Clin. Gastroenterol.* 51, 548–556. Available from: <https://doi.org/10.1097/MCG.0000000000000805>.
- Stokkeland, K., Ludvigsson, J.F., Hultcrantz, R., et al., 2016. Increased risk of preterm birth in women with autoimmune hepatitis—a nationwide cohort study. *Liver Int.* 36, 76–83. Available from: <https://doi.org/10.1111/liv.12901>.
- Strassburg, C.P., Obermayer-Straub, P., Alex, B., et al., 1996. Autoantibodies against glucuronosyltransferases differ between viral hepatitis and autoimmune hepatitis. *Gastroenterology* 111, 1576–1586.
- Strassburg, C.P., Alex, B., Zindy, F., et al., 1996. Identification of cyclin A as a molecular target of antinuclear antibodies (ANA) in hepatic and non-hepatic autoimmune diseases. *J. Hepatol.* 25, 859–866.
- Takeda, K., Hayakawa, Y., Van Kaer, L., et al., 2000. Critical contribution of liver natural killer T cells to a murine model of hepatitis. *Proc. Natl. Acad. Sci. U.S.A.* 97, 5498–5503.
- Tan, E.M., Feltkamp, T.E., Smolen, J.S., et al., 1997. Range of antinuclear antibodies in “healthy” individuals. *Arthritis Rheum.* 40, 1601–1611. Available from: [https://doi.org/10.1002/1529-0131\(199709\)40:9<1601::AID-ART9>3.0.CO;2-T](https://doi.org/10.1002/1529-0131(199709)40:9<1601::AID-ART9>3.0.CO;2-T).
- Tanaka, T., Zhang, W., Sun, Y., et al., 2017. Autoreactive monoclonal antibodies from patients with primary biliary cholangitis recognize environmental xenobiotics. *Hepatology* 66, 885–895. Available from: <https://doi.org/10.1002/hep.29245>.
- Tansel, A., Katz, L.H., El-Serag, H.B., et al., 2017. Incidence and determinants of hepatocellular carcinoma in autoimmune hepatitis: a systematic review and meta-analysis. *Clin. Gastroenterol. Hepatol.* 15, 1207–1217.e4. Available from: <https://doi.org/10.1016/j.cgh.2017.02.006>.
- Tarbell, K.V., Yamazaki, S., Olson, K., et al., 2004. CD25+ CD4+ T cells, expanded with dendritic cells presenting a single autoantigenic peptide, suppress autoimmune diabetes. *J. Exp. Med.* 199, 1467–1477. Available from: <https://doi.org/10.1084/jem.20040180>. jem.20040180 [pii].
- Taubert, R., Hardtke-Wolenski, M., Noyan, F., et al., 2014. Intrahepatic regulatory T cells in autoimmune hepatitis are associated with treatment response and depleted with current therapies. *J. Hepatol.* 61, 1106–1114. Available from: <https://doi.org/10.1016/j.jhep.2014.05.034>.
- Tenca, A., Farkkila, M., Jalanko, H., et al., 2016. Environmental risk factors of pediatric-onset primary sclerosing cholangitis and autoimmune hepatitis. *J. Pediatr. Gastroenterol. Nutr.* 62, 437–442. Available from: <https://doi.org/10.1097/MPG.0000000000000995>.
- Terrabuio, D.R., Abrantes-Lemos, C.P., Carrilho, F.J., et al., 2009. Follow-up of pregnant women with autoimmune hepatitis: the disease behavior along with maternal and fetal outcomes. *J. Clin. Gastroenterol.* 43, 350–356. Available from: <https://doi.org/10.1097/MCG.0b013e318176b8c5>.
- Thomas-Dupont, P., Remes-Troche, J.M., Izquierre-Hernandez, I.Y., et al., 2016. Elevated circulating levels of IL-21 and IL-22 define a cytokine signature profile in type 2 autoimmune hepatitis patients. *Ann. Hepatol.* 15, 550–558.
- Tiegs, G., Hentschel, J., Wendel, A.A., 1992. T cell-dependent experimental liver injury in mice inducible by concanavalin A. *J. Clin. Invest.* 90, 196–203. Available from: <https://doi.org/10.1172/JCI115836>.
- Tiniakos, D.G., Brain, J.G., Bury, Y.A., 2015. Role of histopathology in autoimmune hepatitis. *Dig. Dis.* 33 (Suppl 2), 53–64. Available from: <https://doi.org/10.1159/000440747>.
- Tucker, S.M., Jonas, M.M., Perez-Atayde, A.R., 2015. Hyaline droplets in Kupffer cells: a novel diagnostic clue for autoimmune hepatitis. *Am. J. Surg. Pathol.* 39, 772–778. Available from: <https://doi.org/10.1097/PAS.0000000000000395>.

- Ustundag, G., Kuloglu, Z., Kirsacioglu, C.T., et al., 2008. Complete regression of cirrhosis after immunosuppressive treatment in autoimmune hepatitis. *Pediatr. Int.* 50, 711–713. Available from: <https://doi.org/10.1111/j.1442-200X.2008.02714.x>. DOI: PED2714 [pii].
- Van Thiel, D.H., Wright, H., Carroll, P., et al., 1995. Tacrolimus: a potential new treatment for autoimmune chronic active hepatitis: results of an open-label preliminary trial. *Am. J. Gastroenterol.* 90, 771–776.
- Vento, S., Hegarty, J.E., Bottazzo, G., et al., 1984. Antigen specific suppressor cell function in autoimmune chronic active hepatitis. *Lancet* 1, 1200–1204.
- Verdonk, R.C., Lozano, M.F., van den Berg, A.P., et al., 2016. Bile ductal injury and ductular reaction are frequent phenomena with different significance in autoimmune hepatitis. *Liver Int.* 36, 1362–1369. Available from: <https://doi.org/10.1111/liv.13083>.
- Vergani, D., Choudhuri, K., Bogdanos, D.P., et al., 2002. Pathogenesis of autoimmune hepatitis. *Clin. Liver Dis.* 6, 439–449.
- Vergani, D., Alvarez, F., Bianchi, F.B., et al., 2004. Liver autoimmune serology: a consensus statement from the committee for autoimmune serology of the International Autoimmune Hepatitis Group. *J. Hepatol.* 41, 677–683. Available from: <https://doi.org/10.1016/j.jhep.2004.08.002>.
- Vierling, J.M., 2015. Autoimmune hepatitis and overlap syndromes: diagnosis and management. *Clin. Gastroenterol. Hepatol.* 13, 2088–2108. Available from: <https://doi.org/10.1016/j.cgh.2015.08.012>.
- Villalta, D., Girolami, E., Alessio, M.G., et al., 2016. Autoantibody profiling in a cohort of pediatric and adult patients with autoimmune hepatitis. *J. Clin. Lab. Anal.* 30, 41–46. Available from: <https://doi.org/10.1002/jcla.21813>.
- Waldenstrom JVS, 1950. Blutproteine und Nahrungseiweiß. *Deutsch Z Verdau Stoffwechselk* 15, 113–119.
- Wang, K.K., Czaja, A.J., Beaver, S.J., et al., 1989. Extrahepatic malignancy following long-term immunosuppressive therapy of severe hepatitis B surface antigen-negative chronic active hepatitis. *Hepatology* 10, 39–43.
- Weiler-Normann, C., Lohse, A.W., 2016. Nonalcoholic fatty liver disease in patients with autoimmune hepatitis: further reason for teeth GNASHing? *Dig. Dis. Sci.* 61, 2462–2464. Available from: <https://doi.org/10.1007/s10620-016-4258-3>.
- Weiler-Normann, C., Wiegard, C., Schramm, C., et al., 2009. A case of difficult-to-treat autoimmune hepatitis successfully managed by TNF-alpha blockade. *Am. J. Gastroenterol.* 104, 2877–2878. Available from: <https://doi.org/10.1038/ajg.2009.433>. doi: ajg2009433 [pii].
- Weiler-Normann, C., Schramm, C., Quaas, A., et al., 2013. Infliximab as a rescue treatment in difficult-to-treat autoimmune hepatitis. *J. Hepatol.* 58, 529–534. Available from: <https://doi.org/10.1016/j.jhep.2012.11.010>.
- Wen, L., Peakman, M., Mieli-Vergani, G., et al., 1992. Elevation of activated gamma delta T cell receptor bearing T lymphocytes in patients with autoimmune chronic liver disease. *Clin. Exp. Immunol.* 89, 78–82.
- Werner, M., Almer, S., Prytz, H., et al., 2009. Hepatic and extrahepatic malignancies in autoimmune hepatitis. A long-term follow-up in 473 Swedish patients. *J. Hepatol.* 50, 388–393. Available from: <https://doi.org/10.1016/j.jhep.2008.08.022>.
- Whalley, S., Puvanachandra, P., Desai, A., et al., 2007. Hepatology outpatient service provision in secondary care: a study of liver disease incidence and resource costs. *Clin. Med.* 7, 119–124.
- Wies, I., Brunner, S., Henninger, J., et al., 2000. Identification of target antigen for SLA/LP autoantibodies in autoimmune hepatitis. *Lancet* 355, 1510–1515.
- Wong, G.W., Heneghan, M.A., 2015. Association of extrahepatic manifestations with autoimmune hepatitis. *Dig. Dis.* 33 (Suppl 2), 25–35. Available from: <https://doi.org/10.1159/000440707>.
- Wong, G.W., Yeong, T., Lawrence, D., et al., 2017. Concurrent extrahepatic autoimmunity in autoimmune hepatitis: implications for diagnosis, clinical course and long-term outcomes. *Liver Int.* 37, 449–457. Available from: <https://doi.org/10.1111/liv.13236>.
- Wong, R.J., Gish, R., Frederick, T., et al., 2011. Development of hepatocellular carcinoma in autoimmune hepatitis patients: a case series. *Dig. Dis. Sci.* 56, 578–585. Available from: <https://doi.org/10.1007/s10620-010-1444-6>.
- Wood, I.J., King, W.E., et al., 1948. Non-suppurative hepatitis; a study of acute and chronic forms with special reference to biochemical and histological changes. *Med. J. Aust.* 1, 249–261.
- Woynarowski, M., Nemeth, A., Baruch, Y., et al., 2013. Budesonide versus prednisone with azathioprine for the treatment of autoimmune hepatitis in children and adolescents. *J. Pediatr.* 163, 1347–1353.e1. Available from: <https://doi.org/10.1016/j.jpeds.2013.05.042>.
- Yada, N., Kudo, M., Chung, H., et al., 2013. Autoimmune hepatitis and immunoglobulin G4-associated autoimmune hepatitis. *Dig. Dis.* 31, 415–420. Available from: <https://doi.org/10.1159/000355238>.
- Yamagiwa, S., Kamimura, H., Takamura, M., et al., 2014. Presence of antibodies against self human leukocyte antigen class II molecules in autoimmune hepatitis. *Int. J. Med. Sci.* 11, 850–856. Available from: <https://doi.org/10.7150/ijms.8633>.
- Yang, F., Wang, Q., Bian, Z., et al., 2015. Autoimmune hepatitis: east meets west. *J. Gastroenterol. Hepatol.* 30, 1230–1236. Available from: <https://doi.org/10.1111/jgh.12952>.
- Yeoman, A.D., Al-Chalabi, A.T., Karani, J.B., et al., 2008. Evaluation of risk factors in the development of HCC in AIH: implications for follow-up and screening. *Hepatology Volume* 9999.
- Yeoman, A.D., Westbrook, R.H., Zen, Y., et al., 2011. Early predictors of corticosteroid treatment failure in icteric presentations of autoimmune hepatitis. *Hepatology* 53, 926–934. Available from: <https://doi.org/10.1002/hep.24141>.
- Yeoman, A.D., Westbrook, R.H., Zen, Y., et al., 2014. Prognosis of acute severe autoimmune hepatitis (AS-AIH): the role of corticosteroids in modifying outcome. *J. Hepatol.* 61, 876–882. Available from: <https://doi.org/10.1016/j.jhep.2014.05.021>.
- Yilmaz, B., Unlu, O., Evcen, R., et al., 2016. Acute onset seronegative autoimmune hepatitis: are simplified diagnostic criteria sufficient? *Eur. J. Gastroenterol. Hepatol.* 28, 607–608. Available from: <https://doi.org/10.1097/MEG.0000000000000580>.
- Yoshioka, Y., Taniai, M., Hashimoto, E., et al., 2014. Clinical profile of primary biliary cirrhosis with features of autoimmune hepatitis: Importance of corticosteroid therapy. *Hepatol. Res.* 44, 947–955. Available from: <https://doi.org/10.1111/hepr.12210>.
- Yoshizawa, K., Joshita, S., Matsumoto, A., et al., 2016. Incidence and prevalence of autoimmune hepatitis in the Ueda area, Japan. *Hepatol. Res.* 46, 878–883. Available from: <https://doi.org/10.1111/hepr.12639>.
- Ytting, H., Larsen, F.S., 2015. Everolimus treatment for patients with autoimmune hepatitis and poor response to standard therapy and drug alternatives in use. *Scand. J. Gastroenterol.* 50, 1025–1031. Available from: <https://doi.org/10.3109/00365521.2014.998271>.
- Yuksel, M., Wang, Y., Tai, N., et al., 2015. A novel “humanized mouse” model for autoimmune hepatitis and the association of gut microbiota with liver inflammation. *Hepatology* 62, 1536–1550. Available from: <https://doi.org/10.1002/hep.27998>.

- Yuksel, M., Xiao, X., Tai, N., et al., 2016. The induction of autoimmune hepatitis in the human leucocyte antigen-DR4 non-obese diabetic mice autoimmune hepatitis mouse model. *Clin. Exp. Immunol.* 186, 164–176. Available from: <https://doi.org/10.1111/cei.12843>.
- Zachou, K., Gatselis, N.K., Arvaniti, P., et al., 2016. A real-world study focused on the long-term efficacy of mycophenolate mofetil as first-line treatment of autoimmune hepatitis. *Aliment. Pharmacol. Ther.* 43, 1035–1047. Available from: <https://doi.org/10.1111/apt.13584>.
- Zenouzi, R., Lohse, A.W., 2014. Long-term outcome in PSC/AIH “overlap syndrome”: does immunosuppression also treat the PSC component? *J. Hepatol.* 61, 1189–1191. Available from: <https://doi.org/10.1016/j.jhep.2014.08.002>.
- Zhao, L., Tang, Y., You, Z., et al., 2011. Interleukin-17 contributes to the pathogenesis of autoimmune hepatitis through inducing hepatic interleukin-6 expression. *PLoS One* 6, . Available from: <https://doi.org/10.1371/journal.pone.0018909>. e18909.
- Zhao, Y., Zhang, Y., Liu, Y.M., et al., 2011. Identification of T cell epitopes on soluble liver antigen in Chinese patients with auto-immune hepatitis. *Liver Int.* 31, 721–729. Available from: <https://doi.org/10.1111/j.1478-3231.2011.02487.x>.
- Zhu, B., You, S.L., Wan, Z.H., et al., 2014. Clinical characteristics and corticosteroid therapy in patients with autoimmune-hepatitis-induced liver failure. *World J. Gastroenterol.* 20, 7473–7479. Available from: <https://doi.org/10.3748/wjg.v20.i23.7473>.
- Zimmerman, H.J., Heller, P., Hill, R.P., 1951. Extreme hyperglobulinemia in subacute hepatic necrosis. *N. Engl. J. Med.* 244, 245–249. Available from: <https://doi.org/10.1056/NEJM195102152440702>.
- Zizzo, A.N., Valentino, P.L., Shah, P.S., et al., 2017. Second-line agents in pediatric patients with autoimmune hepatitis: a systematic review and meta-analysis. *J. Pediatr. Gastroenterol. Nutr.* 65, 6–15. Available from: <https://doi.org/10.1097/MPG.0000000000001530>.

# Primary Biliary Cholangitis

Atsushi Tanaka<sup>1</sup> and M. Eric Gershwin<sup>2</sup>

<sup>1</sup>Department of Medicine, Teikyo University, School of Medicine, Tokyo, Japan <sup>2</sup>Division of Rheumatology, Allergy and Clinical Immunology; The Jack and Donald Endowed Professor; University of California School of Medicine, CA, United States

## OUTLINE

<b>Introduction</b> <b>Changing Nomenclature for Primary Biliary Cholangitis—From “Cirrhosis” to “Cholangitis”</b> <b>Epidemiology</b> <b>Etiology</b> Antimitochondrial Autoantibody Epitopes CD4+ and CD8+ T-Cell Epitopes Why Biliary Epithelial Cells?—The “ABC” of Primary Biliary Cholangitis Genetic Predisposition Environmental Triggering Factors <b>Animal Models of Primary Biliary Cholangitis</b> Spontaneous Murine Models Xenobiotics-Triggered Murine Models Adenylate Uridine-Rich Element Del <sup>-/-</sup> Mice as a Novel Primary Biliary Cholangitis Model <b>Diagnosis</b> Serum Biochemistry and Imaging Studies Serological Testing Histopathology	<b>1149</b> <b>Treatment</b> Ursodeoxycholic Acid Obeticholic Acid Fibrates Ileal Bile Acid Transporter Inhibitors <b>1150</b> <b>Management of Symptoms and Extrahepatic Manifestations</b> Fatigue Pruritus <b>1151</b> <b>Disorders Associated With Primary Biliary Cholangitis</b> Primary Biliary Cholangitis With Features With Autoimmune Hepatitis Sicca Syndrome Osteopenia and Osteoporosis Hyperlipidemia and Metabolic Syndrome Hepatocellular Carcinoma <b>1152</b> <b>1153</b> <b>1154</b> <b>1155</b> <b>1156</b> <b>1157</b> <b>1158</b> <b>Stratification of the Risk for Progression</b> Stratification at Baseline Stratification During Treatment <b>Perspectives</b> <b>References</b>	<b>1159</b> <b>1159</b> <b>1159</b> <b>1160</b> <b>1160</b> <b>1160</b> <b>1160</b> <b>1161</b> <b>1161</b> <b>1162</b> <b>1162</b> <b>1162</b> <b>1162</b> <b>1163</b> <b>1163</b> <b>1163</b> <b>1164</b> <b>1164</b>
--	---	--

## INTRODUCTION

Primary biliary cholangitis (PBC), formally known as primary biliary cirrhosis, is a chronic cholestatic liver disease that can affect adult women of all ages but is most common in the middle years of life. It presents as a chronic nonsuppurative destructive cholangitis (CNSDC) with granuloma formation in the liver, and degeneration and necrosis of biliary epithelial cells (BECs) elicit destructive changes and disappearance of small- or

medium-sized intrahepatic bile ducts, leading to chronic and progressive cholestasis. Although the etiology of PBC has not been fully elucidated, robust evidence indicate that autoimmune reactions against intrahepatic BECs play a critical role in the pathogenesis of the disease. Indeed, PBC is considered a model autoimmune disease because of detection of disease-specific autoantibodies (i.e., antimitochondrial autoantibodies (AMAs)), dense infiltration of mononuclear cells into the bile ducts, and a high prevalence of autoimmune diseases as comorbidities. Whereas patients with PBC frequently lack subjective symptoms and the disease is incidentally detected during random blood testing, a substantial number of patients experience a variety of symptoms, including pruritus, fatigue, dryness, and body pain. Other autoimmune diseases such as rheumatoid arthritis, Sjogren's syndrome, and chronic thyroiditis frequently coexist in patients with PBC. Jaundice and other decompensating events of the liver develop as progressive features, eventually resulting in liver failure and the need for liver transplantation (LT). Until recently, ursodeoxycholic acid (UDCA) had been the only approved drug for PBC and clinical trials demonstrated that the administration of UDCA can extend LT-free survival. On the other hand, 30% of the patients with PBC are refractory to UDCA and second-line therapeutic options are strongly needed. In 2016, obeticholic acid (OCA), a farnesoid X receptor (FXR) agonist, was approved for UDCA-refractory or UDCA-intolerant patients; however, the efficacy and safety of OCA are still unsatisfactory. In the near future, an individualized treatment policy based on stratification of the risk for progression should be a goal in the treatment of PBC.

## CHANGING NOMENCLATURE FOR PRIMARY BILIARY CHOLANGITIS— FROM “CIRRHOSIS” TO “CHOLANGITIS”

In 1851 a patient presenting with symptoms resembling PBC was first described in the literature ([Addison and Gull, 1851](#)). The term “primary biliary cirrhosis” first appeared in the title of a published article in 1949 ([Dauphinee and Sinclair, 1949](#)) and was described by [Ahrens et al. \(1950\)](#). Most early descriptions of PBC involved patients at the cirrhotic stage, with jaundice, ascites, and variceal bleeding, and therefore the nomenclature of cirrhosis, which refers to an end-stage liver disease, was correct. However, [Sherlock \(1959\)](#) had already noted that this name should be changed because many patients were free of cirrhosis. In 1965 Hans Popper et al. also suggested that the term “primary biliary cirrhosis” is actually a misnomer, as neither septa nor nodules are present, and suggested “CNSDC” instead ([Rubin et al., 1965](#)). Thereafter, the introduction of biochemistry and AMA tests into clinical settings enabled diagnosing PBC at earlier stages, and the establishment of UDCA as a first-line treatment drug prevented progression to cirrhosis. The serious gap between the disease and its misnomer became wider, and the term “cirrhosis” became not merely an inaccuracy but an active stigma for patients. In 2014 during the 2nd European Association for the Study of the Liver (EASL) monothematic conference on primary biliary cirrhosis, experts gathering from different parts of the world agreed that (1) the name “primary biliary cirrhosis” should be changed and (2) the alternative should be “primary biliary cholangitis,” keeping the acronym “PBC.” The EASL and the American Association for the Study of Liver Diseases governing boards approved this agreement in 2014 and 2015, respectively ([Beuers et al., 2015a, 2015b, 2015c, 2015d, 2015e, 2015f, 2015g, 2015h](#)). The Asian Pacific Association for the Study of Liver (APASL) also officially approved this decision, and the new nomenclature “PBC” is currently used in the official journal of the APASL ([Tanaka et al., 2016](#)). The new name “primary biliary cholangitis,” although still imperfect, is therefore used for this disease worldwide.

## EPIDEMIOLOGY

A systematic review by [Boonstra et al. \(2012\)](#) identified 29 epidemiological studies of PBC and indicated that the incidence and point prevalence ranged from 0.39 to 5.8 per 100,000 populations and from 1.91 to 40.2 per 100,000 populations, respectively. Both the incidence and point prevalence greatly varied depending on the study, probably attributable to either a true epidemiological difference among regions or periods or the study design for case finding or ascertainment. In [Table 58.1](#), the results of epidemiological studies published after 2010 are summarized. It is of note that the epidemiological data of PBC are nearly exclusively limited to countries in Europe, Northern America, and Australia. A study from South Korea including a large number of patients provided a relatively low prevalence compared with those reported in Europe and North America, indicating that the Asian population may be genetically protected from PBC. Population-based studies from other Asian countries are awaited to solve this question. Furthermore, it is notable that the male-to-female ratio seems to be changing in recent decades and the number of male patients with PBC is increasing. Previous large-scale population-based studies demonstrated that the male-to-female ratio was almost 1:10 ([Danielsson et al., 1990; Hamlyn et al., 1983](#);

**TABLE 58.1** Incidence and Prevalence of Primary Biliary Cholangitis (Published After 2010)

Country	No. of patients	Incidence (95% CI)	Prevalence (95% CI)	Male (%)	Year
<b>EUROPE AND AMERICA</b>					
Iceland (Baldursdottir et al., 2012)	168	2.5	38.3	18	2012
Northeast England (McNally et al., 2014)	982	4.51 (4.11–4.91)	NA	10	2014
Netherlands (Boonstra et al., 2014)	992	1.1	13.2	14	2014
North Italy (Lleo et al., 2016)	2970	1.67 (1.44–1.91)	11.1 (10.9–11.3)	33	2016
Denmark (Lleo et al., 2016)	722	1.14 (1.06–1.23)	11.5 (11.3–11.8)	21	2016
USA (Wisconsin) (Kanth et al., 2017)	79	4.9	NA	5	2017
Greece (Gatselis et al., 2017)	482	NA	58.2	13.5	2017
<b>ASIA AND PACIFIC</b>					
Southern China (Liu et al., 2010a)	4	NA	49.2 (12.8–109.3)	25	2010
New Zealand (Ngu et al., 2012)	71	0.8 (0.1–1.6)	9.9 (7.1–12.7)	8	2012
South Korea (Kim et al., 2016)	2824	0.86	4.75	16	2016

CI, Confidence interval; NA, not applicable.

Kim et al., 2000; Myszor and James, 1990). However, recent data suggested that the proportion of male patients have increased to around 15%, indicating a male-to-female ratio of 1:6 (Boonstra et al., 2012; Baldursdottir et al., 2012; Gatselis et al., 2017; Kim et al., 2016). Surprisingly, Lleo et al. (2016) reported that male patients comprise 21% of PBC cases in Denmark, and even up to 33% in Lombardia, Italy. This alteration may be explained by either a true increase of male patients owing to environmental factors or the increased recognition of the disease even in the male population.

Another controversial issue concerning the epidemiology is whether the prevalence of PBC is increasing. Two serial epidemiological studies were performed in Australia. The first population-based study was published in 1995, giving an estimated prevalence of 1.91 per 100,000 persons in 1990 and 1991 (Watson et al., 1995). A second study was published in 2004, indicating a prevalence of 5.1 per 100,000 in 1990–2002 (Sood et al., 2004). The increased prevalence of PBC in recent years was also demonstrated by investigators from the Netherlands (Boonstra et al., 2014) and Lombardia, Italy (Lleo et al., 2016). In contrast, the prevalence remained stable in Iceland (Baldursdottir et al., 2012), Denmark (Lleo et al., 2016), and northeast England (McNally et al., 2014).

## ETIOLOGY

The first finding to unravel the etiological myth of PBC was the molecular identification of autoantigens targeted by AMAs, which are exclusively detected in patients with PBC, leading to the determination of T- and B-cell epitopes. PBC is a multifactorial disease and is considered to be caused by the interaction of both genetic background and environmental triggers. In other words, PBC results from a combination of “bad genes and bad luck,” that is, individuals with a genetic predisposition to the disease develop PBC as a consequence of environmental triggering effects. Recent innovative technologies including genome-wide association studies (GWAS) and traditional case-control studies have identified a number of genetic and environmental factors contributing to susceptibility. Knowledge on the biology of BECs, the major target cells in PBC, has helped in dissecting the interactions between immune cells and BECs. Finally, efforts to establish animal models of PBC have generated novel findings.

### Antimitochondrial Autoantibody Epitopes

AMAs are the most disease-specific autoantibodies in human immunopathology and are detected in 90%–95% of the patients with PBC (Van de Water et al., 1988; Oertelt et al., 2007). Although a high titer of autoantibody in the sera of patients with PBC was observed by Mackay (1958) more than 60 years ago, the

**TABLE 58.2** Molecular Mimicry and Immunodominant Epitopes of Human PDC-E2 155–185<sup>a</sup>

Human PDC-E2	KVGEKLSEGDLAELIETDKATIGFEVQEEGY
B cell	KVGEKLSEGDLAELIETDKATIGFEVQEEGY
CD4 + T cell	KVGEKLSEGDLAELIETDKATIGFEVQEEGY
CD8 + T cell	KVGEKLSEGDLAELIETDKATIGFEVQEEGY
<i>E. coli</i> PDC-E2 <sup>b</sup>	- - d - veaeqs - itv - g - - smevpspqa - I

<sup>a</sup>K denotes lysine 173, which is the attachment site of lipoic acid.

<sup>b</sup>Identical amino acids to human PDC-E2 are denoted as “-”

immunodominant epitopes of AMAs were not determined until the identification by Gershwin et al. (1987) of the pyruvate dehydrogenase complex E2 subunit (PDC-E2) as the mitochondrial autoantigen of PBC by cDNA cloning. AMAs recognize a family of enzymes located at the inner membrane of the mitochondria, named as the 2-oxo-acid dehydrogenase complex (2-OADC), which mainly includes PDC-E2, branched-chain 2-OADC (BCOADC-E2), and 2-oxo-glutaric acid dehydrogenase complex (OGDC-E2), and dihydrolipoamide dehydrogenase-binding protein (E3BP) (Dubel et al., 1999). All of these E2 enzymes have a common structure consisting of an N-terminal domain with a single or multiple attachment sites to a lysine (<sup>173</sup>K in mammalian PDC-E2) of lipoic acid (LA) (Table 58.2). The dominant epitope sites recognized by AMAs are in contiguity with the LA attachment site(s) as the lipoyl domains of these target antigens (Leung et al., 1995; Moteki et al., 1996a; Surh et al., 1990). The amino acid residues critical to maintaining the structural integrity of the PDC-E2 lipoyl domain have been revealed by site-directed mutagenesis (Wang et al., 2013). The high specificity of AMAs for PBC suggests that AMAs are not simply serological markers for diagnosis but are important in the immunopathology of PBC.

### CD4+ and CD8+ T-Cell Epitopes

The histological signature of PBC includes dense infiltration of mononuclear cells in the portal tracts near small- or medium-sized bile ducts. Immunohistochemical examination of these lymphocytes reveals a predominance of CD4+ and CD8+ T cells with B and natural killer cells (Kita et al., 2002b; Shimoda et al., 2011). BECs and hepatocytes in the liver of patients with PBC also express large amounts of human leukocyte antigen (HLA) class I and II molecules (Bjorkland et al., 1991; Krams et al., 1990). Therefore both CD4+ and CD8+ autoreactive T cells play a crucial role in the pathogenesis of PBC. In the case of CD4+ T cells, Shimoda et al. (1995) established HLA DRB4 0101-restricted PDC-E2-specific T-cell clones from the peripheral blood of patients with PBC and mapped immunodominant T-cell epitopes as PDC-E2 peptide 163–176 (GDLLEIETDKATI), which overlapped with the B-cell epitope of human PDC-E2 (Table 58.2). Importantly, the frequency of PDC-E2-specific CD4+ T cells was 100- to 150-fold higher in the liver and hilar lymph nodes than in peripheral blood (Shimoda et al., 1998). Our laboratory also characterized an MHC class I (HLA-A2)-restricted epitope for CD8+ T cells as PDC-E2 peptide 159–167 (KLSEGDLA), which again mapped to the same region of the autoantigen PDC-E2 (Table 58.2) (Kita et al., 2002a). Taken together, AMAs and autoreactive helper and cytotoxic T cells contain a shared peptide sequence of the inner lipoyl domain of human PDC-E2.

### Why Biliary Epithelial Cells?—The “ABC” of Primary Biliary Cholangitis

PDC-E2 is a ubiquitous protein located in nearly all nucleated cells in the human body, and it remains unclear why autoreactive T cells specific for PDC-E2 elicit cytotoxicity against only BECs in the liver. In this regard, it should be noted that PBC recurs even after LT, indicating that the immunopathological susceptibility of BECs in PBC is not MHC specific but a general feature shared with autologous BECs. To answer this question, Lleo et al. demonstrated that only human intrahepatic BECs (HIBECs) could maintain PDC-E2 immunologically intact within apoptotic blebs (apoptoses) during apoptosis, but not control epithelial cells. This supports data showing that AMA-containing sera react with PDC-E2 on apoptotic BECs without permeabilization (Lleo et al., 2009). Lleo et al. (2010) then examined the ability of BECs to induce cytokine secretion from mature monocyte-derived macrophages, with and without AMAs, and observed intense inflammatory cytokine

production in the presence of a unique triad consisting of BEC apoptoses, macrophages from patients with PBC, and AMAs. Macrophages from healthy controls did not produce inflammatory cytokines, even when cocultured with apoptotic bodies from HIBECs and AMAs. Thus we propose that A (AMA, apoptote, and APC), B (blebs from apoptotic BECs), and C (complex formation and cytokine secretion) constitute the crucial triad in the inflammatory cascade of PBC.

## Genetic Predisposition

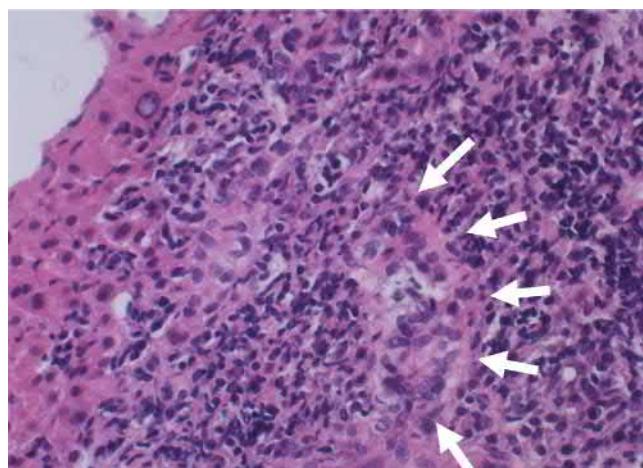
Robust evidence indicate a contribution of genetic background to the development of PBC. An increased prevalence of patients with PBC among first-degree relatives and siblings of an index patient has been repeatedly demonstrated, known as familial clustering of PBC (Abu-Mouch et al., 2003; Corpechot et al., 2010; Mantaka et al., 2012; Yanagisawa et al., 2010). The concordance rate of PBC is 63% in monozygotic twins, higher than that of other autoimmune diseases (Selmi et al., 2004b).

Concerning genetic predisposition, HLA class II alleles are found to be associated with the development of PBC by case-control studies, in particular *DRB1\*08*, *DRB1\*0801*, *DRB1\*0803*, *DRB1\*14*, and *DPB1\*0301* as susceptibility alleles and *DRB1\*11*, *DRB1\*13* as protective alleles, and, as expected, these associations vary depending on the populations studied (Donaldson et al., 2006; Invernizzi et al., 2012; Mella et al., 1995; Onishi et al., 1994). Recent GWAS from North America, European countries, Japan, and China have also identified that HLA alleles possess the strongest link with susceptibility of PBC and additionally demonstrated that more than 40 non-HLA alleles contribute to susceptibility to PBC (Cordell et al., 2015; Hirschfield et al., 2010, 2009, 2012; Juran et al., 2012; Kawashima et al., 2017; Liu et al., 2012, 2010c; Mells et al., 2011; Nakamura et al., 2012; Qiu et al., 2017; Tang et al., 2017). Although risk alleles differ among studies and populations, pathways that involve identified genes are largely shared among populations and are represented as antigen presentation and production of interleukin (IL)-12 (*IRF5*, *SOCS1*, *TNFAIP3*, *NFKB*, *IL-12A*), activation of T cells and interferon (IFN)- $\gamma$  production (*TNFSF15*, *IL12R*, *TYK2*, *STAT4*, *SOCS1*, *NFKB*, *TNFAIP3*), and activation of B cells and production of immunoglobulins (Igs) (*POU2AF1*, *SPIB*, *PRKCB*, *IKZF3*, *ARID3A*). These immune pathways are therefore assumed to be important in the pathogenesis of PBC. However, as with other autoimmune diseases, GWAS have remained disappointing and no “smoking gun” has been identified, leading to the suggestion that epigenetics may play a role.

## Environmental Triggering Factors

Although studies of monozygotic twins demonstrated a high concordance rate, recent epidemiological studies revealed a relatively low risk of developing PBC in first-degree relatives of the indicated patient during 8 years of follow-up, suggesting that genetic predisposition does not define the risk of PBC (Gulamhusein et al., 2016). Large-scale case-control studies have consistently found an association of urinary tract infections and cigarette smoking with PBC (Corpechot et al., 2010; Burroughs et al., 1984; Gershwin et al., 2005; Howel et al., 2000). Bacterial infection may have an impact on the etiology of PBC because PDC-E2, which is an immunodominant target of AMA, has a molecular mimic between human PDC-E2 and *Escherichia coli* PDC-E2 (Table 58.2). In particular, the ExDK sequence, which was reported to be the essential sequence of human PDC-E2 for recognition of CD4+ PDC-E2-specific T cells, is entirely shared by both human and *E. coli* PDC-E2 (Shimoda et al., 1995); thus *E. coli* infection may trigger the breaking of immunological tolerance against human PDC-E2. Another candidate bacterium that may be involved in the etiology of PBC through cross-reactivity is *Novosphingobium aromaticivorans*, a ubiquitous xenobiotic-metabolizing bacterium, because the sera of patients with PBC are highly reactive with lipoylated bacterial proteins from *N. aromaticivorans* (Selmi et al., 2003).

Case-control studies also suggest that frequent use of nail polish is associated with an increased susceptibility to PBC (Gershwin et al., 2005). Furthermore, a geographically uneven distribution of patients with PBC in a particular region is reported, especially near toxic waste sites (McNally et al., 2014; Ala et al., 2006; Prince et al., 2001). These epidemiological data, along with the known crucial role of AMAs in the immunopathology of PBC, prompted researchers to identify environmental mimotopes in the form of xenobiotics. A detailed, quantitative structure-activity relationship analysis with 107 potential xenobiotic mimics coupled to the lysine residue of the immunodominant 15-amino-acid peptide of the PDC-E2 inner lipoyl domain revealed that 2-octynamide, the conjugate derived from 2-octynoic acid (2-OA) present in cosmetics, lipsticks, and some chewing gums, was unique in both its quantitative structure-activity relationship analysis and reactivity with PBC sera (Amano et al., 2005).



**FIGURE 58.1** Chronic nonsuppurative destructive cholangitis in primary biliary cholangitis (arrow, hematoxylin, and eosin staining).

Furthermore, another xenobiotic, 2-nonyamide, provided an optimal chemical structure of the xenobiotics-modified epitope, which demonstrated enhanced recognition by AMA-positive PBC sera (Rieger et al., 2006). Indeed, significant molecular mimicry between lipoamide and 2-nonyamide was observed (Fig. 58.1). These findings illustrate that xenobiotic modification of PDC-E2 with chemicals abundantly found in daily life plays a role in generating immunogenic neoantigens and breaking tolerance in PBC. Cross-reactive monoclonal antibodies with both native PDC-E2 and 2-OA also recognize LA (Tanaka et al., 2017), further supporting the hypothesis that xenobiotically modified LA is the initial target of autoimmunity in PBC.

Genome-wide epigenetic analysis identified significant differences for methylation profiles, copy number variation, and gene expression in three monozygotic twins and eight sibling pairs discordant for PBC (Selmi et al., 2014). Moreover, aberrant demethylation on the CXCR3 promoter of the X chromosome was noted in patients with PBC (Lleo et al., 2015). These findings are still only descriptive, and further studies are needed to unravel the etiological implications of epigenetics. Finally, a role of the gut microbiota is now suggested, and dysbiosis was found in patients with PBC and, interestingly, partially resolved with UDCA treatment (Tang et al., 2017).

## ANIMAL MODELS OF PRIMARY BILIARY CHOLANGITIS

In addition to in vitro studies, murine models are important for understanding the etiology and natural history of PBC. Patients with newly diagnosed PBC are well past the onset of loss of tolerance, and there is likely a long latency period between the appearance of autoantibodies and clinical disease. Animal models that reflect many important aspects of the disease are therefore needed to explore the initiating events and interactions between genetic and environmental factors. The animal model should have the same physiological mechanisms observed in human PBC, such as female predominance, chronic cholestasis, AMA production, histological features including lymphocyte infiltration into the liver, and bile duct involvement. In particular, recognition of a strong sex predominance (females) is essential in understanding PBC. Sex hormones, X-linked genes, and sex-specific microbiota may contribute to the immune difference between males and females (Rubtsova et al., 2015; Fish, 2008; Markle et al., 2013; Rosser and Mauri, 2016). However, the physiological mechanisms accounting for the strong female predominance in PBC remain unclear (Sun et al., 2015). PBC risk factors may function synergistically in accelerating the loss of tolerance. One theory proposes that haploinsufficiency for specific X-linked genes leads to the susceptibility to PBC of women and that enhanced monosomy X in the peripheral lymphocytes of affected women induces PBC (Invernizzi et al., 2004; Selmi et al., 2004a). However, how these genetic and environmental factors interact with the immune system to elicit autoimmunity in PBC remains unclear.

To date, several murine models that develop autoimmune cholangitis resembling PBC have been established spontaneously or through xenobiotic induction (Table 58.3). These mice share some of the important clinical phenotypes of PBC (Katsumi et al., 2015).

**TABLE 58.3** Characteristics of the Primary Biliary Cholangitis Mouse Models

	Spontaneous model				Induced model	
	NOD.c3c4	dnTGF $\beta$ RII	IL-2R $\alpha^{-/-}$	ARE Del $^{-/-}$	2-OA–BSA immunized	
Female dominance	Yes	No	No	Yes	No	
Cholestasis	–	+	–	+	+	
AMA seropositivity	50%–60%	100%	100%	100%	100%	
Portal inflammation	++ +	++ +	++ +	Yes	+	
Granulomas	+	–	–	+	+	
Other features	Biliary polycystic lesions	Moderate colitis	Severe anemia, inflammatory bowel diseases, and short life span		Peritonitis	

2-OA, 2-Octynoic acid; BSA, bovine serum albumin; ARE, adenylate uridine-rich element; AMA, antimitochondrial autoantibody.

### Spontaneous Murine Models

There are three mice models that spontaneously develop autoimmune cholangitis: NOD.c3c4 mice, a dominant-negative form of transforming growth factor (TGF)- $\beta$  receptor type II (dnTGF $\beta$ RII) mice, and IL-2R $\alpha^{-/-}$  mice. The NOD.c3c4 mouse has multiple B6- and B10-derived insulin-dependent diabetes-resistant alleles on chromosomes 3 and 4, respectively. These mice are protected from autoimmune diabetes but spontaneously develop lymphocytic peribiliary infiltrates and AMA positivity (Irie et al., 2006; Koarada et al., 2004). Notably, AMAs were detected in female mice, indicating female predominance as in human PBC. However, pathological examination of the liver revealed biliary polycystic diseases in both the intra- and extrahepatic biliary ducts, and little evidence of CNSDC. The dnTGF $\beta$ RII mice also mimicked phenotypes of human PBC (Oertelt et al., 2006). These mice are transgenic for the expression of a dominantly negative form of TGF- $\beta$  receptor type II directed by the CD4 promoter. dnTGF $\beta$ RII mice spontaneously produce AMAs directed to the same mitochondrial autoantigens as human PBC. Lymphocytic liver infiltration with periportal inflammation is analogous to the histological profile of human PBC. The complexity of the IL-12/IL-23 cytokine milieu in autoimmunity in dnTGF $\beta$ RII mice was examined by generating a series of cytokine knockouts: IFN- $\gamma^{-/-}$ , IL-12p35 $^{-/-}$ , IL-12/IL-23p40 $^{-/-}$ , IL-23p19 $^{-/-}$ , and IL-17A $^{-/-}$  dnTGF $\beta$ RII mice. Collectively, our data indicated that the IL-12/T-helper type 1 (Th1) pathway is essential for biliary disease pathogenesis and IFN- $\gamma$  production is significant for triggering Th1-cell responses in this model (Ando et al., 2012; Tsuda et al., 2013; Wang et al., 2014; Yoshida et al., 2009).

The third spontaneous mouse model is the IL-2R $\alpha^{-/-}$  mice, which lack the IL-2R cytokine crucial for differentiation of regulatory T cells (Tregs) and eventual reduction in Tregs (Wakabayashi et al., 2006). These mice develop portal inflammation, biliary ductular damage, and a Th1 cytokine bias, resembling human PBC. In addition, AMAs are targeted to the inner lipoyl domain of PDC-E2. However, female predominance was not observed.

### Xenobiotics-Triggered Murine Models

We have also examined possible environmental triggers of autoimmune cholangitis in mice, particularly chemical xenobiotics. We immunized C57BL/6 mice with 2-OA, which has been suggested as a candidate xenobiotic present in the environment in our previous study (Amano et al., 2005), coupled to bovine serum albumin (BSA). We found that anti-PDC-E2 antibodies were seropositive as early as 4 weeks after immunization, indicating loss of tolerance to PDC-E2 with xenobiotic immunization. In addition, these mice demonstrated portal infiltration of CD4+ and CD8+ T cells, granulomas, and elevated tumor necrosis factor- $\alpha$  and IFN- $\gamma$  expression levels (Wakabayashi et al., 2008). By using several unique gene-deleted mice immunized with 2-OA–BSA (Kawata et al., 2013), we also found that both IL-12/Th1 and IL-23/Th17 were involved in autoimmune cholangitis. The IL-12/Th1 signaling pathway elicited the pathology, whereas deletion of IFN- $\gamma$  prevented autoimmune cholangitis.

### Adenylate Uridine-Rich Element Del $^{-/-}$ Mice as a Novel Primary Biliary Cholangitis Model

We are currently focusing on IFN- $\gamma$  by using a “designer” mouse with dysregulation of IFN- $\gamma$ , in which the adenylate uridine-rich element (ARE) of the IFN- $\gamma$  3'-untranslated region is deleted and IFN- $\gamma$  is constitutively

produced (Hodge et al., 2014). Through various assays, we found that IFN- $\gamma$  is crucial to the pathogenesis of autoimmune cholangitis in this model (Kawata et al., 2013; Yang et al., 2014). We should note that the activation of naïve CD4 T cells from healthy women produces higher levels of IFN- $\gamma$  and lower levels of IL-17 than in healthy men (Zhang et al., 2012). Increased IFN- $\gamma$  levels have also been observed in patients with autoimmune diseases (Rubtsova et al., 2015; Pelfrey et al., 2002).

ARE Del<sup>-/-</sup> mice spontaneously developed many symptoms similar to human PBC, including liver histology, AMA production, and elevated serum total bile acid levels (Bae et al., 2016). These features were also found predominantly in female mice. In male ARE Del<sup>-/-</sup> mice, portal inflammation was rarely observed and serum titers of AMA were elevated but not significantly compared with those in wild-type mice. The total bile acid levels were comparable. In addition, gene expression analysis revealed that upregulated genes in female ARE Del<sup>-/-</sup> mice specifically overlapped with the gene expression signature of BECs in human PBC. Therefore female ARE Del<sup>-/-</sup> mice closely mimic human PBC.

Female predominance occurs in ARE Del<sup>-/-</sup> mice likely because female hormones and genetics cause immune cells in female mice to favor production of additional IFN- $\gamma$ -producing cells. In contrast, male mice may be protected by androgens, which favor the upregulation of regulatory cells and downregulation of IFN- $\gamma$ -producing cells. Female hormones activate T lymphocytes to express higher levels of IFN- $\gamma$  in female mice in this mouse model. Although numerous murine spontaneous and induced models have been reported as PBC mouse models (Katsumi et al., 2015; Wang et al., 2014), no single model exhibits female dominance as observed in ARE Del<sup>-/-</sup> mice.

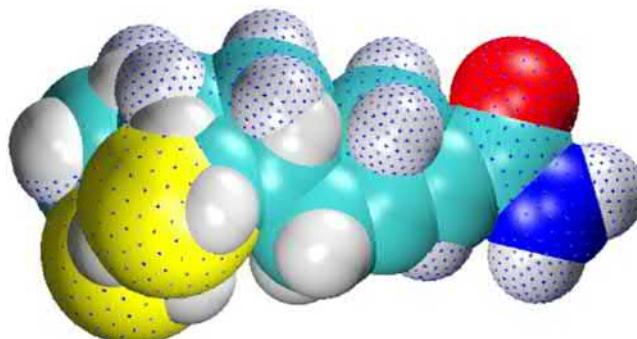
ARE Del<sup>-/-</sup> mice also provide clues about the immunopathology of PBC. IFN- $\gamma$  may play a pathogenic role in BECs during the initiation stage of PBC, and changes in expression levels of IFN- $\gamma$  are critical to the development of PBC in susceptible individuals. Furthermore, we demonstrated that transfer of CD4 T cells from ARE Del<sup>-/-</sup> mice to B5/Rag1<sup>-/-</sup> mice (an immune-deficient strain producing no mature T or B cells) induced moderate portal and parenchymal inflammation, indicating that CD4+ T cells contribute to the induction of cholangitis.

## DIAGNOSIS

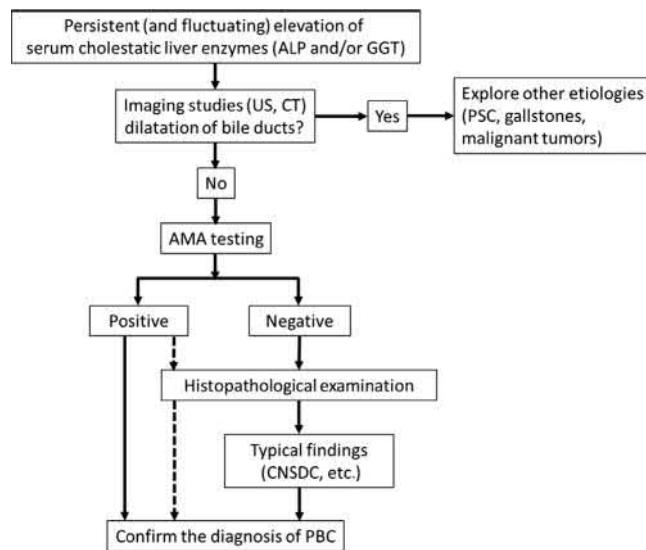
The diagnostic criteria of PBC include the following: (1) elevation of cholestatic liver enzymes, (2) AMA positivity, and (3) typical histopathological findings of the liver, that is, CNSDC of small- or medium-sized intrahepatic bile ducts (Fig. 58.2). The diagnosis of PBC is confirmed if two out of these three features are found (European Association for the Study of the Liver, 2017; Lindor et al., 2009). Because pruritus and fatigue, which are dominant symptoms in PBC, are not disease specific, most patients are suspected as having PBC when routine laboratory studies by chance demonstrate elevation of cholestatic liver enzymes. PBC is very unlikely to develop in childhood or adolescence but pediatric cases are exceptionally reported (Dahlan et al., 2003; Kitic et al., 2012).

### Serum Biochemistry and Imaging Studies

PBC should be suspected if the levels of the cholestatic enzymes alkaline phosphatase (ALP) and/or gamma-glutamylpeptidase are persistently elevated. In Fig. 58.3, a diagnostic flowchart for patients with elevated



**FIGURE 58.2** Molecular mimicry between lipoamide and 2-nonylamine. Superimposed models of lipoamide (dotted) versus 2-nonylamine in corkscrew conformation.



**FIGURE 58.3** Diagnostic flowchart of patients with PBC. *PBC*, Primary biliary cholangitis; *ALP*, alkaline phosphatase;  $\gamma$ -*GT*, gamma glutamyl transferase; *US*, ultrasonography; *CT*, computed tomography; *PSC*, primary sclerosing cholangitis; *AMA*, antimitochondrial autoantibody; *CNSDC*, chronic nonsuppurative destructive cholangitis.

cholestatic enzymes is presented. First, imaging studies of the liver and biliary tracts with ultrasonography or computed tomography scanning should be promptly carried out to rule out obstruction of the biliary tract resulting from gallstones or malignant tumors, which could be fatal and may require further urgent studies such as endoscopic procedures. Another chronic cholestatic liver disease, primary sclerosing cholangitis, is within the differential diagnosis if intra- or extrahepatic bile ducts are dilated. In contrast, PBC is suspected when imaging studies fail to detect abnormalities of the biliary tract in patients with elevated cholestatic liver enzymes. In most cases, cholestatic enzymes fluctuate. When cholestatic enzymes are consistently increasing, other etiologies including gallstones or malignant tumors obstructing the intra- or extrahepatic bile ducts should be explored.

Serum IgM (Kikuchi et al., 2005) and cholesterol levels are often elevated because of chronic cholestasis. Usually, elevation of aminotransferases—aspartate aminotransferase (AST) and alanine aminotransferase (ALT)—is mild and a marked increase of aminotransferases with elevated IgG raise the suspicion for a variant of PBC, such as PBC with features of autoimmune hepatitis (AIH), which requires corticosteroid treatment (Chazouilleres et al., 1998). In cases with advanced hepatic fibrosis, elevation of bilirubin and decreased levels of albumin are observed as cirrhosis due to other etiologies.

## Serological Testing

Serological testing, AMA testing in particular, is a necessary step for the diagnosis of PBC. If AMA is positive at a high titer, the diagnosis of PBC may be confirmed even without histopathological examination. Notably, the presence of other causes for chronic liver disorders (positive hepatitis B surface (HBs) antigen or antihepatitis C virus antibody, history of drug or alcohol use, or fatty liver) does not rule out the diagnosis of PBC in patients with detectable AMAs.

AMAs are highly specific for PBC and considered a hallmark of PBC. AMAs are detectable in about 95% of the patients with PBC and, in contrast, are very rarely found in individuals without PBC (Leung et al., 1997). As previously described, AMAs recognize a family of enzymes located at the inner membrane of the mitochondria (2-OADC), including PDC-E2, BCOADC-E2, OGDC-E2, PDC-E1 $\alpha$ , and E3BP (Table 58.4). The detection rate of AMAs is increased when enzyme-linked immunosorbent assay with recombinant mitochondrial autoantigens is used for AMA testing compared with conventional indirect immunofluorescence (Leung et al., 1992; Moteki et al., 1996b). There are, however, AMA-negative patients in a minority of patients with PBC (Oertelt et al., 2007; Miyakawa et al., 2001). “AMA-negative PBC” was once called “autoimmune cholangitis” as a variant of PBC (Taylor et al., 1994); however, it is identical to AMA-positive PBC and does not represent an independent clinical entity (Selmi et al., 2008) according to serological, immunological, and clinicopathological studies (Invernizzi et al., 1997; Kim et al., 1997; Nakanuma et al., 1997; Shimoda et al., 2008). Recent data indicate that AMA-negative PBC

**TABLE 58.4** Mitochondrial and Nuclear Autoantibodies and Their Frequencies

Antimitochondrial autoantibodies	
PDC-E2	95%
BCOADC-E2	53%–55%
OGDC-E2	39%–88%
PDC-E1 $\alpha$	41% – 66%
E3BP	95%
ANTINUCLEAR AUTOANTIBODIES	
Sp100	9%–27%
gp210	16%–26%

has a significantly worse outcome than conventional PBC; however, this observation was explained by a delay in the diagnosis (Juliusson et al., 2016). On the other hand, AMAs are occasionally detected in less than 1% of healthy individuals or patients without PBC (Mattalia et al., 1998; Shibata et al., 2004). A large-scale cohort study in France demonstrated that the prevalence of AMA-positive patients without evidence of PBC was 16.1 per 100,000, and only 1 in 6 patients with AMA positivity and normal ALP developed PBC in 5 years (Dahlqvist et al., 2017). This low number of patients with PBC raises a question about the relationship between the presence of AMA and the development of PBC, although 5 years of observation may be too short for the development of the disease.

Antinuclear antibodies (ANAs) are occasionally present in patients with PBC. Among them, anti-gp210 and/or anti-sp100 are of interest in both diagnosis and stratification of risk for progression. Two different antinuclear staining patterns with indirect immunofluorescence are observed in PBC: multiple nuclear dots (MNDs) and nuclear pore complex (NPC). Two nuclear proteins, Sp100 and promyelocytic leukemia antigen, are identified as major autoantigens in MND staining, and gp210 protein, the lamin B receptor, and nucleoporin p62 are targeted as autoantigens in NPC. Although the prevalence of anti-Sp100 and anti-gp210 is 9%–27% and 16%–26% in recent studies, respectively (Liu et al., 2010b; Muratori et al., 2008; Nakamura et al., 2007), and much lower than that of AMAs (Table 58.4), the specificities of these antibodies for PBC are very high (Invernizzi et al., 1997; Nakamura et al., 2007; Muratori et al., 2003). Thus these ANAs can be helpful for identifying patients with PBC when they are AMA negative (Bandin et al., 1996), and a novel commercially available assay kit (PBC screen) detecting AMAs against three major mitochondrial autoantigens (PDC-E2, BCOADC-E2, and OGDC-E2), anti-gp210, and anti-sp100 may serve as a first-line assay tool for the diagnosis of PBC (Liu et al., 2010b).

## Histopathology

In typical cases, dense infiltration of inflammatory cells including lymphocytes and mononuclear cells is found around small- or medium-sized intrahepatic bile ducts, coined as CNSDC (Fig. 58.1). Granulomas are occasionally observed and also diagnostic for PBC (Ludwig, 2000). As the disease progresses, the inflammatory infiltrates increase and later involves ductular proliferation, eventually resulting in septal fibrosis and cirrhosis.

Liver biopsy is not mandatory in patients with serologically confirmed PBC, positive AMA, and persistently elevated cholestatic enzymes. In addition, sampling errors and complications that could occur with liver biopsy may diminish the opportunities to carry out this invasive technique. Nevertheless, histological findings of the liver are required for establishing the diagnosis in atypical cases, such as with elevated cholestatic enzymes but negative AMA, and still remain the gold standard for the assessment hepatic fibrosis, which is associated with long-term prognosis. To avoid sampling errors, enough samples containing at least 10–15 portal tracts should be taken and multiple continuous sections should be examined to seek diagnostic findings of PBC.

Histologic lesions have been classically divided into four stages by Scheuer (1967) and Ludwig et al. (1978), as follows: stage 1, portal infiltration of lymphocytes; stage 2, portal/periportal ductular proliferation; stage 3, scarring and bridging fibrosis; and stage 4, nodular cirrhosis. This classification, although very simple and broadly applicable to clinical settings, does not overcome sampling errors in the assessment of stages and does not reflect necroinflammatory activity, which also contributes to the progression of hepatic fibrosis. Recently,

Nakanuma et al. (2010) proposed new histological assessment criteria for PBC, which consist of both staging (scoring of fibrosis, bile duct loss, and deposition of orcein-positive granules) and grading (cholangitis activity and hepatitis activity). These novel assessment criteria for histology, although more complicated, could stratify the risk for progression and outcomes of patients with PBC at baseline (Harada et al., 2013b).

## TREATMENT

### Ursodeoxycholic Acid

UDCA is now globally approved as a first-line therapy for PBC (European Association for the Study of the Liver, 2017; Lindor et al., 2009; Working Subgroup for Clinical Practice Guidelines for Primary Biliary Cirrhosis, 2014). UDCA is used at a dose of 13–15 mg/kg/day and is recommended for all patients with PBC with abnormal liver biochemistry. UDCA is a naturally occurring hydrophilic bile acid that, when orally administered, becomes the dominant bile acid in the enterohepatic circulation, exerting a protective effect to bile duct cells and hepatocytes through its choleretic and bicarbonate-secreting effects (Lindor, 2007). UDCA has been shown to improve serum biochemical abnormalities and also to delay the progression of histological progression and development of varices and to prolong transplant-free survival (Angulo et al., 1999; Combes et al., 1995; Corpechot et al., 2000; Heathcote et al., 1994; Lindor et al., 1996, 1997; Poupon et al., 1991, 1994, 1997, 2003). Indeed, patients who completely respond to UDCA treatment have been shown to have a comparable survival to the general population (Corpechot et al., 2008; Kuiper et al., 2009; Kumagi et al., 2010; Lammers et al., 2015; Pares et al., 2006). As discontinuation of UDCA frequently leads to elevation of serum liver enzymes, treatment with UDCA should be continued throughout life. Although the safety profile of UDCA is excellent in most cases, abdominal fullness, diarrhea, and constipation may infrequently occur and intolerance to UDCA may exist.

On the other hand, up to 30% of the patients with PBC exhibit incomplete responses to UDCA and the outcomes of these patients can be poor. Therefore it is extremely important to define nonresponders to UDCA with easy-to-use criteria. It is generally accepted that a variety of combinations of biochemical markers at 1 year after commencement of UDCA treatment are useful for this purpose, as will be discussed later.

### Obeticholic Acid

OCA is a selective ligand of the FXR. Bile acid toxicity against BECs and hepatocytes is decreased by FXR signaling through impairment of bile acid synthesis and stimulation of choleresis. The endogenous FXR ligand is chenodeoxycholic acid (CDCA), a primary bile acid. Compared with CDCA, OCA has approximately 100 times greater potency for activating FXR (Pellicciari et al., 2002). In 2016 the US Food and Drug Administration (FDA) officially approved OCA based on the results of the POISE trial (phase 3 clinical trial of OCA) (Nevens et al., 2016). In this international, prospective, randomized, placebo-controlled trial, 217 patients with PBC who showed an inadequate response (serum ALP level  $>1.67 \times$  upper limit of normal (ULN)) or an abnormal total bilirubin level ( $<2 \times$  ULN), or were intolerant to UDCA, were enrolled and received 5–10 mg OCA, 10 mg OCA, and placebo for 1 year. The primary end point was an ALP level of  $<1.67 \times$  ULN with  $>15\%$  reduction from the baseline and normal bilirubin level. Of the patients, 46%–47% achieved the primary end point (Nevens et al., 2016). With this result, OCA received accelerated FDA approval on May 27, 2016.

Thus OCA has become the long-awaited second-line drug officially approved for PBC. However, it is unsatisfactory for several reasons. First, the response rate was at most 50%, which means that half of the patients did not respond to OCA. Second, pruritus, a symptom frequently experienced by patients with PBC, appeared as an adverse effect in 56%–68% of the patients treated with OCA. Furthermore, whether the primary end points (ALP level  $<1.67 \times$  ULN with  $>15\%$  reduction from the baseline and normal bilirubin level) are associated with improvement of long-term outcomes has not been confirmed yet. In this regard, follow-up studies of the POISE trial were required by the FDA and a phase 3 study is currently ongoing (COBALT, NCT02308111). Finally, the appropriate treatment duration for OCA should be continued for patients refractory to UDCA; thus OCA may be prescribed lifelong along with UDCA. On the basis of the high cost of OCA (\$69,350 per year), this unlimited prescription of OCA places substantial economic burdens on both patients and societies and is not cost-effective. Samur et al. (2017) recently demonstrated that the price should be decreased to \$18,450 per year to make OCA cost-effective.

## Fibrates

Fibrates (fenofibrate and bezafibrate) were originally indicated for dyslipidemia and used for decreasing serum cholesterol and triglycerides. Fibrates are PPAR- $\alpha$  and PXR agonists, resulting in a reduction of de novo bile acid synthesis and upregulation of bile acid transporters (Honda et al., 2013). Bezafibrate was first reported as biologically effective for patients with PBC who are refractory to UDCA in 1999 (Iwasaki et al., 1999), and prospective, multicenter, randomized open-label studies in Japan demonstrated the significant biochemical efficacy of bezafibrate administered for 1 year (Iwasaki et al., 2008). A large-scale retrospective cohort study in Japan also indicated that patients with good response to bezafibrate (normalization of ALT) exhibited a comparable prognosis to those treated with UDCA alone and significantly better outcomes than those without good responses to bezafibrate (Tanaka et al., 2015). Recently, a prospective randomized study in Japan revealed that long-term outcomes were not significantly different between the UDCA plus bezafibrate and UDCA-only groups; however, the sample size in this study might be too small to establish enough statistical power (Hosonuma et al., 2015). Another prospective, randomized phase 3 study of bezafibrate for patients with PBC with incomplete responses to UDCA is ongoing in France (NCT01654731), and the addition of bezafibrate to UDCA for 2 years was significantly effective for improving liver biochemistries (Corpechot et al., 2017). Bezafibrate may also work for pruritus of PBC (Bolier et al., 2017). Fenofibrate was reported to decrease serum ALP levels in studies in Japan and China (Cheung et al., 2016; Dohmen et al., 2004), whereas adjunct use of fenofibrate with UDCA showed no association with decreased serum ALP levels in a UK cohort (Hegade et al., 2016). Participants are being recruited for a prospective randomized study in China (NCT02965911). However, these two prospective clinical trials provide improvements in liver enzyme levels notably at 12 or 24 months as the primary end point. Even after these trials are terminated, whether long-term outcomes are improved with additional treatment with fibrates is still unknown, and follow-up studies of these trials are needed.

## Ileal Bile Acid Transporter Inhibitors

Bile acids are essential to the human body, and 98% of bile acids excreted into the intestine are reabsorbed from the ileum bile acid transporter (IBAT) and returned to the liver via the portal tract, known as enterohepatic circulation. Therefore another strategy for reducing bile acids is blocking the inhibition of IBAT. One of these compounds, GSK2330672, did not significantly decrease serum ALP levels in patients with incomplete responses to UDCA but was demonstrated to significantly improve pruritus in comparison with placebo (Hegade et al., 2017). A prospective, randomized, placebo-controlled phase 2 study of GSK2330672 was launched in 2017 (NCT02966834).

## MANAGEMENT OF SYMPTOMS AND EXTRAHEPATIC MANIFESTATIONS

PBC is often diagnosed without any clinical symptom (asymptomatic PBC). The reported proportion of asymptomatic PBC at presentation greatly varies, from 13% to 80.7% (Jeffrey et al., 1990; Mahl et al., 1994; Nakano et al., 2002; Nyberg and Loof, 1989; Prince et al., 2002; Wong et al., 2007, 2008), probably owing to the difference of stages at diagnosis among studies or the lower recognition by physicians of the subjective symptoms of patients. The most dominant clinical symptoms at the early stage of PBC are fatigue and pruritus, followed by jaundice.

### Fatigue

Fatigue is the most common and debilitating symptom in PBC, experienced by approximately 50% (ranging from 20% to 80% depending on each study) of the patients (Prince et al., 2002; Huet et al., 2000; Mells et al., 2013). Objective assessment of fatigue can be carried out with the Fatigue Impact Scale (Fisk et al., 1994) or the PBC-40 questionnaire (Jacoby et al., 2005). Although it is difficult to define the cutoff clearly according to the presence of fatigue, it has been repeatedly shown that fatigue has a great impact on impairment of the quality of life of patients with PBC (Mells et al., 2013; Jopson and Jones, 2015). Fatigue is not associated with disease severity and staging but may be related to age at onset and sex (Carbone et al., 2013b).

The cause of fatigue remains unknown but appears to be complex in origin, probably multifactorial in most patients and associated with depression, autonomic dysfunction, and sleep disturbance (Jopson and Jones, 2015). Recent studies with magnetic resonance imaging revealed neuroimaging changes in the brain even in patients in

the early stage of PBC (Grover et al., 2016). Fatigue does not respond to UDCA treatment and there have been no established treatment. Fatigue may be improved by LT but still persists in a substantial part of patients even after LT, making the role of LT as a therapeutic option for severe fatigue questionable (Carbone et al., 2013a). Modafinil, which is officially approved by the FDA for wakefulness disorders, has been used; however, a recent randomized, placebo-controlled clinical trial failed to indicate beneficial effects of modafinil in terms of fatigue improvement in patients with PBC (Silveira et al., 2017).

## Pruritus

Pruritus is another important symptom in PBC, affecting 20%–80% of the patients. Pruritus can occur locally or diffusely, and its presence and severity fluctuate throughout the clinical course. It tends to become more pronounced along with the progression of PBC but could be present even at a very early stage. Pruritus could be highly bothersome and intolerable, such as causing sleep disturbance, and is an important indication for LT. Similar to fatigue, the severity of pruritus is objectively assessable with the PBC-40 questionnaire (Jacoby et al., 2005). The cause of pruritus remains unknown, although several substances are hypothesized to be related to pruritus in cholestatic liver diseases (Beuers et al., 2014). Most notably, lysophosphatidic acid (LPA) may be a potential candidate for the initiation of pruritus (Kremer et al., 2010a), and the activity of serum autotaxin, the serum enzyme converting lysophosphatidylcholine into LPA, is related to the severity of pruritus and responds to therapeutic interventions (Kremer et al., 2010b, 2012). Therefore LPA-autotaxin is an important candidate therapeutic target yet clinically unavailable. Very recently, an ileal bile acid transporter inhibitor compound (GSK2330672) that inhibits reabsorption of bile acids at the ileum was shown to effectively decrease pruritus of patients with PBC in a phase 2a study (Hegade et al., 2017), and the efficacy of this compound is now being further investigated in a global phase 2b study (NCT02966834). Moreover, bezafibrate, originally developed as an antihypertriglyceridemia drug, is now on clinical trial for the treatment of cholestatic pruritus (NCT02701166) (Bolier et al., 2017).

## DISORDERS ASSOCIATED WITH PRIMARY BILIARY CHOLANGITIS

### Primary Biliary Cholangitis With Features With Autoimmune Hepatitis

Although PBC typically presents as an elevation of cholestatic liver enzymes and detectable AMA, variant forms of PBC lacking one or more typical characteristics are occasionally encountered. In particular, some patients synchronously or consecutively present with features of AIH (i.e., elevation of transaminases, serum IgG levels, and positive ANAs). This variant type was formerly referred to as PBC–AIH overlap syndrome but is now termed PBC with features of AIH, because this atypical disorder is neither a single clinical entity nor a combination of PBC and AIH but rather a variant form of classic PBC (Boberg et al., 2011). The Paris criteria (Chazouillères et al., 1998) (Table 58.5) are most commonly used to define this variant, and patients who meet these criteria benefit from corticosteroid treatment in addition to UDCA (European Association for the Study of the Liver, 2017). Nevertheless, it must be kept in mind that the Paris criteria were not intended to define this variant as a single clinical entity.

**TABLE 58.5** The Paris Criteria for Primary Biliary Cholangitis (PBC) With Features of Autoimmune Hepatitis (AIH) (Chazouillères et al., 1998)

PBC criteria	Features of AIH criteria
1. ALP > 2 × ULN or γ-GT > 5 × ULN	1. ALT > 5 × ULN
2. Positive AMA	2. IgG > 2 × ULN or positive SMA
3. Florid bile duct lesion on histology	3. Moderate or severe periportal or periseptal lymphocytic piecemeal necrosis

The presence of at least two of three for each condition was required. ALP, Alkaline phosphatase; ULN, upper limit of normal; γ-GT, gamma glutamyl transferase; AMA, antimitochondrial autoantibody; SMA, smooth muscle antibody.

## Sicca Syndrome

The sicca complex is frequently present in patients with PBC, manifesting as dry eyes and/or dry mouth. External glands including the lachrymal or salivary glands are also affected in PBC. Indeed, a recent retrospective study revealed the prevalence of Sjogren's syndrome as up to 56% in patients with PBC ([Floreani et al., 2015b](#)); however, the sicca complex affects patients with PBC who do not meet the criteria of Sjogren's syndrome. Patients with sicca syndrome may experience a variety of complaints including burning, itching, or irritated eyes; blepharitis; dysphagia; stomatitis; dental caries; and dry cough, resulting in severe impairment of the quality of life. Early recognition of sicca symptoms and consultations to ophthalmologists or dentists are suggested.

## Osteopenia and Osteoporosis

Osteopenic bone disease, including osteopenia and osteoporosis, is a common disorder in PBC mainly affecting middle-aged women and is associated with an increased risk for fragility fracture. The decrease in bone mineral density found in PBC is multifactorial. Chronic cholestasis leads to malabsorption and deficiency of vitamin D, which is essential to bone metabolism. Other factors associated with bone diseases include age, sex, low body mass index, history of fragile fracture, and advanced stage of PBC ([Guanabens et al., 2005, 2010](#)). Intervention with a bisphosphonate for patients with osteoporosis and with a history of fragility fracture is safe and improves bone mineral density ([Guanabens et al., 2013](#)); however, it remains unclear whether it is associated with a decrease in fragility fractures.

## Hyperlipidemia and Metabolic Syndrome

Chronic cholestasis is a main feature of PBC, and therefore hyperlipidemia is common and affects up to 80% of the patients ([Sorokin et al., 2007](#)). Several prospective studies indicated that an increase in serum lipid levels is not associated with a higher risk for cardiovascular diseases related to atherosclerosis, and treatment for hyperlipidemia per se is not necessary. Notably, these studies were carried out in the 1990s when the metabolic syndrome was relatively rare in patients with PBC. A recent study in Italy demonstrated that cardiovascular events developed more frequently in patients with metabolic syndrome ([Floreani et al., 2015a](#)), indicating the importance of treatment intervention for patients with hyperlipidemia if metabolic syndrome exists.

## Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC), although previously very rare, is now occasionally encountered in patients with PBC in whom life expectancy is comparable to the general population with the introduction of UDCA. The reported incidences and risk factors for developing HCC from several large-scale retrospective cohorts are summarized in [Table 58.6](#). Surprisingly, the incidence rates (cases per 1000 patient-years) of HCC in all patients with PBC are similar across different regions: 3.6 in Barcelona, Spain; 3.7 in Padova, Italy ([Cavazza et al., 2009](#)); 3.6 in a nationwide study in Japan ([Harada et al., 2013a](#)); and 3.4 in an international cohort ([Trivedi et al., 2016b](#)). The

**TABLE 58.6** Incidence and Risk Factors for Hepatocellular Carcinoma (HCC) in Patients With Primary Biliary Cholangitis

Country/Region	Number		Incidence <sup>a</sup>			Risk factors
	Total	HCC	All	Male	Female	
Barcelona, Spain ( <a href="#">Cavazza et al., 2009</a> )	389	13	3.6	NA	NA	Advanced histological stage
Padova, Italy ( <a href="#">Cavazza et al., 2009</a> )	327	11	3.7	NA	NA	Advanced histological stage (all), male sex
Japan ( <a href="#">Harada and Nakanuma, 2014</a> )	2946	71	3.6	9.5	2.92.9	Male sex, advanced histological stage (in females)
International ( <a href="#">Trivedi et al., 2016b</a> )	4565	123	3.4	6.7	2.6	Advanced age, male sex, thrombocytopenia at 12 months, biochemical nonresponse
Beijing, China ( <a href="#">Rong et al., 2015</a> )	1865	70	6.6	NA	NA	Advanced age, male sex, coexistence of diabetes, Hx of HBV infection

<sup>a</sup>Cases per 1000 patient-years.

Hx, History; HBV, hepatitis B virus; NA, not applicable.

incidence rate was exceptionally high, 6.6, in a cohort from Beijing, China (Rong et al., 2015), presumably because of the high rate of the population with previous hepatitis B virus (HBV) infection. Indeed, a history of HBV infection was identified as an independent risk factor for HCC in this study. The incidence rate was higher in men than in women. In those studies, male sex and advanced histological stage independently contributed to the development of HCC (Cavazza et al., 2009; Harada et al., 2013a; Trivedi et al., 2016b; Rong et al., 2015). Treatment response was included among possible risk factors only in the international cohort study, and biochemical nonresponse at 1 year of UDCA treatment (Paris-II not fulfilled) significantly increased the future risk of HCC (adjusted hazard ratio, 3.44) (Trivedi et al., 2016b). Taken together, close monitoring of HCC is strongly recommended for high-risk patients with PBC, such as male patients, patients with advanced-stage disease, and nonresponders to UDCA. The mean survival of patients who developed HCC was 36 months after diagnosis, and another cohort indicated 5- and 10-year-survival rates of 49.5% and 31.7%, respectively (Imam et al., 2012).

## STRATIFICATION OF THE RISK FOR PROGRESSION

After the introduction of UDCA as the first-line drug, the prognosis of patients with PBC dramatically improved and now is comparable to those of the general population if the response to UDCA is favorable. On the other hand, a substantial proportion of patients who are already in the cirrhotic stage at presentation or are refractory to UDCA unavoidably progress to liver failure and require orthotopic liver transplantation (OLT). Therefore clinicians must stratify individual patients who are diagnosed as having PBC and estimate the risk of the patient for progression, both at presentation and at any time during treatment, especially at 1 year after the commencement of UDCA treatment (Trivedi et al., 2016a).

### Stratification at Baseline

Sex and age at diagnosis are reported to be associated with response to UDCA treatment and symptom development, and women who are younger than 50 years exhibited the lowest response rate to UDCA and the highest levels of symptoms (Carbone et al., 2013b). The AST/platelet ratio index at baseline is also a predictor of outcomes independent of the UDCA response (Trivedi et al., 2014). The presence of ANAs, especially anti-gp210, at baseline may be associated with a more severe clinical course (Nakamura et al., 2007; Muratori et al., 2003; Invernizzi et al., 2001). A recent retrospective study in China also reinforced the significance of anti-gp210 as a biomarker of worse outcomes (Yang et al., 2017). Advanced histological stages at presentation are obviously associated with poor prognosis. However, assessment of liver histology definitely requires liver biopsy as an invasive procedure, and sampling errors have always been a problem. In this regard, a variety of noninvasive techniques for the evaluation of liver fibrosis have been developed, including liver stiffness measurement by means of vibration-controlled transient elastography, magnetic resonance elastography, and serum biomarker measurements (Poupon, 2015). Recent studies indicated that serum levels of *Wisteria floribunda* agglutinin-positive mac-2-binding protein, soluble CD-14, IL-8, and IFN- $\gamma$ -inducible protein-10 are other candidate serum biomarkers for predicting liver fibrosis and the prognosis of PBC (Nishikawa et al., 2016; Umemura et al., 2015, 2017).

### Stratification During Treatment

It is important to stratify patients with PBC depending on responses to treatment. Different criteria for defining biochemical responsiveness to UDCA have been proposed (Table 58.7). A global consensus has been established to judge responsiveness to UDCA at 1 year after the commencement of UDCA treatment with liver biochemistry tests, which are easy to conduct. Furthermore, although several simple definitions of unresponsiveness (responder or nonresponder) have been established in a nationwide scale in earlier studies (Corpechot et al., 2008, 2011; Kuiper et al., 2009; Kumagi et al., 2010; Pares et al., 2006; Azemoto et al., 2009; Momah et al., 2012) and later in an international consortium (Global PBC Group) to take into account a very large-scale cohort (Lammers et al., 2014), these dichotomous definitions did not serve as predictors that could precisely and quantitatively assess the risk for progression in a given patient. Recently, two large-scale multicenter studies have developed continuous predictive models (GLOBE score, UK-PBC score) with age and liver biochemistries either at baseline or at 1 year after UDCA treatment (Lammers et al., 2015; Carbone et al., 2016). These scores are easy to use with computer-aided calculations and allow to continuously quantify the risk for progression over time in

**TABLE 58.7** Criteria Defining Biochemical Responses to Ursodeoxycholic Acid

Criteria	Number of patients	Duration	Definition
<b>QUALITATIVE DEFINITION</b>			
Barcelona (Pares et al., 2006)	192	1 year	Normal ALP or ALP reduction >40%
Paris-I (Corpechot et al., 2008)	292	1 year	ALP < 3 × ULN, AST < 2 × ULN, normal bilirubin
Rotterdam (Kuiper et al., 2009)	375	1 year	Normal bilirubin, normal albumin
Toronto (Kumagi et al., 2010)	69	2 years	ALP ≤ 1.67 × ULN
Ehime (Azemoto et al., 2009)	83	6 months	Normal γ-GT or γ-GT reduction ≥ 70%
Paris-II (Corpechot et al., 2011)	165	1 year	ALP < 1.5 × ULN, AST < 1.5 × ULN, normal bilirubin
Rochester (Momah et al., 2012)	73	1 year	ALP ≤ 1.67 × ULN, bilirubin ≤ 1 mg/dL
International (Global PBC) (Lammers et al., 2014)	4845	1 year	ALP < 2 × ULN, normal bilirubin
<b>QUANTITATIVE SCORES</b>			
GLOBE score (Lammers et al., 2015)	4119	1 year	Bilirubin, ALP, albumin platelet count at 1 year, age at baseline
UK-PBC score (Carbone et al., 2016)	3165	1 year	ALP, AST/ALT, bilirubin at 1 year, albumin, platelet count at baseline

ALP, Alkaline phosphatase; ULN, upper limit of normal; AST, aspartate aminotransferase; γ-GT, gamma glutamyl transferase.

a single patient. It is of note, however, that these scores were solely developed for a patient cohort treated with UDCA monotherapy, and whether these scores based on biochemical responses at 1 year treatment are also applicable to patients treated with additional drugs such as OCA or bezafibrate should be further validated.

## PERSPECTIVES

Clearly, the introduction of OCA in 2016 as an alternative therapeutic option was an epoch-making event in PBC, and it is very welcome that several new drugs are in clinical trials and under development. As the clinical characteristics of PBC may vary among patients and progression is not always predictable, it is an ultimate goal to stratify patients depending on the risk for progression and individualize the treatment with a variety of therapeutic agents including an option of no treatment. To accomplish this, more details about the etiology of PBC should be uncovered. Animal models of PBC that would allow scrutinizing the initial events of PBC should be established, the interaction between environmental triggers and genetic predispositions needs to be investigated, and the critical pathways in PBC that should be targeted by therapeutic interventions should be elucidated.

## References

- Abu-Mouch, S., Selmi, C., Benson, G.D., Kenny, T.P., Invernizzi, P., Zuin, M., et al., 2003. Geographic clusters of primary biliary cirrhosis. *Clin. Dev. Immunol.* 10, 127–131.
- Addison, T., Gull, W., 1851. On a certain affection of the skin—vitiligo idea (a) plana; (b) tuberosa with remarks. *Guys Hosp. Rep.* 7, 265–276.
- Ahrens Jr, E.H., Payne, M.A., Kunkel, H.G., Eisenmenger, W.J., Blondheim, S.H., 1950. Primary biliary cirrhosis. *Medicine (Baltimore)* 29, 299–364.
- Ala, A., Stanca, C.M., Bu-Ghanim, M., Ahmado, I., Branch, A.D., Schiano, T.D., et al., 2006. Increased prevalence of primary biliary cirrhosis near superfund toxic waste sites. *Hepatology* 43, 525–531.
- Amano, K., Leung, P., Rieger, R., Quan, C., Wang, X., Marik, J., et al., 2005. Chemical xenobiotics and mitochondrial autoantigens in primary biliary cirrhosis: identification of antibodies against a common environmental, cosmetic, and food additive, 2-octynoic acid. *J. Immunol.* 174, 5874–5883.
- Ando, Y., Yang, G.X., Tsuda, M., Kawata, K., Zhang, W., Nakajima, T., et al., 2012. The immunobiology of colitis and cholangitis in interleukin-23p19 and interleukin-17A deleted dominant negative form of transforming growth factor beta receptor type II mice. *Hepatology* 56, 1418–1426.
- Angulo, P., Batts, K.P., Therneau, T.M., Jorgensen, R.A., Dickson, E.R., Lindor, K.D., 1999. Long-term ursodeoxycholic acid delays histological progression in primary biliary cirrhosis. *Hepatology* 29, 644–647.

- Azemoto, N., Abe, M., Murata, Y., Hiasa, Y., Hamada, M., Matsuura, B., et al., 2009. Early biochemical response to ursodeoxycholic acid predicts symptom development in patients with asymptomatic primary biliary cirrhosis. *J. Gastroenterol.* 44, 630–634.
- Bae, H.R., Leung, P.S., Tsuneyama, K., Valencia, J.C., Hodge, D.L., Kim, S., et al., 2016. Chronic expression of interferon-gamma leads to murine autoimmune cholangitis with a female predominance. *Hepatology* 64, 1189–1201.
- Baldursdottir, T.R., Bergmann, O.M., Jonasson, J.G., Ludviksson, B.R., Axelsson, T.A., Bjornsson, E.S., 2012. The epidemiology and natural history of primary biliary cirrhosis: a nationwide population-based study. *Eur. J. Gastroenterol. Hepatol.* 24, 824–830.
- Bandin, O., Courvalin, J.C., Poupon, R., Dubel, L., Homberg, J.C., Johonet, C., 1996. Specificity and sensitivity of gp210 autoantibodies detected using an enzyme-linked immunosorbent assay and a synthetic polypeptide in the diagnosis of primary biliary cirrhosis. *Hepatology* 23, 1020–1024.
- Beuers, U., Kremer, A.E., Bolier, R., Elferink, R.P., 2014. Pruritus in cholestasis: facts and fiction. *Hepatology* 60, 399–407.
- Beuers, U., Gershwin, M.E., Gish, R.G., Invernizzi, P., Jones, D.E., Lindor, K., et al., 2015a. Changing nomenclature for PBC: from ‘cirrhosis’ to ‘cholangitis’. *Clin. Res. Hepatol. Gastroenterol.* 39, e57–e59.
- Beuers, U., Gershwin, M.E., Gish, R.G., Invernizzi, P., Jones, D.E., Lindor, K., et al., 2015b. Changing nomenclature for PBC: from ‘cirrhosis’ to ‘cholangitis’. *Dig. Liver Dis.* 47, 924–926.
- Beuers, U., Gershwin, M.E., Gish, R.G., Invernizzi, P., Jones, D.E., Lindor, K., et al., 2015c. Changing nomenclature for PBC: from ‘cirrhosis’ to ‘cholangitis’. *Am. J. Gastroenterol.* 110, 1536–1538.
- Beuers, U., Gershwin, M.E., Gish, R.G., Invernizzi, P., Jones, D.E., Lindor, K., et al., 2015d. Changing nomenclature for PBC: from ‘cirrhosis’ to ‘cholangitis’. *Clin. Gastroenterol. Hepatol.* 13, 1867–1869.
- Beuers, U., Gershwin, M.E., Gish, R.G., Invernizzi, P., Jones, D.E., Lindor, K., et al., 2015e. Changing nomenclature for PBC: from ‘cirrhosis’ to ‘cholangitis’. *J. Hepatol.* 63, 1285–1287.
- Beuers, U., Gershwin, M.E., Gish, R.G., Invernizzi, P., Jones, D.E., Lindor, K., et al., 2015f. Changing nomenclature for PBC: from ‘cirrhosis’ to ‘cholangitis’. *Gastroenterology* 149, 1627–1629.
- Beuers, U., Gershwin, M.E., Gish, R.G., Invernizzi, P., Jones, D.E., Lindor, K., et al., 2015g. Changing nomenclature for PBC: from ‘cirrhosis’ to ‘cholangitis’. *Gut* 64, 1671–1672.
- Beuers, U., Gershwin, M.E., Gish, R.G., Invernizzi, P., Jones, D.E., Lindor, K., et al., 2015h. Changing nomenclature for PBC: from ‘cirrhosis’ to ‘cholangitis’. *Hepatology* 62, 1620–1622.
- Bjorkland, A., Festin, R., Mendel-Hartvig, I., Nyberg, A., Loof, L., Totterman, T.H., 1991. Blood and liver-infiltrating lymphocytes in primary biliary cirrhosis: increase in activated T and natural killer cells and recruitment of primed memory T cells. *Hepatology* 13, 1106–1111.
- Boberg, K.M., Chapman, R.W., Hirschfield, G.M., Lohse, A.W., Manns, M.P., Schrumpf, E., et al., 2011. Overlap syndromes: the International Autoimmune Hepatitis Group (IAIHG) position statement on a controversial issue. *J. Hepatol.* 54, 374–385.
- Bolier, R., de Vries, E.S., Pares, A., Helder, J., Kemper, E.M., Zwinderman, K., et al., 2017. Fibrates for the treatment of cholestatic itch (FITCH): study protocol for a randomized controlled trial. *Trials* 18, 230.
- Boonstra, K., Beuers, U., Ponsioen, C.Y., 2012. Epidemiology of primary sclerosing cholangitis and primary biliary cirrhosis: a systematic review. *J. Hepatol.* 56, 1181–1188.
- Boonstra, K., Kunst, A.E., Stadhouders, P.H., Tuynman, H.A., Poen, A.C., van Nieuwkerk, K.M., et al., 2014. Rising incidence and prevalence of primary biliary cirrhosis: a large population-based study. *Liver Int.* 34, e31–e38.
- Burroughs, A., Rosenstein, I., Epstein, O., Hamilton-Miller, J., Brumfitt, W., Sherlock, S., 1984. Bacteriuria and primary biliary cirrhosis. *Gut* 25, 133–137.
- Carbone, M., Bufton, S., Monaco, A., Griffiths, L., Jones, D.E., Neuberger, J.M., 2013a. The effect of liver transplantation on fatigue in patients with primary biliary cirrhosis: a prospective study. *J. Hepatol.* 59, 490–494.
- Carbone, M., Mells, G.F., Pells, G., Dawwas, M.F., Newton, J.L., Heneghan, M.A., et al., 2013b. Sex and age are determinants of the clinical phenotype of primary biliary cirrhosis and response to ursodeoxycholic acid. *Gastroenterology* 144, 560–569, e567; quiz 513–564.
- Carbone, M., Sharp, S.J., Flack, S., Paximadas, D., Spiess, K., Adegey, C., et al., 2016. The UK-PBC risk scores: derivation and validation of a scoring system for long-term prediction of end-stage liver disease in primary biliary cholangitis. *Hepatology* 63, 930–950.
- Cavazza, A., Caballeria, L., Floreani, A., Farinati, F., Bruguera, M., Caroli, D., et al., 2009. Incidence, risk factors, and survival of hepatocellular carcinoma in primary biliary cirrhosis: comparative analysis from two centers. *Hepatology* 50, 1162–1168.
- Chazouillères, O., Wendum, D., Serfety, L., Montembault, S., Rosmorduc, O., Poupon, R., 1998. Primary biliary cirrhosis-autoimmune hepatitis overlap syndrome: clinical features and response to therapy. *Hepatology* 28, 296–301.
- Cheung, A.C., Lapointe-Shaw, L., Kowgier, M., Meza-Cardona, J., Hirschfield, G.M., Janssen, H.L., et al., 2016. Combined ursodeoxycholic acid (UDCA) and fenofibrate in primary biliary cholangitis patients with incomplete UDCA response may improve outcomes. *Aliment. Pharmacol. Ther.* 43, 283–293.
- Combes, B., Carithers Jr, R.L., Maddrey, W.C., Lin, D., McDonald, M.F., Wheeler, D.E., et al., 1995. A randomized, double-blind, placebo-controlled trial of ursodeoxycholic acid in primary biliary cirrhosis. *Hepatology* 22, 759–766.
- Cordell, H.J., Han, Y., Mells, G.F., Li, Y., Hirschfield, G.M., Greene, C.S., et al., 2015. International genome-wide meta-analysis identifies new primary biliary cirrhosis risk loci and targetable pathogenic pathways. *Nat. Commun.* 6, 8019.
- Corpechot, C., Carrat, F., Bonnand, A.M., Poupon, R.E., Poupon, R., 2000. The effect of ursodeoxycholic acid therapy on liver fibrosis progression in primary biliary cirrhosis. *Hepatology* 32, 1196–1199.
- Corpechot, C., Abenavoli, L., Rabahi, N., Chretien, Y., Andreani, T., Johonet, C., et al., 2008. Biochemical response to ursodeoxycholic acid and long-term prognosis in primary biliary cirrhosis. *Hepatology* 48, 871–877.
- Corpechot, C., Chretien, Y., Chazouillères, O., Poupon, R., 2010. Demographic, lifestyle, medical and familial factors associated with primary biliary cirrhosis. *J. Hepatol.* 53, 162–169.
- Corpechot, C., Chazouillères, O., Poupon, R., 2011. Early primary biliary cirrhosis: biochemical response to treatment and prediction of long-term outcome. *J. Hepatol.* 55, 1361–1367.
- Corpechot, C., Chazouillères, O., Rousseau, A., Guyader, D., Habersetzer, F., Mathurin, P., et al., 2017. A 2-year multicenter, double-blind, randomized, placebo-controlled study of bezafibrate for the treatment of primary biliary cholangitis in patients with inadequate biochemical response to ursodeoxycholic acid therapy (Bezurso). *J. Hepatol.* 66, S89.

- Dahlan, Y., Smith, L., Simmonds, D., Jewell, L.D., Wanless, I., Heathcote, E.J., et al., 2003. Pediatric-onset primary biliary cirrhosis. *Gastroenterology* 125, 1476–1479.
- Dahlqvist, G., Gaouar, F., Carrat, F., Meurisse, S., Chazouilleres, O., Poupon, R., et al., 2017. Large-scale characterization study of patients with antimitochondrial antibodies but nonestablished primary biliary cholangitis. *Hepatology* 65, 152–163.
- Danielsson, A., Boqvist, L., Uddenfeldt, P., 1990. Epidemiology of primary biliary cirrhosis in a defined rural population in the northern part of Sweden. *Hepatology* 11, 458–464.
- Dauphinee, J.A., Sinclair, J.C., 1949. Primary biliary cirrhosis. *Can. Med. Assoc. J.* 61, 1–6.
- Dohmen, K., Mizuta, T., Nakamura, M., Shimohashi, N., Ishibashi, H., Yamamoto, K., 2004. Fenofibrate for patients with asymptomatic primary biliary cirrhosis. *World J. Gastroenterol.* 10, 894–898.
- Donaldson, P.T., Baragiotta, A., Heneghan, M.A., Floreani, A., Venturi, C., Underhill, J.A., et al., 2006. HLA class II alleles, genotypes, haplotypes, and amino acids in primary biliary cirrhosis: a large-scale study. *Hepatology* 44, 667–674.
- Dubel, L., Tanaka, A., Leung, P., Van de Water, J., Coppel, R., Roche, T., et al., 1999. Autoepitope mapping and reactivity of autoantibodies to the dihydrolipoamide dehydrogenase-binding protein (E3BP) and the glycine cleavage proteins in primary biliary cirrhosis. *Hepatology* 29, 1013–1018.
- European Association for the Study of the Liver, 2017. EASL Clinical Practice Guidelines: the diagnosis and management of patients with primary biliary cholangitis. *J. Hepatol.* 67, 145–172.
- Fish, E.N., 2008. The X-files in immunity: sex-based differences predispose immune responses. *Nat. Rev. Immunol.* 8, 737–744.
- Fisk, J., Ritvo, P., Ross, L., Haase, D., Marrie, T., Schlech, W., 1994. Measuring the functional impact of fatigue: initial validation of the fatigue impact scale. *Clin. Infect. Dis.* 18, S79–S83.
- Floreani, A., Cazzagon, N., Franceschet, I., Canesso, F., Salmaso, L., Baldo, V., 2015a. Metabolic syndrome associated with primary biliary cirrhosis. *J. Clin. Gastroenterol.* 49, 57–60.
- Floreani, A., Franceschet, I., Cazzagon, N., Spinazzese, A., Buja, A., Furlan, P., et al., 2015b. Extrahepatic autoimmune conditions associated with primary biliary cirrhosis. *Clin. Rev. Allergy Immunol.* 48, 192–197.
- Gatselis, N.K., Zachou, K., Lygoura, V., Azariadis, K., Arvaniti, P., Spyrou, E., et al., 2017. Geoepidemiology, clinical manifestations and outcome of primary biliary cholangitis in Greece. *Eur. J. Intern. Med.* 42, 81–88.
- Gershwin, M., Mackay, I., Sturgess, A., Coppel, R., 1987. Identification and specificity of a cDNA encoding the 70 kd mitochondrial antigen recognized in primary biliary cirrhosis. *J. Immunol.* 138, 3525–3531.
- Gershwin, M.E., Selmi, C., Worman, H.J., Gold, E.B., Watnik, M., Utts, J., et al., 2005. Risk factors and comorbidities in primary biliary cirrhosis: a controlled interview-based study of 1032 patients. *Hepatology* 42, 1194–1202.
- Grover, V.P., Southern, L., Dyson, J.K., Kim, J.U., Crossey, M.M., Wylezinska-Arridge, M., et al., 2016. Early primary biliary cholangitis is characterised by brain abnormalities on cerebral magnetic resonance imaging. *Aliment. Pharmacol. Ther.* 44, 936–945.
- Guanabens, N., Pares, A., Ros, I., Caballeria, L., Pons, F., Vidal, S., et al., 2005. Severity of cholestasis and advanced histological stage but not menopausal status are the major risk factors for osteoporosis in primary biliary cirrhosis. *J. Hepatol.* 42, 573–577.
- Guanabens, N., Cerda, D., Monegal, A., Pons, F., Caballeria, L., Peris, P., et al., 2010. Low bone mass and severity of cholestasis affect fracture risk in patients with primary biliary cirrhosis. *Gastroenterology* 138, 2348–2356.
- Guanabens, N., Monegal, A., Cerda, D., Muxi, A., Gifre, L., Peris, P., et al., 2013. A randomized trial comparing monthly ibandronate and weekly alendronate for osteoporosis in patients with primary biliary cirrhosis. *Hepatology* 58, 2070–2078.
- Gulamhusein, A.F., Juran, B.D., Atkinson, E.J., McCauley, B., Schlicht, E., Lazaridis, K.N., 2016. Low incidence of primary biliary cirrhosis (PBC) in the first-degree relatives of PBC probands after 8 years of follow-up. *Liver Int.* 36, 1378–1382.
- Hamlyn, A.N., Macklon, A.F., James, O., 1983. Primary biliary cirrhosis: geographical clustering and symptomatic onset seasonality. *Gut* 24, 940–945.
- Harada, K., Nakanuma, Y., 2014. Prevalence and risk factors of hepatocellular carcinoma in Japanese patients with primary biliary cirrhosis. *Hepatol. Res.* 44, 133–140.
- Harada, K., Hirohara, J., Ueno, Y., Nakano, T., Kakuda, Y., Tsubouchi, H., et al., 2013a. Incidence of and risk factors for hepatocellular carcinoma in primary biliary cirrhosis: national data from Japan. *Hepatology* 57, 1942–1949.
- Harada, K., Hsu, M., Ikeda, H., Zeniya, M., Nakanuma, Y., 2013b. Application and validation of a new histologic staging and grading system for primary biliary cirrhosis. *J. Clin. Gastroenterol.* 47, 174–181.
- Heathcote, E.J., Cauch-Dudek, K., Walker, V., Bailey, R.J., Blendis, L.M., Ghent, C.N., et al., 1994. The Canadian Multicenter Double-blind Randomized Controlled Trial of ursodeoxycholic acid in primary biliary cirrhosis. *Hepatology* 19, 1149–1156.
- Hegade, V.S., Khanna, A., Walker, L.J., Wong, L.L., Dyson, J.K., Jones, D.E., 2016. Long-term fenofibrate treatment in primary biliary cholangitis improves biochemistry but not the UK-PBC risk score. *Dig. Dis. Sci.* 61, 3037–3044.
- Hegade, V.S., Kendrick, S.F., Dobbins, R.L., Miller, S.R., Thompson, D., Richards, D., et al., 2017. Effect of ileal bile acid transporter inhibitor GSK2330672 on pruritus in primary biliary cholangitis: a double-blind, randomised, placebo-controlled, crossover, phase 2a study. *Lancet* 18, 1114–1123.
- Hirschfield, G.M., Liu, X., Xu, C., Lu, Y., Xie, G., Gu, X., et al., 2009. Primary biliary cirrhosis associated with HLA, IL12A, and IL12RB2 variants. *N. Engl. J. Med.* 360, 2544–2555.
- Hirschfield, G.M., Liu, X., Han, Y., Gorlov, I.P., Lu, Y., Xu, C., et al., 2010. Variants at IRF5-TNPO3, 17q12-21 and MMEL1 are associated with primary biliary cirrhosis. *Nat. Genet.* 42, 655–657.
- Hirschfield, G.M., Xie, G., Lu, E., Sun, Y., Juran, B.D., Chellappa, V., et al., 2012. Association of primary biliary cirrhosis with variants in the CLEC16A, SOCS1, SPIB and SIAE immunomodulatory genes. *Genes Immun.* 13, 328–335.
- Hodge, D.L., Berthet, C., Coppola, V., Kastenmuller, W., Buschman, M.D., Schaughency, P.M., et al., 2014. IFN-gamma AU-rich element removal promotes chronic IFN-gamma expression and autoimmunity in mice. *J. Autoimmun.* 53, 33–45.
- Honda, A., Ikegami, T., Nakamura, M., Miyazaki, T., Iwamoto, J., Hirayama, T., et al., 2013. Anticholestatic effects of bezafibrate in patients with primary biliary cirrhosis treated with ursodeoxycholic acid. *Hepatology* 57, 1931–1941.

- Hosonuma, K., Sato, K., Yamazaki, Y., Yanagisawa, M., Hashizume, H., Horiguchi, N., et al., 2015. A prospective randomized controlled study of long-term combination therapy using ursodeoxycholic acid and bezafibrate in patients with primary biliary cirrhosis and dyslipidemia. *Am. J. Gastroenterol.* 110, 423–431.
- Howel, D., Fischbacher, C.M., Bhopal, R.S., Gray, J., Metcalf, J.V., James, O.F., 2000. An exploratory population-based case-control study of primary biliary cirrhosis. *Hepatology* 31, 1055–1060.
- Huet, P.M., Deslauriers, J., Tran, A., Faucher, C., Charbonneau, J., 2000. Impact of fatigue on the quality of life of patients with primary biliary cirrhosis. *Am. J. Gastroenterol.* 95, 760–767.
- Imam, M.H., Silveira, M.G., Sinakos, E., Gossard, A.A., Jorgensen, R., Keach, J., et al., 2012. Long-term outcomes of patients with primary biliary cirrhosis and hepatocellular carcinoma. *Clin. Gastroenterol. Hepatol.* 10, 182–185.
- Invernizzi, P., Crosignani, A., Battezzati, P., Covini, G., Valle, G., Larghi, A., et al., 1997. Comparison of the clinical features and clinical course of antimitochondrial antibody-positive and -negative primary biliary cirrhosis. *Hepatology* 25, 1090–1095.
- Invernizzi, P., Podda, M., Battezzati, P.M., Crosignani, A., Zuin, M., Hitchman, E., et al., 2001. Autoantibodies against nuclear pore complexes are associated with more active and severe liver disease in primary biliary cirrhosis. *J. Hepatol.* 34, 366–372.
- Invernizzi, P., Miozzo, M., Battezzati, P.M., Bianchi, I., Grati, F.R., Simoni, G., et al., 2004. Frequency of monosomy X in women with primary biliary cirrhosis. *Lancet* 363, 533–535.
- Invernizzi, P., Ransom, M., Raychaudhuri, S., Kosoy, R., Lleo, A., Shigeta, R., et al., 2012. Classical HLA-DRB1 and DPB1 alleles account for HLA associations with primary biliary cirrhosis. *Genes Immun.* 13, 461–468.
- Irie, J., Wu, Y., Wicker, L.S., Rainbow, D., Nalesnik, M.A., Hirsch, R., et al., 2006. NOD.c3c4 congenic mice develop autoimmune biliary disease that serologically and pathogenetically models human primary biliary cirrhosis. *J. Exp. Med.* 203, 1209–1219.
- Iwasaki, S., Ohira, H., Nishiguchi, S., Zeniya, M., Kaneko, S., Onji, M., et al., 2008. The efficacy of ursodeoxycholic acid and bezafibrate combination therapy for primary biliary cirrhosis: a prospective, multicenter study. *Hepatol. Res.* 38, 557–564.
- Iwasaki, S., Tsuda, K., Ueta, H., Aono, R., Ono, M., Saibara, T., et al., 1999. Bezafibrate may have a beneficial effect in pre-cirrhotic primary biliary cirrhosis. *Hepatol. Res.* 16, 12–18.
- Jacoby, A., Rannard, A., Buck, D., Bhala, N., Newton, J.L., James, O.F., et al., 2005. Development, validation, and evaluation of the PBC-40, a disease specific health related quality of life measure for primary biliary cirrhosis. *Gut* 54, 1622–1629.
- Jeffrey, G., Reed, W., Shilkin, K., 1990. Primary biliary cirrhosis: clinicopathological characteristics and outcome. *J. Gastroenterol. Hepatol.* 5, 639–645.
- Jopson, L., Jones, D.E., 2015. Fatigue in primary biliary cirrhosis: prevalence, pathogenesis and management. *Dig. Dis.* 33 (Suppl 2), 109–114.
- Juliusson, G., Imam, M., Bjornsson, E.S., Talwalkar, J.A., Lindor, K.D., 2016. Long-term outcomes in antimitochondrial antibody negative primary biliary cirrhosis. *Scand. J. Gastroenterol.* 51, 745–752.
- Juran, B.D., Hirschfield, G.M., Invernizzi, P., Atkinson, E.J., Li, Y., Xie, G., et al., 2012. Immunochip analyses identify a novel risk locus for primary biliary cirrhosis at 13q14, multiple independent associations at four established risk loci and epistasis between 1p31 and 7q32 risk variants. *Hum. Mol. Genet.* 21, 5209–5221.
- Kanth, R., Shrestha, R.B., Rai, I., VanWormer, J.J., Roy, P.K., 2017. Incidence of primary biliary cholangitis in a rural Midwestern population. *Clin. Med. Res.* 15, 13–18.
- Katsumi, T., Tomita, K., Leung, P.S., Yang, G.X., Gershwin, M.E., Ueno, Y., 2015. Animal models of primary biliary cirrhosis. *Clin. Rev. Allergy Immunol.* 48, 142–153.
- Kawashima, M., Hitomi, Y., Aiba, Y., Nishida, N., Kojima, K., Kawai, Y., et al., 2017. Genome-wide association studies identify PRKCB as a novel genetic susceptibility locus for primary biliary cholangitis in the Japanese population. *Hum. Mol. Genet.* 26, 650–659.
- Kawata, K., Tsuda, M., Yang, G.X., Zhang, W., Tanaka, H., Tsuneyama, K., et al., 2013. Identification of potential cytokine pathways for therapeutic intervention in murine primary biliary cirrhosis. *PLoS One* 8, e74225.
- Kikuchi, K., Lian, Z.X., Yang, G.X., Ansari, A.A., Ikehara, S., Kaplan, M., et al., 2005. Bacterial CpG induces hyper-IgM production in CD27(+) memory B cells in primary biliary cirrhosis. *Gastroenterology* 128, 304–312.
- Kim, W., Poterucha, J., Jorgensen, R., Batts, K., Homburger, H., Dickson, E., et al., 1997. Does antimitochondrial antibody status affect response to treatment in patients with primary biliary cirrhosis? Outcomes of ursodeoxycholic acid therapy and liver transplantation. *Hepatology* 26, 22–26.
- Kim, W.R., Lindor, K.D., Locke III, G.R., Therneau, T.M., Homburger, H.A., et al., 2000. Epidemiology and natural history of primary biliary cirrhosis in a US community. *Gastroenterology* 119, 1631–1636.
- Kim, K.A., Ki, M., Choi, H.Y., Kim, B.H., Jang, E.S., Jeong, S.H., 2016. Population-based epidemiology of primary biliary cirrhosis in South Korea. *Aliment. Pharmacol. Ther.* 43, 154–162.
- Kita, H., Lian, Z.X., Van de Water, J., He, X.S., Matsumura, S., Kaplan, M., et al., 2002a. Identification of HLA-A2-restricted CD8(+) cytotoxic T cell responses in primary biliary cirrhosis: T cell activation is augmented by immune complexes cross-presented by dendritic cells. *J. Exp. Med.* 195, 113–123.
- Kita, H., Matsumura, S., He, X.S., Ansari, A.A., Lian, Z.X., Van de Water, J., et al., 2002b. Quantitative and functional analysis of PDC-E2-specific autoreactive cytotoxic T lymphocytes in primary biliary cirrhosis. *J. Clin. Invest.* 109, 1231–1240.
- Kitic, I., Boskovic, A., Stankovic, I., Prokic, D., 2012. Twelve-year-old girl with primary biliary cirrhosis. *Case Rep. Pediatr.* 2012, 937150.
- Koarada, S., Wu, Y., Fertig, N., Sass, D.A., Nalesnik, M., Todd, J.A., et al., 2004. Genetic control of autoimmunity: protection from diabetes, but spontaneous autoimmune biliary disease in a nonobese diabetic congenic strain. *J. Immunol.* 173, 2315–2323.
- Krams, S.M., Van de Water, J., Coppel, R.L., Esquivel, C., Roberts, J., Ansari, A., et al., 1990. Analysis of hepatic T lymphocyte and immunoglobulin deposits in patients with primary biliary cirrhosis. *Hepatology* 12, 306–313.
- Kremer, A.E., Martens, J.J., Kulik, W., Rueff, F., Kuiper, E.M., van Buuren, H.R., et al., 2010a. Lysophosphatidic acid is a potential mediator of cholestatic pruritus. *Gastroenterology* 139, 1008–1018.e1.
- Kremer, A.E., Martens, J.J., Kulik, W., Williamson, C., Moolenaar, W.H., Kondrackiene, J., et al., 2010b. Autotaxin but not bile salts correlate with itch intensity in cholestasis. *J. Hepatol.* 52, S1.
- Kremer, A.E., van Dijk, R., Leckie, P., Schaap, F.G., Kuiper, E.M., Mettang, T., et al., 2012. Serum autotaxin is increased in pruritus of cholestasis, but not of other origin, and responds to therapeutic interventions. *Hepatology* 56, 1391–1400.

- Kuiper, E.M., Hansen, B.E., de Vries, R.A., den Ouden-Muller, J.W., van Ditzhuijsen, T.J., Haagsma, E.B., et al., 2009. Improved prognosis of patients with primary biliary cirrhosis that have a biochemical response to ursodeoxycholic acid. *Gastroenterology* 136, 1281–1287.
- Kumagi, T., Guindi, M., Fischer, S.E., Arenovich, T., Abdalian, R., Coltescu, C., et al., 2010. Baseline ductopenia and treatment response predict long-term histological progression in primary biliary cirrhosis. *Am. J. Gastroenterol.* 105, 2186–2194.
- Lammers, W.J., Hirschfield, G.M., Corpechot, C., Nevens, F., Lindor, K.D., Janssen, H.L., et al., 2015. Development and validation of a scoring system to predict outcomes of patients with primary biliary cirrhosis receiving ursodeoxycholic acid therapy. *Gastroenterology* 149, 1804–1812.e4.
- Lammers, W.J., van Buuren, H.R., Hirschfield, G.M., Janssen, H.L., Invernizzi, P., Mason, A.L., et al., 2014. Levels of alkaline phosphatase and bilirubin are surrogate end points of outcomes of patients with primary biliary cirrhosis: an international follow-up study. *Gastroenterology* 147, 1338–1349.e5; quiz e1315.
- Leung, P.S., Iwayama, T., Prindiville, T., Chuang, D.T., Ansari, A.A., Wynn, R.M., et al., 1992. Use of designer recombinant mitochondrial antigens in the diagnosis of primary biliary cirrhosis. *Hepatology* 15, 367–372.
- Leung, P., Chuang, D., Wynn, R., Cha, S., Danner, D., Ansari, A., et al., 1995. Autoantibodies to BCOADC-E2 in patients with primary biliary cirrhosis recognize a conformational epitope. *Hepatology* 22, 505–513.
- Leung, P., Coppel, R., Ansari, A., Munoz, S., Gershwin, M., 1997. Antimitochondrial antibodies in primary biliary cirrhosis. *Semin. Liver Dis.* 17, 61–69.
- Lindor, K., 2007. Ursodeoxycholic acid for the treatment of primary biliary cirrhosis. *N. Engl. J. Med.* 357, 1524–1529.
- Lindor, K.D., Therneau, T.M., Jorgensen, R.A., Malinchoc, M., Dickson, E.R., 1996. Effects of ursodeoxycholic acid on survival in patients with primary biliary cirrhosis. *Gastroenterology* 110, 1515–1518.
- Lindor, K.D., Jorgensen, R.A., Therneau, T.M., Malinchoc, M., Dickson, E.R., 1997. Ursodeoxycholic acid delays the onset of esophageal varices in primary biliary cirrhosis. *Mayo Clin. Proc.* 72, 1137–1140.
- Lindor, K.D., Gershwin, M.E., Poupon, R., Kaplan, M., Bergasa, N.V., Heathcote, E.J., 2009. American Association for Study of Liver Diseases. Primary biliary cirrhosis. *Hepatology* 50, 291–308.
- Liu, H., Liu, Y., Wang, L., Xu, D., Lin, B., Zhong, R., et al., 2010a. Prevalence of primary biliary cirrhosis in adults referring hospital for annual health check-up in Southern China. *BMC Gastroenterol.* 10, 100.
- Liu, H., Norman, G.L., Shums, Z., Worman, H.J., Krawitt, E.L., Bizzaro, N., et al., 2010b. PBC screen: an IgG/IgA dual isotype ELISA detecting multiple mitochondrial and nuclear autoantibodies specific for primary biliary cirrhosis. *J. Autoimmun.* 35, 436–442.
- Liu, X., Invernizzi, P., Lu, Y., Kosoy, R., Lu, Y., Bianchi, I., et al., 2010c. Genome-wide meta-analyses identify three loci associated with primary biliary cirrhosis. *Nat. Genet.* 42, 658–660.
- Liu, J.Z., Almarri, M.A., Gaffney, D.J., Mells, G.F., Jostins, L., Cordell, H.J., et al., 2012. Dense fine-mapping study identifies new susceptibility loci for primary biliary cirrhosis. *Nat. Genet.* 44, 1137–1141.
- Lleo, A., Selmi, C., Invernizzi, P., Podda, M., Coppel, R.L., Mackay, I.R., et al., 2009. Apoptoses and the biliary specificity of primary biliary cirrhosis. *Hepatology* 49, 871–879.
- Lleo, A., Bowlus, C.L., Yang, G.X., Invernizzi, P., Podda, M., Van de Water, J., et al., 2010. Biliary apoptoses and anti-mitochondrial antibodies activate innate immune responses in primary biliary cirrhosis. *Hepatology* 52, 987–998.
- Lleo, A., Zhang, W., Zhao, M., Tan, Y., Bernuzzi, F., Zhu, B., et al., 2015. DNA methylation profiling of the X chromosome reveals an aberrant demethylation on CXCR3 promoter in primary biliary cirrhosis. *Clin. Epigenetics* 7, 61.
- Lleo, A., Jepsen, P., Morenghi, E., Carbone, M., Moroni, L., Battezzati, P.M., et al., 2016. Evolving trends in female to male incidence and male mortality of primary biliary cholangitis. *Sci. Rep.* 6, 25906.
- Ludwig, J., 2000. The pathology of primary biliary cirrhosis and autoimmune cholangitis. *Baillieres Best Pract. Res. Clin. Gastroenterol.* 14, 601–613.
- Ludwig, J., McDonald, G.S., 1978. Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). *Virchows Arch. A Pathol. Anat. Histol.* 379, 103–112.
- Mackay, I.R., 1958. Primary biliary cirrhosis showing a high titer of autoantibody: report of a case. *N. Engl. J. Med.* 258, 185–188.
- Mahl, T.C., Shockcor, W., Boyer, J.L., 1994. Primary biliary cirrhosis: survival of a large cohort of symptomatic and asymptomatic patients followed for 24 years. *J. Hepatol.* 20, 707–713.
- Mantaka, A., Koulentaki, M., Chlouverakis, G., Enele-Melono, J.M., Darvianaki, A., Tzardi, M., et al., 2012. Primary biliary cirrhosis in a genetically homogeneous population: disease associations and familial occurrence rates. *BMC Gastroenterol.* 12, 110.
- Markle, J.G., Frank, D.N., Mortin-Toth, S., Robertson, C.E., Feazel, L.M., Rolle-Kampczyk, U., et al., 2013. Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. *Science* 339, 1084–1088.
- Mattalia, A., Quaranta, S., Leung, P., Bauducci, M., Van de Water, J., Calvo, P., et al., 1998. Characterization of antimitochondrial antibodies in healthy adults. *Hepatology* 27, 656–661.
- McNally, R.J., James, P.W., Ducker, S., Norman, P.D., James, O.F., 2014. No rise in incidence but geographical heterogeneity in the occurrence of primary biliary cirrhosis in north East England. *Am. J. Epidemiol.* 179, 492–498.
- Mella, J., Roschmann, E., Maier, K.-P., Volk, B., 1995. Association of primary biliary cirrhosis with the allele HLA-DPB1\*0301 in a German population. *Hepatology* 21, 398–402.
- Mells, G.F., Floyd, J.A., Morley, K.I., Cordell, H.J., Franklin, C.S., Shin, S.Y., et al., 2011. Genome-wide association study identifies 12 new susceptibility loci for primary biliary cirrhosis. *Nat. Genet.* 43, 329–332.
- Mells, G.F., Pells, G., Newton, J.L., Bathgate, A.J., Burroughs, A.K., Heneghan, M.A., et al., 2013. Impact of primary biliary cirrhosis on perceived quality of life: the UK-PBC national study. *Hepatology* 58, 273–283.
- Miyakawa, H., Tanaka, A., Kikuchi, K., Matsushita, M., Kitazawa, E., Kawaguchi, N., et al., 2001. Detection of antimitochondrial autoantibodies in immunofluorescent AMA-negative patients with primary biliary cirrhosis using recombinant autoantigens. *Hepatology* 34, 243–248.
- Momah, N., Silveira, M.G., Jorgensen, R., Sinakos, E., Lindor, K.D., 2012. Optimizing biochemical markers as endpoints for clinical trials in primary biliary cirrhosis. *Liver Int.* 32, 790–795.

- Moteki, S., Leung, P., Dickson, E., Van Thiel, D., Galperin, C., Buch, T., et al., 1996a. Epitope mapping and reactivity of autoantibodies to the E2 component of 2-oxoglutarate dehydrogenase complex in primary biliary cirrhosis using recombinant 2-oxoglutarate dehydrogenase complex. *Hepatology* 23, 436–444.
- Moteki, S., Leung, P.S., Coppel, R., Dickson, E., Kaplan, M., Munoz, S., et al., 1996b. Use of a designer triple expression hybrid clone for three different lipoyl domain for the detection of antimitochondrial autoantibodies. *Hepatology* 24, 97–103.
- Muratori, L., Granito, A., Muratori, P., Pappas, G., Bianchi, F.B., 2008. Antimitochondrial antibodies and other antibodies in primary biliary cirrhosis: diagnostic and prognostic value. *Clin. Liver Dis.* 12, 261–276.
- Muratori, P., Muratori, L., Ferrari, R., Cassani, F., Bianchi, G., Lenzi, M., et al., 2003. Characterization and clinical impact of antinuclear antibodies in primary biliary cirrhosis. *Am. J. Gastroenterol.* 98, 431–437.
- Myszor, M., James, O.F., 1990. The epidemiology of primary biliary cirrhosis in north-east England: an increasingly common disease? *Q. J. Med.* 75, 377–385.
- Nakamura, M., Kondo, H., Mori, T., Komori, A., Matsuyama, M., Ito, M., et al., 2007. Anti-gp210 and anti-centromere antibodies are different risk factors for the progression of primary biliary cirrhosis. *Hepatology* 45, 118–127.
- Nakamura, M., Nishida, N., Kawashima, M., Aiba, Y., Tanaka, A., Yasunami, M., et al., 2012. Genome-wide association study identifies TNFSF15 and POU2AF1 as susceptibility loci for primary biliary cirrhosis in the Japanese population. *Am. J. Hum. Genet.* 91, 721–728.
- Nakano, T., Inoue, K., Hirohara, J., Arita, S., Higuchi, K., Omata, M., et al., 2002. Long-term prognosis of primary biliary cirrhosis (PBC) in Japan and analysis of the factors of stage progression in asymptomatic PBC (a-PBC). *Hepatol. Res.* 22, 250–260.
- Nakanuma, Y., Harada, K., Kaji, K., Terasaki, S., Tsuneyama, K., Moteki, S., et al., 1997. Clinicopathological study of primary biliary cirrhosis negative for antimitochondrial antibodies. *Liver* 17, 281–287.
- Nakanuma, Y., Zen, Y., Harada, K., Sasaki, M., Nonomura, A., Uehara, T., et al., 2010. Application of a new histological staging and grading system for primary biliary cirrhosis to liver biopsy specimens: interobserver agreement. *Pathol. Int.* 60, 167–174.
- Nevens, F., Andreone, P., Mazzella, G., Strasser, S.I., Bowlus, C., Invernizzi, P., et al., 2016. A placebo-controlled trial of obeticholic acid in primary biliary cholangitis. *N. Engl. J. Med.* 375, 631–643.
- Ngu, J.H., Gearry, R.B., Wright, A.J., Stedman, C.A., 2012. Low incidence and prevalence of primary biliary cirrhosis in Canterbury, New Zealand: a population-based study. *Hepatol. Int.* 6, 796–800.
- Nishikawa, H., Enomoto, H., Iwata, Y., Hasegawa, K., Nakano, C., Takata, R., et al., 2016. Impact of serum *Wisteria floribunda* agglutinin positive Mac-2-binding protein and serum interferon-gamma-inducible protein-10 in primary biliary cirrhosis. *Hepatol. Res.* 46, 575–583.
- Nyberg, A., Loof, L., 1989. Primary biliary cirrhosis: clinical features and outcome, with special reference to asymptomatic disease. *Scand. J. Gastroenterol.* 24, 57–64.
- Oertelt, S., Lian, Z.X., Cheng, C.M., Chuang, Y.H., Padgett, K.A., He, X.S., et al., 2006. Anti-mitochondrial antibodies and primary biliary cirrhosis in TGF-beta receptor II dominant-negative mice. *J. Immunol.* 177, 1655–1660.
- Oertelt, S., Rieger, R., Selmi, C., Invernizzi, P., Ansari, A.A., Coppel, R.L., et al., 2007. A sensitive bead assay for antimitochondrial antibodies: chipping away at AMA-negative primary biliary cirrhosis. *Hepatology* 45, 659–665.
- Onishi, S., Sakamaki, T., Maeda, T., Iwamura, S., Tomita, A., Saibara, T., et al., 1994. DNA typing of HLA class II genes: DRB1\*0803 increases the susceptibility of Japanese to primary biliary cirrhosis. *J. Hepatol.* 21, 1053–1060.
- Pares, A., Caballeria, L., Rodes, J., 2006. Excellent long-term survival in patients with primary biliary cirrhosis and biochemical response to ursodeoxycholic acid. *Gastroenterology* 130, 715–720.
- Pelfrey, C.M., Cotleur, A.C., Lee, J.C., Rudick, R.A., 2002. Sex differences in cytokine responses to myelin peptides in multiple sclerosis. *J. Neuroimmunol.* 130, 211–223.
- Pellicciari, R., Fiorucci, S., Camaiioni, E., Clerici, C., Costantino, G., Maloney, P.R., et al., 2002. 6Alpha-ethyl-chenodeoxycholic acid (6-ECDCA), a potent and selective FXR agonist endowed with anticholestatic activity. *J. Med. Chem.* 45, 3569–3572.
- Poupon, R., 2015. Non-invasive assessment of liver fibrosis progression and prognosis in primary biliary cholangitis. *Dig. Dis.* 33 (Suppl 2), 115–117.
- Poupon, R.E., Balkau, B., Eschwege, E., Poupon, R., 1991. A multicenter, controlled trial of ursodiol for the treatment of primary biliary cirrhosis. UDCA-PBC Study Group. *N. Engl. J. Med.* 324, 1548–1554.
- Poupon, R.E., Poupon, R., Balkau, B., 1994. Ursodiol for the long-term treatment of primary biliary cirrhosis. The UDCA-PBC Study Group. *N. Engl. J. Med.* 330, 1342–1347.
- Poupon, R.E., Lindor, K.D., Cauch-Dudek, K., Dickson, E.R., Poupon, R., Heathcote, E.J., 1997. Combined analysis of randomized controlled trials of ursodeoxycholic acid in primary biliary cirrhosis. *Gastroenterology* 113, 884–890.
- Poupon, R.E., Lindor, K.D., Pares, A., Chazouilleres, O., Poupon, R., Heathcote, E.J., 2003. Combined analysis of the effect of treatment with ursodeoxycholic acid on histologic progression in primary biliary cirrhosis. *J. Hepatol.* 39, 12–16.
- Prince, M.I., Chetwynd, A., Diggle, P., Jarner, M., Metcalf, J.V., James, O.F., 2001. The geographical distribution of primary biliary cirrhosis in a well-defined cohort. *Hepatology* 34, 1083–1088.
- Prince, M., Chetwynd, A., Newman, W., Metcalf, J.V., James, O.F., 2002. Survival and symptom progression in a geographically based cohort of patients with primary biliary cirrhosis: follow-up for up to 28 years. *Gastroenterology* 123, 1044–1051.
- Qiu, F., Tang, R., Zuo, X., Shi, X., Wei, Y., Zheng, X., et al., 2017. A genome-wide association study identifies six novel risk loci for primary biliary cholangitis. *Nat. Commun.* 8, 14828.
- Rieger, R., Leung, P.S., Jeddelloh, M.R., Kurth, M.J., Nantz, M.H., Lam, K.S., et al., 2006. Identification of 2-nonynoic acid, a cosmetic component, as a potential trigger of primary biliary cirrhosis. *J. Autoimmun.* 27, 7–16.
- Rong, G., Wang, H., Bowlus, C.L., Wang, C., Lu, Y., Zeng, Z., et al., 2015. Incidence and risk factors for hepatocellular carcinoma in primary biliary cirrhosis. *Clin. Rev. Allergy Immunol.* 48, 132–141.
- Rosser, E.C., Mauri, C., 2016. A clinical update on the significance of the gut microbiota in systemic autoimmunity. *J. Autoimmun.* 74, 85–93.
- Rubin, E., Schaffner, F., Popper, H., 1965. Primary biliary cirrhosis. Chronic non-suppurative destructive cholangitis. *Am. J. Pathol.* 46, 387–407.

- Rubtsova, K., Marrack, P., Rubtsov, A.V., 2015. TLR7, IFNgamma, and T-bet: their roles in the development of ABCs in female-biased autoimmunity. *Cell. Immunol.* 294, 80–83.
- Samur, S., Klebanoff, M., Banken, R., Pratt, D.S., Chapman, R., Ollendorf, D.A., et al., 2017. Long-term clinical impact and cost-effectiveness of obeticholic acid for the treatment of primary biliary cholangitis. *Hepatology* 65, 920–928.
- Scheuer, P., 1967. Primary biliary cirrhosis. *Proc. R. Soc. Med.* 60, 1257.
- Selmi, C., Balkwill, D., Invernizzi, P., Ansari, A., Coppel, R., Podda, M., et al., 2003. Patients with primary biliary cirrhosis react against a ubiquitous xenobiotic-metabolizing bacterium. *Hepatology* 38, 1250–1257.
- Selmi, C., Invernizzi, P., Miozzo, M., Podda, M., Gershwin, M.E., 2004a. Primary biliary cirrhosis: does X mark the spot? *Autoimmun. Rev.* 3, 493–499.
- Selmi, C., Mayo, M., Bach, N., Ishibashi, H., Invernizzi, P., Gish, R., et al., 2004b. Primary biliary cirrhosis in monozygotic and dizygotic twins: genetics, epigenetics, and environment. *Gastroenterology* 127, 485–492.
- Selmi, C., Zuin, M., Bowlus, C.L., Gershwin, M.E., 2008. Anti-mitochondrial antibody-negative primary biliary cirrhosis. *Clin. Liver Dis.* 12, 173–185. ix.
- Selmi, C., Cavaciocchi, F., Lleo, A., Cheroni, C., De Francesco, R., Lombardi, S.A., et al., 2014. Genome-wide analysis of DNA methylation, copy number variation, and gene expression in monozygotic twins discordant for primary biliary cirrhosis. *Front. Immunol.* 5, 128.
- Sherlock, S., 1959. Primary biliary cirrhosis (chronic intrahepatic obstructive jaundice). *Gastroenterology* 37, 574–586.
- Shibata, M., Onozuka, Y., Morizane, T., Koizumi, H., Kawaguchi, N., Miyakawa, H., et al., 2004. Prevalence of antimitochondrial antibody in Japanese corporate workers in Kanagawa prefecture. *J. Gastroenterol.* 39, 255–259.
- Shimoda, S., Harada, K., Niijo, H., Shirabe, K., Taketomi, A., Maebara, Y., et al., 2011. Interaction between Toll-like receptors and natural killer cells in the destruction of bile ducts in primary biliary cirrhosis. *Hepatology* 53, 1270–1281.
- Shimoda, S., Miyakawa, H., Nakamura, M., Ishibashi, H., Kikuchi, K., Kita, H., et al., 2008. CD4 T-cell autoreactivity to the mitochondrial autoantigen PDC-E2 in AMA-negative primary biliary cirrhosis. *J. Autoimmun.* 31, 110–115.
- Shimoda, S., Nakamura, M., Ishibashi, H., 1995. HLA DRB4 0101-restricted immunodominant T cell autoepitope of pyruvate dehydrogenase complex in primary biliary cirrhosis: evidence of molecular mimicry in human autoimmune disease. *J. Exp. Med.* 181, 1835–1845.
- Shimoda, S., Van de Water, J., Ansari, A., Nakamura, M., Ishibashi, H., Coppel, R., et al., 1998. Identification and precursor frequency analysis of a common T cell epitope motif in mitochondrial autoantigens in primary biliary cirrhosis. *J. Clin. Invest.* 102, 1831–1840.
- Silveira, M.G., Gossard, A.A., Stahler, A.C., Jorgensen, R.A., Petz, J.L., Ali, A.H., et al., 2017. A randomized, placebo-controlled clinical trial of efficacy and safety: modafinil in the treatment of fatigue in patients with primary biliary cirrhosis. *Am. J. Ther.* 24, e167–e176.
- Sood, S., Gow, P.J., Christie, J.M., Angus, P.W., 2004. Epidemiology of primary biliary cirrhosis in Victoria, Australia: high prevalence in migrant populations. *Gastroenterology* 127, 470–475.
- Sorokin, A., Brown, J.L., Thompson, P.D., 2007. Primary biliary cirrhosis, hyperlipidemia, and atherosclerotic risk: a systematic review. *Atherosclerosis* 194, 293–299.
- Sun, Y., Haapanen, K., Li, B., Zhang, W., Van de Water, J., Gershwin, M.E., 2015. Women and primary biliary cirrhosis. *Clin. Rev. Allergy Immunol.* 48, 285–300.
- Surh, C., Coppel, R., Gershwin, M., 1990. Structural requirement for autoreactivity on human pyruvate dehydrogenase-E2, the major autoantigen of primary biliary cirrhosis. Implication for a conformational autoepitope. *J. Immunol.* 144, 1321–1328.
- Tanaka, A., Hirohara, J., Nakanuma, Y., Tsubouchi, H., Takikawa, H., 2015. Biochemical responses to bezafibrate improve long-term outcome in asymptomatic patients with primary biliary cirrhosis refractory to UDCA. *J. Gastroenterol.* 50, 675–682.
- Tanaka, A., Ma, X., Yokosuka, O., Weltman, M., You, H., Amarapurkar, D.N., et al., 2016. Autoimmune liver diseases in the Asia-Pacific region: proceedings of APASL Symposium on AIH and PBC 2016. *Hepatol. Int.* 10, 909–915.
- Tanaka, T., Zhang, W., Sun, Y., Shuai, Z., Chida, A., Kenny, T.P., et al., 2017. Autoreactive monoclonal antibodies from patients with primary biliary cholangitis recognize environmental xenobiotics. *Hepatology* 66, 885–895.
- Tang, R., Wei, Y., Li, Y., Chen, W., Chen, H., Wang, Q., et al., 2017. Gut microbial profile is altered in primary biliary cholangitis and partially restored after UDCA therapy. *Gut* Epub ahead of print.
- Taylor, S., Dean, P., Riely, C., 1994. Primary autoimmune cholangitis: an alternative to antimitochondrial antibody-negative primary biliary cirrhosis. *Am. J. Surg. Pathol.* 18, 91–99.
- Trivedi, P.J., Bruns, T., Cheung, A., Li, K.K., Kittler, C., Kumagi, T., et al., 2014. Optimising risk stratification in primary biliary cirrhosis: AST/platelet ratio index predicts outcome independent of ursodeoxycholic acid response. *J. Hepatol.* 60, 1249–1258.
- Trivedi, P.J., Corpechot, C., Pares, A., Hirschfield, G.M., 2016a. Risk stratification in autoimmune cholestatic liver diseases: opportunities for clinicians and trialists. *Hepatology* 63, 644–659.
- Trivedi, P.J., Lammers, W.J., van Buuren, H.R., Pares, A., Floreani, A., Janssen, H.L., et al., 2016b. Stratification of hepatocellular carcinoma risk in primary biliary cirrhosis: a multicentre international study. *Gut* 65, 321–329.
- Tsuda, M., Zhang, W., Yang, G.X., Tsuneyama, K., Ando, Y., Kawata, K., et al., 2013. Deletion of interleukin (IL)-12p35 induces liver fibrosis in dominant-negative TGFbeta receptor type II mice. *Hepatology* 57, 806–816.
- Umemura, T., Joshi, S., Sekiguchi, T., Usami, Y., Shibata, S., Kimura, T., et al., 2015. Serum *Wisteria floribunda* agglutinin-positive Mac-2-binding protein level predicts liver fibrosis and prognosis in primary biliary cirrhosis. *Am. J. Gastroenterol.* 110, 857–864.
- Umemura, T., Sekiguchi, T., Joshi, S., Yamazaki, T., Fujimori, N., Shibata, S., et al., 2017. Association between serum soluble CD14 and IL-8 levels and clinical outcome in primary biliary cholangitis. *Liver Int.* 37, 897–905.
- Van de Water, J., Gershwin, M., Leung, P., 1988. The autoepitope of the 74-kD mitochondrial autoantigen of primary biliary cirrhosis corresponds to the functional site of dihydrolipoamide acetyltransferase. *J. Exp. Med.* 167, 1791–1799.
- Wakabayashi, K., Lian, Z.X., Moritoki, Y., Lan, R.Y., Tsuneyama, K., Chuang, Y.H., et al., 2006. IL-2 receptor alpha(-/-) mice and the development of primary biliary cirrhosis. *Hepatology* 44, 1240–1249.
- Wakabayashi, K., Lian, Z.X., Leung, P.S., Moritoki, Y., Tsuneyama, K., Kurth, M.J., et al., 2008. Loss of tolerance in C57BL/6 mice to the autoantigen E2 subunit of pyruvate dehydrogenase by a xenobiotic with ensuing biliary ductular disease. *Hepatology* 48, 531–540.

- Wang, J., Budamagunta, M.S., Voss, J.C., Kurth, M.J., Lam, K.S., Lu, L., et al., 2013. Antimitochondrial antibody recognition and structural integrity of the inner lipoyl domain of the E2 subunit of pyruvate dehydrogenase complex. *J. Immunol.* 191, 2126–2133.
- Wang, J., Yang, G.X., Tsuneyama, K., Gershwin, M.E., Ridgway, W.M., Leung, P.S., 2014. Animal models of primary biliary cirrhosis. *Semin. Liver Dis.* 34, 285–296.
- Watson, R.G., Angus, P.W., Dewar, M., Goss, B., Sewell, R.B., Smallwood, R.A., 1995. Low prevalence of primary biliary cirrhosis in Victoria, Australia. *Melbourne Liver Group. Gut* 36, 927–930.
- Wong, G., Law, F., Wong, V., Hui, A., Chan, F., Sung, J., et al., 2007. Health-related quality of life in Chinese patients with primary biliary cirrhosis. *J. Gastroenterol. Hepatol.* Epub ahead of print.
- Wong, R.K., Lim, S.G., Wee, A., Chan, Y.H., Aung, M.O., Wai, C.T., 2008. Primary biliary cirrhosis in Singapore: evaluation of demography, prognostic factors and natural course in a multi-ethnic population. *J. Gastroenterol. Hepatol.* 23, 599–605.
- Working Subgroup for Clinical Practice Guidelines for Primary Biliary Cirrhosis, 2014. Guidelines for the management of primary biliary cirrhosis: the Intractable Hepatobiliary Disease Study Group supported by the Ministry of Health, Labour and Welfare of Japan. *Hepatol. Res.* 44 (Suppl S1), 71–90.
- Yanagisawa, M., Takagi, H., Takahashi, H., Uehara, M., Otsuka, T., Yuasa, K., et al., 2010. Familial clustering and genetic background of primary biliary cirrhosis in Japan. *Dig. Dis. Sci.* 55, 2651–2658.
- Yang, C.Y., Ma, X., Tsuneyama, K., Huang, S., Takahashi, T., Chalasani, N.P., et al., 2014. IL-12/Th1 and IL-23/Th17 biliary microenvironment in primary biliary cirrhosis: implications for therapy. *Hepatology* 59, 1944–1953.
- Yang, F., Yang, Y., Wang, Q., Wang, Z., Miao, Q., Xiao, X., et al., 2017. The risk predictive values of UK-PBC and GLOBE scoring system in Chinese patients with primary biliary cholangitis: the additional effect of anti-gp210. *Aliment. Pharmacol. Ther.* 45, 733–743.
- Yoshida, K., Yang, G.X., Zhang, W., Tsuda, M., Tsuneyama, K., Moritoki, Y., et al., 2009. Deletion of interleukin-12p40 suppresses autoimmune cholangitis in dominant negative transforming growth factor beta receptor type II mice. *Hepatology* 50, 1494–1500.
- Zhang, M.A., Rego, D., Moshkova, M., Kebir, H., Chruscinski, A., Nguyen, H., et al., 2012. Peroxisome proliferator-activated receptor (PPAR) alpha and -gamma regulate IFNgamma and IL-17A production by human T cells in a sex-specific way. *Proc. Natl. Acad. Sci. U.S.A.* 109, 9505–9510.

# Autoimmune Pancreatitis and Immunoglobulin G4–Related Disease

*Shigeyuki Kawa<sup>1</sup>, Kendo Kiyosawa<sup>2</sup> and Hideaki Hamano<sup>3</sup>*

<sup>1</sup>Department of Internal Medicine, Matsumoto Dental University, Shiojiri, Japan <sup>2</sup>Department of Gastrointestinal Medicine, Aizawa Hospital, Matsumoto, Japan <sup>3</sup>Division of Medical Informatics, Shinshu University Hospital, Matsumoto, Japan

## O U T L I N E

<b>General Introduction</b>	<b>1173</b>	<i>Adaptive Immunity</i>	1179
<i>Gateway From Autoimmune Pancreatitis to Immunoglobulin G4–Related Disease</i>	1173	<b>Genetics</b>	<b>1181</b>
<i>Association Studies Using Polymorphic Markers in Candidate Genes</i>		<i>Association Studies Using Genome-Wide Polymorphic Markers</i>	1181
<i>Association Studies Using Genome-Wide Polymorphic Markers</i>		<i>Animal Models</i>	1182
<b>Historical Progression From Autoimmune Pancreatitis to Immunoglobulin G4–Related Disease</b>	<b>1174</b>	<i>Diagnostic Procedures</i>	1182
<b>Epidemiology</b>	<b>1174</b>	<i>Treatment</i>	1183
<b>Clinical Features and Disease Associations</b>	<b>1175</b>	<i>Perspectives</i>	1183
<i>Autoimmune Pancreatitis</i>	1175	<i>References</i>	1183
<b>Pathological Features</b>	<b>1178</b>		
<b>Autoimmune Features</b>	<b>1179</b>		
<i>Complement Activation System</i>	1179		
<i>Innate Immunity</i>	1179		

## GENERAL INTRODUCTION

### Gateway From Autoimmune Pancreatitis to Immunoglobulin G4–Related Disease

Immunoglobulin G4–related disease (IgG4-RD) is a currently proposed systemic disease that is characteristically associated with IgG4 and is considered to be caused by autoimmune mechanisms. The clinical features of IgG4-RD can be summarized as (1) systemic distribution; (2) imaging findings of swelling, nodules, and/or wall thickening; (3) high serum IgG4 concentration; (4) abundant lymphoplasmacytic and IgG4-bearing plasma cell infiltration in affected organs; (5) a favorable response to corticosteroid therapy; and (6) combination with other IgG4-RDs simultaneously or in a metachronous fashion.

The establishment of this disease concept was based on a culmination of specific discoveries, namely, (1) the proposal of autoimmune pancreatitis (AIP) presumably caused by autoimmune mechanisms, (2) a close association between AIP and high serum IgG4 concentration, (3) systemic other organ involvements (OOIs) in AIP, (4) abundant IgG4-bearing plasma cell infiltration in affected tissues of AIP and its extrapancreatic organs, and (5)

OOIs also exhibiting a favorable response to corticosteroid therapy. These findings led the way to the establishment of the concept of a systemic disease consisting of AIP and related extrapancreatic lesions, that is, IgG4-RD, and opened the gateway from AIP to this new disease entity (Kawa, 2016).

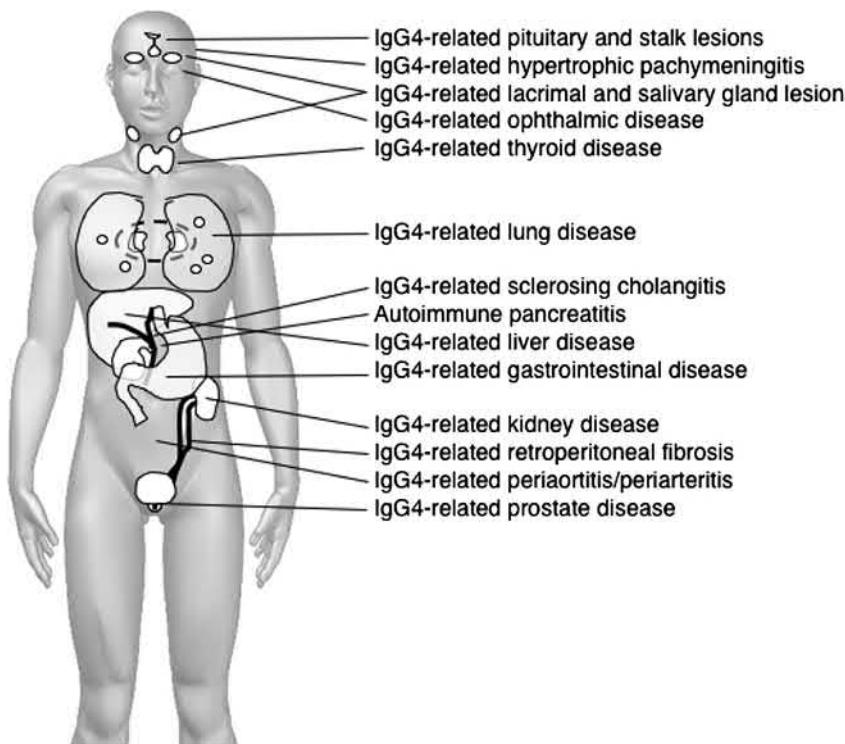
## HISTORICAL PROGRESSION FROM AUTOIMMUNE PANCREATITIS TO IMMUNOGLOBULIN G4–RELATED DISEASE

Although chronic pancreatitis is mainly caused by injury from alcohol abuse, there have long been reports of another type of chronic pancreatitis presumably caused by autoimmune mechanisms. Sarles et al. (1961) first reported a specific type of pancreatitis as chronic inflammatory sclerosis of the pancreas showing hyperglobulinemia and histologically marked lymphocytic infiltration. Nakano et al. (1978) described the first such case successfully treated with corticosteroids. Kawaguchi et al. (1991) designated this condition as lymphoplasmacytic sclerosing pancreatitis based on the detailed pathological study. Toki et al. (1992) reported chronic pancreatitis showing diffuse irregular narrowing of the entire main pancreatic duct (MPD). Yoshida et al. (1995) termed this condition as “AIP” based on the clinical findings of hypergammaglobulinemia, positive tests for autoantibodies and lymphoplasmacytic infiltration in the pancreas, and a favorable response to corticosteroid treatment. Since then, many cases of AIP have been diagnosed based on these characteristic features and a number of single cases and small case series have been published. In 2001 we reported that patients with AIP frequently and specifically exhibited high serum IgG4 concentrations that correlated closely with disease activity, suggesting that the disease was a discrete clinical entity different from ordinary chronic pancreatitis (Hamano et al., 2001). Protein electrophoresis of AIP patient sera revealed a polyclonal band in the rapidly migrating fraction of  $\gamma$ -globulins that was confirmed by the immunolectrophoresis to be caused by an elevated serum IgG4. We witnessed an increased IgG4 elevation in 90% of patients with AIP but scarcely in other conditions, which implicated IgG4 as a sensitive and specific marker for the disease. IgG4 levels also correlated closely with disease activity (Hamano et al., 2001). In 2002 we identified an abundant IgG4-bearing plasma cell infiltration in the pancreas and extrapancreatic sites of AIP, which have remained a histological hallmark of AIP and IgG4-RD (Hamano et al., 2002).

AIP is known to be accompanied by a variety of extrapancreatic lesions (Hamano et al., 2006). Investigation on the prevalence of extrapancreatic lesions in AIP based on the imaging modalities of computed tomography (CT), magnetic resonance imaging (MRI), and fluorodeoxyglucose position emission tomography showed dacryoadenitis/sialadenitis involvement to be the most common (48%), followed next by pulmonary lesions (54%), bile duct lesions (78%), kidney lesions (14%), retroperitoneal fibrosis (20%), and prostate lesions (10%) (Fujinaga et al., 2009). These additional manifestations shared many of the histopathological features of AIP, primarily IgG4-bearing plasma cell infiltration, and generally demonstrated a good clinical response to glucocorticoid treatment (Hamano et al., 2002). They also indicated a common pathophysiological background among the disorders and ultimately led to the proposal of a systemic disease linking IgG4, AIP, and related extrapancreatic lesions (Hamano et al., 2002; Kamisawa et al., 2003a). Elsewhere, experts in the field of rheumatology were proposing a similar systemic disease comprising IgG4, lachrymal and salivary gland lesions, and other extraglandular involvement (Yamamoto et al., 2006; Neild et al., 2006; Masaki et al., 2008). In 2010 the new disease concept of IgG4-RD was proposed for this condition by experts in Japan (Umeshara et al., 2012a), and in 2011 this entity was accepted at an international conference in Boston (Stone, 2012; Stone et al., 2012b). IgG4-RD now includes a wide variety of systemic lesions (Fig. 59.1) (Stone et al., 2012a), the number of which is increasing.

## EPIDEMIOLOGY

The precise epidemiology of IgG4-RD remains unclear due to a lack of large-scale surveys and the fact that most studies have dealt only with a specific disease subset, such as AIP, and not the entity as a whole. A nationwide survey of AIP in Japan revealed an overall prevalence rate of 4.6/100,000 individuals, annual incidence rate of 1.4/100,000 (Kanno et al., 2015). As AIP accounts for only a part of the total number of IgG4-RD cases, the true prevalence of IgG4-RD is certainly higher. Since the frequency of AIP among IgG4-RD cases was reported to be 60% (Inoue et al., 2015), the prevalence of IgG4-RD was estimated as 7.7/100,000 individuals. The genetic contribution to IgG4-RD remains incompletely described. To date, there has been only one reported familial case of IgG4-RD (Watanabe et al., 2013b).



**FIGURE 59.1** Systemic manifestations of IgG4-related disease. IgG4, Immunoglobulin G4.

## CLINICAL FEATURES AND DISEASE ASSOCIATIONS

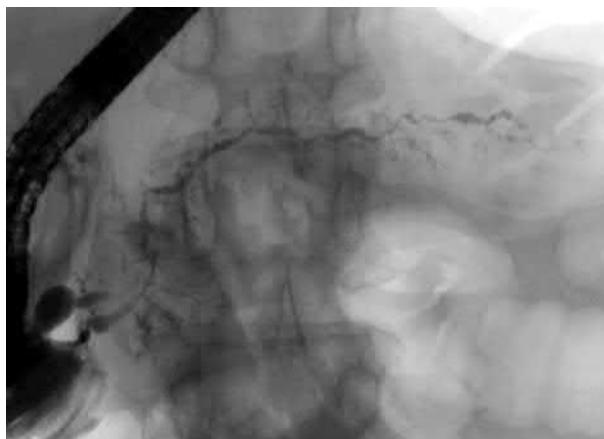
A host of systemic manifestations have been included in IgG4-RD (Fig. 59.1).

### Autoimmune Pancreatitis

The male ratio in AIP was found to be 77%, and the median age of occurrence was 63 years (range: 38–85 years), indicative of an elderly male preponderance. Few patients complained of severe pancreatitis attacks that were different from classical acute pancreatitis. A striking feature was obstructive jaundice in 70% of patients caused by stenosis or obstruction of the intrapancreatic common bile duct. Diabetes mellitus was often observed, the majority of which being type 2 (Yoshida et al., 1995; Kawa, 2016), and some patients improved after steroid therapy (Tanaka et al., 2000). Laboratory tests revealed several abnormal findings related to obstructive jaundice. The tumor-associated antigen CA19-9 was elevated in 50% of subjects, probably due to cholestasis rather than a malignant process. Elevated serum  $\gamma$ -globulin, IgG, and IgG4 were present in 60%, 70%, and 80% of patients, respectively. Although IgG4 was the most sensitive and specific immunological marker, decreased IgA and IgM concentrations were also detected in patients with increased IgG4 levels (Taguchi et al., 2009). Positivity for anti-nuclear antibody was 40% and that for other antibodies was 10%–30%, and serum immune complex (IC) values reflected disease activity as well. The decreased exocrine and endocrine function by the bentiromide test and HbA1c were found in 66% and 51% of cases, respectively (Kawa, 2016). Image finding disclosed a characteristic pancreatogram of diffuse but irregular narrowing (Fig. 59.2).

Patients with AIP sometimes receive an erroneous diagnosis of pancreatic cancer due to the preponderance of the disease in the elderly together with symptoms of obstructive jaundice, elevated serum CA19-9, and pancreatic swelling with irregular narrowing or obstruction of the pancreatic duct. The pathological analysis following pancreaticoduodenectomy (Whipple resection) for a diagnosis of pancreatic cancer disclosed results representative of AIP in 2.2% of patients (Hardacre et al., 2003). Also owing to similar pathological findings, AIP has been misdiagnosed as pancreatic lymphoma (Horiuchi et al., 1996).

Although AIP was initially believed to be an acute, nonprogressive condition (Yoshida et al., 1995), it became evident that some patients experienced pancreatic stone formation, pancreatic atrophy, and/or irregular dilatation of the MPD overtime (Takayama et al., 2004; Maruyama et al., 2012). During the long-term course, a number of AIP cases could progress to chronic pancreatitis with severe dysfunction (Maruyama et al., 2013, 2014; Kanai et al., 2016).



**FIGURE 59.2** Endoscopic retrograde cholangiopancreatography demonstrating the characteristic diffuse irregular narrowing seen in AIP. AIP, Autoimmune pancreatitis.

Recently, the long-term outcomes of AIP and IgG4-RD were described to include the complication of malignancy development (Yamamoto et al., 2012; Shiokawa et al., 2013), although this point is controversial (Hirano et al., 2014; Hart et al., 2014; Inoue et al., 2015). We identified a close association between IgG4-RD and malignancy formation within 12 years after diagnosis, particularly during the first year (Asano et al., 2015).

### **Immunoglobulin G4–Related Pituitary and Stalk Lesions**

IgG4-related pituitary and stalk lesions include hypophysitis presenting with compressive optic neuropathy, panhypopituitarism, pituitary hypothyroidism, adrenocortical insufficiency, and syndrome of inappropriate secretion of antidiuretic hormone (van der Vliet and Perenboom, 2004; Shimatsu et al., 2009; Shikuma et al., 2017). A review of 84 patients with IgG4-related hypophysitis demonstrated manifestations of anterior hypopituitarism in 26.2% of cases, central diabetes insipidus in 17.9%, and panhypopituitarism in 52.4% (Shikuma et al., 2017). IgG4-related hypophysitis should always be considered in the differential diagnosis of primary hypophysitis (Berreuther et al., 2017).

### **Immunoglobulin G4–Related Hypertrophic Pachymeningitis**

Hypertrophic pachymeningitis (HP) is a rare fibroinflammatory lesion that causes thickening of the dura in the cranium and/or spinal canal and presents as radiculomyopathy, headache, or cranial nerve palsy depending on the site of the involvement (Riku et al., 2009; Chan et al., 2009; De Virgilio et al., 2017). A nationwide survey of HP in Japan uncovered IgG4-related HP in 14 (8.8%) of 159 cases (Yonekawa et al., 2014).

### **Immunoglobulin G4–Related Lacrimal and Salivary Gland Lesions**

IgG4-related lacrimal and salivary gland lesions are major members of IgG4-RD including Mikulicz's disease and Küttner tumor and present as symmetrical swelling of lacrimal and salivary glands. Patients show no gender differences, which is different from other IgG4-RDs (Yamamoto et al., 2015). In contrast to Sjögren's syndrome, however, IgG4-related lacrimal and salivary gland lesions show milder exocrine dysfunction, are negative for anti-SS-A/Ro and SS-B/La autoantibodies, and have more frequent submandibular gland lesions (Yamamoto et al., 2005; Umehara et al., 2012a).

### **Immunoglobulin G4–Related Ophthalmic Disease**

IgG4-related ophthalmic disease includes lacrimal gland lesions (Mikulicz's disease) in 50% of cases and ocular adnexa lesions (trigeminal nerve branch enlargement, extraocular muscle enlargement, diffuse orbital fat lesions, orbital mass lesions, eyelid lesions, or nasolacrimal duct lesions) in another 50% (Wallace et al., 2014; Sogabe et al., 2014; Yamamoto et al., 2015). These lesions present as vision abnormalities from optic nerve disturbance, restriction of ocular movement, and exophthalmos (Sogabe et al., 2014) and should be differentiated from mucosa-associated lymphoid tissue (MALT) lymphoma (extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue), diffuse large B-cell lymphoma, and follicular lymphoma (Goto et al., 2015).

### **Immunoglobulin G4–Related Thyroid Disease**

Riedel's thyroiditis is a chronic fibrosing disorder of unknown etiology that is primarily known as a member of multifocal fibrosclerosis, with a portion of cases considered to be IgG4-RD (Dahlgren et al., 2010; Takeshima et al., 2015). AIP patients with complicating hypothyroidism had a significantly higher frequency of antithyroglobulin antibody than those without (Komatsu et al., 2005). The other series of Hashimoto's thyroiditis were classified as either IgG4 or non-IgG4 thyroiditis based on the immunostaining profiles (Li et al., 2012). IgG4 thyroiditis may represent IgG4-RD of the thyroid gland because it shares common histopathological characteristics with IgG4-RD in other organs (Kakudo et al., 2011). IgG4-RD is significantly associated with hypothyroidism that responds favorably to corticosteroid therapy as well, implicating the existence of an IgG4-related thyroid disease or an IgG4-related thyroiditis (Watanabe et al., 2013a).

### **Immunoglobulin G4–Related Lung Disease**

IgG4-related lung disease develops through the lymphatic routes of the lungs and exhibits various clinical characteristics. The Japanese diagnostic criteria for IgG4-related respiratory disease include the imaging findings of any of the following intrathoracic lesions of hilar/mediastinal lymphadenopathy, bronchial wall/bronchovascular bundle thickening, interlobular septal wall thickening, nodular shadow, infiltrative shadow, pleural thickening, and/or effusion (Matsui et al., 2016). There is a variety of IgG4-related respiratory lesions diagnosed with interstitial pneumonia (Taniguchi et al., 2004), inflammatory pseudotumor (Zen et al., 2005), hilar or mediastinal lymphadenopathy (Saegusa et al., 2003), or bronchial wall thickening (Ito et al., 2009).

### **Immunoglobulin G4–Related Sclerosing Cholangitis**

IgG4-related sclerosing cholangitis (IgG4-SC) is closely associated with AIP (60%–70% of AIP patients), and their cooccurrence often aids in diagnosis (Hamano et al., 2006; Tanaka et al., 2014). In contrast, solitary IgG4-SC without AIP closely resembles other conditions, such as primary SC (PSC) and bile duct malignancy (Hamano et al., 2005; Graham et al., 2014). IgG4-SC can be found in any part of the biliary system (Nakazawa et al., 2001, 2016). Lower bile duct lesions should be differentiated from pancreatic cancer and intrahepatic or hilar lesions from PSC and biliary malignancies (Nakazawa et al., 2005; Naitoh et al., 2009).

### **Immunoglobulin G4–Related Liver Disease**

IgG4-related liver disease consists of a variety of histological changes, including portal inflammation, interface hepatitis, large bile duct obstruction, portal sclerosis, lobular hepatitis, and canalicular cholestasis, all of which are collectively designated as IgG4 hepatopathy (Umemura et al., 2007b). Some of these lesions mimic those observed in autoimmune hepatitis (AIH) and have a similar clinical presentation; therefore they also fulfill the diagnostic criteria of AIH (Umemura et al., 2007a; Ishizu et al., 2016; Nakanuma et al., 2016).

### **Immunoglobulin G4–Related Kidney Disease**

Most cases of IgG4-related kidney disease involve the uriniferous tubules, with few being glomerular, and display tubulointerstitial nephritis with hypocomplementemia and deposits of ICs and C3 in tubular basement membranes (Takeda et al., 2004; Uchiyama-Tanaka et al., 2004; Saeki et al., 2010). Roughly, half of the patients have very mild proteinuria or hematuria, and urinalysis abnormalities are generally inconspicuous. However, nephrotic-range proteinuria may be observed when membranous glomerulonephritis or other glomerular lesions overlap. Some patients show an acute or progressive renal failure at presentation, while others are diagnosed based on incidental imaging abnormalities with normal renal function (Saeki et al., 2010; Raissian et al., 2011; Kawano and Yamada, 2016). CT depicts renal cortical lesions as areas of a decreased enhancement that appears as small peripheral cortical nodules and round or wedge-shaped lesions (Takahashi et al., 2007; Fujinaga et al., 2009).

### **Immunoglobulin G4–Related Retroperitoneal Fibrosis**

CT and MRI reveal soft tissue density or masses around the aorta and ureters (Fujinaga et al., 2009). Patients with periureteral lesions sometimes complain of lumbago or back pain due to hydronephrosis, which may result in renal atrophy and failure (Hamano et al., 2002).

### **Immunoglobulin G4–Related Periaortitis/Periarteritis**

Although IgG4-related periaortitis is a major member of IgG4-related retroperitoneal fibrosis, its disease spectrum extends to the thoracic aorta, coronary artery, and iliac artery, which has led to the proposal of the distinct systemic disease entity of IgG4-related periaortitis/periarteritis. Histopathologically, IgG4-related arterial lesions are characterized by arterial wall thickening corresponding to inflammation with IgG4-positive plasmacytes and fibrosis, both mainly in the adventitia (Kasashima et al., 2008; Inoue et al., 2011). Although IgG4-related periaortitis/periarteritis responds well to corticosteroid treatment, cases with prior luminal dilatation have shown exacerbation after therapy (Mizushima et al., 2014; Ozawa et al., 2017).

### **Immunoglobulin G4–Related Prostate Disease**

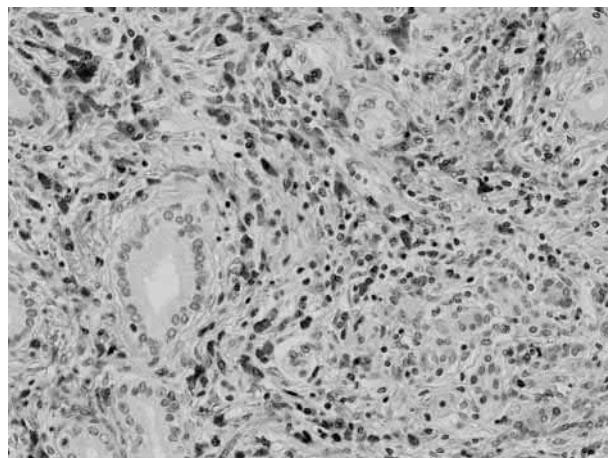
As prostate involvement might not be rare in IgG4-RD, the clinicians should consider this disease entity in patients with IgG4-RD accompanied with prostatic symptoms (Buijs et al., 2014). Patients present with a symmetrically, nontender, swollen prostate highlighted by an imaging analysis to have severe inflammatory lesions mainly in the central and transition zones (Uehara et al., 2008; Buijs et al., 2014).

## PATHOLOGICAL FEATURES

The diagnosis of IgG4-RD rests on the combined presence of a characteristic histopathological appearance and increased numbers of IgG4-bearing plasma cells. The critical histopathological features in IgG4-RD are dense lymphoplasmacytic infiltration, a storiform pattern of fibrosis, and obliterative phlebitis. A terminology scheme for diagnosing IgG4-RD is based primarily on its morphological appearance on biopsy, while tissue IgG4 count and IgG4/IgG ratio are of secondary importance (Deshpande et al., 2012).

The pathological features of IgG4-RD were first investigated intensely in AIP tissues (Kawaguchi et al., 1991; Kloppel et al., 2007). On gross examination, the involved pancreas appears glistening white, is firm or hard, and may be enlarged or shows mass lesions. Lymphoplasmacytic infiltration and fibrosis are the characteristic microscopic features of pancreatic lesions, and in some cases, result in the formation of lymphoid follicles. Infiltrating plasma cells characteristically bear IgG4 (Hamano et al., 2002) (Fig. 59.3). Cell infiltration is prominent around the pancreatic duct, resulting in its stenosis or obstruction, stasis of pancreatic secretions, and damage to the pancreatic lobules. Obliterating phlebitis is another characteristic feature, showing marked cellular infiltration of the venous wall and venous thrombosis (Kawaguchi et al., 1991).

The pathological findings of each type of IgG4-RD share common features that are also present in AIP (Hamano et al., 2002; Kamisawa et al., 2003b; Deshpande et al., 2009, 2012; Deshpande, 2015); however, there are subtle variations among some organs as well as organ-specific features (Zen and Nakanuma, 2010). The unique pathological features include numerous lymph follicles in lacrimal and salivary gland lesions, and obliterative arteritis in lung lesions (Zen and Nakanuma, 2010). Obliterative phlebitis is scarcely found in lacrimal gland



**FIGURE 59.3** IgG4 immunostaining of pancreatic tissue in AIP revealing abundant infiltration of IgG4-bearing plasma cells. AIP, Autoimmune pancreatitis; IgG4, immunoglobulin G4.

lesions. The semiquantitative analysis of IgG4-bearing plasma cells may help to distinguish IgG4-RD from other conditions (Deshpande et al., 2006; Dhall et al., 2010), and the ratio of IgG4-bearing plasma cells to IgG-bearing plasma cells further assists in diagnosing IgG4-RD, with a ratio of >50% being highly suggestive of a diagnosis. The closest histopathological mimics of IgG4-RD are lymphomas, for which clonality studies are necessary to differentiate among these two conditions (Stone et al., 2012b) and multicentric Castleman's disease (Terasaki et al., 2017).

## AUTOIMMUNE FEATURES

### Complement Activation System

Serum concentrations of complements C3 and C4 were reduced in 36% of patients with AIP, suggesting a role of the complement activation system in its pathogenesis. We uncovered a significantly higher serum IgG1 concentration and decreased C3 and C4 values in the elevated IC group. Since IgG4 did not bind to C1q, it appeared to have no contribution to complement activation, while IgG1 seemed strongly implicated via the classical pathway (Muraki et al., 2006). On the other hand, Sugimoto et al. observed that both the classical and mannose-binding lectin pathways might participate in the complement activation system (Sugimoto et al., 2016; Kawa, 2017).

### Innate Immunity

Recent investigations have highlighted the importance of innate immunity in preceding and/or augmenting IgG4 responses driven by adaptive immunity (Akitake et al., 2010; Arai et al., 2015; Fukui et al., 2015; Watanabe et al., 2012, 2013c, 2017). Antigens derived from intestinal microflora as part of the microbe-associated molecular pattern are thought to activate the host's innate immune system via pattern recognition receptors or pathogen recognition receptors (PRRs), such as the Toll-like receptors (TLRs), nucleotide-binding oligomerization-like receptors (NLRs), retinoic acid-inducible gene (RIG)-I-like receptors, or C-type lectin like receptors. Under sterile inflammatory conditions, endogenous nonmicrobial danger molecules, or damage-associated molecular patterns, released from dying and necrotic cells activate PRRs and have been implicated in the development of autoimmune diseases (Kono and Rock, 2008; Watanabe et al., 2017). Peripheral blood mononuclear cells, presumably B cells, isolated from patients with AIP show the enhanced production of IgG4 upon stimulation with NLR and TLR ligands that are associated with the release of B cell-activating factor (BAFF) (Yamanishi et al., 2011; Watanabe et al., 2012). The activation of basophils is also likely involved in the pathogenesis of IgG4-RD since TLR activation in basophils stimulates IgG4 production by B cells in a T-cell independent manner (Watanabe et al., 2013c). As described earlier, the mannose-binding lectin pathway, which is a member of innate immunity, may participate in the complement activation system in IgG4-RD (Sugimoto et al., 2016).

### Adaptive Immunity

#### Autoantibodies

Shiokawa et al. demonstrated that subcutaneous injection of IgG from AIP patients, but not control IgG, caused pancreatic and salivary gland injury in neonatal mice. Although pancreatic injury was also induced by the patient with IgG1 or IgG4, the potent pathogenic activity of IgG1 was significantly inhibited by simultaneous injection of IgG4, suggesting that circulating IgG4 in AIP patients had both pathogenic and protective roles. Moreover, the autoantibodies in AIP serum recognized molecules involved in cell-extracellular matrix adhesion, laminin 511 (Shiokawa et al., 2016, 2018).

*Helicobacter pylori* infection may also trigger AIP, possibly as a result of molecular mimicry. There is substantial homology between human CA II and *H. pylori* alpha-carbonic anhydrase: the homologous segments contain the binding motif of the human leucocyte antigen (HLA) molecule DRB1\*04:05, which is closely associated with AIP (Kawa et al., 2002). The HLA-DRB1\*04:05 allele was also an important risk factor for AIP in functional studies using a transgenic mouse model (Freitag et al., 2010). These data led to the hypothesis that the DRB1\*0405-restricted peptide of CA II was present in genetically predisposed subjects and that reactive T cells injured pancreatic tissue via interaction with the CA II of pancreatic ductal cells (Guarneri et al., 2005).

### Immunoglobulin G4

Since serum IgG4 and IgG4-type ICs are closely associated with disease activity (Hamano et al., 2001), they may play a major role in the pathogenesis of AIP and IgG4-RD. However, their exact nature, that is, whether they are beneficial or harmful, remains unclear. To date, the pathological effects of IgG4 and IgG4-type ICs have been reported in limited settings, such as pemphigus vulgaris (Rock et al., 1989), idiopathic membranous glomerulonephritis (Beck et al., 2009), acquired thrombocytopenic purpura (Ferrari et al., 2009), and muscle-specific kinase myasthenia gravis (Plomp et al., 2012). In AIP, IgG4-type autoantibodies may elicit an inflammatory response as described before (Shiokawa et al., 2016). Recent studies have demonstrated two outstanding characteristics of IgG4, Fab-arm exchange (van der Neut Kolfschoten et al., 2007) and rheumatoid factor (RF)-like activity (Kawa et al., 2008), which enable IgG4 to contribute to defense against disease progression. Specifically, dynamic Fab-arm exchange in the IgG4 molecule results in bispecific activity and the loss of monospecific cross-linking activity and ability to form ICs to impart antiinflammatory effects (van der Neut Kolfschoten et al., 2007; Aalberse et al., 2009; Rispens et al., 2011). IgG4 has also been reported to bind IgG and exert RF activity (Hennig et al., 2000). However, IgG4 Fc, but not IgG4 Fab, binds to IgG Fc, indicating that IgG4 binding to IgG Fc occurs via an Fc–Fc interaction and not RF per se (Kawa et al., 2008). Furthermore, the human IgG4 was seen to bind to the Fc portions of various animal IgGs (Ito et al., 2010). Although the exact role of this novel RF-like IgG4 activity remains unclear, different IgG4 molecules with the same antigen reactivity may associate via Fc–Fc interaction and then exchange half molecules of each IgG4, resulting in Fab-arm exchange with different epitope reactivity (Rispens et al., 2009).

### Cellular Immunity

In some cases of AIP, CD4 + Th1 cells predominate over Th2 cells, suggesting that Th1 cytokines are essential for the induction and maintenance of AIP while Th2 cytokines are involved in disease progression (Okazaki and Chiba, 2002). However, there is also evidence of a Th2-predominant immune response in AIP (Zen et al., 2007), and a deviation of Th1/Th2 balance toward Th2 was described in patients with IgG4-RD (Miyake et al., 2008). The expression profile of cytokines in salivary glands from patients with AIP, IgG4-related SC, and IgG4-related dacryoadenitis/sialadenitis suggested that Th2 immune reactions might play a key role in IgG4 production (Tanaka et al., 2012; Zen et al., 2007). In particular, interleukin (IL)-33 from CD163-positive M2 macrophages appeared to be strongly involved in the activation of a Th2 immune response (Moriyama and Nakamura, 2017). Several studies have observed that abnormal innate immune responses via TLRs expressed by monocytes/macrophages enhanced Th2 immune responses and the immunopathogenesis of IgG4-RDs (Watanabe et al., 2012; Fukui et al., 2015). Recent studies have indicated that particular Th subsets might be involved in the initiation of IgG4-RD (Moriyama and Nakamura, 2017).

Regulatory immune cells play an important role in several immune-related diseases. In particular, abnormalities in regulatory T cell (Treg) and regulatory B cell (Breg) number and function are implicated in multiple immune-related and autoimmune conditions, which has brought about investigation of their roles in IgG4-RD (Uchida and Okazaki, 2017). Transforming growth factor (TGF)- $\beta$  secreted from naïve Th0 cells can induce CD4 + CD25 + Tregs that have potent inhibitory function via the transcription factor forkhead box P3 (Foxp3). In the peripheral blood, Treg numbers were significantly increased in patients with AIP, and this rise was correlated with augmented serum concentrations of IgG4. In contrast, naïve Tregs are significantly decreased in patients with AIP, suggesting that the increased numbers of Tregs may influence IgG4 production and reflect disease progression while diminished numbers of naïve Tregs may be involved in AIP pathogenesis (Miyoshi et al., 2008). The ratio of Foxp3 + cells to infiltrating mononuclear cells (Foxp3/Mono) in AIP patients is significantly higher than in individuals with chronic alcoholic pancreatitis, and the Foxp3/Mono and IgG4/Mono ratios are positively correlated with each other (Kusuda et al., 2011). In the periphery, two functionally different subsets of effector Tregs [inducible costimulatory molecule (ICOS) + or ICOS – effector Tregs] actively produce the immunosuppressive cytokines IL-10 and TGF- $\beta$ , respectively. The levels of ICOS + Tregs and IL-10 + Tregs are significantly higher in patients with AIP, implying that ICOS + Tregs modulate IgG4 production via IL-10 in this disease (Kusuda et al., 2011) and supporting the notion that a decrease in the number of naïve Tregs is involved in AIP onset. Fibrosis may be regulated by TGF- $\beta$ , which is secreted by ICOS – Tregs (Uchida and Okazaki, 2017). The role of Tregs has been also confirmed in other IgG4-RDs, including IgG4-SC (Koyabu et al., 2010), IgG4-related sialadenitis (Tanaka et al., 2012), and IgG4-related kidney disease (Mizushima et al., 2012; Kawamura et al., 2015). Mechanisms underlying IgG4 production was proposed by Tanaka et al. (2012) and Takeuchi et al. (2014), showing that Th2 cell and Treg regulate the production of IgG4 via IL-4 and IL-10 cytokines, which may be initiated or controlled by M2 macrophage or mast cell.

Predominantly identified based on their ability to produce IL-10, Bregs play an important role in suppressing pathological immune responses (Mauri and Bosma, 2012). CD19<sup>+</sup>CD24<sup>high</sup>CD38<sup>high</sup> immature Bregs were significantly increased in the peripheral blood of AIP patients, whereas the CD19<sup>+</sup>CD24<sup>high</sup>CD27<sup>+</sup> B10 cell fraction of AIP patients was lower. The total number of IL-10-producing B cells was not significantly different between AIP patients and healthy controls. However, in untreated AIP patients, the number of CD19<sup>+</sup>CD24<sup>high</sup>CD38<sup>high</sup> immature Bregs did not correlate to IgG4 levels, which differed from findings for Tregs. These results imply that CD19<sup>+</sup>CD24<sup>high</sup>CD38<sup>high</sup> immature Bregs increase reactively to suppress disease activity, which is consistent with the hypothesis that CD19<sup>+</sup>CD24<sup>high</sup>CD27<sup>+</sup> B10 cells are involved in the development of type 1 AIP (Sumimoto et al., 2014; Uchida and Okazaki, 2017).

Taking the above observations into account, Uchida and Okazaki proposed that the decreased numbers of naïve Tregs and CD19<sup>+</sup>CD24<sup>high</sup>CD27<sup>+</sup> Bregs factored prominently in the induction of IgG4-RD. Moreover, effector/inducible Tregs and CD19<sup>+</sup>CD24<sup>+</sup>CD38<sup>high</sup> B10 cells increased in a reactive manner. Since Th2 immune responses promote disease progression, the elevated numbers of inducible Tregs and CD19<sup>+</sup>CD24<sup>+</sup>CD38<sup>high</sup> B10 cells may contribute to the development and progress of IgG4-RD as well. Both innate and adaptive immunity are believed to affect the production of IgG4 and fibrosis development. In adaptive immunity, ICOS + Tregs may regulate IgG4 production via secretion of IL-10. In innate immunity, basophils and monocytes are regulated via BAFF using the TLR and NLR signaling pathways. Fibrosis may in turn be modulated by TGF-β secreted from ICOS - Tregs and M2 macrophages to suppress inflammation. M2 macrophages are also known to accelerate the Th2 immune response (Uchida and Okazaki, 2017).

## GENETICS

As autoimmune diseases are multifactorial, their pathogenesis involves a complex interplay of multiple genetic and environmental factors. Although identifying responsibility genes is complex, two approaches, the candidate-gene approach by association studies and genome-wide association testing of the large numbers of single nucleotide polymorphisms (SNPs), have been employed to elucidate the genetic factors that influence disease susceptibility (Amos et al., 2011). In the candidate-gene approach, an association study is carried out using polymorphic markers (SNPs and microsatellites) that reside around or within biologically functional genes presumably involved in disease development. When the fundamental pathophysiology of a disease is unknown, however, genome-wide studies are better suited to hunt down causative genes (Ota et al., 2017).

### Association Studies Using Polymorphic Markers in Candidate Genes

The HLA serotypes DR4 and DQ4 are the markers most frequently associated with AIP among the major histocompatibility complex class I and class II molecules. Among the DR4 and DQ4 subtypes, the frequencies of *DRB1\*0405* and *DQB1\*0401* are significantly higher in AIP patients. In the Japanese population, *DRB1\*0405* is known to be in strong linkage disequilibrium with *DQB1\*0401*, resulting in the *DRB1\*0405-DQB1\*0401* haplotype. This haplotype is specifically associated with susceptibility to AIP and may play a functional role in antigen presentation and induction of an autoimmune response (Kawa et al., 2002). Moreover, *HLA-DR\*0405* transgenic mice displayed a high prevalence of AIP after sublethal irradiation and adoptive transfer of CD90+ T cells (Freitag et al., 2010). In a case-control study, using microsatellite markers distributed throughout the HLA region, we mapped AIP susceptibility to two areas of the telomeric region of HLA adjacent to the C3-2-11 marker and to HLA-DRB1-DQB1. The region adjacent to C3-2-11 contains the ATP-binding cassette subfamily F (*ABCF1*) gene that is regulated by TNF-α, a major cytokine involved in inflammatory and autoimmune reactions (Ota et al., 2007).

Polymorphisms in Fc receptor-like 3 (*FCRL*) genes alter the binding affinity of nuclear factor κB and regulate *FCRL3* expression. An analysis of genotype frequencies among *FCRL3-110* polymorphisms revealed a significant association between the -110A/A genotype and AIP; serum IgG4 concentrations were significantly and positively correlated with the number of susceptibility alleles (Umemura et al., 2006).

The cytotoxic T lymphocyte antigen (*CTLA-4*) gene product is an inhibitory receptor expressed on the surface of activated memory T cells and CD4+CD25+ Tregs to largely act as a negative regulator of T-cell responses. We observed that the frequency of the +6230G/G genotype was significantly higher in Japanese patients with AIP than in those without the disease and that the +49A/A and +6230A/A genotypes were associated with an

enhanced risk of AIP relapse (Umemura et al., 2008). The *CTLA-4* 49A polymorphism and the -318C/ + 49A/CT60G haplotype have also been linked to AIP in a Chinese population (Chang et al., 2007).

## Association Studies Using Genome-Wide Polymorphic Markers

An association analysis involving 400 microsatellite markers with an average spacing of 10.8 cm revealed an association of AIP with 7 SNPs within the 20 kb region around potassium voltage-gated channel, shaker-related subfamily, member 3 (*KCNA3*). Further analysis of *KCNA3* by SNP genotyping revealed four SNPs that were significantly related to AIP susceptibility (Ota et al., 2011). *KCNA3* is involved in the immunomodulation of auto-reactive effector T cell and memory T cell-mediated autoimmune diseases.

Several genes were implicated in the development of lachrymal/salivary gland lesions in AIP by genome wide association study (GWAS) using the Gene Chip Human Mapping 500K Array Set (Affymetrix, CA) and fine-tuned mapping of specific SNPs (Oguchi et al., 2016).

## ANIMAL MODELS

Several experimental models of AIP have been reported to date (Haruta et al., 2012). Inflammation was found in the pancreas of immunized mice and in all animals receiving whole spleen cells or CD4+ cells. CA II- and lactoferrin (LF)-immunized mice developed apoptotic duct cells and acinar cells, respectively (Uchida et al., 2002). The adoptive transfer of amylase-specific CD4+ T cells in rats also resulted in pancreatitis characterized by mononuclear cell infiltration and destruction of lobular tissue (Davidson et al., 2005). As for microorganism-related induction groups, virus-induced AIP models, such as C57BL/6 mice infected with the murine leukemia retrovirus LP-BM5, exhibited histological findings similar to those in human AIP (Watanabe et al., 2003). The spontaneous development of pancreatitis via an autoimmune mechanism in MRL/Mp mice was accelerated by the administration of polyinosinic:polycytidylic acid, a synthetic double-stranded RNA, and TLR 3 ligand (Qu et al., 2002; Soga et al., 2009; Asada et al., 2010; Nishio et al., 2011). Another animal model for AIP was developed by exposing C57BL/6 mice to heat-killed *Escherichia coli*, which produced the marked cellular infiltration with fibrosis in the exocrine pancreas and salivary glands (Haruta et al., 2010). One representative antigen reacting with the serum of *E. coli*-inoculated mice was identified as FliC (Yanagisawa et al., 2014). These findings indicate that avirulent bacteria and other silently infiltrating microorganisms can engage pathogen-associated molecular patterns and thereby activate the innate immune system so as to elicit a host immune response to the target antigen by molecular mimicry and lead to AIP (Oldstone, 1998; Haruta et al., 2010; Yanagisawa et al., 2014).

## DIAGNOSTIC PROCEDURES

Specific diagnostic criteria for individual organs involved in IgG4-RD have been established, including those for AIP (Pearson et al., 2003; Okazaki et al., 2006; Kim et al., 2006; Chari et al., 2006; Otsuki et al., 2008; Shimosegawa et al., 2011), IgG4-related sialadenitis and dacryoadenitis (Masaki et al., 2010), IgG4-related kidney disease (Kawano et al., 2011), IgG4-related SC (Ohara et al., 2012), and IgG4-related respiratory disease (Matsui et al., 2016). The framework of these criteria is based largely on that of AIP to include combinations of the following: (1) characteristic radiological findings, (2) elevation of serum IgG4 levels, (3) histopathological findings of abundant IgG4-positive plasma cell and lymphocyte infiltration, storiform fibrosis, and/or obliterative phlebitis, (4) association with other IgG4-RDs, and (5) a favorable response to steroids. The diagnostic criteria for AIP are not suitable for identifying other involved organs (Stone et al., 2012a), which requires specialized training often unknown to general clinicians.

The comprehensive diagnostic criteria for IgG4-RD including the involvement of various organs are intended for the practical use of general physicians and nonspecialists (Umehara et al., 2012b). Although many patients with IgG4-RD have lesions, either synchronously or metachronously, in several organs and the pathological features of each organ differ, a consensus has been reached on two diagnostic criteria for a definite diagnosis of IgG4-RD: (1) serum IgG4 concentration >135 mg/dL and (2) >40% of IgG+ plasma cells being IgG4+ and >10 IgG4+ cells per high-power field of biopsy sample. It is also important to differentiate IgG4-RD from malignant tumors affecting individual organs (cancer or lymphoma) and mimicking conditions by additional

histopathological examination. As it can be difficult to obtain tissue samples from certain organs, such as the pancreas, organ-specific diagnostic criteria are available for such lesions in the form of diagnostic algorithms. Together, the comprehensive diagnostic criteria combined with organ-specific diagnostic criteria and algorithms have remarkably increased detection sensitivity (Umehara et al., 2012b). For accurate diagnosis of the overall condition, the international consensus has been achieved for the nomenclature, management, treatment, and pathology of IgG4-RD (Stone et al., 2012a; Khosroshahi et al., 2015; Deshpande et al., 2012).

## TREATMENT

Corticosteroid treatment is regarded as the therapeutic standard for patients with AIP (Ito et al., 2007; Okazaki et al., 2009). According to the international guidance statement on the management and treatment of IgG4-RD, glucocorticoids are the first-line agent for remission induction in all patients with active, untreated IgG4-RD unless contraindications are present. Following a successful course of induction therapy, certain patients benefit from maintenance therapy (Khosroshahi et al., 2015).

A set regimen for corticosteroid treatment has been established for AIP. Aggressive intervention is advocated when vital organs are involved or serious organ dysfunction or failure is likely. However, not all forms of IgG4-RD require immediate treatment but simple observation is justified in some cases (Stone et al., 2012b). Most patients with obstructive jaundice, diffuse enlargement of the pancreas, associated extrapancreatic involvement, and abdominal pain are good candidates for steroid therapy, although spontaneous remission has been observed in patients with low disease activity (Kamisawa et al., 2009). Pancreatic tissue samples obtained by needle biopsy after corticosteroid therapy have shown marked histological improvement (Song et al., 2005; Ko et al., 2010). Some patients with high disease activity may continue maintenance therapy for as many as 3 years.

Azathioprine, mycophenolate mofetil, 6-mercaptopurine, methotrexate, tacrolimus, and cyclophosphamide have all been adopted as steroid-sparing agents for IgG4-RD (Ghazale et al., 2008; Raina et al., 2009; Hart et al., 2013). However, the efficacies of these agents have not been evaluated in prospective trials, and there is little evidence to support the notion that conventional steroid-sparing drugs are effective (Hart et al., 2013).

B-cell depletion with rituximab (RTX) was effective for IgG4-RD, even in patients refractory to conventional steroid-sparing agents (Topazian et al., 2008; Khosroshahi et al., 2010, 2015; Hart et al., 2013). Hart et al. (2013) reported an 83% rate of complete remission following RTX therapy in a group of patients with AIP whose disease had been resistant to, or who had contraindications for, steroids or conventional steroid-sparing agents. CD19<sup>+</sup>CD27<sup>+</sup>CD20<sup>-</sup>CD38<sup>high</sup> plasmablasts, which are largely IgG4<sup>+</sup>, play an important role in IgG4-RD disease activity. The number of CD19<sup>+</sup>CD27<sup>+</sup>CD38<sup>high</sup> plasmablasts decreased sharply after RTX treatment, presumably due to depletion of CD20<sup>+</sup> precursors since CD19<sup>+</sup>CD27<sup>+</sup>CD38<sup>high</sup> plasmablasts did not express CD20 (Mattoo et al., 2014; Wallace et al., 2015; Lanzillotta et al., 2017; Uchida and Okazaki, 2017).

## PERSPECTIVES

The intense study of AIP has opened the door to the new disease concept of IgG4-RD, an intriguing disorder presenting features never before encountered. Researchers need to clarify the following issues in IgG4-RD: (1) pathogenesis based on abnormal immune responses and genetics, (2) the role of IgG4, (3) the detailed characteristics and real global prevalence, (4) a rational treatment strategy, and (5) long-term outcome with respect to structure and function and the predisposition to malignancy. Many affected patients worldwide have been overlooked or categorized as having other diseases. Expanding the awareness of IgG4-RD will result in enhanced diagnosis and the scope for larger international studies in the future.

## References

- Alberse, R.C., Stapel, S.O., Schuurman, J., Rispens, T., 2009. Immunoglobulin G4: an odd antibody. *Clin. Exp. Allergy* 39, 469–477.
- Akitake, R., Watanabe, T., Zaima, C., Uza, N., Ida, H., Tada, S., et al., 2010. Possible involvement of T helper type 2 responses to Toll-like receptor ligands in IgG4-related sclerosing disease. *Gut* 59, 542–545.
- Amos, W., Driscoll, E., Hoffman, J.I., 2011. Candidate genes versus genome-wide associations: which are better for detecting genetic susceptibility to infectious disease? *Proc. Biol. Sci.* 278, 1183–1188.
- Arai, Y., Yamashita, K., Kuriyama, K., Shiokawa, M., Kodama, Y., Sakurai, T., et al., 2015. Plasmacytoid dendritic cell activation and IFN-alpha production are prominent features of murine autoimmune pancreatitis and human IgG4-related autoimmune pancreatitis. *J. Immunol.* 195, 3033–3044.

- Asada, M., Nishio, A., Akamatsu, T., Tanaka, J., Saga, K., Kido, M., et al., 2010. Analysis of humoral immune response in experimental autoimmune pancreatitis in mice. *Pancreas* 39, 224–231.
- Asano, J., Watanabe, T., Oguchi, T., Kanai, K., Maruyama, M., Ito, T., et al., 2015. Association between immunoglobulin G4-related disease and malignancy within 12 years after diagnosis: an analysis after longterm followup. *J. Rheumatol.* 42, 2135–2142.
- Beck, L.H., Bonegio, J.R., Lambeau, R.G., Beck, G., Powell, D.M., Cummins, D.W., et al., 2009. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. *N. Engl. J. Med.* 361, 11–21.
- Bernreuther, C., Illies, C., Flitsch, J., Buchfelder, M., Buslei, R., Glatzel, M., et al., 2017. IgG4-related hypophysitis is highly prevalent among cases of histologically confirmed hypophysitis. *Brain. Pathol.* 27, 839–845.
- Buijs, J., Maillette de Buy Wenniger, L., Van Leenders, G., Verheij, J., Van Onna, I., Hansen, B., et al., 2014. Immunoglobulin G4-related prostatitis: a case-control study focusing on clinical and pathologic characteristics. *Urology* 83, 521–526.
- Chan, S.K., Cheuk, W., Chan, K.T., Chan, J.K., 2009. IgG4-related sclerosing pachymeningitis: a previously unrecognized form of central nervous system involvement in IgG4-related sclerosing disease. *Am. J. Surg. Pathol.* 33, 1249–1252.
- Chang, M.C., Chang, Y.T., Tien, Y.W., Liang, P.C., Jan, I.S., Wei, S.C., et al., 2007. T-cell regulatory gene CTLA-4 polymorphism/haplotype association with autoimmune pancreatitis. *Clin. Chem.* 53, 1700–1705.
- Chari, S.T., Smyrk, T.C., Levy, M.J., Topazian, M.D., Takahashi, N., Zhang, L., et al., 2006. Diagnosis of autoimmune pancreatitis: the Mayo Clinic experience. *Clin. Gastroenterol. Hepatol.* 4, 1010–1016. quiz 934.
- Dahlgren, M., Khosroshahi, A., Nielsen, G.P., Deshpande, V., Stone, J.H., 2010. Riedel's thyroiditis and multifocal fibrosclerosis are part of the IgG4-related systemic disease spectrum. *Arthritis Care Res. (Hoboken)* 62, 1312–1318.
- Davidson, T.S., Longnecker, D.S., Hickey, W.F., 2005. An experimental model of autoimmune pancreatitis in the rat. *Am. J. Pathol.* 166, 729–736.
- De Virgilio, A., De Vincentiis, M., Inghilleri, M., Fabrini, G., Conte, M., Gallo, A., et al., 2017. Idiopathic hypertrophic pachymeningitis: an autoimmune IgG4-related disease. *Immunol. Res.* 65, 386–394.
- Deshpande, V., 2015. IgG4 related disease of the head and neck. *Head Neck Pathol.* 9, 24–31.
- Deshpande, V., Chicano, S., Finkelberg, D., Selig, M.K., Mino-Kenudson, M., Brugge, W.R., et al., 2006. Autoimmune pancreatitis: a systemic immune complex mediated disease. *Am. J. Surg. Pathol.* 30, 1537–1545.
- Deshpande, V., Sainani, N.I., Chung, R.T., Pratt, D.S., Mentha, G., Rubbia-Brandt, L., et al., 2009. IgG4-associated cholangitis: a comparative histological and immunophenotypic study with primary sclerosing cholangitis on liver biopsy material. *Mod. Pathol.* 22, 1287–1295.
- Deshpande, V., Zen, Y., Chan, J.K., Yi, E.E., Sato, Y., Yoshino, T., et al., 2012. Consensus statement on the pathology of IgG4-related disease. *Mod. Pathol.* 25, 1181–1192.
- Dhall, D., Suriawinata, A.A., Tang, L.H., Shia, J., Klimstra, D.S., 2010. Use of immunohistochemistry for IgG4 in the distinction of autoimmune pancreatitis from peritumoral pancreatitis. *Hum. Pathol.* 41, 643–652.
- Ferrari, S., Mudde, G.C., Rieger, M., Veyradier, A., Kremer Hovinga, J.A., Scheiflinger, F., 2009. IgG subclass distribution of anti-ADAMTS13 antibodies in patients with acquired thrombotic thrombocytopenic purpura. *J. Thromb. Haemost.* 7, 1703–1710.
- Freitag, T., Cham, C., Sung, H.H., Beilhack, G.F., Durinovic-Bello, I., Patel, S.D., et al., 2010. The human risk allele HLA-DRB1\*0405 predisposes class II transgenic Ab0 NOD mice to autoimmune pancreatitis. *Gastroenterology* 139, 281–291.
- Fujinaga, Y., Kadoya, M., Kawa, S., Hamano, H., Ueda, K., Momose, M., et al., 2009. Characteristic findings in images of extra-pancreatic lesions associated with autoimmune pancreatitis. *Eur. J. Radiol.* 76, 228–238.
- Fukui, Y., Uchida, K., Sakaguchi, Y., Fukui, T., Nishio, A., Shikata, N., et al., 2015. Possible involvement of Toll-like receptor 7 in the development of type 1 autoimmune pancreatitis. *J. Gastroenterol.* 50, 435–444.
- Ghazale, A., Chari, S.T., Zhang, L., Smyrk, T.C., Takahashi, N., Levy, M.J., et al., 2008. Immunoglobulin G4-associated cholangitis: clinical profile and response to therapy. *Gastroenterology* 134, 706–715.
- Goto, H., Takahira, M., Azumi, A., 2015. Diagnostic criteria for IgG4-related ophthalmic disease. *Jpn. J. Ophthalmol.* 59, 1–7.
- Graham, R.P., Smyrk, T.C., Chari, S.T., Takahashi, N., Zhang, L., 2014. Isolated IgG4-related sclerosing cholangitis: a report of 9 cases. *Hum. Pathol.* 45, 1722–1729.
- Guarneri, F., Guarneri, C., Benvenga, S., 2005. Helicobacter pylori and autoimmune pancreatitis: role of carbonic anhydrase via molecular mimicry? *J. Cell Mol. Med.* 9, 741–744.
- Hamano, H., Kawa, S., Horiuchi, A., Unno, H., Furuya, N., Akamatsu, T., et al., 2001. High serum IgG4 concentrations in patients with sclerosing pancreatitis. *New Engl. J. Med.* 344, 732–738.
- Hamano, H., Kawa, S., Ochi, Y., Unno, H., Shiba, N., Wajiki, M., et al., 2002. Hydronephrosis associated with retroperitoneal fibrosis and sclerosing pancreatitis. *Lancet* 359, 1403–1404.
- Hamano, H., Kawa, S., Uehara, T., Ochi, Y., Takayama, M., Komatsu, K., et al., 2005. Immunoglobulin G4-related lymphoplasmacytic sclerosing cholangitis that mimics infiltrating hilar cholangiocarcinoma: part of a spectrum of autoimmune pancreatitis? *Gastrointest. Endosc.* 62, 152–157.
- Hamano, H., Arakura, N., Muraki, T., Ozaki, Y., Kirosawa, K., Kawa, S., 2006. Prevalence and distribution of extrapancreatic lesions complicating autoimmune pancreatitis. *J. Gastroenterol.* 41, 1197–1205.
- Hardacre, J.M., Iacobuzio-Donahue, C.A., Sohn, T.A., Abraham, S.C., Yeo, C.J., Lillemoe, K.D., et al., 2003. Results of pancreaticoduodenectomy for lymphoplasmacytic sclerosing pancreatitis. *Ann. Surg.* 237, 853–858. discussion 858–9.
- Hart, P.A., Topazian, M.D., Witzig, T.E., Clain, J.E., Gleeson, F.C., Kleibig, R.R., et al., 2013. Treatment of relapsing autoimmune pancreatitis with immunomodulators and rituximab: the Mayo Clinic experience. *Gut* 62, 1607–1615.
- Hart, P.A., Law, R.J., Dierkhising, R.A., Smyrk, T.C., Takahashi, N., Chari, S.T., 2014. Risk of cancer in autoimmune pancreatitis: a case-control study and review of the literature. *Pancreas* 43, 417–421.
- Haruta, I., Yanagisawa, N., Kawamura, S., Furukawa, T., Shimizu, K., Kato, H., et al., 2010. A mouse model of autoimmune pancreatitis with salivary gland involvement triggered by innate immunity via persistent exposure to avirulent bacteria. *Lab. Invest.* 90, 1757–1769.
- Haruta, I., Shimizu, K., Yanagisawa, N., Shiratori, K., Yagi, J., 2012. Commensal flora, is it an unwelcomed companion as a triggering factor of autoimmune pancreatitis? *Front. Physiol.* 3, 77.
- Hennig, C., Rink, L., Kirchner, H., 2000. Evidence for presence of IgG4 anti-immunoglobulin autoantibodies in all human beings. *Lancet* 355, 1617–1618.

- Hirano, K., Tada, M., Sasahira, N., Isayama, H., Mizuno, S., Takagi, K., et al., 2014. Incidence of malignancies in patients with IgG4-related disease. *Intern. Med.* 53, 171–176.
- Horiuchi, A., Kaneko, T., Yamamura, N., Nagata, A., Nakamura, T., Akamatsu, T., et al., 1996. Autoimmune chronic pancreatitis simulating pancreatic lymphoma. *Am. J. Gastroenterol.* 91, 2607–2609.
- Inoue, D., Zen, Y., Abo, H., Gabata, T., Demachi, H., Yoshikawa, J., et al., 2011. Immunoglobulin G4-related periaortitis and periarteritis: CT findings in 17 patients. *Radiology* 261, 625–633.
- Inoue, D., Yoshida, K., Yoneda, N., Ozaki, K., Matsubara, T., Nagai, K., et al., 2015. IgG4-related disease: dataset of 235 consecutive patients. *Medicine (Baltimore)* 94, e680.
- Ishizu, Y., Ishigami, M., Kuzuya, T., Honda, T., Hayashi, K., Nakano, I., et al., 2016. Immunoglobulin G4-associated autoimmune hepatitis later complicated by autoimmune pancreatitis: a case report. *Hepatol. Res.* 46, 601–606.
- Ito, T., Nishimori, I., Inoue, N., Kawabe, K., Gibo, J., Arita, Y., et al., 2007. Treatment for autoimmune pancreatitis: consensus on the treatment for patients with autoimmune pancreatitis in Japan. *J. Gastroenterol.* 42 (Suppl 18), 50–58.
- Ito, M., Yasuo, M., Yamamoto, H., Tsushima, K., Tanabe, T., Yokoyama, T., et al., 2009. Central airway stenosis in a patient with autoimmune pancreatitis. *Eur. Respir. J.* 33, 680–683.
- Ito, T., Kitahara, K., Umemura, T., Ota, M., Shimozuru, Y., Kawa, S., et al., 2010. A novel heterophilic antibody interaction involves IgG4. *Scand. J. Immunol.* 71, 109–114.
- Kakudo, K., Li, Y., Taniguchi, E., Mori, I., Ozaki, T., Nishihara, E., et al., 2011. IgG4-related disease of the thyroid glands [Review]. *Endocr. J.* 59, 273–281.
- Kamisawa, T., Funata, N., Hayashi, Y., Eishi, Y., Koike, M., Tsuruta, K., et al., 2003a. A new clinicopathological entity of IgG4-related autoimmune disease. *J. Gastroenterol.* 38, 982–984.
- Kamisawa, T., Funata, N., Hayashi, Y., Tsuruta, K., Okamoto, A., Amemiya, K., et al., 2003b. Close relationship between autoimmune pancreatitis and multifocal fibrosclerosis. *Gut* 52, 683–687.
- Kamisawa, T., Shimosegawa, T., Okazaki, K., Nishino, T., Watanabe, H., Kanno, A., et al., 2009. Standard steroid treatment for autoimmune pancreatitis. *Gut* 58, 1504–1507.
- Kanai, K., Maruyama, M., Kameko, F., Kawasaki, K., Asano, J., Oguchi, T., et al., 2016. Autoimmune pancreatitis can transform into chronic features similar to advanced chronic pancreatitis with functional insufficiency following severe calcification. *Pancreas* 45, 1189–1195.
- Kanno, A., Masamune, A., Okazaki, K., Kamisawa, T., Kawa, S., Nishimori, I., et al., 2015. Nationwide epidemiological survey of autoimmune pancreatitis in Japan in 2011. *Pancreas* 44, 535–539.
- Kashashima, S., Zen, Y., Kawashima, A., Konishi, K., Sasaki, H., Endo, M., et al., 2008. Inflammatory abdominal aortic aneurysm: close relationship to IgG4-related periaortitis. *Am. J. Surg. Pathol.* 32, 197–204.
- Kawa, S., 2016. Current concepts and diagnosis of IgG4-related pancreatitis (type 1AIP). *Semin. Liver Dis.* 36, 257–273.
- Kawa, S., 2017. The immunobiology of immunoglobulin G4 and complement activation pathways in IgG4-related disease. *Curr. Top. Microbiol. Immunol.* 401, 61–73.
- Kawa, S., Ota, M., Yoshizawa, K., Horiuchi, A., Hamano, H., Ochi, Y., et al., 2002. HLA DRB10405-DQB10401 haplotype is associated with autoimmune pancreatitis in the Japanese population. *Gastroenterology* 122, 1264–1269.
- Kawa, S., Kitahara, K., Hamano, H., Ozaki, Y., Arakura, N., Yoshizawa, K., et al., 2008. A novel immunoglobulin-immunoglobulin interaction in autoimmunity. *PLoS One* 3, e1637.
- Kawaguchi, K., Koike, M., Tsuruta, K., Okamoto, A., Tabata, I., Fujita, N., 1991. Lymphoplasmacytic sclerosing pancreatitis with cholangitis: a variant of primary sclerosing cholangitis extensively involving pancreas. *Hum. Pathol.* 22, 387–395.
- Kawamura, E., Hisano, S., Nakashima, H., Takeshita, M., Saito, T., 2015. Immunohistological analysis for immunological response and mechanism of interstitial fibrosis in IgG4-related kidney disease. *Mod. Rheumatol.* 25, 571–578.
- Kawano, M., Yamada, K., 2016. IgG4-related kidney disease and IgG4-related retroperitoneal fibrosis. *Semin. Liver Dis.* 36, 283–290.
- Kawano, M., Saeki, T., Nakashima, H., Nishi, S., Yamaguchi, Y., Hisano, S., et al., 2011. Proposal for diagnostic criteria for IgG4-related kidney disease. *Clin. Exp. Nephrol.* 15, 615–626.
- Khosroshahi, A., Bloch, D.B., Deshpande, V., Stone, J.H., 2010. Rituximab therapy leads to rapid decline of serum IgG4 levels and prompt clinical improvement in IgG4-related systemic disease. *Arthritis Rheum.* 62, 1755–1762.
- Khosroshahi, A., Wallace, Z.S., Crowe, J.L., Akamizu, T., Azumi, A., Carruthers, M., et al., 2015. International consensus guidance statement on the management and treatment of IgG4-related disease. *Arthritis Rheumatol.* 67, 1688–1699.
- Kim, K.P., Kim, M.H., Kim, J.C., Lee, S.S., Seo, D.W., Lee, S.K., 2006. Diagnostic criteria for autoimmune chronic pancreatitis revisited. *World J. Gastroenterol.* 12, 2487–2496.
- Kloppel, G., Sipos, B., Zamboni, G., Kojima, M., Morohoshi, T., 2007. Autoimmune pancreatitis: histo- and immunopathological features. *J. Gastroenterol.* 42 (Suppl 18), 28–31.
- Ko, S.B., Mizuno, N., Yatabe, Y., Yoshikawa, T., Ishiguro, H., Yamamoto, A., et al., 2010. Corticosteroids correct aberrant CFTR localization in the duct and regenerate acinar cells in autoimmune pancreatitis. *Gastroenterology* 138, 1988–1996.
- Komatsu, K., Hamano, H., Ochi, Y., Takayama, M., Muraki, T., Yoshizawa, K., et al., 2005. High prevalence of hypothyroidism in patients with autoimmune pancreatitis. *Dig. Dis. Sci.* 50, 1052–1057.
- Kono, H., Rock, K.L., 2008. How dying cells alert the immune system to danger. *Nat. Rev. Immunol.* 8, 279–289.
- Koyabu, M., Uchida, K., Miyoshi, H., Sakaguchi, Y., Fukui, T., Ikeda, H., et al., 2010. Analysis of regulatory T cells and IgG4-positive plasma cells among patients of IgG4-related sclerosing cholangitis and autoimmune liver diseases. *J. Gastroenterol.* 45, 732–741.
- Kusuda, T., Uchida, K., Miyoshi, H., Koyabu, M., Satoi, S., Takaoka, M., et al., 2011. Involvement of inducible costimulator- and interleukin 10-positive regulatory T cells in the development of IgG4-related autoimmune pancreatitis. *Pancreas* 40, 1120–1130.
- Lanzillotta, M., Della-Torre, E., Stone, J.H., 2017. Roles of plasmablasts and B Cells in IgG4-related disease: implications for therapy and early treatment outcomes. *Curr. Top. Microbiol. Immunol.* 401, 85–92.
- Li, Y., Zhou, G., Ozaki, T., Nishihara, E., Matsuzuka, F., Bai, Y., et al., 2012. Distinct histopathological features of Hashimoto's thyroiditis with respect to IgG4-related disease. *Mod. Pathol.* 25, 1086–1097.

- Maruyama, M., Arakura, N., Ozaki, Y., Watanabe, T., Ito, T., Yoneda, S., et al., 2012. Risk factors for pancreatic stone formation in autoimmune pancreatitis over a long-term course. *J. Gastroenterol.* 47, 553–560.
- Maruyama, M., Arakura, N., Ozaki, Y., Watanabe, T., Ito, T., Yoneda, S., et al., 2013. Type 1 autoimmune pancreatitis can transform into chronic pancreatitis: a long-term follow-up study of 73 Japanese patients. *Int. J. Rheumatol.* 2013, 8.
- Maruyama, M., Watanabe, T., Kanai, K., Oguchi, T., Asano, J., Ito, T., et al., 2014. Autoimmune pancreatitis can develop into chronic pancreatitis. *Orphanet J. Rare Dis.* 9, 77.
- Masaki, Y., Dong, L., Kurose, N., Kitagawa, K., Morikawa, Y., Yamamoto, M., et al., 2008. Proposal for a new clinical entity, IgG4-positive multi-organ lymphoproliferative syndrome: analysis of 64 cases of IgG4-related disorders. *Ann. Rheum. Dis.* 68, 1310–1315.
- Masaki, Y., Sugai, S., Umehara, H., 2010. IgG4-related diseases including Mikulicz's disease and sclerosing pancreatitis: diagnostic insights. *J. Rheumatol.* 37, 1380–1385.
- Matsui, S., Yamamoto, H., Minamoto, S., Waseda, Y., Mishima, M., Kubo, K., 2016. Proposed diagnostic criteria for IgG4-related respiratory disease. *Respir. Invest.* 54, 130–132.
- Mattoo, H., Mahajan, V.S., Della-Torre, E., Sekigami, Y., Carruthers, M., Wallace, Z.S., et al., 2014. De novo oligoclonal expansions of circulating plasmablasts in active and relapsing IgG4-related disease. *J. Allergy Clin. Immunol.* 134, 679–687.
- Mauri, C., Bosma, A., 2012. Immune regulatory function of B cells. *Annu. Rev. Immunol.* 30, 221–241.
- Miyake, K., Moriyama, M., Aizawa, K., Nagano, S., Inoue, Y., Sadanaga, A., et al., 2008. Peripheral CD4+ T cells showing a Th2 phenotype in a patient with Mikulicz's disease associated with lymphadenopathy and pleural effusion. *Mod. Rheumatol.* 18, 86–90.
- Miyoshi, H., Uchida, K., Taniguchi, T., Yazumi, S., Matsushita, M., Takaoka, M., et al., 2008. Circulating naive and CD4+CD25 high regulatory T cells in patients with autoimmune pancreatitis. *Pancreas* 36, 133–140.
- Mizushima, I., Yamada, K., Fujii, H., Inoue, D., Umehara, H., Yamagishi, M., et al., 2012. Clinical and histological changes associated with corticosteroid therapy in IgG4-related tubulointerstitial nephritis. *Mod. Rheumatol.* 22, 859–870.
- Mizushima, I., Inoue, D., Yamamoto, M., Yamada, K., Saeki, T., Ubara, Y., et al., 2014. Clinical course after corticosteroid therapy in IgG4-related aortitis/periaortitis and periarteritis: a retrospective multicenter study. *Arthritis Res. Ther.* 16, R156.
- Moriyama, M., Nakamura, S., 2017. Th1/Th2 immune balance and other T helper subsets in IgG4-related disease. *Curr. Top. Microbiol. Immunol.* 401, 75–83.
- Muraki, T., Hamano, H., Ochi, Y., Komatsu, K., Komiyama, Y., Arakura, N., et al., 2006. Autoimmune pancreatitis and complement activation system. *Pancreas* 32, 16–21.
- Naitoh, I., Nakazawa, T., Ohara, H., Ando, T., Hayashi, K., Tanaka, H., et al., 2009. Endoscopic transpapillary intraductal ultrasonography and biopsy in the diagnosis of IgG4-related sclerosing cholangitis. *J. Gastroenterol.* 44, 1147–1155.
- Nakano, S., Takeda, I., Kitamura, K., Watahiki, H., Iimura, Y., Takenaka, M., 1978. Vanishing tumor of the abdomen in patient with Sjögren's syndrome. *Dig. Dis.* 23, 75–79.
- Nakanuma, Y., Ishizu, Y., Zen, Y., Harada, K., Umemura, T., 2016. Histopathology of IgG4-related autoimmune hepatitis and IgG4-related hepatopathy in IgG4-related disease. *Semin. Liver Dis.* 36, 229–241.
- Nakazawa, T., Ohara, H., Yamada, T., Ando, H., Sano, H., Kajino, S., et al., 2001. Atypical primary sclerosing cholangitis cases associated with unusual pancreatitis. *Hepato-gastroenterology* 48, 625–630.
- Nakazawa, T., Ohara, H., Sano, H., Ando, T., Aoki, S., Kobayashi, S., et al., 2005. Clinical differences between primary sclerosing cholangitis and sclerosing cholangitis with autoimmune pancreatitis. *Pancreas* 30, 20–25.
- Nakazawa, T., Shimizu, S., Naitoh, I., 2016. IgG4-related sclerosing cholangitis. *Semin. Liver Dis.* 36, 216–228.
- Neild, G.H., Rodriguez-Justo, M., Wall, C., Connolly, J.O., 2006. Hyper-IgG4 disease: report and characterisation of a new disease. *BMC. Med.* 4, 23.
- Nishio, A., Asada, M., Uchida, K., Fukui, T., Chiba, T., Okazaki, K., 2011. The role of innate immunity in the pathogenesis of experimental autoimmune pancreatitis in mice. *Pancreas* 40, 95–102.
- Oguchi, T., Ota, M., Ito, T., Hamano, H., Arakura, N., Katsuyama, Y., et al., 2016. Correction: investigation of susceptibility genes triggering lacrimal/salivary gland lesion complications in Japanese patients with type 1 autoimmune pancreatitis. *PLoS One* 11, e0146738.
- Ohara, H., Okazaki, K., Tsubouchi, H., Inui, K., Kawa, S., Kamisawa, T., et al., 2012. Clinical diagnostic criteria of IgG4-related sclerosing cholangitis 2012. *J. Hepatobiliary Pancreat Sci.* 19, 536–542.
- Okazaki, K., Chiba, T., 2002. Autoimmune related pancreatitis. *Gut* 51, 1–4.
- Okazaki, K., Kawa, S., Kamisawa, T., Naruse, S., Tanaka, S., Nishimori, I., et al., 2006. Clinical diagnostic criteria of autoimmune pancreatitis: revised proposal. *J. Gastroenterol.* 41, 626–631.
- Okazaki, K., Kawa, S., Kamisawa, T., Ito, T., Inui, K., Irie, H., et al., 2009. Japanese clinical guidelines for autoimmune pancreatitis. *Pancreas* 38, 849–866.
- Oldstone, M.B., 1998. Molecular mimicry and immune-mediated diseases. *FASEB J.* 12, 1255–1265.
- Ota, M., Katsuyama, Y., Hamano, H., Umemura, T., Kimura, A., Yoshizawa, K., et al., 2007. Two critical genes (HLA-DRB1 and ABCF1) in the HLA region are associated with the susceptibility to autoimmune pancreatitis. *Immunogenetics* 59, 45–52.
- Ota, M., Ito, T., Umemura, T., Katsuyama, Y., Yoshizawa, K., Hamano, H., et al., 2011. Polymorphism in the KCNA3 gene is associated with susceptibility to autoimmune pancreatitis in the Japanese population. *Dis. Markers* 31, 223–229.
- Ota, M., Umemura, T., Kawa, S., 2017. Immunogenetics of IgG4-related AIP. *Curr. Top. Microbiol. Immunol.* 401, 35–44.
- Otsuki, M., Chung, J.B., Okazaki, K., Kim, M.H., Kamisawa, T., Kawa, S., et al., 2008. Asian diagnostic criteria for autoimmune pancreatitis: consensus of the Japan-Korea symposium on autoimmune pancreatitis. *J. Gastroenterol.* 43, 403–408.
- Ozawa, M., Fujinaga, Y., Asano, J., Nakamura, A., Watanabe, T., Ito, T., et al., 2017. Clinical features of IgG4-related periaortitis/periarteritis based on the analysis of 179 patients with IgG4-related disease: a case-control study. *Arthritis Res. Ther.* 19, 223.
- Pearson, R.K., Longnecker, D.S., Chari, S.T., Smyrk, T.C., Okazaki, K., Frulloni, L., et al., 2003. Controversies in clinical pancreatology: autoimmune pancreatitis: does it exist? *Pancreas* 27, 1–13.
- Plomp, J.J., Huijbers, M.G., Van Der Maarel, S.M., Verschuuren, J.J., 2012. Pathogenic IgG4 subclass autoantibodies in MuSK myasthenia gravis. *Ann. N. Y. Acad. Sci.* 1275, 114–122.
- Qu, W.M., Miyazaki, T., Terada, M., Okada, K., Mori, S., Kanno, H., et al., 2002. A novel autoimmune pancreatitis model in MRL mice treated with polyinosinic:polycytidylic acid. *Clin. Exp. Immunol.* 129, 27–34.

- Raina, A., Yadav, D., Krasinskas, A.M., McGrath, K.M., Khalid, A., Sanders, M., et al., 2009. Evaluation and management of autoimmune pancreatitis: experience at a large US center. *Am. J. Gastroenterol.* 104, 2295–2306.
- Raijani, Y., Nasr, S.H., Larsen, C.P., Colvin, R.B., Smyrk, T.C., Takahashi, N., et al., 2011. Diagnosis of IgG4-related tubulointerstitial nephritis. *J. Am. Soc. Nephrol.* 22, 1343–1352.
- Riku, S., Hashizume, Y., Yoshida, M., Riku, Y., 2009. [Is hypertrophic pachymeningitis a dural lesion of IgG4-related systemic disease?]. *Rinsho Shinkeigaku* 49, 594–596.
- Rispens, T., Ooijevaar-De Heer, P., Vermeulen, E., Schuurman, J., Van Der Neut kolfschoten, M., Aalberse, R.C., 2009. Human IgG4 binds to IgG4 and conformationally altered IgG1 via Fc-Fc interactions. *J. Immunol.* 182, 4275–4281.
- Rispens, T., Ooijevaar-de Heer, P., Bende, O., Aalberse, R.C., 2011. Mechanism of immunoglobulin G4 Fab-arm exchange. *J. Am. Chem. Soc.* 133, 10302–10311.
- Rock, B., Martins, C.R., Theofilopoulos, A.N., Balderas, R.S., Anhalt, G.J., Labib, R.S., et al., 1989. The pathogenic effect of IgG4 autoantibodies in endemic pemphigus foliaceus (fogo selvagem). *N. Engl. J. Med.* 320, 1463–1469.
- Saegusa, H., Momose, M., Kawa, S., Hamano, H., Ochi, Y., Takayama, M., et al., 2003. Hilar and pancreatic gallium-67 accumulation is characteristic feature of autoimmune pancreatitis. *Pancreas* 27, 20–25.
- Saeki, T., Nishi, S., Imai, N., Ito, T., Yamazaki, H., Kawano, M., et al., 2010. Clinicopathological characteristics of patients with IgG4-related tubulointerstitial nephritis. *Kidney Int.* 78, 1016–1023.
- Sarles, H., Sarles, J.C., Muratore, R., Guien, C., 1961. Chronic inflammatory sclerosis of the pancreas—an autonomous pancreatic disease? *Am. J. Dig. Dis.* 6, 688–698.
- Shikuma, J., Kan, K., Ito, R., Hara, K., Sakai, H., Miwa, T., et al., 2017. Critical review of IgG4-related hypophysitis. *Pituitary* 20, 282–291.
- Shimatsu, A., Oki, Y., Fujisawa, I., Sano, T., 2009. Pituitary and stalk lesions (infundibulo-hypophysitis) associated with immunoglobulin G4-related systemic disease: an emerging clinical entity. *Endocr. J.* 56, 1033–1041.
- Shimosegawa, T., Chari, S.T., Frulloni, L., Kamisawa, T., Kawa, S., Mino-Kenudson, M., et al., 2011. International consensus diagnostic criteria for autoimmune pancreatitis: guidelines of the international association of pancreatology. *Pancreas* 40, 352–358.
- Shiokawa, M., Kodama, Y., Yoshimura, K., Kawanami, C., Mimura, J., Yamashita, Y., et al., 2013. Risk of cancer in patients with autoimmune pancreatitis. *Am. J. Gastroenterol.* 108, 610–617.
- Shiokawa, M., Kodama, Y., Kuriyama, K., Yoshimura, K., Tomono, T., Morita, T., et al., 2016. Pathogenicity of IgG in patients with IgG4-related disease. *Gut* 65, 1322–1332.
- Shiokawa, M., Kodama, Y., Sekiguchi, K., Kuwada, T., Tomono, T., Kuriyama, K., et al., 2018. Laminin 511 is a target antigen in autoimmune pancreatitis. *Sci. Transl. Med* 10, 453.
- Soga, Y., Komori, H., Miyazaki, T., Arita, N., Terada, M., Kamada, K., et al., 2009. Toll-like receptor 3 signaling induces chronic pancreatitis through the Fas/Fas ligand-mediated cytotoxicity. *Tohoku J. Exp. Med.* 217, 175–184.
- Sogabe, Y., Ohshima, K., Azumi, A., Takahira, M., Kase, S., Tsuji, H., et al., 2014. Location and frequency of lesions in patients with IgG4-related ophthalmic diseases. *Graefes Arch. Clin. Exp. Ophthalmol.* 252, 531–538.
- Song, M.H., Kim, M.H., Lee, S.K., Seo, D.W., Lee, S.S., Han, J., et al., 2005. Regression of pancreatic fibrosis after steroid therapy in patients with autoimmune chronic pancreatitis. *Pancreas* 30, 83–86.
- Stone, J.H., 2012. IgG4-related disease: nomenclature, clinical features, and treatment. *Semin. Diagn. Pathol.* 29, 177–190.
- Stone, J.H., Khosroshahi, A., Deshpande, V., Chan, J.K., Heathcote, J.G., Aalberse, R., et al., 2012a. Recommendations for the nomenclature of IgG4-related disease and its individual organ system manifestations. *Arthritis Rheum.* 64, 3061–3067.
- Stone, J.H., Zen, Y., Deshpande, V., 2012b. IgG4-related disease. *N. Engl. J. Med.* 366, 539–551.
- Sugimoto, M., Watanabe, H., Asano, T., Sato, S., Takagi, T., Kobayashi, H., et al., 2016. Possible participation of IgG4 in the activation of complement in IgG4-related disease with hypocomplementemia. *Mod. Rheumatol.* 26, 251–258.
- Sumimoto, K., Uchida, K., Kusuda, T., Mitsuyama, T., Sakaguchi, Y., Fukui, T., et al., 2014. The role of CD19+ CD24high CD38high and CD19+ CD24high CD27+ regulatory B cells in patients with type 1 autoimmune pancreatitis. *Pancreatology* 14, 193–200.
- Taguchi, M., Kihara, Y., Nagashio, Y., Yamamoto, M., Otsuki, M., Harada, M., 2009. Decreased production of immunoglobulin M and A in autoimmune pancreatitis. *J. Gastroenterol.* 44, 1133–1139.
- Takahashi, N., Kawashima, A., Fletcher, J.G., Chari, S.T., 2007. Renal involvement in patients with autoimmune pancreatitis: CT and MR imaging findings. *Radiology* 242, 791–801.
- Takayama, M., Hamano, H., Ochi, Y., Saegusa, H., Komatsu, K., Muraki, T., et al., 2004. Recurrent attacks of autoimmune pancreatitis result in pancreatic stone formation. *Am. J. Gastroenterol.* 99, 932–937.
- Takeda, S., Haratake, J., Kasai, T., Takaeda, C., Takazakura, E., 2004. IgG4-associated idiopathic tubulointerstitial nephritis complicating autoimmune pancreatitis. *Nephrol. Dial. Transplant.* 19, 474–476.
- Takeshima, K., Inaba, H., Ariyasu, H., Furukawa, Y., Doi, A., Nishi, M., et al., 2015. Clinicopathological features of Riedel's thyroiditis associated with IgG4-related disease in Japan. *Endocr. J.* 62, 725–731.
- Takeuchi, M., Sato, Y., Ohno, K., Tanaka, S., Takata, K., Gion, Y., et al., 2014. T helper 2 and regulatory T-cell cytokine production by mast cells: a key factor in the pathogenesis of IgG4-related disease. *Mod. Pathol.* 27, 1126–1136.
- Tanaka, S., Kobayashi, T., Nakanishi, K., Okubo, M., Murase, T., Hashimoto, M., et al., 2000. Corticosteroid-responsive diabetes mellitus associated with autoimmune pancreatitis. *Lancet* 356, 910–911.
- Tanaka, A., Moriyama, M., Nakashima, H., Miyake, K., Hayashida, J.N., Maehara, T., et al., 2012. Th2 and regulatory immune reactions contribute to IgG4 production and the initiation of Mikulicz disease. *Arthritis Rheum.* 64, 254–263.
- Tanaka, A., Tazuma, S., Okazaki, K., Tsubouchi, H., Inui, K., Takikawa, H., 2014. Nationwide survey for primary sclerosing cholangitis and IgG4-related sclerosing cholangitis in Japan. *J. Hepatobiliary Pancreat. Sci.* 21, 43–50.
- Taniguchi, T., Ko, M., Seko, S., Nishida, O., Inoue, F., Kobayashi, H., et al., 2004. Interstitial pneumonia associated with autoimmune pancreatitis. *Gut* 53, 770. author reply 770-1.
- Terasaki, Y., Ikushima, S., Matsui, S., Hebisawa, A., Ichimura, Y., Izumi, S., et al., 2017. Comparison of clinical and pathological features of lung lesions of systemic IgG4-related disease and idiopathic multicentric Castleman's disease. *Histopathology* 70, 1114–1124.

- Toki, F., Kozu, T., Oi, I., Nakasato, T., Suzuki, M., Hanyu, F., 1992. An unusual type of chronic pancreatitis showing diffuse irregular narrowing of the entire main pancreatic duct on ERCP-A report of four cases. *Endoscopy* 24, 640.
- Topazian, M., Witzig, T.E., Smyrk, T.C., Pulido, J.S., Levy, M.J., Kamath, P.S., et al., 2008. Rituximab therapy for refractory biliary strictures in immunoglobulin G4-associated cholangitis. *Clin. Gastroenterol. Hepatol.* 6, 364–366.
- Uchida, K., Okazaki, K., 2017. Roles of regulatory T and B cells in IgG4-related disease. *Curr. Top. Microbiol. Immunol.* 401, 93–114.
- Uchida, K., Okazaki, K., Nishi, T., Uose, S., Nakase, H., Ohana, M., et al., 2002. Experimental immune-mediated pancreatitis in neonatally thymectomized mice immunized with carbonic anhydrase II and lactoferrin. *Lab. Invest.* 82, 411–424.
- Uchiyama-Tanaka, Y., Mori, Y., Kimura, T., Sonomura, K., Umemura, S., Kishimoto, N., et al., 2004. Acute tubulointerstitial nephritis associated with autoimmune-related pancreatitis. *Am. J. Kidney Dis.* 43, e18–e25.
- Uehara, T., Hamano, H., Kawakami, M., Koyama, M., Kawa, S., Sano, K., et al., 2008. Autoimmune pancreatitis-associated prostatitis: distinct clinicopathological entity. *Pathol. Int.* 58, 118–125.
- Umeshara, H., Okazaki, K., Masaki, Y., Kawano, M., Yamamoto, M., Saeki, T., et al., 2012a. A novel clinical entity, IgG4-related disease (IgG4RD): general concept and details. *Mod. Rheumatol.* 22, 1–14.
- Umeshara, H., Okazaki, K., Masaki, Y., Kawano, M., Yamamoto, M., Saeki, T., et al., 2012b. Comprehensive diagnostic criteria for IgG4-related disease (IgG4-RD). *Mod. Rheumatol.* 22, 21–30.
- Umemura, T., Ota, M., Hamano, H., Katsuyama, Y., Kiyosawa, K., Kawa, S., 2006. Genetic association of Fc receptor-like 3 polymorphisms with autoimmune pancreatitis in Japanese patients. *Gut* 55, 1367–1368.
- Umemura, T., Zen, Y., Hamano, H., Kawa, S., Nakanuma, Y., Kiyosawa, K., 2007a. IgG4-hepatopathy: association of IgG4-bearing plasma cells in liver with autoimmune pancreatitis. *Gastroenterology* 132, A45–A46.
- Umemura, T., Zen, Y., Hamano, H., Kawa, S., Nakanuma, Y., Kiyosawa, K., 2007b. Immunoglobulin G4-hepatopathy: association of immunoglobulin G4-bearing plasma cells in liver with autoimmune pancreatitis. *Hepatology* 46, 463–471.
- Umemura, T., Ota, M., Hamano, H., Katsuyama, Y., Muraki, T., Arakura, N., et al., 2008. Association of autoimmune pancreatitis with cytotoxic T-lymphocyte antigen 4 gene polymorphisms in Japanese patients. *Am. J. Gastroenterol.* 103, 588–594.
- van der Neut Kolfschoten, M., Schuurman, J., Losen, M., Bleeker, W.K., Martinez-Martinez, P., Vermeulen, E., et al., 2007. Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange. *Science* 317, 1554–1557.
- van der Vliet, H.J., Perenboom, R.M., 2004. Multiple pseudotumors in IgG4-associated multifocal systemic fibrosis. *Ann. Intern. Med.* 141, 896–897.
- Wallace, Z.S., Deshpande, V., Stone, J.H., 2014. Ophthalmic manifestations of IgG4-related disease: single-center experience and literature review. *Semin. Arthritis Rheum.* 43, 806–817.
- Wallace, Z.S., Mattoo, H., Carruthers, M., Mahajan, V.S., Della Torre, E., Lee, H., et al., 2015. Plasmablasts as a biomarker for IgG4-related disease, independent of serum IgG4 concentrations. *Ann. Rheum. Dis.* 74, 190–195.
- Watanabe, S., Suzuki, K., Kawauchi, Y., Yamagiwa, S., Yoneyama, H., Kawachi, H., et al., 2003. Kinetic analysis of the development of pancreatic lesions in mice infected with a murine retrovirus. *Clin. Immunol.* 109, 212–223.
- Watanabe, T., Yamashita, K., Fujikawa, S., Sakurai, T., Kudo, M., Shiokawa, M., et al., 2012. Involvement of activation of toll-like receptors and nucleotide-binding oligomerization domain-like receptors in enhanced IgG4 responses in autoimmune pancreatitis. *Arthritis Rheum.* 64, 914–924.
- Watanabe, T., Maruyama, M., Ito, T., Fujinaga, Y., Ozaki, Y., Maruyama, M., et al., 2013a. Clinical features of a new disease concept, IgG4-related thyroiditis. *Scand. J. Rheumatol.* 42, 325–330.
- Watanabe, T., Maruyama, M., Ito, T., Kanai, K., Oguchi, T., Muraki, T., et al., 2013b. Two siblings with type 1 autoimmune pancreatitis. *Intern. Med.* 52, 895–899.
- Watanabe, T., Yamashita, K., Sakurai, T., Kudo, M., Shiokawa, M., Uza, N., et al., 2013c. Toll-like receptor activation in basophils contributes to the development of IgG4-related disease. *J. Gastroenterol.* 48, 247–253.
- Watanabe, T., Yamashita, K., Kudo, M., 2017. IgG4-related disease and innate immunity. *Curr. Top. Microbiol. Immunol.* 401, 115–128.
- Yamamoto, M., Takahashi, H., Sugai, S., Imai, K., 2005. Clinical and pathological characteristics of Mikulicz's disease (IgG4-related plasmacytic exocrinopathy). *Autoimmun. Rev.* 4, 195–200.
- Yamamoto, M., Takahashi, H., Ohara, M., Suzuki, C., Naishiro, Y., Yamamoto, H., et al., 2006. A new conceptualization for Mikulicz's disease as an IgG4-related plasmacytic disease. *Mod. Rheumatol.* 16, 335–340.
- Yamamoto, M., Takahashi, H., Tabeya, T., Suzuki, C., Naishiro, Y., Ishigami, K., et al., 2012. Risk of malignancies in IgG4-related disease. *Mod. Rheumatol.* 22, 414–418.
- Yamamoto, M., Yajima, H., Takahashi, H., Yokoyama, Y., Ishigami, K., Shimizu, Y., et al., 2015. Everyday clinical practice in IgG4-related dacryoadenitis and/or sialadenitis: results from the SMART database. *Mod. Rheumatol.* 25, 199–204.
- Yamanishi, H., Kumagi, T., Yokota, T., Azemoto, N., Koizumi, M., Kobayashi, Y., et al., 2011. Clinical significance of B cell-activating factor in autoimmune pancreatitis. *Pancreas* 40, 840–845.
- Yanagisawa, N., Haruta, I., Shimizu, K., Furukawa, T., Higuchi, T., Shibata, N., et al., 2014. Identification of commensal flora-associated antigen as a pathogenetic factor of autoimmune pancreatitis. *Pancreatology* 14, 100–106.
- Yonekawa, T., Murai, H., Utsuki, S., Matsushita, T., Masaki, K., Isobe, N., et al., 2014. A nationwide survey of hypertrophic pachymeningitis in Japan. *J. Neurol. Neurosurg. Psychiatry* 85, 732–739.
- Yoshida, K., Toki, F., Takeuchi, T., Watanabe, S., Shiratori, K., Hayashi, N., 1995. Chronic pancreatitis caused by an autoimmune abnormality. Proposal of the concept of autoimmune pancreatitis. *Dig. Dis. Sci.* 40, 1561–1568.
- Zen, Y., Nakanuma, Y., 2010. IgG4-related disease: a cross-sectional study of 114 cases. *Am. J. Surg. Pathol.* 34, 1812–1819.
- Zen, Y., Kitagawa, S., Minato, H., Kurumaya, H., Katayanagi, K., Masuda, S., et al., 2005. IgG4-positive plasma cells in inflammatory pseudotumor (plasma cell granuloma) of the lung. *Hum. Pathol.* 36, 710–717.
- Zen, Y., Fuji, T., Harada, K., Kawano, M., Yamada, K., Takahira, M., et al., 2007. Th2 and regulatory immune reactions are increased in immunoglobulin G4-related sclerosing pancreatitis and cholangitis. *Hepatology* 45, 1538–1546.

# Autoimmune Bullous Skin Diseases: Pemphigus and Pemphigoid

*Donna A. Culton, Zhi Liu and Luis A. Diaz*

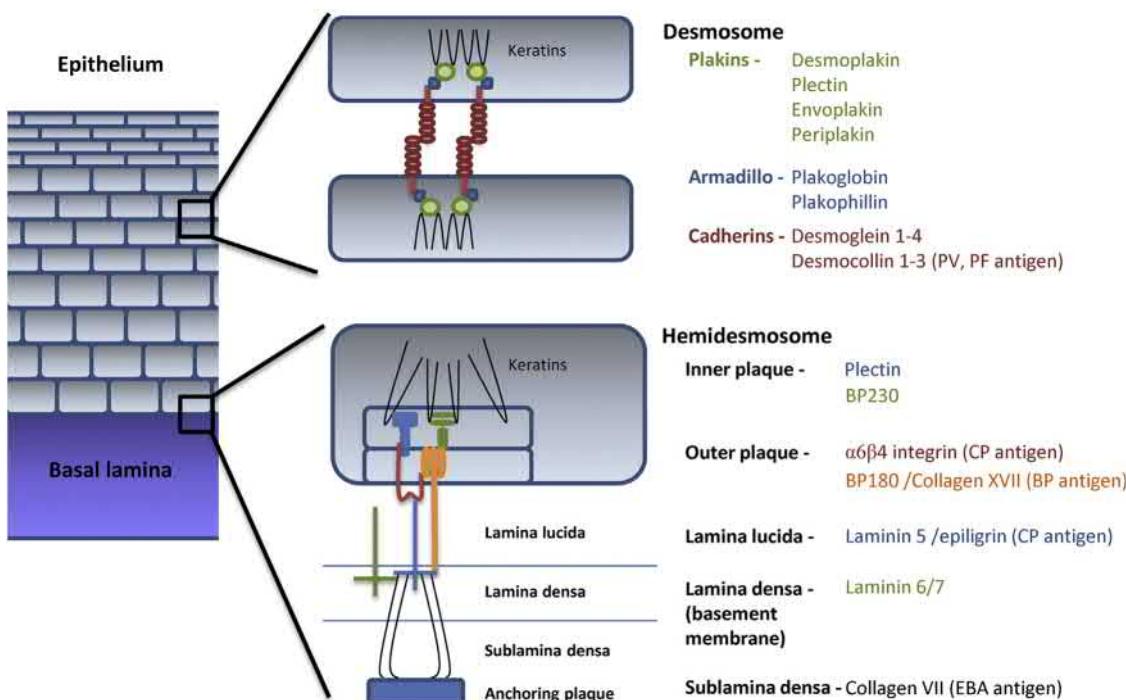
Department of Dermatology, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

## OUTLINE

<b>Introduction</b>	1191	<b>Bullous Pemphigoid</b>	1200
<b>Pemphigus Vulgaris</b>	1193	Clinical, Pathologic, and Epidemiologic features	1200
Clinical, Pathologic, and Epidemiologic Features	1193	Autoimmune Features	1200
Autoimmune Features	1194	Genetic Features	1201
Genetic Features	1195	In Vivo and In Vitro Models	1201
In Vivo and In Vitro Models	1195	Pathologic Effector Mechanisms	1201
Pathologic Effector Mechanisms	1197	Autoantibodies as Potential Immunologic Markers	1202
Autoantibodies as Potential Immunologic Markers	1197	<b>Other Subepidermal Bullous Diseases</b>	1202
<b>Pemphigus Foliaceus</b>	1197	Herpes Gestationis (Pemphigoid Gestationis)	1202
Clinical, Pathologic, and Epidemiologic features	1197	Cicatricial Pemphigoid	1202
Autoimmune Features	1197	Linear IgA disease	1203
Genetic Features	1198	Epidermolysis Bullosa Acquisita	1203
In Vivo and In Vitro Models	1198	Dermatitis Herpetiformis	1203
Pathologic Effector Mechanisms	1198	<b>Treatment of Autoimmune Bullous Diseases</b>	1204
Autoantibodies as Potential Immunologic Markers	1198	<b>Concluding Remarks</b>	1204
Environmental Factors Involved in Fogo Selvagem	1199	<b>References</b>	1204
<b>Other Types of Pemphigus</b>	1199		
Paraneoplastic Pemphigus	1199		
Drug-Induced Pemphigus	1200		
IgA Pemphigus	1200		

## INTRODUCTION

Autoimmune bullous diseases are rare disorders affecting skin and mucous membranes. These diseases are mediated by pathogenic autoantibodies directed against keratinocyte adhesion molecules (Diaz and Giudice, 2000). In the epidermis, neighboring keratinocytes adhere to each other through organelles known as desmosomes, whereas dermal–epidermal junction adhesion is mediated by hemidesmosomes. The majority of antigens



**FIGURE 60.1** Diagram of the desmosome and the hemidesmosome.

recognized by these autoantibodies are desmosomal and hemidesmosomal transmembrane glycoproteins involved in epidermal cell–cell and epidermal–dermal adherence.

The desmosome contains two parallel intracellular plaques, which are located just beneath the cell membranes of neighboring cells (Fig. 60.1). Transmembrane glycoproteins emerge from the desmosomal plaques and meet in the narrow extracellular space shared by the two cells, constituting the desmosomal core. The desmosomal plaques are composed of plakin family proteins and serve as insertion sites for intracellular keratins, whereas the core is composed of transmembrane calcium-dependent cell adhesion molecules known as desmosomal cadherins. The desmosomal cadherins include desmogleins (Dsgs)1–4 and desmocollins (Dscs)1–3 (Getsios et al., 2004), the isoforms of which vary in expression throughout the epidermis and in different squamous epithelial tissues. For example, in the skin Dsg1 is expressed throughout the epidermis with predominance in the upper layers of this tissue, whereas Dsg3 is expressed mainly in the suprabasal layers of the epidermis.

The hemidesmosomes, located on the dermal pole of the epidermal basal keratinocytes, also contain an intracellular plaque and an extracellular core structure (Fig. 60.1). The hemidesmosomal plaque, linked to the keratin network, contains the intracellular proteins BP230 and plectin (forming the inner plaque) and the transmembrane proteins BP180,  $\alpha 6\beta 4$  integrin (forming the outer plaque). The extracellular space termed the lamina lucida contains the ecto-domains of BP180,  $\alpha 6\beta 4$  integrin as well as laminin 5. The lamina lucida (corresponding to the desmosomal core) separates the basal keratinocytes from the underlying lamina densa (composed of collagen IV) (Diaz and Giudice, 2000). The sublamina dense region contains other matrix molecules and the anchoring fibrils (collagen VII).

The pemphigus group includes diseases that are characterized by autoantibodies against desmosomal cadherins (Dsg and Dsc) (Anhalt and Diaz, 2001; Beutner and Jordon, 1964), and intraepidermal cell–cell detachment known as acantholysis (Civatte, 1943). There are two classic forms of pemphigus: pemphigus vulgaris (PV) and pemphigus foliaceus (PF). PV is characterized by suprabasilar acantholysis and anti-Dsg3 IgG autoantibodies, whereas PF is characterized by subcorneal acantholysis and anti-Dsg1 IgG autoantibodies. Other less common forms of pemphigus include paraneoplastic pemphigus (PNP), drug-induced pemphigus, and IgA pemphigus (Table 60.1).

The pemphigoid group includes bullous pemphigoid (BP) and other rare subepidermal autoimmune blistering diseases (Table 60.1). The pemphigoid group is characterized by autoantibodies against hemidesmosomal proteins and separation of the epidermis from the dermis. BP is the most common autoimmune bullous disease and is the prototypic subepidermal blistering disorder (Lever, 1965). BP is characterized by subepidermal blisters and autoantibodies against the hemidesmosomal proteins BP180 and BP230 (Labib et al., 1986; Mutambo et al., 1985; Stanley et al., 1981). Other subepidermal blistering diseases such as cicatricial pemphigoid (CP), herpes

**TABLE 60.1** Autoimmune Blistering Diseases of the Skin

Diseases	Cleavage site	Skin organelle	Autoantigens	Pathogenic autoantibodies
Pemphigus vulgaris	Suprabasilar acantholysis	Desmosome	Dsg3	Passive transfer
Pemphigus foliaceus	Subcorneal acantholysis	Desmosome	Dsg1	Passive transfer
Paraneoplastic pemphigus	Suprabasilar acantholysis	Desmosome, Hemidesmosome	Dsg3, Dsg1, and plakin family	Passive transfer (Dsg3)
Drug-induced pemphigus	Subcorneal acantholysis (commonly)	Desmosome	Dsg1	?
IgA pemphigus	Subcorneal/intraepidermal pustules	Desmosome	Desmocollin 1	?
Bullous pemphigoid	Subepidermal	Hemidesmosome	BP180 and BP230	Passive transfer (BP180)
Herpes gestationis	Subepidermal	Hemidesmosome	BP180	Passive transfer
Cicatricial pemphigoid	Subepidermal	Hemidesmosome	BP180, laminin 5, $\alpha 6 \beta 4$ integrin	Passive transfer (laminin 5)
Linear IgA dermatosis	Subepidermal	Hemidesmosome	BP180 fragments	?
Epidermolysis bullosa acquisita	Subepidermal	Anchoring fibers	Type VII collagen	Passive transfer
Dermatitis herpetiformis	Subepidermal	Dermal papilla	Transglutaminase	?

Dsg, Desmoglein; BP, bullous pemphigoid.

gestationis (HG), and linear IgA dermatosis (LAD) exhibit distinctive clinical, histological, and immunologic features, yet share a humoral autoimmune response to BP180 and occasionally other antigens. The rest of acquired subepidermal blistering diseases show autoantibody responses to the structural molecules of the dermal extracellular matrix. They include epidermolysis bullosa acquisita (EBA) with a target antigen of collagen VII and dermatitis herpetiformis (DH) with a target antigen of epidermal transglutaminase.

While the autoantibody response in most of these autoimmune skin diseases belongs to the IgG class, there are exceptions. For example, IgE autoantibodies have been detected in endemic PF and BP, and IgA is found in IgA pemphigus, LAD, and DH.

In this chapter we shall review the current clinical, histological, and immunological features of the most common forms of autoimmune bullous diseases (PV, PF, and BP) (Fig. 60.2) and briefly discuss other less common forms of pemphigus and subepidermal bullous diseases.

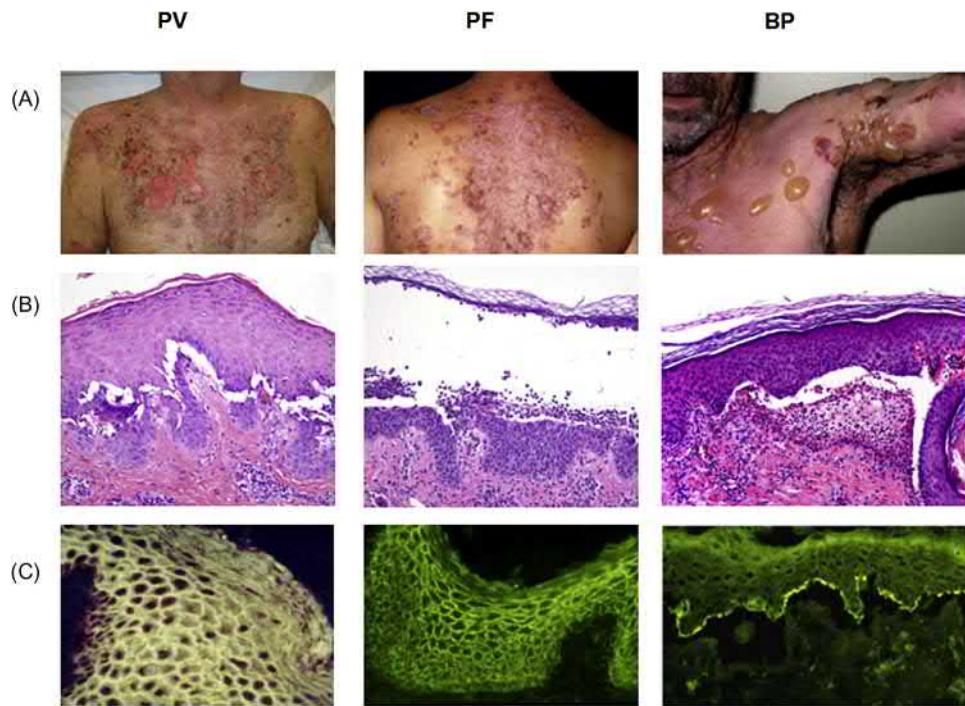
## PEMPHIGUS VULGARIS

### Clinical, Pathologic, and Epidemiologic Features

PV is the most severe and common form of pemphigus (Lever, 1965). The disease usually begins with painful erosions of the oral mucosa (mucosal PV), which may last for several months. Oral lesions are typically followed by involvement of the skin as well (mucocutaneous PV) where the disease produces flaccid blisters and erosions. Other squamous epithelial tissues (e.g., nasal, esophageal, larynx, pharynx, conjunctival, vaginal, and rectal) may also be involved.

Histologically, the lesions of PV show intraepidermal separation just above the basal cell layer of the epidermis with acantholysis, or rounding up of individual cells (Fig. 60.2, left panel). The basal cells remain attached to the dermis but laterally detached from each other producing the histological sign known as “row of tombstones” (Civatte, 1943). Inflammatory infiltration is usually mild and consists of a variable superficial and perivasular mixed infiltrate as well as lymphocytes, eosinophils, and occasionally neutrophils in the epidermis and blister cavity.

The incidence of PV ranges but is on average 1–7 new cases per million persons per year, with a mean age of onset of 50–60 years of age (Chams-Davatchi et al., 2005; Langan et al., 2008; Alpsoy et al., 2015; Shah et al., 2015). Although found in all ethnic and racial groups, the disease is more prevalent in patients harboring certain



**FIGURE 60.2** Clinical, histologic, and immunofluorescent features of pemphigus vulgaris, pemphigus foliaceus, and bullous pemphigoid human disease. The clinical (A), histological (B), and immunofluorescent (C) features of PV (left panel), PF (middle panel), and BP (right panel) are shown. *BP*, Bullous pemphigoid; *PF*, pemphigus foliaceus; *PV*, pemphigus vulgaris.

human leukocyte antigen (HLA) class II alleles (see the “Genetic features” subsection of “Pemphigus vulgaris” section).

## Autoimmune Features

### Autoantibodies

The serum of PV patients contains IgG autoantibodies that stain the epidermal intercellular spaces (ICS) by indirect immunofluorescence (IF) producing titers that roughly correlate with disease activity (Beutner and Jordon, 1964). Direct IF of perilesional skin reveals IgG bound to keratinocyte cell surfaces. These autoantibodies are predominantly of the IgG4 subclass (Jones et al., 1988; Futei et al., 2001). Interestingly, nonpathogenic autoantibodies have been detected in the first-degree relatives of PV patients, which are mainly IgG1 subclass (Bhol et al., 1995; Kricheli et al., 2000; Torzecka et al., 2007).

The target antigen recognized by PV autoantibodies is Dsg3, a 130-kDa desmosomal core glycoprotein (Amagai et al., 1991). While patients with limited mucosal lesions have autoantibodies to Dsg3 exclusively, patients who develop skin lesions possess autoantibodies against Dsg1 as well (Ding et al., 1997). It is well established that about 60% of the PV patients have autoantibodies against Dsg3 and Dsg1.

Dsg1 and Dsg3 belong to the cadherin family of calcium-dependent cell-adhesion molecules and share high-sequence homology (Getsios et al., 2004). The ectodomain of these glycoproteins is composed of four cadherin repeats (EC1-4) and a variable extracellular anchor (EC5). The six putative calcium-binding motifs of the ectodomain are involved in maintaining the conformation and adhesive function of Dsg3. A putative adhesion site (amino acid sequence RAL) is located on the EC1 domain. The ectodomain of Dsg3 has been expressed in the baculovirus system and can be used to adsorb pathogenic autoantibodies from PV serum. These Dsg3 affinity-purified autoantibodies are sufficient to induce suprabasilar acantholysis when passively transferred to neonatal mice (Amagai et al., 1992; Ding et al., 1999). These pathogenic autoantibodies recognize conformational and calcium-dependent epitopes located on the EC1 and EC2 domains of Dsg3.

([Sekiguchi et al., 2001](#)), which are believed to be involved in the *trans* and *cis* adhesive functions of the molecule ([Di Zenzo et al., 2012](#)).

The autoantibody response in PV is thought to be oligoclonal in nature. Indeed, studies utilizing antibody phage display indicate that a limited set of anti-Dsg3 B-cell clones persist in patients over time and through relapsing disease ([Hammers et al., 2015](#)).

### **T-Cell Activation**

Induction of anti-Dsg3 autoantibodies is T-cell dependent as supported by several lines of evidence. In vitro anti-Dsg3 antibody production by autoreactive B cells is abolished upon depletion of CD4<sup>+</sup> T cells ([Nishifuji et al., 2000](#)). In addition, T-cell clones derived from PV patients proliferate when stimulated with various Dsg3 peptides ([Lin et al., 1997; Veldman et al., 2004; Wucherpfennig et al., 1995](#)). The HLA restrictions and cytokine profiles of the T-cell clones have been characterized. CD4<sup>+</sup> T cells from PV patients responsive to three polypeptides (residues 145–192, 240–303, and 570–614) were restricted to HLA-DR and exhibit a Th2-like cytokine profile ([Lin et al., 1997](#)). These studies suggest that Th2 cells are relevant in the induction of Dsg3-specific autoantibodies. Not only autoreactive Th2 cells, but also Th1 cells are found in PV patients ([Veldman et al., 2004](#)). Both Th1 and Th2 cells may be involved in the production of PV autoantibodies, since the ratio of Dsg3-specific Th1/Th2 cells in PV patients correlated well with the serum autoantibody titers of the patients. In addition, recent studies have shown that an imbalance of Dsg3-specific type 1 regulatory T cells and Th2 cells may be critical for the loss of tolerance against Dsg3 in PV ([Veldman et al., 2009](#)).

Interestingly, Dsg3-specific T cells are not only detected in PV patients, but also detected in healthy individuals who carry PV susceptible HLA alleles ([Veldman et al., 2004](#)). However, in contrast to PV patients, Dsg3-responsive T-cell clones from the healthy donors exhibit exclusively Th1 cytokine profiles.

Recent studies have shown that Dsg3-specific CD4<sup>+</sup> T cells can induce PV in mice, further underscoring the importance of T cells in initiation of disease ([Takahashi et al., 2011](#)).

### **Genetic Features**

HLA alleles may play an important role in the development and progression of PV ([Delgado et al., 1997; Sinha et al., 1988; Sinha, 2011](#)). Two haplotypes, HLA-DR4 and HLA-DR6, are strongly associated with PV in different ethnic groups ([Todd et al., 1988](#)). In the non-Jewish population, DRB1\*0402 and DQB1\*0503 are the two candidate alleles most likely associated with disease susceptibility, whereas in the Ashkenazi Jewish population DRB1\*0402 seems to be singularly associated with PV ([Lee et al., 2006; Sinha, 2011](#)).

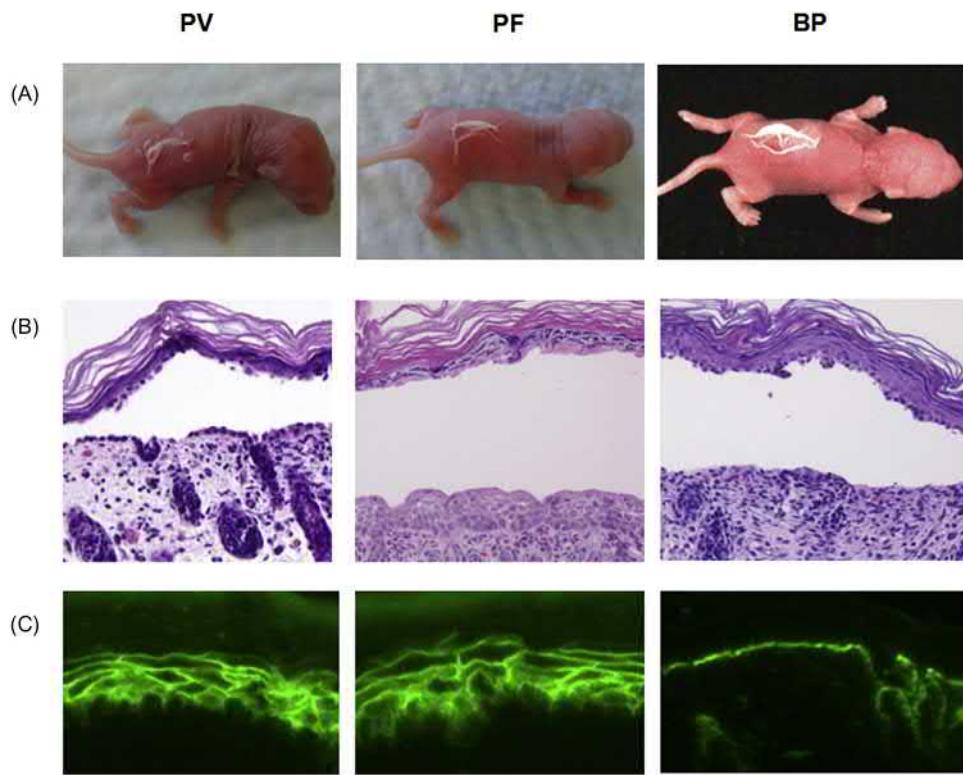
### **In Vivo and In Vitro Models**

#### ***In Vitro Models***

Normal human keratinocytes or keratinocyte cell lines cultured to a confluent monolayer have been used in pathogenicity assays for many years. Sera, purified IgG, or monoclonal anti-Dsg3 antibodies are added to the keratinocyte monolayer and allowed to incubate for a period of time. Dispase treatment releases the monolayer from the culture dish, and the keratinocyte sheets are subjected to mechanical stress. Pathogenic autoantibodies induce the monolayer to dissociate leading to fragmented cell sheets ([Ishii et al., 2005](#)). Human skin explants can also be used in vitro for testing. These assays are frequently used for the testing pathogenicity of monoclonal antibodies, which are often available in limited quantities ([Payne et al., 2005; Yamagami et al., 2010](#)).

#### ***Autoantibody Passive Transfer Model***

As described in the previous sections, neonatal mice have been used for passive transfer experiments of PV IgG ([Anhalt et al., 1982](#)). The small size of the animals and the lack of hair allow the use of smaller amounts of IgG and the lesions are easily visible on the hairless skin. PV IgG transferred to neonatal mice reproduces the clinical, histological, and immunological features of human disease within the first 24 hours postinjection ([Fig. 60.3, left panel](#)). Similarly, IgG from patients with mucosal PV can be passively transferred to the buccal mucosa of adult mice. Interestingly, mucosal PV IgG only induce blister formation in human Dsg3 transgenic mice (not WT mice), suggesting that the pathogenic epitopes targeted in mucosal PV are unique to human Dsg3 ([Culton et al., 2015](#)). The disease induced in these passive transfer animal models is dose dependent and correlates with the titers of PV autoantibodies detected in the sera of the injected mice.



**FIGURE 60.3** Clinical, histologic, and immunofluorescent features of the murine models of pemphigus vulgaris, pemphigus foliaceus, and bullous pemphigoid. The clinical (A), histological (B), and immunofluorescent (C) features of PV (left panel), PF (middle panel), and BP (right panel) murine models are shown. The animals passively transferred with human IgG (and IgG4) develop skin lesions (A), which histologically (B) are similar to the human disease. The human autoantibodies are detected bound to lesional skin (C) and circulating in the mouse serum. *BP*, Bullous pemphigoid; *PF*, pemphigus foliaceus; *PV*, pemphigus vulgaris.

### Murine Models of Pemphigus Vulgaris

The efforts to induce PV in adult mice by conventional active immunization with human Dsg3 have been largely unsuccessful. While the immunized animals produce anti-Dsg3 antibodies that were able to induce skin blisters when passively transferred into neonatal mice, the animals themselves do not develop disease (Fan et al., 1999). Amagai et al. (2000) developed an active mouse model of PV using a novel strategy to overcome the barrier of self-tolerance. Dsg3 knockout mice (Koch et al., 1997) were immunized with murine Dsg3, and splenocytes were subsequently harvested and adoptively transferred from these immunized animals to immunodeficient Rag-2<sup>-/-</sup> mice which express Dsg3 in their epidermis. The recipient mice produce anti-Dsg3 antibodies and show mucosal suprabasilar acantholysis histologically. Rag-2<sup>-/-</sup> mice that receive naïve Dsg3 knockout splenocytes also produce anti-Dsg3 IgG and develop the PV phenotype (Aoki-Ota et al., 2004). This active disease model has allowed for the characterization of anti-Dsg3 antibodies and Dsg3 reactive CD4<sup>+</sup> T-cell clones (Tsunoda et al., 2003; Takahashi et al., 2008).

Immunization of humanized HLA DRB1\*0402 transgenic mice with human Dsg3 induces human Dsg3-specific autoantibodies. Sera from these mice induce loss of adhesion in human keratinocyte dissociation assays, suggesting autoantibody pathogenicity. Although these antihuman Dsg3 antibodies are detected in the sera of immunized mice, they show very little reactivity with murine Dsg3 and, thus, do not induce the PV phenotype in these animals. This model has provided evidence that the HLA-DRB1\*0402 restricted T-cell recognition of human Dsg3 epitopes leads to activation of anti-Dsg3 B cells and subsequent anti-Dsg3 antibody production but also highlights the differences between human and murine Dsg3 in terms of both B and T-cell dominant epitopes (Eming et al., 2014).

## Pathologic Effector Mechanisms

### ***Pathogenic Role of Autoantibodies***

Several studies demonstrate the correlation of PV autoantibody titers and disease extent and activity. In vitro studies also show that PV IgG can induce acantholysis in skin organ cultures and cell detachment in primary keratinocyte cell cultures. The in vivo passive transfer studies demonstrate that PV IgG is able to faithfully reproduce the disease in neonatal mice and attribute a pathogenic role for PV autoantibodies (Fig. 60.3, left panel) (Anhalt et al., 1982). These pioneer studies were extended to demonstrate that PV autoantibodies are able to induce disease by passive transfer in a process that is independent of complement or plasminogen activator (Anhalt et al., 1986; Mahoney et al., 1999; Mascaro et al., 1997).

While it is well established that PV IgG cause disease, the current research is addressing the molecular mechanisms of how PV autoantibodies trigger acantholysis and cell detachment. A long-standing theory for autoantibody-mediated pathogenesis is the steric hindrance theory, in which binding of the autoantibodies to Dsg3 directly impairs the adhesive ability of the molecules, thereby causing cell separation (Diaz and Marcelo, 1978). PV autoantibodies bind Dsg3 on the keratinocyte cell surfaces forming clusters of that are internalized and fused with lysosomes (Calkins et al., 2006; Mao et al., 2009) and subsequent Dsg3 degradation. Thus, it seems that PV IgG may alter the assembly/disassembly of desmosomes by impairing the dynamics of the soluble and insoluble pools of Dsg3, which, in turn, may lead to acantholysis (Jennings et al., 2011).

Substantial evidence is accumulating in support of a complimentary hypothesis in which binding of PV autoantibodies to Dsg3 leads to that activation of intracellular signaling pathways that may augment acantholysis. Several signaling pathways have been shown to be activated upon autoantibody binding to Dsg3 including p38MAPK/HSP27 (Jolly et al., 2010; Berkowitz et al., 2008a; Berkowitz et al., 2008b), epidermal growth factor receptor (EGFR), Rho family GTPase (Waschke et al., 2006), c-myc (Williamson et al., 2006), and caspases (Li et al., 2009; Luyet et al., 2015). While modulation of certain signaling pathways can prevent autoantibody-induced acantholysis, the induction of individual signaling pathways is not sufficient to cause disease.

## Autoantibodies as Potential Immunologic Markers

As PV autoantibodies are the direct effectors of keratinocyte dissociation and blister formation, anti-Dsg3 and anti-Dsg1 autoantibodies are not merely immunologic markers of PV but are critical for the diagnosis.

---

## PEMPHIGUS FOLIACEUS

---

### Clinical, Pathologic, and Epidemiologic features

Unlike PV, PF affects skin only and the disease is manifested by superficial blisters and erosions, which may lead to crusting and formation of keratotic plaques (Fig. 60.2, middle panel). The skin lesions initially involve the central areas of the face, scalp, the mid chest, and the upper back. From these areas, the disease may spread to involve the entire body, occasionally producing an exfoliative erythroderma. Histological examination of these lesions reveals subcorneal vesicles and acantholysis, predominantly in the upper layers of the stratum spinosum (Lever, 1965).

The classic form of PF is seen sporadically in different parts of the world with an incidence of less than one case per million persons per year. A second form of PF is endemic and originally described in certain states of Brazil with the name of *fogo selvagem* (FS) and a prevalence of 1%–3% (Diaz et al., 1989). Endemic forms of PF have also been reported in Colombia and Tunisia. Epidemiological studies suggest environmental triggers for FS development (Aoki et al., 2004).

## Autoimmune Features

### ***Autoantibodies***

Similar to PV, PF patients are also characterized by antiepidermal ICS autoantibodies, predominantly of the IgG4 subclass (Rock et al., 1989). These autoantibodies are detected bound to diseased epidermis and circulating in the serum of the patients, with titers that roughly correlate with disease extent and activity.

Dsg1, expressed mostly in the superficial layers of the epidermis, is the target antigen of PF autoantibodies. Affinity-purified anti-Dsg1 antibodies from PF serum are able to induce disease in neonatal mice (Amagai et al., 1995). The majority of PF autoantibodies recognize conformational and calcium-dependent epitopes residing in the NH<sub>2</sub>-terminal amino acid 1-161 of Dsg1 (Sekiguchi et al., 2001). In the preclinical stage of FS, during disease-remission, and in some normal individuals, the anti-Dsg1 autoantibodies recognize the EC5 domain only. On the contrary, FS anti-Dsg1 autoantibodies from patients with active disease or during disease relapses recognize the EC1/EC2 domains (Li et al., 2003). Although the majority of PF/FS autoantibodies are of IgG4 subclass, it appears that epitope specificity, rather than the subclass of IgG, is the driver of the pathogenicity of the autoantibodies (Li et al., 2002). Considering these findings, we hypothesize that an environmental agent(s) cross-reacts with the EC5 domain of Dsg1 and triggers an initial nonpathogenic anti-EC5 autoimmune response (Diaz et al., 2004). In genetically predisposed individuals, the autoimmune response may undergo intramolecular epitope spreading toward pathogenic epitopes on the EC1/EC2 domains of Dsg1, which leads to disease onset (see the “Environmental factors involved in fogo selvagem” section).

### T-Cell Activation

It has been demonstrated that CD4<sup>+</sup> T-cell lines and clones derived from peripheral blood of FS patients show a proliferative response when incubated with the Dsg1 ectodomain (Lin et al., 2000a). The stimulation of these CD4<sup>+</sup> T cells is HLA-DR restricted. Moreover, these cells secrete Th2-like cytokines.

### Genetic Features

HLA alleles strongly associated with FS are DRB1\*0404, \*1406, \*1402 (relative risk = 14), and \*0102 (relative risk = 7.3) (Moraes et al., 1997). A common epitope of LLEQRRAA, the residues 67–74 of the third hypervariable region of the DRB1 molecule, is shared by these susceptibility alleles. Similarly, a strong association of DRB1\*0102 and DRB1\*0404 is also found in nonendemic PF patients in France (Loiseau et al., 2000). Interestingly, two susceptible alleles in PV, the DRB1\*1401 and DQB1\*0503, have been also reported with high frequencies in Italian and Japanese PF patients (Lombardi et al., 1999; Miyagawa et al., 1999).

### In Vivo and In Vitro Models

Keratinocyte cell culture and human skin explants have been utilized for in vitro testing PF autoantibody pathogenicity as described for PV. The classic animal model of PF was developed by passively transferring IgG from patients into neonatal mice (Roscoe et al., 1985). The animals develop skin blisters, which show the typical histological features of the human disease, that is, subcorneal vesicles (Fig. 60.3, middle panel). The extent of the disease correlates well with the indirect IF titers of human autoantibodies detected in the mouse. These animals develop classic subcorneal acantholysis (Futamura et al., 1989). The disease is complement-independent and can be induced by monovalent PF IgG fragments as well (Espana et al., 1997). Despite the availability of recombinant human and murine Dsg1, the studies on inducing disease by active immunization have been unsuccessful.

### Pathologic Effector Mechanisms

The IgG4 autoantibodies are pathogenic as demonstrated by passive transfer studies (Fig. 60.3, middle panel) (Rock et al., 1989). Activation of the complement cascade and plasminogen activator is not required for the induction of acantholysis by these autoantibodies in the mouse model (Espana et al., 1997; Mahoney et al., 1999).

### Autoantibodies as Potential Immunologic Markers

The importance of anti-Dsg1 autoantibodies as markers of disease has been illustrated in the FS population, where IgG4 anti-Dsg1 autoantibodies serve as a novel classifier/predictor that identifies FS patients with high sensitivity and specificity (92% and 97%, respectively). In an FS prone population with an incidence of 3%, detection of IgG4 anti-Dsg1 autoantibodies has a positive predictive value of 49% and a negative predictive value of 99.7% (Qaqish et al., 2009).

## Environmental Factors Involved in Fogo Selvagem

A remarkable characteristic of FS is its epidemiology. Several independent lines of evidence indicate that FS is precipitated by exposure to an environmental factor(s) (Aoki et al., 2004). Anti-Dsg1 autoantibodies are detected in 55% of the normal individuals living in an area exhibiting a high prevalence (3.4%) of FS (Warren et al., 2000). Remarkably, anti-Dsg1 autoantibodies are also detected in FS patients from one to several years before the clinical onset of disease (Qaqish et al., 2009). These preclinical antibodies are mixture of IgG1 and IgG4 subclasses and recognize the EC5 domain of Dsg1 (Li et al., 2003; Warren et al., 2003).

Early case-control epidemiological studies point to a hematophagous insects as a prime etiological agent of FS (Aoki et al., 2004). For example, anti-Dsg1 EC5 antibodies have been detected in the sera of patients with onchocerciasis, leishmaniasis, and Chagas disease, though the patients exhibit no skin lesions of FS (Diaz et al., 2004). Recent studies have revealed that FS patients harbor anti-Dsg1 IgG4 and IgE antibodies that cross-react with LJM11, a sand fly salivary gland antigen (Qian et al., 2012, 2015, 2016b). It is hypothesized that LJM11 induces an anti-Dsg1 autoantibody response through cross-reactivity/molecular mimicry. Genetic analysis confirms that these cross-reactive antibodies evolve from the same naïve B-cell clones. The cross-reactive antibodies may be pathogenic or may evolve via epitope spreading to a pathogenic autoantibody response in genetically susceptible individuals leading to FS disease onset (Diaz et al., 2008; Qian et al., 2011, 2016a).

## OTHER TYPES OF PEMPHIGUS

### Paraneoplastic Pemphigus

PNP is a rare, severe mucocutaneous disease that runs a usually lethal course in patients with underlying lymphoproliferative malignancy, that is, non-Hodgkin's lymphoma, chronic lymphocytic leukemia, and Castleman's diseases (Anhalt, 2004; Anhalt et al., 1990). Patients exhibit severe stomatitis that is often hemorrhagic and skin lesions that may be vesiculobullous in some patients or show erythema multiforme or lichen planus features in other patients. Approximately 30% of the PNP patients will also develop bronchiolitis obliterans with severe respiratory insufficiency.

Histological examination of the skin lesions reveals keratinocyte necrosis, lichenoid interface dermatitis, basal cell vacuolization, and suprabasilar acantholysis. Direct IF shows deposition of IgG and C3 in the epidermal ICS (as in pemphigus) but also along the basement membrane zone (BMZ). The unique and characteristic immunological finding in PNP patients is the polyclonal autoantibody response against structural antigens of the desmosome and hemidesmosome. The antigens that have been characterized besides Dsg3 and Dsg1 are members of the plakin family of proteins that includes desmoplakin I and II, BP230, envoplakin, periplakin, and plectin. Removal of anti-Dsg3 autoantibodies from PNP sera abrogates the pathogenicity of the IgG fraction (Amagai et al., 1998). Affinity-purified anti-Dsg3 antibodies and monoclonal anti-Dsg3 antibodies derived from PNP patients (Saleh et al., 2012) are able to induce skin lesions in neonatal mice further confirming anti-Dsg3 pathogenicity. The relevance of the antiplakin antibodies is not known. However, autoantibody-mediated disease explains only part of the complex epithelial injury found in these patients. Other mechanisms of tissue-injury are those mediated by cytotoxic T cells.

It has been proposed that the autoantibody response in PNP is primarily directed against tumor antigens that cross-react with epithelial structural proteins. In addition the tumor may produce cytokines that modulate the autoimmune response. Interestingly, Wang et al. (2004) have demonstrated recently that tumor B cells are able to produce antiepidermal antibodies. In those patients that receive rituximab, the B cell-mediated aspect of the disease and therefore autoantibodies wane. In these cases, the T cell-mediated aspect predominates and the disease takes on a distinct lichenoid pattern with absent or low PNP autoantibodies (Cummins et al., 2007).

The diagnosis of PNP is made clinically, histologically, and immunologically. By indirect IF, the serum of PNP patients typically stains plakin-rich rat bladder epithelium, which produce negative results when stained with the serum of PV or PF. Immunoprecipitation using radiolabeled keratinocyte extracts reveal reactivity with the plakin proteins, but this technique is rarely used in the clinical setting. Multivariant enzyme linked immunosorbent assay (ELISA) and biochip assays are now available to detect antiplakin autoantibodies (van Beek et al., 2017a; Probst et al., 2009).

## Drug-Induced Pemphigus

Certain drugs, particularly thiol-containing drugs, such as penicillamine and captopril, may induce clinical and histological features of PF and less commonly PV (Feng et al., 2011; Yoshimura et al., 2014). The majority of drug-induced pemphigus patients exhibit circulating autoantibodies to the epidermal ICS and epidermal-bound IgG. In cases of drug-induced PF the autoantibodies recognize Dsg1. Some of these drugs have been shown to cause acantholysis in vitro directly. Drug-induced pemphigus may be transient and usually resolves upon withdrawal of the medication, although in rare cases the disease may run a chronic course. The mechanisms involved in the induction of the autoimmune response by drugs in pemphigus remain obscure (Brenner and Goldberg, 2011).

## IgA Pemphigus

This variant of pemphigus is unique because of its clinical and histological phenotype. Clinically, the patients show superficial clusters of small vesicles and pustules, in some cases producing annular patterns. The Nikolsky sign is positive and the great majority of patients show no mucosal lesions. The histological features show, in addition to the acantholysis, an intense neutrophilic infiltrate in the epidermis. This infiltrate may be subcorneal [subcorneal pustular dermatosis (SPD)-type] or located in the mid-epidermis [intraepidermal neutrophilic (IEN)-type]. The immunological hallmark of this form of pemphigus is the presence of IgA class autoantibodies directed to the epidermal ICS. The antigen recognized in the SPD-type has been shown to be another desmosomal cadherin, Dsc1, whereas for the IEN-type, the target antigen appears to be a nondesmosomal cell-surface protein (Hashimoto, 2001). The pathogenic role of these IgA autoantibodies has not been demonstrated. Further, the intense neutrophilic infiltrate of the epidermis and a rapid response of the disease to dapsone (a neutrophilic targeted drug) might indicate a unique IgA-mediated pathway of tissue damage (Tsuruta et al., 2011).

# BULLOUS PEMPHIGOID

## Clinical, Pathologic, and Epidemiologic features

BP is the most common autoimmune bullous disease affecting the skin (Lever, 1965). The incidence of disease is 7–14 cases per million per year in Germany and Scotland (Gudi et al., 2005; Zillikens et al., 1995). Recent studies suggest that the incidence is increasing and may be as high as 22–24 per million per year in the United States and France and 43 cases per million per year in the United Kingdom (Langan et al., 2008; Joly et al., 2012; Brick et al., 2014). BP occurs most frequently in elderly (60 years of age and older) and affects men and women equally. The skin lesions usually begin as urticarial plaques or erythematous papules, which evolve into large, tense bullae filled with clear fluid (Fig. 60.2, right panel). The lesions are frequently associated with significant itch. Histological examination shows subepidermal blister formation with detachment of the epidermis from the dermis. The upper dermis exhibits an inflammatory infiltrate including eosinophils, neutrophils, lymphocytes, and monocytes/macrophages, and these cells may also be present in the blister cavity. The predominant inflammatory cells in early lesions are usually eosinophils and eosinophilic spongiosis may be present. Histological evidence of mast-cell degranulation has also been reported.

## Autoimmune Features

### Autoantibodies

BP is characterized by circulating and tissue-bound IgG autoantibodies directed against the BMZ. While these autoantibodies were originally detected by IF techniques (Jordon et al., 1967), molecular studies have demonstrated that the autoantibodies are directed against two hemidesmosomal proteins (Mutusim et al., 1985) known as BP180 (BPAG2, type XVII collagen) and BP230 (BPAG1) (Jordon et al., 1967; Labib et al., 1986; Stanley et al., 1981).

BP180 antigen is a hemidesmosomal transmembrane protein belonging to collagen family (Giudice et al., 1992). The BP180 protein shows a type II orientation, with its amino-terminal region toward the intracellular hemidesmosomal plaque and its carboxy-terminal half projecting into the extracellular space of the BMZ. The anti-BP180 autoantibodies from BP patients recognize multiple epitopes that cluster within the noncollagen (NC)

16A domain of the BP180 ectodomain (Giudice et al., 1993). The serum levels of autoantibodies to BP180 NC16A in patients directly correlate to disease severity (Haase et al., 1998; Tsuji-Abe et al., 2005). The BP230 antigen is an intracellular hemidesmosomal plaque protein belonging to plakin family (Tanaka et al., 1991). Intra- and intermolecular epitope spreading has been reported in BP and may shape the course of individual disease (Di Zenzo et al., 2011). Only antibodies to BP180 have been demonstrated to be pathogenic in neonatal mice (Fig. 60.3, right panel) (Liu et al., 1993).

The autoantibodies in BP sera are predominantly of IgG1 and IgG4 subclasses (Laffitte et al., 2001). Recent evidence suggests that BP autoantibodies of the IgG1 subclass are pathogenic, while the IgG4 subclass may be inhibitory (Zuo et al., 2016). Epitope specificity is also thought to play a role in autoantibody pathogenicity (Wada et al., 2016). In addition to IgG autoantibodies IgE autoantibodies to BP180 are found in the majority of untreated BP patients (Dimson et al., 2003). Anti-NC16A autoantibodies of both the IgG and IgE class correlate with disease activity as measured by the BP disease activity index (van Beek et al., 2017b).

### **T-Cell Activation**

BP180-specific autoreactive T cells recognize epitopes located predominantly on the NC16A domain of the molecule (Budinger et al., 1998; Lin et al., 2000b). These T lymphocytes express CD4 memory T-cell surface markers and exhibit a Th1/Th2 mixed cytokine profile. Regulatory T cells may also play a role in suppressing inflammation in BP (Bieber et al., 2017; Gambichler et al., 2017).

### **Genetic Features**

Early HLA studies involving American, Japanese, and British BP patients showed no significant association between the disease and HLA-A, B, C, and DR loci, while a marked increase in the HLA-DR5 allele was found in BP patients from France. Recent studies have demonstrated that HLA-DQB1\*0301 is associated with several ethnic groups including Caucasian, Chinese, and Iranian (Delgado et al., 1996; Gao et al., 2002; Esmaili et al., 2013), whereas DRB1\*0403, 0406, or DRB1\*1101 have high frequency in Japanese BP (Okazaki et al., 2000).

### **In Vivo and In Vitro Models**

Early studies on passive transfer of human BP IgG containing anti-BP180 and anti-BP230 autoantibodies to neonatal mice were unsuccessful. It was later found that human anti-BP180 antibodies did not react with the murine BP180 protein due to differences at the amino acid level of the NC16A region of the molecule. This problem was overcome by raising rabbit antibodies against a segment of murine BP180 homologous to the human epitope. It was demonstrated that this anti-BP180 antibodies were pathogenic if passively transferred into neonatal mice. The animals recapitulate the key clinical and histological features of the human disease, (Fig. 60.3, right panel) (Liu et al., 1993).

In contrast to PV and PF mouse models, the subepidermal blistering in mice induced by anti-BP 180 antibodies depends on complement activation and a subsequent cascade of inflammatory events including mast-cell degranulation and neutrophil infiltration. Proteolytic enzymes released from recruited neutrophils are the final effector molecules that cause the epidermal–dermal separation seen in skin lesions (Liu, 2004).

To directly test the pathogenicity of anti-BP180 IgG autoantibodies from BP patients, humanized BP180 mice were generated which harbor the human BP180 or NC16A domain in place of the murine protein (Liu et al., 2008; Nishie et al., 2007). Injection of BP patient anti-BP180 IgG into these humanized mice leads to subepidermal blister formation in process dependent on complement, mast cells, and neutrophils (Liu et al., 2008; Nishie et al., 2007).

IgE purified from BP patient sera was also shown to be pathogenic upon passive transfer into athymic nude or SCID mice with human skin grafts. The human skin grafts developed urticarial plaques and subepidermal splitting at higher doses (Fairley et al., 2007; Zone et al., 2007).

### **Pathologic Effector Mechanisms**

As opposed to the pemphigus group where the autoantibodies directly induce blister formation, BP is the result of autoantibody deposition leading to a complex inflammatory cascade. In addition to autoantibody deposition at the BMZ, inflammatory cells are present in the upper dermis and bullous cavity, including both intact

and degranulating eosinophils, neutrophils, and mast cells (Borrego et al., 1996; Czech et al., 1993; Dvorak et al., 1982; Wintrob et al., 1978). Proteinases including plasmin, matrix metalloproteinase (MMP)-9, collagenase, and elastase are present in blister fluid (Ujie et al., 2011). Animal models of BP demonstrate that anti-BP180 IgG-induced subepidermal blistering requires mast cells and neutrophils. Plasmin, MMP-9, and neutrophil elastase work in concert to degrade BP180 and other extracellular matrix proteins, causing dermal–epidermal separation (Chen et al., 2001; Liu et al., 1997, 1998, 2000a, 2000b, 2005). The deposition of complement components at the BMZ of the lesional/perilesional skin of BP is common, but whether the complement activation is actively involved in BP or a bystander remains controversial.

## Autoantibodies as Potential Immunologic Markers

The detection of anti-BP180 autoantibodies by direct immunofluorescence (DIF), indirect IF, and/or ELISA is critical for the diagnosis of BP. IgG antibodies against BP180 NC16A correlate with disease activity (Schmidt et al., 2000; van Beek et al., 2017b; Amo et al., 2001). Interestingly, a study of 337 patients with various non-BP dermatologic disorders suggests that 4% of the individuals without BP show a low positive level of anti-BP180 by ELISA (Wieland et al., 2010).

## OTHER SUBEPIDERMAL BULLOUS DISEASES

### Herpes Gestationis (Pemphigoid Gestationis)

Pemphigoid gestationis (PG), formerly known as herpes gestationis (HG), is a variant of BP affecting pregnant women or women immediately postpartum. The incidence is about 1 in 50,000 pregnancies (Ambros-Rudolph et al., 2006). The disease usually develops during the second or third trimester of pregnancy or in the immediate postpartum period. PG has also been associated with underlying trophoblastic tumors, hydatidiform mole, and choriocarcinoma (Jenkins et al., 1999). Similar to BP, PG patients develop subepidermal blisters and linear deposition of IgG and C3 at the BMZ (Provost and Tomasi, 1973). The sera of these patients is usually negative by conventional indirect IF assays but become positive when fresh complement fixation to the skin is assayed (HG factor) (Provost and Tomasi, 1973). The HG factor was found to be a complement fixing autoantibody against the BP180 antigen (Giudice et al., 1993; Jordon et al., 1976; Katz et al., 1976; Morrison et al., 1988). While indirect IF is frequently negative in PG, the BP180 NC16A ELISA shows a high sensitivity and specificity for the diagnosis of PG (97% and 100%, respectively) and may be a useful test to distinguish between PG and other pregnancy-related dermatoses (Powell et al., 2005; Al Saif et al., 2017). Autoantibodies are predominantly IgG4 and recognize the same epitope (within the BP180 NC16A domain) as BP autoantibodies (Patton et al., 2006). BP180-specific T cells also recognize the BP180 NC16A and express a CD4<sup>+</sup> Th1 memory phenotype (Lin et al., 1999). PG is associated with maternal HLA-DR3 and/or HLA-DR4 (Shornick et al., 1981). The mechanism of autoimmunity in PG and the role of pregnancy in the pathogenesis of this unique form of pemphigoid are not fully understood (Sadik et al., 2016).

### Cicatricial Pemphigoid

CP, also known as mucous membrane pemphigoid, is a group of heterogeneous diseases characterized by subepithelial blistering involving mucous membranes predominantly (Korman and Cooper, 2000; Chan et al., 2002). The oral and ocular surfaces are most commonly involved, while skin lesions occur only in one-third of CP patients. A striking clinical feature of CP is that healing of the lesions leads to scarring and dysfunction of the affected organs. For example, ocular involvement may cause blindness due to corneal scarring and fibrosis. Direct IF reveals linear deposition of IgG, IgA, or C3 along the epithelial BMZ (Egan et al., 2003). CP is also associated with DQB1\*0301 (Delgado et al., 1996; Chan et al., 1997).

There are several antigenic targets in CP including BP180 NC16A, C-terminal domain of BP180, laminin 5 (also known as laminin 332 or epiligrin), integrin subunits  $\alpha$ 6 and  $\beta$ 4, and NC1 domain of collagen VII. Patients with primarily oral disease often show circulating IgG autoantibodies against BP180 NC16A antigen (Balding et al., 1996), but some show autoantibodies that recognize the C-terminal domain of BP 180, which is different from BP. A subset of CP patients exhibit circulating autoantibodies against laminin 5 (also known as laminin 332 or epiligrin) (Domloge-Hultsch et al., 1994; Egan et al., 1999). Experimentally, it was shown that antilaminin 5 antibodies are able to induce subepidermal blisters in neonatal mice (Lazarova et al., 1996). An association of

cancer with some of CP with antilaminin 5 autoantibodies has been reported (Egan et al., 2003). Many patients with ocular involvement have autoantibodies against  $\alpha 6\beta 4$  integrin (Tyagi et al., 1996). The pathogenic role of the various autoantibodies found in CP and their link to scarring remains to be elucidated (Kurosh and Yancey, 2011).

### Linear IgA disease

LAD is a subepidermal blistering disorder characterized by pruritic lesions and linear deposition of IgA autoantibodies at the BMZ (Nemzer et al., 2000; Venning, 2011). Mucosal membrane involvement is common and can be predominant (60%–80%). LAD can be subdivided into adult-onset, childhood-onset (also known as chronic bullous disease of childhood), and drug-induced LAD (Zone et al., 2004). The histology of lesional and perilesional skin reveals subepidermal vesicles with neutrophilic infiltration along the BMZ and in the superficial dermis in all forms of the disease. In drug-induced LAD, vancomycin is the most frequently implicated drug (Venning, 2011; Fortuna et al., 2012).

IgA autoantibodies in LAD serum react with multiple antigenic peptides derived of the BP180 ectodomain. The most common antigenic peptides for the major type of LAD (lamina lucida type), detected by immunoblotting using epidermal extracts, are the 97-kDa protein (LABD97) and the 120-kDa antigens (LAD-1), which are considered to be the proteolytic fragments of the BP180 polypeptide (Zone et al., 2004). These findings suggest that the majority of LAD autoantibodies recognize epitopes on LABD97 and LAD-1, which are different from those bound by BP autoantibodies on the intact BP180 molecule.

### Epidermolysis Bullosa Acquisita

EBA is an acquired subepithelial blistering disease of the skin and mucous membranes mediated by IgG autoantibodies against type VII collagen (O'Toole and Woodley, 2000). Lesions occur predominantly in areas of trauma and often heal with scarring, like CP. There are two clinical forms of the disease: inflammatory and non-inflammatory (or mechanobullous). Subepidermal blisters are formed as a result of detachment of the epidermis at the level of the sublamina densa area as demonstrated by ultrastructural studies (Fig. 60.1). EBA is associated with HLA-DR2 (Gammon et al., 1988).

The target antigen of EBA is type VII collagen (C-VII), which is confined to anchoring fibers of the sublamina densa region of the skin. EBA autoantibodies react with four major epitopes within the amino-terminal noncollagenous NC1 domain of C-VII. It is hypothesized that binding of these autoantibodies may interfere with dimer formation of the C-VII molecule or may impair the association of C-VII with its ligands, laminin 5, and fibronectin. It has also been suggested that complement activation by the autoantibodies induces inflammation and blistering in a process that is Fc-dependent (Sitaru et al., 2005). Rabbit antihuman type VII collagen NC1 domain IgG induced EBA-like lesions in adult hairless mice and nude mice bearing human skin grafts (Chen et al., 2004). Furthermore, rabbit antibodies specific to a murine portion of type VII collagen induced subepidermal blistering when passively transferred into adult mice, thereby reproducing the clinical, histological, and immunopathological features of human disease in a complement-dependent fashion (Sitaru et al., 2005). Neutrophils are the key effector cells in the pathogenesis of EBA, particularly in the inflammatory form of the disease (Chiriac et al., 2007).

### Dermatitis Herpetiformis

DH is an IgA-mediated skin blistering disease characterized by intensely pruritic erythematous papules and vesicles symmetrically distributed over extensor surfaces (Bagheri and Hall, 2000). The skin rash is gluten-dependent and responsive to gluten-free diet. Skin biopsies from DH lesions show a characteristic neutrophilic infiltration in the upper dermis involving the dermal papillae. Perilesional and normal skin in DH show a granular deposition of IgA along the BMZ with accentuation in the dermal papillae. Recently it has been shown that DH patients possess in their sera IgA autoantibodies that recognize epidermal transglutaminase (Sardy et al., 2002; Donaldson et al., 2007). It is unknown if these antitransglutaminase autoantibodies are pathogenic or an epiphenomenon, but the autoantibodies can be used as a sensitive serologic marker for DH. A remarkable feature of DH is its high association with celiac disease, another IgA-mediated gluten-sensitive disorder involving small intestine. Patients with DH and gluten sensitive enteropathy (GSE) share an increased expression of the HLA-A1, HLA-B8, HLA-DR3, and HLA-DQA1\*0505 and DQB1\*02 genes (Sollid, 2000) and a humoral response to transglutaminases. Both diseases

are exacerbated by the intake of gluten-containing food. The mechanisms of gluten-induced autoimmunity and the relationship of the IgA autoantibody response to transglutaminase in DH remain unclear (Cardones and Hall, 2011).

## TREATMENT OF AUTOIMMUNE BULLOUS DISEASES

The aim of the therapy in all autoimmune blistering diseases of the skin is to abrogate the pathogenic autoantibodies and to decrease the tissue inflammatory response triggered by these autoantibodies. The elimination of pathogenic autoantibodies from the patient is accomplished by a variety of approaches ranging from general immunosuppression to B cell–directed therapies to reduction/elimination of the autoantibodies themselves. In addition, the resultant inflammatory response in the skin may be modulated by using topical or systemic steroids or drugs that are known to impair the effector function of infiltrating inflammatory cells (e.g., dapsone-modulating neutrophils). It is understood that systemic steroids may modulate the antibody production and also benefit the local inflammatory response in the skin.

Systemic corticosteroids are the first line of therapy for patients with most clinical forms of pemphigus and pemphigoid. Doses of prednisone in the range of 1.0 mg/kg daily are used initially. If patients continue to develop new lesions, the dose of prednisone can be increased incrementally to 2.0 mg/kg; however, in our practice we rarely exceed a total prednisone dose of 80 mg daily. The use of azathioprine, cyclophosphamide, methotrexate, or mycophenolate mofetil, as adjunctive therapy, is beneficial in controlling the disease of these patients (Frew et al., 2011). The use of these drugs enhances the chances of inducing a prolonged remission of the disease. These immunomodulatory agents have shown excellent steroid sparing ability, thereby reducing severe complications that arise from long-term use of systemic corticosteroid therapy, that is, osteoporosis, diabetes, hypertension, obesity. The doses and side effects of these drugs are described in detail in dermatological textbooks.

Some patients are resistant to or develop serious side effects to these therapeutic modalities. These patients may benefit from plasmapheresis or parenteral infusions of human immunoglobulin aimed at eliminating/reducing autoantibodies. B cell–targeted therapy with anti-CD20 humanized monoclonal antibodies has been shown to induce clinical and serological remissions in severe cases of pemphigus and CP/MMP (Maley et al., 2016; Joly et al., 2007) and is effective as a first-line agent in pemphigus with high rates of disease remission (Joly et al., 2017).

Dapsone is the drug of choice in DH; it exerts its antiinflammatory effects by direct action on the neutrophil by interfering with the myeloperoxidase–hydrogen peroxide–halide-mediated cytotoxic system in neutrophils. Not only patients with DH, but also LAD and IgA pemphigus show a remarkable clinical response to dapsone. In addition, CP and certain cases of BP respond favorably to dapsone. Dapsone can cause hemolysis and methemoglobinemia; therefore it is mandatory to assay the levels of the enzyme glucose 6-phosphate dehydrogenase (G6PD) prior to beginning dapsone as G6PD deficient patients may experience severe hemolysis.

Therapy for patients with EBA is limited since most of the drugs used to control other autoimmune blistering diseases do not change the course of the disease in EBA patients, particularly in the noninflammatory mechanobullous subtype of EBA. The use of systemic steroids, immunosuppressive drugs, or dapsone may be individualized in each patient. Treatment of underlying malignancy, steroids, immunosuppressive drugs, and supportive therapy has been attempted in PNP patients. The prognosis of these patients however is poor.

## CONCLUDING REMARKS

The autoimmune blistering disorders represent classic antibody-mediated organ-specific autoimmune diseases that can serve as models for understanding the development and pathogenesis of antibody-mediated autoimmune disease in general. New treatments continue to emerge based on our current understanding of disease pathogenesis. Furthermore, unique disease responses to some of these treatments have led investigators back to the bench to further explore the immunologic aberrations present in these patients.

## References

- Al Saif, F., Jouen, F., Hebert, V., Chiavelli, H., Darwish, B., Duvert-Lehembre, S., et al., 2017. Sensitivity and specificity of BP180 NC16A enzyme-linked immunosorbent assay for the diagnosis of pemphigoid gestationis. *J. Am. Acad. Dermatol.* 76, 560–562.  
Alpsoy, E., Akman-Karakas, A., Uzun, S., 2015. Geographic variations in epidemiology of two autoimmune bullous diseases: pemphigus and bullous pemphigoid. *Arch. Dermatol. Res.* 307, 291–298.

- Amagai, M., Klaus-Kovtun, V., Stanley, J.R., 1991. Autoantibodies against a novel epithelial cadherin in pemphigus vulgaris, a disease of cell adhesion. *Cell* 67, 869–877.
- Amagai, M., Karpati, S., Prussick, R., Klaus-Kovtun, V., Stanley, J.R., 1992. Autoantibodies against the amino-terminal cadherin-like binding domain of pemphigus vulgaris antigen are pathogenic. *J. Clin. Invest.* 90, 919–926.
- Amagai, M., Hashimoto, T., Green, K.J., Shimizu, N., Nishikawa, T., 1995. Antigen-specific immunoabsorption of pathogenic autoantibodies in pemphigus foliaceus. *J. Invest. Dermatol.* 104, 895–901.
- Amagai, M., Nishikawa, T., Nousari, H.C., Anhalt, G.J., Hashimoto, T., 1998. Antibodies against desmoglein 3 (pemphigus vulgaris antigen) are present in sera from patients with paraneoplastic pemphigus and cause acantholysis in vivo in neonatal mice. *J. Clin. Invest.* 102, 775–782.
- Amagai, M., Tsunoda, K., Suzuki, H., Nishifuji, K., Koyasu, S., Nishikawa, T., 2000. Use of autoantigen-knockout mice in developing an active autoimmune disease model for pemphigus. *J. Clin. Invest.* 105, 625–631.
- Ambros-Rudolph, C.M., Mullegger, R.R., Vaughan-Jones, S.A., Kerl, H., Black, M.M., 2006. The specific dermatoses of pregnancy revisited and reclassified: results of a retrospective two-center study on 505 pregnant patients. *J. Am. Acad. Dermatol.* 54, 395–404.
- Amo, Y., Ohkawa, T., Tatsuta, M., Hamada, Y., Fujimura, T., Katsuoka, K., et al., 2001. Clinical significance of enzyme-linked immunosorbent assay for the detection of circulating anti-BP180 autoantibodies in patients with bullous pemphigoid. *J. Dermatol. Sci.* 26, 14–18.
- Anhalt, G.J., 2004. Paraneoplastic pemphigus. *J. Invest. Dermatol. Symp. Proc.* 9, 29–33.
- Anhalt, G.J., Diaz, L.A., 2001. Prospects for autoimmune disease: research advances in pemphigus. *JAMA* 285, 652–654.
- Anhalt, G.J., Labib, R.S., Voorhees, J.J., Beals, T.F., Diaz, L.A., 1982. Induction of pemphigus in neonatal mice by passive transfer of IgG from patients with the disease. *N. Engl. J. Med.* 306, 1189–1196.
- Anhalt, G.J., Till, G.O., Diaz, L.A., Labib, R.S., Patel, H.P., Eaglstein, N.F., 1986. Defining the role of complement in experimental pemphigus vulgaris in mice. *J. Immunol.* 137, 2835–2840.
- Anhalt, G.J., Kim, S.C., Stanley, J.R., Korman, N.J., Jabs, D.A., Kory, M., et al., 1990. Paraneoplastic pemphigus. An autoimmune mucocutaneous disease associated with neoplasia. *N. Engl. J. Med.* 323, 1729–1735.
- Aoki, V., Millikan, R.C., Rivitti, E.A., Hans-Filho, G., Eaton, D.P., Warren, S.J., et al., 2004. Environmental risk factors in endemic pemphigus foliaceus (fogo selvagem). *J. Invest. Dermatol. Symp. Proc.* 9, 34–40.
- Aoki-Ota, M., Tsunoda, K., Ota, T., Iwasaki, T., Koyasu, S., Amagai, M., et al., 2004. A mouse model of pemphigus vulgaris by adoptive transfer of naive splenocytes from desmoglein 3 knockout mice. *Br. J. Dermatol.* 151, 346–354.
- Bagheri, B., Hall III, R.P., 2000. Dermatitis herpetiformis. In: Jordon, R.E. (Ed.), *Atlas of Bullous Disease*. Churchill Livingstone, New York.
- Balding, S.D., Prost, C., Diaz, L.A., Bernard, P., Bedane, C., Aberdam, D., et al., 1996. Cicatricial pemphigoid autoantibodies react with multiple sites on the BP180 extracellular domain. *J. Invest. Dermatol.* 106, 141–146.
- Berkowitz, P., Chua, M., Liu, Z., Diaz, L.A., Rubenstein, D.S., 2008a. Autoantibodies in the autoimmune disease pemphigus foliaceus induce blistering via p38 mitogen-activated protein kinase-dependent signaling in the skin. *Am. J. Pathol.* 173, 1628–1636.
- Berkowitz, P., Diaz, L.A., Hall, R.P., Rubenstein, D.S., 2008b. Induction of p38MAPK and HSP27 phosphorylation in pemphigus patient skin. *J. Invest. Dermatol.* 128, 738–740.
- Beutner, E.H., Jordon, R.E., 1964. Demonstration of skin antibodies in sera of pemphigus vulgaris patients by indirect immunofluorescent staining. *Proc. Soc. Exp. Biol. Med.* 117, 505–510.
- Bhol, K., Natarajan, K., Nagarwalla, N., Mohimen, A., Aoki, V., Ahmed, A.R., 1995. Correlation of peptide specificity and IgG subclass with pathogenic and nonpathogenic autoantibodies in pemphigus vulgaris: a model for autoimmunity. *Proc. Natl. Acad. Sci. U.S.A.* 92, 5239–5243.
- Bieber, K., Sun, S., Witte, M., Kasprick, A., Beltsiou, F., Behnen, M., et al., 2017. Regulatory T cells suppress inflammation and blistering in pemphigoid diseases. *Front. Immunol.* 8, 1628.
- Borrego, L., Maynard, B., Peterson, E.A., George, T., Iglesias, L., Peters, M.S., et al., 1996. Deposition of eosinophil granule proteins precedes blister formation in bullous pemphigoid. Comparison with neutrophil and mast cell granule proteins. *Am. J. Pathol.* 148, 897–909.
- Brenner, S., Goldberg, I., 2011. Drug-induced pemphigus. *Clin. Dermatol.* 29, 455–457.
- Brick, K.E., Weaver, C.H., Lohse, C.M., Pittelkow, M.R., Lehman, J.S., Camilleri, M.J., et al., 2014. Incidence of bullous pemphigoid and mortality of patients with bullous pemphigoid in Olmsted County, Minnesota, 1960 through 2009. *J. Am. Acad. Dermatol.* 71, 92–99.
- Budinger, L., Borradori, L., Yee, C., Eming, R., Ferencik, S., Grosse-Wilde, H., et al., 1998. Identification and characterization of autoreactive T cell responses to bullous pemphigoid antigen 2 in patients and healthy controls. *J. Clin. Invest.* 102, 2082–2089.
- Calkins, C.C., Setzer, S.V., Jennings, J.M., Summers, S., Tsunoda, K., Amagai, M., et al., 2006. Desmoglein endocytosis and desmosome disassembly are coordinated responses to pemphigus autoantibodies. *J. Biol. Chem.* 281, 7623–7634.
- Cardones, A.R., Hall III, R.P., 2011. Pathophysiology of dermatitis herpetiformis: a model for cutaneous manifestations of gastrointestinal inflammation. *Dermatol. Clin.* 29, 469–477. x.
- Chams-Davatchi, C., Valikhani, M., Daneshpazhooh, M., Esmaili, N., Balighi, K., Hallaji, Z., et al., 2005. Pemphigus: analysis of 1209 cases. *Int. J. Dermatol.* 44, 470–476.
- Chan, L.S., Hammerberg, C., Cooper, K.D., 1997. Significantly increased occurrence of HLA-DQB1\*0301 allele in patients with ocular cicatricial pemphigoid. *J. Invest. Dermatol.* 108, 129–132.
- Chan, L.S., Ahmed, A.R., Anhalt, G.J., Bernauer, W., Cooper, K.D., Elder, M.J., et al., 2002. The first international consensus on mucous membrane pemphigoid: definition, diagnostic criteria, pathogenic factors, medical treatment, and prognostic indicators. *Arch. Dermatol.* 138, 370–379.
- Chen, M., Saadat, P., Atha, T., Lipman, K., Ram, R., Woodley, D.T., 2004. A passive transfer model of epidermolysis bullosa acquisita using antibodies generated against the noncollagenous (NC1) domain of human type VII collagen on human skin grafted onto mice. *J. Invest. Dermatol.* 122, A11.
- Chen, R., Ning, G., Zhao, M.L., Fleming, M.G., Diaz, L.A., Werb, Z., et al., 2001. Mast cells play a key role in neutrophil recruitment in experimental bullous pemphigoid. *J. Clin. Invest.* 108, 1151–1158.

- Chiriac, M.T., Roesler, J., Sindrilaru, A., Scharffetter-Kochanek, K., Zillikens, D., Sitaru, C., 2007. NADPH oxidase is required for neutrophil-dependent autoantibody-induced tissue damage. *J. Pathol.* 212, 56–65.
- Civatte, A., 1943. Diagnostic histopathologique de la dermatite polymorphe douloureuse ou maladie de Düring-Brocq. *Ann. Dermatol. Syph.* 3, 1–30.
- Culton, D.A., Mccray, S.K., Park, M., Roberts, J.C., Li, N., Zedek, D.C., et al., 2015. Mucosal pemphigus vulgaris anti-Dsg3 IgG is pathogenic to the oral mucosa of humanized Dsg3 mice. *J. Invest. Dermatol.* 135, 1590–1597.
- Cummins, D.L., Mimouni, D., Tzu, J., Owens, N., Anhalt, G.J., Meyerle, J.H., 2007. Lichenoid paraneoplastic pemphigus in the absence of detectable antibodies. *J. Am. Acad. Dermatol.* 56, 153–159.
- Czech, W., Schaller, J., Schopf, E., Kapp, A., 1993. Granulocyte activation in bullous diseases: release of granular proteins in bullous pemphigoid and pemphigus vulgaris. *J. Am. Acad. Dermatol.* 29, 210–215.
- Delgado, J.C., Turbay, D., Yunis, E.J., Yunis, J.J., Morton, E.D., Bhol, K., et al., 1996. A common major histocompatibility complex class II allele HLA-DQB1\*0301 is present in clinical variants of pemphigoid. *Proc. Natl. Acad. Sci. U.S.A.* 93, 8569–8571.
- Delgado, J.C., Hameed, A., Yunis, J.J., Bhol, K., Rojas, A.I., Rehman, S.B., et al., 1997. Pemphigus vulgaris autoantibody response is linked to HLA-DQB1\*0503 in Pakistani patients. *Hum. Immunol.* 57, 110–119.
- Di Zenzo, G., Thoma-Uszynski, S., Calabresi, V., Fontao, L., Hofmann, S.C., Lacour, J.P., et al., 2011. Demonstration of epitope-spreading phenomena in bullous pemphigoid: results of a prospective multicenter study. *J. Invest. Dermatol.* 131, 2271–2280.
- Di Zenzo, G., Di Lullo, G., Corti, D., Calabresi, V., Sinistro, A., Vanzetta, F., et al., 2012. Pemphigus autoantibodies generated through somatic mutations target the desmoglein-3 cis-interface. *J. Clin. Invest.* 122, 3781–3790.
- Diaz, L.A., Giudice, G.J., 2000. End of the century overview of skin blisters. *Arch. Dermatol.* 136, 106–112.
- Diaz, L.A., Marcelo, C.L., 1978. Pemphigoid and pemphigus antigens in cultured epidermal cells. *Br. J. Dermatol.* 98, 631–637.
- Diaz, L.A., Sampaio, S.A., Rivitti, E.A., Martins, C.R., Cunha, P.R., Lombardi, C., et al., 1989. Endemic pemphigus foliaceus (Fogo Selvagem): II. Current and historic epidemiologic studies. *J. Invest. Dermatol.* 92, 4–12.
- Diaz, L.A., Arteaga, L.A., Hilario-Vargas, J., Valenzuela, J.G., Li, N., Warren, S., et al., 2004. Anti-desmoglein-1 antibodies in onchocerciasis, leishmaniasis and Chagas disease suggest a possible etiological link to Fogo selvagem. *J. Invest. Dermatol.* 123, 1045–1051.
- Diaz, L.A., Prisayanh, P.S., Dasher, D.A., Li, N., Evangelista, F., Aoki, V., et al., 2008. The IgM anti-desmoglein 1 response distinguishes Brazilian pemphigus foliaceus (fogo selvagem) from other forms of pemphigus. *J. Invest. Dermatol.* 128, 667–675.
- Dimson, O.G., Giudice, G.J., Fu, C.L., Van Den Bergh, F., Warren, S.J., Janson, M.M., et al., 2003. Identification of a potential effector function for IgE autoantibodies in the organ-specific autoimmune disease bullous pemphigoid. *J. Invest. Dermatol.* 120, 784–788.
- Ding, X., Aoki, V., Mascaro JR, J.M., Lopez-Swiderski, A., Diaz, L.A., Fairley, J.A., 1997. Mucosal and mucocutaneous (generalized) pemphigus vulgaris show distinct autoantibody profiles. *J. Invest. Dermatol.* 109, 592–596.
- Ding, X., Diaz, L.A., Fairley, J.A., Giudice, G.J., Liu, Z., 1999. The anti-desmoglein 1 autoantibodies in pemphigus vulgaris sera are pathogenic. *J. Invest. Dermatol.* 112, 739–743.
- Domloge-Hultsch, N., Anhalt, G.J., Gammon, W.R., Lazarova, Z., Briggaman, R., Welch, M., et al., 1994. Antiepiligrin cicatricial pemphigoid. A subepithelial bullous disorder. *Arch. Dermatol.* 130, 1521–1529.
- Donaldson, M.R., Zone, J.J., Schmidt, L.A., Taylor, T.B., Neuhausen, S.L., Hull, C.M., et al., 2007. Epidermal transglutaminase deposits in peri-lesional and uninvolved skin in patients with dermatitis herpetiformis. *J. Invest. Dermatol.* 127, 1268–1271.
- Dvorak, A.M., Mihm JR, M.C., Osage, J.E., Kwan, T.H., Austen, K.F., Wintroub, B.U., 1982. Bullous pemphigoid, an ultrastructural study of the inflammatory response: eosinophil, basophil and mast cell granule changes in multiple biopsies from one patient. *J. Invest. Dermatol.* 78, 91–101.
- Egan, C.A., Hanif, N., Taylor, T.B., Meyer, L.J., Petersen, M.J., Zone, J.J., 1999. Characterization of the antibody response in oesophageal cicatricial pemphigoid. *Br. J. Dermatol.* 140, 859–864.
- Egan, C.A., Lazarova, Z., Darling, T.N., Yee, C., Yancey, K.B., 2003. Anti-epiligrin cicatricial pemphigoid: clinical findings, immunopathogenesis, and significant associations. *Medicine (Baltimore)* 82, 177–186.
- Eming, R., Hennerici, T., Backlund, J., Feliciani, C., Visconti, K.C., Willenborg, S., et al., 2014. Pathogenic IgG antibodies against desmoglein 3 in pemphigus vulgaris are regulated by HLA-DRB1\*04:02-restricted T cells. *J. Immunol.* 193, 4391–4399.
- Esmaili, N., Mortazavi, H., Chams-Davatchi, C., Daneshpazhooh, M., Damavandi, M.R., Aryanian, Z., et al., 2013. Association between HLA-DQB1\*03:01 and Bullous pemphigoid in Iranian patients. *Iran. J. Immunol.* 10, 1–9.
- Espana, A., Diaz, L.A., Mascaro JR, J.M., Giudice, G.J., Fairley, J.A., Till, G.O., et al., 1997. Mechanisms of acantholysis in pemphigus foliaceus. *Clin. Immunol. Immunopathol.* 85, 83–89.
- Fairley, J.A., Burnett, C.T., Fu, C.L., Larson, D.L., Fleming, M.G., Giudice, G.J., 2007. A pathogenic role for IgE in autoimmunity: bullous pemphigoid IgE reproduces the early phase of lesion development in human skin grafted to nu/nu mice. *J. Invest. Dermatol.* 127, 2605–2611.
- Fan, J.L., Memar, O., McCormick, D.J., Prabhakar, B.S., 1999. BALB/c mice produce blister-causing antibodies upon immunization with a recombinant human desmoglein 3. *J. Immunol.* 163, 6228–6235.
- Feng, S., Zhou, W., Zhang, J., Jin, P., 2011. Analysis of 6 cases of drug-induced pemphigus. *Eur. J. Dermatol.* 21, 696–699.
- Fortuna, G., Salas-Alanis, J.C., Guidetti, E., Marinkovich, M.P., 2012. A critical reappraisal of the current data on drug-induced linear immunoglobulin A bullous dermatosis: a real and separate nosological entity? *J. Am. Acad. Dermatol.* 66, 988–994.
- Frew, J.W., Martin, L.K., Murrell, D.F., 2011. Evidence-based treatments in pemphigus vulgaris and pemphigus foliaceus. *Dermatol. Clin.* 29, 599–606.
- Futamura, S., Martins, C., Rivitti, E.A., Labib, R.S., Diaz, L.A., Anhalt, G.J., 1989. Ultrastructural studies of acantholysis induced in vivo by passive transfer of IgG from endemic pemphigus foliaceus (fogo selvagem). *J. Invest. Dermatol.* 93, 480–485.
- Futei, Y., Amagai, M., Ishii, K., Kuroda-Kinoshita, K., Ohya, K., Nishikawa, T., 2001. Predominant IgG4 subclass in autoantibodies of pemphigus vulgaris and foliaceus. *J. Dermatol. Sci.* 26, 55–61.
- Gambichler, T., Tsitlakidon, A., Skrygan, M., Hoxtermann, S., Susok, L., Hessam, S., 2017. T regulatory cells and other lymphocyte subsets in patients with bullous pemphigoid. *Clin. Exp. Dermatol.* 42, 632–637.

- Gammon, W.R., Heise, E.R., Burke, W.A., Fine, J.D., Woodley, D.T., Briggaman, R.A., 1988. Increased frequency of HLA-DR2 in patients with autoantibodies to epidermolysis bullosa acquisita antigen: evidence that the expression of autoimmunity to type VII collagen is HLA class II allele associated. *J. Invest. Dermatol.* 91, 228–232.
- Gao, X.H., Winsey, S., Li, G., Barnardo, M., Zhu, X.J., Chen, H.D., et al., 2002. HLA-DR and DQ polymorphisms in bullous pemphigoid from northern China. *Clin. Exp. Dermatol.* 27, 319–321.
- Getsios, S., Huen, A.C., Green, K.J., 2004. Working out the strength and flexibility of desmosomes. *Nat. Rev. Mol. Cell Biol.* 5, 271–281.
- Giudice, G.J., Emery, D.J., Diaz, L.A., 1992. Cloning and primary structural analysis of the bullous pemphigoid autoantigen BP180. *J. Invest. Dermatol.* 99, 243–250.
- Giudice, G.J., Emery, D.J., Zelickson, B.D., Anhalt, G.J., Liu, Z., Diaz, L.A., 1993. Bullous pemphigoid and herpes gestationis autoantibodies recognize a common non-collagenous site on the BP180 ectodomain. *J. Immunol.* 151, 5742–5750.
- Gudi, V.S., White, M.I., Cruickshank, N., Herriot, R., Edwards, S.L., Nimmo, F., et al., 2005. Annual incidence and mortality of bullous pemphigoid in the Grampian Region of North-east Scotland. *Br. J. Dermatol.* 153, 424–427.
- Haase, C., Budinger, L., Borradori, L., Yee, C., Merk, H.F., Yancey, K., et al., 1998. Detection of IgG autoantibodies in the sera of patients with bullous and gestational pemphigoid: ELISA studies utilizing a baculovirus-encoded form of bullous pemphigoid antigen 2. *J. Invest. Dermatol.* 110, 282–286.
- Hammers, C.M., Chen, J., Lin, C., Kacir, S., Siegel, D.L., Payne, A.S., et al., 2015. Persistence of anti-desmoglein 3 IgG(+) B-cell clones in pemphigus patients over years. *J. Invest. Dermatol.* 135, 742–749.
- Hashimoto, T., 2001. Immunopathology of IgA pemphigus. *Clin. Dermatol.* 19, 683–689.
- Ishii, K., Harada, R., Matsuo, I., Shirakata, Y., Hashimoto, K., Amagai, M., 2005. In vitro keratinocyte dissociation assay for evaluation of the pathogenicity of anti-desmoglein 3 IgG autoantibodies in pemphigus vulgaris. *J. Invest. Dermatol.* 124, 939–946.
- Jenkins, R.E., Hern, S., Black, M.M., 1999. Clinical features and management of 87 patients with pemphigoid gestationis. *Clin. Exp. Dermatol.* 24, 255–259.
- Jennings, J.M., Tucker, D.K., Kottke, M.D., Saito, M., Delva, E., Hanakawa, Y., et al., 2011. Desmosome disassembly in response to pemphigus vulgaris IgG occurs in distinct phases and can be reversed by expression of exogenous Dsg3. *J. Invest. Dermatol.* 131, 706–718.
- Jolly, P.S., Berkowitz, P., Bektas, M., Lee, H.E., Chua, M., Diaz, L.A., et al., 2010. p38MAPK signaling and desmoglein-3 internalization are linked events in pemphigus acantholysis. *J. Biol. Chem.* 285, 8936–8941.
- Joly, P., Mouquet, H., Roujeau, J.C., D'incan, M., Gilbert, D., Jacquot, S., et al., 2007. A single cycle of rituximab for the treatment of severe pemphigus. *N. Engl. J. Med.* 357, 545–552.
- Joly, P., Baricault, S., Sparsa, A., Bernard, P., Bedane, C., Duvert-Lehembre, S., et al., 2012. Incidence and mortality of bullous pemphigoid in France. *J. Invest. Dermatol.* 132, 1998–2004.
- Joly, P., Maho-Vaillant, M., Prost-Squarcioni, C., Hebert, V., Houivet, E., Calbo, S., et al., 2017. First-line rituximab combined with short-term prednisone versus prednisone alone for the treatment of pemphigus (Ritux 3): a prospective, multicentre, parallel-group, open-label randomised trial. *Lancet* 389, 2031–2040.
- Jones, C.C., Hamilton, R.G., Jordon, R.E., 1988. Subclass distribution of human IgG autoantibodies in pemphigus. *J. Clin. Immunol.* 8, 43–49.
- Jordon, R.E., Beutner, E.H., Witebsky, E., Blumental, G., Hale, W.L., Lever, W.F., 1967. Basement zone antibodies in bullous pemphigoid. *JAMA* 200, 751–756.
- Jordon, R.E., Heine, K.G., Tappeiner, G., Bushkell, L.L., Provost, T.T., 1976. The immunopathology of herpes gestationis. Immunofluorescence studies and characterization of "HG factor". *J. Clin. Invest.* 57, 1426–1431.
- Katz, S.I., Hertz, K.C., Yaoita, H., 1976. Herpes gestationis. Immunopathology and characterization of the HG factor. *J. Clin. Invest.* 57, 1434–1441.
- Koch, P.J., Mahoney, M.G., Ishikawa, H., Pulkkinen, L., Uitto, J., Shultz, L., et al., 1997. Targeted disruption of the pemphigus vulgaris antigen (desmoglein 3) gene in mice causes loss of keratinocyte cell adhesion with a phenotype similar to pemphigus vulgaris. *J. Cell Biol.* 137, 1091–1102.
- Korman, N.J., Cooper, K.D., 2000. Cicatricial pemphigoid. In: Jordon, R.E. (Ed.), *Atlas of Bullous Disease*. Churchill Livingstone, New York.
- Kouros, A.S., Yancey, K.B., 2011. Pathogenesis of mucous membrane pemphigoid. *Dermatol. Clin.* 29, 479–484. x.
- Kricheli, D., David, M., Frusic-Zlotkin, M., Goldsmith, D., Rabinov, M., Sulkes, J., et al., 2000. The distribution of pemphigus vulgaris-IgG subclasses and their reactivity with desmoglein 3 and 1 in pemphigus patients and their first-degree relatives. *Br. J. Dermatol.* 143, 337–342.
- Labib, R.S., Anhalt, G.J., Patel, H.P., Mutasim, D.F., Diaz, L.A., 1986. Molecular heterogeneity of the bullous pemphigoid antigens as detected by immunoblotting. *J. Immunol.* 136, 1231–1235.
- Laffitte, E., Skaria, M., Jaunin, F., Tamm, K., Saurat, J.H., Favre, B., et al., 2001. Autoantibodies to the extracellular and intracellular domain of bullous pemphigoid 180, the putative key autoantigen in bullous pemphigoid, belong predominantly to the IgG1 and IgG4 subclasses. *Br. J. Dermatol.* 144, 760–768.
- Langan, S.M., Smeeth, L., Hubbard, R., Fleming, K.M., Smith, C.J., West, J., 2008. Bullous pemphigoid and pemphigus vulgaris—incidence and mortality in the UK: population based cohort study. *BMJ* 337, a180.
- Lazarova, Z., Yee, C., Darling, T., Briggaman, R.A., Yancey, K.B., 1996. Passive transfer of anti-laminin 5 antibodies induces subepidermal blisters in neonatal mice. *J. Clin. Invest.* 98, 1509–1518.
- Lee, E., Lendas, K.A., Chow, S., Pirani, Y., Gordon, D., Dionisio, R., et al., 2006. Disease relevant HLA class II alleles isolated by genotypic, haplotypic, and sequence analysis in North American Caucasians with pemphigus vulgaris. *Hum. Immunol.* 67, 125–139.
- Lever, W.F., 1965. *Pemphigus and Pemphigoid*. Charles C. Thomas Publisher, Springfield, IL.
- Li, N., Liu, Z., Diaz, L.A., 2002. Pemphigus foliaceus autoantibodies recognize two dominant pathogenic epitopes located in EC1 and EC2 domains of desmoglein-1. *J. Invest. Dermatol.* 119, A305.
- Li, N., Aoki, V., Hans-Filho, G., Rivitti, E.A., Diaz, L.A., 2003. The role of intramolecular epitope spreading in the pathogenesis of endemic pemphigus foliaceus (fogo selvagem). *J. Exp. Med.* 197, 1501–1510.
- Li, N., Zhao, M., Wang, J., Liu, Z., Diaz, L.A., 2009. Involvement of the apoptotic mechanism in pemphigus foliaceus autoimmune injury of the skin. *J. Immunol.* 182, 711–717.

- Lin, M.S., Swartz, S.J., Lopez, A., Ding, X., Fernandez-Vina, M.A., Stastny, P., et al., 1997. Development and characterization of desmoglein-3 specific T cells from patients with pemphigus vulgaris. *J. Clin. Invest.* 99, 31–40.
- Lin, M.S., Gharia, M.A., Swartz, S.J., Diaz, L.A., Giudice, G.J., 1999. Identification and characterization of epitopes recognized by T lymphocytes and autoantibodies from patients with herpes gestationis. *J. Immunol.* 162, 4991–4997.
- Lin, M.S., Fu, C.L., Aoki, V., Hans-Filho, G., Rivitti, E.A., Moraes, J.R., et al., 2000a. Desmoglein-1-specific T lymphocytes from patients with endemic pemphigus foliaceus (fogo selvagem). *J. Clin. Invest.* 105, 207–213.
- Lin, M.S., Fu, C.L., Giudice, G.J., Olague-Marchan, M., Lazaro, A.M., Stastny, P., et al., 2000b. Epitopes targeted by bullous pemphigoid T lymphocytes and autoantibodies map to the same sites on the bullous pemphigoid 180 ectodomain. *J. Invest. Dermatol.* 115, 955–961.
- Liu, Z., 2004. Bullous pemphigoid: using animal models to study the immunopathology. *J. Invest. Dermatol. Symp. Proc.* 9, 41–46.
- Liu, Z., Diaz, L.A., Troy, J.L., Taylor, A.F., Emery, D.J., Fairley, J.A., et al., 1993. A passive transfer model of the organ-specific autoimmune disease, bullous pemphigoid, using antibodies generated against the hemidesmosomal antigen, BP180. *J. Clin. Invest.* 92, 2480–2488.
- Liu, Z., Giudice, G.J., Zhou, X., Swartz, S.J., Troy, J.L., Fairley, J.A., et al., 1997. A major role for neutrophils in experimental bullous pemphigoid. *J. Clin. Invest.* 100, 1256–1263.
- Liu, Z., Shipley, J.M., Vu, T.H., Zhou, X., Diaz, L.A., Werb, Z., et al., 1998. Gelatinase B-deficient mice are resistant to experimental bullous pemphigoid. *J. Exp. Med.* 188, 475–482.
- Liu, Z., Shapiro, S.D., Zhou, X., Twining, S.S., Senior, R.M., Giudice, G.J., et al., 2000a. A critical role for neutrophil elastase in experimental bullous pemphigoid. *J. Clin. Invest.* 105, 113–123.
- Liu, Z., Zhou, X., Shapiro, S.D., Shipley, J.M., Twining, S.S., Diaz, L.A., et al., 2000b. The serpin alpha1-proteinase inhibitor is a critical substrate for gelatinase B/MMP-9 in vivo. *Cell* 102, 647–655.
- Liu, Z., Li, N., Diaz, L.A., Shipley, M., Senior, R.M., Werb, Z., 2005. Synergy between a plasminogen cascade and MMP-9 in autoimmune disease. *J. Clin. Invest.* 115, 879–887.
- Liu, Z., Sui, W., Zhao, M., Li, Z., Li, N., Thresher, R., et al., 2008. Subepidermal blistering induced by human autoantibodies to BP180 requires innate immune players in a humanized bullous pemphigoid mouse model. *J. Autoimmun.* 31, 331–338.
- Loiseau, P., Lecleach, L., Prost, C., Lepage, V., Busson, M., Bastuji-Garin, S., et al., 2000. HLA class II polymorphism contributes to specify desmoglein derived peptides in pemphigus vulgaris and pemphigus foliaceus. *J. Autoimmun.* 15, 67–73.
- Lombardi, M.L., Mercuro, O., Ruocco, V., Lo Schiavo, A., Lombari, V., Guerrera, V., et al., 1999. Common human leukocyte antigen alleles in pemphigus vulgaris and pemphigus foliaceus Italian patients. *J. Invest. Dermatol.* 113, 107–110.
- Luyet, C., Schulze, K., Sayar, B.S., Howald, D., Muller, E.J., Galichet, A., 2015. Preclinical studies identify non-apoptotic low-level caspase-3 as therapeutic target in pemphigus vulgaris. *PLoS One* 10, e0119809.
- Mahoney, M.G., Wang, Z.H., Stanley, J.R., 1999. Pemphigus vulgaris and pemphigus foliaceus antibodies are pathogenic in plasminogen activator knockout mice. *J. Invest. Dermatol.* 113, 22–25.
- Maley, A., Warren, M., Haberman, I., Swerlick, R., Kharod-Dholakia, B., Feldman, R., 2016. Rituximab combined with conventional therapy versus conventional therapy alone for the treatment of mucous membrane pemphigoid (MMP). *J. Am. Acad. Dermatol.* 74, 835–840.
- Mao, X., Choi, E.J., Payne, A.S., 2009. Disruption of desmosome assembly by monovalent human pemphigus vulgaris monoclonal antibodies. *J. Invest. Dermatol.* 129, 908–918.
- Mascaro JR., J.M., Espana, A., Liu, Z., Ding, X., Swartz, S.J., Fairley, J.A., et al., 1997. Mechanisms of acantholysis in pemphigus vulgaris: role of IgG valence. *Clin. Immunol. Immunopathol.* 85, 90–96.
- Miyagawa, S., Amagai, M., Niizeki, H., Yamashina, Y., Kaneshige, T., Nishikawa, T., et al., 1999. HLA-DRB1 polymorphisms and autoimmune responses to desmogleins in Japanese patients with pemphigus. *Tissue Antigens* 54, 333–340.
- Moraes, M.E., Fernandez-Vina, M., Lazaro, A., Diaz, L.A., Filho, G.H., Friedman, H., et al., 1997. An epitope in the third hypervariable region of the DRB1 gene is involved in the susceptibility to endemic pemphigus foliaceus (fogo selvagem) in three different Brazilian populations. *Tissue Antigens* 49, 35–40.
- Morrison, L.H., Labib, R.S., Zone, J.J., Diaz, L.A., Anhalt, G.J., 1988. Herpes gestationis autoantibodies recognize a 180-kD human epidermal antigen. *J. Clin. Invest.* 81, 2023–2026.
- Mutasim, D.F., Takahashi, Y., Labib, R.S., Anhalt, G.J., Patel, H.P., Diaz, L.A., 1985. A pool of bullous pemphigoid antigen(s) is intracellular and associated with the basal cell cytoskeleton-hemidesmosome complex. *J. Invest. Dermatol.* 84, 47–53.
- Nemzer, P.G., Egan, C.A., Zone, J.J., 2000. Linear IgA bullous dermatosis. In: Jordon, R.E. (Ed.), *Atlas of Bullous Disease*. Churchill Livingstone, New York.
- Nishie, W., Sawamura, D., Goto, M., Ito, K., Shibaki, A., Mcmillan, J.R., et al., 2007. Humanization of autoantigen. *Nat. Med.*, 13. pp. 378–383.
- Nishifumi, K., Amagai, M., Kuwana, M., Iwasaki, T., Nishikawa, T., 2000. Detection of antigen-specific B cells in patients with pemphigus vulgaris by enzyme-linked immunospot assay: requirement of T cell collaboration for autoantibody production. *J. Invest. Dermatol.* 114, 88–94.
- Okazaki, A., Miyagawa, S., Yamashina, Y., Kitamura, W., Shirai, T., 2000. Polymorphisms of HLA-DR and -DQ genes in Japanese patients with bullous pemphigoid. *J. Dermatol.* 27, 149–156.
- O'toole, E.A., Woodley, D.T., 2000. Epidermolysis bullosa acquisita. In: Jordon, R.E. (Ed.), *Atlas of Bullous Disease*. Churchill Livingstone, New York.
- Patton, T., Plunkett, R.W., Beutner, E.H., Deng, J.S., Jukic, D.M., 2006. IgG4 as the predominant IgG subclass in pemphigoides gestationis. *J. Cutan. Pathol.* 33, 299–302.
- Payne, A.S., Ishii, K., Kacir, S., Lin, C., Li, H., Hanakawa, Y., et al., 2005. Genetic and functional characterization of human pemphigus vulgaris monoclonal autoantibodies isolated by phage display. *J. Clin. Invest.* 115, 888–899.
- Powell, A.M., Sakuma-Oyama, Y., Oyama, N., Albert, S., Bhogal, B., Kaneko, F., et al., 2005. Usefulness of BP180 NC16a enzyme-linked immunosorbent assay in the serodiagnosis of pemphigoid gestationis and in differentiating between pemphigoid gestationis and pruritic urticarial papules and plaques of pregnancy. *Arch. Dermatol.* 141, 705–710.

- Probst, C., Schlumberger, W., Stocker, W., Recke, A., Schmidt, E., Hashimoto, T., et al., 2009. Development of ELISA for the specific determination of autoantibodies against envoplakin and periplakin in paraneoplastic pemphigus. *Clin. Chim. Acta* 410, 13–18.
- Provost, T.T., Tomasi JR., T.B., 1973. Evidence for complement activation via the alternate pathway in skin diseases, I. Herpes gestationis, systemic lupus erythematosus, and bullous pemphigoid. *J. Clin. Invest.* 52, 1779–1787.
- Qaqish, B.F., Prisayanh, P., Qian, Y., Andraca, E., Li, N., Aoki, V., et al., 2009. Development of an IgG4-based predictor of endemic pemphigus foliaceus (fogo selvagem). *J. Invest. Dermatol.* 129, 110–118.
- Qian, Y., Prisayanh, P., Andraca, E., Qaqish, B.F., Aoki, V., Hans-Filho, G., et al., 2011. IgE, IgM, and IgG4 anti-desmoglein 1 autoantibody profile in endemic pemphigus foliaceus (fogo selvagem). *J. Invest. Dermatol.* 131, 985–987.
- Qian, Y., Jeong, J.S., Maldonado, M., Valenzuela, J.G., Gomes, R., Teixeira, C., et al., 2012. Cutting Edge: Brazilian pemphigus foliaceus anti-desmoglein 1 autoantibodies cross-react with sand fly salivary LJMJ11 antigen. *J. Immunol.* 189, 1535–1539.
- Qian, Y., Jeong, J.S., Abdeladhim, M., Valenzuela, J.G., Aoki, V., Hans-Filho, G., et al., 2015. IgE anti-LJM11 sand fly salivary antigen may herald the onset of fogo selvagem in endemic Brazilian regions. *J. Invest. Dermatol.* 135, 913–915.
- Qian, Y., Culton, D.A., Jeong, J.S., Trupiano, N., Valenzuela, J.G., Diaz, L.A., 2016a. Non-infectious environmental antigens as a trigger for the initiation of an autoimmune skin disease. *Autoimmun. Rev.* 15, 923–930.
- Qian, Y., Jeong, J.S., Ye, J., Dang, B., Abdeladhim, M., Aoki, V., et al., 2016b. Overlapping IgG4 responses to self- and environmental antigens in endemic pemphigus foliaceus. *J. Immunol.* 196, 2041–2050.
- Rock, B., Martins, C.R., Theofilopoulos, A.N., Balderas, R.S., Anhalt, G.J., Labib, R.S., et al., 1989. The pathogenic effect of IgG4 autoantibodies in endemic pemphigus foliaceus (fogo selvagem). *N. Engl. J. Med.* 320, 1463–1469.
- Roscoe, J.T., Diaz, L., Sampaio, S.A., Castro, R.M., Labib, R.S., Takahashi, Y., et al., 1985. Brazilian pemphigus foliaceus autoantibodies are pathogenic to BALB/c mice by passive transfer. *J. Invest. Dermatol.* 85, 538–541.
- Sadik, C.D., Lima, A.L., Zillikens, D., 2016. Pemphigoid gestationis: toward a better understanding of the etiopathogenesis. *Clin. Dermatol.* 34, 378–382.
- Saleh, M.A., Ishii, K., Yamagami, J., Shirakata, Y., Hashimoto, K., Amagai, M., 2012. Pathogenic anti-desmoglein 3 mAbs cloned from a paraneoplastic pemphigus patient by phage display. *J. Invest. Dermatol.* 132, 1141–1148.
- Sardy, M., Karpati, S., Merkl, B., Paulsson, M., Smyth, N., 2002. Epidermal transglutaminase (TGase 3) is the autoantigen of dermatitis herpetiformis. *J. Exp. Med.* 195, 747–757.
- Schmidt, E., Obe, K., Brocker, E.B., Zillikens, D., 2000. Serum levels of autoantibodies to BP180 correlate with disease activity in patients with bullous pemphigoid. *Arch. Dermatol.* 136, 174–178.
- Sekiguchi, M., Futei, Y., Fujii, Y., Iwasaki, T., Nishikawa, T., Amagai, M., 2001. Dominant autoimmune epitopes recognized by pemphigus antibodies map to the N-terminal adhesive region of desmogleins. *J. Immunol.* 167, 5439–5448.
- Shah, A.A., Seiffert-Sinha, K., Sirois, D., Werth, V.P., Rengarajan, B., Zrnchik, W., et al., 2015. Development of a disease registry for autoimmune bullous diseases: initial analysis of the pemphigus vulgaris subset. *Acta Derm. Venereol.* 95, 86–90.
- Shornick, J.K., Stastny, P., Gilliam, J.N., 1981. High frequency of histocompatibility antigens HLA-DR3 and DR4 in herpes gestations. *J. Clin. Invest.* 68, 553–555.
- Sinha, A.A., 2011. The genetics of pemphigus. *Dermatol. Clin.* 29, 381–391. vii.
- Sinha, A.A., Brautbar, C., Szafer, F., Friedmann, A., Tzfon, E., Todd, J.A., et al., 1988. A newly characterized HLA DQ beta allele associated with pemphigus vulgaris. *Science* 239, 1026–1029.
- Sitaru, C., Mihai, S., Otto, C., Chiriac, M.T., Hausser, I., Dotterweich, B., et al., 2005. Induction of dermal-epidermal separation in mice by passive transfer of antibodies specific to type VII collagen. *J. Clin. Invest.* 115, 870–878.
- Sollid, L.M., 2000. Molecular basis of celiac disease. *Annu. Rev. Immunol.* 18, 53–81.
- Stanley, J.R., Hawley-Nelson, P., Yuspa, S.H., Shevach, E.M., Katz, S.I., 1981. Characterization of bullous pemphigoid antigen: a unique basement membrane protein of stratified squamous epithelia. *Cell* 24, 897–903.
- Takahashi, H., Amagai, M., Nishikawa, T., Fujii, Y., Kawakami, Y., Kuwana, M., 2008. Novel system evaluating in vivo pathogenicity of desmoglein 3-reactive T cell clones using murine pemphigus vulgaris. *J. Immunol.* 181, 1526–1535.
- Takahashi, H., Kouno, M., Nagao, K., Wada, N., Hata, T., Nishimoto, S., et al., 2011. Desmoglein 3-specific CD4+ T cells induce pemphigus vulgaris and interface dermatitis in mice. *J. Clin. Invest.* 121, 3677–3688.
- Tanaka, T., Parry, D.A., Klaus-Kovtun, V., Steinert, P.M., Stanley, J.R., 1991. Comparison of molecularly cloned bullous pemphigoid antigen to desmoplakin I confirms that they define a new family of cell adhesion junction plaque proteins. *J. Biol. Chem.* 266, 12555–12559.
- Todd, J.A., Acha-Orbea, H., Bell, J.I., Chao, N., Fronek, Z., Jacob, C.O., et al., 1988. A molecular basis for MHC class II-associated autoimmunity. *Science* 240, 1003–1009.
- Torzecka, J.D., Wozniak, K., Kowalewski, C., Waszczykowska, E., Sysa-Jedrzejowska, A., Pas, H.H., et al., 2007. Circulating pemphigus autoantibodies in healthy relatives of pemphigus patients: coincidental phenomenon with a risk of disease development? *Arch. Dermatol. Res.* 299, 239–243.
- Tsuji-Abe, Y., Akiyama, M., Yamanaka, Y., Kikuchi, T., Sato-Matsumura, K.C., Shimizu, H., 2005. Correlation of clinical severity and ELISA indices for the NC16A domain of BP180 measured using BP180 ELISA kit in bullous pemphigoid. *J. Dermatol. Sci.* 37, 145–149.
- Tsunoda, K., Ota, T., Aoki, M., Yamada, T., Nagai, T., Nakagawa, T., et al., 2003. Induction of pemphigus phenotype by a mouse monoclonal antibody against the amino-terminal adhesive interface of desmoglein 3. *J. Immunol.* 170, 2170–2178.
- Tsuruta, D., Ishii, N., Hamada, T., Ohyama, B., Fukuda, S., Koga, H., et al., 2011. IgA pemphigus. *Clin. Dermatol.* 29, 437–442.
- Tyagi, S., Bhol, K., Natarajan, K., Livir-Rallatos, C., Foster, C.S., Ahmed, A.R., 1996. Ocular cicatricial pemphigoid antigen: partial sequence and biochemical characterization. *Proc. Natl. Acad. Sci. U.S.A.* 93, 14714–14719.
- Ujie, H., Nishie, W., Shimizu, H., 2011. Pathogenesis of bullous pemphigoid. *Dermatol. Clin.* 29, 439–446. ix.
- Van Beek, N., Dahnrich, C., Johannsen, N., Lemcke, S., Goletz, S., Hubner, F., et al., 2017a. Prospective studies on the routine use of a novel multivariant enzyme-linked immunosorbent assay for the diagnosis of autoimmune bullous diseases, *J. Am. Acad. Dermatol.*, 76. pp. 889–894.e5.

- Van Beek, N., Luttmann, N., Huebner, F., Recke, A., Karl, I., Schulze, F.S., et al., 2017b. Correlation of serum levels of IgE autoantibodies against BP180 with bullous pemphigoid disease activity. *JAMA Dermatol.* 153, 30–38.
- Veldman, C., Pahl, A., Hertl, M., 2009. Desmoglein 3-specific T regulatory 1 cells consist of two subpopulations with differential expression of the transcription factor Foxp3. *Immunology* 127, 40–49.
- Veldman, C.M., Gebhard, K.L., Uter, W., Wassmuth, R., Grotzinger, J., Schultz, E., et al., 2004. T cell recognition of desmoglein 3 peptides in patients with pemphigus vulgaris and healthy individuals. *J. Immunol.* 172, 3883–3892.
- Venning, V.A., 2011. Linear IgA disease: clinical presentation, diagnosis, and pathogenesis. *Dermatol. Clin.* 29, 453–458. ix.
- Wada, M., Nishie, W., Ujije, H., Izumi, K., Iwata, H., Natsuga, K., et al., 2016. Epitope-dependent pathogenicity of antibodies targeting a major bullous pemphigoid autoantigen collagen XVII/BP180. *J. Invest. Dermatol.* 136, 938–946.
- Wang, L., Bu, D., Yang, Y., Chen, X., Zhu, X., 2004. Castleman's tumours and production of autoantibody in paraneoplastic pemphigus. *Lancet* 363, 525–531.
- Warren, S.J., Lin, M.S., Giudice, G.J., Hoffmann, R.G., Hans-Filho, G., Aoki, V., et al., 2000. The prevalence of antibodies against desmoglein 1 in endemic pemphigus foliaceus in Brazil. Cooperative Group on Fogo Selvagem Research. *N. Engl. J. Med.* 343, 23–30.
- Warren, S.J., Arteaga, L.A., Rivitti, E.A., Aoki, V., Hans-Filho, G., Qaqish, B.F., et al., 2003. The role of subclass switching in the pathogenesis of endemic pemphigus foliaceus. *J. Invest. Dermatol.* 120, 104–108.
- Waschke, J., Spindler, V., Bruggeman, P., Zillikens, D., Schmidt, G., Drenckhahn, D., 2006. Inhibition of Rho A activity causes pemphigus skin blistering. *J. Cell Biol.* 175, 721–727.
- Wieland, C.N., Comfere, N.I., Gibson, L.E., Weaver, A.L., Krause, P.K., Murray, J.A., 2010. Anti-bullous pemphigoid 180 and 230 antibodies in a sample of unaffected subjects. *Arch. Dermatol.* 146, 21–25.
- Williamson, L., Raess, N.A., Caldelari, R., Zakher, A., De Bruin, A., Posthaus, H., et al., 2006. Pemphigus vulgaris identifies plakoglobin as key suppressor of c-Myc in the skin. *EMBO J.* 25, 3298–3309.
- Wintroub, B.U., Mihm JR, M.C., Goetzl, E.J., Soter, N.A., Austen, K.F., 1978. Morphologic and functional evidence for release of mast-cell products in bullous pemphigoid. *N. Engl. J. Med.* 298, 417–421.
- Wucherpfennig, K.W., Yu, B., Bhol, K., Monos, D.S., Argyris, E., Karr, R.W., et al., 1995. Structural basis for major histocompatibility complex (MHC)-linked susceptibility to autoimmunity: charged residues of a single MHC binding pocket confer selective presentation of self-peptides in pemphigus vulgaris. *Proc. Natl. Acad. Sci. U.S.A.* 92, 11935–11939.
- Yamagami, J., Payne, A.S., Kacir, S., Ishii, K., Siegel, D.L., Stanley, J.R., 2010. Homologous regions of autoantibody heavy chain complementarity-determining region 3 (H-CDR3) in patients with pemphigus cause pathogenicity. *J. Clin. Invest.* 120, 4111–4117.
- Yoshimura, K., Ishii, N., Hamada, T., Abe, T., Ono, F., Hashikawa, K., et al., 2014. Clinical and immunological profiles in 17 Japanese patients with drug-induced pemphigus studied at Kurume University. *Br. J. Dermatol.* 171, 544–553.
- Zillikens, D., Wever, S., Roth, A., Weidenthaler-Barth, B., Hashimoto, T., Brocker, E.B., 1995. Incidence of autoimmune subepidermal blistering dermatoses in a region of central Germany. *Arch. Dermatol.* 131, 957–958.
- Zone, J.J., Egan, C.A., Taylor, T.B., Meyer, L.J., 2004. IgA autoimmune disorders: development of a passive transfer mouse model. *J. Invest. Dermatol. Symp. Proc.* 9, 47–51.
- Zone, J.J., Taylor, T., Hull, C., Schmidt, L., Meyer, L., 2007. IgE basement membrane zone antibodies induce eosinophil infiltration and histological blisters in engrafted human skin on SCID mice. *J. Invest. Dermatol.* 127, 1167–1174.
- Zuo, Y., Evangelista, F., Culton, D., Guilabert, A., Lin, L., Li, N., et al., 2016. IgG4 autoantibodies are inhibitory in the autoimmune disease bullous pemphigoid. *J. Autoimmun.* 73, 111–119.

# 61

## Nonbullous Skin Diseases: Alopecia Areata, Vitiligo, Psoriasis, and Urticaria

*Stanca A. Birlea, Marc Serota and David A. Norris*

Department of Dermatology, University of Colorado School of Medicine, Anschutz Medical Campus, University of Colorado, Denver, CO, United States

### OUTLINE

<b>Alopecia Areata</b>	1211	<b>Psoriasis</b>	1221
Clinical, Pathologic, and Epidemiologic Features	1211	Clinical, Pathologic, and Epidemiologic Features	1221
Autoimmune Features	1212	Autoimmune Features	1222
Genetic Features	1213	Genetic Features	1224
In Vivo and In Vitro Models	1214	In Vivo and In Vitro Models	1224
Pathologic Effector Mechanisms	1215	Concluding Remarks—Future Prospects	1226
Autoantibodies as Potential Immunologic			
Markers	1215		
Concluding Remarks—Future Prospects	1215		
<b>Vitiligo</b>	1215	<b>Chronic Urticaria</b>	1226
Clinical, Pathologic, and Epidemiologic Features	1215	Clinical, Pathologic, and Epidemiologic Features	1226
Autoimmune Features	1216	Autoimmune Features	1227
Genetic Features	1218	In Vivo and In Vitro Models	1227
In Vivo and In Vitro Models	1218	Pathologic Effector Mechanisms	1228
Pathogenetic Mechanism	1219	Autoantibodies as Potential Immunologic	
Autoantibodies as Potential Immunologic		Markers	1228
Markers	1220	Concluding Remarks—Future Prospects	1228
Concluding Remarks—Future Prospects	1220	<b>References</b>	1229
		<b>Further Reading</b>	1234

### ALOPECIA AREATA

#### Clinical, Pathologic, and Epidemiologic Features

Alopecia areata (AA) is perhaps the most common autoimmune disease, affecting 5.3 million Americans including males and females across all ethnic groups (Petukhova et al., 2011), with a lifetime risk of 1.7% (Safavi et al., 1995). The most common presentation of AA is reversible loss of one or a few patches of hair called transient AA. More extensive persistent hair loss is classified as patchy persistent AA (Fig. 61.1). Loss of all hair of the scalp is called alopecia totalis (AT), and loss of all scalp and body hair is termed alopecia universalis (AU).



**FIGURE 61.1** Patchy persistent, extensive alopecia areata.

Loss of hair localized to the retroauricular area and occipital areas is called ophiasis and has a poor prognosis. Onset early in life, severe and long-lasting course (Chu et al., 2011), extensive disease, and associated nail dystrophy (pits, ridges, or trachonychia) also indicate poor prognosis.

In AA a mononuclear cell infiltrate attacks the hair follicle (HF) bulb, the factory which produces the hair shaft. T-cell (Tc) cytokines and cytotoxic Tcs produce cytotoxic damage (Whiting, 2003) and disrupt the normal function of the HF. This leads to the production of thin, fragile hairs, which easily break off or detach from the follicle and fall out. Since the hairs which fall out in AA are in actively growing anagen follicles, AA is classified as a form of anagen effluvium.

AA is commonly associated with atopy, especially in severe forms, where the incidence of atopy may be 50% or more (Barahmani et al., 2009). AA is highly associated with other autoimmune diseases, the most common being hypothyroidism, especially autoimmune thyroiditis (Goh et al., 2006). Because the immune damage is localized to the hair bulb, the stem-cell populations are preserved and normal cycling HF can be regenerated, and the regrowth of the hair can occur even years after total hair loss.

## Autoimmune Features

There are multiple observations supporting the concept that AA is an autoimmune disease:

1. AA HFs are surrounded by an immune infiltrate of activated T helper(h) cells, cytotoxic Tcs, natural killer (NK) cells, and a Th1-type cytokine profile (Petukhova et al., 2011).
2. The target of the immune response in AA appears to be the pigmented anagen HF. Anagen is the growing phase of the hair cycle. Neither resting HFs (telogen phase) nor white hair-containing follicles are affected in AA.
3. Autoantibodies are common both in human AA and in the C3H/HeJ mouse model of AA, and their specificities are quite different from the antibody specificities in human vitiligo (Cui et al., 1992; Tobin et al., 1997) where melanocytes are the targets. Antibodies in the C3H/HeJ mouse and in human AA appear to react to proteins of 40–46 kDa, possibly HF-specific keratins (Tobin et al., 1997).

4. Other candidate targets are trichohyalin, an HF keratin-associated protein, and keratin 16 (Tobin, 2003; Leung et al., 2010). Attempts to identify antibodies specific for HF melanocytes have not been successful.
5. Autoreactive Tcs and NK cells are necessary for hair loss in AA, as demonstrated in a xenograft model in which hair-bearing skin from AA patients is grafted onto the severe combined immunodeficiency mouse. This is followed by injection into the graft of leukocytes from the AA patient stimulated in vitro with various melanocyte antigens (Gilhar et al., 1998). In this model CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes transferred the disease after they were primed in vitro by the melanocyte antigens gp100, MART-1, or tyrosinase (Gilhar et al., 2001). In a report on this model leukocytes from AA patients were first stimulated with interleukin (IL)-2 (not melanocyte antigens), producing hair loss dependent on NK cells, CD8<sup>+</sup>NG2D<sup>+</sup>, and CD8<sup>+</sup>CD56<sup>+</sup> cells (Gilhar et al., 2012, 2013).
6. CD8<sup>+</sup> Tcs are necessary for local HF cytotoxicity, while CD4<sup>+</sup>CD25<sup>+</sup> Tcs can transfer the disease systemically in the C3H/HeJ mouse (McElwee et al., 2005).
7. The adhesion pair Cadm-1/class 1-restricted Tc-associated molecule (CRTAM)-1 appears to be necessary to mediate CD8<sup>+</sup> Tc cytotoxicity in AA. Cadm-1 is expressed by epidermal cells and mediates heterotypic adhesion to lymphocytes expressing the CRTAM.
8. The overexpression of Cadm-1 in a mouse model of alopecia favored the progression of alopecia (Giangreco et al., 2012).
9. It has been hypothesized that low expression of class I and II major histocompatibility complex (MHC) molecules in the HF, together with local expression of potent immunosuppressants, maintains a state of immune privilege of HF, in which the cytotoxic immune attack on the HF is suppressed (Ito et al., 2008). Several reports indicated that AA occurs because of the breakdown in this immune privilege in the follicle (Paus et al., 2003; Ito et al., 2008; Kang et al., 2010). According to this hypothesis, proinflammatory signals such as interferon (IFN)- $\gamma$  and substance P cause upregulation of class I MHC molecules and allow the presentation of previously sequestered HF antigens to preexisting autoreactive CD8<sup>+</sup> Tcs. Costimulatory signals from the CD4<sup>+</sup> Tcs and mast cells also facilitate the attack of lymphocytic infiltrates on the HF (Gilhar et al., 2012).
10. Whole genome gene expression analysis of 96 human scalp skin biopsy specimens from AA or normal control subjects revealed distinct expression clusters based on the presence or absence of disease as well as disease phenotype (patchy disease compared with AT or AU). This approach demonstrated the graded immune activity in samples of increasing phenotypic severity, leading to a quantitative gene expression scoring system (alopecia areata disease severity index (ALADIN)) that classified samples based on IFN and cytotoxic T lymphocyte immune signatures critical for disease pathogenesis (Jabbari et al., 2016).
11. High-throughput T cell receptor (TCR) sequencing in the C3H/HeJ mouse model of AA and in human AA patients revealed clonal CD8<sup>+</sup> Tc expansions in lesional skin (de Jong et al., 2018). In the C3H/HeJ model there was interindividual sharing of TCR $\beta$  chain protein sequences, which strongly support a model of antigenic drive in AA. The overlap between the lesional TCR repertoire and a population of CD8<sup>+</sup>NKG2D<sup>+</sup> Tcs in skin-draining lymph nodes identified this subset as pathogenic effectors. In AA patients the treatment with the oral janus kinase inhibitor tofacitinib resulted in a decrease in clonally expanded CD8<sup>+</sup> Tcs in the scalp. However, many expanded lesional Tc clones did not completely disappear from either skin or blood during the treatment with tofacitinib, which may explain in part the relapse of disease after stopping treatment (de Jong et al., 2018).

## Genetic Features

As the approaches to studying the genetics of AA have broadened from observed heritability in first-degree relatives, twin studies, and family-based linkage studies (Martinez-Mir et al., 2007) to genome-wide association studies or deep sequencing of key areas of the genome, it has been increasingly clear that genetic control of innate and acquired immunity is the most powerful factor in determining the susceptibility to all variants of AA, from patchy disease to AU. The two genome wide association study performed in AA to date (Petukhova et al., 2010; Jagielska et al., 2012) provided compelling evidence for the implication of numerous individual genes, most of them with immune function, and have established the major determinants of autoimmunity in AA (Table 61.1) (Petukhova et al., 2010).

The first metaanalysis in AA confirmed that the strongest region of association is the MHC, containing 4 well-mapped independent effects, all implicating human leukocyte antigen (HLA)-DR as a key etiologic driver.

**TABLE 61.1** Alopecia Areata Major Susceptibility Genes With Role in Innate or Acquired Immunity

Mechanism	Genes
<b>INNATE IMMUNITY</b>	
NK cell activation	NKG2D ligands: <i>ULBP6</i> , <i>ULBP3</i> , <i>MICA</i>
Cytokine	<i>IL-2</i> , <i>IL-21</i> , <i>IL2RA</i>
<b>ACQUIRED IMMUNITY</b>	
Antigen presentation	<i>HLA-DRA</i> , <i>HLA-DQA1</i> , <i>HLA-DQA2</i> , <i>HLA-DQB2</i> , <i>HLA-DOB9</i>
T cell proliferation	<i>CTLA4</i> , <i>ICOS</i> , <i>IL-21</i> , <i>IL-2</i> , <i>IL2RA</i> , <i>IKZF4</i> , <i>BTNL2</i>
T cell differentiation	<i>NOTCH4</i>
Tregs	<i>CTLA4</i> , <i>IKZF4</i> , <i>IL-2</i> , <i>IL2RA</i>
B cell proliferation	<i>IL13</i>
<b>END ORGAN</b>	
Antioxidant	<i>PRDX5</i>
Premature graying	<i>STX1</i>
With supposed immune function	<i>KIAA0350/CLEC16A</i>

NK, Natural killer; *IL*, interleukin; Tregs, regulatory T cells.

Outside the MHC, there were two novel loci that exceed statistical significance, containing acyl-CoA oxidase like/BCL2L11(BCL2-interacting protein) (2q13); GARP (LRRC32) (11q13.5), as well as a third nominally significant region SH2B3 (lymphocyte-specific adapter protein)/ATXN2 (12q24.12). Candidate susceptibility gene expression analysis in these regions demonstrates expression in relevant immune cells and the HF. This work uncovered new molecular pathways disrupted in AA, including autophagy/apoptosis, transforming growth factor - $\beta$ /regulatory T cells (Tregs), and JAK kinase signaling, and supported the causal role of aberrant immune processes in AA (Betz et al., 2015).

## In Vivo and In Vitro Models

Since AA is a complex immune process attacking the human anagen HF, and since the exact targets of autoimmune damage are not known, in vitro experiments based on single-cell populations have not been very useful. However, both naturally occurring and engineered animal models have been extremely useful in defining disease mechanisms in AA, in identifying potential drug targets, and more recently, in testing new drugs for future use in human experimentation.

The C3H/HeJ mouse has been an indispensable tool in defining the immunoregulatory AA genes (Sundberg et al., 2004). As in humans the alopecia developed in these mice is strongly IFN- $\gamma$  and IL-2 dependent (Freyschmidt-Paul et al., 2005) and is characterized by a diffuse inflammatory hair loss, which closely mimics in terms of histology, gene expression, and response to treatment, the human disease AA. The AA phenotype could be adoptively transferred to a whole colony of other syngeneic C3H/HeJ mice by transplanting involved alopecic skin, providing the ability to synchronize AA development in large cohorts of mice, and an exciting basic and clinical research platform (Sun et al., 2008). Newer engineered mouse models promise to validate Tc and NK cell roles in AA pathogenesis (Alli et al., 2012; Gilhar et al., 2012).

The xenograft mouse model using hairy skin from AA patients and autologous peripheral blood mononuclear cells (PBMC) sensitized in vitro has already been described in a review by Gilhar et al. (1998). This model has been instrumental in showing that melanocyte-specific CD4 $^{+}$ , CD8 $^{+}$ , and NK cells are effectors of hair loss in AA.

**TABLE 61.2** Inflammatory Response Underlying Alopecia Areata

Type of response	Cells and signaling molecules
Innate/NKG2D response	Alarms: hair follicle TNF- $\alpha$ , IL-15, IFN- $\gamma$ , MICA, UBLP-3, UBLP-6, Rae-1 First responders: DETC, NK, NKT, $\gamma$ /8T
APC/Sentinel	Cellular sentinels: Langerhans cells, dermal dendritic cells, dermal macrophage, mast cells Determinants: HLA, TAP, IFN- $\gamma$
Adaptive immunity	Immune cells: CD4+ T cells, CD8+ T cells, Tregs, B cells, CTLA4, ICOS, IL-2, IL-2R, IL-21 Cytokine network: IL-2, IL-6, IL-17, IL-21, IFN- $\gamma$ , IFN- $\alpha$ Downstream signaling pathways: JAK 1/2, Syk

NK, Natural killer; IL, interleukin; IFN, interferon; TAP, transporter associated with antigen processing; ICOS, inducible T cell co-stimulator; TNF, tumor necrosis factor; Tregs, regulatory T cells.

## Pathologic Effector Mechanisms

At the recent AA Research Summit “From Basepairs to Bedside: Innovations in the Immunology and Clinical Science of Alopecia Areata,” Raphael Clynes and Angela Christiano presented their recent investigations on the inflammatory cascade in AA. As summarized in Table 61.2, the effector cascade in AA encompasses danger signals, NK/innate immune first responders, antigen-presenting cell (APC)/sentinel presentation of HF antigens and adaptive responses dependent on the interplay of multiple T and B-cell populations, and a complex cytokine network (Petukhova et al., 2011). Their studies on gene expression in AA lesions show that 16 of the top 20 signals are IFN- $\gamma$  response genes.

Targeting the IFN- $\gamma$  response genes in AA with JAK inhibitors is one of the great clinical success stories in medicine. The impressive array of case reports and now ongoing clinical trials using multiple JAK inhibitors in AA are summarized in a recent review in the proceeding of the Montagna Symposium on the Biology of Skin (Wang et al., 2018).

## Autoantibodies as Potential Immunologic Markers

There is preliminary evidence that autoantibodies to hair keratins and trichohyalin are commonly found in AA serum (Tobin, 2003). Research is under way to use validated techniques to identify and quantitate autoantibodies in AA.

## Concluding Remarks—Future Prospects

Until recently, the treatments for AA have been inadequate. As stated in a previous Cochrane review, “There is no good trial evidence that any treatments provide long-term benefit to patients with alopecia areata, totalis and universalis” (Delamere et al., 2008). Initial studies of biologics that are highly effective in psoriasis were stunningly unsuccessful in AA. The National Alopecia Areata Foundation has organized a program to develop and test new treatments in the AA. This includes an intensive effort to design and validate a uniform clinical research platform for testing effectiveness of the new treatments. Multiple new approaches using drugs effective in other autoimmune diseases are being developed and deployed: anti-CTLA4, anti-JAK (1/2), anti-Syk, anti-IL-15, anti-NKG2D (Colonna et al., 2010; Petukhova et al., 2011).

It is unknown whether it is necessary to purposefully reactivate affected HF in AA.

Future research programs will determine the effectiveness of direct HF stimuli to reactivate hair growth in the AA.

## VITILIGO

### Clinical, Pathologic, and Epidemiologic Features

Vitiligo is the most common acquired type of leukoderma, causing significant social and psychological difficulties in people with darker skin phototypes. The hallmark of the disease is the white patch with well-

**TABLE 61.3** Classification of Vitiligo

Types and subtypes of disease	Features
1. NSV	
1.1. Generalized/vulgaris	Presents as multiple scattered lesions over the body
1.2. Acrofacial	Begins typically on the fingers and feet and around facial orifices
1.3. Universalis	Depigmentation over the whole body
2. SV	
2.1. Uni/plurisegmental	Follows a dermatomal distribution; stops at the midline; most common type in childhood
2.2. Mucosal	
2.3. Focal	One/few macules in one site. Lesions on multiple sites
3. Mixed	NSV + SV
4. Unclassified	
4.1. Focal	At onset; an earlier stage of generalized type
4.2. Multifocal asymmetrical NSV	Becomes generalized/mixed overtime
4.3. Mucosal	Lesions on one site

NSV, Nonsegmental vitiligo; SV, segmental vitiligo.

Modified from Taieb, A., Alomar, A., Böhm, M., Dell'Anna, M.L., De Pase, A., Eleftheriadou, V., et al., 2013. Guidelines for the management of vitiligo: the European Dermatology Forum consensus, In: Vitiligo European Task Force (VETF); European Academy of Dermatology and Venereology (EADV); Union Européenne des Médecins Spécialistes (UEMS). *Br. J. Dermatol.* 168, 5–19.

defined borders, affecting the skin and mucous membranes (Birlea et al., 2012). Based on the lesions' distribution, extension, and number, the disease is divided into nonsegmental vitiligo (SV) (NSV), SV, mixed, and unclassified (Taieb et al., 2013) (Table 61.3).

In general, NSV is characterized by symmetrical depigmentation (Fig. 61.2) with earlier onset and progression overtime. SV is characterized by a unilateral distribution that may be dermatomal, rapid in onset, and with the involvement of both epidermal and HF melanocytes (Taieb et al., 2013). Vitiligo often occurs in photodistribution and commonly affects body folds and periorificial areas. The most common triggers include sunburn, physical trauma, repeated rubbing of the skin (so-called Koebner phenomenon), psychological stress, and pregnancy. The clinical course of vitiligo is unpredictable; in general, NSV is gradually progressive, while the most SV cases rapidly stabilize.

In atypical cases of vitiligo, the pathologic exam of lesional skin is necessary to confirm the diagnosis. It shows an epidermal basal layer completely devoid of melanocytes in the center of the lesions (Le Poole et al., 1993a); cells expressing melanocyte markers are observed at the margins of lesions. In early vitiligo or in episodes of progression, the dermis at the margin of lesions may contain sparse perivascular and perifollicular infiltrates, consistent with the cell-mediated immune processes destroying melanocytes in situ (Kim et al., 2008).

Vitiligo affects all races and geographic areas with a frequency of 0.3%–0.5% (Birlea et al., 2012). Generalized vitiligo (GV), the most common subtype, occurs with equal frequency in males and females and may manifest at any time in life, with the average age of onset of 24 years (Spritz, 2010).

## Autoimmune Features

Several clinical and experimental observations support the autoimmune basis of vitiligo:

1. In GV subjects of white European ancestry, there is a 15%–25% incidence of at least one additional concomitant autoimmune disease, particularly autoimmune thyroid disease, pernicious anemia, rheumatoid arthritis, psoriasis, type 1 diabetes, Addison's disease, or systemic lupus erythematosus. These diseases also occur at increased frequencies in first-degree relatives of patients with GV (Spritz, 2010), indicating a heritable autoimmune diathesis in vitiligo. In contrast with GV, in SV the occurrence



**FIGURE 61.2** Generalized symmetric vitiligo of the anterior forearms with perifollicular repigmentation process (*brown dots*).

of other autoimmune diseases is uncommon (el-Mofty and el-Mofty, 1980; Koga and Tango, 1988; Park et al., 1988).

2. Circulating antimelanocyte antibodies (antityrosinase, antityrosinase-related protein-1, antidopachrome tautomerase) have been described in GV. Vitiligo antibodies were initially described by immunoprecipitation studies using melanoma cell extracts; they were most commonly directed against antigens with the molecular weights of 35, 40–45, 75, 90, and 150 kDa; the antigens of 35 and 90 kDa were preferentially expressed on melanocytes (Cui et al., 1992). Antigens of 45, 65, and 110 kDa have been identified in immunoblotting studies with melanocyte extracts (Hann et al., 1996b; Park et al., 1996), while the vitiligo-associated antibodies have been demonstrated to recognize melanoma cell proteins of 68, 70, 88, 90, 110, and 165 kDa (Hann et al., 1996a; Rocha et al., 2002). The other vitiligo-associated antibody targets have been reported (lamin A, MCHR-1, PMEL-17, SOX-9, SOX-10, tyrosine hydroxylase).
3. The presence of circulating skin-homing cytotoxic Tcs (Ogg et al., 1998) and infiltrates containing activated cytotoxic Tc and macrophages was described at the margins of active vitiligo lesions (Gross et al., 1987; Badri et al., 1993; Le Poole et al., 1993b, 1996). The Tcs were shown to target melanocyte-specific antigens [including Melan-A (MART-1), gp100 (Pmel-17), and tyrosinase] (Ogg et al., 1998; Lang et al., 2001; Palermo et al., 2001) and to express high levels of the skin-homing receptor cutaneous lymphocyte-associated antigen. The frequency of Tc correlated with both the extent and activity of the disease (Lang et al., 2001). In progressive disease the CD4/CD8 ratio was reported decreased among the skin-infiltrating Tcs, which exhibited a predominant Th1 cytokine secretion profile (Wankowicz-Kalinska et al., 2003). It is accepted that skin-homing, autoreactive, melanocyte-specific Tcs cause melanocyte destruction in vitiligo and that regulatory cells ineffectively restrain autoreactive Tcs in GV (Klarquist et al., 2010).
4. Recent evidence suggests that immune responses may also contribute to the pathogenesis of SV, in which the melanocyte loss, which remains more localized, seems to be related to a lymphocytic infiltrate of IFN- $\gamma$ -producing CD8 $^{+}$  and of some CD4 $^{+}$  Tcs found at the lesional margin of early stages, similar to those observed in GV (van Geel et al., 2011).

5. An immune mechanism in vitiligo was also implied by the experimental observations in occupational vitiligo ([Manga et al., 2006](#)). So-called chemical leukoderma can initially occur on upper extremities of subjects coming in contact with cleaning solutions containing phenolic compounds (such as 4-tertiary butyl phenol). It was hypothesized that these agents are directly toxic to melanocytes, in the context of a possible genetic susceptibility of patients' melanocytes to chemical injuries, which can lead to melanocyte death, release of intracellular proteins, and subsequent autoimmunity.
6. Vitiligo-like depigmentation occurring during IL-2 immunotherapy for malignant melanoma was proposed to follow an autoimmune mechanism. Since some melanoma-associated antigens (such as MART-1, gp100, and tyrosinase) are shared by normal melanocytes and melanoma; the onset of vitiligo suggests that the cytotoxic Tcs activated by IL-2 ([Feliciani et al., 1996](#); [Verheyen et al., 2001](#)) can also attack normal tissue ([Phan et al., 2001](#)), producing vitiligo-like depigmentation.
7. Lymphocyte-mediated destruction of melanocytes can occur in other nonvitiligo scenarios: (1) intratumor depigmentation during melanoma regression, (2) leukoderma acquisitum centrifugum around melanoma and nevi (the latter defining the "halo nevi" phenomenon), and (3) vitiligo-like depigmentation, observed at distant sites from primary melanoma ([Birlea et al., 2012](#)).
8. Immunosuppressive drugs may induce repigmentation in vitiligo. As such, topical calcineurin inhibitors (tacrolimus or pimecrolimus) are first-line therapy in vitiligo and exert an immunosuppressive effect on Tcs by blocking the action of the cytokine gene-activating cofactor calcineurin ([Homey et al., 1998](#); [Boone et al., 2007](#); [Hartmann et al., 2008](#)). Topical corticosteroids have antiinflammatory and immunosuppressive effects and are recommended typically in children and adults with SV or NSV of recent onset ([Abu Tahir et al., 2010](#); [Gawkroger et al., 2010](#)). Systemic corticosteroids, which represent a second-line alternative, can halt the progression of the rapid-spreading vitiligo. Unfortunately, well-established depigmented vitiligo lesions often do not repigment in response to immunosuppressives or corticosteroids. In these circumstances, ultraviolet light-based therapies are necessary to trigger repigmentation ([Fig. 61.2](#)), through a regenerative process involving HF melanocyte precursors ([Birlea et al., 2012, 2018](#)).

## Genetic Features

Epidemiologic studies showed that 15%–20% of the vitiligo patients have one or more affected first-degree relatives, although the most vitiligo cases occur sporadically. A single-locus Mendelian pattern of transmission was not supported for vitiligo. Most reports favored a polygenic, multifactorial model involving multiple genes and also environmental risk factors, features that define a "complex trait" ([Spritz, 2011](#)). In addition to the genetic factors, a considerable effect of nongenetic, environmental triggers has been implicated by the observation of a low concordance rate of 23% in monozygotic twins ([Alkhateeb et al., 2003](#)). Different earlier approaches, such as candidate gene association studies ([Birlea et al., 2011](#)) or genome-wide linkage studies ([Spritz, 2011](#)), tested the involvement of susceptibility genes in vitiligo pathogenesis, some of which could not be confirmed to date. More successful in gene identification were the genome-wide association studies; they have produced a rich yield of validated GV susceptibility genes that encode components of biological pathways reaching from immune cells to the melanocyte ([Birlea et al., 2010, 2013](#); [Jin et al., 2010a,b, 2012a](#); [Quan et al., 2010](#)). Among the most informative signals in Caucasians ([Jin et al., 2010a](#)) were those within MHC class I single-nucleotide polymorphisms rs12206499 which tags the HLA-A\*02a marker that was associated with a favorable response to melanoma therapy. Also significant, and within the tyrosinase gene locus, was the R402Q polymorphism which is associated with susceptibility to malignant melanoma ([Spritz, 2011](#)). Currently, the corresponding underlying causal variants for the two abovementioned signals have already been identified by next generation resequencing ([Jin et al., 2012b](#)).

## In Vivo and In Vitro Models

Several animal models of naturally occurring vitiligo (the Smyth line chicken, the gray horse, the vitiligo mouse, and the Sinclair swine) were identified and described ([Boissy and Lamoreux, 1988](#)). Of these, the Smyth line chicken has been studied extensively because it recapitulates the entire spectrum of clinical and biological manifestations of the human disease (mainly, an inherent defect in the melanocytes in feathers and ocular tissue, and an associated autoimmune response that eliminates pigment cells). A spontaneous mouse model for autoimmune vitiligo was created by introducing a human Tc receptor to human tyrosinase (h3T) into mice, combined

with transgenic expression of the associated human HLA-A\*0201 molecule (Mehrotra et al., 2012; Mosenson et al., 2013). The ensuing h3TA2 mice develop rapid, symmetrical, and progressive depigmentation of the pelage during adolescence.

Research in vitiligo using in vitro models has greatly advanced over the past two decades, providing a range of methodological options to study discrete mechanistic questions, as cited by Dell'anna et al. (2012):

1. Histological, immunofluorescence, and molecular techniques to test the destruction of melanocyte in vitiligo, and the migration of melanocytes to the interfollicular epidermis during vitiligo repigmentation.
2. Monolayer cell culture studies from vitiligo skin to characterize the response of vitiligo melanocytes to various pharmacological agents, and to study melanocyte differential susceptibility to noxious stimuli (UVB, cumene, hydroperoxide, and *tert*-butylphenol).
3. Three-dimensional skin models, such as reconstructed epidermis with keratinocytes and melanocytes on dead deepidermized dermis offer the possibility of reproducing the epidermal melanin unit.
4. The analysis of PBMC in vitiligo consists in characterization of the immunological status of such cells, their ability to recognize specific melanocyte antigens, their cytotoxic effects to melanocytes or melanoma cell lines, and their redox status and responses to DNA damage.
5. Computer simulation models where structural and functional features can be reproduced to define better the sequence of events that possibly lead to functional defects.
6. Fluorescence-based assays, such as microscopy and flow cytometry, represent easy methods to study some functional parameters and cell morphology.
7. A new mouse model of vitiligo has at last provided an experimental platform for demonstrating the molecular details of vitiligo pathomechanisms (Riding et al., 2018). This genetically engineered mouse has epidermal melanocytes that can be killed by immune mechanisms like those in human vitiligo. Adoptive transfer of melanocyte-specific CD8+ Tcs into recipient mice and the subsequent activation of these Tcs using a viral vector produces depigmentation of the epidermis within 5–7 weeks in a patchy pattern similar to patients with vitiligo.
8. New understanding of the mechanisms of vitiligo repigmentation has been possible using a new research platform using narrow band UVB to repigment human vitiligo skin (Goldstein et al., 2015, 2016). This model of repigmentation of human vitiligo by narrow band ultraviolet B treatment is associated with proliferation, migration, and differentiation of melanocyte precursors in the HF bulge, infundibulum, and interfollicular epidermis. The primary melanocyte germ was present in the untreated and treated HF bulge, whereas a possible secondary melanocyte germ composed of C-KIT+ melanocytes was found in the infundibulum and interfollicular epidermis of UV-treated vitiligo. This is an exceptional model for studying the mobilization of melanocyte stem cells in human skin. The improved understanding of this process is essential for designing better treatments for vitiligo, ultimately based on melanocyte stem-cell activation and mobilization (Birlea et al., 2018).

Using careful microdissection and RNA-sequencing to measure gene activation, it has been shown that the repigmentation of human vitiligo skin by narrow band ultraviolet B is controlled by transcription of GLI1 and activation of the  $\beta$ -Catenin pathway in the HF bulge stem cells (Goldstein et al., 2018).

## Pathogenetic Mechanism

One of the most important factors in vitiligo pathogenesis is the unique nature of the human melanocyte as a target of cytotoxic damage. Melanocytes are factories for melanin, a heterogeneous protein product that absorbs light over a broad range of wavelengths. The melanization apparatus is enclosed in the melanosome, an organelle that separates the toxic intermediates of melanization from the cytoplasm of melanocytes. These melanocytes are transferred to surrounding keratinocytes to produce an intact pigment network. Melanocytes are terminally differentiated cells of neuroendocrine lineage. They have limited regenerative capacity and are highly resistant to apoptotic cell death because of high expression of Bcl-2, Bcl-X, and Mcl-1 (Bowen et al., 2003). As discussed in the "Autoimmune features" Section, melanocyte-specific Tc can cause the progressive depigmentation seen in vitiligo (van den Boorn et al., 2009). Moreover, vitiligo-associated antibodies are able to destroy melanocytes and melanoma cells in vitro and in vivo by complement-mediated damage and antibody-dependent cellular cytotoxicity (Fishman et al., 1993; Gilhar et al., 1995; Norris et al., 1988a; Gottumukkala et al., 2006). However, the current opinion is that these autoantibodies reflect a humoral response secondary to melanocyte destruction,

rather than a primary cause of GV (Kemp et al., 2007). There is also extensive evidence that other mechanisms participate in the development and progression of disease and the induction of autoimmunity (Le Poole et al., 1993a; Norris et al., 1994). In vitro studies have connected cellular oxidative stress (Schallreuter et al., 1991, 2001, 2005; Dell'anna and Picardo, 2006) with the immune response in vitiligo; stressed melanocytes were found to mediate dendritic-cell activation with the consequent dendritic cell effector functions playing a role in the destruction of melanocytes (Kroll et al., 2005). These experimental observations suggest that, like other autoimmune diseases, intrinsic damage to melanocytes could be the initiating event in vitiligo, followed by a secondary immune response by Tc lymphocytes which exacerbates the destruction of melanocytes (Le Poole and Luiten, 2008; Hariharan et al., 2010; van den Boorn et al., 2011).

The observation that depigmented lesions develop at a site previously exposed to a physical trauma (Le Poole and Luiten, 2008) suggests that immune response follows melanocyte damage. Cryptic epitopes of several vitiligo-associated intracellular melanocyte autoantigens such as tyrosinase and gp100 could be released during apoptosis and lead to the Tc activation (Namazi, 2007; Westerhof and d'Ischia, 2007; Kemp et al., 2007). Antibodies could then be produced following the stimulation of B lymphocytes by the activated helper Tc (Namazi, 2007); the activated Tc could directly attack melanocytes that express antigenic peptides on their surface in the context of MHC class I molecules (Le Poole et al., 1993b; Hedley et al., 1998; Kemp et al., 2007). The selective destruction of melanocytes in vitiligo might occur because they are intrinsically more sensitive to immune-mediated injury than other skin cells (Norris et al., 1988b).

The use of a new genetically engineered mouse that closely mimics human vitiligo has demonstrated that autoimmunity in vitiligo is driven by the IFN- $\gamma$ -CXCL10 cytokine signaling pathway. Activated melanocyte-specific CD8+ Tcs secrete IFN- $\gamma$ , which signals through the IFN- $\gamma$  receptor to activate JAK1/2 and STAT1. This induces the production of CXCL9 and CXCL10, which signal through their receptor CXCR3 to recruit more auto-reactive Tcs to the epidermis, resulting in widespread melanocyte destruction. Targeting this cytokine pathway represents an emerging treatment strategy for vitiligo (Rashighi and Harris, 2017; Richmond et al., 2017).

There is great enthusiasm to use inhibitors of these mechanistic pathways to treat vitiligo, following the lead of the application of JAK inhibitors to treat AA. The initial experience with one JAK inhibitor suggests that ultraviolet radiation might be necessary as a partner treatment with the JAK inhibitors to treat vitiligo (Liu et al., 2017). Other treatments such as IL-15 inhibition are also under active consideration, based on the mechanistic studies done using this new engineered mouse model (Richmond et al., 2017).

## Autoantibodies as Potential Immunologic Markers

The use of melanocyte antibodies as markers of vitiligo activity is a controversial subject. While a recent study reported no correlation between the presence of antibodies and recent disease activity or different clinical parameters (e.g., age, gender, extension, and duration of vitiligo) (Kroon et al., 2012), other reports found the incidence and/or level of melanocyte antibodies linked to the activity and extent of vitiligo (Naughton et al., 1986; Aronson and Hashimoto, 1987; Hanning et al., 1991; Yu et al., 1993; Kemp et al., 2007).

Following treatment with systemic steroids, a reduction in antimelanocyte antibody levels and in antibody-mediated antimelanocyte cytotoxicity has been reported (Hann et al., 1993; Takei et al., 1984). After therapy with psoralen and ultraviolet A (PUVA), decreased expression of vitiligo-associated melanocyte antigens was noted, leading to a blocking of antibody-dependent cell-mediated cytotoxicity against melanocytes (Kao and Yu, 1992; Viac et al., 1997). The presence or levels of antimelanocyte antibodies have not yet been effectively developed as diagnostic tools or markers of disease activity.

## Concluding Remarks—Future Prospects

Vitiligo has been observed and treated since antiquity, but it still remains an enigmatic disorder in which combined genetic and nongenetic factors can cause the autoimmune-mediated destruction of melanocytes from epidermis, but often sparing HF melanocytes. The treatment in vitiligo must consider two distinct aspects: (1) limitation of the immune process which halts the progression of depigmentation and (2) stimulation of repigmentation of vitiligo lesions by mobilizing the stem-cell populations to proliferate, migrate, and differentiate to regenerate the interfollicular epidermal melanocytes. The effective treatment of vitiligo involves topical medications and phototherapy to both suppress the immune response and to activate melanocyte regeneration from HF reservoirs of pluripotent stem cells (Birlea et al., 2012). New research initiatives are focusing on the

repopulation of affected interfollicular epidermis with melanocyte precursors from the bulge region of the HF. This process is a classic example of regenerative medicine, based on stem-cell activation. The key to improving therapeutic approaches for vitiligo is targeted stimulation of the principal repigmentation genes/proteins or designing new compounds which are potent and selective inducers of melanocyte activation, migration, and differentiation.

## PSORIASIS

### Clinical, Pathologic, and Epidemiologic Features

Psoriasis is a common, chronic, immune-mediated inflammatory skin disease with polygenic inheritance, characterized by erythematous scaly plaques that can range from a few scattered lesions to the involvement of the entire body surface. Psoriasis may progressively worsen with age or wax and wane in severity (Stern et al., 2004; Gelfand et al., 2005; Kurd and Gelfand, 2009; Parisi et al., 2013). The disease prevalence is 2%–4% of the population in Western countries (Parisi et al., 2013), ranging from 0.91% (United States) (Robinson et al., 2006) to 8.5% (Norway) (Bø et al., 2008). Psoriasis can present at any age (see Table 61.4); type I psoriasis presents in younger patients and has a poor prognosis, while type II psoriasis presents in elderly patients and is easier to control. When psoriasis has extensive distribution, especially involving the groin and hands or feet, it produces

**TABLE 61.4** Classification of Psoriasis

Types and subtypes of disease	Features
1. Plaque psoriasis/vulgaris type	Sharply circumscribed, round–oval, or nummular (coin-sized) plaques Plaques occur on elbows, knee, trunk, back Most common type, occurring in 85%–90% of patients
2. Guttate psoriasis	Small, 2–10 mm diameter; acute onset, centripetal distribution Lesions occur shortly after an acute streptococcal infection of the pharynx or tonsils Common in children; occasionally in adults
3. Flexural (inverse) psoriasis	Affects the flexures (inframammary, perineal, and axillary) Lesions devoid of scale appear as red, shiny, well-demarcated plaques Differential diagnosis: candidal intertrigo, dermatophyte infections
4. Erythroderma	Total or subtotal involvement of the body skin
a. Chronic plaque psoriasis	May impair the thermoregulatory capacity of the skin, leading to hypothermia, high output cardiac failure, and metabolic changes
b. Unstable psoriasis	a. Lesions gradually progress as plaques and become confluent and extensive b. Precipitated by infection, tar, drugs, withdrawal of corticosteroids; becomes rapidly extensive
5. Generalized pustular psoriasis (von Zumbusch)	Monomorphic, sterile pustules, which may coalesce to form sheets Active, unstable disease; sometimes requires hospital admission Precipitated by withdrawal of systemic or potent topical corticosteroids and by infections
6. Palmoplantar pustulosis	Sterile, yellow pustules on erythematous-squamous background Frequently with nail involvement Prevalent in women, in the fourth to sixth decade, and in smokers
7. Psoriatic nail disease	Small pits, onycholysis, “oil spots” thickening, dystrophy, discoloration, subungual hyperkeratosis



**FIGURE 61.3** Typical plaque of psoriasis.

significant morbidity and a decrease in the quality of life (Nestle et al., 2009). The prototypic lesion is plaque psoriasis, characterized by raised, well-demarcated, erythematous, oval plaques with adherent silvery-white, dry scales (Fig. 61.3). The histopathology of psoriasis characteristically shows a thickened epidermis with an expanded proliferative compartment, premature maturation of keratinocytes, and incomplete cornification with retention of nuclei in the stratum corneum (parakeratosis) (Nestle et al., 2009). The dermal inflammatory infiltrate consists mainly of dendritic cells, macrophages, and Tcs, while the epidermis contains neutrophils and some Tcs. Erythema in lesions is due to the increased numbers of tortuous capillaries in the rete ridges, covered by a very thin epidermis (Nestle et al., 2009). Psoriasis may be triggered by trauma or injury (Koebner's phenomenon), infections (Leung et al., 1995, 1998), xerosis, and reactions to various drugs (Gudjonsson and Elder, 2008). Psoriatic arthritis presents as distal interphalangeal disease, axial arthritis, or symmetrical arthritis (Winchester, 2008). Nail involvement may be present, particularly associated with arthritis. The various types and presentations of psoriasis are outlined in Table 61.4 (Langley et al., 2005), and guttate and pustular psoriasis are presented in Figs. 61.4 and 61.5.

## Autoimmune Features

It is now generally accepted that psoriatic lesions are caused by abnormal reactivity of specific Tcs in the skin. It is also accepted that alterations in the epidermal barrier, in innate immune defenses, and in processing of inflammatory signals may all contribute to the triggering of nonspecific innate immune mechanisms. The effector mechanisms in the epidermis include the increased production of IL-23 and Th1- and Th17-type cytokines (Gudjonsson and Johnston, 2009).

The concept of an autoimmune basis for psoriasis notwithstanding the presence of a dysregulated immune system (Nestle et al., 2009) is supported by the following:

1. Increased numbers of immune cells (mainly dendritic and Tcs) within the lesions, and the appearance of oligoclonal Tcs in lesions overtime.
2. Oligoclonal Tc activation in psoriatic lesions (Lin et al., 2001).
3. Efficacy of Tc-targeted treatments (cyclosporine, efalizumab, alefacept) (Lowes et al., 2007), drugs that inhibit Th1 and Th17 cells and their cytokines (ustekinumab), or therapies that are thought to target the adaptive immune system rather than modify keratinocyte function (corticosteroids, ultraviolet B (UVB)) (Schwarz, 2008; Bergboer et al., 2012).
4. Genetic associations that are largely immunologic (discussed next).
5. Xenograft animal models (Bergboer et al., 2012) that have focused on the functional role of Tcs and cytokines (Nestle et al., 2009).
6. The observation that bone marrow transplantation into psoriasis patients may cure the disease and that psoriasis can be transferred from a psoriatic transplant donor to the recipient (Nestle et al., 2009).



**FIGURE 61.4** Guttate psoriasis of the trunk.



**FIGURE 61.5** Pustular psoriasis of the neck and upper chest.

However, as yet there is no convincing evidence identifying the specificity of autoreactive Tc clones in psoriasis. Although it has been proposed that CD4 Tc activation initiates psoriasis and CD8 intraepidermal Tcs cause lesion persistence, there is no evidence that these cells are activated by self-antigens (Lin et al., 2001). Many of the triggers for psoriasis are not antigen specific (Leung et al., 1993, 1998), and several of the most effective treatments such as antitumor necrosis factor (TNF) biologics may affect both innate and acquired immunity as well as inflammation in general. It has been proposed that psoriasis is a hybrid of autoimmune and autoinflammatory diseases (Liang et al., 2017).

## Genetic Features

The importance of a hereditary component in the pathogenesis of psoriasis has been established through the population-based studies (Gudjonsson and Elder, 2008). They indicate that the incidence of psoriasis is greater among first-degree and second-degree relatives of patients than among the general population (Nestle et al., 2009). Twin studies showed a concordance rate of 35%–72% in monozygotic twins and 12%–23% in dizygotic twins (Bowcock and Krueger, 2005). About 71% of the patients with childhood psoriasis report a positive family history (Morris et al., 2001). In the past decades, several linkage studies and genome-wide association studies (Bergboer et al., 2012) identified susceptibility loci for psoriasis. Genome-wide linkage analysis has identified at least nine chromosomal loci with statistically significant linkage to psoriasis (called PSORS1–PSORS9) (Nestle et al., 2009). Genomic signatures in psoriatic lesions point to dendritic cells as a key cell type, and IFN- $\gamma$  and TNF- $\alpha$  as key cytokines; this reinforces the concept that cells and mediators of the immune system have essential roles in susceptibility to and maintenance of psoriasis (Nestle et al., 2009). The psoriasis genome-wide association signals confirmed (Bergboer et al., 2012) are listed in Table 61.5.

## In Vivo and In Vitro Models

### ***In Vivo Models***

#### **SPONTANEOUS MOUSE MODELS**

A number of spontaneous mouse mutations give rise to psoriasiform inflammatory and scaly phenotypes (Sundberg and King, 1996; Raychaudhuri et al., 2001), which usually represent only a limited set of psoriatic features (Gudjonsson et al., 2007; Nestle et al., 2009).

#### **CONSTRUCTED MOUSE MODELS**

Over the last four decades, more than 40 unique mouse models for psoriasis have been described or constructed. These models can be categorized into three major types: acute (inducible), genetically engineered (transgenic), and xenograft (humanized) (Hawkes et al., 2018a,b).

#### **ACUTE MODELS**

Since the initial description of the imiquimod (IMQ)-induced psoriasiform dermatitis model (van der Fits et al., 2009), acute or inducible mouse models have rapidly emerged as one of the most widely used systems for studying human psoriasis, in spite of serious reservations about how valid a facsimile the IMQ model really is.

#### **GENETICALLY ENGINEERED MOUSE MODELS**

Transgenic mice have been generated that overexpress adhesion molecules, cytokines, transcription factors, and inflammatory mediators in both keratinocytes and immunocytes to define the important cell types in psoriasis disease initiation. Epidermal overexpression of molecules under the control of promoters in the epidermis (e.g., keratin (K)5, K14, and K10 or involucrin) induces the development of a psoriasis-like disease in several mouse models. The K5-STAT3C mouse develops a psoriasis-like disease, with keratinocyte hyperplasia, loss of stratum granulosum, and parakeratosis.

Knockout or hypomorphic mice, in which a specific genetic element has been removed or attenuated, have also been used to study a given mediator in psoriasis. Mice develop a condition that resembles psoriatic skin disease after *ITGB2*, *ITGAE*, *IL1RN*, and *IRF2* genes are knocked out (Wagner et al., 2010).

**TABLE 61.5** Top Psoriasis Signals With Immune Function

Putative biological pathway	Gene/locus
Adaptive immunity	<i>IL23R</i> , <i>ERAP1</i> , <i>IL-12B</i> , <i>TNF</i> , <i>TRAG3IP2</i> , <i>IL-4</i> , <i>IL-13</i> , <i>IL-23A</i> , <i>ZNF313/RNF114</i> , <i>HLA-C</i>
Barrier skin function	<i>LCERB</i> , <i>LCE3C</i> , <i>CDSN</i> , <i>DEFB</i> , <i>GJB2</i>
Innate immunity	<i>IF1H1</i> , <i>REL</i> , <i>TNIP1</i> , <i>TNFAIP3</i> , <i>IL-28RA</i> , <i>NFKBIA</i> , <i>FBXL19</i> , <i>NOS2</i> , <i>TYK2</i>

*CDSN*, corneodesmosin; *DEFB*, defensin beta; *IL*, Interleukin; *NFKBIA*, NF-kappa-B inhibitor alpha; *TNF*, tumor necrosis factor.

## XENOGRAFT MODELS (Nestle et al., 2009; Gudjonsson et al., 2007)

Psoriasis-like findings were observed in *Prkdc<sup>scid</sup>* mice receiving CD4<sup>+</sup>-CD45-RBhi Tcs from donors who were MHC matched but mismatched for minor histocompatibility antigens (Schön et al., 1997). Humanized xenotransplantation models most closely resemble psoriasis. Their advantage is that both human skin and immune cell infiltrate are transplanted and can be studied directly.

### In Vitro Models

Because of the complex nature of psoriasis, in vivo models are the preferred platform for investigation of most aspects of disease. However, in vitro studies have been useful in isolating infiltrating lymphocytes from lesional areas, in studying selected functions in psoriatic keratinocytes, and showing the effects of deepidermized psoriatic dermis on the disease process.

### Pathogenic Mechanism

The mechanism of evolution of a psoriatic lesion from initiation to persistence of disease has been thoroughly studied (Nestle et al., 2009, Hawkes et al., 2018a,b).

1. Molecular control of inflammation in psoriasis centers on the IL-23/IL-17 pathway, and the most effective modern therapies for psoriasis target some specific component of this pathway (Hawkes et al., 2018a,b).
2. Initial triggers (e.g., physical trauma or bacterial products) start a cascade of events that include the formation of DNA-LL-37 complexes, activation of plasmacytoid dendritic cells, and secretion of the cytokine IFN- $\alpha$  (both found increased in early psoriatic lesions). Clinical observation points to an important role of IFN- $\alpha$  as an inducer of psoriasis (Funk et al., 1991).
3. Psoriatic keratinocytes are a rich source of antimicrobial peptides, including LL-37,  $\beta$ -defensins, and psoriasin. In addition, keratinocytes are responsive to key dendritic cell-derived and Tc-derived cytokines, including IFNs, TNF, IL-17, and IL-20, and in turn will produce.
4. Proinflammatory cytokines including IL-1, IL-6, and TNF- $\alpha$ .
5. Myeloid dermal dendritic cells are increased in psoriatic lesions and induce proliferation of Tcs and production of Th1 cytokines. The activated myeloid dendritic cells migrate into draining lymph nodes and stimulate differentiation of naïve Tcs into effector cells, Th17 or type 17 cytotoxic Tcs17, Th1, or Tc1 (Nestle et al., 1994). They have proinflammatory effects, producing TNF- $\alpha$  and nitric oxide synthase (NOS) (Lowes et al., 2005).
6. Effector cells recirculate and slow down in skin capillaries in the presence of selectin- and integrin-guided receptor-ligand interactions. Immune cells expressing the chemokine receptors CCR6, CCR4, and CXCR3 emigrate into skin tissue along these chemokine gradients. The key processes during disease maintenance are the presentation of putative autoantigens to Tcs and the release of IL-23 by dermal dendritic cells, the production of proinflammatory mediators such as TNF- $\alpha$  and nitric oxide (NO) by TNF- $\alpha$  and inducible NOS-producing dendritic cells, and the production of IL-17 and IL-22 by Th17 and Tc17 cells, and of IFN- $\gamma$  and TNF- $\alpha$  by Th1 and Tc1 cells. These mediators act on keratinocytes, leading to the activation, proliferation, and production of antimicrobial peptides (e.g., LL-37, cathelicidin, and  $\beta$ -defensins), chemokines (e.g., CXCL1, CXCL9–CXCL11, and CCL20), and S100 proteins by keratinocytes. Dendritic and Tcs form perivascular clusters in the presence of CCL19 produced by macrophages. An essential event is the migration of Tcs from the dermis into the epidermis, controlled through the interaction of  $\alpha 1\beta 1$  integrin (very late antigen 1) on Tcs with collagen IV at the basement membrane.
7. NK Tcs contribute to the disease process.
8. Feedback loops involving keratinocytes, fibroblasts, and endothelial cells contribute to tissue reorganization with endothelial cell activation and proliferation and deposition of extracellular matrix. Neutrophils in the epidermis are attracted by chemokines, including CXCL8 and CXCL1.
9. Studies have indicated a defect in the overall suppressive activity of Tregs (Sugiyama et al., 2005), with decreased contraregulation of the proinflammatory state (Nestle et al., 2009).

### Autoantibodies as Potential Immunologic Markers

Autoantibodies to keratin-associated intermediate filaments (Shigenobu et al., 1989), to U1 and U2 small nuclear ribonucleoproteins (Reeves et al., 1986), to calpastatin (Matsushita et al., 2005), and to other epidermal proteins have been described in psoriasis, but none has proven reliable as a biomarker of disease activity or has been accepted as a participant in disease pathogenesis.

## Concluding Remarks—Future Prospects

Psoriasis is a common inflammatory skin disease of undetermined etiology in which an autoimmune basis is suspected but is far from established. There is no cure, although multiple treatments can produce worthwhile disease remissions (UV light phototherapy, methotrexate, biologics, and small molecules); all of the treatments that induce remissions decrease the population of activated Tcs within the lesions. This heterogeneous, cutaneous, inflammatory disorder is histopathologically characterized by prominent epidermal hyperplasia and a distinct inflammatory infiltrate. Cross talk between immunocytes and keratinocytes, which results in the production of cytokines, chemokines, and growth factors, is thought to mediate the disease. Given that psoriasis is only observed in humans, numerous genetic approaches to model psoriasis in mice have been undertaken (Wagner et al., 2010). Existing and new mouse models are needed in order to dissect this complex disease and to provide novel insights into the molecular mechanisms and pathways that incite the disease initiating events, and also those which are responsible for recurrences following the successful treatment. An impressive array of modern biological therapies and small molecules are already providing remarkable clinical responses. However, the high cost of these treatments, the loss of effectiveness with time, and uncommon but dangerous side effects compel us to seek better treatments. It is also important to seek alternative treatments that can maintain disease remissions achieved by modern biology therapies combination treatments intended to reduce the immune activation and angiogenesis in psoriasis and to restore differentiation of keratinocytes in a disease stage-dependent manner will be beneficial.

## CHRONIC URTICARIA

### Clinical, Pathologic, and Epidemiologic Features

Chronic urticaria (CU) is a relatively common type of urticaria, defined as hives lasting longer than 6 weeks (Kaplan, 2004). It affects around 0.1% of the population including both adults and children (Schocket, 2006), with a higher predilection in women than in men (2:1), and with significant adverse effects on quality of life (Schocket, 2006). CU is currently divided into three subcategories (Greaves and Tan, 2007): (1) urticarial vasculitis (leukocytoclastic vasculitis of the small vessels with hives duration of more than 24 hours which can accompany autoimmune rheumatic diseases, inflammatory bowel disease, viral hepatitis, and paraproteinemia); (2) physical urticarias with several subtypes, for example, solar, cold, aquagenic, delayed pressure, vibratory, cholinergic, and dermographism (these are commonly referred to as chronic inducible urticaria—CindU); and (3) chronic autoimmune urticaria (which is caused by anti-high-affinity receptor for the Fc region of immunoglobulin E (Fc $\epsilon$ RI) antibodies, and less often by anti-IgE antibodies).

Chronic spontaneous urticaria (with more than 6 weeks' duration of symptoms) is a subtype of urticaria in which there is no identifiable cause (Zuberbier, 2012). Spontaneous urticaria is about three times as frequent as CindU.

The characteristic clinical lesion of both acute urticaria and CU is the hive; hives present as pruritic, erythematous, blanching, circumscribed macular, or raised lesions, involving the superficial layers of the skin (Joint Task Force on Practice, 2000). Up to 40% of the CU patients have associated angioedema (swelling of the deeper structures) (Kaplan, 2004). Generally, an individual CU lesion does not persist in the same location for greater than 24 hours (Joint Task Force on Practice, 2000). The untreated CU should virtually always be associated with pruritus. In the absence of pruritus the clinician should consider alternative diagnoses. A thorough history is critical to identifying known causes of urticaria.

CU is diagnosed clinically. The patient history is critical in making the diagnosis, identifying a potential trigger, and ruling out other diseases. For both acute and CU, important specific points of history include physical triggers, recent changes in medications, recent travel, infections (including parasitic), history of atopy, and a complete review of systems. Additional dermatologic conditions should be considered in the differential diagnosis: erythema annulare centrifugum, erythema chronicum migrans, and erythema multiforme.

The principal histologic finding in CU is dermal edema. There is variable cellular infiltrate around vessels, with the predominance of neutrophils and eosinophils in early lesions and of lymphocytes accompanied by neutrophils and eosinophils in advanced lesions (Lee et al., 2002).

## Autoimmune Features

The presence of autoimmunity in a significant proportion of CU patients was initially suggested by the demonstration of skin reactivity to the injection of the patient's own serum, the so-called autologous serum skin test (ASST) (Grattan et al., 1986, 1990). Later, it was shown that the serum and purified IgG of a subset of patients with chronic idiopathic urticaria release histamine from basophils and dermal mast cells when incubated with leukocytes prepared from peripheral blood from the two healthy human donors. This release could be inhibited by preincubation with the recombinant Fc $\epsilon$ R1 $\alpha$ , the  $\alpha$ -chain of the high-affinity IgE receptor. In a small proportion of patients (5%) the histamine-releasing factor was inhibited not by the  $\alpha$ -chain of Fc $\epsilon$ R1 but by IgE itself (Hide et al., 1993; Niimi et al., 1996).

CU has been shown to be associated with an autoimmune mechanism in 30%–50% of idiopathic urticaria cases. In addition, other concomitant autoimmune disorders including autoimmune thyroid disorders, rheumatoid arthritis, type 1 diabetes mellitus, celiac disease, Sjögren's syndrome, and systemic lupus erythematosus were significantly more prevalent in patients with CU when compared to the general population (Confino-Cohen et al., 2012). Moreover, in the same population, CU patients showed a higher prevalence of other serologic markers of autoimmunity, notably rheumatoid factor and antinuclear antibodies, as compared with the non-CU controls (Confino-Cohen et al., 2012). The association of CU with thyroid autoimmunity was described earlier (Leznoff et al., 1983), followed by the observation that suppression of thyroid activity results in CU remission (Schocket, 2006).

The presence of an inflammatory perivascular infiltrate in many CU patients also supports the immune basis of this disease. This infiltrate is predominantly composed of T lymphocytes, specifically CD4+, T helper cells, and a small number of CD8+ cytotoxic Tcs (Mekori et al., 1983).

## Genetic Features

Autoimmune CU is a complex multifactorial disease with probably genetic and environmental components. CU clusters in families, being more frequent among the first-degree relatives of the affected individuals than in the general population (Asero, 2002). There is a paucity of literature on the proposed genetic susceptibilities, mainly consisting of small candidate genes studies, of which results are still unconfirmed. These studies targeted protein tyrosine phosphatase-22 (Brzoza et al., 2012), formyl peptide receptor-like 1 (Yang et al., 2010), HLA-DRB1, and HLA-DQB1 (Chen et al., 2005).

## In Vivo and In Vitro Models

While it is understood that autoimmune mechanisms are responsible for a subset of CU, no single test is diagnostic, and therefore all require clinical correlation. Biopsy and histologic evaluation are only valuable to confirm features consistent with urticaria when the diagnosis is in doubt, or when alternative diagnoses are being strongly considered. While there is evidence suggesting the presence of immunoglobulin G (IgG) directed against both the IgE receptor  $\alpha$  subunit and the Fc region of IgE (Sabroe and Greaves, 2006), there remains significant debate as to the clinical utility of the available testing modalities. One of the earliest tests performed on patients with CU is the ASST. This involves intradermal injection of autologous serum on the volar surface of an unaffected arm along with positive and negative controls (typically saline and histamine).

The test is read 30 minutes later and is considered positive if the wheal at the serum site is 1.5 mm greater than that of the wheal at the negative control. The sensitivity was calculated at 65%–81% and specificity 71%–78% (Sabroe et al., 1999). Other authors have shown unacceptably high false-positive reactions in healthy controls with the ASST as high as 56% (Taskapan et al., 2008).

The other testing modalities including basophil activation and commercial assays for detecting anti-Fc $\epsilon$ R1 $\alpha$  antibodies (sometimes referred to as the CU index) are available, although there remains much debate regarding their clinical utility. More recently, an assay focusing on a unique connective tissue mast cell line has been proposed (Posthumus et al., 2012).

Animal models for contact urticaria were previously described; however, a suitable model for CU has not been reported so far.

## Pathologic Effector Mechanisms

Histamine-releasing IgG autoantibodies directed against both the IgE receptor  $\alpha$  subunit and the Fc region of IgE remain the basis of the autoimmune subset within CU (Hide et al., 1993; Niimi et al., 1996; Kaplan 2004; Mlynek et al., 2008). It was also suggested that the sera from patients with CU containing Fc $\epsilon$ RI $\alpha$  antibody release mediators and TNF- $\alpha$  by activating human foreskin mast cells (Lee et al., 2002). The mast cells are considered to be the primary effector cells in CU, by releasing a variety of inflammatory mediators such as leukotrienes, tryptase, prostaglandins, histamine, IL-1, IL-6, IL-8, and TNF- $\alpha$  (Lee et al., 2002), which regulate the emigration of leukocytes; these mediators induce a sequential upregulation of endothelial adhesion molecules (P-selectin, E-selectin, intercellular adhesion molecules 1) and vascular cell adhesion molecule-1 (Lee et al., 2002), which facilitate the binding of the activated leukocytes to endothelial cells and then transmigrate into tissues. Recent literature has suggested alternative mast cell–activating factors relate to the coagulation cascade. The activation of the classical complement pathway and formation of C5a are important for dermal mast-cell activation and for neutrophil and eosinophil chemoattraction (Greaves and Tan, 2007). Autologous sera from some patients with CU retain the ability to induce a wheal-and-flare reaction when injected intracutaneously even after depletion of IgG (Mlynek et al., 2008). Other studies have demonstrated that thrombin causes edema development by both direct endothelial and indirect inflammatory mediator mechanisms (Asero et al., 2006, 2007a,b). This effect is reduced by antihistamines and completely absent if mast-cell granules are eliminated. One recent study showed high serum levels of the Th17 cell profile of cytokines including IL-17, IL-23, and TNF- $\alpha$  among the chronic spontaneous urticaria patients (Atwa et al., 2013). Other recent works have focused on a possible hormonal component given that CU is twice as prevalent in women. To date, there is no evidence to suggest a hormonal mechanism.

## Autoantibodies as Potential Immunologic Markers

Numerous attempts to develop immunoassays that measure serum levels of IgG and anti-Fc $\epsilon$ R1 or anti-immunglobulin E (IgE) antibodies in urticaria, although successful, have shown low specificity and poor correlation with in vitro serum histamine release and disease activity (Greaves and Tan, 2007).

However, specificity of IgE in spontaneous CU is producing important results. IL-24 is a common and specific autoantigen of IgE in patients with chronic spontaneous urticaria (Schmetzler et al., 2018). In chronic spontaneous urticaria, IgE against staphylococcal enterotoxins is common and functional (Altrichter et al., 2018).

## Concluding Remarks—Future Prospects

Regardless of the mechanism, the first-line treatment for CU remains H1 antihistamines. The second-line treatments include addition of an H2 antihistamine, or of a leukotriene modifier (Tilles, 2005). Using oral glucocorticoids is a common practice for refractory symptoms. In light of the likely adverse effects with long-term use, most clinicians recommend using them for short periods (usually 3–7 days) at the minimally effective dose to control symptoms. The third-line treatments include immunomodulators, but the evidence for efficacy with these agents is largely based on the clinical experience and small-scale studies. Management guidelines also recommend dapsone as the next line of therapy. In patients with anemia, sulfasalazine is used as an alternative. In patients with only modest impairment in quality of life, the use of hydroxychloroquine was suggested given its slow onset of action (Reeves et al., 2004). Alternative immunomodulators used for refractory cases include other calcineurin inhibitors (Trojan and Khan, 2012), mycophenolate mofetil (Zimmerman et al., 2012), immunoglobulin (Asero, 2000), TNF inhibitors, colchicine (Pho et al., 2011), methotrexate (Gach et al., 2001; Perez et al., 2010; Sagi et al., 2011), and cyclophosphamide (Bernstein et al., 2002; Asero, 2005). Phototherapy has also been shown to be effective and is a treatment option for patients desiring to avoid systemic medications (Berroeta et al., 2004; Engin et al., 2008; Aydogan et al., 2012). More recently, omalizumab, a human monoclonal antibody against the high-affinity IgE receptor Fc $\epsilon$ RI, has been shown to be effective, although cost remains a limiting factor (Maurer et al., 2013).

More recent studies have shown that omalizumab has substantial benefits in various CIndUs; the evidence is strongest for symptomatic dermatographism, cold urticaria, and solar urticarial (Maurer et al., 2013).

Although CU is a relatively common condition, identifying the precise trigger in individual patients is usually not possible. Better testing for antigen specificities is a high priority to correct this important issue and is essential

for the best treatment outcomes. Blocking mast-cell degranulation or blocking the effects of released mast-cell mediators is often an inadequate approach to treatment.

The addition of immunosuppressive drugs and specific anti-IgE biologics has enhanced the effectiveness of treatment for recalcitrant CU patients. Nevertheless, better tools for immunologic characterization of the autoimmune mechanism in individual patients, better drugs for mast-cell stabilization, and better drugs to block the autoimmune mechanisms of CU are all important to enhance the effectiveness of treating these patients.

## References

- Abu Tahir, M., Pramod, K., Ansari, S.H., Ali, J., 2010. Current remedies for vitiligo. *Autoimmun. Rev.* 9, 516–520.
- Alkhateeb, A., Fain, P.R., Thody, A., Bennett, D.C., Spritz, R.A., 2003. Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their relatives. *Pigment Cell Res.* 16, 208–214.
- Alli, R., Nguyen, P., Boyd, K., Sundberg, J.P., Geiger, T.L., 2012. A mouse model of clonal CD81 T lymphocyte-mediated alopecia areata progressing to alopecia universalis. *J. Immunol.* 188, 477–486.
- Altrichter, S., Hawro, T., Liedtke, M., Holtappels, G., Bachert, C., Skov, P.S., et al., 2018. In chronic spontaneous urticaria, IgE against staphylococcal enterotoxins is common and functional. *Allergy* 73, 1497–1504.
- Aronson, P.J., Hashimoto, K., 1987. Association of IgA anti-melanoma antibodies in the sera of vitiligo patients with active disease. *J. Invest. Dermatol.* 88, 475.
- Asero, R., 2000. Are IVIG for chronic unremitting urticaria effective? *Allergy* 55, 1099–1101.
- Asero, R., 2002. Chronic idiopathic urticaria: a family study. *Ann. Allergy Asthma Immunol.* 89, 195–196.
- Asero, R., 2005. Oral cyclophosphamide in a case of cyclosporin and steroid-resistant chronic urticaria showing autoreactivity on autologous serum skin testing. *Clin. Exp. Dermatol.* 30, 582–583.
- Asero, R., Tedeschi, A., Riboldi, P., Cugno, M., 2006. Plasma of patients with chronic urticaria shows signs of thrombin generation, and its intradermal injection causes wheal-and-flare reactions much more frequently than autologous serum. *J. Allergy Clin. Immunol.* 117, 1113–1117.
- Asero, R., Riboldi, P., Tedeschi, A., Cugno, M., Meroni, P., 2007a. Chronic urticaria: a disease at a crossroad between autoimmunity and coagulation. *Autoimmun. Rev.* 7, 71–76.
- Asero, R., Tedeschi, A., Coppola, R., Griffini, S., Paparella, P., Riboldi, P., et al., 2007b. Activation of the tissue factor pathway of blood coagulation in patients with chronic urticaria. *J. Allergy Clin. Immunol.* 119, 705–710.
- Atwa, M.A., Emara, A.S., Youssef, N., Bayoumy, N.M., 2013. Serum concentration of IL-17, IL-23 and TNF-alpha among patients with chronic spontaneous urticaria: association with disease activity and autologous serum skin test. *J. Eur. Acad. Dermatol. Venereol.* 28, 469–474.
- Aydogan, K., Karadogan, S.K., Tunali, S., Saricaoglu, H., 2012. Narrowband ultraviolet B (311 nm, TL01) phototherapy in chronic ordinary urticaria. *Int. J. Dermatol.* 51, 98–103.
- Badri, A.M., Todd, P.M., Garioch, J.J., Gudgeon, J.E., Stewart, D.G., Goudie, R.B., 1993. An immunohistological study of cutaneous lymphocytes in vitiligo. *J. Pathol.* 170, 149–155.
- Barahmani, N., Schabath, M.B., Duvic, M., 2009. History of atopy or autoimmunity increases risk of alopecia areataNational Alopecia Areata Registry. *J. Am. Acad. Dermatol.* 61, 581–591.
- Bergboer, J.G., Zeeuwen, P.L., Schalkwijk, J., 2012. Genetics of psoriasis: evidence for epistatic interaction between skin barrier abnormalities and immune deviation. *J. Invest. Dermatol.* 132, 2320–2321.
- Bernstein, J.A., Garramone, S.M., Lower, E.G., 2002. Successful treatment of autoimmune chronic idiopathic urticaria with intravenous cyclophosphamide. *Ann. Allergy Asthma Immunol.* 89, 212–214.
- Berroeta, L., Clark, C., Ibbotson, S.H., Ferguson, J., Dawe, R.S., 2004. Narrow-band (TL-01) ultraviolet B phototherapy for chronic urticaria. *Clin. Exp. Dermatol.* 29, 97–98.
- Betz, R.C., Petukhova, L., Ripke, S., Huang, H., Menelaou, A., Redler, S., et al., 2015. Genome-wide meta-analysis in alopecia areata resolves HLA associations and reveals two new susceptibility loci. *Nat Commun.* 22 (6), 5966.
- Birlea, S.A., Gowan, K., Fain, P.R., Spritz, R.A., 2010. Genome-wide association study of generalized vitiligo in an isolated European founder population identifies SMOC2, in close proximity to IDDM8. *J. Invest. Dermatol.* 130, 798–803.
- Birlea, S.A., Jin, Y., Bennett, D.C., Herbstman, D.M., Wallace, M.R., McCormack, W.T., et al., 2011. Comprehensive association analysis of candidate genes for generalized vitiligo supports XBP1, FOXP1, and TSLP. *J. Invest. Dermatol.* 131, 371–381.
- Birlea, S.A., Spritz, R.A., Norris, D.A., 2012. Vitiligo. In: Wolff, K., Goldsmith, L.A., Katz, S.I., Gilchrest, B.A., Paller, A.S., Leffell, D.J. (Eds.), *Fitzpatrick's Dermatology in General Medicine*, eighth ed. McGraw-Hill, New York, pp. 792–803.
- Birlea, S.A., Ahmad, F.J., Uddin, R.M., Ahmad, S., Pal, S.S., Begum, R., et al., 2013. Association of generalized vitiligo with MHC Class II Loci in patients from the Indian subcontinent. *J. Invest. Dermatol.* 133, 1369–1372.
- Boissy, R.E., Lamoreux, M.L., 1988. Animal models of an acquired pigmentary disorder: vitiligo. In: Bagnara, J. (Ed.), *Advances in Pigment Cell Research*. Alan R. Liss Inc., New York, pp. 207–218.
- Boone, B., Ongenae, K., Van Geel, N., Vernijns, S., De Keyser, S., Naeyaert, J.M., 2007. Topical pimecrolimus in the treatment of vitiligo. *Eur. J. Dermatol.* 17, 55–61.
- van den Boorn, J.G., Konijnenberg, D., Dellemijn, T.A., van der Veen, J.P., Bos, J.D., Melief, C.J., et al., 2009. Autoimmune destruction of skin melanocytes by perilesional T cells from vitiligo patients. *J. Invest. Dermatol.* 129, 2220–2232.
- van den Boorn, J.G., Picavet, D.I., van Swieten, P.F., van Veen, H.A., Konijnenberg, D., van Veelen, et al., 2011. Skin-depigmenting agent monobenzone induces potent T-cell autoimmunity toward pigmented cells by tyrosinase hapteneation and melanosome autophagy. *J. Invest. Dermatol.* 131, 1240–1251.
- Bowcock, A.M., Krueger, J.G., 2005. Getting under the skin: the immunogenetics of psoriasis. *Nat. Rev. Immunol.* 5, 699–711.

- Bowen, A.R., Hanks, A.N., Allen, S.M., Alexander, A., Diedrich, M.J., Grossman, D., 2003. Apoptosis regulators and responses in human melanocytic and keratinocytic cells. *J. Invest. Dermatol.* 120, 48–55.
- Birlea, S. A., Costin, G. E., Roop, D.R., Norris, D.A., 2018. Trends in regenerative medicine: repigmentation in vitiligo through melanocyte stem cell mobilization. *Med. Res. Rev.* 37, 907–935.
- Brzoza, Z., Grzeszczak, W., Rogala, B., Trautsolt, W., Moczulski, D., 2012. PTPN22 polymorphism presumably plays a role in the genetic background of chronic spontaneous autoreactive urticaria. *Dermatology* 224, 340–345.
- Bø, K., Thoresen, M., Dalgard, F., 2008. Smokers report more psoriasis, but not atopic dermatitis or hand eczema: results from a Norwegian population survey among adults. *Dermatology* 216, 40–45.
- Chen, J., Tan, Z., Li, J., Xiong, P., 2005. Association of HLA-DRB1, DQB1 alleles with chronic urticaria. *J. Huazhong Univ. Sci. Technol. Med. Sci.* 25, 354–356.
- Chu, S.Y., Chen, Y.J., Tseng, W.C., et al., 2011. Comorbidity profiles among patients with alopecia areata: the importance of onset age, a nationwide population-based study. *J. Am. Acad. Dermatol.* 65, 949–956.
- Colonna, L., Catalano, G., Chew, C., et al., 2010. Therapeutic targeting of Syk in autoimmune diabetes. *J. Immunol.* 185, 1532–1543.
- Confino-Cohen, R., Chodick, G., Shalev, V., Leshno, M., Kimhi, O., Goldberg, A., 2012. Chronic urticaria and autoimmunity: associations found in a large population study. *J. Allergy Clin. Immunol.* 129, 1307–1313.
- Cui, J., Hanning, R., Henn, M., Bystryn, J.C., 1992. Identification of pigment cell antigens defined by vitiligo antibodies. *J. Invest. Dermatol.* 98, 162–165.
- Delamere, F.M., Sladden, M.M., Dobbins, H.M., Leonardi-Bee, J., 2008. Interventions for alopecia areata. *Cochrane Database Syst. Rev.* 16, CD004413.
- Dell'anna, M.L., Picardo, M., 2006. A review and a new hypothesis for non-immunological pathogenetic mechanisms in vitiligo. *Pigment Cell Res* 19, 406–411.
- Dell'anna, M.L., Cario-André, M., Bellei, B., Taieb, A., Picardo, M., 2012. In vitro research on vitiligo: strategies, principles, methodological options and common pitfalls. *Exp. Dermatol.* 21, 490–496.
- Engin, B., Ozdemir, M., Balevi, A., Mevlitoglu, I., 2008. Treatment of chronic urticaria with narrowband ultraviolet B phototherapy: a randomized controlled trial. *Acta Derm. Venereol.* 88, 247–251.
- Feliciani, C., Gupta, A.K., Sauder, D.N., 1996. Keratinocytes and cytokine/growth factors. *Crit. Rev. Oral Biol. Med.* 7, 300–318.
- Fishman, P., Azizi, E., Shoenfeld, Y., Sredni, B., Yecheskel, G., Ferrone, S., et al., 1993. Vitiligo autoantibodies are effective against melanoma. *Cancer* 72, 2365–2369.
- van der Fits, L., Mourits, S., Voerman, J.S., Kant, M., Boon, L., Laman, J.D., et al., 2009. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. *J. Immunol.* 182, 5836–5845.
- Freyschmidt-Paul, P.M., Zoller, K.J., McElwee, J., et al., 2005. The functional relevance of the type 1 cytokines IFN-gamma and IL-2 in alopecia areata of C3H/HeJ mice. *J. Invest. Dermatol. Symp. Proc.* 10, 282–283.
- Funk, J., Langeland, T., Schrumpf, E., Hanssen, L.E., 1991. Psoriasis induced by interferon-alpha. *Br. J. Dermatol.* 125, 463–465.
- Gach, J.E., Sabroe, R.A., Greaves, M.W., Black, A.K., 2001. Methotrexate-responsive chronic idiopathic urticaria: a report of two cases. *Br. J. Dermatol.* 145, 340–343.
- Gawkroger, D.J., Ormerod, A.D., Shaw, L., Mauri-Sole, I., Whitton, M.E., Watts, M.J., et al., 2010. Vitiligo: concise evidence based guidelines on diagnosis and management. *Postgrad. Med. J.* 86, 466–471.
- van Geel, N., De Lille, S., Vandenhoute, S., Gauthier, Y., Mollet, I., Brochez, L., et al., 2011. Different phenotypes of segmental vitiligo based on a clinical observational study. *J. Eur. Acad. Dermatol. Venereol.* 25, 673–678.
- Gelfand, J.M., Weinstein, R., Porter, S.B., Neumann, A.L., Berlin, J.A., Margolis, D.J., 2005. Prevalence and treatment of psoriasis in the United Kingdom: a population-based study. *Arch Dermatol.* 141, 1537–1541.
- Giangreco, A., Hoste, E., Takai, Y., Rosewell, I., Watt, F.M., 2012. Epidermal Cadm1 expression promotes autoimmune alopecia via enhanced T cell adhesion and cytotoxicity. *J. Immunol.* 188, 1514–1522.
- Gilhar, A., Zelickson, B., Ulman, Y., Etzioni, A., 1995. In vivo destruction of melanocytes by the IgG fraction of serum from patients with vitiligo. *J. Invest. Dermatol.* 105, 683–686.
- Gilhar, A., Ullmann, Y., Berkutzki, T., Assy, B., Kalish, R.S., 1998. Autoimmune hair loss alopecia areata transferred by T lymphocytes to human scalp explants on SCID mice. *J. Clin. Invest.* 101, 62–67.
- Gilhar, A., Landau, M., Assy, B., Shalaginov, R., Serafimovich, S., Kalish, R.S., 2001. Mediation of alopecia areata by cooperation between CD4+ and CD8+ T lymphocytes: transfer to human scalp explants on Prkdc (scid) mice. *J. Invest. Dermatol.* 117, 1357–1362.
- Gilhar, A., Ftzion, A.I., Paus, R., 2012. Alopecia areata. *N. Engl. J. Med.* 366, 1515–1525.
- Gilhar, A., Keren, A., Shemer, A., d'Ovidio, R., Ullmann, Y., Paus, R., 2013. Autoimmune disease induction in a healthy human organ: a humanized mouse model of alopecia areata. *J. Invest. Dermatol.* 133, 844–847.
- Goh, C., Finkel, M., Christos, P.J., Sinha, A.A., 2006. Profile of 513 patients with alopecia areata: associations of disease subtypes with atopy, autoimmune disease and positive family history. *J. Eur. Acad. Dermatol. Venereol.* 20, 1055–1060.
- Goldstein, N.B., Koster, M.I., Hoaglin, L.G., Spoelstra, N.S., Kechris, K.J., Robinson, S.E., et al., 2015. Narrow band ultraviolet B treatment for human vitiligo is associated with proliferation, migration, and differentiation of melanocyte precursors. *J. Invest. Dermatol.* 135, 2068–2076.
- Goldstein, N.B., Koster, M.I., Hoaglin, L.G., Wright, M.J., Robinson, S.E., Robinson, W.A., et al., 2016. Isolating RNA from precursor and mature melanocytes from human vitiligo and normal skin using laser capture microdissection. *Exp. Dermatol.* 25, 805–811.
- Goldstein, N.B., Koster, M.I., Jones, K.L., Gao, B., Hoaglin, L.G., Robinson, S.E., et al., 2018. Repigmentation of human vitiligo skin by NBUVB is controlled by transcription of GLI1 and activation of the β-Catenin pathway in the hair follicle bulge stem cells. *J. Invest. Dermatol.* 138, 657–668.
- Gottumukkala, R.V.S.R.K., Gavalas, N.G., Akhtar, S., Metcalfe, R.A., Gawkroger, D.J., Haycock, J.W., et al., 2006. Function blocking autoantibodies to the melanin-concentrating hormone receptor in vitiligo patients. *Lab. Invest.* 86, 781–789.
- Grattan, C.E., Wallington, T.B., Warin, R.P., Kennedy, C.T., Bradfield, J.W., 1986. A serological mediator in chronic idiopathic urticarial – a clinical, immunological and histological evaluation. *Br. J. Dermatol.* 114, 583–590.

- Grattan, C.E., Boon, A.P., Eady, R.A., Winkelmann, R.K., 1990. The pathology of the autologous serum skin test response in chronic urticaria resembles IgE-mediated late-phase reactions. *Int. Arch. Allergy Appl. Immunol.* 93, 198–204.
- Greaves, M.W., Tan, K.T., 2007. Chronic urticaria: recent advances. *Clin. Rev. Allergy Immunol.* 33, 134–143.
- Gross, A., Tapia, F.J., Mosca, W., Perez, R.M., Briceño, L., Henriquez, J.J., et al., 1987. Mononuclear cell subpopulations and infiltrating lymphocytes in erythema dyschromicum perstans and vitiligo. *Histol. Histopathol.* 2, 277–283.
- Gudjonsson, J.E., Johnston, A., 2009. Current understanding of the genetic basis of psoriasis. *Expert Rev. Clin. Immunol.* 5, 433–443.
- Gudjonsson, J.E., Johnston, A., Dyson, M., Valdimarsson, H., Elder, J.T., 2007. Mouse models of psoriasis. *J. Invest. Dermatol.* 127, 1292–1308.
- Gudjonsson, J.E., Elder, J.T., 2008. Psoriasis. In: Wolff, K., et al., (Eds.), *Fitzpatrick's Dermatology in General Medicine*, seventh ed McGraw-Hill, New York, pp. 169–193.
- Hann, S.K., Kim, H.I., Im, S., Park, Y.K., Cui, J., Bystryn, J.C., 1993. The change of melanocyte cytotoxicity after systemic steroid treatment in vitiligo patients. *J. Dermatol. Sci.* 6, 201–205.
- Hann, S.K., Koo, S.W., Kim, J.B., Park, Y.K., 1996a. Detection of antibodies to human melanoma cells in vitiligo and alopecia areata by Western blot analysis. *J. Dermatol.* 23, 100–103.
- Hann, S.K., Shin, H.K., Park, S.H., Reynolds, S.R., Bystryn, J.C., 1996b. Detection of antibodies to melanocytes in vitiligo by western immunoblotting. *Yonsei Med. J.* 37, 365–370.
- Hariharan, V., Klarquist, J., Reust, M.J., Koshofer, A., McKee, M.D., Boissy, R.E., et al., 2010. Monobenzyl ether of hydroquinone and 4-tertiary butyl phenol activate markedly different physiological responses in melanocytes: relevance to skin depigmentation. *J. Invest. Dermatol.* 130, 211–220.
- Harning, R., Cui, J., Bystryn, J.C., 1991. Relation between the incidence and level of pigment cell antibodies and disease activity in vitiligo. *J. Invest. Dermatol.* 97, 1078–1080.
- Hartmann, A., Brocker, E.B., Hamm, H., 2008. Occlusive treatment enhances efficacy of tacrolimus 0.1% ointment in adult patients with vitiligo: results of a placebo controlled 12-month prospective study. *Acta Derm. Venereol.* 88, 474–479.
- Hawkes, J.E., Adalsteinsson, J.A., Gudjonsson, J.E., Ward, N.L., 2018a. Research techniques made simple: murine models of human psoriasis. *J. Invest. Dermatol.* 138, e1–e8.
- Hawkes, J.E., Yan, B.Y., Chan, T.C., Krueger, J.G., 2018b. Discovery of the IL-23/IL-17 signaling pathway and the treatment of psoriasis. *J. Immunol.* 201, 1605–1613.
- Hedley, S.J., Metcalfe, R., Gawkrodger, D.J., Weetman, A.P., MacNeil, S., 1998. Vitiligo melanocytes in long-term culture show normal constitutive and cytokine-induced expression of intercellular adhesion molecule-1 and major histocompatibility complex class I and class II molecules. *Br. J. Dermatol.* 139, 965–973.
- Hide, M., Francis, D.M., Grattan, C.E., Hakimi, J., Kochan, J.P., Greaves, M.W., 1993. Autoantibodies against the high-affinity IgE receptor as a cause of histamine release in chronic urticaria. *N. Engl. J. Med.* 328, 1599–1604.
- Homey, B., Assmann, T., Vohr, H.W., Ulrich, P., Lauermann, A.I., Ruzicka, T., et al., 1998. Topical FK506 suppresses cytokine and costimulatory molecule expression in epidermal and local draining lymph node cells during primary skin immune responses. *J. Immunol.* 160, 5331–5340.
- Ito, T., Ito, N., Saatoff, M., Hashizume, H., Fukamizu, H., Nickoloff, B.J., et al., 2008. Maintenance of hair follicle immune privilege is linked to prevention of NK cell attack. *J. Invest. Dermatol.* 128, 1196–1206.
- Jabbari, A., Cerise, J.E., Chen, J.C., Mackay-Wiggan, J., Duvic, M., Price, V., et al., 2016. Molecular signatures define alopecia areata subtypes and transcriptional biomarkers. *EBioMedicine* 7, 240–247.
- Jagielska, D., Redler, S., Brockschmidt, F.F., Herold, C., Pasternack, S.M., Garcia Bartels, N., 2012. Follow-up study of the first genome-wide association scan in alopecia areata: IL13 and KIAA0350 as susceptibility loci supported with genome-wide significance. *J. Invest. Dermatol.* 132, 2192–2197.
- Jin, Y., Birlea, S.A., Fain, P.R., Gowan, K., Riccardi, S.L., Holland, P.J., et al., 2010a. Variant of TYR and autoimmunity susceptibility loci in generalized vitiligo. *N. Engl. J. Med.* 362, 1686–1697.
- Jin, Y., Birlea, S.A., Fain, P.R., Mailloux, C.M., Riccardi, S.L., Gowan, K., et al., 2010b. Common variants in FOXP1 are associated with generalized vitiligo. *Nat. Genet.* 42, 576–578.
- Jin, Y., Birlea, S.A., Fain, P.R., Ferrara, T.M., Ben, S., Riccardi, S.L., et al., 2012a. Genome-wide association analyses identify 13 new susceptibility loci for generalized vitiligo. *Nat. Genet.* 44, 676–680.
- Jin, Y., Ferrara, T., Gowan, K., Holcomb, C., Rastrou, M., Erlich, H.A., et al., 2012b. Next-generation DNA re-sequencing identifies common variants of TYR and HLA-A that modulate the risk of generalized vitiligo via antigen presentation. *J. Invest. Dermatol.* 132, 1730–1733.
- Joint Task Force on Practice Parameters, 2000. The diagnosis and management of urticaria: a practice parameter part I: acute urticaria/angioedema part II: chronic urticaria/angioedema. *Ann. Allergy Asthma Immunol.* 85, 521–544.
- de Jong, A., Jabbari, A., Dai, Z., Xing, L., Lee, D., Li, M.M., et al., 2018. High-throughput T cell receptor sequencing identifies clonally expanded CD8+ T cell populations in alopecia areata. *JCI Insight* 3, 19.
- Kang, H., Wu, W.Y., Lo, B.K.K., Yu, M., Leung, G., Shapiro, J., et al., 2010. Hair follicles from alopecia areata patients exhibit alterations in immune privilege-associated gene expression in advance of hair loss. *J. Invest. Dermatol.* 130, 2677–2680.
- Kao, C.H., Yu, H.S., 1992. Comparison of the effect of 8-methoxysoralen (8-MOP) plus UVA (PUVA) on human melanocytes in vitiligo vulgaris and in vitro. *J. Invest. Dermatol.* 98, 734–740.
- Kaplan, A.P., 2004. Chronic urticaria: pathogenesis and treatment. *J. Allergy Clin. Immunol.* 114, 465–474.
- Kemp, E.H., Gavalska, N.G., Gawkrodger, D.J., Weetman, A.P., 2007. Autoantibody responses to melanocytes in the depigmented skin disease vitiligo. *Autoimmun. Rev.* 6, 138–142.
- Kim, Y.C., Kim, Y.J., Kang, H.Y., Sohn, S., Lee, E.S., 2008. Histopathologic features in vitiligo. *Am. J. Dermatopathol.* 30, 112–116.
- Klarquist, J., Denman, C.J., Hernandez, C., Wainwright, D.A., Strickland, F.M., Overbeck, A., et al., 2010. Reduced skin homing by functional Treg in vitiligo. *Pigment Cell Melanoma Res.* 23, 276–286.
- Koga, M., Tango, T., 1988. Clinical features and course of type A and type B vitiligo. *Br. J. Dermatol.* 118, 223–228.
- Kroll, T.M., Bommiasamy, H., Boissy, R.E., Hernandez, C., Nickoloff, B.J., Mestril, R., et al., 2005. 4-Tertiary butyl phenol exposure sensitizes human melanocytes to dendritic cell-mediated killing: relevance to vitiligo. *J. Invest. Dermatol.* 124, 798–806.

- Kroon, M.W., Kemp, E.H., Wind, B.S., Krebbers, G., Bos, J.D., Gawkrodger, D.J., et al., 2012. Melanocyte antigen-specific antibodies cannot be used as markers for recent disease activity in patients with vitiligo. *J. Eur. Acad. Dermatol. Venereol.* 27, 1172–1175.
- Kurd, S.K., Gelfand, J.M., 2009. The prevalence of previously diagnosed and undiagnosed psoriasis in US adults: results from NHANES 2003–2004. *J. Am. Acad. Dermatol.* 60, 218–224.
- Lang, K.S., Caroli, C.C., Muhm, D., Wernet, D., Moris, A., Schittek, B., et al., 2001. HLA-A2 restricted, melanocyte-specific CD8<sup>+</sup> T lymphocytes detected in vitiligo patients are related to disease activity and are predominantly directed against MelanA/MART1. *J. Invest. Dermatol.* 116, 891–897.
- Langley, R.G., Krueger, G.G., Griffiths, C.E., 2005. Psoriasis: epidemiology, clinical features, and quality of life. *Ann. Rheum. Dis.* 64, 18–23.
- Le Poole, I.C., Luiten, R.M., 2008. Autoimmune etiology of generalized vitiligo. *Curr. Dir. Autoimmun.* 10, 227–243.
- Le Poole, I.C., van den Wijngaard, R.M., Westerhof, W., Dutrieux, R.P., Das, P.K., 1993a. Presence or absence of melanocytes in vitiligo lesions: an immunohistochemical investigation. *J. Invest. Dermatol.* 100, 816–822.
- Le Poole, I.C., Das, P.K., van den Wijngaard, R.M., Bos, J.D., Westerhof, W., 1993b. Review of the etiopathomechanism of vitiligo: a convergence theory. *Exp. Dermatol.* 2, 145–153.
- Le Poole, I.C., van den Wijngaard, R.M., Westerhof, W., Das, P.K., 1996. Presence of T cells and macrophages in inflammatory vitiligo skin parallels melanocyte disappearance. *Am. J. Pathol.* 148, 1219–1228.
- Lee, K.H., Kim, J.Y., Kang, D.S., Choi, Y.J., Lee, W.J., Ro, J.Y., 2002. Increased expression of endothelial cell adhesion molecules due to mediator release from human foreskin mast cells stimulated by auto-antibodies in chronic urticaria sera. *J. Invest. Dermatol.* 118, 658–663.
- Leung, D.Y., Hauk, P., Strickland, I., Travers, J.B., Norris, D.A., 1998. The role of superantigens in human diseases: therapeutic implications for the treatment of skin diseases. *Br. J. Dermatol.* 139, 17–29.
- Leung, D.Y.M., Travers, J.B., Giorno, R., Norris, D.A., Skinner, R., Aelion, J., et al., 1995. Evidence for a streptococcal superantigen-driven process in acute guttate psoriasis. *J. Clin. Invest.* 96, 2106–2112.
- Leung, M.C., Sutton, C.W., Fenton, D.A., Tobin, D.J., 2010. Trichohyalin is a potential major autoantigen in human alopecia areata. *J. Proteome Res.* 9, 5153–5163.
- Leznoff, A., Josse, R.G., Denburg, J., Dolovich, J., 1983. Association of chronic urticaria and angioedema with thyroid autoimmunity. *Arch. Dermatol.* 119, 636–640.
- Liang, Y., Sarkar, M.K., Tsoi, L.C., Gudjonsson, J.E., 2017. Psoriasis: a mixed autoimmune and autoinflammatory disease. *Curr. Opin. Immunol.* 49, 1–8.
- Lin, W.J., Norris, D.A., Achziger, M., Kotzin, B.L., Tomkinson, B., 2001. Oligoclonal expansion of intraepidermal T cells in psoriasis lesions. *J. Invest. Dermatol.* 117, 1546–1553.
- Liu, L.Y., Strassner, J.P., Refat, M.A., Harris, J.E., King, B.A., 2017. Repigmentation in vitiligo using the Janus kinase inhibitor tofacitinib may require concomitant light exposure. *J. Am. Acad. Dermatol.* 77, 675–682.e1.
- Lowes, M.A., Chamian, F., Abello, M.V., Fuentes-Duculan, J., Lin, S.L., Nussbaum, R., et al., 2005. Increase in TNF-alpha and inducible nitric oxide synthase-expressing dendritic cells in psoriasis and reduction with efalizumab (anti-CD11a). *Proc. Natl. Acad. Sci. U.S.A.* 102, 19057–19062.
- Lowes, M.A., Bowcock, A.M., Krueger, J.G., 2007. Pathogenesis and therapy of psoriasis. *Nature* 445, 866–873.
- Manga, P., Sheyn, D., Yang, F., Sarangarajan, R., Boissy, R.E., 2006. A role for tyrosinase-related protein 1 in 4-*tert*-butylphenol-induced toxicity in melanocytes: Implications for vitiligo. *Am. J. Pathol.* 169, 1652–1662.
- Martinez-Mir, A., Zlotogorski, A., Gordon, D., et al., 2007. Genomewide scan for linkage reveals evidence of several susceptibility loci for alopecia areata. *Am. J. Hum. Genet.* 80, 316–328.
- Matsushita, Y., Shimada, Y., Kawara, S., Takehara, K., Sato, S., 2005. Autoantibodies directed against the protease inhibitor calpastatin in psoriasis. *Clin. Exp. Immunol.* 139, 355–362.
- Maurer, M., Rosen, K., Hsieh, H.J., Saini, S., Grattan, C., Gimenez-Arnau, A., et al., 2013. Omalizumab for the treatment of chronic idiopathic or spontaneous urticaria. *N. Engl. J. Med.* 368, 924–935.
- McElwee, K.J., Freyschmidt-Paul, P., Hoffmann, R., Kissling, S., Hummel, S., Vitacolonna, M., et al., 2005. Transfer of CD8(1) cells induces localized hair loss whereas CD4(1)/CD25(2) cells promote systemic alopecia areata and CD4(1)/CD25(1) cells blockade disease onset in the C3H/HeJ mouse model. *J. Invest. Dermatol.* 124, 947–957.
- Mehrotra, S., Al-Khami, A.A., Klarquist, J., Husain, S., Naga, O., Eby, J.M., et al., 2012. A coreceptor-independent transgenic human TCR mediates anti-tumor and anti-self-immunity in mice. *J. Immunol.* 189, 1627–1638.
- Mekori, Y.A., Giorno, R.C., Anderson, P., Kohler, P.F., 1983. Lymphocyte subpopulations in the skin of patients with chronic urticaria. *J. Allergy Clin. Immunol.* 72, 681–684.
- Mlynak, A., Maurer, M., Zalewska, A., 2008. Update on chronic urticaria: focusing on mechanisms. *Curr. Opin. Allergy Clin. Immunol.* 8, 433–437.
- Morris, A., Rogers, M., Fischer, G., Williams, K., 2001. Childhood psoriasis: a clinical review of 1262 cases. *Pediatr. Dermatol.* 18, 188–198.
- Mosenson, J.A., Zloza, A., Nieland, J.D., Garrett-Mayer, E., Eby, J.M., Huelsmann, E.J., et al., 2013. Mutant HSP70 reverses autoimmune depigmentation in vitiligo. *Sci. Transl. Med.* 5, 174.
- Namazi, M.R., 2007. Neurogenic dysregulation, oxidative stress, and melanocytorrhyg in vitiligo: can they be interconnected? *Pigment Cell Res.* 20, 360–363.
- Naughton, G.K., Reggiardo, M.D., Bystryn, J.C., 1986. Correlation between vitiligo antibodies and extent of depigmentation in vitiligo. *J. Am. Acad. Dermatol.* 15, 978–981.
- Nestle, F.O., Turka, L.A., Nickoloff, B.J., 1994. Characterization of dermal dendritic cells in psoriasis: autostimulation of T lymphocytes and induction of Th1 type cytokines. *J. Clin. Invest.* 94, 202–209.
- Nestle, F.O., Kaplan, D.H., Barker, J., 2009. Psoriasis. *N. Engl. J. Med.* 361, 496–509.
- Niimi, N., Francis, D.M., Kermani, F., O'Donnell, B.F., Hide, M., Kobza-Black, A., et al., 1996. Dermal mast cell activation by auto-antibodies against the high affinity IgE receptor in chronic urticaria. *J. Invest. Dermatol.* 106, 1001–1006.

- Norris, D.A., Kissinger, R.M., Naughton, G.M., Bystryn, J.C., 1988a. Evidence for immunologic mechanisms in human vitiligo: patients' sera induce damage to human melanocytes in vitro by complement-mediated damage and antibody dependent cellular cytotoxicity. *J. Invest. Dermatol.* 90, 783–789.
- Norris, D.A., Capin, L., Muglia, J.J., Osborn, R.L., Zerbe, G.O., Bystryn, J.C., et al., 1988b. Enhanced susceptibility of melanocytes to different immunologic effector mechanisms in vitro: potential mechanisms for post-inflammatory hypopigmentation and vitiligo. *Pigment Cell Res.* 1, 113–123.
- Norris, D.A., Horikawa, T., Morelli, J.G., 1994. Melanocyte destruction and repopulation in vitiligo. *Pigment Cell Res.* 7, 193–203.
- Ogg, G.S., Dunbar, P.R., Romero, P., Chen, J.L., Cerundolo, V., 1998. High frequency of skin-homing melanocyte-specific cytotoxic T lymphocytes in autoimmune vitiligo. *J. Exp. Med.* 188, 1203–1208.
- Palermo, B., Campanelli, R., Garbelli, S., Mantovani, S., Lantelme, E., Brazzelli, V., et al., 2001. Specific cytotoxic T lymphocyte responses against Melan-A/MART1, tyrosinase and gp100 in vitiligo by the use of major histocompatibility complex/peptide tetramers: the role of cellular immunity in the etiopathogenesis of vitiligo. *J. Invest. Dermatol.* 117, 326–332.
- Parisi, R., Symmons, D.P., Griffiths, C.E., Ashcroft, D.M., 2013. Identification and Management of Psoriasis and Associated Comorbidity (IMPACT) project team, Global epidemiology of psoriasis: a systematic review of incidence and prevalence. *J. Invest. Dermatol.* 133, 377–385.
- Park, K.C., Youn, J.I., Lee, Y.S., 1988. Clinical study of 326 cases of vitiligo. *Korean J. Dermatol.* 26, 200–205.
- Park, Y.K., Kim, N.S., Hann, S.K., Im, S.J., 1996. Identification of auto-antibody to melanocytes and characterization of vitiligo antigen in vitiligo patients. *Dermatol. Sci.* 11, 111–120.
- Paus, R., Ito, N., Takigawa, M., Ito, T., 2003. The hair follicle and immune privilege. *J. Invest. Dermatol. Symp. Proc.* 8, 188–194.
- Perez, A., Woods, A., Grattan, C.E., 2010. Methotrexate: a useful steroid-sparing agent in recalcitrant chronic urticaria. *Br. J. Dermatol.* 162, 191–194.
- Petukhova, L., Duvic, M., Hordinsky, M., Norris, D., Price, V., Shimomura, Y., et al., 2010. Genome-wide association study in alopecia areata implicates both innate and adaptive immunity. *Nature* 466, 113–117.
- Petukhova, L., Cabral, R.M., Mackay-Wiggan, J., Clynes, R., Christiano, A.M., 2011. The genetics of alopecia areata: what's new and how will it help our patients? *Dermatol. Ther.* 24, 326–336.
- Phan, G.Q., Attia, P., Steinberg, S.M., White, D.E., Rosenberg, S.A., 2001. Factors associated with response to high-dose interleukin-2 in patients with metastatic melanoma. *J. Clin. Oncol.* 19, 3477–3482.
- Pho, L.N., Eliason, M.J., Regruto, M., Hull, C.M., Powell, D.L., 2011. Treatment of chronic urticaria with colchicine. *J. Drugs Dermatol.* 10, 1423–1428.
- Posthumus, J., Tinana, A., Mozena, J.D., Steinke, J.W., Borish, L., 2012. Autoimmune mechanisms in chronic idiopathic urticaria. *J. Allergy Clin. Immunol.* 130, 814–816.
- Quan, C., Ren, Y.Q., Xiang, L.H., Sun, L.D., Xu, A.E., Gao, X.H., et al., 2010. Genome-wide association study for vitiligo identifies susceptibility loci at 6q27 and the MHC. *Nat. Genet.* 42, 614–618.
- Rashighi, M., Harris, J.E., 2017. Vitiligo pathogenesis and emerging treatments. *Dermatol. Clin.* 35, 257–265.
- Raychaudhuri, S.P., Dutt, S., Raychaudhuri, S.K., Sanyal, M., Farber, E.M., 2001. Severe combined immunodeficiency mouse-human skin chimeras: a unique animal model for the study of psoriasis and cutaneous inflammation. *Br. J. Dermatol.* 144, 931–939.
- Reeves, G.E., Boyle, M.J., Bonfield, J., Dobson, P., Loewenthal, M., 2004. Impact of hydroxychloroquine therapy on chronic urticaria: chronic autoimmune urticaria study and evaluation. *Intern. Med. J.* 34, 182–186.
- Reeves, W.H., Fisher, D., Wisniewolski, R., Gottlieb, A.B., Chiorazzi, N., 1986. Psoriasis Raynaud's phenomenon associated with autoantibodies to U1 and U2 small nuclear ribonucleoproteins. *N. Engl. J. Med.* 315, 105–111.
- Richmond, J.M., Bangari, D.S., Essien, K.I., Currimbhoy, S.D., Groom, J.R., Pandya, A.G., et al., 2017. Keratinocyte-derived chemokines orchestrate T-cell positioning in the epidermis during vitiligo and may serve as biomarkers of disease. *J. Invest. Dermatol.* 137, 350–358.
- Riding, R.L., Richmond, J.M., Harris, J.E., 2018. Mouse model for human vitiligo. *Curr. Protoc. Immunol.* 25, e63.
- Robinson Jr., D., Hackett, M., Wong, J., Kimball, A.B., Cohen, R., et al., 2006. Co-occurrence and comorbidities in patients with immune-mediated inflammatory disorders: an exploration using US healthcare claims data, 2001–2002. *Curr. Med. Res. Opin.* 22, 989–1000.
- Rocha, I.M., Oliveira, L.J., De Castro, L.C., De Araujo Pereira, L.I., Chaul, A., Guerra, J.G., et al., 2002. Recognition of melanoma cell antigens with antibodies present from patients with vitiligo. *Int. J. Dermatol.* 39, 840–843.
- Sabroe, R.A., Greaves, M.W., 2006. Chronic idiopathic urticaria with functional autoantibodies: 12 years on. *Br. J. Dermatol.* 154, 813–819.
- Sabroe, R.A., Grattan, C.E., Francis, D.M., Barr, R.M., Kobza Black, A., Greaves, M.W., 1999. The autologous serum skin test: a screening test for autoantibodies in chronic idiopathic urticaria. *Br. J. Dermatol.* 140, 446–452.
- Safavi, K.H., Muller, S.A., Suman, V.J., Moshell, A.N., Melton III, L.J., 1995. Incidence of alopecia areata in Olmsted County, Minnesota, 1975 through 1989. *Mayo Clin. Proc.* 70, 628–633.
- Sagi, L., Solomon, M., Baum, S., Lyakhovitsky, A., Trau, H., Barzilai, A., 2011. Evidence for methotrexate as a useful treatment for steroid-dependent chronic urticaria. *Acta Derm. Venereol.* 91, 303–306.
- Schallreuter, K.U., Wood, J.M., Berger, J., 1991. Low catalase levels in the epidermis of patients with vitiligo. *J. Invest. Dermatol.* 97, 1081–1085.
- Schallreuter, K.U., Moore, J., Wood, J.M., Beazley, W.D., Peters, E.M.J., Marles, L.K., et al., 2001. Epidermal H<sub>2</sub>O<sub>2</sub> accumulation alters tetrahydrobiopterin (6BH4) recycling in vitiligo: identification of a general mechanism in regulation of all 6BH4-dependent processes? *J. Invest. Dermatol.* 116, 167–174.
- Schallreuter, K.U., Chavan, B., Rokos, H., Hibberts, N., Panske, A., Wood, J.M., 2005. Decreased phenylalanine uptake and turnover in patients with vitiligo. *Mol. Genet. Metabol.* 86, S27–S33.
- Schmetz, O., Lakin, E., Topal, F.A., Preusse, P., Freier, D., Church, M.K., et al., 2018. IL-24 is a common and specific autoantigen of IgE in patients with chronic spontaneous urticaria. *J. Allergy Clin. Immunol.* 142, 876–882.
- Schocket, A.L., 2006. Chronic urticaria: pathophysiology and etiology, or the what and why. *Allergy Asthma Proc.* 27, 90–95.
- Schön, M.P., Detmar, M., Parker, C.M., 1997. Murine psoriasis-like disorder induced by naive CD41 T cells. *Nat. Med.* 3, 183–188.
- Schwarz, T., 2008. 25 years of UV-induced immunosuppression mediated by T cells—from disregarded T suppressor cells to highly respected regulatory T cells. *Photochem. Photobiol.* 84, 10–18.

- Shigenobu, A., Yaoita, H., Kitajima, Y., 1989. An elevated level of auto-antibodies against 48- to 50-kd keratins in the serum of patients with psoriasis. *J. Invest. Dermatol.* 92, 179–183.
- Spritz, R.A., 2010. The genetics of generalized vitiligo: autoimmune pathways and an inverse relationship with malignant melanoma. *Genome Med.* 2, 78.
- Spritz, R.A., 2011. Recent progress in the genetics of generalized vitiligo. *J. Genet. Genomics.* 38, 271–278.
- Stern, R.S., Nijsten, T., Feldman, S.R., Margolis, D.J., Rolstad, T., 2004. Psoriasis is common, carries a substantial burden even when not extensive, and is associated with widespread treatment dissatisfaction. *J. Invest. Dermatol. Symp. Proc.* 9, 136–139.
- Sugiyama, H., Gyulai, R., Toichi, E., Garaczi, E., Shimada, S., Stevens, S.R., et al., 2005. Dysfunctional blood and target tissue CD41 CD25 high regulatory T cells in psoriasis: mechanism underlying unrestrained pathogenic effector T cell proliferation. *J. Immunol.* 174, 164–173.
- Sun, J., Silva, K.A., McElwee, K.J., King Jr., L.E., Sundberg, J.P., 2008. The C3H/HeJ mouse and DEBR rat models for alopecia areata: review of preclinical drug screening approaches and results. *Exp. Dermatol.* 17, 793–805.
- Sundberg, J.P., King Jr., L.E., 1996. Mouse mutations as animal models and biomedical tools for dermatological research. *J. Invest. Dermatol.* 106, 368–376.
- Sundberg, J.P., Silva, K.A., Li, R., Cox, G.A., King, L.E., 2004. Adult-onset alopecia areata is a complex polygenic trait in the C3H/HeJ mouse model. *J. Invest. Dermatol.* 123, 294–297.
- Taieb, A., Alomar, A., Böhm, M., Dell'anna, M.L., De Pase, A., Eleftheriadou, V., et al., 2013. Guidelines for the management of vitiligo: the European Dermatology Forum consensus, in: Vitiligo European Task Force (VETF); European Academy of Dermatology and Venereology (EADV); Union Européenne des Médecins Spécialistes (UEMS). *Br. J. Dermatol.* 168, 5–19.
- Takei, M., Mishima, Y., Uda, H., 1984. Immunopathology of vitiligo vulgaris, Sutton's leukoderma and melanoma-associated vitiligo in relation to steroid effects. I. Circulating antibodies for cultured melanoma cells. *J. Cutan. Pathol.* 11, 107–113.
- Taskapan, O., Kutlu, A., Karabudak, O., 2008. Evaluation of autologous serum skin test results in patients with chronic idiopathic urticaria, allergic/non-allergic asthma or rhinitis and healthy people. *Clin. Exp. Dermatol.* 33, 754–758.
- Tilles, S.A., 2005. Approach to therapy in chronic urticaria: when benadryl is not enough. *Allergy Asthma Proc.* 26, 9–12.
- Tobin, D.J., 2003. Characterization of hair follicle antigens targeted by the anti-hair follicle response. *J. Invest. Dermatol. Symp. Proc.* 8, 176–181.
- Tobin, D.J., Sundberg, J.P., King Jr., L.E., Boggess, D., Bystryn, J.C., 1997. Autoantibodies to hair follicles in C3H/HeJ mice with alopecia areata-like hair loss. *J. Invest. Dermatol.* 109, 329–333.
- Trojan, T.D., Khan, D.A., 2012. Calcineurin inhibitors in chronic urticaria. *Curr. Opin. Allergy Clin. Immunol.* 12, 412–420.
- Verheyen, J., Bonig, H., Banning, U., Shin, D.I., Mauz-Körholz, C., Korholz, D., 2001. Co-operation of IL-1 and IL-2 on T-cell activation in mononuclear cell cultures. *Immunol. Invest.* 30, 289–302.
- Viac, J., Groujon, C., Misery, L., Staniak, V., Faure, M., Schmitt, D., et al., 1997. Effect of UVB 311 nm irradiation on normal human skin. *Photodermatol. Photoimmunol. Photomed.* 13, 103–108.
- Wagner, E.F., Schonthalter, H.B., Guinea-Viniegra, J., Tschachler, E., 2010. Psoriasis: what we have learned from mouse models. *Nat. Rev. Rheumatol.* 6, 704–714.
- Wang, E.H.C., Sallee, B.N., Tejeda, C.I., Christiano, A.M., 2018. JAK inhibitors for treatment of alopecia areata. *J. Invest. Dermatol.* 138, 1911–1916.
- Wankowicz-Kalinska, A., van den Wijngaard, R.M., Tigges, B.J., Westerhof, W., Ogg, G.S., Cerundolo, V., et al., 2003. Immunopolarization of CD41 and CD81 T cell to type-1-like is associated with melanocyte loss in human vitiligo. *Lab. Invest.* 83, 683–695.
- Westerhof, W., d'Ischia, M., 2007. Vitiligo puzzle: the pieces fall in place. *Pigment Cell Res.* 20, 345–359.
- Whiting, D.A., 2003. Histopathologic features of alopecia areata: a new look. *Arch. Dermatol.* 139, 1555–1559.
- Winchester, R., 2008. Psoriatic arthritis. In: Wolff, K., et al., (Eds.), *Fitzpatrick's Dermatology in General Medicine*, seventh ed. McGraw-Hill, New York, pp. 194–207.
- Yang, E.M., Kim, S.H., Kim, N.H., Park, H.S., 2010. The genetic association of the FPRL1 promoter polymorphism with chronic urticaria in a Korean population. *Ann. Allergy Asthma Immunol.* 105, 96–97.
- Yu, H.S., Kao, C.H., Yu, C.L., 1993. Coexistence and relationship of antikeratinocyte and antimelanocyte antibodies in patients with non-segmental-type vitiligo. *J. Invest. Dermatol.* 100, 823–828.
- Zimmerman, A.B., Berger, E.M., Elmariah, S.B., Soter, N.A., 2012. The use of mycophenolate mofetil for the treatment of autoimmune and chronic idiopathic urticaria: experience in 19 patients. *J. Am. Acad. Dermatol.* 66, 767–770.
- Zuberbier, T., 2012. Chronic urticaria. *Curr. Allergy Asthma Rep.* 12, 267–272.
- el-Mofty, A.M., el-Mofty, M., 1980. Vitiligo. A symptom complex. *Int. J. Dermatol.* 19, 237–244.

## Further Reading

- Birlea, S.A., Costin, G.E., Roop, D.R., Norris, D.A., 2017. Trends in regenerative medicine: repigmentation in vitiligo through melanocyte stem cell mobilization. *Med. Res. Rev.* 37, 907–935.
- Dressler, C., Werner, R.N., Eisert, L., Zuberbier, T., Nast, A., Maurer, M., 2018. Chronic inducible urticaria: a systematic review of treatment options. *J. Allergy Clin. Immunol.* 141, 1726–1734.
- Fukuda, K., Harris, J.E., 2018. Vitiligo-like depigmentation in patients receiving programmed cell death-1 inhibitor reflects active vitiligo. *J. Am. Acad. Dermatol.* 78, e15–e16.
- Maurer, M., Metz, M., Brehler, R., Hillen, U., Jakob, T., Mahler, V., et al., 2018a. Omalizumab treatment in patients with chronic inducible urticaria: A systematic review of published evidence. *J. Allergy Clin. Immunol.* 141, 638–649.
- Maurer, M., Zuberbier, T., Siebenhaar, F., Krause, K., 2018b. Chronic urticaria – what does the new guideline tell us? *J. Dtsch. Dermatol. Ges.* 16, 584–593.
- Richmond, J.M., Strassner, J.P., Zapata Jr, L., Garg, M., Riding, R.L., Refat, M.A., et al., 2018. Antibody blockade of IL-15 signaling has the potential to durably reverse vitiligo. *Sci. Transl. Med.* 10, 450.

# Autoimmune Orchitis and Autoimmune Oophoritis

*Livia Lustig<sup>1</sup>, Vanesa A. Guazzone<sup>1</sup> and Kenneth S.K. Tung<sup>2</sup>*

<sup>1</sup>University of Buenos Aires, National Scientific and Technical Research Council, Institute of Biomedical Research (INBIOMED), School of Medicine, Buenos Aires, Argentina <sup>2</sup>Department of Pathology and Beirne B Carter Center for Immunology Research, University of Virginia, Charlottesville, VA, United States

## O U T L I N E

<b>Introduction</b>	<b>1235</b>	Tolerance Mechanism for Ovary Autoantigens	1242
<b>Autoimmune Disease of the Testis</b>	<b>1236</b>	Experimental Autoimmune Ovarian Disease	1243
Tolerance Mechanism for Testis Autoantigens	1236	<b>Concluding Remarks</b>	1245
Experimental Autoimmune Disease of the Testis	1237	Acknowledgments	1245
Clinical Autoimmune Disease of the Testis	1241	<b>References</b>	1246
<b>Autoimmune Oophoritis</b>	<b>1242</b>		

## INTRODUCTION

Experimental studies predict a frequent occurrence of human gonadal autoimmunity. In spontaneous multiorgan models of autoimmune diseases, the testis and ovary are frequent targets. Although progress in clinical research has been slow, in part explained by the success of assisted reproduction techniques, experimental studies have yielded exceptional information on the fundamental mechanisms of tolerance and autoimmunity. They include the discovery and functional analysis of the CD4+ CD25+ Foxp3+ regulatory T cells (Tregs) as a major tolerance mechanism, the sequel of autoimmune regulator (AIRE) gene deficiency, molecular mimicry as a basis of autoreactive T-cell response, the epitope spreading phenomenon in autoantibody production, and neonatal propensity in autoimmunity development. In addition, they provide guidelines for translational research into human disease and better understanding of chronic inflammatory conditions of the gonads associated with subfertility and infertility. Finally, knowledge of the local immune regulation in testis as an immune privileged site, and the recently discovered systemic tolerance mechanism, have contributed to a more complete understanding of the immunological control against gonadal autoantigens, as well as to the numerous human cancer antigens that they share with normal germ-cell antigens in testes and ovaries (cancer/testis antigens). In this chapter, we will present the experimental autoimmune models, followed by a description of existing data supporting an autoimmune basis in human testicular and ovarian diseases.

## AUTOIMMUNE DISEASE OF THE TESTIS

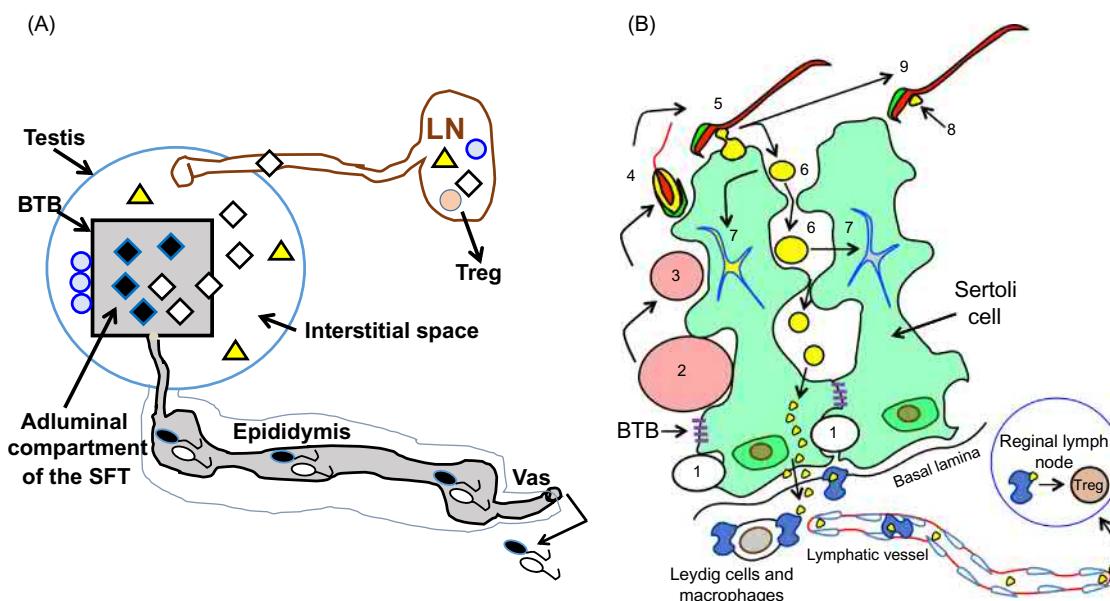
### Tolerance Mechanism for Testis Autoantigens

Peripheral systemic tolerance requires continuous exposure of the immune system to tissue antigens (Samy et al., 2005; Setiady et al., 2006; Wheeler et al., 2009). Therefore it is important to examine their location in the testis relative to the strong blood–testis barrier (BTB).

### The Landscape of Testicular Autoantigen Expression

The BTB is a basal tight junction between adjacent Sertoli cells completely encircling the adluminal compartment of the seminiferous tubules that are lined by the basement membrane and peritubular cells. The adluminal compartment is occupied by all the developing meiotic germ cells. External to this compartment reside the stem cells and premeiotic germ cells (spermatogonia and the preleptotene spermatocytes), Leydig cells, and macrophages (interstitial space) (Fig. 62.1A and B). Blood vessels and afferent lymphatics traverse the interstitial space, providing full access of the interstitial testis antigens to the immune system. They also allow circulating antibodies and activated leukocytes to enter the interstitial space. The interstitial space contains two classes of resident macrophages with essential nonimmune functions (Hutson, 1998; Smith et al., 2015): the yolk sac–derived macrophages in close proximity to the Leydig cells (Mossadegh-Keller et al., 2017) and the postnatal bone marrow–derived macrophages close to the spermatogonia (DeFalco et al., 2015). Macrophages are also the major antigen-presenting cells in the murine testis (Winnall and Hedger, 2013).

In contrast, all the autoantigens expressed by sperm and other meiotic germ cells in the adluminal compartment have been considered to be totally sequestered in a closed environment, invisible to the immune system (Mital et al., 2011; Hedger, 2002; Fijak and Meinhardt, 2006; Li et al., 2012), and would not be protected



**FIGURE 62.1** (A) A landscape of cellular antigens in testicular compartments, epididymis and regional lymph node. The blood–testis barrier (BTB) divides the normal testis into the seminiferous tubule compartment and the interstitial compartment. Antigenic resident cells in interstitial space are: spermatogonia and preleptotene spermatocytes (circle) and Leydig cells (triangle). Seminiferous tubule contains all meiotic germ cells (diamonds). Some meiotic germ cell antigens (white diamond) continuously egress into interstitial space to reach the regional lymph node while other antigens are sequestered (filled diamond). (B) A cartoon showing the location and fate of male germ cell development adjacent to two Sertoli cells (in green). Spermatogonia (1) transverses the BTB to become spermatocytes (2 and 3) and early spermatid (4) that develops acrosome (in green), cytoplasm (in yellow) and tail (in red). Spermiation occurs when late spermatid (5) becomes sperm (9). Some meiotic germ cell cytoplasm becomes the residual body (6) and others become cytoplasmic droplet on sperm (6 in 9). Most residual bodies are engulfed and destroyed by Sertoli cell (7). However, some residual bodies stay outside Sertoli cells until reaching their base, and enter interstitial space. The testis autoantigens protected by the Treg-dependent tolerance mechanism include those in: 1) Leydig cell, 2) spermatogonia and preleptotene spermatocytes, 3) meiotic germ cell antigens in residual body (example: LDH3). The sequestered meiotic germ cell antigens (example: zonadhesin in sperm acrosome) are not protected by systemic tolerance. (Fig 1B is reproduced from Tung et al., 2017, with permission from the Publisher.)

by systemic tolerance. However, recent studies have challenged and shifted this complete testis antigen sequestration paradigm.

First, a cohort of meiotic germ-cell antigens, located inside the seminiferous tubule behind the BTB, is continuously “exported” into the interstitial space despite an intact BTB (Fig. 62.1A and B). Importantly, these exposed autoantigens are protected by systemic tolerance conferred by the CD4+ CD25+ Foxp3+ Treg (Tung et al., 2017). Second, the testis autoantigens in the interstitial compartments are also relevant to testicular autoimmunity: (1) Leydig cell steroidogenic enzymes commonly targeted by autoantibodies in the autoimmune polyendocrine syndromes (APS) (Sotsiou et al., 1980; Elder et al., 1981); (2) antigens expressed in the spermatogonia, expressed in many human cancers as cancer/testis antigens, are currently utilized in human cancer vaccines (Simpson et al., 2005); and (3) autoimmunogenic antigens are known to exist in preleptotene spermatocytes (Yule et al., 1988).

Testis antigens with capacity to induce experimental autoimmune orchitis (EOA) are localized in meiotic germ cells including the spermatozoa. When strictly defined as antigens capable of inducing EAO at the low µg range, only very few orchitogenic antigens have so far been identified (reviewed by Frayne and Hall, 1999). They included the meiotic germ cell–specific guinea pig PH20 (Tung et al., 1997) and the murine zonadhesin (ZAN) (Wheeler et al., 2011). However, mice with EAO also produce autoantibodies that target somatic antigens but their orchitogenicity has not been analyzed (Terayama et al., 2016).

### **Systemic Tolerance to the Exposed Meiotic Germ-Cell Antigens**

Discovery of systemic tolerance to the exposed meiotic germ-cell antigens involves a study on the cytoplasmic lactate dehydrogenase 3 (LDH3) (Hintz and Goldberg, 1977), the acrosomal membrane antigen ZAN (Hardy and Garbers, 1994), and transgenic ovalbumin (OVA)—a surrogate cytoplasmic meiotic germ-cell antigen, based on the following findings (Tung et al., 2017). First, ZAN, normally hidden and not tolerogenic, elicits a strong orchitogenic response only when exposed in the epididymal interstitium after vasectomy (Wheeler et al., 2011). Second, tolerogenicity of LDH3 is documented in a study on the DEREG mice that express the human diphtheria toxin receptor exclusively on the Foxp3+ Treg (Lahl et al., 2007). Transient Treg depletion by diphtheria toxin leads to spontaneous EAO and production of antibodies that target LDH3 but not ZAN. A similar study also identified the transgenic OVA as an exposed meiotic germ-cell antigen (Fig. 62.2A and B). Third, LDH3 (Fig. 62.2A) and OVA but not ZAN (Fig. 62.2B) are contents in the residual bodies (Fig. 62.1B); these residual bodies stay outside the Sertoli cell and escape degradation (Fig. 62.1B). Fourth, and most directly, LDH3 escape is documented in normal wild-type mice injected intravenously with rabbit LDH3 antibody, which forms immune complexes with LDH3 on the seminiferous tubule boundary outside the BTB (Fig. 62.2C), and this is not observed in mice injected with ZAN antibody (Fig. 62.2D).

### **Local Regulation in the Testis (Immune Privilege)**

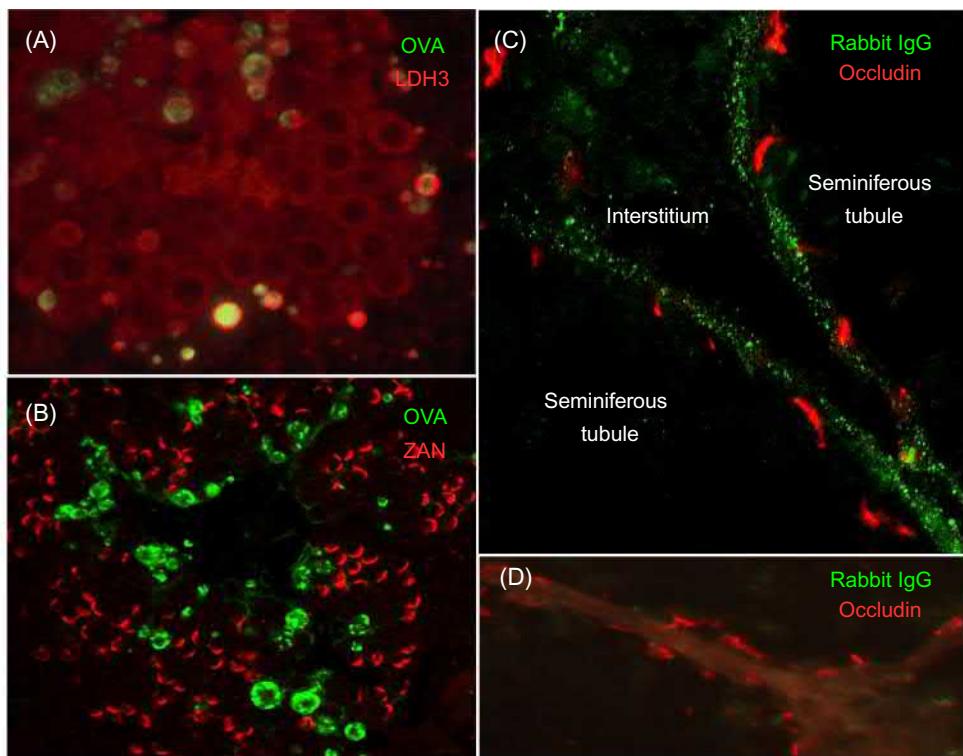
Systemic tolerance and local immune privilege are partners for the complete immune protection of sperm autoantigens. Local regulation of antigen exposure and tolerogenic presentation by unique antigen-presenting cells ensures successful maintenance of Treg and systemic tolerance. In addition, local regulation per se provides barrier function and controls immune mediators that operate in the efferent arm of autoimmunity, or from any other causes of testis inflammation, including infections.

Head et al. (1983) discovered the prolonged survival of an allogeneic organ grafted in the testicular interstitial space and called attention to the testis as an immunoprivileged organ (reviewed by Fijak et al., 2011; Li et al., 2012). Since then, many soluble and cell-based immunosuppressive factors have been discovered in the testis interstitium, largely based on in vitro studies. We summarize the major cell types and soluble factors involved in testicular immune privilege in Table 62.1.

## **Experimental Autoimmune Disease of the Testis**

### **Classical Experimental Autoimmune Orchitis Induced by Immunization with Testis Antigen in Adjuvant**

EOA in guinea pigs was first described by Voisin et al. (1951) and by Freund et al. (1953). Attempts to transfer disease by serum antibody to normal guinea pig failed but the study clarified regional differences in the barrier properties in different spermatogenic compartments (Tung et al., 1971a, 1987b; Johnson, 1973). Lymphocytes retrieved directly from immunized guinea pig transferred EAO only by subcapsular injection but not by intravenous injection (Tung et al., 1971b). This suggested T cells need to express tissue homing receptor to enter



**FIGURE 62.2** LDH3 (A) but not ZAN (B) is co-localized with ovalbumin (OVA) in the residual bodies of mice expressing a transgenic OVA. OVA, a surrogate meiotic germ cell antigen expressed under the protamine 1 promoter, serves as residual body marker (Tung et al., 2017). (C and D) Normal wild type mice, injected intravenously with rabbit antibody to LDH3 (C), but not antibody to ZAN (D), develop immune complexes as punctate rabbit IgG surrounding the seminiferous tubule, outside the occludin-positive BTB. Therefore, mouse LDH3 but not ZAN egresses normal seminiferous tubule. (Confocal microscopy  $\times 1600$ .)

the testis. Further progress come from mouse and rat EAO models. In general, EAO induction requires the injection of testis antigen in complete Freund's adjuvant (CFA) and/or pertussis toxin (Kohno et al., 1983; Doncel et al., 1989); however, murine EAO can also be induced by subcutaneous injections of viable testicular cells without adjuvant (Sakamoto et al., 1985; Itoh et al., 1991, 2005). A detailed description of the protocols for testicular autoimmune disease was described.

EAO induced by active immunization is initiated in subcapsular seminiferous tubules, adjacent to the major lymphatic vessels (Hirai et al., 2012). In contrast, when orchitogenic T cells are adoptively transferred to normal recipients, they initially target the straight tubules and the ductus efferentes that are not lined by Sertoli cells; subsequently, they spread to the seminiferous tubules (Tung et al., 1987b; Itoh et al., 2005). After migration into the testis interstitium, the macrophages, dendritic cells (DCs), and T cells form multiple cell clusters around the seminiferous tubule. In many species, with the exception of rat EAO, these cells invade the BTB and enter seminiferous tubules. It is important to understand how T cells enter the testis, damage the BTB, and induce sloughing and apoptosis of germ cells.

EAO mechanisms have been elucidated by investigations on rat with active EAO induction. First, chemokines and cytokines upregulate adhesion molecules on the endothelial cell (EC) and support T-cell attachment to EC and extravasation into the interstitial space. Also required is contact between the activated form of CD44 on lymphocytes and its major ligand hyaluronic acid expressed on EC (Guazzone et al., 2005). Second, chemokines (which include CCL2/MCP1, CCL3/MIP1 $\alpha$ , and CCL4/MIP1 $\beta$ ) expressed by testicular cells convert the leukocyte rolling into cell arrest. In addition, the higher percentage of leukocytes expressing CD49d integrin and the increased expression of CD106/VCAM ligand in EC mediate the step of firm adhesion. Third, inflammatory cell transmigration occurs via the interaction of CD106 and CD31/PECAM-1 (Guazzone et al., 2009, 2012). Finally, germ-cell apoptosis involves several mechanisms, including TNF $\alpha$ /TNFR1, IL-6/IL6R, and FasL/Fas (Suescum et al., 2003; Theas et al., 2003, 2008; Rival et al., 2006b), as well as the death receptor and mitochondrial apoptotic pathways (Theas et al., 2006).

**TABLE 62.1** Immune and Somatic Cells Involved in Testis Immune Privilege

Cells	Functions
Foxp3 Tregs	Treg maintain systemic tolerance to nonsequestered meiotic germ-cell antigens in normal mice (Tung et al., 2017) <sup>a</sup>
Dendritic cells	DCs in rat testis are functionally tolerogenic: do not stimulate T-cell proliferation (Rival et al., 2007)
Macrophages	Resident macrophages, highly represented in testis (Rival et al., 2008), have an impaired capacity to act as costimulators for T cell–activation responses (Winnall et al., 2011) but are able to expand Tregs (Wang et al., 2017)
Sertoli cells	As implant in ectopic site, prevent allograft rejection and suppress diabetes (Selawry and Cameron, 1993; Mital et al., 2010) <sup>a</sup> Express/secrete immunoregulatory molecules <ul style="list-style-type: none"> <li>• TGF<math>\beta</math> expression by murine prepuberal Sertoli cells was associated with protection of islet allografts from autoimmune destruction, with generation of Treg (Suarez-Pinzon et al., 2000; Campese et al., 2014)</li> <li>• Inhibition of IDO expression abrogates the ability of porcine prepuberal Sertoli cells to protect allografts in mice (Fallarino et al. 2009). IDO is downregulated in rats with autoimmune orchitis (Guazzone VA, <i>personal communication</i>)</li> <li>• Prepuberal murine Sertoli cells expressing Galectin1 promote the in vitro differentiation of tolerogenic dendritic cells (Gao et al., 2016). Adult mice Sertoli cells also express galectin1. However, mice genetically deficient in Galectin1 present a decreased incidence and severity of orchitis versus wild-type mice (Pérez et al., 2015)<sup>a</sup></li> <li>• Prepuberal murine Sertoli cells expressing PD-L1 reduce CD8<math>^{+}</math> T-cell proliferation (Dal Secco et al., 2008)</li> </ul> <i>BTBS</i> ertoli cell tight junction proteins play a key role in structure and function of BTB that blocks antibodies and lymphoid cells entering seminiferous tubules (Mruk and Cheng, 2015) <sup>a</sup> Negative regulator of innate system TAM receptor tyrosine kinase subfamily is a negative regulator of innate immune system (Lu et al 1999; Lemke and Rothlin, 2008; Zhang et al. 2013; Li et al., 2015). Also enhance Sertoli cell and macrophage phagocytosis of apoptotic germ cells and reduce accessible testis autoantigens (Xiong et al., 2008)
Leydig cells	Secret TT regulates BTB permeability by modulation of claudin 3 expression (Meng et al, 2005) <sup>a</sup> T stimulates the differentiation of Treg (Fijak et al., 2015)
Germ cells	PD-L1 expression in spermatocytes and spermatids constitutively. Its inhibition abrogates long-term survival of intratesticular islets allografts (Cheng et al., 2009) <sup>a</sup>

<sup>a</sup>In vivo studies.

IDO, Indolamine 2,3-dioxygenase; PD-L1, programmed death-ligand 1; BTB, blood–testis barrier; TAM, tyro 3-Axl-Mer; T, testosterone.

Interstitial macrophages and DCs are critical participants in EAO; in vivo depletion of these cells in rat EAO by clodronate-containing liposomes significantly reduces EAO incidence and severity (Rival et al., 2006a, 2007, 2008). The interstitial macrophages express high levels of MHC class II, CD80, and CD86; their numbers are increased (Rival et al., 2008); and they produce proinflammatory cytokines, mainly TNF $\alpha$ , IL6, IFN $\gamma$  (Suescum et al., 2003; Rival et al., 2006b), and nitric oxide (Jarazo-Dietrich et al., 2012, 2015). The mediators also alter the integrity and function of Sertoli adherens and tight junctions facilitating germ-cell sloughing (Lee and Cheng, 2003; Pérez et al., 2011, 2012, 2014). The DCs from testicular draining lymph nodes (LNs) are mature and express more IL-12p35 mRNA than do DCs from nondraining LN (Guazzone et al., 2011). Excessive male germ-cell antigens released into the interstitium during EAO further stimulate T-cell response in the testis draining LN.

CD4 $^{+}$  but not CD8 T cells are pivotal. Polyclonal or monoclonal murine CD4 $^{+}$  T cells adoptively transfer severe EAO (Tung et al., 1987b; Yule and Tung, 1993). In rat EAO, detection of an increase in the testicular IL17 and IL23 suggests the involvement of Th17 subsets (Jacobo et al., 2011a). At the chronic stage, variable number and cytokine profile (Th1 and Th17) of CD8 $^{+}$  T cells are also identified (Jacobo et al., 2009, 2011a). In mouse EAO, Th subset requirement is strictly strain dependent, with IL17 being necessary and sufficient for C57BL/6 mice, but requiring both IL17 and IFNg in BALB/c mice (Rival and Tung, unpublished data).

The changes in Treg have been investigated in rats and mice with EAO. They are distributed as clusters in the subcapsular seminiferous tubules of rats with classical EAO (Jacobo et al., 2009). In a transgenic mouse EAO model (Paul and Tung, unpublished data), these clusters were found to include antigen-specific Treg. In rat EAO, the Tregs isolated from regional LN are more potent suppressors of polyclonal T-cell response than the Treg from other LN in vitro (Jacobo et al., 2015). However, Treg fails to effectively suppress ongoing inflammation in vivo. This may be caused by effector T-cell resistance to suppression or to the reduction of the

Treg-suppressive function (Jacobo et al., 2011b). In a study on transgenic mouse EAO, Tregs in LN and testis with EAO were found to lose Foxp3 expression, produce specific proinflammatory cytokines that match the cytokines produced by the host antigen-specific effector T cells in the inflamed testis (Paul and Tung, unpublished data).

Although antibody per se does not transfer EAO to normal recipients, it participates as immune complex formation in the tubular basement membrane. In murine EAO, spontaneous EAO, and vasectomized rabbits, immune complexes are characterized by granular deposition of IgG and complement C3. In addition to complement activation, the antibody can also synergize with effector T cells to induce severe EAO (Wheeler et al., 2011; Paul and Tung, unpublished data).

### **Autoimmune Orchitis in the Dark Mink**

Inbreeding of mink for a dark fur has coselected male infertility in this seasonal breeder (Tung et al., 1981) but also in mink with other fur colors (Pelletier, 1986). There are two histopathologic patterns: one with massive granulomatous inflammation and the other extensive germ cell loss with little inflammation. In the latter, antibodies to sperm acrosome form a massive immune complex, with IgG and complement C3 depositing in the basement membrane outside the BTB. Interestingly, an association of autoimmune orchitis with abnormal hypothalamic–pituitary–testicular function is suggested by the finding that EAO is inhibited by exogenous gonadotropins (Tung et al., 1984). Pelletier et al. (2009) have critically analyzed the spermatogenic cycle in this seasonal breeder and provided evidence for defects in the apoptotic cell clearance, a mechanism that may cause breakdown of self-tolerance in mink leading to spontaneous autoimmune orchitis.

### **Autoimmune Orchitis in Rats Expressing Transgenic Human HLA B27 and Human $\beta$ 2 Microglobulin**

Lewis rats with transgenic human HLA B27/ $\beta$ 2m spontaneously develop spondyloarthritis and rheumatoid arthritis that mimic the human diseases (Taurog, 2009). Unexpectedly, severe autoimmune orchitis was discovered in the transgenic rats several months before the onset of joint disease (Taurog et al., 2012). Inflammation first appears in the ductus efferentes when the first wave of apoptotic testicular germ cells transits this region to reach the epididymis. Three months later, severe inflammation shifts to the testis and ultimately eliminates sperm production. Interestingly, bilateral excision of the orchitic testes prevents arthritis development and establishes a causal link between two organ-specific autoimmune diseases (Taurog et al., 2012). Although autoimmune orchitis has not been reported in men with spondyloarthritis, sperm abnormalities are reported in these patients (Villiger et al., 2010).

### **Testis Antigen-Specific Tolerance Occurs in Vasectomized Mice and Concomitant Treg Depletion is Required for Postvasectomy EAO Induction**

Vasectomy is a major global contraceptive method adopted by over 0.5 million men in the United States alone. Autoimmune orchitis with immune complex deposition occurs in vasectomized rabbits, guinea pigs, monkeys, and others (Bigazzi, 1981), and they develop sperm antibody, generally of late onset. A recent study in unilaterally vasectomized mice focused on the immunological events over the first 10 weeks. It revealed that the release of sequestered sperm cell antigens rapidly induced a Treg response that prevents subsequent active EAO induction. Tregs are testis antigen specific; they do not prevent EAE induction (Wheeler et al., 2011). Indeed, postvasectomy orchitis and sperm antibody response within the first 10 weeks do not occur unless the Tregs are partially (60%) depleted. Depletion had to occur at the time of vasectomy, but not at 1 week after vasectomy. Importantly, the antibodies targeted the sequestered Zan but not the exposed LDH3 (Wheeler et al., 2011; Tung et al., 2017). Therefore even the sequestered sperm antigens, when released into the epididymis microenvironment, can rapidly initiate Treg-dependent tolerance. However, after 3–4 months, mice with vasectomy alone begin to show detectable serum sperm antibody that fluctuates over time; this is likely dependent on the balance of effector Treg and effector T-cell actions (Rival et al., 2013).

### **Autoimmune Orchitis Associated With Bacterial and Viral Infections**

Autoimmune orchitis may be relevant to the development and the sequel of infectious orchitis. Microbial antigens may cross-react with testis antigen via molecular mimicry at the T- or B-cell level. Alternatively, autoimmune response and orchitis may result from severe infection. In mice with *Listeria monocytogenes* infection only in one testis, the contralateral testis develops orchitis without detectable microorganisms, suggesting an

autoimmune component in an infectious orchitis (Mukasa et al., 1995). Indeed, CD4+ T cells from the infected donors transfer orchitis to uninfected recipients. In this study,  $\gamma\delta$  T cells in both testis are found to regulate  $\alpha\beta$  pathogenic T cells by cytokines, notably involving IL10 and TGF $\beta$  (Mukasa et al., 1997, 1998). The injection of uropathogenic *Escherichia coli* (UPEC) into the vas deferens of rats results in a severe epididymo-orchitis. This experimental model mimics one of the most frequent acute epididymo-orchitis in men. The UPEC elicited differential responses in testicular and peritoneal macrophages, with findings that suggest bifunctional testicular macrophages: one that can initiate immune responses to bacteria, while maintaining testicular immune privilege (Bhushan et al., 2011).

Viruses have been shown to directly infect somatic cells or germ cells and modify their functions, leading to "autoimmune" tissue damage. Orchitis develops in rabbits infected with myxoma virus (Fountain et al., 1997) and Sendai virus (Melaine et al., 2003). More recently, in vitro experiments showed that mumps virus triggers innate immune responses in mouse Sertoli and Leydig cells through TLR2 and retinoic acid-inducible gene-I signaling, which results in the production of proinflammatory cytokines and chemokines (Wu et al., 2016). Herpes simplex virus infection is also evaluated in an experimental model of ascending infection in mice resulting in orchitis, irreversible atrophy of the germinal epithelium, and infertility (Malolina et al., 2014). Mice infected with a mouse-adapted Zika virus (ZIKV) develop testicular and epididymal damage that can progress to reduce key sex hormones, destroy germ, and somatic cells in the testis, inducing loss of mature sperm and infertility. It was suggested that the spermatogonia and other germ cells as well as the Sertoli cells are key targets for ZIKV in the testis (Govero et al., 2016).

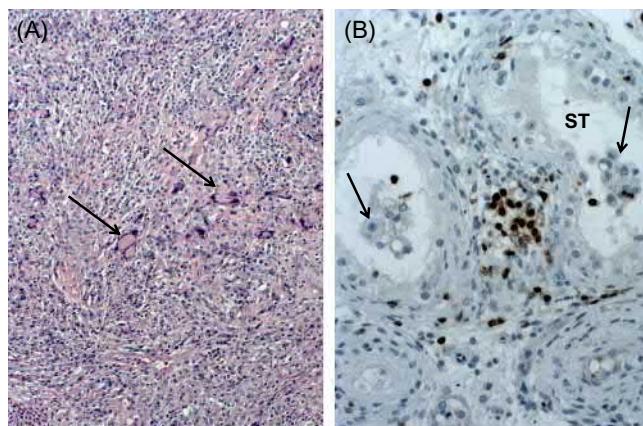
## Clinical Autoimmune Disease of the Testis

### ***Idiopathic Male Infertility***

Antisperm antibodies (ASA) are found in 3%–12% of men with infertility, compared to 0%–2% of the general male population (reviewed by Turek and Lipshultz, 1994). Testicular biopsy indicates that 50% of the patients have focal orchitis (Schuppe et al., 2008). In principle, ASA may cause infertility by interfering with sperm transport and with many steps of fertilization in vitro, such as cervical mucus penetration, acrosome reaction, zona binding, zona penetration, oolemma binding, and pronucleus formation in the fertilized oocyte (reviewed by Tung et al., 2002). The detection by the immunobead assay, of ASA (immunoglobulin A (IgA) and IgG isotypes) in sperm head present in local genital secretions shows a strong collaboration with male infertility (reviewed by Marshburn and Kutteh, 1994), but the nature of the cognate sperm antigens has not been defined (Frayne and Hall, 1999).

Diagnosis of human autoimmune orchitis requires immunopathologic evaluation by testis biopsy which is rarely performed (Schuppe et al., 2008). Most helpful is the detection of orchitis and immune complexes in the testis and sperm or testis autoantibodies in the serum. Immune complexes, detected on frozen section, are found in many types of EAO (Tung, 1978; Tung et al., 1981; Bigazzi, 1981; Lustig et al., 1987, 2000). In human orchitis, light and electron microscope studies showed deposits of electron-dense material in the tubular basement membrane that reacts with antibody to human IgG and complement C3 (Salomon et al., 1982; Lehmann et al., 1987). There are other human orchitides with possible autoimmune basis. One is idiopathic granulomatous orchitis. Clinically, patients present mild scrotal pain or swelling, or a hard scrotal mass. Histologically, the seminiferous tubules are replaced by granulomatous inflammation consisting of T cells, macrophages, and multi-nucleated giant cells (Fig. 62.3A). These findings mimic the changes in rat EAO (Doncel et al., 1989; Taurog et al., 2012). Another and more common type of orchitis contains multiple foci of monocytic inflammation (Suominen and Söderström, 1982; Chan and Schlegel, 2002a,b) and resembles the early lesions in EAO of mice and guinea pig. T-cell and macrophage clusters located at the boundary of seminiferous tubules penetrate the tubule via disrupted BTB (Fig. 62.3B) (Kohno et al., 1983; Schuppe et al., 2008). A recent study detected IL17, IL21, and IL23 among the inflammatory cells and suggests involvement of the proinflammatory Th17 pathway (Duan et al., 2011). Notably, the possible relationship between the infertile patients with circulating sperm antibodies with those whose disease is diagnosed by biopsy-proven orchitis has not been resolved.

Finally, lymphomonocyte infiltration frequently accompanies testis infection and other diseases that damage the testicular parenchyma, including testis cancer, trauma, toxic agents, and cryptorchidism (Jahnukainen et al., 1995; Klein et al., 2016; Nistal et al., 2002). Continuous release of orchitogenic antigens from damaged seminiferous tubules may disrupt tolerance and induce autoimmune orchitis.



**FIGURE 62.3** Human orchitis. (A) Granulomatous orchitis with heavy monocytic inflammation and numerous multinuclear giant cells (arrows); the normal testis histology is effaced. (H and E x200.) (B) Focal “autoimmune orchitis” in testis biopsy of an infertile man. Note CD3+ T cells in a cluster of mononuclear cells that abut the wall of seminiferous tubule (ST). Note macrophage-like cells inside lumen (arrows). These findings phenocopy the histopathology of rodent EAO. (immunoperoxidase x800) (B is photographed from a slide from Dr. H.C. Schuppe and Dr. A. Meinhardt)

### **Infertility and Antisperm Antibodies Coexist With Other Autoimmune Diseases**

The major evidence for an autoimmune basis of human autoimmune orchitis comes from patients with APS. APS-type 1 (APS-1) is a rare autosomal recessive disease caused by AIRE gene mutation (Kisand and Peterson, 2011). About 30% of the male patients develop testis failure with autoantibody to steroidogenic enzymes and other antigens expressed in the Leydig cells (Maclare et al., 2001).

### **Antibody Response in Vasectomy and Cystic Fibrosis**

Patients with vasectomy commonly produce ASA, detectable several months after surgery. Postvasectomy ASA may be a cause of infertility in men with vasovasostomy and adequate sperm count (Lee et al., 2009). Focal orchitis has also been reported in the testes of vasectomized men (McDonald, 1997) and by epidemiological analysis (Goldacre et al., 2007). However, a systematic immunopathologic analysis of the human testes and epididymis in vasectomized subjects is still lacking. Epididymal sperm granuloma commonly occurs in vasectomized mice (>80%) and also occurs in men, but the real incidence in human is unknown in the absence of histopathologic data. Finally, ASA response also occurs in patients with congenital absence of the vas deferens and seminal vesicles associated with cystic fibrosis (D'Cruz et al., 1991).

### **Orchitis Associated With Bacterial and Virus Infections**

Many infectious agents target the testis and cause epididymo-orchitis and infertility. They include bacteria, mainly *E. coli* and *Neisseria gonorrhoea*, and viruses, mainly mumps virus, human immunodeficiency virus (HIV), and ZIKV. The attendant tissue injury may expose germ-cell antigens, which could invoke secondarily an autoimmune response. Indeed, sperm antibodies are detectable in patients with HIV (reviewed by Dejucq and Jégou, 2001; Xia et al., 2009). The immune privilege status of the testis may facilitate the latency of infectious agents (Winnall et al., 2015). HIV and ZIKV, and bacteria that enter Sertoli cells or spermatogonia, would facilitate sexual transmission (Baud et al., 2017; Ma et al., 2017). Finally, idiopathic orchitis such as granulomatous orchitis may have an infectious etiology that has not yet been identified.

## **AUTOIMMUNE OOPHORITIS**

### **Tolerance Mechanism for Ovary Autoantigens**

Suppression of murine ovarian autoimmunity induced by thymectomy on day 3 of life (d3tx), by lymphoid organs implanted from normal donors, eventually led to the discovery of Treg (Nishizuka and Sakakura, 1969). Ovarian antigen-specific Tregs have been shown to accumulate strategically in the ovarian regional LN of normal mice. Whereas 0.5 million Tregs pooled from all LN are required to suppress autoimmune ovarian disease

(AOD), suppression was accomplished with 30,000 Tregs from the ovary-draining LN alone. The enrichment of disease-specific Treg in regional LN applies to the suppression of other autoimmune diseases (Setiady et al., 2006; Samy et al., 2008; Wheeler et al., 2009). Importantly, the regional LN-specific enrichment of organ-specific Treg function is maintained by tissue antigens from the normal target organ. Thus the ablation of prostate antigen led to the loss of prostate LN-specific Treg enrichment, but enrichment is restored upon prostate antigen reexpression (Setiady et al., 2006). The preferential distribution of antigen specific Treg in regional LN is now confirmed at the level of antigen-specific Treg numbers and Treg function (Leventhal et al., 2016; Malchow et al., 2016). In addition, T cell–receptor analysis in individual T cells indicates that the repertoire of Treg is unique for an individual draining LN, but the repertoire for T effectors is shared among all LNs (Hsieh et al., 2012). Therefore the ovarian autoimmune disease is prevented by antigen-specific polyclonal Treg, whose function and/or number are continuously enhanced by interaction with endogenous tissue autoantigens in the strategic location of the regional LN.

## Experimental Autoimmune Ovarian Disease

### ***Spontaneous Autoimmune Ovarian Disease in the AIRE Null Mice***

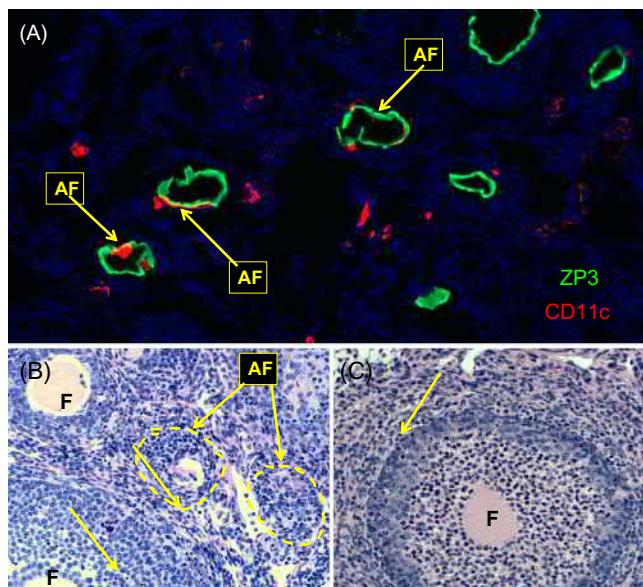
Mice with *AIRE* deficiency resemble humans with the APS-1 syndrome including a high incidence of spontaneous AOD and female infertility (Anderson et al., 2002; Kuroda et al., 2005; Cheng et al., 2007). *AIRE* regulates the transcription of many tissue-specific antigens that are ectopically expressed in thymic medullary epithelial cells. These antigens, presented by unique DC, participate in central tolerance by promoting the development of antigen-specific natural Treg and deleting high-affinity effector T cells (Anderson et al., 2002; Derbinski et al., 2005). In *AIRE* null mice, the absence of thymic ovarian antigen allows the emergence of pathogenic effector T cells against ovarian autoantigen. The major target autoantigen resides in oocyte cytoplasm (Cheng and Nelson, 2011), including NALP5 (or Mater) (Tung, unpublished data). NALP5 antibody is also detected in APS-1 patients.

### ***Autoimmune Ovarian Disease in Day 3 Thymectomized (d3tx) Mice***

Autoimmune disease in d3tx B6AF1 mice commonly targets the ovary, lacrimal gland, prostate, and epididymis (Kojima and Prehn, 1981; Tung et al., 1987a). The depletion of Treg of late ontogeny was initially considered as the mechanism of disease. However, this has been modified by recent studies (Dujardin et al., 2004; Ang et al., 2007; Samy et al., 2008). Tregs with the capacity to suppress autoimmune disease are detectable in normal 3 day-old mice. After d3tx, the mice accumulate more instead of less Treg; in fact, additional Treg depletion from the d3tx mice greatly enhanced disease repertoire, frequency, and severity (Samy et al., 2008). Monteiro et al. (2008) also reported that enhanced effector T cells in a lymphopenic environment are associated with the imbalance in Treg and effectors, and possibly modified thymic T-cell repertoire. Importantly, diseases in the d3tx mice are effectively prevented by CD4+ T cells transferred from normal donors (Sakaguchi et al., 1982; Smith et al., 1991). This key experiment ultimately led to the discovery of the CD25+ Foxp3+ CD4+ Treg (Sakaguchi et al., 1995; Fontenot et al., 2003; Hori et al., 2003). In d3tx mice, effector CD4+ T-cell activation is detected in the regional LN (Alard et al., 2001). This is the same location where the antigen-specific exogenous Tregs accumulate and suppress AOD and effector T-cell and B-cell responses (Samy et al., 2005).

### ***Autoimmune Ovarian Disease in Adult Mice Immunization with Zp3 Peptide (pZP3) with T- and B-Cell Epitopes in Adjuvant***

AOD was first induced in rabbits and dogs by immunization with heterologous zona pellucida (ZP) in CFA (Wood et al., 1981; Mahi-Brown et al., 1982). Since then, significant disease mechanisms have been elucidated by studies in mouse AOD inducible by a novel 13-mer peptide from mouse ZP 3 (pZP3). The pZP3 contains a pathogenic T-cell epitope and a native B-cell epitope (Rhim et al., 1992). First, microbial and other tissue peptides sharing a limited number of critical residues with pZP3 also induced AOD, documenting molecular mimicry at the effector T-cell level (Luo et al., 1993; Garza and Tung, 1995). Second, AOD is inducible by adoptive transfer of pZP3-specific Th1 or Th2 clones. Th1 pathogenic T cells require both CD28 and CD40 costimulatory pathways for activation and disease induction (Griggs et al., 1996). pZP3-specific Th2 cells produce IL4 and IL5 and elicit AOD with dominant eosinophilic inflammation (Lou et al., 2000; Agersborg et al., 2001). A strong murine Th2 autoimmune response to pZP3 is driven by the enteric nematode (the rodent pinworm), a common environmental factor, with neonatal mice being the most susceptible. Neonates injected with pZP3 in water without adjuvant



**FIGURE 62.4** Locations of tissue inflammation and types of inflammatory cells in AOD induced by passive transfer of murine ZP3 specific T-cell clones. (A) T-cell clones alone target the atretic follicles (AF) (arrows with outline) and spare the intact ovarian follicles (no arrow). The zona pellucida that surround oocytes is decorated by a fluorescent Ab to ZP3. (B) Concomitant transfer of T cells and IgG ZP3 antibody results in retargeting of the inflammation into the intact ovarian follicle (C). The transfer of Th1 clones in (B) results in monocytic AOD, whereas transfer of Th2 clones in (C) results in eosinophil-rich AOD. (A: immunofluorescence staining; ZP3 green, CD11c red, x200; B and C: H and E, x200.)

developed severe Th2 AOD and Th2 memory (Agersborg et al., 2001; O’Leary et al., 2008; Lloyd et al., 2010). Notably, monocyte-dominant and eosinophil-dominant ovarian inflammations also exist in human AOD (Lewis, 1993). Research on the pZP3 model also uncovered unique mechanisms of autoantibody induction and its role in disease. Third, mice immunized with a truncated pZP3 T-cell epitope that lacks B-cell epitope rapidly produced antibodies to ZP3 determinants outside the pZP3, including other ZP antigens (Lou et al., 1996; Bagavant et al., 1999). This amplified antibody response is driven by endogenous ovarian antigens and occurs within 7 days. This suggests that autoreactive B cells are not tolerant of the ovarian antigens. The study provided the first definitive evidence for a diversified autoantibody response driven by endogenous antigens (antibody epitope spreading) (Lou and Tung, 1993). Although AOD is not induced in adult mice by ZP antibodies alone, the antibody can strongly influence the distribution of AOD induced by CD4+ T cells. Normal atretic follicles in the ovarian interstitial space, containing the ZP3 antigen and CD11c+ DCs, are major targets of pathogenic CD4+ T cells (Fig. 62.4A). By sparing the mature ovarian follicles, a pure T cell-mediated interstitial oophoritis does not affect mouse fertility (Fig. 62.4B) (Bagavant et al., 1999). However, when ZP3 antibody is bound to the ZP, it redirects the interstitial T-cell inflammation into the mature ovarian follicles (Fig. 62.4C), causing ovarian atrophy and infertility (Lou et al., 2000). Finally, although the antibody does not mediate adult AOD, it induces a unique and severe AOD in the neonatal mice (nAOD).

#### **Neonatal AOD Induction Involves Innate and/or Adaptive Immune Responses Requiring the Neonatal NK Cell with Unique Pathogenic Capacity**

AOD is induced in neonatal mice by antibody injection, or by transplacental transfer of IgG antibody against a ZP3 B-cell epitope (Setiady et al., 2003, 2004; Rival et al., 2014). First, ZP3 nAOD occurs only in neonatal mice that receive the ZP3 antibody between day 1 and day 5 of life. Second, nAOD occurs in both wild-type mice and RAG null mice; the wild-type mice develop a de novo neonatal CD4+ Th1 pathogenic response to ovarian antigens. Third, the neonatal natural killer (NK) cells are absolutely required in AOD that occurs in wild-type or RAG null mice. The NK cells are activated by the ovarian immune complexes via their Fc $\gamma$ RIII. Third, the unique pathogenicity of neonatal NK cells is due to their deficiency of LY49I inhibitory receptor, which is increased after 7–10 days of life. Fourth, the increased susceptibility of newborns to nAOD is unrelated to ontogenetic differences in the ovarian microenvironment (Setiady et al., 2003). And finally, the resistance of nAOD beyond the neonatal time window is caused by Treg suppression, and the window of nAOD susceptibility is extended beyond day 7 after Treg depletion (Setiady et al., 2003, 2004).

### Clinical Autoimmune Disease of the Ovary

Premature ovarian insufficiency (POI) occurs in 1% of women before the age of 40, with amenorrhea, sex steroid deficiency, and elevated levels of gonadotropins (Conway, 2002; Rees and Purdie, 2006; Rebar, 2009; La Marca et al., 2010). POI has known genetic, developmental, and environmental causes, but many are idiopathic. The evidence for an autoimmune basis include circulating autoantibodies against ovarian targets, coexistence with other autoimmune diseases, response to immunosuppressive therapy, and ovarian lymphocytic infiltration (Kim et al., 1995; Hoek et al., 1997).

Human AOD is best documented in POI patients with adrenal autoimmunity; they, in fact, produce antibody to antigens shared by the two organs (Hoek et al., 1997; Bakalov et al., 2005). A percentage of 2–10 POI cases show this association (LaBarbera et al., 1988; Betterle et al., 1993; Kim et al., 1997; Bakalov et al., 2002), which can manifest as part of the APS-1 or type 2. APS-1 patients have mutations in the *AIRE* gene on chromosome 21 (Nagamine et al., 1997; Finnish-German APECED Consortium, 1997). Kriegel et al. (2004) demonstrated that patients with this disease exhibit a defective Treg function. Antibodies against antigenic enzymes common to ovarian theca cells, Leydig cells, and adrenal gland include cytochrome P450 side-chain cleavage enzyme, 17- $\alpha$  hydroxylase, 20-lyase, and 21-hydroxylase (CYP21). Indirect immunofluorescence on frozen adrenal gland sections and CYP21 antibody detection by radioimmunoassays are sensitive diagnostic tests (Falorni et al., 2002a,b; Dal Pra et al., 2003). Female APS-1 patients with infertility and parathyroid disease also develop a high prevalence of autoantibody that targets the oocyte cytoplasmic NALP5 antigen (Alimohammadi et al., 2008). POI also occurs as an independent AOD, which can result in unsuccessful in vitro fertilization (IVF) embryo transfer therapy. Recent studies have identified serum antibodies from POI patients that target ovary-nonspecific antigens including alpha actinin 4, heat shock 70 protein 5, and actin beta (Mande et al., 2011); and mice immunized with their peptides develop loss of ovarian structure and partial fertility reduction (Mande et al., 2012).

Histopathologically, the ovaries from POI patients may show a complete or partial loss of ovarian follicles (Hoek et al., 1997). Oophoritis is represented by infiltration of monocytes, lymphocytes (mainly effector CD4+ T cells), macrophages, and plasma cells in the theca layer of large, antral follicles; in contrast, the earlier stage follicles are consistently free of lymphocytic infiltration (Bakalov et al., 2005; La Marca et al., 2010). There is upregulation of class II MHC antigen expression in granulosa cells and an increase in the number of CD8+ T cells and NK cells (Hill et al., 1990; Giglio et al., 1994; Wu et al., 2004). Because the primordial follicles are unaffected in POI, fertility may be partially conserved in autoimmune oophoritis but may progress to severe POI and infertility (Gloor and Hurlmann, 1984; Bannatyne et al., 1990).

## CONCLUDING REMARKS

Recent basic research on EAO and AOD has unraveled exciting new approaches to investigate human gonadal autoimmunity and chronic inflammation of the gonads associated with subfertility and infertility. In this chapter, we have described in detail autoimmune diseases that occur in several animal models associated with global perturbation of immune regulation, including: d3tx, the Dereg mice with Treg depletion, vasectomy with Treg depletion, *AIRE*-deficient mice, hypothalamic–pituitary deficiency, and transgenic HLA B27/ $\beta$ 2m expression. It is surprising that in all instances of global immune perturbation, the testis and the ovary are the frequent and major targets. Moreover, Treg and *AIRE* are the major perturbed regulatory elements. We propose that the gonads, and their unique functions, demand exceptional protection from autoimmune destruction, and this requires the thymic Treg. We also hypothesize that Treg protection may extend to the other events in normal reproduction to assure successful procreation. They include gamete production, gamete transfer, embryo implantation, and maintenance of the pregnancy itself (Trowsdale and Betz, 2006; Mold et al., 2008; Guerin et al., 2009; Kahn and Baltimore, 2010; Samstein et al., 2012; Jiang et al., 2014).

## Acknowledgments

We wish to thank the following graduate students and postdoctoral fellows for their contribution to the chapter: Sallie Agersborg, Harini Bagavant, Kristina Garza, Vanesa A. Guazzzone, Jessica Harakal, Patricia Jacobo, Sabrina Jarazo-Dietrich, Yahuan Lou, An Ming Luo, Cecilia Pérez, Claudia Rival, Eileen Samy, Yulius Setiady, Hedy Smith, Cristian M. Sobarzo, María S. Theas, Karen Wheeler, and Terecita Yule. The work is supported by NIH grant RO1 AI 41236 to KSKT and by Universidad de Buenos Aires and Consejo Nacional de Investigaciones Científicas y Técnicas to LL and VAG.

## References

- Agersborg, S.S., Garza, K.M., Tung, K.S., 2001. Intestinal parasitism terminates self tolerance and enhances neonatal induction of autoimmune disease and memory. *Eur. J. Immunol.* 31, 851–859.
- Alard, P., Thompson, C., Agersborg, S.S., Thatte, J., Setiady, Y., Samy, E., et al., 2001. Endogenous oocyte antigens are required for rapid induction and progression of autoimmune ovarian disease following day-3 thymectomy. *J. Immunol.* 166, 4363–4369.
- Alimohammadi, M., Björklund, P., Hallgren, A., Pöntynen, N., Szinnai, G., Shikama, N., et al., 2008. Auto-immune polyendocrine syndrome type 1 and NALP5, a parathyroid autoantigen. *N. Engl. J. Med.* 358 (10), 1018–1028.
- Anderson, M.S., Venanzi, E.S., Klein, L., Chen, Z., Berzins, S.P., Turley, S.J., et al., 2002. Projection of an immunological self shadow within the thymus by the aire protein. *Science* 298, 1395–1401.
- Ang, D.K., Brodnicki, T.C., Jordan, M.A., Wilson, W.E., Silveira, P., Gliddon, B.L., et al., 2007. Two genetic loci independently confer susceptibility to autoimmune gastritis. *Int. Immunol.* 19, 1135–1144.
- Bagavant, H., Adams, S., Terranova, P., Chang, A., Kraemer, F.W., Lou, Y., et al., 1999. Autoimmune ovarian inflammation triggered by proinflammatory (Th1) T cells is compatible with normal ovarian function in mice. *Biol. Reprod.* 61, 635–642.
- Bakalov, V.K., Vanderhoof, V.H., Bondy, C.A., Nelson, L.M., 2002. Adrenal antibodies detect asymptomatic auto-immune adrenal insufficiency in young women with spontaneous premature ovarian failure. *Hum. Reprod.* 17, 2096–2100.
- Bakalov, V.K., Anasti, J.N., Calis, K.A., Vanderhoof, V.H., Premkumar, A., Chen, S., et al., 2005. Autoimmune oophoritis as a mechanism of follicular dysfunction in women with 46, XX spontaneous premature ovarian failure. *Fertil. Steril.* 84, 958–965.
- Bannatyne, P., Russell, P., Shearman, R.P., 1990. Autoimmune oophoritis: a clinicopathologic assessment of 12 cases. *Int. J. Gynecol. Pathol.* 9, 191–207.
- Baud, D., Gubler, D.J., Schaub, B., Lanteri, M.C., Musso, D., 2017. An update on Zika virus infection. *Lancet*. Available from: [https://doi.org/10.1016/S0140-6736\(16\)31450-2](https://doi.org/10.1016/S0140-6736(16)31450-2).
- Betterle, C., Rossi, A., Dalla, P.S., Artifoni, A., Pedini, B., Gavasso, S., 1993. Premature ovarian failure: autoimmunity and natural history. *Clin. Endocrinol.* 39, 35–43.
- Bigazzi, P., 1981. Immunologic effects of vasectomy in men and experimental animals. *Prog. Clin. Biol. Res.* 70, 461–476.
- Bhushan, S., Hossain, H., Lu, Y., Geisler, A., Tchatalbachev, S., Mikulski, Z., et al., 2011. Uropathogenic *E. coli* induce different immune response in testicular and peritoneal macrophages: implications for testicular immune privilege. *PLoS One* 6 (12), e28452. Available from: <https://doi.org/10.1371/journal.pone.0028452>.
- Campese, A.F., Grazioli, P., de Cesaris, P., Riccioli, A., Bellavia, D., Pelullo, M., et al., 2014. Mouse Sertoli cells sustain de novo generation of regulatory T cells by triggering the notch pathway through soluble JAGGED1. *Biol. Reprod.* 90 (53), 1–10.
- Chan, P.T., Schlegel, P.N., 2002a. Inflammatory conditions of the male excurrent ductal system. Part I. *J. Androl.* 23, 453–460.
- Chan, P.T., Schlegel, P.N., 2002b. Inflammatory conditions of the male excurrent ductal system. Part II. *J. Androl.* 23, 461–469.
- Cheng, M.H., Shum, A.K., Anderson, M.S., 2007. What's new in the Aire. *Trends Immunol.* 28, 321–327.
- Cheng, X., Dai, H., Wan, N., Moore, Y., Vankayalapati, R., Dai, Z., 2009. Interaction of programmed death-1 and programmed death-1 ligand-1 contributes to testicular immune privilege. *Transplantation* 87, 1778–1786.
- Cheng, M.H., Nelson, L.M., 2011. Mechanisms and models of immune tolerance breakdown in the ovary. *Semin. Reprod. Med.* 29, 308–316.
- Conway, S., 2002. Primary ovarian failure. In: Wass, A.H., Shalet, S.M. (Eds.), *Endocrinology and Diabetes*. Oxford University Press, Oxford, UK, pp. 1107–1113.
- Dal Pra, C., Chen, S., Furmaniak, J., Smith, B.R., Pedini, B., Moscon, A., et al., 2003. Autoantibodies to steroidogenic enzymes in patients with premature ovarian failure with and without Addison's disease. *Eur. J. Endocrinol.* 148, 565–570.
- Dal Secco, V., Riccioli, A., Padula, F., Ziparo, E., Filippini, A., 2008. Mouse Sertoli cells display phenotypical and functional traits of antigen-presenting cells in response to interferon gamma. *Biol. Reprod.* 7, 234–242.
- DeFalco, T., Potter, S.J., Williams, A.V., Waller, B., Kan, M.J., Capel, B., 2015. Macrophages contribute to the spermatogonial niche in the adult testis. *Cell Rep.* 12 (7), 1107–1119.
- Dejucq, N., Jégou, B., 2001. Viruses in the mammalian male genital tract and their effects on the reproductive system. *Microb. Mol. Biol. Rev.* 65, 208–231.
- Derbinski, J., Gabler, J., Brors, B., Tierling, S., Jonnakuty, S., Hergenhahn, M., et al., 2005. Promiscuous gene expression in thymic epithelial cells is regulated at multiple levels. *J. Exp. Med.* 202, 33–45.
- Doncel, G.F., Di Paola, J.A., Lustig, L., 1989. Sequential study of the histopathology and cellular and humoral immune response during the development of an autoimmune orchitis in Wistar rats. *Am. J. Reprod. Immunol.* 20, 44–51.
- Duan, Y.G., Yu, C.F., Novak, N., Bieber, T., Zhu, C.H., Schuppe, H.C., et al., 2011. Immunodeviation towards a Th17 immune response associated with testicular damage in azoospermic men. *Int. J. Androl.* 34, 536–545.
- Dujardin, H.C., Burlen-Defranoux, O., Boucontet, L., Vieira, P., Cumano, A., Bandeira, A., 2004. Regulatory potential and control of Foxp3 expression in newborn CD4 1 T cells. *Proc. Natl. Acad. Sci. U.S.A.* 101, 14473–14478.
- D'Cruz, O.J., Haas, G.G., de La Rocha, R., Lambert, H., 1991. Occurrence of serum antisperm antibodies in patients with cystic fibrosis. *Fertil. Steril.* 56, 519–527.
- Elder, M., Maclare, N., Riley, W.J., 1981. Gonadal autoantibodies in patients with hypogonadism and/or Addison's disease. *Clin. Endocrinol. Metab.* 52 (6), 1137–1142.
- Falorni, A., Laureti, S., Santeusario, F., 2002a. Autoantibodies in autoimmune polyendocrine syndrome type II. *Endocrinol. Metab. Clin. North Am.* 31, 369–389.
- Falorni, A., Laureti, S., Candeloro, P., Perrino, S., Coronella, C., Bizzarro, A., et al., 2002b. Steroid-cell auto-antibodies are preferentially expressed in women with premature ovarian failure who have adrenal autoimmunity. *Fertil. Steril.* 78, 270–279.
- Fallarino, F., Luca, G., Calvitti, M., Mancuso, F., Nastruzzi, M.C., Fioretti, C., et al., 2009. Therapy of experimental type 1 diabetes by isolated Sertoli cell xenografts alone. *J. Exp. Med.* 206, 2511–2526.
- Fijak, M., Meinhardt, A., 2006. The testis in immune privilege. *Immunol. Rev.* 213, 66–81.
- Fijak, M., Bhushan, S., Meinhardt, A., 2011. Immunoprivileged sites: the testis. *Methods Mol. Biol.* 67, 459–470.

- Fijak, M., Damm, L.J., Wenzel, J.P., Aslani, F., Walecki, M., Wahle, E., et al., 2015. Influence of testosterone on inflammatory response in testicular cells and expression of transcription Factor Foxp3 in T Cells. *Am. J. Reprod. Immunol.* 74 (1), 12–25.
- Finnish-German APECED Consortium, 1997. An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. *Nat. Genet.* 17, 393–398.
- Fontenot, J.D., Gavin, M.A., Rudensky, A.Y., 2003. Foxp3 programs the development and function of CD4 1 CD25 1 regulatory T cells. *Nat. Immunol.* 4, 330–336.
- Fountain, S., Holland, M.K., Hinds, L.A., Janssens, P.A., Kerr, P., 1997. Interstitial orchitis with impaired steroidogenesis and spermatogenesis in the testes of rabbits infected with an attenuated strain of myxoma virus. *J. Reprod. Fertil.* 110, 161–169.
- Frayne, J., Hall, L., 1999. The potential use of sperm antigens as targets for immunocontraception: past, present and future. *J. Reprod. Immunol.* 43, 1–33.
- Freund, J., Lipton, M.M., Thompson, G.E., 1953. Aspermatogenesis in guinea pig induced by testicular tissue and adjuvant. *J. Exp. Med.* 97, 711–725.
- Gao, J., Wang, X., Wang, Y., Han, F., Cai, W., Zhao, B., et al., 2016. Murine Sertoli cells promote the development of tolerogenic dendritic cells: a pivotal role of galectin-1. *Immunology* 148, 253–265.
- Garza, K.M., Tung, K.S., 1995. Frequency of molecular mimicry among T cell peptides as the basis for autoimmune disease and autoantibody induction. *J. Immunol.* 155, 5444–5448.
- Giglio, T., Imro, M.A., Filaci, G., Scudeletti, M., Puppo, F., De Cecco, L., et al., 1994. Immune cell circulating subsets are affected by gonadal function. *Life Sci.* 54, 1305–1312.
- Gloor, E., Hurlimann, J., 1984. Autoimmune oophoritis. *Am. J. Clin. Pathol.* 81, 105109.
- Goldacre, M.J., Wotton, C.J., Seagroatt, V., Yeates, D., 2007. Immunerelated disease before and after vasectomy: an epidemiological database study. *Hum. Reprod.* 22, 1273–1278.
- Govero, J., Esakkay, P., Scheaffer, S., Fernandez, E., Drury, A., Platt, D.J., 2016. Zika virus infection damages the testes in mice. *Nature* 540 (7633), 438–442.
- Griggs, N.D., Agersborg, S.S., Noelle, R.J., Ledbetter, J.A., Linsley, P.S., Tung, K.S., 1996. The relative contribution of the CD28 and gp39 costimulatory pathways in the clonal expansion and pathogenic acquisition of self-reactive T cells. *J. Exp. Med.* 1183, 801–810.
- Guazzone, V., Denduchis, B., Lustig, L., 2005. Involvement of CD44 in testicular leukocyte recruitment in experimental autoimmune orchitis. *Reproduction* 129, 603–609.
- Guazzone, V.A., Jacobo, P., Theas, M.S., Lustig, L., 2009. Cytokines and chemokines in testicular inflammation: a brief review. *Microsc. Res. Tech.* 72, 620–628.
- Guazzone, V.A., Hollwegs, S., Mardirosian, M., Jacobo, P., Hackstein, H., Wygrecka, M., et al., 2011. Characterization of dendritic cells in testicular draining lymph nodes in a rat model of experimental autoimmune orchitis. *Int. J. Androl.* 34, 276–289.
- Guazzone, V.A., Jacobo, P., Denduchis, B., Lustig, L., 2012. Expression of cell adhesion molecules, chemokines and chemokine receptors involved in leukocyte traffic in rats undergoing autoimmune orchitis. *Reproduction* 143, 651–662.
- Guerin, L.R., Prins, J.R., Robertson, S.A., 2009. Regulatory T-cells and immune tolerance in pregnancy: a new target for infertility treatment? *Hum. Reprod. Update* 15, 517–535.
- Hardy, D.M., Garbers, D.L., 1994. Species-specific binding of sperm proteins to the extracellular matrix (zona pellucida) of the egg. *J. Biol. Chem.* 269 (29), 19000–19004.
- Head, J.R., Neaves, W.B., Billingham, R.E., 1983. Immune privilege in the testis. Basic parameters of allograft survival. *Transplantation* 36, 423–431.
- Hedger, M.P., 2002. Macrophages and the immune responsiveness of the testis. *J. Reprod. Immunol.* 57 (1-2), 19–34.
- Hill, J.A., Welch, W.R., Faris, H.M., Anderson, D.J., 1990. Induction of class II major histocompatibility complex antigen expression in human granulosa cells by interferon gamma: a potential mechanism contributing to autoimmune ovarian failure. *Am. J. Obstet. Gynaecol.* 162, 534–540.
- Hintz, M., Goldberg, E., 1977. Immunohistochemical localization of LDH-x during spermatogenesis in mouse testes. *Dev. Biol.* 57 (2), 375–384.
- Hirai, S., Naito, M., Terayama, H., Qu, N., Kuerban, M., Musha, M., et al., 2012. The origin of lymphatic capillaries in murine testes. *J. Androl.* 33 (4), 745–751.
- Hoek, A., Schoemaker, J., Drexhage, H.A., 1997. Premature ovarian failure and ovarian autoimmunity. *Endocr. Rev.* 18, 107–134.
- Hori, S., Nomura, T., Sakaguchi, S., 2003. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 299, 1057–1061.
- Hsieh, C.S., Lee, H.M., Lio, C.W., 2012. Selection of regulatory T cells in the thymus. *Nat. Rev. Immunol.* 12, 157–167.
- Hutson, J.C., 1998. Interactions between testicular macrophages and Leydig cells. *J. Androl.* 19 (4), 394–398.
- Itoh, M., Hiramine, C., Hojo, K., 1991. A new murine model of autoimmune orchitis induced by immunization with viable syngeneic testicular germ cells alone. *Immunological and histological studies. Clin. Exp. Immunol.* 83, 137–142.
- Itoh, M., Terayama, H., Naito, M., Ogawa, Y., Tainosho, S., 2005. Tissue microcircumstances for leukocytic infiltration into the testis and epididymis in mice. *J. Reprod. Immunol.* 67, 57–67.
- Jacobo, P., Guazzone, V.A., Jarazo-Dietrich, S., Theas, M.S., Lustig, L., 2009. Differential changes in CD4+ and CD8+ effector and regulatory T lymphocyte subsets in the testis of rats undergoing autoimmune orchitis. *J. Reprod. Immunol.* 81, 44–54.
- Jacobo, P.V., Pérez, C.V., Theas, M.S., Guazzone, V.A., Lustig, L., 2011a. CD4+ and CD8+ T cells producing Th1 and Th17 cytokines are involved in the pathogenesis of autoimmune orchitis. *Reproduction* 141, 249–258.
- Jacobo, P., Guazzone, V.A., Theas, M.S., Lustig, L., 2011b. Testicular autoimmunity. *Autoimmun. Rev.* 10, 201–204.
- Jacobo, P., Guazzone, V.A., Pérez, C.V., Lustig, L., 2015. CD4+ Foxp3+ regulatory T cells in autoimmune orchitis: phenotypic and functional characterization. *Am. J. Reprod. Immunol.* 73, 109–125.
- Jahnukainen, K., Jorgensen, N., Pöllänen, P., Giwercman, A., Skakkebaek, N.E., 1995. Incidence of testicular mononuclear cell infiltrates in normal human males and in patients with germ cell neoplasia. *Int. J. Androl.* 18, 313–320.
- Jarazo-Dietrich, S., Jacobo, P., Pérez, C.V., Guazzone, V.A., Lustig, L., Theas, M.S., 2012. Up regulation of nitric oxide synthase-nitric oxide system in the testis of rats undergoing autoimmune orchitis. *Immunobiology* 217, 778–787.

- Jarazo Dietrich, S., Fass, M.I., Jacobo, P.V., Sobralo, C.M., Lustig, L., Theas, M.S., 2015. Inhibition of NOS-NO system prevents autoimmune orchitis development in rats: relevance of NO released by testicular macrophages in germ cell apoptosis and testosterone secretion. *PLoS One* 10 (6), e0128709. Available from: <https://doi.org/10.1371/journal.pone.0128709>.
- Jiang, T.T., Chaturvedi, V., Ertelt, J.M., Kinder, J.M., Clark, D.R., Valent, A.M., et al., 2014. Regulatory T cells: new keys for further unlocking the enigma of fetal tolerance and pregnancy complications. *J. Immunol.* 192 (11), 4949–4956.
- Johnson, M.H., 1973. Physiological mechanisms for the immunological isolation of spermatozoa. *Adv. Reprod. Physiol.* 6, 297–324.
- Kahn, D.A., Baltimore, D., 2010. Pregnancy induces a fetal antigen-specific maternal T regulatory cell response that contributes to tolerance. *Proc. Natl. Acad. Sci. U.S.A.* 107, 9299–9304.
- Kim, J.G., Moon, S.Y., Chang, Y.S., Lee, J.Y., 1995. Autoimmune premature ovarian failure. *J. Obstet. Gynaecol.* 21, 59–66.
- Kim, T.J., Anasti, J.N., Flack, M.R., Kimzey, L.M., Defensor, R.A., Nelson, L.M., 1997. Routine endocrine screening for patients with karyotypically normal spontaneous premature ovarian failure. *Obstet. Gynecol.* 89, 777–779.
- Kisand, K., Peterson, P., 2011. Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy: known and novel aspects of the syndrome. *Ann. N.Y. Acad. Sci.* 1246, 77–91.
- Klein, B., Haggeneck, T., Fietz, D., Indumathy, S., Loveland, K.L., Hedger, M., et al., 2016. Specific immune cell and cytokine characteristics of human testicular germ cell neoplasia. *Hum. Reprod.* 31 (10), 2192–2202.
- Kohno, S., Munoz, J.A., Williams, T.M., Teuscher, C., Bernard, C.C.A., Tung, K.S.K., 1983. Immunopathology of murine experimental allergic orchitis. *J. Immunol.* 130, 2675–2682.
- Kojima, A., Prehn, R.T., 1981. Genetic susceptibility to post-thymectomy autoimmune diseases in mice. *Immunogenetics* 14 (1–2), 15–27.
- Kriegel, M.A., Lohmann, T., Gabler, C., Blank, N., Kalden, J.R., Lorenz, H.M., 2004. Defective suppressor function of human CD4 1 CD25 1 regulatory T cells in autoimmune polyglandular syndrome Type II. *J. Exp. Med.* 199, 1285–1291.
- Kuroda, N., Mitani, T., Takeda, N., Ishimaru, N., Arakaki, R., Hayashi, Y., et al., 2005. Development of autoimmunity against transcriptionally unexpressed target antigen in the thymus of AIRE-deficient mice. *J. Immunol.* 174, 1862–1870.
- Lahl, K., Loddenkemper, C., Drouin, C., Freyer, J., Arnason, J., Eberl, G., et al., 2007. Selective depletion of Foxp3 + regulatory T cells induces a scurfy-like disease. *J. Exp. Med.* 204 (1), 57–63.
- La Marca, A., Brozzetti, A., Sighinolfi, G., Marzotti, S., Volpea, A., Falorni, A., 2010. Primary ovarian insufficiency: autoimmune causes. *Curr. Opin. Obst. Gynecol.* 22, 277–282.
- LaBarbera, A.R., Miller, M.M., Ober, C., Rebar, R.W., 1988. Autoimmune etiology in premature ovarian failure. *Am. J. Reprod. Immunol.* Microbiol. 16, 115–122.
- Lee, N.P., Cheng, C.Y., 2003. Regulation of Sertoli cell tight junction dynamics in the rat testis via the nitric oxide synthase/soluble guanylate cyclase/3',5'-cyclic guanosine monophosphate/protein kinase G signaling pathway: an in vitro study. *Endocrinology* 144, 3114–3129.
- Lee, R., Goldstein, M., Ullery, B.W., Ehrlich, J., Soares, M., Razzano, R.A., et al., 2009. Value of serum antisperm antibodies in diagnosing obstructive azoospermia. *J. Urol.* 181, 264–269.
- Lehmann, D., Temminch, D., Da Rugna, D., Leibundgut, B., Sulmoni, A., Muller, H.J., 1987. Role of immunological factors in male infertility: immunohistochemical and serological evidence. *Lab. Invest.* 57, 21–28.
- Lemke, G., Rothlin, C.V., 2008. Immunobiology of the TAM receptors. *Nat. Rev. Immunol.* 8 (5), 327–336.
- Leventhal, D.S., Gilmore, D.C., Berger, J.M., Nishi, S., Lee, V., Malchow, S., et al., 2016. Dendritic cells coordinate the development and homeostasis of organ-specific regulatory T cells. *Immunity* 44 (4), 847–8759.
- Lewis, J., 1993. Eosinophilic perifolliculitis: a variant of autoimmune oophoritis? *Int. J. Gynecol. Pathol.* 12, 360364.
- Li, N., Wang, T., Han, D., 2012. Structural, cellular and molecular aspects of immune privilege in the testis. *Front. Immunol.* 3, 152. Available from: <https://doi.org/10.3389/fimmun.2012.00152>.
- Li, N., Liu, Z., Zhang, Y., Chen, Q., Liu, P., Cheng, C.Y., et al., 2015. Mice lacking Axl and Mer tyrosine kinase receptors are susceptible to experimental autoimmune orchitis induction. *Immunol. Cell Biol.* 93 (3), 311–320.
- Lloyd, M.L., Papadimitriou, J.M., O'Leary, S., Robertson, S.A., Shellam, G.R., 2010. Immunoglobulin to zona pellucida 3 mediates ovarian damage and infertility after contraceptive vaccination in mice. *J. Autoimmun.* 35, 77–85.
- Luo, A.M., Garza, K.M., Hunt, D., Tung, K.S., 1993. Antigen mimicry in autoimmune disease sharing of amino acid residues critical for pathogenic T cell activation. *J. Clin. Invest.* 92, 2117–2123.
- Lou, Y.H., Tung, K.S.K., 1993. T cell peptide of a self protein elicits autoantibody to the protein antigen: Implications for specificity and pathogenic role of antibody in autoimmunity. *J. Immunol.* 151, 5790–5799.
- Lou, Y.H., McElveen, M.F., Garza, K.M., Tung, K.S., 1996. Rapid induction of autoantibodies by endogenous ovarian antigens and activated T cells: implication in autoimmune disease pathogenesis and B cell tolerance. *J. Immunol.* 156, 3535–3540.
- Lou, Y.H., Park, K.K., Agersborg, S., Alard, P., Tung, K.S., 2000. Retargeting T cell-mediated inflammation: a new perspective on autoantibody action. *J. Immunol.* 2164, 5251–5257.
- Lu, Q., Gore, M., Zhang, Q., Camenisch, T., Boast, S., Casagranda, F., et al., 1999. Tyro-3 family receptors are essential regulators of mammalian spermatogenesis. *Nature* 398, 723–728.
- Lustig, L., Doncel, G.F., Berensztein, E., Denduchis, B., 1987. Testis lesions, cell and humoral immune response induced in rats by immunization with laminin. *Am. J. Reprod. Immunol.* 14, 123–128.
- Lustig, L., Denduchis, B., Ponzio, R., Lauzon, M., Pelletier, R.M., 2000. Passive immunization with anti-laminin immunoglobulin G modifies the integrity of the seminiferous epithelium and induces arrest of spermatogenesis in the guinea pig. *Biol. Reprod.* 62, 1505–1514.
- Ma, W., Li, S., Ma, S., Jia, L., Zhang, F., Zhang, Y., et al., 2017. Zika virus causes testis damage and leads to male infertility in mice. *Cell* 167 (6), 1511–1524.
- MacLaren, N., Chen, Q.Y., Kukreja, A., Marker, J., Zhang, C.H., Sun, Z.S., 2001. Autoimmune hypogonadism as part of an autoimmune polyglandular syndrome. *J. Soc. Gynecol. Investig.* 8 (1 Suppl. Proceedings), S52–S54.
- Mahi-Brown, C.A., Huang Jr., T.T., Yanagimachi, R., 1982. Infertility in bitches induced by active immunization with porcine zonae pellucidae. *J. Exp. Zool.* 222, 89–95.

- Malchow, S., Leventhal, D.S., Lee, V., Nishi, S., Socci, N.D., Savage, P.A., 2016. Aire enforces immune tolerance by directing autoreactive T cells into the regulatory T cell lineage. *Immunity* 44, 1102–1113.
- Malolina, E.A., Kulibin, A.Y., Naumenko, V.A., Gushchina, E.A., Zavalishina, L.E., Kushch, A.A., 2014. Herpes simplex virus inoculation in murine rete testis results in irreversible testicular damage. *Int. J. Exp. Pathol.* 95, 120–130.
- Mande, P.V., Parikh, F.R., Hinduja, I., Zaveri, K., Vaidya, R., Gajbhaye, R., et al., 2011. Identification and validation of candidate biomarkers involved in human ovarian autoimmunity. *Reprod. Biomed.* 23, 471–483.
- Mande, P.V., Thomas, S., Khan, S., Jadhav, S., Khole, V.V., 2012. Immunization with ovarian autoantigens leads to reduced fertility in mice following follicular dysfunction. *Reproduction* 143, 309–323.
- Marshburn, P.B., Kutteh, W.H., 1994. The role of antisperm antibody in infertility. *Fertil. Steril.* 5, 799811.
- McDonald, S.W., 1997. Is vasectomy harmful to health? *Br. J. Gen. Pract.* 47, 381–386.
- Melaine, N., Ruffault, A., Dejucq-Rainsford, N., Jégou, B., 2003. Experimental inoculation of the adult rat testis with Sendai virus: effect on testicular morphology and leukocyte population. *Human Reprod.* 18, 1574–1579.
- Meng, J., Holdcraft, R.W., Shima, J.E., Griswold, M.D., Braun, R.E., et al., 2005. Androgens regulate the permeability of the blood testis barrier. *Proc. Natl. Acad. Sci. U.S.A.* 102 (46), 16696–16700.
- Mital, P., Kaur, G., Dufour, J.M., 2010. Immunoprotective Sertoli cells: making allogeneic and xenogeneic transplantation feasible. *Reproduction* 139, 485–504.
- Mital, P., Hinton, B.T., Dufour, J.M., 2011. The blood-testis and blood-epididymis barriers are more than just their tight junctions. *Biol. Reprod.* 84 (5), 851–858.
- Mold, J.E., Michae lsson, J., Burt, T.D., Muench, M.O., Beckerman, K.P., Busch, M.P., et al., 2008. Maternal alloantigens promote the development of tolerogenic fetal regulatory T cells in utero. *Science* 322, 1562–1565.
- Monteiro, J.P., Farache, J., Mercadante, A.C., Mignaco, J.A., Bonamino, M., Bonomo, A., 2008. Pathogenic effector T cell enrichment overcomes regulatory T cell control and generates autoimmune gastritis. *J. Immunol.* 181, 5895–5903.
- Mossadegh-Keller, N., Gentek, R., Gimenez, G., Bigot, S., Mailfert, S., Sieweke, M.H., 2017. Developmental origin and maintenance of distinct testicular macrophage populations. *J. Exp. Med.* 214 (10), 2829–2841.
- Mruk, D.D., Cheng, C.Y., 2015. The mammalian blood-testis barrier: its biology and regulation. *Endocr. Rev.* 36, 564–591.
- Mukasa, A., Hiromatsu, K., Matsuzaki, G., O'Brien, R., Born, W., Nomoto, K., 1995. Bacterial infection of the testis leading to autoaggressive immunity triggers apparently opposed responses of  $\alpha\beta$  and  $\gamma\delta$  T cells. *J. Immunol.* 155, 2047–2056.
- Mukasa, A., Lahn, M., Pflum, E.K., Born, W., O'Brien, R.L., 1997. Evidence that the same gamma delta T cells respond during infection-induced and autoimmune inflammation. *J. Immunol.* 159 (12), 5787–5794.
- Mukasa, A., Yoshida, H., Kobayashi, N., Matsuzaki, G., Nomoto, K., 1998.  $\gamma\delta$  T cells in infection-induced and autoimmune-induced testicular inflammation. *Immunology* 95, 395–401.
- Nagamine, K., Peterson, P., Scott, H., 1997. Positional cloning of the APECED gene. *Nat. Genet.* 17, 393–397.
- Nishizuka, Y., Sakakura, T., 1969. Thymus and reproduction: sex-linked dysgenesis of the gonad after neonatal thymectomy in mice. *Science* 166, 753–755.
- Nistal, M., Riestra, M.L., Paniagua, R., 2002. Focal orchitis in undescended testes. *Arch. Pathol. Lab. Med.* 126, 64–69.
- O'Leary, S., Lloyd, M.L., Shellam, G.R., Robertson, S.A., 2008. Immunization with recombinant murine cytomegalovirus expressing murine zona pellucida 3 causes permanent infertility in BALB/c mice due to follicle depletion and ovulation failure. *Biol. Reprod.* 79, 849–860.
- Pelletier, R.M., 1986. Cyclic formation and decay of the blood-testis barrier in the mink (*Mustela vison*), a seasonal breeder. *Am. J. Anat.* 175, 91–117.
- Pelletier, R.M., Yoon, S.R., Akpovi, C.D., Silvas, E., Vitale, M.L., 2009. Defects in the regulatory clearance mechanisms favor the breakdown of self-tolerance during spontaneous autoimmune orchitis. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 296, 743–762.
- Pérez, C.V., Sobarzo, C., Jacobo, P., Jarazo-Dietrich, S., Theas, M., Denduchis, B., et al., 2011. Impaired expression and distribution of adherens and gap junction proteins in the seminiferous tubules of rats undergoing autoimmune orchitis. *Int. J. Androl.* 34, e566–e577.
- Pérez, C.V., Sobarzo, C.M., Jacobo, P.V., Pellizzari, E.H., Cigorraga, S.B., Denduchis, B., et al., 2012. Loss of occludin expression and impairment of blood-testis barrier permeability in rats with autoimmune orchitis: effect of interleukin 6 on Sertoli cell tight junctions. *Biol. Reprod.* 87 (5), 122. 1-12.
- Pérez, C.V., Pellizzari, E.H., Cigorraga, S.B., Galardo, M.N., Naito, M., Lustig, L., et al., 2014. IL17A impairs blood-testis barrier integrity and induces testicular inflammation. *Cell Tissue Res.* 358, 885–898.
- Pérez, C.V., Gómez, L.G., Gualdoni, G.S., Lustig, L., Rabinovich, G.A., Guazzone, V.A., 2015. Dual roles of endogenous and exogenous galectin-1 in the control of testicular immunopathology. *Sci. Rep.* 5, 12259. Available from: <https://doi.org/10.1038/srep12259>.
- Rebar, R.W., 2009. Premature ovarian failure. *Obstet. Gynecol.* 113 (6), 1355–1363.
- Rees, M., Purdie, D., 2006. Premature menopause, Management of the Menopause: The Handbook, fourth ed. Royal Society of Medicine Press Ltd, London, pp. 142–149.
- Rhim, S.H., Millar, S.E., Robey, F., Luo, A.M., Lou, Y.H., Yule, T., et al., 1992. Autoimmune disease of the ovary induced by a ZP3 peptide from the mouse zona pellucida. *J. Clin. Invest.* 89, 28–35.
- Rival, C., Lustig, L., Iosub, R., Guazzone, V.A., Schneider, E., Meinhardt, A., et al., 2006a. Identification of a dendritic cell population in normal testis and in chronically inflamed testis of rats with autoimmune orchitis. *Cell Tissue Res.* 324 (2), 311–318.
- Rival, C., Theas, M.S., Guazzone, V.A., Lustig, L., 2006b. Interleukin-6 and IL-6 receptor cell expression in testis of rats with autoimmune orchitis. *J. Reprod. Immunol.* 70, 43–54.
- Rival, C., Guazzone, V.A., von Wulffen, W., Hackstein, H., Schneider, E., Lustig, L., et al., 2007. Expression of co-stimulatory molecules, chemokine receptors and proinflammatory cytokines in dendritic cells from normal and chronically inflamed rat testis. *Mol. Human Reprod.* 13, 853–861.
- Rival, C., Theas, M.S., Suescum, M.O., Jacobo, P., Guazzone, V.A., van Rooijen, N., et al., 2008. Functional and phenotypic characteristics of testicular macrophages in experimental autoimmune orchitis. *J. Pathol.* 215, 108–117.

- Rival, C., Wheeler, K., Jeffrey, S., Qiao, H., Luu, B., Tewalt, E.F., et al., 2013. Regulatory T cells and vasectomy. *J. Reprod. Immunol.* 100 (1), 66–75.
- Rival, C., Setiady, Y., Samy, E.T., Harakal, J., Tung, K.S., 2014. The unique neonatal NK cells: a critical component required for neonatal autoimmune disease induction by maternal autoantibody. *Front. Immunol.* 5, 242. Available from: <https://doi.org/10.3389/fimmu.2014.00242>.
- Sakaguchi, S., Takahashi, T., Nishizuka, Y., 1982. Study on cellular events in post-thymectomy autoimmune oophoritis in mice. II. Requirement of Lyt-1 cells in normal female mice for the prevention of oophoritis. *Exp. Med.* 156 (6), 1577–1586.
- Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M., Toda, M., 1995. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol.* 155, 1151–1164.
- Sakamoto, H., Himeno, K., Sanui, H., Yoshida, S., Nomoto, K., 1985. Experimental allergic orchitis in mice. I. A new model induced by immunization without adjuvants. *Clin. Immunol. Immunopathol.* 37, 360–368.
- Salomon, F., Saremaslani, P.P., Jakob, M., Hedinger, C.F., 1982. Immune complex orchitis in infertile men. *Lab. Invest.* 47, 555–567.
- Samstein, R.M., Josefowicz, S.Z., Arvey, A., Treuting, P.M., Rudensky, A.Y., 2012. Extrathymic generation of regulatory T cells in placental mammals mitigates maternal-fetal conflict. *Cell.* 150, 29–38.
- Samy, E.T., Parker, L.A., Sharp, C.P., Tung, K.S., 2005. Continuous control of autoimmune disease by antigen-dependent polyclonal CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells in the regional lymph node. *J. Exp. Med.* 202, 771–781.
- Samy, E.T., Wheeler, K.M., Roper, R.J., Teuscher, C., Tung, K.S.K., 2008. Cutting edge: autoimmune disease in day 3 thymectomized mice is actively controlled by endogenous disease-specific regulatory T cells. *J. Immunol.* 180, 4366–4370.
- Schuppe, H.C., Meinhhardt, A., Allam, J.P., Bergmann, M., Weidner, W., Haidl, G., 2008. Chronic orchitis: a neglected cause of male infertility? *Andrologia* 40, 84–91.
- Selawry, H.P., Cameron, D.F., 1993. Sertoli cell-enriched fractions in successful islet cell transplantation. *Cell Transplant.* 2, 123–129.
- Setiady, Y.Y., Samy, E.T., Tung, K.S., 2003. Maternal autoantibody triggers de novo T cell-mediated neonatal autoimmune disease. *J. Immunol.* 170, 4656–4664.
- Setiady, Y., Pramoonjago, P., Tung, K.S., 2004. Requirements of NK cells and proinflammatory cytokines in T cell-dependent neonatal autoimmune ovarian disease triggered by immune complex. *J. Immunol.* 173, 1051–1058.
- Setiady, Y.Y., Ohno, K., Samy, E.T., Bagavant, H., Qiao, H., Sharp, C., et al., 2006. Physiologic self antigens rapidly capacitate autoimmune disease-specific polyclonal CD4<sup>+</sup> CD25<sup>+</sup> regulatory T cells. *Blood* 107 (3), 1056–1062.
- Simpson, A.J., Caballero, O.L., Jungbluth, A., Chen, Y.T., Old, L.J., 2005. Cancer/testis antigens, gametogenesis and cancer. *Nat. Rev. Cancer* 5 (8), 615–625.
- Smith, H., Sakamoto, Y., Kasai, K., Tung, K.S., 1991. Effector and regulatory cells in autoimmune oophoritis elicited by neonatal thymectomy. *J. Immunol.* 147 (9), 2928–2933.
- Smith, L.B., O'Shaughnessy, P.J., Reboulet, D., 2015. Cell-specific ablation in the testis: what have we learned? *Andrology* 3 (6), 1035–1049.
- Sotsiou, F., Bottazzo, G.F., Doniach, D., 1980. Immunofluorescence studies on autoantibodies to steroid-producing cells, and to germline cells in endocrine disease and infertility. *Clin. Exp. Immunol.* 39 (1), 97–111.
- Suarez-Pinzon, W., Korbutt, G.S., Power, R., Hooton, J., Rajotte, R.V., Rabinovitch, A., 2000. Testicular Sertoli cells protect islet beta-cells from autoimmune destruction in NOD mice by a transforming growth factor-beta1-dependent mechanism. *Diabetes* 49, 1810–1818.
- Suescum, M.O., Rival, C., Theas, M.S., Calandra, R.S., Lustig, L., 2003. Involvement of tumor necrosis factor-alpha in the pathogenesis of autoimmune orchitis in rats. *Biol. Reprod.* 68, 2114–2121.
- Suominen, J., Söderström, K.O., 1982. Lymphocyte infiltration in human testicular biopsies. *Int. J. Androl.* 5, 461–466.
- Taurog, J.D., 2009. Animal models of spondyloarthritis. *Adv. Exp. Med. Biol.* 649, 245254.
- Taurog, J.D., Rival, C., van Duivenvoorde, L.M., Satumtira, N., Dorris, M.L., Sun, M., et al., 2012. Autoimmune epididymo-orchitis is essential to the pathogenesis of male-specific spondyloarthritis in HLA-B27 transgenic rats. *Arthritis Rheum.* 64, 2518–2528.
- Terayama, H., Hirai, S., Naito, M., Qu, N., Katagiri, C., Nagahori, C., et al., 2016. Specific autoantigens identified by sera obtained from mice that are immunized with testicular germ cells alone. *Sci. Rep.* 6, 35599. Available from: <https://doi.org/10.1038/srep35599>.
- Theas, M.S., Rival, C., Lustig, L., 2003. Germ cell apoptosis in autoimmune orchitis: involvement of the Fas-Fas L system. *Am. J. Reprod. Immunol.* 50, 166–176.
- Theas, M.S., Rival, C., Jarazo-Dietrich, S., Guazzone, V.A., Lustig, L., 2006. Death receptor and mitochondrial pathways are involved in germ cell apoptosis in an experimental model of autoimmune orchitis. *Hum. Reprod.* 21, 1734–1742.
- Theas, M.S., Rival, C., Jarazo-Dietrich, S., Jacobo, P., Guazzone, V.A., Lustig, L., 2008. Tumour necrosis factor-alpha released by testicular macrophages induces apoptosis of germ cells in autoimmune orchitis. *Hum. Reprod.* 23, 1865–1872.
- Trowsdale, J., Betz, A.G., 2006. Mother's little helpers: mechanisms of maternal fetal tolerance. *Nat. Immunol.* 7, 241–246.
- Tung, K.S.K., Unanue, E.R., Dixon, F.J., 1971a. Pathogenesis of experimental allergic orchitis. I. Transfer with immune lymph node cells. *J. Immunol.* 106, 1453–1462.
- Tung, K.S.K., Unanue, E.R., Dixon, F.J., 1971b. Pathogenesis of experimental allergic orchitis. II. The role of antibody. *J. Immunol.* 106, 1463–1472.
- Tung, K.S., 1978. Autoimmunity to sperm. *Andrologia* 10, 247–249.
- Tung, K.S.K., Ellis, L., Teuscher, C., Meng, A., Blaustein, J.C., Kohno, S., et al., 1981. The black mink (*Mustela vison*): a natural model of immunologic male infertility. *J. Exp. Med.* 154, 1016–1032.
- Tung, K.S., Ellis, L.E., Childs, G.V., Dufau, M., 1984. The dark mink: a model of male infertility. *Endocrinology* 114, 922–929.
- Tung, K.S., Smith, S., Teuscher, C., Cook, C., Anderson, R.E., 1987a. Murine autoimmune oophoritis, epididymoorchitis, and gastritis induced by day 3 thymectomy. *Immunopathology*. *Am. J. Pathol.* 126 (2), 293–302.
- Tung, K.S.K., Yule, T.D., Mahi-Brown, C.A., Listrom, M.B., 1987b. Distribution of histopathology and Ia positive cells in actively induced and adoptively-transferred experimental autoimmune orchitis. *J. Immunol.* 138, 752–759.
- Tung, K.S., Primakoff, P., Woolman-Gamer, L., Myles, D.G., 1997. Mechanism of infertility in male guinea pigs immunized with sperm PH-20. *Biol. Reprod.* 56, 1133–1141.

- Tung, K.S.K., Fusi, F., Teuscher, C., 2002. Autoimmune disease of the spermatozoa, ovary and testis. In: Theofilopoulos, A.N., Bona, C.A. (Eds.), *The Molecular Pathology of Autoimmune Diseases*. Taylor & Francis, New York, pp. 1031–1045.
- Tung, K.S., Harakal, J., Qiao, H., Rival, C., Li, J.C., Paul, A.G., et al., 2017. Egress of sperm autoantigen from seminiferous tubules maintains systemic tolerance. *J. Clin. Invest.* 127, 1046–1060.
- Turek, P.J., Lipshultz, L.I., 1994. Immunologic infertility. *Urol. Clin. North Am.* 21, 447–468.
- Villiger, P.M., Caliezi, G., Cottin, V., Forger, F., Senn, A., Ostensen, M., 2010. Effects of TNF antagonists on sperm characteristics in patients with spondyloarthritis. *Ann. Rheum. Dis.* 69, 1842–1844.
- Voisin, G.A., Delauney, A., Barber, M., 1951. Sur les lésions testiculaires provoquées chez les cobayes par iso- et autosensibilisation. *Ann. Inst. Pasteur. (Paris)* 81, 48–63.
- Wang, M., Fijak, M., Hossain, H., Markmann, M., Nüsing, R.M., Lochnit, G., et al., 2017. Characterization of the micro-environment of the testis that shapes the phenotype and function of testicular macrophages. *J. Immunol.* 198 (11), 4327–4340.
- Wheeler, K.M., Samy, E.T., Tung, K.S., 2009. Cutting edge: normal regional lymph node enrichment of antigen-specific regulatory T cells with autoimmune disease-suppressive capacity. *J. Immunol.* 183, 7635–7638.
- Wheeler, K.M., Tardif, S., Rival, C., Luu, B., Bui, E., Del Rio, R., et al., 2011. Regulatory T cells control tolerogenic versus autoimmune response to sperm in vasectomy. *Proc. Natl. Acad. Sci. U.S.A.* 108, 7511–7516.
- Winnall, W.R., Muir, J.A., Hedger, M.P., 2011. Rat resident testicular macrophages have an alternatively activated phenotype and constitutively produce interleukin-10 in vitro. *J. Leukoc. Biol.* 90, 133–143.
- Winnall, W.R., Hedger, M.P., 2013. Phenotypic and functional heterogeneity of the testicular macrophage population: a new regulatory model. *J. Reprod. Immunol.* 97, 147–158.
- Winnall, W.R., Lloyd, S.B., De Rose, R., Alcantara, S., Amarasingha, T.H., Hedger, M.P., et al., 2015. Simian immunodeficiency virus infection and immune responses in the pig-tailed macaque testis. *J. Leukoc. Biol.* 97 (3), 599–609.
- Wood, D.M., Liu, C., Dunbar, B.S., 1981. Effect of alloimmunization and heteroimmunization with zonae pellucidae on fertility in rabbits. *Biol. Reprod.* 25, 439–450.
- Wu, H., Shi, L., Wang, Q., Cheng, L., Zhao, X., Chen, Q., et al., 2016. Mumps virus-induced innate immune responses in mouse Sertoli and Leydig cells. *Sci. Rep.* 18 (6), 19507. Available from: <https://doi.org/10.1038/srep19507>.
- Wu, R., Van der Hoek, K.H., Ryan, N.K., Norman, R.J., Robker, R.L., 2004. Macrophage contributions to ovarian function. *Hum. Reprod. Update* 10, 119–133.
- Xia, W., Wong, E.W., Mruk, D.D., Cheng, C.Y., 2009. TGF-beta 3 and TNF-alpha perturb blood testis barrier (BTB) dynamics by accelerating the clathrin-mediated endocytosis of integral membrane proteins: a new concept of BTB regulation during spermatogenesis. *Dev. Biol.* 327, 48–61.
- Xiong, W., Chen, Y., Wang, H., Wang, H., Wu, H., Lu, Q., et al., 2008. Gas6 and the Tyro 3 receptor tyrosine kinase subfamily regulate the phagocytic function of Sertoli cells. *Reproduction* 135, 77–87.
- Yule, T.D., Montoya, G.D., Russell, L.D., Williams, T.M., Tung, K.S., 1988. Autoantigenic germ cells exist outside the blood testis barrier. *J. Immunol.* 141 (4), 1161–1167.
- Yule, T.D., Tung, K.S.K., 1993. Experimental autoimmune orchitis induced by testis and sperm antigen-specific T cell clones: an important pathogenic cytokine is tumor necrosis factor. *Endocrinology* 133, 1098–1107.
- Zhang, Y., Li, N., Chen, Q., Yan, K., Liu, Z., Zhang, X., et al., 2013. Breakdown of immune homeostasis in the testis of mice lacking Tyro3, Axl and Mer receptor tyrosine kinases. *Immunol. Cell Biol.* 91 (6), 416–426.

# Rheumatic Fever and Rheumatic Heart Disease

*Luiza Guilherme<sup>1,2</sup> and Jorge Kalil<sup>1,2,3</sup>*

<sup>1</sup>Heart Institute (InCor), School of Medicine, University of São Paulo, São Paulo, Brazil <sup>2</sup>Immunology Investigation Institute, National Institute for Science and Technology, University of São Paulo, São Paulo, Brazil <sup>3</sup>Clinical Immunology and Allergy Division, School of Medicine, University of São Paulo, São Paulo, Brazil

## OUTLINE

Clinical, Pathological, and Epidemiologic Features	1255	In Vivo and In Vitro Models	1262
Autoimmune Features	1257	In Vivo Model of Myocarditis and Valvulitis	1262
Genetic Features	1258	In Vitro Model of Rheumatic Heart Disease	
Innate Immune Response	1260	Autoimmune Reactions	1262
Adaptive Immune Response	1260		
Major Histocompatibility Complex: DRB1, DRB3, DQB1, DQA1 Genes	1261	Pathologic Effector Mechanisms	1263
Both Innate and Adaptive Immune Response	1261	Autoantibodies as Potential Immunologic Markers	1264
		Concluding Remarks—Future Prospects	1265
		References	1265

## CLINICAL, PATHOLOGICAL, AND EPIDEMIOLOGIC FEATURES

The clinical profile of rheumatic fever (RF) was first described by Cheadle in 1889, and the manifestation of the disease follows defined criteria established by Jones in 1944, which were updated in 1992 and remain useful today (Dajani et al., 1992). Briefly, the disease follows an untreated *Streptococcus pyogenes* infection in children and teenagers that present some genetic factors that predispose to the diverse clinical manifestations. The diagnosis is made in a clinical basis. The major manifestations include polyarthritis, carditis, chorea, subcutaneous nodules, and *erythema marginatum*. The minor manifestations are fever, arthralgia (clinical), and prolonged PR interval, increased erythrocyte sedimentation rate, and presence of C-reactive protein.

Polyarthritis and carditis are the most frequent manifestations of the disease and occur in around 70% of the children. Arthritis is one of the earliest and most common features of the disease, present in 60%–80% of the patients. It usually affects the peripheral large joints; small joints and the axial skeleton are rarely involved. Knees, ankles, elbows, and wrists are most frequently affected. The arthritis is usually migratory and very painful. Carditis is the most serious manifestation of the disease, occurring a few weeks after the infection, and usually present as a pancarditis. Endocarditis is the most severe sequel and frequently leads to chronic rheumatic heart disease (RHD). Mitral and aortic regurgitation (AR) are the most common events caused by valvulitis. Sydenham's chorea is less common (30%–40%) characterized by involuntary movements, especially of the face

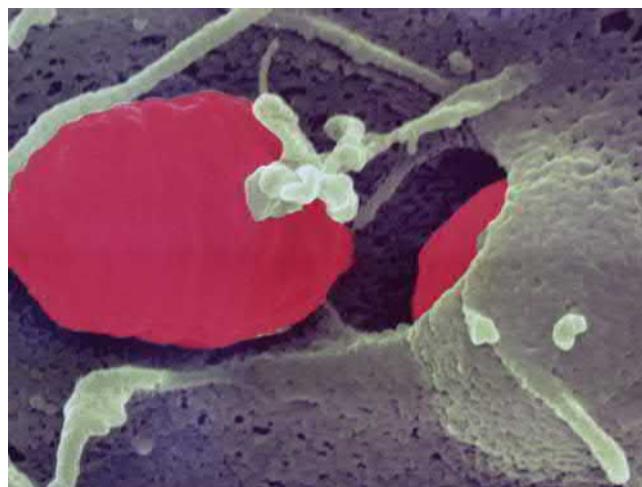
and limbs, muscular weakness, and disturbances of speech, gait, and voluntary movements. It is usually a delayed manifestation, and often the sole manifestation of acute RF. Other manifestations such as subcutaneous nodules and erythema marginatum can also occur during RF episodes and are characterized by nodules on the surface of joints and skin lesions, respectively (Mota et al., 2009).

*S. pyogenes*, or group A streptococci, was identified in 1941 by Rebecca Lancefield through serology based on its cell wall polysaccharide that is composed by carbohydrates such as N-acetyl- $\beta$ -D-glucosamine linked to a polymeric rhamnose backbone. Group A streptococci contain M, T, and R surface proteins and lipoteichoic acid, involved in bacterial adherence and invasion to throat epithelial cells (Fig. 63.1). The M protein, which extends from the cell wall, is composed by two polypeptide chains with approximately 450 amino acid residues, in an alpha-helical coiled-coil configuration. The amino-terminal (N-terminal) portion is composed by two regions, A and B, which present variable numbers of amino acid residues. The A region shows high polymorphism and defines the different M types, currently more than 225 according to CDC (Centers for Disease Control and Prevention; <http://www.cdc.gov/ncidod/biotech/strep/streblast.htm>). These M types were more recently grouped in 48 emm-clusters based on their structural and binding properties. The C-terminal portion (regions C and D) is highly conserved (Smeesters et al., 2010).

Epidemiological studies indicate the *emm1*, *emm* 12, and *emm* 28 as the most common *emm* types found in both high- and low-income countries (Steer et al., 2009b).

It is interesting to note that some strains are predominant in different regions of the world (Table 63.1) and could be related with population migrations and genetic background (Arya et al., 2014; Arêas et al., 2014; Baroux et al., 2014; Boyd et al., 2016; Dundar et al., 2010; Engel et al., 2014; Espinosa et al., 2003; Freschi de Barros et al., 2015; Friães et al., 2012; Hraoui et al., 2011; Imöhl et al., 2010; Koutouzi et al., 2015; Lindsay et al., 2016; Lopardo et al., 2005; Luca-Harari et al., 2008; Ma et al., 2009; Meisal et al., 2010; O'Brien et al., 2002; O'Grady et al., 2007; Rogers et al., 2007; Seale et al., 2016; Shea et al., 2011; Shulman et al., 2009; Smeesters et al., 2006; Steer et al., 2009a; Tamayo et al., 2014; Tanaka et al., 2016; Tapia et al., 2015; Tartof et al., 2010; Turner et al., 2016; Williamson et al., 2014).

The incidence of ARF in some developing countries exceeds 50 per 100,000 children. The worldwide incidence of RHD is of at least 15.6 million cases and the major cause of around 233,000 deaths/year. However, since these estimates are based on conservative assumptions, the actual disease burden is probably substantially higher. The incidence of ARF can vary from 0.7 to 508 per 100,000 children per year in different populations from several countries (Carapetis et al., 2005). More recently, it was showed that the prevalence of RHD varied from less than 50,000 cases to more than 8,000,000 depending on the region of the world (Carapetis et al., 2016). In Brazil, according to the WHO epidemiological model and data from IBGE (Brazilian Institute of Geography and Statistics), the number of *Streptococcal pharyngitis* infections is around 10 million cases, which could lead to 30,000 new cases of RF, of which around 15,000 could develop to cardiac lesions (Barbosa and Müller, 2009).



**FIGURE 63.1** *Streptococcus pyogenes* throat colonization. *S. pyogenes* (red) invasion and adherence into throat epithelial cells leads to colonization and invasion into human pharynx cells. Source: Image obtained by emission scanning EM, kindly provided by Prof. Dr M. Rhode, HZI, Braunschweig, Germany.

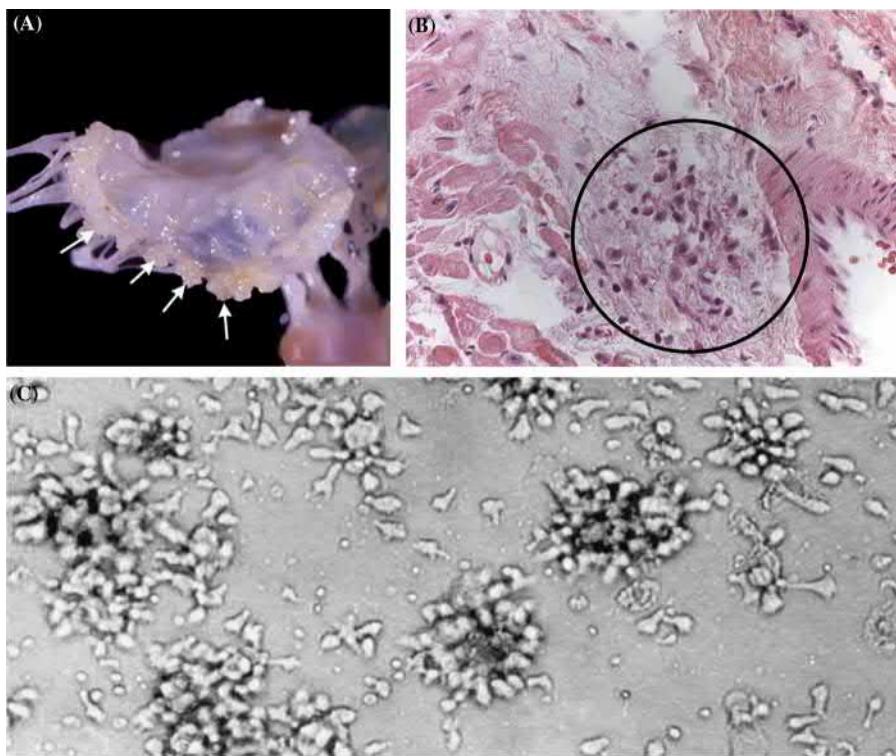
**TABLE 63.1** *Streptococcus pyogenes* Distribution Around the World

		References
North America	<i>Canada</i> : 1, 2, 3, 4, 6, 12, 28, 77, 89 <i>United States</i> : 1, 2, 3, 4, 12, 28 <i>Mexico</i> : 1, 2, 3, 4, 12, 75, 77	Shea et al. (2011) Espinosa et al. (2003) Shulman et al. (2009) O'Brien et al. (2002) Espinosa et al. (2003)
Europe/United Kingdom	<i>Norway</i> : 1, 3, 4, 12, 28, 82 <i>Denmark</i> : 1, 3, 12, 28, 89 <i>Germany</i> : 1, 3, 4, 12, 28, 89 <i>Scotland</i> : 1, 4, 12, 28, 76, 89 <i>England</i> : 1, 3, 4, 12, 28, 89 <i>Portugal</i> : 1, 3, 4, 6, 12, 28, 89 <i>Spain</i> : 1, 3, 4, 6, 12, 75, 89 <i>Greece</i> : 1, 3, 4, 12, 77, 89	Meisal et al. (2010) Luca-Harari et al. (2008) Imöhl et al. (2010) Lindsay et al. (2016) Turner et al. (2016) Friäes et al. (2012) Tamayo et al. (2014) Koutouzi et al. (2015)
Africa	<i>Tunisia</i> : 1, 28, 42, 76, 103, 118, st432 <i>Mali</i> : 18, 25, 42, 55, 58, 65, 109 <i>Kenya</i> : 8, 11, 18, 44, 65, 90, stg866 <i>South Africa</i> : 1, 4, 12, 48, 75, 89	Hraoui et al. (2011) Tapia et al. (2015) Seale et al. (2016) Engel et al. (2014) Arêas et al. (2014)
South America	<i>Brazil</i> : 1, 4, 6, 8, 12, 22, 49, 53, 58, 66, 77, 83, 87, 183 <i>Argentina</i> : 12, 75, 82, 87	Freschi de Barros et al. (2015) Smeesters et al. (2006) Tartof et al. (2010) Lopardo et al. (2005)
Asia	<i>China</i> : 1, 4, 12, 75 <i>Japan</i> : 1, 4, 12, 75, 89 <i>India</i> : 1, 8, 15, 42, 48, 49 <i>Turkey</i> : 1, 4, 12, 77, 89	Ma et al. (2009) Tanaka et al. (2016) Arya et al. (2014) Dundar et al. (2010)
Oceania	<i>Australia</i> : 1, 3, 4, 12, 22, 28, 44, 75, 81, 92, 113, 197 <i>New Zealand</i> : 1, 3, 4, 12, 22, 28, 75, 89 <i>Fiji Island</i> : 1, 12, 22, 28, 75, 89 <i>New Caledonia</i> : 1, 25, 76, 95	Boyd et al. (2016) O'Grady et al. (2007) Rogers et al. (2007) Williamson et al. (2014) Steer et al. (2009) Baroux et al. (2014)

## AUTOIMMUNE FEATURES

RHD is the most serious complication of RF and depends on several host factors that mediate a heart tissue–driven autoimmune response triggered by a defensive immune response against *S. pyogenes*.

Genetic predisposition is one of the leading factors contributing to the development of autoimmunity. In the last 5 years, using molecular biology tools, several new single-nucleotide polymorphisms (SNPs) of genes



**FIGURE 63.2** Acute phase rheumatic lesions (A and B) and cultured intraleisional T lymphocytes. (A) Mitral valve surgical fragment obtained from a RHD patient, with verrucae lesions (arrows). (B) Immunohistochemistry of myocardium exhibiting Aschoff bodies, granulomatous structures with mononuclear cells infiltration (circle). Magnification: 200 $\times$ . (C) In vitro proliferation of infiltrating T CD4 $^{+}$  isolated from rheumatic lesions. Magnification: 200 $\times$ .

involved with the activation of both innate and adaptive immune responses were associated to the development of RF/RHD (see the “Genetic features” section).

The first genetic associations described in the 1980s focused on human leukocytes antigens (HLA) class II alleles coded by HLA-DRB1 and DQB1 genes. The HLA class II molecules are expressed in the surface of antigen-presenting cells (APCs), for example, macrophages, dendritic cells, and B lymphocytes and trigger the activation of the immune system. In the case of RF/RHD, T-cell populations activated upon specific self-antigen stimulation will trigger autoimmune reactions. The production of several inflammatory cytokines will perpetuate the heart tissue damage. These observations are corroborated by the fact that during the acute phase of disease, Aschoff bodies, a granulomatous lesion containing macrophages, Anitschkow cells, multinucleated cells, and polymorphonuclear leukocytes develop in the myocardium and/or endocardium of RHD patients. Inflammatory cytokines such as IL-1, TNF $\alpha$ , and IL-2 have been found, depending on the developmental phase of the Aschoff bodies (Fraser et al., 1997) and as mentioned above, probably initiate the inflammatory process leading to heart tissue rheumatic lesions.

More recently, other molecules were described involved with the inflammatory process such as integrins and chemokine and cytokines such as IFN $\gamma$ , IL-23, and IL-17 that play a role in the recruitment of both T and B lymphocytes leading to the autoimmune reactions observed in rheumatic heart lesions (reviewed by Guilherme et al., 2011b) (Guilherme et al., 2011a). T and B lymphocytes react against self-antigens through molecular mimicry, first in the periphery and later in the heart tissue. The mechanisms of T-cell receptor (TCR) degeneracy and epitope spreading amplify the autoimmune reactions (see the “Pathologic effector mechanisms” section). All these steps are represented in Fig. 63.2.

## GENETIC FEATURES

RF and RHD occur in 1%–5% of the untreated children with genetic predisposition. The disease is associated with several genes, some of which are related to the innate or adaptive immune response or both (Table 63.2) (Azevedo et al., 2010; Beltrame et al., 2014; Berdelli et al., 2005, 2006; Catarino et al., 2014; Chou et al., 2004; Col-Araz et al., 2013; Düzung et al., 2009; Guilherme and Kalil, 2010; Hernández-Pacheco et al., 2003b; Hirsch et al., 1996; Kamal et al., 2010; Messias Reason et al. 2006, 2009; Ramasawmy et al., 2007, 2008; Sallakci et al., 2005; Settin et al., 2007).

**TABLE 63.2** Genetic Polymorphism Associated With Development of Rheumatic Fever (RF)/Rheumatic Heart Disease (RHD)

Immune response	Gene	Chromosome localization	Polymorphism	Allele/Genotype/Haplotype associated with disease	Clinical picture	Population studied	References
<i>Innate</i>	<i>MBL2</i>	10q11.2-q21	–221 X,Y A (52C, 54G, 57G), O (52T, 54A, 57A)	YA/YA, YA/XA	RHD–MS	Brazilian	<a href="#">Messias Reason et al. (2006)</a>
				O, O/O (52T, 54A, 57A)	RHD–AR	Brazilian	<a href="#">Ramasawmy et al. (2008)</a>
	<i>TLR2</i>	4q32	2258A/G (753 Arg/Gln)	753Gln, Arg753Gln	ARF	Turkish	<a href="#">Berdeli et al. (2005)</a>
	<i>FCN2</i>	9q34	–986G/A, –602G/A, –4G/A	G/G/A	RHD	Brazilian	<a href="#">Messias Reason et al. (2006)</a>
				G/G/A			<a href="#">Beltrame et al. (2014)</a>
	<i>MIF</i>	22q11-23	–173G/C	173C/C	RF	Turkish	<a href="#">Col-Araz et al. (2013)</a>
	<i>MASP2</i>	1p36.23-31	p.371D, p.377V, p.439R	AG	RHD	Brazilian	<a href="#">Catarino et al. (2014)</a>
	<i>FCγRIIA</i>	1q21-q23	494A/G (131H/R)	131R, R/R (high risk), R/H (intermediate risk)	ARF	Turkish	<a href="#">Hirsch et al. (1996)</a>
<i>Adaptive</i>	<i>MHC</i>	6p21.31	DRB1, DRB3, DQB1, DQA1	Several alleles	RF/RHD	Several	<a href="#">Guilherme and Kalil (2010)</a>
	<i>CTLA4</i>	2q33.2	+49A/G	G/G	RHD	Turkish	<a href="#">Düzungün et al. (2009)</a>
	<i>Both innate and adaptive</i>	6p21.3	–308G/A  –238G/A	A	RHD	Mexican	<a href="#">Hernandez-Pacheco et al. (2003b)</a>
				A/A, G/G	RHD–MVL, MVD	Egyptian	<a href="#">Sallakci et al. (2005)</a>
				A	ARF/RHD	Brazilian	<a href="#">Ramasawmy et al. (2007)</a>
				A	ARF/RHD	Turkish	<a href="#">Berdeli et al. (2006)</a>
				G, G/G	RHD	Mexican	<a href="#">Hernandez-Pacheco et al. (2003b)</a>
				A	ARF/RHD	Brazilian	<a href="#">Ramasawmy et al. (2007)</a>
				A1/A1	RHD	Egyptian	<a href="#">Settin et al. (2007)</a>
				A1, A1/A1	RHD	Brazilian	<a href="#">Azevedo et al. (2010)</a>
				T, T/T	RHD	Egyptian	<a href="#">Kamal et al. (2010)</a>
				C/C	RHD	Egyptian	<a href="#">Chou et al. (2004)</a>
	<i>IL-10</i>	1q31-q32	–1082G/A	G/G	RHD–MVD	Egyptian	<a href="#">Settin et al. (2007)</a>
				A/A	RHD–MVL	Egyptian	<a href="#">Settin et al. (2007)</a>

AR, Aortic regurgitation; ARF, acute rheumatic fever; *CTLA4*, cytotoxic T cell Lymphocyte antigen 4, *FCN2*, ficolin 2; *FCγRIIA*, IgG Fc receptor; *IL-1RA*, IL-1 receptor antagonist; *MASP2*, mannan-binding lectin serine protease; *MASP2*, mannan-binding lectin; *MBL*, mannan-binding lectin; *MHC*, major histocompatibility complex; *MIF*, macrophage inhibitory factor; *MS*, mitral stenosis; *MVD*, mitral valve disease; *MVL*, multivalvular lesions; *RHD*, rheumatic heart disease; *TGFβ*, transforming growth factor beta; *TLR2*, toll-like receptor 2; *TNFα*, tumor necrosis factor alpha.

In order to facilitate the comprehension of the role of implicated genes, known up to now, we describe the associated genes/alleles based on their role.

## Innate Immune Response

### **MBL2 Gene**

MBL (mannan-binding lectin) is an acute phase inflammatory protein and functions as a soluble pathogen recognition receptor. It binds to a wide variety of sugars on the surface of pathogens and plays a major role in innate immunity due to its ability to opsonize pathogens, enhancing their phagocytosis and activating the complement cascade via the lectin pathway (Jack et al., 2001). Different variants of the promoter and exon 1 regions of the *MBL2* gene, which encodes MBL, have been reported in patients with RF/RHD. Interestingly, the A allele that codes for high production of MBL was associated with the development of mitral stenosis (MS) and most of these patients presented high serum levels of MBL (Messias Reason et al., 2006). In contrast, RHD patients with AR presented the O allele that codes for low production of MBL, and the patients presented low serum levels of MBL (Ramasawmy et al., 2008).

### **TLR-2 Gene**

Toll-like receptors (TLRs) are sensors of foreign microbial products, which initiate host defense responses in multicellular organisms. A polymorphism of TLR-2 at codon 753 generally leads to the replacement of arginine to glutamine. The genotype 753 Arg/Gln was more frequent in a Turkish ARF cohort when compared to controls (Berdeli et al., 2005).

### **Ficolin Gene**

Ficolin trigger the innate immune response by either binding collectin cellular receptors or initiating the complement lectin pathway (Beltrame et al., 2014; Messias-Reason et al., 2009). In Brazilian chronic RHD patients, with prolonged time of infection or repeated streptococcal infections, the haplotype G/G/A (-986/-602/-4) was found to be more frequent than in controls and was also correlated with low expression levels of this protein.

### **Fc $\gamma$ RIIA Gene**

The protein plays a role in the clearance of immune complexes by macrophages, neutrophils, and platelets (Hirsch et al., 1996). ARF patients presented histidine (H) in the codon 131, which typically encodes for arginine (A), consequently RF/RHD patients present a protein with low binding capacity to the immune complex, favoring the inflammatory response.

### **Masp2 Gene**

MBL-associated serine protease (Masp) results from this gene. It is a protease that plays a role during innate immune response process through recognition of the pathogen and complement activation. Three polymorphisms (p.371D, p.377V and p.439R) increase the susceptibility to develop RHD (Catarino et al., 2014).

### **MIF Gene**

This gene codes for a macrophage inhibitory factor (MIF), expressed in monocytes/macrophages and other type of cells and tissues and has been associated with several inflammatory diseases (Col-Araz et al., 2013). A significant increase in the frequency of MIF-173CC genotype was found in children with RF (Col-Araz et al., 2013).

## Adaptive Immune Response

The HLA system is located in the short arm of the human chromosome 6 and codes for diverse proteins; it is considered the most polymorphic system, composed by several genes with several alleles. The class I proteins are present in all nucleated cells; however, the class II are expressed only in specialized cells of the immune system (B lymphocytes, activated T lymphocytes, monocytes/macrophages, and dendritic cells). These proteins are involved with antigen recognition and presentation of self and foreign (microbes) antigens.

## Major Histocompatibility Complex: DRB1, DRB3, DQB1, DQA1 Genes

Several HLA class II alleles were described in association with RF/RHD. Patarroyo et al. (1979) described an alloantigen on the surface of B cells, designated 883, probably related to the HLA class II molecules, which was present in a high frequency in RF patients. Later, a monoclonal antibody (D8/17 MoAb) was produced against B cells from RF patients bearing the 883 alloantigen. Studies performed by Zabriskie et al., (1985) showed an increased frequency of this alloantigen in RF patients.

The susceptibility of developing RF/RHD was first associated with the alleles of HLA class II genes (*DRB1*, *DRB3*, *DQB*, and *DQA*), which are located on human chromosome 6. Briefly, HLA-DR7 was the allele most consistently associated with RF (Guilherme et al., 1991; Guédez et al., 1999; Ozkan et al., 1993; Stanevicha et al., 2003; Visentainer et al., 2000; Weidebach et al., 1994). In addition the association of DR7 with different DQB or DQA alleles seems to be related with the development of multiple valvular lesions (MVL) or mitral valve regurgitation in RHD patients (Guédez et al., 1999; Stanevicha et al., 2003). HLA-DR53 coded by the *DRB3* gene is another HLA class II molecule in linkage disequilibrium with HLA-DR4, DR7, and DR9. This allele was strongly associated with RF/RHD in two studies with Mulatto Brazilian patients (Guilherme et al., 1991; Weidebach et al., 1994), but not in Brazilian Caucasian patients (Visentainer et al., 2000). Although DR53 has not been described in previous studies, DR4 and DR9 were associated with RF in American Caucasian and Arabian patients (Ayoub, 1984; Rajapakse et al., 1987), whereas in Egyptian and Latvian patients, DR7 was associated with the disease (Guédez et al., 1999; Stanevicha et al., 2003) (Table 63.1). In Japanese RHD patients, susceptibility to MS seems to be in part controlled by the HLA-DQA gene or by genes in close disequilibrium linkage with HLA-DQA\*0104 and DQB1\*05031 (Koyanagi et al., 1996). HLA-DQA\*0501 DQB\*0301 with DRB1\*1601 (DR2) were associated with RHD in a Mexican Mestizo population (Hernández-Pacheco et al., 2003a).

The molecular mechanism by which MHC class II molecules confers susceptibility to autoimmune diseases is not clear. However, since the role of HLA molecules is to present antigens to the TCR, it is probable that the associated alleles facilitate the presentation of some streptococcal peptides that will later trigger autoimmune reactions mediated by molecular mimicry mechanisms.

### CTLA4 Gene

This gene is an essential inhibitor of T-cell responses. It is a strong candidate susceptibility gene in autoimmunity, and several studies suggest disease-associated polymorphisms (reviewed by Gough et al., 2005).

### Both Innate and Adaptive Immune Response

More recently, with new technologies that have allowed the description of gene variability by SNPs, other associations have been established that could clarify some reactions related with both innate and adaptive immune response leading the autoimmune reactions in RF/RHD.

- *TNF- $\alpha$*  gene, also located in the chromosome 6, between HLA class I and II genes, codes for a proinflammatory cytokine that plays a role during the *S. pyogenes* infection and later in the inflammatory process in the valves. Polymorphisms at -308G/A and -238G/A were associated with the susceptibility of RHD patients from several countries (Berdeli et al., 2006; Hernández-Pacheco et al., 2003b; Ramasawmy et al., 2007; Sallakci et al., 2005).
- *IL-10* gene is responsible for the production of IL-10, an antiinflammatory cytokine. The genotype -1082G/A, misrepresented in RHD patients, is apparently associated with the development of MVL and with the severity of RHD (Settin et al., 2007).
- *TGF-B1* is a gene that controls the proliferation and differentiation of cells. The polymorphism of both the SNPs 869T and -509T alleles were considered possible risk factors for the development of valvular RHD lesions in Egyptian and Taiwan RHD patients (Chou et al., 2004; Kamal et al., 2010).
- *IL-1Ra* gene, for which the most frequent alleles are 1 and 2, encodes the antagonist of IL-1 $\alpha$  and IL-1 $\beta$ , which are inflammatory cytokines. Two studies in Brazilian and Egyptian RHD patients with severe carditis showed low frequencies of allele 1, suggesting lack of inflammatory control (Azevedo et al., 2010; Settin et al., 2007).

## IN VIVO AND IN VITRO MODELS

### In Vivo Model of Myocarditis and Valvulitis

Humans are unique hosts for *S. pyogenes* infections. However, several studies have been performed to determine a suitable animal model, and numerous different species (mice, rats, hamsters, rabbits, and primates) have been tested for the development of autoimmune reactions that resemble those observed in RF/RHD patients (Unny and Middlebrooks, 1983), all with little success.

In the last decade a model that appears to be useful for the study of RF/RHD has been developed with Lewis rats. These rats have already been used to induce experimental autoimmune myocarditis and to study the pathogenesis of RF/RHD (Li et al., 2004).

Immunization of Lewis rats with recombinant M6 protein induced focal myocarditis, myocyte necrosis, and valvular heart lesions in three out of six animals. The disease in these animals included verruca-like nodules and the presence of Anitschkow cells, which are large macrophages (also known as caterpillar cells), in mitral valves. Lymph node cells from these animals showed a proliferative response against cardiac myosin, but not skeletal myosin or actin. A CD4<sup>+</sup> T-cell line responsive to both the M protein and cardiac myosin was also obtained. Taken together, these results confirmed the cross-reactivity between the M protein and cardiac myosin triggered by molecular mimicry, as observed in humans, possibly causing a break in tolerance and consequently leading to autoimmunity (Quinn et al., 2001). In another study done by the same group, Lewis rats were immunized with a pool of synthetic peptides from the conserved region of the M5 protein. Mononuclear spleen cells from these animals were able to proliferate in response to peptides from both the C-terminal region of M5 protein and the N-terminal region of a heterologous protein (M1) and myosin. These rats developed a focal infiltration of mononuclear cells predominantly in the aortic valve, although no evidence of Aschoff bodies, the hallmark of RF lesions, or Anitschkow cells was observed (Lymbury et al., 2003).

Another study immunized Lewis rats with recombinant M5 or synthetic peptides from the B- and C-regions of group A *streptococcus* (GAS) M5 (Gorton et al., 2009). Sera and T cells from these animals recognized a peptide (M5-B.6) from the B repeat of the N-terminal portion of M5 protein and induced heart lesions (Gorton et al., 2010), confirming the previous results. The immunized rats (five out of seven) developed mononuclear cell infiltration in the myocardial or valvular tissue. Histopathological analysis of valve lesions showed the presence of both CD4<sup>+</sup> T cells and CD68<sup>+</sup> macrophages (Gorton et al., 2010), consistent with human studies (Guilherme et al., 1995).

Altogether, these studies indicated that the Lewis rats could be a model of autoimmune valvulitis.

In the last decade, however, other animal models as mice and guinea pig were also used by several researchers and appear to be also useful as experimental models of RF/RHD as reviewed by Rush et al. (2014).

### In Vitro Model of Rheumatic Heart Disease Autoimmune Reactions

The major sequels of RF are heart tissue lesions that lead to chronic RHD, which is characterized by permanent valvular lesions. The heart disease starts by pericarditis, followed by myocarditis episodes in which the healing process results in varied degrees of valvular damage (Mota et al., 2009).

By isolating infiltrating T lymphocytes from damaged valvular tissue, we could establish the mechanism by which the immune response in the heart leads to autoimmune reactions (Guilherme et al., 1995). Fig. 63.2 shows a damaged mitral valve in which verrucae lesions are observed, indicative of an acute RF episode. Furthermore, the presence of Aschoff bodies in the myocardium tissue allowed for histological diagnosis of an active episode of rheumatic disease. In vitro tissue culture of small pieces of the surgical fragment allowed to the isolation of infiltrating T cells.

The in vitro analysis of these tissue-infiltrating T cells showed their ability to recognize several streptococcal-M protein peptides and self-antigens by molecular mimicry mechanisms. We identified some mitral valve-derived proteins such as vimentin, PDIA3 (protein disulfide isomerase ER-60 precursor) and HSPA5 (78 kDa glucose-regulated protein precursor) that were recognized by both peripheral and intralesional T-cell clones (Faé et al., 2008).

The identification of heart-M protein cross-reactive T cell clones directly from rheumatic valvular lesions established their involvement in the pathogenesis of the disease.

## PATHOLOGIC EFFECTOR MECHANISMS

The term of “molecular mimicry” was introduced in 1964 by Damian to define the mechanism by which self-antigens are recognized after an infection by cross-reactivity (Damian, 1964).

Pathogen and self-antigens can be recognized by T lymphocytes and antibodies through molecular mimicry by four different mechanisms. They can recognize (1) identical amino acid sequences, (2) homologous but nonidentical sequences, (3) common or similar amino acid sequences of different molecules (proteins, carbohydrates), and (4) structural similarities between the microbe or environmental agent and its host (Peterson and Fujimani, 2007).

RF/RHD is the most convincing example of molecular mimicry in human pathological autoimmunity, in light of the cross-reactions between streptococcal antigens and human tissue proteins, mainly heart tissue proteins that follow throat infection by *S. pyogenes* in susceptible individuals.

The inflammatory process that follows a *S. pyogenes* throat infection in individuals with genetic predisposition leads to intense cytokine production by monocytes and macrophages that trigger the activation of B and T lymphocytes.

Several heart-reactive antibodies, first described from 1945 (reviewed by Cunningham, 2000 and Guilherme et al., 2011b) (Guilherme et al., 2011a), also play a role in the development of the disease.

Streptococcal and heart tissue cross-reactive antibodies activate the heart tissue valvular endothelial cells increasing the expression of adhesion molecules such as VCAM1, which facilitates cellular infiltration by neutrophils, monocytes, B and T cells (Yeğin et al., 1997). The “rolling” of leukocytes through vessels is triggered by chemokines expressed by activated endothelial cells that induce the expression of integrins, selectins, and subsequent transendothelial migration. Recently, we identified increased expression of intercellular adhesion molecule (ICAM), another adhesion molecule, a few chemokines (CCL-1, CCL-3, and CCL9) (Faé et al., 2013) as well as some integrins (P- and E-selectins and) in the myocardium and valvular tissue of RHD patients. All of these molecules are involved with the inflammatory process and T and B lymphocytes infiltration leading to rheumatic valvular tissue damage.

CD4<sup>+</sup> infiltrating T cells are predominant in the heart rheumatic lesions (Kemeny et al., 1989; Raizada et al., 1983), and the first evidence of the molecular mimicry between *streptococcus* and heart tissue was obtained through an analysis of these heart tissue–infiltrating T cells. Three immunodominant regions of the M5 protein (residues 1-25, 81-103, and 163-177), heart tissue proteins (myocardium and valve-derived proteins, as well as vimentin), and synthetic peptides of the beta chain of cardiac myosin-light meromyosin region (LMM) were recognized by cross-reactivity by intralesional T-cell clones (Ellis et al., 2005; Faé et al., 2006; Guilherme et al., 1995, 2001). Peripheral T-cell clones also recognized human-purified myosin, tropomyosin, laminin, and cardiac myosin-derived peptides from LMM and S2 regions (Guilherme et al., 1995).

Employing a proteomics approach, we characterized a number of mitral valve proteins identified by molecular weight and isoelectric point (pI). Four valve-derived proteins with molecular masses ranging between 52 and 79 kDa and different pI cross-reacted with the M5 immunodominant peptides and were recognized in proliferation assays by intralesional T-cell clones from patients with severe RHD. Vimentin was one of the identified proteins, a result that reinforces the role of this protein as a putative autoantigen involved in the rheumatic lesions. Novel heart tissue proteins were also identified, including disulfide isomerase ER-60 precursor (PDIA3) protein and a 78-kDa glucose-regulated protein precursor (HSPA5). The role of PDIA3 in RHD pathogenesis and other autoimmune diseases is not clear (Table 63.3) (Faé et al., 2008).

A mass spectrometry analysis allowed the identification of several valve proteins that suffered expression alterations probably due to the autoimmune process. Briefly, we identified abundant expression of two isoforms of vimentin (45 and 42 kDa) with reduced expression of the full size of protein (54 kDa). Vitronectin was other altered protein that presented increased expression and reduced collagen VI expression (Martins et al., 2014). Immunohistochemical analysis of their distribution in valve tissue lesions was also analyzed, and a disorganized distribution of these proteins in RHD valves was found and correlates with clinical manifestations such as valve regurgitation or stenosis. Confocal microscopy analysis revealed a diverse pattern of distribution of collagen VI and lumican into RHD and myxomatous degeneration (MXD) valves (Martins et al., 2014).

The analysis of the TCRs of autoreactive T lymphocytes that infiltrate both myocardium and valves allowed us to evaluate the V $\beta$  chains usage of TCR and the degree of clonality of heart tissue–infiltrating T cells (Guilherme et al., 2000). In the heart tissue (myocardium and valves) of both chronic and acute RHD patients, several expanded T-cell populations with an oligoclonal profile were found. Such oligoclonal expansions were identified by TCRs analyses (Guilherme et al., 2000). The finding of oligoclonal T-cell populations is in contrast with the peripheral blood scenario, which contains polyclonal TCR-BV families. The fact that a high number of

**TABLE 63.3** Mitral Valve Proteins Identified by 2-D Gel Electrophoresis and Mass Spectrometry Analysis Recognized by Peripheral and Intralesional T Cells

Protein	Accession number	Coverage (%)	Masses matched/total	MW/pI
Vimentin	<u>P08670</u>	34	20/23	53.0/5.4 53.7/5.1
Vimentin	<u>P08670</u>	49	23/87	51.0/5.9
<b>PDIA3</b> Protein disulfide isomerase ER-60 precursor	<u>P30101</u>	45	19/92	56.0/6.7 56.0/6.0
<b>HSPA5</b> 78 kD glucose-regulated protein precursor	<u>P11021</u>	43	27/69	68.0/5.9

MW, molecular weight; *pl*, isoelectrical point; coverage (%), percentage of matched peptides identification in terms of amino acid residues.

Adapted from Faé, K.C., Diefenbach da Silva, D., Bilate, A.M., Tanaka, A.C., Pomerantzoff, P.M., Kiss, M.H., et al., 2008. PDIA3, HSPA5 and vimentin, proteins identified by 2-DE in the valvular tissue, are the target antigens of peripheral and heart infiltrating T cells from chronic rheumatic heart disease patients. *J. Autoimmun.*, 31(2), 136–141.

T-cell oligoclonal expansions could be found in the valvular tissue indicates that specific and cross-reactive T cells migrate to the valves and proliferate upon specific cytokine stimulation at the site of the lesions (Guilherme et al., 2000).

Cytokines are important secondary signals following an infection because they trigger effective immune responses in most individuals and probably deleterious responses in patients with autoimmune diseases. Three subsets of T helper cytokines are currently described. Antigen-activated CD4<sup>+</sup> T cells polarize to the Th1, Th2, or Th17 subsets, depending on the cytokine secreted. Th1 is involved with the cellular immune response and produces IL-2, IFN $\gamma$ , and TNF $\alpha$ . Th2 cells mediate humoral and allergic immune responses and produce IL-4, IL-5, and IL-13.

Another lineage of CD4<sup>+</sup> T cells, namely Th17 cells, produces a set of cytokines identified as IL-17, TGF $\beta$ , IL-6, and IL-23, and this subset of cells is involved with inflammatory reactions and in association with several autoimmune diseases (Volin and Shahrara, 2011).

In RHD, in both myocardium and valvular tissue, we found large numbers of infiltrating mononuclear cells secreting the inflammatory cytokines IFN $\gamma$  and TNF $\alpha$ . However, mononuclear cells secreting IL-10 and IL-4, which are regulatory cytokines, were also found in the myocardium tissue; nonetheless, in the valvular tissue, only a few cells secrete IL-4, suggesting that low numbers of IL-4-producing cells may contribute to the progression of valvular RHD lesions (Guilherme et al., 2004).

Increased numbers of Th17 cells in peripheral blood were found in a cohort of RHD patients from Turkey, in which high levels of IL-17A cytokine in the sera were observed (Bas et al., 2014). We also identified large numbers of IL-17- and IL-23-producing cells in both myocardium and valvular endothelium of RHD, confirming that Th17 cells also play an important role in the inflammatory process in RHD heart lesions.

## AUTOANTIBODIES AS POTENTIAL IMMUNOLOGIC MARKERS

Several streptococcal and human cross-reactive antibodies have been found in the sera of RF patients and immunized rabbits and mice over the last 60 years and have been recently reviewed (Carapetis et al., 2016). N-Acetyl- $\beta$ -D-glucosamine that is present in both the streptococcal cell wall and heart valvular tissue is one of the major targets of the humoral response in RF/RHD, and antibodies against this polysaccharide displayed cross-reactivity with laminin, an extracellular matrix alpha-helical coiled-coil protein that surrounds heart cells and is also present in the valves (Cunningham, 2000; Cunningham et al., 1989).

Cardiac myosin is the most important protein in the myocardium and by using affinity purified antimyosin antibody, Cunningham's group identified a five-amino acid residue (Gln-Lys-Ser-Lys-Gln) epitope of the N-terminal M5 and M6 proteins as being cross-reactive with cardiac myosin (Cunningham et al., 1989).

The permanent rheumatic lesions that damage the valves and antibodies against vimentin, an abundant protein in the valvular tissue, probably play a role in the valvular lesions (Cunningham, 2000). In agreement with this observation the permanent rheumatic lesions that damage the valves and antibodies against vimentin, an

abundant protein in the valvular tissue, probably play a role in the valvular lesions (Cunningham, 2000). In agreement with this observation, as mentioned before, two isoforms of vimentin (45 and 42 kDa) were recently identified by proteomics approach in damaged valves probably due to the autoimmune process (Martins et al., 2014).

In conclusion, antibodies against *N*-acetyl- $\beta$ -D-glucosamine, some epitopes of cardiac myosin, vimentin, collagen, and vitronectin can be considered the immunological markers of the disease.

## CONCLUDING REMARKS—FUTURE PROSPECTS

RF/RHD is the most convincing example of molecular mimicry in which the response against *S. pyogenes* triggers autoimmune reactions with human tissues. RF/RHD lesions result from a complex network of several genes that control both innate and adaptive immune responses after a *S. pyogenes* throat infection. An inflammatory process permeates the development of heart lesions, in which adhesion molecules and specific chemokines facilitate the valvular-tissue infiltration by B and T cells. CD4 $^{+}$  T lymphocytes are the prime effectors of heart lesions. Several self-antigens such as vimentin, collagen VI, vitronectin, myosin proteins, and other human targets proteins are recognized by molecular mimicry mechanism between streptococcal immunodominant peptides, particularly in individuals with genetic predisposition. The production of inflammatory cytokines (IFN $\gamma$ , TNF $\alpha$ , IL-17, and IL-23), and low numbers of IL-4-producing cells, a regulatory cytokine, lead to local inflammation.

All these information create a new scenario for the development of RHD, opening new possibilities for immunotherapy. Molecular knowledge of the autoimmune reactions mediated by antibodies and peripheral and intra-lesional T cells will certainly assist in the choice of streptococcal protective epitopes for the construction of an effective and safe vaccine.

Anti-GAS vaccine candidates based on the M protein and other alternative streptococcal antigens, including A-CHO, C5a peptidase (SCPA), cysteine protease (Spe), binding proteins, *streptococcus* pili, and other antigens are under investigation as reviewed by Steer and Carapetis (2009).

Briefly, a recombinant N-terminal protein candidate vaccine including the 30 most prevalent *S. pyogenes* serotypes in the US strains was constructed and has entered into phase I clinical trials (Dale et al., 2011).

J8 is a candidate vaccine that incorporates a minimal C-terminal protective epitope and induced protective antibodies in mouse models (Batzloff et al., 2003). StreptInCor, composed of 55 amino acid residues is a vaccine epitope based on the selection of a large panel of human sera and peripheral blood cells. This epitope can undergo processing by APCs (monocytes and/or macrophages) and generates a universal, robust, and safe immune response (Guilherme et al., 2009, 2011b). Experimental assays using several animal models showed that the candidate vaccine induced high titers of opsonic, neutralizing, and protective antibodies (De Amicis et al., 2014; Postol et al., 2013). The immunogenicity and safety of the StreptInCor vaccine epitope was also evaluated for a period of 1 year in a model of HLA class II transgenic mice. Specific and nonautoreactive antibodies were produced without autoimmune or pathological reactions in the heart or other organs (Guerino et al., 2011).

In conclusion, of a vaccine to protect against *S. pyogenes* without triggering autoimmune reactions remains a challenge. The knowledge of the mechanisms that lead to RF and/or RHD allows and favors the construction of a safe and efficacious anti-*S. pyogenes* vaccine.

## References

- Arêas, G.P., Schuab, R.B., Neves, F.P., Barros, R.R., 2014. Antimicrobial susceptibility patterns, emm type distribution and genetic diversity of *Streptococcus pyogenes* recovered in Brazil. Mem. Inst. Oswaldo Cruz 109 (7), 935–939.
- Arya, D.K., Sharma, A., Mehta, G., Dua, M., Johri, A.K., 2014. Molecular epidemiology and virulence characteristics of prevalent group A streptococci recovered from patients in northern India. J. Infect. Dev. Ctries. 8 (3), 271–281.
- Ayoub, E.M., 1984. The search for host determinants of susceptibility to rheumatic fever: the missing link. T. Duckett Jones Memorial Lecture. Circulation 69 (1), 197–201.
- Azevedo, P.M., Bauer, R., Caparbo, V. e F., Silva, C.A., Bonfá, E., Pereira, R.M., 2010. Interleukin-1 receptor antagonist gene (IL1RN) polymorphism possibly associated to severity of rheumatic carditis in a Brazilian cohort. Cytokine 49 (1), 109–113.
- Barbosa, P.J.B., Müller, R.E., 2009. Diretrizes Brasileiras para o Diagnóstico, Tratamento e Prevenção da Febre Reumática. Arquivo Brasileiro de Cardiologia, 1–18.
- Baroux, N., D'Ortenzio, E., Amédéo, N., Baker, C., Ali Alsuwayyid, B., Dupont-Rouzeyrol, M., et al., 2014. The emm-cluster typing system for Group A *Streptococcus* identifies epidemiologic similarities across the Pacific region. Clin. Infect. Dis. 59 (7), e84–e92.
- Bas, H.D., Baser, K., Yavuz, E., Bolayir, H.A., Yaman, B., Unlu, S., et al., 2014. A shift in the balance of regulatory T and T helper 17 cells in rheumatic heart disease. J. Invest. Med. 62 (1), 78–83.

- Batzloff, M.R., Hayman, W.A., Davies, M.R., Zeng, M., Pruksakorn, S., Brandt, E.R., et al., 2003. Protection against group A *streptococcus* by immunization with J8-diphtheria toxoid: contribution of J8- and diphtheria toxoid-specific antibodies to protection. *J. Infect. Dis.* 187 (10), 1598–1608.
- Beltrame, M.H., Catarino, S.J., Goeldner, I., Boldt, A.B., de Messias-Reason, I.J., 2014. The lectin pathway of complement and rheumatic heart disease. *Front. Pediatr.* 2, 148.
- Berdeli, A., Celik, H.A., Ozyürek, R., Dogrusoz, B., Aydin, H.H., 2005. TLR-2 gene Arg753Gln polymorphism is strongly associated with acute rheumatic fever in children. *J. Mol. Med. (Berl.)* 83 (7), 535–541.
- Berdeli, A., Tabel, Y., Celik, H.A., Ozyürek, R., Dogrusoz, B., Aydin, H.H., 2006. Lack of association between TNFalpha gene polymorphism at position -308 and risk of acute rheumatic fever in Turkish patients. *Scand. J. Rheumatol.* 35 (1), 44–47.
- Boyd, R., Patel, M., Currie, B.J., Holt, D.C., Harris, T., Krause, V., 2016. High burden of invasive group A streptococcal disease in the Northern Territory of Australia. *Epidemiol. Infect.* 144 (5), 1018–1027.
- Carapetis, J.R., Steer, A.C., Mulholland, E.K., Weber, M., 2005. The global burden of group A streptococcal diseases. *Lancet Infect. Dis.* 5 (11), 685–694.
- Carapetis, J.R., Beaton, A., Cunningham, M.W., Guilherme, L., Karthikeyan, G., Mayosi, B.M., et al., 2016. Acute rheumatic fever and rheumatic heart disease. *Nat. Rev. Dis. Primers* 2, 15084.
- Catarino, S.J., Boldt, A.B., Beltrame, M.H., Nishihara, R.M., Schafranski, M.D., de Messias-Reason, I.J., 2014. Association of MASP2 polymorphisms and protein levels with rheumatic fever and rheumatic heart disease. *Hum. Immunol.* 75 (12), 1197–1202.
- Chou, H.T., Chen, C.H., Tsai, C.H., Tsai, F.J., 2004. Association between transforming growth factor-beta1 gene C-509T and T869C polymorphisms and rheumatic heart disease. *Am. Heart J.* 148 (1), 181–186.
- Col-Araz, N., Pehlivan, S., Baspinar, O., Sever, T., Oguzkan-Balci, S., Balat, A., 2013. Association of macrophage migration inhibitory factor and mannose-binding lectin-2 gene polymorphisms in acute rheumatic fever. *Cardiol. Young* 23 (4), 486–490.
- Cunningham, M.W., 2000. Pathogenesis of group A streptococcal infections. *Clin. Microbiol. Rev.* 13 (3), 470–511.
- Cunningham, M.W., McCormack, J.M., Fenderson, P.G., Ho, M.K., Beachey, E.H., Dale, J.B., 1989. Human and murine antibodies cross-reactive with streptococcal M protein and myosin recognize the sequence GLN-LYS-SER-LYS-GLN in M protein. *J. Immunol.* 143 (8), 2677–2683.
- Dajani, A., Ayoub, E., Bierman, F., Bisno, A., Denny, F., Durack, D., et al., 1992. Guidelines for the diagnosis of rheumatic fever: Jones criteria, 1992 update. *JAMA* 268 (15), 2069–2073.
- Dale, J.B., Penfound, T.A., Chiang, E.Y., Walton, W.J., 2011. New 30-valent M protein-based vaccine evokes cross-opsonic antibodies against non-vaccine serotypes of group A streptococci. *Vaccine* 29 (46), 8175–8178.
- Damian, R., 1964. Molecular mimicry. Antigen sharing by parasite and host and its consequences. *Am. Nat.* 98 (900), 21.
- De Amicis, K.M., Freschi de Barros, S., Alencar, R.E., Postól, E., Martins, C.O., Arcuri, H.A., et al., 2014. Analysis of the coverage capacity of the StreptInCor candidate vaccine against *Streptococcus pyogenes*. *Vaccine* 32 (32), 4104–4110.
- Dundar, D., Sayan, M., Tamer, G.S., 2010. Macrolide and tetracycline resistance and emm type distribution of *Streptococcus pyogenes* isolates recovered from Turkish patients. *Microb. Drug Resist.* 16 (4), 279–284.
- Düzungün, N., Duman, T., Haydardedeoğlu, F.E., Tutkak, H., 2009. Cytotoxic T lymphocyte-associated antigen-4 polymorphism in patients with rheumatic heart disease. *Tissue Antigens* 74 (6), 539–542.
- Ellis, N.M., Li, Y., Hildebrand, W., Fischetti, V.A., Cunningham, M.W., 2005. T cell mimicry and epitope specificity of cross-reactive T cell clones from rheumatic heart disease. *J. Immunol.* 175 (8), 5448–5456.
- Engel, M.E., Muhammed, B., Whitelaw, A.C., Musvosvi, M., Mayosi, B.M., Dale, J.B., 2014. Group A streptococcal emm type prevalence among symptomatic children in Cape Town and potential vaccine coverage. *Pediatr. Infect. Dis. J.* 33 (2), 208–210.
- Espinosa, L.E., Li, Z., Gomez Barreto, D., Calderon Jaimes, E., Rodriguez, R.S., Sakota, V., et al., 2003. M protein gene type distribution among group A streptococcal clinical isolates recovered in Mexico City, Mexico, from 1991 to 2000, and Durango, Mexico, from 1998 to 1999: overlap with type distribution within the United States. *J. Clin. Microbiol.* 41 (1), 373–378.
- Faé, K.C., da Silva, D.D., Oshiro, S.E., Tanaka, A.C., Pomerantz, P.M., Douay, C., et al., 2006. Mimicry in recognition of cardiac myosin peptides by heart-intralesional T cell clones from rheumatic heart disease. *J. Immunol.* 176 (9), 5662–5670.
- Faé, K.C., Diefenbach da Silva, D., Bilate, A.M., Tanaka, A.C., Pomerantz, P.M., Kiss, M.H., et al., 2008. PDIA3, HSPA5 and vimentin, proteins identified by 2-DE in the valvular tissue, are the target antigens of peripheral and heart infiltrating T cells from chronic rheumatic heart disease patients. *J. Autoimmun.* 31 (2), 136–141.
- Faé, K.C., Palacios, S.A., Nogueira, L.G., Oshiro, S.E., Demarchi, L.M., Bilate, A.M., et al., 2013. CXCL9/Mig mediates T cells recruitment to valvular tissue lesions of chronic rheumatic heart disease patients. *Inflammation* 36 (4), 800–811.
- Fraser, W.J., Haffejee, Z., Jankelow, D., Wadee, A., Cooper, K., 1997. Rheumatic Aschoff nodules revisited. II: Cytokine expression corroborates recently proposed sequential stages. *Histopathology* 31 (5), 460–464.
- Freschi de Barros, S., De Amicis, K.M., Alencar, R., Smeesters, P.R., Trunkel, A., Postól, E., et al., 2015. *Streptococcus pyogenes* strains in São Paulo, Brazil: molecular characterization as a basis for StreptInCor coverage capacity analysis. *BMC Infect. Dis.* 15, 308.
- Friães, A., Pinto, F.R., Silva-Costa, C., Ramirez, M., Melo-Cristino, J., 2012. The Portuguese Group for the Study of Streptococcal Infections. Group A streptococci clones associated with invasive infections and pharyngitis in Portugal present differences in emm types, superantigen gene content and antimicrobial resistance. *BMC Microbiol* 12, 280.
- Gorton, D., Govan, B., Olive, C., Ketheesan, N., 2009. B- and T-cell responses in group a *streptococcus* M-protein- or peptide-induced experimental carditis. *Infect. Immun.* 77 (5), 2177–2183.
- Gorton, D., Blyth, S., Gorton, J.G., Govan, B., Ketheesan, N., 2010. An alternative technique for the induction of autoimmune valvulitis in a rat model of rheumatic heart disease. *J. Immunol. Methods* 355 (1–2), 80–85.
- Gough, S.C., Walker, L.S., Sansom, D.M., 2005. CTLA4 gene polymorphism and autoimmunity. *Immunol. Rev.* 204, 102–115.
- Guédez, Y., Kotby, A., El-Demellawy, M., Galal, A., Thomson, G., Zaher, S., et al., 1999. HLA class II associations with rheumatic heart disease are more evident and consistent among clinically homogeneous patients. *Circulation* 99 (21), 2784–2790.
- Guerino, M.T., Postol, E., Demarchi, L.M., Martins, C.O., Mundel, L.R., Kalil, J., et al., 2011. HLA class II transgenic mice develop a safe and long lasting immune response against StreptInCor, an anti-group A *streptococcus* vaccine candidate. *Vaccine* 29 (46), 8250–8256.

- Guilherme, L., Kalil, J., 2010. Rheumatic fever and rheumatic heart disease: cellular mechanisms leading autoimmune reactivity and disease. *J. Clin. Immunol.* 30 (1), 17–23.
- Guilherme, L., Weidebach, W., Kiss, M.H., Snitcowsky, R., Kalil, J., 1991. Association of human leukocyte class II antigens with rheumatic fever or rheumatic heart disease in a Brazilian population. *Circulation* 83 (6), 1995–1998.
- Guilherme, L., Cunha-Neto, E., Coelho, V., Snitcowsky, R., Pomerantzeff, P.M., Assis, R.V., et al., 1995. Human heart-infiltrating T-cell clones from rheumatic heart disease patients recognize both streptococcal and cardiac proteins. *Circulation* 92 (3), 415–420.
- Guilherme, L., Dulphy, N., Douay, C., Coelho, V., Cunha-Neto, E., Oshiro, S.E., et al., 2000. Molecular evidence for antigen-driven immune responses in cardiac lesions of rheumatic heart disease patients. *Int. Immunol.* 12 (7), 1063–1074.
- Guilherme, L., Oshiro, S.E., Faé, K.C., Cunha-Neto, E., Renesto, G., Goldberg, A.C., et al., 2001. T-cell reactivity against streptococcal antigens in the periphery mirrors reactivity of heart-infiltrating T lymphocytes in rheumatic heart disease patients. *Infect. Immun.* 69 (9), 5345–5351.
- Guilherme, L., Cury, P., Demarchi, L.M., Coelho, V., Abel, L., Lopez, A.P., et al., 2004. Rheumatic heart disease: proinflammatory cytokines play a role in the progression and maintenance of valvular lesions. *Am. J. Pathol.* 165 (5), 1583–1591.
- Guilherme, L., Postol, E., Freschi de Barros, S., Higa, F., Alencar, R., Lastre, M., et al., 2009. A vaccine against *S. pyogenes*: design and experimental immune response. *Methods* 49 (4), 316–321.
- Guilherme, L., Köhler, K.F., Kalil, J., 2011a. Rheumatic heart disease: mediation by complex immune events. *Adv. Clin. Chem.* 53, 31–50.
- Guilherme, L., Alba, M.P., Ferreira, F.M., Oshiro, S.E., Higa, F., Patarroyo, M.E., et al., 2011b. Anti-group A streptococcal vaccine epitope: structure, stability, and its ability to interact with HLA class II molecules. *J. Biol. Chem.* 286 (9), 6989–6998.
- Hernández-Pacheco, G., Aguilar-García, J., Flores-Domínguez, C., Rodríguez-Pérez, J.M., Pérez-Hernández, N., Alvarez-Leon, E., et al., 2003a. MHC class II alleles in Mexican patients with rheumatic heart disease. *Int. J. Cardiol.* 92 (1), 49–54.
- Hernández-Pacheco, G., Flores-Domínguez, C., Rodríguez-Pérez, J.M., Pérez-Hernández, N., Fragoso, J.M., Saul, A., et al., 2003b. Tumor necrosis factor-alpha promoter polymorphisms in Mexican patients with rheumatic heart disease. *J. Autoimmun.* 21 (1), 59–63.
- Hirsch, E., Irikura, V.M., Paul, S.M., Hirsh, D., 1996. Functions of interleukin 1 receptor antagonist in gene knockout and overproducing mice. *Proc. Natl. Acad. Sci. U.S.A.* 93 (20), 11008–11013.
- Hraoui, M., Boutiba-Ben Boubaker, I., Doloy, A., Ben Redjeb, S., Bouvet, A., 2011. Molecular mechanisms of tetracycline and macrolide resistance and emm characterization of *Streptococcus pyogenes* isolates in Tunisia. *Microb. Drug Resist.* 17 (3), 377–382.
- Imöhl, M., Reinert, R.R., Ocklenburg, C., van der Linden, M., 2010. Epidemiology of invasive *Streptococcus pyogenes* disease in Germany during 2003–2007. *FEMS Immunol. Med. Microbiol.* 58 (3), 389–396.
- Jack, D.L., Klein, N.J., Turner, M.W., 2001. Mannose-binding lectin: targeting the microbial world for complement attack and opsonophagocytosis. *Immunol. Rev.* 180, 86–99.
- Kamal, H., Hussein, G., Hassoba, H., Mosaad, N., Gad, A., Ismail, M., 2010. Transforming growth factor-beta1 gene C-509T and T869C polymorphisms as possible risk factors in rheumatic heart disease in Egypt. *Acta Cardiol.* 65 (2), 177–183.
- Kemeny, E., Grieve, T., Marcus, R., Sareli, P., Zabriskie, J.B., 1989. Identification of mononuclear cells and T cell subsets in rheumatic valvulitis. *Clin. Immunol. Immunopathol.* 52 (2), 225–237.
- Koutouzi, F., Tsakris, A., Chatzichristou, P., Koutouzis, E., Daikos, G.L., Kirikou, E., et al., 2015. *Streptococcus pyogenes* emm types and clusters during a 7-year period (2007 to 2013) in pharyngeal and nonpharyngeal pediatric isolates. *J. Clin. Microbiol.* 53 (7), 2015–2021.
- Koyanagi, T., Koga, Y., Nishi, H., Toshima, H., Sasazuki, T., Imaizumi, T., et al., 1996. DNA typing of HLA class II genes in Japanese patients with rheumatic heart disease. *J. Mol. Cell Cardiol.* 28 (6), 1349–1353.
- Li, Y., Heuser, J.S., Kosanke, S.D., Hemric, M., Cunningham, M.W., 2004. Cryptic epitope identified in rat and human cardiac myosin S2 region induces myocarditis in the Lewis rat. *J. Immunol.* 172 (5), 3225–3234.
- Lindsay, D.S., Brown, A.W., Scott, K.J., Denham, B., Thom, L., Rundell, G., et al., 2016. Circulating emm types of *Streptococcus pyogenes* in Scotland: 2011–2015. *J. Med. Microbiol.* 65 (10), 1229–1231.
- Lopardo, H.A., Vidal, P., Sparo, M., Jeric, P., Centron, D., Facklam, R.R., et al., 2005. Six-month multicenter study on invasive infections due to *Streptococcus pyogenes* and *Streptococcus dysgalactiae* subsp. *equisimilis* in Argentina. *J. Clin. Microbiol.* 43 (2), 802–807.
- Luca-Harari, B., Ekelund, K., van der Linden, M., Staum-Kaltoft, M., Hammerum, A.M., Jasir, A., 2008. Clinical and epidemiological aspects of invasive *Streptococcus pyogenes* infections in Denmark during 2003 and 2004. *J. Clin. Microbiol.* 46 (1), 79–86.
- Lymbury, R.S., Olive, C., Powell, K.A., Good, M.F., Hirst, R.G., LaBrooy, J.T., et al., 2003. Induction of autoimmune valvulitis in Lewis rats following immunization with peptides from the conserved region of group A streptococcal M protein. *J. Autoimmun.* 20 (3), 211–217.
- Ma, Y., Yang, Y., Huang, M., Wang, Y., Chen, Y., Deng, L., et al., 2009. Characterization of emm types and superantigens of *Streptococcus pyogenes* isolates from children during two sampling periods. *Epidemiol. Infect.* 137 (10), 1414–1419.
- Martins, C. e O., Santos, K.S., Ferreira, F.M., Teixeira, P.C., Pomerantzeff, P.M., Brandão, C.M., et al., 2014. Distinct mitral valve proteomic profiles in rheumatic heart disease and myxomatous degeneration. *Clin. Med. Insights Cardiol.* 8, 79–86.
- Meisal, R., Andreasson, I.K., Høiby, E.A., Aaberge, I.S., Michaelsen, T.E., Caugant, D.A., 2010. *Streptococcus pyogenes* isolates causing severe infections in Norway in 2006 to 2007: emm types, multilocus sequence types, and superantigen profiles. *J. Clin. Microbiol.* 48 (3), 842–851.
- Messias Reason, I.J., Schafranski, M.D., Jensenius, J.C., Steffensen, R., 2006. The association between mannose-binding lectin gene polymorphism and rheumatic heart disease. *Hum. Immunol.* 67 (12), 991–998.
- Messias-Reason, I.J., Schafranski, M.D., Kremsner, P.G., Kun, J.F., 2009. Ficolin 2 (FCN2) functional polymorphisms and the risk of rheumatic fever and rheumatic heart disease. *Clin Exp Immunol.* 157 (3), 395–399.
- Mota, C., Aiello, D., Anderson, R., 2009. Chronic rheumatic heart disease. In: Anderson, R., Baker, E., Penny, D. (Eds.), *Pediatric Cardiology*, third ed. Churchill Livingstone/Elsevier, Philadelphia, PA, pp. 1091–1133.
- O'Brien, K.L., Beall, B., Barrett, N.L., Cieslak, P.R., Reingold, A., Farley, M.M., et al., 2002. Epidemiology of invasive group a *streptococcus* disease in the United States, 1995–1999. *Clin. Infect. Dis.* 35 (3), 268–276.
- O'Grady, K.A., Kelpie, L., Andrews, R.M., Curtis, N., Nolan, T.M., Selvaraj, G., et al., 2007. The epidemiology of invasive group A streptococcal disease in Victoria, Australia. *Med. J. Aust.* 186 (11), 565–569.
- Ozkan, M., Carin, M., Sönmez, G., Senocak, M., Ozdemir, M., Yakut, C., 1993. HLA antigens in Turkish race with rheumatic heart disease [see comment]. *Circulation* 87 (6), 1974–1978.

- Patarroyo, M.E., Winchester, R.J., Vejerano, A., Gibofsky, A., Chalem, F., Zabriskie, J.B., et al., 1979. Association of a B-cell alloantigen with susceptibility to rheumatic fever. *Nature* 278 (5700), 173–174.
- Peterson, L., Fujimani, R., 2007. Molecular mimicry. In: Shoenfeld, Y., Gershwin, M., Meroni, P. (Eds.), *Autoantibodies*. Elsevier, Burlington, NJ, pp. 13–19.
- Postol, E., Alencar, R., Higa, F.T., Freschi de Barros, S., Demarchi, L.M., Kalil, J., et al., 2013. StreptInCor: a candidate vaccine epitope against *S. pyogenes* infections induces protection in outbred mice. *PLoS One* 8 (4), e60969.
- Quinn, A., Kosanke, S., Fischetti, V.A., Factor, S.M., Cunningham, M.W., 2001. Induction of autoimmune valvular heart disease by recombinant streptococcal M protein. *Infect. Immun.* 69 (6), 4072–4078.
- Raizada, V., Williams, R.C., Chopra, P., Gopinath, N., Prakash, K., Sharma, K.B., et al., 1983. Tissue distribution of lymphocytes in rheumatic heart valves as defined by monoclonal anti-T cell antibodies. *Am. J. Med.* 74 (1), 90–96.
- Rajapakse, C.N., Halim, K., Al-Orainey, I., Al-Nozha, M., Al-Aska, A.K., 1987. A genetic marker for rheumatic heart disease. *Br. Heart J.* 58 (6), 659–662.
- Ramasawmy, R., Faé, K.C., Spina, G., Victora, G.D., Tanaka, A.C., Palácios, S.A., et al., 2007. Association of polymorphisms within the promoter region of the tumor necrosis factor-alpha with clinical outcomes of rheumatic fever. *Mol. Immunol.* 44 (8), 1873–1878.
- Ramasawmy, R., Spina, G.S., Fae, K.C., Pereira, A.C., Nishihara, R., Messias Reason, I.J., et al., 2008. Association of mannose-binding lectin gene polymorphism but not of mannose-binding serine protease 2 with chronic severe aortic regurgitation of rheumatic etiology. *Clin. Vaccine Immunol.* 15 (6), 932–936.
- Rogers, S., Commons, R., Danchin, M.H., Selvaraj, G., Kelpie, L., Curtis, N., et al., 2007. Strain prevalence, rather than innate virulence potential, is the major factor responsible for an increase in serious group A *streptococcus* infections. *J. Infect. Dis.* 195 (11), 1625–1633.
- Rush, C.M., Govan, B.L., Sikder, S., Williams, N.L., Ketheesan, N., 2014. Animal models to investigate the pathogenesis of rheumatic heart disease. *Front. Pediatr.* 2, 116.
- Sallakci, N., Akcurin, G., Köksoy, S., Kardelen, F., Uguz, A., Coskun, M., et al., 2005. TNF-alpha G-308A polymorphism is associated with rheumatic fever and correlates with increased TNF-alpha production. *J. Autoimmun.* 25 (2), 150–154.
- Seale, A.C., Davies, M.R., Anampiu, K., Morpeth, S.C., Nyongesa, S., Mwarumba, S., et al., 2016. Invasive group A *Streptococcus* infection among children, rural Kenya. *Emerg Infect. Dis.* 22 (2), 224–232.
- Settin, A., Abdel-Hady, H., El-Baz, R., Saber, I., 2007. Gene polymorphisms of TNF-alpha(-308), IL-10(-1082), IL-6(-174), and IL-1Ra(VNTR) related to susceptibility and severity of rheumatic heart disease. *Pediatr. Cardiol.* 28 (5), 363–371.
- Shea, P.R., Ewbanks, A.L., Gonzalez-Lugo, J.H., Martagon-Rosado, A.J., Martinez-Gutierrez, J.C., Rehman, H.A., et al., 2011. Group A *Streptococcus* emm gene types in pharyngeal isolates, Ontario, Canada, 2002–2010. *Emerg Infect. Dis.* 17 (11), 2010–2017.
- Shulman, S.T., Tanz, R.R., Dale, J.B., Beall, B., Kabat, W., Kabat, K., et al., 2009. Seven-year surveillance of North American pediatric group a streptococcal pharyngitis isolates. *Clin. Infect. Dis.* 49 (1), 78–84.
- Smeesters, P.R., Vergison, A., Campos, D., de Aguiar, E., Miendje Deyi, V.Y., Van Melderen, L., 2006. Differences between Belgian and Brazilian group A *Streptococcus* epidemiologic landscape. *PLoS One* 1, e10.
- Smeesters, P.R., McMillan, D.J., Srivastava, K.S., 2010. The streptococcal M protein: a highly versatile molecule. *Trends Microbiol.* 18 (6), 275–282.
- Stanovich, V., Eglite, J., Sochnevs, A., Gardovska, D., Zavadska, D., Shantere, R., 2003. HLA class II associations with rheumatic heart disease among clinically homogeneous patients in children in Latvia. *Arthritis Res. Ther.* 5 (6), R340–346.
- Steer, A.C., Carapetis, J.R., 2009. Prevention and treatment of rheumatic heart disease in the developing world. *Nat. Rev. Cardiol.* 6 (11), 689–698.
- Steer, A.C., Kado, J., Wilson, N., Tuiketei, T., Batzloff, M., Waqatakierewa, L., et al., 2009a. High prevalence of rheumatic heart disease by clinical and echocardiographic screening among children in Fiji. *J. Heart Valve Dis.* 18 (3), 327–335. discussion 336.
- Steer, A.C., Law, I., Matatolu, L., Beall, B.W., Carapetis, J.R., 2009b. Global emm type distribution of group A streptococci: systematic review and implications for vaccine development. *Lancet Infect. Dis.* 9 (10), 611–616.
- Tamayo, E., Montes, M., García-Arenzana, J.M., Pérez-Trallero, E., 2014. *Streptococcus pyogenes* emm-types in northern Spain; population dynamics over a 7-year period. *J. Infect.* 68 (1), 50–57.
- Tanaka, Y., Gotoh, K., Teramachi, M., Ishimoto, K., Tsumura, N., Shindou, S., et al., 2016. Molecular epidemiology, antimicrobial susceptibility, and characterization of macrolide-resistant *Streptococcus pyogenes* in Japan. *J. Infect. Chemother.* 22, 727–732.
- Tapia, M.D., Sow, S.O., Tamboura, B., Keita, M.M., Berthe, A., Samake, M., et al., 2015. *Streptococcal pharyngitis* in schoolchildren in Bamako, Mali. *Pediatr. Infect. Dis. J.* 34 (5), 463–468.
- Tartof, S.Y., Reis, J.N., Andrade, A.N., Ramos, R.T., Reis, M.G., Riley, L.W., 2010. Factors associated with Group A *Streptococcus* emm type diversification in a large urban setting in Brazil: a cross-sectional study. *BMC Infect. Dis.* 10, 327.
- Turner, C.E., Pyzio, M., Song, B., Lamagni, T., Meltzer, M., Chow, J.Y., et al., 2016. Scarlet fever upsurge in England and molecular-genetic analysis in north-west London, 2014. *Emerg Infect. Dis.* 22 (6), 1075–1078.
- Unny, S.K., Middlebrooks, B.L., 1983. Streptococcal rheumatic carditis. *Microbiol Rev* 47 (1), 97–120.
- Visentainer, J.E., Pereira, F.C., Dalalio, M.M., Tsuneto, L.T., Donadio, P.R., Moliterno, R.A., 2000. Association of HLA-DR7 with rheumatic fever in the Brazilian population. *J. Rheumatol.* 27 (6), 1518–1520.
- Volin, M.V., Shahrara, S., 2011. Role of TH-17 cells in rheumatic and other autoimmune diseases. *Rheumatology (Sunnyvale)* 1 (104).
- Weidebach, W., Goldberg, A.C., Chiarella, J.M., Guilherme, L., Snitcowsky, R., Pileggi, F., et al., 1994. HLA class II antigens in rheumatic fever. Analysis of the DR locus by restriction fragment-length polymorphism and oligotyping. *Hum. Immunol.* 40 (4), 253–258.
- Williamson, D.A., Moreland, N.J., Carter, P., Upton, A., Morgan, J., Proft, T., et al., 2014. Molecular epidemiology of group A *streptococcus* from pharyngeal isolates in Auckland, New Zealand, 2013. *N. Z. Med. J.* 127 (1388), 55–60.
- Yegin, O., Coşkun, M., Ertuğ, H., 1997. Cytokines in acute rheumatic fever. *Eur. J. Pediatr.* 156 (1), 25–29.
- Zabriskie, J.B., Lavenchy, D., Williams, R.C., Fu, S.M., Yeadon, C.A., Fotino, M., et al., 1985. Rheumatic fever-associated B cell alloantigens as identified by monoclonal antibodies. *Arthritis Rheum.* 28 (9), 1047–1051.

# Myocarditis and Dilated Cardiomyopathy

Ziya Kaya<sup>1,2</sup>, Patricia Raczek<sup>1,2</sup> and Noel R. Rose<sup>3</sup>

<sup>1</sup>Department of Cardiology, Medical University Hospital Heidelberg, Heidelberg, Germany <sup>2</sup>Germany Centre for Cardiovascular Research, DZHK, Heidelberg, Germany <sup>3</sup>Department of Pathology, Brigham and Women's Hospital/Harvard Medical School, Boston, MA, United States

## OUTLINE

Historical Background	1269	Genetic Features	1276
Myocarditis—Clinical, Pathologic, and Epidemiologic Features	1270	Animal Models	1277
Treatment	1272	Perspectives	1279
Dilated Cardiomyopathy—Clinical, Pathologic, and Epidemiologic Features	1273	Acknowledgments	1279
Treatment	1274	References	1279
Autoimmune Features	1274	Further Reading	1284

## HISTORICAL BACKGROUND

The role of autoimmunity in cardiovascular disease has long been a topic of investigation in the clinic and the laboratory. Years of research effort were devoted to establishing a link between streptococcal infection and rheumatic heart disease based on an autoimmune response (see Chapter 63). Chagas disease is still believed to be based on a cross-reaction of antibodies to *Trypanosoma cruzi* with myocardial or cardiac conductive tissue (Coura and Borges-Pereira, 2012). Finally, postpericardiotomy syndrome and postmyocardial infarction syndrome are sometimes cited as instances of an autoimmune response instigated by damaged or necrotic tissue (Maisch et al., 1979). This chapter reviews the evidence linking autoimmunity with two important forms of heart disease, myocarditis and dilated cardiomyopathy (DCM). It must be stated, ab initio, that immunologic testing has so far not been effective in allowing a clear distinction between autoimmune and other etiologies of these diseases.

The classic description of myocarditis was given by Corvisart in 1812 (referenced in Gravanis and Sternby, 1991), but for many years, progress in studying the disease was impeded by the uncertainties of clinical diagnosis. Definitive diagnosis was dependent upon autopsy examination. Interest in the disease increased in recent years because of the introduction of antemortem diagnostic tools, especially the endomyocardial biopsy (EBM), greater understanding of the role of cardiotropic viruses, and the availability of new modalities of therapy.

## MYOCARDITIS—CLINICAL, PATHOLOGIC, AND EPIDEMIOLOGIC FEATURES

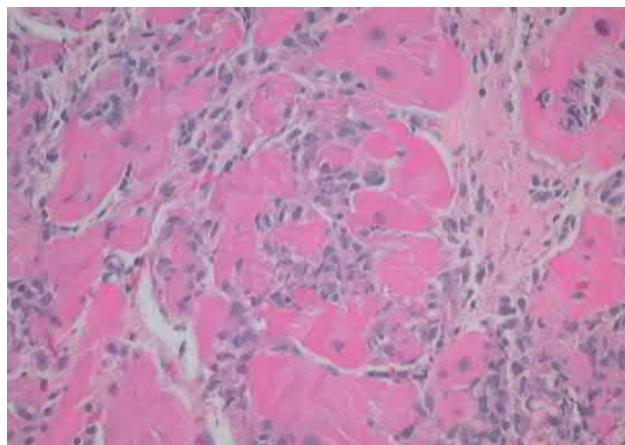
Myocarditis is inflammation of the heart muscle leading to impaired function of the myocardium. The several types of myocarditis can be classified by etiology, histology, immunohistochemistry, clinical pathology, or clinical criteria. Clinical findings can span asymptomatic cases to severe disease with associated arrhythmia and heart failure. Even in the same patient, symptoms vary as the disease progresses. The most serious manifestation of myocarditis is heart failure due to dysfunction of the left ventricle (LV) (Hufnagel et al., 2000). This dysfunction might develop gradually with mild symptoms or rapidly, leading to cardiogenic shock. Supraventricular and/or ventricular arrhythmias are other symptoms linked to myocarditis. Patients might present with palpitations, dizziness, or syncope. Conduction disturbances or serious ventricular arrhythmia suggest giant cell myocarditis, cardiac sarcoidosis, or *Borrelia burgdorferi*–associated myocarditis. Sudden cardiac death in previously healthy young adults can be caused by heart failure or severe arrhythmia. Another major symptom is angina-like chest pain. In these cases, acute coronary syndrome should be ruled out by catheterization. After further exclusion of other pathologies (such as aortic dissection, atrial or ventricular tachycardia, ulcer diseases, or severe anemia), myocarditis should be considered (Baccouche et al., 2009). After initial systolic dysfunction, about half of the cases improve spontaneously or after standard heart failure treatment. Therefore it is recommended to postpone invasive therapeutic interventions, such as the implantation of a cardioverter or defibrillator, for about 3–6 months after onset of the disease or start of treatment. Cases presenting predominantly with heart failure tend to have a worse prognosis than those presenting with arrhythmia or chest pain (Caforio et al., 2007).

The most common cause of myocarditis is viral infection. Patients may recall a recent viral illness with symptoms of malaise, chills and fever, upper respiratory or gastrointestinal symptoms, myalgia, and chest pain. Most cases, however, cannot be traced back to an obvious preceding illness. Adenovirus and enterovirus have mostly been associated with cardiomyocyte infection, and parvovirus B19 attacks cardiac endothelial cells. Today, human herpes virus 6 and HIV are the major pathogens causing myocarditis (Breinholt et al., 2010). Other causes are diverse and include bacteria, protozoa, alcohol, drugs, and toxins. In South and Central America, Chagas disease is a major cause of heart muscle inflammation. The hemoflagellate *T. cruzi* is transmitted to humans via the bite of the reduviid bug triatomine. Most patients initially have only mild, influenza-like symptoms, but 10%–30% of the infected individuals develop fulminant myocarditis.

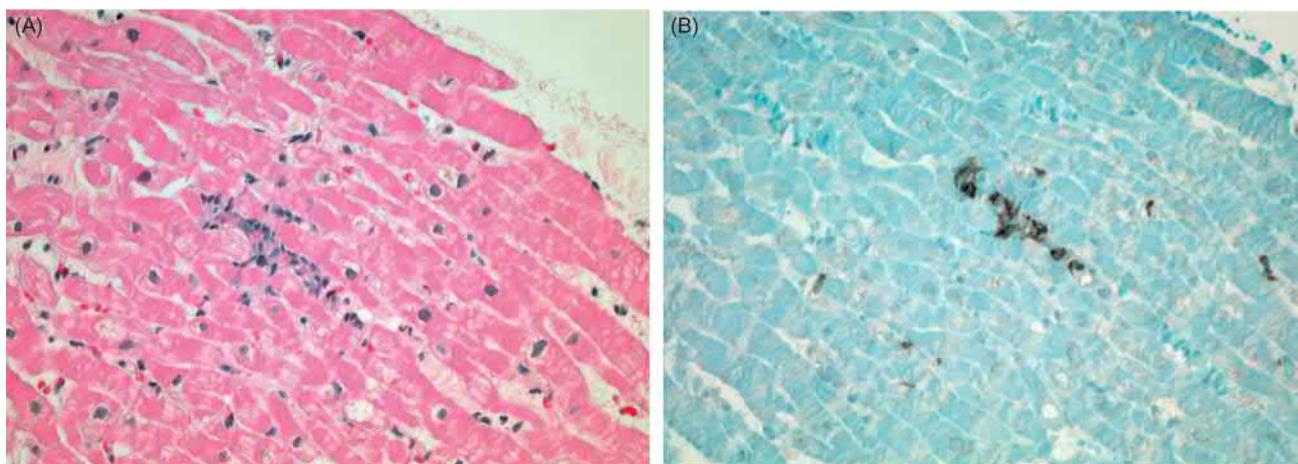
For a long time, conclusive diagnosis of myocarditis was difficult because suitable diagnostic methods were lacking. The diagnosis was supported by the exclusion of other diseases that could explain the symptoms. Today, even though our diagnostic possibilities have expanded and improved, there is still room for further modifications and more specific criteria to diagnose myocarditis. The first indication is clinical symptoms such as chest pain, heart failure, and arrhythmia. Electrocardiogram (ECG) and echocardiography are the basic diagnostic tools for the heart, but they do not provide specific signs for myocarditis. Their value lies more in excluding other causes and in assessing heart function. Even normal findings in a patient do not exclude myocarditis. Echocardiography can help to record disease progression because temporal changes in systolic function, chamber size and thickness can be evaluated regularly (Caforio et al., 2013).

The most important noninvasive method to date is magnetic resonance imaging (MRI). It is helpful in the evaluation of numerous morphological and functional aspects of myocardial impairment and allows a thorough tissue characterization (Friedrich et al., 2009; Olimulder et al., 2009; Bruder et al., 2013; Lurz et al., 2012). Tissue pathologies, such as myocardial edema and hyperemia, capillary leak, necrosis, and fibrosis as well as contraction abnormalities and pericardial effusion can be detected (Kuchynka et al., 2015). The Lake Louise criteria have been proposed to standardize the evaluation of findings in MRI and improve diagnostic accuracy (Friedrich et al., 2009). They combine three different cardiac magnetic resonance techniques and are based on myocardial edema as a sign of acute inflammation, early gadolinium enhancement linked to hyperemia and late gadolinium enhancement linked to increased myocardial necrosis or fibrosis. Late gadolinium enhancement is also an important prognostic factor as well as a significant predictor of cardiovascular mortality (Grün et al., 2012). MRI is, in general, better suited for acute cases with inflammation than chronic cases with less inflammatory activity.

Even though MRI diagnosis has improved, EBM still constitutes the gold standard in the diagnosis of myocarditis, since it is the only method for a definitive diagnosis *in vivo*. It dates to the 1980s, when the Dallas criteria were used as a standardized method to evaluate samples (Aretz et al., 1987). Today, the application of the Dallas criteria has been limited due to low sensitivity and high interobserver variability. Even though endomyocardial biopsy (EMB) is invasive and sampling errors may occur, it offers important information for diagnosis, prognosis, and therapy (Hufnagel et al., 2000; Caforio et al., 2015; Lassner et al., 2014). By immunohistochemistry,



**FIGURE 64.1** Lymphocytic myocarditis. There is a heavy infiltrate of large activated lymphocytes throughout the myocardium. Myocyte necrosis is noted in the middle of this image. Fibrosis is present on the right side of the image. (H&E 400 $\times$ ).

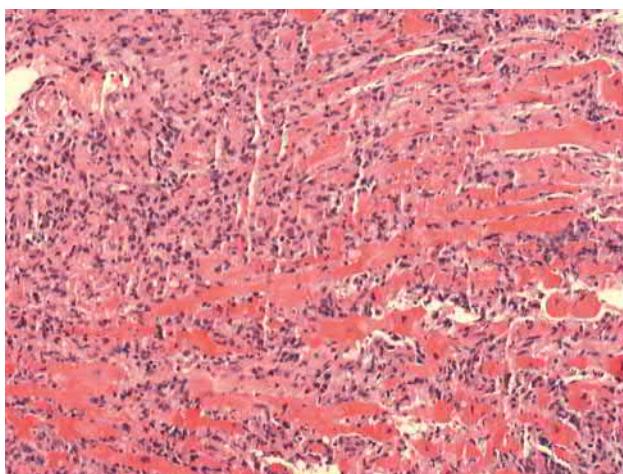


**FIGURE 64.2** Borderline myocarditis. (A) A single cluster of perivascular lymphocytes is present. No myocardial damage is identified. (H&E 400 $\times$ ). (B) A CD8 immunohistochemical stain highlights the infiltrating T lymphocytes. (H&E 400 $\times$ ).

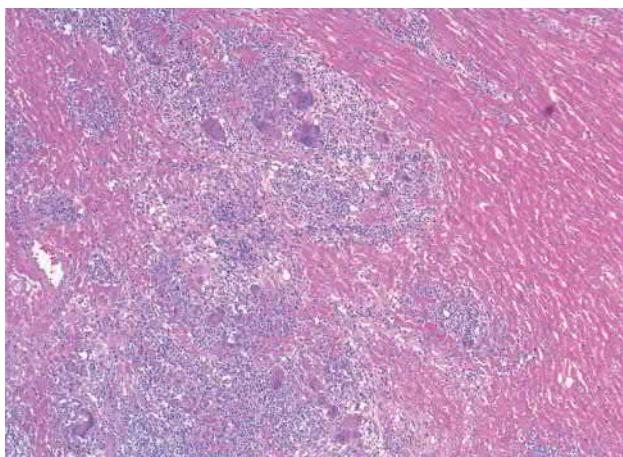
inflammatory cell infiltrates as well as activated immunological processes can be identified and characterized (Maisch and Pankuweit, 2012; Malik et al., 2015; Palecek et al., 2010). EMB allows classification of the types of myocarditis, pathogens can be detected by polymerase chain reaction, and a quantitative assessment of viral load is possible. Perforin-positive cells and human leukocyte antigen (HLA) expression can be evaluated (Figs. 64.1–64.4).

Myocarditis typically has a three-phased progression (Cooper, 2009; Kindermann et al., 2012; Dennert et al., 2008). In the first, or acute phase, viral pathogens enter cardiomyocytes via the coxsackie–adenoviral receptor under participation of the coreceptors (Noutsias et al., 2003). This phase lasts several days to weeks as the virus replicates, and a reaction of nonspecific immunity takes place. Viral and inflammatory mediators lead to myocardial impairment. Patients in this phase are often asymptomatic.

The second phase begins 2–4 weeks after onset of the disease and is characterized by a specific immune reaction with cellular and antibody-mediated response. In this phase the start of an autoimmune reaction is possible. In this reaction, antibodies against heart-specific structures such as myosin or troponin are produced, which cause additional damage to the cardiac tissue. The onset of the third phase after several weeks or months is marked either by an improvement of cardiac function or a deterioration into chronic cardiomyopathy. In between 50% and 70% of the cases the inflammation retreats and LV function increases. If improvement does not occur in this phase, a persistent dysfunction and development of postinflammatory DCM is usually the long-term consequence. Many factors, such as the degree of initial damage, intensity and duration of inflammation, and persistence of viral pathogens influence the course of the disease (Schultheiss et al., 2011; D'Ambrosio et al., 2001).



**FIGURE 64.3 Fulminant myocarditis.** The myocardium is replaced by a marked polymorphous inflammatory infiltrate composed predominantly of lymphocytes and macrophages with rarer eosinophils and neutrophils. Global myocyte injury and loss is noted. No giant cells are present. (H&E 100 $\times$ ).



**FIGURE 64.4 Giant cell myocarditis.** The myocardium is infiltrated by a patchy and diffuse inflammatory infiltrate composed primarily of lymphocytes and macrophages. Multiple collections of giant cells are seen within the infiltrate along with eosinophils. There is significant injury and loss of myocytes in areas of inflammation, while adjacent myocardium is relatively unininvolved. (H&E 50 $\times$ ).

The extent of myocardial damage in the acute phase is one of the most important factors determining the recovery of LV function in later periods (Baccouche et al., 2009; Maisch and Pankuweit, 2012; Cooper and Myocarditis, 2009; Kindermann et al., 2012; Dennert et al., 2008).

Most people do not develop continuing myocarditis after exposure to viruses, which suggests a genetic susceptibility. A higher prevalence in patients with a family history of myocarditis has also been observed, which would seem to support this theory (Hufnagel et al., 2000; Dennert et al., 2008).

## TREATMENT

With the diagnosis of myocarditis, physical activity should be limited for 6 months or until the regression of inflammation and restitution of LV function (Hufnagel et al., 2000). Standard heart failure treatment seems to have a potential positive influence on inflammatory changes and outcome of myocarditis patients and is widely recommended (Bahk et al., 2008; Saegusa et al., 2007; Yuan et al., 2004; Pauschinger et al., 2005). This includes angiotensin-converting enzyme (ACE) inhibitors/angiotensin receptor blockers, beta blockers, and aldosterone antagonists (McMurray et al., 2012; Yancy et al., 2013a,b). However, nonsteroidal anti-inflammatory drugs

(NSAIDs) and digoxin have not been recommended. In critical cases mechanical circulatory support can aid as a temporary solution until the decision between treatment and transplantation is made. Heart transplantation is the last option in severe cases of heart failure.

Devices for treating arrhythmia should be postponed since a significant improvement of LV function and retreat of arrhythmia can often be seen after a few weeks with the regression of myocardial inflammation. The optimal treatment depends on the type of myocarditis present. This is one reason why it is important to have a classification by EMB. For giant cell myocarditis, eosinophilic myocarditis, and cardiac sarcoidosis, immunosuppressive therapy is indicated, but the dose and duration of treatment have not yet been standardized. Lu et al. (2016) have stated that immunosuppressive therapy does not affect mortality or the need for heart transplantation but has a favorable effect on the improvement of LV systolic function. The results of smaller studies still should be verified in larger, multicenter studies. Another approach is treatment with intravenous immunoglobulins and immunoabsorption, but the effect is still disputed.

In the first phase of myocarditis, antiviral treatment is used to stop viral replication and contain damage to cardiomyocytes. Interferon beta treatment seems to be beneficial in the cases of adenovirus and enterovirus infection but not necessarily in other types (Kühl et al., 2003). Specifically for enterovirus, interferon beta therapy may be associated with long-term prognostic benefit (Kühl et al., 2012).

## DILATED CARDIOMYOPATHY—CLINICAL, PATHOLOGIC, AND EPIDEMIOLOGIC FEATURES

DCM is characterized by left ventricular dilation and contractile dysfunction with the absence of abnormal loading conditions and severe coronary artery disease. It is one of the most common causes of heart failure and the most common indication for heart transplant worldwide (Maron et al., 2006). The prevalence is estimated to be around 40 cases per 100,000 individuals with an annual incidence of 7 cases per 100,000 (Maron et al., 2006; Manolio et al., 1992; Taylor et al., 2006). Racial differences have been detected, whereas sex does not seem to make a difference in susceptibility (Manolio et al., 1992; Yancy et al., 2013a,b). About 60% of all childhood cardiomyopathies are the cases of DCM (Liupshultz et al., 2003; Nugent et al., 2003) and infants younger than 12 months have the highest incidence (Nugent et al., 2003; Towbin et al., 2006). The overall mortality is higher in children than in adults; therefore, age is an important risk factor for the deaths of DCM. Symptoms usually include congestive heart failure with excessive sweating, ankle edema, orthopnea, fatigue after mild exertion, palpitations, and syncope. Circulatory collapse, arrhythmia, and thromboembolic events are also possible. There is a risk of sudden death, particularly in infants (Nugent et al., 2003), caused by electromechanical dissociation or ventricular arrhythmia.

There are varying causes for DCM. Genetic mutations in genes for cytoskeletal, sarcomere, nuclear envelope proteins, transcriptional pathways, and mitochondrial proteins make up about 35% of the cases (Grunig et al., 1998; Michels et al., 1992). The inheritance is mostly an autosomal dominant trait (Michels et al., 1992; McNally et al., 2013). Hereditary DCM can be classified in predominantly cardiac types, mutations associated with neuromuscular diseases such as Duchenne and Becker muscular dystrophy or DCM as part of a syndrome. The most common mutations for predominant cardiac phenotypes are in titin and lamin A/C genes (Gerull et al., 2002; Fatkin et al., 1999; Parks et al., 2008).

Acquired causes include myocarditis, alcohol, drugs, and toxins. About 20% of the myocarditis patients develop a chronic DCM while the disease progresses (D'Ambrosio et al., 2001). Alcohol, cocaine, and methamphetamine abuse have a cardiotoxic effect and can lead to DCM. The same applies to anthracycline, a cytostatic used in therapy for cancer, whose cardiotoxicity can occur during treatment or many years afterward. In addition, certain metabolic and endocrine disturbances have been linked to DCM. A special case is peripartum cardiomyopathy. It can occur in the last month of pregnancy or within 5 months of delivery; however, the exact pathologic mechanisms and cause are still unknown (Pearson et al., 2000).

In DCM patients the LV assumes a spherical shape. Hypertrophy and fibrosis restrict the heart function, and ventricular relaxation and filling are reduced. There is a significant decrease in stroke volume and cardiac output. Preload and afterload as well as end-diastolic pressure are increased, resulting in elevated wall stress. Compensatory changes in the vascular system such as an increase in systemic vascular resistance, a decrease in arterial compliance, and an increase in venous pressure will usually occur. These exacerbating circumstances cause secondary neurohormonal changes, for example, an increase in sympathetic adrenergic activity and a

reduction in vagal activity further add to the wall stress and elevate myocardial oxygen demand. Myocytes incur damage, leading to a further reduction in myocardial performance.

Because of its heritability, genetic testing and screening at-risk family members is important in affected families. In patients with DCM, ECG can show nonspecific repolarization abnormalities, left ventricular hypertrophy, pathological Q waves, poor R wave progression, prolongation in the PR interval, AV block, left bundle branch block, or left anterior hemiblock. In cardiac radiography, cardiomegaly and pulmonary venous redistribution may be visible. Like ECG and radiography, echocardiography can detect abnormalities of the heart but none specific for DCM. Global LV hypokinesis is common, sometimes regional wall motion abnormalities, intracardiac thrombi, and functional mitral regurgitation due to annular dilation can occur. Doppler parameters can assist in quantifying the severity of diastolic dysfunction ([Nishimura and Tajik, 1997](#)). MRI is a noninvasive option for examining ventricular volume, wall thickness, contractile function, and tissue characterization.

Histologically, irregular myocyte hypertrophy with or without the areas of fibrosis and myocyte damage can be detected. Lymphocytic infiltrates are the regular markers of inflammation. Furthermore, specific disorders may be identified which can be helpful in choosing the correct treatment. Polymerase chain reaction is a possible method for identifying viral genome in affected patients. Biomarkers such as B-type natriuretic peptide (BNP) and N-terminal BNP play an important prognostic role as their elevation is proportional to the severity of heart failure ([Maisel et al., 2002](#)).

## TREATMENT

The most important goals in the treatment of DCM are the improvement of survival and reduction of hospital admissions. Heart failure guidelines should be applied. Standard first-line drugs are ACE inhibitors and beta blockers. Mineralocorticoid antagonists and the If-channel inhibitor ivabradine provide additional survival benefits when combined with ACE inhibitors and beta blockers ([Pitt et al., 1999](#); [Zannad et al., 2011](#); [Swedberg et al., 2010](#)). Studies have shown that sacubitril–valsartan therapy has better outcomes than treatment with enalapril. Digoxin is useful for patients with sustained atrial fibrillation or refractory heart failure symptoms. To control symptoms loop diuretics are recommended, as well as salt and fluid restriction. Other vasodilatory, natriuretic, and inotropic drugs were tested in clinical trials, but they do not seem to have a positive influence on the outcome ([Chen et al., 2013](#)). New drugs are being tested in ongoing trials. A specific genetic diagnosis might indicate additional or alternative drug therapy. Device therapy can be used for patients with arrhythmia and to prevent sudden death. Implantation of implantable cardioverter defibrillators are recommended for patients at highest risk. Patients with combined DCM and symptomatic bradycardia are eligible for biventricular pacing. Heart transplantation or implantation of long-term mechanical circulatory support such as an extracorporeal membrane oxygenation are to be seen as last measures only. For all patients with DCM an enrollment in a multidisciplinary heart failure service is advised. Its primary function is in educating patients about their disease and giving advice for living with DCM, as well as offering support and monitoring at-risk patients.

## AUTOIMMUNE FEATURES

In recent years, autoimmunity has been accepted as a significant contributing factor in the pathology of inflammatory cardiovascular diseases. Patients suffering from classic autoimmune diseases have a higher risk for developing cardiovascular diseases ([Jastrzebska et al., 2013](#)), which emphasizes the connection between autoimmunity and cardiovascular diseases.

Cardiac autoantibodies have many different points of origin; they can be directed against contractile elements, stress (“heat shock”) proteins, mitochondrial and extracellular matrix antigens, and cardiac receptors. At present, antibodies against cardiac myosin, troponin, the  $\beta_1$ -receptor and muscarinic 2-receptor (M2-receptor) are thought to have the greatest impact in the development of cardiomyopathies ([Satta and Vuilleumier, 2015](#); [Bornholz et al., 2017](#); [Müller et al., 2016b](#); [Caforio et al., 2008a,b](#); [Lappé et al., 2008](#); [Nussinovitch and Shoenfeld, 2010, 2012, 2013a,b](#); [Kaya et al., 2010](#)). They can be divided into two types of autoantibodies. The first ones are classic autoantibodies, which signal an immune response that injures the target tissue. Relevant antibodies are directed against cardiac myosin and cardiac troponin. The second class is “functional autoantibodies.” They are directed against G-protein-coupled receptors (GPCR) and influence receptor-mediated signal cascades by binding to the receptor

and acting as ligands. They thus have an impact on physiological functions similar to the physiologically specific receptor ligand but usually without appropriate controlling feedback mechanisms. In cardiomyopathy patients, GPCR-autoantibodies (GPCR-AABs) are associated primarily with electrical cardiac abnormalities, arrhythmia, ventricular tachycardia, sudden death, and myocarditis (Caforio et al., 2008a; Root-Bernstein and Fairweather, 2015; Wallukat et al., 1992; Chiale et al., 1995; Brisinda et al., 2012; Iwata et al., 2001). Mainly  $\beta_1$ -adrenergic and M2-receptor autoantibodies seem to play an important role in cardiomyopathy.

$\beta_1$ -Autoantibody effects ( $\beta_1$ -AABs) cause the activation of the adenylate cyclase (cAMP increase) (Dandel et al., 2012), activation of protein kinase A (Krause et al., 1996), elongation of the action potential, increase of the L-type calcium ion current (Christ et al., 2001), change of mitochondrial structure and membrane potential (Wang et al., 2013), induction of apoptosis and cell death (Staudt et al., 2003; Jane-wit et al., 2007; Haberland et al., 2011), and maturation and degranulation of cardiac mast cells (Okruhlikova et al., 2007).  $\beta_1$ -AABs also activate the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway. They may contribute to cardiac hypertrophy (Tutor et al., 2007). Changes in T-cell proliferation and secretion have also been observed (Du et al., 2012).

M<sub>2</sub>-Autoantibody effects (M2-AABs) are associated with a negative chronotropic effect and the blocking of cardiac parasympathetic innervation (Wallukat et al., 1999; Goin et al., 1997) by inhibiting the L-type calcium ion channels. This could explain electric abnormalities often observed in the heart in the presence of these antibodies (Lazzerini et al., 2008; Hong et al., 2009; Dobrev et al., 2004). M2-AABs also seem to influence the regulation of COX-2 and iNOS mRNA, hereby possibly contributing to proinflammatory conditions (Ganzinelli et al., 2009).

The first autoantibodies in DCM patients were identified in 1992, with the detection of IgG class antibodies directed against alpha- and beta-myosin heavy chains (Caforio et al., 1992). Similar antibodies were found in some patients with Chagas cardiomyopathy (Ballinas-Vedugo et al., 2003) and women with peripartum cardiomyopathy (Haghikia et al., 2015). Myosin is a motor protein in the contractile apparatus of the cardiomyocyte. It consists of two heavy chains and four light chains. For the heavy chain two isoforms exist, the alpha-myosin heavy chain and beta-myosin heavy chain. The beta-myosin heavy chain is not heart specific; it appears in the heart muscle as well as in the skeletal muscle, whereas alpha-myosin heavy chain can, to our knowledge, only be found in the myocardium. Therefore anti-alpha-myosin autoantibodies are heart specific (Becker et al., 2017). In 2007 antocardiac troponin I autoantibodies were detected in patients with DCM, in the following years also in patients with Chagas cardiomyopathy and peripartum myopathy. In animal models, those antibodies were generated and administered to mice resulting in dilatation and dysfunction of the heart (Okazaki et al., 2003).

Treatment strategies against functional autoantibodies show promising results in early clinical trials. Patients with  $\beta_1$ -AABs showed benefits from immunoabsorption for antibody removal (Dandel et al., 2012). To detect and measure GPCR-AABs, two groups of assays have been applied (Bornholz et al., 2017). The first group (bioassay) uses changes of a second messenger signal in (living) cells that occur after the binding of GPCR-ABs to the receptor. The second method, enzyme-linked immunosorbent assay (ELISA), is used to directly detect GPCR-AABs after binding to epitope mimics. ELISA provides information about the type or amount of antibodies in a sample but cannot measure their functionality. For both assays data about sensitivity and specificity are often unclear and validation and standardization deficient. Therefore numbers for the prevalence of autoantibodies in cardiomyopathy patients and healthy subjects vary from study to study. Antimyosin antibodies were found in up to 5% of healthy individuals (Caforio et al., 2008a; Nussinovitch and Shoenfeld, 2013a,b), whereas DCM patients had a prevalence of 50%–66%. In comparison, patients with ischemic or valvular cardiomyopathy showed the same prevalence of anti-c myosin-AABs as healthy individuals (Caforio et al., 1992; Konstadoulakis et al., 1993). For  $\beta_1$ -AABs and M2-AABs a prevalence of around 10% in healthy individuals is estimated, increasing with age. A coexistence of both antibodies was detected in 65% of healthy subjects (Liu et al., 1999). Different studies delivered a wide range for the presence of  $\beta_1$ -AABs in DCM patients between 26% and 95% (Störk et al., 2006), again with a high coexistence of M2-AABs (Hoebke et al., 1994). Myocarditis patients even had a prevalence of up to 96%. In contrast, ischemic cardiomyopathy patients had a prevalence of  $\beta_1$ -AABs lower than 15% (Nussinovitch and Shoenfeld, 2013a,b; Störk et al., 2006; Nikolaev et al., 2007). Levels of  $\beta_1$ -AABs were correlated with a negative prognosis, higher mortality and increased risk for electric abnormalities and sudden death in some studies (Caforio et al., 2008b; Nussinovitch and Shoenfeld, 2013a,b). However, other studies could not prove this connection. It is unclear if antibody titers can give information about the severity of the disease progression. For the detection of functional  $\beta_1$ -AABs a new screening technology is currently being tested in the Etiology, Titre-Course, and Survival (ETiCS) study on patients with EMB-proven new-onset myocarditis. This assay is called functional fluorescence energy transfer and uses novel cAMP sensors (Nikolaev et al., 2007; Deubner et al., 2010; Beavo and

Brunton, 2002; Staudt et al., 2004). M2-AABs were found in 15%–50% of DCM patients (Nussinovitch and Shoenfeld, 2012). The occurrence of M2-AABs in DCM patients is associated with electric abnormalities (Lazzerini et al., 2008; Lee et al., 2011). The data are based on bioassay and ELISA measurements. About half of the patients with Chagas cardiomyopathy were tested positive for anti-c myosin and anti-c troponin T autoantibodies, but about the same amount of Chagas patients presenting without cardiac symptoms were also positive for these antibodies  $\beta_1$ - and M2-AABs are frequently found in Chagas heart patients (Talvani et al., 2006). A correlation between levels of autoantibodies and severity of the disease was not found.

Because of the high prevalence of autoantibodies in DCM patients, treatment options which target pathogenic autoantibodies are promising. Autoantibodies can be removed from the patients' circulation or attacked and destroyed in vivo. Unselected plasmapheresis (therapeutic plasma exchange, TPE) and apheresis technologies (immunoabsorption) have been proposed. During TPE the patients' blood is transferred to a device that separates plasma and blood cells. The cells are returned to the patients' circulation, and the plasma, which contains the autoantibodies, is replaced by donor plasma or a plasma surrogate. TPE is a common technique in the treatment of autoimmune disorders [Joint United Kingdom (UK) Blood Transfusion and Tissue Transplantation Services Professional Advisory Committee]. In DCM patients TPE was so far only used in case studies. These did, however, show good results. Immunoabsorption also begins with connecting the patients' circulation to a machine that separates plasma and blood cells. The plasma is passed through a column containing ligands that enable the binding of immunoglobulins, specific IgG subclasses, or specific antibodies. The targeted molecules are left behind while the cleared plasma joins the blood cells and is transferred back into the patients' circulation. With this method, specific antibodies can be filtered from the plasma. Immunoabsorption was first applied to DCM patients in 1996 (Wallukat et al., 1996). Of the 8 patients 7 showed benefits and shifted to lower NYHA classes. Follow-up studies demonstrated an increase in LV ejection fraction, cardiac index, and stroke volume index immediately after immunoabsorption (Felix et al., 2000, 2002; Mobini et al., 2003). In addition, lasting positive effects such as increased cardiac function, decreased diastolic diameter, improved echocardiographic and cardio-pulmonary exercise parameters, and improved endothelial function for months or even years following treatment were documented (Reinthalter et al., 2015; Müller et al., 2000; Cooper et al., 2007; Knebel et al., 2004; Staudt et al., 2006a,b; Herda et al., 2010; Bulut et al., 2010, 2011, 2013; Doesch et al., 2009, 2010; Trimpert et al., 2010). For patients who benefited from immunoabsorption a significant increase in regulatory T-cells was observed, which was associated with long-term patient health improvement (Bulut et al., 2010, 2011, 2013). The studies mentioned above focused primarily on  $\beta_1$ -AABs. Subgroup analysis revealed that mostly patients with high levels of autoantibodies benefit from immunoabsorption therapy (Dandel et al., 2012). Therefore it is important in clinical practice to differentiate between cardiomyopathy patients who tested positive or negative for cardiac autoantibodies to make adequate therapy decisions. Immunoabsorption is a relatively expensive procedure, but worth the gain in patient benefits and survival rate. New therapeutic possibilities that directly attack and neutralize autoantibodies in vivo are being tested. Methods such as intravenous IgG treatment, B-cell depletion and aptamer-based neutralization of GPCRs might offer more therapy options for DCM patients in the future.

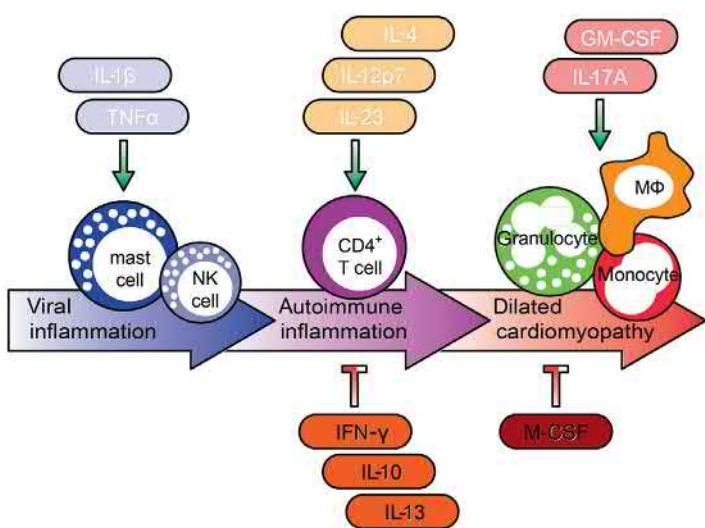
## GENETIC FEATURES

Because of the possible autoimmune origin of myocarditis and DCM in humans, and the well-documented association of experimental myocarditis with the major histocompatibility complex (MHC) in mice (Rose et al., 1988), a number of studies to determine the relationship with the human MHC (HLA) have been carried out. Anderson et al. (1984) reported that DCM patients had an increased frequency of HLA-DR4 and a decreased frequency of HLA-DR6. These findings were corroborated by Limas et al. (1990), who also demonstrated an increased frequency of HLA-DR4 in DCM patients. A genetic predisposition toward cardiac autoimmunity was demonstrated, in that 72% of HLA-DR4 + patients had anti- $\beta_1$  adrenergic receptor antibodies compared with 21% of HLA-DR4 – patients. In the largest study to date, Carlquist et al. (1991) reconfirmed these findings and also found that the DR4-DQw4 haplotype conferred heightened risk of disease. In a metaanalysis of five studies, they confirmed that the DR4 association with myocarditis was sustained among different patient populations. No differences in disease phenotypes have been reported. The availability of EBMs has allowed genetic studies of CVB3 infection in viral myocarditis in humans. Tschopp et al. (2017) reported that non-obese diabetic 2 (NOD2), a nucleotide-binding domain, mediates viral uptake and inflammation in the heart.

## ANIMAL MODELS

Since enteroviruses are often implicated in human myocarditis and DCM, these agents have been widely used to investigate the pathogenic mechanisms. Although infections by CVB3 are relatively common, the development of clinically significant, ongoing myocardial disease in humans is relatively uncommon, suggesting that differences in host response play a critical role in disease susceptibility. These differences are likely to be genetically determined and may relate to the expression of virus-specific receptor on heart tissue or to the immune response of the host. Because it is difficult to examine the role that genetic polymorphisms play in humans, investigators have developed models of coxsackievirus-induced myocarditis in mice, for which many genetically different, inbred strains are available. A model of the time course of viral myocarditis is illustrated in Fig. 64.5.

All strains of mice tested developed acute myocarditis starting 2 or 3 days after CVB3 infection. Viral disease reached its peak on day 7 and gradually resolved so that by day 21 the heart was histologically normal. No infectious virus was found after day 9. In a few strains of mice, however, the myocarditis persisted (Rose et al., 1987; Cihakova and Rose, 2008), but the histologic picture shifted. The first phase was characterized by the focal necrosis of myocytes and accompanying a focal acute inflammatory response with a mixed cell infiltrate consisting of polymorphonuclear and mononuclear cells. In those mice that developed the second phase of disease, the inflammatory process was diffuse rather than focal and consisted mainly of mononuclear interstitial infiltrates, including both T- and B-lymphocytes and little or no myocyte necrosis. In the mice that developed the second phase of disease, heart-reactive autoantibodies were present and shown to be specific for the cardiac isoform of myosin (Neu et al., 1987a). This finding suggested that the second phase represented an autoimmune response initiated by the viral infection. Direct evidence to support this hypothesis was produced by immunizing the susceptible strains of mice with purified cardiac myosin and showing that they developed a very similar histologic picture of myocarditis (Neu et al., 1987b). No heart disease was found in animals immunized in a similar manner with skeletal myosin, and none appeared in the strains of mice that were not genetically susceptible to the second phase of virus-induced myocarditis. Further evidence that the disease was due to an immune response to cardiac myosin was assembled by inducing specific tolerance to cardiac myosin (Wang et al., 2000; Fousteri et al., 2011). The finding first suggested that the second phase represents an autoimmune response initiated by molecular mimicry between viral and heart antigens (Cunningham et al., 2004). Other evidence showed that the autoimmune response depends upon virus-induced damage to the heart. (Horwitz et al., 2000) found that transgenic mice expressing interferon gamma (IFN- $\gamma$ ) in their pancreatic cells failed to produce CVB3-induced myocarditis, even though the virus proliferated greatly in other sites. The virus infection may serve as an adjuvant for cardiac antigens that have been expressed or liberated during the viral infection of the heart (Rose, 2000). Other viruses unrelated to coxsackieviruses such as cytomegalovirus produce a similar autoimmune myocarditis following infection. The experiments showing that myocarditis can be produced by immunization with cardiac myosin in animals with no viral infection establish that the disease does not depend upon persisting virus even though it is possible to demonstrate traces of viral RNA.



**FIGURE 64.5** Schematic of the pathogenesis of viral myocarditis.

Unless subjected to exercise stress, most mice survived autoimmune myocarditis whether induced by virus infection or by immunization with cardiac myosin. Gradually the disease waned in severity (Cihakova and Rose, 2008; Rose and Hill, 1996). In some mice, however, the histologic picture changed to produce a mainly fibrotic disease. As the process continued, there was a thinning of the ventricular cell walls and a large increase in size of the left ventricle. By day 35, after infection or immunization, there were definite signs of cardiac insufficiency and by day 60, most of the animals died of heart failure. This form of the disease, whether induced by viral infection or myosin autoimmunity, replicated the major characteristics of DCM, suggesting that DCM can represent an end stage of autoimmune myocarditis.

A striking finding in the investigations described above was that strains of mice highly susceptible to the autoimmune myocarditis following viral infection were susceptible to the myosin-induced disease. Other strains were resistant to both forms of myocarditis. These observations indicated that the susceptibility to autoimmune myocarditis was under a large measure of genetic control. As in most autoimmune diseases, genes of the MHC have an important influence on the development and course of autoimmune disease (Li et al., 2008a). In both the viral and myosin-induced models the H-2s, H-2a, and H-2b alleles were associated with increased morbidity of autoimmune myocarditis, whereas other H-2 alleles were associated with low susceptibility. The genetic findings in the mouse can be related to human myocarditis through experiments by Hayward et al. (2006) and Taneja and David (2009), who demonstrated a spontaneous myocarditis model in NOD mice carrying the transgenically introduced human HLA-DQ8 allele associated with greater susceptibility to human DCM. Like most autoimmune diseases, non-MHC genes play a determining role in susceptibility to myocarditis. Genome-wide linkage analysis revealed at least two prominent loci that had significant effects on susceptibility to autoimmune myocarditis. A putative susceptibility gene, eam1, was located on the proximal end of chromosome 1 and eam2 on the distal region of chromosome 6 (Guler et al., 2005; Li et al., 2008b). Both chromosomal segments bore genes determining susceptibility to a number of other autoimmune diseases such as autoimmune encephalomyelitis and autoimmune arthritis as well as spontaneous diabetes.

In addition to lending themselves to genetic studies the experimental models of autoimmune myocarditis provide the opportunity of following the inflammatory process from the beginning to the end (Rose, 2011). The first major question to be considered was the basis of the susceptibility to autoimmune myocarditis following the virus infection. Studies show that two critical cytokines, IL-1 $\beta$  and TNF- $\alpha$ , were both necessary and sufficient for the progression from viral myocarditis to autoimmune myocarditis (Lane 1992). Blocking either one of these two cytokines prevented the transition from viral to autoimmune myocarditis. Even more significant was the demonstration that providing either of these two cytokines in recombinant form to genetically resistant mice caused them to develop the autoimmune myocarditis. Early signs of susceptibility to autoimmune myocarditis become evident early in the course of viral infection. Significantly, the elevations of IL-1 $\beta$  were found as early as 8 hours after viral infection (Fairweather et al., 2004a; Fairweather et al., 2004b). The innate immune response to the virus determined later susceptibility to autoimmune disease. Adoptive transfer experiments using myosin or myosin peptide-induced disease have shown that the induction of autoimmune myocarditis depends upon myosin-specific CD4 T-cells (Smith and Allen, 1991; Chen et al., 2012; Li et al., 2008b).

The course of the inflammation during autoimmune myocarditis can be traced to the relative proportions of certain key cytokines emanating from different CD4 T-cell families. Differing forms of autoimmune myocarditis are associated with greater production of IL-12p35 P40 (a Th1 signal), IL-4 (a Th2 signal), and IL-23p19p40 (a signal of the Th17 response) (Rose, 2011). For example, IFN- $\gamma$ , a signature of Th1 responses, retards the development of myocarditis, and its deficiency produces a particularly severe form lymphocytic disease. A rapidly developing, fatal form of eosinophilic myocarditis occurs in the absence of both IFN- $\gamma$  and IL-17A (Barin et al., 2013). These findings are striking examples of the cytokine "interactome"; they further suggest that a balance of cytokines affects not only the severity but the profile of inflammation. IL-4 promotes a particularly severe form of giant-cell myocarditis in mice, and eosinophil-derived IL-4 drives progression of myocarditis to DCM (Diny et al., 2017). IL-17A, a cytokine associated with neutrophilic inflammation, has little impact on the severity of overall inflammation in the myosin-induced autoimmune disease. It is, however, critical for the later progression to DCM; animals deprived of IL-17A fail to develop postmyocarditis cardiac remodeling and the subsequent fibrotic disease (Baldeviano et al., 2010). The disease can be prevented by administering antibody to IL-17A earlier during inflammation. This key role of IL-17 may be related to its established ability to increase granulocyte proliferation as well as to activate macrophages. Wu et al. (2014) found that IL-17A stimulates cardiac fibroblasts to produce GM-CSF, which, in turns, stimulates both leukocytes and Ly6Chigh-bearing monocytes/macrophages. Similar studies in humans supported the rationale for targeting Th-17-related cytokines for the treatment of DCM (Myers et al., 2016). An issue critical to understanding the pathogenesis of autoimmune myocarditis is the dynamic

balance of mediators tending to favor inflammation and cardiomyocyte injury with mediators that reduce or retard inflammation.

As mentioned previously, other cardiac-specific antigens can induce autoimmune myocarditis. Goser et al. (2006) demonstrated the provocation of an autoimmune response to cardiac troponin I, which induces severe inflammation in the myocardium of mice followed by fibrosis and heart failure, with marked mortality. These investigators identified two sequence motifs of cardiac troponin I that induced inflammation and fibrosis in the myocardium (Kaya et al., 2008). Interestingly, these same animals eventually developed immunity to cardiac myosin following at least 90 days of inflammation. Thus autoimmune myocarditis, like most autoimmune diseases, is characterized by the production of multiple organ-specific autoantibodies.

## PERSPECTIVES

When the first edition of "The Autoimmune Diseases" was published in 1985, research on the inflammatory cardiopathologies was taking on new life. Cardiac transplantation and EBM focused greater attention on the details of histopathology of the heart muscle and years of study of cardiotropic coxsackievirus melded into an opportunity to appraise a recurrent question in the investigations of many autoimmune diseases: how could a viral infection trigger an autoimmune disorder? Myocarditis has proven to be an accessible model for studying the question. The disease can be reasonably replicated in the mouse with the candidate virus, and viral peptides were defined that tracked the course of virus-induced myocarditis. The door was opened to trace the inflammatory process with respect to both cellular composition and mediator balance from initiation of the immune response to heart failure. Together, these studies are leading to promise of earlier diagnosis and more specific therapies. The lessons learned may well be applied to other immune-mediated disorders.

## Acknowledgments

The figures were prepared by Dr. Marc Halushka and Dr. Jobert Barin, Department of Pathology, Johns Hopkins University.

## References

- Anderson, J.L., Carlquist, J.F., Lutz, J.R., DeWitt, C.W., Hammond, E.H., 1984. HLA A, B and DR typing in idiopathic dilated cardiomyopathy: a search for immune response factors. *Am. J. Cardiol.* 53, 473–487.
- Aretz, H.T., Billingham, M.E., Edwards, W.D., et al., 1987. Myocarditis. A histopathologic definition and classification. *Am. J. Cardiovasc. Pathol.* 1 (1), 3–14.
- Baccouche, H., Mahrholdt, H., Meinhardt, G., et al., 2009. Diagnostic synergy of non-invasive cardiovascular magnetic resonance and invasive endomyocardial biopsy in troponin-positive patients without coronary artery disease. *Eur. Heart J.* 30 (23), 2869–2879.
- Bahk, T.J., Daniels, M.D., Leon, J.S., Wang, K., Engman, D.M., 2008. Comparison of angiotensin converting enzyme inhibition and angiotensin II receptor blockade for the prevention of experimental autoimmune myocarditis. *Int. J. Cardiol.* 125 (1), 85–93.
- Baldeviano, G.C., Barin, J.G., Talor, M.V., Srinivasan, S., Bedja, D., Zheng, D., 2010. Interleukin-17A is dispensable for myocarditis but essential for the progression to dilated cardiomyopathy. *Circ. Res.* 106 (10), 1646–1655.
- Ballinas-Vedugo, M.A., Alejandro-Aguilar, R., Aranda-Frausto, A., Reyes, P.A., Monteon, V.M., 2003. Anti-myosin autoantibodies are more frequent in non-chagasic cardiomyopathy than in chagasic cardiomyopathy patients. *Int. J. Cardiol.* 92, 101–102.
- Barin, J.G., et al., 2013. Fatal eosinophilic myocarditis develops in the absence of IFN- and IL-17A. *J. Immunol.* 191 (8), 4038–4047.
- Beavo, J.A., Brunton, L.L., 2002. Epac-a directly cAMP-activated exchange protein. *Nat. Rev. Mol. Cell. Biol.* 3, 710–718.
- Becker, N., Müller, J., Göttel, P., Wallukat, G., Schimke, I., 2017. Cardiomyopathy—an approach to the autoimmune background. *Autoimmun. Rev.* 16 (3), 269–286.
- Bornholz, B., Wallukat, G., Roggenbuck, D., Schimke, I., 2017. Autoantibodies against G-protein-coupled receptors in cardiovascular diseases: basics and diagnostics. In: Nussinovitch, U. (Ed.), *The Heart in Rheumatologic, Inflammatory and Autoimmune Diseases: Pathophysiology, Clinical Aspects and Therapeutic Approaches*. Elsevier.
- Breinholt, J.P., Moulik, M., Dreyer, W.J., et al., 2010. Viral epidemiologic shift in inflammatory heart disease: the increasing involvement of parvovirus B19 in the myocardium of pediatric cardiac transplant patients. *J. Heart Lung Transplant.* 29 (7), 739–746.
- Brisinda, D., Sorbo, A.R., Venuti, A., Ruggieri, M.P., Manna, R., Fenici, P., et al., 2012. Anti-β-adrenoceptor autoimmunity causing idiopathic arrhythmias and cardiomyopathy. *Circ. J.* 76, 1345–1353.
- Bruder, O., Wagner, A., Lombardi, M., et al., 2013. European cardiovascular magnetic resonance (EuroCMR) registry—multi national results from 57 centers in 15 countries. *J. Cardiovasc. Magn. Reson.* 15, article 9.
- Bulut, D., Scheeler, M., Wichmann, T., Börgel, J., Miebach, T., Mügge, A., 2010. Effect of protein A immunoabsorption on T cell activation in patients with inflammatory dilated cardiomyopathy. *Clin. Res. Cardiol.* 99, 633–638.
- Bulut, D., Scheeler, M., Niedballa, L.M., Miebach, T., Mügge, A., 2011. Effects of immunoabsorption on endothelial function, circulating endothelial progenitor cells and circulating microparticles in patients with inflammatory dilated cardiomyopathy. *Clin. Res. Cardiol.* 100, 603–610.

- Bulut, D., Creutzberg, G., Mügge, A., 2013. The number of regulatory T cells correlates with hemodynamic improvement in patients with inflammatory dilated cardiomyopathy after immunoabsorption therapy. *Scand. J. Immunol.* 77, 54–61.
- Caforio, A.L., Grazzini, M., Mann, J.M., Keeling, P.J., Bottazzo, G.F., McKenna, W.J., et al., 1992. Identification of alpha- and beta-cardiac myosin heavy chain isoforms as major autoantigens in dilated cardiomyopathy. *Circulation* 85, 1734–1742.
- Caforio, L.P., Calabrese, F., Angelini, A., et al., 2007. A prospective study of biopsy-proven myocarditis: prognostic relevance of clinical and aetiopathogenetic features at diagnosis. *Eur. Heart J.* 28 (11), 1326–1333.
- Caforio, A.L., Tona, F., Bottaro, S., Vinci, A., Dequal, G., Daliento, L., et al., 2008a. Clinical implications of anti-heart autoantibodies in myocarditis and dilated cardiomyopathy. *Autoimmunity* 41, 35–45.
- Caforio, A.L., Vinci, A., Iliceto, S., 2008b. Anti-heart autoantibodies in familial dilated cardiomyopathy. *Autoimmunity* 41, 462–469.
- Caforio, A.L.P., Marcolongo, R., Jahns, R., et al., 2013. Immune-mediated and autoimmune myocarditis: clinical presentation, diagnosis and management. *Heart Fail. Rev.* 18, 715. Available from: <https://doi.org/10.1007/s10741-012-9364-5>.
- Caforio, L.P., Marcolongo, R., Bassi, C., Iliceto, S., 2015. Clinical presentation and diagnosis of myocarditis. *Heart* 101 (16), 1332–1344.
- Carlquist, J.E., Menlove, Murray, M.B., O'Connell, J.R., Anderson, J.L., 1991. VILA class II (DR and DQ) antigen associations in idiopathic dilated cardiomyopathy. *Circulation* 83, 515–522.
- Chen, P., Baldeviano, G.C., Ligons, D.L., Talor, M.V., Barin, J.G., Rose, N.R., et al., 2012. Susceptibility to autoimmune myocarditis is associated with intrinsic differences in CD4(1) T cells. *Clin. Exp. Immunol.* 169, 79–88.
- Chen, H.H., Anstrom, K.J., Givertz, M.M., et al., 2013. Low-dose dopamine or low-dose nesiritide in acute heart failure with renal dysfunction: the ROSE acute heart failure randomized trial. *JAMA* 310, 2533–2543.
- Chiale, P.A., Rosenbaum, M.B., Elizari, M.V., Hjalmarson, Å., Magnusson, Y., Wallukat, G., et al., 1995. High prevalence of antibodies against beta1- and beta2-adrenoceptors in patients with primary electrical abnormalities. *J. Am. Coll. Cardiol.* 26, 864–869.
- Christ, T., Wetwer, E., Dobrew, D., Adolph, E., KnautM, Wallukat, G., et al., 2001. Autoantibodies against the beta1 adrenoceptor from patients with dilated cardiomyopathy prolong action potential duration and enhance contractility in isolated cardiomyocytes. *J. Mol. Cell. Cardiol.* 33, 1280–1287.
- Cihakova, D., Rose, N.R., 2008. Pathogenesis of myocarditis and dilated cardiomyopathy. *Adv. Immunol.* 99, 95–114.
- Cooper Jr., L.T., Myocarditis, 2009. *N. Engl. J. Med.* 360 (15), 1526–1538.
- Cooper, L.T., Belohlavek, M., Korinek, J., Yoshifuku, S., Sengupta, P.P., Burgstaler, E.A., et al., 2007. A pilot study to assess the use of protein A immunoabsorption for chronic dilated cardiomyopathy. *J. Clin. Apher.* 224, 210–214.
- Coura, J.R., Borges-Pereira, J., 2012. Chagas disease: what is known and what should be improved: a systemic review. *Rev. Soc. Bras. Med. Trop.* 45, 286–296.
- Cunningham, M.W., 2004. T cell mimicry in inflammatory heart disease. *Mol. Immunol.* 40, 1121–1127.
- D'Ambrosio, A., Patti, G., Manzoli, A., et al., 2001. The fate of acute myocarditis between spontaneous improvement and evolution to dilated cardiomyopathy: a review. *Heart* 85, 499–504.
- Dandel, M., Wallukat, G., Englert, A., Lehmkuhl, H.B., Knosalla, C., Hetzer, R., 2012. Longterm benefits of immunoabsorption in β(1)-adrenoceptor autoantibody-positive transplant candidates with dilated cardiomyopathy. *Eur. J. Heart Fail.* 14, 1374–1388.
- Dennert, R., Crijns, H.J., Heymans, S., 2008. Acute viral myocarditis. *Eur. Heart J.* 29 (17), 2073–2082.
- Deubner, N., Berliner, D., Schlippe, A., Gelbrich, G., Caforio, A.L.P., Felix, S.B., et al., 2010. Cardiac beta1-adrenoceptor autoantibodies in human heart disease: rationale and design of the etiology, titre-course, and survival (ETiCS) Study—on behalf of the ETiCS-study group. *Eur. J. Heart Fail.* 12, 753–762.
- Diny, N.L., Baldeviano, G.C., Talor, M.V., Barin, J.G., Ong, S., Bedja, D., et al., 2017. Eosinophil-derived IL-4 drives progression of myocarditis to inflammatory dilated cardiomyopathy. *J. Exp. Med.* 214 (4), 943–957.
- Dobrev, D., Christ, T., Ravens, U., 2004. Muscarinic subtype-2 receptor autoantibodies: actors or bystanders in human atrial fibrillation? *Eur. Heart J.* 25, 1091–1092.
- Doesch, A.O., Konstandin, M., Celik, S., Kristen, A., Frankenstein, L., Hardt, S., et al., 2009. Effects of protein A immunoabsorption in patients with advanced chronic dilated cardiomyopathy. *J. Clin. Apher.* 2009 (24), 141–149.
- Doesch, A.O., Mueller, S., Konstandin, M., Celik, S., Kristen, A., Frankenstein, L., et al., 2010. Effects of protein A immunoabsorption in patients with chronic dilated cardiomyopathy. *J. Clin. Apher.* 25, 315–322.
- Du, Y., Yan, L., Wang, J., Zhan, W., Song, K., Han, X., et al., 2012. β1-Adrenoceptor autoantibodies from DCM patients enhance the proliferation of T lymphocytes through the β1-AR/cAMP/PKA and p38 MAPK pathways. *PLoS One* 7, e52911.
- Fairweather, D., Frisancho-Kiss, S., Gatewood, S., Njoku, D., Steele, R., Barrett, M., et al., 2004a. Mast cells and innate cytokines are associated with susceptibility to autoimmune heart disease following coxsackievirus B3 infection. *Autoimmunity* 37, 131–145.
- Fairweather, D., Afanasyeva, M., Rose, N.R., 2004b. Cellular immunity: a role for cytokines. In: Doria, A., Pauletti, P. (Eds.), *Handbook of Systemic Autoimmune Diseases, 1: The Heart in Systemic Autoimmune Diseases*. Elsevier, Amsterdam, pp. 3–17.
- Fatkin, D., MacRae, C., Sasaki, T., et al., 1999. Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease. *N. Engl. J. Med.* 341, 1715–1724.
- Felix, S.B., Staudt, A., Dorffel, W.V., Stangl, V., Merkel, K., Pohl, M., et al., 2000. Hemodynamic effects of immunoabsorption and subsequent immunoglobulin substitution in dilated cardiomyopathy: three-month results from a randomized study. *J. Am. Coll. Cardiol.* 35, 1590–1598.
- Felix, S.B., Staudt, A., Landsberger, M., Grosse, Y., Stangl, V., Spielhagen, T., et al., 2002. Removal of cardiotropic antibodies in dilated cardiomyopathy by immunoabsorption. *J. Am. Coll. Cardiol.* 20 (39), 646–652.
- Fousteri, G., Dave, A., Morin, B., Omid, S., Croft, M., von Herrath, M.G., 2011. Nasal cardiac myosin peptide treatment and OX40 blockade protect mice from acute and chronic virally-induced myocarditis. *J. Autoimmun.* 36, 210–220.
- Friedrich, M.G., Sechtem, U., Schulz-Menger, J., et al., 2009. Cardiovascular magnetic resonance in myocarditis: a JACC White Paper. *J. Am. Coll. Cardiol.* 53 (17), 1475–1487.
- Fu, M.L., Hoebelke, J., Matsui, S., Matoba, M., Magnusson, Y., Hedner, T., 1994. Autoantibodies against cardiac G-protein-coupled receptors define different populations with cardiomyopathies but not with hypertension. *Clin. Immunol. Immunopathol.* 72 (1), 15–20.

- Ganzinelli, S., Borda, E., Joensen, L., Sterin-Borda, L., 2009. Chagasic antibodies induce cardiac COX-2/iNOS mRNA expression with PGE2/NO production. *Int. J. Cardiol.* 134, 212–223.
- Gerull, B., Gramlich, M., Atherton, J., et al., 2002. Mutations of TTN, encoding the giant muscle filament titin, cause familial dilated cardiomyopathy. *Nat. Genet.* 30, 201–204.
- Goin, J.C., Leiros, C.P., Sterin-Borda, L., 1997. Interaction of human chagasic IgG with the second extracellular loop of the human heart muscarinic acetylcholine receptor: functional and pathological implications. *FASEB J.* 11, 77–83.
- Goser, S., Andrassy, M., Buss, S.J., Leuschner, F., Volz, C.H., Ottl, R., et al., 2006. Cardiac troponin I but not cardiac troponin T induces severe autoimmune inflammation in the myocardium. *Circulation* 114, 1693–1702.
- Gravanis, M.B., Sternby, N.H., 1991. Incidence of myocarditis: a 10-year autopsy study from Malmö, Sweden. *Arch. Pathol. Lab. Med.* 115, 390–392.
- Grün, S., Schumm, J., Greulich, S., et al., 2012. Long-term follow-up of biopsy-proven viral myocarditis: predictors of mortality and incomplete recovery. *J. Am. Coll. Cardiol.* 59 (18), 1604–1615.
- Grunig, E., Tasman, J.A., Kucherer, H., Franz, W., Kubler, W., Katus, H.A., 1998. Frequency and phenotypes of familial dilated cardiomyopathy. *J. Am. Coll. Cardiol.* 31, 186–194.
- Guler, M.L., et al., 2005. Two autoimmune diabetes loci influencing T cell apoptosis control susceptibility to experimental autoimmune myocarditis. *J. Immunol.* 174 (4), 2167–2173.
- Haberland, A., Wallukat, G., Dahmen, C., Kage, A., Schimke, I., 2011. Aptamer neutralization of beta1-adrenoceptor autoantibodies isolated from patients with cardiomyopathies. *Circ. Res.* 109, 986–992.
- Haghikia, A., Kaya, Z., Schwab, J., Westenfeld, R., Ehlermann, P., Bachelier, K., et al., 2015. Evidence of autoantibodies against cardiac troponin I and sarcomeric myosin in peripartum cardiomyopathy. *Basic Res. Cardiol.* 110 (6), 60. Available from: <https://doi.org/10.1007/s00395-015-00517-2> [Epub 2015 Oct 30].
- Hayward, S.L., Bautista-Lopez, N., Suzuki, K., Atrazhev, A., Dickie, P., Elliott, J.F., 2006. CD4 T cells play major effector role and CD8 T cells initiating role in spontaneous autoimmune myocarditis of HLA-DQ8 transgenic IAB knockout nonobese diabetic mice. *J. Immunol.* 176, 7715–7725.
- Herda, L.R., Trimpert, C., Nauke, U., Landsberger, M., Hummel, A., Beug, D., et al., 2010. Effects of immunoabsorption and subsequent immunoglobulin G substitution on cardiopulmonary exercise capacity in patients with dilated cardiomyopathy. *Am. Heart J.* 159, 809–816.
- Hong, C.M., Zheng, Q.S., Liu, X.T., Shang, F.J., Wang, H.T., Jiang, W.R., 2009. Effects of autoantibodies against M2 muscarinic acetylcholine receptors on rabbit atria in vivo. *Cardiology* 112, 180–187.
- Horwitz, M.S., La Cava, A., Fine, C., Rodriguez, E., Ilic, A., Sarvetnick, N., 2000. Pancreatic expression of interferon-gamma protects mice from lethal coxsackievirus B3 infection and subsequent myocarditis. *Nat. Med.* 6, 693–697.
- Hufnagel, G., Pankweit, S., Richter, A., Schönian, U., Maisch, B., 2000. The European Study of Epidemiology and Treatment of Cardiac Inflammatory Diseases (ESETCID): first epidemiological results. *Herz* 25 (3), 279–285.
- Iwata, M., Yoshikawa, T., Baba, A., Anzai, T., Mitamura, H., Ogawa, S., 2001. Autoantibodies against the second extracellular loop of the β1-adrenergic receptor predict ventricular tachycardia and sudden death in patients with idiopathic dilated cardiomyopathy. *J. Am. Coll. Cardiol.* 37, 418–424.
- Jane-wit, D., Alfuntas, C.Z., Johnson, J.M., Yong, S., Wickley, P.J., Wang, C., et al., 2007. Beta1-adrenergic receptor autoantibodies mediate dilated cardiomyopathy by agonistically inducing cardiomyocyte apoptosis. *Circulation* 116, 399–410.
- Jastrzebska, M., Czok, M.E., Guzik, P., 2013. Autoimmune diseases, their pharmacological treatment and the cardiovascular system. *Cardiol. J.* 20, 569–576.
- Joint United Kingdom (UK) Blood Transfusion and Tissue Transplantation Services Professional Advisory Committee. <<http://www.transfusionguidelines.org.uk/transfusion-handbook/11-therapeutic-apheresis/11-1-therapeutic-plasma-exchange-tpe>>.
- Kaya, Z., Goser, S., Buss, S.J., Leuschner, F., Ottl, R., Li, J., et al., 2008. Identification of cardiac troponin I sequence motifs leading to heart failure by induction of myocardial inflammation and fibrosis. *Circulation* 118, 2063–2072.
- Kaya, Z., Katus, H.A., Rose, N.R., 2010. Cardiac troponins and autoimmunity: their role in the pathogenesis of myocarditis and of heart failure. *Clin. Immunol.* 134, 80–88.
- Kindermann, Barth, C., Mahfoud, F., et al., 2012. Update on myocarditis. *J. Am. Coll. Cardiol.* 59 (9), 779–792.
- Knebel, F., Böhm, M., Staudt, A., Borges, A.C., Tepper, M., Jochmann, N., et al., 2004. Reduction of morbidity by immunoabsorption therapy in patients with dilated cardiomyopathy. *Int. J. Cardiol.* 97, 517–520.
- Konstadoulakis, M.M., Kroumbouzou, H., Tsiamis, E., Trikas, A., Toutouzas, P., 1993. Clinical significance of antibodies against tropomyosin, actin and myosin in patients with dilated cardiomyopathy. *J. Clin. Lab. Immunol.* 40, 61–67.
- Krause, E.G., Bartel, S., Beyerdörfer, I., Wallukat, G., 1996. Activation of cyclic AMP-dependent protein kinase in cardiomyocytes by anti-beta 1-adrenoceptor autoantibodies from patients with idiopathic dilated cardiomyopathy. *Blood Press. (Suppl. 3)*, 37–40.
- Kuchynka, P., Palecek, T., Nemecek, E., Fikrle, M., Linhart, A., 2015. New therapeutic aspects on inflammatory cardiomyopathy. *Curr. Pharm. Des.* 21 (4), 459–465.
- Kühl, U., Pauschinger, M., Schwimmbeck, P.L., et al., 2003. Interferon-β treatment eliminates cardiotropic viruses and improves left ventricular function in patients with myocardial persistence of viral genomes and left ventricular dysfunction. *Circulation* 107 (22), 2793–2798.
- Kühl, U., Lassner, D., von Schlippenbach, J., Poller, W., Schultheiss, H.-P., 2012. Interferon-beta improves survival in enterovirus associated cardiomyopathy. *J. Am. Coll. Cardiol.* 60 (14), 1295–1296.
- Lane, J.R., 1992. Interleukin 1 or tumor necrosis factor can promote coxsackie B3-induced myocarditis in resistant B10.A mice. *J. Exp. Med.* 175 (4), 1123–1129.
- Lappé, J.M., Pelfrey, C.M., Tang, W.H., 2008. Recent insights into the role of autoimmunity in idiopathic dilated cardiomyopathy. *J. Card. Fail.* 14, 521–530.
- Lassner, D., Rohde, M., Siegmund, C.S., et al., 2014. Myocarditis—personalized medicine by expanded endomyocardial biopsy diagnostics. *World J. Cardiovasc. Dis.* 4 (6), 325–340.
- Lazzerini, P.E., Capecchi, P.L., Guideri, F., Acampa, M., Selvi, E., Bisogno, S., et al., 2008. Autoantibody-mediated cardiac arrhythmias: mechanisms and clinical implications. *Basic Res. Cardiol.* 103, 1–11.

- Lee, H.C., Huang, K.T., Wang, X.L., Shen, W.K., 2011. Autoantibodies and cardiac arrhythmias. *Heart Rhythm* 8, 1788–1795.
- Li, H.S., Ligons, D.L., Rose, N.R., 2008a. Genetic complexity of autoimmune myocarditis. *Autoimmun. Rev.* 7, 168–173.
- Li, H.S., Ligons, D.L., Rose, N.R., Guler, M.L., 2008b. Genetic differences in bone marrow-derived lymphoid lineages control susceptibility to experimental autoimmune myocarditis. *J. Immunol.* 180, 7480–7484.
- Limas, C.J., Limas, C., Kubo, S.H., Olivari, M.T., 1990. Anti-beta receptor antibodies in human dilated cardiomyopathy and correlation with HLA-DR antigens. *Am. J. Cardiol.* 65, 483–487.
- Lipshultz, S.E., Sleeper, L.A., Towbin, J.A., Lowe, A.M., Orav, E.J., Cox, G.F., et al., 2003. The incidence of pediatric cardiomyopathy in two regions of the United States. *N Engl. J. Med.* 348 (17), 1647–1655.
- Liu, H.R., Zhao, R.R., Zhi, J.M., Wu, B.W., Fu, M.L., 1999. Screening of serum autoantibodies to cardiac beta1-adrenoceptors and M2-muscarinic acetylcholine receptors in 408 healthy subjects of varying ages. *Autoimmunity* 29, 43–51.
- Lu, C., Qui, F., Yan, Y., Liu, T., Li, J., Chen, H., 2016. Immunosuppressive treatment for myocarditis: a meta-analysis of randomized controlled trials. *J. Cardiovasc. Med.* 17, 631–637.
- Lurz, P., Eitel, I., Adam, J., et al., 2012. Diagnostic performance of CMR imaging compared with EMB in patients with suspected myocarditis. *JACC Cardiovasc. Imaging* 5 (5), 513–524.
- Maisch, B., Pankweitz, S., 2012. Current treatment options in (peri)myocarditis and inflammatory cardiomyopathy. *Herz* 37 (6), 644–656.
- Maisch, B., Berg, P.A., Kochsiek, K., 1979. Clinical significance of immunopathological findings in patients with post-pericardiotomy syndrome. I. Relevance of antibody pattern. *Clin. Exp. Immunol.* 38, 189–197.
- Maisel, A.S., Krishnasamy, P., Nowak, R.M., et al., 2002. Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure. *N. Engl. J. Med.* 347, 161–167.
- Malik, L.H., Singh, G.D., Amsterdam, E.A., 2015. The epidemiology, clinical manifestations, and management of chagas heart disease. *Clin. Cardiol.* 38 (9), 565–569.
- Manolio, T.A., Baughman, K.L., Rodeheffer, R., et al., 1992. Prevalence and etiology of idiopathic dilated cardiomyopathy (summary of a National Heart, Lung, and Blood Institute workshop). *Am. J. Cardiol.* 69, 1458–1466.
- Maron, B.J., Towbin, J.A., Thiene, G., et al., 2006. Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. *Circulation* 113, 1807–1816.
- McMurray, J.J.V., Adamopoulos, S., Anker, S.D., et al., 2012. ESC guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: the task force for the diagnosis and treatment of acute and chronic heart failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. *Eur. Heart J.* 33 (14), 1787–1847.
- McNally, E.M., Golbus, J.R., Puckelwartz, M.J., 2013. Genetic mutations and mechanisms in dilated cardiomyopathy. *J. Clin. Invest.* 123, 19–26.
- Michels, V.V., Moll, P.P., Miller, F.A., et al., 1992. The frequency of familial dilated cardiomyopathy in a series of patients with idiopathic dilated cardiomyopathy. *N. Engl. J. Med.* 326, 77–82.
- Mobini, R., Staudt, A., Felix, S.B., Baumann, G., Wallukat, G., Deinum, J., et al., 2003. Hemodynamic improvement and removal of autoantibodies against beta1-adrenergic receptor by immunoabsorption therapy in dilated cardiomyopathy. *J. Autoimmun.* 20, 345–350.
- Müller, J., Wallukat, G., Dandel, M., Bieda, H., Brandes, K., Spiegelsberger, S., et al., 2000. Immunoglobulin adsorption in patients with idiopathic dilated cardiomyopathy. *Circulation* 101, 385–391.
- Müller, J., Wallukat, G., Schimke, I., 2016b. Autoantibody directed therapy in cardiovascular diseases. In: Nussinovitch, U. (Ed.), *The Heart in Rheumatologic, Inflammatory and Autoimmune Diseases: Pathophysiology, Clinical Aspects and Therapeutic Approaches*. Elsevier.
- Myers, J.M., et al., 2016. Cardiac myosin-Th17 responses promote heart failure in human myocarditis. *JCI Insight* 1 (9), pii: e85851.
- Neu, N., Beisel, K.W., Traystman, M.D., Rose, N.R., Craig, S.W., 1987a. Autoantibodies specific for the cardiac myosin isoform are found in mice susceptible to coxsackievirus B3-induced myocarditis. *J. Immunol.* 138, 2488–2492.
- Neu, N., Rose, N.R., Beisel, K.W., Herskowitz, A., Gurri-Glass, G., Craig, S.W., 1987b. Cardiac myosin induces myocarditis in genetically predisposed mice. *J. Immunol.* 139, 3630–3636.
- Nikolaev, V.O., Boivin, V., Störk, S., Angermann, C.E., Ertl, G., Lohse, M.J., et al., 2007. A novel fluorescent method for the rapid detection of functional beta1-adrenergic receptor autoantibodies in heart failure. *J. Am. Coll. Cardiol.* 50, 423–431.
- Nishimura, R.A., Tajik, A.J., 1997. Evaluation of diastolic filling of left ventricle in health and disease: Doppler echocardiography is the clinician's Rosetta Stone. *J. Am. Coll. Cardiol.* 30, 8–18.
- Noutsias, M., Pauschinger, M., Poller, W.-C., Schultheiss, H.-P., Kühl, U., 2003. Current insights into the pathogenesis, diagnosis and therapy of inflammatory cardiomyopathy. *Heart Fail. Monit.* 3 (4), 127–135.
- Nugent, A.W., Wilkinson, L.C., Daubeney, P.E.F., et al., 2003. The epidemiology of childhood cardiomyopathy in Australia. *N. Engl. J. Med.* 348, 1639.
- Nussinovitch, U., Shoenfeld, Y., 2010. Anti-troponin autoantibodies and the cardiovascular system. *Heart* 96, 1518–1524.
- Nussinovitch, U., Shoenfeld, Y., 2012. The diagnostic and clinical significance of anti-muscarinic receptor autoantibodies. *Clin. Rev. Allergy Immunol.* 42, 298–308.
- Nussinovitch, U., Shoenfeld, Y., 2013a. The clinical significance of anti-beta-1 adrenergic receptor autoantibodies in cardiac disease. *Clin. Rev. Allergy Immunol.* 44, 75–83.
- Nussinovitch, U., Shoenfeld, Y., 2013b. The clinical and diagnostic significance of anti-myosin autoantibodies in cardiac disease. *Clin. Rev. Allergy Immunol.* 44, 98–108.
- Okazaki, T., Tanaka, Y., Nishio, R., Mitsuiye, T., Mizoguchi, A., Wang, J., et al., 2003. Autoantibodies against cardiac troponin I are responsible for dilated cardiomyopathy in PD-1-deficient mice. *Nat. Med.* 9, 1477–1483.

- Okruhlikova, L., Morwinski, R., Schulze, W., Bartel, S., Weismann, P., Tribulova, N., et al., 2007. Autoantibodies against G-protein coupled receptors modulate heart mast cells. *Cell. Mol. Immunol.* 4, 127–133.
- Olimulder, M.A.G.M., van Es, J., Galjee, M.A., 2009. The importance of cardiac MRI as a diagnostic tool in viral myocarditis induced cardiomyopathy. *Netherlands Heart J.* 17 (12), 481–486.
- Palecek, T., Kuchynka, P., Hulinska, D., et al., 2010. Presence of *Borrelia burgdorferi* in endomyocardial biopsies in patients with new-onset unexplained dilated cardiomyopathy. *Med. Microbiol. Immunol.* 199 (2), 139–143.
- Parks, S.B., Kushner, J.D., Nauman, D., et al., 2008. Lamin A/C mutation analysis in a cohort of 324 unrelated patients with idiopathic or familial dilated cardiomyopathy. *Am. Heart J.* 156, 161–169.
- Pauschinger, M., Rutschow, S., Chandrasekharan, K., Westermann, D., Weitz, A., Peter Schwimmbeck, L., et al., 2005. Carvedilol improves left ventricular function in murine coxsackievirus-induced acute myocarditis: association with reduced myocardial interleukin-1 $\beta$  and MMP-8 expression and a modulated immune response. *Euro. J. Heart Fail* 7 (4), 444–452.
- Pearson, G.D., Veille, J.C., Rahimtoola, S., et al., 2000. Peripartum cardiomyopathy: National Heart, Lung, and Blood Institute and office of rare diseases (National Institutes of Health) workshop recommendations and review. *JAMA* 283, 1183–1188.
- Pitt, B., Zannad, F., Remme, W.J., et al., 1999. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *N. Engl. J. Med.* 341, 709–717.
- Reinthalter, M., Empen, K., Herda, L.R., Schwabe, A., Rühl, M., Dörfler, M., et al., 2015. The effect of a repeated immunoabsorption in patients with dilated cardiomyopathy after recurrence of severe heart failure symptoms. *J. Clin. Apher.* 30, 217–223.
- Root-Bernstein, R., Fairweather, D., 2015. Unresolved issues in theories of autoimmune disease using myocarditis as a framework. *J. Theor. Biol.* 375, 101–123.
- Rose, N.R., 2000. Viral damage or “molecular mimicry”: placing the blame in myocarditis. *Nat. Med.* 6, 5–6.
- Rose, N.R., 2011. Critical cytokine pathways to cardiac inflammation. *J. Interferon Cytokine Res.* 31 (10), 705–710.
- Rose, N.R., Hill, S.L., 1996. The pathogenesis of postinfectious myocarditis. *Clin. Immunol. Immunopathol.* 80, S92–S99.
- Rose, N.R., Beisel, K.W., Herskowitz, A., Neu, N., Wolfgram, L.J., Alvarez, F.L., et al., 1987. Cardiac myosin and autoimmune myocarditis. In: Evered, D., Whelan, J. (Eds.), *Ciba Symposium 129*. John Wiley & Sons, Chichester, pp. 3–24.
- Rose, N.R., Neumann, D.A., Hetskowitz, A., Traystman, M., Beisel, K.W., 1988. Genetics of susceptibility to viral myocarditis in Illice. *Pathol. Innuunopathol. Res.* 7, 266–278.
- Saegusa, S., Fei, Y., Takahashi, T., et al., 2007. Oral administration of candesartan improves the survival of mice with viral myocarditis through modification of cardiac adiponectin expression. *Cardiovasc. Drugs Ther.* 21 (3), 155–160.
- Satta, N., Vuilleumier, N., 2015. Auto-antibodies as possible markers and mediators of ischemic, dilated, and rhythmic cardiopathies. *Curr. Drug Targets* 16 (4), 342–360.
- Schlutheiss, H.-P., Khl, U., Cooper, L.T., 2011. The management of myocarditis. *Eur. Heart J.* 32 (21), 2616–2625.
- Smith, S.C., Allen, P.M., 1991. Myosin-induced acute myocarditis is a T cell-mediated disease. *J. Immunol.* 147, 2141–2147.
- Staudt, Y., Mobini, R., Fu, M., Felix, S.B., Kuhn, J.P., Staudt, A., 2003. Beta1-adrenoceptor antibodies induce apoptosis in adult isolated cardiomyocytes. *Eur. J. Pharmacol.* 466, 1–6.
- Staudt, A., Staudt, Y., Dörr, M., Böhm, M., Knebel, F., Hummel, A., et al., 2004. Potential role of humoral immunity in cardiac dysfunction of patients suffering from dilated cardiomyopathy. *J. Am. Coll. Cardiol.* 44, 829–836.
- Staudt, A., Hummel, A., Ruppert, J., Dörr, M., Trimpert, C., Birkenmeier, K., et al., 2006a. Immunoabsorption in dilated cardiomyopathy: 6-month results from a randomized study. *Am. Heart J.* 152, 712.e1–712.e6.
- Staudt, A., Staudt, Y., Hummel, A., Empen, K., Dörr, M., Trimpert, C., et al., 2006b. Effects of immunoabsorption on the nt-BNP and nt-ANP plasma levels of patients suffering from dilated cardiomyopathy. *Ther. Apher. Dial.* 10, 42–48.
- Störk, S., Boivin, V., Horf, R., Hein, L., Lohse, M.J., Angermann, C.E., et al., 2006. Stimulating autoantibodies directed against the cardiac beta1-adrenergic receptor predict increased mortality in idiopathic cardiomyopathy. *Am. Heart J.* 152, 697–704.
- Swedberg, K., Komajda, M., Böhm, M., Borer, J.S., Ford, I., Dubost-Brama, A., Lerebours, G., Tavazzi, L., SHIFT Investigators, 2010. Ivabradine and outcomes in chronic heart failure (SHIFT): a randomised placebo-controlled study. *Lancet* 376, 875–885.
- Talvani, A., Rocha, M.O., Ribeiro, A.L., Borda, E., Sterin-Borda, L., Teixeira, M.M., 2006. Levels of anti-M2 and anti-beta1 autoantibodies do not correlate with the degree of heart dysfunction in Chagas’ heart disease. *Microbes Infect.* 8, 2459–2464.
- Taneja, V., David, C.S., 2009. Spontaneous autoimmune myocarditis and cardiomyopathy in HLA-DQ8.NODAbo transgenic mice. *J. Autoimmun.* 33, 260–269.
- Taylor, M.R., Carniel, E., Mestroni, L., 2006. Cardiomyopathy, familial dilated. *Orphanet J. Rare Dis.* 1, 27.
- Towbin, J.A., Lowe, A.M., Colan, S.D., et al., 2006. Incidence, causes, and outcomes of dilated cardiomyopathy in children. *JAMA* 296, 1867–1876.
- Trimpert, C., Herda, L.R., Eckerle, L.G., Pohle, S., Müller, C., Landsberger, M., et al., 2010. Immunoabsorption in dilated cardiomyopathy: long-term reduction of cardiotropic antibodies. *Eur. J. Clin. Invest.* 40, 685–691.
- Tschope, C., Muller, I., Xia, Y., Savvatis, K., Papritz, K., Pinkert, S., et al., 2017. NOD2 (Nucleotide-Binding Oligomerization Domain 2) is a major pathogenic mediator of coxsackievirus B3-induced myocarditis. *Circ. Heart Fail.* 10 (9).
- Tutor, A.S., Penela, P., Mayor Jr, F., 2007. Anti-beta1-adrenergic receptor autoantibodies are potent stimulators of the ERK1/2 pathway in cardiac cells. *Cardiovasc. Res.* 76, 51–60.
- Wallukat, G., Morwinski, R., Magnusson, Y., Hoebeke, J., Wollenberger, A., 1992. Autoantibodies against the beta 1-adrenergic receptor in myocarditis and dilated cardiomyopathy: localization of two epitopes. *Z. Kardiol.* 81 (Suppl. 4), 79–83.
- Wallukat, G., Reinke, P., Dörfel, W.V., Luther, H.P., Bestvater, K., Felix, S.B., et al., 1996. Removal of autoantibodies in dilated cardiomyopathy by immunoabsorption. *Int. J. Cardiol.* 54, 191–195.
- Wallukat, G., Fu, H.M., Matsui, S., Hjalmarsson, Å., Fu, M.L., 1999. Autoantibodies against M2 muscarinic receptors in patients with cardiomyopathy display non-desensitizing agonist-like effects. *Life Sci.* 64, 465–469.
- Wang, Y., Afanasyeva, M., Hill, S.L., Kaya, A., Rose, N.R., 2000. Nasal administration of cardiac myosin suppresses autoimmune myocarditis in mice. *J. Am. Coll. Cardiol.* 36, 1992–1999.

- Wang, L., Lu, K., Hao, H., Li, X., Wang, J., Wang, K., et al., 2013. Decreased autophagy in rat heart induced by anti- $\beta$ 1-adrenergic receptor autoantibodies contributes to the decline in mitochondrial membrane potential. *PLoS One* 8, e81296.
- Wu, L., Ong, S., Talor, M.V., Barin, J.G., Baldeviano, G.C., Kass, D.A., et al., 2014. Cardiac fibroblasts mediate IL-17A-driven inflammatory dilated cardiomyopathy. *J. Exp. Med.* 211 (7), 1449–1464.
- Yancy, C.W., Jessup, M., Bozkurt, B., et al., 2013a. ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on practice guidelines. *Circulation* 128 (16), e240–e327. 2013.
- Yancy, C.W., Jessup, M., Bozkurt, B., et al., 2013b. ACCF/AHA guideline for the management of heart failure: executive summary: a report of the American College of Cardiology Foundation/American Heart Association Task Force on practice guidelines. *J. Am. Coll. Cardiol.* 2013 (62), 1495–1539.
- Yuan, Z., Shioji, K., Kihara, Y., Takenaka, H., Onozawa, Y., Kishimoto, C., 2004. Cardioprotective effects of carvedilol on acute autoimmune myocarditis: anti-inflammatory effects associated with antioxidant property. *Am. J. Physiol.—Heart Circ. Physiol.* 286 (1), H83–H90.
- Zannad, F., McMurray, J.J., Krum, H., ... and for the EMPHASIS-HF Study Group, 2011. Eplerenone in patients with systolic heart failure and mild symptoms. *N. Engl. J. Med.* 364, 11–21.

## Further Reading

- Ciháková, D., Sharma, R.B., Fairweather, D., Afanasyeva, M., Rose, N.R., 2004. Animal models for autoimmune myocarditis and autoimmune thyroiditis. *Methods Mol. Med.* 102, 175–193.
- Dörffel, W.V., Wallukat, G., Dörffel, Y., Felix, S.B., Baumann, G., 2004. Immunoadsorption in idiopathic dilated cardiomyopathy, a 3-year follow-up. *Int. J. Cardiol.* 97, 529–534.
- Fu, M.L., Hoebelke, J., Matsui, S., Matoba, M., Magnusson, Y., Hedner, T., et al., 1994. Autoantibodies against cardiac G-protein-coupled receptors define different populations with cardiomyopathies but not with hypertension. *Clin. Immunol. Immunopathol.* 72, 15–20.
- Krejci, J., Mlejnek, D., Sochorova, D., Nemec, P., 2016. Inflammatory cardiomyopathy: a current view on the pathophysiology, diagnosis, and treatment. *BioMed. Res. Int.* Available from: <https://doi.org/10.1155/2016/4087632> Article ID 4087632, 11 pages, 2016.
- Lipshultz, S., Sleeper, L., Towbin, J., et al., 2003. The incidence of pediatric cardiomyopathy in two regions of the United States. *N. Engl. J. Med.* 348, 1647.
- Matsumoto, Y., Park, I.K., Kohyama, K., 2007. B-cell epitope spreading is a critical step for the switch from C-protein-induced myocarditis to dilated cardiomyopathy. *Am. J. Pathol.* 170, 43–51.
- Müller, A.M., Bockstahler, M., Hristov, G., Weiß, C., Fischer, A., Korkmaz-Icöz, S., et al., 2016a. Identification of novel antigens contributing to autoimmunity in cardiovascular diseases. *Clin. Immunol.* 173, 64–75.
- Xiao, J., Shimada, M., Liu, W., Hu, D., Matsumori, A., 2009. Anti-inflammatory effects of eplerenone on viral myocarditis. *Eur. J. Heart Fail.* 11 (4), 349–353.

# Necrotizing Arteritis and Small-Vessel Vasculitis

Marco A. Alba<sup>1</sup>, J. Charles Jennette<sup>1</sup> and Ronald J. Falk<sup>2</sup>

<sup>1</sup>Department of Pathology and Laboratory Medicine, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States <sup>2</sup>Department of Medicine, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

## O U T L I N E

<b>Historical Background</b>	1286	Epidemiology	1293
<i>Necrotizing Arteritis</i>	1286	Clinical Features and Disease Associations	1293
<i>Purpura and Small-Vessel Vasculitis</i>	1288	Pathological Features	1294
<b>Polyarteritis Nodosa</b>	1288	Pathogenesis	1295
Definition	1288	Autoimmune Features	1296
Epidemiology	1288	Genetic Features and Environmental Influences	1297
Clinical Features and Disease Association	1289	Animal Models	1298
Pathological Features	1289	Diagnostic Procedures	1299
Pathogenesis	1289	Treatment	1299
Autoimmune Features	1290	<b>Cryoglobulinemic Vasculitis</b>	1300
Environmental Influences and Genetic Features	1290	Definition	1300
Animal Models	1290	Epidemiology	1300
Diagnostic Procedures	1290	Clinical Features and Disease Associations	1300
Treatment	1290	Pathological Features	1301
<b>Kawasaki's Disease</b>	1291	Pathogenesis	1301
Definition	1291	Autoimmune Features	1301
Epidemiology	1291	Genetic Features and Environmental Influences	1302
Clinical Features and Disease Associations	1291	Animal Models	1302
Pathological Features	1291	Diagnostic Procedures	1302
Pathogenesis	1291	Treatment	1302
Autoimmune Features	1292	<b>IgA Vasculitis (Henoch–Schönlein Purpura)</b>	1302
Genetic Features and Environmental Influences	1292	Definition	1302
Animal Models	1292	Epidemiology	1302
Diagnostic Procedures	1292	Clinical Features and Disease Associations	1303
Treatment	1292	Pathological Features	1303
<b>Antineutrophil Cytoplasmic Autoantibody Vasculitis</b>	1293	Pathogenesis	1303
Definition	1293	Autoimmune Features	1303
		Genetic Features and Environmental Influences	1303

Animal Models	1304	Concluding Remarks—Future Prospects	1304
Diagnostic Procedures	1304	References	1305
Treatment	1304		

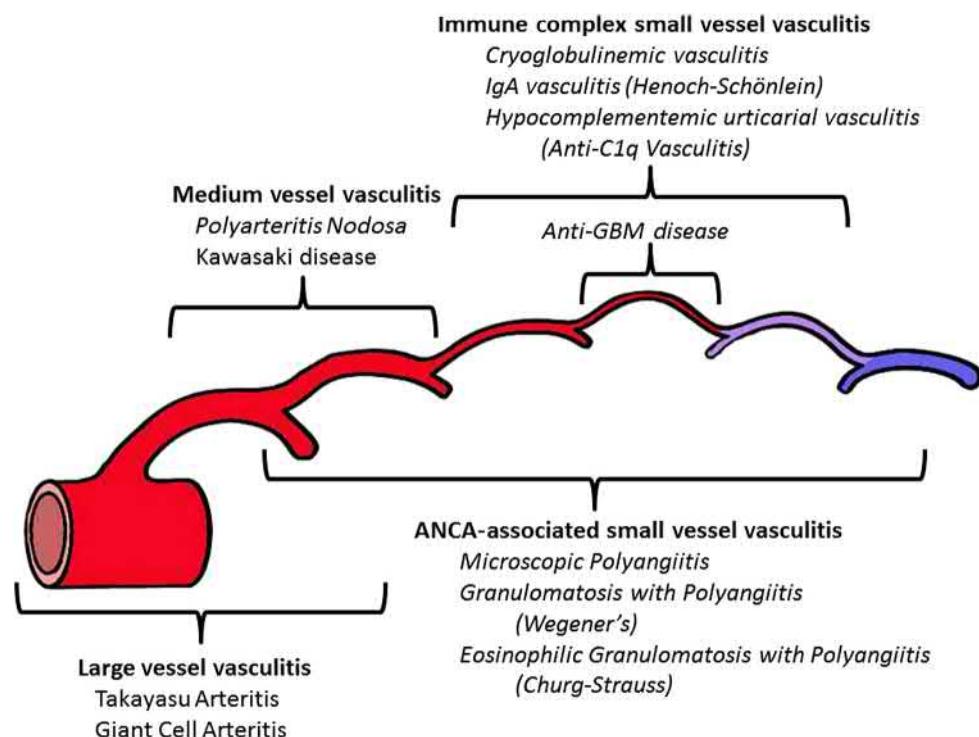
## HISTORICAL BACKGROUND

Vasculitis is inflammation of blood vessel walls (Jennette et al., 2013a). Major categories of systemic vasculitides are based on the predominant type of vessels involved (and the pattern of initial injury), that is, large-vessel vasculitis (chronic granulomatous arteritis), medium-vessel vasculitis (necrotizing arteritis), and small-vessel vasculitis (necrotizing polyangiitis) (Fig. 65.1 and Box 65.1).

Necrotizing arteritis was first recognized because of the grossly discernible segmental inflammatory nodular lesions that occur along major arteries (Kussmaul and Maier, 1866). Small-vessel vasculitis (SVV) was first recognized because of the palpable purpura that is caused by inflammation of dermal venules (Willan, 1808).

### Necrotizing Arteritis

Kussmaul and Maier (1866) provided the first definitive pathologic and clinical description of a patient with necrotizing arteritis. They reported the case of a 27-year-old journeyman tailor, who developed a fulminant disease characterized by fever, anorexia, muscle weakness, paresthesias, myalgias, abdominal pain, and oliguria. Kussmaul and Maier (1866) introduced the term *periarteritis nodosa* (PAN) as a reference to the major findings identified during this patient's autopsy, that is, multiple inflammatory nodules affecting medium-sized arteries. For years after the original description, the generic term PAN was given to any patient with necrotizing vasculitis



**FIGURE 65.1** Major categories of systemic vasculitis include large-vessel vasculitis, medium-vessel vasculitis, and small-vessel vasculitis. A substantial overlap with respect to arterial involvement can be observed in all three major categories. The aorta, a large artery, medium artery, small artery/arteriole, capillary, venule, and vein are shown from left to right. Source: Reprinted with permission from Jennette, J.C., Falk, R.J., Bacon, P.A., Basu, N., Cid, M.C., Ferrario, F., et al., 2013a. 2012 Revised international Chapel Hill consensus conference nomenclature of vasculitides. *Arthritis Rheum.* 65 (1), 1–11.

## BOX 65.1

**NAME FOR VASCULITIDES PROPOSED BY THE 2012  
INTERNATIONAL CHAPEL HILL CONSENSUS CONFERENCE ON THE  
NOMENCLATURE OF VASCULITIDES**

- Large-vessel vasculitis (LVV)
  - Takayasu arteritis (TAK)
  - Giant cell arteritis (GCA)
- Medium-vessel vasculitis (MVV)
  - Polyarteritis nodosa (PAN)
  - Kawasaki's disease (KD)
- Small-vessel vasculitis (SVV)
  - Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV)
    - Microscopic polyangiitis (MPA)
    - Granulomatosis with polyangiitis (Wegener's) (GPA)
    - Eosinophilic granulomatosis with polyangiitis (Churg–Strauss) (EGPA)
  - Immune complex SVV
    - Antiglomerular basement membrane (anti-GBM) disease
    - Cryoglobulinemic vasculitis (CV)
    - IgA vasculitis (Henoch–Schönlein) (IgAV)
    - Hypocomplementemic urticarial vasculitis (HUV) (anti-C1q vasculitis)
- Variable vessel vasculitis (VVV)
  - Behcet's disease (BD)
  - Cogan's syndrome (CS)
- Single-organ vasculitis (SOV)
  - Vasculitis associated with systemic disease
    - Lupus vasculitis
    - Rheumatoid vasculitis
    - Others
  - Vasculitis associated with probable etiology
    - Hepatitis C virus-associated cryoglobulinemic vasculitis
    - Hepatitis B virus-associated vasculitis
    - Drug-associated immune complex vasculitis
    - Drug-associated ANCA-associated vasculitis
    - Cancer-associated vasculitis
    - Others

*Modified from Jennette, J.C., Falk, R.J., Bacon, P.A., Basu, N., Cid, M.C., Ferrario, F., et al., 2013a. 2012 Revised international Chapel Hill consensus conference nomenclature of vasculitides. Arthritis Rheum.* 65 (1), 1–11.

([Jennette and Falk, 1997](#)). The name later evolved into the more pathologically correct term of PAN, as it became clear that the inflammation arose in the walls of arteries rather than in the perivascular tissue.

By the 1950s, distinction of PAN and a *microscopic form* of the disease was gradually recognized ([Chung and Seo, 2010](#)). Although [Wohlfwill \(1923\)](#) described a variant of PAN that was characterized by the involvement of small-vessels, Davson and Zeek were probably the first to suggest the separation of PAN into different disorders based on the presence of widespread glomerular inflammation (glomerulonephritis, GN) ([Davson et al., 1948; Zeek et al., 1948](#)). Today, the *microscopic form* of PAN is designated as microscopic polyangiitis (MPA) ([Jennette et al., 1994](#)).

In 1931, medical student Heinz Klinger published the first case of a patient with granulomatosis with polyangiitis (GPA) ([Klinger, 1931](#)). The detailed features of this multisystemic disorder were provided initially by the German pathologist Friedrich Wegener ([Wegener, 1939](#)). A few years later, the diagnostic triad of Wegener's granulomatosis was identified, that is, systemic necrotizing angiitis, necrotizing inflammatory involvement of the respiratory tract, and necrotizing GN ([Godman and Churg, 1954](#)).

[Churg and Strauss \(1951\)](#) described a series of 13 patients with asthma, eosinophilia, necrotizing vasculitis, and granulomatous inflammation. Three years later, [Godman and Churg \(1954\)](#) concluded that this disease, now designated as eosinophilic GPA (EGPA), the *microscopic form of periarteritis nodosa* (MPA), and Wegener's granulomatosis (GPA), were all pathologically and clinically distinct from PAN and probably have a related etiology and pathogenesis. This concept has been borne out by the discovery that GPA, MPA, and EGPA are associated with, and are probably caused by, antineutrophil cytoplasmic autoantibodies (ANCAs), whereas PAN is not ([Jennette and Falk, 1997; Jennette et al., 2013a](#)).

[Kawasaki \(1967\)](#) discovered an additional form of necrotizing arteritis that was associated with the mucocutaneous lymph node syndrome. This disease has been called *infantile polyarteritis nodosa* because it almost always occurs in young children ([Magilavy et al., 1977](#)); however, the clinical, pathologic, and pathogenetic features of Kawasaki's disease (KD) are clearly distinct from those of PAN ([Jennette et al., 2013a](#)).

## Purpura and Small-Vessel Vasculitis

Purpura was the first manifestation of SVV that was described in the medical literature. The term probably derives from the Greek *porphyra*, which refers to the purple color produced by the mollusk *Purpura lapillus* (Jones and Tocantins, 1933). English dermatologist Willan (1808) was one of the first to separate purpura caused by systemic febrile infections from noninfectious purpura, which were associated with systemic diseases.

Schönlein (1837) and Henoch (1868) described the occurrence of purpura in children in association with arthralgias and arthritis, abdominal pain, nephritis, and small visceral hemorrhages (Henoch, 1868; Schönlein, 1837). Osler (1895, 1914) recognized that peripheral neuropathy, pulmonary hemorrhage, epistaxis, iritis, and rapidly progressive renal disease were all symptoms caused by necrotizing inflammation of small vessels (Jennette and Falk, 1997; Osler, 1914). In 1919 Goodpasture reported a patient with pulmonary hemorrhage and rapidly progressive GN (pulmonary–renal syndrome) who had hemorrhagic alveolar capillaritis and vasculitis of glomerular capillaries as main pathology findings (Goodpasture, 2009).

By the 1950s, the pathologic features in SVV of extensive acute inflammation with numerous neutrophils and conspicuous leukocytoclasia had been well described (Jennette and Falk, 1997). Similarity of these findings with the injury pattern of the *Arthus* reaction in addition to the association of some cases of necrotizing vasculitis with serum sickness or exposure to certain drugs led to the suggestion of a possible “hypersensitivity” or allergic cause for vasculitis (Alarcon Segovia and Brown, 1964; Winkelmann, 1958; Zeek et al., 1948).

In the 1960s, the widespread application of immunofluorescence microscopy revealed that certain forms of SVV had substantial vascular localization of immunoglobulins and complement, suggesting an immune complex pathogenesis. Landmark discoveries using this technique include the identification of linear deposits of immunoglobulin along glomerular and pulmonary capillary basement membranes in a subset of patients with pulmonary–renal syndrome (Sturgill and Westervelt, 1965), later shown to be pathogenic autoantibodies directed against basement membrane collagen (Lerner et al., 1967); the demonstration of granular deposits of IgM, IgG, and complement in vessels walls of patients with cryoglobulinemic vasculitis (CV) (Meltzer and Franklin, 1966; Meltzer et al., 1966), and the finding of deposits of IgA and C3 in dermal venules and glomerular capillaries of children with Henoch–Schönlein purpura (now termed IgA vasculitis) (Faille-Kuyper et al., 1976).

By the end of the 1970s, there was a widespread belief that most if not all SVV was mediated by immune complexes (Fauci et al., 1978). However, evaluation of a wide range of vasculitides failed to identify substantial vessel wall deposition of immunoglobulins or complement. This was especially true in cases of GPA, MPA, and EGPA (Ronco et al., 1983; Weiss and Crissman, 1984). Davies et al. (1982) reported a new type of autoantibody that reacted with neutrophil cytoplasm in eight patients with pauci-immune necrotizing GN and SVV. Three years later, van der Woude et al. (1985) identified these autoantibodies in patients with GPA and suggested its value as a diagnostic and prognostic marker. Numerous subsequent studies documented that ANCAs were closely associated with GPA, MPA, and EGPA. The two major antigen specificities in patients with ANCA vasculitis were described by Falk and Jennette (1988), and Niles et al. (1989), that is, antimyeloperoxidase (MPO) ANCA (MPO-ANCA) and antiproteinase 3 (anti-PR3) (PR3-ANCA) (Falk and Jeneette 1988; Jennette and Nachman, 2017; Niles et al., 1989). In addition to the diagnostic value of these autoantibodies there is now strong evidence that ANCA participate in disease pathogenesis by direct activation of neutrophils (Jennette et al., 2013a).

## POLYARTERITIS NODOSA

### Definition

PAN is a systemic necrotizing arteritis of medium-sized or small arteries without GN or vasculitis in arterioles, capillaries, or venules. PAN is typically not associated with ANCAs (Jennette et al., 2013a).

### Epidemiology

The annual incidence of PAN in European countries is approximately 2–10 cases per million population. Prevalence has been estimated between 2 and 31 cases/million (Mahr et al., 2004; Mohammad et al., 2007; Watts and Scott, 2004). The peak incidence occurs in the sixth decade of life (Mahr et al., 2004; Mohammad et al., 2007; Watts and Scott, 2004).

## Clinical Features and Disease Association

Presentation includes constitutional symptoms, such as fever, weight loss, arthralgia, and myalgia (30%–93% of the cases); tender erythematous nodules, livedo reticularis, and ulcers caused by dermal and subcutaneous arteritis (28%–49%); peripheral neuropathy (e.g., mononeuritis multiplex) caused by arteritis in epineural arteries (38%–74%); and gastrointestinal manifestations, that is, abdominal pain, bowel bleeding, or perforation (14%–38%) (Hernandez-Rodriguez et al., 2014; Pagnoux et al., 2010). Kidney involvement is observed in 15% of the patients and is the result of tissue infarction or rupture of renal arterial aneurysms rather than GN. Signs and symptoms of renal involvement are renal insufficiency, new-onset hypertension, hematuria, or low-level proteinuria (Agard et al., 2003; Pagnoux et al., 2010). Less common complications of PAN include myocardial infarction, orchitis, and ischemic retinopathy.

A PAN-like disease caused by deficiency of adenosine deaminase 2 (DADA2) has been described. DAD2 is a monogenic autoinflammatory disorder characterized by early-onset polyarteritis, recurrent ischemic or hemorrhagic strokes, livedo reticularis, fever, elevation of acute phase reactants, and in some cases peripheral neuropathy, gastrointestinal ischemic symptoms, and hypogammaglobulinemia (Navon Elkan et al., 2014; Zhou et al., 2014).

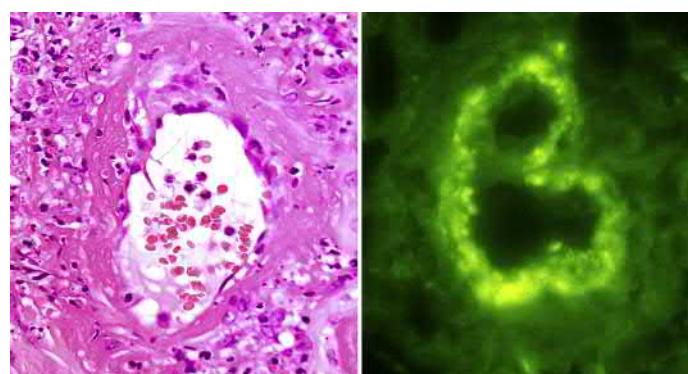
## Pathological Features

The hallmark of PAN is transmural inflammation and necrosis of muscular arteries (Fig. 65.2). The vascular inflammation initially contains predominantly neutrophils, but within a few days, the infiltrates contain predominantly monocytes, macrophages, and T-lymphocytes (Jennette, 2002). Typically, lesions are observed at arterial branching sites with acute necrotizing vasculitis usually coexisting with chronic vascular fibrotic changes. Segmental inflammation and necrosis may produce pseudoaneurysms by eroding through the vessel wall into the surrounding tissue. Thrombosis can cause acute ischemia, including infarction. Rupture of pseudoaneurysms results in hemorrhage, which may be severe and life threatening. The histopathological features of newly described DADA2 are indistinguishable from those of classic PAN (Caorsi et al., 2016).

## Pathogenesis

The pathogenesis of PAN remains poorly understood. It is probable that both humoral and cellular inflammatory systems participate in the development of necrotizing arteritis (Ozen, 2017). In a particular subset of patients, that is, those with hepatitis B virus–associated PAN (HBV-PAN), deposition of immune complexes is believed to play a role (Fig. 65.2). When arterial wall immune complexes are present, they cause inflammation by activating the complement, kinin, plasmin, and coagulation humoral systems, and the neutrophil, mononuclear phagocyte, lymphocyte, and platelet cellular systems. This complex interplay between the innate and adaptive immune systems results in the influx of inflammatory cells (especially neutrophils), necrosis, and sometimes thrombosis.

ADA2 acts as a growth factor for endothelial and hematopoietic cells and also participates in monocyte proliferation and macrophage differentiation (Zavialov et al., 2010). In this sense deficiency of this enzyme has been associated with compromised vascular endothelial integrity and with defects in the differentiation of M2 antiinflammatory monocytes, leading to an increased polarization of M1 proinflammatory cells (Caorsi et al., 2016; Zhou et al., 2014). In addition DADA2 may lead to an increased neutrophil activation (Caorsi et al., 2016; Zhou et al., 2014).



**FIGURE 65.2** Left panel: Light microscopy of necrotizing arteritis consistent with PAN in the wall of the small intestine (hematoxylin and eosin). The muscular media of the artery has been destroyed and replaced by fibrinoid material with admixed leukocytes and leukocyte nuclear debris (leukocytoclasis). Right panel: Direct immunofluorescence photomicrograph demonstrating granular IgG deposits in an artery from the subcutaneous tissue of a patient with hepatitis B–associated polyarteritis nodosa, showing granular vessel wall staining for C3. *PAN*, periarteritis nodosa.

## Autoimmune Features

Most patients with PAN do not have recognized evidence for an autoimmune pathogenesis. Exceptions are the few patients with systemic lupus erythematosus (SLE) who have a vasculitis that is pathologically similar to idiopathic PAN (lupus arteritis) (Korbet et al., 1984). ANCAs are typically absent in PAN patients (Jennette et al., 2013a).

## Environmental Influences and Genetic Features

There is a strong association between PAN and HBV, as approximately one-third of PAN cases are secondary to this infectious disease (Guillevin et al., 2005; Pagnoux et al., 2010). In comparison with the idiopathic form of this vasculitis HBV infected patients usually exhibit a more severe phenotype, frequently involving the gastrointestinal and peripheral nervous system and the heart (Pagnoux et al., 2010). Anecdotal reports of PAN-like vasculitis associated with hepatitis C virus (HCV), human immunodeficiency virus (HIV), parvovirus B19, cytomegalovirus, Mediterranean fever, and hematological neoplasias have been published (Hasler et al., 1995; Ozen et al., 2001).

There is no evidence that genetic factors play a substantial role in the development of classic PAN neither than genetic features of HBV correlates with the induction of the disease. In contrast DADA2 is clearly associated with a genetic defect, that is, loss-of-function homozygous or compound heterozygous mutations in CECR1 gene (Caorsi et al., 2017).

## Animal Models

Pearl Zeek, who was one of the pioneers in delineating the clinical and pathologic features of PAN, studied an animal model of systemic arteritis that resembled PAN and that was induced by implanting pieces of silk in the perirenal tissue of rats (Zeek et al., 1948). More recently, necrotizing inflammation of mesenteric, pancreatic, and testicular arteries has been reported to occur spontaneously in certain rodent strains and in transgenic rats carrying the *env-pX* gene of human T-cell leukemia virus type I (Fugo et al., 2002; Yoshiki, 2002). The relevance of these models to PAN in patients is not clear because these experimental animals often have involvement of small-sized vessel (e.g., GN), as well as the presence of several pathogenic autoantibodies that are not observed in most patients with PAN (e.g., anti-DNA and antinuclear antibodies) (Fugo et al., 2002).

## Diagnostic Procedures

Biopsies from muscle, peripheral nerve, or skin (subcutaneous tissue) may document necrotizing arteritis. Conventional angiography is an alternative to biopsy (Hernandez-Rodriguez et al., 2014; Pagnoux et al., 2010). Typical findings include multiple aneurysms (pseudoaneurysms), and stenosis of mesenteric, renal, and hepatic arteries. The diagnosis PAN is supported by the serologic identification of HBV (Agard et al., 2003; Janssen et al., 2004). The diagnosis of PAN in a patient with necrotizing arteritis requires the exclusion of other diseases with arteritis, such as KD or ANCA vasculitis (Agard et al., 2003; Jennette et al., 2013a; Mahr et al., 2004). In this sense the presence of GN, alveolar capillaritis, or positive MPO-ANCA or PR3-ANCA rule out a diagnosis of PAN and indicate some form of SVV, for example, MPA or GPA (Agard et al., 2003; Jennette et al., 2013a).

## Treatment

Patients with nonorgan and non-life-threatening disease may be treated with glucocorticoids (GC) alone. A recent clinical trial showed that the addition of azathioprine to GC for remission-induction of nonsevere PAN did not improve remission rates or lower the relapse risk of these patients (Puechal et al., 2017). Under this category are included those who present with constitutional symptoms, mild cutaneous involvement or those who lack any of the poor prognostic variables described in the five factor score (FFS), that is, renal insufficiency, proteinuria >1 g/day, cardiac involvement, central nervous system involvement, or gastrointestinal involvement. Half of these patients will require an additional immunosuppressant for remission-maintenance (Samson et al., 2014). Moderate or severe manifestations, that is, one or more features of the FFS, presence of mononeuritis multiplex, or limb ischemia, are treated with high-dose GC in combination with cyclophosphamide (Gayraud et al.,

2001; Guillemin et al., 1995). Cases with HBV-associated PAN should receive antiviral therapy with interferon (IFN)- $\alpha$  or lamivudine in addition to immunosuppressive agents (Guillemin, 1999; Han, 2004; Janssen et al., 2004).

Experience treating DADA2 is limited. High-dose GC, tumor necrosis factor (TNF)-blockers, and enzymatic replacement therapy have all reported to be of benefit (Caorsi et al., 2016).

## KAWASAKI'S DISEASE

### Definition

KD is an acute vasculitis of childhood associated with the mucocutaneous lymph node syndrome that predominantly affects medium and small arteries. Coronary arteries are often involved (Jennette et al., 2013a).

### Epidemiology

In the United States, the estimated incidence of KD among children <5 years of age is in the range of 19–25 cases per 100,000 (Watts and Scott, 2004). Incidence varies by race, being markedly more frequent in East Asia, particularly in Japan. Approximately 90% of the patients are between ages 6 months and 5 years, with a male-to-female ratio of 1.5:1 (Yanagawa et al., 1995). In children from developed countries KD is the leading cause of acquired heart disease (Newburger and Fulton, 2004).

### Clinical Features and Disease Associations

Major clinical manifestations (also considered diagnostic criteria) include persistent fever in combination with (1) extremity changes (50%–85% of the cases), that is, erythema and edema of the palms and soles, periungual desquamation; (2) nonexudative bilateral conjunctivitis (70%–90%); (3) cutaneous involvement (70%–90%), that is, maculopapular eruption, diffuse erythroderma, or erythema multiforme-like rash; (4) oral mucosal lesions (90%), that is, erythema and cracking of the lips, “strawberry tongue,” or erythema of pharyngeal mucosa; and (5) cervical lymphadenopathy (25%–70%) (Fukushige et al., 1994; Kawasaki, 1967; McCrindle et al., 2017; Newburger and Fulton, 2004; Ozdemir et al., 2010). Cardiovascular complications, the major determinant of long-term prognosis, include coronary artery aneurysms, myocardial infarction, myocarditis, and valvular abnormalities (McCrindle et al., 2017).

### Pathological Features

The vasculitis of KD involves medium-sized and small arteries, most notably the coronary arteries. KD arteriopathy usually evolves from an acute necrotizing arteritis, characterized histologically by segmental mural necrosis with infiltration by predominantly mononuclear leukocytes with less conspicuous neutrophils, to a subacute vasculitis with infiltration of lymphocytes, plasma cells, and eosinophils, that is followed by myofibroblastic proliferation that may result in narrowing of the lumen, arterial stenosis, and ischemia (Jennette, 2002; McCrindle et al., 2017). Pseudoaneurysms are most common in the proximal coronary arteries and may be occluded by thrombus, resulting in myocardial infarction. In addition to the coronary arteries, renal, iliac, mesenteric, hepatic, and peri-pancreatic arteries may be involved (Cohen and Sundel, 2016). In comparison with PAN less fibrinoid material, more edema, and more macrophages are observed in necrotizing lesions of KD (Jennette, 2002).

### Pathogenesis

As in PAN, activation of both the innate and adaptive immune systems may play a role in KD. Previous studies have demonstrated a marked increase in serum concentration of proinflammatory cytokines IL-1 $\beta$ , IL-6, IL-8, IL-17, and TNF- $\alpha$  during the acute phase of KD (Franco et al., 2010; Matsubara et al., 2005; Sohn et al., 2003; Wang et al., 2013). In addition KD patients have a deficit of regulatory T cells and increased activity of CD4+ T-lymphocytes (Hirabayashi et al., 2013; Sohn et al., 2011). Although not fully verified, antiendothelial cell antibodies have been incriminated in the pathogenesis of vascular injury in patients with KD (Grunebaum et al., 2002; Kaneko et al., 2004; Leung et al., 1986a, 1986b, 1989).

## Autoimmune Features

Autoantibodies that react with activated endothelial cells and cardiac myosin have been identified in patients with KD (Cunningham et al., 1999; Grunebaum et al., 2002; Kaneko et al., 2004; Leung et al., 1986a, 1986b, 1989). The relevance of this finding is still to be determined.

## Genetic Features and Environmental Influences

Infectious, environmental, and genetic factors have been implicated in the development of KD. An infectious etiology of KD has been suggested on the basis of the temporal clustering and seasonality of reported cases and the similarity of clinical presentation with childhood febrile exanthems. Although several microorganisms have been incriminated, that is, coxsackie virus, cytomegalovirus, parainfluenza virus, novel RNA viruses, or bacterial superantigens, none has been confirmed (Burns et al., 2005). Proposed associations with environmental factors include dust mites or pollen exposure, residence near standing water, and large-scale wind currents originating from Asia (Cohen and Sundel, 2016; McCrindle et al., 2017; Rodo et al., 2011).

A role for genetic factors in the pathogenesis of KD is supported by the observation that children of parents who had KD in childhood are at greater risk for developing the disease (Uehara et al., 2003). In addition a child is at 10-fold greater risk of developing the disease within 1 year of onset of the disease in a sibling (Fujita et al., 1989). Genome-wide association studies (GWAS) and family linkage studies have identified an increased susceptibility for the development of KD in the presence of single-nucleotide polymorphisms of several genes that are involved in the regulation of immune response, that is, BLK, CD40, ITPKC, CASP3, and FC $\gamma$ R2A (Lee et al., 2012; McCrindle et al., 2017; Onouchi et al., 2008, 2012).

## Animal Models

Takahashi et al. (2004) have developed an animal model of vasculitis that has a remarkable pathologic similarity to the arteritis of KD. They injected a *Candida albicans* extract intraperitoneally for 5 consecutive days into a variety of mouse strains. Arteritis developed in 66% of the CD-1 mice and most often affected the coronary arteries and aortic root close to the orifice of coronary arteries. The gross distribution and histologic pattern of injury closely mimics coronary arteritis in patients with KD. Not all strains of mice developed disease, indicating a genetic susceptibility in certain strains. Duong et al. (2003) have described a similar mouse model of coronary arteritis induced by the injection of *Lactobacillus casei* cell wall extract.

## Diagnostic Procedures

Diagnosis of KD and incomplete (atypical) forms is based on clinical criteria and exclusion of other systemic vasculitis and infectious conditions (McCrindle et al., 2017). Leukocytosis, anemia, and raised erythrocyte sedimentation rate (ESR) and C-reactive protein are common during the first days of disease. Echocardiography of coronary arteries should be performed in all patients (McCrindle et al., 2017). Until now, no autoantibodies have been recognized that can be used for routine diagnosis of KD (Newburger and Fulton, 2004).

## Treatment

Management of KD is aimed to prevent the development of coronary aneurysms. Recommended initial treatment includes the administration of a single dose of intravenous gamma-globulin (IVIg, 2 g/kg) in addition to high-dose aspirin (Furusho et al., 1984; McCrindle et al., 2017; Nagashima et al., 1987; Newburger and Fulton, 2004). After normalization of fever and in the absence of coronary aneurysms, low-dose aspirin should be continued for 4–6 weeks.

Patients who are refractory to initial IVIg dose (10%–20% of the cases) have an increased risk of developing aneurysms. For these children, options include a repeated infusion of IVIg, infliximab, GC, cyclosporine, cyclophosphamide, or plasma exchange (McCrindle et al., 2017).

## ANTINEUTROPHIL CYTOPLASMIC AUTOANTIBODY VASCULITIS

### Definition

ANCAAs are autoantibodies specific for antigens located in the cytoplasmic granules of neutrophils and lysosomes of monocytes (Jennette and Nachman, 2017). The main autoantigen targets of ANCAAs are MPO (MPO-ANCA) and PR3 (PR3-ANCA). ANCA vasculitis is characterized by necrotizing vasculitis, with few or no immune deposits, predominantly affecting small vessels, that is, capillaries, venules, arterioles, and small arteries (Jennette et al., 2013a). ANCA vasculitis has three major clinicopathologic expressions, that is, GPA (formerly called Wegener's granulomatosis), MPA, and EGPA (formerly Churg–Strauss syndrome) (Jennette et al., 2013a) (Box 65.1). GPA has necrotizing granulomatous inflammation superimposed on the vasculitis. EGPA has asthma, eosinophilia, and granulomatous inflammation in addition to the vasculitis. MPA has only the vasculitis, without granulomatous inflammation, asthma, or eosinophilia (Jennette et al., 2013a).

### Epidemiology

The overall incidence and prevalence of ANCA vasculitis has been estimated at 13–23 cases/million adults per year and 46–184 cases/million, respectively (Mohammad et al., 2007, 2014; Watts et al., 2015; Watts and Scott, 2004). The annual incidence in 1,000,000 adults ranges from 2 to 12 for GPA, from 3 to 12 for MPA, and from 1 to 3 for EGPA (Watts et al., 2015). Reported prevalence of GPA is 24–160 cases/million inhabitants, between 9 and 94 for MPA, and 2 and 38 for EGPA (Mohammad et al., 2007, 2014; Watts et al., 2015; Watts and Scott, 2004). GPA is more prevalent in Nordic countries, whereas MPA is the most common ANCA vasculitis in Southern Europe and Japan (Ntatsaki et al., 2010). Mean age at disease onset is 45–60 years for GPA, 55–74 for MPA, and 38–52 for EGPA (Guillevin et al., 1999; Smyth et al., 2004).

### Clinical Features and Disease Associations

Constitutional symptoms such as fever and arthralgias are observed in a large majority of patients (Agard et al., 2003). Small vessels in any organ or tissue can be affected (Table 65.1). Frequent examples include vasculitis affecting dermal venules causing palpable purpura; necrotizing arteritis in small dermal and subcutaneous arteries causing ulcers, and nodules; vasculitis affecting small epineurial arteries and arterioles causing peripheral neuropathy (usually mononeuritis multiplex or sensory neuropathy); vasculitis of the small vessels of the eye causing scleritis and uveitis; and vasculitis in small vessels in the gastrointestinal mucosa and submucosa causing abdominal pain and blood in the stool (Jennette and Nachman, 2017).

Inflammation of glomerular capillaries causes focal and segmental, necrotizing, crescentic GN, and pulmonary capillaritis causes pulmonary hemorrhage (Jennette and Falk, 1997). Vasculitis of small vessels in the upper respiratory tract, present in approximately 90% of the GPA patients, causes sinusitis, otitis, bloody nasal discharge, or collapse of the nasal septum causing saddle nose deformity (Hoffman et al., 1992). A smaller proportion of MPA patients have upper respiratory tract inflammation that is less destructive than that of GPA.

Patients with GPA may have clinical manifestations of necrotizing granulomatous inflammation, that is, pulmonary nodules and cavities, subglottic stenosis, orbital masses, and nasolacrimal duct obstruction (Hoffman

**TABLE 65.1** Differential Diagnostic Features of Several Forms of Small-Vessel Vasculitis (SVV).

	MPA	GPA	EGPA	IgAV	Cryo V	PAN
SVV signs and symptoms	Yes	Yes	Yes	Yes	Yes	No
IgA-dominant deposits	No	No	No	Yes	No	No
Cryoglobulins	No	No	No	No	Yes	No
Necrotizing granulomas	No	Yes	Yes	No	No	No
Asthma and eosinophilia	No	No	Yes	No	No	No

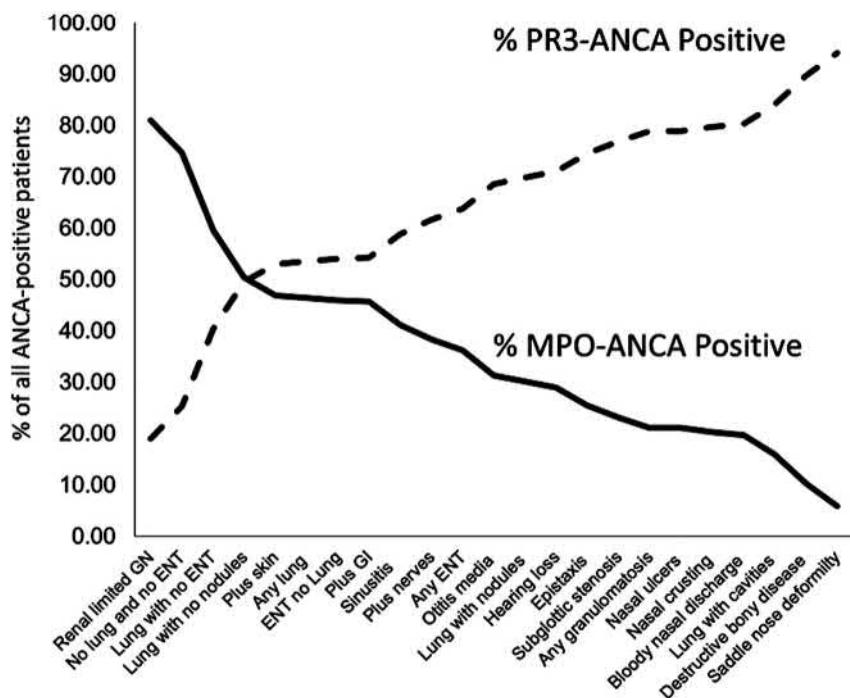
All these SVVs can manifest any or all of the shared features of SVV, such as purpura, nephritis, abdominal pain, peripheral neuropathy, myalgias, and arthralgias. Each is distinguished by the presence, and just as importantly the absence, of certain specific features. Both ANCA vasculitis and PAN can cause arteritis, but PAN lacks features of SVV and ANCA. GPA and EGPA are distinguished from MPA by granulomatous inflammation, and EGPA is distinguished by asthma and blood eosinophilia. ANCA, Antineutrophil cytoplasmic autoantibody; GPA, granulomatosis with polyangiitis; EGPA, eosinophilic granulomatosis with polyangiitis; PAN, periarteritis nodosa.

et al., 1992), whereas those with EGPA may suffer from organ damage associated with eosinophilic infiltration, for example, cardiomyopathy, or gastroenteritis (Comarmond et al., 2013).

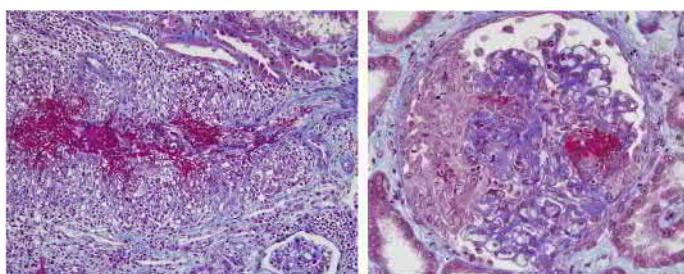
The frequency of different organ system involvement, and the clinical signs and symptoms of disease, are influenced by the ANCA antigen specificity (serotype), that is, whether the patient has MPO-ANCA or PR3-ANCA (Lionaki et al., 2012). As noted earlier, this also is influenced by geography. Fig. 65.3 demonstrates the correlation of organ system involvement with ANCA serotype in a cohort of patients from the southeastern United States (Jennette and Nachman, 2017). Note that patients with renal-limited disease have predominantly MPO-ANCA, whereas patients with evidence for destructive upper respiratory tract disease have predominantly PR3-ANCA.

## Pathological Features

Patients with all clinicopathologic expressions of ANCA vasculitis share a common pathologic manifestation of small-vessel inflammation (Jennette, 1991, 2002). The acute vascular lesion is similar in all vessels and is characterized by mural fibrinoid necrosis with karyorrhexis and infiltrating leukocytes (Fig. 65.4) (Jennette et al., 2013b). Neutrophils predominate in early lesions and are later replaced by inflammation with a



**FIGURE 65.3** Frequency of PR3-ANCA and MPO-ANCA positivity in ANCA-positive patients with a particular organ system involvement in an inception cohort of 502 ANCA vasculitis patients with MPA, GPA, or RLV evaluated at the University of North Carolina Kidney Center (excluding patients with EGPA). Organ groupings are not mutually exclusive. 'No lung and no ENT' has vasculitis in some other organs. Plus means there is vasculitis in an additional organ. ANCA, antineutrophil cytoplasmic autoantibody; EGPA, eosinophilic granulomatosis with polyangiitis; ENT, ear, nose, and throat; GI, gastrointestinal tract; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; MPO, myeloperoxidase; PR3, proteinase 3; RLV, renal-limited vasculitis. Source: Modified from Jennette, J.C., Nachman, P.H., 2017. ANCA glomerulonephritis and vasculitis. *Clin J. Am. Soc. Nephrol.* 12 (10), 1680–1691, with permission.



**FIGURE 65.4** (A, left panel) Necrotizing arteritis in a small artery and (B, right panel) necrotizing glomerulonephritis in a patient with microscopic polyangiitis. The artery and glomerulus have bright red staining for fibrinoid necrosis with a Masson trichrome stain. The artery and adjacent tissue are infiltrated by neutrophils and mononuclear leukocytes. The glomerulus has a cellular crescent on the left and segmental fibrinoid necrosis on the right.

predominance of monocytes, macrophages, and T-lymphocytes. Late-stage lesions have fibrotic scars (Jennette et al., 2013b). Acute and chronic vasculitic lesions usually coexist in tissue samples as a result of ongoing waves of new acute lesions superimposed on earlier lesions.

Focal segmental necrotizing GN with crescent formation is a frequent lesion in patients with ANCA vasculitis (Fig. 65.4). By immunofluorescence microscopy, the absence or paucity of staining for immunoglobulin distinguishes ANCA GN from immune complex GN.

In addition to SVV, GPA is characterized by the presence of necrotizing granulomatous inflammation that most often affects the upper and lower respiratory tract (Jennette et al., 2013a). Earliest granulomatous lesions are histologically characterized by the presence of a neutrophil-rich inflammatory infiltrate that resembles abscess formation (Gaudin et al., 1995; Travis et al., 1991). As the lesion progresses, geographic necrosis, palisading elongated macrophages, and scattered multinucleated giant cells are observed (Travis et al., 1991). More chronic lesions have extensive fibroblastic proliferation and interstitial deposition of collagen. In contrast to GPA the necrotizing granulomas of EGPA have more intense infiltration of eosinophils along with neutrophils.

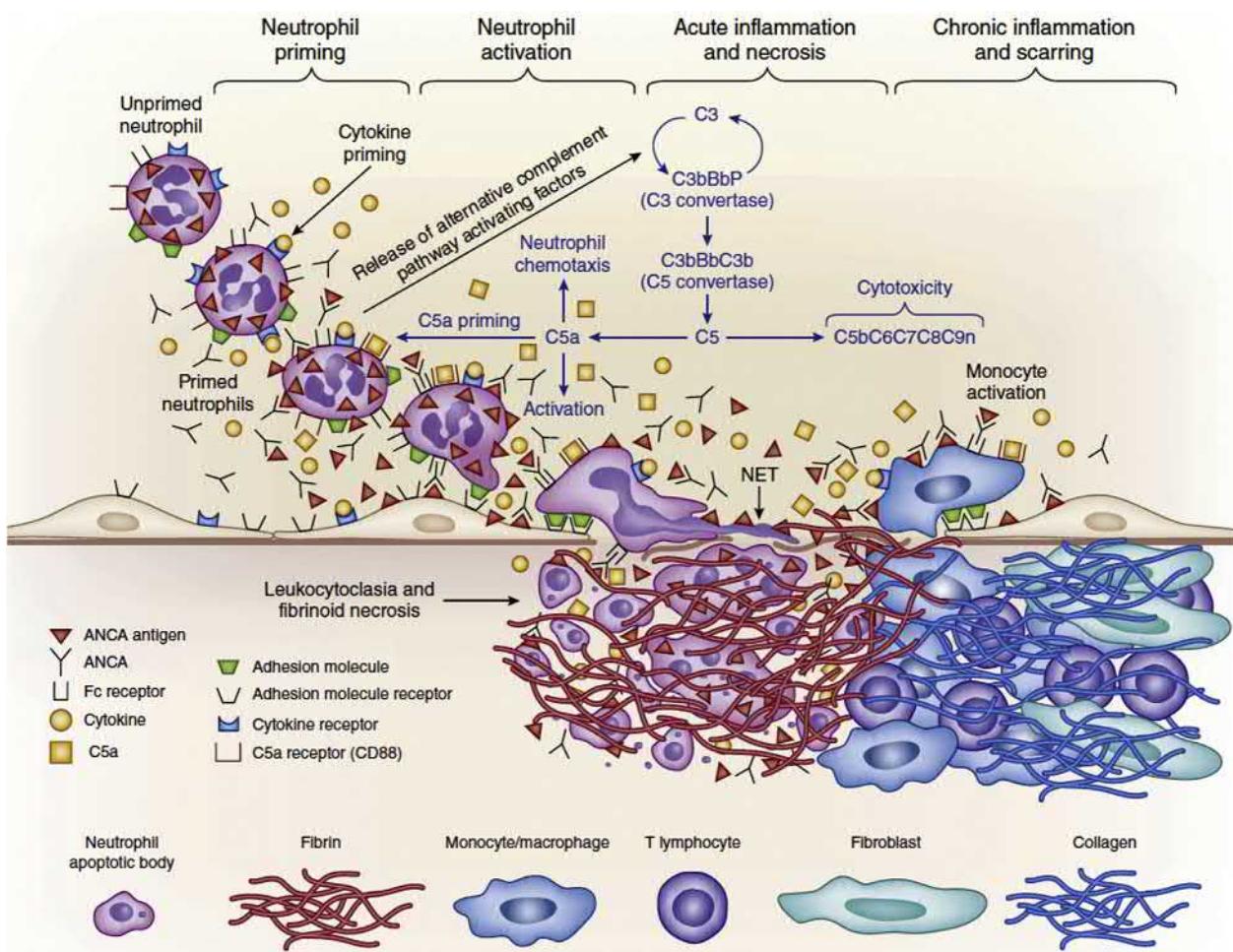
## Pathogenesis

The leading theory of the pathogenesis of vascular inflammation in ANCA vasculitis proposes that interaction of ANCAs with antigens (PR3 and MPO) expressed at the surface of cytokine-primed neutrophils causes full neutrophil activation [through both FcR engagement and F(ab')2 attachment to antigens] with generation of proinflammatory cytokines, oxygen radicals, destructive enzymes, and extrusion of neutrophil extracellular traps, in addition to the activation of the alternative complement pathway, resulting in the attachment to, injury and death of vascular endothelial cells, and other vessel wall components (Fig. 65.5) (Jennette and Nachman, 2017).

Extensive in vitro evidence supports this scenario (Jennette and Falk, 1998; Rarok et al., 2003; Williams et al., 2005). Incubation of ANCA IgG with primed neutrophils induces the release of toxic reactive oxygen species and lytic granule enzymes (Falk et al., 1990b). Neutrophil priming, as occurs with exposure to certain cytokines, results in the expression of small amounts of ANCA antigens at the surface of neutrophils where they can interact with ANCA (Falk et al., 1990b). In vitro ANCA-activated neutrophils are able to kill primed endothelial cells (Ewert et al., 1992; Savage et al., 1992). Also, exposure to ANCA IgG causes rolling neutrophils to adhere to endothelial cells in culture through integrin-mediated adhesion (Radford et al., 2000). Further, it has been demonstrated that MPO-ANCA and PR3-ANCA antigens may become planted in vessel walls by a charge-dependent mechanism, allowing their interaction with ANCA to form immune complexes *in situ* (Vargunam et al., 1992). If the latter occurs *in vivo*, the magnitude of vessel wall immune complex formation must be substantially less than in conventional immune complex disease because of the absence or paucity of staining for immunoglobulin in vessel walls in ANCA SVV.

In addition to in vitro experimental data, clinical observations and *in vivo* models (see the “Animal models” subsection of “Antineutrophil cytoplasmic autoantibody vasculitis” section) strongly support the pathogenic potential of ANCAs (Jennette and Falk, 2014). Clinical evidence include the report of a neonate who developed pulmonary hemorrhage and nephritis following transplacental transfer of maternal MPO-ANCA IgG; the presence of ANCA in the circulation and the correlation in *some* patients of ANCA titers with disease activity and recurrences of disease; the efficacy of anti-B-cell therapy (rituximab) and plasma exchange; and the induction of ANCA and pauci-immune SVV by drug exposure (Bansal and Tobin, 2004; Falk and Jennette, 2010; Han et al., 2003; Kallenberg et al., 1994). In addition it is interesting to note that approximately 90% of the patients report a “flu-like illness” shortly before the onset of the signs and symptoms of ANCA vasculitis (Falk et al., 1990a). An inflammatory process, such as a viral respiratory tract infection, may cause increased levels of circulating cytokines, which in turn prime neutrophils to interact with circulating ANCAs to induce vasculitis (Jennette and Falk, 1998). Experimental support for this hypothesis is provided by the observation that injection of bacterial lipopolysaccharide (LPS) into mice prior to induction of GN with anti-MPO IgG causes more severe injury (Huugen et al., 2005).

The pathogenic mechanisms involved in the development of extravascular granulomatous lesions observed in GPA are unknown. One hypothesis proposes that these lesions are consequence of an exaggerated antigen-independent innate response, that is, activation of primed-neutrophils by ANCAs located in the extravascular compartment results in an intense localized necrotizing inflammation that attract an influx of mononuclear cell, later evolving into more typical granulomatous inflammation (Jennette and Falk, 2014; Jennette et al., 2013b). An alternative proposal suggest that ANCA granulomatosis is induced by an antigen-specific T-cell immune response directed against PR3 or MPO (Lamprecht and Gross, 2007).

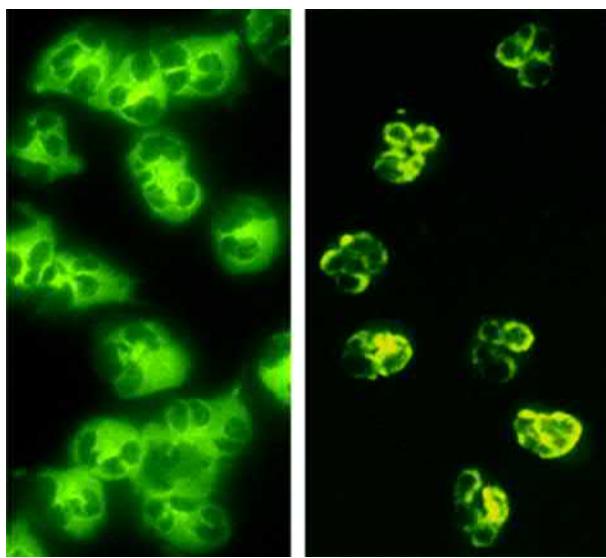


**FIGURE 65.5** Putative pathogenic mechanisms for ANCA-induced vasculitis. ANCA antigens (granule proteins) that are normally within the cytoplasm of neutrophils are transferred to the surface by cytokine priming, where they can interact with ANCAs. Some antigens also are released and can bind to endothelial cells and other vessel wall structures. These free and bound antigens can also react with ANCAs. The interaction of neutrophils with ANCAs, especially Fc receptor engagement, induces full activation with respiratory burst and degranulation, which releases factors from ANCA-activated neutrophils that activate the alternative pathway of complement activation. The resultant inflammation causes vascular injury (vasculitis). The response to this injury results in chronic inflammation and scarring. ANCA, antineutrophil cytoplasmic autoantibody. Source: Reproduced with permission from Jennette, J.C., Nachman, P.H., 2017. ANCA glomerulonephritis and vasculitis. *Clin J Am Soc Nephrol.* 12 (10), 1680–1691.

## Autoimmune Features

Over 90% of the patients with active untreated GPA or MPA, and approximately 45% of the EGPA have circulating ANCAs (Falk and Jennette, 2010; Finkelman et al., 2007; Jennette and Falk, 1997). Standard methods for the detection of ANCAs include indirect immunofluorescence (IIF) microscopy and enzyme-linked immunosorbent assays (ELISA) (Lim et al., 1999; Savige et al., 1999). When detected by IIF using alcohol-fixed neutrophils as substrate, the two major antigen specificities cause two different staining patterns: cytoplasmic (C-ANCA) and perinuclear (P-ANCA) (Fig. 65.6). The perinuclear pattern is an artifact of substrate preparation caused by diffusion of antigens from the cytoplasm to the nucleus (Charles et al., 1989). When analyzed by specific immunoassays, the most frequent C-ANCA antigen specificity is for PR3 (PR3-ANCA) (Goldschmeding et al., 1989; Jennette et al., 1990; Niles et al., 1989), and the most frequent P-ANCA specificity is for MPO (MPO-ANCA) (Jennette and Nachman, 2017). ANCA specificity is a major determinant of clinical presentation, independently of the clinicopathologic variant (Lionaki et al., 2012) (Fig. 65.3); therefore a diagnosis for ANCA vasculitis should include both the serotype as well as the phenotype, for example, PR3-ANCA MPA.

It is important to realize that a minority of patients with typical clinicopathologic features of ANCA vasculitis are ANCA-negative using clinical serologic assays (Eisenberger et al., 2005). This may change with the



**FIGURE 65.6** Indirect immunofluorescence microscopy photomicrograph of (A) C-ANCA and (B) P-ANCA—staining patterns on alcohol-fixed human neutrophils; produced, respectively, by PR3-ANCA and MPO-ANCA. C-ANCA, cytoplasmic antineutrophil cytoplasmic autoantibody; MPO, myeloperoxidase; P-ANCA, perinuclear antineutrophil cytoplasmic autoantibody; PR3, proteinase 3.

development of more sensitive and activity-specific assays. For example, using an epitope-specific assay, Roth et al. were able to detect MPO-ANCA with very restricted epitope specificity in many patients who were ANCA-negative by conventional clinical assays (Roth et al., 2013). In addition they identified three categories of MPO-ANCA epitope specificity: (1) epitope specificity confined to ANCA-associated vasculitis patients with active disease, (2) epitope specificity detected in patients with active disease and in remission, and (3) epitope specificity that was seen in patients as well as in very low titer in healthy controls (natural ANCA) (Roth et al., 2013).

Approximately 5%–15% of the ANCA-vasculitis patients have concurrent antiglomerular basement membrane (anti-GBM) antibodies (Jennette, 2003; McAdoo et al., 2017; Rutgers et al., 2005). When both autoantibodies are present, a patient is at risk for manifesting vasculitic features of ANCA-associated disease that do not occur with anti-GBM disease alone, such as cutaneous, skeletal muscle, or gut vasculitis. In addition these patients have a worse prognosis than those with ANCA alone (Jennette, 2003; McAdoo et al., 2017; Rutgers et al., 2005). The presence of antiplasminogen antibodies is associated with venous thrombotic events in patients with PR3-ANCA vasculitis (Bautz et al., 2008). In addition antibodies directed against lysosomal membrane protein 2 have been reported in some patients with ANCA-necrotizing GN (Kain et al., 2008).

## Genetic Features and Environmental Influences

The precise etiology of the autoimmune response that causes pathogenic ANCAs is unknown, but evidence suggests that multiple environmental and genetic factors may play a relevant role in this process (Jennette et al., 2013b). Several infections have been implicated in the induction of ANCA vasculitis, for example, Ross River virus, *Entamoeba histolytica*, and *Staphylococcus aureus* (Stegeman et al., 1996). Some authors suggest that molecular mimicry between microbial proteins and self-antigens is responsible for the induction of the autoimmune ANCA response (Kain et al., 2008). Another theory proposes that pathogens can initiate an antibody response through induction of an appropriate antibody response to microbial proteins that have an amino acid sequence that mimics the antisense sequence (complementary sequence) of the autoantigen (Pendergraft et al., 2004). These antibodies to the complementary peptide in turn induce antiidiotypic antibodies that cross-react with the autoantigen (i.e., are autoantibodies). In support of this theory patients with PR3-ANCA disease have circulating antibodies that react with peptides that have an amino acid sequence that is complementary to PR3, and these antibodies react with anti-PR3 antibodies as an antiidiotypic pair. Further, immunization of mice with a complementary PR3 peptide induces not only an antibody response to the complementary PR3 peptide but also to native PR3. Interestingly, *S. aureus*, Ross River virus, and *E. histolytica*, which have been associated with PR3-ANCA disease, contain proteins with amino acid sequences that mimic complementary peptides of PR3 (Pendergraft et al., 2004).

ANCA disease can also be caused by a variety of drugs, including propylthiouracil, allopurinol, minocycline, hydralazine, or cocaine contaminated with levamisole (Choi et al., 2000; Pendergraft and Niles, 2014). Other environmental exposures that have been associated with the development of ANCA vasculitis include silica, mercury, and lead exposure (Albert et al., 2004; Hogan et al., 2001; Lane et al., 2003).

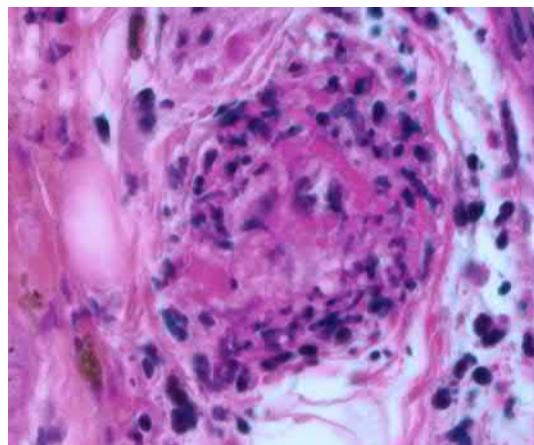
A GWAS of a large cohort of European patients has revealed a genetic influence on ANCA disease that correlated best with MPO-ANCA and PR3-ANCA autoantigen specificity rather than clinicopathologic phenotype (Lyons et al., 2012). In this study PR3-ANCA was associated with the human leukocyte antigen (HLA)-DP and genes encoding  $\alpha$ 1-antitrypsin (SERPINA1) and PR3 (PTRN3). MPO-ANCA was associated with HLA-DQ. Similar results were recently obtained in a North American cohort of GPA/MPA patients, where additionally a gene variant of PTPN22 was associated with an increasing susceptibility to ANCA disease (Merkel et al., 2017).

The predilection for the disease in white persons and the low prevalence in African Americans suggests that a genetic background contributes to disease induction. A previous study showed that African Americans with PR3-ANCA ANCA vasculitis had 73.3-fold higher odds of having HLA-DRB1\*15 alleles than healthy controls. Interestingly, DRB1\*1501 protein binds with high affinity to amino acid sequences of both sense-PR3 and anti-sense (complementary) PR3, suggesting that the major histocompatibility complex (MHC) antigen binding site is important in disease induction (Cao et al., 2011).

Epigenetic factors can also influence ANCA pathogenesis. Epigenetic modification of ANCA autoantigen-encoding genes results in aberrant overexpression of PR3 and MPO in neutrophils of ANCA patients (Ciavatta et al., 2010).

## Animal Models

Xiao et al. (2002) described the first convincing animal model of ANCA-induced SVV. This model is based on the passive transfer of purified anti-MPO IgG or splenocytes, obtained from MPO-deficient mice immunized with murine MPO, into wild-type or immunodeficient  $Rag2^{-/-}$  mice (lacking functional T and B cells) (Xiao et al., 2002). Over the course of 6 days, all mice that received anti-MPO developed necrotizing and crescentic GN that closely mimic human disease. In addition some mice exhibited extrarenal systemic SVV, for example, pulmonary capillaritis or necrotizing granulomatous inflammation, leukocytoclastic angiitis in the skin, or necrotizing arteritis in multiple viscera (Fig. 65.7). Studies using this model, or its variants, have demonstrated that (1) MPO-ANCA antibodies alone, in the absence of functional T cells, are sufficient to cause acute disease, (2) neutrophils are the mainstay effectors of disease induction, (3) bone marrow-derived cells are sufficient and necessary to induce ANCA GN, (4) genetic background plays an important role in the susceptibility and severity of disease, (5) circulating proinflammatory cytokines are able to exacerbate ANCA disease, (6) FC receptors are involved in pathogenesis and disease modulation, and (7) activation of alternative complement pathway is required to induce ANCA-associated GN (Huugen et al., 2005; Schreiber et al., 2006; Xiao et al., 2002, 2005, 2007, 2013).



**FIGURE 65.7** Necrotizing arteritis in a small artery in the dermis of a mouse 6 days after intravenous injection of mouse antimyeloperoxidase IgG. There is a central area of deeply eosinophilic fibrinoid necrosis surrounded by leukocytes with leukocytoclasis (H&E stain).

**TABLE 65.2** Clinicopathologic Phenotypes (RLV = Renal Limited Vasculitis, MPA = Microscopic Polyangiitis, GPA = Granulomatosis With Polyangiitis) and Serotypes [MPO-ANCA (Antineutrophil-Cytoplasmic-Autoantibody—Myeloperoxidase), PR3-ANCA (Proteinase-3—Antineutrophil-Cytoplasmic-Autoantibody)] of 502 ANCA-Positive Patients From an Inception Cohort Evaluated at the University of North Carolina Kidney Center (Excluding Patients With Eosinophilic Granulomatosis With Polyangiitis, EGPA)

Phenotype	MPO-ANCA (%)	PR3-ANCA (%)	
RLV ( <i>n</i> = 121)	81	19	
MPA ( <i>n</i> = 264)	59	41	
GPA ( <i>n</i> = 117)	26	74	
Serotype	RLV (%)	MPA (%)	GPA (%)
All ( <i>n</i> = 502)	24	53	23
MPO-ANCA ( <i>n</i> = 283)	35	55	11
PR3-ANCA ( <i>n</i> = 219)	10	50	40

## Diagnostic Procedures

Diagnosis of ANCA vasculitis is based on a combination of compatible clinical manifestations, positive laboratory test results, supportive radiology findings, and, when possible, the pathology confirmation of SVV (Table 65.2) (Jayne, 2009). As an SVV, ANCA vasculitis must be distinguished from other forms of SVV that can cause similar signs and symptoms, and have similar pathologic lesions by light microscopy. Immunopathologic features in tissue and serum are important in distinguishing among different forms of SVV (Table 65.1). ANCA vasculitis can involve arteries causing lesion that are indistinguishable histologically for the arteritis of PAN; however, PAN does not cause venulitis and capillaritis and is not associated with ANCA.

Laboratory workup for suspected ANCA vasculitis must include serum creatinine levels and urinalysis in all cases, in addition to determination of ANCA, antinuclear, and anti-GBM antibodies. Although one approach to ANCA testing is to first perform a screening by IIF and if positive (or inconclusive) to test for MPO-ANCA/PR3-ANCA by ELISA (Bossuyt et al., 2017; Savige et al., 1999), there is increasing evidence that high-quality immunoassays may be used for the primary screening, without the categorical need of IIF (Csernok et al., 2016; Damoiseaux et al., 2017). In addition epitope-specific assays may be a future option for the diagnosis and follow-up of these patients (Roth et al., 2013).

Serology alone cannot make the distinction between MPA, GPA, EGPA, or isolated pauci-immune crescentic GN because each syndrome can be associated with either C-ANCA (PR3-ANCA) or P-ANCA (MPO-ANCA) (Lim et al., 1999; Savige et al., 1999). However, the relative frequencies of ANCA specificities vary among the disease variants. For example, most patients with GPA have C-ANCA (PR3-ANCA), and most patients with renal-limited disease or MPA have P-ANCA (MPO-ANCA) (Table 65.2). Dual positivity is usually observed in drug-induced ANCA vasculitis (Savige et al., 2000). Diseases other than pauci-immune SVV can be associated with ANCAs, especially with ANCAs that are not specific for MPO or PR3, for example, autoimmune hepatitis, rheumatoid arthritis, ulcerative colitis, or sclerosing cholangitis (Bartunkova et al., 2002; Jennette and Falk, 1993).

Radiologic evaluation of the chest, orbits, brain, or ear, nose, and throat (ENT) structures may add diagnostic information and may be useful during follow-up. Computed tomography is preferred for evaluation of ANCA-associated lung disease. Typical findings include multiple, bilateral nodules, masses, and cavitations in cases of GPA and ground-glass opacities, usually reflecting alveolar hemorrhage, in patients with MPA (Cordier et al., 1990). Pulmonary fibrosis may be observed in approximately 7%–10% of these patients, particularly those with MPO-ANCA (Alba et al., 2017). Additional procedures may be performed if clinically indicated, for example, nerve-conduction studies, bronchoscopy, or ophthalmologic examination.

## Treatment

Treatment of ANCA vasculitis is composed of a remission induction phase with administration of highly potent immunosuppressants followed by a remission-maintenance period that aims to prevent relapses, still present in 40%–50% of the patients, and accrual of damage associated with vasculitis activity (Yates et al., 2016).

Remission-induction therapy of organ- or life-threatening disease consists in a combination of high-dose GC and either rituximab or cyclophosphamide (de Groot et al., 2009; Stone et al., 2010). Plasma exchange needs to be considered in patients with rapidly progressive GN and serum creatinine level >5.6 mg/dL, concomitant anti-GBM autoantibodies, or in those with severe diffuse pulmonary hemorrhage (Jayne et al., 2007; Klemmer et al., 2003; Yates et al., 2016). Methotrexate and mycophenolate mofetil are alternatives for less severe disease, for example, GPA with limited ENT involvement or patients with cutaneous or articular involvement (De Groot et al., 2005; Han et al., 2011). EGPA patients with nonorgan or life-threatening manifestations are usually managed with GC alone (Groh et al., 2015). The addition of IL-5 antagonist mepolizumab is effective in relapsing/refractory EGPA disease (Wechsler et al., 2017).

Current therapy strategies achieve remission of approximately 80%–90% of the patients, usually within 3–6 months after the initiation of treatment (Holle et al., 2011). Once remission is induced, patients are switched to maintenance therapy with low-dose GC in combination with rituximab, azathioprine, or methotrexate (Jayne et al., 2003; Pagnoux et al., 2008, 2015). The optimal duration of this phase is unknown. Ideally, extent of maintenance therapy should be individualized and should take into account aspects such as age, previous morbidity, or the presence of factors that have been associated with a relapsing course, for example, ANCA-PR3 seropositivity, lung or ENT involvement, or a clinical diagnosis of GPA (Hogan et al., 2005).

Despite the efficacy of immunosuppressive therapy, morbidity remains common in ANCA vasculitides. Patients are at increased risk of cardiovascular events and malignancy in addition to development of end-stage renal disease or treatment-associated adverse effects, for example, osteoporosis or opportunistic infections (Westman et al., 2015).

## CRYOGLOBULINEMIC VASCULITIS

### Definition

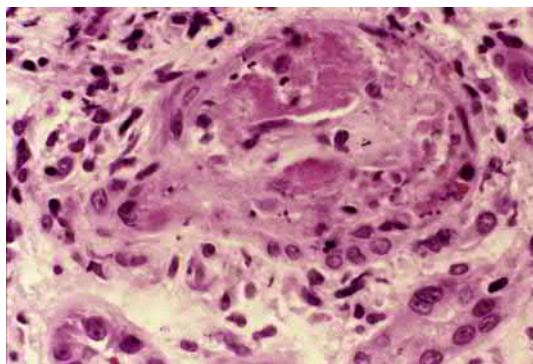
Cryoglobulins are circulating immunoglobulins that precipitate at cold temperatures and dissolve with rewarming. CV is defined as vasculitis with cryoglobulin immune deposits that affects small vessels (predominantly capillaries, venules, or arterioles) with associated serum cryoglobulins (Jennette et al., 2013a).

### Epidemiology

CV is more common in southern Europe than in northern Europe or North America and is closely associated with HCV infection (Ferri et al., 2004). Cryoglobulins are detected in 12%–50% of the HCV-infected patients, although less than 30% develop overt systemic vasculitis. HCV antibodies and RNA are detected in the serum of 75%–95% of the patients with vasculitis and GN caused by mixed cryoglobulins (D'Amico and Fornasieri, 1995; Ferri et al., 2004; Ramos-Casals et al., 2012). CV is most frequent in middle-aged individuals, predominantly women (female-to-male ratio 3:1) (Ferri et al., 2004).

### Clinical Features and Disease Associations

The clinical picture of CV may overlap with those of other systemic SVV (Table 65.1) and ranges from mild involvement of the skin and joints to potentially life-threatening disease, for example, alveolar hemorrhage. Patients with type I cryoglobulinemia (see the “Autoimmune features” subsection of “Cryoglobulinemic Vasculitis” section) may develop symptoms of vascular occlusion, for example, acrocytosis, livedo reticularis, skin necrosis, digital gangrene, or manifestations suggestive of hyperviscosity syndrome, that is, blurred vision, mucosal bleeding, headache, confusion, hearing loss, or heart failure (Harel et al., 2015; Terrier et al., 2013). Features of mixed CV (type II/III cryoglobulinemia) include constitutional symptoms (weakness, fever), palpable purpura, cutaneous ulcers, arthralgia/arthritis, mononeuritis multiplex, or distal sensory peripheral neuropathy; and kidney involvement, which usually presents as microscopic hematuria with proteinuria and less frequently as acute renal failure, nephrotic syndrome, or systemic hypertension. Sicca syndrome and abnormal liver function tests are common in HCV-associated CV.



**FIGURE 65.8** Cryoglobulinemic vasculitis affecting a small artery in the kidney. The deeply acidophilic material in the vessel probably is a mixture of thrombus, fibrinoid necrosis, and aggregated cryoglobulins. The vessel wall and adjacent tissue are infiltrated by leukocytes with leukocytoclasis.

## Pathological Features

CV is characterized pathologically by inflammation in small vessels that is associated with deposits of cryoglobulins and complement in vessel lumens and walls (Fig. 65.8). CV affects vessels of many types, including postcapillary venules (e.g., in the dermis), capillaries (e.g., glomerular and rarely pulmonary alveolar capillaries), arterioles, and rarely small arteries (Ferri et al., 2004). Immunofluorescence microscopy reveals granular deposits of immunoglobulins and complement in vessel walls, and sometimes luminal aggregates of cryoglobulins and complement. Type I membranoproliferative GN is observed in 70%–80% of the kidney biopsies; highly cellular glomerular infiltrates, thickening of GBM, hyaline intraluminal thrombi (precipitated cryoglobulins), and subendothelial dense deposits are typical features (D'Amico et al., 1989; Sinico et al., 1988).

## Pathogenesis

The pathogenic effect of cryoglobulins is the result of two different mechanisms, that is, immunoglobulin precipitation in the microcirculation, and deposition of mixed cryoglobulins in small vessels (Ramos-Casals et al., 2012). In the latter acute inflammation of blood vessels develops when immune complexes, composed of mixed cryoglobulins, activate the complement cascade, particularly the classic pathway (Sansonno et al., 2009). Of interest, it has been previously reported that immune complexes in HCV-associated CV contain nonenveloped nucleocapsid proteins and whole HCV virions (Sansonno and Dammacco, 2005). In this regard although mechanisms of cryoglobulin induction by HCV are not fully elucidated, one hypothesis proposes that infection of B cells by HCV triggers the production of polyclonal and monoclonal rheumatoid factors (RFs) that participate in cryoglobulin formation (D'Amico and Fornasieri, 1995). Another proposes that HCV lipoprotein induces an IgM response that is initially reactive with a virus-self complex but subsequently mutates to have RF activity (Agnello, 1995).

## Autoimmune Features

Cryoglobulins are divided into three major types: monoclonal (type I, either IgM or IgG), mixed monoclonal–polyclonal (type II, monoclonal IgM in combination with polyclonal IgG), and polyclonal (type III) (Brouet et al., 1974). Type I cryoglobulinemia (6% of the cases) is associated with B-cell lymphoproliferative disorders. Type II cryoglobulinemia (62% of the cases) is almost always caused by chronic HCV infection, although HBV and HIV are detected in a small percentage. Type III cryoglobulinemia (32% of the cases) is caused by HCV, lymphoproliferative malignancies or autoimmune diseases such as Sjögren syndrome or SLE (Monti et al., 1995).

Monoclonal cryoglobulins are not effective activators of inflammatory mediator systems and, therefore, rarely cause overt vasculitis, and morbidity is primarily by precipitation within vessels with the resultant occlusion (Muchtar et al., 2017). In contrast mixed cryoglobulins are immune complexes that are capable of activating inflammatory mediators and, therefore, characteristically cause systemic vasculitis (Muchtar et al., 2017). Most type II/III cryoglobulins contain autoantibodies directed against immunoglobulins, that is, RFs (Ferri et al., 2004; Sansonno and Dammacco, 2005).

## Genetic Features and Environmental Influences

As described previously, there is a strong association between CV and HCV infection, though not all patients with HCV develop cryoglobulinemia (D'Amico and Fornasieri, 1995; Ferri et al., 2004; Sansonno and Dammacco, 2005). The likelihood of developing circulating cryoglobulins is closely linked to the duration of HCV infection and the presence of certain HLA polymorphisms (Amoroso et al., 1998; Congia et al., 1996).

## Animal Models

The production of high titers of IgG-RF by secreting hybridomas was the base of two experimental mouse models of CV (Gyotoku et al., 1987; Kikuchi et al., 2002). In the first model investigators established monoclonal IgG-RF-secreting hybridomas from MRL/lpr mice (Gyotoku et al., 1987). These monoclonal RF antibodies were capable of forming cryoglobulins, and, when injected into normal mice, caused peripheral vasculitis and GN resembling that seen in patients with mixed cryoglobulinemia. In an alternative model mice were implanted with a hybridoma that secreted an IgG3 anti-IgG2a RF. These animals developed circulating cryoglobulins, acute GN, and cutaneous leukocytoclastic vasculitis (Kikuchi et al., 2002).

## Diagnostic Procedures

Classification criteria for CV have been proposed (De Vita et al., 2011). The laboratory hallmark of CV is the detection of cryoglobulins in the circulation (Table 65.2). Blood collection for cryoglobulin identification should be performed in prewarmed tubes. The type of cryoglobulins is later identified by immunoelectrophoresis and immunofixation (Ferri, 2008). Hypocomplementemia, characteristically low C4 but normal or near normal C3, is typically observed in mixed cryoglobulinemia as is the presence of high titers of RF (Ferri, 2008). Evaluation for possible HCV infection is mandatory. Additional work-up in selected cases may include HBV and HIV testing and determination of antinuclear, anti-DNA, and anti-Ro/La antibodies (Ramos-Casals et al., 2012). Tissue samples of the skin, peripheral nerve, or kidney may confirm the presence of a SVV.

## Treatment

Treatment of type I cryoglobulinemia is directed against the underlying lymphoproliferative disease (Muchtar et al., 2017). In patients with hyperviscosity syndrome plasma exchange may be of benefit. Management of noninfectious mixed cryoglobulinemia consists in the administration of high-dose GC and rituximab (Terrier et al., 2012). In organ- or life-threatening disease combination of intravenous methylprednisolone, rituximab (or cyclophosphamide), and plasmapheresis is recommended (D'Amico and Fornasieri, 1995).

Suppression of vascular inflammation and clearance of HCV are necessary in patients with HCV-associated CV. In these cases GC and rituximab are used in combination with pegylated IFN-ribavirin or preferentially, direct-acting antiviral agents, for example, sofosbuvir, simeprevir, or daclatasvir (Gragnani et al., 2016; Saadoun et al., 2017; Sise et al., 2016; Sneller et al., 2012). The choice of hepatitis C antiviral therapy should follow international guidelines (2016).

## IgA VASCULITIS (HENOCH–SCHÖNLEIN PURPURA)

### Definition

IgA vasculitis is a systemic vasculitis with IgA-dominant vascular immune deposits affecting small vessels. The disease occurs commonly in children and often involves the skin, the gastrointestinal tract, and the kidneys (Jennette et al., 2013a).

### Epidemiology

IgA vasculitis is the most common form of vasculitis in children. The disease has an incidence of 13–20 per 100,000 and is observed more frequently in patients of 4–7 years old (Gardner-Medwin et al., 2002). Male-to-

female ratio is about 1.5–1.8 to 1 (Gardner-Medwin et al., 2002; Hocevar et al., 2014). IgA vasculitis is less common in adults, that is, annual incidence of 3.4 new cases per million inhabitants (Watts et al., 1998).

## Clinical Features and Disease Associations

The classic manifestations of IgA vasculitis include palpable purpura, abdominal pain, arthritis/arthralgia, and nephritis. Purpura is typically symmetric and involves the lower extremities and buttocks. In adults purpura may show necrotic or hemorrhagic areas (Audemard-Verger et al., 2017). Arthritis of IgA vasculitis is typically nonerosive, migratory, and oligoarticular. Gastrointestinal involvement may present as nausea and vomiting, abdominal pain, bleeding, or, uncommonly, perforation or intussusception. Renal involvement is usually manifested as microscopic hematuria with low-level proteinuria, although nephrotic-range proteinuria, hypertension, and renal failure may occur. In contrast to the excellent overall prognosis observed in children 10%–30% of the adult patients suffer from more severe kidney involvement that may progress to end-stage renal disease (ESRD) (Coppo et al., 2006; Pillebout et al., 2002; Shrestha et al., 2006). In addition approximately 20% of adult patients have chronic relapsing disease (Coppo et al., 2006; Pillebout et al., 2002; Shrestha et al., 2006). As in other SVV, alveolar hemorrhage and peripheral neuropathy may be observed.

## Pathological Features

IgA vasculitis predominantly affects capillaries, venules, or arterioles (Jennette et al., 2013a). Immunofluorescence microscopy of dermal venules and renal glomeruli reveals granular vessel wall deposits of predominantly IgA1 and C3, with variable amounts of IgG and IgM, supporting a pathogenic mechanism that involves IgA-dominant immune deposits and activation of the alternative pathway of the complement system (Heineke et al., 2017).

## Pathogenesis

IgA vasculitis is an immune-mediated vasculitis that probably results from a dysregulated mucosal immune response, possibly initiated by environmental or infectious stimuli in a genetically predisposed individual (Heineke et al., 2017). IgA1-dominant immune complex deposits in vessel walls are the putative mediators of the inflammation. The IgA1 deposits may contain predominantly self-aggregated IgA1 or IgA1 complexed with anti-IgA1 autoantibodies (Heineke et al., 2017). Human IgA1 has an O-glycosylated hinge region not present in other immunoglobulin classes, and this hinge region has reduced galactosyl residues in patients with IgA nephropathy and IgA vasculitis (Allen et al., 1998; Heineke et al., 2017; Lau et al., 2007; Suzuki et al., 2009). This reduced galactosylation may be due to a functional defect in plasma cell  $\beta$ -1,3-galactosyltransferase. The abnormal glycosylation alters IgA1 structure and function, resulting in IgA1 aggregation, greater affinity for matrix proteins in vessel walls (including glomerular mesangium), and greater complement activation, which could result in localization of pathogenic IgA1-dominant deposits in vessel walls with resultant complement activation and vasculitis. Increased serum levels of IgA antiphospholipid antibodies (APA) directed against cardiolipin and  $\beta$ 2-glycoprotein have been reported in some patients (Kawakami et al., 2006; Yang et al., 2000, 2012). These antibodies are able to induce complement-dependent lysis of endothelial cells (Heineke et al., 2017).

## Autoimmune Features

Several antibodies have been detected in the serum of children and adults with active IgA vasculitis nephritis and IgA nephropathy, for example, antibodies directed against abnormally glycosylated IgA1, IgA anti- $\alpha$ -galactosyl, IgA antiendothelial cell antibodies, and APA (Davin et al., 1987; Heineke et al., 2017; Kawakami et al., 2006; Yang et al., 2000, 2012). The precise role of these antibodies in the pathogenesis of this vasculitis remains uncertain.

## Genetic Features and Environmental Influences

IgA vasculitis has a multifactorial etiology in which both genetic and environmental factors are probably involved. An infectious etiology of IgA vasculitis has long been suspected due to the seasonal pattern of the

disease, and the finding that approximately 40%–50% of the affected children suffered from an upper respiratory tract infection near the time of disease onset (Gonzalez-Gay et al., 2004; Masuda et al., 2003; Saulsbury, 2002; Ting and Hashkes, 2004). In this sense group A *Streptococcus*, methicillin-resistant *S. aureus*, and *Helicobacter pylori* have all been implicated as potential triggers for IgA vasculitis. In addition anecdotal cases have reported the association of this vasculitis with certain drugs, vaccination, and malignancy (Podjasek et al., 2014). Infections and other mucosal immune stimuli may be able to incite the production of pathogenic IgA1 that is not dependent on an antigen-specific immune response.

There is evidence from family studies that there may be a genetic predisposition for having circulating potentially pathogenic abnormally glycosylated IgA1 (Boyd and Barratt, 2011; Kiryluk et al., 2011). More importantly, a recent GWAS performed in 308 European patients identified that polymorphisms of HLA-DRB1 gene were associated with IgA vasculitis susceptibility (Lopez-Mejias et al., 2017). These findings are in line with previous studies that reported an increased prevalence of HLA-DRB1 and IL-1 allele polymorphisms in IgA vasculitis patients (Amoli et al., 2004).

## Animal Models

There is no good animal model for IgA vasculitis, although there are several animal models of IgA nephropathy, which is the glomerular lesion of IgA vasculitis. Many of these involve oral or nasal immunization with dietary or infectious antigens (Amore et al., 2004). Uteroglobin gene knockout and uteroglobin antisense transgenic mice develop pathologic features of human IgA nephropathy, but the relevance of this to human disease is unclear (Coppo et al., 2002; Lin et al., 2015; Zheng et al., 1999). Okazaki et al. (2012) used ddY mice as a model of spontaneously developing IgA nephropathy and have shown that aberrant IgA glycosylation influences the progression of kidney disease.

## Diagnostic Procedures

The immunohistologic identification of IgA-dominant immune deposits in vessels is currently the only accepted diagnostic marker for IgA nephropathy and is a defining feature of IgA vasculitis (Table 65.1) (Jennette et al., 2013a). Although a kidney biopsy may confirm the diagnosis, these are reserved for patients (usually adults) with nontypical presentations or those with severe involvement. The amount of circulating abnormally glycosylated IgA1 or IgG autoantibodies specific for abnormally glycosylated IgA1 may become a useful diagnostic marker in the future (Heineke et al., 2017). Differential diagnosis of IgA vasculitis includes other SVV, viral infections, thrombocytopenic purpura, and SLE. Patients with SVV that has vascular IgA-dominant immune deposits who have unusually aggressive vasculitis or GN should be evaluated for concurrent ANCA disease.

## Treatment

In children, IgA vasculitis usually is a mild, self-limited, vasculitis that does not warrant corticosteroid or cytotoxic therapy (Heineke et al., 2017; Robson and Leung, 1994). Some patients will require symptomatic relief of articular or abdominal pain with nonsteroidal antiinflammatory drugs. Colchicine and dapsone may be used in cases with chronic purpuric lesions (Audemard-Verger et al., 2015). Approximately 5% of the IgA vasculitis patients will develop organ- or life-threatening disease, for example, rapidly progressive GN, significant gastrointestinal bleeding or severe central nervous system (CNS) disease. In these cases high-dose methylprednisolone should be administered; cyclophosphamide, cyclosporine, rituximab, or plasmapheresis may be tried as adjuvant therapy (Audemard-Verger et al., 2015; Gedalia, 2004; Niaudet and Habib, 1998; Pillebout et al., 2010; Tarshish et al., 2004; Ting and Hashkes, 2004).

## CONCLUDING REMARKS—FUTURE PROSPECTS

A variety of pathogenic immunologic mechanisms, including autoimmune processes, mediate necrotizing arteritis and SVV. Clinically, and even pathologically, identical disease can be produced by distinctly different etiologies and pathogenic mechanisms, and a given etiology can produce more than one clinical and pathologic pattern of vasculitis. Because different organs can be affected in different patients, the clinical manifestations of

even relatively specific types of vasculitis are extremely variable among patients. Therefore the diagnosis of systemic vasculitis, including autoimmune vasculitis, is difficult and requires knowledgeable and skillful integration of clinical, pathologic, and laboratory data. Although difficult, precise diagnosis is essential for proper management, because the prognosis and appropriate treatment vary substantially among different categories of vasculitis. As knowledge of pathogenic immunologic mechanisms and inflammatory mediator systems increases, more effective treatments for autoimmune-mediated vasculitis will emerge, which will make precise diagnosis even more important.

## References

- Agard, C., Mounthon, L., Mahr, A., Guillevin, L., 2003. Microscopic polyangiitis and polyarteritis nodosa: how and when do they start? *Arthritis Rheum.* 49 (5), 709–715.
- Agnello, V., 1995. The aetiology of mixed cryoglobulinaemia associated with hepatitis C virus infection. *Scand. J. Immunol.* 42 (2), 179–184.
- Alarcon Segovia, D., Brown Jr, A.L., 1964. Classification and etiologic aspects of necrotizing angiitides: an analytic approach to a confused subject with a critical review of the evidence for hypersensitivity in polyarteritis nodosa. *Mayo Clin. Proc.* 39, 205–222.
- Alba, M.A., Flores-Suarez, L.F., Henderson, A.G., Xiao, H., Hu, P., Nachman, P.H., et al., 2017. Interstitial lung disease in ANCA vasculitis. *Autoimmun. Rev.* 16 (7), 722–729.
- Albert, D., Clarkin, C., Komoroski, J., Brensinger, C.M., Berlin, J.A., 2004. Wegener's granulomatosis: possible role of environmental agents in its pathogenesis. *Arthritis Rheum.* 51 (4), 656–664.
- Allen, A.C., Willis, F.R., Beattie, T.J., Feehally, J., 1998. Abnormal IgA glycosylation in Henoch-Schonlein purpura restricted to patients with clinical nephritis. *Nephrol. Dial. Transplant.* 13 (4), 930–934.
- Amoli, M.M., Calvino, M.C., Garcia-Porrúa, C., Llorca, J., Ollier, W.E., Gonzalez-Gay, M.A., 2004. Interleukin 1beta gene polymorphism association with severe renal manifestations and renal sequelae in Henoch-Schonlein purpura. *J. Rheumatol.* 31 (2), 295–298.
- Amore, A., Coppo, R., Nedrud, J.G., Sigmund, N., Lamm, M.E., Emancipator, S.N., 2004. The role of nasal tolerance in a model of IgA nephropathy induced in mice by Sendai virus. *Clin. Immunol.* 113 (1), 101–108.
- Amoroso, A., Berrino, M., Canale, L., Cornaglia, M., Guarnera, S., Mazzola, G., et al., 1998. Are HLA class II and immunoglobulin constant region genes involved in the pathogenesis of mixed cryoglobulinemia type II after hepatitis C virus infection? *J. Hepatol.* 29 (1), 36–44.
- Audemard-Verger, A., Pillebout, E., Guillevin, L., Thervet, E., Terrier, B., 2015. IgA vasculitis (Henoch-Schonlein purpura) in adults: diagnostic and therapeutic aspects. *Autoimmun. Rev.* 14 (7), 579–585.
- Audemard-Verger, A., Terrier, B., Dechartres, A., Chanal, J., Amoura, Z., Le Gouellec, N., et al., 2017. Characteristics and management of IgA vasculitis (Henoch-Schonlein purpura) in adults: data from the 260 patients included in the IGAVAS survey. *Arthritis Rheum.* 69, 1862–1870.
- Bansal, P.J., Tobin, M.C., 2004. Neonatal microscopic polyangiitis secondary to transfer of maternal myeloperoxidase-antineutrophil cytoplasmic antibody resulting in neonatal pulmonary hemorrhage and renal involvement. *Ann. Allergy Asthma Immunol.* 93 (4), 398–401.
- Bartunkova, J., Kolarova, I., Sediva, A., Holzelova, E., 2002. Antineutrophil cytoplasmic antibodies, anti-saccharomyces cerevisiae antibodies, and specific IgE to food allergens in children with inflammatory bowel diseases. *Clin. Immunol.* 102 (2), 162–168.
- Bautz, D.J., Preston, G.A., Lionaki, S., Hewins, P., Wolberg, A.S., Yang, J.J., et al., 2008. Antibodies with dual reactivity to plasminogen and complementary PR3 in PR3-ANCA vasculitis. *J. Am. Soc. Nephrol.* 19 (12), 2421–2429.
- Bossuyt, X., Cohen Tervaert, J.W., Arimura, Y., Blockmans, D., Flores-Suarez, L.F., Guillevin, L., et al., 2017. Position paper: Revised 2017 international consensus on testing of ANCAs in granulomatosis with polyangiitis and microscopic polyangiitis. *Nat. Rev. Rheumatol.* 13 (11), 683–692.
- Boyd, J.K., Barratt, J., 2011. Inherited IgA glycosylation pattern in IgA nephropathy and HSP nephritis: where do we go next? *Kidney Int.* 80 (1), 8–10.
- Brouet, J.C., Clauvel, J.P., Danon, F., Klein, M., Seligmann, M., 1974. Biologic and clinical significance of cryoglobulins. A report of 86 cases. *Am. J. Med.* 57 (5), 775–788.
- Burns, J.C., Cayan, D.R., Tong, G., Bainto, E.V., Turner, C.L., Shike, H., et al., 2005. Seasonality and temporal clustering of Kawasaki syndrome. *Epidemiology* 16 (2), 220–225.
- Cao, Y., Schmitz, J.L., Yang, J., Hogan, S.L., Bunch, D., Hu, Y., et al., 2011. DRB1\*15 allele is a risk factor for PR3-ANCA disease in African Americans. *J. Am. Soc. Nephrol.* 22 (6), 1161–1167.
- Caorsi, R., Penco, F., Grossi, A., Insalaco, A., Omenetti, A., Alessio, M., et al., 2017. ADA2 deficiency (DADA2) as an unrecognised cause of early onset polyarteritis nodosa and stroke: a multicentre national study. *Ann. Rheum. Dis.* 76, 1648–1656.
- Caorsi, R., Penco, F., Schena, F., Gattorno, M., 2016. Monogenic polyarteritis: the lesson of ADA2 deficiency. *Pediatr. Rheumatol. Online J.* 14 (1), 51.
- Charles, L.A., Falk, R.J., Jennette, J.C., 1989. Reactivity of anti-neutrophil cytoplasmic autoantibodies with HL-60 cells. *Clin. Immunol. Immunopathol.* 53 (2 Pt 1), 243–253.
- Choi, H.K., Merkel, P.A., Walker, A.M., Niles, J.L., 2000. Drug-associated antineutrophil cytoplasmic antibody-positive vasculitis: prevalence among patients with high titers of antimyeloperoxidase antibodies. *Arthritis Rheum.* 43 (2), 405–413.
- Chung, S.A., Seo, P., 2010. Microscopic polyangiitis. *Rheum. Dis. Clin. North Am.* 36 (3), 545–558.
- Churg, J., Strauss, L., 1951. Allergic granulomatosis, allergic angiitis, and periarteritis nodosa. *Am. J. Pathol.* 27 (2), 277–301.
- Ciavatta, D.J., Yang, J., Preston, G.A., Badhwar, A.K., Xiao, H., Hewins, P., et al., 2010. Epigenetic basis for aberrant upregulation of autoantigen genes in humans with ANCA vasculitis. *J. Clin. Invest.* 120 (9), 3209–3219.
- Cohen, E., Sundel, R., 2016. Kawasaki disease at 50 years. *JAMA Pediatr.* 170 (11), 1093–1099.

- Comarmond, C., Pagnoux, C., Khellaf, M., Cordier, J.F., Hamidou, M., Viallard, J.F., et al., 2013. Eosinophilic granulomatosis with polyangiitis (Churg-Strauss): clinical characteristics and long-term followup of the 383 patients enrolled in the French Vasculitis Study Group cohort. *Arthritis Rheum.* 65 (1), 270–281.
- Congia, M., Clemente, M.G., Dessi, C., Cucca, F., Mazzoleni, A.P., Frau, F., et al., 1996. HLA class II genes in chronic hepatitis C virus-infection and associated immunological disorders. *Hepatology* 24 (6), 1338–1341.
- Coppo, R., Andrulli, S., Amore, A., Gianoglio, B., Conti, G., Peruzzi, L., et al., 2006. Predictors of outcome in Henoch-Schonlein nephritis in children and adults. *Am. J. Kidney Dis.* 47 (6), 993–1003.
- Coppo, R., Chiesa, M., Cirina, P., Peruzzi, L., Amore, A., European Ig ACESG, 2002. In human IgA nephropathy uteroglobin does not play the role inferred from transgenic mice. *Am. J. Kidney Dis.* 40 (3), 495–503.
- Cordier, J.F., Valeyre, D., Guillemin, L., Loire, R., Brechot, J.M., 1990. Pulmonary Wegener's granulomatosis. A clinical and imaging study of 77 cases. *Chest* 97 (4), 906–912.
- Csernok, E., Damoiseaux, J., Rasmussen, N., Hellmich, B., van Paassen, P., Vermeersch, P., et al., 2016. Evaluation of automated multi-parametric indirect immunofluorescence assays to detect anti-neutrophil cytoplasmic antibodies (ANCA) in granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA). *Autoimmun. Rev.* 15 (7), 736–741.
- Cunningham, M.W., Meissner, H.C., Heuser, J.S., Pietra, B.A., Kurahara, D.K., Leung, D.Y., 1999. Anti-human cardiac myosin autoantibodies in Kawasaki syndrome. *J. Immunol.* 163 (2), 1060–1065.
- D'Amico, G., Colasanti, G., Ferrario, F., Sinico, R.A., 1989. Renal involvement in essential mixed cryoglobulinemia. *Kidney Int.* 35 (4), 1004–1014.
- D'Amico, G., Fornasieri, A., 1995. Cryoglobulinemic glomerulonephritis: a membranoproliferative glomerulonephritis induced by hepatitis C virus. *Am. J. Kidney Dis.* 25 (3), 361–369.
- Damoiseaux, J., Csernok, E., Rasmussen, N., Moosig, F., van Paassen, P., Baslund, B., et al., 2017. Detection of antineutrophil cytoplasmic antibodies (ANCA): a multicentre European Vasculitis Study Group (EUVAS) evaluation of the value of indirect immunofluorescence (IIF) versus antigen-specific immunoassays. *Ann. Rheum. Dis.* 76 (4), 647–653.
- Davies, D.J., Moran, J.E., Niall, J.F., Ryan, G.B., 1982. Segmental necrotising glomerulonephritis with antineutrophil antibody: possible arbovirus aetiology? *Br. Med. J. (Clin. Res. Ed.)* 285 (6342), 606.
- Davin, J.C., Malaise, M., Foidart, J., Mahieu, P., 1987. Anti-alpha-galactosyl antibodies and immune complexes in children with Henoch-Schonlein purpura or IgA nephropathy. *Kidney Int.* 31 (5), 1132–1139.
- Davson, J., Ball, J., Platt, R., 1948. The kidney in periarteritis nodosa. *Q. J. Med.* 17 (67), 175–202.
- De Groot, K., Rasmussen, N., Bacon, P.A., Tervaert, J.W., Feighery, C., Gregorini, G., et al., 2005. Randomized trial of cyclophosphamide versus methotrexate for induction of remission in early systemic antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum.* 52 (8), 2461–2469.
- de Groot, K., Harper, L., Jayne, D.R., Flores Suarez, L.F., Gregorini, G., Gross, W.L., et al., 2009. Pulse versus daily oral cyclophosphamide for induction of remission in antineutrophil cytoplasmic antibody-associated vasculitis: a randomized trial. *Ann. Intern. Med.* 150 (10), 670–680.
- De Vita, S., Soldano, F., Isola, M., Monti, G., Gabrielli, A., Tzioufas, A., et al., 2011. Preliminary classification criteria for the cryoglobulinaemic vasculitis. *Ann. Rheum. Dis.* 70 (7), 1183–1190.
- Duong, T.T., Silverman, E.D., Bissessar, M.V., Yeung, R.S., 2003. Superantigenic activity is responsible for induction of coronary arteritis in mice: an animal model of Kawasaki disease. *Int. Immunol.* 15 (1), 79–89.
- Eisenberger, U., Fakhouri, F., Vanhille, P., Beaufils, H., Mahr, A., Guillemin, L., et al., 2005. ANCA-negative pauci-immune renal vasculitis: histology and outcome. *Nephrol. Dial. Transplant.* 20 (7), 1392–1399.
- Ewert, B.H., Jennette, J.C., Falk, R.J., 1992. Anti-myeloperoxidase antibodies stimulate neutrophils to damage human endothelial cells. *Kidney Int.* 41 (2), 375–383.
- Faille-Kuyper, E.H., Kater, L., Kuijten, R.H., Kooiker, C.J., Wagenaar, S.S., van der Zouwen, P., et al., 1976. Occurrence of vascular IgA deposits in clinically normal skin of patients with renal disease. *Kidney Int.* 9 (5), 424–429.
- Falk, R.J., Jennette, J.C., 1988. Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis. *N. Engl. J. Med.* 318, 1651–1657.
- Falk, R.J., Hogan, S., Carey, T.S., Jennette, J.C., 1990a. Clinical course of anti-neutrophil cytoplasmic autoantibody-associated glomerulonephritis and systemic vasculitis. The Glomerular Disease Collaborative Network. *Ann. Intern. Med.* 113 (9), 656–663.
- Falk, R.J., Terrell, R.S., Charles, L.A., Jennette, J.C., 1990b. Anti-neutrophil cytoplasmic autoantibodies induce neutrophils to degranulate and produce oxygen radicals in vitro. *Proc. Natl. Acad. Sci. U.S.A.* 87 (11), 4115–4119.
- Falk, R.J., Jennette, J.C., 2010. ANCA disease: where is this field heading? *J. Am. Soc. Nephrol.* 21 (5), 745–752.
- Fauci, A.S., Haynes, B., Katz, P., 1978. The spectrum of vasculitis: clinical, pathologic, immunologic and therapeutic considerations. *Ann. Intern. Med.* 89 (5 Pt 1), 660–676.
- Ferri, C., 2008. Mixed cryoglobulinemia. *Orphanet J. Rare Dis.* 3, 25.
- Ferri, C., Sebastiani, M., Giuggioli, D., Cazzato, M., Longombardo, G., Antonelli, A., et al., 2004. Mixed cryoglobulinemia: demographic, clinical, and serologic features and survival in 231 patients. *Semin. Arthritis Rheum.* 33 (6), 355–374.
- Finkelman, J.D., Lee, A.S., Hummel, A.M., Viss, M.A., Jacob, G.L., Homburger, H.A., et al., 2007. ANCA are detectable in nearly all patients with active severe Wegener's granulomatosis. *Am. J. Med.* 120 (7), 643.e9–643.14.
- Franco, A., Shimizu, C., Tremoulet, A.H., Burns, J.C., 2010. Memory T-cells and characterization of peripheral T-cell clones in acute Kawasaki disease. *Autoimmunity* 43 (4), 317–324.
- Fugo, K., Ishizu, A., Ikeda, H., Hayase, H., Sugaya, T., Higuchi, M., et al., 2002. The role of the thymus in development of necrotizing arteritis in transgenic rats carrying the env-pX gene of human T-cell leukemia virus type-I. *Am. J. Pathol.* 161 (3), 755–761.
- Fujita, Y., Nakamura, Y., Sakata, K., Hara, N., Kobayashi, M., Nagai, M., et al., 1989. Kawasaki disease in families. *Pediatrics* 84 (4), 666–669.
- Fukushige, J., Takahashi, N., Ueda, Y., Ueda, K., 1994. Incidence and clinical features of incomplete Kawasaki disease. *Acta Paediatr.* 83 (10), 1057–1060.

- Furusho, K., Kamiya, T., Nakano, H., Kiyosawa, N., Shinomiya, K., Hayashidera, T., et al., 1984. High-dose intravenous gammaglobulin for Kawasaki disease. *Lancet* 2 (8411), 1055–1058.
- Gardner-Medwin, J.M., Dolezalova, P., Cummins, C., Southwood, T.R., 2002. Incidence of Henoch-Schonlein purpura, Kawasaki disease, and rare vasculitides in children of different ethnic origins. *Lancet* 360 (9341), 1197–1202.
- Gaudin, P.B., Askin, F.B., Falk, R.J., Jennette, J.C., 1995. The pathologic spectrum of pulmonary lesions in patients with anti-neutrophil cytoplasmic autoantibodies specific for anti-proteinase 3 and anti-myeloperoxidase. *Am. J. Clin. Pathol.* 104 (1), 7–16.
- Gayraud, M., Guillemin, L., le Toumelin, P., Cohen, P., Lhote, F., Casassus, P., et al., 2001. Long-term followup of polyarteritis nodosa, microscopic polyangiitis, and Churg-Strauss syndrome: analysis of four prospective trials including 278 patients. *Arthritis Rheum.* 44 (3), 666–675.
- Gedalia, A., 2004. Henoch-Schonlein purpura. *Curr. Rheumatol. Rep.* 6 (3), 195–202.
- Godman, G.C., Churg, J., 1954. Wegener's granulomatosis: pathology and review of the literature. *AMA Arch. Pathol.* 58 (6), 533–553.
- Goldschmeding, R., van der Schoot, C.E., ten Bokkel Huinink, D., Hack, C.E., van den Ende, M.E., Kallenberg, C.G., et al., 1989. Wegener's granulomatosis autoantibodies identify a novel diisopropylfluorophosphate-binding protein in the lysosomes of normal human neutrophils. *J. Clin. Invest.* 84 (5), 1577–1587.
- Gonzalez-Gay, M.A., Calvino, M.C., Vazquez-Lopez, M.E., Garcia-Porrúa, C., Fernandez-Iglesias, J.L., Dierssen, T., et al., 2004. Implications of upper respiratory tract infections and drugs in the clinical spectrum of Henoch-Schonlein purpura in children. *Clin. Exp. Rheumatol.* 22 (6), 781–784.
- Goodpasture, E.W., 2009. Landmark publication from The American Journal of the Medical Sciences: the significance of certain pulmonary lesions in relation to the etiology of influenza. *Am. J. Med. Sci.* 338 (2), 148–151.
- Gragnani, L., Visentini, M., Fognani, E., Urraro, T., De Santis, A., Petraccia, L., et al., 2016. Prospective study of guideline-tailored therapy with direct-acting antivirals for hepatitis C virus-associated mixed cryoglobulinemia. *Hepatology* 64 (5), 1473–1482.
- Groh, M., Pagnoux, C., Baldini, C., Bel, E., Bottero, P., Cottin, V., et al., 2015. Eosinophilic granulomatosis with polyangiitis (Churg-Strauss) (EGPA) Consensus Task Force recommendations for evaluation and management. *Eur. J. Intern. Med.* 26 (7), 545–553.
- Grunebaum, E., Blank, M., Cohen, S., Afek, A., Kopolovic, J., Meroni, P.L., et al., 2002. The role of anti-endothelial cell antibodies in Kawasaki disease—in vitro and in vivo studies. *Clin. Exp. Immunol.* 130 (2), 233–240.
- Guillemin, L., 1999. Treatment of classic polyarteritis nodosa in 1999. *Nephrol. Dial. Transplant.* 14 (9), 2077–2079.
- Guillemin, L., Lhote, F., Cohen, P., Jarrousse, B., Lortholary, O., Genereau, T., et al., 1995. Corticosteroids plus pulse cyclophosphamide and plasma exchanges versus corticosteroids plus pulse cyclophosphamide alone in the treatment of polyarteritis nodosa and Churg-Strauss syndrome patients with factors predicting poor prognosis. A prospective, randomized trial in sixty-two patients. *Arthritis Rheum.* 38 (11), 1638–1645.
- Guillemin, L., Durand-Gasselin, B., Cevallos, R., Gayraud, M., Lhote, F., Callard, P., et al., 1999. Microscopic polyangiitis: clinical and laboratory findings in eighty-five patients. *Arthritis Rheum.* 42 (3), 421–430.
- Guillemin, L., Mahr, A., Callard, P., Godmer, P., Pagnoux, C., Leray, E., et al., 2005. Hepatitis B virus-associated polyarteritis nodosa: clinical characteristics, outcome, and impact of treatment in 115 patients. *Medicine (Baltimore)* 84 (5), 313–322.
- Gyotoku, Y., Abdelmoula, M., Spertini, F., Izui, S., Lambert, P.H., 1987. Cryoglobulinemia induced by monoclonal immunoglobulin G rheumatoid factors derived from autoimmune MRL/Mpj-lpr/lpr mice. *J. Immunol.* 138 (11), 3785–3792.
- Han, F., Liu, G., Zhang, X., Li, X., He, Q., He, X., et al., 2011. Effects of mycophenolate mofetil combined with corticosteroids for induction therapy of microscopic polyangiitis. *Am. J. Nephrol.* 33 (2), 185–192.
- Han, S.H., 2004. Extrahepatic manifestations of chronic hepatitis B. *Clin. Liver Dis.* 8 (2), 403–418.
- Han, W.K., Choi, H.K., Roth, R.M., McCluskey, R.T., Niles, J.L., 2003. Serial ANCA titers: useful tool for prevention of relapses in ANCA-associated vasculitis. *Kidney Int.* 63 (3), 1079–1085.
- Harel, S., Mohr, M., Jahn, I., Aucoeurier, F., Galicier, L., Asli, B., et al., 2015. Clinico-biological characteristics and treatment of type I monoclonal cryoglobulinaemia: a study of 64 cases. *Br. J. Haematol.* 168 (5), 671–678.
- Hasler, P., Kistler, H., Gerber, H., 1995. Vasculitides in hairy cell leukemia. *Semin. Arthritis Rheum.* 25 (2), 134–142.
- Heineke, M.H., Ballering, A.V., Jamin, A., Ben Mkaddem, S., Monteiro, R.C., Van Egmond, M., 2017. New insights in the pathogenesis of immunoglobulin A vasculitis (Henoch-Schonlein purpura). *Autoimmun. Rev.* 16 (12), 1246–1253.
- Henoch, E., 1868. Über den Zusammenhang von Purpura und intestinal-stoerungen. *Berl. Klin. Wochenschr.* 5, 517–519.
- Hernandez-Rodriguez, J., Alba, M.A., Prieto-Gonzalez, S., Cid, M.C., 2014. Diagnosis and classification of polyarteritis nodosa. *J. Autoimmun.* 48–49, 84–89.
- Hirabayashi, Y., Takahashi, Y., Xu, Y., Akane, K., Villalobos, I.B., Okuno, Y., et al., 2013. Lack of CD4(+)CD25(+)FOXP3(+) regulatory T cells is associated with resistance to intravenous immunoglobulin therapy in patients with Kawasaki disease. *Eur. J. Pediatr.* 172 (6), 833–837.
- Hocevar, A., Rotar, Z., Ostrovrsnik, J., Jurcic, V., Vizjak, A., Dolenc Voljc, M., et al., 2014. Incidence of IgA vasculitis in the adult Slovenian population. *Br. J. Dermatol.* 171 (3), 524–527.
- Hoffman, G.S., Kerr, G.S., Leavitt, R.Y., Hallahan, C.W., Lebovics, R.S., Travis, W.D., et al., 1992. Wegener granulomatosis: an analysis of 158 patients. *Ann. Intern. Med.* 116 (6), 488–498.
- Hogan, S.L., Satterly, K.K., Dooley, M.A., Nachman, P.H., Jennette, J.C., Falk, R.J., et al., 2001. Silica exposure in anti-neutrophil cytoplasmic autoantibody-associated glomerulonephritis and lupus nephritis. *J. Am. Soc. Nephrol.* 12 (1), 134–142.
- Hogan, S.L., Falk, R.J., Chin, H., Cai, J., Jennette, C.E., Jennette, J.C., et al., 2005. Predictors of relapse and treatment resistance in antineutrophil cytoplasmic antibody-associated small-vessel vasculitis. *Ann. Intern. Med.* 143 (9), 621–631.
- Holle, J.U., Gross, W.L., Latza, U., Nolle, B., Ambrosch, P., Heller, M., et al., 2011. Improved outcome in 445 patients with Wegener's granulomatosis in a German vasculitis center over four decades. *Arthritis Rheum.* 63 (1), 257–266.
- Huugen, D., Xiao, H., van Esch, A., Falk, R.J., Peutz-Kootstra, C.J., Buurman, W.A., et al., 2005. Aggravation of anti-myeloperoxidase antibody-induced glomerulonephritis by bacterial lipopolysaccharide: role of tumor necrosis factor-alpha. *Am. J. Pathol.* 167 (1), 47–58.
- Janssen, H.L., van Zonneveld, M., van Nunen, A.B., Niesters, H.G., Schalm, S.W., de Man, R.A., 2004. Polyarteritis nodosa associated with hepatitis B virus infection. The role of antiviral treatment and mutations in the hepatitis B virus genome. *Eur. J. Gastroenterol. Hepatol.* 16 (8), 801–807.

- Jayne, D., 2009. The diagnosis of vasculitis. *Best Pract. Res. Clin. Rheumatol.* 23 (3), 445–453.
- Jayne, D., Rasmussen, N., Andrassy, K., Bacon, P., Tervaert, J.W., Dadoniene, J., et al., 2003. A randomized trial of maintenance therapy for vasculitis associated with antineutrophil cytoplasmic autoantibodies. *N. Engl. J. Med.* 349 (1), 36–44.
- Jayne, D.R., Gaskin, G., Rasmussen, N., Abramowicz, D., Ferrario, F., Guillemin, L., et al., 2007. Randomized trial of plasma exchange or high-dosage methylprednisolone as adjunctive therapy for severe renal vasculitis. *J. Am. Soc. Nephrol.* 18 (7), 2180–2188.
- Jennette, J.C., 1991. Antineutrophil cytoplasmic autoantibody-associated diseases: a pathologist's perspective. *Am. J. Kidney Dis.* 18 (2), 164–170.
- Jennette, J.C., 2002. Implications for pathogenesis of patterns of injury in small- and medium-sized-vessel vasculitis. *Cleve Clin. J. Med.* 69 (Suppl. 2), SII33–SII38.
- Jennette, J.C., 2003. Rapidly progressive crescentic glomerulonephritis. *Kidney Int.* 63 (3), 1164–1177.
- Jennette, J.C., Falk, R.J., 1993. Antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease. *Am. J. Clin. Pathol.* 99 (3), 221–223.
- Jennette, J.C., Falk, R.J., 1997. Small-vessel vasculitis. *N. Engl. J. Med.* 337 (21), 1512–1523.
- Jennette, J.C., Falk, R.J., 1998. Pathogenesis of the vascular and glomerular damage in ANCA-positive vasculitis. *Nephrol. Dial. Transplant.* 13 (Suppl. 1), 16–20.
- Jennette, J.C., Falk, R.J., 2014. Pathogenesis of antineutrophil cytoplasmic autoantibody-mediated disease. *Nat. Rev. Rheumatol.* 10 (8), 463–473.
- Jennette, J.C., Nachman, P.H., 2017. ANCA glomerulonephritis and vasculitis. *Clin. J. Am. Soc. Nephrol.* 12 (10), 1680–1691.
- Jennette, J.C., Hoidal, J.R., Falk, R.J., 1990. Specificity of anti-neutrophil cytoplasmic autoantibodies for proteinase 3. *Blood* 75 (11), 2263–2264.
- Jennette, J.C., Falk, R.J., Andrassy, K., Bacon, P.A., Churg, J., Gross, W.L., et al., 1994. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum.* 37 (2), 187–192.
- Jennette, J.C., Falk, R.J., Bacon, P.A., Basu, N., Cid, M.C., Ferrario, F., et al., 2013a. 2012 Revised international Chapel Hill consensus conference nomenclature of vasculitides. *Arthritis Rheum.* 65 (1), 1–11.
- Jennette, J.C., Falk, R.J., Hu, P., Xiao, H., 2013b. Pathogenesis of antineutrophil cytoplasmic autoantibody-associated small-vessel vasculitis. *Annu. Rev. Pathol.* 8, 139–160.
- Jones, H., Tocantins, L., 1933. The history of purpura hemorrhagica. *Ann. Med. Hist.* 15 (5), 349–364.
- Kain, R., Exner, M., Brandes, R., Ziebermayr, R., Cunningham, D., Alderson, C.A., et al., 2008. Molecular mimicry in pauci-immune focal necrotizing glomerulonephritis. *Nat. Med.* 14 (10), 1088–1096.
- Kallenberg, C.G., Brouwer, E., Weening, J.J., Tervaert, J.W., 1994. Anti-neutrophil cytoplasmic antibodies: current diagnostic and pathophysiological potential. *Kidney Int.* 46 (1), 1–15.
- Kaneko, M., Ono, T., Matsubara, T., Yamamoto, Y., Ikeda, H., Yoshiki, T., et al., 2004. Serological identification of endothelial antigens predominantly recognized in Kawasaki disease patients by recombinant expression cloning. *Microbiol. Immunol.* 48 (9), 703–711.
- Kawakami, T., Watabe, H., Mizoguchi, M., Soma, Y., 2006. Elevated serum IgA anticardiolipin antibody levels in adult Henoch-Schonlein purpura. *Br. J. Dermatol.* 155 (5), 983–987.
- Kawasaki, T., 1967. Acute febrile mucocutaneous syndrome with lymphoid involvement with specific desquamation of the fingers and toes in children. *Arerugi* 16 (3), 178–222.
- Kikuchi, S., Pastore, Y., Fossati-Jimack, L., Kuroki, A., Yoshida, H., Fulpius, T., et al., 2002. A transgenic mouse model of autoimmune glomerulonephritis and necrotizing arteritis associated with cryoglobulinemia. *J. Immunol.* 169 (8), 4644–4650.
- Kiryuk, K., Moldoveanu, Z., Sanders, J.T., Eison, T.M., Suzuki, H., Julian, B.A., et al., 2011. Aberrant glycosylation of IgA1 is inherited in both pediatric IgA nephropathy and Henoch-Schonlein purpura nephritis. *Kidney Int.* 80 (1), 79–87.
- Klemmer, P.J., Chalermkulrat, W., Reif, M.S., Hogan, S.L., Henke, D.C., Falk, R.J., 2003. Plasmapheresis therapy for diffuse alveolar hemorrhage in patients with small-vessel vasculitis. *Am. J. Kidney Dis.* 42 (6), 1149–1153.
- Klinger, H., 1931. Grenzformen der periarteritis nodosa. *Frankfurt Z. Pathol.* 42, 455.
- Korbet, S.M., Schwartz, M.M., Lewis, E.J., 1984. Immune complex deposition and coronary vasculitis in systemic lupus erythematosus. Report of two cases. *Am. J. Med.* 77 (1), 141–146.
- Kussmaul, A., Maier, R., 1866. Über eine bisher nicht beschriebene eigenthümliche Arterienerkrankung (Periarteritis nodosa), die mit Morbus Brightii und rapid fortschreitender allgemeiner Muskellähmung einhergeht. *Dtsch. Arch. Klin. Med.* 1, 484–518.
- Lamprecht, P., Gross, W.L., 2007. Current knowledge on cellular interactions in the WG-granuloma. *Clin. Exp. Rheumatol.* 25 (1Suppl. 44), S49–S51.
- Lane, S.E., Watts, R.A., Benthall, G., Innes, N.J., Scott, D.G., 2003. Are environmental factors important in primary systemic vasculitis? A case-control study. *Arthritis Rheum.* 48 (3), 814–823.
- Lau, K.K., Wyatt, R.J., Moldoveanu, Z., Tomana, M., Julian, B.A., Hogg, R.J., et al., 2007. Serum levels of galactose-deficient IgA in children with IgA nephropathy and Henoch-Schonlein purpura. *Pediatr. Nephrol.* 22 (12), 2067–2072.
- Lee, Y.C., Kuo, H.C., Chang, J.S., Chang, L.Y., Huang, L.M., Chen, M.R., et al., 2012. Two new susceptibility loci for Kawasaki disease identified through genome-wide association analysis. *Nat. Genet.* 44 (5), 522–525.
- Lerner, R.A., Glassock, R.J., Dixon, F.J., 1967. The role of anti-glomerular basement membrane antibody in the pathogenesis of human glomerulonephritis. *J. Exp. Med.* 126 (6), 989–1004.
- Leung, D.Y., Collins, T., Lapierre, L.A., Geha, R.S., Poer, J.S., 1986a. Immunoglobulin M antibodies present in the acute phase of Kawasaki syndrome lyse cultured vascular endothelial cells stimulated by gamma interferon. *J. Clin. Invest.* 77 (5), 1428–1435.
- Leung, D.Y., Geha, R.S., Newburger, J.W., Burns, J.C., Fiers, W., Lapierre, L.A., et al., 1986b. Two monokines, interleukin 1 and tumor necrosis factor, render cultured vascular endothelial cells susceptible to lysis by antibodies circulating during Kawasaki syndrome. *J. Exp. Med.* 164 (6), 1958–1972.
- Leung, D.Y., Cotran, R.S., Kurt-Jones, E., Burns, J.C., Newburger, J.W., Poer, J.S., 1989. Endothelial cell activation and high interleukin-1 secretion in the pathogenesis of acute Kawasaki disease. *Lancet* 2 (8675), 1298–1302.
- Lim, L.C., Taylor 3rd, J.G., Schmitz, J.L., Folds, J.D., Wilkman, A.S., Falk, R.J., et al., 1999. Diagnostic usefulness of antineutrophil cytoplasmic autoantibody serology. Comparative evaluation of commercial indirect fluorescent antibody kits and enzyme immunoassay kits. *Am. J. Clin. Pathol.* 111 (3), 363–369.

- Lin, D., Li, S., Xu, H., Chen, H., Dong, Z., 2015. Association of uteroglobin G38A gene polymorphism with IgA nephropathy risk: an updated meta-analysis. *J. Recept. Signal Transduct. Res.* 35 (2), 115–121.
- Lionaki, S., Blyth, E.R., Hogan, S.L., Hu, Y., Senior, B.A., Jennette, C.E., et al., 2012. Classification of antineutrophil cytoplasmic autoantibody vasculitides: the role of antineutrophil cytoplasmic autoantibody specificity for myeloperoxidase or proteinase 3 in disease recognition and prognosis. *Arthritis Rheum.* 64 (10), 3452–3462.
- Lopez-Mejias, R., Carmona, F.D., Castaneda, S., Genre, F., Remuzgo-Martinez, S., Sevilla-Perez, B., et al., 2017. A genome-wide association study suggests the HLA Class II region as the major susceptibility locus for IgA vasculitis. *Sci. Rep.* 7 (1), 5088.
- Lyons, P.A., Rayner, T.F., Trivedi, S., Holle, J.U., Watts, R.A., Jayne, D.R., et al., 2012. Genetically distinct subsets within ANCA-associated vasculitis. *N. Engl. J. Med.* 367 (3), 214–223.
- Magilavy, D.B., Petty, R.E., Cassidy, J.T., Sullivan, D.B., 1977. A syndrome of childhood polyarteritis. *J. Pediatr.* 91 (1), 25–30.
- Mahr, A., Guillemin, L., Poissonnet, M., Ayme, S., 2004. Prevalences of polyarteritis nodosa, microscopic polyangiitis, Wegener's granulomatosis, and Churg-Strauss syndrome in a French urban multiethnic population in 2000: a capture-recapture estimate. *Arthritis Rheum.* 51 (1), 92–99.
- Masuda, M., Nakanishi, K., Yoshizawa, N., Iijima, K., Yoshikawa, N., 2003. Group A streptococcal antigen in the glomeruli of children with Henoch-Schonlein nephritis. *Am. J. Kidney Dis.* 41 (2), 366–370.
- Matsubara, T., Ichiyama, T., Furukawa, S., 2005. Immunological profile of peripheral blood lymphocytes and monocytes/macrophages in Kawasaki disease. *Clin. Exp. Immunol.* 141 (3), 381–387.
- McAdoo, S.P., Tanna, A., Hruskova, Z., Holm, L., Weiner, M., Arulkumaran, N., et al., 2017. Patients double-seropositive for ANCA and anti-GBM antibodies have varied renal survival, frequency of relapse, and outcomes compared to single-seropositive patients. *Kidney Int.* 92 (3), 693–702.
- McCredie, B.W., Rowley, A.H., Newburger, J.W., Burns, J.C., Bolger, A.F., Gewitz, M., et al., 2017. Diagnosis, treatment, and long-term management of Kawasaki disease: a scientific statement for health professionals from the American Heart Association. *Circulation* 135 (17), e927–e999.
- Meltzer, M., Franklin, E.C., 1966. Cryoglobulinemia—a study of twenty-nine patients. I. IgG and IgM cryoglobulins and factors affecting cryoprecipitability. *Am. J. Med.* 40 (6), 828–836.
- Meltzer, M., Franklin, E.C., Elias, K., McCluskey, R.T., Cooper, N., 1966. Cryoglobulinemia—a clinical and laboratory study. II. Cryoglobulins with rheumatoid factor activity. *Am. J. Med.* 40 (6), 837–856.
- Merkel, P.A., Xie, G., Monach, P.A., Ji, X., Ciavatta, D.J., Byun, J., et al., 2017. Identification of functional and expression polymorphisms associated with risk for antineutrophil cytoplasmic autoantibody-associated vasculitis. *Arthritis Rheum.* 69 (5), 1054–1066.
- Mohammad, A.J., Jacobsson, L.T., Mahr, A.D., Sturfelt, G., Segelmark, M., 2007. Prevalence of Wegener's granulomatosis, microscopic polyangiitis, polyarteritis nodosa and Churg-Strauss syndrome within a defined population in southern Sweden. *Rheumatology (Oxford)* 46 (8), 1329–1337.
- Mohammad, A.J., Nilsson, J.A., Jacobsson, L.T., Merkel, P.A., Turesson, C., 2014. Incidence and mortality rates of biopsy-proven giant cell arteritis in southern Sweden. *Ann. Rheum. Dis.* 74, 993–997.
- Monti, G., Galli, M., Invernizzi, F., Pioltelli, P., Saccardo, F., Monteverde, A., et al., 1995. Cryoglobulinaemias: a multi-centre study of the early clinical and laboratory manifestations of primary and secondary disease. GISCI. Italian Group for the Study of Cryoglobulinaemias. *QJM* 88 (2), 115–126.
- Muchtar, E., Magen, H., Gertz, M.A., 2017. How I treat cryoglobulinemia. *Blood* 129 (3), 289–298.
- Nagashima, M., Matsushima, M., Matsuoka, H., Ogawa, A., Okumura, N., 1987. High-dose gammaglobulin therapy for Kawasaki disease. *J. Pediatr.* 110 (5), 710–712.
- Navon Elkan, P., Pierce, S.B., Segel, R., Walsh, T., Barash, J., Padeh, S., et al., 2014. Mutant adenosine deaminase 2 in a polyarteritis nodosa vasculopathy. *N. Engl. J. Med.* 370 (10), 921–931.
- Newburger, J.W., Fulton, D.R., 2004. Kawasaki disease. *Curr. Opin. Pediatr.* 16 (5), 508–514.
- Niaudet, P., Habib, R., 1998. Methylprednisolone pulse therapy in the treatment of severe forms of Schonlein-Henoch purpura nephritis. *Pediatr. Nephrol.* 12 (3), 238–243.
- Niles, J.L., McCluskey, R.T., Ahmad, M.F., Arnaout, M.A., 1989. Wegener's granulomatosis autoantigen is a novel neutrophil serine proteinase. *Blood* 74 (6), 1888–1893.
- Ntatsaki, E., Watts, R.A., Scott, D.G., 2010. Epidemiology of ANCA-associated vasculitis. *Rheum. Dis. Clin. North Am.* 36 (3), 447–461.
- Okazaki, K., Suzuki, Y., Otsuji, M., Suzuki, H., Kihara, M., Kajiyama, T., et al., 2012. Development of a model of early-onset IgA nephropathy. *J. Am. Soc. Nephrol.* 23 (8), 1364–1374.
- Onouchi, Y., Gunji, T., Burns, J.C., Shimizu, C., Newburger, J.W., Yashiro, M., et al., 2008. ITPKC functional polymorphism associated with Kawasaki disease susceptibility and formation of coronary artery aneurysms. *Nat. Genet.* 40 (1), 35–42.
- Onouchi, Y., Ozaki, K., Burns, J.C., Shimizu, C., Terai, M., Hamada, H., et al., 2012. A genome-wide association study identifies three new risk loci for Kawasaki disease. *Nat. Genet.* 44 (5), 517–521.
- Osler, W., 1914. The visceral lesions of purpura and allied conditions. *Br. Med. J.* 1 (2775), 517–525.
- Ozdemir, H., Ciftci, E., Tapisiz, A., Ince, E., Tutar, E., Atalay, S., et al., 2010. Clinical and epidemiological characteristics of children with Kawasaki disease in Turkey. *J. Trop. Pediatr.* 56 (4), 260–262.
- Ozen, S., 2017. The changing face of polyarteritis nodosa and necrotizing vasculitis. *Nat. Rev. Rheumatol.* 13 (6), 381–386.
- Ozen, S., Ben-Chetrit, E., Bakkaloglu, A., Gur, H., Tinaztepe, K., Calguneri, M., et al., 2001. Polyarteritis nodosa in patients with Familial Mediterranean Fever (FMF): a concomitant disease or a feature of FMF? *Semin. Arthritis Rheum.* 30 (4), 281–287.
- Pagnoux, C., Guillemin, L., French Vasculitis Study, G., investigators, M., 2015. Rituximab or azathioprine maintenance in ANCA-associated vasculitis. *N. Engl. J. Med.* 372 (4), 386–387.
- Pagnoux, C., Mahr, A., Hamidou, M.A., Boffa, J.J., Ruivard, M., Ducroix, J.P., et al., 2008. Azathioprine or methotrexate maintenance for ANCA-associated vasculitis. *N. Engl. J. Med.* 359 (26), 2790–2803.
- Pagnoux, C., Seror, R., Henegar, C., Mahr, A., Cohen, P., Le Guern, V., et al., 2010. Clinical features and outcomes in 348 patients with polyarteritis nodosa: a systematic retrospective study of patients diagnosed between 1963 and 2005 and entered into the French Vasculitis Study Group Database. *Arthritis Rheum.* 62 (2), 616–626.

- Pendergraft 3rd, W.F., Niles, J.L., 2014. Trojan horses: drug culprits associated with antineutrophil cytoplasmic autoantibody (ANCA) vasculitis. *Curr. Opin. Rheumatol.* 26 (1), 42–49.
- Pendergraft 3rd, W.F., Preston, G.A., Shah, R.R., Tropsha, A., Carter Jr., C.W., Jennette, J.C., et al., 2004. Autoimmunity is triggered by cPR-3 (105-201), a protein complementary to human autoantigen proteinase-3. *Nat. Med.* 10 (1), 72–79.
- Pillebout, E., Alberti, C., Guillemin, L., Ouslimani, A., Thervet, E., group Cs, 2010. Addition of cyclophosphamide to steroids provides no benefit compared with steroids alone in treating adult patients with severe Henoch Schonlein Purpura. *Kidney Int.* 78 (5), 495–502.
- Pillebout, E., Thervet, E., Hill, G., Alberti, C., Vanhille, P., Nochy, D., 2002. Henoch-Schonlein Purpura in adults: outcome and prognostic factors. *J. Am. Soc. Nephrol.* 13 (5), 1271–1278.
- Podjasek, J.O., Wetter, D.A., Wieland, C.N., Camilleri, M.J., Lohse, C.M., 2014. Histopathological findings in cutaneous small-vessel vasculitis associated with solid-organ malignancy. *Br. J. Dermatol.* 171 (6), 1397–1401.
- Puechal, X., Pagnoux, C., Baron, G., Quemeneur, T., Neel, A., Agard, C., et al., 2017. Adding azathioprine to remission-induction glucocorticoids for eosinophilic granulomatosis with polyangiitis (Churg-Strauss), microscopic polyangiitis, or polyarteritis nodosa without poor prognosis factors: a randomized, controlled trial. *Arthritis Rheum.* 69 (11), 2175–2186.
- Radford, D.J., Savage, C.O., Nash, G.B., 2000. Treatment of rolling neutrophils with antineutrophil cytoplasmic antibodies causes conversion to firm integrin-mediated adhesion. *Arthritis Rheum.* 43 (6), 1337–1345.
- Ramos-Casals, M., Stone, J.H., Cid, M.C., Bosch, X., 2012. The cryoglobulinaemias. *Lancet* 379 (9813), 348–360.
- Rarok, A.A., Limburg, P.C., Kallenberg, C.G., 2003. Neutrophil-activating potential of antineutrophil cytoplasm autoantibodies. *J. Leukoc. Biol.* 74 (1), 3–15.
- Robson, W.L., Leung, A.K., 1994. Henoch-Schonlein purpura. *Adv. Pediatr.* 41, 163–194.
- Rodo, X., Ballester, J., Cayan, D., Melish, M.E., Nakamura, Y., Uehara, R., et al., 2011. Association of Kawasaki disease with tropospheric wind patterns. *Sci. Rep.* 1, 152.
- Ronco, P., Verroust, P., Mignon, F., Kourilsky, O., Vanhille, P., Meyrier, A., et al., 1983. Immunopathological studies of polyarteritis nodosa and Wegener's granulomatosis: a report of 43 patients with 51 renal biopsies. *Q. J. Med.* 52 (206), 212–223.
- Roth, A.J., Ooi, J.D., Hess, J.J., van Timmeren, M.M., Berg, E.A., Poulton, C.E., et al., 2013. Epitope specificity determines pathogenicity and detectability in ANCA-associated vasculitis. *J. Clin. Invest.* 123 (4), 1773–1783.
- Rutgers, A., Slot, M., van Paassen, P., van Breda Vriesman, P., Heeringa, P., Tervaert, J.W., 2005. Coexistence of anti-glomerular basement membrane antibodies and myeloperoxidase-ANCA in crescentic glomerulonephritis. *Am. J. Kidney Dis.* 46 (2), 253–262.
- Saadoun, D., Pol, S., Ferfar, Y., Alric, L., Hezode, C., Si Ahmed, S.N., et al., 2017. Efficacy and safety of sofosbuvir plus daclatasvir for treatment of HCV-associated cryoglobulinemia vasculitis. *Gastroenterology* 153 (1), 49–52.e5.
- Samson, M., Puechal, X., Devilliers, H., Ribi, C., Cohen, P., Bienvenu, B., et al., 2014. Long-term follow-up of a randomized trial on 118 patients with polyarteritis nodosa or microscopic polyangiitis without poor-prognosis factors. *Autoimmun. Rev.* 13 (2), 197–205.
- Sansonno, D., Dammacco, F., 2005. Hepatitis C virus, cryoglobulinaemia, and vasculitis: immune complex relations. *Lancet Infect. Dis.* 5 (4), 227–236.
- Sansonno, D., Tucci, F.A., Ghebrehiwet, B., Lauleta, G., Peerschke, E.I., Conteduca, V., et al., 2009. Role of the receptor for the globular domain of C1q protein in the pathogenesis of hepatitis C virus-related cryoglobulin vascular damage. *J. Immunol.* 183 (9), 6013–6020.
- Saulsbury, F.T., 2002. Epidemiology of Henoch-Schonlein purpura. *Cleve Clin. J. Med.* 69 (Suppl 2), SII87–SII89.
- Savage, C.O., Pottinger, B.E., Gaskin, G., Pusey, C.D., Pearson, J.D., 1992. Autoantibodies developing to myeloperoxidase and proteinase 3 in systemic vasculitis stimulate neutrophil cytotoxicity toward cultured endothelial cells. *Am. J. Pathol.* 141 (2), 335–342.
- Savige, J., Davies, D., Falk, R.J., Jennette, J.C., Wiik, A., 2000. Antineutrophil cytoplasmic antibodies and associated diseases: a review of the clinical and laboratory features. *Kidney Int.* 57 (3), 846–862.
- Savige, J., Gillis, D., Benson, E., Davies, D., Esnault, V., Falk, R.J., et al., 1999. International consensus statement on testing and reporting of antineutrophil cytoplasmic antibodies (ANCA). *Am. J. Clin. Pathol.* 111 (4), 507–513.
- Schönlein, J., 1837. Allgemeine und spezielle, Pathologie und Therapie., third ed. Literatur-Comptoir, Herisau.
- Schreiber, A., Xiao, H., Falk, R.J., Jennette, J.C., 2006. Bone marrow-derived cells are sufficient and necessary targets to mediate glomerulonephritis and vasculitis induced by anti-myeloperoxidase antibodies. *J. Am. Soc. Nephrol.* 17 (12), 3355–3364.
- Shrestha, S., Sumigan, N., Tan, J., Alhous, H., McWilliam, L., Ballardie, F., 2006. Henoch Schonlein purpura with nephritis in adults: adverse prognostic indicators in a UK population. *QJM* 99 (4), 253–265.
- Sinico, R.A., Winearls, C.G., Sabadini, E., Fornasieri, A., Castiglione, A., D'Amico, G., 1988. Identification of glomerular immune deposits in cryoglobulinemia glomerulonephritis. *Kidney Int.* 34 (1), 109–116.
- Sise, M.E., Bloom, A.K., Wisocky, J., Lin, M.V., Gustafson, J.L., Lundquist, A.L., et al., 2016. Treatment of hepatitis C virus-associated mixed cryoglobulinemia with direct-acting antiviral agents. *Hepatology* 63 (2), 408–417.
- Smyth, L., Gaskin, G., Pusey, C.D., 2004. Microscopic polyangiitis. *Semin. Respir. Crit. Care Med.* 25 (5), 523–533.
- Sneller, M.C., Hu, Z., Langford, C.A., 2012. A randomized controlled trial of rituximab following failure of antiviral therapy for hepatitis C virus-associated cryoglobulinemic vasculitis. *Arthritis Rheum.* 64 (3), 835–842.
- Sohn, M.H., Noh, S.Y., Chang, W., Shin, K.M., Kim, D.S., 2003. Circulating interleukin 17 is increased in the acute stage of Kawasaki disease. *Scand. J. Rheumatol.* 32 (6), 364–366.
- Sohn, S.Y., Song, Y.W., Yeo, Y.K., Kim, Y.K., Jang, G.Y., Woo, C.W., et al., 2011. Alteration of CD4CD25Foxp3 T cell level in Kawasaki disease. *Korean J. Pediatr.* 54 (4), 157–162.
- Stegeman, C.A., Tervaert, J.W., de Jong, P.E., Kallenberg, C.G., 1996. Trimethoprim-sulfamethoxazole (co-trimoxazole) for the prevention of relapses of Wegener's granulomatosis. Dutch Co-Trimoxazole Wegener Study Group. *N. Engl. J. Med.* 335 (1), 16–20.
- Stone, J.H., Merkel, P.A., Spiera, R., Seo, P., Langford, C.A., Hoffman, G.S., et al., 2010. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. *N. Engl. J. Med.* 363 (3), 221–232.
- Sturgill, B.C., Westervelt, F.B., 1965. Immunofluorescence studies in a case of Goodpasture's syndrome. *JAMA* 194 (8), 914–916.
- Suzuki, H., Fan, R., Zhang, Z., Brown, R., Hall, S., Julian, B.A., et al., 2009. Aberrantly glycosylated IgA1 in IgA nephropathy patients is recognized by IgG antibodies with restricted heterogeneity. *J. Clin. Invest.* 119 (6), 1668–1677.

- Takahashi, K., Oharaseki, T., Wakayama, M., Yokouchi, Y., Naoe, S., Murata, H., 2004. Histopathological features of murine systemic vasculitis caused by *Candida albicans* extract—an animal model of Kawasaki disease. *Inflamm. Res.* 53 (2), 72–77.
- Tarshish, P., Bernstein, J., Edelmann Jr., C.M., 2004. Henoch-Schonlein purpura nephritis: course of disease and efficacy of cyclophosphamide. *Pediatr. Nephrol.* 19 (1), 51–56.
- Terrier, B., Krastinova, E., Marie, I., Launay, D., Lacraz, A., Belenotti, P., et al., 2012. Management of noninfectious mixed cryoglobulinemia vasculitis: data from 242 cases included in the CryoVas survey. *Blood* 119 (25), 5996–6004.
- Terrier, B., Karras, A., Kahn, J.E., Le Guenno, G., Marie, I., Benarous, L., et al., 2013. The spectrum of type I cryoglobulinemia vasculitis: new insights based on 64 cases. *Medicine (Baltimore)* 92 (2), 61–68.
- Ting, T.V., Hashkes, P.J., 2004. Update on childhood vasculitides. *Curr. Opin. Rheumatol.* 16 (5), 560–565.
- Travis, W.D., Hoffman, G.S., Leavitt, R.Y., Pass, H.I., Fauci, A.S., 1991. Surgical pathology of the lung in Wegener's granulomatosis. Review of 87 open lung biopsies from 67 patients. *Am. J. Surg. Pathol.* 15 (4), 315–333.
- Uehara, R., Yashiro, M., Nakamura, Y., Yanagawa, H., 2003. Kawasaki disease in parents and children. *Acta Paediatr.* 92 (6), 694–697.
- van der Woude, F.J., Rasmussen, N., Lobatto, S., Wiik, A., Permin, H., van Es, L.A., et al., 1985. Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker of disease activity in Wegener's granulomatosis. *Lancet* 1 (8426), 425–429.
- Vargunam, M., Adu, D., Taylor, C.M., Michael, J., Richards, N., Neuberger, J., et al., 1992. Endothelium myeloperoxidase-antimyeloperoxidase interaction in vasculitis. *Nephrol. Dial. Transplant.* 7 (11), 1077–1081.
- Wang, Y., Wang, W., Gong, F., Fu, S., Zhang, Q., Hu, J., et al., 2013. Evaluation of intravenous immunoglobulin resistance and coronary artery lesions in relation to Th1/Th2 cytokine profiles in patients with Kawasaki disease. *Arthritis Rheum.* 65 (3), 805–814.
- Watts, R.A., Jolliffe, V.A., Grattan, C.E., Elliott, J., Lockwood, M., Scott, D.G., 1998. Cutaneous vasculitis in a defined population—clinical and epidemiological associations. *J. Rheumatol.* 25 (5), 920–924.
- Watts, R.A., Mahr, A., Mohammad, A.J., Gatenby, P., Basu, N., Flores-Suarez, L.F., 2015. Classification, epidemiology and clinical subgrouping of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis. *Nephrol. Dial. Transplant.* 30 (Suppl. 1), i14–i22.
- Watts, R.A., Scott, D.G., 2004. Epidemiology of the vasculitides. *Semin Respir Crit Care Med* 25 (5), 455–464.
- Wechsler, M.E., Akuthota, P., Jayne, D., Khouri, P., Klion, A., Langford, C.A., et al., 2017. Mepolizumab or placebo for eosinophilic granulomatosis with polyangiitis. *N. Engl. J. Med.* 376 (20), 1921–1932.
- Wegener, F., 1939. Über eine eigenartige rhinogene Granulomatose mit besonderer Beteiligung des Arteriensystems unter der Nieren. *Beiträge Zur Pathologischen Anatomie* 102, 36–68.
- Weiss, M.A., Crissman, J.D., 1984. Renal biopsy findings in Wegener's granulomatosis: segmental necrotizing glomerulonephritis with glomerular thrombosis. *Hum. Pathol.* 15 (10), 943–956.
- Westman, K., Flossmann, O., Gregorini, G., 2015. The long-term outcomes of systemic vasculitis. *Nephrol. Dial. Transplant.* 30 (Suppl. 1), i60–i66.
- Willan, R., 1808. On Cutaneous Diseases. Kimber & Conrad, pp. 452–471.
- Williams, J.M., Kamesh, L., Savage, C.O., 2005. Translating basic science into patient therapy for ANCA-associated small vessel vasculitis. *Clin. Sci. (Lond.)* 108 (2), 101–112.
- Winkelmann, R.K., 1958. Clinical and pathologic findings in the skin in anaphylactoid purpura (allergic angiitis). *Proc. Staff Meet. Mayo Clin.* 33 (11), 277–288.
- Wohlwill, F., 1923. Ueber die nur mikroskopisch erkennbare form der periarteriitis nodosa. *Virchows Arch. Pathol. Anat.* 246, 377–411.
- Xiao, H., Heeringa, P., Hu, P., Liu, Z., Zhao, M., Aratani, Y., et al., 2002. Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. *J. Clin. Invest.* 110 (7), 955–963.
- Xiao, H., Heeringa, P., Liu, Z., Huugen, D., Hu, P., Maeda, N., et al., 2005. The role of neutrophils in the induction of glomerulonephritis by anti-myeloperoxidase antibodies. *Am. J. Pathol.* 167 (1), 39–45.
- Xiao, H., Schreiber, A., Heeringa, P., Falk, R.J., Jennette, J.C., 2007. Alternative complement pathway in the pathogenesis of disease mediated by anti-neutrophil cytoplasmic autoantibodies. *Am. J. Pathol.* 170 (1), 52–64.
- Xiao, H., Ciavatta, D., Aylor, D.L., Hu, P., de Villena, F.P., Falk, R.J., et al., 2013. Genetically determined severity of anti-myeloperoxidase glomerulonephritis. *Am. J. Pathol.* 182 (4), 1219–1226.
- Yanagawa, H., Yashiro, M., Nakamura, Y., Kawasaki, T., Kato, H., 1995. Epidemiologic pictures of Kawasaki disease in Japan: from the nationwide incidence survey in 1991 and 1992. *Pediatrics* 95 (4), 475–479.
- Yang, Y.H., Huang, M.T., Lin, S.C., Lin, Y.T., Tsai, M.J., Chiang, B.L., 2000. Increased transforming growth factor-beta (TGF-beta)-secreting T cells and IgA anti-cardiolipin antibody levels during acute stage of childhood Henoch-Schonlein purpura. *Clin. Exp. Immunol.* 122 (2), 285–290.
- Yang, Y.H., Chang, C.J., Chuang, Y.H., Hsu, H.Y., Yu, H.H., Lee, J.H., et al., 2012. Identification and characterization of IgA antibodies against beta2-glycoprotein I in childhood Henoch-Schonlein purpura. *Br. J. Dermatol.* 167 (4), 874–881.
- Yates, M., Watts, R.A., Bajema, I.M., Cid, M.C., Crestani, B., Hauser, T., et al., 2016. EULAR/ERA-EDTA recommendations for the management of ANCA-associated vasculitis. *Ann. Rheum. Dis.* 75 (9), 1583–1594.
- Yoshiki, T., 2002. 1. Etiopathogenesis of necrotizing vasculitis. *Intern. Med.* 41 (1), 39–40.
- Zavialov, A.V., Gracia, E., Glaichenhaus, N., Franco, R., Zavialov, A.V., Lauvau, G., 2010. Human adenosine deaminase 2 induces differentiation of monocytes into macrophages and stimulates proliferation of T helper cells and macrophages. *J. Leukoc. Biol.* 88 (2), 279–290.
- Zeek, P.M., Smith, C.C., Weeter, J.C., 1948. Studies on periarteritis nodosa; the differentiation between the vascular lesions of periarteritis nodosa and of hypersensitivity. *Am. J. Pathol.* 24 (4), 889–917.
- Zheng, F., Kundu, G.C., Zhang, Z., Ward, J., DeMayo, F., Mukherjee, A.B., 1999. Uteroglobin is essential in preventing immunoglobulin A nephropathy in mice. *Nat. Med.* 5 (9), 1018–1025.
- Zhou, Q., Yang, D., Ombrello, A.K., Zavialov, A.V., Toro, C., Zavialov, A.V., et al., 2014. Early-onset stroke and vasculopathy associated with mutations in ADA2. *N. Engl. J. Med.* 370 (10), 911–920.

# Large and Medium-Vessel Vasculitides

Cornelia M. Weyand<sup>1,2</sup> and Jörg J. Goronzy<sup>1,2</sup>

<sup>1</sup>Department of Medicine, Division of Immunology and Rheumatology, Stanford University School of Medicine, Stanford, CA, United States <sup>2</sup>Department of Medicine, Veterans Affairs Palo Alto Health Care System, Palo Alto, CA, United States

## OUTLINE

Vasculitides of Large and Medium-Sized Blood Vessels	1313	Immuno-stromal Interactions in Vasculitis	1323
Giant Cell Arteritis	1315	Extravascular Giant Cell Arteritis	1323
Historic Background	1316	Treatment, Monitoring, and Outcome	1324
Clinical, Pathologic, and Epidemiologic Features	1316	Takayasu's Arteritis	1325
The Vascular Lesion	1318	Historic Background	1325
Epidemiology	1319	Clinical, Pathologic, and Epidemiologic Features	1326
Genetic Features	1319	Genetic Features	1327
Pathogenic Mechanisms	1319	Pathogenic Mechanisms	1328
T Cells and Antigen-Presenting Cells in Giant Cell Arteritis	1320	Treatment and Outcome	1328
Macrophages in Giant Cell Arteritis	1322	Concluding Remarks—Future Perspectives	1329
Neoangiogenesis of Microvascular Networks and Intimal Hyperplasia	1323	Acknowledgment	1330
		References	1330

## VASCULITIDES OF LARGE AND MEDIUM-SIZED BLOOD VESSELS

Vasculitides, inflammatory diseases of blood vessels, are recognized for their potential to rapidly progress to serious, and at times life-threatening, complications. Accordingly, arteritides of medium-sized and large arteries that cause aortitis and aortic branch vasculitis are considered medical emergencies that require prompt diagnosis and therapeutic management. Whereas the vasculitides of the capillaries and small arterioles may result in tissue damage, large-vessel vasculitis (LVV) puts the host at risk for fatal complications. One outcome is the inflammatory destruction of the vessel wall, leading to dissection, aneurysm formation, rupture, and hemorrhage (Weyand and Goronzy, 2003b). Alternatively, arteritis promotes luminal stenosis with subsequent occlusion, tissue ischemia, and infarct.

Inflammation in venous vessels is rare, whereas the layered wall structure of large arteries is a preferred site for granulomatous vasculitis, such as giant-cell arteritis (GCA) and Takayasu's arteritis (TA) (Table 66.1). The overlap of GCA and TA in affected vascular territories is only partial, and each entity also manifests in unique

**TABLE 66.1** Principal Features of Giant Cell Arteritis and Takayasu's Arteritis

	Giant cell arteritis	Takayasu's arteritis
Clinical	Subacute disease onset Restricted to individuals older than 50 years Highest incidence in individuals with Northern European ancestry Often combined with polymyalgia rheumatica	Subacute disease onset 90% of patients are female Highest risk in Asian and South American populations Systemic inflammatory syndrome with malaise, fever, arthralgias, and chest pain
Vascular component	Extracranial branches of the carotid artery Subclavian–axillary junction Aorta	Aorta Innominate, carotid, subclavian Mesenteric, renal arteries
Extravascular component	Hepatic acute phase response Elevated IL-6 Polymyalgia rheumatica Malaise Weight loss Anemia	Hepatic acute phase response Elevated innate cytokines (e.g., IL-6) Myalgias Arthralgias Fever Failure-to-thrive
Severe complications	Blindness Stroke Aortic aneurysm	Stroke Pulselessness Visual impairment Aortic regurgitation Hypertension
Pathogenesis	Loss of the artery's immune privilege Deficiency of immunosuppressive CD8 T regulatory cells in the periphery Aberrant expression of Jagged1 on adventitial microvascular cells Breakdown of the immuno-protective PD-1 checkpoint Intramural granulomatous lesions: <ul style="list-style-type: none"> <li>• Arterial wall dendritic cells</li> <li>• Tissue-injurious macrophages</li> <li>• Multiple lineages of effector T cells (Th1, Th17, Th9, Tfh, etc.)</li> </ul> Trigger of immune-mediated vascular inflammation unknown	Peripheral immune defects unknown Key cellular elements in the vascular lesions: <ul style="list-style-type: none"> <li>• Perforin-producing T cells</li> <li>• Natural killer cells</li> </ul> Instigator of vessel wall inflammation unknown

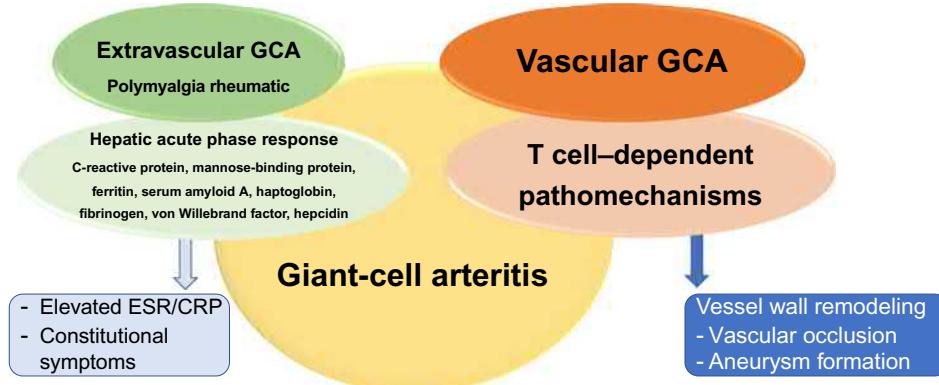
arterial beds. In rare cases, aortitides can be associated with other syndromes, such as relapsing polychondritis, sarcoidosis, inflammatory bowel disease, connective tissue disease, and infection (Rojo-Leyva et al., 2000). Some patients with aortitis, typically diagnosed from resected tissue after aortic repair, cannot be assigned to an underlying syndrome and appear to have isolated aortic disease. Noninvasive imaging modalities have greatly expanded diagnostic approaches in suspected large and medium-vessel vasculitis. Most patients require immunosuppressive therapy over prolonged periods and the prevailing paradigm of therapy-induced remission is being challenged and replaced by recognition that most patients have persistent smoldering vessel wall inflammation.

This chapter will focus on GCA and TA, the most frequent types of aortitis and aortic branch vasculitis. A singular etiologic agent for GCA or TA has not been identified, but affected patients have defined abnormalities in their innate and adaptive immune responses. Typical features include the assembly of granulomatous infiltrates within the vessel wall; the importance of host age (the young are exclusively susceptible to TA, the old exclusively develop GCA); the chronicity of inflammation; and the combination of vascular autoimmunity with an extravascular component. While GCA, TA, and atherosclerosis share the tissue tropism for medium and large arteries and have some overlap in pathogenic mechanisms, they differ in the intensity of inflammation and in the patterning of ischemic complications.

## GIANT CELL ARTERITIS

GCA has two major disease components, vascular GCA leading to aneurysm formation and arterial stenotic disease and extravascular GCA, characterized by an intense hepatic acute phase response and systemic inflammation (Fig. 66.1). The relationship of both disease components is not entirely understood, but emerging data suggest that the two components may be distinct in pathogenesis and therapeutic responsiveness.

Vascular GCA occurs due to the breakdown of the natural immune privilege that protects medium and large arteries from inflammatory attack. The immune-privileged niche of the vessel wall. Walls of medium and large arteries are composed of three layers; the intima, a thin endothelial cell layer; the media, built from contractile smooth-muscle cells; and the adventitia, a supportive structure containing the only microvascular access to the wall. An endogenous population of dendritic cells is placed at the adventitia–media border. Due to the vital function of large arteries, the wall is an immuno-privileged niche, naturally protected from inflammatory attack. Innate and adaptive immune responses are often associated with bystander damage to host tissue. Such damage is acceptable if it outweighs the risk of infection or malignancy. Since damage to the aorta and its large branches is incompatible with host survival, it is in the host's best interest to forgo immune responses that threaten vital structures. Named immune privilege, this principle is enforced by multiple mechanisms and applies to such vital organs as the eye and the myocardium. In the case of GCA, vital arteries have lost this protective mechanism, rendering them susceptible to inflammation. Typical histomorphologic lesions of GCA are granulomatous infiltrates composed of T cells and macrophages that invade into the wall layers of the aorta and its second to fifth branch vessels. It is now understood that immune cells gain access through the adventitial vasa vasorum. Once in the arterial wall, T cells interact with tissue-resident dendritic cells (DC) and differentiate into multiple functional linages. Activated by interferon- $\gamma$  (IFN- $\gamma$ )-producing T cells, macrophages release a broad portfolio of effector molecules, ranging from cytokines to proteases, growth and angiogenesis factors, and reactive oxygen species. Inflammatory damage to the wall elicits a maladaptive remodeling program, culminating in the hyperplasia of the intimal layer, luminal occlusion, and tissue ischemia. Since the upper



**FIGURE 66.1** Disease components of giant cell arteritis. Extravascular GCA is characterized by an intense hepatic acute phase response causing elevation of ESR and CRP. Vascular GCA manifests as transmural granulomatous vasculitis. Vasculitic effector T cells activate multiple macrophage subpopulations. Downstream consequences include cellular injury, neoangiogenesis, and intimal hyperplasia causing aneurysm formation in the aorta and luminal occlusion in aortic branches. CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; GCA, giant cell arteritis.

extremity and cranial branches of the aorta are at highest risk, ischemic complications occur in the eye [ischemic optic neuropathy (ION)], the brain (vertebrobasilar strokes), and the subclavian-axillary bed (pulselessness). Almost all patients are older than 50 years of age, thus susceptible to immune aging and associated complications (Goronzy and Weyand, 2017). The highest incidence has been reported in persons of Northern European ancestry. If diagnosed early and treated appropriately, the clinical outcome is excellent (Weyand and Goronzy, 2003a,b). While extravascular GCA is promptly responsive to immunosuppression, vascular GCA appears more refractory, persisting long-term in a smoldering form (Maleszewski et al., 2017). It is currently unknown whether and to which degree such smoldering vasculitis requires treatment. The diagnosis can be made by biopsy of the temporal artery and given the potential side effects of long-term immunosuppressive therapy, diagnostic confirmation by histology should be sought whenever possible.

## Historic Background

Horton et al. (1932) reported on two patients admitted to the Mayo Clinic with “fever, weakness, anorexia, weight loss, anemia and painful, tender areas over the scalp and along the temporal vessels.” Histomorphology of the removed temporal artery described periarteritis and arteritis with granulation tissue in the adventitia. Horton recognized that this type of arteritis was distinct from all other vasculitic syndromes and named it “temporal arteritis.” Later the vasculitis also became known as cranial arteritis, granulomatous arteritis, polymyalgia arteritis, and Horton’s disease. Prior descriptions of temporal artery disease suggest that GCA may be a very old disease. The English surgeon Hutchinson (1890) described a patient with a red and swollen temporal artery who had difficulties wearing his hat. In the grave of Pa-Aton-Ern-Hebs, built during the Amarna period (around 1350 AD), a blind harpist was shown with nodularity and swelling of the temporal artery. And Ali Ibn Isa (940–1010 BC), an ophthalmologist in Baghdad, recommended removal of the temporal artery not only to treat headaches, but also inflammation of the scalp muscles associated with blindness (Henriet et al., 1989).

Horton deserves credit for recognizing the coexistence of the constitutional symptoms and the arteritis of the temporal vessels and for introducing temporal artery biopsy as a diagnostic test.

## Clinical, Pathologic, and Epidemiologic Features

The clinical manifestations of vascular GCA are a consequence of stenosis or occlusion of the artery’s lumen, which leads to impaired blood flow, ischemia, and tissue infarction. In case of aortic involvement, the damage pattern is that of dissection and aneurysm formation, often requiring emergency aortic repair (Table 66.2). In contrast, extravascular GCA presents with constitutional symptoms related to systemic inflammation, such as anorexia, weight loss, malaise, depression, and failure-to-thrive. In some patients, fever is the presenting symptom. Predictably, such constitutional symptoms are associated with laboratory abnormalities indicative of an intense hepatic acute phase response; for example, elevated C-reactive protein (CRP), serum amyloid A (SAA), fibrinogen (Fig. 66.1) (Evans and Hunder, 2000; Weyand and Goronzy, 2003a). Traditionally, systemic inflammatory manifestations were believed to precede vascular inflammation. A novel paradigm conceptualizes vascular and extravascular GCA as parallel processes or places extravascular GCA as downstream of the vascular pathology. In that model, vascular inflammation and remodeling could progress in the absence of systemic inflammation and both disease domains would require a differential diagnostic and therapeutic approach. For clinical purposes, it is helpful to subdivide vascular GCA into different subtypes, including cranial and large-vessel GCA, and separate these subtypes from PMR, a typical manifestation of extravascular disease.

Cranial GCA results from vasculitis in the head- and neck-supplying branches of the aorta. Most often affected are the superficial temporal artery, the vertebral artery, the ophthalmic and posterior ciliary arteries, and, less frequently, the internal and external carotid artery. Clinical consequences include headaches, scalp tenderness, claudication of the masseter muscles, and vision loss due to ischemia in the visual pathway (Table 66.2). The headaches are often intense, throbbing, and sharp in character, at times combined with temporal tenderness. On physical examination, temporal vessels are thickened, nodular, and pulseless. Other scalp arteries can be involved. Occlusion in branches of the ophthalmic artery leads to ION that causes sudden and pain-free blindness. Amaurosis fugax can precede complete vision loss. Blindness in one eye indicates high risk to suffer additional visual loss in the other eye. Vascular insufficiency of the orbital arteries can cause a wide spectrum of ophthalmic complications, but ION remains the most frequent ophthalmic manifestation (Chan et al., 2005).

**TABLE 66.2** The Clinical Profile in Giant Cell Arteritis (GCA)

Sign/symptom	Frequency (%)	Pathology
<b>VASCULAR GCA</b>		
Headaches	80	Vascular stenosis, ischemia
Scalp tenderness	70	
Jaw claudication	30	Vascular stenosis, ischemia
Ocular symptoms (blindness, amaurosis fugax, motor deficit)	<20	Vascular stenosis, ischemia
Painful dysphagia	<10	Vascular stenosis, ischemia
Cough	<10	Vascular stenosis, ischemia
Limb claudication	10	Vascular stenosis, ischemia
Absent pulses	<10	Vascular stenosis, ischemia
Asymmetrical blood pressure readings	<10	Vascular stenosis, ischemia
CNS ischemia	<5	Vascular stenosis, ischemia
Peripheral neuropathy	<5	
Aortic regurgitation	<5	Vascular dilatation
Synovitis	<5	
<b>EXTRAVASCULAR GCA</b>		
Intense acute phase response (elevated ESR, CRP, IL-6)	90	Systemic inflammation
Anemia	70	
Polymyalgia rheumatica	40	
Wasting syndrome	30	

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IL-6, interleukin 6; CNS, central nervous system.

Intermittent claudication of the masseter and temporal muscles are relatively disease-specific but only affect a fraction of patients (Smetana and Shmerling, 2002). Typically, chewing or prolonged talking induces jaw claudication. Tongue claudication and painful dysphagia can occur. Impaired blood flow in the vertebrobasilar and carotid arteries causes ischemia of the central nervous system, for example, transient ischemic attacks, or stroke. Chronic nonproductive cough has been attributed to vasculitis of pulmonary artery branches.

In a subset of patients, the primary targets of vasculitis are the subclavian, axillary, and carotid arteries, and the aorta itself (Brack et al., 1999; Salvarani, 2003). Presenting symptoms of upper extremity vasculitis are arm claudication, loss of pulses, paresthesias, and, rarely, frank gangrene. Temporal artery biopsies can be negative, and, in most patients, symptoms indicative of cranial ischemia are lacking (Brack et al., 1999). Aortitis can coexist with cranial arteritis or large-vessel involvement and predominantly manifests in the thoracic aorta. Silent aneurysm, aortic dissection, or rupture is a consequence of destruction of the elastic membranes (Evans et al., 1994; Lie, 1995). Not unusual, histomorphologic evidence of vasculitis is found during surgical repair of an aortic aneurysm. Aortic involvement is typically a late complication of GCA, suggesting a smoldering disease process and is associated with increased mortality (Kermani et al., 2012).

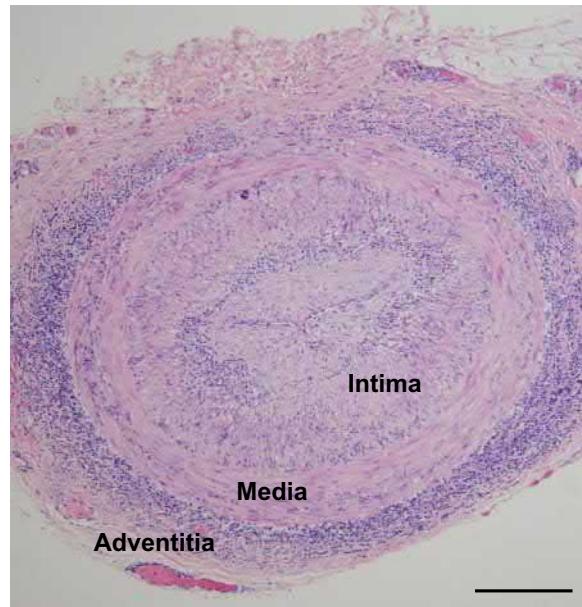
The extravascular component of GCA can appear in isolation, with the vascular component remaining subclinical (Fig. 66.1; Table 66.2). Such patients are diagnosed with PMR due to the predominance of muscle pain and stiffness. PMR is about twofold to threefold more frequent in incidence than GCA (Doran et al., 2002; Kermani and Warrington, 2013). Alternatively, patients present with fever-of-unknown-origin and wasting. Malaise, depression, anorexia, and weight loss are frequent complaints (Weyand and Goronzy, 2003a). Signs and symptoms of impaired blood flow are missing, and clinical findings are dominated by myalgias and stiffness.

The precise relationship between the extravascular and the vascular component of GCA is not understood. Vascular GCA is diagnosed by tissue histology and in some cases by typical involvement pattern of LVV of aortitis captured by noninvasive imaging. The challenge of diagnosing the extravascular component is much higher.

Lead symptoms are those of shoulder girdle and pelvic girdle pain and stiffness. Synovitis of hip and shoulder joints has been described in some patients with PMR and recent provisional classification criteria have been expanded to include cases with bursitis and synovitis (Dasgupta et al., 2012). The diagnosis of PMR remains founded on nonspecific findings, such as pain and, in some cases, inflammation in joint-related structures. A dominant laboratory finding is that of an intense hepatic acute phase response, that leads to elevated acute phase proteins, elevated erythrocyte sedimentation rate (ESR), and elevated CRP. All of these findings are nonspecific, likely to occur in multiple clinical settings, particularly in individuals older than 50 years of age. Urgent need exists to replace the nonspecific clinical classification criteria with objective, histological, and molecularly based criteria. Until that goal is reached, PMR remains a diagnosis of exclusion and includes an assortment of conditions. Patients with diffuse myalgias associated with statin therapy may also fulfill the criteria for PMR (de Jong et al., 2012). Patients and physicians need to be aware of the provisional character of this diagnosis and stay alert for the possibility of an alternate disease processes.

## The Vascular Lesion

In its typical form, GCA is a panarteritis with T lymphocytes and macrophages infiltrating into all layers of the vessel wall (Fig. 66.2) (Maleszewski et al., 2017). In about half of the cases, granulomatous formations are seen. Giant cells, which give the name to the syndrome, are not necessarily present. They tend to accumulate at the media-intima border, often close to the fragmented internal elastic lamina. Inflammatory infiltrates are usually segmental but can be circumferential. Focal placement of lesions produces the so-called skip pattern of vasculitis. In some patients, the infiltrates are predominately composed of lymphocytes without granuloma formation, particularly so after corticosteroid therapy (Maleszewski et al., 2017). The intima is hyperplastic, obstructing the lumen. T cells and macrophage infiltrates are also encountered in the adventitia, which is the port-of-entrance for inflammatory cells. Small clusters of lymphocytes and macrophages in the adventitia may not be indicative of a vasculitic process and, in isolation, should not be considered sufficient to make the diagnosis (Bjornsson, 2002). In the early stages of vasculitis the small vessels in the adventitia may be the primary target for the inflammatory infiltrates, and microvascular networks in the adventitia have recently been shown to provide instructive cues for circulating T cells (Chatelain et al., 2008; Wen et al., 2017).



**FIGURE 66.2** Giant cell arteritis. Cross-section of the temporal artery from a 76-year-old patient. Transmural inflammation with granuloma formation in the media and along the medial–intimal junction. Dense T-cell infiltrates at the adventitia–media border. Multinucleated giant cells positioned along the fragmented lamina elastic interna. Complete occlusion of the lumen by hyperplastic intima.

## Epidemiology

Among the vasculitic syndromes, GCA is the most frequent in the Western world. Age is the most significant risk factor; the disease almost exclusively occurs in individuals older than 50 years of age, and susceptibility increases with age (Machado et al., 1988; Salvarani et al., 2004), reaching highest incidence rates in those 71–80 years old (Mohammad et al., 2015). The female-to-male ratio of patients is 3:1. A characteristic feature of GCA is pronounced geographical variations in prevalence. The range of incidence rates spans between 1 and 25 cases per 100,000 persons aged 50 years and older. Highest incidence rates have been reported in Northern Europe, including Iceland, Denmark, and Sweden (Baldursson et al., 1994; Nordborg et al., 2003), as well as in areas of North America settled by populations of Scandinavian origin (Machado et al., 1988), such as Minnesota. Conversely, blacks and Hispanics are infrequently affected (Gonzalez et al., 1989; Smith et al., 1983).

A diagnosis of GCA does not impair long-term survival, and mortality rates are not increased in chronic patients (Hill et al., 2017; Mohammad et al., 2015). In a high-incidence Swedish population, 15% of GCA patients and 10.8% of a matched-reference cohort had large-vessel involvement diagnosed by noninvasive imaging studies (Naderi et al., 2017). In this patient population, 18 of 840 GCA patients had complete unilateral or bilateral vision loss, similar to data from Olmsted County, Minnesota, where seven of 204 patients suffered vision loss (Singh et al., 2015), confirming that the feared complication of complete vision loss is fortunately infrequent.

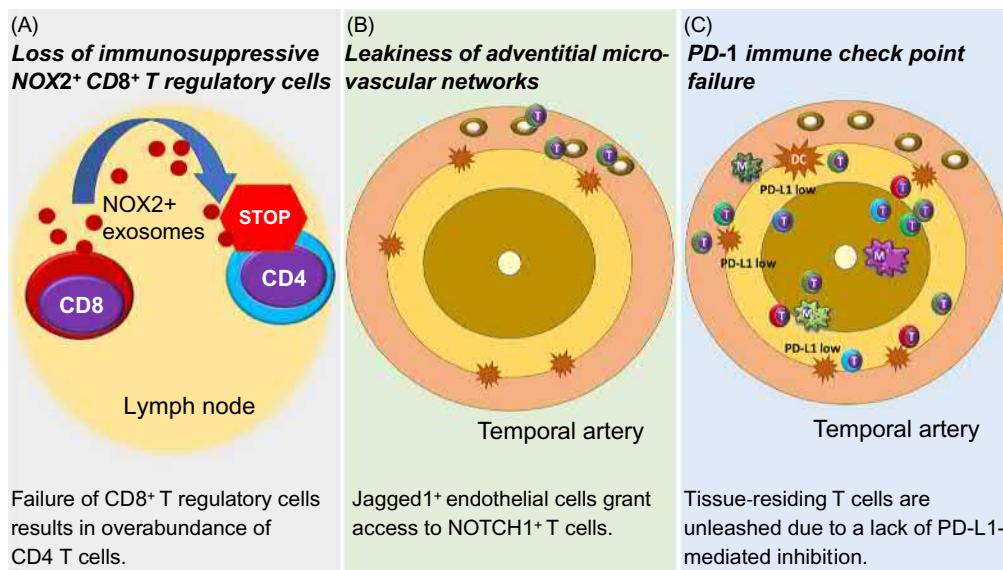
## Genetic Features

GCA is an human leukocyte antigen (HLA) class II–associated disease (Weyand et al., 1992, 1994a), as recently confirmed in a large scale genetic analysis (Carmona et al., 2015). HLA-DR4 confers the highest risk to develop arteritis. Several allelic variants of HLA-DRB1\*04 are overrepresented among affected individuals. Sharing of sequence polymorphisms in the antigen-binding groove of HLA-DR molecules have been cited as evidence for a role of antigenic peptides in disease initiation. Minor effects of polymorphisms in multiple non-HLA genes, such as corticotrophin-releasing hormone, CCL5 (RANTES), intracellular adhesion molecule 1, and interleukin (IL)-6, have been reported and larger studies are awaited to confirm or refute such associations (Alvarez-Rodriguez et al., 2011; Rodriguez-Rodriguez et al., 2011a,b). A genome-wide association study has identified risk alleles in plasminogen and P4HA2, molecules involved in vascular remodeling and angiogenesis (Carmona et al., 2017).

## Pathogenic Mechanisms

Progress has been made in deciphering the pathogenic mechanisms relevant for the inflammatory injury of the vessel wall (Weyand, 2000; Weyand and Goronzy, 2003b; Weyand et al., 2012). Data on the extravascular component, representing an intense upregulation of hepatic acute phase responses, remain limited. Acute-phase serum amyloid A can enhance IL-6 and IL-8 production and amplify tissue remodeling (O'Neill et al., 2015). However, it is unresolved whether the hepatic acute phase response, including the production of CRP, mannose-binding protein, ferritin, serum amyloid A, haptoglobin, fibrinogen, von Willebrand factor, hepcidin, etc., lies upstream or downstream of the vasculitis and may simply be an indicator of a nonhealing tissue lesion.

Principally, in vascular GCA, granulomatous lesions composed of T cells and macrophages occupy the vascular wall niche and elicit a maladaptive repair response, ultimately leading to neointima growth and luminal occlusion (Fig. 66.2). Studies in GCA patients are supporting a disease model that conceptualizes the vasculitis as a failure of a physiologic wall-protective mechanism. Given the life-sustaining functions of medium and large arteries, nature has created a high threshold for inflammatory attack, as the risks to the host are disproportional to potential benefits (Fig. 66.1). The immune privilege of arteries as vital organs is multipronged, combining a series of tolerance mechanisms to prevent vasculitis. Recent studies have revealed that the arterial-wall immune privilege fails in GCA patients on at least three levels (Fig. 66.3): (1) GCA patients lack suppressive mechanisms in peripheral immunity, resulting in uncontrolled CD4 T-cell reactivity (Wen et al., 2016) (Fig. 66.3A); (2) microvascular endothelial cells (mvEC) in the adventitia of inflamed arteries actively engage T cells, enabling T-cell invasion into a protected tissue niche (Wen et al., 2017) (Fig. 66.3B); (3) the immunoinhibitory PD-1 checkpoint is defective in GCA, allowing for unopposed T-cell activity (Zhang et al., 2017) (Fig. 66.3C). Obviously, all three mechanisms render T cells more responsive. Antigens recognized by intralesional T cells continue to be undefined; possibly, multiple antigens function as enablers. Age represents the dominant risk factor to develop GCA and may contribute through the process of immune aging (Goronzy and Weyand, 2017; Wen et al., 2016) as well as vascular



**FIGURE 66.3** Immune defects in GCA. Left panel: the T-cell zones of lymph nodes are populated by specialized CD8 T cells that carry NOX2 in their membranes. CD8<sup>+</sup> NOX2<sup>+</sup> T cells release NOX2<sup>+</sup> exosomes and transfer them to neighboring CD4 T cells. This provides a strong negative signal, preventing CD4 T cell expansion. Patients with GCA lack such immunosuppressive CD8 Treg cells. Middle panel: in GCA patients, adventitial microvessels (*vasa vasorum*) express Jagged1. Jagged1<sup>+</sup> endothelial cells directly communicate with Notch1<sup>+</sup> T cells, granting such T cells access to the otherwise inaccessible and immunoprivileged tissue site. Right panel: in healthy arteries, the immunoinhibitory ligand PD-L1 is highly expressed. In GCA arteries, PD-L1 expression on dendritic cells is low; paralyzing negative signals that stop T-cell activation in the tissue niche. Wall-infiltrating T cells are unleashed and are capable of multiple inflammatory effector functions. *GCA*, giant-cell arteritis; *NOX2*, NADPH oxidase 2; *DC*, dendritic cell.

aging. In population-based prospective health cohorts, GCA was predicted by a low body mass index (Jakobsson et al., 2015), compatible with long-term effects in disease susceptibility.

## T CELLS AND ANTIGEN-PRESENTING CELLS IN GIANT CELL ARTERITIS

The vast majority of lesional T cells are CD4<sup>+</sup> memory cells (Andersson et al., 1987). CD4<sup>+</sup> T cells enter the vessel wall through the *vasa vasorum* and infiltrate into the adventitia recruited by chemokines that derive from DCs positioned at the adventitia–media border (Krupa et al., 2002), an indigenous vessel wall cell population (Ma-Krupa et al., 2004). Vascular DC forms a circumferential ring along the lamina elastica externa. They express typical DC markers, such as CD11c, fascin, and S-100, but lack CD1a and thus are distinct from Langerhans cells in the skin. Functional profiling has demonstrated that they possess Toll-like receptors (TLR) and respond to pathogen-associated molecular patterns (Pryshchev et al., 2008). Different arterial beds in the human body carry vascular DC with distinct profiles of pattern recognition receptors (Pryshchev et al., 2008), providing a molecular basis for target tissue variations and a possible mechanism for the unusual patterning of GCA.

TLR are characteristically found on cells of the innate immune system and typical ligands are lipopolysaccharides, flagellin, viral RNA, bacterial DNA, and heat-shock proteins. TLR triggering initiates the maturation of immature DCs. Mature DCs switch their chemokine receptor profile, become migratory, and travel via lymphatic vessels to lymph nodes. Homing to the T-cell zones, they become stationary, produce T cell-attracting chemokines, and upregulate antigen-presenting HLA molecules and costimulatory molecules.

The physiological role of adventitial DCs and what they are checking for is unknown. Implantation of human temporal arteries into immunodeficient mice revealed that adventitial DCs respond to blood-borne lipopolysaccharide (LPS) (Ma-Krupa et al., 2004). Lesional DCs are highly activated, resemble lymph node-positioned DCs (Krupa et al., 2002), produce high amounts of chemokines, are no longer migratory, and can present antigen to T cells. A major abnormality of lesion-residing DC is the lack of PD-L1 expression (Weyand et al., 2018; Zhang et al., 2017). PD-L1 is an immunoinhibitory ligand that binds the PD-1 receptor on T cells, thereby sending

negative signals and inhibiting T-cell activation. Cancer cells abuse this immune tolerance mechanism to evade antitumor T-cell immunity and the PD-1 checkpoint is targeted by the revolutionary checkpoint inhibition therapies (Davies and Duffield, 2017). Tissue-resident and circulating DC in GCA patients express low amounts of PD-L1 and PD-L1 expression inversely correlates to disease activity (Watanabe et al., 2017b). Essentially, vessel-wall DC in GCA patients favor T-cell activation over T-cell suppression. Molecular mechanisms underlying this defect are unknown.

DC activation is a very early step in the disease process as evidenced by studies in PMR patients whose arteries lack T-cell infiltrates but contain activated DC. If appropriate T cells are made available, they will invade the wall and undergo *in situ* activation (Ma-Krupa et al., 2004). Thus, PMR patients appear protected from frank vasculitis, unless additional tolerance mechanisms fail.

A mechanism protecting the artery's immune privilege appears to lie in the control of cellular access to the wall. The earliest lesion captured by noninvasive imaging reveals adventitial thickening, where microvascular vasa vasorum networks grant access for inflammatory cells. The tissue transcriptome of GCA-affected aorta and temporal artery contains high expression levels of Jagged1, a ligand for the NOTCH receptor. Jagged1 expression was localized to the mvEC in the adventitia; the NOTCH receptor was located to CD4 T cells. In mechanistic studies, Jagged1-expressing mvEC triggered T-cell activation, activating mTOR-dependent signaling events, thereby opening the gate for incoming T cells and educating circulating T cells (Wen et al., 2017).

Once CD4<sup>+</sup> T cells have made their entrance into the immuno-protected microenvironment of the arterial wall, they undergo local activation (Brack et al., 1997; Weyand et al., 1994b). Studies in bilateral temporal artery specimens from the same patient have shown that identical T-cell clonotypes emerge in physically separated lesions (Weyand et al., 1994b), providing strong support for antigen-driven selection (Weyand and Goronzy, 1995). It is now clear that several functional T-cell lineages participate in the vasculitic lesions (Watanabe et al., 2017a). A major subset of tissue-infiltrating CD4 T cells are IFN- $\gamma$ -producing Th1 cells (Deng et al., 2010; Samson et al., 2012; Terrier et al., 2012; Wagner et al., 1996). In contrast to Th17 cells, lesion-residing Th1 cells are relatively resistant to standard immunosuppression, as indicated by the persistent IFN- $\gamma$  production in long-term treated patients (Deng et al., 2010; Maleszewski et al., 2017). How exactly T-cell effector molecules other than IFN- $\gamma$  and IL-17, for example, IL-9 and IL-21, contribute to the disease process has not been clarified (Table 66.3). Almost all T cells assembled in the granulomatous lesions are PD-1 positive (Zhang et al., 2017), yet are neither exhausted nor inhibited. Considering the diversity of T-cell effector populations in the lesions, the infiltrates are not suggestive for a single antigen-driving vasculitogenic immunity. Rather, vasculitogenic T cells appear unopposed, uninhibited by suppressive immune checkpoints that prevent uncontrolled and tissue damaging immunity (Weyand et al., 2018).

The lack of appropriate inhibition holds true for peripheral T cells as well. GCA patients lack a population of regulatory CD8 T cells (Wen et al., 2016) that are positioned in secondary lymphoid tissues. Such CD8 Tregs express on their surface the NADPH oxidase-2 (NOX2), package this enzyme into microvesicles and transfer NOX2-containing exosomes onto neighboring CD4 T cells. NOX2 inhibits the T-cell receptor activation cascade in close-by CD4 T cells, resulting in dampened CD4 T-cell expansion. Ultimately, NOX2<sup>+</sup> CD8 Tregs control

**TABLE 66.3** T-Cell Effector Functions in the Vascular Lesions

T-cell effector functions in the vascular lesions	
Functional lineage	Effector pathway
TH1; IFN- $\gamma$	Activation of macrophages, endothelial cells, fibroblasts, etc.
TH17; IL-17	Activation of immune and nonimmune cells
TH9; IL-9	??
TFH; IL-21	??
TH0; IL-2	T-cell survival, expansion
TH22	??

the size of the host's CD4 compartment and thus the propensity for CD4-dependent immune responses. The availability of NOX2<sup>+</sup> CD8 Tregs is closely linked to age, connecting immune aging and GCA susceptibility (Wen et al., 2016).

In essence, multiple defects in adaptive T-cell immunity are combined to predispose individuals for the development of GCA. Uncontrolled expansion of peripheral CD4 T cells, failure of barriers protecting the vessel wall, and breakdown of inhibitory signals in the tissue microenvironment are all permissive to allow T cells to infiltrate into the wall layers and establish granulomatous lesions.

## MACROPHAGES IN GIANT CELL ARTERITIS

There are no data to suggest that T lymphocytes accumulating in the vasculitic infiltrates have direct involvement in wall injury. Rather, a critical effector function of tissue-invasive T cells is the regulation of macrophage recruitment and differentiation. Macrophages rely on IFN- $\gamma$ -dependent signals, and the intensity of tissue IFN- $\gamma$  has been correlated to disease manifestations. IFN- $\gamma$ -high patients have more intense intimal hyperplasia, more visual symptoms, and cranial ischemia. Patients with distinctly lower tissue IFN- $\gamma$  often lack vessel occlusion and tend to present with fever of unknown origin (Weyand et al., 1997). Multiple pathways have been identified through which macrophages mediate tissue damage (Table 66.4). Macrophage function is closely related to the positioning in the wall, suggesting a tight interaction between infiltrating macrophages and the matrix components they encounter in the tissue niche (Weyand et al., 1996).

Adventitial macrophages focus on the production of proinflammatory cytokines, such as IL-1 $\beta$  and IL-6. Tumor necrosis factor (TNF)- $\alpha$  has been reported to be present in inflamed arteries with the staining pattern, suggesting production in the media (Hernandez-Rodriguez et al., 2004). Medial macrophages specialize in generating reactive oxygen intermediates (Rittner et al., 1999b). Toxic aldehydes, products of lipid peroxidation, have been detected in the smooth-muscle cell layer (Rittner et al., 1999b). The formation of nitrotyrosine, a marker of nitrosative stress, has also been localized to the media (Borkowski et al., 2002), where endothelial cells of medial neovessels are affected by nitrotyrosine formation. A noteworthy aspect of the medial oxidative stress is the induction of protective mechanisms, such as the induction of aldose reductase, a ketoreductase with a role in metabolizing toxic aldehydes (Rittner et al., 1999a). Blocking aldose reductase in GCA lesions increased apoptosis rates of smooth-muscle cells, identifying a protective role of the enzyme. It is unknown whether patients vary in the induction of such protective mechanisms, making them more or less susceptible to oxidative damage.

Other functional properties of media-located macrophages include the secretion of matrix metalloproteinases (MMPs) (Sorbi et al., 1996; Tomita and Imakawa, 1998). CD68-expressing macrophages close to the lamina elastica interna are prone to produce MMP-2 and MMP-9. Such metalloproteinases produce the typical fragmentation

**TABLE 66.4** Macrophage Effector Functions in the Vascular Lesions



Effector molecule	Functional impact
Chemokine production	Assemble granulomatous lesions
Cytokine production (IL-1, IL-6, TNF- $\alpha$ etc.)	Amplify inflammation
Reactive oxygen species	Oxidative tissue damage
Aldose reductase	Antioxidant defense
Growth factors (PDGF, FGF)	Proliferation of myofibroblasts-neointima
Angiogenesis factors (VEGF)	Neoangiogenesis
Metalloproteinases (MMP-2, MMP-9)	Digestion of collagen and elastic membranes

*IL*, Interleukin; *MMP*, matrix metalloproteinase; *PDGF*, platelet-derived growth factor; *FGF*, fibroblast growth factor; *TNF*, tumor necrosis factor; *VEGF*, vascular endothelial growth factor.

of the elastic membranes and digest collagen but may have pathogenic potential beyond their elastinolytic and gelatinolytic activities. So far, macrophages captured in the intima have not been assigned to particular injury pathways (Weyand et al., 1996). They have been described to stain positive for NOS-2, but downstream effects of nitrosative stress have not been detected in the intima itself.

Progress in macrophage biology has led to the identification of functionally different macrophage populations, including proinflammatory M1 macrophages and reparative M2 macrophages. IL-33, a cytokine associated with M2 polarization, has been localized in temporal artery biopsies (Ciccia et al., 2013). Why tissue-damaging macrophages functions dominate the vasculitic lesions remains unexplored.

## NEOANGIOGENESIS OF MICROVASCULAR NETWORKS AND INTIMAL HYPERPLASIA

Blockage of the vascular lumen by hyperplastic intima is the mechanism through which GCA causes vascular insufficiency and tissue ischemia (Fig. 66.2). Proliferating myofibroblasts create an expanding neointimal layer and deposit matrix. They migrate to the intima from deeper wall layers, either the media or the adventitia. Factors regulating myofibroblasts migration and differentiation thus have gatekeeper function in vascular remodeling. Disarming the PD-1 immune checkpoint increased the neointima thickness, directly implicating T cells in the regulation of this process (Zhang et al., 2017).

Inflamed temporal artery walls produce platelet-derived growth factor, which derives from at least two cell types, CD68<sup>+</sup> macrophages and resident wall cells (Kaiser et al., 1999). Numerically, the most important sources are medial macrophages, followed by multinucleated giant cells. Fibroblast growth factor has been described to be present in vasculitic lesions; its function is unexplored.

Wall remodeling, including neointimal expansion, needs to be supported by the formation of new blood vessels (Cid, 2002; Kaiser et al., 1998). Neoangiogenesis typically affects the adventitia, where new capillaries sprout out of vasa vasorum. Once wall remodeling has progressed, microvessels appear in the otherwise avascular media and intima. Multinucleated giant cells have been found to secrete vascular endothelial growth factor (VEGF) (Kaiser et al., 1998), implicating tissue-infiltrating macrophages in the restructuring of the arterial wall. Interestingly, VEGF production is correlated with IFN- $\gamma$  production, thereby connecting adaptive, innate, and tissue repair immunity. Dismantling of the PD-1 checkpoint accelerates formation of neovascular lumina, placing T cells at the pinnacle of the vasculitis-induced maladaptive tissue repair process (Zhang et al., 2017).

## IMMUNO-STROMAL INTERACTIONS IN VASCULITIS

While the immune system participates in vasculitic responses through a multitude of mechanisms, the blood vessel wall is not just an innocent bystander. Vascular cells, including vascular smooth-muscle cells (VSMC) and endothelial cells, contribute to the intramural inflammation. Receptors and ligands of the NOTCH–NOTCH ligand family appears to be particularly important in facilitating the communication between immune, mesenchymal, and endothelial cells. In GCA patients, circulating and wall-invading T cells spontaneously express NOTCH1, enabling them to interact with VSMC (Piggott et al., 2011) as well as mvEC (Wen et al., 2017). Endothelial cell–T-cell interactions have been implicated in “opening the gate” for CD4 T cells to enter the tissue. Once in the wall, NOTCH ligand-expressing VSMC could regulate the functional activity and the survival of NOTCH1<sup>+</sup> T cells.

## EXTRAVASCULAR GIANT CELL ARTERITIS

Mechanisms driving the systemic inflammatory component of GCA are less well understood. Patients with PMR in whom vascular inflammatory infiltrates are undetectable by standard histology are equally affected by systemic inflammation as GCA patients with full-fledged vasculitis (Wagner et al., 1994; Weyand et al., 1999). Thus, extravascular GCA is not simply a “spillover” from the vasculitis. In most cases, PMR presents as an isolated problem, making it unlikely that the cytokine storm of extravascular GCA induces wall inflammation.

Typical findings include a massive acute phase response with downstream effects in multiple-organ systems (Weyand and Goronzy, 2003a). Abnormalities in hematopoiesis, such as anemia and thrombocytosis, are not unusual. The liver function is often abnormal and shows elevation of alkaline phosphatase levels. By affecting the

central nervous system, the acute phase response causes fever, malaise, and depression. Profound elevation of the ESR, a typical laboratory marker of GCA, is also a consequence of excessive production of acute phase proteins.

Innate cytokines, including IL-6, IL-8, IL-12p70, MCP-1, MIP-1 $\beta$ , eotaxin, and pentraxin 3, are known to induce acute phase proteins in hepatocytes. It has long been known that IL-6 is highly elevated in the sera of PMR and GCA patients (Roche et al., 1993; Wagner et al., 1994; Weyand et al., 2000). Circulating monocytes in GCA and PMR patients are spontaneously activated and produce IL-6 (Wagner et al., 1994). Pentraxin 3 is abundant in the serum of GCA patients (Baldini et al., 2012), but the cellular origin is unresolved. Upstream signals leading to the production of innate cytokines remain unknown. Elegant studies in interstitial fluids collected from PMR-affected muscles have shown local enrichment of proinflammatory cytokines and pain-inducing substances, such as glutamate and PGE<sub>2</sub> (Kreiner and Galbo, 2011; Kreiner et al., 2010).

Extravascular GCA can exist without a vasculitic component and presents as PMR. Evidence for a causative role in the inflammatory response affecting the arterial wall is lacking, suggesting parallel pathogenic cascades that may require separate therapeutic interventions.

## Treatment, Monitoring, and Outcome

The gold standard for treatment of GCA is the use of corticosteroids. Patients respond explicitly well, with prompt and substantial improvement of symptoms within 24–48 hours. Initial doses of 60 mg of prednisone (or approx. 1 mg/kg body weight) have been found to be effective in almost all patients. Current discussions center on the question of whether at least some patients could be successfully treated with lower doses. Corticosteroids are fast and effective in suppressing the extravascular component, whereas vessel wall inflammation may require more chronic therapy (Maleszewski et al., 2017).

Once patients are stabilized on high doses of corticosteroids, daily prednisolone doses should be tapered. A reduction of 10%–20% every 2 weeks has proven to be a clinically useful guidance. Both clinical symptoms and laboratory markers of inflammation are monitored to guide the tapering process. A frequent clinical dilemma is a discrepancy between clinical and laboratory findings. To avoid overutilization of corticosteroids, it has been recommended that treatment decisions should not be solely based on laboratory results.

Many patients develop signs of flaring disease when corticosteroids are reduced. Fortunately, severe manifestations, such as sight-threatening ischemic complications, appear to be rare (Martinez-Lado et al., 2011; Weyand et al., 2000). Instead, most disease flares present as PMR or constitutional symptoms, indicative for hepatic acute phase responses and extravascular GCA. Dose adjustments of corticosteroids can effectively recapture disease control.

Corticosteroids can be discontinued in many patients after about 2 years of therapy. There is evidence that the disease process does not enter remission but continues with smoldering activity (Uddhammar, 2000; Weyand et al., 2000, 2012). Importantly, inflammatory infiltrates in the vessel wall appear to be long-lasting. Whether chronic-smoldering vasculitis requires continued immunosuppression or can be managed by watchful monitoring remains a matter of discussion.

One of the serious long-term consequences of GCA is the development of large-vessel involvement, including aortic aneurysm and dissection (Evans et al., 1994; Kermani et al., 2012; Naderi et al., 2017). In a high-incidence Swedish population, 15% of patients and 10.8% of a reference cohort had imaging evidence for large-vessel involvement, which developed after an average of 3.7 years (Naderi et al., 2017). Patients need to be informed about this complication, and monitoring for aortic wall thickness and aortic dimensions is recommended. Differences in the temporal artery histomorphology in patients with and without aortic involvement are suggestive for distinct disease pathways. Whether continuous immunosuppression can avoid this complication is entirely unclear.

With an average age of almost 75 years at disease onset and the duration of therapy for several years, patients are prone to show steroid side effects (Proven et al., 2003). Monitoring of blood glucose and blood pressure is obvious. Also, bone-sparing therapy with calcium, vitamin D substitution, bisphosphonates, etc. should be a fixed component of management.

Given the high rate of steroid side effects, efforts have been made to identify steroid-sparing therapies. In an experimental model of GCA with human temporal arteries implanted into immunodeficient mice, aspirin effectively suppressed IFN- $\gamma$  production and augmented the antiinflammatory effects of corticosteroids (Weyand et al., 2002). In contrast to other chronic inflammatory diseases, patients with GCA seem to have little benefit from methotrexate as an immunosuppressant. A meta-analysis has suggested a mild steroid-sparing effect of methotrexate (MTX) after 48 weeks of combination therapy for female but not for male patients

(Mahr et al., 2007). Similar modest effects have been reported for azathioprine as a steroid-sparing agent (De Silva and Hazleman, 1986). While anti-TNF biologics provide powerful immunosuppression in patients with rheumatoid arthritis, clinical trials testing the use of a combination therapy with corticosteroids plus infliximab compared to corticosteroids alone have been disappointing (Hoffman et al., 2007; Salvarani et al., 2007), and TNF- $\alpha$  blockade is not considered a first-line therapy in this vasculitis.

In a recent clinical trial, patients were treated with tocilizumab, an IL-6 receptor-alpha inhibitor while rapidly tapering corticosteroids. A total of 52% of tocilizumab-treated patients, but only 14% in the placebo group, were glucocorticoid-free at week 52 (Stone et al., 2017). Longer follow-up studies are necessary to determine the durability of the steroid-sparing effect and the safety of tocilizumab. The most important question is whether IL-6 blockade can suppress the vascular component of GCA or whether its effect is restricted to inhibiting the hepatic acute phase response. IL-6 blockade promptly suppresses ESR and IL-6, eliminating these two biomarkers for the monitoring of disease load. Tissue studies are needed to quantify the effect of IL-6 blockade on vascular GCA.

Difficulties in assessing disease activity in the vascular wall are exemplified by a recent study utilizing high-resolution positron emission tomography (PET). Based on FDG-PET scans, most patients with LVV in clinical remission had active disease (Grayson et al., 2018).

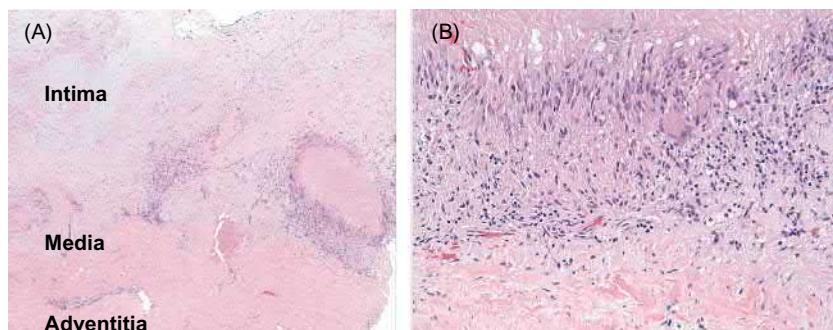
Despite concerns about the persistence of vasculitis, the overall outcome of GCA is good. In a cohort of 840 patients with biopsy-proven GCA the long-term survival was not impaired (Mohammad et al., 2015).

## TAKAYASU'S ARTERITIS

TA is a systemic arteritis that predominantly manifests in the aorta and its major branches. It is also known as pulseless disease or occlusive thromboarthritis. The typical patient is a female of Asian or South American origin presenting with vascular insufficiency and generalized inflammation in the second or third decades of life. But care must be taken not to miss the diagnosis in males and middle-aged individuals and in non-Asian populations. Inflammatory infiltrates in the wall of large elastic arteries induce thickening of the adventitia, destruction of the media, and hyperplasia of the intima (Fig. 66.4) (Gravanis, 2000). Recently, diagnosis and management of TA have benefited from advances in noninvasive imaging methods and more aggressive use of surgical procedures (Luqmani, 2012). Over the last decade, the prognosis of TA has continued to improve with more rapid diagnosis, more aggressive immunosuppression and less vaso-occlusive complications (Kim and Beckman, 2018; Ohigashi et al., 2012).

### Historic Background

In 1830, Yamamoto reported the first case of TA. In 1905, M. Takayasu was the first to describe peculiar optic fundus abnormalities with coronal anastomosis (Takayasu, 1908), which 40 years later were interpreted as neovascularization and anastomosis secondary to ischemia caused by the occlusion of cervical vessels. Shimizu and Sano (1951) described a cohort of 31 cases and made the connection between pulselessness, coronal anastomosis of retinal vessels, and carotid abnormalities, and called it pulseless disease.



**FIGURE 66.4** Takayasu's arteritis. Cross-section of a surgical specimen from the aorta of a 24 year-old female. (A) Inflammatory infiltrates in all wall layers. Massive fibrosis of the adventitia. Loss of medial smooth-muscle cells intimal thickening. The media is destroyed and the intimal is thickened. (B) Detailed view of highly activated histiocytes and multinucleated giant cells.

## Clinical, Pathologic, and Epidemiologic Features

TA is a rare disease with incidence rates of 1–2 cases in 1 million individuals per year (Watts et al., 2009). The most significant risk factors are female sex, age less than 40 years, and selected ethnic origin. The highest prevalence rates have been reported for Asian countries, including Japan, Korea, China, India, Thailand, and Turkey (Koide, 1992). South American countries, such as Mexico, Brazil, Columbia and Peru, are now also considered higher incidence areas (Dabague and Reyes, 1996). The disease does occur in Whites, can affect males, and needs to be kept in mind as a differential diagnosis in older individuals with aortitis or LVV.

Prominent histomorphologic findings include the thickening of the aortic wall which may involve all three layers (Fig. 66.4) but often is most significant in the adventitia (Bjornsson, 2002; Gravanis, 2000). Extension of inflammatory infiltrates and fibrosis into the periaortic tissues may occur. Most often, the aortic lumen is compromised, but stenotic lesions can alternate with aneurysmal dilatation giving rise to fusiform or saccular aneurysms. Characteristically, densely inflamed regions are alternating with normal vessel wall, creating the typical “inflamed” lesions. Besides the aorta and its primary branches, coronary and pulmonary arteries can be involved.

Microscopic examination shows inflammatory infiltrates composed of lymphocytes and plasma cells primarily around the vasa vasorum, causing vasa vasoritis with luminal stenosis. Lymphocytes, histiocytes, and occasional giant cells accumulate in the media, sometimes complicating the distinction between GCA and TA. Patches of medial necrosis can occur, and destruction of elastic membranes is typical. Growth of fibroblasts and smooth-muscle cells and deposition of acid mucopolysaccharides result in widening of the intimal layer, representing fibromuscular hyperplasia. Vascular insufficiency in the aortic branches is related to ostial stenosis or more extended involvement.

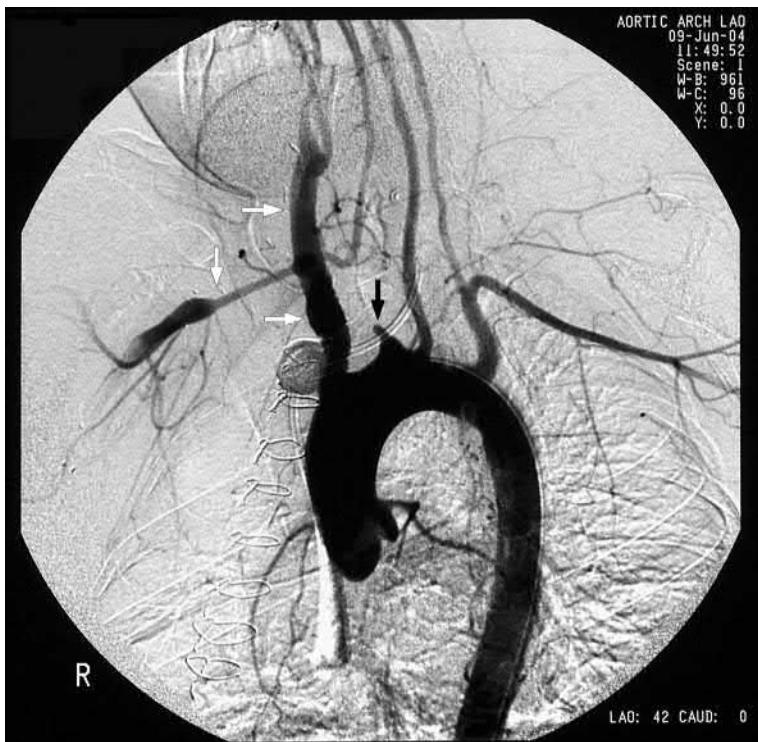
The clinical spectrum of TA may vary in distinct geographic areas (Numano, 1997) (Table 66.5). Japanese, Korean, and North American patients are more likely to present with impairment of cervical, cerebral, and upper extremity blood flow and aortic regurgitation, due to involvement of the ascending aorta and the primary aortic arch branches. In Indian patients, the abdominal aorta, including the renal arteries, appears to be the preferred target resulting in renovascular hypertension. Overall, blindness and severe retinal ischemia are less common now than they used to be (Numano, 2002).

Initial presentation may be dominated by a generalized inflammatory syndrome with fever, night sweats, weakness, arthralgias, and chest pain (Kerr et al., 1994) (Table 66.5). Direct complications of vessel wall inflammation include headaches, syncope, visual disturbances, and face and neck pain from insufficient blood flow in cervical vessels. Stenotic lesions in the brachiocephalic and subclavian arteries lead to arm claudication,

**TABLE 66.5** The Clinical Spectrum in Takayasu's Arteritis

Organ system	Sign/symptom	Frequency (%)
Vascular Takayasu's	Bruit	70
	Claudication	70
	Reduced/absent pulses	60
	Asymmetrical blood pressure	50
CNS	Dizziness	40
	Visual abnormalities	30
	Stroke/TIA	10
Cardiac	Aortic regurgitation	20
	Angina	10
	Congestive heart failure	<5
Extravascular Takayasu's	Malaise	70
	Fever	30
	Weight loss	20

TIA, transient ischemic attack.



**FIGURE 66.5** Takayasu's arteritis. Angiography of the aortic arch and the cervical vessels. The native brachiocephalic artery (black arrow) is occluded just distal to its origin. A graft has been placed (white arrows) from the ascending aorta to the right common carotid artery with a separate limb connecting to the right subclavian artery. The left carotid artery and both vertebral arteries are normal.

pulselessness, and discrepant blood pressure measurements (Fig. 66.5). Ischemic heart disease is a result of reduction in coronary blood flow. A feared complication of TA is aortic regurgitation caused by dilatation of the ascending aorta. Congestive heart failure is not unusual (Endo et al., 2003).

Marked progress has been made in imaging modalities, compensating at least partially for the difficulties in accessing tissue to establish the diagnosis of TA and follow its course for patients receiving therapy. Besides conventional angiography, which has been the standard imaging tool for diagnosing and evaluating patients with TA (see Fig. 66.5), a number of alternatives have emerged, including magnetic resonance (MR) imaging and MR angiography, computed tomography (CT), Doppler ultrasound, and metabolic imaging with PET (Cheng et al., 2013; Kissin and Merkel, 2004; Litmanovich et al., 2012; Tezuka et al., 2012). Ultrasound is particularly useful in evaluating the carotid arteries, with high sensitivity for submillimeter changes in wall thickness. The diffuse circumferential thickening (halo) of the carotid artery wall in TA has been named the "macaroni sign" (Keo et al., 2013). CT and MR imaging provide excellent visualization of vessels and their relationship to neighboring structures and demonstrate both luminal and mural abnormalities. Contrast enhancement is necessary for the CT to depict the vessel lumen, but it can provide fast information about the aorta and its wall; MR imaging has inherent multiplanar imaging capabilities, can be used with or without contrast enhancement, and lends itself for the evaluation of the aorta and its major branches (Matsunaga et al., 2003; Nastri et al., 2004). With the potential to assess mural edema and vascularity, it has been hoped that it would be an ideal instrument to monitor disease activity and progress in patients on immunosuppression. However, a recent study has cast doubt on the utility of edema-weighted MR as a sole means to estimate disease activity (Tso et al., 2002). A role for PET, which detects areas of active glucose metabolism in the vascular wall has been suggested (Webb et al., 2004), but no large-scale controlled studies are available. A recent study has reported only moderate sensitivity of 18FDG-PET in estimating disease activity in TA (Cheng et al., 2013), whereas a study of 39 Japanese patients with TA suggested superiority of FDG-PET/CT over ESR and CRP in detecting active disease (Tezuka et al., 2012).

## Genetic Features

In contrast to GCA, which is an HLA class II–associated disease, the strongest genetic risk factor for TA has been localized to the HLA class I region. Specifically, HLA-B\*52 is most enriched amongst TA patients, holding in several ethnicities (Terao, 2016). Through genome-wide associations studies, several non-HLA susceptibility

loci have been added to the list, including IL12B, IL-6, FCGR2A/3A, RPS9/LILRB3, supporting the concept of a complex genetic predisposition for this LVV (Renauer and Sawalha, 2017). Interestingly, TA patients share a significant proportion of their genetic background with patients diagnosed with ulcerative colitis, including enrichment of HLA-B\*52:01 and cooccurrence of ulcerative colitis with TA (Terao et al., 2015).

## Pathogenic Mechanisms

Despite considerable overlap in the histomorphology and clinical presentation of GCA and TA, evidence suggests that pathogenic mechanisms are distinct (Seko, 2002). Whereas CD4<sup>+</sup> T cells producing IFN- $\gamma$  have been identified as key regulators in the vascular lesions of GCA, they appear to be less important in TA. In the vascular infiltrates of TA,  $\gamma\delta$  T cells account for 31% of the cells, natural killer cells for 20%, and CD8<sup>+</sup> cytotoxic T cells for 15% (Seko, 2000). CD4<sup>+</sup> T cells, macrophages, and B cells were less frequent. In support for the concept that cytotoxic cells directly damage resident cells in the aorta, Seko et al. (1994) have shown cellular expression of perforin and deposition of perforin directly onto wall resident cells. Heat-shock proteins, in particular HSP65, have been proposed as the antigen stimulating cytotoxic lymphocytes (Seko et al., 2000). Restricted usage of T-cell receptor AV and BV genes in tissue infiltrating T cells also support the concept of selective expansion of antigen-reactive T cells in the vessel wall infiltrates (Seko et al., 1996). Dense expression of costimulatory molecules is in line with a role for adaptive immune responses driving the disease process. Data suggesting a possible role of metalloproteinases as biomarkers of disease activities refocus interest on macrophage-dependent disease mechanisms (Matsuyama et al., 2003). Destruction of elastic membranes points toward release of elastolytic enzymes, likely derived from tissue-infiltrating macrophages.

Much less is known about the pathomechanisms underlying extravascular TA. Cytokine levels of IL-6 and RANTES have been reported to be elevated and to correlate with clinical activity (Noris et al., 1999). Serum IL-18 levels have been correlated with disease activity (Park et al., 2006). IL-18 is a Th1 immunity promoting cytokine, suggesting a role for IFN- $\gamma$ -producing T cells in this vasculitis. Possible, not yet validated, biomarkers of disease activity include pentraxin-3 and MMP-9.

## Treatment and Outcome

Immunosuppressive therapy remains the mainstay of treatment for this arteritis, but, in contrast to GCA, surgical procedures are gaining in importance for managing patients with TA (Perrotta et al., 2012). While endovascular treatment of TA has been associated with poor outcomes with respect to patency, more recent experience with newer endovascular techniques appear to be associated with better results (Qureshi et al., 2011).

As with most rare diseases, randomized controlled treatment trials are explicitly difficult to perform, are missing, or are based on small patient numbers. The immunosuppressants of choice are corticosteroids, which are started at a dose of 40–60 mg/day prednisone. Clinicians in Japan have advocated doses of only 20–30 mg/day (Numano, 2002). Once acute disease activity is controlled, an effort needs to be made to reduce corticosteroids. Tapering by 5 mg/day every 2–3 weeks has been adopted as a useful guideline. However, there has been agreement that a target maintenance dose of 5–10 mg/day should be kept stable over a prolonged period to avoid exacerbation of vascular and generalized inflammation. Although not formally tested, most patients receive aspirin or an alternative agent to reduce platelet aggregation and thrombus formation.

About 50% of patients may be considered to be insufficiently treated with corticosteroid monotherapy (Kerr et al., 1994). Some of these patients may benefit from methotrexate as a steroid-sparing agent (Hoffman, et al., 1994). Mycophenolate mofetil has been reported to show clinical efficiency in a small patient cohort (Daina, et al., 1999). TNF- $\alpha$  blocking agents have been reported to induce sustained remission in a subset of patients, but about 50% of patients fail treatment with anti-TNF- $\alpha$  (Schmidt, et al., 2012). Given the role of IL-6 in driving inflammation in TA, rescue therapy with anti-IL-6 blocking antibodies has been reported for selected cases (Salvarani, et al., 2012). More recent reports of persistent aortic wall inflammation despite IL-6-blocking therapy have raised concerns that this therapeutic approach may be less effective than expected (Xenitidis et al., 2013). In a recent randomized, double-blind, placebo-controlled, phase 3 trial testing the efficacy and safety of tocilizumab in patients with refractory TA, the primary endpoint of the study was not met, questioning the usefulness of IL-6 blockade in this patient population (Nakaoka et al., 2018).

Monitoring for and managing hypertension is prudent in patients with TA. There is an ongoing discussion about whether vascular inflammation predisposes for the accelerated development and progression of

atherosclerosis, although it may be difficult to separate these disease processes (Numano et al., 2000). Accelerated atherosclerosis demands appropriate monitoring for risk factors and treatment of dyslipidemia.

Critical renal artery stenosis, limiting claudication of extremities, cerebrovascular ischemia, coronary ischemia, and aortic regurgitation may represent indications for surgical intervention. If clinically possible, vascular inflammation should be quiescent prior to surgery. Prevention of stroke may be possible if critical stenosis of cervical vessels is bypassed with grafts originating from the ascending aorta. Percutaneous transluminal angioplasty has emerged as an alternative to bypass surgery, specifically for renal artery stenosis (Weaver et al., 2004), and may be useful for preserving blood flow in other territories.

The best outcome data for TA are available from Japan where all patients with TA are registered by the government. Analysis of 897 patients through 1998 showed that more than 70% of patients had well-controlled disease, enjoying almost normal lives. Twenty-five percent of patients had severe complications. Cardiac manifestations have become the most common cause of death among TA patients (Numano, 2002). Modern imaging techniques are enabling close monitoring of vessel wall inflammation. The time between disease onset and diagnosis is shortening (Isobe, 2013), and the overall prognosis is improving. In a recent analysis of 251 TA patients in Southern India, upfront immunosuppressive combination therapy was associated with only 7% of refractory disease, and relapse-free survival was above 50% at 10 years (Goel et al., 2018).

## CONCLUDING REMARKS—FUTURE PERSPECTIVES

Vasculitides are infrequent yet clinically challenging diseases because they threaten blood supply to vital organs. Damage to large and medium-sized arteries immediately puts the host at risk for severe clinical consequences, as compensatory mechanisms for losing the function of the aorta and its major branches are very limited. Large-vessel arteritides are characterized by a combination of extravascular and vascular disease. Pathogenic mechanisms relevant for these two disease dimensions are distinct, require distinct diagnostic approaches, and respond differentially to therapy. Extravascular LVV is characterized by the induction of an abrupt and massive acute phase response. Hepatic acute phase proteins elevate ESR and CRP. Upstream inducers appear to be innate cytokines, for example, IL-6. Such cytokines are druggable, but whether their blockade has any impact on vessel wall inflammation is unknown. Possibly, the extravascular component lies downstream of the vasculitic events in the arteries, serving as a biomarker, but not a driver of arteritis.

The aorta and its large branches are immune-privileged tissues, in line with their life-sustaining function. Three mechanisms have been identified, which lead to breakdown of arterial wall immune protection and arteritis: unopposed expansion of CD4 T cells in the peripheral immune system (Wen et al., 2016); leakiness of microvascular networks that are supposed to keep T cells out of the vessel wall (Wen et al., 2017); and uncontrolled T-cell activation in the wall due to failure of the immunoinhibitory PD-1 checkpoint (Zhang et al., 2017).

Once T cells have made it into the wall structure, they become the key regulators of tissue injury. They either mediate direct cellular damage, as in the case of TA, or orchestrate the functional activity of tissue-injurious macrophages, as in GCA. Arterial wall injury causes hyperproliferation of myofibroblasts and results in thickening of the intima, the underlying mechanism of vessel stenosis/occlusion.

GCA and TA are unique among the chronic inflammatory diseases in that they respond explicitly well to corticosteroids. However, even prolonged therapy can usually not induce complete remission and the vessel wall inflammation enters a phase of chronic-smoldering persistence. Conventional immunosuppressive appear amazingly ineffective in depleting inflammatory cells from the vessel wall. Better understanding of mechanisms that foster tissue-residence of T cells and of the maladaptive repair mechanism that sustain wall remodeling is needed to target core elements of vasculitis and its consequences.

Despite advances in noninvasive imaging methods, monitoring patients for disease activity continues to be a challenge. Structural changes, such as neoangiogenesis and neointimal growth, may not be reversible. Biomarkers reflecting persistence of adaptive immunity in the wall and of maladaptive tissue remodeling need to be developed to capture these elements of vasculitis. It is relatively easy to follow the hepatic acute phase response linked to extravascular vasculitis, as it leads to laboratory abnormalities such as elevation of ESR and CRP, anemia, thrombocytosis, etc.

The ultimate challenge in understanding GCA and TA remains the identification of the initial triggers of the disease process. Predictably, infectious microorganisms are suspected to start vasculitis, but unequivocal evidence is missing. Local factors intrinsic to the artery itself, such as loss of protective mechanisms, acquisition of immune-stimulatory functions of nonimmune vascular cells, or misfiring of vascular dendritic cells, may be

instrumental in breaking tolerance and giving rise to misplaced immune responses (Watanabe et al., 2017b). Also, age is a major risk factor in GCA and TA, suggesting that age-related changes in T-cell function may be critical determinants of disease susceptibility (Goronzy and Weyand, 2017).

## Acknowledgment

Supported in part by the National Institutes of Health (R01 AR042527, R01 HL117913, R01 AI108906 and P01 HL129941 to CMW and R01 AI108891, R01 AG045779, U19 AI057266, R01 AI129191 and I01 BX001669 to JJG).

## References

- Alvarez-Rodriguez, L., Munoz Cacho, P., Lopez-Hoyos, M., Beares, I., Mata, C., Calvo-Alen, J., et al., 2011. Toll-like receptor 4 gene polymorphism and giant cell arteritis susceptibility: a cumulative meta-analysis. *Autoimmun. Rev.* 10, 790–792.
- Andersson, R., Jonsson, R., Tarkowski, A., Bengtsson, B.A., Malmvall, B.E., 1987. T cell subsets and expression of immunological activation markers in the arterial walls of patients with giant cell arteritis. *Ann. Rheum. Dis.* 46, 915–923.
- Baldini, M., Maugeri, N., Ramirez, G.A., Giacomassi, C., Castiglioni, A., Prieto-Gonzalez, S., et al., 2012. Selective up-regulation of the soluble pattern-recognition receptor pentraxin 3 and of vascular endothelial growth factor in giant cell arteritis: relevance for recent optic nerve ischemia. *Arthritis Rheum.* 64, 854–865.
- Baldursson, O., Steinsson, K., Bjornsson, J., Lie, J.T., 1994. Giant cell arteritis in Iceland. An epidemiologic and histopathologic analysis. *Arthritis Rheum.* 37, 1007–1012.
- Bjornsson, J., 2002. Histopathology of primary vasculitis disorders. In: Hoffman, G.S., Weyand, C.M. (Eds.), *Inflammatory Diseases of Blood Vessels*. Marcel Dekker, Inc, New York.
- Borkowski, A., Younge, B.R., Szweda, L., Mock, B., Bjornsson, J., Moeller, K., et al., 2002. Reactive nitrogen intermediates in giant cell arteritis: selective nitration of neocapillaries. *Am. J. Pathol.* 161, 115–123.
- Brack, A., Geisler, A., Martinez-Taboada, V.M., Younge, B.R., Goronzy, J.J., Weyand, C.M., 1997. Giant cell vasculitis is a T cell-dependent disease. *Mol. Med.* 3, 530–543.
- Brack, A., Martinez-Taboada, V., Stanson, A., Goronzy, J.J., Weyand, C.M., 1999. Disease pattern in cranial and large-vessel giant cell arteritis. *Arthritis Rheum.* 42, 311–317.
- Carmona, F.D., Mackie, S.L., Martin, J.E., Taylor, J.C., Vaglio, A., Eyre, S., et al., 2015. A large-scale genetic analysis reveals a strong contribution of the HLA class II region to giant cell arteritis susceptibility. *Am. J. Hum. Genet.* 96, 565–580.
- Carmona, F.D., Vaglio, A., Mackie, S.L., Hernandez-Rodriguez, J., Monach, P.A., Castaneda, S., et al., 2017. A genome-wide association study identifies risk alleles in plasminogen and P4HA2 associated with giant cell arteritis. *Am. J. Hum. Genet.* 100, 64–74.
- Chan, C.C., Paine, M., O'Day, J., 2005. Predictors of recurrent ischemic optic neuropathy in giant cell arteritis. *J. Neuroophthalmol.* 25, 14–17.
- Chatelain, D., Duhaut, P., Loire, R., Bosshard, S., Pellet, H., Piette, J.C., et al., 2008. Small-vessel vasculitis surrounding an uninflamed temporal artery: a new diagnostic criterion for polymyalgia rheumatica? *Arthritis Rheum.* 58, 2565–2573.
- Cheng, Y., Lv, N., Wang, Z., Chen, B., Dang, A., 2013. 18FDG-PET in assessing disease activity in Takayasu arteritis: a meta-analysis. *Clin. Exp. Rheumatol.* 31, S22–S27.
- Ciccia, F., Alessandro, R., Rizzo, A., Raimondo, S., Giardina, A., Raiata, F., et al., 2013. IL-33 is overexpressed in the inflamed arteries of patients with giant cell arteritis. *Ann. Rheum. Dis.* 72, 258–264.
- Cid, M.C., 2002. Endothelial cell biology, perivascular inflammation, and vasculitis. *Cleve. Clin. J. Med.* 69 (Suppl 2), SII45–SII49.
- Dabague, J., Reyes, P.A., 1996. Takayasu arteritis in Mexico: a 38-year clinical perspective through literature review. *Int. J. Cardiol.* 54 (Suppl), S103–109.
- Daina, E., Schieppati, A., Remuzzi, G., 1999. Mycophenolate mofetil for the treatment of Takayasu arteritis: report of three cases. *Ann. Intern. Med.* 130, 422–426.
- Dasgupta, B., Cimmino, M.A., Kremers, H.M., Schmidt, W.A., Schirmer, M., Salvarani, C., et al., 2012. 2012 provisional classification criteria for polymyalgia rheumatica: a European League Against Rheumatism/American College of Rheumatology collaborative initiative. *Arthritis Rheum.* 64, 943–954.
- Davies, M., Duffield, E.A., 2017. Safety of checkpoint inhibitors for cancer treatment: strategies for patient monitoring and management of immune-mediated adverse events. *Immunotargets Ther.* 6, 51–71.
- de Jong, H.J., Saldi, S.R., Klungel, O.H., Vandebriel, R.J., Souverein, P.C., Meyboom, R.H., et al., 2012. Statin-associated polymyalgia rheumatica. An analysis using WHO global individual case safety database: a case/non-case approach. *PLoS One* 7, e41289.
- De Silva, M., Hazleman, B.L., 1986. Azathioprine in giant cell arteritis/polymyalgia rheumatica: a double-blind study. *Ann. Rheum. Dis.* 45, 136–138.
- Deng, J., Younge, B.R., Olshan, R.A., Goronzy, J.J., Weyand, C.M., 2010. Th17 and Th1 T-cell responses in giant cell arteritis. *Circulation* 121, 906–915.
- Doran, M.F., Crowson, C.S., O'Fallon, W.M., Hunder, G.G., Gabriel, S.E., 2002. Trends in the incidence of polymyalgia rheumatica over a 30 year period in Olmsted County, Minnesota, USA. *J. Rheumatol.* 29, 1694–1697.
- Endo, M., Tomizawa, Y., Nishida, H., Aomi, S., Nakazawa, M., Tsurumi, Y., et al., 2003. Angiographic findings and surgical treatments of coronary artery involvement in Takayasu arteritis. *J. Thor. Cardiovasc. Surg.* 125, 570–577.
- Evans, J.M., Hunder, G.G., 2000. Polymyalgia rheumatica and giant cell arteritis. *Rheum. Dis. Clin. N. Am.* 26, 493–515.
- Evans, J.M., Bowles, C.A., Bjornsson, J., Mullany, C.J., Hunder, G.G., 1994. Thoracic aortic aneurysm and rupture in giant cell arteritis. A descriptive study of 41 cases. *Arthritis Rheum.* 37, 1539–1547.

- Goel, R., Danda, D., Joseph, G., Ravindran, R., Kumar, S., Jayaseelan, V., et al., 2018. Long-term outcome of 251 patients with Takayasu arteritis on combination immunosuppressant therapy: Single centre experience from a large tertiary care teaching hospital in Southern India. *Semin. Arthritis Rheum.* 47, 718–726.
- Gonzalez, E.B., Varner, W.T., Lisse, J.R., Daniels, J.C., Hokanson, J.A., 1989. Giant cell arteritis in the southern United States. An 11-year retrospective study from the Texas Gulf Coast. *Arch. Intern. Med.* 149, 1561–1565.
- Goronzy, J.J., Weyand, C.M., 2017. Successful and maladaptive T cell aging. *Immunity* 46, 364–378.
- Gravanis, M.B., 2000. Giant cell arteritis and Takayasu aortitis: morphologic, pathogenetic and etiologic factors. *Int. J. Cardiol.* 75 (Suppl 1), S21–S33. discussionS35–26.
- Grayson, P.C., Alehashemi, S., Bagheri, A.A., Civelek, A.C., Cupps, T.R., Kaplan, M.J., et al., 2018. Positron emission tomography as an imaging biomarker in a prospective, longitudinal cohort of patients with large vessel vasculitis. *Arthritis Rheumatol.* 70, 439–449.
- Henriet, J.P., Marin, J., Gosselin, J., Hamel-Desnos, C., Ducrocq, M., Brard, G., et al., 1989. [The history of Horton's disease or ... 10 centuries of a fascinating adventure]. *J. Mal. Vasc.* 14 (Suppl C), 93–97.
- Hernandez-Rodriguez, J., Segarra, M., Vilardell, C., Sanchez, M., Garcia-Martinez, A., Esteban, M.J., et al., 2004. Tissue production of pro-inflammatory cytokines (IL-1beta, TNFalpha and IL-6) correlates with the intensity of the systemic inflammatory response and with corticosteroid requirements in giant-cell arteritis. *Rheumatology* 43, 294–301.
- Hill, C.L., Black, R.J., Nossent, J.C., Ruediger, C., Nguyen, L., Ninan, J.V., et al., 2017. Risk of mortality in patients with giant cell arteritis: a systematic review and meta-analysis. *Semin. Arthritis Rheum.* 46, 513–519.
- Hoffman, G.S., Leavitt, R.Y., Kerr, G.S., Rottem, M., Sneller, M.C., Fauci, A.S., 1994. Treatment of glucocorticoid-resistant or relapsing Takayasu arteritis with methotrexate. *Arthritis Rheum.* 37, 578–582.
- Hoffman, G.S., Cid, M.C., Rendt-Zagar, K.E., Merkel, P.A., Weyand, C.M., Stone, J.H., et al., 2007. Infliximab for maintenance of glucocorticosteroid-induced remission of giant cell arteritis: a randomized trial. *Ann. Intern. Med.* 146, 621–630.
- Horton, B.T., Magath, T.B., Brown, G.E., 1932. An undescribed form of arteritis of the temporal vessels. *Proc. Staff Meet. Mayo Clinic* 7, 700–701.
- Hutchinson, J., 1890. Diseases of the arteries. On a peculiar form of thrombotic arteritis of the aged which is sometimes productive of gangrene. *Arch. Surg.* 1, 323–329.
- Isobe, M., 2013. Takayasu arteritis revisited: current diagnosis and treatment. *Int. J. Cardiol.* 168, 3–10.
- Jakobsson, K., Jacobsson, L., Warrington, K., Matteson, E.L., Liang, K., Melander, O., et al., 2015. Body mass index and the risk of giant cell arteritis: results from a prospective study. *Rheumatology* 54, 433–440.
- Kaiser, M., Weyand, C.M., Bjornsson, J., Goronzy, J.J., 1998. Platelet-derived growth factor, intimal hyperplasia, and ischemic complications in giant cell arteritis. *Arthritis Rheum.* 41, 623–633.
- Kaiser, M., Younge, B., Bjornsson, J., Goronzy, J.J., Weyand, C.M., 1999. Formation of new vasa vasorum in vasculitis. Production of angiogenic cytokines by multinucleated giant cells. *Am. J. Pathol.* 155, 765–774.
- Keo, H.H., Caliezi, G., Baumgartner, I., Diehm, N., Willenberg, T., 2013. Increasing echogenicity of diffuse circumferential thickening ("macaroni sign") of the carotid artery wall with decreasing inflammatory activity of Takayasu arteritis. *J. Clin. Ultrasound: JCU* 41, 59–62.
- Kermani, T.A., Warrington, K.J., 2013. Polymyalgia rheumatica. *Lancet* 381, 63–72.
- Kermani, T.A., Warrington, K.J., Crowson, C.S., Ytterberg, S.R., Hunder, G.G., Gabriel, S.E., et al., 2012. Large-vessel involvement in giant cell arteritis: a population-based cohort study of the incidence-trends and prognosis. *Ann. Rheum. Dis.* 1–6. *Epub*.
- Kerr, G.S., Hallahan, C.W., Giordano, J., Leavitt, R.Y., Fauci, A.S., Rottem, M., et al., 1994. Takayasu arteritis. *Ann. Intern. Med.* 120, 919–929.
- Kim, E.S.H., Beckman, J., 2018. Takayasu arteritis: challenges in diagnosis and management. *Heart* 104, 558–565.
- Kissin, E.Y., Merkel, P.A., 2004. Diagnostic imaging in Takayasu arteritis. *Curr. Opin. Rheumatol.* 16, 31–37.
- Koide, K., 1992. Takayasu arteritis in Japan. *Heart Vessels Suppl.* 7, 48–54.
- Kreiner, F., Galbo, H., 2011. Elevated muscle interstitial levels of pain-inducing substances in symptomatic muscles in patients with polymyalgia rheumatica. *Pain* 152, 1127–1132.
- Kreiner, F., Langberg, H., Galbo, H., 2010. Increased muscle interstitial levels of inflammatory cytokines in polymyalgia rheumatica. *Arthritis Rheum.* 62, 3768–3775.
- Krupa, W.M., Dewan, M., Jeon, M.S., Kurtin, P.J., Younge, B.R., Goronzy, J.J., et al., 2002. Trapping of misdirected dendritic cells in the granulomatous lesions of giant cell arteritis. *Am. J. Pathol.* 161, 1815–1823.
- Lie, J.T., 1995. Aortic and extracranial large vessel giant cell arteritis: a review of 72 cases with histopathologic documentation. *Semin. Arthritis Rheum.* 24, 422–431.
- Litmanovich, D.E., Yildirim, A., Bankier, A.A., 2012. Insights into imaging of aortitis. *Insights Imaging* 3, 545–560.
- Luqmani, R., 2012. Large vessel vasculitides: update for the cardiologist. *Curr. Opin. Cardiol.* 27, 578–584.
- Ma-Krupa, W., Jeon, M.S., Spoerl, S., Tedder, T.F., Goronzy, J.J., Weyand, C.M., 2004. Activation of arterial wall dendritic cells and breakdown of self-tolerance in giant cell arteritis. *J. Exp. Med.* 199, 173–183.
- Machado, E.B., Michet, C.J., Ballard, D.J., Hunder, G.G., Beard, C.M., Chu, C.P., et al., 1988. Trends in incidence and clinical presentation of temporal arteritis in Olmsted County, Minnesota, 1950–1985. *Arthritis Rheum.* 31, 745–749.
- Mahr, A.D., Jover, J.A., Spiera, R.F., Hernandez-Garcia, C., Fernandez-Gutierrez, B., Lavallee, M.P., et al., 2007. Adjunctive methotrexate for treatment of giant cell arteritis: an individual patient data meta-analysis. *Arthritis Rheum.* 56, 2789–2797.
- Maleszewski, J.J., Younge, B.R., Fritzzen, J.T., Hunder, G.G., Goronzy, J.J., Warrington, K.J., et al., 2017. Clinical and pathological evolution of giant cell arteritis: a prospective study of follow-up temporal artery biopsies in 40 treated patients. *Mod. Pathol.* 30, 788–796.
- Martinez-Lado, L., Calvino-Diaz, C., Pineiro, A., Dierssen, T., Vazquez-Rodriguez, T.R., Miranda-Filloy, J.A., et al., 2011. Relapses and recurrences in giant cell arteritis: a population-based study of patients with biopsy-proven disease from northwestern Spain. *Medicine* 90, 186–193.
- Matsunaga, N., Hayashi, K., Okada, M., Sakamoto, I., 2003. Magnetic resonance imaging features of aortic diseases. *Top. Magn. Reson. Imaging: TMRI* 14, 253–266.

- Matsuyama, A., Sakai, N., Ishigami, M., Hiraoka, H., Kashine, S., Hirata, A., et al., 2003. Matrix metalloproteinases as novel disease markers in Takayasu arteritis. *Circulation* 108, 1469–1473.
- Mohammad, A.J., Nilsson, J.A., Jacobsson, L.T., Merkel, P.A., Turesson, C., 2015. Incidence and mortality rates of biopsy-proven giant cell arteritis in southern Sweden. *Ann. Rheum. Dis.* 74, 993–997.
- Naderi, N., Mohammad, A.J., Turesson, C., 2017. Large vessel involvement in biopsy-proven giant cell arteritis: incidence, distribution, and predictors. *Scand. J. Rheumatol.* 46, 215–221.
- Nakaoka, Y., Isobe, M., Takei, S., Tanaka, Y., Ishii, T., Yokota, S., et al., 2018. Efficacy and safety of tocilizumab in patients with refractory Takayasu arteritis: results from a randomised, double-blind, placebo-controlled, phase 3 trial in Japan (the TAKT study). *Ann. Rheum. Dis.* 77, 348–354.
- Nastri, M.V., Baptista, L.P., Baroni, R.H., Blasbalg, R., de Avila, L.F., Leite, C.C., et al., 2004. Gadolinium-enhanced three-dimensional MR angiography of Takayasu arteritis. *Radiographics* 24, 773–786.
- Nordborg, C., Johansson, H., Petursdottir, V., Nordborg, E., 2003. The epidemiology of biopsy-positive giant cell arteritis: special reference to changes in the age of the population. *Rheumatology* 42, 549–552.
- Noris, M., Daina, E., Gamba, S., Bonazzola, S., Remuzzi, G., 1999. Interleukin-6 and RANTES in Takayasu arteritis: a guide for therapeutic decisions? *Circulation* 100, 55–60.
- Numano, F., 1997. Differences in clinical presentation and outcome in different countries for Takayasu's arteritis. *Curr. Opin. Rheumatol.* 9, 12–15.
- Numano, F. (Ed.), 2002. *Takayasu's Arteritis: Clinical Aspects*. Marcel Dekker, Inc, New York.
- Numano, F., Kishi, Y., Tanaka, A., Ohkawara, M., Kakuta, T., Kobayashi, Y., 2000. Inflammation and atherosclerosis. Atherosclerotic lesions in Takayasu arteritis. *Ann. N. Y. Acad. Sci.* 902, 65–76.
- O'Neill, L., Rooney, P., Molloy, D., Connolly, M., McCormick, J., McCarthy, G., et al., 2015. Regulation of inflammation and angiogenesis in giant cell arteritis by acute-phase serum amyloid A. *Arthritis Rheumatol.* 67, 2447–2456.
- Ohigashi, H., Haraguchi, G., Konishi, M., Tezuka, D., Kamiishi, T., Ishihara, T., et al., 2012. Improved prognosis of Takayasu arteritis over the past decade—comprehensive analysis of 106 patients. *Circ. J.* 76, 1004–1011.
- Park, M.C., Lee, S.W., Park, Y.B., Lee, S.K., 2006. Serum cytokine profiles and their correlations with disease activity in Takayasu's arteritis. *Rheumatology* 45, 545–548.
- Perrotta, S., Radberg, G., Perrotta, A., Lentini, S., 2012. Aneurysmatic disease in patients with Takayasu disease: a case review. *Herz* 37, 347–353.
- Piggott, K., Deng, J., Warrington, K., Younge, B., Kubo, J.T., Desai, M., et al., 2011. Blocking the NOTCH pathway inhibits vascular inflammation in large-vessel vasculitis. *Circulation* 123, 309–318.
- Proven, A., Gabriel, S.E., Orces, C., O'Fallon, W.M., Hunder, G.G., 2003. Glucocorticoid therapy in giant cell arteritis: duration and adverse outcomes. *Arthritis Rheum.* 49, 703–708.
- Pryshchev, O., Ma-Krupa, W., Younge, B.R., Goronzy, J.J., Weyand, C.M., 2008. Vessel-specific Toll-like receptor profiles in human medium and large arteries. *Circulation* 118, 1276–1284.
- Qureshi, M.A., Martin, Z., Greenberg, R.K., 2011. Endovascular management of patients with Takayasu arteritis: stents versus stent grafts. *Semin. Vasc. Surg.* 24, 44–52.
- Renauer, P., Sawalha, A.H., 2017. The genetics of Takayasu arteritis. *Presse Med.* 46, e179–e187.
- Rittner, H.L., Hafner, V., Klimiuk, P.A., Szweda, L.I., Goronzy, J.J., Weyand, C.M., 1999a. Aldose reductase functions as a detoxification system for lipid peroxidation products in vasculitis. *J. Clin. Invest.* 103, 1007–1013.
- Rittner, H.L., Kaiser, M., Brack, A., Szweda, L.I., Goronzy, J.J., Weyand, C.M., 1999b. Tissue-destructive macrophages in giant cell arteritis. *Circ. Res.* 84, 1050–1058.
- Roche, N.E., Fulbright, J.W., Wagner, A.D., Hunder, G.G., Goronzy, J.J., Weyand, C.M., 1993. Correlation of interleukin-6 production and disease activity in polymyalgia rheumatica and giant cell arteritis. *Arthritis Rheum.* 36, 1286–1294.
- Rodriguez-Rodriguez, L., Carmona, F.D., Castaneda, S., Miranda-Filloy, J.A., Morado, I.C., Narvaez, J., et al., 2011a. Role of rs1343151 IL23R and rs3790567 IL12RB2 polymorphisms in biopsy-proven giant cell arteritis. *J. Rheumatol.* 38, 889–892.
- Rodriguez-Rodriguez, L., Castaneda, S., Vazquez-Rodriguez, T.R., Morado, I.C., Gomez-Vaquero, C., Mari-Alfonso, B., et al., 2011b. Role of the rs6822844 gene polymorphism at the IL2-IL21 region in biopsy-proven giant cell arteritis. *Clin. Exp. Rheumatol.* 29, S12–S16.
- Rojo-Leyva, F., Ratliff, N.B., Cosgrove III, D.M., Hoffman, G.S., 2000. Study of 52 patients with idiopathic aortitis from a cohort of 1,204 surgical cases. *Arthritis Rheum.* 43, 901–907.
- Salvarani, C., 2003. Large vessel vasculitis. *Clin. Exp. Rheumatol.* 21, S133–S134.
- Salvarani, C., Crowson, C.S., O'Fallon, W.M., Hunder, G.G., Gabriel, S.E., 2004. Reappraisal of the epidemiology of giant cell arteritis in Olmsted County, Minnesota, over a fifty-year period. *Arthritis Rheum.* 51, 264–268.
- Salvarani, C., Macchioni, P., Manzini, C., Paolazzi, G., Trotta, A., Manganelli, P., et al., 2007. Infliximab plus prednisone or placebo plus prednisone for the initial treatment of polymyalgia rheumatica: a randomized trial. *Ann. Intern. Med.* 146, 631–639.
- Salvarani, C., Magnani, L., Catanoso, M.G., Pipitone, N., Versari, A., Dardani, L., et al., 2012. Rescue treatment with tocilizumab for Takayasu arteritis resistant to TNF-alpha blockers. *Clin. Exp. Rheumatol.* 30, S90–S93.
- Samson, M., Audia, S., Fraszczak, J., Trad, M., Ornetti, P., Lakomy, D., et al., 2012. Th1 and Th17 lymphocytes expressing CD161 are implicated in giant cell arteritis and polymyalgia rheumatica pathogenesis. *Arthritis Rheum.* 64, 3788–3798.
- Schmidt, J., Kermani, T.A., Bacani, A.K., Crowson, C.S., Matteson, E.L., Warrington, K.J., 2012. Tumor necrosis factor inhibitors in patients with Takayasu arteritis: experience from a referral center with long-term followup. *Arthritis Care Res.* 64, 1079–1083.
- Seko, Y., 2000. Takayasu arteritis: insights into immunopathology. *Jpn. Heart J.* 41, 15–26.
- Seko, Y., 2002. Takayasu's arteritis: pathogenesis. In: Hoffman, G.S. (Ed.), *Inflammatory Diseases of Blood Vessels*. Marcel Dekker, New York.
- Seko, Y., Minota, S., Kawasaki, A., Shinkai, Y., Maeda, K., Yagita, H., et al., 1994. Perforin-secreting killer cell infiltration and expression of a 65-kD heat-shock protein in aortic tissue of patients with Takayasu's arteritis. *J. Clin. Invest.* 93, 750–758.

- Seko, Y., Sato, O., Takagi, A., Tada, Y., Matsuo, H., Yagita, H., et al., 1996. Restricted usage of T-cell receptor Valpha-Vbeta genes in infiltrating cells in aortic tissue of patients with Takayasu's arteritis. *Circulation* 93, 1788–1790.
- Seko, Y., Takahashi, N., Tada, Y., Yagita, H., Okumura, K., Nagai, R., 2000. Restricted usage of T-cell receptor Vgamma-Vdelta genes and expression of costimulatory molecules in Takayasu's arteritis. *Int. J. Cardiol.* 75 (Suppl 1), S77–S83. discussion S85-77.
- Shimizu, K., Sano, K., 1951. Pulseless disease. *J. Neuropath. Clin. Neurol.* 1, 37–47.
- Singh, A.G., Kermani, T.A., Crowson, C.S., Weyand, C.M., Matteson, E.L., Warrington, K.J., 2015. Visual manifestations in giant cell arteritis: trend over 5 decades in a population-based cohort. *J. Rheumatol.* 42, 309–315.
- Smetana, G.W., Shmerling, R.H., 2002. Does this patient have temporal arteritis? *JAMA* 287, 92–101.
- Smith, C.A., Fidler, W.J., Pinals, R.S., 1983. The epidemiology of giant cell arteritis. Report of a ten-year study in Shelby County, Tennessee. *Arthritis Rheum.* 26, 1214–1219.
- Sorbi, D., French, D.L., Nuovo, G.J., Kew, R.R., Arbeit, L.A., Gruber, B.L., 1996. Elevated levels of 92-kd type IV collagenase (matrix metalloproteinase 9) in giant cell arteritis. *Arthritis Rheum.* 39, 1747–1753.
- Stone, J.H., Tuckwell, K., Dimonaco, S., Klearman, M., Aringer, M., Blockmans, D., et al., 2017. Trial of tocilizumab in giant-cell arteritis. *N. Engl. J. Med.* 377, 317–328.
- Takayasu, M., 1908. A case with peculiar changes of the central retinal vessels. *Acta Soc. Ophthalm. Jpn.* 12, 554–555.
- Terao, C., 2016. Revisited HLA and non-HLA genetics of Takayasu arteritis—where are we? *J. Hum. Genet.* 61, 27–32.
- Terao, C., Matsumura, T., Yoshifiji, H., Kirino, Y., Maejima, Y., Nakao, Y., et al., 2015. Takayasu arteritis and ulcerative colitis: high rate of co-occurrence and genetic overlap. *Arthritis Rheumatol.* 67, 2226–2232.
- Terrier, B., Geri, G., Chaara, W., Allenbach, Y., Rosenzwaig, M., Costedoat-Chalumeau, N., et al., 2012. Interleukin-21 modulates Th1 and Th17 responses in giant cell arteritis. *Arthritis Rheum.* 64, 2001–2011.
- Tezuka, D., Haraguchi, G., Ishihara, T., Ohigashi, H., Inagaki, H., Suzuki, J., et al., 2012. Role of FDG PET-CT in Takayasu arteritis: sensitive detection of recurrences. *JACC Cardiovasc. Imaging* 5, 422–429.
- Tomita, T., Imakawa, K., 1998. Matrix metalloproteinases and tissue inhibitors of metalloproteinases in giant cell arteritis: an immunocytochemical study. *Pathology* 30, 40–50.
- Tso, E., Flamm, S.D., White, R.D., Schwartzman, P.R., Mascha, E., Hoffman, G.S., 2002. Takayasu arteritis: utility and limitations of magnetic resonance imaging in diagnosis and treatment. *Arthritis Rheum.* 46, 1634–1642.
- Uddhammar, A.C., 2000. Von Willebrand factor in polymyalgia rheumatica and giant cell arteritis. *Clin. Exp. Rheumatol.* 18, S32–S33.
- Wagner, A.D., Goronzy, J.J., Weyand, C.M., 1994. Functional profile of tissue-infiltrating and circulating CD68+ cells in giant cell arteritis. Evidence for two components of the disease. *J. Clin. Invest.* 94, 1134–1140.
- Wagner, A.D., Bjornsson, J., Bartley, G.B., Goronzy, J.J., Weyand, C.M., 1996. Interferon-gamma-producing T cells in giant cell vasculitis represent a minority of tissue-infiltrating cells and are located distant from the site of pathology. *Am. J. Pathol.* 148, 1925–1933.
- Watanabe, R., Hosgur, E., Zhang, H., Wen, Z., Berry, G., Goronzy, J.J., et al., 2017a. Pro-inflammatory and anti-inflammatory T cells in giant cell arteritis. *Joint Bone Spine* 84, 421–426.
- Watanabe, R., Zhang, H., Berry, G., Goronzy, J.J., Weyand, C.M., 2017b. Immune checkpoint dysfunction in large and medium vessel vasculitis. *Am. J. Physiol. Heart Circ. Physiol.* 312, H1052–H1059.
- Watts, R., Al-Taiar, A., Mooney, J., Scott, D., Macgregor, A., 2009. The epidemiology of Takayasu arteritis in the UK. *Rheumatology* 48, 1008–1011.
- Weaver, F.A., Kumar, S.R., Yellin, A.E., Anderson, S., Hood, D.B., Rowe, V.L., et al., 2004. Renal revascularization in Takayasu arteritis-induced renal artery stenosis. *J. Vasc. Surg.* 39, 749–757.
- Webb, M., Chambers, A., A L-Nahas, A., Mason, J.C., Maudlin, L., Rahman, L., et al., 2004. The role of 18F-FDG PET in characterising disease activity in Takayasu arteritis. *Eur. J. Nucl. Med. Mol. Imaging* 31, 627–634.
- Wen, Z., Shimojima, Y., Shirai, T., Li, Y., Ju, J., Yang, Z., et al., 2016. NADPH oxidase deficiency underlies dysfunction of aged CD8+ Tregs. *J. Clin. Invest.* 126, 1953–1967.
- Wen, Z., Shen, Y., Berry, G., Shahram, F., Li, Y., Watanabe, R., et al., 2017. The microvascular niche instructs T cells in large vessel vasculitis via the VEGF-Jagged1-Notch pathway. *Sci. Transl. Med.* 9. Available from: <https://doi.org/10.1126/scitranslmed.aal3322>.
- Weyand, C.M., 2000. The Dunlop-Dottridge Lecture: the pathogenesis of giant cell arteritis. *J. Rheumatol.* 27, 517–522.
- Weyand, C.M., Goronzy, J.J., 1995. Giant cell arteritis as an antigen-driven disease. *Rheum. Dis. Clin. N. Am.* 21, 1027–1039.
- Weyand, C.M., Goronzy, J.J., 2003a. Giant cell arteritis and polymyalgia rheumatica. *Ann. Inter. Med.* 139, 505–515.
- Weyand, C.M., Goronzy, J.J., 2003b. Medium- and large-vessel vasculitis. *N. Engl. J. Med.* 349, 160–169.
- Weyand, C.M., Hicok, K.C., Hunder, G.G., Goronzy, J.J., 1992. The HLA-DRB1 locus as a genetic component in giant cell arteritis. Mapping of a disease-linked sequence motif to the antigen binding site of the HLA-DR molecule. *J. Clin. Invest.* 90, 2355–2361.
- Weyand, C.M., Hunder, N.N., Hicok, K.C., Hunder, G.G., Goronzy, J.J., 1994a. HLA-DRB1 alleles in polymyalgia rheumatica, giant cell arteritis, and rheumatoid arthritis. *Arthritis Rheum.* 37, 514–520.
- Weyand, C.M., Schonberger, J., Oppitz, U., Hunder, N.N., Hicok, K.C., Goronzy, J.J., 1994b. Distinct vascular lesions in giant cell arteritis share identical T cell clonotypes. *J. Exp. Med.* 179, 951–960.
- Weyand, C.M., Wagner, A.D., Bjornsson, J., Goronzy, J.J., 1996. Correlation of the topographical arrangement and the functional pattern of tissue-infiltrating macrophages in giant cell arteritis. *J. Clin. Invest.* 98, 1642–1649.
- Weyand, C.M., Tetzlaff, N., Bjornsson, J., Brack, A., Younge, B., Goronzy, J.J., 1997. Disease patterns and tissue cytokine profiles in giant cell arteritis. *Arthritis Rheum.* 40, 19–26.
- Weyand, C.M., Fulbright, J.W., Evans, J.M., Hunder, G.G., Goronzy, J.J., 1999. Corticosteroid requirements in polymyalgia rheumatica. *Arch. Intern. Med.* 159, 577–584.
- Weyand, C.M., Fulbright, J.W., Hunder, G.G., Evans, J.M., Goronzy, J.J., 2000. Treatment of giant cell arteritis: interleukin-6 as a biologic marker of disease activity. *Arthritis Rheum.* 43, 1041–1048.
- Weyand, C.M., Kaiser, M., Yang, H., Younge, B., Goronzy, J.J., 2002. Therapeutic effects of acetylsalicylic acid in giant cell arteritis. *Arthritis Rheum.* 46, 457–466.

- Weyand, C.M., Liao, Y.J., Goronzy, J.J., 2012. The immunopathology of giant cell arteritis: diagnostic and therapeutic implications. *J. Neuroophthalmol.* 32, 259–265.
- Weyand, C.M., Berry, G.J., Goronzy, J.J., 2018. The immunoinhibitory PD-1/PD-L1 pathway in inflammatory blood vessel disease. *J. Leukoc. Biol.* 103, 565–575.
- Xenitidis, T., Horger, M., Zeh, G., Kanz, L., Henes, J.C., 2013. Sustained inflammation of the aortic wall despite tocilizumab treatment in two cases of Takayasu arteritis. *Rheumatology* 52, 1729–1731.
- Zhang, H., Watanabe, R., Berry, G.J., Vaglio, A., Liao, Y.J., Warrington, K.J., et al., 2017. Immunoinhibitory checkpoint deficiency in medium and large vessel vasculitis. *Proc. Natl. Acad. Sci. U. S. A.* 114, E970–E979.

# Idiopathic and Autoimmune Interstitial Lung Disease

Brian Gelbman<sup>1</sup> and Ronald G. Crystal<sup>1,2</sup>

<sup>1</sup>Division of Pulmonary and Critical Care Medicine, Department of Medicine, Weill Medical College of Cornell University, New York, NY, United States <sup>2</sup>Department of Genetic Medicine, Weill Medical College of Cornell University, New York, NY, United States

## O U T L I N E

<b>Introduction</b>			
<b>History</b>			
Cryptogenic Organizing Pneumonia	1335	In Vivo and In Vitro Models	1344
Idiopathic Pulmonary Fibrosis	1336	Cryptogenic Organizing Pneumonia	1344
Idiopathic Pulmonary Fibrosis	1336	Idiopathic Pulmonary Fibrosis	1345
<b>Clinical, Pathological and Epidemiological Features</b>			
Cryptogenic Organizing Pneumonia	1336	<b>Pathologic Effector Mechanisms</b>	1345
Idiopathic Pulmonary Fibrosis	1337	Cryptogenic Organizing Pneumonia	1345
Idiopathic Pulmonary Fibrosis	1337	Idiopathic Pulmonary Fibrosis	1346
<b>Autoimmune Features</b>			
Cryptogenic Organizing Pneumonia	1340	<b>Treatment and Outcome</b>	1348
Idiopathic Pulmonary Fibrosis	1340	Cryptogenic Organizing Pneumonia	1348
Antifibrotic Agents	1341	Idiopathic Pulmonary Fibrosis	1348
<b>Genetic Features</b>			
Cryptogenic Organizing Pneumonia	1343	Antifibrotic Agents	1349
Idiopathic Pulmonary Fibrosis	1344	<b>Conclusions</b>	1349
		References	1349

## INTRODUCTION

Interstitial lung disease is a broad category of heterogenous diseases which share the common feature of inflammatory and fibrotic changes that primarily affect the alveoli and small airways. The two most common manifestations are cryptogenic organizing pneumonia (COP) and idiopathic pulmonary fibrosis (IPF), both of which can occur as “idiopathic” conditions or in association with several autoimmune diseases. Although both are classified as “lung disorders of unknown etiology,” there are multiple clues suggesting that autoimmune mechanisms play a role in the pathogenesis of both disorders. The focus of this chapter is to explore the link between autoimmune mechanisms underlying COP and IPF as models for autoimmune disorders of the lung. To do so, we first discuss the history and the clinical features of COP and IPF, followed by a summary of the autoimmune, genetic, environmental features, current concepts of pathogenesis, and the therapies currently available and under investigation for both disorders.

## HISTORY

Although both COP and IPF are inflammatory/fibrotic disorders, they have different clinical, radiologic, and pathologic features and therefore are considered to be different disorders.

### Cryptogenic Organizing Pneumonia

The term “bronchiolitis obliterans” was first used by [Lange \(1901\)](#), when he described two patients in whom the bronchioles were blocked by the plugs of granulation tissue, though those patients likely had what is now known as “COP.” In [Baar and Galindo \(1966\)](#) used the term “bronchiolitis fibrosa obliterans” to describe concentric rings of fibrotic tissue in the wall of the airways. In [Gosink et al. \(1973\)](#) employed “bronchiolitis obliterans” to describe a group of patients that had submucosal and peribronchiolar infiltrate of granulation tissue resulting in extrinsic narrowing of the bronchiolar lumen. During the 1970s, several rheumatological conditions, particularly rheumatoid arthritis (RA), were recognized to be associated with bronchiolitis obliterans, as were adverse reactions to therapies used for RA such as gold and penicillamine ([Geddes et al., 1977](#)).

In [Davison et al. \(1983\)](#) first used the term “cryptogenic organizing pneumonitis” to describe eight patients that had pulmonary infiltrates and histology similar to bronchopneumonia, yet no organism was identified, and they responded to corticosteroids. In [Epler et al. \(1985\)](#) similarly described 50 patients not only with bronchiolitis obliterans but also with granulation tissue in airways, airway ducts, and alveoli and coined the term bronchiolitis obliterans organizing pneumonia (BOOP). COP and BOOP are now believed to represent the same disease, and COP is the preferred term. Current usage of the term “bronchiolitis obliterans” is reserved primarily for transplant-related lung injury that occurs almost exclusively at the bronchiole.

### Idiopathic Pulmonary Fibrosis

The first recognition of the broad category of idiopathic interstitial lung diseases, which includes IPF, is credited to [Osler \(1892\)](#), when he described a chronic fibrinoid change occurring between the alveolus and the blood vessels. Osler demonstrated great foresight by also noting that there were diverse patterns of the disease that made classification difficult.

IPF, also referred to as “cryptogenic fibrosing alveolitis,” “idiopathic interstitial pneumonitis,” “usual interstitial pneumonitis” (UIP), and “idiopathic interstitial pneumonia,” was first described by the Czech pathologist Sandoz (1907), but this went largely unnoticed. [Hamman and Rich \(1935\)](#) are frequently credited with the first description of IPF in 1935, despite the fact that their initial cases were more acute in presentation, unlike classic IPF, and may have been the descriptions of acute respiratory distress syndrome (ARDS). In [Scadding \(1964\)](#) coined the term “cryptogenic fibrosing alveolitis” to describe IPF as a diffuse inflammatory and fibrotic lung disease affecting primarily the alveoli. Scadding advanced the concept that IPF was a slow, progressive disease that was distinctly different from the relatively acute process described by Hamman and Rich.

IPF was further defined by Liebow and Carrington in the 1960s using histologic criteria. These investigators separated IPF into two morphologic patterns, which they referred to as “usual interstitial pneumonia” (now often referred to as “usual interstitial pneumonitis”) and desquamative interstitial pneumonia (DIP; [Liebow and Carrington, 1969](#)). The inflammatory component of IPF was characterized by our group using bronchoalveolar lavage (BAL) to distinguish IPF from other interstitial lung disorders ([Crystal et al., 1976](#); [Reynolds et al., 1977](#)). The pathological pattern of UIP was further refined over the next several decades, including the description of nonspecific interstitial pneumonia (NSIP) by [Katzenstein and Fiorelli \(1994\)](#) as a distinct entity. The classification system developed by the American Thoracic Society (ATS) and European Respiratory Society (ERS) in 2001 separates IPF from NSIP and DIP based primarily on pathologic findings (American Thoracic Society and European Respiratory Society, 2002). The most recent guidelines on idiopathic interstitial pneumonias were published by ATS/ERS/Japanese Respiratory Society in 2011 and highlight the importance of a multidisciplinary discussion to render a diagnosis ([Raghu et al., 2011](#)).

## CLINICAL, PATHOLOGICAL AND EPIDEMIOLOGICAL FEATURES

### Cryptogenic Organizing Pneumonia

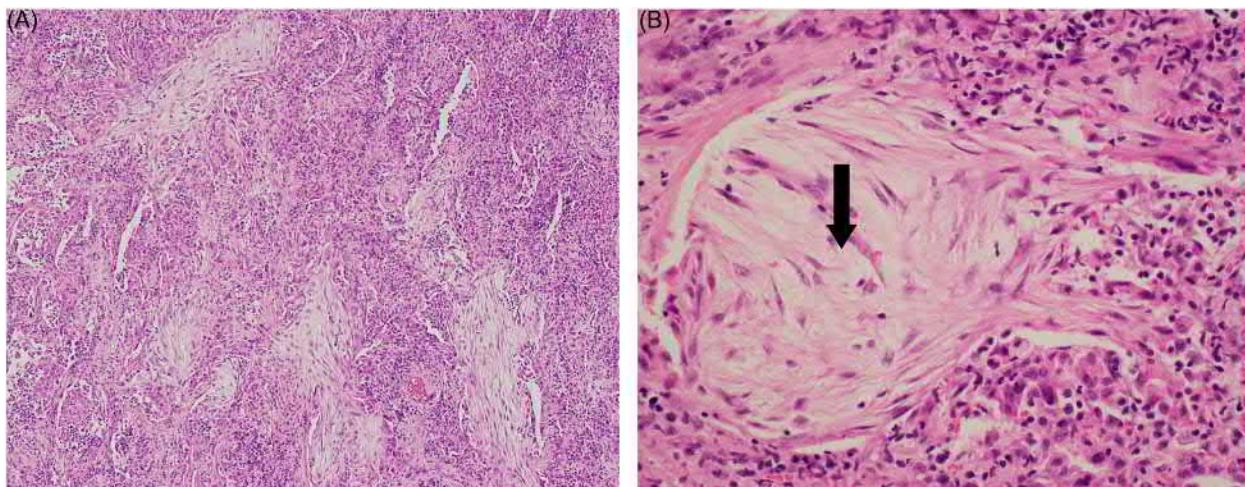
COP is identified pathologically by an accumulation of granulation tissue resulting in near obstruction of the small airways and that can extend into the alveoli with a surrounding interstitial lympho-plasmacytic infiltrate (Murray and Nadel, 2000). Overall, there is preservation of the underlying lung architecture and apparent temporal homogeneity, with very little fibrotic changes present (Fig. 67.1). These findings may also be present during the “organizing” phase of a bacterial or viral pneumonia, but when they occur in the absence of an infectious disease, it is termed “COP.” Thus the definition relies on both pathologic and clinical features.

Individuals with COP usually present with persistent cough and worsening dyspnea on exertion, which can mimic bronchopneumonia. The physical exam is notable for crackles in the affected lobe(s). Most patients will have a mild obstructive pattern on pulmonary function testing, although the disease can present with normal, restrictive, or mixed pattern (King and Mortenson, 1992). Routine chest radiographs may reveal bilateral air-space opacities, peripherally located, that can be migratory. High-resolution computed tomography (HRCT) reveals patchy air-space consolidations or ground glass opacities that can be indistinguishable from bacterial pneumonia or eosinophilic pneumonia. Fibrosis or honeycombing is rarely seen. The areas affected by COP are scattered and patchy in distribution. For this reason, transbronchial lung biopsy is insensitive as a diagnostic tool, as it often misses the involved area, and thus a surgical lung biopsy is often necessary to make the diagnosis.

There have been no formal epidemiological studies that quantify the overall prevalence of COP, although one study found a yearly cumulative incidence of six to seven cases per 100,000 hospital admissions (Alasaly et al., 1995). COP is generally considered a rare disease, though it is likely that it is underdiagnosed and may account for a small percentage of cases misdiagnosed as bacterial or viral pneumonia.

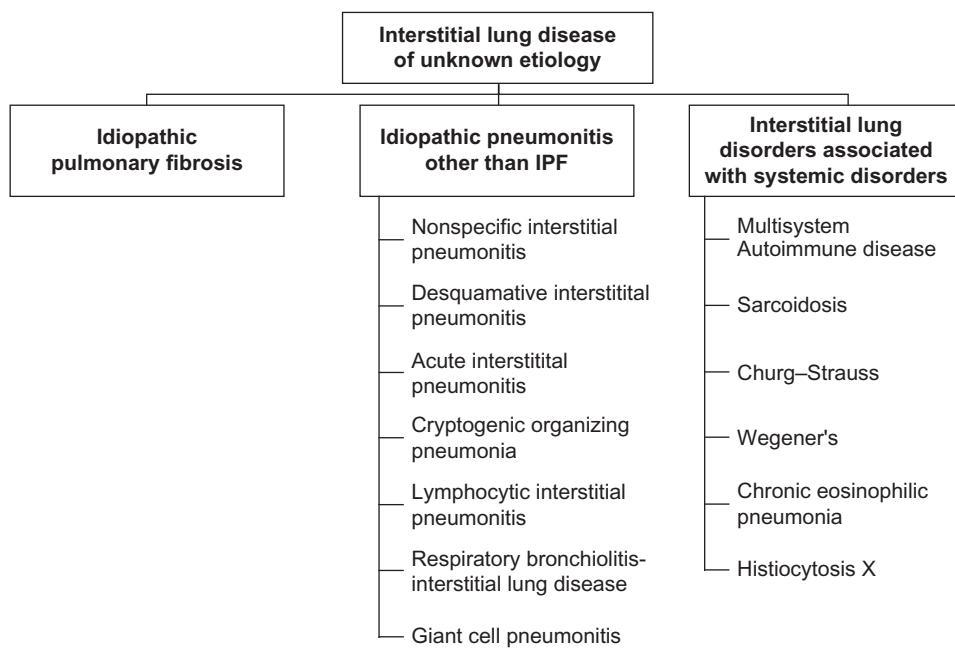
### Idiopathic Pulmonary Fibrosis

IPF (sometimes referred to as “cryptogenic fibrosing alveolitis” in Europe) refers to a distinctive type of chronic inflammatory/fibrotic interstitial lung disorder of unknown cause that is limited to the lungs and



Images Courtesy of Alain Borczuk, M.D.  
Department of Pathology,  
Weill Cornell Medical Center

**FIGURE 67.1** Histologic pattern of cryptogenic organizing pneumonia. (A) Low power view of lung tissue, showing that the normal lung architecture with intraalveolar fibroblastic plugs filling several airspaces, surrounded by lymphoplasmacytic infiltrates. (B) High power view demonstrating fibroblastic proliferation occurs amidst loose extracellular matrix filling the airspace (arrow).



**FIGURE 67.2** Relationship of idiopathic pulmonary fibrosis to other idiopathic interstitial disorders. Some of the rare disorders have been left off the lists [see (American Thoracic Society and European Respiratory Society, 2002; Cantin and Crystal, 1985; Schoenberger and Crystal, 1983) for further details].

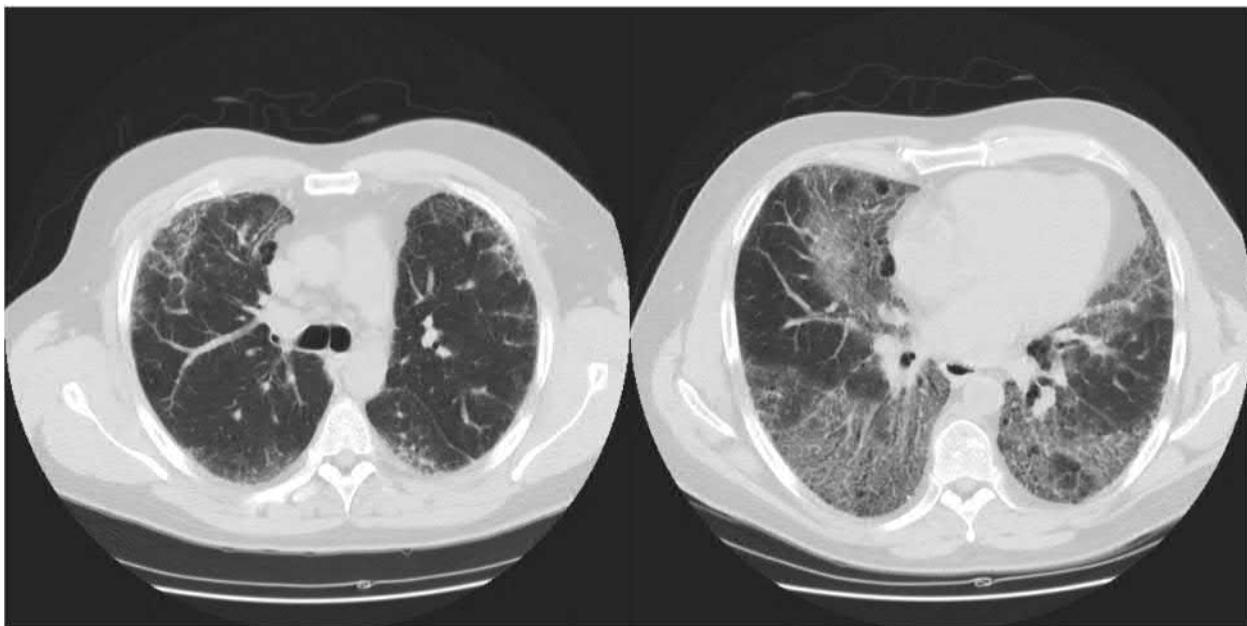
associated with a histologic pattern of UIP (Fig. 67.2; American Thoracic Society and European Respiratory Society, 2002). The pattern of UIP on surgical lung biopsy is also seen in patients with multisystem autoimmune disease who present with similar pulmonary-related clinical features as IPF (Harrison et al., 1991).

Individuals with IPF typically present with the gradual onset of symptoms, most commonly dyspnea on exertion and nonproductive cough. Most will have had at least 6 months of symptoms before presentation, with an average duration of 24 months (American Thoracic Society and European Respiratory Society, 2002). The clinical course typically involves gradual deterioration, occasionally punctuated by periods of rapid decline, although some of the diseases stabilize in some individuals. The best estimates for life expectancy from time of diagnosis to death are derived from case-control cohort studies that are not biased like prevalence studies which tend to be overrepresented by survivors. According to two such studies, the average survival from the time of diagnosis to death is 3–4 years (Hubbard et al., 1998; Mapel et al., 1998). This survival average likely represents the more aggressive phases of the disease. With the increased utilization of CT scans, more cases of IPF have been identified at the earlier stages of disease and have much slower progression.

On physical exam, patients with IPF may have digital clubbing (25%–50%) and fine, inspiratory, Velcro-like crackles confined to the bases of the lungs. As there is progressive loss of alveoli, and the diffusing capacity drops below 50% predicted, pulmonary hypertension develops, first with exercise, and later at rest (Crystal et al., 1976; McLees et al., 1979). Once established at rest, the pulmonary hypertension is associated with an increased pulmonary component of the S2 on cardiac exam, and eventual fixed split of the second sound. In the late stages of the disease, there can be signs of right heart failure, such as peripheral edema.

Pulmonary function testing usually shows a restrictive pattern of ventilatory defects and a decrease in diffusing capacity, although in the early stages of the disease, these tests can be normal (Cherniack et al., 1995; Fulmer et al., 1979; Keogh and Crystal, 1980). Individuals with IPF typically have mild-to-moderate hypoxemia with concomitant low resting oxygen saturation that falls with exercise (Keogh and Crystal, 1980).

Chest radiographs of individuals with IPF are routinely abnormal, with a reticular pattern seen at the periphery and in the bases. Later, in the disease, there is honeycombing and volume loss in the lower lobes (Staples et al., 1987). In the early stages of the disease, chest radiographs may be normal. HRCT is more sensitive and is the preferred imaging modality. The typical HRCT findings are reticular–nodular opacities (Fig. 67.3). Later, in the disease, traction bronchiectasis is commonly seen in the peripheral and basal segments of the lower lobes. Volume loss and ground glass opacities are frequently present. As the disease progresses, honeycomb cysts develop and enlarge overtime (Staples et al., 1987). The chest X-ray and HRCT appearance of IPF is nearly



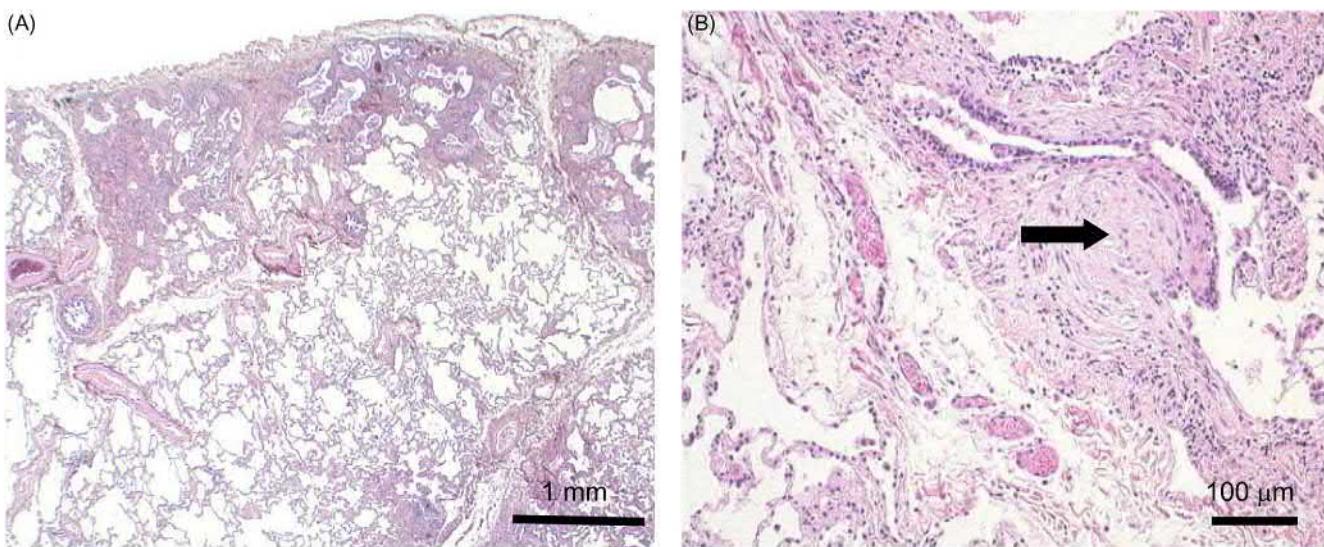
**FIGURE 67.3** Diffuse reticular–nodular pattern on high-resolution chest tomography commonly observed in idiopathic pulmonary fibrosis. Note areas of honeycombing.

identical to that seen in pulmonary fibrosis associated with the rheumatic disorders, with the lone exception of possibly more basal involvement seen on HRCT in IPF (Chan et al., 1997).

Transbronchial biopsies are insufficient to diagnose IPF because the biopsy specimen is too small and does not preserve the architecture of the lung. However, transbronchial biopsies may be useful to rule out other conditions, such as sarcoidosis, that can mimic IPF. Bronchoscopic lung cryobiopsy is a new technique that permits a larger sample of lung to be obtained through the flexible bronchoscope that provides diagnostic information with lower risk (Lentz et al., 2017). A surgical lung biopsy has been required in the past to firmly establish the diagnosis. However, the consensus statement by the ATS and ERS permits the diagnosis of IPF when there are typical clinical and HRCT findings of IPF (American Thoracic Society and European Respiratory Society, 2002; Hunninghake et al., 2001; Raghu et al., 2011). Surgical lung biopsies are still recommended whenever there are atypical clinical or HRCT features.

The histological pattern characteristic for IPF is referred to as UIP (Fig. 67.4). This pattern is distinct from the other forms of idiopathic interstitial lung disorders such as DIP, lymphocytic interstitial pneumonia, COP, nonspecific interstitial pneumonia, and acute interstitial pneumonia (American Thoracic Society and European Respiratory Society, 2002; Katzenstein and Fiorelli, 1994; Liebow and Carrington, 1969). There is typically a heterogeneous distribution of interstitial fibrosis interspersed within the areas of normal lung, suggesting different temporal stages of involvement. There is patchy inflammation, dominated by alveolar macrophages, and to a lesser extent, lymphocytes, neutrophils, and sometimes eosinophils. The alveolar epithelium undergoes marked changes, referred to as “cuboidalization,” with a loss of type I epithelial cells, and their replacement with cuboidal cells, both alveolar type II epithelial cells and bronchiolar epithelium. One hallmark finding in IPF is the subepithelial “fibroblastic focus,” which is a nodule of spindle shaped, mesenchymal cells that produce an abundant deposition of extracellular matrix. These “foci” are believed to be the “leading edge” of the fibrotic process. Occasionally, the interstitial fibrosis extends through breaks in the epithelium into the alveolar space to form intraalveolar fibrosis (Basset et al., 1986). Later, in the disease, there is architectural destruction and fibrosis with honeycombing. Frequently, the subpleural parenchyma is the most severely involved region. When a biopsy shows areas of both UIP and nonspecific interstitial pneumonitis, the default pathologic diagnosis becomes the clinical diagnosis IPF, as these patients have been shown to behave similarly to IPF with UIP-only pattern (Raghu et al., 2011; Flaherty et al., 2001).

The histological features of pulmonary fibrosis associated with multisystem autoimmune disease can be classified into the same pathological descriptions of idiopathic interstitial pneumonias put forth by the ATS and ERS, including UIP, NSIP, DIP, and COP (Fig. 67.2). The UIP pattern that is seen with multisystem autoimmune



**FIGURE 67.4** Histologic pattern of idiopathic pulmonary fibrosis. The patterns are often referred to as “usual interstitial pneumonitis.” (A) Subpleural, paraseptal, and interstitial collagen deposition together with the areas of mildly abnormal alveolar parenchyma (hematoxylin and eosin, bar = 1 mm). (B) Thickened interstitium with collagen deposition, mild-to-moderate alveolar septal mononuclear cell infiltrates, mainly lymphocytes, cuboidalization of the epithelium and large fibroblastic foci (thick arrow). The fibroblastic foci are composed of spindled mesenchymal cells and are thought to represent the “leading edge” of the progressive fibroinflammatory process (hematoxylin and eosin; bar = 100 m).

diseases is essentially identical to the pattern seen in the IPF. In polymyositis, dermatomyositis, and scleroderma, NSIP is the most frequently seen histologic pattern followed by UIP (Bouros et al., 2002; Douglas et al., 2001). The prevalence of the different histologic subtypes of idiopathic interstitial pneumonias has not been well characterized in the other multisystem autoimmune diseases.

The estimated annual incidence of IPF is 7 per 100,000 for women and 10 per 100,000 for men. Most patients present between 50 and 70 years of age; however, the incidence, prevalence, and death rate rise with age (Coults et al., 1994).

Several studies have tried to establish the prevalence of pulmonary fibrosis in patients with the multisystem autoimmune diseases, but the results have varied depending on the method by which patients are screened for pulmonary involvement. Studies that utilize autopsy or lung biopsy data are subject to overdiagnosis, since they have been shown to detect subclinical disease that fail to progress (Cervantes-Perez et al., 1980). Likewise, HRCT screening studies may also overestimate the prevalence of disease by detecting parenchymal changes that may not be clinically significant. In RA, cross-sectional studies using chest radiographs estimate the prevalence to be less than 5% of patients (Hyland et al., 1983). When HRCT is used to screen patients, the prevalence appears to be between 20% and 50%, although these studies are limited by selection bias (Remy-Jardin et al., 1994; Gabbay et al., 1997). Pulmonary fibrosis is most prevalent in scleroderma, where autopsy series have shown it to be present in 70% of the cases and chest radiograph series estimate the prevalence between 25% and 65% (Wiedemann and Matthay, 1989; Minai et al., 1998). The prevalence of clinically overt pulmonary fibrosis is 30% in polymyositis and dermatomyositis, 5% in systemic lupus erythematosus, and 10% in Sjogren’s syndrome (Schwarz, 1998; Gardiner, 1993; Eisenberg et al., 1973).

## AUTOIMMUNE FEATURES

### Cryptogenic Organizing Pneumonia

COP has been reported in association with many different types of autoimmune disorders, including RA, lupus erythematosus, ankylosing spondylitis, Sjogren’s syndrome, and scleroderma (Ryu et al., 2003; Colby, 1998). COP is most frequently seen with RA. In one case series of 40 RA patients undergoing open lung biopsy, COP was the second most common diagnosis behind rheumatoid nodules of the lung (Yousem et al., 1985). In RA, bronchiolar involvement typically presents in the fifth and sixth decades of life and is more frequently seen

in women (Epler et al., 1979; Geddes et al., 1977; Herzog et al., 1981). Most patients with COP in association with RA have long-standing symptoms of arthritis, though occasionally the pulmonary involvement predates the rheumatologic disease. Occasionally, COP will appear at the same time as a flare in arthritic symptoms, and there is a case report of it occurring simultaneously with the onset of pernicious anemia, an autoimmune disease. Further support for the autoimmune basis is the fact that most cases of COP in RA will have a positive rheumatoid factor (85%) and usually have very high titers (Mori et al., 2008). Immunofluorescence stains in the affected regions of the lung of patients with RA have IgM and IgG depositions in the alveolar septum (Yousem et al., 1985; Begin et al., 1982), and many cells are positive for S-100 protein (Yoshinouchi et al., 1999).

The BAL fluid from patients with COP is notable for a marked activation of macrophages and lymphocytes, and an overall Th1 helper response (Cordier, 2006). BAL obtained from patients with COP has elevated levels of tryptase, mast cells, and interleukins-10, -12, and -18 (Forlani et al., 2002; Pesci et al., 1996). Platelet-derived growth factor and interleukin-8 also appear to play a role in the pathogenesis of COP (Carre et al., 1994; Aubert et al., 1997).

Indirect evidence for autoimmune mechanisms in COP comes from the bronchiolitis obliterans syndrome associated with both lung and allogeneic stem cell transplants. These cases, which can be viewed as *in vivo* human models of bronchiolar disease, have been more extensively studied than COP or rheumatic disease-associated COP. The bronchiolitis obliterans syndrome is the main cause of morbidity and mortality in association with lung transplantation (Arcasoy and Kotloff, 1999). It is a form of chronic lung rejection that is mediated by B-cell and T-cell activation against mismatched HLA class I and II antigens (Kelly and Hertz, 1997). Bronchiolitis obliterans associated with allogeneic bone marrow transplants is also thought to represent graft versus host disease. The disease progresses from inflammatory changes around the bronchioles to fibrosis and scarring (Ratanatharathorn et al., 2001). In bronchiolitis obliterans syndrome associated with lung transplant or with bone marrow transplant, the bronchioles are the more frequent site for chronic rejection, not the lung parenchyma.

## Idiopathic Pulmonary Fibrosis

Although the etiology of IPF remains unknown, it has been hypothesized for 30 years that autoimmune mechanisms to external stimuli are the driving force for repeated injury and inflammation that ultimately lead to fibrosis (Crystal et al., 1976, 1981, 1984). Although no clear infectious or environmental agent has been found as the etiology of IPF (Gross and Hunninghake, 2001), there are extensive data regarding autoimmune cellular and humoral processes ongoing in this disorder.

Initial studies in IPF relevant to autoimmunity focused on identifying autoantibodies that target the pulmonary parenchyma. Turner-Warwick demonstrated that 40% patients with IPF had circulating, nonspecific autoantibodies such as antinuclear antibodies (ANA) and rheumatoid factor (Turner-Warwick and Doniach, 1965). Increases in circulating ANA and rheumatoid factor were observed in 30% of our patients (Crystal et al., 1976). Similar studies which evaluated the presence of autoantibodies to nuclear antigens, DNA topoisomerase, and cytokeratin detected the antibodies in some patients (Table 67.1). More recent studies have focused on antibodies that are specific to lung proteins, although these studies have also shown variable expression of autoantibodies (Robinson et al., 2001; Wallace et al., 1994). These results raise the possibility that autoantibodies are a secondary consequence of the ongoing immune activation in the setting of inflammation and tissue damage, rather than the causative mechanism.

Similarly, investigations have evaluated the role that immune complex deposition plays in the pathogenesis of inflammation and fibrosis in IPF. Early studies observed the elevated levels of circulating immune complexes in certain subsets of patients with IPF; however, these were performed prior to the current classification system and included patients with other forms of interstitial lung disease (Haslam et al., 1979; Dreisin et al., 1978). Hunninghake et al. (1981) demonstrated a correlation between the level of immune complexes in BAL fluid and the levels of neutrophil chemotactic factor released by alveolar macrophages in patients with IPF. Other studies have also seen the elevated levels of immune complex in lavage fluid, but the levels are variable and do not correlate with disease activity.

More recently, attention has turned to immune-based microvascular injury as the ongoing trigger for pulmonary fibrosis. In scleroderma-associated pulmonary fibrosis, studies have shown complement and immunoglobulin deposition in the microvasculature. Some have hypothesized that perhaps antiendothelial cell antibodies, which are present in circulation in several autoimmune conditions, may be responsible for the injury.

**TABLE 67.1** Autoantibodies Associated with Idiopathic Pulmonary Fibrosis<sup>a</sup>

Antigens	Number of patients	Result	Reference
Fresh unfixed lung tissue, rheumatoid factor, nuclear, thyroglobulin, gastric parietal cells	48 IPF	Sensitivity: positive titers for: RF 49%, ANA 28%, rat liver homogenate 19%, gastric cells 0%; no autoantibodies specific to lung were detected by immunofluorescence	Turner-Warwick and Doniach (1965)
Nuclear ANA, ds-DNA, ss-DNA, RF <sup>b</sup>	53 IPF 33 SLE <sup>c</sup> 50 controls	Sensitivity: IPF patients had 42% ANA (titer > 1/10), 25% anti-dsDNA, and 100% anti-ssDNA titers that were two standard deviations above the normal range, levels did not correlate with disease activity	Holgate et al. (1983)
Nuclear <sup>d</sup> ANA, nRNP, dsDNA, Sm, SS-A and SS-B	68 IPF 54 PF-AID 47 controls	Sensitivity: ANA present in 21% of IPF and 46% of PF-AID. Anti-nRNP present in 15% of IPF and PF-AID. Other antibodies were not significantly different from control group  Specificity: ANA 94%, anti-nRNP 98%	Chapman et al. (1984)
Histidyl-tRNA synthetase (Jo-1 antigen)	62 IPF 19 PF-AID (myositis) 53 myositis alone	Sensitivity: Antibody present in 68% of PF-AID (myositis) patients compared to 3% IPF alone and 7.5% myositis alone  Specificity: >99% normal and 98% autoimmune controls	Bernstein et al. (1984)
Topoisomerase II	41 IPF	Sensitivity: 44% positive. Remained elevated in 17 of 19 follow up patients. Did not correlate with disease activity	Meliconi et al. (1993)
Collagen type I, II, III, IV	16 IPF 29 controls	Sensitivity: 75% of IPF patients had antibody titers to at least one type of collagen >1:16  Specificity: 83% using cutoff titer 1:16  Negative correlation between antibody level and duration of disease	Nakos et al. (1993)
Lung proteins derived from IPF, sarcoid, and normal lung	17 IPF 17 controls	Sensitivity: 71% of IPF patients had IgG that reacted to lung proteins in the 70–90 kDa range by western blot; IgG reacted to alveolar lining cells  Specificity: 82%	Wallace et al. (1994)
Cytokeratin 19	26 IPF 11 PF-AID 52 controls	Significantly higher mean serum levels in IPF compared to control. No cutoff value determined by ELISA because of overlap between groups	Fujita et al. (1999)
Expressed cDNA library from lung cancer cell line	11 IPF	Serum from index patient used to probe expressed cDNA library. Antigens recognized were unique to index patient (including anti-alanyl tRNA synthetase), but not shared with sera from other 10 patients	Robinson et al. (2001)
Endothelial cell protein extract	45 PF-AID (scleroderma) 16 controls	Sensitivity: 93% PF-AID had positive staining using an indirect immunofluorescent assay against rodent lung tissue  Specificity: 88%	Wusirika et al. (2003)
RNA helicase melanoma differentiation-associated gene 5 (MDA5)	61 amyotrophic dermatomyositis 61 dermatomyositis controls	Anti-MDA-5-positivity was significantly associated with ILD, since 50% of MDA-5-positive subjects (8 of 16) had ILD versus 25.5% of MDA-5-negative subjects (27 of 106; $P = .04$ )	Sato et al. (2009)

<sup>a</sup>Summary of autoantibody studies in patients with idiopathic pulmonary fibrosis (IPF). PF-AID—pulmonary fibrosis associated with autoimmune disease.<sup>b</sup>RF—rheumatoid factor.<sup>c</sup>SLE—systemic lupus erythematosus.<sup>d</sup>Nuclear antigens: ANA—anticellular antibodies; nRNP—nuclear ribonucleoprotein, Sm—Smith antigen, SS-A and SS-B—Sjogren's syndrome A and B antigens.

Several studies have correlated the presence of antiendothelial cell antibodies with the development and severity of IPF (Magro et al., 2007).

The lack of clear evidence for a humoral mechanism as the primary cause for IPF shifted the focus toward cellular immunity. One popular theory is that an unknown stimulus triggers a dysregulated activation of cell-mediated immune response that results in the fibrotic process analogous to that observed in abnormal wound healing. This theory rests on the belief that a normal cell-mediated defense to a pulmonary insult would be a Th1 response, as seen in most infections and in hypersensitivity pneumonitis (Kunkel, 2004; Lukacs et al., 2001; Wallace et al., 1995). The Th1 response is characterized by the release of interferon- $\gamma$  and the activation of neutrophils and macrophages for the efficient clearing of the antigen, as well as the suppression of fibroblast activation and collagen deposition. Cytokine profiles in IPF are closer to Th2 response which are typified by elevated IL-4, IL-5, and IL-13 levels and result in fibroblast activation (Hancock et al., 1998; Wallace and Howie, 1999). Early evidence for this T-cell-mediated mechanism in IPF was demonstrated by Kravis et al. (1976), who demonstrated that circulating lymphocytes from patients with IPF released migration inhibitor factor after exposure to collagen and lysed collagen-coated sheep red blood cells.

One experimental model used to demonstrate a cell-mediated autoimmune mechanism is the adoptive transfer, hapten-immune pulmonary interstitial fibrosis model (Stein-Streilein et al., 1987). In this model, donor mice are sensitized by a hapten, and then lymph nodes and spleen are transferred to recipient mice. When the recipient mice are then challenged with intratracheal administration of the hapten, they develop pulmonary fibrosis in 7–14 days. Interestingly, when the adoptive transfer with Th1 cells was used, an alveolitis developed, but not fibrosis, thus supporting the Th2 theory regarding the pathogenesis of the fibrotic component of IPF (Irifune et al., 2003).

In pulmonary fibrosis associated with the multisystem autoimmune disease, several serological and genetic markers are linked to the development of lung disease. For instance, patients with RA that have high levels of rheumatoid factor and prominent rheumatoid nodules are at increased risk for developing pulmonary fibrosis (Hyland et al., 1983). In systemic sclerosis the presence of antitopoisomerase antibodies and diffuse cutaneous involvement is associated with pulmonary fibrosis (Fanning et al., 1998).

Perhaps the best evidence for associating autoantibodies to pulmonary fibrosis is in patients with polymyositis and dermatomyositis, in which antibodies against aminoacyl-tRNA synthetases are highly correlated with pulmonary fibrosis (Targoff, 1993; Bernstein et al., 1984). At least five known forms of the autoantibodies have been identified, which include anti-alanyl-tRNA synthetase (PL), anti-histidyl-tRNA synthetase (Jo-1), anti-isoleucyl-tRNA synthetase (OJ), anti-glycyl tRNA synthetase, and anti-threonyl tRNA synthetase. The strongest correlation appears to be with anti-Jo-1 antibodies, which have been reported to have a frequency of interstitial lung disease between 50% (Hochberg et al., 1984) and 100% (Yoshida et al., 1983). Patients with polymyositis or dermatomyositis can frequently present with the “antisynthetase syndrome” characterized by pulmonary fibrosis in 50%–75% of the patients of arthritis, Raynaud’s phenomenon, and fevers. Occasionally, the antisynthetase syndrome can occur in the absence of clinical myositis (Marguerie et al., 1990). There are also several case series of patients with isolated pulmonary fibrosis occurring in association with aminoacyl-tRNA synthetase antibodies (Sauty et al., 1997; Friedman et al., 1996). These patients have been shown to have a CD8 lymphocyte predominant BAL and nonspecific interstitial pneumonitis pattern on lung biopsy. Most of the patients described in these case series have been more responsive to therapy with cyclosporine and azathioprine than patients with IPF.

Prior studies have suggested that the prognosis may be favorable for patients with pulmonary fibrosis associated with systemic sclerosis (Wells et al., 1994). However, Hubbard and Venn (2002) demonstrated with actuarial data that the mortality rates for patients with pulmonary fibrosis associated with all rheumatic disorders, predominantly RA in their study, are remarkably similar to those that are idiopathic in origin.

## GENETIC FEATURES

### Cryptogenic Organizing Pneumonia

There are no studies that demonstrate a genetic predisposition to this condition. The same is true for the autoimmune associated forms of COP.

## Idiopathic Pulmonary Fibrosis

Up to 3% of the cases of IPF occur in clusters of families, suggesting that a genetic predisposition may be responsible for the susceptibility to disease (Hodgson et al., 2002). A simple pattern of Mendelian inheritance has not been observed. Familial cases appear to be inherited in an autosomal dominant pattern, although the penetrance is variable. In familial cases of IPF, children of individuals with fibrotic lung disease can have the evidence of inflammatory alveolitis in bronchial lavage fluid, without having any clinical evidence of the disease (Bitterman et al., 1986). There have been several reports of pulmonary fibrosis occurring in separately raised monozygotic twins, underscoring the role of genetic factors (Jawaheri et al., 1980; Peabody et al., 1950). Using a candidate gene approach in one large family kindred with IPF, Thomas et al. (2002) identified a mutation in the highly conserved coding region of surfactant protein C, resulting in aberrant cellular distribution of SP-C in the lung tissue of affected individuals. From the Vanderbilt Familial Pulmonary Fibrosis Registry a telomerase gene mutation was found to be associated with the familiar form of IPF (Armanios et al., 2007).

In addition to the familial form of the disease, there is evidence for a genetic basis for the more common, sporadic form of the disease. A study from Finland demonstrated a clustering of sporadic cases of IPF in areas where familial cases were found, suggesting a founder's effect (Hodgson et al., 2002). One prevailing hypothesis is that susceptibility does not lie in one gene locus, but rather within multiple genes that interact through inflammatory and fibrotic mechanisms, creating a background genotype of fibrotic potential, yet still requiring an insult to create the profibrotic phenotype. This theory would explain why the familial form of the disease has an earlier age of onset (55 vs 67), yet still presents relatively later in life, implying that a reduced threshold for the development of fibrosis exists in familial types (Marshall et al., 2000). This theory is also supported by the fact that only a small proportion of individuals who receive drugs known to cause pulmonary fibrosis (such as bleomycin or amiodarone) actually develop the disease (Tisdale et al., 1995).

One major limitation in the search for genetic sources for susceptibility is that the sizes of the association studies are limited because of the rarity of the disease. Polymorphisms in several genes have been explored as potential causes for the inherited susceptibility to IPF; however, these studies utilized a traditional candidate gene approach which is limited by the current understanding of the disease and may lead to false positive associations. The best evidence for a genetic linkage to IPF susceptibility has been found with polymorphisms in the gene for MUC5B, which has been shown to convey increased risk in the general population (Seibold et al., 2011). The biologic role of MUC5B is related to mucin secretion in bronchial epithelial cells. Its role in pathogenesis is not clear, but MUC5B was found to be present in the epithelial cells of IPF but not in the fibrotic regions of scleroderma-associated NSIP (Borie et al., 2013).

Most of the genetic linkage studies are focused on genes involved in the inflammatory pathways, such as interleukin-1 $\alpha$  and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and have shown conflicting results (Pantelidis et al., 2001; Whyte et al., 2000). Xaubet et al. (2003) demonstrated that transforming growth factor- $\beta$  (TGF- $\beta$ ) polymorphisms were related to disease progression, but they found no association to susceptibility to IPF.

Given recent advances in genomics, microarray analysis, and proteomics, there is an excellent opportunity to perform genome-wide searches for linkage in patients with IPF to identify novel genes that are involved in pathogenesis and may represent therapeutic targets. Many of these genetic approaches are simply hypothesis generators; however, this may be a necessary first step to decipher the complex interactions of multiple loci. In addition, microarray technology can be used for "molecular fingerprinting" to identify subclassifications within the general category of IPF.

## IN VIVO AND IN VITRO MODELS

Animal models of the classical human Mendelian genetic diseases are relatively easy to produce because the genotype typically involves one affected gene, and knockout models of that gene, in mice for instance, can usually approximate the phenotype. Generating an animal model of a complex polygenic disease poses far more challenges because it involves multiple genes, each exerting a relatively small effect.

## Cryptogenic Organizing Pneumonia

The best animal model for COP involves inoculating CBA/J mice with reovirus serotype 1 via intranasal route (Bellum et al., 1997). These CBA/J mice develop intraluminal fibrosis and lymphocytic, peribronchial infiltrates

that are identical to the pathologic findings of COP in human disease. Interestingly, the COP pathological changes appear when titers of  $10^6$  are used; however, when  $10^7$  titers of the same virus are used, the CBA/J mice develop diffuse alveolar damage, the pathological finding of ARDS. This suggests that the tendency toward COP versus ARDS may be due to severity of the initial insult (London et al., 2002a, 2002b).

Other animal models for COP are designed to mimic bronchiolitis obliterans, the transplant-associated form of the disease. One model frequently used is the heterotopic murine tracheal transplant, whereby grafts of trachea and main bronchi are placed subcutaneously into allogeneic mismatched recipients (Hertz et al., 1993). By 21 days, grafts demonstrate fibroproliferation in the airway lumen, a characteristic for the human chronic rejection process. Subsequent studies on this model have shown that this is mediated by cellular and humoral immunity (Kelly and Hertz, 1997). Although this model can successfully mimic the phenotypic changes of bronchiolitis obliterans, it has limited applicability to understand COP because the entire tracheo-bronchial graft is an immunogenic stimulus, which is not likely the case in the idiopathic or autoimmune-associated forms of the disease.

## Idiopathic Pulmonary Fibrosis

There is no animal model for IPF that clearly represents human IPF. However, there are several animal models for susceptibility to developing experimental pulmonary fibrosis. The susceptibility to bleomycin-induced interstitial lung disease has been correlated with both immune-related (TH2 type) and nonimmune-related genes in different strains of mice (Rossi et al., 1987). Transforming growth factor- $\beta$  has been shown to induce pulmonary fibrotic changes when overexpressed via intratracheal administration of an adenoviral vector (Liu et al., 2001). Likewise, when mice are treated with an adenoviral vector expressing SMAD-7, an inhibitory regulator of TGF- $\beta$  production, they appear to be protected against bleomycin-induced lung injury (Nakao et al., 1999).

Many different transgenic and knockout mice have also been developed to investigate the molecular pathways that lead to pulmonary fibrosis. One example is mice that overexpress TNF- $\alpha$  that develops pulmonary fibrosis and have some of the features of human IPF (Miyazaki et al., 1995). Knockout mice with targeted deletions of genes necessary for fibrosis (e.g., adhesion molecules, ICAM-1 and L-selectin, which facilitate the accumulation of leukocytes) show significantly decreased fibrosis in response to bleomycin (Hamaguchi et al., 2002).

Many of these models are focused on only a select number of candidate genes, which may or may not have relevance to human disease. While these studies have been useful to identify genes that may play a role in susceptibility, the actual human form of the disease is likely far more complex and polygenic, which may prohibit creating a comprehensive animal model that reflects the underlying pathogenesis.

## PATHOLOGIC EFFECTOR MECHANISMS

### Cryptogenic Organizing Pneumonia

The sequence of events that ultimately leads to organizing pneumonia has been established in multiple studies. The common initial event is airway epithelial injury, with necrosis and denudation of the pneumocytes, but preservation of basal laminae and endothelial cells. This is distinct from ARDS, where there is a breakdown in the basal membrane leading to hyaline membrane formation. The first alveolar changes in COP involve the formation of inflammatory cell clusters (macrophages, lymphocytes, and neutrophils) bound together by fibrin. The next stage involves the migration of fibroblasts through the basal lamina and proliferation of fibrotic and inflammatory "buds" in the airway. The final stage is when these buds progress to a mature stage, and there is less inflammatory component, and the alveolar space begins to clear of fibrin (Kuhn and McDonald, 1991; Myers and Colby, 1993; Peyrol et al., 1990). Vascular endothelial growth factor and basic fibroblast growth factor are highly expressed in COP, and this results in a proliferation of capillary tissue as is normally seen in response to wound healing (Lappi-Blanco et al., 2002).

Environmental factors are clearly linked to the development of COP in general, although specific causative environmental factors for COP are difficult to identify. Perhaps the best documented environmental cases of COP occurred after exposure to aerosolized textile dye Acramin (Sole et al., 1996; Romero et al., 1998; Moya et al., 1994; Camus and Nemery, 1998). In this outbreak, 14 textile workers in Valencia Spain developed a severe respiratory illness following exposure to this dye.

Inhalational injuries can progress into bronchiolitis obliterans, which is similar pathologically to COP, except the injury is predominantly bronchiolar in location, without necessarily an inflammatory alveolar infiltrate.

The mechanism is believed to be due to inflammation caused by the irritant in the bronchioles that progresses into irreversible fibrosis and airway obstruction. Frequently, this occurs after exposure to water-insoluble gases (such as oxides of nitrogen in the case of silo-filler's lung) and organic dusts or fumes, which are not rapidly absorbed in the mucus membranes of the upper airways (Fleming et al., 1979; Ramirez and Dowell, 1971). Nitrous fumes are a significant industrial hazard and can be found in the agriculture, firefighting, and chemical industries. The timing of the injury can be delayed from the exposure because the gases are slowly hydrolyzed into acids that act as powerful oxidants which eventually penetrate the small airways and cause severe tissue injury. Clinical symptoms may present as acute, subacute, or chronic. The chronic form usually presents as a new clinical illness, weeks to months after recovery from the initial acute illness (King, 2003).

There have been many case reports of industrial exposures followed by the development of bronchiolitis obliterans in unrelated industries such as battery workers, food flavoring workers, and nylon flock workers (Eschenbacher et al., 1999; Konichezky et al., 1993; Kreiss et al., 2002). It is difficult to prove causation when only a few workers are affected. Kreiss et al. (2002) reported a high incidence of bronchiolitis obliterans in 7% (8 out of 117) of workers at a microwave popcorn plant, which was attributed to inhalation of the volatile agent, diacetyl in the butter flavoring. This observation was supported by toxicity studies of diacetyl inhalation in rats (Hubbs et al., 2002). Bronchiolitis obliterans has also been described in a truck driver who inhaled fly ash, as well as in a young man with smoke inhalation from a fire (Tasaka et al., 1995; Boswell and McCunney, 1995). Both cases recovered from the acute illness only to have their symptoms recur and progress weeks to months later.

Organizing pneumonia can also occur as a sequela from prior pulmonary infections, most commonly seen with adenovirus, but also associated with other viruses (Respiratory syncytial virus (RSV), influenza) and mycoplasma (Wright et al., 1992; Penn and Liu, 1993; Chan et al., 1997). When an organizing pneumonia pattern is observed after an infection, it is referred to as "secondary organizing pneumonia." COP has been reported to occur in the contralateral lung in women undergoing breast radiation for breast cancer (Stover et al., 2001). Some medications have been implicated as etiological agents in the development of COP, including amiodarone, amphotericin, bleomycin, mesalazine, methotrexate, phenytoin, and sirolimus (Cordier, 2006). Both gold and penicillamine have been suspected; however, it is difficult to determine if the COP results from the medication or the underlying disease (Geddes et al., 1977).

## Idiopathic Pulmonary Fibrosis

The original theory for the pathogenesis of IPF rested on the premise that inflammation of the alveoli was followed by fibrosis (Crystal et al., 1976, 1981, 1984; Hunninghake et al., 1979). There are many observations that support this hypothesis. Early studies that used bronchial lavage demonstrated increased numbers of alveolar macrophages, neutrophils, eosinophils, and lymphocytes (Weinberger et al., 1978; Reynolds et al., 1977; Crystal et al., 1976). Gallium scans, which are nonspecific markers of inflammation with macrophages and neutrophils, are positive in about 70% of all patients with IPF (Crystal et al., 1976; Line et al., 1978). Biopsies that are performed in the early stages of the disease reveal large amounts of inflammation and alveolar wall derangement compared to biopsies taken in the later stages of disease when fibrosis predominates (Carrington et al., 1978). Alveolar macrophages are believed to play a central role in the inflammatory process through the release of cytokines that affect other cells. Alveolar macrophages from patients with IPF have been shown to secrete neutrophil chemotactic factor which does not occur in normal nonactivated alveolar macrophages (Hunninghake et al., 1980, 1981). Animal models have shown the presence of inflammation preceding fibrosis, and the suppression of the inflammatory response attenuates the progression to fibrosis (Snider, 1986).

More recently, there has been a focus on the fibrotic aspect of the disease as also playing an important role. These theories about the pathogenesis of IPF involve the idea of recurrent, ongoing stimulus and injury to the lung, with abnormal wound healing. The abnormal wound healing is believed to result from a complex interplay of the genetic background of the individual, the predominant inflammatory phenotype (Th1 or Th2), and the environmental triggers. The stimulus that acts as the driving force remains a mystery, as does the mechanism that promotes a pathological fibrotic response instead of the normal reparative response.

The lung parenchyma regulates its immune and fibrotic processes through cytokine signaling between the cells of the interstitium, including epithelial cells, endothelial cells, fibroblasts, and macrophages, and it is likely that these cells contribute to the pathogenesis of IPF (Standiford et al., 1991; Selman et al., 2001; Martinet et al., 1987). Although the exact sequence of chemokine signaling that leads to the progression of fibrosis is not understood, it

has been shown that cytokines are responsible for the cell-to-cell communication and fibroblast activation, proliferation and collagen production.

One theory is that an imbalance in inflammatory phenotype, shifted toward a Th2 response, is responsible for the fibrotic phenotype. Support for this theory comes from the observation that IL-4 and IL13, major Th2-type cytokines, have been shown to be major stimuli for fibroblast-derived extracellular matrix deposition (Hancock et al., 1998; Furue et al., 1997). Interferon- $\gamma$ , one of the major Th1-type cytokines, has been shown to suppress the production of collagen and fibronectin by fibroblasts (Goldring et al., 1986). The increased presence of eosinophils, which have been associated with Th2 cytokine expression in asthma and parasitic infections, in association with fibrotic changes in IPF is consistent with the concepts of the Th2 paradigm of abnormal healing. Davis et al. (1984) demonstrated that eosinophils represented greater than 5% of the cells in the lavage fluid of 20% of patients with IPF compared to less than 1% of the normal controls. These eosinophils were shown to have collagenase activity and have the capacity to injure lung parenchymal cells.

The pathogenetic mechanisms that lead to the development of pulmonary fibrosis associated with rheumatic diseases have been most extensively studied in scleroderma. The most common theory, which may or may not be applicable to the idiopathic form, is that an initial environmental injury triggers an ongoing, amplified immune response in the lung. Both TNF- $\alpha$  and TGF- $\beta$  have been shown to be upregulated early in the course of the disease (Corrin et al., 1994; Bolster et al., 1997), and there is a cytokine shift from Th1 to Th2 in helper T cells (Bolster et al., 1997). BAL fluid from patients with scleroderma has increased levels of several inflammatory cytokines such as IL-8, TNF- $\alpha$ , and macrophage inflammatory protein (Southcott et al., 1995). Lavage is also believed to have prognostic value in patients with systemic sclerosis. Scleroderma patients with a neutrophil and eosinophil predominant BAL have been associated with more extensive pulmonary fibrosis seen on CT and more rapid clinical deterioration (Witt et al., 1999; Silver et al., 1990; Rossi et al., 1987; Friedman et al., 1996; Behr et al., 1996).

The stimulus that leads to the inflammatory response in the lungs of patients with scleroderma is also unknown, but one hypothesis suggests that aspiration of refluxed esophageal and gastric contents may be the cause. The premise behind this theory arises from the fact that many patients with systemic sclerosis have both esophageal dysmotility and pulmonary fibrosis. This association was initially observed in a relatively small cohort of 12 patients (Johnson et al., 1989a). Subsequent studies have found conflicting results when trying to correlate pulmonary function parameters meant to be a surrogate marker of pulmonary fibrosis (such as total lung capacity and diffusion capacity) with esophageal manometry (Troshinsky et al., 1994; Lock et al., 1998). These studies are limited by confounding because both esophageal dysmotility and pulmonary fibrosis may be the independent markers of scleroderma disease severity, without having a causal link.

Several environmental factors have been investigated for an etiological role in IPF. The premise of an environmental stimulus that leads to repeated lung injury followed by abnormal wound healing fits nicely into one theory of the pathogenesis of IPF (see below). The search for these stimuli is challenging because the offending agent may be different between individuals based on individual differences in genetic susceptibility. Nevertheless, epidemiological studies have identified links between IPF and occupational exposures, medications, and infectious agents.

Four separate case-controlled studies have looked at the association between occupational exposure and IPF. Although these studies are limited because of recall bias, there were some consistent observations. Metal dust exposure was significantly associated with IPF in every study and in a dose-response relationship (Baumgartner et al., 1997, 2000; Hubbard et al., 1996; Iwai et al., 1994). Two studies showed a significant association with livestock exposure, farming, and agricultural exposure (Baumgartner et al., 2000; Iwai et al., 1994). Paraquat, an herbicide used in agriculture, has been found to cause fatal pulmonary fibrosis in humans and experimental animals after oral, inhalational, or cutaneous exposure (Schoenberger et al., 1984). Anecdotal case reports have linked the cases of IPF with occupations that result in toxic dust or fume exposure such as diamond polishing, dairy work, welding, gold extraction, and dental work (Baumgartner et al., 1997).

Pulmonary fibrosis is a well-known side effect of several medications, most notably bleomycin, amiodarone, methotrexate, and nitrofurantoin (Holmberg and Boman, 1981; Israel-Biet et al., 1991; Martin and Rosenow, 1988; Schoenberger and Crystal, 1983; Sleijfer, 2001). It has also been found as a rare complication of more commonly used medications such as beta blockers, antidepressants, anticonvulsants, and nonsteroidal antiinflammatory drugs. However, these associations have been difficult to prove given the ubiquity of these medications and the relative rarity of IPF (Coulas et al., 1994).

The effect of smoking on the natural history of IPF is controversial, but of four case-control studies examining the association of smoking with pulmonary fibrosis, three showed a significant link between smoking and the development of IPF (Iwai et al., 1994; Hubbard et al., 1996; Baumgartner et al., 1997, 2000). Some studies have

shown improved survival in smokers with IPF compared to nonsmokers (King et al., 2001; Cherniack et al., 1995). However, this observation may be due to lead time bias in the smoking group and simply reflect a greater severity of disease in the nonsmoking groups.

## TREATMENT AND OUTCOME

### Cryptogenic Organizing Pneumonia

One of the defining features of COP is that it usually has an excellent response to corticosteroids. Although there have not been any randomized clinical trials to prove their efficacy, there is widespread consensus based on individual experience that corticosteroids are highly efficacious. When patients respond, there is typically complete radiographic and clinical resolution of the organizing pneumonia. There is no consensus on duration of treatment, with some authorities suggesting 6–8 weeks, while others advocate for 1 year. Spontaneous remissions are unusual, except when a precipitating factor is identified and withdrawn, for example, new medication. Second-line agents, for example cytotoxic agents, or macrolide antibiotics, can be used when steroids are contraindicated, but high-quality data for these are lacking.

### Idiopathic Pulmonary Fibrosis

IPF usually portends a poor prognosis and is typically poorly responsive to therapy. Early diagnosis and treatment have traditionally been advocated so that therapy can be initiated and, hopefully, prevent irreversible fibrosis. Therefore, the typical medications used to treat IPF work through antiinflammatory and immunosuppressant mechanisms. However, these traditional approaches have failed to show that the inflammatory or fibrotic process can be altered or reversed. As the understanding of the pathogenesis of the disease improves, newer agents are being developed based on cellular mechanisms to inhibit fibrogenesis. There are currently two FDA-approved antifibrotic therapies for IPF, pirfenidone and nintedanib. Both medications have been shown to slow progression; however, no therapy for IPF has been shown to improve survival.

Pirfenidone is an oral antifibrotic agent that was the first drug approved for the treatment of IPF in 2014. Pirfenidone initially performed two parallel phase 3 trials, called CAPACITY (studies 004 and 006) which evaluated the safety and efficacy of pirfenidone after 72 weeks in about 350 IPF patients, with the primary end point being change in forced vital capacity (FVC% predicted). CAPACITY 004 showed a significant reduction in the rate of decline in FVC% predicted, while CAPACITY 006 showed no significant decline. This prompted a third phase 3 trial called ASCEND, which enrolled 555 IPF patients and demonstrated a reduction in decline of FVC% predicted at 52 weeks and an improved progression free survival (Noble et al., 2016).

Nintedanib is an intracellular inhibitor of multiple tyrosine kinases that block many of the growth factors thought to be involved in the pathogenesis of IPF. Two replicate double-blinded phase 3 trials with 1066 patients total, demonstrated that nintedanib reduced the decline in FVC over a 52-week period. Both nintedanib and pirfenidone are currently approved for IPF, their efficacy in patients with pulmonary fibrosis associated with autoimmune diseases has not been studied to date (Richeldi et al., 2014).

### Corticosteroids

Prior to the approval of nintedanib and pirfenidone, the most common medication used to treat IPF was corticosteroids, despite the fact that they have not been properly evaluated in a large clinical trial. Early studies showed that 10%–30% of patients with IPF appear to improve or survive longer when treated with corticosteroids (Stack et al., 1972; Turner-Warwick et al., 1980). However, these studies likely included other forms of idiopathic interstitial pneumonia, such as nonspecific interstitial pneumonia or COP, which tend to have a more favorable prognosis than IPF. When histopathological criteria are used for the diagnosis of IPF, the clinical response (0%–16%) and survival rates are much worse than for the other pathological patterns (Ziesche et al., 1999; Nicholson et al., 2000; Daniil et al., 1999). Despite the lack of evidence for a beneficial role of corticosteroids, some pulmonologists use a 3–6-months trial of corticosteroids with close monitoring for radiographic or physiologic improvement. Patients who improve with corticosteroids or who remain stable are then kept on a maintenance dose of 10–20 mg of prednisone per day (American Thoracic Society and European Respiratory Society, 2002). Intravenous pulse corticosteroids for 3–5 days are generally recommended for an acute exacerbation of IPF, as Keogh et al. (1983) demonstrated that the higher doses of corticosteroids can significantly reduce the neutrophil accumulation during an active alveolitis of IPF.

### Cytotoxic Agents

Other antiinflammatory and cytotoxic agents have been investigated for a beneficial role in IPF. Cyclophosphamide, when used in combination with corticosteroids, had promising results toward a survival advantage compared to corticosteroids alone in an early randomized control trial (Johnson et al., 1989b; Baughman and Lower, 1992). Cyclophosphamide has been shown to reduce the neutrophil component of the active alveolitis after 3 and 6 months of therapy (O'Donnell et al., 1987). However, subsequent studies have shown cyclophosphamide to have limited efficacy and a high frequency of side effects when used to treat patients with IPF who failed to respond to corticosteroid therapy (Zisman et al., 2000). Cyclophosphamide may be effective in patients with scleroderma-associated pulmonary fibrosis, as it was shown to improve pulmonary function testing in this population. Scleroderma patients with a neutrophil predominant BAL seemed to have the best outcomes, although these studies were limited by either small sample size, lack of a control group or selection bias from retrospective review (Schnabel et al., 1998; Silver et al., 1990; White et al., 2000). Cyclophosphamide is currently considered a second-line drug for patients with progressive IPF. Side effects include leukopenia, thrombocytopenia, and hemorrhagic cystitis.

### Antifibrotic Agents

Many other novel strategies have been investigated in the treatment of IPF, including colchicine, interferon  $\beta$ , cyclosporine A, sildenafil, bosentan, and etanercept (Raghu et al., 2011). These therapies focus on different aspects of the pathogenesis of the disease, such as antioxidants; inhibitors of cytokines, proteases, and fibroblast growth factors. However, all of these agents have failed to demonstrate clinically meaningful responses; and therefore, the joint statement by the ATS and ERS recommended against using corticosteroids, colchicine, interferon  $\beta$ , cyclosporine, bosentan, or etanercept for the treatment of IPF (Raghu et al., 2011).

Lung transplantation should be considered for individuals with IPF who progress despite optimized medical management. Patients should be referred to a transplant center relatively early in their course, since wait times on the list can exceed 2 years due to limited donor availability. Those who receive a successful transplant can experience significant improvement in arterial oxygenation, pulmonary hypertension, and right ventricular dysfunction. Unfortunately, the 5-year survival rates for lung transplant are only about 60%, with death frequently due to graft failure, infection, or bronchiolitis obliterans (Lu and Bhorade, 2004).

## CONCLUSIONS

The autoimmune disorders of the lung, notably COP and IPF, have long been recognized as inflammatory and fibrotic processes that are associated with variable prognosis, whether idiopathic in nature or in association with the multisystem autoimmune diseases. Both diseases appear to be mediated through repetitive cell-mediated injury directed at the bronchioles and alveoli/interstitium, followed by wound healing. Recent advances in the classification and diagnostic testing for these diseases have improved our ability to study their incidence, prognosis, and response to therapy. While much progress has been made, the search for antigenic stimuli and genetic polymorphisms that are responsible for disease pathogenesis continues. Only through a better understanding of the disease pathogenesis we will be able to offer new therapies for these diseases.

## References

- American Thoracic Society and European Respiratory Society, 2002. American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. Am. J. Respir. Crit Care Med. 165, 277–304.
- Alasaly, K., Muller, N., Ostrow, D.N., et al., 1995. Cryptogenic organizing pneumonia. A report of 25 cases and a review of the literature. Medicine (Baltimore) 74, 201–211.
- Arcasoy, S.M., Kotloff, R.M., 1999. Lung transplantation. N. Engl. J. Med. 340, 1081–1091.
- Armanios, M.Y., Chen, J.J., Cogan, J.D., et al., 2007. Telomerase mutations in families with idiopathic pulmonary fibrosis. N. Engl. J. Med. 356, 1317–1326.
- Aubert, J.D., Pare, P.D., Hogg, J.C., et al., 1997. Platelet-derived growth factor in bronchiolitis obliterans-organizing pneumonia. Am. J. Respir. Crit. Care Med. 155, 676–681.
- Baar, H.S., Galindo, J., 1966. Bronchiolitis fibrosa obliterans. Thorax 21, 209–214.

- Basset, F., Ferrans, V.J., Soler, P., et al., 1986. Intraluminal fibrosis in interstitial lung disorders. *Am. J. Pathol.* 122, 443–461.
- Baughman, R.P., Lower, E.E., 1992. Use of intermittent, intravenous cyclophosphamide for idiopathic pulmonary fibrosis. *Chest* 102, 1090–1094.
- Baumgartner, K.B., Samet, J.M., Stidley, C.A., et al., 1997. Cigarette smoking: a risk factor for idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 155, 242–248.
- Baumgartner, K.B., Samet, J.M., Coultas, D.B., et al., 2000. Occupational and environmental risk factors for idiopathic pulmonary fibrosis: a multicenter case-control study. *Collaborating Centers. Am. J. Epidemiol.* 152, 307–315.
- Begin, R., Masse, S., Cantin, A., et al., 1982. Airway disease in a subset of nonsmoking rheumatoid patients. Characterization of the disease and evidence for an autoimmune pathogenesis. *Am. J. Med.* 72, 743–750.
- Behr, J., Vogelmeier, C., Beinert, T., et al., 1996. Bronchoalveolar lavage for evaluation and management of scleroderma disease of the lung. *Am. J. Respir. Crit. Care Med.* 154, 400–406.
- Bellum, S.C., Dove, D., Harley, R.A., et al., 1997. Respiratory reovirus 1/L induction of intraluminal fibrosis. A model for the study of bronchiolitis obliterans organizing pneumonia. *Am. J. Pathol.* 150, 2243–2254.
- Bernstein, R.M., Morgan, S.H., Chapman, J., et al., 1984. Anti-Jo-1 antibody: a marker for myositis with interstitial lung disease. *Br. Med. J. (Clin. Res. Ed.)* 289, 151–152.
- Bitterman, P.B., Rennard, S.I., Keogh, B.A., et al., 1986. Familial idiopathic pulmonary fibrosis. Evidence of lung inflammation in unaffected family members. *N. Engl. J. Med.* 314, 1343–1347.
- Bolster, M.B., Ludwicka, A., Sutherland, S.E., et al., 1997. Cytokine concentrations in bronchoalveolar lavage fluid of patients with systemic sclerosis. *Arthritis Rheum.* 40, 743–751.
- Borie, R., Crestani, B., Dieude, P., et al., 2013. The MUC5B variant is associated with idiopathic pulmonary fibrosis but not with systemic sclerosis interstitial lung disease in the European Caucasian population. *PLoS One* 8, e70621.
- Boswell, R.T., McCunney, R.J., 1995. Bronchiolitis obliterans from exposure to incinerator fly ash. *J. Occup. Environ. Med.* 37, 850–855.
- Bouros, D., Wells, A.U., Nicholson, A.G., et al., 2002. Histopathologic subsets of fibrosing alveolitis in patients with systemic sclerosis and their relationship to outcome. *Am. J. Respir. Crit. Care Med.* 165, 1581–1586.
- Cantin, A., Crystal, R.G., 1985. Interstitial pathology': an overview of the chronic interstitial lung disorders. *Int Arch Allergy Appl Immunol* 76 (Suppl 1), 83–91.
- Camus, P., Nemery, B., 1998. A novel cause for bronchiolitis obliterans organizing pneumonia: exposure to paint aerosols in textile workshops. *Eur. Respir. J.* 11, 259–262.
- Carre, P.C., King Jr., T.E., Mortensen, R., et al., 1994. Cryptogenic organizing pneumonia: increased expression of interleukin-8 and fibronectin genes by alveolar macrophages. *Am. J. Respir. Cell Mol. Biol.* 10, 100–105.
- Carrington, C.B., Gaensler, E.A., Coutu, R.E., et al., 1978. Natural history and treated course of usual and desquamative interstitial pneumonia. *N. Engl. J. Med.* 298, 801–809.
- Cervantes-Perez, P., Toro-Perez, A.H., Rodriguez-Jurado, P., 1980. Pulmonary involvement in rheumatoid arthritis. *JAMA* 243, 1715–1719.
- Chan, T.Y., Hansell, D.M., Rubens, M.B., et al., 1997. Cryptogenic fibrosing alveolitis and the fibrosing alveolitis of systemic sclerosis: morphological differences on computed tomographic scans. *Thorax* 52, 265–270.
- Chapman, J.R., Charles, P.J., Venables, P.J., et al., 1984. Definition and clinical relevance of antibodies to nuclear ribonucleoprotein and other nuclear antigens in patients with cryptogenic fibrosing alveolitis. *Am. Rev. Respir. Dis.* 130, 439–443.
- Cherniack, R.M., Colby, T.V., Flint, A., et al., 1995. Correlation of structure and function in idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 151, 1180–1188.
- Colby, T.V., 1998. Bronchiolitis. Pathologic considerations. *Am. J. Clin. Pathol.* 109, 101–109.
- Cordier, J.F., 2006. Cryptogenic organising pneumonia. *Eur. Respir. J.* 28, 422–446.
- Corrin, B., Butcher, D., McAnulty, B.J., et al., 1994. Immunohistochemical localization of transforming growth factor-beta 1 in the lungs of patients with systemic sclerosis, cryptogenic fibrosing alveolitis and other lung disorders. *Histopathology* 24, 145–150.
- Coultas, D.B., Zumwalt, R.E., Black, W.C., et al., 1994. The epidemiology of interstitial lung diseases. *Am. J. Respir. Crit. Care Med.* 150, 967–972.
- Crystal, R.G., Fulmer, J.D., Roberts, W.C., et al., 1976. Idiopathic pulmonary fibrosis. Clinical, histologic, radiographic, physiologic, scintigraphic, cytologic, and biochemical aspects. *Ann. Intern. Med.* 85, 769–788.
- Crystal, R.G., Gadek, J.E., Ferrans, V.J., et al., 1981. Interstitial lung disease: current concepts of pathogenesis, staging and therapy. *Am. J. Med.* 70, 542–568.
- Crystal, R.G., Bitterman, P.B., Rennard, S.I., et al., 1984. Interstitial lung diseases of unknown cause. Disorders characterized by chronic inflammation of the lower respiratory tract. *N. Engl. J. Med.* 310, 235–244.
- Daniil, Z.D., Gilchrist, F.C., Nicholson, A.G., et al., 1999. A histologic pattern of nonspecific interstitial pneumonia is associated with a better prognosis than usual interstitial pneumonia in patients with cryptogenic fibrosing alveolitis. *Am. J. Respir. Crit. Care Med.* 160, 899–905.
- Davis, W.B., Fells, G.A., Sun, X.H., et al., 1984. Eosinophil-mediated injury to lung parenchymal cells and interstitial matrix. A possible role for eosinophils in chronic inflammatory disorders of the lower respiratory tract. *J. Clin. Invest.* 74, 269–278.
- Davison, A.G., Heard, B.E., McAllister, W.A., et al., 1983. Cryptogenic organizing pneumonitis. *Q. J. Med.* 52, 382–394.
- Douglas, W.W., Tazelaar, H.D., Hartman, T.E., et al., 2001. Polymyositis-dermatomyositis-associated interstitial lung disease. *Am. J. Respir. Crit. Care Med.* 164, 1182–1185.
- Dreisin, R.B., Schwarz, M.I., Theofilopoulos, A.N., et al., 1978. Circulating immune complexes in the idiopathic interstitial pneumonias. *N. Engl. J. Med.* 298, 353–357.
- Eisenberg, H., Dubois, E.L., Sherwin, R.P., et al., 1973. Diffuse interstitial lung disease in systemic lupus erythematosus. *Ann. Intern. Med.* 79, 37–45.
- Epler, G.R., Snider, G.L., Gaensler, E.A., et al., 1979. Bronchiolitis and bronchitis in connective tissue disease. A possible relationship to the use of penicillamine. *JAMA* 242, 528–532.
- Epler, G.R., Colby, T.V., McLoud, T.C., et al., 1985. Bronchiolitis obliterans organizing pneumonia. *N. Engl. J. Med.* 312, 152–158.

- Eschenbacher, W.L., Kreiss, K., Lougheed, M.D., et al., 1999. Nylon flock-associated interstitial lung disease. *Am. J. Respir. Crit. Care Med.* 159, 2003–2008.
- Fanning, G.C., Welsh, K.I., Bunn, C., et al., 1998. HLA associations in three mutually exclusive autoantibody subgroups in UK systemic sclerosis patients. *Br. J. Rheumatol.* 37, 201–207.
- Flaherty, K.R., Travis, W.D., Colby, T.V., et al., 2001. Histopathologic variability in usual and nonspecific interstitial pneumonias. *Am. J. Respir. Crit Care Med.* 164, 1722–1727.
- Fleming, G.M., Chester, E.H., Montenegro, H.D., 1979. Dysfunction of small airways following pulmonary injury due to nitrogen dioxide. *Chest* 75, 720–721.
- Forlani, S., Ratta, L., Bulgheroni, A., et al., 2002. Cytokine profile of broncho-alveolar lavage in BOOP and UIP. *Sarcoidosis Vasc. Diffuse Lung Dis.* 19, 47–53.
- Friedman, A.W., Targoff, I.N., Arnett, F.C., 1996. Interstitial lung disease with autoantibodies against aminoacyl-tRNA synthetases in the absence of clinically apparent myositis. *Semin. Arthritis Rheum.* 26, 459–467.
- Fujita, J., Dobashi, N., Ohtsuki, Y., et al., 1999. Elevation of anti-cytokeratin 19 antibody in sera of the patients with idiopathic pulmonary fibrosis and pulmonary fibrosis associated with collagen vascular disorders. *Lung* 177, 311–319.
- Fulmer, J.D., Roberts, W.C., von Gal, E.R., et al., 1979. Morphologic-physiologic correlates of the severity of fibrosis and degree of cellularity in idiopathic pulmonary fibrosis. *J. Clin. Invest.* 63, 656–676.
- Furue, H., Yamasaki, H., Suga, M., et al., 1997. Altered accessory cell function of alveolar macrophages: a possible mechanism for induction of Th2 secretory profile in idiopathic pulmonary fibrosis. *Eur. Respir. J.* 10, 787–794.
- Gabbay, E., Tarala, R., Will, R., et al., 1997. Interstitial lung disease in recent onset rheumatoid arthritis. *Am. J. Respir. Crit. Care Med.* 156, 528–535.
- Gardiner, P., 1993. Primary Sjogren's syndrome. *Baillieres Clin. Rheumatol.* 7, 59–77.
- Geddes, D.M., Corrin, B., Brewerton, D.A., et al., 1977. Progressive airway obliteration in adults and its association with rheumatoid disease. *Q. J. Med.* 46, 427–444.
- Goldring, M.B., Sandell, L.J., Stephenson, M.L., et al., 1986. Immune interferon suppresses levels of procollagen mRNA and type II collagen synthesis in cultured human articular and costal chondrocytes. *J. Biol. Chem.* 261, 9049–9055.
- Gosink, B.B., Friedman, P.J., Liebow, A.A., 1973. Bronchiolitis obliterans. Roentgenologic-pathologic correlation. *Am. J. Roentgenol. Radium Ther. Nucl. Med.* 117, 816–832.
- Gross, T.J., Hunninghake, G.W., 2001. Idiopathic pulmonary fibrosis. *N. Engl. J. Med.* 345, 517–525.
- Hamaguchi, Y., Nishizawa, Y., Yasui, M., et al., 2002. Intercellular adhesion molecule-1 and L-selectin regulate bleomycin-induced lung fibrosis. *Am. J. Pathol.* 161, 1607–1618.
- Hamman, L., Rich, A.R., 1935. Fulminating diffuse interstitial fibrosis of the lungs. *Trans. Am. Clin. Climatol. Assoc.* 51, 154–163.
- Hancock, A., Armstrong, L., Gama, R., et al., 1998. Production of interleukin 13 by alveolar macrophages from normal and fibrotic lung. *Am. J. Respir. Cell Mol. Biol.* 18, 60–65.
- Harrison, N.K., Myers, A.R., Corrin, B., et al., 1991. Structural features of interstitial lung disease in systemic sclerosis. *Am. Rev. Respir. Dis.* 144, 706–713.
- Haslam, P.L., Thompson, B., Mohammed, I., et al., 1979. Circulating immune complexes in patients with cryptogenic fibrosing alveolitis. *Clin. Exp. Immunol.* 37, 381–390.
- Hertz, M.I., Jessurun, J., King, M.B., et al., 1993. Reproduction of the obliterative bronchiolitis lesion after heterotopic transplantation of mouse airways. *Am. J. Pathol.* 142, 1945–1951.
- Herzog, C.A., Miller, R.R., Hoidal, J.R., 1981. Bronchiolitis and rheumatoid arthritis. *Am. Rev. Respir. Dis.* 124, 636–639.
- Hochberg, M.C., Feldman, D., Stevens, M.B., et al., 1984. Antibody to Jo-1 in polymyositis/dermatomyositis: association with interstitial pulmonary disease. *J. Rheumatol.* 11, 663–665.
- Hodgson, U., Laitinen, T., Tukiainen, P., 2002. Nationwide prevalence of sporadic and familial idiopathic pulmonary fibrosis: evidence of founder effect among multiplex families in Finland. *Thorax* 57, 338–342.
- Holgate, S.T., Haslam, P., Turner-Warwick, M., 1983. The significance of antinuclear and DNA antibodies in cryptogenic fibrosing alveolitis. *Thorax* 38, 67–70.
- Holmberg, L., Boman, G., 1981. Pulmonary reactions to nitrofurantoin. 447 cases reported to the Swedish Adverse Drug Reaction Committee 1966–1976. *Eur. J. Respir. Dis.* 62, 180–189.
- Hubbard, R., Venn, A., 2002. The impact of coexisting connective tissue disease on survival in patients with fibrosing alveolitis. *Rheumatology (Oxford)* 41, 676–679.
- Hubbard, R., Lewis, S., Richards, K., et al., 1996. Occupational exposure to metal or wood dust and aetiology of cryptogenic fibrosing alveolitis. *Lancet* 347, 284–289.
- Hubbard, R., Johnston, I., Britton, J., 1998. Survival in patients with cryptogenic fibrosing alveolitis: a population-based cohort study. *Chest* 113, 396–400.
- Hubbs, A.F., Battelli, L.A., Goldsmith, W.T., et al., 2002. Necrosis of nasal and airway epithelium in rats inhaling vapors of artificial butter flavoring. *Toxicol. Appl. Pharmacol.* 185, 128–135.
- Hunninghake, G.W., Gadek, J.E., Kawanami, O., et al., 1979. Inflammatory and immune processes in the human lung in health and disease: evaluation by bronchoalveolar lavage. *Am. J. Pathol.* 97, 149–206.
- Hunninghake, G.W., Gadek, J.E., Fales, H.M., et al., 1980. Human alveolar macrophage-derived chemotactic factor for neutrophils: stimuli and partial characterization. *J. Clin. Invest.* 66, 473–483.
- Hunninghake, G.W., Gadek, J.E., Lawley, T.J., et al., 1981. Mechanisms of neutrophil accumulation in the lungs of patients with idiopathic pulmonary fibrosis. *J. Clin. Invest.* 68, 259–269.
- Hunninghake, G.W., Zimmerman, M.B., Schwartz, D.A., et al., 2001. Utility of a lung biopsy for the diagnosis of idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 164, 193–196.

- Hyland, R.H., Gordon, D.A., Broder, I., et al., 1983. A systematic controlled study of pulmonary abnormalities in rheumatoid arthritis. *J. Rheumatol.* 10, 395–405.
- Irifune, K., Yokoyama, A., Kohno, N., et al., 2003. T-helper 1 cells induce alveolitis but do not lead to pulmonary fibrosis in mice. *Eur. Respir. J.* 21, 11–18.
- Israel-Biet, D., Labrune, S., Huchon, G.J., 1991. Drug-induced lung disease: 1990 review. *Eur. Respir. J.* 4, 465–478.
- Iwai, K., Mori, T., Yamada, N., et al., 1994. Idiopathic pulmonary fibrosis. Epidemiologic approaches to occupational exposure. *Am. J. Respir. Crit. Care Med.* 150, 670–675.
- Javaheri, S., Lederer, D.H., Pella, J.A., Mark, G.J., Levine, B.W., 1980. Idiopathic pulmonary fibrosis in monozygotic twins. The importance of genetic predisposition. *Chest* 78 (4), 591–594.
- Johnson, D.A., Drane, W.E., Curran, J., et al., 1989a. Pulmonary disease in progressive systemic sclerosis. A complication of gastroesophageal reflux and occult aspiration? *Arch. Intern. Med.* 149, 589–593.
- Johnson, M.A., Kwan, S., Snell, N.J., et al., 1989b. Randomised controlled trial comparing prednisolone alone with cyclophosphamide and low dose prednisolone in combination in cryptogenic fibrosing alveolitis. *Thorax* 44, 280–288.
- Katzenstein, A.L., Fiorelli, R.F., 1994. Nonspecific interstitial pneumonia/fibrosis. Histologic features and clinical significance. *Am. J. Surg. Pathol.* 18, 136–147.
- Kelly, K., Hertz, M.I., 1997. Obliterative bronchiolitis. *Clin. Chest Med.* 18, 319–338.
- Keogh, B.A., Crystal, R.G., 1980. Clinical significance of pulmonary function tests. Pulmonary function testing in interstitial pulmonary disease. What does it tell us? *Chest* 78, 856–865.
- Keogh, B.A., Bernardo, J., Hunninghake, G.W., et al., 1983. Effect of intermittent high dose parenteral corticosteroids on the alveolitis of idiopathic pulmonary fibrosis. *Am. Rev. Respir. Dis.* 127, 18–22.
- King Jr., T.E., 2003. Bronchiolitis. In: Schwarz, M.I., King Jr., T.E. (Eds.), *Interstitial Lung Disease*. BD Decker Inc., London, pp. 787–824.
- King Jr., T.E., Mortenson, R.L., 1992. Cryptogenic organizing pneumonitis. The North American experience. *Chest* 102, 8S–13S.
- King Jr., T.E., Tooze, J.A., Schwarz, M.I., et al., 2001. Predicting survival in idiopathic pulmonary fibrosis: scoring system and survival model. *Am. J. Respir. Crit. Care Med.* 164, 1171–1181.
- Konichezky, S., Schattner, A., Ezri, T., et al., 1993. Thionyl-chloride-induced lung injury and bronchiolitis obliterans. *Chest* 104, 971–973.
- Kravis, T.C., Ahmed, A., Brown, T.E., et al., 1976. Pathogenic mechanisms in pulmonary fibrosis: collagen-induced migration inhibition factor production and cytotoxicity mediated by lymphocytes. *J. Clin. Invest.* 58, 1223–1232.
- Kreiss, K., Gomaa, A., Kullman, G., et al., 2002. Clinical bronchiolitis obliterans in workers at a microwave-popcorn plant. *N. Engl. J. Med.* 347, 330–338.
- Kuhn, C., McDonald, J.A., 1991. The roles of the myofibroblast in idiopathic pulmonary fibrosis. Ultrastructural and immunohistochemical features of sites of active extracellular matrix synthesis. *Am. J. Pathol.* 138, 1257–1265.
- Kunkel, S.L., 2004. Cytokine phenotypes and the progression of chronic pulmonary fibrosis. In: Lenfant, C. (Ed.), *Idiopathic Pulmonary Fibrosis*. Maecl Decker, Inc, New York, pp. 303–320.
- Lange, W., 1901. Über eine eigentümliche Erkrankung der kleinen Bronchien obliterans. *Dtsch. Arch. Klin. Med.* 70, 342–364.
- Lappi-Blanco, E., Soini, Y., Kinnula, V., et al., 2002. VEGF and bFGF are highly expressed in intraluminal fibromyxoid lesions in bronchiolitis obliterans organizing pneumonia. *J. Pathol.* 196, 220–227.
- Lentz, R.J., Argento, A.C., Colby, T.V., et al., 2017. Transbronchial cryobiopsy for diffuse parenchymal lung disease: a state-of-the-art review of procedural techniques, current evidence, and future challenges. *J. Thorac. Dis.* 9, 2186–2203.
- Liebow, A.A., Carrington, D., 1969. The interstitial pneumonias. In: Simon, M., Potchen, E., LeMay, M. (Eds.), *Frontiers of Pulmonary Radiology*. Grune & Stratton, New York, pp. 102–141.
- Line, B.R., Fulmer, J.D., Reynolds, H.Y., et al., 1978. Gallium-67 citrate scanning in the staging of idiopathic pulmonary fibrosis: Correlation and physiologic and morphologic features and bronchoalveolar lavage. *Am. Rev. Respir. Dis.* 118, 355–365.
- Liu, J.Y., Sime, P.J., Wu, T., et al., 2001. Transforming growth factor-beta(1) overexpression in tumor necrosis factor-alpha receptor knockout mice induces fibroproliferative lung disease. *Am. J. Respir. Cell Mol. Biol.* 25, 3–7.
- Lock, G., Pfeifer, M., Straub, R.H., et al., 1998. Association of esophageal dysfunction and pulmonary function impairment in systemic sclerosis. *Am. J. Gastroenterol.* 93, 341–345.
- London, L., Majeski, E.I., Paintlia, M.K., et al., 2002a. Respiratory reovirus 1/L induction of diffuse alveolar damage: a model of acute respiratory distress syndrome. *Exp. Mol. Pathol.* 72, 24–36.
- London, L., Majeski, E.I., tman-Hamamdzic, S., et al., 2002b. Respiratory reovirus 1/L induction of diffuse alveolar damage: pulmonary fibrosis is not modulated by corticosteroids in acute respiratory distress syndrome in mice. *Clin. Immunol.* 103, 284–295.
- Lu, B.S., Bhorade, S.M., 2004. Lung transplantation for interstitial lung disease. *Clin. Chest Med.* 25, 773–782.
- Lukacs, N.W., Hogaboam, C., Chensue, S.W., et al., 2001. Type 1/type 2 cytokine paradigm and the progression of pulmonary fibrosis. *Chest* 120, 5S–8S.
- Magro, C.M., Ross, P., Marsh, C.B., et al., 2007. The role of anti-endothelial cell antibody-mediated microvascular injury in the evolution of pulmonary fibrosis in the setting of collagen vascular disease. *Am. J. Clin. Pathol.* 127, 237–247.
- Mapel, D.W., Hunt, W.C., Utton, R., et al., 1998. Idiopathic pulmonary fibrosis: survival in population based and hospital based cohorts. *Thorax* 53, 469–476.
- Marguerie, C., Bunn, C.C., Beynon, H.L., et al., 1990. Polymyositis, pulmonary fibrosis and autoantibodies to aminoacyl-tRNA synthetase enzymes. *Q. J. Med.* 77, 1019–1038.
- Marshall, R.P., Puddicombe, A., Cookson, W.O., et al., 2000. Adult familial cryptogenic fibrosing alveolitis in the United Kingdom. *Thorax* 55, 143–146.
- Martin, W.J., Rosenow III, E.C., 1988. Amiodarone pulmonary toxicity. Recognition and pathogenesis (Part I). *Chest* 93, 1067–1075.
- Martinet, Y., Rom, W.N., Grotendorst, G.R., et al., 1987. Exaggerated spontaneous release of platelet-derived growth factor by alveolar macrophages from patients with idiopathic pulmonary fibrosis. *N. Engl. J. Med.* 317, 202–209.

- McLees, B.D., Adair, N., Moss, J., Fulmer, J.D., Keogh, B., Crystal, R.G., 1979. Patterns of pulmonary hemodynamic dysfunction: similarities between idiopathic pulmonary fibrosis and panacinar emphysema. *Am. Rev. Respir. Dis.* 119, 380. Ref Type: Abstract.
- Meliconi, R., Negri, C., Borzi, R.M., et al., 1993. Antibodies to topoisomerase II in idiopathic pulmonary fibrosis. *Clin. Rheumatol.* 12, 311–315.
- Minai, O.A., Dweik, R.A., Arroliga, A.C., 1998. Manifestations of scleroderma pulmonary disease. *Clin. Chest Med.* 19, 713–732.
- Miyazaki, Y., Araki, K., Vesin, C., et al., 1995. Expression of a tumor necrosis factor-alpha transgene in murine lung causes lymphocytic and fibrosing alveolitis. A mouse model of progressive pulmonary fibrosis. *J. Clin. Invest.* 96, 250–259.
- Mori, S., Cho, I., Koga, Y., et al., 2008. A simultaneous onset of organizing pneumonia and rheumatoid arthritis, along with a review of the literature. *Mod. Rheumatol.* 18, 60–66.
- Moya, C., Anto, J.M., Taylor, A.J., 1994. Outbreak of organising pneumonia in textile printing sprayers. Collaborative Group for the Study of Toxicity in Textile Aerographic Factories. *Lancet* 344, 498–502.
- Murray, J.F., Nadel, J.A., 2000. Idiopathic interstitial pneumonias. In: Murray, J.F., Nadel, J.A. (Eds.), *Murray and Nadel's Textbook of Respiratory Medicine*. Saunders, pp. 1686–1687.
- Myers, J.L., Colby, T.V., 1993. Pathologic manifestations of bronchiolitis, constrictive bronchiolitis, cryptogenic organizing pneumonia, and diffuse panbronchiolitis. *Clin. Chest Med.* 14, 611–622.
- Nakao, A., Fujii, M., Matsumura, R., et al., 1999. Transient gene transfer and expression of Smad7 prevents bleomycin-induced lung fibrosis in mice. *J. Clin. Invest.* 104, 5–11.
- Nakos, G., Adams, A., Andriopoulos, N., 1993. Antibodies to collagen in patients with idiopathic pulmonary fibrosis. *Chest* 103, 1051–1058.
- Nicholson, A.G., Colby, T.V., du Bois, R.M., et al., 2000. The prognostic significance of the histologic pattern of interstitial pneumonia in patients presenting with the clinical entity of cryptogenic fibrosing alveolitis. *Am. J. Respir. Crit. Care Med.* 162, 2213–2217.
- Noble, P.W., Albera, C., Bradford, W.Z., et al., 2016. Pirfenidone for idiopathic pulmonary fibrosis: analysis of pooled data from three multinational phase 3 trials. *Eur. Respir. J.* 47, 243–253.
- O'Donnell, K., Keogh, B., Cantin, A., et al., 1987. Pharmacologic suppression of the neutrophil component of the alveolitis in idiopathic pulmonary fibrosis. *Am. Rev. Respir. Dis.* 136, 288–292.
- Osler, W., 1892. *The Principles and Practice of Medicine*. Appleton, New York.
- Pantelidis, P., Fanning, G.C., Wells, A.U., et al., 2001. Analysis of tumor necrosis factor-alpha, lymphotoxin-alpha, tumor necrosis factor receptor II, and interleukin-6 polymorphisms in patients with idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 163, 1432–1436.
- Peabody, J.W., Peabody Jr, J.W., Hayes, E.W., Hayes Jr, E.W., 1950. Idiopathic pulmonary fibrosis; its occurrence in identical twin sisters. *Dis Chest* 18 (4), 330–344.
- Penn, C.C., Liu, C., 1993. Bronchiolitis following infection in adults and children. *Clin. Chest Med.* 14, 645–654.
- Pesci, A., Majori, M., Piccoli, M.L., et al., 1996. Mast cells in bronchiolitis obliterans organizing pneumonia. Mast cell hyperplasia and evidence for extracellular release of tryptase. *Chest* 110, 383–391.
- Peyrol, S., Cordier, J.F., Grimaud, J.A., 1990. Intra-alveolar fibrosis of idiopathic bronchiolitis obliterans-organizing pneumonia. Cell-matrix patterns. *Am. J. Pathol.* 137, 155–170.
- Raghu, G., Collard, H.R., Egan, J.J., et al., 2011. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am. J. Respir. Crit. Care Med.* 183, 788–824.
- Ramirez, J., Dowell, A.R., 1971. Silo-filler's disease: nitrogen dioxide-induced lung injury. Long-term follow-up and review of the literature. *Ann. Intern. Med.* 74, 569–576.
- Ratanathathorn, V., Ayash, L., Lazarus, H.M., et al., 2001. Chronic graft-versus-host disease: clinical manifestation and therapy. *Bone Marrow Transplant.* 28, 121–129.
- Remy-Jardin, M., Remy, J., Cortet, B., et al., 1994. Lung changes in rheumatoid arthritis: CT findings. *Radiology* 193, 375–382.
- Reynolds, H.Y., Fulmer, J.D., Kazmierowski, J.A., et al., 1977. Analysis of cellular and protein content of broncho-alveolar lavage fluid from patients with idiopathic pulmonary fibrosis and chronic hypersensitivity pneumonitis. *J. Clin. Invest.* 59, 165–175.
- Richeldi, L., du Bois, R.M., Raghu, G., et al., 2014. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. *N. Engl. J. Med.* 370, 2071–2082.
- Robinson, C., Callow, M., Stevenson, S., et al., 2001. Private specificities can dominate the humoral response to self-antigens in patients with cryptogenic fibrosing alveolitis. *Respir. Res.* 2, 119–124.
- Romero, S., Hernandez, L., Gil, J., et al., 1998. Organizing pneumonia in textile printing workers: a clinical description. *Eur. Respir. J.* 11, 265–271.
- Rossi, G.A., Szapiel, S., Ferrans, V.J., et al., 1987. Susceptibility to experimental interstitial lung disease is modified by immune- and non-immune related genes. *Am. Rev. Respir. Dis.* 135, 448–455.
- Ryu, J.H., Myers, J.L., Swensen, S.J., 2003. Bronchiolar disorders. *Am. J. Respir. Crit. Care Med.* 168, 1277–1292.
- Sandoz, E., 1907. Über zwei Fälle von "Fotaler Bronchektasie". *Beitr. Pathol. Anat.* 41, 495–516.
- Sato, S., Hoshino, K., Satoh, T., et al., 2009. RNA helicase encoded by melanoma differentiation-associated gene 5 is a major autoantigen in patients with clinically amyopathic dermatomyositis: Association with rapidly progressive interstitial lung disease. *Arthritis Rheum.* 60, 2193–2200.
- Sauty, A., Rochat, T., Schoch, O.D., et al., 1997. Pulmonary fibrosis with predominant CD8 lymphocytic alveolitis and anti-Jo-1 antibodies. *Eur. Respir. J.* 10, 2907–2912.
- Scadding, J.G., 1964. Fibrosing alveolitis. *Br. Med. J.* 5410, 686.
- Schnabel, A., Reuter, M., Gross, W.L., 1998. Intravenous pulse cyclophosphamide in the treatment of interstitial lung disease due to collagen vascular diseases. *Arthritis Rheum.* 41, 1215–1220.
- Schoenberger, C.I., Crystal, R.G., 1983. Drug induced lung disease. In: Isselbacher, K.J., Adams, R.D., Braunwald, E.M.J.B., Petersdorf, R.G., Wilson, J.D. (Eds.), *Harrison's Principles of Internal Medicine Update IV*. McGraw-Hill, New York, pp. 49–74.
- Schoenberger, C.I., Rennard, S.I., Bitterman, P.B., et al., 1984. Paraquat-induced pulmonary fibrosis. Role of the alveolitis in modulating the development of fibrosis. *Am. Rev. Respir. Dis.* 129, 168–173.
- Schwarz, M.I., 1998. The lung in polymyositis. *Clin. Chest Med.* 19, 701–712.

- Seibold, M.A., Wise, A.L., Speer, M.C., et al., 2011. A common MUC5B promoter polymorphism and pulmonary fibrosis. *N. Engl. J. Med.* 364, 1503–1512.
- Selman, M., King, T.E., Pardo, A., 2001. Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. *Ann. Intern. Med.* 134, 136–151.
- Silver, R.M., Miller, K.S., Kinsella, M.B., et al., 1990. Evaluation and management of scleroderma lung disease using bronchoalveolar lavage. *Am. J. Med.* 88, 470–476.
- Sleijfer, S., 2001. Bleomycin-induced pneumonitis. *Chest* 120, 617–624.
- Snider, G.L., 1986. Interstitial pulmonary fibrosis. *Chest* 89, 115S–121S.
- Sole, A., Cordero, P.J., Morales, P., et al., 1996. Epidemic outbreak of interstitial lung disease in aerographics textile workers—the “Ardystil syndrome”: a first year follow up. *Thorax* 51, 94–95.
- Southcott, A.M., Jones, K.P., Li, D., et al., 1995. Interleukin-8. Differential expression in lone fibrosing alveolitis and systemic sclerosis. *Am. J. Respir. Crit. Care Med.* 151, 1604–1612.
- Stack, B.H., Choo-Kang, Y.F., Heard, B.E., 1972. The prognosis of cryptogenic fibrosing alveolitis. *Thorax* 27, 535–542.
- Standiford, T.J., Kunkel, S.L., Phan, S.H., et al., 1991. Alveolar macrophage-derived cytokines induce monocyte chemoattractant protein-1 expression from human pulmonary type II-like epithelial cells. *J. Biol. Chem.* 266, 9912–9918.
- Staples, C.A., Muller, N.L., Vedral, S., et al., 1987. Usual interstitial pneumonia: correlation of CT with clinical, functional, and radiologic findings. *Radiology* 162, 377–381.
- Stein-Streilein, J., Lipscomb, M.F., Fisch, H., et al., 1987. Pulmonary interstitial fibrosis induced in hapten-immune hamsters. *Am. Rev. Respir. Dis.* 136, 119–123.
- Stover, D.E., Milite, F., Zakowski, M., 2001. A newly recognized syndrome—radiation-related bronchiolitis obliterans and organizing pneumonia. A case report and literature review. *Respiration* 68, 540–544.
- Targoff, I.N., 1993. Humoral immunity in polymyositis/dermatomyositis. *J. Invest. Dermatol.* 100, 116S–123S.
- Tasaka, S., Kanazawa, M., Mori, M., et al., 1995. Long-term course of bronchiectasis and bronchiolitis obliterans as late complication of smoke inhalation. *Respiration* 62, 40–42.
- Thomas, A.Q., Lane, K., Phillips III, J., et al., 2002. Heterozygosity for a surfactant protein C gene mutation associated with usual interstitial pneumonitis and cellular nonspecific interstitial pneumonitis in one kindred. *Am. J. Respir. Crit. Care Med.* 165, 1322–1328.
- Tisdale, J.E., Follin, S.L., Ordelova, A., et al., 1995. Risk factors for the development of specific noncardiovascular adverse effects associated with amiodarone. *J. Clin. Pharmacol.* 35, 351–356.
- Troshinsky, M.B., Kane, G.C., Varga, J., et al., 1994. Pulmonary function and gastroesophageal reflux in systemic sclerosis. *Ann. Intern. Med.* 121, 6–10.
- Turner-Warwick, M., Doniach, D., 1965. Auto-antibodies studies in interstitial pulmonary fibrosis. *Br. Med. J.* 5439, 886–891.
- Turner-Warwick, M., Burrows, B., Johnson, A., 1980. Cryptogenic fibrosing alveolitis: clinical features and their influence on survival. *Thorax* 35, 171–180.
- Wallace, W.A., Howie, S.E., 1999. Immunoreactive interleukin 4 and interferon-gamma expression by type II alveolar epithelial cells in interstitial lung disease. *J. Pathol.* 187, 475–480.
- Wallace, W.A., Schofield, J.A., Lamb, D., et al., 1994. Localisation of a pulmonary autoantigen in cryptogenic fibrosing alveolitis. *Thorax* 49, 1139–1145.
- Wallace, W.A., Ramage, E.A., Lamb, D., et al., 1995. A type 2 (Th2-like) pattern of immune response predominates in the pulmonary interstitium of patients with cryptogenic fibrosing alveolitis (CFA). *Clin. Exp. Immunol.* 101, 436–441.
- Weinberger, S.E., Kelman, J.A., Elson, N.A., et al., 1978. Bronchoalveolar lavage in interstitial lung disease. *Ann. Intern. Med.* 89, 459–466.
- Wells, A.U., Cullinan, P., Hansell, D.M., et al., 1994. Fibrosing alveolitis associated with systemic sclerosis has a better prognosis than lone cryptogenic fibrosing alveolitis. *Am. J. Respir. Crit. Care Med.* 149, 1583–1590.
- White, B., Moore, W.C., Wigley, F.M., et al., 2000. Cyclophosphamide is associated with pulmonary function and survival benefit in patients with scleroderma and alveolitis. *Ann. Intern. Med.* 132, 947–954.
- Whyte, M., Hubbard, R., Meliconi, R., et al., 2000. Increased risk of fibrosing alveolitis associated with interleukin-1 receptor antagonist and tumor necrosis factor-alpha gene polymorphisms. *Am. J. Respir. Crit. Care Med.* 162, 755–758.
- Wiedemann, H.P., Matthay, R.A., 1989. Pulmonary manifestations of the collagen vascular diseases. *Clin. Chest Med.* 10, 677–722.
- Witt, C., Borges, A.C., John, M., et al., 1999. Pulmonary involvement in diffuse cutaneous systemic sclerosis: bronchoalveolar fluid granulocytosis predicts progression of fibrosing alveolitis. *Ann. Rheum. Dis.* 58, 635–640.
- Wright, J.L., Cagle, P., Churg, A., et al., 1992. Diseases of the small airways. *Am. Rev. Respir. Dis.* 146, 240–262.
- Wusirika, R., Ferri, C., Marin, M., et al., 2003. The assessment of anti-endothelial cell antibodies in scleroderma-associated pulmonary fibrosis. A study of indirect immunofluorescent and western blot analysis in 49 patients with scleroderma. *Am. J. Clin. Pathol.* 120, 596–606.
- Xaubet, A., Marin-Arguedas, A., Lario, S., et al., 2003. Transforming growth factor-beta1 gene polymorphisms are associated with disease progression in idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 168, 431–435.
- Yoshida, S., Akizuki, M., Mimori, T., et al., 1983. The precipitating antibody to an acidic nuclear protein antigen, the Jo-1, in connective tissue diseases. A marker for a subset of polymyositis with interstitial pulmonary fibrosis. *Arthritis Rheum.* 26, 604–611.
- Yoshinouchi, T., Ohtsuki, Y., Ueda, R., et al., 1999. Myofibroblasts and S-100 protein positive cells in idiopathic pulmonary fibrosis and rheumatoid arthritis-associated interstitial pneumonia. *Eur. Respir. J.* 14, 579–584.
- Yousem, S.A., Colby, T.V., Carrington, C.B., 1985. Follicular bronchitis/bronchiolitis. *Hum. Pathol.* 16, 700–706.
- Ziesche, R., Hofbauer, E., Wittmann, K., et al., 1999. A preliminary study of long-term treatment with interferon gamma-1b and low-dose prednisolone in patients with idiopathic pulmonary fibrosis. *N. Engl. J. Med.* 341, 1264–1269.
- Zisman, D.A., Lynch III, J.P., Toews, G.B., et al., 2000. Cyclophosphamide in the treatment of idiopathic pulmonary fibrosis: a prospective study in patients who failed to respond to corticosteroids. *Chest* 117, 1619–1626.

## Autoimmune Diseases in the Kidney

Jing Gong<sup>1,2</sup>, Ami Tamhaney<sup>1</sup>, Mohanraj Sadasivam<sup>3</sup>, Hamid Rabb<sup>1</sup>  
and Abdel Rahim A. Hamad<sup>3</sup>

<sup>1</sup>Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, United States <sup>2</sup>Department of Pediatric Nephrology, The Second Affiliated Hospital of Nanjing Medical University, Nanjing, P.R. China

<sup>3</sup>Department of Pathology, School of Medicine, Johns Hopkins University, Baltimore, MD, United States

### OUTLINE

Introduction	1355	Pathological Features	1359
History	1355	Autoimmune Responses (Allograft Rejection) in Kidney Transplantation	1360
Epidemiology	1356	Clinical Features and Disease Associations	1361
Genetics	1357	Summary	1363
Autoimmune Features	1357	References	1363
Animal Models	1358		

### INTRODUCTION

The kidney, like most other organs, is a target of autoimmunity. This can happen as a consequence of systemic autoimmunity, such as systemic lupus erythematosus (SLE) which causes nephritis in 35%–55% of patients due to an abnormal inflammation in the glomerulus (Bombard, 2017). In other instances, kidney disorders are caused by deposits of nonspecific autoantibodies in the glomerulus as in cases of antineutrophil cytoplasmic antibody (ANCA), complement 3 (C3), and immunoglobulin (Ig)A glomerulopathies (Sethi et al., 2016). The kidney is also the target of autoantibodies that attack specific cell types in the glomerulus (McAdoo and Pusey, 2017). These include membranous nephropathy (MN) and glomerular basement membrane (GBM) nephropathy. Besides these established autoimmune diseases, acute kidney injury (AKI) and chronic kidney disease (CKD) have autoimmune features and are increasingly being considered to have important autoimmune components (Jang and Rabb, 2015). This chapter briefly summarizes the main autoimmune diseases of the kidney, treatments, and connections to the general concept of autoimmunity.

### HISTORY

The original idea that nephrotoxic substances, later identified as autoantibodies, mediated nephritis was suggested by early studies. A study in Japan in 1933 showed that injecting rat kidney adjuvants into rabbits caused disease signs that resembled human glomerulonephritis (GN), implicating nephrotoxic substances in disease

pathogenesis (Redakcja naukowa et al., 2009). Smadel et al. (Farr and Smadel, 1939) followed up by a study in 1939 where they induced acute nephritis in rats injected with anti-rat kidney serum obtained from immunized rabbits. Another study by Schwentker and Comployer (1939) showed that the injection of rabbits with homologous kidney emulsions mixed with *Staphylococcus* toxins generated complement-fixing antibodies that were reactive with rabbit kidney. Given the presence of antibody in patients with scarlet fever, the authors speculated that the toxin combined with kidney tissues formed a complete antigen that induced kidney-specific antibodies (Mackay, 2010).

A major breakthrough came in 1959 when Marianne and Bielschowsky developed the first lupus model by crossbreeding of New Zealand black (NZB) and New Zealand white (NZW) mice (Bielschowsky and Bielschowsky, 1964). The F1 generation developed spontaneous autoimmune hemolytic anemia with renal lesions comparable to those found in SLE patients. Subsequent studies showed the presence of the same antibodies in the NZB/NZW mice and SLE patients as reviewed by Mackay (2010). In the same year, Stanton et al. showed that the Goodpasture's disease or antiglomerular basement disease has autoimmune features (Hellmark and Segelmark, 2014). Using direct immunofluorescence (IF), the authors were able to visualize Ig deposits along the GBM in multiple patients. The pathogenic roles of these antibodies were demonstrated by inducing Goodpasture-like symptoms and lesions in primates injected with antibodies isolated from patients (Hellmark and Segelmark, 2014). Subsequent studies brought in a new era of renal histological methods and the use of transmission electron microscopy. In 1959 two children with scarlet fever and nephritis were found to have low hemolytic activity and low C3 serum content (Cunningham, 2000). Using electron microscopy and IF techniques, Mueller-Eberhard and Nilsson identified a C3 nephritic factor which when mixed with normal human serum caused GN (Nilsson and Mueller-Eberhard, 1965).

Besides antibodies, T cells have been gradually implicated in the pathogenesis of autoimmune disorders of the kidney. CD4 T cells can directly help B cells to produce antibodies. In addition, they have been shown to cause kidney pathology (Tsokos, 2011). CD4<sup>+</sup> T cells mediate renal ischemia-reperfusion injury, making AKI another likely autoimmune disease (Rabb et al., 2016). Autoreactive T cells in patients with Goodpasture's disease are specific for peptides in the autoantigen that has a high affinity for the disease-associated human leukocyte antigen (HLA) Class II molecule, DR15 (Phelps et al., 1996). Furthermore, while B-cell hyperactivity in SLE is T-cell dependent, CD4<sup>+</sup> T-cell activation is essential to SLE pathogenesis. Most recently, T-cell Ig and immunoreceptor tyrosine-based inhibition motif (ITIM) domain (TIGIT), an inhibitory receptor, was identified as a powerful negative regulator of CD4<sup>+</sup> T cells in SLE (Mao et al., 2017).

## EPIDEMIOLOGY

*Environmental factors.* Environmental factors play important roles in the development of renal autoimmune diseases. These factors range from infectious agents (Krawczak et al., 2017), chemicals/metals and toxins (Stevenson et al., 1995), stress (Richter et al., 2018), diet (Johnson et al., 2015), smoking (Donaghy and Rees, 1983), exposure to prolonged sunlight exposure (Ngo et al., 2014), and xenobiotics.

*Infectious agents.* Bacteria, viruses, and parasites have been associated with autoimmune glomerular diseases. There is a strong correlation between IgA nephropathy and infections, especially upper respiratory tract infections (Roberts, 2014). *Escherichia coli* is the most common bacterial cause of hemolytic uremic syndrome (HUS) (Jokiranta, 2017). Hepatitis B and C viruses can cause membranous GN (Gupta and Quigg, 2015), while HIV is associated with a glomerular immune complex disease (Nebuloni et al., 2009). Furthermore, molecular mimicry between self-antigens and microbial determinants has been associated with the anti-GBM disease and acute dengue infection (Lizarraga et al., 2015). On the other hand, infectious agents can be protective, as it has been reported that infecting BWF1 mice with malaria parasite protects from lupus by reducing B-cell autoreactivity (Badr et al., 2015).

*Chemicals/metals.* Penicillamine, an antirheumatic drug and exogenous chemical, is associated with MN (Dische et al., 1984). Exposure to gold and mercury, in the environment or for therapeutic reasons, causes autoimmune responses in kidneys and other tissues in some individuals (Bigazzi, 1999). Lithotripsy-associated kidney trauma is associated with the development of anti-GBM disease (Xenocostas et al., 1999). On the other hand, intermittent exposure to ultraviolet radiation with sun-simulation has been reported to protect and even treat vitamin D deficiency in chronic and end-stage kidney disease patients (Krause, 2013). However, under the experimental conditions, exposure to ultraviolet radiation has been shown to have detrimental effects on rat kidney tissue cells (Türker and Yel, 2014).

*Ethnic/gender.* As with most other autoimmune diseases, there are significant gender differences in the prevalence of kidney autoimmune diseases with females generally more susceptible than males (Ngo et al., 2014).

The only notable exception is IgA nephropathy where the disease incidence in men is double than in women (O'Shaughnessy et al., 2017). There are also major differences in prevalence depending on ancestry. For example, lupus nephritis (LN) affects 68.9% of African-Americans, 60.6% of Hispanics patients compared to only 29.1% of Caucasians (Bastian et al., 2002). On the other hand, IgA nephropathy is less common in African-Americans and Caucasians but is the most common form of glomerular disease in Asian, accounting for 30%–40% of all cases (Falk PHNJCJRJ, 2008). Age is also another important factor; whereas IgA nephropathy declines with age, LN peaks in early adulthood, and ANCA GN dramatically surges after the age of 50 years (Jennette and Nachman, 2017).

## GENETICS

Genetic background contributes to autoimmune diseases of the kidney. For example, two variants of the APOL1 gene have been linked to the development of multiple glomerular disorders in African-Americans (Genovese et al., 2010). The low-affinity Fc gamma receptors II and III (FcγR2a, FcγR2b, and FcγR3a) are important regulators of immune responses that are encoded for in the FCRG locus. Polymorphisms and variations in copy numbers of genes in this locus are associated with LN and to impair IgA binding and clearance, resulting in increasing amounts of circulating IgGs (Relle et al., 2015). Studies in SLE mouse models showed that deletion of CAMK4 decreased IL-2 production by T cells and reduced the severity of GN, making it a potential genetic link with LN (Otomo et al., 2015).

There are multiple different pathways that can cause IgA nephropathy: abnormal antigen presentation, the complement system, regulation of global mucosal Ig production, and innate immunity (Magistroni et al., 2015). Each of these pathways is controlled by certain genetic loci. For examples, *ITGAM-ITGAX*, *VAV3*, *CARD9*, *HLA-DQB1*, and *DEFA* are tightly linked to IgA nephropathy (Magistroni et al., 2015). *CFHR1/3* and *ITGAM-ITGAX* loci are linked with the complement system, whereas *TNFSF13* and *LIF/OSM* loci regulate the IgA production in mucosal membranes (Magistroni et al., 2015).

Occurrence of MN varies among individuals depending on their ethnic backgrounds (Sim et al., 2016). Polymorphisms in *HLA-DQA* and phospholipase A2 receptor (*PLA2R1*) have been correlated with the MN. European Caucasians have been documented with the most cases of circulating anti-PLA2R antibodies and incidences of MN compared to other racial ethnicities (Ronco and Debiec, 2015). Furthermore, a truncating mutation in the neutral endopeptidase (NEP) results in complete absence of the NEP protein in homozygous mothers who can become alloimmunized during pregnancy/miscarriage and generate high titers of anti-NEP antibodies upon reexposure to antigens from fetal or placenta tissues to cause antenatal MN due to fetomaternal alloimmunization (Beck, 2015).

There are also positive correlations between the expression of HLA-DRB1<sup>\*</sup>1501 and DRB1<sup>\*</sup>1502 and the over-production of anti-GBM antibodies in 80% of the patients studied (Cui and Zhao, 2011). On the other hand, HLA-DR7 and DR1 have protective effects and negative genetic linkage with the anti-GBM disease (Hellmark and Segelmark, 2014). Recent studies in congenic rats have shown that the Fcgr3 gene and the expression of the activator protein (AP)-1 transcription factor, JunD, could also affect the severity of the anti-GBM disease by modulating macrophage activity (McAdoo and Pusey, 2017).

Immunoglobulin G4-related disease (IgG4-RD) is a systemic fibro-inflammatory disorder that affects most organs, including the kidney (Pradhan et al., 2015). Tubulointerstitial nephritis (IgG4-TIN) is the most common form of IgG4-RD with the majority of patients having autoantibodies free in serum and bound to podocytes (Cortazar and Stone, 2015).

Genome-wide association studies that aimed at identifying genetic loci influencing susceptibility to SLE (Harley et al., 2008) have identified >50 genes associated with SLE (Chen et al., 2017). Inclusion of patients with LN in these studies has provided additional important insights into the potential pathogenic pathways that lead to both SLE and LN (Gualtierotti et al., 2010).

## AUTOIMMUNE FEATURES

Adaptive immune cells, B and T cells, are responsible for the major pathogenic features of kidney autoimmunity. A pivotal role for B cells is the production of pathogenic autoantibodies. Anti-DNA antibodies produced by autoreactive antibodies deposit in the GBM through interactions with DNA, nucleosomes trapped in the GBM,

and/or binding to intrinsic non-DNA glomerular antigens (Yung and Chan, 2008). Anti-dsDNA antibodies also play pathogenic roles in LN (Misra and Gupta, 2015). Natural autoantibodies against myeloperoxidase (MPO) and proteinase 3 are detected in patients with ANCA-associated disease (Cui and Zhao, 2011). B cells also serve as potent antigen-capturing cells, particularly to stimulate activated/memory T cells and perpetuate injurious autoimmune processes (Silveira et al., 2004).

Despite their perceived importance and growing evidence, the pathogenic role of T cells in mediating renal diseases is yet to be thoroughly characterized. Follicular helper T CD4 T cells, reside in the germinal center and are specialized in helping B cells, produce antibodies, participate in renal autoimmune injury (Crotty, 2014), and play a central role in the pathogenesis of SLE (Blanco et al., 2016). On the other hand, regulatory T cells (Tregs), which usually play a protective role against autoimmune responses (Schaerli et al., 2000), have been described to be deficient in SLE patients and Tregs bearing exon 2-deficient FoxP3 splice variant are reported to predominate in ANCA-associated vasculitis (AAV) (Free et al., 2013).

## ANIMAL MODELS

*LN model.* There are several spontaneous mouse models that develop the lupus-like nephritis (LN). These include the F1 hybrid between the NZB and NZW strains (NZB/W) F1, New Zealand Mixed (NZM)/Aeg2410 (NZM2410), MRL/lpr, and BXSB/Yaa strains (Peng, 2012). NZB/W F1 mice develop high titers of antinuclear antibody (ANA), hemolytic anemia, splenomegaly, and early onset immune complex-mediated GN (Peng, 2012). The NZM2410 and NZM2328 strains have lupus-like disease, and their phenotypes are similar to those of (NZB/W) F1 mice, with anti-DNA autoantibodies and nephritis (Waters et al., 2001). Lupus-like disease in the NZM2328 strain shows a strong gender bias toward females similar to the NZB/W F1 mice, whereas NZM2410 mice exhibit nephritis with similar severity in males and females (Waters et al., 2001). The BXSB/Yaa mouse represents a recombinant inbred strain derived from backcrossing of (B6 × SB/Le) F1 and SB/Le (Li et al., 2017). These mice develop high levels of ANA and severe GN (Li et al., 2017). MRL/lpr mice and MRL/Mp mice are also used for the study of LN (Beck et al., 2000). MRL/lpr mice produce autoantibodies against dsDNA and Sm, leading to large amounts of immune complexes that induce renal disorder (Andrews et al., 1978). The SNF1 model is produced by crossing the Swiss Webster (SW) strain with the NZB strain. The autoantibodies derived from SNF1 mice are cationic IgG2b anti-dsDNA and are nephritogenic with restricted idiotypes, and they cluster along the GBM to initiate renal injury (Gavalchin et al., 1985).

*ANCA-induced GN model.* The anti-MPO model is based on the use of MPO-deficient (MPO – / –) mice to produce high-titer, high-affinity, and anti-MPO IgG that are pathogenic when transferred into wild-type or immune-deficient mice (Xiao et al., 2002). They cause necrotizing crescentic GN (NCGN), demonstrating a pathogenic role for MPO-ANCA and that anti-MPO IgG alone is sufficient to induce focal NCGN in naive subjects (Xiao et al., 2002). Jennette et al. demonstrated that an oral small-molecule antagonist of human C5aR blocks induction of anti-MPO NCGN in 129S6/B6 mice bearing a transgenic human C5aR (Xiao et al., 2014).

*IgA nephropathy—the ddY mouse model.* The ddY mouse was first reported in 1985 (Imai et al., 1985). It is a model of spontaneous IgA nephropathy, which develops GN with a striking deposition of IgA in the mesangium, as well as codeposits of IgG, IgM, and C3 after the age of 40 weeks. Recently, Tomino et al. used a novel strain of ddY mice termed “grouped ddY mice” by selective mating to establish the early-onset IgA nephropathy (Okazaki et al., 2012).

*Anti-GBM nephritis model.* Human anti-GBM nephritis presents a clinical course of rapidly progressive GN with diffuse crescent formation (Ohashi et al., 2003). A number of animal models of anti-GBM nephritis have been developed. One model is developed by immunization of mice with homologous or heterologous GBM in complete Freund’s adjuvant (Reynolds et al., 1998). A second model, which was originally developed in sheep (the T.D. Hewitson Steblay nephritis model), is also easily inducible in rabbits but less reliably in rats (Steblay, 1962). Another model, first described in the 1930s, is induced by passive administration of heterologous anti-GBM antibodies in rats (El Nahas, 1993). Furthermore, anti-GBM nephritis can be induced in mice and rats, but with great variations in susceptibility between strains (Yang et al., 2010).

*AKI model.* AKI is a cause of renal damage in diverse clinical circumstances (Basile et al., 2012). Three different models of ischemic AKI are commonly used for research. Bilateral clamping of the renal pedicle has been used by many groups to simulate AKI induced by acute severe hypotension (Wang et al., 2017). Clamping of a single renal artery, which induces a unilateral disease, derives its value from allowing the use of contralateral kidney as control, and overall survival is higher than with bilateral clamping (Wei and Dong, 2012). Finally, the removal of

one kidney prior to occlusion of the contralateral renal artery avoids the confusion caused by functioning and nonfunctioning kidneys and also more closely mimics the situation often occurring in renal transplantation (Burne-Taney et al., 2003).

The degree of AKI in these models is proportional to the period of ischemia and has to be determined for the individual animal strain used and age. As a guide, 30–50 minutes of the occlusion is generally used in uninephrectomized rats and mice. However, the functional effects remain highly variable, with small differences in ischemia time significantly changing the characteristics of the model. These differences can make comparative studies problematic (Wei and Dong, 2012).

*MN-Heymann nephritis (HN) rat model.* The study of rat HN identified fundamental disease mechanisms that revolutionized our understanding of human MN (Foster, 2016). Human membranous nephritis is a significant cause of end-stage kidney disease (Ronco and Debiec, 2015). Active HN is an autoimmune model of membranous nephritis induced in Lewis rats by immunization with a crude renal tubular antigen (Fx1A) or megalin (gp330). The pathogenesis involves binding of anti-Fx1A autoantibodies to the autoantigen expressed on glomerular epithelial cells, causing severe glomerular injury and proteinuria. The pathological features of HN include immune deposits in glomeruli and infiltration of glomeruli and the tubulointerstitium by macrophages and T cells (Wang et al., 2015).

*Nephrotoxic models.* Drugs affect renal function in a variety of ways. Adriamycin results in nephrotic syndrome and cisplatin as well as mercuric chloride and gentamicin are associated with tubular toxicity (Servais et al., 2008). In most cases, outcomes are directly related to the duration of exposure. With the injection of cisplatin, AKI induced in rats or mice generates renal pathology and tubular dysfunction comparable to those seen in humans (Deng et al., 2001). Repeated injection of cisplatin in rats results in inflammatory and fibrotic processes that are being regulated by IL-10 (Deng et al., 2001). Exposure to mercury is an environmental cause of heavy metal–induced renal toxicity in humans that results in acute proximal tubule injury within 2–3 days, followed by a regenerative process (Anders and Schlondorff, 2000). Intraperitoneal injection of gentamicin in mice is used as a highly clinically relevant model that leads to myeloid bodies in proximal tubule cells, a morphologic feature of gentamicin exposure in humans (Zager et al., 2007).

## PATHOLOGICAL FEATURES

*LN.* The histopathological features specific to LN include immune complex deposits in the mesangial, subendothelial and subepithelial glomerulus, extraglomerular deposits within tubular basement membranes (TBMs), and tubuloreticular inclusions in the glomerular endothelial cells. Tissues were stained for the “full house” of three IgGs (IgG, IgA, and IgM) and two complement factors (C3 and C1q), which when all detected together are strong indicators of SLE (Barisoni, 2015). There are multiple stages of LN, depending on the presence, placement, and severity of endocapillary proliferation, glomerular leukocyte infiltration, wire loop deposits, fibrinoid necrosis, karyorrhexis, cellular crescents, and interstitial inflammation (Gerald et al., 2008). There are six classes of LN: Class I, minimal mesangial LN; Class II, mesangial proliferative LN; Class III, focal LN; Class IV, diffuse LN; Class V, membranous LN; and Class VI, advanced sclerosing LN. Class I and II LN point to mesangial proliferation which could progress to membranous LN in the epithelium. Class III LN is characterized by more damage in the glomerular area, including necrotizing or sclerosing lesions. Class IV LN lesions have more necrosis and crescents, with severe interstitial lesions and fibrosis. The various forms of LN may overlap with cases of Class V membranous nephritis, which is characterized by subepithelial deposits on the loops of glomeruli (Anders and Fogo, 2014).

*Crescentic GN.* Although light microscopy lesions may be similar in all the three types of crescentic GN, IF is useful to distinguish diseases (Barisoni, 2015). Linear deposits of IgG along the GBM are typical of Goodpasture syndrome (Cui and Zhao, 2011). Diabetic nephropathy and fibrillary GN are often confused with anti-GBM disease because of the IgG staining; however, they do not show evidence of crescents under light microscope. In later stages, it can be characterized by breaks in the Bowman’s capsule and fibrinous necrosis and adjacent segments of glomeruli (Hellmark and Segelmark, 2014).

Among type 2 crescentic GN, the diagnosis of IgA nephropathy essentially depends on microscopic examination. Light microscopy shows proliferation of mesangial cells, whereas IF reveals “dominant” (greater than any other Ig) mesangial granular IgA staining, whereas mesangial deposits are seen on electron microscope (EM). Postinfectious GN (PIGN) is also an immune-mediated GN associated with extrarenal bacterial infections. Its pathologic features include glomerular endocapillary hypercellularity by light-microscopy (LM), granular IgG

and C3 staining in capillary walls by IF, and subepithelial “hump”-like deposits by EM (Roberts, 2014). The characteristic lesion in pauci-immune GN is glomerular capillary necrosis (often segmental) with the cellular crescent formation and few to no immune deposits by IF and EM.

*Autoimmune MN.* It is characterized by glomerular capillary wall thickening on LM, prominent granular (“lumpy-bumpy”) capillary wall staining for IgG and C3 on IF, and subepithelial deposits on EM (Barisoni, 2015). Cases of early MN may show no overt changes by LM and quite a subtle staining by IF, whereas EM readily establishes the diagnosis in such early cases by revealing small subepithelial deposits. Detection of enhanced PLA2R expression in glomeruli by IF can serve as a footprint of PLA2R-associated MN. MN has been reported in association with IgG4-RD. In IgG4-related MN, unlike primary MN, IF staining for PLA2R is negative while IgG4 is primary (Barisoni, 2015).

*C3 glomerulopathy (C3G).* The pathology of C3G includes glomerular disorders with a membranoproliferative LM pattern and IF characterized by C3 deposition and little to no Igs. C3G includes both C3GN and the rare dense deposit disease (DDD, previously known as Membranoproliferative glomerulonephritis (MPGN) type II). These two disorders are best distinguished on EM: mesangial and capillary wall (usually subendothelial) deposits in C3GN and dense osmiophilic intramembranous deposits in DDD (Barbour et al., 2013).

*TIN.* The pathology of acute interstitial nephritis (AIN) is characterized by the interstitial infiltration of inflammatory cells and edema, which may be sparse, focal, or intense. The most numerous cell types are lymphocytes, monocytes, and macrophages, but they can also be associated with plasma cells, eosinophils, and neutrophils. EM may show that the TBM is discontinuous, partially thickened, or even disrupted (Muriithi et al., 2014).

Chronic TIN is characterized by renal interstitial fibrosis with mononuclear cell infiltration, tubular atrophy, luminal dilation, flattened epithelial cells, and thickening of TBM. The cellular infiltrate in chronic interstitial disease is composed of lymphocytes, macrophages, and B cells, with occasional neutrophils, plasma cells, and eosinophils. Infiltrate is chronic TIN typically less marked than in AIN (Barisoni, 2015).

## AUTOIMMUNE RESPONSES (ALLOGRAFT REJECTION) IN KIDNEY TRANSPLANTATION

*Hyperacute rejection.* Microscopy shows diffuse glomerular and peritubular capillary (PTC) thrombosis with interstitial hemorrhages, ischemic acute tubular necrosis, and infarction. Capillary neutrophils and platelets are abundant. IF shows focal IgG, IgM, and C3 in the PTC. C4d may be detectable in PTC. Diffuse capillary endothelial swelling and detachment from the basement membrane, platelet-fibrin thrombi, neutrophils, macrophages, and capillary rupture may be seen by EM (Han et al., 2015).

*Acute rejection.* Acute rejection can be divided into 30%–60% cell-mediated rejection, 10% humoral-mediated rejection, and about 30% mixed rejection. Light microscopy of cell-mediated rejection manifested mainly as interstitial mononuclear inflammation, edema, tubulitis, intimal arteritis (acute endarteritis), ± acute transplant glomerulitis. Antibody-mediated rejection is mainly manifested as acute capillaritis, glomerular + PTC, acute tubular necrosis (ATN), Thrombotic Microangiopathy (TMA) (rare), and necrotizing arteritis. Indirect IF reveals diffuse PTC C4d in humoral acute rejection (AR). Direct IF reveals little Ig or other complement components in vasculature. EM is useful to assess basement membranes of interstitial capillaries, multilayered in chronic antibody-mediated rejection (AMR) (Han et al., 2015).

*Chronic rejection.* Chronic transplant glomerulopathy is characterized by diffuse, often segmental, GBM duplication on PAS-stained sections and variable mesangial sclerosis with segmental adhesion of the glomerular tufts to Bowman capsule. Global and segmental glomerular sclerosis and hyalinosis may be prominent. PTCs have basement membrane thickening and multilayering. Arteries have intimal fibrous thickening with small amounts of infiltrating T cells and foamy macrophages, which are features diagnostic of chronic endarteritis. Interstitial inflammation and fibrosis with tubular atrophy are variable. IF reveals little IgG, IgM, and C3 in glomeruli. PTC, glomerular capillary, and mesangial C4d deposition are frequent in biopsies with chronic transplant glomerulopathy. Diffuse PTC C4d correlates with the presence of circulating donor-specific antibodies in chronic rejection. EM shows glomerular capillaries with swollen endothelium, fenestral loss, detachment from the basement membrane, and formation of new subendothelial strands of the basement membrane. Cellular interposition could also be evident, whereas electron-dense deposits of immune complexes absent. On the other hand, mesangial sclerosis will be prominent, podocyte foot processes effaced, PTCs will have swollen endothelium and multiple periendothelial layers of new basement membrane (Han et al., 2015).

## CLINICAL FEATURES AND DISEASE ASSOCIATIONS

Pathogeneses of these autoimmune diseases often involve loss of self-tolerance and induction and mobilization of immune effector cells that cause cell injury and organ dysfunction.

**LN.** SLE is the classic autoimmune disease of the kidney. Although SLE has diverse organ involvement, the kidney is among the most commonly affected organs. Nearly 50% of the Afro-Caribbean women with SLE develop LN that results in significant morbidity and mortality (Saxena et al., 2011). Abnormal proteinuria, abnormal urinary sediment, or decreased glomerular filtration rate (GFR) are present at the time of diagnosis in at least one-half of the SLE patients. According to the American College of Rheumatology's guideline, the use of renal biopsy is important for a demonstration of immune complex-mediated GN indicative of LN (Petri et al., 2012). Indicators such as anti-dsDNA, C3, C4, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) provide the most useful clinical information. Raised serum levels of antibody to dsDNA and serum complement component levels are generally reduced in LN.

**Treatment.** Patients with SLE are treated with nonsteroidal antiinflammatory drugs, antimalarial agents, glucocorticoids, and immunosuppressive drugs, including cyclophosphamide, azathioprine, methotrexate, and mycophenolate mofetil (Yildirim-Toruner and Diamond, 2011). Inhibitors of cyclooxygenase-2, which have antiinflammatory effects, have been claimed to promote the death of autoreactive T cells (Esmaeili et al., 2017). The choice of drug depends largely on the severity of the disease and functions of the organs involved. Blockade of BLyS with an anti-BLyS antibody, which has been approved by the Food and Drug Administration for the treatment of lupus (Navarra et al., 2011), results in a small but significant beneficial clinical effect within the first year of treatment in patients with mild or moderate disease. The role of B-cell depletion therapy using anti-CD20 antibody (rituximab) in the treatment of SLE is unclear. However, the use of BLyS after B-cell depletion still sounds reasonable. Restorations of T-cell tolerance with peptide components of putative autoantigens and anti-CD40L antibodies are undergoing clinical trials (Tsokos, 2011).

**Crescentic GN.** Crescentic GN often presents as a rapid progressive GN (RPGN) with the histological manifestation of severe glomerular damage rather than the specific disease. It is characterized by the accumulation of proliferating and dedifferentiated visceral and parietal cells in the Bowman's space, which surround and compress the glomerular tuft. Crescentic GN is usually associated with macroscopic or microscopic hematuria, erythrocyte casts, variable degrees of proteinuria that result in loss of renal function within 3 months after the clinical onset (Falk PHNJCRJ, 2008). There are three categories of crescentic GN based on the presence and the distribution of immune deposits at IF. Type 1 accounts for about 10% of crescentic GN. Goodpasture syndrome is a typical example of this category. Type 2 accounts for 15%–20% of crescentic GN. It is a heterogeneous group of RPGN characterized by granular deposits of IgG (Singh et al., 2008). Different immune complex diseases could lead to type 2 crescentic GN. These include PI acute GN, LN, Henoch–Schonlein purpura, mixed cryoglobulinemia, IgA nephritis, immune complex-mediated membranoproliferative GN, diabetic glomerulosclerosis, and primitive or secondary amyloidosis. Type 3 is the most common form of crescentic GN as it accounts for 60%–80% of all cases which is now reclassified as AAV.

Almost half of crescentic GN patients have AKI as their clinical presentation (Chen et al., 2016). Nephritic syndrome and RPGN are common features in all types of crescentic GN. Circulating anti-GBM antibodies are typical of Goodpasture syndrome. Low C3 levels are frequent in PIGN. ANCA test is the immunologic marker of AAV, present in 90% of the cases.

**Treatment.** Early treatment is paramount for crescentic GN patients (Moroni and Ponticelli, 2014). The current approach utilizes a combination of corticosteroids and cytotoxic drugs to suppress active inflammation and reduce cellular response and the antibody production. Plasmapheresis can be used to remove circulating antibodies and immune complexes (Syed et al., 2015). Prognosis depends largely on the level of serum creatinine at presentation (Perez-Valdivieso et al., 2007). In one European survey, an estimated GFR of <15 mL/min, advancing age, lower hemoglobin, higher Birmingham Vasculitis Activity Score, and higher white cell count were identified as significant negative prognostic factors for a patient's long-term survival. While the natural course of crescentic GN often leads to the end-stage renal disease (ESRD), an appropriate treatment could halt progression and even lead to complete remission. Prompt diagnosis and treatment are thus essential for favorable prognosis (Moroni and Ponticelli, 2014).

**Autoimmune MN.** MN is a rare disease that is considered a model of organ-specific autoimmune disorder that targets the glomerulus (Sinico et al., 2016). It is the most common cause of the nephrotic syndrome (defined by massive urinary protein loss) in White patients (Hull and Goldsmith, 2008), accounting for about 30% of the cases (annual incidence, 1.7 per 100,000; approximately 1300 and 10,000 new cases in France and European Union each

year, respectively). Autoimmune idiopathic MN involves *in situ* formation of subepithelial deposits, resulting from binding of circulating anti-PLA2R autoantibodies to podocyte's PLA2R. Approximately 70%–80% of the patients with idiopathic (primary) MN have circulating anti-PLA2R autoantibodies while secondary forms of MN (e.g., membranous LN) generally lack these autoantibodies, hence an excellent biomarker of the disease (Beck and Salant, 2014). Furthermore, MN is one of the most frequent etiologies of nephrotic syndrome after renal transplantation which affects 10%–40% of the patients and can cause graft loss (Golgert et al., 2008).

**Treatment.** Clinical outcome is extremely variable and unpredictable with more than one-third of the patients with idiopathic MN experience spontaneous remission of the nephrotic syndrome. However, a significant number of patients will have a poor response to immunosuppressive therapy and progress to ESRD.

**C3G.** C3Gs are a group of severe renal diseases with distinct patterns of glomerular inflammation and C3 deposition caused by complement dysregulation (Medjeral-Thomas et al., 2014). The clinical features of C3 GN are heterogeneous with up to 50% of the patients retain normal renal function while as many as 15% progress to ESRD. Forms of C3G include DDD, C3 GN, familial MPGN3, CFhr5 nephropathy, and immune complex-mediated MPGN. Recent advances in the assessment of CFHR proteins have revealed roles in atypical HUS (aHUS) and C3Gs (Tortajada et al., 2013). aHUS is an acute and severe disease belonging to thrombotic microangiopathies, whereas C3Gs are chronic diseases characterized by glomerular C3 deposition with no or limited Ig deposition.

**Treatment.** Before 2012, the treatment has invariably included antacellular immune suppression targeting T and/or B cells (e.g., cyclophosphamide, mycophenolate, or rituximab) with or without plasma therapy. More recently, the treatment plans have sometimes included anticomplement C5 therapy (Wong and Kavanagh, 2015). Plasma therapy is used on a case-by-case basis in C3Gs.

**TIN.** AIN is caused by multiple factors. Drugs such as antibiotics and non-steroidal anti-inflammatory drugs (NSAIDs) are the most common cause of acute TIN; however, other causes include autoimmune and systemic disorders, infections, and metabolic etiologies (Perazella and Markowitz, 2010). Cellular and humoral immunity are involved in the pathogenesis of the disease with renal biopsy showing significant renal interstitial infiltration by T cells (Hooke et al., 1987). The typical clinical manifestations of AIN include fever, rash, joint pain, and other systemic inflammatory manifestations. Kidney manifests as abnormal urinalysis, renal impairment, and tubular injury. In addition to drugs, infection is another common cause of AIN. Viruses such as hantavirus and polyomavirus manifest as AKI at an early stage, with persistent tubular necrosis leading to interstitial fibrosis, and tubule atrophy ultimately to renal failure (Gnemmi et al., 2015). AIN can also be caused by a variety of autoimmune diseases such as SLE, Sjogren's syndrome, sarcoidosis, Wegener granulomatosis, primary biliary cirrhosis, and cryoglobulinemia while chronic interstitial nephritis (CIN) may develop chronically following acute TIN or may be the initial manifestation of an autoimmune or systemic process (Guillevin and Dorner, 2007). Besides renal tubular dysfunction, clinical manifestations of CIN include kidney endocrine dysfunction, such as reduced production of erythropoietin, vitamin D metabolic disorders, and chronic renal failure (Kokot et al., 1990).

Diagnosis includes clinical assessment and serum tests, and urine examination includes dipstick urinalysis, urine eosinophils, and urine microscopy. Imaging tests, such as kidney ultrasonography/computed tomography (CT) scan, gallium-scintigraphy, and fluorodeoxyglucose-positron emission tomography (FDG-PET) scan, offer limited benefits (Perazella, 2017). Unfortunately, none of these allows a definitive diagnosis, making kidney biopsy a real necessity to accurately diagnose and manage AIN and CIN.

**Treatment.** AIN and CIN treatment include immediate halting of using suspicious drugs, control of infection, and the primary disease (Perazella, 2017; González et al., 2008). Hemodialysis or plasmapheresis can be used as supportive care. Tubulointerstitial diseases are a relatively common cause of both acute and CKDs. The extent of tubulointerstitial fibrosis is closely linked to kidney function and is an important predictor of kidney functional recovery.

**Autoimmune responses (allograft rejection) in kidney transplantation.** Allograft rejection is classified into three major categories: hyperacute, acute, and chronic (Haas, 2014).

**Hyperacute rejection.** Begins immediately after perfusion, the diagnosis is confirmed by an intraoperative biopsy of the kidney that shows polymorphonuclear leukocytes in the glomeruli and PTCs with widespread vascular thrombosis (Boehmig et al., 1971). This type of rejection results from preformed cytotoxic antibodies reactive to endothelial cells of the graft.

**Treatment.** The treatment of hyperacute rejection is disappointing and is generally associated with graft loss.

**Acute rejection.** It occurs in 10%–40% of allografts within the first year after transplantation (Boehmig et al., 1971). With the current immunosuppression, the only presenting sign is a rise in the serum creatinine level. Allograft biopsy is an effective method of diagnosis. Approximately 90% of acute rejections are cell-mediated

and are characterized by lymphocytic invasion of the interstitial and tubular epithelium. Ten percent of acute rejections show evidence of antibody-mediated rejection, characterized by complement deposition (C4d) in the PTCs. Vascular rejection signifies arterial inflammation and can result from either cell-mediated or antibody-mediated rejection (Haas, 2014). Acute rejection within the first year after transplantation can be successfully reversed approximately 80% of the time.

*Treatment.* Acute cellular rejection is treated by high-dose methylprednisolone, antilymphocytic antibodies, or increasing the dosage of calcineurin inhibitor medications. For antibody-mediated acute rejection, most clinicians favor the rapid removal of the circulating antibodies by plasmapheresis and the neutralization of the remaining antibodies with intravenous Ig.

*Chronic rejection.* This is identified in most renal transplants after a few years. Renal allograft biopsies show the evidence of accelerated atherosclerosis, interstitial fibrosis, and tubular atrophy (Orandi et al., 2016). Both antigen-dependent and antigen-independent mechanisms of vascular injury are implicated. The serum creatinine concentration slowly "creeps" up until the allograft fails. Although immune factors including acute rejection play a role in the development of chronic rejection, chronic allograft nephropathy more accurately describes the multi-factorial nature of this disease. Hypercholesterolemia, hypertension, immunosuppression nephrotoxicity, ischemia-reperfusion injury, and infection have all been associated with the same pathologic findings.

*Treatment.* Many clinical trials are now being designed to determine whether this process can be stopped or prevented. Increasing immunosuppressive treatment is usually not advised.

## SUMMARY

Autoimmune and alloimmune diseases of the kidney are common, serious, and increasingly treatable because of advances in basic and translational research. Further benefits will come from the application of novel technologies and approaches such as precision medicine, population-based genetics, and better experimental models.

## References

- Anders, H., Schlondorff, D., 2000. Murine models of renal disease: possibilities and problems in studies using mutant mice. *Exp. Nephrol.* 8, 181–193.
- Anders, H.J., Fogo, A.B., 2014. Immunopathology of lupus nephritis. *Semin. Immunopathol.* 36, 443–459.
- Andrews, B.S., Eisenberg, R.A., Theofilopoulos, A.N., Izui, S., Wilson, C.B., McConahey, P.J., et al., 1978. Spontaneous murine lupus-like syndromes. Clinical and immunopathological manifestations in several strains. *J. Exp. Med.* 148, 1198–1215.
- Badr, G., Sayed, A., Abdel-Maksoud, M.A., Mohamed, A.O., El-Amir, A., Abdel-Ghaffar, F.A., et al., 2015. Infection of female BWF1 lupus mice with malaria parasite attenuates B cell autoreactivity by modulating the CXCL12/CXCR4 axis and its downstream signals PI3K/AKT, NFκB and ERK. *PLoS One* 10, e0125340.
- Barbour, T.D., Pickering, M.C., Terence Cook, H., 2013. Dense deposit disease and C3 glomerulopathy. *Semin. Nephrol.* 33, 493–507.
- Barisoni L., 2015. LJAAaDBT. Introduction to Renal Biopsy. *Genitourinary Pathology: Foundations in Diagnostic Pathology*, second ed, vol. 7, pp. 378–455.
- Basile, D.P., Anderson, M.D., Sutton, T.A., 2012. Pathophysiology of acute kidney injury. *Compr. Physiol.* 2, 1303–1353.
- Bastian, H.M., Roseman, J.M., McGwin Jr, G., Alarcon, G.S., Friedman, A.W., Fessler, B.J., et al., 2002. Systemic lupus erythematosus in three ethnic groups. XII. Risk factors for lupus nephritis after diagnosis. *Lupus* 11, 152–160.
- Beck, L.H., 2015. Lessons from a rare disease: IgG subclass and disease severity in alloimmune antenatal membranous nephropathy. *Kidney Int.* 87, 494–497.
- Beck Jr, L.H., Salant, D.J., 2014. Membranous nephropathy: from models to man. *J. Clin. Invest.* 124, 2307–2314.
- Beck, J.A., Lloyd, S., Hafezparast, M., Lennon-Pierce, M., Eppig, J.T., Festing, M.F., et al., 2000. Genealogies of mouse inbred strains. *Nat. Genet.* 24, 23–25.
- Bielschowsky, M., Bielschowsky, F., 1964. Observation on NZB/B1 mice; differential fertility in reciprocal crosses and the transmission of the auto-immune haemolytic anaemia to NZB/B1 X NZC/B1 hybrids. *Aust. J. Exp. Biol. Med. Sci.* 42, 561–568.
- Bigazzi, P.E., 1999. Metals and kidney autoimmunity. *Environ. Health Perspect.* 107 (Suppl 5), 753–765.
- Blanco, P., Ueno, H., Schmitt, N., 2016. T follicular helper (Tfh) cells in lupus: activation and involvement in SLE pathogenesis. *Eur. J. Immunol.* 46, 281–290.
- Boehmig, H.J., Giles, G.R., Amemiya, H., Wilson, C.B., Coburg, A.J., Genton, E., et al., 1971. Hyperacute rejection of renal homografts: with particular reference to coagulation changes, humoral antibodies, and formed blood elements. *Transplant. Proc.* 3, 1105–1117.
- Bombard, A.S., 2017. After 4 decades of lupus nephritis trials, is there a "best" therapy? *Am. J. Kidney Dis.* 70, 309–310.
- Burne-Taney, M.J., Kofler, J., Yokota, N., Weisfeldt, M., Traystman, R.J., Rabb, H., 2003. Acute renal failure after whole body ischemia is characterized by inflammation and T cell-mediated injury. *Am. J. Physiol. Renal. Physiol.* 285, F87–F94.
- Chen, S., Tang, Z., Xiang, H., Li, X., Chen, H., Zhang, H., et al., 2016. Etiology and outcome of crescentic glomerulonephritis from a single center in China: a 10-year review. *Am. J. Kidney Dis.* 67, 376–383.
- Chen, L., Morris, D.L., Vyse, T.J., 2017. Genetic advances in systemic lupus erythematosus: an update. *Curr. Opin. Rheumatol.* 29, 423–433.

- Cortazar, F.B., Stone, J.H., 2015. IgG4-related disease and the kidney. *Nat. Rev. Nephrol.* 11, 599–609.
- Crotty, S., 2014. T follicular helper cell differentiation, function, and roles in disease. *Immunity* 41, 529–542.
- Cui, Z., Zhao, M.H., 2011. Advances in human antiglomerular basement membrane disease. *Nat. Rev. Nephrol.* 7, 697–705.
- Cunningham, M.W., 2000. Pathogenesis of group A streptococcal infections. *Clin. Microbiol. Rev.* 13, 470–511.
- Deng, J., Kohda, Y., Chiao, H., Wang, Y., Hu, X., Hewitt, S.M., et al., 2001. Interleukin-10 inhibits ischemic and cisplatin-induced acute renal injury. *Kidney Int.* 60, 2118–2128.
- Dische, F.E., Swinson, D.R., Hamilton, E.B., Parsons, V., 1984. Immunopathology of penicillamine-induced glomerular disease. *J. Rheumatol.* 11, 584–585.
- Donaghy, M., Rees, A.J., 1983. Cigarette smoking and lung haemorrhage in glomerulonephritis caused by autoantibodies to glomerular basement membrane. *Lancet* 2, 1390–1393.
- El Nahas, A.M., 1993. Masugi nephritis: a model for all seasons. In: *Experimental and Genetic Rat Models of Chronic Renal Failure*. Basel, Karger, pp. 49–67.
- Esmaeili, S.-A., Mahmoudi, M., Momtazi, A.A., Sahebkar, A., Doulabi, H., Rastin, M., 2017. Tolerogenic probiotics: potential immunoregulators in systemic lupus erythematosus. *J. Cell. Physiol.* 232, 1994–2007.
- Falk, P.H.N.J.C.J.R.J., 2008. Primary Glomerular Disease – Brenner and Rector's The Kidney, eighth ed. Chapter 30, pp. 1024–1032.
- Farr, L.E., Smadel, J.E., 1939. The effect of dietary protein on the course of nephrotoxic nephritis in rats. *J. Exp. Med.* 70, 615–627.
- Foster, M.H., 2016. Optimizing the translational value of animal models of glomerulonephritis: insights from recent murine prototypes. *Am. J. Physiol. Renal. Physiol.* 311, F487–F495.
- Free, M.E., Bunch, D.O., McGregor, J.A., Jones, B.E., Berg, E.A., Hogan, S.L., et al., 2013. Patients with antineutrophil cytoplasmic antibody-associated vasculitis have defective Treg cell function exacerbated by the presence of a suppression-resistant effector cell population. *Arthritis Rheum.* 65, 1922–1933.
- Gavalchin, J., Nicklas, J.A., Eastcott, J.W., Madaio, M.P., Stollar, B.D., Schwartz, R.S., et al., 1985. Lupus prone (SWR x NZB)F1 mice produce potentially nephritogenic autoantibodies inherited from the normal SWR parent. *J. Immunol.* 134, 885–894.
- Genovese, G., Tonna, S.J., Knob, A.U., Appel, G.B., Katz, A., Bernhardy, A.J., et al., 2010. A risk allele for focal segmental glomerulosclerosis in African Americans is located within a region containing APOL1 and MYH9. *Kidney Int.* 78, 698–704.
- Gerald, B., Appel, J.R., D'Agati, V., 2008. Secondary Glomerular Disease Brenner and Rector's The Kidney, eighth ed. pdf, pp. 1067–1146.
- Gnemmi, V., Verine, J., Vrigneaud, L., Glowacki, F., Ratsimbazafy, A., Copin, M.C., et al., 2015. Microvascular inflammation and acute tubular necrosis are major histologic features of hantavirus nephropathy. *Hum. Pathol.* 46, 827–835.
- Golert, W.A., Appel, G.B., Hariharan, S., 2008. Recurrent glomerulonephritis after renal transplantation: an unsolved problem. *Clin. J. Am. Soc. Nephrol.* 3, 800–807.
- González, E., Gutiérrez, E., Galeano, C., Chevia, C., de Sequera, P., Bernis, C., et al., 2008. Early steroid treatment improves the recovery of renal function in patients with drug-induced acute interstitial nephritis. *Kidney Int.* 73, 940–946.
- Gualtierotti, R., Biggioggero, M., Penatti, A.E., Meroni, P.L., 2010. Updating on the pathogenesis of systemic lupus erythematosus. *Autoimmun. Rev.* 10, 3–7.
- Guillevin, L., Dorner, T., 2007. Vasculitis: mechanisms involved and clinical manifestations. *Arthritis Res. Ther.* 9 (Suppl 2), S9.
- Gupta, A., Quigg, R.J., 2015. Glomerular diseases associated with hepatitis B and C. *Adv. Chronic Kidney Dis.* 22, 343–351.
- Haas, M., 2014. An updated Banff schema for diagnosis of antibody-mediated rejection in renal allografts. *Curr. Opin. Organ Transplant.* 19, 315–322.
- Han, Y., Guo, H., Cai, M., Xiao, L., Wang, Q., Xu, X., et al., 2015. Renal graft biopsy assists diagnosis and treatment of renal allograft dysfunction after kidney transplantation: a report of 106 cases. *Int. J. Clin. Exp. Med.* 8, 4703–4707.
- Harley, J.B., Alarcon-Riquelme, M.E., Criswell, L.A., Jacob, C.O., Kimberly, R.P., Moser, K.L., et al., 2008. Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in ITGAM, PXK, KIAA1542 and other loci. *Nat. Genet.* 40, 204–210.
- Hellmark, T., Segelmark, M., 2014. Diagnosis and classification of Goodpasture's disease (anti-GBM). *J. Autoimmun.* 48–49, 108–112.
- Hooke, D.H., Gee, D.C., Atkins, R.C., 1987. Leukocyte analysis using monoclonal antibodies in human glomerulonephritis. *Kidney Int.* 31, 964–972.
- Hull, R.P., Goldsmith, D.J.A., 2008. Nephrotic syndrome in adults. *Br. Med. J.* 336, 1185–1189.
- Imai, H., Nakamoto, Y., Asakura, K., Miki, K., Yasuda, T., Miura, A.B., 1985. Spontaneous glomerular IgA deposition in ddY mice: an animal model of IgA nephritis. *Kidney Int.* 27, 756–761.
- Jang, H.R., Rabb, H., 2015. Immune cells in experimental acute kidney injury. *Nat. Rev. Nephrol.* 11, 88–101.
- Jennette, J.C., Nachman, P.H., 2017. ANCA glomerulonephritis and vasculitis. *Clin. J. Am. Soc. Nephrol.* 12, 1680–1691.
- Johnson, B.M., Gaudreau, M.C., Al-Gadban, M.M., Gudi, R., Vasu, C., 2015. Impact of dietary deviation on disease progression and gut microbiome composition in lupus-prone SNF1 mice. *Clin. Exp. Immunol.* 181, 323–337.
- Jokiranta, T.S., 2017. HUS and atypical HUS. *Blood* 129, 2847–2856.
- Kokot, F., Grzeszczak, W., Zukowska-Szczechowska, E., Wiecek, A., 1990. Endocrine alterations in kidney transplant patients. *Blood Purif.* 8, 76–86.
- Krause, R., 2013. Vitamin D and UV exposure in chronic kidney disease. *Dermato-endocrinology* 5, 109–116.
- Krawczak, K., Donskow-Lyoniewska, K., Doligalska, M., 2017. Regulatory function of parasites in autoimmune disease—outcome from experimental model infection. *Ann. Parasitol.* 63, 7–14.
- Li, W., Titov, A.A., Morel, L., 2017. An update on lupus animal models. *Curr. Opin. Rheumatol.* 29, 434–441.
- Lizarraga, K.J., Florindez, J.A., Daftarian, P., Andrews, D.M., Ortega, L.M., Mendoza, J.M., et al., 2015. Anti-GBM disease and ANCA during dengue infection. *Clin. Nephrol.* 83, 104–110.
- Mackay, I.R., 2010. Travels and travails of autoimmunity: a historical journey from discovery to rediscovery. *Autoimmun. Rev.* 9, A251–A258.
- Magistroni, R., D'Agati, V.D., Appel, G.B., Kiryluk, K., 2015. New developments in the genetics, pathogenesis, and therapy of IgA nephropathy. *Kidney Int.* 88, 974–989.

- Mao, L., Hou, H., Wu, S., Zhou, Y., Wang, J., Yu, J., et al., 2017. TIGIT signalling pathway negatively regulates CD4(+) T-cell responses in systemic lupus erythematosus. *Immunology* 151, 280–290.
- McAdoo, S.P., Pusey, C.D., 2017. Anti-glomerular basement membrane disease. *Clin. J. Am. Soc. Nephrol.* 12, 1162–1172.
- Medjeral-Thomas, N.R., O'Shaughnessy, M.M., O'Regan, J.A., Traynor, C., Flanagan, M., Wong, L., et al., 2014. C3 glomerulopathy: clinicopathologic features and predictors of outcome. *Clin. J. Am. Soc. Nephrol.* 9, 46–53.
- Misra, R., Gupta, R., 2015. Biomarkers in lupus nephritis. *Int. J. Rheum. Dis.* 18, 219–232.
- Moroni, G., Ponticelli, C., 2014. Rapidly progressive crescentic glomerulonephritis: early treatment is a must. *Autoimmun. Rev.* 13, 723–729.
- Muriithi, A.K., Leung, N., Valeri, A.M., Cornell, L.D., Sethi, S., Fidler, M.E., et al., 2014. Biopsy-proven acute interstitial nephritis, 1993–2011: a case series. *Am. J. Kidney Dis.* 64, 558–566.
- Navarra, S.V., Guzman, R.M., Gallacher, A.E., Hall, S., Levy, R.A., Jimenez, R.E., et al., 2011. Efficacy and safety of belimumab in patients with active systemic lupus erythematosus: a randomised, placebo-controlled, phase 3 trial. *Lancet* 377, 721–731.
- Nebuloni, M., Barbiano di Belgioioso, G., Genderini, A., Tosoni, A., Riani, L.N., et al., 2009. Glomerular lesions in HIV-positive patients: a 20-year biopsy experience from Northern Italy. *Clin. Nephrol.* 72, 38–45.
- Ngo, S.T., Steyn, F.J., McCombe, P.A., 2014. Gender differences in autoimmune disease. *Front. Neuroendocrinol.* 35, 347–369.
- Nilsson, U.R., Mueller-Eberhard, H.J., 1965. Isolation of beta Ig-globulin from human serum and its characterization as the fifth component of complement. *J. Exp. Med.* 122, 277–298.
- Ohashi, N., Sugiura, T., Isozaki, T., Yamamoto, T., Hishida, A., 2003. Anti-glomerular basement membrane antibody-induced glomerulonephritis with periglomerular granulomatous reaction and massive renal eosinophilic infiltration. *Am. J. Kidney Dis.* 42, E28–E35.
- Okazaki, K., Suzuki, Y., Otsuji, M., Suzuki, H., Kihara, M., Kajiyama, T., et al., 2012. Development of a model of early-onset IgA nephropathy. *J. Am. Soc. Nephrol.: JASN* 23, 1364–1374.
- Orandi, B.J., Luo, X., Massie, A.B., Garonzik-Wang, J.M., Lonze, B.E., Ahmed, R., et al., 2016. Survival benefit with kidney transplants from HLA-incompatible live donors. *N. Engl. J. Med.* 374, 940–950.
- O'Shaughnessy, M.M., Hogan, S.L., Poulton, C.J., Falk, R.J., Singh, H.K., Nickeleit, V., et al., 2017. Temporal and demographic trends in glomerular disease epidemiology in the Southeastern United States, 1986–2015. *Clin. J. Am. Soc. Nephrol.* 12, 614–623.
- Otomo, K., Koga, T., Mizui, M., Yoshida, N., Kriegel, C., Bickerton, S., et al., 2015. Cutting edge: nanogel-based delivery of an inhibitor of CaMK4 to CD4+ T cells suppresses experimental autoimmune encephalomyelitis and lupus-like disease in mice. *J. Immunol.* 195, 5533–5537.
- Peng, S.L., 2012. Experimental use of mouse models of systemic lupus erythematosus. *Methods Mol. Biol.* 900, 135–168.
- Perazella, M.A., 2017. Clinical approach to diagnosing acute and chronic tubulointerstitial disease. *Adv. Chronic Kidney Dis.* 24, 57–63.
- Perazella, M.A., Markowitz, G.S., 2010. Drug-induced acute interstitial nephritis. *Nat. Rev. Nephrol.* 6, 461–470.
- Perez-Valdivieso, J.R., Bes-Rastrollo, M., Monedero, P., de Irala, J., Lavilla, F.J., 2007. Prognosis and serum creatinine levels in acute renal failure at the time of nephrology consultation: an observational cohort study. *BMC Nephrol.* 8, 14.
- Petri, M., Orbai, A.M., Alarcon, G.S., Gordon, C., Merrill, J.T., Fortin, P.R., et al., 2012. Derivation and validation of the systemic lupus international collaborating clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum.* 64, 2677–2686.
- Phelps, R.G., Turner, A.N., Rees, A.J., 1996. Direct identification of naturally processed autoantigen-derived peptides bound to HLA-DR15. *J. Biol. Chem.* 271, 18549–18553.
- Pradhan, D., Pattnaik, N., Silowash, R., Mohanty, S.K., 2015. IgG4-related kidney disease—a review. *Pathol. Res. Pract.* 211, 707–711.
- Rabb, H., Griffin, M.D., McKay, D.B., Swaminathan, S., Pickkers, P., Rosner, M.H., et al., 2016. Inflammation in AKI: current understanding, key questions, and knowledge gaps. *J. Am. Soc. Nephrol.* 27, 371–379.
- Redakcja naukowa, A.G., Kwasnik, Z., Zukow, W., et al., 2009. Acute (Diffuse) Glomerulonephritis. *Pathology* 266–267.
- Relle, M., Weinmann-Menke, J., Scorletti, E., Cavagna, L., Schwarting, A., 2015. Genetics and novel aspects of therapies in systemic lupus erythematosus. *Autoimmun. Rev.* 14, 1005–1018.
- Reynolds, J., Mavromatis, K., Cashman, S.J., Evans, D.J., Pusey, C.D., 1998. Experimental autoimmune glomerulonephritis (EAG) induced by homologous and heterologous glomerular basement membrane in two substrains of Wistar-Kyoto rat. *Nephrol. Dial. Transplant.* 13, 44–52.
- Richter, J.G., Muth, T., Li, J., Brinks, R., Chehab, G., Koch, T., et al., 2018. Elevated psychosocial stress at work in patients with systemic lupus erythematosus and rheumatoid arthritis. *J. Rheumatol.* 45, 227–234.
- Roberts, I.S., 2014. Pathology of IgA nephropathy. *Nat. Rev. Nephrol.* 10, 445–454.
- Ronco, P., Debiec, H., 2015. Pathophysiological advances in membranous nephropathy: time for a shift in patient's care. *Lancet* 385, 1983–1992.
- Saxena, R., Mahajan, T., Mohan, C., 2011. Lupus nephritis: current update. *Arthritis Res. Ther.* 13, 240.
- Schaerli, P., Willimann, K., Lang, A.B., Lipp, M., Loetscher, P., Moser, B., 2000. CXCR chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. *J. Exp. Med.* 192, 1553–1562.
- Schwentker, F.F., Comploier, F.C., 1939. The production of kidney antibodies by injection of hemologous kidney plus bacterial toxins. *J. Exp. Med.* 70, 223–230.
- Servais, H., Ortiz, A., Devuyst, O., Denamur, S., Tulkens, P.M., Mingeot-Leclercq, M.P., 2008. Renal cell apoptosis induced by nephrotoxic drugs: cellular and molecular mechanisms and potential approaches to modulation. *Apoptosis* 13, 11–32.
- Sethi, S., Haas, M., Markowitz, G.S., D'Agati, V.D., Rennke, H.G., Jennette, J.C., et al., 2016. Mayo clinic/renal pathology society consensus report on pathologic classification, diagnosis, and reporting of GN. *J. Am. Soc. Nephrol.* 27, 1278–1287.
- Silveira, P.A., Dombrowsky, J., Johnson, E., Chapman, H.D., Nemazee, D., Serreze, D.V., 2004. B cell selection defects underlie the development of diabetogenic APCs in nonobese diabetic mice. *J. Immunol.* 172, 5086–5094.
- Sim, J.J., Batech, M., Hever, A., Harrison, T.N., Avelar, T., Kanter, M.H., et al., 2016. Distribution of biopsy-proven presumed primary glomerulonephropathies in 2000–2011 among a racially and ethnically diverse US population. *Am. J. Kidney Dis.* 68, 533–544.
- Singh, N.P., Gulati, S., Garg, V., Beniwal, P., Garg, S., 2008. Nephrotic range proteinuria in c-ANCA-positive crescentic glomerulonephritis with linear immune deposits. *Indian J. Nephrol.* 18, 169–172.

- Sinico, R.A., Mezzina, N., Trezzi, B., Ghiggeri, G.M., Radice, A., 2016. Immunology of membranous nephropathy: from animal models to humans. *Clin. Exp. Immunol.* 183, 157–165.
- Steblay, R.W., 1962. Glomerulonephritis induced in sheep by injections of heterologous glomerular basement membrane and Freund's complete adjuvant. *J. Exp. Med.* 116, 253–272.
- Stevenson, A., Yaqoob, M., Mason, H., Pai, P., Bell, G.M., 1995. Biochemical markers of basement membrane disturbances and occupational exposure to hydrocarbons and mixed solvents. *Q. J. Med.* 88, 23–28.
- Syed, R., Rehman, A., Valecha, G., El-Sayegh, S., 2015. Pauci-immune crescentic glomerulonephritis: an ANCA-associated vasculitis. *Biomed Res. Int.* 2015, 402826.
- Tortajada, A., Yebenes, H., Abarregui-Garrido, C., Anter, J., Garcia-Fernandez, J.M., Martinez-Barricarte, R., Alba-Dominguez, M., et al., 2013. C3 glomerulopathy-associated CFHR1 mutation alters FHR oligomerization and complement regulation. *J. Clin. Invest.* 123, 2434–2446.
- Tsokos, G.C., 2011. Systemic lupus erythematosus. *N. Engl. J. Med.* 365, 2110–2121.
- Türker, H., Yel, M., 2014. Effects of ultraviolet radiation on mole rats kidney: a histopathologic and ultrastructural study. *J. Rad. Res. Appl. Sci.* 7, 182–187.
- Wang, Y.M., Lee, V.W., Wu, H., Harris, D.C., Alexander, S.I., 2015. Heymann nephritis in Lewis rats. *Curr. Protoc. Immunol.* 109 (1-6), 15.29.
- Wang, L., Song, J., Buggs, J., Wei, J., Wang, S., Zhang, J., et al., 2017. A new mouse model of hemorrhagic shock-induced acute kidney injury. *Am. J. Physiol. Renal. Physiol.* 312, F134–F142.
- Waters, S.T., Fu, S.M., Gaskin, F., Deshmukh, U.S., Sung, S.S., Kannapell, C.C., et al., 2001. NZM2328: a new mouse model of systemic lupus erythematosus with unique genetic susceptibility loci. *Clin. Immunol.* 100, 372–383.
- Wei, Q., Dong, Z., 2012. Mouse model of ischemic acute kidney injury: technical notes and tricks. *Am. J. Physiol. Renal. Physiol.* 303, F1487–F1494.
- Wong, E.K., Kavanagh, D., 2015. Anticomplement C5 therapy with eculizumab for the treatment of paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome. *Transl. Res.* 165, 306–320.
- Xenocostas, A., Jothy, S., Collins, B., Loertscher, R., Levy, M., 1999. Anti-glomerular basement membrane glomerulonephritis after extracorporeal shock wave lithotripsy. *Am. J. Kidney Dis.* 33, 128–132.
- Xiao, H., Heeringa, P., Hu, P., Liu, Z., Zhao, M., Aratani, Y., et al., 2002. Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. *J. Clin. Invest.* 110, 955–963.
- Xiao, H., Dairaghi, D.J., Powers, J.P., Ertl, L.S., Baumgart, T., Wang, Y., et al., 2014. C5a receptor (CD88) blockade protects against MPO-ANCA GN. *J. Am. Soc. Nephrol.* 25, 225–231.
- Yang, H.C., Zuo, Y., Fog, A.B., 2010. Models of chronic kidney disease. *Drug Discov. Today Dis. Models* 7, 13–19.
- Yildirim-Toruner, C., Diamond, B., 2011. Current and novel therapeutics in treatment of SLE. *J. Allergy Clin. Immunol.* 127, 303–314.
- Yung, S., Chan, T.M., 2008. Anti-DNA antibodies in the pathogenesis of lupus nephritis—the emerging mechanisms. *Autoimmun. Rev.* 7, 317–321.
- Zager, R.A., Johnson, A.C., Geballe, A., 2007. Gentamicin suppresses endotoxin-driven TNF-alpha production in human and mouse proximal tubule cells. *Am. J. Physiol. Renal. Physiol.* 293, F1373–F1380.

# Autoantibody Assays: Performance, Interpretation, and Standardization

Marvin J. Fritzler

Cumming School of Medicine, University of Calgary, Calgary, AB, Canada

## OUTLINE

Introduction	1369	Laboratory Reports, Electronic Medical Records, and Cost Analysis	1379
Spectrum of Autoantibodies	1373	Standardization and Quality Assurance	1381
Approaches to and Standardizing Autoantibody Testing	1374	Summary	1383
Clinical Interpretation and Application of Autoantibody Testing	1377	References	1383
Clinical Practice Guidelines	1378		

## INTRODUCTION

The history of autoantibodies (aab) dates back more than a century to Ehrlich's description of "horror autotoxicus" (Ehrlich, 1900) and the development of the Wassermann test for syphilis (Wassermann et al., 1906). Progress in the identification and detection of aab lagged from about 1900 to 1950, with the notable exception of organ-specific autoimmune diseases (reviewed in Conrad et al., 2017). In stark contrast, over the following 65 years, aab discoveries and novel testing platforms flourished and achieved prominence in both laboratory and clinical settings. The more recent advances in aab testing date to the seminal discovery of the lupus erythematosus (LE) cell in 1948 (Hargraves et al., 1948) and the introduction of the LE cell test (Conn, 1994). In the ensuing 20 years, a number of techniques such as indirect immunofluorescence (IIF), immunodiffusion (ID), hemagglutination, and complement fixation were developed and then refined in the following 20 years (reviewed in Olsen et al., 2017). IIF was described by Coons, Kaplan, and Weller in the early 1950s (Warde, 2011) and this immunoassay stands out as one of the few aab technologies that is still in wide use today, particularly as a screening test in the diagnosis of systemic autoimmune rheumatic diseases (SARD) (Agmon-Levin et al., 2014; Mahler et al., 2014a; Pisetsky, 2017).

In the years following the introduction of IIF, a variety of assay substrates were utilized, but cryopreserved sections of rodent organs became the mainstay for the first 25 years (Holborow et al., 1957; Kunkel and Tan, 1964; Beck, 1961). In the mid-1970s, human tissue culture cells such as HeLa and HEp-2 became the substrate of choice because they were superior to organ sections in the identification of many aab, primarily because these cells had larger nuclei, target antigens were expressed in various stages of the cell cycle, and the assay kits using these substrates were relatively economical to manufacture.

Despite the advantages of HEp-2 cells, the increased sensitivity of the IIF techniques using these cells became an issue of concern that lead to a study by the Serology Subcommittee of the International Union of Immunology Societies/World Health Organization/Arthritis Foundation (IUIS/WHO/AF) who recommended that adult sera should be screened at dilutions of 1/40 and 1/160 but that a cutoff of 1/160 was the most appropriate to achieve a balance of sensitivity and specificity for the diagnosis of SARD (Tan et al., 1997). This recommendation achieved limited acceptance because many laboratories preferred to screen at serum dilutions that provided the appropriate balance of sensitivity and specificity in their own clinical environment. In addition, many manufacturers “fine-tuned” their kits and components (i.e., secondary antibodies) so that a “one-size-fits-all” recommendation seemed inappropriate. Eventually, there was a move away from HEp-2 IIF screening techniques to higher throughput, enzyme-linked immunoassays (ELISA) and addressable laser bead immunoassays (ALBIA). Some clinicians expressed concerns that this trend was troubled by an unacceptable loss of diagnostic sensitivity and subsequent clinical errors. After deliberation, a study committee commissioned by the American College of Rheumatology declared that the antinuclear antibody (ANA) IIF test on HEp-2 cell substrates should continue to be regarded the “gold standard” for the detection of ANA (Meroni and Schur, 2010; American College of Rheumatology, 2011; Fritzler, 2016). Nevertheless, following this “declaration,” there was little evidence that the majority of high-throughput laboratories reverted to the IIF test. Furthermore, with continuous improvement of ELISA and ALBIA, there is mounting evidence that these high-throughput screening assays now outperform the ANA IIF test (Pisetsky, 2017; Pérez et al., 2018). In this context, it is important to emphasize that there is no evidence-based consensus on approaches to or interpretation of aab testing by IIF (or any diagnostic technology) in children, although key observations are emerging (Mahler and Fritzler, 2014).

The use of HEp-2 cell substrates to detect aab in SARD ushered in the “golden age” of aab discovery (Fritzler, 2012) leading to the identification of novel intracellular autoantigen targets but, equally important, provided cell and molecular biologists with valuable probes for studies of novel cell structures and macromolecules (Tan, 1989; Backes et al., 2011) such as anti-proliferating cell nuclear antigen (PCNA) and other cell cycle targets (Mahler et al., 2010) and centromere (Fritzler et al., 2010), as well as targets in the nucleoli (Reimer et al., 1987; Welting et al., 2003), nuclear envelope (Enarson et al., 2004), and cytoplasm (Stinton et al., 2004; Fritzler et al., 2007). The identification of these novel targets and their disease relevance occurred in parallel with emerging technologies, such as immunoblotting (IB), expression cloning, and rapid and economical DNA sequencing.

The remarkable advances in identifying and cataloguing the molecular targets of aab lead to a new generation of technology platforms such as ELISA (Halbert et al., 1981; Reichlin and Harley, 1986; Tonutti et al., 2004), dot blots (Stott, 1989; Nezlin and Mozes, 1995), line immunoassays (LIA) (Pottel et al., 2004; Damoiseaux et al., 2005), multiplexed immunoassays such as ALBIA (Fritzler and Fritzler, 2009) and chemiluminescence (CLA) (Mahler et al., 2011b) (Table 69.1). Some of these newer technologies are gaining favor by modern diagnostic laboratories because they are automated, amenable to high throughput and shortened turnaround times, can be linked to elaborate and secure laboratory information systems and the patient’s electronic medical record (EMR), all resulting in reduced reporting errors and considerable laboratory cost-savings. Other emerging but as yet not widely adopted technologies include lateral flow (Renger et al., 2010), antigen arrays on planar surfaces (Baker et al., 2004; Balboni et al., 2008; Chandra et al., 2011), nanobarcodes (Freeman et al., 2005), and electrochemical sensors (Rubin et al., 2014; Konstantinov and Rubin, 2017). These technology platforms seem to be more suited to point of care diagnostics (Fritzler, 2015; Rubin and Konstantinov, 2016; Dincer et al., 2017; Joh et al., 2017), a field that has been slow to be adopted in autoimmune diseases.

The continued use of IIF has advantages, but the limitations of this technology in the context of ever changing clinical algorithms should not be overlooked (Pisetsky, 2017; Fritzler, 2012, 2016). It has become clear that the results from any immunoassay can be at considerable variance with the other. This has prompted concerns about the relative value of old and new diagnostic platforms where the implications of false-negative and false-positive test results are debated (Fritzler, 2011c, 2016). Many clinicians prefer to adhere to assay results that are easily understood, fit within existing diagnostic paradigms, and are clinically relevant. Hence, some prefer test results derived from IIF on specified substrates (i.e., HEp-2) and regard them as the “gold standard” for aab testing (Meroni and Schur, 2010; Satoh et al., 2009).

One of the arguments supporting continued use of IIF on HEp-2 substrates is that this substrate is essentially a mini-array containing more than 100 different target antigens whereas newer screening technologies such as ELISA, LIA, ALBIA, or CLA typically are configured to detect less than 20 different aab targets (Meroni and Schur, 2010; American College of Rheumatology, 2011). While the claim that numerous target autoantigens are expressed in HEp-2 cells is true from a molecular biology perspective, it should not be taken to mean that they are necessarily detectable by IIF on cells that are stabilized with a variety of fixatives, especially if certain

**TABLE 69.1** Contemporary and Emerging Technologies Used to Detect Autoantibodies in Human Sera

Assay	Essential technology	Applications
IIF	<ul style="list-style-type: none"> <li>Tissue or cell substrates</li> <li>Fluorochrome-labeled secondary antibodies</li> <li>Microscope fitted with UV source and optics</li> </ul>	<ul style="list-style-type: none"> <li>Screening test</li> <li>Specific aab detection by staining pattern</li> </ul>
LIA	<ul style="list-style-type: none"> <li>Native or recombinant antigens on solid phase substrate</li> <li>Enzyme-labeled secondary antibodies</li> <li>Densitometry/Scanner</li> </ul>	<ul style="list-style-type: none"> <li>Test aab reactivity to specific targets</li> <li>Arrays based on disease groups</li> </ul>
ALBIA	<ul style="list-style-type: none"> <li>Antigen of interest bound to addressable microbeads</li> <li>Dual laser flow (Luminex)</li> </ul>	<ul style="list-style-type: none"> <li>Test aab reactivity to specific targets</li> <li>Arrays based on disease groups</li> </ul>
CBA	<ul style="list-style-type: none"> <li>Cell lines transfected with and express cDNA encoding antigen of interest</li> <li>IIF protocols</li> </ul>	<ul style="list-style-type: none"> <li>Detect aab directed to antigens of low expression</li> <li>Detect aab directed to highly conformational epitopes</li> </ul>
CLA	<ul style="list-style-type: none"> <li>Antigen of interest bound to addressable microbeads</li> <li>Fluorochrome-labeled secondary antibodies</li> <li>Chemiluminescence</li> <li>Laser flow technology (Bio Flash)</li> </ul>	<ul style="list-style-type: none"> <li>Test aab reactivity to specific targets</li> <li>Arrays based on disease groups</li> </ul>
Lateral flow assays	<ul style="list-style-type: none"> <li>Similar to LIA but two- or three-step protocol</li> <li>Line array enclosed in a portable cassette</li> <li>Small handheld densitometer</li> </ul>	<ul style="list-style-type: none"> <li>Point-of-care diagnostics</li> </ul>
<sup>a</sup> Antigen arrays on planar surfaces	<ul style="list-style-type: none"> <li>High-density autoantigen arrays printed on glass or other matrices</li> </ul>	<ul style="list-style-type: none"> <li>Personalized medicine</li> </ul>
<sup>a</sup> Microfluidics or “lab on a chip”	<ul style="list-style-type: none"> <li>Portable microdevices with all components embedded</li> </ul>	<ul style="list-style-type: none"> <li>Point-of-care diagnostics</li> </ul>
<sup>a</sup> Electrochemiluminescence arrays	<ul style="list-style-type: none"> <li>Antigen arrays on solid phase</li> <li>Specialized detection systems</li> </ul>	<ul style="list-style-type: none"> <li>Personalized medicine</li> </ul>
<sup>a</sup> Nanotechnology	<ul style="list-style-type: none"> <li>High-density autoantigen arrays (“nanobarcodes”) printed or absorbed to nanoscale devices</li> </ul>	<ul style="list-style-type: none"> <li>Personalized medicine</li> <li>Point-of-care diagnostics</li> </ul>
<sup>a</sup> Mass and NMR spectroscopy	<ul style="list-style-type: none"> <li>Early development depends on decreasing footprint and cost of technology</li> </ul>	<ul style="list-style-type: none"> <li>Personalized medicine</li> <li>High-throughput diagnostics</li> </ul>

<sup>a</sup>Not in wide use, in developmental stage.

aab, Autoantibodies; IIF, indirect immunofluorescence; LIA, line immunoassay; NMR, nuclear magnetic resonance; ALBIA, addressable laser bead immunoassays; CBA, cell-based assays; CLA, chemiluminescence assay.

conformational epitopes are required for aab binding. Indeed, there is substantial evidence that IIF does not detect all aab in a given human sera even when they are directed to an autoantigen that is expressed in HEp-2 cells (Fritzler, 2016). For example, a significant proportion of sera that have aab directed to Jo-1 (histidyl tRNA synthetase) (Suzuki et al., 2014), ribosomal P proteins (Mahler et al., 2004; Somani et al., 2008), PCNA (Mahler et al., 2010), GW Bodies (Stinton et al., 2010), SSA/Ro60 (Op De Beeck et al., 2011), Ro52/TRIM21 (Peene et al., 2002; Schulte-Pelkum et al., 2009), and PM/Scl (Mahler and Fritzler, 2009) (to name a few) detected by ELISA, ALBIA, LIA, and CLA are often not detected by IIF on conventional HEp-2 substrates. It has been suggested that these “false-negative” IIF results are attributable to low aab titers, hidden or cryptic epitopes, technical features of antihuman secondary antibodies, and/or characteristics of the substrate (i.e., cell density, growth media, and fixation protocols). However, evidence to support any of these contentions has not been thoroughly studied or proven.

Further limitations of IIF testing are highlighted by the evidence that when five different manufacturer substrates and a characterized set of SARD sera were used and then read and interpreted by the same cadre of technologists, the agreement of test results was only 78% (Copple et al., 2012). And within the specific groups of serum samples, agreement ranged from only 44% for systemic sclerosis (SSc) samples to 72% agreement for the systemic LE (SLE) sera but, reassuringly, 93% for healthy control sera. In this particular study, variations in slide and substrate quality (i.e., clarity, consistency of fluorescence, cell size, number, and quality of mitotic cells) from different manufacturers were also noted. Therefore, along with problems of subjective interpretation, IIF on

**TABLE 69.2** Autoantibodies and Related Technology Platforms Used in Diagnosis of Autoimmune Diseases

Autoantibody	Primary disease associations	Commercial immunoassay
dsDNA	SLE DIL <sup>a</sup>	IIF, ELISA, IP, ALBIA, CLA
Sm (U2-U4-6 RNP)	SLE	ELISA, ID, IP, ALBIA, LIA, CLA
U1RNP	MCTD SLE	ELISA, ID, IP, ALBIA, LIA, CLA
SS-A/Ro 60	SjS SLE	ELISA, ID, IP, ALBIA, LIA, CLA
SS-B/La	SjS SLE	ELISA, ID, IP, ALBIA, LIA, CLA
Ro52/TRIM21	SARD	ELISA, LIA, ALBIA, CLA
Histone/Chromatin/Nucleosome	SLE/DIL	ELISA, IB, ALBIA, LIA, CLA
Topoisomerase I/Scl-70	SSc	ELISA, IB, IP, ALBIA, LIA, CLA
U3 RNP/Fibrillarin	SSc	IIF, IP, LIA
CENP-B	SSc	IIF, ELISA, ALBIA, LIA, CLA
Th/To	SSc	ELISA, LIA, CLA
Jo-1/Histidyl tRNA synthetase, other tRNA synthetases (OJ, PL-7, PL-12, etc.)	PM/Synthetase syndrome	ELISA, IB, IP, ALBIA, LIA, CLA
SRP	NAM	LIA, ELISA, ALBIA
HMGCR	NAM	CLA, ELISA, LIA
MDA-5	DM	LIA, CLA
Mup44/NT5C1	sIBM	ELISA, ALBIA
Citrullinated peptides and carbamylated proteins	RA	ELISA
Proteinase 3 (cANCA)	GPA	ELISA, IB, LIA, CLA, LFA
Myeloperoxidase (pANCA)	MPA	ELISA, IIF, CLA
Pyruvate dehydrogenase complex (M2)	PBC	IIF, IB, ELISA, LIA, CLA
Smooth muscle	Chronic active hepatitis	IIF, ELISA
F-actin		
Intrinsic factor	Pernicious anemia	IIF, ELISA
Human tissue transglutaminase	Celiac disease	IIF, ELISA
Cardiolipin	APS	ELISA
β2-Glycoprotein I (domain 1)	APS	ELISA
PS/PT	APS	ELISA
Basement membrane (α4 domain of type IV collagen)	Anti-GBM disease (renal–pulmonary syndrome)	IIF, ELISA, LFA
Acetylcholine receptor	Myasthenia gravis	ELISA
Thyroid microsomes (thyroid peroxidase)	Hashimoto's thyroiditis	IIF, ELISA
Cadherins	Pemphigus vulgaris	ELISA
Skin basement membrane zone	Bullous pemphigoid	ELISA
Yo/Purkinje cell	PCD	IIF, LIA
Hu	PEM	IIF, IB, IP, LIA

(Continued)

**TABLE 69.2** (Continued)

Autoantibody	Primary disease associations	Commercial immunoassay
AQP4	NMOSD/Devic's disease	CBA, ELISA
NMDA/NR1 receptor	Autoimmune encephalitis	CBA, ELISA
PLA2R	IMN	ELISA, CBA, ALBIA
THSD-17	IMN	CBA, ELISA
ADAMTS13	Autoimmune thrombocytopenia, atypical hemolytic uremic syndrome	ELISA

<sup>a</sup>Some patients treated with anti-TNF and other biological therapeutics develop features of drug-induced lupus, anti-dsDNA and other aab.

ADAMTS, A disintegrin and metalloproteinase with thrombospondin motifs; ALBIA, addressable laser bead immunoassay; APS, antiphospholipid syndrome; CBA, cell-based assay; CENP, centromere protein; CLA, chemiluminescence assay; DIL, drug-induced lupus; DM, dermatomyositis; ELISA, enzyme-linked immunoassay; GBM, glomerular basement membrane; GPA, granulomatosis with polyangiitis—formerly Wegener's syndrome; IIF, indirect immunofluorescence; IMN, idiopathic membranous nephritis; IP, immunoprecipitation; LFA, lateral flow assay; LIA, line immunoassay; MCTD, mixed connective tissue disease; MPA, microscopic polyangiitis; MSR: more studies required; NAM, necrotizing autoimmune myopathy; NMDAR, N-methyl-D-aspartate (glutamate) receptor; NMOSD, neuromyelitis optica spectrum disorders; PBC, primary biliary cirrhosis; PCD, paraneoplastic cerebellar degeneration; PEM, paraneoplastic encephalomyelitis; PLA2R, phospholipase A2 receptor; PM, polymyositis; PS/PT, phosphatidyl serine/prothrombin complex; RNP, ribonucleoprotein; SARD, systemic autoimmune rheumatic diseases; sIBM, sporadic inclusion body myopathy; SjS, Sjögren's syndrome; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; Th/To, mitochondrial RNase P complex comprising hPOP1, Rpp25, Rpp38; THSD-17, Thrombospondin type-1 domain containing 7A; TRIM, tripartite motif AQP4, aquaporin 4.

HEp-2 substrates is subject to problematic issues of intermanufacturer and interlaboratory assay standardization ([Copple et al., 2007](#)).

Some of the issues plaguing IIF assays, particularly subjective reader bias, are partially addressed through the development of technology platforms that provide automated, digital reading of IIF ([Mahler et al., 2014a](#); [Hiemann et al., 2009](#); [Egerer et al., 2010](#)). These technologies have advantages of machine-learning algorithms and archival of digital images of the IIF result for subsequent review. In addition, some of these platforms require only a single serum dilution to derive a digital end point titer, resulting in significant cost savings ([Copple et al., 2014](#)). However, a current limitation of this technology is an inability to correctly classify complex IIF patterns in sera where multiple or less common aab are present.

At this juncture, it is important to refer to the emergence of cell-based IIF assays (CBA) that have taken a significant place in the repertoire of aab techniques ([Table 69.1](#)). In the CBA, a cell host is chosen and transfected with the cDNA of the desired antigen target and after the cDNA is overexpressed, the cells are stabilized and then used in a conventional IIF protocol to detect antibodies to the desired target. Untransfected cells or cells transfected with an irrelevant cDNA serve as controls. This approach has been particularly useful to produce a substrate to detect antibodies directed to targets in low abundance (i.e., SS-A/Ro60) ([Keech et al., 1996](#)) and/or highly conformational (i.e., PLA2R, NMDAR1 AQP4) targets ([Table 69.2](#)) ([Beck et al., 2009](#); [Gerstein et al., 2014](#); [Dalmau, 2016](#)).

In summary, the rapid proliferation of aab specificities along with the emergence and adoption of novel technologies has created a dilemma for standardization of aab testing protocols and the results that are communicated to clinicians ([Fritzler et al., 2003a](#); [Wiik et al., 2004](#)). Rapid advances in diagnostic technologies have made it difficult for even the most modern laboratory to keep abreast of the changes, not to mention clinicians who are hard pressed to keep abreast of new diagnostic paradigms that follow the adoption of the newer technologies.

## SPECTRUM OF AUTOANTIBODIES

In a single chapter, it is a challenge to cover the spectrum of aab that are now regarded disease-specific or disease-related diagnostic and prognostic biomarkers. Selected aab and their primary disease associations are shown in [Table 69.2](#) to illustrate the spectrum of diagnostic technologies used to detect them ([Table 69.2](#)). The task of a more comprehensive discussion of disease-related aab has been left to authors of other chapters in this book and is also detailed in other publications ([Conrad et al., 2017](#); [Conrad et al., 2015](#)). Given the variation of observations with the same technology or between different technologies, the spectrum of aab and their associated sensitivity or specificity is typically wide and needs to be interpreted in the context of the clinician's own clinical and laboratory setting. As an example of the complexity of data sets, there are now well over 10 different

diagnostics platforms (Table 69.1) and in autoimmune conditions such as SLE there are now over 200 aab catalogued (Sherer et al., 2004; Sherer and Shoenfeld, 2007), over 30 in SSc (Mehra et al., 2013; Kayser and Fritzler, 2015), and a growing number in autoimmune inflammatory myopathies (Tansley et al., 2013; Casciola-Rosen and Mammen, 2012; Mahler et al., 2014b; Lundberg et al., 2016). In contrast to SARD, in most organ-localized autoimmune diseases, the aab are typically directed to only one or a few antigens that tend to be harbored in the affected organ (Conrad et al., 2017).

A challenge in performing and interpreting aab test results is related to the complexity of the B-cell repertoire and the limited ability of assays to capture a complete profile of different aab subclasses, isotypes, and posttranslational modifications of both antibodies and antigens. Detection of this aab spectrum is highly dependent on the secondary antibodies and the target antigens used in various immunoassays. Hence, the relative clinical value of IgG, IgM, and IgA aab is not always clearly defined. In addition, there is evidence that IgG1, IgG3, and various immunoglobulin glycosylation patterns (galactosylation, sialylation) have greater pathogenic significance in some clinical settings (Feehally, 2017). One of the relatively new arrivals on the aab scene is a class of autoimmune diseases referred to as IgG4-related diseases (IgG4-RD) that encompasses a variety of clinical entities once regarded as being separate diseases (Mahajan et al., 2014; Wolfson and Hamilos, 2017; Bozzalla and Stone, 2017). Clinical manifestations of IgG4-RD have been reported in virtually all organ systems where they display consistent histopathological similarities: diffuse lymphoplasmacytic infiltrates, abundant IgG4-positive plasma cells, modest tissue eosinophilia, and extensive fibrosis (Khosroshahi et al., 2011). Polyclonal elevations of serum IgG4 are found in approximately 70% of patients and in some cases IgG4 aab directed to specific targets are found. In general, the strength of aab associations with autoimmune disease subgroups can vary according to the diagnostic techniques being used but also varies in accord with demographic, environmental, and genetic factors (Fritzler, 2006; Whelan and Isenberg, 2009).

## APPROACHES TO AND STANDARDIZING AUTOANTIBODY TESTING

As mentioned above, the IIF technique using HEp-2 or other cellular substrates is currently regarded by some as the preferred screening immunoassay to detect aab, especially in SARD. However, it is also important to appreciate that a negative IIF screening test does not necessarily exclude the presence of a wide spectrum of aab in SARD (Fritzler, 2012, 2016; Mahler et al., 2007; Salazar et al., 2014). Therefore, in the setting of high clinical suspicion of a SARD or another autoimmune disease (i.e., autoimmune hepatitis, juvenile arthritis with uveitis) and a negative IIF test, the identification of aab should include a more specific and sensitive assay that includes an array of relevant autoantigens. These newer technologies (Olsen et al., 2017; Fritzler and Fritzler, 2009; Mahler et al., 2011a) are described in more detail below. In general, the approach to requesting and screening for clinically relevant aab should be highly dependent on the differential diagnosis (Table 69.3) and a selected few IIF patterns are highly associated with specific autoantigens and diseases (Table 69.4). For example, the IIF test is a reliable screening test if the diagnosis of SLE, SSc, mixed connective disease, autoimmune hepatitis, and uveitis in juvenile arthritis is being considered. In this clinical context, if the ANA IIF screening test is negative and the clinical suspicion is high (i.e., high pretest probability), multiplexed disease-related aab testing is recommended (Table 69.3). By comparison, IIF is not a reliable screening test for Sjögren's syndrome, autoimmune inflammatory myopathies, antiphospholipid syndrome, vasculopathies, autoimmune encephalopathies, or autoimmune kidney diseases, to name a few. In these conditions, disease-specific and disease-related multiplexed assays should be considered the initial screening test of choice.

Because the evolution of clinically recognizable SARD and other autoimmune conditions can span decades before a diagnosis is firmly established, long-term longitudinal studies are ideally required to reach definitive conclusions about aab specificities. In the setting of very early SARD (i.e., SSc, SLE), screening for disease-specific aab may be more clinically relevant than ANA IIF screening (Choi et al., 2016; Choi and Fritzler, 2016).

With the emergence of the more sensitive immunoassays, care must be taken to ensure that cutoffs are based on age- and gender-matched healthy and comparative disease controls (Fritzler et al., 2003a; Bossuyt et al., 2008; Shoenfeld et al., 2007). Also, the source and characteristics (i.e., recombinant vs native, peptide vs full length) of the autoantigen used in different immunoassays can affect the results. Despite years of substantial effort, standardization of aab assays continues to be a major challenge (Satoh et al., 2009; Mahler et al., 2011a; Kessenbrock et al., 2007; Mahler and Fritzler, 2010).

For routine aab detection, many laboratories rely on commercial aab assay kits that employ a variety of technologies such as IIF, ID, IP, IB, LIA, ELISA, ALBIA, and CLA (Olsen et al., 2017; Mahler et al., 2011b). In the

**TABLE 69.3** Screening Autoantibody Tests for Systemic Autoimmune Rheumatic and Related Diseases

Disease: differential diagnosis	Screening test	Comments
Systemic lupus erythematosus	IIF HEp-2	Sensitivity >80%. If serum has single specificity antiribosomal P, SSA/Ro60 or anti-Ro52/TRIM21 IIF often negative; some anti-dsDNA positive sera can have negative ANA
Systemic sclerosis	IIF HEp-2	Sensitivity >85%. Newer autoantibodies (i.e., BICD2) not detected by IIF
Mixed connective tissue disease	IIF-HEp-2	Sensitivity >90%
Rheumatoid arthritis	RA-specific testing	In juvenile RA/inflammatory arthritis, ANA IIF is an important biomarker for development of uveitis
Autoimmune inflammatory myopathies	AIM-related array(s)	Approximately 50% of anti-Jo-1 (histidyl tRNA synthetase) can have cytoplasmic staining by IIF
Sjögren's syndrome	SjS-related array	HEp-2000 (ImmunoConcepts) engineered to detect anti-Ro60 by IIF in transfected HEp-2 cells (sensitivity ~85%)
Vasculopathies	Vasculitis-related testing: ANCA, PR3, MPO	In special cases, testing for "atypical" ANCA may be considered (i.e., antielastase for levamisole/cocaine-related vasculopathy)
Antiphospholipid syndrome	Anticardiolipin, anti-β2 glycoprotein I (domain 1), anti-PS/PT	
Autoimmune liver diseases	Disease-related arrays	IIF screen of antimitochondrial antibodies sensitivity of 80% for PBC; IIF ANA IIF may be helpful in diagnosis of autoimmune hepatitis; antibodies to "rods and rings" seen in patients with ribavirin-treated viral hepatitis

ANA, Antinuclear antibody; ANCA, antineutrophil cytoplasmic antibodies; BICD2, bicaudal D2; IIF, indirect immunofluorescence; PS/PT, phosphatidyl serine/prothrombin complex; MPO, myeloperoxidase; PBC, primary biliary cholangitis; PR3, proteinase 3; TRIM21, tripartite motif 21.

**TABLE 69.4** Autoantibodies Determined by IIF Patterns That May Be Useful in Clinical Diagnostics

Cellular structure/IIF staining pattern/ICAP pattern designation <sup>a</sup>	Molecular targets	Disease associations
CENP-B/AC-3	Centromere B protein	lcSSc, PBC
Coiled bodies/AC-7	p80 coilin	Localized SSc, Raynaud's syndrome, also seen in association with DFS pattern (see below)
Dense fine speckled/AC-2	DFS70/LEDGF	Rare in SARD; in isolation, may rule out diagnosis of SARD
Golgi complex/AC-22	Golgins, giantin	SLE, SjS, RA overlap syndromes, malignancy, viral infection
GW bodies/AC-18	GW182, hAgo2, Ge-1/Hedls, RAP55	SjS, sensory/motor neuropathy, SLE, PBC
Mitotic spindle apparatus	Enolase, pericentrin, ninein	SSc, SjS, postviral syndromes
Centrioles/AC-24	NuMa 235	<i>Mycoplasma</i> infection
NuMa pattern/AC-26	HsEg5	SLE, SjS
Spindle microtubules/AC-25		SjS, SLE
Multiple nuclear dots/AC-6	SP-100	Primary biliary cirrhosis
Nuclear envelope/AC-11	Lamins A/C, B1, B2, LAP1/2	SLE, SjS, CAH, APS, SNP
Nuclear pore complex/AC-12	p62, gp210, Tpr	PBC, SjS
Rods and rings/AC-23		Ribavirin therapy of viral hepatitis
Scl-70/Topoisomerase I pattern/AC-29	The topoisomerase I IIF pattern must meet four IIF staining criteria: speckled staining of interphase nuclei, staining of metaphase chromatin, nucleoli, at higher serum dilutions staining of cytoplasm	dcSSc

<sup>a</sup><https://www.anapatterns.org/trees.php>.

APS, Antiphospholipid antibody syndrome; CAH, chronic active hepatitis; dcSSc, diffuse cutaneous systemic sclerosis; DFS, dense fine speckles; ICAP, International Consensus on Autoantibody Patterns (see Fig. 69.1); lcSSc, limited cutaneous systemic sclerosis; LEDGF, lens epithelium-derived growth factor; NuMA, nuclear mitotic apparatus; PBC, primary biliary cirrhosis; RA, rheumatoid arthritis; SARD, systemic autoimmune rheumatic diseases; SjS, Sjögren's syndrome; SLE, systemic lupus erythematosus; SNP, seronegative polyarthritis; SSc, systemic sclerosis; Tpr, translocated promoter region; IIF, indirect immunofluorescence.

quest to understand relationships between multiple aab, higher density antigen arrays are being used (Olsen et al., 2017; Sokolove et al., 2012; Dorner and Lipsky, 2016; Cohen, 2016). The use and application of these diagnostic platforms has been attended by certain considerations that are not always apparent to the clinician (Box 69.1). One of the more popular assay platforms is the ELISA because it offers high sensitivity, efficient throughput, only modest equipment required to perform the assay, all resulting in a relatively economical assay. Unfortunately, little has been done to standardize these kits (Feltkamp, 1996; Tan et al., 1999; Fritzler et al., 2003b) and postmarketing surveillance and quality assurance are largely left to the manufacturers (Fritzler et al., 2003a). Although the ELISA and other array technology kits are constantly being improved, high titer aab to a variety of autoantigens, especially those bearing discontinuous conformation epitopes, are often not detected. Hence, a report that indicates a “negative ELISA” screen should not be interpreted as “negative” for all relevant aab, because they might otherwise be detected by IIF, CBA, CLA, and/or other immunoassays.

Like ELISA, the performance of ALBIA also continues to improve and many studies show good agreement with other platforms (Fritzler and Fritzler, 2009; Shovman et al., 2005a; Hanly et al., 2010; Ronnelid, 2015). However, some ALBIA have limitations especially when comparing the results for specific analytes such as anti-dsDNA (Shovman et al., 2005b; Caramaschi et al., 2007; Avaniss-Aghajani et al., 2007; Bardin et al., 2009). In general, the same limitations and observations regarding progressive improvement of diagnostic accuracy apply to LIA (Almeida et al., 2010; Lee et al., 2012; Van Praet et al., 2011; Chandratilleke et al., 2016), although the use of LIA as a screening assay in individuals who have at least one criterion for SARD was found to be cost-effective (Wallace, 2006). An advantage of ALBIA and CLA over LIA and ELISA is that the target antigen is bound to a bead and the assay is then performed so that the aab can bind its target in the fluid phase rather than on a solid surface bearing the adsorbed antigen.

It has been suggested that because there is considerable variation in the sensitivity of the various commercial diagnostic kits, the identity of the particular assay used to generate the test result should be made available to the clinician (Meroni and Schur, 2010; American College of Rheumatology, 2011; Yazdany et al., 2013). In addition, because of the growing trend to use automated immunoassay systems that provide quantitative results, laboratories should follow standards of chemistry instrumentation including a requirement to demonstrate commutability, traceability, analytical measured ranges, and periodic calibrations (Meroni et al., 2014). Clinical laboratories that adopt multiplexed assays (i.e., ALBIA, CLA, and LIA) must establish reference ranges and cutoff values for each analyte, and the sensitivity and specificity must be established with care as the laboratory findings may present a difficult problem for the clinician who has to interpret the results.

#### **BOX 69.1**

#### **CONSIDERATIONS REGARDING THE CLINICAL INTERPRETATION OF AUTOANTIBODY TESTING**

- The definition of a positive autoantibody test is based on an empirically defined threshold.
- Autoantibodies are found in the sera of first degree relatives of patients, infectious diseases, malignancies, and normal individuals.
- Disease-specific autoantibodies can antedate diagnosis and in the context of early signs and symptoms of autoimmune diseases can be used as biomarkers to develop strategies for disease prevention and precision medicine.
- The approach to and interpretation of autoantibody testing in pediatric populations requires more thorough evaluation and evidence-based consensus.
- Autoantibody tests, once regarded as a tool for confirmation of a specific disease (i.e., IIF on HEp-2 cells for SLE), are used by a wide spectrum of health-care providers and for an ever-widening spectrum of autoimmune conditions.
- Limitations of assays based on subjective interpretation, such as indirect immunofluorescence, are being mitigated by the introduction of automated digital technologies.
- New high-throughput immunoassays and diagnostic assay platforms continue to emerge but new assays should only be adopted after validation of local performance.
- The advent of electronic medical records is enhancing the autoantibody reporting system.
- Short-term cost and budget restraints have an impact on autoantibody testing, while the long-term impact of autoantibody testing on total health-care expenditures and quality of life is mostly unreported.

## CLINICAL INTERPRETATION AND APPLICATION OF AUTOANTIBODY TESTING

As referred to earlier, it is important to appreciate some issues and limitations that impact on the interpretation and clinical application of aab tests (Box 69.1). Many of the perceived shortcomings of aab testing are due to a lack of thorough knowledge of autoimmunity, such as the perpetuation and perturbation of aab production, and the continuum of B-cell responses that span innate to acquired immunity (Fritzler, 2012). It is firmly established that any given human serum contains varying concentrations of a wide range of aab (Rose, 1996). As mentioned earlier, it is also important to understand that the measurement and assignment of an abnormal or elevated aab test are based on an empirically defined threshold, which, in turn, is dependent on numerous variables such as the equipment used to perform the assays, antihuman secondary antibodies, and adherence to manufacturer's protocols (Fritzler et al., 2003a; Wiik et al., 2004).

One of the most misused and misunderstood terms in aab testing is that the result is a "false positive." First, it needs to be recognized that there is substantial evidence that a number of aab can antedate the diagnosis of SARD and organ-specific autoimmune diseases by as much as 20 years (Arbuckle et al., 2003; Rose, 2007; Bizzaro et al., 2007; Senecal et al., 2008; Fritzler, 2008, 2011a; Meroni and Shoenfeld, 2008; Kallenberg, 2011; Villalta et al., 2012; Frech et al., 2016; Graf et al., 2012; Watad et al., 2014; Elnady et al., 2016; Nihtyanova and Denton, 2017). For example, antibodies to centromere proteins may antedate the clinical diagnosis of SSc (Choi and Fritzler, 2016; Wigley et al., 1992; Kallenberg et al., 1988) and antibodies to SS-A/Ro6 and dsDNA antedate the clinical diagnosis of SLE (Choi et al., 2016; Arbuckle et al., 2001, 2003) by many years. Second, even ANA-positive, apparently "healthy" individuals have been shown to exhibit dysregulation in multiple immune pathways (Slight-Webb et al., 2016). Therefore, unless there is a systematic laboratory error in performance of the test, the evidence indicates that the use of the term "false-positive aab test" should be used with caution and the dismissal of a person that may have an evolving autoinflammatory condition carefully considered. The performance characteristics of each test must be known to avoid misinterpretation, incorrect diagnosis, and potentially harmful treatment, outcomes that are commonly attributed to so-called false-positive tests. As diagnostic paradigms move more and more to precision medicine and disease prevention (Choi et al., 2016; Choi and Fritzler, 2016), an evidence-based understanding of aab and other biomarkers as predictors of disease needs to be more clearly defined. It is equally important to consider the impact of "false-negative" tests that can lead to delayed diagnoses and unnecessary morbidity (Fritzler, 2011c). Therefore, to achieve significant clinical utility, it is important to perform aab tests that discriminate between disease and the absence of disease, between emerging or subclinical disease, or between disease and confounding clinical conditions (Conrad et al., 2012).

Decades of clinical experience and research have led to the development of classification and diagnostic criteria to support the diagnosis of SARD and other autoimmune diseases, many of which include aab biomarkers (Kasukawa, 1987; Nishimaki et al., 1991; Hochberg, 1997; Petri et al., 2012; Jennette et al., 2013; Aletaha et al., 2010; Ferreira et al., 2015; Bossuyt et al., 2017). Since SARD frequently involve multiple organ systems, multiple criteria are required to support accurate classification. As a consequence, it is rare that a single criterion can be translated into certainty that a given diagnosis is correct or that a certain disease is emerging. To ensure that results from the immunology laboratory gain maximum utility for clinicians, it is important to study the performance of each aab assay as an aid to diagnostics in early disease because that is the time at which a serologic result would impact prognostic considerations the most (Fritzler, 2016; Conrad et al., 2012; Fenger et al., 2004). Since the clinical diagnosis may be established only after months or years of clinical follow-up, the more knowledgeable the clinician is with regards to the early clinical and laboratory characteristics of diseases, the greater the chance that a correct diagnosis will be made. Studies based on literature review and metanalysis have been published as "evidence-based guidelines" (Kavanaugh and Solomon, 2002; Solomon et al., 2002; Reveille and Solomon, 2003) but the translation of this information is limited because of the newer assays and assay parameters that have come into use since these studies and recommendations were published. The ongoing need for prospective, unbiased, and multicenter studies is needed to ensure that the clinical accuracy of contemporary aab testing is much clearer.

Many of the diseases listed in Table 69.2 have clinical subgroups with somewhat dissimilar manifestations and, hence, prognosis. These subgroups are often associated with different aab profiles and specificities (Cervera et al., 1993, 2002; Kuwana et al., 1994; Permin et al., 1978; Mustila et al., 2000; Wiik, 2001; Scussell-Lonzetti et al., 2002; Targoff, 2002). Therefore, certain aab are valuable biomarkers of a disease subgroup with markedly different clinical features, end organ involvement, and prognosis. Indeed, SARD subtypes and phenotypes based on aab profiles have been suggested to be more clinically relevant than other distinguishing features

or parameters (Mahler et al., 2014b; Walker et al., 2007; Agmon-Levin et al., 2012; Biesen et al., 2016; Allenbach et al., 2017). Accordingly, the information imparted by aab profiles, in combination with other biomarkers, is likely to be valuable in the future as an approach to tailoring therapeutic strategies (i.e., personalized or precision medicine) (Biesen et al., 2016; Fritzler, 2011b; Andrade, 2009; Plenge and Bridges, 2011; Tak, 2012).

With the introduction and adoption of newer diagnostic technology platforms, aab that were thought to be specific for one disease based on older or even outdated technologies may subsequently turn out to be associated with a variety of autoimmune diseases or other conditions (van Eenennaam et al., 2002; Vermeersch et al., 2009). The appreciation that multiple disease-related aab occur in a single serum and that aab expression may change over time in individual patients (von Muhlen and Tan, 1995; Blass et al., 1999; Vasiliauskienė et al., 2001; Visser et al., 2002) suggests that aab serology in combination with other biomarkers (i.e., genomics, cytokines, metabolomics, transcriptomics) may lead to an earlier and more accurate diagnosis, and by extension, more effective therapeutic interventions (Fritzler, 2016; Choi et al., 2016; Choi and Fritzler, 2016; Plenge and Bridges, 2011). While some evidence indicates that certain aab are stable over the disease course (Ippolito et al., 2011), other evidence indicates that aab profiles as determined by array technologies will likely change over time (reviewed in Fritzler, 2012). Evidence supporting this notion includes observations that aab to RNA helicase occur only early in the course of SLE (Yamasaki et al., 2007) and only clinically distinct neuropsychiatric events attributed to SLE that occurred around the time of diagnosis were found to be associated with anti-P antibodies and the lupus anticoagulant (antiphospholipid, anticardiolipin) (Hanly et al., 2008). Hence, multiplexed and autoantigen array technologies that are now emerging provide more extensive aab profiles in a given patient (Kessenbrock et al., 2007; Mahler and Fritzler, 2010) and are altering approaches to diagnostics and therapeutics (Schachna et al., 2002; Robinson et al., 2002; Fritzler, 2002).

## CLINICAL PRACTICE GUIDELINES

Because of the complexity of modern autoimmune serology, there is a pressing need for clinical practice guidelines (CPG) that outline the appropriate and economic use of serologic testing. If a limited number and spectrum of clinicians are involved in ordering diagnostic testing, it is easier to achieve a consensus on testing strategies. However, there is a growing trend toward amalgamation of local laboratories into regional and even national laboratories that provide high-throughput service to a widening spectrum of health-care providers. Clearly articulated CPG that establish criteria for aab screening and an evidence-based testing algorithm that reduces unnecessary laboratory testing and health-care expenditures (HCE) have been thought to be a productive approach (Wiik et al., 2004), including a proposal to reduce testing as part of the “Choosing Wisely” campaign (Yazdany et al., 2013; Chow et al., 2015). It has been suggested that a related strategy is to develop aab order forms in such a way that the doctor chooses between tentative diagnoses, after which only evidence-based tests are done. It is important to realize that the pretest probability to detect useful diagnostic laboratory results increases dramatically when each clinical feature or diagnostic criterion has been incorporated into the tentative diagnosis (Keren and Nakamura, 1997). However, if CPGs indicate that testing should only be done on patients that already have a high pretest probability of an autoimmune disease based on other clinical findings, it can be argued that the test is not likely to add much clinical value and probably shouldn’t be done at all (Fritzler, 2016). In addition, if significant inroads are going to be made into tempering HCE, it is clear that there needs to be a more concerted approach to prevention of SARD, which means that screening tests such as ANA, extractable nuclear antigen (ENA), and other disease-related biomarkers will likely be needed to identify patients with pre-clinical disease when inroads to prevention of morbidity due to end organ failure can be made (Fritzler, 2016).

A common misperception among clinicians and some health administrators is that in vitro diagnostics, such as ANA, ENA, and other biomarkers, account for a high proportion of HCE, when in actual practice the entire spectrum of in vitro diagnostics is in the range of 2%–5% HCE per year (Rohr et al., 2016). It should be no surprise that aab testing accounts for a rather small proportion of expenditures on in vitro diagnostics (Fritzler, 2016). Taken together, a balance needs to be achieved in aab testing where it can be used to help confirm a diagnosis where the clinician is pressed with the “intent to treat” the disease but also needs to include early disease identification with an “intent to prevent” disease (Fritzler, 2016). There is a serious deficiency in our knowledge of the actual costs incurred through inappropriate laboratory testing, although the actual costs of laboratory diagnostic testing can readily be calculated (Fritzler, 2016; Rohr et al., 2016).

The implications of HCE are intimately linked to the three overarching tenets of clinical medicine: first, disease prevention is of utmost importance; second, an early and accurate diagnosis is the next most important; and third

appropriate, effective treatment follows the first two. Many health-care providers and payers give close attention to the first and third tenets without due consideration of the second. An estimation of long-term HCE related to an early and accurate diagnosis, attended by evidence-based interventions (Choi et al., 2016; Choi and Fritzler, 2016) as compared to a missed or a wrong diagnosis, with or without treatment, needs to be performed to highlight the value of high-quality laboratory diagnostics (Fritzler, 2016).

## **LABORATORY REPORTS, ELECTRONIC MEDICAL RECORDS, AND COST ANALYSIS**

Many laboratories use an aab testing algorithm that includes a rapid and inexpensive screening test (i.e., IIF) followed by more specific tests (i.e., LIA, ALBIA, and ELISA) as an approach to evidence-based screening for serum aab. For example, the IIF test or whole cell lysate ELISAs are often used to detect aab in SARD and other diseases (reviewed in Fritzler, 2011c, 2012; Stinton and Fritzler, 2007). This serves two purposes: first, as an approach to triage samples for further reflex testing; second, if the IIF or screening ELISA test is negative, unless there is a compelling clinical evidence to do so, no further testing is required and the result is reported as such. However, as discussed earlier, there is a caveat insomuch as the sensitivity of IIF testing is not as high as some presume and is attended by significant “false-negative” results (Pisetsky, 2017; Fritzler, 2011c). Although a few aab can be quite accurately identified solely by IIF screening (Table 69.4), as more and more aab are identified in SARD and other autoimmune conditions with overlapping clinical features, the usefulness of IIF as a screening test is diminishing. Hence, some laboratories have inverted the testing algorithm and screen with technologies that include a wide spectrum of target autoantigens and if that test is negative, then the test might be reflexed to an IIF test (Meroni and Schur, 2010; Fritzler, 2011a,c). More recent analysis, ahs indicated that combining IIF with solid phase immunoassays as a screen for SARD may be the most effective approach (Bizzaro et al., 2018)

It is very important to validate aab test results through regular quality assurance and quality control programs. In this context, borderline (low positive) test results can be especially troublesome. It is recommended that borderline-positive results must be confirmed or refuted to ensure that only certified positive results are reported. If uncertainty about the result persists, the laboratory should add a note of caution to the test report that the result may have limited significance in supporting a diagnosis and/or may recommend retesting a new serum sample in 2–6 months, or sooner if clinical features continue to progress or accumulate. Some regulatory agencies mandate that borderline-positive tests should be reported as positive until proven otherwise after repeat or follow-up testing at appropriate intervals. High and intermediate positive results of a single credible technique can be reported without independent confirmation by a second technique. To aid in the interpretation of laboratory results, the chosen limit for positivity should be stated in the report along with ranges of low, intermediate, and strong positivity.

A persistent problem is that easy to perform and high-throughput techniques are often adopted by clinical laboratories without proper clinical validation. As discussed above, screening for aab by ELISA that have adsorbed complex mixtures of native and/or recombinant autoantigens or nuclear extracts is now used by many laboratories instead of IIF ANA screening. This practice continues despite data showing that many patients with Sjögren's syndrome, SSc, autoimmune myopathies, and juvenile rheumatoid arthritis score negative for ANA using such composite ELISA screening techniques (Keren and Nakamura, 1997; Fawcett et al., 1999). While these alternative screening assays have improved remarkably over the last few years, HEp-2-IIF screening of these *false-negative* sera reveals that many of these sera contain aab to a variety of targets including nucleoli, nuclear matrix, nuclear envelope, nuclear pores, coiled bodies, promyelocyte leukemia domains, cell cycle-specific antigens such as PCNA and mitotic spindle apparatus components, or other cytoplasmic organelles and structures such as mitochondria, Golgi apparatus, signal recognition particle, or ribosomal proteins (Stinton et al., 2004; Bayer et al., 1999; Wiik, 2003a,b) (Tables 69.2 and 69.4).

Studies using older screening ELISAs have shown that some of these aab are readily recognized by experienced technicians (Wiik and Lam, 2001) but are missed by ELISAs for ANA screening (Bayer et al., 1999). Some of these aab have defined clinical associations and should be identified as such (Table 69.4). It needs to be emphasized that newer technologies and wider testing have not supported clinical associations of some aab. For example, anti-PCNA aab once thought to be highly specific for SLE have recently been shown to lack specific disease associations (Vermeersch et al., 2009; Merkel et al., 2012). Aab that lack proven clinical value should be reported but it must be clearly stated that their diagnostic specificity or value has not been clearly established.

At this point, it is important to point out that not all aab are diagnostic of or associated with clinically apparent autoimmune diseases. This includes established evidence that disease-specific aab can be detected in first degree relatives of autoimmune diseases patients, in individuals harboring a variety of bacterial and viral infections, or in apparently unrelated conditions and healthy individuals. Several lines of evidence indicate that approximately 20% of serum samples from apparently healthy individuals have a positive ANA test, a significant proportion of which produce an IIF pattern known as dense fine speckles (DFS) (Mahler et al., 2016; Ochs et al., 2016). Of note, the DFS staining pattern has been reported in 33% of ANA-positive healthy individuals, but not in ANA-positive SARD sera (Mariz et al., 2011). Since their first description, anti-DFS70 antibodies have been found in the sera of patients with a variety of chronic inflammatory conditions, such as interstitial cystitis, atopic dermatitis, as well as cancer and healthy individuals (reviewed in Mahler et al., 2016; Ochs et al., 2016). The typical IIF DFS staining pattern is observed as small speckles that are somewhat uniformly distributed throughout the nucleus and, notably, on metaphase chromatin. The target autoantigen is a 70-kDa protein and the target antigen was initially termed DFS70, but the primary target autoantigen was eventually identified as the lens epithelium-derived growth factor (LEDGF) (reviewed in Ochs et al., 2016). Since the intended use of the ANA HEp-2 test is to primarily serve as a screening test to aid in the diagnosis of SARD, the reporting of anti-DFS70 antibodies as a positive test significantly reduces the specificity and the positive likelihood of the ANA IIF test. This has important implications for ANA test algorithms because if these results are widely validated, it suggests that aab associated with DFS70/LEDGF can be used to rule out the diagnosis of SARD (Fitch-Rogalsky et al., 2014; Mahler et al., 2012; Mahler et al., 2019).

When aab are detected, the positive result is usually communicated in a printed or digital report that is sent directly to the requesting physician or the referring laboratory. Unfortunately, in some jurisdictions, regulatory constraints (i.e., Health Protection Act and Freedom of Information Acts) prohibit the transmission of a digital report directly to the referring physician's computer or other digital devices. Nevertheless, there is a trend in many jurisdictions to rapidly adopt EMRs that will accommodate uploading encrypted or otherwise secure data files directly to online or "cloud" storage of patient medical records. For many laboratories, this has added another layer of protocols and standard operating procedures that are intended to protect patient confidentiality. Efforts to develop consensus on the format and structure of the EMR, along with consensus terminology and content of aab results, would be an important step forward in standardizing the aab testing system (Pincus et al., 2009; Malaviya and Gogia, 2010; Wiik et al., 2010; Vogt et al., 2004). A number of advantages and disadvantages of the EMR have been identified (van der Vaart et al., 2013; Liao et al., 2010 Aug) (Box 69.2). The questions and concerns regarding unlimited access of patients to their EMR is unsettled (van der Vaart et al., 2013). While information on diagnosis, treatment plan, and consultations might be released for patient access, more complex data, such as physical examinations, laboratory results, and radiographic images, is more controversial. There is some thought that providing patients random access to their EMR might be an important step to empowering and enhancing patient service, provided that clinical and personal data and information security is optimal, and content and presentation of data are constructed so that they are clearly understood by patient and physician alike.

#### BOX 69.2

#### ADVANTAGES AND DISADVANTAGES OF THE EMR

##### Advantages

- Improved turnaround time and accessibility to laboratory results
- Eliminating part of "paper trail" improves accuracy of reporting
- Increased knowledge of the patient's disease status
- Improved interaction of patient with health-care providers
- Increased patient safety
- More knowledgeable patients involved in self-management

- Enhanced communication with health-care team
- Elimination of lower security multiple paper records and mailed/faxed paper reports
- Higher security of health information

##### Disadvantages

- Challenges interpreting reports and data
- Additional security protocols and standard operating procedures adds extra workload
- Consultation content changed
- Altered face-to-face patient-provider interactions

A description of the most common diagnostic associations related to an aab found in diagnostic testing should be included with the result. Also, the level (titer) of the aab or the strength of expression should be mentioned together with how these relate to cutoff values and how they were established (i.e., disease controls, normal controls, and the range of credible measurements).

## STANDARDIZATION AND QUALITY ASSURANCE

There is little debate that the most important issue that impacts on aab testing is standardization of test performance, and test result nomenclature and interpretation. Thankfully, there are substantial coordinated and cooperative efforts in various jurisdictions that are addressing these and related issues (Box 69.3). An example of this activity is ongoing concerted efforts of a subcommittee of the Autoantibody Standardization Committee, the International Consensus on Autoantibody Patterns (<https://anapatterns.org/index.php>) to achieve a standardized nomenclature of the different ANA IIF patterns observed on HEp-2 substrates (Damoiseaux and von Muhlen, 2016; Chan et al., 2016). This important activity allows laboratory personnel, diagnosticians, and clinicians alike to use a common nomenclature in research as well as diagnostic laboratory reports. The goal, now at least partially attained, was to assign both common nomenclature and a code to each of the most common aab IIF patterns observed in diagnostic laboratories (Fig. 69.1). On the surface, this may seem

### BOX 69.3

#### INTERNATIONAL EFFORTS TO STANDARDIZE AUTOANTIBODY TESTING

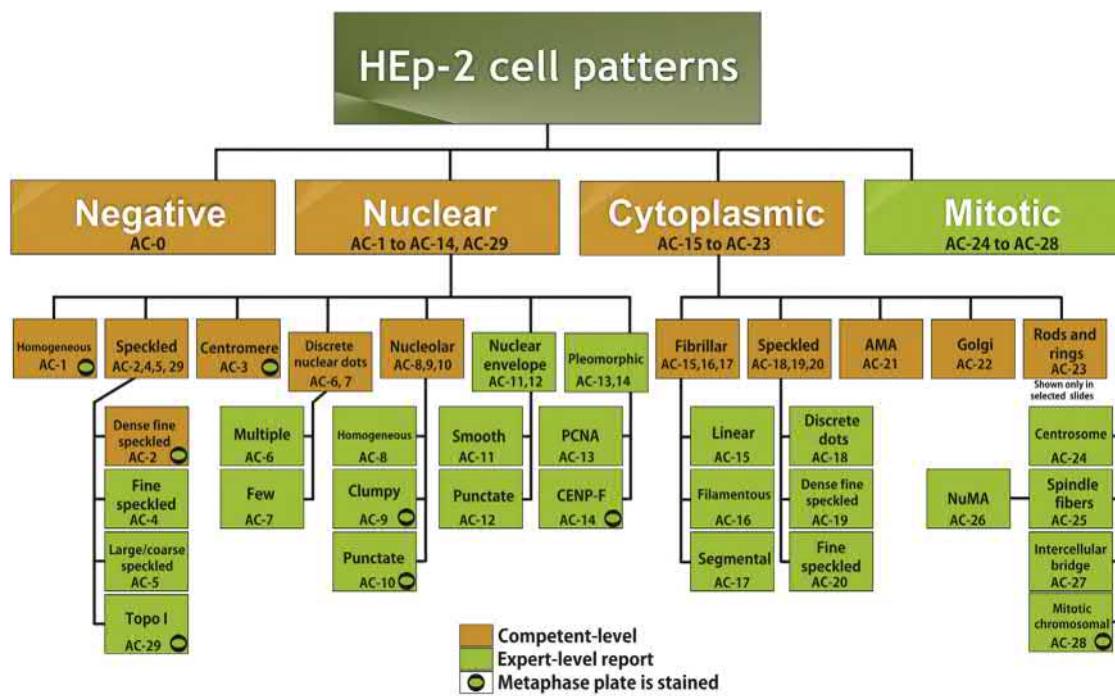
##### Committees and Leadership

- Royal College of Pathologists of Australasia—Quality Assurance Program (RCPAQAP): Louise Wienholt, Sydney, Australia
- Autoantibody Standardization Committee/International Union of Immunology Societies (ASSC/IUIS): Edward K.L. Chan, Gainesville, Florida, USA
- European Autoantibody Standardization Initiative (EASI): Jan Damoiseaux, Maastricht University Medical Centre, Maastricht, Netherlands.
- European Consensus Finding Study Group on Autoantibodies (ECFSGA): Johan Rönnelid, Uppsala, Sweden
- International Federation of Clinical Chemistry Committee on Harmonization of Autoimmune Tests (IFCC C-HAT): Joanna Sheldon, London, UK
- National Institute for Biological Standards and Control/World Health Organization (NIBSC/WHO): Bernard Fox and Lucy Studholme, Potters Bar, Herts, United Kingdom

##### Issues Under Consideration

- There is a need to coordinate the efforts of all the Committees.

- Training and ongoing competency of technologists and clinicians who read ANA slides I accord with expectations of ISO 15189.
- As various jurisdictions move to electronic medical records, standardized nomenclature and reporting of test results are needed.
- Since reference materials have different performance characteristics, their performance in different diagnostic platforms and protocols should be specified.
- New reference samples should be identified and made available.
- Address logistics (i.e., preparation, storage, quality assurance/quality control, distribution, financial support to assure sustainability) of making reliable reference materials available.
- Given continuously changing platforms for autoantibody detection, a strategy for ongoing validation is needed.
- Industry and other parties need samples from well-defined disease and control cohorts.
- Diagnostic manufacturers should be encouraged and incented to calibrate their kits according to the reference standards and the units of activity assigned to them.
- Effective communication and education of the medical, scientific, and industry communities.



[www.anapatterns.org](http://www.anapatterns.org)

**FIGURE 69.1** Consensus nomenclature of IIF staining patterns on HEp-2 substrate established by the ICAP (<https://www.anapatterns.org/>), a subcommittee of the Autoantibody Standardization Committee ([www.AutoAb.org](http://www.AutoAb.org)) (Damoiseaux and von Muhlen, 2016; Chan et al., 2015, 2016). ICAP, International Consensus on Autoantibody Patterns; IIF, indirect immunofluorescence.

like a straight forward exercise, but it has taken more than 5 years and four meetings to arrive at the current level of consensus, and even then some challenges remain. Most notably, the focus has shifted to the goal of standardizing reports and also the appropriate recognition of complex (multiple) IIF patterns that are often found in single sera (Damoiseaux and von Muhlen, 2016).

A number of jurisdictions have also recognized the importance of standardizing the technical aspects of aab testing (Shoenfeld et al., 2007; Wiik et al., 2006) and a set of standardized sera provided by the IUIS/AF/WHO/CDC Serology Committee ([www.autoab.org](http://www.autoab.org)) are currently available. Over the past two decades, these samples were available through the Centers for Disease Control (CDC) in Atlanta (Chan et al., 2007) but are now made available through the auspices of Plasma Services Group in Huntingdon Valley, Pennsylvania, USA (<https://www.plasmaservicesgroup.com/>). These reference sera have been continuously monitored and more recent additions to the reference sera available include those with defined aab directed to a number of aab targets (Box 69.3). Sera used as standards for a particular methodology or technology platform need to be reviewed from time to time as exemplified by the reevaluation of AF/CDC reference sera by a variety of contemporary techniques (Copple et al., 2012; Smolen et al., 1997). Although it would likely decrease interlaboratory variation in performance, standardized secondary antibodies are not widely utilized.

All clinical laboratories should participate in quality assurance and quality improvement programs such as the one administered by the College of American Pathologists ([www.cap.org](http://www.cap.org)) and the Royal College of Pathologists of Australasia, to name only two. The Clinical Laboratory Improvement Amendments of 1988 set standards for all laboratories engaged in clinical testing. These standards include requirements for trained and competent supervisory and testing personnel, record keeping and instrument maintenance, daily quality control practices, result reporting, and laboratory inspection and maintenance. It is not clear that these standards are being met in routine practice. In Europe and some other jurisdictions, the most widely used quality assurance and management program (DS/EN ISO/IEC 17025:2000) additionally sets laboratory standards aimed to prove that an assay actually gives results that are useful for clinical diagnostics.

## SUMMARY

Aab can serve as valuable biomarkers for early disease detection and case finding as well as confirmation of a diagnosis when it is the clinician's intention to treat the disease. Aab testing also drives the clinician's attention to involved tissues or organs using classical diagnostic tools such as histopathology, imaging techniques, and organ function testing. CPG to facilitate the communication between clinicians and laboratories need to be formulated, along with mutually accepted algorithms for test ordering and interpretation. The emergence of the EMR is a significant step forward but is attended by some logistic and ethical challenges.

In contemporary medical practice, changes in laboratory diagnostics and new technologies to detect aab are being introduced at a rapid pace. This is attended by concerns that certain key matters, such as clinical utility, are not thoroughly addressed. In many cases, the manufacturer has often been assumed to be the root cause for shortcomings in aab testing; however, the cost-effective use of commercial kits and their appropriate application in a clinical setting involves a rather complex set of contingencies and considerations (Fritzler, 2012, 2016; Fritzler et al., 2003a). It has been suggested that a higher level of commitment and partnership between all of the participants is required to achieve the goal of improving the quality of patient care through the use of aab testing and accurate interpretation of the results (Fritzler et al., 2003a; Wiik et al., 2004).

## References

- Agmon-Levin, N., Mosca, M., Petri, M., Shoenfeld, Y., 2012. Systemic lupus erythematosus one disease or many? *Autoimmun. Rev.* 11, 593–595.
- Agmon-Levin, N., Damoiseaux, J., Kallenberg, C., Sack, U., Witte, T., Herold, M., et al., 2014. International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. *Ann. Rheum. Dis.* 73, 17–23.
- Aletaha, D., Neogi, T., Silman, A.J., Funovits, J., Felson, D.T., Bingham III, C.O., et al., 2010. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum.* 62, 2569–2581.
- Allenbach, Y., Benveniste, O., Goebel, H.H., Stenzel, W., 2017. Review: integrated classification of inflammatory myopathies. *Neuropathol. Appl. Neurobiol.* 43, 662–673.
- Almeida, G.D., Cabrera de, L.A., Rodriguez Perez, M.D., Brito, D.B., Gonzalez, H.A., Garcia, G.D., et al., 2010. Efficiency of different strategies to detect autoantibodies to extractable nuclear antigens. *J. Immunol. Methods* 360, 89–95.
- American College of Rheumatology, 2011. Position Paper: Methodology of Testing for Antinuclear Antibodies. Ref Type: Online Source.
- Andrade, L.E., 2009. Future perspective for diagnosis in autoimmune diseases. *An. Acad. Bras. Cienc.* 81, 367–380.
- Arbuckle, M.R., James, J.A., Kohlhase, K.F., Rubertone, M.V., Dennis, G.J., Harley, J.B., 2001. Development of anti-dsDNA autoantibodies prior to clinical diagnosis of systemic lupus erythematosus. *Scand. J. Immunol.* 54, 211–219.
- Arbuckle, M.R., McClain, M.T., Rubertone, M.V., Scofield, R.H., Dennis, G.J., James, J.A., et al., 2003. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N. Engl. J. Med.* 349, 1526–1533.
- Avaniss-Aghajani, E., Berzon, S., Sarkissian, A., 2007. Clinical value of multiplexed bead based immunoassays for detection of autoantibodies to nuclear antigens. *Clin. Vaccine Immunol.* 14, 505–509.
- Backes, C., Ludwig, N., Leidinger, P., Harz, C., Hoffmann, J., Keller, A., et al., 2011. Immunogenicity of autoantigens. *BMC Genomics* 12, 340.
- Baker, C.A., Lu, Z.Y., Manuelidis, L., 2004. Early induction of interferon-responsive mRNAs in Creutzfeldt–Jakob disease. *J. Neurovirol.* 10, 29–40.
- Balboni, I., Limb, C., Tenenbaum, J.D., Utz, P.J., 2008. Evaluation of microarray surfaces and arraying parameters for autoantibody profiling. *Proteomics* 8, 3443–3449.
- Bardin, N., Desplat-Jego, S., Daniel, L., Jourde, C.N., Sanmarco, M., 2009. BioPlex 2200 multiplexed system: simultaneous detection of anti-dsDNA and anti-chromatin antibodies in patients with systemic lupus erythematosus. *Autoimmunity* 42, 63–68.
- Bayer, P.M., Bauerfeind, S., Bienvenu, J., Fabien, N., Frei, P.C., Gilburd, B., et al., 1999. Multicenter evaluation study on a new HEp2 ANA screening enzyme immune assay. *J. Autoimmun.* 13, 89–93.
- Beck, J.S., 1961. Variations in the morphological patterns of "autoimmune" nuclear fluorescence. *Lancet* 1, 1203–1207.
- Beck Jr., L.H., Bonegio, R.G., Lambeau, G., Beck, D.M., Powell, D.W., Cummins, T.D., et al., 2009. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. *N. Engl. J. Med.* 361, 11–21.
- Biesen, R., Rose, T., Hoyer, B.F., Alexander, T., Hiepe, F., 2016. Autoantibodies, complement and type I interferon as biomarkers for personalized medicine in SLE. *Lupus* 25, 823–829.
- Bizzaro, N., Tozzoli, R., Shoenfeld, Y., 2007. Are we at a stage to predict autoimmune rheumatic diseases? *Arthritis Rheum.* 56, 1736–1744.
- Bizzaro, N., Brusca, I., Previtali, G., Alessio, M.G., Daves, M., et al., 2018. The association of solid-phase assays to immunofluorescence increases the diagnostic accuracy for ANA screening in patients with autoimmune rheumatic diseases. *Autoimmun. Rev.* 17, 541–547.
- Blass, S., Engel, J.-M., Burmester, G.R., 1999. The immunologic homunculus in rheumatoid arthritis. *Arthritis Rheum.* 42, 2499–2506.
- Bossuyt, X., Louche, C., Wiik, A., 2008. Standardisation in clinical laboratory medicine: an ethical reflection. *Ann. Rheum. Dis.* 67, 1061–1063.
- Bossuyt, X., Cohen Tervaert, J.W., Arimura, Y., Blockmans, D., Flores-Suarez, L.F., Guillemin, L., et al., 2017. Position paper: revised 2017 international consensus on testing of ANCAs in granulomatosis with polyangiitis and microscopic polyangiitis. *Nat. Rev. Rheumatol.* 13, 683–692.
- Bozzalla, C.E., Stone, J.H., 2017. IgG4-related disease. *Curr. Opin. Rheumatol.* 29, 223–227.

- Caramaschi, P., Ruzzenente, O., Pieropan, S., Volpe, A., Carletto, A., Bambara, L.M., et al., 2007. Determination of ANA specificity using multiplexed fluorescent microsphere immunoassay in patients with ANA positivity at high titres after infliximab treatment: preliminary results. *Rheumatol. Int.* 27, 649–654.
- Casciola-Rosen, L., Mammen, A.L., 2012. Myositis autoantibodies. *Curr. Opin. Rheumatol.* 24, 602–608.
- Cervera, R., Khamashta, M.A., Font, J., Sebastiani, G.D., Gil, A., Lavilla, P., et al., 1993. Systemic lupus erythematosus: clinical and immunologic patterns of disease expression in a cohort of 1,000 patients. *Medicine (Baltimore)* 72, 113–124.
- Cervera, R., Piette, J.C., Font, J., Khamashta, M.A., Cervera, R., Piette, J.C., et al., 2002. Antiphospholipid syndrome—clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients. *Arthritis Rheum.* 46, 1019–1027.
- Chan, E.K.L., Fritzler, M.J., Wiik, A., Andrade, L.E., Reeves, W.H., Tincani, A., et al., 2007. AutoAbSC. Org—Autoantibody Standardization Committee in 2006. *Autoimmun. Rev.* 6, 577–580.
- Chan, E.K., Damoiseaux, J., Carballo, O.G., Conrad, K., de Melo, C.W., Francescantonio, P.L., et al., 2015. Report of the first international consensus on standardized nomenclature of antinuclear antibody HEp-2 cell patterns 2014–2015. *Front. Immunol.* 6, 412.
- Chan, E.K., Damoiseaux, J., de Melo, C.W., Carballo, O.G., Conrad, K., Francescantonio, P.L., et al., 2016. Report on the second international consensus on ANA pattern (ICAP) workshop in Dresden 2015. *Lupus* 25, 797–804.
- Chandra, P.E., Sokolove, J., Hipp, B.G., Lindstrom, T.M., Elder, J.T., Reveille, J.D., et al., 2011. Novel multiplex technology for diagnostic characterization of rheumatoid arthritis. *Arthritis Res. Ther.* 13, R102.
- Chandratilleke, D., Silvestrini, R., Culican, S., Campbell, D., Byth-Wilson, K., Swaminathan, S., et al., 2016. Comparison of two extractable nuclear antigen testing algorithms: ALBIA versus ELISA/line immunoassay. *Pathology* 48, 491–497.
- Choi, M.Y., Fritzler, M.J., 2016. Progress in understanding the diagnostic and pathogenic role of autoantibodies associated with systemic sclerosis. *Curr. Opin. Rheumatol.* 28, 589–594.
- Choi, M.Y., Barber, M.R., Barber, C.E., Clarke, A.E., Fritzler, M.J., 2016. Preventing the development of SLE: identifying risk factors and proposing pathways for clinical care. *Lupus* 25, 838–849.
- Chow, S.L., Thorne, J.C., Bell, M.J., Ferrari, R., Bagheri, Z., Boyd, T., et al., 2015. Choosing wisely: the Canadian Rheumatology Association's list of 5 items physicians and patients should question. *J. Rheumatol.* 42, 682–689.
- Cohen, I.R., 2016. Antigen-microarray profiling of antibodies in SLE: a personal view of translation from basic science to clinic. *Lupus: Open Access* 1, 118.
- Conn, R.B., 1994. Practice parameter—the lupus erythematosus cell test. *Am. J. Clin. Pathol.* 101, 65–66.
- Conrad, K., Roggenbuck, D., Reinhold, D., Sack, U., 2012. Autoantibody diagnostics in clinical practice. *Autoimmun. Rev.* 11, 207–211.
- Conrad, K., Schlosser, U., Hiepe, F., Fritzler, M.J., 2015. Autoantibodies in Systemic Autoimmune Diseases: A Diagnostic Reference. Third edition. Pabst Scientific Publishers, Berlin.
- Conrad, K., Schlosser, W., Hiepe, F., Fritzler, M.J., 2017. Autoantibodies in Organ Specific Autoimmune Diseases: A Diagnostic Reference. Third edition. Pabst Science Publishers, Berlin. Autoantigens, Autoantibodies, Autoimmunity. 2015.
- Copple, S.S., Martins, T.B., Masterson, C., Joly, E., Hill, H.R., 2007. Comparison of three multiplex immunoassays for detection of antibodies to extractable nuclear antibodies using clinically defined sera. *Ann. N. Y. Acad. Sci.* 1109, 464–472.
- Copple, S.S., Giles, S.R., Jaskowski, T.D., Gardiner, A.E., Wilson, A.M., Hill, H.R., 2012. Screening for IgG antinuclear autoantibodies by HEp-2 indirect fluorescent antibody assays and the need for standardization. *Am. J. Clin. Pathol.* 137, 825–830.
- Copple, S.S., Jaskowski, T.D., Giles, R., Hill, H.R., 2014. Interpretation of ANA indirect immunofluorescence test outside the darkroom using NOVA view compared to manual microscopy. *J. Immunol. Res.* 2014, 149316.
- Dalmau, J., 2016. NMDA receptor encephalitis and other antibody-mediated disorders of the synapse: the 2016 Cotzias Lecture. *Neurology* 87, 2471–2482.
- Damoiseaux, J., von Muhlen, C.A., Garcia-de la Torre, I., Carballo, O.G., de Melo, C.W., Francescantonio, P.L., et al., 2016. International consensus on ANA patterns (ICAP): the bumpy road towards a consensus on reporting ANA results. *Autoimmun. Highlights* 7, 1.
- Damoiseaux, J., Boesten, K., Giesen, J., Austen, J., Tervaert, J.W., 2005. Evaluation of a novel line blot immunoassay for the detection of antibodies to extractable nuclear antigens. *Ann. N. Y. Acad. Sci.* 1050, 340–347.
- Dincer, C., Bruch, R., Kling, A., Dittrich, P.S., Urban, G.A., 2017. Multiplexed point-of-care testing—xPOCT. *Trends Biotechnol.* 35, 728–742.
- Dorner, T., Lipsky, P.E., 2016. Beyond pan-B-cell-directed therapy—new avenues and insights into the pathogenesis of SLE. *Nat. Rev. Rheumatol.* 12, 645–657.
- Egerer, K., Roggenbuck, D., Hiemann, R., Weyer, M.G., Buettner, T., Radau, B., et al., 2010. Automated evaluation of autoantibodies on human epithelial-2 cells as an approach to standardize cell-based immunofluorescence tests. *Arthritis Res. Ther.* 12, R40.
- Ehrlich, P., 1900. On immunity with special reference to cell life. *Proc. R. Soc. Lond.* 66, 424–448.
- Elnady, B.M., Kamal, N.M., Shaker, R.H., Soliman, A.F., Hasan, W.A., Alghamdi, H.A., et al., 2016. Prevalence and clinical significance of non-organ specific antibodies in patients with autoimmune thyroiditis as predictor markers for rheumatic diseases. *Medicine (Baltimore)* 95, e4336.
- Enarson, P., Ratner, J.B., Ou, Y., Miyachi, K., Horigome, T., Fritzler, M.J., 2004. Autoantigens of the nuclear pore complex. *J. Mol. Med.* 82, 423–433.
- Fawcett, P.T., Rose, C.D., Gibney, K.M., Emerich, M.J., Athreya, B.H., Doughty, R.A., 1999. Use of ELISA to measure antinuclear antibodies in children with juvenile rheumatoid arthritis. *J. Rheumatol.* 26, 1822–1826.
- Feehally, J., 2017. Immunosuppression in IgA nephropathy: guideline medicine versus personalized medicine. *Semin. Nephrol.* 37, 464–477.
- Feltkamp, T.E.W., 1996. Antinuclear antibody determination in a routine laboratory. *Ann. Rheum. Dis.* 55, 723–727.
- Fenger, M., Wiik, A., Hoier-Madsen, M., Lykkegaard, J.J., Rozenfeld, T., Hansen, M.S., et al., 2004. Detection of antinuclear antibodies by solid-phase immunoassays and immunofluorescence analysis. *Clin. Chem.* 50, 2141–2147.
- Ferreira, B.I., Hill, R., Link, W., 2015. Special review: caught in the crosshairs: targeted drugs and personalized medicine. *Cancer J.* 21, 441–447.
- Fitch-Rogalsky, C., Steber, W., Mahler, M., Lupton, T., Martin, L., Barr, S.G., et al., 2014. Clinical and serological features of patients referred through a rheumatology triage system because of positive antinuclear antibodies. *PLoS One* 9, e93812.

- Frech, T.M., Pauling, J.D., Murtaugh, M.A., Kendall, K., Domsic, R.T., 2016. Sublingual abnormalities in systemic sclerosis. *J. Clin. Rheumatol.* 22, 19–21.
- Freeman, R.G., Raju, P.A., Norton, S.M., Walton, I.D., Smith, P.C., He, L., et al., 2005. Use of nanobarcodes particles in bioassays. *Methods Mol. Biol.* 303, 73–83.
- Fritzler, M.J., 2002. New technologies in the detection of autoantibodies. In: Conrad, K., Fritzler, M.J., Meurer, M., Sack, U., Shoenfeld, Y. (Eds.), *Autoantigens, Autoantibodies, Autoimmunity*, Third ed. Pabst Scientific Publishers, Lengerich, pp. 50–63.
- Fritzler, M.J., 2006. Advances and applications of multiplexed diagnostic technologies in autoimmune diseases. *Lupus* 15, 422–427.
- Fritzler, M.J., 2008. Challenges to the use of autoantibodies as predictors of disease onset, diagnosis and outcomes. *Autoimmun. Rev.* 7, 616–620.
- Fritzler, M.J., 2011a. Autoantibody testing: current challenges and future opportunities. In: Conrad, K., Chan, E.K.L., Fritzler, M.J., Humbel, R.L., Meroni, P.L., Shoenfeld, Y. (Eds.), *From Prediction to Prevention of Autoimmune Diseases*. Pabst Science Publishers, Berlin, pp. 584–596.
- Fritzler, M.J., 2011b. Personalized medicine approaches in rheumatoid arthritis and other systemic autoimmune rheumatic diseases. In: Conrad, K., Chan, E.K.L., Fritzler, M.J., Humbel, R.L., Meroni, P.L., Shoenfeld, Y. (Eds.), *From Prediction to Prevention of Autoimmune Diseases*. Pabst Science Publishers, Berlin, pp. 127–137.
- Fritzler, M.J., 2011c. The antinuclear antibody (ANA) test: last or lasting gasp? *Arthritis Rheum.* 63, 19–22.
- Fritzler, M.J., 2012. Toward a new autoantibody diagnostic orthodoxy: understanding the bad, good and indifferent. *Autoimmun. Highlights* 3, 51–58.
- Fritzler, M.J., 2015. Perspectives on the imperatives, opportunities and challenges for point of care diagnostics in systemic lupus erythematosus. *Int. J. Clin. Rheumatol.* 9, 449–456.
- Fritzler, M.J., 2016. Choosing wisely: review and commentary on anti-nuclear antibody (ANA) testing. *Autoimmun. Rev.* 15, 272–280.
- Fritzler, M.J., Fritzler, M.L., 2009. Microbead-based technologies in diagnostic autoantibody detection. *Expert Opin. Med. Diag.* 3, 81–89.
- Fritzler, M.J., Wiik, A., Fritzler, M.L., Barr, S.G., 2003a. The use and abuse of commercial kits used to detect autoantibodies. *Arthritis Res. Ther.* 5, 192–201.
- Fritzler, M.J., Wiik, A., Tan, E.M., Smolen, J.S., McDougal, J.S., Chan, E.K.L., et al., 2003b. A critical evaluation of enzyme immunoassay kits for detection of antinuclear antibodies of defined specificities. III. Comparative performance characteristics of academic and manufacturers' laboratories. *J. Rheumatol.* 30, 2374–2381.
- Fritzler, M.J., Stinton, L.M., Chan, E.K.L., 2007. Autoantibodies to cytoplasmic autoantigens in endosomes, exosomes and the Golgi complex. In: Conrad, K., Chan, E.K.L., Fritzler, M.J., Sack, U., Shoenfeld, Y., Wiik, A. (Eds.), *From Etiopathogenesis to the Prediction of Autoimmune Diseases: Relevance of Autoantibodies*, Fifth ed. Pabst Science Publishers, Lengerich, Germany, pp. 194–209.
- Fritzler, M.J., Ratner, J.B., Luft, L.M., Edworthy, S.M., Casiano, C.A., Peebles, C., et al., 2010. Historical perspectives on the discovery and elucidation of autoantibodies to centromere proteins (CENP) and the emerging importance of antibodies to CENP-F. *Autoimmun. Rev.* 10, 194–200.
- Gerstein, M., Sukhdeo, S., Levy, D.M., Feldman, B.M., Benseler, S.M., Ng, L.W., et al., 2014. A15: predicting macrophage activation syndrome in pediatric systemic lupus erythematosus patients at diagnosis. *Arthritis Rheumatol.* 66 (Suppl 11), S25. Available from: <https://doi.org/10.1002/art.38431:S25>.
- Graf, S.W., Hakendorf, P., Lester, S., Patterson, K., Walker, J.G., Smith, M.D., et al., 2012. South Australian Scleroderma Register: autoantibodies as predictive biomarkers of phenotype and outcome. *Int. J. Rheum. Dis.* 15, 102–109.
- Halbert, S.P., Karsh, J., Anken, M., 1981. Studies on autoantibodies to deoxyribonucleic acid and deoxyribonucleoprotein with enzyme-immunoassay (ELISA). *J. Lab. Clin. Med.* 97, 97–111.
- Hanly, J.G., Urowitz, M.B., Siannis, F., Farewell, V., Gordon, C., Bae, S.C., et al., 2008. Autoantibodies and neuropsychiatric events at the time of systemic lupus erythematosus diagnosis: results from an international inception cohort study. *Arthritis Rheum.* 58, 843–853.
- Hanly, J.G., Su, L., Farewell, V., Fritzler, M.J., 2010. Comparison between multiplex assays for autoantibody detection in systemic lupus erythematosus. *J. Immunol. Methods* 358, 75–80.
- Hargraves, M.M., Richmond, H., Morton, R., 1948. Presentation of two bone marrow elements: the "tart" cells and the "L.E." cell. *Mayo Clin. Proc.* 23, 25–28.
- Hiemann, R., Buttner, T., Krieger, T., Roggenbuck, D., Sack, U., Conrad, K., 2009. Challenges of automated screening and differentiation of non-organ specific autoantibodies on HEp-2 cells. *Autoimmun. Rev.* 9, 17–22.
- Hochberg, M.C., 1997. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* 40, 1725.
- Holborow, E.J., Weir, D.M., Johnson, G.D., 1957. A serum factor in lupus erythematosus with affinity for tissue nuclei. *Br Med. J.* 2, 732–734.
- Ippolito, A., Wallace, D.J., Gladman, D., Fortin, P.R., Urowitz, M., Werth, V., et al., 2011. Autoantibodies in systemic lupus erythematosus: comparison of historical and current assessment of seropositivity. *Lupus* 20, 250–255.
- Jennette, J.C., Falk, R.J., Bacon, P.A., Basu, N., Cid, M.C., Ferrario, F., et al., 2013. 2012 revised international Chapel Hill consensus conference nomenclature of vasculitides. *Arthritis Rheum.* 65, 1–11.
- Joh, D.Y., Hucknall, A.M., Wei, Q., Mason, K.A., Lund, M.L., Fontes, C.M., et al., 2017. Inkjet-printed point-of-care immunoassay on a nano-scale polymer brush enables subpicomolar detection of analytes in blood. *Proc. Natl. Acad. Sci. U.S.A.* 114, E7054–E7062.
- Kallenberg, C.G., 2011. Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis: where to go? *Clin. Exp. Immunol.* 164 (Suppl 1), 1–3. Available from: <https://doi.org/10.1111/j.1365-2249.2011.04355.x>:1-3.
- Kallenberg, C.G., Wouda, A.A., Hoet, M.H., Van Venrooij, W.J., 1988. Development of connective tissue disease in patients presenting with Raynaud's phenomenon: a six year follow up with emphasis on the predictive value of antinuclear antibodies as detected by immunoblotting. *Ann. Rheum. Dis.* 47, 634–641.
- Kasukawa, R., 1987. Preliminary diagnostic criteria for classification of mixed connective tissue disease. In: Kasukawa, R., Sharp, G.C. (Eds.), *Mixed Connective Tissue Disease and Anti-Nuclear Antibodies*. Excerpta Medica, Amsterdam, pp. 41–47.
- Kavanaugh, A.F., Solomon, D.H., American College of Rheumatology Ad Hoc Committee on Immunologic Testing Guidelines, 2002. Guidelines for immunologic laboratory testing in the rheumatic diseases: anti-DNA antibody tests. *Arthritis Rheum.* 47, 546–555.

- Kayser, C., Fritzler, M.J., 2015. Autoantibodies in systemic sclerosis: unanswered questions. *Front Immunol.* 6, 167.
- Keech, C.L., Howarth, S., Coates, T., Rischmueller, M., McCluskey, J., Gordon, T.P., 1996. Rapid and sensitive detection of anti-Ro (SS-A) antibodies by indirect immunofluorescence of 60 kDa Ro HEp-2 transfectants. *Pathology* 28, 54–57.
- Keren, D.F., Nakamura, R.M., 1997. Progress and controversies in autoimmune disease testing. *Clin. Lab. Med.* 17, 483–497.
- Kessenbrock, K., Rajmakers, R., Fritzler, M.J., Mahler, M., 2007. Synthetic peptides: the future of patient management in systemic rheumatic diseases? *Curr. Med. Chem.* 14, 2831–2838.
- Khosroshahi, A., Deshpande, V., Stone, J.H., 2011. The clinical and pathological features of IgG(4)-related disease. *Curr. Rheumatol. Rep.* 13, 473–481.
- Konstantinov, K.N., Rubin, R.L., 2017. The universe of ANA testing: a case for point-of-care ANA testing. *Autoimmun. Highlights* 8, 4.
- Kunkel, H.G., Tan, E.M., 1964. Autoantibodies and disease. *Adv. Immunol.* 4, 351–372.
- Kuwana, M., Kaburaki, J., Okano, Y., Tojo, T., Homma, M., 1994. Clinical and prognostic associations based on serum antinuclear antibodies in Japanese patients with systemic sclerosis. *Arthritis Rheum.* 37, 75–83.
- Lee, S.A., Kahng, J., Kim, Y., Park, Y.J., Han, K., Kwok, S.K., et al., 2012. Comparative study of immunofluorescent antinuclear antibody test and line immunoassay detecting 15 specific autoantibodies in patients with systemic rheumatic disease. *J. Clin. Lab. Anal.* 26, 307–314.
- Liao, K.P., Cai, T., Gainer, V., Goryachev, S., Zeng-treitler, Q., Raychaudhuri, S., et al., 2010. Electronic medical records for discovery research in rheumatoid arthritis. *Arthritis Care Res. (Hoboken)* 62, 1120–1127.
- Lundberg, I.E., Miller, F.W., Tjarnlund, A., Bottai, M., 2016. Diagnosis and classification of idiopathic inflammatory myopathies. *J. Intern. Med.* 280, 39–51.
- Mahajan, V.S., Mattoo, H., Deshpande, V., Pillai, S.S., Stone, J.H., 2014. IgG4-related disease. *Annu. Rev. Pathol.* 9, 315–347.
- Mahler, M., Fritzler, M.J., 2009. The changing landscape of the clinical value of the PM/Scl autoantibody system. *Arthritis Res. Ther.* 11, 106.
- Mahler, M., Fritzler, M.J., 2010. Epitope specificity and significance in systemic autoimmune diseases. *Ann. N. Y. Acad. Sci.* 1183, 267–287.
- Mahler, M., Fritzler, M.J., 2014. Antinuclear antibodies in children. *J. Rheumatol.* 41, 1260–1262.
- Mahler, M., Kessenbrock, K., Raats, J., Fritzler, M.J., 2004. Technical and clinical evaluation of anti-ribosomal P protein immunoassays. *J. Clin. Lab. Anal.* 18, 215–223.
- Mahler, M., Rajmakers, R., Fritzler, M.J., 2007. Challenges and controversies associated with systemic rheumatic diseases. *Curr. Rheumatol. Rev.* 3, 67–78.
- Mahler, M., Silverman, E.D., Fritzler, M., 2010. Novel diagnostic and clinical aspects of anti-PCNA antibodies detected by novel detection methods. *Lupus* 19, 1527–1533.
- Mahler, M., Binder, W.L., Fritzler, M.J., 2011a. Recent advances in peptide resolved diagnostics of systemic autoimmune diseases. In: Conrad, K., Chan, E.K.L., Fritzler, M.J., Humber, R.L., Meroni, P.L., Shoenfeld, Y. (Eds.), *From Prediction to Prevention of Autoimmune Diseases*. Pabst Science Publishers, Berlin, pp. 598–625.
- Mahler, M., Radice, A., Sinico, R.A., Damoiseaux, J., Seaman, A., Buckmelter, K., et al., 2011b. Performance evaluation of a novel chemiluminescence assay for detection of anti-GBM antibodies: an international multicenter study. *Nephrol. Dial. Transplant.* 27, 243–252.
- Mahler, M., Hanly, J.G., Fritzler, M.J., 2012. Importance of the dense fine speckled pattern on HEp-2 cells and anti-DFS70 antibodies for the diagnosis of systemic autoimmune diseases. *Autoimmun. Rev.* 11, 642–645.
- Mahler, M., Meroni, P.L., Bossuyt, X., Fritzler, M.J., 2014a. Current concepts and future directions for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. *J. Immunol. Res.* 2014, 315179.
- Mahler, M., Miller, F.W., Fritzler, M.J., 2014b. Idiopathic inflammatory myopathies and the anti-synthetase syndrome: a comprehensive review. *Autoimmun. Rev.* 13, 367–371.
- Mahler, M., Meroni, P.L., Andrade, L.E., Khamashta, M., Bizzaro, N., Casiano, C.A., et al., 2016. Towards a better understanding of the clinical association of anti-DFS70 autoantibodies. *Autoimmun. Rev.* 15, 198–201.
- Mahler, M., Andrade, L.E., Casiano, C.A., Malyavantham, K., Fritzler, M.J., 2019. Anti-DFS70 antibodies: an update on our current understanding and their clinical usefulness. *Expert. Rev. Clin. Immunol.* 15, 241–250.
- Malaviya, A.N., Gogia, S.B., 2010. Development, implementation and benefits of a rheumatology-specific electronic medical record application with automated display of outcome measures. *Int. J. Rheum. Dis.* 13, 347–360.
- Mariz, H.A., Sato, E.I., Barbosa, S.H., Rodrigues, S.H., Dellavance, A., Andrade, L.E., 2011. Pattern on the antinuclear antibody-HEp-2 test is a critical parameter for discriminating antinuclear antibody-positive healthy individuals and patients with autoimmune rheumatic diseases. *Arthritis Rheum.* 63, 191–200.
- Mehra, S., Walker, J., Patterson, K., Fritzler, M.J., 2013. Autoantibodies in systemic sclerosis. *Autoimmun. Rev.* 12, 350–354.
- Merkel, P., Silliman, N., Clements, P., Denton, C., Furst, D., Mayes, M., et al., 2012. Patterns and predictors of change in outcome measures in clinical trials in scleroderma: an individual patient meta-analysis of 629 subjects with diffuse cutaneous systemic sclerosis. *Arthritis Rheum.* 64 (10), 3420–3429.
- Meroni, P.L., Biggioggero, M., Pierangeli, S.S., Sheldon, J., Zegers, I., Borghi, M.O., 2014. Standardization of autoantibody testing: a paradigm for serology in rheumatic diseases. *Nat. Rev. Rheumatol.* 10, 35–43.
- Meroni, P.L., Shoenfeld, Y., 2008. Predictive, protective, orphan autoantibodies: The example of the anti-phospholipid antibodies. *Autoimmun. Rev.* 7, 585–587.
- Merrell, P.L., Schur, P.H., 2010. ANA screening: an old test with new recommendations. *Ann. Rheum. Dis.* 69, 1420–1422.
- Mustila, A., Paimela, L., Leirisalo-Repo, M., Huhtala, H., Miettinen, A., 2000. Antineutrophil cytoplasmic antibodies in patients with early rheumatoid arthritis - An early marker of progressive erosive disease. *Arthritis Rheum.* 43, 1371–1377.
- Nezlin, R., Mozes, E., 1995. Detection of antigens in immune complexes by a dot blot assay. *J. Immunol. Methods* 184, 273–276.
- Nihtyanova, S.I., Denton, C.P., 2017. Scleroderma lung involvement, autoantibodies, and outcome prediction: the confounding effect of time. *J. Rheumatol.* 44, 404–406.
- Nishimaki, T., Aotsuka, S., Kunieda, T., Yokohari, R., 1991. Preliminary criteria for the diagnosis of pulmonary hypertension in mixed connective tissue disease. *Ryumachi* 31, 159–166.

- Ochs, R.L., Mahler, M., Basu, A., Rios-Colon, L., Sanchez, T.W., Andrade, L.E., et al., 2016. The significance of autoantibodies to DFS70/LEDGFp75 in health and disease: integrating basic science with clinical understanding. *Clin. Exp. Med.* 16, 273–293.
- Olsen, N.J., Choi, M.Y., Fritzler, M.J., 2017. Emerging technologies in autoantibody testing for rheumatic diseases. *Arthritis Res. Ther.* 19, 172.
- Op De Beeck, K., Vermeersch, P., Verschueren, P., Westhovens, R., Marien, G., Blockmans, D., et al., 2011. Detection of antinuclear antibodies by indirect immunofluorescence and by solid phase assay. *Autoimmun. Rev.* 10, 801–808.
- Peene, I., Meheus, L., De, K.S., H umbel, R., Veys, E.M., De, K.F., 2002. Anti-Ro52 reactivity is an independent and additional serum marker in connective tissue disease. *Ann. Rheum. Dis.* 61, 929–933.
- Pérez, D., Gilburd, B., Cabrera-Marante, Ó., Martínez-Flores, J.A., Serrano, M., Naranjo, L., et al., 2018. Predictive autoimmunity using autoantibodies: screening for anti-nuclear antibodies. *Clin. Chem. Lab. Med.* 56, 1771–1777.
- Permin, H., Hørbov, S., Wiik, A., Knudsen, J.V., 1978. Antinuclear antibodies in juvenile chronic arthritis. *Acta Paediatr. Scand.* 67, 181–185.
- Petri, M., Orbai, A.M., Alarcon, G.S., Gordon, C., Merrill, J.T., Fortin, P.R., et al., 2012. Derivation and validation of systemic lupus international collaborating clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum.* 64, 2677–2686.
- Pincus, T., Mandelin, A.M., Swearingen, C.J., 2009. Flowsheets that include MDHAQ physical function, pain, global, and RAPID3 scores, laboratory tests, and medications to monitor patients with all rheumatic diseases: an electronic database for an electronic medical record. *Rheum. Dis. Clin. North Am.* 35, 829–842. x–xi.
- Pisetsky, D.S., 2017. Antinuclear antibody testing—misunderstood or misbegotten? *Nat. Rev. Rheumatol.* 13, 495–502.
- Plenge, R.M., Bridges Jr., S.L., 2011. Personalized medicine in rheumatoid arthritis: miles to go before we sleep. *Arthritis Rheum.* 63, 590–593.
- Pottel, H., Wiik, A., Locht, H., Gordon, T., Roberts-Thomson, P., Abraham, D., et al., 2004. Clinical optimization and multicenter validation of antigen-specific cut-off values on the INNO-LIA ANA update for the detection of autoantibodies in connective tissue disorders. *Clin. Exp. Rheumatol.* 22, 579–588.
- Reichlin, M., Harley, J.B., 1986. Detection by ELISA of antibodies to small RNA protein particles in SLE patients whose sera lack precipitins. *Trans. Assoc. Am. Physicians* 99, 161–171.
- Reimer, G., Raska, I., Tan, E.M., Scheer, U., 1987. Human autoantibodies: probes for nucleolus structure and function. *Virchows Arch. B* 54, 131–143.
- Renger, F., Bang, H., Feist, E., Fredenhagen, G., Natusch, A., Backhaus, M., et al., 2010. Immediate determination of ACPA and rheumatoid factor—a novel point of care test for detection of anti-MCV antibodies and rheumatoid factor using a lateral-flow immunoassay. *Arthritis Res. Ther.* 12, R120.
- Reveille, J.D., Solomon, D.H., The American College of Rheumatology Ad hoc Committee on Immunologic Testing Guidelines, 2003. Evidence-based guidelines for the use of immunologic tests: anticentromere, Scl-70, and nucleolar antibodies. *Arthritis Rheum.* 49, 399–412.
- Robinson, W.H., Steinman, L., Utz, P.J., 2002. Proteomics technologies for the study of autoimmune disease. *Arthritis Rheum.* 46, 885–893.
- Rohr, U.P., Binder, C., Dieterle, T., Giusti, F., Messina, C.G., Toerien, E., et al., 2016. The value of in vitro diagnostic testing in medical practice: a status report. *PLoS One* 11, e0149856.
- Ronnelid, J., 2015. The choice of laboratory methodology influences autoantibody test results. *Front Immunol.* 6, 392.
- Rose, N.R., 1996. Foreword—the uses of autoantibodies. In: Peter, J.B., Shoenfeld, Y. (Eds.), *Autoantibodies*. Elsevier, Amsterdam, pp. xxvii–xxix.
- Rose, N.R., 2007. Prediction and prevention of autoimmune disease: a personal perspective. *Ann. N. Y. Acad. Sci.* 1109, 117–128.
- Rubin, R.L., Konstantinov, K.N., 2016. Biosensor for total antinuclear antibody determination at the point-of-care. *Biosens. Bioelectron.* 83, 306–311.
- Rubin, R.L., Wall, D., Konstantinov, K.N., 2014. Electrochemical biosensor for quantitation of anti-DNA autoantibodies in human serum. *Biosens. Bioelectron.* 51, 177–183.
- Salazar, G.A., Assassi, S., Wigley, F., Hummers, L., Varga, J., Hinchcliff, M., et al., 2014. Antinuclear antibody-negative systemic sclerosis. *Semin. Arthritis Rheum.* 44, 680–686.
- Satoh, M., Vazquez-Del, M.M., Chan, E.K.L., 2009. Clinical interpretation of antinuclear antibody tests in systemic rheumatic diseases. *Mod. Rheumatol.* 19, 219–228.
- Schachna, L., Wigley, F.M., Morris, S., Gelber, A.C., Rosen, A., Casciola-Rosen, L., 2002. Recognition of granzyme B-generated autoantigen fragments in scleroderma patients with ischemic digital loss. *Arthritis Rheum.* 46, 1873–1884.
- Schulte-Pelkum, J., Fritzler, M., Mahler, M., 2009. Latest update on the Ro/SS-A autoantibody system. *Autoimmun. Rev.* 8, 632–637.
- Scussel-Lonzetti, L., Joyal, F., Raynauld, J.P., Roussin, A., Rich, É., Goulet, J.R., et al., 2002. Predicting mortality in systemic sclerosis—analysis of a cohort of 309 French Canadian patients with emphasis on features at diagnosis as predictive factors for survival. *Medicine (Baltimore)* 81, 154–167.
- Senecal, J.L., Dieudé, M., Koenig, M., 2008. Predictive value of antinuclear autoantibodies: the lessons of the systemic sclerosis autoantibodies. *Autoimmun. Rev.* 7, 588–593.
- Sherer, Y., Shoenfeld, Y., 2007. Autoantibody explosion in lupus—155 different autoantibodies in SLE. *Lupus* 16 (supp 1), 42.
- Sherer, Y., Gorstein, A., Fritzler, M.J., Shoenfeld, Y., 2004. Autoantibody explosion in systemic lupus erythematosus. *Semin. Arthritis Rheum.* 34, 501–537.
- Shoenfeld, Y., Cervera, R., Haass, M., Kallenberg, C., Khamashta, M., Meroni, P., et al., 2007. EASI—The European Autoimmunity Standardisation Initiative: a new initiative that can contribute to agreed diagnostic models of diagnosing autoimmune disorders throughout Europe. *Ann. N. Y. Acad. Sci.* 1109, 138–144.
- Shovman, O., Gilburd, B., Barzilai, O., Shinar, E., Larida, B., Zandman-Goddard, G., et al., 2005a. Evaluation of the BioPlex™ 2200 ANA screen: analysis of 510 healthy subjects: incidence of natural/predictive autoantibodies. *Ann. N. Y. Acad. Sci.* 1050, 380–388.
- Shovman, O., Gilburd, B., Zandman-Goddard, G., Yehiely, A., Langevitz, P., Shoenfeld, Y., 2005b. Multiplexed AthENA multi-lyte immunoassay for ANA screening in autoimmune diseases. *Autoimmunity* 38, 105–109.
- Slight-Webb, S., Lu, R., Ritterhouse, L.L., Munroe, M.E., Maecker, H.T., Fathman, C.G., et al., 2016. Autoantibody-positive healthy individuals display unique immune profiles that may regulate autoimmunity. *Arthritis Rheumatol.* 68, 2492–2502.

- Smolen, J.S., Butcher, B., Fritzler, M.J., Gordon, T., Hardin, J., Kalden, J.R., et al., 1997. Reference sera for antinuclear antibodies. II. Further definition of antibody specificities in international antinuclear antibody reference sera by immunofluorescence and Western immunoblotting. *Arthritis Rheum.* 40, 413–418.
- Sokolove, J., Lindstrom, T.M., Robinson, W.H., 2012. Development and deployment of antigen arrays for investigation of B-cell fine specificity in autoimmune disease. *Front Biosci. (Elite Ed)* 4, 320–330.
- Solomon, D.H., Kavanaugh, A.J., Schur, P.H., 2002. Evidence-based guidelines for the use of immunologic tests: antinuclear antibody testing. *Arthritis Rheum.* 47, 434–444.
- Somani, A.K., Swick, A.R., Cooper, K.D., McCormick, T.S., 2008. Severe dermatomyositis triggered by interferon beta-1a therapy and associated with enhanced type I interferon signaling. *Arch. Dermatol.* 144, 1341–1349.
- Stinton, L.M., Eystathioy, T., Selak, S., Chan, E.K.L., Fritzler, M.J., 2004. Autoantibodies to protein transport and messenger RNA processing pathways: endosomes, lysosomes, Golgi complex, proteasomes, assemblyosomes, exosomes and GW Bodies. *Clin. Immunol.* 110, 30–44.
- Stinton, L.M., Fritzler, M.J., 2007. A clinical approach to autoantibody testing in systemic autoimmune rheumatic disorders. *Autoimmun. Rev.* 7, 77–84.
- Stinton, L.M., Swain, M., Myers, R.P., Shaheen, A.A., Fritzler, M.J., 2010. Autoantibodies to GW bodies and other autoantigens in primary biliary cirrhosis. *Clin. Exp. Immunol.* 163, 147–156.
- Stott, D.I., 1989. Immunoblotting and dot blotting. *J. Immunol. Methods* 119, 153–187.
- Suzuki, S., Yonekawa, T., Kuwana, M., Hayashi, Y.K., Okazaki, Y., Kawaguchi, Y., et al., 2014. Clinical and histological findings associated with autoantibodies detected by RNA immunoprecipitation in inflammatory myopathies. *J. Neuroimmunol.* 74, 202–208.
- Tak, P.P., 2012. A personalized medicine approach to biological treatment of rheumatoid arthritis: a preliminary treatment algorithm. *Rheumatology (Oxford)* 51, 600–609.
- Tan, E.M., 1989. Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. *Adv. Immunol.* 44, 93–151.
- Tan, E.M., Feltkamp, T.E.W., Smolen, J.S., Butcher, B., Dawkins, R., Fritzler, M.J., et al., 1997. Range of antinuclear antibodies in "healthy" individuals. *Arthritis Rheum.* 40, 1601–1611.
- Tan, E.M., Smolen, J., McDougal, J.S., Butcher, B.T., Conn, D., Dawkins, R., et al., 1999. A critical evaluation of enzyme immunoassays for the detection of antinuclear antibodies of defined specificities. I. Precision, sensitivity and specificity. *Arthritis Rheum.* 42, 455–464.
- Tansley, S.L., Betteridge, Z.E., McHugh, N.J., 2013. The diagnostic utility of autoantibodies in adult and juvenile myositis. *Curr. Opin. Rheumatol.* 25, 772–777.
- Targoff, I.N., 2002. Laboratory testing in the diagnosis and management of idiopathic inflammatory myopathies. *Rheum. Dis. Clin. North Am.* 28, 859–890. viii.
- Tonutti, E., Bassetti, D., Piazza, A., Visentini, D., Poletto, M., Bassetto, F., et al., 2004. Diagnostic accuracy of ELISA methods as an alternative screening test to indirect immunofluorescence for the detection of antinuclear antibodies. Evaluation of five commercial kits. *Autoimmunity* 37, 171–176.
- van der Vaart, V., Drossaert, C.H., Taal, E., van de Laar, M.A., 2013. Giving rheumatology patients online home access to their electronic medical record (EMR): advantages, drawbacks and preconditions according to care providers. *Rheumatol. Int.* 33, 2405–2410.
- van Eenennaam, H., Vogelzangs, J.H.P., Bisschops, L., Te Boome, L.C.J., Seelig, H.P., Renz, M., et al., 2002. Autoantibodies against small nucleolar ribonucleoprotein complexes and their clinical associations. *Clin. Exp. Immunol.* 130, 532–540.
- Van Praet, J.T., Van, S.K., Smith, V., De, B.G., Mimori, T., Bonroy, C., et al., 2011. Specific anti-nuclear antibodies in systemic sclerosis patients with and without skin involvement: an extended methodological approach. *Rheumatology (Oxford)* 50, 1302–1309.
- Vasiliauskienė, L., Wiik, A., Hoier-Madsen, M., 2001. Prevalence and clinical significance of antikeratin antibodies and other serological markers in Lithuanian patients with rheumatoid arthritis. *Ann. Rheum. Dis.* 60, 459–466.
- Vermeersch, P., De Beeck, K.O., Lauwers, B.R., Van den Berghe, K., Develder, M., Marien, G., et al., 2009. Antinuclear antibodies directed against proliferating cell nuclear antigen are not specifically associated with systemic lupus erythematosus. *Ann. Rheum. Dis.* 68, 1791–1793.
- Villalta, D., Imbastaro, T., Di, G.S., Lauriti, C., Gabini, M., Turi, M.C., et al., 2012. Diagnostic accuracy and predictive value of extended auto-antibody profile in systemic sclerosis. *Autoimmun. Rev.* 12, 114–120.
- Visser, H., Cessie, S., Vos, K., Breedveld, F.C., Hazes, J.M.W., 2002. How to diagnose rheumatoid arthritis early. *Arthritis Rheum.* 46, 357–365.
- Vogt, T.M., Aickin, M., Ahmed, F., Schmidt, M., 2004. The prevention index: using technology to improve quality assessment. *Health Serv. Res.* 39, 511–530.
- von Muhlen, C.A., Tan, E.M., 1995. Autoantibodies in the diagnosis of systemic rheumatic disease. *Semin. Arthritis Rheum.* 24, 323–358.
- Walker, U.A., Tyndall, A., Czirjak, L., Denton, C.P., Farge, B.D., Kowal-Bielecka, O., et al., 2007. Clinical risk assessment of organ manifestations in systemic sclerosis—a report from the EULAR Scleroderma Trials And Research (EUSTAR) group data base. *Ann. Rheum. Dis.* 66, 754–763.
- Wallace, D.J., 2006. Commentary: new methods for anti-nuclear antibody testing: does it cut costs and corners without jeopardizing clinical reliability? *Nat. Clin. Pract. Rheumatol.* 2, 410–411.
- Warde, N., 2011. Connective tissue diseases: agonistic autoantibodies: do they have a role in the pathophysiology of SSc? *Nat. Rev. Rheumatol.* 7, 71.
- Wassermann, V.A., Neisser, A., Bruck, C., 1906. Eine serodiagnostische reaktion bei syphilis. *Dtsch. Med. Wochenschr.* 32 (19), 745–746.
- Watad, A., Agmon-Levin, N., Gilburd, B., Lidar, M., Amital, H., Shoenfeld, Y., 2014. Predictive value of anti-citrullinated peptide antibodies: a real life experience. *Immunol. Res.* 60, 348–355.
- Welting, T.J., Raijmakers, R., Pruijn, G.J., 2003. Autoantigenicity of nucleolar complexes. *Autoimmun. Rev.* 2, 313–321.
- Whelan, B.R., Isenberg, D.A., 2009. Poor response of anti-SRP-positive idiopathic immune myositis to B-cell depletion. *Rheumatology (Oxford)* 48, 594–595.
- Wigley, F.M., Wise, R.A., Miller, R., Needlemann, B.W., Spence, R.J., 1992. Anticentromere antibody as a predictor of digital ischemic loss in patients with systemic sclerosis. *Arthritis Rheum.* 35, 688–693.
- Wiik, A., 2001. Methods for the detection of anti-neutrophil cytoplasmic antibodies. Recommendations for clinical use of ANCA serology and laboratory efforts to optimize the informative value of ANCA test results. *Springer Semin. Immunopathol.* 23, 217–229.

- Wiik, A., 2003a. Testing for ANA and ANCA-diagnostic value and pitfalls. In: Hochberg, M.C., Silman, A.J., Smolen, J.S., Weinblatt, M.E., Weisman, M.H. (Eds.), *Rheumatology*, Third ed. Mosby, Edinburgh, pp. 215–226.
- Wiik, A.S., 2003b. Appropriateness of autoantibody testing in clinical medicine. *Clin. Chim. Acta* 333, 177–180.
- Wiik, A., Lam, K., 2001. Report to the European Commission: on the usability of extended DOORS software for education and training, quality assurance and consensus formation. Report No.: Deliverable D 09, version 2.1.
- Wiik, A.S., Gordon, T.P., Kavanaugh, A.F., Lahita, R.G., Reeves, W., Van Venrooij, W.J., et al., 2004. Cutting edge diagnostics in rheumatology: on the role of patients, clinicians, and laboratory scientists in optimizing the use of autoimmune serology. *Arthritis Care Res.* 51, 291–298.
- Wiik, A., Cervera, R., Haass, M., Kallenberg, C., Khamashta, M., Meroni, P.L., et al., 2006. European attempts to set guidelines for improving diagnostics of autoimmune rheumatic disorders. *Lupus* 15, 391–396.
- Wiik, A.S., Hoier-Madsen, M., Forslid, J., Charles, P., Meyrowitsch, J., 2010. Antinuclear antibodies: a contemporary nomenclature using HEp-2 cells. *J. Autoimmun.* 35, 276–290.
- Wolfson, A.R., Hamilos, D.L., 2017. Recent advances in understanding and managing IgG4-related disease. *F1000Res* 6, F1000–F1185.
- Yamasaki, Y., Narain, S., Yoshida, H., Hernandez, L., Barker, T., Hahn, P.C., et al., 2007. Autoantibodies to RNA helicase A: a new serologic marker of early lupus. *Arthritis Rheum.* 56, 596–604.
- Yazdany, J., Schmajuk, G., Robbins, M., Daikh, D., Beall, A., Yelin, E., et al., 2013. Choosing wisely: the American College of Rheumatology's top 5 list of things physicians and patients should question. *Arthritis Care Res. (Hoboken)* 65, 329–339.

# Prevention of Autoimmune Disease: The Type 1 Diabetes Paradigm

*Leonard C. Harrison and John M. Wentworth*

Walter & Eliza Hall Institute of Medical Research, Parkville, VIC, Australia

## OUTLINE

Overview of Type 1 Diabetes	1391	Mucosa-Mediated Antigen-Specific Tolerance	1403
Autoimmune Pathology	1391	Trials of Islet Autoantigen-Specific Vaccination in	
Nature and Nurture	1393	Humans	1404
Prevention of Type 1 Diabetes	1395	Epilogue	1406
Primary Prevention	1400	Acknowledgments	1407
Diet and Gut Microbiome Modification	1401	References	1407
Virus Vaccination	1402		
Antigen-Specific Immunotherapy	1402	Further Reading	1413
Secondary Prevention	1402		

## OVERVIEW OF TYPE 1 DIABETES

### Autoimmune Pathology

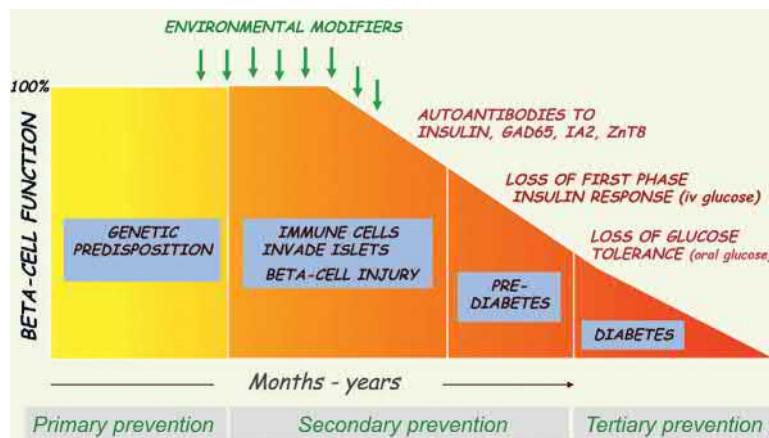
From the 1970s, it has gradually become clearer that type 1 diabetes (T1D) is an autoimmune disease (Eisenbarth, 1986). The evidence includes infiltration of the islets by immune cells (Gepts, 1965; Foulis et al., 1986), strong genetic risk associated with specific classes I and II human leukocyte antigen (HLA) genes coding for molecules that present peptide antigens to T cells, as well as with the insulin gene and a range of immune genes (Concannon et al., 2009), circulating autoantibodies to beta-cell antigens (Verge et al., 1996; Colman et al., 2000; Bingley et al., 1999; Ziegler et al., 2013), T cells in the blood (Harrison et al., 1993; Mannerling et al., 2005) and pancreas (Pathiraja et al., 2015) that recognize insulin and other beta-cell antigens, a report of recurrent immune beta-cell destruction after pancreatic isograft transplantation without immunosuppression between identical twins discordant for T1D (Sutherland et al., 1989), occasional reports of T1D after bone marrow transplantation from a T1D donor (Lampeter et al., 1998a,b) and induction of T1D by immune checkpoint inhibitor antibodies (Kapke et al., 2017). Beta-cell destruction in T1D is mediated by autoreactive T cells, the ultimate effectors being CD8 cytotoxic T cells. The evidence for this is unequivocal in the inbred nonobese mouse (NOD) model of T1D, which shares a number of key features with T1D in outbred humans (Leiter et al., 1987; Adorini et al., 2002).

The molecular mechanisms of beta-cell death, gleaned mostly from the NOD mouse, encompass a combination of apoptosis induced by activation of extrinsic (e.g., TNF receptor or Fas ligation) or intrinsic (e.g., endoplasmic reticulum stress) death pathways and necroptosis induced by cytotoxic CD8 T-cell granule components

(granzymes and perforin), reactive oxygen species, or ischemia. The NOD mouse has also served to demonstrate proof-of-principle for preventative therapies that could translate to humans.

Two major distinctions between T1D and other autoimmune diseases, that allow those at risk to be identified and, therefore, are requisite for prevention, are the major contribution of HLA genes to risk and the ability to identify children many months to years before eventual loss of most beta-cell function leads to clinical presentation (Fig. 70.1). Birth cohort studies of children with a T1D relative have shown that the development of diabetes by the age of 18 years is almost always associated with the appearance of islet autoantibodies in the first years of life. Of children with two or more islet autoantibodies before the age of 3 years, 57% (95% CI: 51.7%–62.3%) and 74.8% (95% CI: 69.7%–79.9%) progressed to diabetes by 6 and 10 years, respectively; with a single islet autoantibody, 14.5% progressed to diabetes by 10 years (Ziegler et al., 2013). In order to prevent T1D, a paradigm shift is necessary to redefine the disease as an autoimmune beta-cell disorder from the beginning of the asymptomatic phase of islet autoimmunity (Stage 1), rather than as a metabolic disorder of end-stage pathology (Stage 3) (Couper and Harrison, in press).

In children, the diagnosis of T1D at clinical presentation is usually based on symptoms and signs, but can be confirmed, and in older individuals established, by detecting circulating islet autoantibodies to beta-cell antigens, to insulin (IAA), glutamic acid decarboxylase 65,000 mol. wt. isoform (GADA), insulinoma-like antigen-2 (IA-2A), and zinc transporter-8. One or more autoantibody specificities is present in at least 90% of Caucasian children with T1D compared to ~1% of the general population, but in only ~50% of the Hispanic-American and African-American children diagnosed with T1D. IAA are more often the first sign of islet autoimmunity in children followed from birth and is the most predictive autoantibody. A family history of T1D in close relatives is present in only 10%–15% of newly diagnosed cases. However, affected families have provided major insights into the genetics and natural history of T1D. In T1D relatives the rate of progression to clinical diabetes is positively associated with the number and titer of islet autoantibodies (Verge et al., 1996; Bingley et al., 1999; Colman et al., 2000; Ziegler et al., 2013), the number and type of HLA classes I and II risk alleles (Honeyman et al., 1995; Tait et al., 1995) and the degree of insulin resistance (Fourlanos et al., 2004) and is negatively associated with age (Table 70.1).



**FIGURE 70.1** Stages in the natural history of type 1 diabetes.

**TABLE 70.1** Markers of Risk for Diabetes in Islet Autoantibody-Positive Relatives

- Number of antigen specificities of islet autoantibodies
- Antigen specificity of islet autoantibody
- Level of islet autoantibody
- Age at detection of islet autoantibody
- FPIR to i.v. glucose
- Insulin resistance, for example, estimated as HOMA-R
- HLA alleles for risk or protection
- HLA haplotype sharing with proband
- Kinship with proband
- Body mass index

FPIR, First phase insulin response; HOMA-R, Homeostatic model assessment-insulin resistance.

## Nature and Nurture

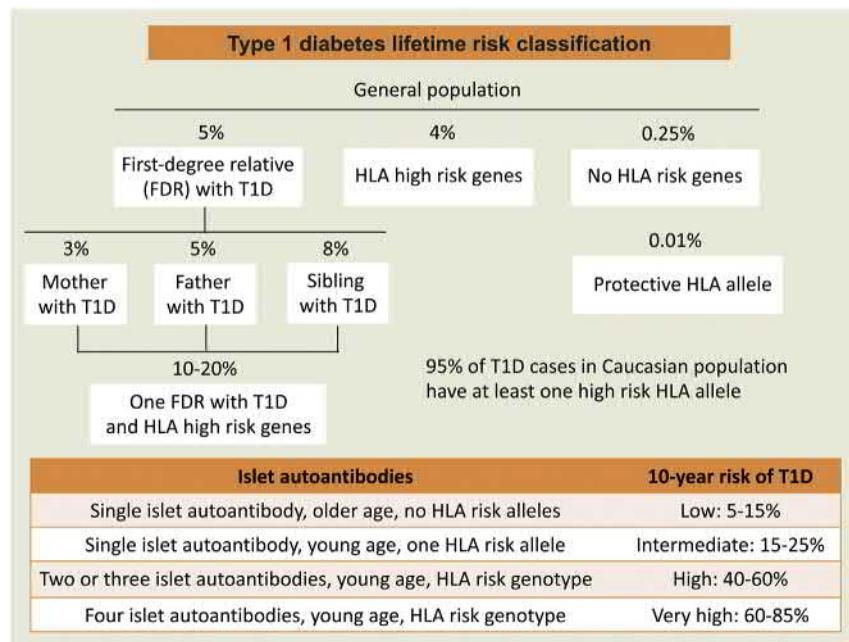
The incidence of T1D is highest in Caucasian northwest Europeans. This reflects the distribution of specific risk HLA genes, which account for up to 50% of the lifetime risk for T1D (Table 70.2).

However, the incidence of T1D has been rising on a background of lower risk HLA alleles. In Western European societies, the incidence in childhood has more than doubled since the 1980s and has been rising at ~3% annually, particularly in younger children (Gale, 2002). The same trend is beginning to be seen in countries such as Kuwait and Saudi Arabia, and areas of India and China that have adopted Western lifestyles, but where the prevalence of high-risk HLA genotypes for T1D is much lower. Environmental factors may increase the penetrance of risk genes for T1D. In the case of HLA genes, the increasing incidence of T1D is accounted for by children with intermediate (DR 4, 4 or DR 3, 3) or low (DR 4, X or DR 3, X) risk phenotypes, not the highest risk HLA phenotypes (DR 3, 4; DQ 2, 8) (Fourlanos et al., 2008). Interestingly, these lower risk HLA phenotypes are the ones seen in non-Caucasians and in adults presenting with T1D.

The environment in Western societies has changed dramatically during the last century including in ways that have been associated either epidemiologically or in animal studies with a rising incidence of T1D (Wentworth et al., 2009). A marker of the modern “exposome” is obesity, associated with insulin resistance and type 2 diabetes, and with alterations in the gut microbiome. When children at increased genetic risk for T1D (with an affected first-degree relative) were monitored from birth, weight gain in the first 2–3 years of life was a risk factor for islet autoimmunity (Couper et al., 2009). In at-risk children who developed islet autoantibodies, insulin resistance was an independent marker of those who progressed most rapidly to clinical diabetes (Fourlanos et al., 2004). Whether insulin resistance promotes the development of islet autoimmunity is an important question that can be answered by an ongoing pregnancy–birth cohort study (Penn et al., 2013). Thus, insulin resistance associated with obesity could synergize with impaired beta-cell function to accelerate the development of T1D, justifying attention to environment–lifestyle factors to forestall or prevent T1D.

Obesity is the outcome of increased energy consumption and changed diet composition, both readily provided by the modern “Western” diet. This diet lacks diversity of components, lacks plant-derived prebiotics and complex carbohydrates (starches and fiber), is high in saturated fats, sucrose, and fructose, and contains artificial preservatives, emulsifiers, and sweeteners. All of these alter the composition of the gut microbiome and reduce its diversity, which are features of the gut microbiome in children at risk (Dunne et al., 2014;

**TABLE 70.2** Lifetime Risks for Type 1 Diabetes



**Knip and Siljander, 2016).** Diets containing a diverse range of plant products (cereals, fresh fruits vegetables, nuts, seeds) provide complex carbohydrates for fermentation by colonic bacteria to short-chain fatty acids such as butyrate, propionate, and acetate, and other antiinflammatory products (Thorburn et al., 2014).

Microbial colonization of the gut is required for development of a normal immune system and the maintenance of gut epithelial homeostasis and “barrier function” mediated by products such as butyrate and mucins (Yu et al. 2012). It is no surprise therefore that the microbiome—the trillions of microorganisms (bacteria, fungi, archaea, protozoa, viruses) and their millions of genes and proteins that reside within our mucosae, skin, and secretions at the interface with the world—has come under increasing focus as a bellwether of health and disease.

In the NOD mouse, the incidence of autoimmune diabetes is markedly altered by changes in the microbiome in combination with diet. The incidence of spontaneous diabetes in NOD mice differs greatly between colonies around the world and is inversely correlated with exposure to microbial infection (Pozzilli et al., 1993). The high incidence of diabetes in NOD mice housed under pathogen-free conditions is reduced by conventional conditions of housing and feeding (Suzuki, 1987). Under such conditions, bacterial colonization of the intestine is accompanied by maturation of mucosal immune function (Kawaguchi-Miyashita et al., 1996). In germ-free on a defined, sterile diet the incidence of disease compared to specific pathogen-free (SPF) mice is accelerated and increased from 70% to 100% (Mariño et al., 2017). Provision of a diet high in butyrate and acetate to NOD mice in SPF conditions almost totally prevented diabetes (Mariño et al., 2017). The counterpart in humans may be modern Finland and its Russian neighbor Karelia. Finland has the highest incidence of T1D in the world, currently 57.6 cases/100,000 population  $\leq$  14 years ([www.diabetesatlas.org](http://www.diabetesatlas.org)). In 2005, there was a 6-fold difference in the incidence of T1D between Finland and Karelia, despite overlap in ethnic background and a similar distribution of high-risk HLA genotypes (Kondrashova et al., 2005). This marked difference in the incidence of T1D is associated in Finnish children with decreased gut bacterial microbiome diversity, a dominance of the phylum Bacteroidetes over Firmicutes and a deficiency of butyrate- and mucin-producing bacteria (Kostic et al., 2015). These changes were seen after the appearance of autoantibodies, suggesting that they followed rather than preceded the disease process. However, a further small study in Finnish children identified a relative abundance of *Bacteroides dorei*, which peaked around 7–8 months of age with the introduction of solids and preceded the appearance of islet autoantibodies (Davis-Richardson et al., 2014). Gut *Bacteroides* species are abundant in Finnish children, including *B. dorei*, which produces a lipopolysaccharide (LPS) endotoxin that inhibits the immunostimulatory activity of *Escherichia coli* LPS, known to protect against diabetes development in NOD mice (Vatanen et al., 2016). Individuals with T1D and even those with islet autoantibodies at risk for T1D, have impaired barrier function with increased “leakiness” through intercellular gap junctions (Bosi et al., 2006), consistent with the descriptions of the altered composition and decreased diversity of the microbiome in T1D. We noted some years ago that “gut leakiness,” reflected by increased titers of antibodies to food components, was present even in T1D and celiac disease relatives with the HLA A1-B8-DR3-DQ2 risk haplotype (Harrison and Honeyman, 1999). This is consistent with more recent evidence for a relationship between the microbiome and host genome. The question, does “gut leakiness” precede the development of islet autoimmunity, and how does it relate to the microbiome and to T1D genetics, has not been fully answered. We found that among asymptomatic children with islet autoantibodies those who progressed most rapidly to diabetes had lower gut microbial diversity with deficiency of the *Prevotella* genus and increased gut permeability (Harbison et al., 2018).

The presentation of clinical T1D peaks in winter (Moltchanova et al., 2009), attributed to environmental factors such as increased number of virus infections and a decrease in vitamin D. However, given the long presymptomatic stage of disease, these would appear to represent nonspecific precipitants. Viruses, in particular enteroviruses, are proposed as a cause of T1D but the evidence remains circumstantial (Honeyman 2005; Roivainen and Klingel 2010). Viral mechanisms in T1D could be direct or indirect, for example, infection of  $\beta$  cells, infection of the exocrine pancreas with bystander death of  $\beta$  cells, mimicry between T-cell epitopes in a viral protein and beta-cell autoantigens, or activation of endogenous retroviruses in  $\beta$  cells by environmental agents. If an exogenous virus was clearly identified then protective vaccination in early in life would be an approach to primary prevention. However, if a specific enterovirus strain was shown to be diabetogenic, creating a vaccine may be challenging because among the many thousands of strain variants, the only one for which a vaccine currently exists is poliovirus. The first virus to be associated with T1D was rubella (Forrest et al., 1971). Children with congenital rubella born to mothers who contracted rubella early in pregnancy had evidence of infection in the brain, pancreas, and other tissues and 20% developed insulin-dependent diabetes (Menser et al., 1978). Subsequently, almost twice this proportion was reported to develop islet cell antibodies (ICAs) (Ginsberg-Fellner et al., 1985). Children with congenital rubella and ensuing diabetes had a higher frequency of the T1D susceptibility HLA class I phenotype A1 (Menser et al., 1974), on the risk haplotype HLA A1-B8-[DR3-DQ2]. Rubella vaccine virtually eliminated

congenital rubella but obviously not T1D; clearly many other environmental factors must be involved, mumps virus epidemics have been associated with T1D onset after 2–4 years and introduction of a mumps vaccine was associated with a plateau in the rising incidence of T1D in Finland, but this was temporary and mumps vaccination has clearly not prevented T1D. Rotavirus (RV) is the commonest cause of gastroenteritis in young children. The discovery of strong sequence similarities between T-cell epitopes in the VP7 protein of rotavirus and GAD and IA-2 islet antigens in autoantibody-positive children led to speculation that mimicry with rotavirus might contribute to islet autoimmunity. Subsequently, in the Australian BabyDiab Study, rotavirus infection was associated with the first appearance of or an increase in islet autoantibodies in children (Honeyman et al., 2000). Moreover, rotavirus infects  $\beta$  cells in islets of mice, pigs, and monkeys, and was recently shown to cause transient involution of the pancreas and hyperglycemia in a toll-like receptor (TLR)-3-dependent manner in mice (Honeyman et al., 2014). Ubiquitous rotavirus infections that drive cross-reactive immunity to islet autoantigens are unlikely to be diabetogenic but could complement and sustain the immune response following direct infection of  $\beta$  cells. Recently, in Australian children aged less than 4 years, it was shown that the number of incident cases of T1D has decreased by 14% (RR 0.86) following the introduction of oral RV vaccine in 2007 (Perrett et al., 2019). As with mumps, the significance of this observation requires ongoing surveillance, and confirmation from an ongoing case-control linkage study.

## PREVENTION OF TYPE 1 DIABETES

Progress in understanding mechanisms of beta-cell destruction, the ability to identify individuals at high risk for T1D, and proof-of-principle for preventative therapies in the NOD mouse model set the scene for preventing or arresting autoimmune beta-cell damage in humans. This goal is relevant not only to individuals at risk but to those with clinical diabetes, in order to preserve residual beta-cell function, permit possible beta-cell regeneration and prevent recurrent autoimmune disease after therapeutic beta-cell replacement or regeneration. It will be eminently more achievable with increasing acceptance that T1D is an autoimmune disease that begins early in life, which is when intervention for primary or secondary prevention should logically begin and not at the time of end-stage pathology and clinical presentation. Newer biologic agents that are disease-sparing in autoimmune diseases such as rheumatoid arthritis would never be expected to reverse end-stage joint pathology. The caveat is that any form of treatment given to asymptomatic, at-risk children must have an excellent safety profile—*“Primum non nocere”* (first do no harm)—because even in islet autoantibody-positive children prediction of clinical disease is not 100%.

Population heterogeneity is a critical consideration in the design and interpretation of clinical trials. In addition to HLA genes, over 50 genetic loci are associated with T1D, but very little is known about how they contribute to disease development in different environments or influence prevention strategies. Although a restricted set of HLA genes is shared among individuals with T1D, HLA-based heterogeneity in age at clinical presentation is well known (Honeyman et al., 1995; Tait et al., 1995). This suggests that T1D comprises disease subtypes and that prevention will most likely require a more “personalized” approach. Indeed, it is known that the natural history of declining beta-cell function after diagnosis depends on age, HLA status, autoimmune status including number and level of islet autoantibodies, residual beta-cell function, and insulin resistance (Greenbaum and Harrison, 2003). Inclusion of T1D relatives in secondary prevention trials has been based on age (<40) and islet autoantibodies ( $\geq 2$ ), for a predicted 5-year incidence of ~40%, but more refinement is possible by building subtype analysis into trial design. Up to 10% of adults presenting with diabetes have what appears to be a slowly progressive form of T1D, associated mainly with GAD65 autoantibodies, which initially is noninsulin-requiring (Gottsater et al., 1995; Hagopian et al., 1993; Tuomi et al., 1993; Turner et al., 1997). They have higher residual beta-cell function at diagnosis than younger patients with classical T1D, which implies a wider and perhaps more penetrable therapeutic window for secondary prevention (Fourlanos et al., 2005). The “personalized” approach requires new robust surrogate assays of disease mechanisms to identify people most likely to benefit from a specific therapy and allow the design of more practical, cheaper, and efficient boutique trials, rather than larger, expensive trials powered on the endpoint of diabetes.

The number of candidate agents that fulfill scientific and ethical criteria for primary or secondary prevention trials is limited and recruiting individuals for these trials is a significant logistical exercise. Consequently, of the many clinical trials undertaken for “prevention” of T1D since the 1980s, most have been tertiary trials in individuals with recent-onset clinical T1D. A comprehensive listing of these trials is provided (Table 70.3), in which classification by agent necessitates combining the categories of secondary and tertiary prevention. The primary outcome measure in

**TABLE 70.3** Trials for Prevention of Type 1 Diabetes

	Participants (n)	Follow-up (months)	Outcome	Reference
<b>PRIMARY PREVENTION</b>				
Cow's milk elimination (trial to reduce IDDM in the genetically at-risk TRIGR)	FDRs with HLA risk (150)	≥ 36	No change in incidence of islet autoantibodies or diabetes	Hummel et al. (2011)
Elimination of bovine insulin from infant formula (FINDIA study)	HLA at-risk infants (1113)	36	Decrease in incidence of islet autoantibodies	Vaarala et al. (2012)
Weaning to hydrolyzed casein formula vs cow's milk + 20%casein formula (TRIGR study)	HLA at-risk infants (2159)	138 m (median)	No change in incidence of islet antibodies or of diabetes	Knip et al. (2011), Knip et al. (2014), and Knip et al. (2018)
<b>SECONDARY AND TERTIARY PREVENTION</b>				
<b>Nonspecific immune suppression</b>				
Azathioprine (2 mg/kg/day for up to 12 months)	RD (24)	12	Increased basal and glucagon-stimulated C-peptide, and more remissions	Harrison et al. (1985)
Cyclosporine (7.5 mg/kg/day for up to 9 months)	RD (122)	9	More remissions	Feutren et al. (1986)
Azathioprine (2 mg/kg/day for a year) + prednisolone (decreasing dose over 10 weeks)	RD (46)	12	Increased meal-stimulated C-peptide and decreased insulin dose	Silverstein et al. (1988)
Cyclosporine (20 mg/kg/day for 1 year)	RD (188)	12	Increased glucagon-stimulated C-peptide and more remissions, especially in recently diagnosed	Canadian–European Randomized Control Trial Group (1988)
Azathioprine (2 mg/kg/day for 1 year)	RD (49)	12	Increased meal-stimulated C-peptide	Cook et al. (1989)
Azathioprine, thymostimulin, or the combination	RD (45)	12	Increased glucagon-stimulated C-peptide and more remissions in combination group	Moncada et al. (1990)
Cyclosporine (10 mg/kg/day for up to 2 years)	RD (219)	24	Increased meal-stimulated C-peptide and more remissions	Assan et al. (1990)
Cyclosporine (10 mg/kg/day for 4 months)	RD (43)	36	No difference in glucagon-stimulated C-peptide, HbA1C, or insulin dose	Chase et al. (1990a)
Prednisolone (15 mg/day for 8 months) or indomethacin (100 mg/day for 8 months)	RD (25)	24	Decreased insulin dose and increased urine C-peptide in prednisolone group	Secchi et al. (1990)
Cyclosporine (10 mg/kg/day for 1 year)	RD (23)	12	Increased meal—but not glucagon—or glucose-stimulated C-peptide. No difference in insulin dose	Skyler and Rabinovitch, (1992)
Anti-CD5 mAb/ricin A chain anti-T-cell therapy (not blinded)	RD (15)	12	Dose-dependent preservation of meal-stimulated C-peptide	Skyler et al. (1993)
Prednisone (1 mg/kg/day tapered over 50 days)	RD (32)	12	Increased glucagon-stimulated C-peptide, but no remissions	Goday et al. (1993)
Anti-CD4 mAb + prednisolone	RD (12)	12	No difference in insulin dose, or islet antibody titers	Kohnert et al. (1996)
Methotrexate (5 mg/m <sup>2</sup> /week; not blinded)	RD (10)	36	No effect on basal or meal-stimulated C-peptide. Insulin dose increased.	Buckingham and Sandborg (2000)
Teplizumab (OKT3γ1(Ala-Ala) anti-CD3 mAb; not blinded)	RD (18)	12	Increase in meal-stimulated C-peptide in first year. IL-10 detected in serum	Herold et al. (2002)
Teplizumab [OKT3γ1(Ala-Ala) anti-CD3 mAb]	RD (42)	24	Improved C-peptide response to mixed meal, decreased HbA1c and decreased insulin dose	Herold et al. (2005)

(Continued)

**TABLE 70.3** (Continued)

	Participants (n)	Follow-up (months)	Outcome	Reference
Otelixizumab (CHAgly anti-CD3 mAb)	RD (80)	48	Improved C-peptide response to glucose. Clamp/glucagon up to 36 months. Decreased insulin dose with similar HbA1c	Keymeulen et al. (2005, 2010)
Rituximab (anti-CD20 mAb)	RD (87)	24	Increased C-peptide response to mixed meal at 12 but not 24 months. Decreased HbA1c and insulin dose	Pescovitz et al. (2014) and Pescovitz et al. (2009)
Mycophenolate mofetil ± daclizumab (anti IL-2 receptor mAb)	RD (126)	24	No effect on basal or meal-stimulated C-peptide. Similar HbA1c and insulin dose	Gottlieb et al. (2010)
Abatacept (CTLA4-Fc fusion protein)	RD (112)	24	Increased C-peptide response to mixed meal. Decreased HbA1c and insulin dose	Orban et al. (2011)
IL-2 + rapamycin	RD (7)	12	Transient decrease in C-peptide response to mixed meal associated with increased numbers of circulating regulatory T cells	Long et al. (2012)
Teplizumab [OKT3 $\gamma$ 1 (Ala-Ala) anti-CD3 mAb]	RD (516)	24	Increased C-peptide response to a mixed meal at 18 and 24 months with decreased insulin use and HbA1c	Roep et al. (2013) and Sherry et al. (2011)
IL-1 antagonism with canakinumab or anakinra	RD (138)	12	No difference in C-peptide response to a mixed meal, HbA1c or insulin dose	Moran et al. (2013)
Teplizumab [OKT3 $\gamma$ 1 (Ala-Ala) anti-CD3]	RD (52)	24	Increased C-peptide response to mixed meal. Decreased HbA1c and insulin dose	Herold et al. (2013)
Alefacept (anti-CD2)	RD (49)	24	Increased C-peptide response to mixed meal and decreased insulin dose and rate of severe hypoglycemia	Rigby et al. (2013) and Rigby et al. (2015)
Ladarixin (IL-8 antagonist)	RD (72)	12	Ongoing	NCT02814838
Teplizumab [OKT3 $\gamma$ 1 (Ala-Ala) anti-CD3]	AR (170)	48–72	Ongoing	NCT01030861
<b>Nonspecific immune stimulation</b>				
BCG vaccine	RD (26)	18	No effect on glucagon-stimulated C-peptide, insulin dose, or HbA1C	Elliott et al. (1998)
BCG vaccine	RD (94)	24	No effect on mixed meal-stimulated C-peptide, insulin dose, or HbA1C	Allen et al. (1999)
Q fever vaccine	RD (39)	12	No effect on glucagon-stimulated C-peptide or insulin dose	Schmidli et al. (unpublished)
<b>Nonspecific immune regulation</b>				
Thymopoietin	RD (32)	6	Decreased insulin antibodies and insulin dose. More remissions. No difference in C-peptide or HbA1c	Giordano et al. (1990)
Gammaglobulin	RD (16)	6	Increased basal C-peptide Decreased insulin dose, unchanged HbA1c	Panto et al. (1990)
Linomide	RD (63)	12	Decreased HbA1c and insulin dose. No difference in glucagon-stimulated C-peptide	Coutant et al. (1998)
HSP60 p277 peptide (DiaPep)	RD (35)	10	Decrease in glucagon-stimulated C-peptide and insulin dose in placebo but not treated group	Raz et al. (2001)
HSP60 p277 peptide (DiaPep)	RD (48 and 99)	18	No effect on C-peptide response to a mixed meal or on insulin requirement	Schloot et al. (2007)

(Continued)

**TABLE 70.3** (Continued)

	Participants (n)	Follow-up (months)	Outcome	Reference
1,25-dihydroxy vitamin D3	RD (20)	18	No effect on C-peptide response to a mixed meal or insulin requirement	Walter et al. (2010)
HSP60 p277 peptide (DiaPep)	RD (146)	12	No effect on C-peptide response to a mixed meal	Buzzetti et al. (2011)
Antithymocyte globulin (6.5 mg/kg)	RD (58)	24	No effect on C-peptide response to a mixed meal	Gitelman et al. (2016) and Gitelman et al. (2013)
Antithymocyte globulin (2.5 mg/kg) + g-CSF (6 mg fortnightly for 6 doses)	RD (25)	24	Borderline ( $P = .05$ ) improvement in C-peptide response to a mixed meal at 12 but not 24 months	Haller et al. (2016) and Haller et al. (2015)
Antithymocyte globulin (ATG; 2.5 mg/kg) ± G-CSF (6 mg fortnightly for 6 doses)	RD (89)	12	Increase in C-peptide response to a mixed meal and decrease in HbA1c; addition of G-CSF did not enhance effects	Haller et al. (2018)
Rapamycin ± vildagliptin	RD (60)	3	Ongoing	NCT02803892
CD34 <sup>+</sup> stem cell mobilization with plerixafor (anti-CXCR4, CXCR7 agonist), alemtuzumab (anti-CD52), anakinra (anti-IL-1), etanercept (anti-TNF), liraglutide	RD (60)	24	Ongoing	NCT03182426
<b>Antigen-specific immune regulation</b>				
Parenteral insulin (i.v. vs. s.c. 2 weeks)	RD (26)	12	Increased meal-stimulated C-peptide, decreased HbA1c	Shah et al. (1989)
Parenteral (s.c.) insulin	RD (49)	60	Increased glucagon-stimulated C-peptide and improved insulin sensitivity and glycemic control	Linn et al. (1996)
Parenteral (s.c.) insulin and sulfonylurea (glipizide)	RD (27)	12	Increased basal and glucagon-stimulated C-peptide, more remissions	Selam et al. (1993)
Parenteral (s.c.) insulin	RD (10)		Increased C-peptide response to oral glucose, HbA1c unchanged	Kobayashi et al. (1996)
Parenteral insulin (i.v. vs s.c. 2 weeks)	RD (19)	12	Increased meal and glucagon-stimulated C-peptide and decreased HbA1c	Schnell et al. (1997)
Parenteral (s.c.) insulin	AR (14)	84	Delay in onset of diabetes. No effect on islet antibody levels	Füchtenbusch et al. (1998)
Oral insulin	RD (80)	12	No effect on basal C-peptide, HbA1c, insulin dose, or insulin antibodies	Pozzilli et al. (2000)
Oral insulin	RD (131)	12	No effect on basal, glucagon-or meal-stimulated C-peptide, HbA1c, insulin dose, or islet antibody levels	Chaillous et al. (2000)
Gluten elimination	AR (7)	24	No effect on islet antibody levels	Hummel et al. (2002)
Parenteral (s.c.) insulin (DPT-1)	AR (339)	44	No effect on diabetes development	Diabetes Prevention Trial-Type 1 Diabetes Study Group (2002)
Intranasal insulin (Melbourne INIT I)	AR (38)	48	Increased antibody and decreased T-cell responses to insulin	Harrison et al. (2004)
Oral insulin (DPT-1)	AR (372)	52	No effect on diabetes development overall. Post hoc analysis revealed >4-year delay in diabetes onset in participants with insulin autoantibodies	Skyler et al. (2005)

(Continued)

**TABLE 70.3** (Continued)

	Participants (n)	Follow-up (months)	Outcome	Reference
Parenteral (s.c.) GAD65-alum	RD (47)	6	Increase in fasting and stimulated plasma C-peptide with intermediate dose of 20 µg	Agardh et al. (2005)
Intranasal insulin (DIPP)	AR (224)	21	No effect to delay progression to diabetes	Nanto-Salonen et al. (2008)
Parenteral (s.c.) GAD65-alum	RD (70)	30	Delay in loss of C-peptide secretion, in those treated within 6 months of clinical diagnosis	Ludvigsson et al. (2008)
Parenteral (s.c.) insulin B chain 9–23 “altered peptide ligand” NBI-6024-0101 (Neurocrine)	RD (188)	25	No effect on C-peptide response to mixed meal	Walter et al. (2009)
Parenteral (s.c.) insulin B chain in incomplete Freund’s adjuvant	RD (12)	24	No effect on C-peptide response to mixed meal. Development of sustained insulin-specific antibody and T-cell responses	Orban et al. (2010)
Intranasal insulin (INIT III)	RD (52)	24	No effect on metabolic parameters. Suppression of T-cell responses to insulin and antibody responses to subcutaneous insulin	Fourlanos et al. (2011)
Parenteral (s.c.) GAD65-alum	RD (334)	15	No effect on metabolic parameters	Ludvigsson et al. (2012)
Parenteral (s.c.) GAD65-alum	RD (145)	12	No effect on C-peptide response to mixed meal	Wherrett et al. (2011)
Parenteral (i.m.) proinsulin plasmid DNA	RD (80)	12	Transient improvement in C-peptide response to mixed meal concomitant with a decrease in the CD8 T-cell response to proinsulin	Roep et al. (2013)
Oral insulin (TrialNet Study TN07)	AR (560)	72–96	No effect of oral insulin overall, but significant delay in T1D in participants with islet cell antibody and FPIR < 60 µU/mL	Krischer et al. (2017)
Intralymphatic GAD65-alum + oral vitamin D	RD (6)	15	Stable C-peptide response to mixed meal and decreased HbA1c and insulin dose when compared to historical control group	Ludvigsson et al. (2017)
Intranasal insulin (INIT II)	AR (110)	≥ 60	Nasal insulin dose-related insulin antibody response, then suppressed consistent with tolerance induction, but no effect on diabetes incidence	Harrison et al. (2018)
Gluten-free diet	AR (60)	24	Ongoing	NCT02605148
Parenteral (s.c.) GAD65-alum	AR (50)	60	Ongoing	NCT02387164
<b>β-Cell protection</b>				
Nicotinamide	RD (20)	12	Increased glucagon-stimulated C-peptide at 45 days, then decline. No difference in remissions	Mendola et al. (1989)
Nicotinamide	RD (23)	9	Increased basal and glucagon-stimulated C-peptide	Vague et al. (1989)
Nicotinamide	RD (35)	12	No difference in basal or glucagon-stimulated C-peptide	Chase et al. (1990b)
Nicotinamide ± cyclosporine	RD (90)	12	Decreased insulin dose. No difference in remissions	Pozzilli et al. (1994)
Nicotinamide	RD (56)	12	Increased glucagon-stimulated C-peptide in subjects >15 years old	Pozzilli et al. (1995)
Nicotinamide versus vitamin E (no control group)	RD (84)	12	No difference in basal or glucagon-stimulated C-peptide, HbA1c, or insulin dose	Pozzilli et al. (1997)

(Continued)

**TABLE 70.3** (Continued)

	Participants (n)	Follow-up (months)	Outcome	Reference
Nicotinamide (DENIS)	AR (55)	36	No effect on diabetes development	Lampeter et al. (1998a,b)
Nicotinamide (ENDIT)	AR (552)		No effect on diabetes development	Philips et al. (2002)
Octreotide	RD (20)	12	Increased glucagon-stimulated C-peptide at 6 and 12 months; no difference in HbA1c or insulin dose	Grunt et al. (1994)
Diazoxide	RD adults (40)	18	Increased basal C-peptide	Bjork et al. (1996)
Nicotinamide ± parenteral insulin	RD (34)	12	No difference in glucagon-stimulated C-peptide	Vidal et al. (2000)
Diazoxide	RD children (56)		Increased stimulated C-peptide at 12, not 24, months	Bjork et al. (2001)
Oral antioxidants	RD (46)	30	No difference in meal-stimulated C-peptide, insulin dose or HbA1c	Ludvigsson et al. (2001)
Lansoprazole and sitagliptin	RD (68)	12	No difference in meal-stimulated C-peptide, insulin dose or HbA1c	Griffin et al. (2014)
Liraglutide	AR (42)	12	Ongoing	NCT02611232
Liraglutide	AR (82)	12	Ongoing	NCT02898506
Liraglutide	RD (10)	12	Ongoing	NCT02908087
Albiglutide	RD (67)	12	Ongoing	NCT02284009
Metformin	AR (90)	21	Ongoing	NCT02881528
<b>Mechanism uncertain</b>				
Imatinib	RD (67)	12	Improved meal-stimulated C-peptide at 12 months	NCT01781975
Hydroxychloroquine	AR (205)	60	Ongoing	NCT03428945

AR, Islet autoantibody-positive first-degree relative; DENIS, Deutsche Nicotinamide Intervention Study; DIPP, Diabetes Prediction and Prevention Project; DPT-1, Diabetes Prevention Trial Type 1; ENDIT, European Nicotinamide Diabetes Intervention Trial; FDR, first-degree relative; FPIR, first phase insulin response to i.v. glucose; GAD, glutamic acid decarboxylase; IDDM, insulin-dependent diabetes mellitus; INIT, Intranasal Insulin Trial; mAb, monoclonal antibody; RD, person with recently diagnosed diabetes. T1D, type 1 diabetes. Participant numbers are shown in parentheses.

primary and secondary trials is (the absence of) clinical diabetes, in tertiary trials the retention, or increase of residual beta-cell function. Trials of tertiary prevention with more than 70 different agents since the early 1980s (Table 70.3) have failed to demonstrate sustained preservation of residual beta-cell function, although several biologic agents, anti-CD20 (rituximab) and anti-CD3 monoclonal antibodies, anti-thymocyte globulin (ATG) and CTLA-4-Ig (abatacept), slowed the decline of beta-cell function for at least 1–2 years after diagnosis providing hope that earlier intervention might prevent or delay progression to clinical disease. Published randomized primary, secondary, and tertiary trials are summarized in Table 70.4; recent, ongoing trials are registered on ClinicalTrials.gov. In the following we will adhere to the strict definition of prevention and discuss only approaches to prevent clinical T1D.

## PRIMARY PREVENTION

Evidence for an etiological role of environment in T1D is persuasive and primary prevention could be targeted at environmental factors thought to initiate or promote islet autoimmunity. In countries with a high prevalence of T1D, neonatal screening for the highest risk HLA class II genes can identify over half the children destined to develop T1D (Kimpimäki et al., 2001). However, the modest predictive value of genetic testing would justify a primary intervention only if it was safe.

**TABLE 70.4** Comparison of Human Type 1 Diabetes With the NOD Mouse Model

Feature	Human	NOD mouse
Preclinical stage	Yes	Yes
Gender	M > F after puberty	F > M
Genetic susceptibility		
MHC class II aa57 non-Asp	Yes (HLA DQ8)	Yes (I-Ag <sup>7</sup> )
Polygenic non-MHC	Yes	Yes
Environmental influence on gene penetrance	Yes	Yes
Disease transmission via bone marrow	Yes	Yes
Mononuclear cell infiltration of islets (insulitis)	Moderate	Marked
Other organs	Sometimes	Yes
Impaired immune regulation	Yes	Yes
Autoantigens		
(Pro)insulin	Yes	Yes
GAD	Yes	Yes
Clinical response to autoantigen-specific therapy	Not yet shown	Yes

GAD, Glutamic acid decarboxylase; MHC, major histocompatibility complex.

## Diet and Gut Microbiome Modification

In the 1980s it was proposed that early exposure of the infant to cow's milk and/or the lack of breastfeeding predisposed to T1D. Two metaanalyses of multiple studies in which T1D prevalence was associated retrospectively with infant feeding revealed only a marginal increase in relative risk (Gerstein, 1994; Norris and Scott, 1996). In the Denver-based Diabetes Autoimmunity Study in the Young, infant feeding patterns retrospectively analyzed up to 6 months of age were not related to the development of islet autoantibodies up to 7 years of age (Norris et al., 1996). Furthermore, in the Australian BabyDiab Study (Couper et al., 1999) and the German BabyDiab Study (Hummel et al., 2000), no association was found between infant feeding patterns and the development of islet autoantibodies. Nevertheless, to answer whether cow's milk exposure is a risk, the multicountry Trial to Reduce IDDM in the Genetically At-Risk was initiated. Newborns with a T1D first-degree relative and HLA risk alleles, initially exclusively breast-fed, were randomized to either a casein hydrolysate formula (Neutramigen) comprising milk proteins of reduced complexity or a conventional cow's milk-based formula until 6–8 months of age and were followed for 10 years. This approach was based on protection from diabetes in NOD mice fed a hydrolyzed casein diet (Lefebvre et al., 2006). Initially, in a preliminary analysis, hydrolyzed casein-based formula was claimed to reduce the rate of islet autoantibody seroconversion (Knip et al., 2010), implying it protected against T1D. However, in the final study report (Knip et al., 2018), this dietary modification was not associated with any change in the incidence of islet autoantibodies or diabetes. Human milk has many components and properties beneficial to the developing infant, including nondigestable human milk oligosaccharides that have a prebiotic effect to promote antiinflammatory Bifidobacteria in the colon (O'Callaghan and van Sinderen, 2016). Breast milk also contains endogenous insulin (Shehadeh et al., 2001), which might induce "oral tolerance" to insulin and so protect against the development of T1D (discussed below). Thus, rather than cow's milk promoting T1D, human milk may be protective through a variety of mechanisms.

As discussed, the gut microbiome differs in composition, diversity, and function between children at risk for T1D and case-controls. Microbiome "dysbiosis" may be partly reversible by "un-westernizing" the modern diet to increase the amount and diversity of natural, unprocessed food types known to promote an "antiinflammatory" gut microbiome. This could be boosted by supplementation with prebiotics as demonstrated with butyrate/acetate in the NOD mouse (Mariño et al., 2017) and with bespoke probiotics based on knowledge of microbiota known to be deficient in children at risk for T1D. Scientific studies of these approaches are on the drawing board.

Dietary gluten has also been implicated as an environmental trigger of T1D. Antibodies to wheat gluten proteins are found in a proportion of T1D patients at the time of diagnosis (MacFarlane et al., 2002) and coeliac disease and T1D share the HLA risk haplotype A1-B8-DR3-DQ2 and often coexist. In addition, in individuals with coeliac disease the prevalence of autoimmune diseases including T1D was reported to correlate with duration of exposure to gluten (Ventura et al., 1999). However, the German BABYDIET Study found that delaying the introduction of gluten beyond 12 months of age had no effect on the cumulative incidence of islet autoantibodies or clinical T1D (Hummel et al., 2002). At the population level, compelling evidence links vitamin D deficiency to T1D and other autoimmune diseases. Vitamin D is derived primarily from ultraviolet B light-induced synthesis in the skin and its deficiency is increasingly recognized, not just in populations living furthest from the equator but in people anywhere who avoid sunlight, work and play mainly indoors, are dark-skinned, and living in temperate climes or cover their skin for cultural or religious reasons. The recommended daily allowance of vitamin D has decreased over the past 50 years from 5000 to 400 IU (Hypponen et al., 2001). This is the minimum dose required to prevent rickets following adequate prenatal intake but is inadequate for the physiological immune modulating and antiinflammatory actions of vitamin D (Holick, 2004). Three European studies demonstrated an inverse relationship between vitamin D intake and the incidence of T1D. In a birth cohort study from Northern Finland, an area with only 1900 hours direct sunlight annually and the highest incidence of T1D in the world, T1D status was related to prerecorded data on infants 7–24 months of age given vitamin D in doses below, above, or at the then recommended 2000 IU daily (Hypponen et al., 2001, p. 170). The 2000 IU dose was associated with a low relative risk of 0.12 (95% CI 0.03–0.47). In a multinational European case-control study, the odds ratio for T1D was significantly reduced in children given vitamin D (EURODIAB Substudy 2 Study Group, 1999). The risk for T1D in Norwegian children was significantly lower if their mothers had taken cod liver oil (a source of vitamin D) during pregnancy (Stene et al., 2000). Randomized controlled trials of vitamin D supplementation are required in individuals genetically at-risk for T1D but are unlikely to ever be undertaken given the general public awareness of vitamin D deficiency and the widespread availability of vitamin D.

## Virus Vaccination

As discussed, if a virus or viruses are shown to initiate or promote islet autoimmunity, vaccination would be the means of primary prevention. Enteroviruses and rotavirus remain candidates. However, even though rubella and mumps viruses were implicated vaccination did not alter the incidence of T1D, which either questions the original evidence or indicates that multiple environmental agents in addition to viruses contribute to the etiology of T1D.

## Antigen-Specific Immunotherapy

This approach employs islet antigens as tools to induce therapeutic immune tolerance, based on extensive proof-of-concept studies in the NOD mouse. Shown to be safe but not yet efficacious for secondary prevention, it is likely to have greater potential in the context of primary prevention, as discussed below.

## SECONDARY PREVENTION

Secondary prevention has focused on first-degree relatives of a T1D proband, who have autoantibodies against one or more islet antigens. A prerequisite for intervention in asymptomatic individuals is a high likelihood of developing clinical disease. Prediction is determined by measuring autoantibody and metabolic markers of T1D (Table 70.1). In young first-degree relatives, the 5-year risk of diabetes is of the order <25%, 25%–50%, and >50% if autoantibodies are present to 1, 2, and 3 islet antigens, respectively. For single specificities, autoantibodies to insulin (IAA) are the most predictive. A measure of insulin secretion, first-phase insulin response (FPIR) to intravenous glucose, further refines risk prediction. In addition, in autoantibody-positive relatives with normal FPIR, the highest risk was shown to be independently associated with insulin resistance (Fourlanos et al., 2004). Importantly, stratification of autoantibody-positive individuals based on insulin resistance to better identify “progressors” could improve trial design and power.

While detection of autoantibodies is fundamental to preclinical diagnosis and disease prediction, about 10% of new-onset T1D patients of European descent have no detectable antibodies. Moreover, while first-degree relatives who share susceptibility genes and environmental risk factors have at least a 10-fold higher prevalence of T1D

than the background population, they represent no more than 15% of people diagnosed with T1D. Identifying the other 85% using available predictive tests is more challenging because the lower prevalence of disease in the general population may in turn lower the predictive value of screening tests compared to relatives (Bayes' theorem). Emerging studies to screen young children in the general population for islet autoantibodies (Ziegler et al., 2016) are based on the fact that preclinical diagnosis averts the classic clinical presentation of life-threatening ketoacidosis (Winkler et al., 2012). The predictive value of islet autoantibodies in the general population has not been widely investigated but will be important if effective means of secondary prevention are found.

The ideal prevention strategy in autoimmune disease is autoantigen-based immunotherapy, in which an autoantigen is administered to induce protective immune tolerance; this has been called "negative vaccination" (Harrison, 2008). The rationale is that autoantigen-driven immunoregulatory mechanisms are physiological and can be boosted or restored to prevent pathological autoimmunity. Approaches include administration of an autoantigen by a "tolerogenic" route (e.g., mucosal), cell type (e.g., resting dendritic cell), mode (e.g., with blockade of costimulation molecules) or form (e.g., as an "altered peptide ligand"), all of which have been shown to prevent or suppress experimental autoimmune diseases in rodents (Faria and Weiner, 1999; Harrison and Hafler, 2000; Krause et al., 2000). Mechanisms encompass deletion and/or induction of anergy in potentially pathogenic effector T or induction of regulatory T cells (Tregs) (iTregs). Autoreactive T cells that are activated strongly by antigen may undergo apoptotic cell death and deletion, while those that survive or respond "partially" may become anergic (von Herrath and Harrison, 2003). Of potential importance clinically is the ability of iTreg generated to specific antigen to exert antigen-nonspecific "bystander suppression." Thus, in response to specific antigen iTreg can, by direct cell contact or release of soluble immunosuppressive factors, impair the ability of antigen-presenting dendritic cells to elicit effector T-cell responses to any antigen locally at the site of the lesion or in the draining lymph nodes. Bystander suppression does not require that the autoantigen used to induce tolerance is necessarily the major or primary pathogenic autoantigen.

## Mucosa-Mediated Antigen-Specific Tolerance

The mucosal immune system shaped by the microbiome actively generates physiological immune tolerance. Most attempts to induce clinical autoantigen-specific tolerance have been mucosa-based. Numerous studies have shown that NOD mice can be partially protected from diabetes by mucosal administration of islet autoantigens. A large body of evidence indicates that (pro)insulin is a key target antigen driving beta-cell destruction but, paradoxically, can be used as an immunotherapeutic tool (Narendran et al., 2003). Zhang et al. (1991) initially reported protection after oral porcine insulin. Bergerot et al. (1994) then showed that human insulin induced CD4 Tregs that transferred protection to naïve mice. Protection following oral insulin was found to be associated with decreased expression of IFN- $\gamma$ -secreting Th1 T cells in the pancreas and pancreatic lymph nodes (Hancock et al., 1995; Ploix et al., 1998). Oral insulin-induced CD4 Treg have also been shown to prevent immune-mediated diabetes induced by lymphocytic choriomeningitis virus (LCMV) infection of mice expressing the viral nucleoprotein of LCMV under control of the rat insulin promoter in their  $\beta$  cells (Homann et al., 1999). The majority of T cells in the islets of oral insulin-treated mice without diabetes were shown to secrete Th2 (IL-4, IL-10) and Th3 (TGF- $\beta$ ) cytokines, in contrast to IFN- $\gamma$ -secreting Th1 cells in islets of mice that developed diabetes. The protective effect of oral insulin was enhanced by simultaneous feeding with IL-10 (Slavin et al., 2001), bacterial component OM-89 (Bellmann et al., 1997; Hartmann et al., 1997), or Schistosome egg antigen (Maron et al., 1998), all of which promote Th2 responses. Fusion of insulin to cholera toxin B-subunit (CTB) significantly improved the ability of oral insulin to prevent diabetes (Bergerot et al., 1997). Oral CTB–insulin conjugates in NOD mice induced a shift from a Th1 to a Th2 immunity associated with the induction of regulatory CD4 T cells (Ploix et al., 1999). NOD mice were protected from diabetes by feeding potatoes that transgenically expressed CTB–insulin conjugates (Arakawa et al., 1998). Oral GAD65 has also been reported to suppress diabetes development in NOD mice (Ma et al., 1997). Although it is generally believed that neonates are less susceptible to mucosal tolerance induction, oral administration of insulin, insulin B-chain, or GAD65 peptide during the neonatal period suppressed diabetes development in NOD mice (Maron et al., 2001). This suggests that mucosal administration of islet autoantigen in milk could be used to treat very young infants at risk of developing T1D.

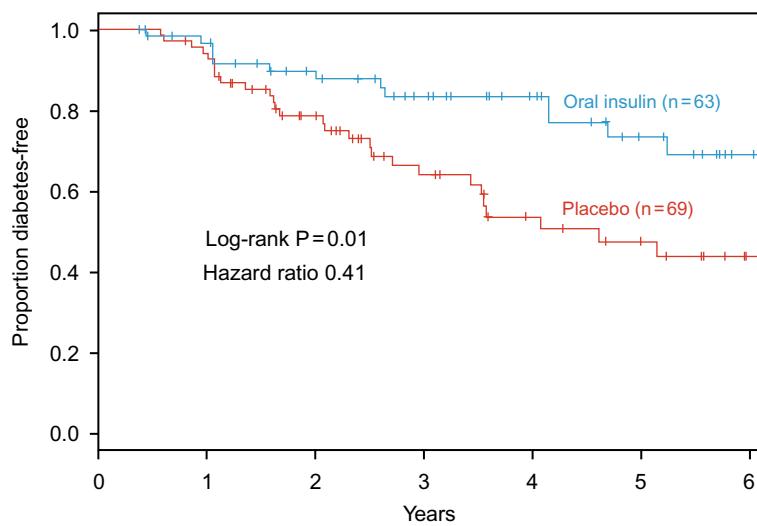
NOD mice are also protected from diabetes by naso-respiratory administration of islet autoantigens. This route of administration to the mucosa is direct avoids antigen degradation in the stomach. When insulin was administered as an aerosol to NOD mice at 8 weeks of age, after the onset of subclinical disease, insulitis, and diabetes incidence were both significantly reduced (Harrison et al., 1996). Aerosol insulin-induced novel antidiabetic CD8

$\gamma\delta$  T cells that suppressed the adoptive transfer of diabetes to nondiabetic mice by T cells from diabetic mice. The type of Treg induced by (pro)insulin depends on the route and form of antigen. Naso-respiratory insulin, which remains nondegraded and conformationally intact, induces CD8  $\gamma\delta$  Treg. On the other hand, oral insulin which is degraded to peptides, or intranasal or oral (pro)insulin peptides, induce CD4 Treg (Hänninen and Harrison, 2000; Martinez et al., 2003). Intranasal administration of the insulin B chain peptide (aa9-23), an epitope recognized by islet-infiltrating CD4 T-cell clones that adoptively transfer diabetes to naïve mice, induce CD4 Treg that protect NOD mice from diabetes (Daniel and Wegmann, 1996). A peptide spanning the B–C chain junction in proinsulin also induced CD4 Treg after intranasal administration (Martinez et al., 2003). This peptide, such as insulin B9-23, binds to the NOD mouse class II major histocompatibility complex, I-A<sup>g7</sup> (Harrison et al., 1997), and is a T-cell epitope in NOD mice (Chen et al., 2001) and humans at risk for T1D (Rudy et al., 1995). T-cell epitope peptides from GAD65 administered intranasally are also protective and associated with the induction of regulatory CD4 Treg and with reduced IFN- $\gamma$  responses to GAD65 (Tian et al., 1996). These “proof-of-principle” studies in the NOD mouse indicate that islet autoantigen proteins or peptides are candidate mucosal “vaccines” for prevention of T1D in humans.

### Trials of Islet Autoantigen-Specific Vaccination in Humans

The large multicenter Diabetes Prevention Trial (DPT)-1 was launched in the United States in 1994 to determine whether antigen-specific therapy with either systemic or oral insulin would delay or prevent diabetes onset in asymptomatic first-degree relatives with islet autoantibodies. Previously, intensive systemic insulin therapy had been reported to prolong the “honeymoon phase” after diagnosis (Shah et al., 1989) and a pilot study of prophylactic systemic insulin had suggested that this approach might be of benefit in at-risk relatives (Keller et al., 1993). Whether systemic insulin would act only as a hormone to control blood glucose and “rest”  $\beta$  cells (making them less sensitive to immune attack) or also as an antigen to induce immune tolerance was not clear, and read-outs to identify immune mechanisms were not employed. In DPT-1, low-dose systemic insulin (annual intravenous insulin infusions and daily subcutaneous injections) was given to high-risk relatives (>50% risk of diabetes over 5 years), matched with an untreated but closely monitored control group, but it had no effect on diabetes incidence (Diabetes Prevention Trial-Type 1 Diabetes Study Group, 2002). In the subsequent randomized controlled DPT-1 trial of oral insulin, islet autoantibody-positive relatives with a 25%–50% 5-year risk of diabetes were given 7.5 mg human insulin or placebo daily for a median of 4.3 years. There was no effect overall, but post hoc hypothesis testing revealed a significant delay of approximately 4 years in diabetes onset in participants who were unequivocally positive for insulin autoantibodies at entry (Skyler et al., 2005) (Fig. 70.2).

That oral insulin only benefited participants with insulin autoimmunity suggests that allelism at the insulin gene susceptibility locus (IDDM2) can shape not only the immune response to endogenous insulin as a target autoantigen but to oral insulin as a potential therapeutic tool. To attempt to confirm the post hoc DPT-1 findings,

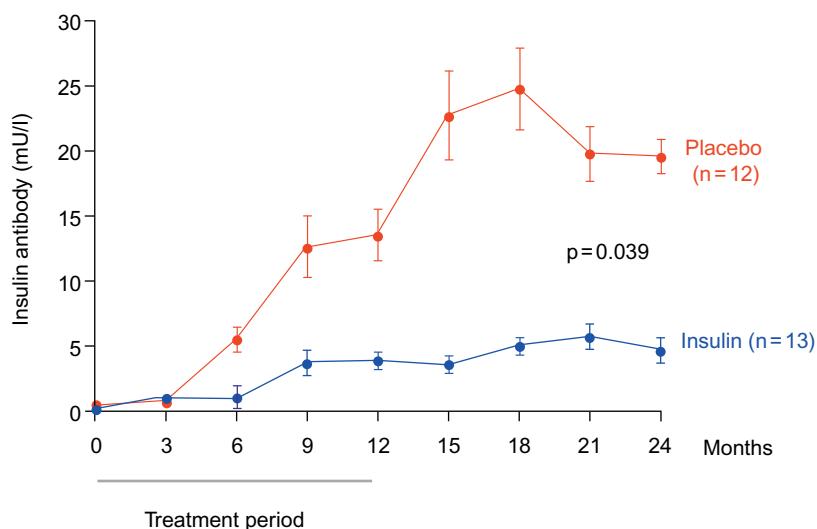


**FIGURE 70.2** Oral insulin vaccination delays development of diabetes in at-risk T1D relatives. *T1D*, type 1 diabetes.

a follow-up international trial of 7.5 mg/d oral insulin was performed by TrialNet between 2007 and 2016. More than 100,000 relatives were screened, of whom 560 met the inclusion criteria, which included the presence of IAA and one or more of GADA, IA-2A, or ICA as measured traditionally by immunofluorescence on pancreas tissue sections. Participants were stratified according to FPIR and presence or absence of ICA. The primary stratum, comprising 389 individuals with ICA and FPIR above threshold, most faithfully represented the post hoc DPT-1 population. The rate of progression to diabetes was highly similar between the placebo groups of the DPT-1 and TrialNet studies. However, oral insulin did not prevent diabetes in the TrialNet primary stratum. Unexpectedly, in the secondary stratum of 55 participants with ICA and low FPIR, oral insulin delayed progression to diabetes by about 2 years. This finding suggests, counterintuitively, that oral insulin might be more effective late rather than early in preclinical T1D. However, given the relatively low number of secondary stratum participants, confirmation of this finding by a dedicated trial will be necessary. Ideally, the TrialNet oral insulin study would have incorporated a higher insulin dose because on a body weight basis the 7.5 mg dose equates to only a few micrograms in the mouse, and milligrams of gavaged insulin were required to induce antidiabetogenic CD4 Treg in NOD mice. Two trials of oral insulin (up to 7.5 mg daily for 12 months) in recently diagnosed patients, attempting tertiary prevention, showed no protective effect on residual beta-cell function (Chaillous et al., 2000; Pozzilli et al., 2000).

Why have trials of oral insulin in T1D, as well as oral myelin basic protein in multiple sclerosis (Weiner et al., 1993) and oral collagen in rheumatoid arthritis (McKown et al., 1999; Trentham et al., 1993) failed to show clinical effects? The answer is probably a combination of reasons: relative ineffectiveness of iTreg against effector T cells in established disease; inadequate dose or bioavailability; coinduction of pathogenic T cells; genetic heterogeneity. Antigen-specific tolerance on its own is clinically ineffective in end-stage disease. If a balance between pathogenic and protective T cells determines clinical outcome then antigen-specific tolerance should be most effective in preventing the onset of disease, not after. The question of dose is discussed below but may be related to route of administration. Oral administration may not be optimal for mucosa-mediated tolerance because proteins are degraded after ingestion and the concentration or form of peptide reaching the upper small intestine may be insufficient to induce mucosa-mediated tolerance. Even with a small peptide, mucosal responses occurred after naso-respiratory but not oral administration (Metzler and Wraith, 1993). In the mouse, nasal administration of the model antigen, ovalbumin, elicited antigen-specific T-cell responses in cervical, mediastinal, and mesenteric mucosal lymph nodes, whereas oral administration elicited responses only in the mesenteric nodes (Hänninen et al., 2001). Irrespective of route of administration, antigen presentation in the mucosa may be a “double-edged” sword simultaneously inducing both iTreg and pathogenic cytotoxic CD8<sup>+</sup> T cells so that a clinical effect is not seen without suppression of the latter, for example, by costimulation blockade with anti-CD40 ligand antibody (Hänninen et al., 2002). Insulin contains potentially pathogenic cytotoxic T-cell epitopes but whether mucosal insulin induces cytotoxic CD8<sup>+</sup> T cells as well as protective Treg is unknown. In the NOD mouse, a proinsulin B-C chain peptide, a “combitope” of CD4<sup>+</sup> (I-A<sup>g7</sup>-restricted) and CD8<sup>+</sup> (K<sup>d</sup>-restricted) T-cell epitopes that induced CD4<sup>+</sup> Treg, was significantly more protective after nasal administration when the C-terminal p9 anchor residue for binding to K<sup>d</sup> was deleted or mutated (Martinez et al., 2003). This indicates that the nature of T-cell epitopes is critical in mucosa-based immunotherapy.

None of the oral autoantigen trials sought evidence for an immune effect. There is a pressing need to evaluate immune responses to mucosal autoantigens in human trials; otherwise, it is not possible to know if an antigen dose is bioactive/available. Induction of insulin antibodies was a marker of bioavailability after aerosol insulin in NOD mice (Harrison et al., 1996) or intranasal insulin in humans (Harrison et al., 2004, 2018; Fourlanos et al., 2011). Irrespective of whether or not they are a marker of immunoprotection, induction of insulin antibodies demonstrates the insulin dose was bioactive. Insulin autoantibodies are a risk marker for T1D and an increase in insulin antibodies after naso-respiratory insulin seems counterintuitive. However, in both the NOD mouse and humans at risk for T1D naso-respiratory insulin, although associated with an increase in insulin antibodies, was also associated with a decrease in T-cell responses to insulin. These findings are entirely consistent with the earliest descriptions of mucosal tolerance and with later landmark studies in humans using keyhole limpet hemocyanin (KLH) as a model antigen. When KLH was administered nasally to human volunteers, it elicited a modest antibody response, but after challenge with subcutaneous KLH both antibody and T-cell responses decreased (Waldo et al., 1994). In a recent randomized trial of nasal insulin in people with recent-onset T1D who did not initially require insulin treatment, those who received nasal insulin had blunted insulin antibody responses to subsequent subcutaneous insulin (Fourlanos et al., 2011) (Fig. 70.3). It will be important to determine if nasal insulin induces insulin-specific Treg and to demonstrate that like nasal KLH nasal insulin suppresses T-cell responses to rechallenge indicative of T-cell tolerance.



**FIGURE 70.3** Nasal insulin vaccination suppresses insulin antibody response to subcutaneous insulin.

The evidence for nasal insulin-induced immune tolerance in humans (Fourlanos et al., 2011) cannot necessarily be extrapolated to endogenous “autoantigenic” insulin but provides a mechanistic rationale for randomized trials of nasal insulin vaccination in individuals at-risk for T1D. Two such trials have been performed with progression to clinical diabetes as the primary outcome. In the T1D Prediction and Prevention Project (DIPP) trial in Finland (Nanto-Salonen et al., 2008), nasal insulin (1 U/kg daily) had no effect on progression to diabetes in islet autoantibody-positive children less than 3 years of age. These children were a very high-risk group and many appear to have had borderline beta-cell function judged by low FPIR to i.v. glucose. In the Australian Type 1 DPT, also known as the Intranasal Insulin Trial II (INIT II) (Harrison et al., 2018) nasal insulin at two doses (40 and 440 U) or nasal placebo was administered daily for 7 days and then weekly for a year, with a further 4 years follow-up, in T1D relatives aged 4–30 with autoantibodies to at least two islet antigens (~40% risk of diabetes over 5 years). The insulin dose in INIT II was substantially higher than in the DIPP trial and the participants were older and had less advanced preclinical disease. Again, nasal insulin induced a significant dose-dependent increase in serum insulin antibody concentration, which peaked after several months then dropped to pretreatment concentrations within the treatment year, consistent with immune tolerance to exogenous insulin. However, this bioeffect did not translate into protection against diabetes and, while unexplained, the rate of diabetes development in the placebo group was substantially lower than expected. As reasoned above, antigen-specific vaccination is most likely to be effective before the onset of the disease process and trials of mucosal insulin in islet autoantibody-negative genetically at-risk children are underway (Bonifacio et al., 2015).

Based on evidence in the NOD mouse that the incidence of diabetes was lowered by systemic GAD65 (Petersen et al., 1994; Tisch et al., 2001) or nasal GAD65 peptides (Tian et al., 1996), the Swedish company, Diamyd P/L, produced recombinant GAD65, and initiated trials of a subcutaneous GAD65-alum (aluminum hydroxide) vaccine (summarized in Table 70.4). Although the initial trials were encouraging, subsequent larger trials failed to substantiate a clinical effect of the vaccine. Again, it is not surprising that the GAD-alum vaccine had no effect after the onset of clinical diabetes. However, these trials appear to have established the safety of the vaccine and, based on GAD65 antibody responses, its bioactivity, thereby justifying an ongoing secondary prevention trial (DIAPREV-IT) of the vaccine in islet autoantibody-positive at-risk children.

## EPILOGUE

T1D prevention trials have taught us that treatment should begin as early as possible in the preclinical stage that “magic bullet” monotherapy is unlikely to be successful and that trials would greatly benefit from mechanistic response markers, especially islet autoantigen-reactive T cells, and noninvasive means of evaluating islet pathology and beta-cell function. Progress in preventing T1D is likely to be incremental, analogous to the evolution of combination treatment regimens for cancer or HIV infection, but more constrained by regulatory

considerations and the relatively slow rate of disease progression. Environmental agents that precipitate or exacerbate autoimmune disease are likely to be ubiquitous and therefore a single strategy, for example, vaccination against a specific virus, is unlikely to be the answer. A prerequisite is the identification of at-risk individuals in early life, for which T1D is a paradigm. Prevention strategies must be safe as well as effective. In this regard, autoantigen-specific vaccination as applied in the oral and nasal insulin trials in T1D provide a glimmer of promise. Lessons learnt from the preclinical diagnosis, prediction, and prevention of T1D should be applicable to other autoimmune diseases.

## Acknowledgments

This work was supported by the National Health and Medical Research Council of Australia [Program Grant 1037321 and Fellowship 1080887 (LCH)] and made possible through Victorian State Government Operational Infrastructure Support and Australian Government NHMRC IRIIS.

## References

- Adorini, L., Gregori, S., Harrison, L.C., 2002. Understanding autoimmune diabetes: insights from mouse models. *Trends Mol. Med.* 8, 31–38.
- Agardh, C.D., Cilio, C.M., Lethagen, A., Lynch, K., Leslie, R.D., Palmer, M., et al., 2005. Clinical evidence for the safety of GAD65 immunomodulation in adult-onset autoimmune diabetes. *J. Diabetes Complications* 19, 238–246.
- Allen, H.F., Klingensmith, G.J., Jensen, P., Simoes, E., Hayward, A., Chase, H.P., 1999. Effect of bacillus Calmette–Guerin vaccination on new-onset type 1 diabetes. A randomized clinical study. *Diabetes Care* 22, 1703–1707.
- Arakawa, T., Yu, J., Chong, D.K., Hough, J., Engen, P.C., Langridge, W.H., 1998. A plant-based cholera toxin B subunit-insulin fusion protein protects against the development of autoimmune diabetes. *Nat. Biotechnol.* 16, 934–938.
- Assan, R., Feutren, G., Sirmaï, J., Laborie, C., Boitard, C., Vexiau, P., et al., 1990. Plasma C-peptide levels and clinical remissions in recent-onset type I diabetic patients treated with cyclosporin A and insulin. *Diabetes* 39, 768–774.
- Bellmann, K., Kolb, H., Hartmann, B., Rothe, H., Rowsell, P., Rastegar, S., et al., 1997. Intervention in autoimmune diabetes by targeting the gut immune system. *Int. J. Immunopharmacol.* 19, 573–577.
- Bergerot, I., Fabien, N., Maguer, V., Thivolet, C., 1994. Oral administration of human insulin to NOD mice generates CD4+ T cells that suppress adoptive transfer of diabetes. *J. Autoimmun.* 7, 655–663.
- Bergerot, I., Ploix, C., Petersen, J., Moulin, V., Rask, C., Fabien, N., et al., 1997. A cholera toxoid-insulin conjugate as an oral vaccine against spontaneous autoimmune diabetes. *Proc. Natl. Acad. Sci. U.S.A.* 94, 4610–4614.
- Bingley, P.J., Williams, A.J., Gale, E.A., 1999. Optimized autoantibody-based risk assessment in family members. Implications for future intervention trials. *Diabetes Care* 22, 1796–1801.
- Bjork, E., Berne, C., Kampe, O., Wibell, L., Oskarsson, P., Karlsson, F.A., 1996. Diazoxide treatment at onset preserves residual insulin secretion in adults with autoimmune diabetes. *Diabetes* 45, 1427–1430.
- Bjork, E., Ortquist, E., Wallensteen, M., Ludvigsson, J., Aman, J., Johansson, C., et al., 2001. Diazoxide treatment at onset in childhood type 1 diabetes. *Diabetes* 50, A90.
- Bonifacio, E., Ziegler, A.G., Klingensmith, G., Schober, E., Bingley, P.J., Rottenkolber, M., et al., 2015. Effects of high-dose oral insulin on immune responses in children at high risk for type 1 diabetes: the Pre-POINT randomized clinical trial. *JAMA* 313, 1541–1549.
- Bosi, E., Molteni, L., Radaelli, M.G., Folini, L., Fermo, I., Bazzigaluppi, E., et al., 2006. Increased intestinal permeability precedes clinical onset of type 1 diabetes. *Diabetologia* 49, 2824–2827.
- Buckingham, B.A., Sandborg, C.I., 2000. A randomized trial of methotrexate in newly diagnosed patients with type 1 diabetes mellitus. *Clin. Immunol.* 96, 86–90.
- Buzzetti, R., Cernea, S., Petrone, A., Capizzi, M., Spoletini, M., Zampetti, S., et al., 2011. C-peptide response and HLA genotypes in subjects with recent-onset type 1 diabetes after immunotherapy with DiaPep277: an exploratory study. *Diabetes* 60, 3067–3072.
- Canadian–European Randomized Control Trial Group, 1988. Cyclosporin-induced remission of IDDM after early intervention. Association of 1 yr of cyclosporin treatment with enhanced insulin secretion. *Diabetes* 37, 1574–1582.
- Chaillous, L., Lefevre, H., Thivolet, C., Boitard, C., Lahliou, N., Atlan-Gepner, C., et al., 2000. Oral insulin administration and residual beta-cell function in recent-onset type 1 diabetes: a multicentre randomised controlled trial. *Diabète Insuline Orale Group. Lancet* 356, 545–549.
- Chase, H.P., Butler-Simon, N., Garg, S.K., Hayward, A., Klingensmith, G.J., Hamman, R.F., et al., 1990a. Cyclosporine A for the treatment of new-onset insulin-dependent diabetes mellitus. *Pediatrics* 85, 241–245.
- Chase, H.P., Butler-Simon, N., Garg, S., McDuffie, M., Hoops, S.L., O'Brien, D., 1990b. A trial of nicotinamide in newly diagnosed patients with type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 33, 444–446.
- Chen, W., Bergerot, I., Elliott, J.F., Harrison, L.C., Delovitch, T.L., 2001. Evidence that a peptide spanning the B–C junction of proinsulin is a primary autoantigen epitope in the pathogenesis of type 1 diabetes. *J. Immunol.* 167, 4926–4935.
- Colman, P.G., Steele, C., Couper, J.J., Beresford, S.J., Powell, T., Kewming, K., et al., 2000. Islet autoimmunity in infants with a type I diabetic relative is common but is frequently restricted to one autoantibody. *Diabetologia* 43, 203–209.
- Concannon, P., Rich, S.S., Nepom, G.T., 2009. Genetics of type 1A diabetes. *N. Engl. J. Med.* 360, 1646–1654.
- Cook, J.J., Hudson, I., Harrison, L.C., Dean, B., Colman, P.G., Werther, G.A., et al., 1989. A double-blind controlled trial of azathioprine in children with newly-diagnosed type 1 diabetes. *Diabetes* 38, 779–783.
- Couper, J.J., Steele, C., Beresford, S., Powell, T., McCaul, K., Pollard, A., et al., 1999. Lack of association between duration of breast-feeding or introduction of cow's milk and development of islet autoimmunity. *Diabetes* 48, 2145–2149.

- Couper, J.J., Beresford, S., Hirte, C., Baghurst, P., Pollard, A., Tait, B.D., et al., 2009. Weight gain in early life predicts risk of islet autoimmunity in children with a first degree relative with type 1 diabetes. *Diabetes Care* 32, 94–99.
- Couper, J.J., Harrison, L.C., Re-defining type 1 diabetes as an autoimmune beta-cell disorder, *Med. J. Aust.* (in press).
- Coutant, R., Landais, P., Rosilio, M., Johnsen, C., Lahoul, N., Chatelain, P., et al., 1998. Low dose linomide in type I juvenile diabetes of recent onset: a randomised placebo-controlled double blind trial. *Diabetologia* 41, 1040–1046.
- Daniel, D., Wegmann, D., 1996. Protection of nonobese diabetic mice from diabetes by intranasal or subcutaneous administration of insulin peptide B(9–23). *Proc. Natl. Acad. Sci. U.S.A.* 93, 956–960.
- Davis-Richardson, A.G., Ardissonne, A.N., Dias, R., Simell, V., Leonard, M.T., Kemppainen, K.M., et al., 2014. *Bacteroides dorei* dominates gut microbiome prior to autoimmunity in Finnish children at high risk for type 1 diabetes. *Front. Microbiol.* 5, 678. Available from: <https://doi.org/10.3389/fmicb.2014.00678>.
- Diabetes Prevention Trial-Type 1 Diabetes Study Group, 2002. Effects of insulin in relatives of patients with type 1 diabetes mellitus. *N. Engl. J. Med.* 346, 1685–1691.
- Dunne, J.L., Triplett, E.W., Gevers, D., Xavier, R., Insel, R., Danska, J., et al., 2014. The intestinal microbiome in type 1 diabetes. *Clin. Exp. Immunol.* 177, 30–37.
- EURODIAB Substudy 2 Study Group, 1999. Vitamin D supplement in early childhood and risk for Type I (insulin-dependent) diabetes mellitus. *Diabetologia* 42, 51–54.
- Eisenbarth, G.S., 1986. Type 1 diabetes mellitus. A chronic autoimmune disease. *N. Engl. J. Med.* 314, 1360–1368.
- Elliott, J.F., Marlin, K.L., Couch, R.M., 1998. Effect of bacille Calmette–Guerin vaccination on C-peptide secretion in children newly diagnosed with IDDM. *Diabetes Care* 21, 1691–1693.
- Faria, A.M., Weiner, H.L., 1999. Oral tolerance: mechanisms and therapeutic applications. *Adv. Immunol.* 73, 153–164.
- Feutren, G., Papoz, L., Assan, R., Vialettes, B., Karsenty, G., Vexiau, P., et al., 1986. Cyclosporin increases the rate and length of remissions in insulin-dependent diabetes of recent onset. Results of a multicentre double-blind trial. *Lancet* 2, 119–124.
- Forrest, J.M., Menser, M.A., Burgess, J.A., 1971. High frequency of diabetes mellitus in young adults with congenital rubella. *Lancet* 2, 332–334.
- Foulis, A.K., Liddle, C.N., Farquharson, M.A., Richmond, J.A., Weir, R.S., 1986. The histopathology of the pancreas in type 1 (insulin-dependent) diabetes mellitus: a 25-year review of deaths in patients under 20 years of age in the United Kingdom. *Diabetologia* 29, 267–274.
- Fourlanos, S., Narendran, P., Byrnes, G., Colman, P., Harrison, L.C., 2004. Insulin resistance is a risk factor for progression to type 1 diabetes. *Diabetologia* 47, 1661–1667.
- Fourlanos, S., Dotta, F., Greenbaum, C.K., Palmer, J.P., Rolandsson, O., Colman, P.G., et al., 2005. Latent autoimmune diabetes in adults (LADA) should be less latent. *Diabetologia* 48, 2206–2212.
- Fourlanos, S., Varney, M., Tait, B.D., Morahan, G., Honeyman, M.C., Colman, P.G., et al., 2008. The rising incidence of type 1 diabetes is accounted for by cases with lower risk HLA genotypes. *Diabetes Care* 31, 1546–1549.
- Fourlanos, S., Perry, C., Gellert, S.A., Martinuzzi, E., Mallone, R., Butler, J., et al., 2011. Evidence that nasal insulin induces immune tolerance to insulin in adults with autoimmune diabetes. *Diabetes* 60, 1237–1245.
- Füchtenbusch, M., Rabl, W., Grassl, B., Bachmann, W., Standl, E., Ziegler, A.G., 1998. Delay of type I diabetes in high risk, first degree relatives by parenteral antigen administration: the Schwabing Insulin Prophylaxis Pilot Trial. *Diabetologia* 41, 536–541.
- Gale, E.A.M., 2002. The rise of childhood type 1 diabetes in the 20th century. *Diabetes* 51, 3353–3361.
- Gepts, W., 1965. Pathologic anatomy of the pancreas in juvenile diabetes mellitus. *Diabetes* 14, 619–633.
- Gerstein, H., 1994. Cow's milk exposure and type 1 diabetes mellitus. *Diabetes Care* 17, 13–19.
- Ginsberg-Fellner, F., Witt, M.E., Fedun, B., Taub, F., Dobersen, M.J., McEvoy, R.C., et al., 1985. Diabetes mellitus and autoimmunity in patients with the congenital rubella syndrome. *Rev. Infect. Dis.* 7 (Suppl 1), S170–S176.
- Giordano, C., Panto, F., Amato, M.P., Sapienza, N., Pugliese, A., Galluzzo, A., 1990. Early administration of an immunomodulator and induction of remission in insulin-dependent diabetes mellitus. *J. Autoimmun.* 3, 611–617.
- Gitelman, S.E., Gottlieb, P.A., Rigby, M.R., Felner, E.I., Willi, S.M., Fisher, L.K., et al., 2013. Antithymocyte globulin treatment for patients with recent-onset type 1 diabetes: 12-month results of a randomised, placebo-controlled, phase 2 trial. *Lancet Diabetes Endocrinol.* 1, 306–316.
- Gitelman, S.E., Gottlieb, P.A., Felner, E.I., Willi, S.M., Fisher, L.K., Moran, A., et al., 2016. Antithymocyte globulin therapy for patients with recent-onset type 1 diabetes: 2 year results of a randomised trial. *Diabetologia* 59, 1153–1161.
- Goday, A., Pujol-Borrell, R., Fernandez, J., Casamitjana, R., Rios, M., Vilardell, E., et al., 1993. Effects of a short prednisone regime at clinical onset of type 1 diabetes. *Diabetes Res. Clin. Pract.* 20, 39–46.
- Gottlieb, P.A., Quinlan, S., Krause-Steinrauf, H., Greenbaum, C.J., Wilson, D.M., Rodriguez, H., et al., 2010. Failure to preserve beta-cell function with mycophenolate mofetil and daclizumab combined therapy in patients with new-onset type 1 diabetes. *Diabetes Care* 33, 826–832.
- Gotttsater, A., Landin-Olsson, M., Lernmark, A., Fernlund, P., Sundkvist, G., Hagopian, W.A., 1995. Glutamic acid decarboxylase antibody levels predict role of β-cell decline in adult onset diabetes. *Diab. Rev. Clin. Prac.* 27, 133–140.
- Greenbaum, C., Harrison, L.C., 2003. Guidelines for intervention trials in subjects with newly-diagnosed type 1 diabetes. *Diabetes* 52, 1059–1065.
- Griffin, K.J., Thompson, P.A., Gottschalk, M., Kyllo, J.H., Rabinovitch, A., 2014. Combination therapy with sitagliptin and lansoprazole in patients with recent-onset type 1 diabetes (REPAIR-T1D): 12-month results of a multicentre, randomised, placebo-controlled, phase 2 trial. *Lancet Diabetes Endocrinol.* 2, 710–718.
- Grunt, J.A., al-Hakim, H., Willoughby, L., Howard, C.P., 1994. A randomized trial of a somatostatin analog for preserving beta cell function in children with insulin dependent diabetes mellitus. *J. Pediatr. Endocrinol.* 7, 331–334.
- Hagopian, W.A., Karlsen, A.E., Gottsater, A., Landin-Olsson, M., Grubin, C.E., Sundkvist, G., et al., 1993. Quantitative assay using recombinant human islet glutamic acid decarboxylase (GAD65) shows that 64K autoantibody positivity at onset predicts diabetes type. *J. Clin. Invest.* 91, 368–374.

- Haller, M.J., Gitelman, S.E., Gottlieb, P.A., Michels, A.W., Rosenthal, S.M., Shuster, J.J., et al., 2015. Anti-thymocyte globulin/G-CSF treatment preserves beta cell function in patients with established type 1 diabetes. *J. Clin. Invest.* 125, 448–455.
- Haller, M.J., Gitelman, S.E., Gottlieb, P.A., Michels, A.W., Perry, D.J., Schultz, A.R., et al., 2016. Antithymocyte globulin plus G-CSF combination therapy leads to sustained immunomodulatory and metabolic effects in a subset of responders with established type 1 diabetes. *Diabetes* 65, 3765–3775.
- Haller, M.J., Schatz, D.A., Skyler, J.S., Krischer, J.P., Bundy, B.N., Miller, J.L., et al., 2018. Low-dose anti-thymocyte globulin (ATG) preserves beta-cell function and improves HbA1c in new-onset type 1 diabetes. *Diabetes Care* 41, 1917–1925.
- Hancock, W.W., Polanski, M., Zhang, J., Blogg, N., Weiner, H.L., 1995. Suppression of insulitis in non-obese diabetic (NOD) mice by oral insulin administration is associated with selective expression of interleukin-4 and -10, transforming growth factor-beta and prostaglandin-E. *Am. J. Pathol.* 147, 1193–1199.
- Hänninen, A., Harrison, L.C., 2000. Gamma delta T cells as mediators of mucosal tolerance: the autoimmune diabetes model. *Immunol. Rev.* 173, 109–119.
- Hänninen, A., Braakhuis, A., Heath, W.R., Harrison, L.C., 2001. Mucosal antigen primes diabetogenic cytotoxic T-lymphocytes regardless of dose or delivery route. *Diabetes* 50, 771–775.
- Hänninen, A., Martinez, N.R., Davey, G.M., Heath, W.R., Harrison, L.C., 2002. Transient blockade of CD40 ligand dissociates pathogenic from protective mucosal immunity. *J. Clin. Invest.* 109, 261–267.
- Harbison, J.E., Roth-Schulze, A.-J., Barry, S.C., Giles, L.C., Tran, C.D., Ngui, K.M., et al., 2018. Gut microbiome dysbiosis and increased intestinal permeability in Australian children with islet autoimmunity and type 1 diabetes. In: 230-OR ADA Presidents' Select Abstract. American Diabetes Association 78th Scientific Session, Orlando, FL.
- Harrison, L.C., 2008. Vaccination against self to prevent autoimmune disease: the type 1 diabetes model. *Immunol. Cell Biol.* 89, 139–145.
- Harrison, L.C., Hafler, D.A., 2000. Antigen-specific therapy for autoimmune disease. *Curr. Opin. Immunol.* 12, 704–711.
- Harrison, L.C., Honeyman, M.C., 1999. Cow's milk and type 1 diabetes: the real debate is about mucosal immune function. *Diabetes* 48, 1501–1507.
- Harrison, L.C., Colman, P.G., Dean, B., Baxter, R., Martin, F.I., 1985. Increase in remission rate in newly diagnosed type I diabetic subjects treated with azathioprine. *Diabetes* 34, 1306–1308.
- Harrison, L.C., Honeyman, M.C., DeAizpurua, H.J., Schmidli, R.S., Tait, B.D., Cram, D.S., et al., 1993. Inverse relationship between humoral and cellular immunity to glutamic acid decarboxylase in humans at-risk for insulin-dependent diabetes. *Lancet* 341, 1365–1369.
- Harrison, L.C., Dempsey-Collier, M., Kramer, D.R., Takahashi, K., 1996. Aerosol insulin induces regulatory CD8 gamma delta T cells that prevent murine insulin-dependent diabetes. *J. Exp. Med.* 184, 2167–2174.
- Harrison, L.C., Honeyman, M.C., Trembleau, S., Gregori, S., Gallazzi, F., Augstein, P., et al., 1997. A peptide-binding motif for I-A(g7), the class II major histocompatibility complex (MHC) molecule of NOD and Biozzi AB/H mice. *J. Autoimmun.* 10, 165–173.
- Harrison, L.C., Honeyman, M.C., Steele, C.E., Stone, N.L., Sarugeri, E., Bonifacio, E., et al., 2004. Pancreatic beta-cell function and immune responses to insulin after administration of intranasal insulin to humans at risk for type 1 diabetes. *Diabetes Care* 27, 2348–2355.
- Harrison, L.C., Hall, C.R., Couper, J.J., Donaghue, K.C., Davis, E.A., Cotterill, A.M., et al., 2018. A randomised controlled trial of intranasal insulin to prevent type 1 diabetes: intranasal insulin trial II (INIT II). In: Proc. Aust. Diab. Congr. Adelaide.
- Hartmann, B., Bellmann, K., Ghiea, I., Kleemann, R., Kolb, H., 1997. Oral insulin for diabetes prevention in NOD mice: potentiation by enhancing Th2 cytokine expression in the gut through bacterial adjuvant. *Diabetologia* 40, 902–909.
- Herold, K.C., Hagopian, W., Auger, J.A., Poumian-Ruiz, E., Taylor, L., Donaldson, D., et al., 2002. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N. Engl. J. Med.* 346, 1692–1698.
- Herold, K.C., Gitelman, S.E., Masharani, U., Hagopian, W., Bisikirska, B., Donaldson, D., et al., 2005. A single course of anti-CD3 monoclonal antibody hOKT3gamma1(Ala-Ala) results in improvement in C-peptide responses and clinical parameters for at least 2 years after onset of type 1 diabetes. *Diabetes* 54, 1763–1769.
- Herold, K.C., Gitelman, S.E., Ehlers, M.R., Gottlieb, P.A., Greenbaum, C.J., Hagopian, W., et al., 2013. Teplizumab (anti-CD3 mAb) treatment preserves C-peptide responses in patients with new-onset type 1 diabetes in a randomized controlled trial: metabolic and immunologic features at baseline identify a subgroup of responders. *Diabetes* 62, 3766–3774.
- von Herrath, M.G., Harrison, L.C., 2003. Antigen-induced regulatory T cells in autoimmunity. *Nat. Rev. Immunol.* 3, 223–232.
- Holick, M.F., 2004. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *Am. J. Clin. Nutr.* 79, 362–371.
- Homann, D., Dyrberg, T., Petersen, J., Oldstone, M.B., von Herrath, M.G., 1999. Insulin in oral immune "tolerance": a one-amino acid change in the B chain makes the difference. *J. Immunol.* 163, 1833–1838.
- Honeyman, M., 2005. How robust is the evidence for viruses in the induction of type 1 diabetes? *Curr. Opin. Immunol.* 17, 616–623.
- Honeyman, M.C., Harrison, L.C., Drummond, B., Colman, P.G., Tait, B.D., 1995. Analysis of families at risk for insulin-dependent diabetes reveals that HLA antigens influence progression to preclinical disease. *Mol. Med.* 1, 576–582.
- Honeyman, M.C., Coulson, B.S., Stone, N.L., Gellert, S.A., Goldwater, P.N., Steele, C.E., et al., 2000. Association between rotavirus infection and pancreatic islet autoimmunity in children at risk of developing type 1 diabetes. *Diabetes* 49, 1319–1324.
- Honeyman, M.C., Laine, D., Londrigan, S., Kirkwood, C., Harrison, L.C., 2014. Rotavirus infection induces transient pancreatic involution and hyperglycemia in weanling mice. *PLoS One* e106560. Available from: <https://doi.org/10.1371/journal.pone.0106560>.
- Hummel, M., Fuchtenbusch, M., Schenker, M., Ziegler, A.G., 2000. No major association of breast-feeding, vaccinations, and childhood viral diseases with early islet autoimmunity in the German BABYDIAB Study. *Diabetes Care* 23, 969–974.
- Hummel, M., Bonifacio, E., Naserke, H.E., Ziegler, A.G., 2002. Elimination of dietary gluten does not reduce titers of type 1 diabetes-associated autoantibodies in high-risk subjects. *Diabetes Care* 25, 1111–1116.
- Hummel, S., Pfluger, M., Hummel, M., Bonifacio, E., Ziegler, A.G., 2011. Primary dietary intervention study to reduce the risk of islet autoimmunity in children at increased risk for type 1 diabetes: the BABYDIET study. *Diabetes Care* 34, 1301–1305.
- Hypponen, E., Laara, E., Reunanen, A., Jarvelin, M.R., Virtanen, S.M., 2001. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. *Lancet* 358, 1500–1503.

- Kapke, J., Shaheen, Z., Kilari, D., Knudson, P., Wong, S., 2017. Immune checkpoint inhibitor-associated type 1 diabetes mellitus: case series, review of the literature, and optimal management. *Case Rep. Oncol.* 10, 897–909.
- Kawaguchi-Miyashita, M., Shimizu, K., Nanno, M., Shimada, S., Watanabe, T., Koga, Y., et al., 1996. Development and cytolytic function of intestinal intraepithelial T lymphocytes in antigen-minimized mice. *Immunology* 89, 268–273.
- Keller, R.J., Eisenbarth, G.S., Jackson, R.A., 1993. Insulin prophylaxis in individuals at high risk of type 1 diabetes. *Lancet* 341, 927–928.
- Keymeulen, B., Vandemeulebroucke, E., Ziegler, A.G., Mathieu, C., Kaufman, L., Hale, G., et al., 2005. Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. *N. Engl. J. Med.* 352, 2598–2608.
- Keymeulen, B., Walter, M., Mathieu, C., Kaufman, L., Gorus, F., Hilbrands, R., et al., 2010. Four-year metabolic outcome of a randomised controlled CD3-antibody trial in recent-onset type 1 diabetic patients depends on their age and baseline residual beta cell mass. *Diabetologia* 53, 614–623.
- Kimpimäki, T., Kupila, A., Hämäläinen, A.-M., Kukko, M., Kulmala, P., Savola, K., et al., 2001. The first signs of β-cell autoimmunity appear in infancy in genetically susceptible children from the general population: The Finnish Type 1 Diabetes Prediction and Prevention Study. *J. Clin. Endocrinol. Metab.* 86, 4782–4788.
- Knip, M., Siljander, H., 2016. The role of the intestinal microbiota in type 1 diabetes mellitus. *Nat. Rev. Endocrinol.* 12, 154–167.
- Knip, M., Virtanen, S.M., Seppä, K., Ilonen, J., Savilahti, E., Vaarala, O., et al., 2010. Dietary intervention in infancy and later signs of beta-cell autoimmunity. *N. Engl. J. Med.* 363, 1900–1908.
- Knip, M., Virtanen, S.M., Becker, D., Dupre, J., Krischer, J.P., Åkerblom, H.K., 2011. Early feeding and risk of type 1 diabetes: experiences from the Trial to Reduce Insulin-dependent diabetes mellitus in the Genetically at Risk (TRIGR). *Am. J. Clin. Nutr.* 94, 1814S–1820S.
- Knip, M., Åkerblom, H.K., Becker, D., Dosch, H.M., Dupre, J., Fraser, W., et al., 2014. Hydrolyzed infant formula and early beta-cell autoimmunity: a randomized clinical trial. *JAMA* 311, 2279–2287.
- Writing Group for the TRIGR Study Group, Knip, M., Åkerblom, H.K., Al Taji, E., Becker, D., Bruining, J., Castano, L., et al., 2018. Effect of hydrolyzed infant formula vs conventional formula on risk of type 1 diabetes: the TRIGR randomized clinical trial. *JAMA* 319, 38–48.
- Kobayashi, T., Nakanishi, K., Murase, T., Kosaka, K., 1996. Small doses of subcutaneous insulin as a strategy for preventing slowly progressive b-cell failure in islet cell antibody-positive patients with clinical features of NIDDM. *Diabetes* 45, 622–626.
- Kohnert, K.D., Hehmke, B., Keilacker, H., Ziegler, M., Emmrich, F., Laube, F., et al., 1996. Antibody response to islet autoantigens in anti-CD4/prednisolone immune intervention of type 1 diabetes. *Int. J. Clin. Lab. Res.* 26, 55–59.
- Kondrashova, A., Reunanan, A., Romanov, A., Karvonen, A., Viskari, H., Vesikari, T., et al., 2005. A six-fold gradient in the incidence of type 1 diabetes at the eastern border of Finland. *Ann. Med.* 37, 67–72.
- Kostic, A.D., Gevers, D., Siljander, H., Vatanen, T., Hyötyläinen, T., Hämäläinen, A.M., et al., 2015. The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. *Cell Host Microbe* 17, 260–273.
- Krause, I., Blank, M., Shoenfeld, Y., 2000. Immunomodulation of experimental autoimmune diseases via oral tolerance. *Crit. Rev. Immunol.* 20, 1–16.
- Krischer, J.P., Writing Committee for the Type 1 Diabetes TrialNet Oral Insulin Study Group, Schatz, D.A., Bundy, B., Skyler, J.S., Greenbaum, C.J., 2017. Effect of oral insulin on prevention of diabetes in relatives of patients with type 1 diabetes: a randomized clinical trial. *JAMA* 318, 1891–1902.
- Lampeter, E.F., McCann, S.R., Kolb, H., 1998a. Transfer of insulin-dependent diabetes by bone marrow transplantation. *Lancet* 351, 568–569.
- Lampeter, E.F., Klinghammer, A., Scherbaum, W.A., Heinze, E., Haastert, B., Giani, G., et al., 1998b. The Deutsche Nicotinamide Intervention Study: an attempt to prevent type 1 diabetes. DENIS Group. *Diabetes* 47, 980–984.
- Lefebvre, D.E., Powell, K.L., Strom, A., Scott, F.W., 2006. Dietary proteins as environmental modifiers of type 1 diabetes mellitus. *Ann. Rev. Nutr.* 26, 175–202.
- Leiter, E.H., Prochazka, M., Coleman, D.L., 1987. The non-obese diabetic (NOD) mouse. *Am. J. Pathol.* 128, 380–383.
- Linn, T., Ortac, K., Laube, H., Federlin, K., 1996. Intensive therapy in adult insulin-dependent diabetes mellitus is associated with improved insulin sensitivity and reserve: a randomized, controlled, prospective study over 5 years in newly diagnosed patients. *Metabolism* 45, 1508–1513.
- Long, S.A., Rieck, M., Sanda, S., Bollyky, J.B., Samuels, P.L., Goland, R., et al., 2012. Rapamycin/IL-2 combination therapy in patients with type 1 diabetes augments Tregs yet transiently impairs beta-cell function. *Diabetes* 61, 2340–2348.
- Ludvigsson, J., Samuelsson, U., Johansson, C., Stenhammar, L., 2001. Treatment with antioxidants at onset of type 1 diabetes in children: a randomized, double-blind placebo-controlled study. *Diabetes Metab. Res. Rev.* 17, 131–136.
- Ludvigsson, J., Faresjo, M., Hjorth, M., Axelsson, S., Cheramy, M., Pihl, M., et al., 2008. GAD treatment and insulin secretion in recent-onset type 1 diabetes. *N. Engl. J. Med.* 359, 1909–1920.
- Ludvigsson, J., Krisky, D., Casas, R., Battelino, T., Castano, L., Greening, J., et al., 2012. GAD65 antigen therapy in recently diagnosed type 1 diabetes mellitus. *N. Engl. J. Med.* 366, 433–442.
- Ludvigsson, J., Wahlberg, J., Casas, R., 2017. Intralymphatic injection of autoantigen in type 1 diabetes. *N. Engl. J. Med.* 376, 697–699.
- Ma, S.W., Zhao, D.L., Yin, Z.Q., Mukherjee, R., Singh, B., Qin, H.Y., et al., 1997. Transgenic plants expressing autoantigens fed to mice to induce oral immune tolerance. *Nat. Med.* 3, 793–796.
- MacFarlane, A.J., Burghardt, K.M., Kelly, J., Simell, T., Simell, O., Altosaar, I., et al., 2002. A type 1 diabetes-related protein from wheat (*Triticum aestivum*): cDNA clone of a wheat storage globulin, Glb1, linked to islet damage. *J. Biol. Chem.* 278, 54–63.
- Mannering, S.I., Harrison, L.C., Williamson, N.A., Morris, J.S., Tearle, D.J., Jensen, K.P., et al., 2005. The insulin A-chain epitope recognized by human T cells is post-translationally modified. *J. Exp. Med.* 202, 1191–1197.
- Mariño, E., Richards, J.L., McLeod, K.H., Yap, Y.A., Stanley, D., Kranich, J., et al., 2017. Gut microbial metabolites limit autoimmune T cell frequencies and protect against type 1 diabetes. *Nat. Immunol.* 18, 1271. Available from: <https://doi.org/10.1038/ni1117-1271c>.
- Maron, R., Palanivel, V., Weiner, H.L., Harn, D.A., 1998. Oral administration of schistosome egg antigens and insulin B-chain generates and enhances Th2-type responses in NOD mice. *Clin. Immunol. Immunopathol.* 87, 85–92.

- Maron, R., Guerau-de-Arellano, M., Zhang, X., Weiner, H.L., 2001. Oral administration of insulin to neonates suppresses spontaneous and cyclophosphamide induced diabetes in the NOD mouse. *J. Autoimmun.* 16, 21–28.
- Martinez, N.R., Augstein, P., Moustakas, A.K., Papadopoulos, G.K., Gregori, S., Adorini, L., et al., 2003. Disabling an integral CTL epitope allows suppression of autoimmune diabetes by intranasal proinsulin peptide. *J. Clin. Invest.* 111, 1365–1371.
- McKown, K.M., Carbone, L.D., Kaplan, S.B., Aelion, J.A., Lohr, K.M., Cremer, M.A., et al., 1999. Lack of efficacy of oral bovine type II collagen added to existing therapy in rheumatoid arthritis. *Arthritis Rheum.* 42, 1204–1208.
- Mendola, G., Casamitjana, R., Gomis, R., 1989. Effect of nicotinamide therapy upon B-cell function in newly diagnosed type 1 (insulin-dependent) diabetic patients. *Diabetologia* 32, 160–162.
- Menser, M.A., Forrest, J.M., Honeyman, M.C., Burgess, J.A., 1974. Letter: Diabetes, HL-A antigens, and congenital rubella. *Lancet* 2, 1508–1509.
- Menser, M.A., Forrest, J.M., Bransby, R.D., 1978. Rubella infection and diabetes mellitus. *Lancet* 1, 57–60.
- Metzler, B., Wraith, D.C., 1993. Inhibition of experimental autoimmune encephalomyelitis by inhalation but not oral administration of the encephalitogenic peptide: influence of MHC binding affinity. *Int. Immunol.* 5, 1159–1165.
- Moltchanova, E.V., Schreier, N., Lammi, N., Karvonen, M., 2009. Seasonal variation of diagnosis of type 1 diabetes mellitus in children worldwide. *Diabetic Med.* 26, 673–678.
- Moncada, E., Subira, M.L., Oleaga, A., Goni, F., Sanchez-Ibarrola, A., Monreal, M., et al., 1990. Insulin requirements and residual beta-cell function 12 months after concluding immunotherapy in type I diabetic patients treated with combined azathioprine and thymostimulin administration for one year. *J. Autoimmun.* 3, 625–638.
- Moran, A., Bundy, B., Becker, D.J., DiMeglio, L.A., Gitelman, S.E., Goland, R., et al., 2013. Interleukin-1 antagonism in type 1 diabetes of recent onset: two multicentre, randomised, double-blind, placebo-controlled trials. *Lancet* 381, 1905–1915.
- Nanto-Salonen, K., Kupila, A., Simell, S., Siljander, H., Salonsaari, T., Hekkala, A., et al., 2008. Nasal insulin to prevent type 1 diabetes in children with HLA genotypes and autoantibodies conferring increased risk of disease: a double-blind, randomised controlled trial. *Lancet* 372, 1746–1755.
- Narendran, P., Mannerling, S.I., Harrison, L.C., 2003. Proinsulin – a pathogenic autoantigen in type 1 diabetes. *Autoimmun. Rev.* 2, 204–210.
- Norris, J.M., Scott, F.W., 1996. A meta analysis of infant diet and insulin-dependent diabetes mellitus: do biases play a role. *Epidemiology* 7, 87–92.
- Norris, J.M., Beaty, B., Klingensmith, G., Yu, L., Hoffman, M., Chase, H.P., et al., 1996. Lack of association between early exposure to cow's milk protein and β cell autoimmunity: Diabetes Autoimmunity Study in the Young (DAISY). *JAMA* 276, 609–614.
- O'Callaghan, A., van Sinderen, D., 2016. Bifidobacteria and their role as members of the human gut microbiota. *Front. Microbiol.* 7, 925. Available from: <https://doi.org/10.3389/fmicb.2016.00925>.
- Orban, T., Farkas, K., Jalahej, H., Kis, J., Treszl, A., Falk, B., et al., 2010. Autoantigen-specific regulatory T cells induced in patients with type 1 diabetes mellitus by insulin B-chain immunotherapy. *J. Autoimmun.* 34, 408–415.
- Orban, T., Bundy, B., Becker, D.J., DiMeglio, L.A., Gitelman, S.E., Goland, R., et al., 2011. Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial. *Lancet* 378, 412–419.
- Panto, F., Giordano, C., Amato, M.P., Pugliese, A., Donatelli, M., D'Acquisto, G., et al., 1990. The influence of high dose intravenous immunoglobulins on immunological and metabolic pattern in newly diagnosed type I diabetic patients. *J. Autoimmun.* 3, 587–592.
- Pathiraja, V., Kuehlich, J.P., Campbell, P.D., Krishnamurthy, B., Loudovaris, T., Coates, P.T., et al., 2015. Proinsulin-specific, HLA-DQ8 and HLA-DQ8-transdimer-restricted CD4<sup>+</sup> T cells infiltrate islets in type 1 diabetes. *Diabetes* 64, 172–182.
- Penno, M.A.S., Couper, J.J., Craig, M.E., Colman, P.G., Rawlinson, W.D., Cotterill, A.M., et al., 2013. Environmental determinants of islet autoimmunity (ENDIA): a pregnancy to early life cohort study in children at-risk of type 1 diabetes. *BMC Pediatr.* 13, 124. Available from: <https://doi.org/10.1186/1471-2431-13-124>.
- Perrett, K., Jachno, K., Nolan, T., Harrison, L.C., 2019. Rotavirus vaccination and the incidence of type 1 diabetes in children. *JAMA Ped.* Available from: <https://doi:10.1001/jamapediatrics.2018.4578>.
- Pescovitz, M.D., Greenbaum, C.J., Krause-Steinrauf, H., Becker, D.J., Gitelman, S.E., Goland, R., et al., 2009. Rituximab, B-lymphocyte depletion, and preservation of beta-cell function. *N. Engl. J. Med.* 361, 2143–2152.
- Pescovitz, M.D., Greenbaum, C.J., Bundy, B., Becker, D.J., Gitelman, S.E., Goland, R., et al., 2014. B-lymphocyte depletion with rituximab and beta-cell function: two-year results. *Diabetes Care* 37, 453–459.
- Petersen, J.S., Karlsen, A.E., Markholst, H., Worsaae, A., Dyrberg, T., Michelsen, B., 1994. Neonatal tolerization with glutamic acid decarboxylase but not with bovine serum albumin delays the onset of diabetes in NOD mice. *Diabetes* 43, 1478–1484.
- Philips, J.C., Scheen, A.J., Le Registre Belge du Diabète, 2002. Info-congress. Study of the prevention of type 1 diabetes with nicotinamide: positive lessons of a negative clinical trial (ENDIT). [Article in French]. *Rev. Med. Liege* 57, 672–675.
- Ploix, C., Bergerot, I., Fabien, N., Perche, S., Moulin, V., Thivolet, C., 1998. Protection against autoimmune diabetes with oral insulin is associated with the presence of IL-4 type 2 T-cells in the pancreas and pancreatic lymph nodes. *Diabetes* 47, 39–44.
- Ploix, C., Bergerot, I., Durand, A., Czerniksky, C., Holmgren, J., Thivolet, C., 1999. Oral administration of cholera toxin B-insulin conjugates protects NOD mice from autoimmune diabetes by inducing CD4<sup>+</sup> regulatory T-cells. *Diabetes* 48, 2150–2156.
- Pozzilli, P., Signore, A., Williams, A.J., Beales, P.E., 1993. NOD mouse colonies around the world—recent facts and figures. *Immunol. Today* 14, 193–196.
- Pozzilli, P., Visalli, B., Boccuni, M.L., Baroni, M.G., Buzzetti, R., Fioriti, E., et al., 1994. Randomized trial comparing nicotinamide and nicotinamide plus cyclosporin in recent onset insulin-dependent diabetes (IMDIAB 1). The IMDIAB Study Group. *Diabet. Med.* 11, 98–104.
- Pozzilli, P., Visalli, N., Signore, A., Baroni, M.G., Buzzetti, R., Cavallo, M.G., et al., 1995. Double blind trial of nicotinamide in recent-onset IDDM (the IMDIAB III study). *Diabetologia* 38, 848–852.
- Pozzilli, P., Visalli, N., Cavallo, M.G., Signore, A., Baroni, M.G., Buzzetti, R., et al., 1997. Vitamin E and nicotinamide have similar effects in maintaining residual beta cell function in recent onset insulin-dependent diabetes (the IMDIAB IV study). *Eur. J. Endocrinol.* 137, 234–239.
- Pozzilli, P., Pitocco, D., Visalli, N., Cavallo, M.G., Buzzetti, R., Crino, A., et al., 2000. No effect of oral insulin on residual beta-cell function in recent-onset type 1 diabetes (the IMDIAB VII). IMDIAB Group. *Diabetologia* 43, 1000–1004.

- Raz, I., Elias, D., Avron, A., Tamir, M., Metzger, M., Cohen, I.R., 2001. Beta-cell function in new-onset type 1 diabetes and immunomodulation with a heat-shock protein peptide (DiaPep277): a randomised, double-blind, phase II trial. *Lancet* 358, 1749–1753.
- Rigby, M.R., DiMeglio, L.A., Rendell, M.S., Felner, E.I., Dostou, J.M., Gitelman, S.E., et al., 2013. Targeting of memory T cells with alefacept in new-onset type 1 diabetes (T1DAL study): 12 month results of a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Diabetes Endocrinol.* 1, 284–294.
- Rigby, M.R., Harris, K.M., Pinckney, A., DiMeglio, L.A., Rendell, M.S., Felner, E.I., et al., 2015. Alefacept provides sustained clinical and immunological effects in new-onset type 1 diabetes patients. *J. Clin. Invest.* 125, 3285–3296.
- Roep, B.O., Solvason, N., Gottlieb, P.A., Abreu, J.R.F., Harrison, L.C., Eisenbarth, G.S., et al., 2013. Plasmid-encoded proinsulin preserves C-peptide while specifically reducing proinsulin-specific CD8(+) T cells in type 1 diabetes. *Sci. Transl. Med.* 5, 191ra182.
- Rovainen, M., Klingel, K., 2010. Virus infections and type 1 diabetes risk. *Curr. Diab. Rep.* 10, 350–356.
- Rudy, G., Stone, N., Harrison, L.C., Colman, P.G., McNair, P., Brusic, V., et al., 1995. Similar peptides from two beta-cell autoantigens, proinsulin and glutamic acid decarboxylase, stimulate T cells of individuals at risk for insulin-dependent diabetes. *Mol. Med.* 1, 625–633.
- Schloot, N.C., Meierhoff, G., Lengyel, C., Vandorfi, G., Takacs, J., Panczel, P., et al., 2007. Effect of heat shock protein peptide DiaPep277 on beta-cell function in paediatric and adult patients with recent-onset diabetes mellitus type 1: two prospective, randomized, double-blind phase II trials. *Diabetes Metab. Res. Rev.* 23, 276–285.
- Schnell, O., Eisfelder, B., Standl, E., Ziegler, A.G., 1997. High-dose intravenous insulin infusion versus intensive insulin treatment in newly diagnosed IDDM. *Diabetes* 46, 1607–1611.
- Secchi, A., Pastore, M.R., Sergi, A., Pontiroli, A.E., Pozza, G., 1990. Prednisone administration in recent onset type I diabetes. *J. Autoimmun.* 3, 593–600.
- Selam, J.L., Woertz, L., Lozano, J., Robinson, M., Chan, E., Charles, M.A., 1993. The use of glipizide combined with intensive insulin treatment for the induction of remissions in new onset adult type I diabetes. *Autoimmunity* 16, 281–288.
- Shah, S.C., Malone, J.I., Simpson, N.E., 1989. A randomized trial of intensive insulin therapy in newly diagnosed insulin-dependent diabetes mellitus. *N. Engl. J. Med.* 320, 550–554.
- Shehadeh, N., Gelertner, L., Blazer, S., Perlman, R., Etzioni, A., 2001. The importance of insulin content in infant diet: suggestion for a new infant formula period. *Acta Paediatr.* 90, 93–95.
- Sherry, N., Hagopian, W., Ludvigsson, J., Jain, S.M., Wahnen, J., Ferry Jr., R.J., et al., 2011. Teplizumab for treatment of type 1 diabetes (Protege study): 1-year results from a randomised, placebo-controlled trial. *Lancet* 378, 487–497.
- Silverstein, J., Maclaren, N., Riley, W., Spillar, R., Radjenovic, D., Johnson, S., 1988. Immunosuppression with azathioprine and prednisone in recent-onset insulin-dependent diabetes mellitus. *N. Engl. J. Med.* 319, 599–604.
- Skyler, J.S., Rabinovitch, A., 1992. Cyclosporine in recent onset type I diabetes mellitus. Effects on islet beta cell function. Miami Cyclosporine Diabetes Study Group. *J. Diabetes Complications* 6, 77–88.
- Skyler, J.S., Lorenz, T.J., Schwartz, S., Eisenbarth, G.S., Einhorn, D., Palmer, J.P., et al., 1993. Effects of an anti-CD5 immunoconjugate (CD5-plus) in recent onset type I diabetes mellitus: a preliminary investigation. The CD5 Diabetes Project Team. *J. Diabetes Complications* 7, 224–232.
- Skyler, J.S., Krischer, J.P., Wolfsdorf, J., Cowie, C., Palmer, J.P., Greenbaum, C., et al., 2005. Effects of oral insulin in relatives of patients with type 1 diabetes: The Diabetes Prevention Trial—Type 1. *Diabetes Care* 28, 1068–1076.
- Slavin, A.J., Maron, R., Weiner, H.L., 2001. Mucosal administration of IL-10 enhances oral tolerance in autoimmune encephalomyelitis and diabetes. *Int. Immunol.* 13, 825–833.
- Stene, L.C., Ulriksen, J., Magnus, P., Joner, G., 2000. Use of cod liver oil during pregnancy associated with lower risk of type I diabetes in the offspring. *Diabetologia* 43, 1093–1098.
- Sutherland, D.E., Goetz, F.C., Sibley, R.K., 1989. Recurrence of disease in pancreas transplants. *Diabetes* 38 (suppl 1), 85–87.
- Suzuki, T., 1987. Diabetogenic effects of lymphocyte transfusion on the NOD or NOD nude mouse. In: Rygaard, J., Brunner, N., Graem, N., Spang-Thomson, M. (Eds.), *Immune Deficient Animals in Biomedical Research*. Karger, Basel, pp. 112–116.
- Tait, B.D., Harrison, L.C., Drummond, B.P., Stewart, V., Varney, M.D., Honeyman, M.C., 1995. HLA antigens and age at diagnosis of insulin-dependent diabetes mellitus. *Human Immunol.* 42, 116–122.
- Thorburn, A.N., Macia, L., Mackay, C.R., 2014. Diet, metabolites, and “western lifestyle” inflammatory diseases. *Immunity* 40, 833–842.
- Tian, J., Atkinson, M.A., Clare-Salzler, M., Herschenfeld, A., Forsthuber, T., Lehmann, P.V., et al., 1996. Nasal administration of glutamate decarboxylase (GAD65) peptides induces Th2 responses and prevents murine insulin-dependent diabetes. *J. Exp. Med.* 183, 1561–1567.
- Tisch, R., Wang, B., Weaver, D.J., Liu, B., Bui, T., Arthos, J., et al., 2001. Antigen-specific mediated suppression of beta cell autoimmunity by plasmid DNA vaccination. *J. Immunol.* 166, 2122–2132.
- Trentham, D.A., Dynesius-Trentham, R.A., Orav, E.J., 1993. Effects of oral administration of type II collagen on rheumatoid arthritis. *Science* 261, 1727–1730.
- Tuomi, T., Groop, L.C., Zimmet, P.Z., Rowley, M.J., Knowles, W., Mackay, I.R., 1993. Antibodies to glutamic acid decarboxylase reveal latent autoimmune diabetes mellitus in adults with a non-insulin-dependent onset of disease. *Diabetes* 42, 359–362.
- Turner, R., Stratton, I., Horton, V., Manley, S., Zimmet, P., Mackay, I.R., et al., 1997. UKPDS 25: autoantibodies to islet-cell cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes. UK Prospective Diabetes Study Group. *Lancet* 350, 1288–1293.
- Vaarala, O., Ilonen, J., Ruohola, T., Pesola, J., Virtanen, S.M., Harkonen, T., et al., 2012. Removal of bovine insulin from cow's milk formula and early initiation of beta-cell autoimmunity in the FINDIA pilot study. *Arch. Pediatr. Adolesc. Med.* 166, 608–614.
- Vague, P., Picq, R., Bernal, M., Lassmann-Vague, V., Viallettes, B., 1989. Effect of nicotinamide treatment on the residual insulin secretion in type 1 (insulin-dependent) diabetic patients. *Diabetologia* 32, 316–321.
- Vatanen, T., Kostic, A.D., d'Hennezel, E., Siljander, H., Franzosa, E.A., Yassour, M., et al., 2016. Variation in microbiome LPS immunogenicity contributes to autoimmunity in humans. *Cell* 165, 842–853.
- Ventura, A., Magazzu, G., Greco, L., 1999. Duration of exposure to gluten and risk for autoimmune disorders in patients with celiac disease (SIGEP study group for autoimmune disorders in celiac disease). *Gastroenterology* 117, 297–303.

- Verge, C.F., Gianani, R., Kawasaki, E., Yu, L., Pietropaolo, M., Jackson, R.A., et al., 1996. Prediction of type I diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-2 autoantibodies. *Diabetes* 45, 926–933.
- Vidal, J., Fernandez-Balsells, M., Sesmilo, G., Aguilera, E., Casamitjana, R., Gomis, R., et al., 2000. Effects of nicotinamide and intravenous insulin therapy in newly diagnosed type 1 diabetes. *Diabetes Care* 23, 360–364.
- Waldo, F.B., van den Wall Bake, A.W., Mestecky, J., Husby, S., 1994. Suppression of the immune response by nasal immunization. *Clin. Immunol. Immunopathol.* 72, 30–34.
- Walter, M., Philotheou, A., Bonnici, F., Ziegler, A.G., Jimenez, R., Group, N.B.I.S., 2009. No effect of the altered peptide ligand NBI-6024 on beta-cell residual function and insulin needs in new-onset type 1 diabetes. *Diabetes Care* 32, 2036–2040.
- Walter, M., Kaupper, T., Adler, K., Foersch, J., Bonifacio, E., Ziegler, A.G., 2010. No effect of the 1alpha,25-dihydroxyvitamin D3 on beta-cell residual function and insulin requirement in adults with new-onset type 1 diabetes. *Diabetes Care* 33, 1443–1448.
- Weiner, H.L., Mackin, G.A., Matsui, M., 1993. Double-blind pilot trial of oral tolerization with myelin antigens in multiple sclerosis. *Science* 259, 1321–1324.
- Wentworth, J.M., Fourlanos, S., Harrison, L.C., 2009. Deconstructing the stereotypes of diabetes within the modern diabetogenic environment. *Nat. Rev. Endocrinol.* 5, 483–489.
- Wherrett, D.K., Bundy, B., Becker, D.J., DiMeglio, L.A., Gitelman, S.E., Goland, R., et al., 2011. Antigen-based therapy with glutamic acid decarboxylase (GAD) vaccine in patients with recent-onset type 1 diabetes: a randomised double-blind trial. *Lancet* 378, 319–327.
- Winkler, C., Schober, E., Ziegler, A.G., Holl, R.W., 2012. Markedly reduced rate of diabetic ketoacidosis at onset of type 1 diabetes in relatives screened for islet autoantibodies. *Pediatr. Diabetes* 13, 308–313.
- Yu, L.C., Wang, J.T., Wei, S.C., Ni, Y.H., 2012. Host-microbial interactions and regulation of intestinal epithelial barrier function: from physiology to pathology. *World J. Gastrointest. Pathophysiol.* 3, 27–43.
- Zhang, Z.H., Davidson, L., Eisenbarth, G., Weiner, H.L., 1991. Suppression of diabetes in nonobese diabetic mice by oral administration of porcine insulin. *Proc. Natl. Acad. Sci. U.S.A.* 88, 10252–10256.
- Ziegler, A.G., Rewers, M., Simell, O., Lempiainen, J., Steck, A., Winkler, C., et al., 2013. Seroconversion to multiple islet antibodies and risk of progression to diabetes in children. *JAMA* 309, 2473–2479.
- Ziegler, A.G., Danne, T., Dunger, D.B., Berner, R., Puff, R., Kiess, W., et al., 2016. Primary prevention of beta-cell autoimmunity and type 1 diabetes—The Global Platform for the Prevention of Autoimmune Diabetes (GPPAD) perspectives. *Mol. Metab.* 5, 255–262.

## Further Reading

- Atkinson, M.A., Leiter, E.H., 1999. The NOD mouse model of type 1 diabetes: as good as it gets? *Nat. Med.* 5, 601–604.
- Bennett, S.T., Lucassen, A.M., Gough, S.C., Powell, E.E., Undlien, D.E., Pritchard, L.E., et al., 1995. Susceptibility to human type 1 diabetes at IDDM2 is determined by tandem repeat variation at the insulin gene minisatellite locus. *Nat. Genet.* 9, 284–292.
- Brown, C.T., Davis-Richardson, A.G., Giongo, A., Gano, K.A., Crabb, D.B., Mukherjee, N., et al., 2011. Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. *PLoS One* 6, e25792.
- Coulson, B.S., Witterick, P.D., Tan, Y., Hewish, M.J., Mountford, J.N., Harrison, L.C., et al., 2002. Growth of rotaviruses in primary pancreatic cells. *J. Virol.* 76, 9537–9544.
- DeStefano, F., Mullooly, J.P., Okoro, C.A., Chen, R.T., Marcy, S.M., Ward, J.I., et al., 2001. Childhood vaccinations, vaccination timing, and risk of type 1 diabetes mellitus. *Pediatrics* 108, E112.
- Durinovic-Bello, I., Wu, R.P., Gersuk, V.H., Sanda, S., Shilling, H.G., Nepom, G.T., 2010. Insulin gene VNTR genotype associates with frequency and phenotype of the autoimmune response to proinsulin. *Genes Immun.* 11, 188–193.
- Harrison, L.C., 2001. Risk assessment, prediction and prevention of type 1 diabetes. *Pediatr. Diabetes* 2, 71–82.
- Honeyman, M.C., Stone, N.L., Falk, B.A., Nepom, G., Harrison, L.C., 2010. Evidence for molecular mimicry between human T cell epitopes in rotavirus and pancreatic islet autoantigens. *J. Immunol.* 184, 2204–2210.
- Honeyman, M.C., Stone, N.L., Harrison, L.C., 1998. T-cell epitopes in type 1 diabetes autoantigen tyrosine phosphatase IA-2: potential for mimicry with rotavirus and other environmental agents. *Mol. Med.* 4, 231–239.
- Leonard, M.T., Davis-Richardson, A.G., Ardissono, A.N., Kemppainen, K.M., Drew, J.C., Ilonen, J., et al., 2014. The methylome of the gut microbiome: disparate Dam methylation patterns in intestinal *Bacteroides dorei*. *Front. Microbiol.* 5, 361. Available from: <https://doi.org/10.3389/fmicb.2014.00361>.
- Leslie, D., Lipsky, P., Notkins, A.B., 2001. Autoantibodies as predictors of disease. *J. Clin. Invest.* 108, 1417–1422.
- Lindberg, B., Ahlfors, K., Carlsson, A., Ericsson, U.B., Landin-Olsson, M., Lernmark, A., et al., 1999. Previous exposure to measles, mumps, and rubella—but not vaccination during adolescence—correlates to the prevalence of pancreatic and thyroid autoantibodies. *Pediatrics* 104, e12.
- Ou, D., Mitchell, L.A., Metzger, D.L., Gillam, S., Tingle, A.J., 2000. Cross-reactive rubella virus and glutamic acid decarboxylase (65 and 67) protein determinants recognised by T cells of patients with type I diabetes mellitus. *Diabetologia* 43, 750–762.
- Pugliese, A., Zeller, M., Fernandez Jr, A., Zalcberg, L.J., Bartlett, R.J., Ricordi, C., et al., 1997. The insulin gene is transcribed in the human thymus and transcription levels correlated with allelic variation at the INS VNTR-IDDM2 susceptibility locus for type 1 diabetes. *Nat. Genet.* 15, 293–297.
- Rose, N.R., 2008. Predictors of autoimmune disease: autoantibodies and beyond. *Autoimmunity* 41, 419–428.
- Sarugeri, E., Dozio, N., Belloni, C., Meschi, F., Pastore, M.R., Bonifacio, E., 1998. Autoimmune responses to the beta cell autoantigen, insulin, and the INS VNTR-IDDM2 locus. *Clin. Exp. Immunol.* 114, 370–376.
- Yu, L.C., Wang, J.T., Wei, S.C., Ni, Y.H., 2012. Host-microbial interactions and regulation of intestinal epithelial barrier function: from physiology to pathology. *World J. Gastrointest. Pathophysiol.* 3, 27–43.

# Treatment of Autoimmune Disease: Established Therapies

Benedict K. Tiong, Bevra H. Hahn and Thanda Aung

David Geffen School of Medicine, Division of Rheumatology, University of California Los Angeles, Los Angeles, CA,  
United States

## OUTLINE

<b>Principles of Immune Suppression</b>	<b>1418</b>	<i>IL-1 Antagonists</i>	<b>1426</b>
<b>General Considerations</b>	<b>1418</b>	<i>Secukinumab (Anti-IL17A: Cosentyx)</i>	<b>1426</b>
<b>Nonspecific Antiinflammatory Drugs</b>	<b>1419</b>	<i>Ustekinumab (Anti-p40 for IL-12 and IL-23 Signaling: Stelara)</i>	<b>1426</b>
Nonsteroidal Antiinflammatory Drugs	1419	<i>B-Cell-Targeted Therapies</i>	1427
Glucocorticoids	1419	<i>Rituximab (Rituxan)</i>	1427
<b>Established Treatments of Rheumatic Diseases</b>	<b>1420</b>	<i>Belimumab (Anti-BLyS: Benlysta)</i>	<b>1427</b>
Antimalarials	1420	<i>T-Cell-Targeted Therapies</i>	1427
Sulfasalazine	1420	<b>Other Treatment Options</b>	<b>1428</b>
Leflunomide	1421	<i>Apremilast (Otezla)</i>	1428
Methotrexate	1421	<i>Tofacitinib (Inhibitor of Janus Kinase Activation Pathway: Xeljanz)</i>	1428
Cyclophosphamide	1422	<b>Intravenous Immunoglobulin</b>	<b>1428</b>
Mycophenolate Mofetil ( <i>Cellcept</i> )	1423	<b>Comment Regarding Costs of Therapies:</b>	
Azathioprine ( <i>Imuran</i> )	1423	<i>Biosimilars</i>	<b>1429</b>
Calcineurin Inhibitors: Cyclosporin A, Tacrolimus, and Voclosporin	1424	<b>Moving Toward More Biological and Molecular Therapies</b>	<b>1429</b>
<b>Biologic Agents</b>	<b>1424</b>	<b>References</b>	<b>1429</b>
Cytokine-Targeted Therapies	1424		
Tumor Necrosis Factor Inhibitors	1425		
Tocilizumab (Anti-IL6R: <i>Actemra</i> )	1425		

Rapid advances in immunology and greater understanding of disease etiology and pathogenesis have made the treatment of autoimmune diseases a dynamic and swiftly evolving field. Established therapies such as glucocorticoids (GCs), nonspecific immunosuppressive agents, and disease-modifying rheumatoid drugs (DMARD) are being supplemented or replaced by newer, more targeted biologic and molecular therapies. Progress in elucidation of basic mechanisms of autoimmune diseases has led to rapid development of targets on antigen-presenting cells (APCs), T/B lymphocytes, cytokines, and costimulatory molecules. In addition, improved measures of defining clinical response and identification of biomarkers reflecting clinical outcomes and prognosis have accelerated the development of treatment options for autoimmune diseases. The goal of this

chapter is to review established therapies of autoimmune diseases, with a focus on rheumatic diseases. We will highlight traditional therapies that form the foundation of current clinical practice (see **Table 71.1**), while laying out a context for which newer biologic and molecular targets are now incorporated into the current standard of care.

**TABLE 71.1** Established Immunosuppressive Treatments for Autoimmune Rheumatic Diseases

Drug	Primary mechanism of action	Side effects	Indications
<b>CONVENTIONAL DMARDs</b>			
Antimalarials	Inhibition of TLR-3/7, raising of lysozyme pH affecting antigen processing	Retinopathy and gray skin/nails discoloration. Rarely headache, pruritus, rash, neuropathy, corneal deposition, peripheral myopathy, cardiomyopathy	<i>Systemic lupus erythematosus, rheumatoid arthritis, juvenile idiopathic arthritis, Sjogren's, juvenile dermatomyositis, palindromic rheumatism</i>
Sulfasalazine	Inhibition of prostaglandin synthesis and cyclooxygenase, inhibition of NF $\kappa$ B transcription, reduction of TNF, suppression of B cells	Elevated liver enzymes, leukopenia, agranulocytosis, megaloblastic anemia, oligospermia, sperm dysmotility, GI or CNS side effects	<i>Inflammatory bowel disease (ulcerative colitis), mild rheumatoid arthritis, psoriatic arthritis, juvenile idiopathic arthritis</i>
Leflunomide	Inhibits dihydroorotate dehydrogenase, affecting de novo pyrimidine synthesis	Elevated liver enzymes, diarrhea, rash, hair loss, hypertension, interstitial pneumonitis, class X teratogen	<i>Rheumatoid arthritis</i>
Methotrexate	Inhibits dihydrofolate reductase, interfering with purine and pyrimidine metabolism and amino acid synthesis	Elevated liver enzymes, oral ulcers, diarrhea, mild hair loss, pneumonitis, infections, bone marrow suppression	<i>Rheumatoid arthritis, psoriasis and psoriatic arthritis, seronegative spondyloarthropathies, arthritic/skin manifestations of systemic lupus erythematosus, granulomatosis with polyangiitis, steroid sparing agent</i>
Cyclophosphamide	Alkylating agent that inhibits cell division by cross-linking DNA and reducing DNA synthesis	Infections, bladder toxicity with hemorrhagic cystitis or carcinoma, secondary malignancy, premature ovarian failure, infertility, neutropenia, hair loss, bone marrow suppression, oral ulcers	Systemic lupus erythematosus nephritis or other life-threatening manifestations, <i>rheumatoid arthritis transverse myelitis, systemic sclerosis-related ILD, granulomatosis with polyangiitis, polyarteritis nodosa, rheumatoid vasculitis</i>
Mycophenolate mofetil	Inhibits inosine monophosphate dehydrogenase, affecting de novo purine synthesis in activated lymphocytes	Diarrhea, abdominal cramps, nausea, infection, bone marrow suppression, neoplasia, rash, tremor	Systemic lupus erythematosus nephritis or other serious manifestations, <i>myasthenia gravis, systemic vasculitis</i>
Azathioprine	Purine antagonist, inhibits synthesis of DNA, RNA, proteins, cellular metabolism	Bone marrow suppression, infection, gastrointestinal upset, nausea, neoplasia	Maintenance therapy for systemic lupus erythematosus nephritis, treatment and/or maintenance for other manifestations of SLE, <i>ulcerative colitis, Crohn's disease rheumatoid arthritis, ANCA-positive vasculitis</i>
Cyclosporine	Calcineurin inhibitors; inhibit transcription of IL-2 production, proliferation of T lymphocytes; inhibits production of IL2, IL-4, and CD40 ligand. Voclosporin is in clinical trials for lupus nephritis	Renal toxicity, hypertension, neurologic side effects, skin or lymphoproliferative disorders, significant drug–drug interactions	Refractory ocular and mucocutaneous Behcet's disease, adult systemic lupus nephritis, systemic sclerosis, severe ulcerative colitis, myasthenia gravis; typically not first-line therapy
Tacrolimus			
Voclosporin			
<b>BIOLOGIC DMARDs</b>			
Infliximab	Tumor necrosis factor inhibitor; downregulates inflammatory cytokines, inhibits immunoregulatory functions of TNF	Infusion reactions to IV infliximab (but not to subcutaneous), rash, arthralgias, fatigue, infection, increased risk of lymphoma, skin cancers, neurologic disorders	<i>Rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, plaque psoriasis (inflix, etan, ada), juvenile idiopathic arthritis (etanercept and adalimumab), Crohn's disease, and ulcerative colitis. Uveitis (adalimumab)</i>
Adalimumab			
Golimumab			
Etanercept			
Certolizumab			

(Continued)

**TABLE 71.1** (Continued)

Drug	Primary mechanism of action	Side effects	Indications
Tocilizumab	IL-6R antagonist leading to reduced cytokine and proinflammatory pathways	Neutropenia, thrombocytopenia, elevated liver enzymes, GI perforation, infections, hyperlipidemia, rash	<i>Rheumatoid arthritis, giant cell arteritis, systemic and polyarticular juvenile idiopathic arthritis</i>
Anakinra	Antagonists of IL-1R, therefore inhibits inflammasome	Nausea, diarrhea, vomiting, eosinophilia, leukopenia, headache, infection	<i>Rheumatoid arthritis, neonatal onset multisystem inflammatory disease (anakinra), systemic juvenile idiopathic arthritis, acute or uncontrolled gout.</i>
Canakinumab			<i>Canakinumab is approved for <i>Familial Mediterranean Fever, cryopyrin-associated periodic syndromes, and TNF receptor-associated periodic syndromes</i></i>
Rituximab	Anti-CD20 monoclonal antibody against B cells	Infusion reaction, infections. Reduced vaccination response, mucocutaneous reactions, malignancy, rare PML due to JC virus, cytopenias	<i>Rheumatoid arthritis, granulomatosis with polyangiitis, microscopic polyangiitis, systemic lupus erythematosus</i>
Belimumab	Inhibits cytokine B lymphocyte stimulator protein (BLys, also called BAFF) necessary for B-cell proliferation, survival, and differentiation	Infections. Nausea, diarrhea, fever, insomnia, depression, leukopenia, angioedema, hypersensitivity to IV infusion. Recently available in the United States in subcutaneous injectable form	<i>Mild-to-moderate autoantibody-positive SLE (has not completed testing in lupus nephritis and has not been used in patients with active central nervous system lupus)</i>
Secukinumab	Inhibits IL-17A and the release of IL-17-stimulated chemokines and proinflammatory cytokines	Infections. Neutropenia, nausea, diarrhea, anaphylaxis to IV doses, urticaria, can induce inflammatory bowel disease	<i>Psoriasis, psoriatic arthritis, and ankylosing spondylitis</i>
Ustekinumab	Inhibits IL-12 and IL-23-mediated cell signaling and cytokine production	Infections. Nasopharyngitis, increased risk of nonmelanoma skin cancers, injection site erythema, headache, reversible posterior leukoencephalopathy syndrome, arthralgia, headache, back pain, nausea, diarrhea	<i>Crohn's disease, plaque psoriasis, and psoriatic arthritis</i>
Abatacept	CTLA4Ig fusion protein: binds CD80 and 86, preventing their binding to CD28, thereby inhibiting T-cell activation	Infections. Headache, nausea, malignancy, may induce multiple sclerosis (very rare), vasculitis, nasopharyngitis, dizziness, back pain, extremity pain	<i>Rheumatoid arthritis, juvenile idiopathic arthritis, psoriatic arthritis, lupus arthritis</i>
Apremilast	Inhibits PDE4, results in increased intracellular cAMP levels	Headache, depression, diarrhea, nausea, decreased appetite, infection, rash, weight loss	<i>Psoriasis, psoriatic arthritis</i>
Tofacitinib Baricitinib	Inhibits JAK enzymes, preventing cytokine signaling of immune cells	Infection. Increased liver enzymes, infection, anemia, lymphopenia, hyperlipidemia, headache	<i>Rheumatoid arthritis</i>

**OTHER THERAPIES**

IVIG	Interference with Fc receptors, antiidiotypic antibodies, inhibition of reticuloendothelial clearance	Rate-related infusion reactions, headache, aseptic meningitis, acute renal failure, thrombosis, urticaria, anaphylaxis, especially in IgA-deficient patients	<i>Guillain–Barré syndrome, myasthenia gravis, autoimmune peripheral neuropathies, rheumatic hematologic autoimmune thrombocytopenia, or hemolytic anemia, refractory dermatomyositis or polymyositis, Kawasaki disease in children</i>
------	---	--	---

US FDA has approved therapies for diseases listed in italics or are expected to soon do so. DMARD, Disease-modifying antirheumatic drugs; GI, gastrointestinal; ILD, interstitial lung disease; IL, Interleukin; IVIG, intravenous immunoglobulin; JC, John Cunningham virus; JAK, Janus kinase; NF $\kappa$ B, nuclear factor kappa B; PML, progressive multifocal leukoencephalopathy; TLR, Toll-like receptor; TNF, tumor necrosis factor.

## PRINCIPLES OF IMMUNE SUPPRESSION

As with our oncology colleagues, the goals for physicians treating autoimmune diseases are to induce improvement (remission or low disease activity), while arresting irreversible organ damage and minimizing treatment side effects. Although definitive cure and restoration of permanent immunological tolerance would be ideal, most treatments now do not achieve that goal. Treatments are often nonspecifically antiinflammatory and/or immunosuppressive. Thus the substantive side effects that accompany such treatments require balancing the risks of infection, bone marrow suppression, and other organ toxicity with potential efficacy.

In recent years, the paradigm of “tight control” has emerged, in which goal-directed treatments are based on quantitative and disease-specific measurements that guide providers to rapidly achieve low disease activity or remission. Notably, “tight control” in rheumatoid arthritis (RA) was illustrated in the TICORA study (Grigor et al., 2004) in which 111 patients were randomly assigned to either an intensive or a routine management group. Intensive management consisted of monthly office visits, measurements of disease activity scores (DAS), steroid injections of swollen joints, and every 3 month escalation of treatment by a defined protocol if moderate or high disease activity persisted. In contrast, the routine management group was seen every 3 months, without DAS measurement, and steroid injections and treatment escalation were based on the clinical judgment of the clinician, in contrast to predefined formal targets. After 18 months, patients in the tight control/intensive group showed significantly improved disease activity, physical function, and quality of life, as well as less radiographic progression of arthritis—all at no additional cost compared to routinely managed patients. The BeST study (Goekoop-Ruiterman et al., 2007; Goekoop-Ruiterman et al., 2005) and others (Mottonen et al., 1999; Rantalaiho et al., 2009) similarly illustrated greater remission rates and earlier functional improvement in RA arthritis patients with early, goal-directed therapy emphasizing aggressive treatment to achieve tight control of disease. Given the clear advantages of intensive treatment, the tight control paradigm is reflected in the 2015 update of the American College of Rheumatology’s recommendations for the use of disease-modifying antirheumatic drugs (DMARD) and biologics in the treatment of RA (Singh et al., 2016). With greater integration of quantitative measurements and DAS to define targets for treatment remission, goal-directed therapy as demonstrated in RA may serve as a harbinger for tight control treatment algorithms under study in other autoimmune diseases. Already, there is interest in working groups for establishing similarly objective “tight control” measures for seronegative spondyloarthropathies, systemic lupus erythematosus (SLE), and others.

## GENERAL CONSIDERATIONS

For many autoimmune disorders, chronic inflammation can lead to a variety of nonspecific, constitutional symptoms that include fatigue, muscle pains, weight loss, and/or fever. In fact, 40%–80% of the patients with SLE (lupus) experience such constitutional symptoms at any one time (Von Feldt, 1995). Furthermore, constitutional symptoms are present in a variety of other disorders such as multiple sclerosis, RA, multisystem autoimmune diseases, and vasculitis. General therapeutic principles focus on treating reversible causes of fatigue, weakness, and weight loss, such as anemia, drug toxicities, and ruling out other potential etiologies such as malignancy, infections, or thyroid/endocrine disorders. Proper muscle conditioning with appropriate aerobic exercise, good sleep management, and pain control may improve symptoms. Some studies have shown that stress may induce or exacerbate preexisting lupus symptoms (Blumenfield, 1978; Otto and Mackay, 1967), in which overall stress reduction may be beneficial (Greco et al., 2004).

In addition, management of precipitating environmental factors such as sun exposure and tobacco smoke is important. UV light in SLE patients induces increased apoptosis in skin cells, thus enhancing self-antigenicity of keratinocytes to express self-antigens on their surface that may stimulate an inflammatory response and autoantibody production leading to photosensitivity, cutaneous SLE, and/or generalized flares (Casciola-Rosen et al., 1994; Jones, 1992). Furthermore, genetically prone RA patients that carry an epitope in the hypervariable region of the human leukocyte antigen-DR (HLA-DR) chain, known as the “shared epitope,” have a higher risk of developing RA if they smoke cigarettes (Padyukov et al., 2004). The relative risk for development of RA in current smokers is 2.3–5.6-fold higher than for nonsmokers. In another study, the relative risk for developing RA was 20-fold higher in those with two alleles of the shared epitope, a smoking history, and anti-cyclic citrullinated peptide (anti-CCP) antibodies (Klareskog et al., 2006). Smoking may also increase disease severity (Saag et al., 1997; Wolfe, 2000). The mechanism of interaction between smoking and the shared epitope may primarily affect

citrullination of proteins in inflamed synovial tissue. The association between smoking and RA was most robust in patients with anti-CCP autoantibodies detected in blood (Klarekog et al., 2006). Studies have shown an association with smoking and development of autoantibodies, such as antinuclear antibodies (ANA) (Regius et al., 1988). Smoking may also be associated with increased risk for development of SLE (Ghassy et al., 2001) and Crohn's disease, although it may lessen the risk of developing ulcerative colitis (UC) (Mahid et al., 2006; Tobin et al., 1987). Hence, simple but important lifestyle changes such as reducing sun exposure (for SLE) and avoiding tobacco (for RA, SLE, and Crohn's disease) are important therapeutic considerations in management of autoimmune diseases.

## NONSPECIFIC ANTIINFLAMMATORY DRUGS

### Nonsteroidal Antiinflammatory Drugs

Nonsteroidal antiinflammatory drugs (NSAIDs) are widely used throughout the world either over the counter or via prescription. All major NSAID classes share the common mechanism of inhibiting cyclooxygenase, which metabolizes arachidonic acid to cyclic endoperoxidases to form prostaglandins. Prostaglandins' role in inflammation includes induction of swelling, erythema, neutrophil trafficking, changes in vascular permeability, and inhibition of apoptosis (Harris, 2002; Lu et al., 1995). In addition, other nonprostaglandin mechanisms include NSAID inhibition of nuclear factor kappa B (NF $\kappa$ B)-dependent transcription (Amin et al., 1995), thus decreasing nitric oxide that normally may lead to increased vascular permeability and immune cell trafficking. NSAIDs are used commonly in multisystem autoimmune diseases to treat constitutional symptoms, fever, arthritis, serositis, and headache. The potential adverse effects (AEs) of NSAIDs often limit their use, particularly induction of dyspepsia, peptic ulcer disease (often with bleeding), renal vasoconstriction with acute renal injury, and exacerbation of hypertension and heart failure (Solomon, 2012), with increased risk for myocardial infarction. Thus, for safety concerns, they are recommended most commonly for as-needed (for pain and/or joint inflammation) rather than continual use. According to Epocrates searched on March 2018, a monthly dose of ibuprofen (400 mg tid) costs \$24.00.

### Glucocorticoids

GCs have a broad range of antiinflammatory and immunosuppressive effects on both the innate and adaptive immune system. GC binds to an intracellular receptor, where they can directly affect gene transcription, resulting in inhibition of production of inflammatory cytokines, and effects on posttranslational mRNA stability. Antiinflammatory effects include downregulation of nitric oxide synthesis resulting in reduced blood vessel permeability, decreased leukocyte migration to peripheral tissues, inhibition of inflammatory mediators such as eicosanoids, inhibition of collagenases, and suppression of inflammatory cytokines. Effects of GC on immune cells include inhibition of signaling for T-cell activation and interleukin (IL)-2 synthesis, downregulation of APCs via blockade of costimulatory molecules, immune deviation toward Th2 cytokines, and induction of T-cell apoptosis (Kirouka, 2007). GCs are often used to control acute manifestations of inflammatory and autoimmune disorders, dosed on a mg/kg basis depending on the severity of the disease. Typical dosing regimens may be divided as follows from most potent to least: (1) pulse intravenous (IV) therapy with methylprednisolone 500–1000 mg/day, (2) very high-dose oral GC at 1–2 mg/kg/day prednisone or equivalent, (3) high-dose oral GC at 0.6–1 mg/kg/day prednisone or equivalent, (4) medium-dose GC at 0.125–0.5 mg/kg/day prednisone or equivalent, or (5) low-dose GC at 0.125 mg/kg/day prednisone or equivalent (King and Hahn, 2007). Organ threatening manifestations of disease in SLE, dermatomyositis, large and small vessel vasculitis, and multiple sclerosis often require pulse IV steroids as part of the initial induction therapy for acute flare control. Other common indications include polymyalgia rheumatica, crystalline disease flares, active RA, and Sjögren's extrasalivary gland manifestations.

GCs have numerous AEs on many organ systems, more common with use of GC in high doses or over a long period of time. Major AEs with high dose (prednisone >20 mg/day) include infection, mental disturbances, osteonecrosis, osteoporosis with fractures, and impact on safety with live vaccination. For use of prednisone doses >10 mg/day, there is increased risk of glucose intolerance, peptic ulcer disease, myopathy, glaucoma, and hypertension. Weight gain is found as a common side effect even with the low dose, prednisone >5 mg/day and osteoporosis risk with the doses  $\geq$  5–7.5 mg/day for 3 months (Saag et al., 2011). Recent data (Al Sawah et al., 2015) show that doses of prednisone  $\geq$  7.5 mg of prednisone daily are associated with measurable damage over

time, but doses >20 mg a day cause damage at twice that rate. Most authorities recommend tapering to doses between 0 and 7.5 mg a day for this reason.

According to Epocrates in March 2018, a monthly dose of prednisone 15 mg daily costs \$48.00.

## ESTABLISHED TREATMENTS OF RHEUMATIC DISEASES

### Antimalarials

Antimalarial medications such as hydroxychloroquine, chloroquine, and quinacrine can be used to treat mild-to-moderate manifestations of SLE or RA and other rheumatic disorders. Various mechanisms of action may contribute to their utility in immune modulation, including (1) inhibition of activation of intracellular Toll-like receptor (TLR)-3 and TLR-7, (2) blockade of antigen processing by raising intracytoplasmic pH in lysozymes with resultant decreased lymphocyte proliferation, autoantibody production, and natural killer cells (NK) activity, and (3) inhibition of formation of immune complexes (Fox, 1993; Fox and Kang, 1993; Kyburz et al., 2006). In addition, antiinflammatory effects include inhibition of phospholipases, prostaglandins, blockade of superoxide secretion, suppression of destructive proteolytic enzymes by synoviocytes, and blockade of UV light to protect keratinocytes from increased antigenicity (Wallace, 2007). Hormonal actions may impair insulin release, antiproliferative effects may inhibit graft versus host disease, and intercalation with DNA may block synthesis to allow degradation of ribosomal RNA (Wallace, 2007). Hydroxychloroquine and chloroquine can also inhibit platelet aggregation and adhesion (Edwards et al., 1997; Ernst et al., 1984; Jancinova et al., 1994), adding to their utility for SLE patients with coexisting antiphospholipid syndrome and/or platelet abnormalities.

In SLE, antimalarials are most useful in treating constitutional symptoms, skin lesions, and arthritis, and for reduction of disease flare. In addition, the 2007 LUMINA trial illustrated an association between hydroxychloroquine and improved survival (Alarcon et al., 2007). Pregnant mothers with anti-Ro may use hydroxychloroquine to reduce risk of antibody-associated cardiac neonatal lupus (Izmirly et al., 2010). In RA, antimalarials are typically used in combination with other disease-modifying agents, such as methotrexate (MTX) or sulfasalazine. A large observational study indicated reduced risk of diabetes mellitus among patients who were taking hydroxychloroquine for RA compared to those not taking the medication (Wasko et al., 2007). Antimalarials may also decrease antibody levels in Sjögren's syndrome, as well as improve sicca symptoms by inhibiting glandular cholinesterase (Dawson et al., 2005). Hydroxychloroquine has also been used in systemic onset juvenile idiopathic arthritis (Still's disease), juvenile dermatomyositis, and palindromic rheumatism. One study found that the use of antimalarials in palindromic rheumatism patients was associated with a 20% decreased risk of progression to RA or other connective tissue disease (Gonzalez-Lopez et al., 2000). Side effects vary with specific antimalarials (in general the highest risk of important AEs is associated with chloroquine). These side effects include neuromuscular and cardiac toxicity, skin changes (particularly hyperpigmentation with all, and yellow discoloration with quinacrine), aplastic anemia (quinacrine), and rare ocular effects, including macular damage. Regular ocular screening of patients treated for more than 6 months with chloroquine or hydroxychloroquine is recommended (Marmor et al., 2011). For maximal safety regarding retinal toxicity, doses of hydroxychloroquine should not exceed 5 mg/kg daily (real weight); and risk for retinal toxicity increases after a total cumulative dose of 1000 g. According to Epocrates searched on March 2018, a monthly dose of hydroxychloroquine (400 mg daily) costs \$240.00.

### Sulfasalazine

Sulfasalazine (azulfidine) was originally proposed for use in RA, although ultimately great benefit was seen in treatment for inflammatory bowel disease (IBD). After oral ingestion, the majority of the intact drug reaches the large intestine and is reduced to sulfapyridine and 5-aminosalicylic acid (5-ASA). For IBD, and specifically UC, 5-ASA acts locally in the colon to decrease inflammatory responses via inhibition of prostaglandin synthesis. Interestingly, in contrast to UC, the active metabolite in RA patients is sulfapyridine, although the exact mechanism of action has not been clearly identified. Patients with mild/moderate UC treated with azulfidine or 5-ASA have 50%–65% response rates (MacDermott, 2017). Patients with RA show approximately 50% response rates (20% improvement or better) to sulfasalazine alone, and better response rates (nearly 80%) for triple therapy with sulfasalazine plus MTX plus hydroxychloroquine (Weisman and Rinaldi, 2017). In addition, studies suggest that the parent sulfasalazine drug inhibits NF $\kappa$ B transcription, may inhibit tumor necrosis factor (TNF)- $\alpha$  in

macrophages by inducing apoptosis, and may suppress B-cell function (Hirohata et al., 2002; Lee et al., 2004; Rodenburg et al., 2000; Wahl et al., 1998). In RA, sulfasalazine is often used in combination therapy with other DMARDs for optimal treatment. AEs include elevated liver function tests, leukopenia, agranulocytosis, megaloblastic anemia, and gastrointestinal or central nervous system effects. In men, transient infertility has been observed with qualitative and quantitative abnormalities in sperm including oligospermia and sperm dysmotility. These effects were reversible 1–3 months after discontinuation of sulfasalazine (Ostensen, 2017; Sands et al., 2015).

According to Epocrates searched on March 2018, the monthly cost of sulfasalazine at 1 g bid is \$41.00. The monthly cost of triple therapy with sulfasalazine plus MTX at 15 mg a week orally plus hydroxychloroquine at 400 mg daily is approximately \$300.00, which is considerably less expensive than the widely used MTX-plus-adalimumab (biologic inhibitor of TNF- $\alpha$  which is given 40 mg subcutaneously every 2 weeks which costs approximately \$5000 per month), a combination which costs approximately \$5125.00 per month.

### Leflunomide

Leflunomide (LF) is an oral medication that, once absorbed, is metabolized into its active form known as teriflunomide. The main mechanism of action of teriflunomide is to inhibit the mitochondrial enzyme dihydroorotate dehydrogenase (DHODH), which is an enzyme involved in the de novo pyrimidine synthesis pathway of ribonucleotide uridine monophosphate pyrimidine. Disruption of DHODH prevents activated lymphocytes from moving from the G1 to S phase (Fox, 1998). In addition, the immunomodulatory effects of LF are broad, including inhibition of leukocyte adhesion to endothelial cells and infiltration into synovium, which may be of particular importance in RA (Dimitrijevic and Bartlett, 1996; Grisar et al., 2004; Salmi et al., 1997). In addition, LF preferentially inhibits memory self-reactive lymphocytes, affects dendritic cell antigen presentation, and blocks NF $\kappa$ B activation (Manna and Aggarwal, 1999; Zhang et al., 1997). LF blocks protein tyrosine kinases Jak1 and Jak3, which affect T-cell stimulation via IL-2 receptor activation (Siemasko et al., 1998). LF may also increase the production of transforming growth factor beta, which is a known antiinflammatory cytokine (Cao et al., 1996). In patients with RA, LF is similar in efficacy to MTX; each treatment used alone results in 20% or greater improvement in approximately 50% of the patients. Common side effects include diarrhea, rash, hair loss, and elevated liver enzymes. Less common side effects include hypertension, interstitial pneumonitis, leukopenia, hematologic toxicities, and peripheral neuropathy. LF is contraindicated in pregnant and nursing women and in patients with preexisting liver disease.

LF is approved by the United States Food and Drug Administration (US FDA) for the treatment of RA. In RA, trials have demonstrated efficacy of LF as monotherapy, with comparable outcomes to those of MTX or sulfasalazine monotherapy (Emery et al., 2000; Smolen et al., 1999). Other trials have shown efficacy in psoriatic arthritis (PsA), juvenile polyarthritis, and resistant dermatomyositis, but not ankylosing spondylitis (AS). In [www.goodrx.com](http://www.goodrx.com) searched on March 2018, a month supply of LF at a dose of 20 mg daily orally costs \$200–400.

### Methotrexate

MTX is a folate antagonist that has been used effectively in a variety of autoimmune conditions. Competitive binding blocks the enzyme dihydrofolate reductase from reducing dihydrofolic acid to folinic acid, the active intracellular metabolite involved in purine/pyrimidine metabolism, and amino acid/polyamine synthesis. However, at the doses used in rheumatic conditions, which are often lower than in oncologic chemotherapeutic regimens, the exact mechanism of action is uncertain. Animal models suggest that MTX increases extracellular concentrations of adenosine in inflammatory tissue, which has antiinflammatory effects via dephosphorylation of adenine nucleotides (Cronstein, 1996; Morabito et al., 1998). Other possible mechanisms include inhibition of DNA methylation necessary for cell proliferation, induction of apoptosis of activated peripheral T cells, regulation of IL-1 $\beta$ , increased IL-10 synthesis, inhibition of leukotriene B2 formation and cyclooxygenase-2, and interference with neutrophil function (Cronstein, 1996; Genestier et al., 1998; Mello et al., 2000; Seitz et al., 2001). MTX can be taken orally, subcutaneously, or intramuscularly. In RA, approximately 50% of the patients with RA show 20% or better improvement in joint scores. Side effects include elevation of liver enzymes, especially when concurrently ingesting alcohol, oral ulcers, postingestion nausea, diarrhea, and hair loss. More severe complications include pneumonitis, infections due to immune suppression, bone marrow suppression, and hepatic fibrosis. Folic or folinic acid is often used as supplementation to reduce hematologic and other side effects, although folinic acid may interfere with efficacy. Pregnancy should be avoided, as MTX is a known teratogen.

MTX is used in a variety of rheumatic conditions, most prominently in RA, but also in psoriasis/PsA, peripheral joint disease in spondyloarthropathies, SLE, granulomatosis with polyangiitis (GPA) (formerly called Wegener's granulomatosis), and other large vessel vasculitides. Barring contraindications or allergy, MTX can be considered one of the cornerstones of DMARDs for the treatment of RA. Studies have shown both short-term and long-term efficacy in RA disease measurements, such as joint pain and swelling, quality of life, objective laboratory markers of inflammation, and radiologic progression of disease (Rich et al., 1999; Weinblatt et al., 1992, 1994), as well as possible improvement in survival (Choi et al., 2002). MTX monotherapy is first-line treatment for RA. If there is not adequate improvement in disease activity after several weeks, it is used in combination therapy with other DMARDs, such as sulfasalazine or hydroxychloroquine, or biologics (O'Dell et al., 1996; Weinblatt, 2013). Numerous trials have demonstrated the value of MTX in combination therapy on disease activity measures, reduction of radiographic progression, and functionality. MTX is often used in other disorders to treat peripheral joint arthritis symptoms, such as in psoriasis (35%–40% have 75% or greater clearing of skin lesions), seronegative spondylarthritis disorders, and joint manifestations in lupus patients. In vasculitides such as GPA, oral MTX can be used as initial therapy for nonorgan threatening, nonrenal disease, although its use is associated with a higher relapse rate compared to cyclophosphamide (CYC). Thus MTX is a reasonable alternative for patients who cannot tolerate other more toxic (but more effective) treatment regimens (De Groot et al., 2005; Mukhtyar et al., 2009). In addition, MTX may be used as a steroid-sparing agent in the large vessel vasculitis in giant cell arteritis, although there is conflicting evidence of its efficacy in this setting (Hoffman et al., 2002; Jover et al., 2001). According to Epocrates searched on March 2018, the monthly cost of 15 mg daily of MTX once a week orally is approximately \$22.00.

## Cyclophosphamide

CYC is a potent alkylating agent, used most often to treat life-threatening or organ-threatening manifestations of autoimmune and inflammatory diseases. It can be administered orally or intravenously and is metabolized by the liver mitochondrial P-450 enzyme into several active metabolites with both therapeutic and toxic effects. CYC has direct effects on DNA resulting in cell death and modulates T-cell activation (Fox and McCune, 1994; McCune and Fox, 1989). Significant side effects often limit more widespread use and include an increased risk for opportunistic infections such as *Pneumocystis jiroveci* pneumonia and fungal infections, bladder toxicity with hemorrhagic cystitis and carcinoma of the bladder, neutropenia, increased risk of infertility or premature ovarian failure, and development of malignancy. Short-term therapeutic use of CYC aims for rapid control of the underlying inflammatory process (over a period of a few weeks to months), while seeking replacement of CYC when possible with an acceptable alternative to avoid the significant long-term toxicities.

CYC has been used effectively in the treatment of lupus nephritis, interstitial lung disease in multisystem autoimmune disease, and medium and small vessel vasculitis. In patients with severe lupus nephritis, initial landmark trials from the National Institute of Health demonstrated the superiority of monthly high-dose IV CYC (500–1000 mg/m<sup>2</sup>) for 6 months, followed by two quarterly pulses with GCs, versus GC therapy alone in preserving renal function (Austin et al., 1986; Boumpas et al., 1992; Gourley et al., 1996; Illei et al., 2001). Subsequent studies have investigated the role of low-dose CYC regimens (500 mg IV every 2 weeks for 3–6 months) and found that this regimen compared to the higher dose CYC produces similar rates of renal remission in European patients—for up to 10 years (Houssiau et al., 2010b, 2002). It is not known whether the low-dose regimen is effective in African Americans, Asians, or Latinos with lupus nephritis. Small studies suggest a role for CYC in treatment of central nervous system manifestations of SLE, such as transverse myelitis (Barile and Lavalle, 1992; Kovacs et al., 2000; Neuwelt et al., 1995). CYC has also demonstrated improvement in pulmonary function, dyspnea, skin thickening, and health-related quality of life in patients with symptomatic systemic sclerosis-related interstitial lung disease (Tashkin et al., 2006). CYC is a second-line agent alternative to mycophenolate for most cases of systemic sclerosis-related interstitial lung disease due to its AEs (Tashkin et al., 2016). Systemic vasculitides such as GPA, microscopic polyangiitis, anti-neutrophilic-cytoplasm antibody (ANCA)-positive vasculitis, rheumatoid vasculitis, polyarteritis nodosa, autoimmune-associated mononeuritis multiplex, and optic neuritis are other indications for CYC in which significant clinical improvement and/or survival benefits have been shown (Galindo-Rodriguez et al., 1999; Ribi et al., 2010; Scott and Bacon, 1984; Stone, 2010). CYC may be administered IV at 3–4 week intervals (or longer) or orally on a daily basis: the incidence of urinary bladder toxicity is probably higher with daily oral dosing. Medicare reimbursement for a 1 g/kg dose of CYC IV is approximately \$1200.00 (Afifi et al., 2016).

## Mycophenolate Mofetil (Cellcept)

Mycophenolate mofetil (MMF) was used initially in the 1990s for the prevention of allograft rejection in renal transplantation. In the past decade, MMF has been shown to be an effective therapy in both induction and maintenance of improvement in SLE nephritis, thus allowing for use of an alternative regimen to CYC. MMF reversibly inhibits the enzyme inosine monophosphate dehydrogenase, which is necessary for de novo purine synthesis in activated lymphocytes. Blockade with MMF results in reduced T- and B-cell proliferation, less antibody production, induction of apoptosis of activated T lymphocytes, and hindrance of production and function of adhesion molecules important for lymphocyte migration to inflammatory tissues (Allison and Eugui, 2000). Side effects include diarrhea, nausea, gastrointestinal upset, infections, bone marrow toxicity, neoplasia, and rash. MMF compared to CYC is less likely to cause alopecia and amenorrhea, but rates of infection, serious infection, and death are similar with the two treatments (Touma et al., 2011). MMF is teratogenic and should be stopped if pregnancy is being considered.

The use of MMF has profoundly changed the outcomes and therapeutic landscape in induction therapy of improvement in diffuse proliferative and membranous serious SLE-related glomerulonephritis. Initial studies in Chinese patients suggested that MMF was an effective alternative to CYC (Chan et al., 2000; Hu et al., 2002). Subsequent clinical trials compared CYC to MMF. A recent international, randomized controlled trial (ALMS trial) of 370 SLE patients with active nephritis compared MMF (2–3 g/day) to IV CYC (0.5–1 g/m<sup>2</sup>) monthly for 6 months. This induction therapy showed similar efficacy between the two treatments (Appel et al., 2009). Interestingly, however, proportions of African-Americans and Latino Americans responding to CYC were lower compared to Caucasians and Asians, whereas responses in all four racial groups to MMF were similar (Isenberg et al., 2010). A subsequent metaanalysis confirmed MMF and CYC to be equivalent in efficacy and side-effect profiles (Touma et al., 2011), although MMF was not superior to CYC as suggested by an earlier randomized but not blinded clinical trial (Ginzler et al., 2005). A follow-up analysis from the ALMS trial examining nonrenal lupus activity in the same nephritis patients showed similar efficacy between MMF and CYC on nonrenal manifestations, thus suggesting MMF as a reasonable alternative to CYC for renal and nonrenal SLE (Ginzler et al., 2010). Furthermore, in addition to the above trials demonstrating MMF's utility in induction therapy in SLE, a recent study has demonstrated MMF's superiority in safety and efficacy as maintenance therapy for SLE nephritis compared to CYC (Contreras et al., 2004) and azathioprine (AZA) (Dooley et al., 2011; Houssiau, 2016). The American College of Rheumatology's 2012 guidelines for treatment and management of classes III, IV, and V glomerulonephritis reflect the current state of the art regarding use of MMF (Hahn et al., 2012). It is a first choice for induction therapy (with GCs) and maintenance therapy in lupus nephritis patients who are not Caucasian or Asian, since similar proportions respond in all racial groups. Short-term therapy (6 months) with IV CYC can be chosen instead; higher proportions of Caucasians and Asians respond compared to Blacks and Latinas, but there are good responders in all groups. MMF has been successfully used for treating systemic sclerosis, myositis, uveitis, and vasculitis (GPA maintenance therapy). Because of its antilymphocyte and antifibrotic effects, it has been effective in treating interstitial lung disease associated with many rheumatic diseases (Fischer et al., 2013; Morganroth et al., 2010). MMF has also been used as a steroid-sparing agent for the treatment of other rheumatic diseases and for the treatment of autoimmune hepatitis. MMF is approved by the FDA in adults and children for maintenance of renal, liver, and heart transplants. An induction dose of MMF, 3 g orally daily, costs approximately \$1300 per month according to Epocrates, searched on March 2018.

## Azathioprine (Imuran)

AZA is a prodrug that is metabolized to its active component 6-mercaptopurine (6-MP), a purine antagonist that inhibits DNA synthesis that leads to both cytotoxicity and decreased cellular proliferation. Intracellular metabolism of 6-MP results in decreased numbers of circulating lymphocytes, IL-2 secretion, and immunoglobulin production (Wilke, 2010). It is approved by the US FDA for prevention of renal transplant rejection and the treatment of RA; however, off-label use is common for remission maintenance of many rheumatic diseases such as SLE, polymyositis/dermatomyositis, Behcet's disease, ANCA-associated vasculitis, or as a steroid-sparing agent in many other rheumatic diseases. AEs include nausea, vomiting, bone marrow suppression (particularly anemia), increased risk of infection, and malignancy. Individuals with a homozygous genetic polymorphism of the enzyme thiopurine methyltransferase that reduces the metabolism of AZA may be at greater risk for AZA bone marrow toxicity. Some authorities recommend checking for this genetic polymorphism prior to initiation of therapy. A number of studies have shown efficacy of AZA over placebo in the

treatment of RA (Cade et al., 1976; Urowitz et al., 1973; Woodland et al., 1981), although more recent combination therapies of MTX plus biologics or other DMARDs are better. Recent data in RA suggested combined MTX and AZA had statistically more withdrawals due to adverse events than oral MTX alone (Hazlewood et al., 2016). In addition, AZA can be used for effective maintenance therapy in lupus nephritis (Contreras et al., 2004; Houssiau et al., 2010a), UC and Crohn's bowel disease (Prefontaine et al., 2009; Timmer et al., 2007), ANCA-positive vasculitis (Pagnoux et al., 2008), and autoimmune hepatitis. According to Epocrates, searched on March 2018, a dose of 150 mg daily of AZA costs approximately \$110.00 a month.

### Calcineurin Inhibitors: Cyclosporin A, Tacrolimus, and Voclosporin

Cyclosporin A (CSA) is a cyclic peptide of 11 amino acids that binds with high affinity to cyclophilins, which competitively bind to calcineurin. This leads to inhibition of translocation of transcription factors, NF-AT, thus reducing transcription of early cytokine genes that encode IL-2, TNF- $\alpha$ , interferon (IFN)- $\gamma$ , IL-3, IL-4, CD40, and granulocyte-macrophage colony-stimulating factor (Schreiber and Crabtree, 1992; Timmerman et al., 1996; Wiederrecht et al., 1993). Furthermore, CSA interferes with antigen presentation by APCs. The ultimate net effect is reduction of lymphocyte proliferation. CSA is metabolized by the cytochrome P450 3A4 liver enzymes and has a variety of drug-drug interactions that may interfere with blood concentration levels, thus requiring monitoring of CSA levels. Side effects include renal toxicity, hypertension, neurologic side effects such as tremor, encephalopathy, increased risk of skin or lymphoproliferative malignancies, and heightened risk for infection. However, unlike many alkylating agents and purine antagonists, CSA lacks clinically significant bone marrow suppression. CSA has been used in a variety of established and suspected autoimmune disorders, including RA, PsA, ocular and mucocutaneous Behcet's disease, adult lupus membranous nephritis, systemic sclerosis, atopic dermatitis, severe UC, pemphigus vulgaris, and myasthenia gravis (Magee, 2012). However, concern over long-term side effects, especially of renal toxicity, hypertension, and a myriad of drug interactions, has limited utility in chronic autoimmune diseases, except in patients who have failed more conventional therapies. Tacrolimus, another oral medication that inhibits T lymphocyte activation similarly to cyclosporine, is used widely for prevention of allograft rejection, because its potential AEs are slightly less than those of cyclosporine, with fewer hypertensive effects. Further, in vivo, the immunosuppressive effects are 10–20 times greater than cyclosporine. It is being used for some autoimmune diseases, including induction and maintenance therapy for lupus nephritis, RA, and autoimmune hepatitis (Lee et al., 2011; Li et al., 2012). In Chinese and Japanese studies, tacrolimus was equivalent to high-dose IV CYC in lupus nephritis (Wang et al., 2012) and in combination with low-dose mycophenolate was very effective in inducing partial or complete remissions (in 83% compared to 63% on IV CYC) (Liu et al., 2015). A dose of 5 mg daily costs approximately \$3000 per month according to Epocrates, searched on March 2018.

Voclosporin, another calcineurin inhibitor, is part of the next generation of treatments in this class of drugs. Previously known by other names, including ISA247, its structure is the same as cyclosporine except at the amino acid-1 residue. This difference allows for higher binding to calcineurin, and higher potency compared to cyclosporine (Papp et al., 2008). The AEs are similar to other calcineurin inhibitors, including headache, hypertension, diarrhea, and respiratory infections. Voclosporin has been used as therapy in plaque psoriasis, noninfectious uveitis, and renal allograft rejection (Schultz, 2013). There are ongoing studies for its use in lupus nephritis.

## BIOLOGIC AGENTS

With increasing understanding of the pathogenesis of autoimmune rheumatic diseases, several biologic agents have been developed. They can be classified based on their mechanism of actions, cytokine-targeted therapies, B-cell-targeted therapies, and T-cell-targeted therapies.

### Cytokine-Targeted Therapies

Therapies interfering with TNF, IL 1, IL6, IL 12, IL 23 are currently available for rheumatic diseases particularly for RA, AS, juvenile chronic arthritis, psoriasis, and PsA.

## Tumor Necrosis Factor Inhibitors

TNF inhibitors are a class of biologic drugs utilizing monoclonal antibodies (mABs) or TNF-binding fusion proteins to neutralize and block various proteins in autoimmune disease processes. The target protein of these therapeutic mABs is TNF- $\alpha$ , a pleiotropic cytokine with proinflammatory and immunoregulatory functions. TNF- $\alpha$  interacts with two receptors, either p55 (TNF receptor 1) or p75 (TNF receptor 2), resulting in various downstream signaling pathways as part of this inflammatory process (Taylor, 2010). TNF inhibitors have a structure based on the immunoglobulin molecule, including two identical heavy chains and two identical light chains. There are two functional regions in each antibody: the variable region (Fab) and the constant region (Fc). Interaction of the Fc receptors with the Fc portion of the mAb results in blocking the interaction between TNF- $\alpha$  and its receptors as the therapeutic target of this class of molecules. Currently, there are five FDA-approved TNF inhibitors in the United States. Infliximab is a chimeric IgG1 mAb made of a murine Fab region linked to a human IgG1 kappa constant region. Adalimumab is a fully human recombinant Fab with a human IgG1 kappa constant region. Golimumab also is fully humanized with a human IgG1 kappa constant region. Etanercept is a recombinant fusion protein consisting of the TNF receptor 2 and the Fc fraction of a human IgG1 constant region. Certolizumab is a humanized IgG4 Fab fragment linked to polyethylene glycol. They are administered intravenously or subcutaneously. TNF inhibitors are indicated for autoimmune disease including RA, PsA, AS, plaque psoriasis, juvenile idiopathic arthritis, Crohn's disease, and UC (Willrich et al., 2015). In RA, the TNFi usually used with MTX results in at least 20% improvement in 60%–70% of the patients, 50% improvement in 40%–50%, and 70% improvement in 20%–30%. Placebos result in improvement rates of 1%–12%. In RA, TNFi are better than MTX alone; most TNFi work better when combined with MTX. In patients with early disease (less than 3 years), remissions (defined as very low disease activity using international standard measures) occur in as many as 50% with etanercept plus MTX. They also reduce the damage that RA causes in joints (erosions and cartilage loss) (Schur, 2017). For treatment of psoriasis, the mAb anti-TNFs are more effective than the fusion protein (etanercept). The most common AEs of TNFi include acute and delayed infusion/injection reactions such as rash, arthralgias, fatigue, and myalgias. Infection with bacteria, fungus, and viruses also occurs due to blockage of the immune response, with a notable increased incidence in mycobacterial tuberculosis infections. In fact, the physician must rule out active tuberculosis before administering any of the TNF inhibitors. Other less common AEs include an increased risk of malignancy and neurological disorders. TNF inhibitors should be avoided in patients with known demyelinating disease. The monthly costs of TNFi are high. According to [www.goodrx.com](http://www.goodrx.com) searched on March 2018, approximate monthly cost of etanercept usual dose of 50 mg a week subcutaneous (autoinjector) is \$5400, adalimumab 40 mg every 2 weeks sc is \$5000, remicade IV at 10 mg/kg (given at 6–8 week intervals) is \$6000, and certolizumab (preferred for patients during pregnancy) is \$4000.

## Tocilizumab (Anti-IL6R: Actemra)

Tocilizumab is a humanized anti-IL-6 receptor mAB targeting the IL-6 pathway. IL-6, a pleiotropic cytokine, is important in the pathogenesis of RA, and other autoimmune diseases. There are two different IL-6-mediated signaling pathways (Calabrese and Rose-John, 2014). In classical signaling, IL-6 binds to the membrane bound receptor (mIL-6R) via the signal transduction protein gp130, leading to dimerization and further intracellular signaling. The second pathway relies on the proteolytic cleavage of mIL-6R, leading to the generation of soluble receptor for IL-6 (sIL-6R). Tocilizumab competitively inhibits IL-6 binding to mIL-6R and sIL-6R thereby blunting downstream proinflammatory effects. A recent clinical trial in giant cell arteritis showed tocilizumab treatment induced improvement in 56% compared to 14% on placebo, at both 6 and 12 months of treatment (Stone et al., 2017). In patients with RA, tocilizumab used in combination with MTX has been studied in randomized controlled trials compared to placebo. Findings showed the efficacy of tocilizumab in combination with MTX compared to placebo, as remission of disease based on 20%, 50%, and 70% improvement was 2.5, 3.2, and 6 times higher, respectively, in this group (Singh et al., 2011). In patients with RA, tocilizumab as monotherapy was studied in a randomized clinical trial compared to MTX, yielding 69% with 20% improvement, 44% with 50% improvement, and 28% with 70% improvement (Jones et al., 2010a). The AEs most commonly reported have been infections including pneumonia, cellulitis, herpes zoster, gastroenteritis, and diverticulitis. Other side effects include an elevation in lipid levels and cytopenias. Effective blockade of IL-6R can also decrease hepcidin levels and result in an elevation in hemoglobin production (Navarro-Millan et al., 2012). In addition to use in RA, tocilizumab has been recently approved by the US FDA for giant cell arteritis. It may also be used in other forms of

vasculitis, and in systemic and polyarticular juvenile idiopathic arthritis. Dosed at 162 mg subcutaneously every 2 weeks (prefilled syringe), the price of a 1 month supply is approximately \$2200 according to [www.goodrx.com](http://www.goodrx.com) searched on March 2018.

## IL-1 Antagonists

IL-1 is one of the proinflammatory cytokines underlying the inflammatory symptoms of RA, systemic juvenile inflammatory arthritis (JIA), and autoinflammatory diseases. Anakinra (Kineret), rilonacept (Arcalyst), and canakinumab (Ilaris) are currently available IL-1 antagonists. Anakinra is a recombinant human IL-1 receptor antagonist; it blocks IL-1 activity by competitively inhibiting IL-1 binding to the IL-1 receptor (IL-1R). Blocking IL1 also serves to inhibit clinical response to inflammasome activation; thus these agents can be useful in inflammasome-mediated conditions such as acute gout. Rilonacept is dimeric fusion protein that incorporates in extracellular domains of both IL-1R and IL-1 R accessory protein fused to the Fc portion of an IgG molecule. Canakinumab is a human mAB that targets IL-1  $\beta$ . All these agents are administered by subcutaneous injection. Anakinra is effective in systemic JIAs ([Nigrovic et al., 2011](#); [Vastert et al., 2014](#)). Anakinra can be used in combination with MTX. However, it is less effective than other biologic DMARDs in adult RA ([Singh et al., 2009](#)). Increasing data suggest that IL-1 inhibition is an effective alternative for patients with familial Mediterranean fever who do not respond to or cannot tolerate colchicine ([Gul et al., 2015](#); [Hashkes et al., 2012](#); [van der Hilst et al., 2016](#)). The choice of IL-1 inhibitor depends upon a combination of factors including regulatory or insurance requirements, route of administration, and cost. Canakinumab may be preferred due to its efficacy and convenience since it is given every 4–8 weeks. Beneficial effects of IL-1 inhibition were seen in patients with acute gout in open-label pilot studies using anakinra, 100 mg daily given subcutaneously until symptoms of acute gouty arthritis improved ([So et al., 2007](#)). Rilonacept and canakinumab are approved by the US FDA for the treatment of cryopyrin-associated periodic syndromes or catastrophic antiphospholipid syndrome (CAPS). Anakinra was also used off-label with success in CAPS. AEs include anaphylaxis/hypersensitivity reactions, infections, injection site reaction, and neutropenia. Canakinumab can also cause nasopharyngitis, headache, vertigo, and diarrhea. The monthly cost of Anakinra dosed at 100 mg subcutaneously daily is \$4156 according to [www.goodrx.com](http://www.goodrx.com) searched on March 2018.

## Secukinumab (Anti-IL17A: Cosentyx)

Secukinumab is a fully humanized (IgG1 $\kappa$ ) mAB directed against IL-17A. IL-17A is a cytokine and the primary effector of Th-17 cells as part of the pathogenesis of several autoimmune diseases including psoriasis, PsA, and AS. More specifically, IL-17A has several functions in these diseases such as keratinocyte trafficking, acting on chemokines such as CCL20 and CXCLs ([Maldonado-Ficco et al., 2016](#)). Selective blocking of IL-17A by secukinumab has demonstrated efficacy in these autoimmune diseases. For example, secukinumab in patients with moderate-to-severe plaque psoriasis induced 75% or greater improvement in the surface area of plaques in almost 80% of the individuals, compared to 44% with etanercept and 5% on placebo (using the psoriasis activity scoring index (PASI) score, a standard measurement of extent of psoriasis). The efficacy of secukinumab (and other IL17 inhibitors that are currently available) in plaque psoriasis is probably the best to date among the biologics ([Feldman, 2017](#)). Secukinumab showed significant but not dramatic response in patients with PsA in previous clinical trials with 20% improvement noted in 54% and 51% of the 300 and 150 mg dosed groups, respectively, as compared to placebo ([McInnes et al., 2015](#)). AEs of secukinumab include neutropenia, and infections notable for candidiasis ([Baeten et al., 2015](#)). According to [www.goodrx.com](http://www.goodrx.com) (searched on April 2018), a monthly dose of secukinumab at 150 mg subcutaneously weekly costs \$4700. However, the biologic can be withheld for a few weeks after the first 5 weeks if there is good response, so the high monthly cost may be intermittent.

## Ustekinumab (Anti-p40 for IL-12 and IL-23 Signaling: Stelara)

Ustekinumab binds to the p40 subunit of IL-12 and IL-23. This then prevents binding to the IL-12 and IL-23 receptors, inhibiting IL-12 and IL-23-mediated cell signaling, activation, and cytokine production, including Th1 and Th17 production of IFN- $\gamma$ , IL-17, and IL-22 ([Yeilding et al., 2012](#)). In patients with active PsA, multicenter placebo-controlled trials showed significant improvement compared to placebo at doses of 45 and 90 mg with 42% and 49%, respectively, achieving 20% improvement, compared to 22% on placebo with 20% improvement

(McInnes et al., 2013). In psoriasis, ustekinumab treatment of moderate-to-severe plaque disease results in 67% of the patients achieving 75% or greater reduction of total skin involved (PASI score) (Feldman, 2017). AEs include infections, such as nasopharyngitis. There is also increased risk of nonmelanoma skin cancers as demonstrated in postmarketing data (Baker and Isaacs, 2017). Ustekinumab has been FDA-approved for use in Crohn's disease, plaque psoriasis, and PsA. A single injection of ustekinumab subcutaneous is approximately \$10,600, but it is given once every 12 weeks for psoriasis and PsA, making the monthly cost \$3533 ([www.goodrx.com](http://www.goodrx.com) searched on April 2018).

## B-Cell-Targeted Therapies

Targeting autoreactive B cells or B-cell maturation signals has become successful therapy for autoimmune rheumatic conditions and will be discussed briefly here and in further detail in the next chapter.

### Rituximab (Rituxan)

Rituximab is a chimeric anti-CD20 mAB, which causes depletion of B cells via various mechanisms, including Fc gamma receptor-mediated antibody-dependent cytotoxicity, antibody-dependent complement-mediated cell lysis, and B-cell apoptosis (Cragg et al., 2005). Rituximab selectively depletes CD20+ B cells, which play a role in autoantibody-mediated diseases including the chronic synovitis associated with RA (Tsokos, 2004). Results of the RAVE and RITUXVAS trials in patients with ANCA vasculitis show that rituximab is an effective and safer alternative to CYC, especially in patients with relapsing disease (Jones et al., 2010b; Stone et al., 2010). Of note, in prospective, controlled randomized trials, rituximab was not shown to be superior to placebo for treatment of SLE patients with active disease (renal or nonrenal) who were receiving GC plus hydroxychloroquine plus an immunosuppressive drug (Merrill et al., 2010; Rovin et al., 2012). However, many open-label studies attest to the efficacy of rituximab in SLE. In addition, there may be patients with lupus nephritis who respond to rituximab infusions in combination with MMF, without additional daily GCs (Beckwith and Lightstone, 2014). Prospective trials are in progress. The US FDA has approved rituximab for non-Hodgkin's lymphoma, B-cell chronic lymphocytic leukemia, RA, GPA, and microscopic polyangiitis. Adverse reactions include infusion reaction, infections, hypogammaglobulinemia, and late onset neutropenia. A "round" of rituximab which consists of once-a-week-for-4-weeks or the same total dose given once every 2 weeks during the 4 weeks is approximately \$38,000 (not including infusion costs). Some patients maintain improvement for many months or years and do not need a second dose; in many rheumatic disease patients, the round is repeated every 6 months.

### Belimumab (Anti-BLyS: Benlysta)

Belimumab is a fully human mAB directed against soluble B lymphocyte stimulator protein (BLyS)/B-cell activating factor (also called B cell-activating-factor (BAFF)); hence, it depletes maturing B cells by inhibition of BLyS, which is required for survival and maturation of most B-cell subsets. In 2011, belimumab became the first drug in 50 years to be approved for the treatment of active SLE (excluding patients with active renal or central nervous system (CNS) disease) (Furie et al., 2011; Hahn, 2013; Navarra et al., 2011). Of note, belimumab is meant for lupus patients who are antinuclear antibody or anti-double stranded DNA positive and interestingly showed less response in African-American patients. A recent study showed that responses are durable for a 4-year period—the follow-up period of the study (Merrill et al., 2012). AEs with belimumab include infusion reactions to the IV biologic (a subcutaneous version has just become available in 2017), infections, and depression. To date, blockade of both soluble and cell-bound BLyS and of the April receptor for BlyS plus the BAFF receptor have not been effective and/or safe enough to replace belimumab. The cost of IV belimumab once a month is approximately \$4070 ([www.goodrx.com](http://www.goodrx.com), searched on April 2018). Prices were not available at the time of this writing for the subcutaneous form, which will probably come into wide usage.

## T-Cell-Targeted Therapies

### Abatacept (CTLA4-Ig Blocks Second Signals: Ocrevus)

Abatacept (CTLA4-Ig) is a fully human soluble fusion protein that is effective for the treatment of RA. It consists of the extracellular domain of CTLA4 and the Fc portion of IgG1. CTLA4-Ig binds CD80 (B7-1) and

CD86 (B7-2) on APCs preventing these molecules from binding to their ligand, CD28, on T cells. This interferes with optimal T-cell activation resulting in decreased production of proinflammatory cytokines (Lenschow et al., 1996). Abatacept is effective, safe and produced statistically significantly less radiographic progression in RA. The US FDA has approved it for use in RA and polyarticular JIA patients who are inadequate responders to DMARDs (Maxwell and Singh, 2010). Abatacept may be used as an alternative to a TNF inhibitor. Abatacept can be administered intravenously every 4 weeks or it can be administered subcutaneously weekly. AEs include infusion reactions, infections, and headache. According to [www.goodrx.com](http://www.goodrx.com) searched on April 2018, the monthly cost of abatacept 125 mg subcutaneously weekly is \$4200.

## OTHER TREATMENT OPTIONS

### Apremilast (Otezla)

Apremilast is an orally available small molecule that specifically targets phosphodiesterase (PDE4) which results in an increased level of cAMP intracellularly in multiple cell types (T cells, mononuclear cells, others). Consequently, the increase in intracellular cAMP decreases the production of several proinflammatory mediators (TNF- $\alpha$ , IL-12, IL-23, IFN- $\gamma$ , and inducible nitric oxide synthase), and it increases the production of antiinflammatory cytokines (IL-10) (Schafer et al., 2010). This compound is effective in psoriasis and PsA and is US FDA approved for the treatment of active PsA or plaque psoriasis (moderate to severe), and/or PsA (Edwards et al., 2016). Apremilast is started at low dose with titration up to 30 mg twice daily. Modest efficacy has been shown in AS in a double-blind, placebo-controlled unpowered phase II study, but there was no statistical significance (Pathan et al., 2013). Its efficacy was also demonstrated in Behcet's disease (oral and genital ulcers) (Hatemi et al., 2015). The drug is well tolerated with diarrhea, nausea, headache, and weight loss being the most common side effects. No specific lab monitoring is required. The monthly cost of Apremilast at 30 mg bid orally is \$3100 according to [www.goodrx.com](http://www.goodrx.com) searched on April 2018.

### Tofacitinib (Inhibitor of Janus Kinase Activation Pathway: Xeljanz)

Tofacitinib is a novel orally administered disease-modifying agent which inhibits Janus kinase (JAK) enzymes, therefore decreasing signaling by a number of cytokine and growth factor receptors, resulting in reduction of a variety of inflammatory mediators. Tofacitinib was shown to be effective and can be used as monotherapy or combined with MTX (our usual approach) or other nonbiologic DMARDs in patients with moderately to severely active RA who have had an inadequate response to MTX (Fleischmann et al., 2012; Kremer et al., 2013). It is taken in a dose of 5 mg twice daily or 11 mg extended release daily. The relative safety of tofacitinib appeared similar to that of other biologic disease modifying agents, including increased risk of infection and abnormal liver function tests. Other side effects include neutropenia, lymphopenia, hyperlipidemia, and, possibly, increased serum creatinine and gastrointestinal perforations. The ACR recommends that tofacitinib be used in patients with RA who have failed to respond adequately to MTX plus a TNF-inhibiting or non-TNF-inhibiting biologic (including abatacept, rituximab, tocilizumab) (Singh et al., 2016). Thus it is not yet viewed as first-line therapy for most patients with RA. Several additional Jak–Stat inhibitors, some more specific for molecules that do not inhibit hematopoiesis, are in development. Baricitinib has recently been approved by the FDA for treatment of RA. According to [www.goodrx.com](http://www.goodrx.com) searched on April 2018, the monthly cost of tofacitinib at 5 mg bid orally is \$4100.

## INTRAVENOUS IMMUNOGLOBULIN

The use of intravenous IV immunoglobulin (IVIG) in autoimmune diseases has several possible mechanisms of action in suppression of inflammatory and autoimmune processes, including interference with Fc receptors on effector cells, supply of antiidiotypic antibody activity against serum autoantibodies, inhibition of reticuloendothelial clearance of antibody-covered platelets via Fc receptors, and regulation of expression of proinflammatory cytokines and blockade of adhesion molecules in inflammatory states (Silvergleid and Berger, 2011). In addition, other effects of IVIG include solubilization and clearance of immune complexes, altered T-cell subsets, and increased T regulatory cells. IVIG is useful in a wide range of autoimmune and autoinflammatory diseases such as Guillain–Barré syndrome, myasthenia gravis, autoimmune peripheral neuropathies, Kawasaki disease,

hematologic conditions such as autoimmune thrombocytopenia, hemolytic anemia or neutropenia, and antibody-mediated CNS diseases resulting from interference with neurotransmitter function. Small studies have shown that IVIG is a reasonable second-line therapy for refractory dermatomyositis or polymyositis (Cherin et al., 2002; Dalakas et al., 1993). AEs of IVIG treatment include allergic reactions, including anaphylaxis (particularly in males who are IgA deficient), headache, nausea, chills or flushing, aseptic meningitis, thrombosis, and renal dysfunction. Most of these reactions are mild and transient, reversible events. Serious reactions are rare (Silvergleid and Berger, 2011). According to Epocrates, searched on April 2018, the cost of a single “round” of IV Ig varies from \$3500 to \$8000, depending on the dose and the supplier. This does not include infusion costs.

## COMMENT REGARDING COSTS OF THERAPIES: BIOSIMILARS

As shown above, the cost of medications for treatment of a common inflammatory rheumatic disease, RA, can vary from \$300 to \$5000 per month. All of the recently developed treatments—biologics and small molecules—are on that expensive side. The costs are also quite variable depending on what contract has been made between the payer, the pharmacy provider, and the pharmacy benefits manager companies. Biosimilars have been developed which USA and European oversight bodies have approved for patient use. To date, biosimilars do not differ from the biologic they mimic in efficacy or toxicity (Cohen et al., 2017). Now the question becomes can they be priced to an advantage for the patients and providers—perhaps 30% less in cost? The answer appears complex, with a small number of European countries paying for only one biosimilar and not the original biologic for patients with RA. A recent study from the United Kingdom showed that using infliximab and etanercept biosimilars (in rheumatic diseases) saved the National Health Service 38.8 million pounds over 2 years (Aladel et al., 2017). In the United States, there is friction between choice, profit, and affordability, with some issues being decided in part by the legal system. Somewhere in the not-too-distant future, a compromise will have to be reached that allows care to be increasingly excellent but more affordable for patients, providers, and societies.

## MOVING TOWARD MORE BIOLOGICAL AND MOLECULAR THERAPIES

Over the past few decades, progressive advancement in our understandings of immunopathogenesis has led to a significant expansion of potential therapeutic targets. This scientific progress has ushered in widespread use of new biologic and molecular treatments specifically targeting B and T cells, costimulation and signal transduction molecules, maturation factors, cytokines, and TLRs on APCs. For example, anti-TNF therapy is now routinely used in RA and seronegative spondyloarthritides, as monotherapy or in combination therapy with traditional DMARDs as discussed above (Singh et al., 2016). Such new therapeutic targets are the subject of the next chapter and continue to improve the standard treatments and outcomes for many autoimmune diseases.

## References

- Afifi, S., Adel, N.G., Devlin, S., et al., 2016. Upfront plerixafor plus G-CSF versus cyclophosphamide plus G-CSF for stem cell mobilization in multiple myeloma: efficacy and cost analysis study. *Bone Marrow Transpl.* 51, 546–552.
- Aladel, M.I., Fitzpatrick, R.X., Chapman, S.R., 2017. Impact of infliximab and etanercept biosimilars on biological disease-modifying antirheumatic drugs utilization and NHS budget in the UK. *BioDrugs* 31, 533–544.
- Al Sahaw, S., Zhang, X., Zhu, B., Magder, L.S., Foster, S.A., Iikuni, N., et al., 2015. Effect of corticosteroid use by dose on the risk of developing organ damage over time in systemic lupus erythematosus—the Hopkins Lupus Cohort. *Lupus Sci. Med.* 2 (1), e000066. Available from: <https://doi.org/10.1136/lupus-2014-000066>.
- Alarcon, G.S., McGwin, G., Bertoli, A.M., Fessler, B.J., Calvo-Alen, J., Bastian, H.M., et al., 2007. Effect of hydroxychloroquine on the survival of patients with systemic lupus erythematosus: data from LUMINA, a multiethnic US cohort (LUMINA L). *Ann. Rheum. Dis.* 66 (9), 1168–1172. Available from: <https://doi.org/10.1136/ard.2006.068676>.
- Allison, A.C., Eugui, E.M., 2000. Mycophenolate mofetil and its mechanisms of action. *Immunopharmacology* 47 (2–3), 85–118.
- Amin, A.R., Vyas, P., Attur, M., Leszczynska-Piziak, J., Patel, I.R., Weissmann, G., et al., 1995. The mode of action of aspirin-like drugs: effect on inducible nitric oxide synthase. *Proc. Natl. Acad. Sci. U.S.A.* 92 (17), 7926–7930.
- Appel, G.B., Contreras, G., Dooley, M.A., Ginzler, E.M., Isenberg, D., Jayne, D., et al., 2009. Mycophenolate mofetil versus cyclophosphamide for induction treatment of lupus nephritis. *J. Am. Soc. Nephrol.* 20 (5), 1103–1112. Available from: <https://doi.org/10.1681/ASN.2008101028>.
- Austin III, H.A., Klippen, J.H., Balow, J.E., le Riche, N.G., Steinberg, A.D., Plotz, P.H., et al., 1986. Therapy of lupus nephritis. Controlled trial of prednisone and cytotoxic drugs. *N. Engl. J. Med.* 314 (10), 614–619. Available from: <https://doi.org/10.1056/NEJM198603063141004>.

- Baeten, D., Sieper, J., Braun, J., Baraliakos, X., Dougados, M., Emery, P., et al., 2015. Secukinumab, an interleukin-17A inhibitor, in ankylosing spondylitis. *N. Engl. J. Med.* 373 (26), 2534–2548. Available from: <https://doi.org/10.1056/NEJMoa1505066>.
- Baker, K.F., Isaacs, J.D., 2017. Novel therapies for immune-mediated inflammatory diseases: What can we learn from their use in rheumatoid arthritis, spondyloarthritis, systemic lupus erythematosus, psoriasis, Crohn's disease and ulcerative colitis? *Ann. Rheum. Dis.* Available from: <https://doi.org/10.1136/annrheumdis-2017-211555>.
- Barile, L., Lavalle, C., 1992. Transverse myelitis in systemic lupus erythematosus—the effect of IV pulse methylprednisolone and cyclophosphamide. *J. Rheumatol.* 19 (3), 370–372.
- Beckwith, H., Lightstone, L., 2014. Rituximab in systemic lupus erythematosus and lupus nephritis. *Nephron Clin. Pract.* 128 (3–4), 250–254. Available from: <https://doi.org/10.1159/000368585>.
- Blumenfield, M., 1978. Psychological aspects of systemic lupus erythematosus. *Prim. Care* 5 (1), 159–171.
- Boumpas, D.T., Austin 3rd, H.A., Vaughn, E.M., Klippel, J.H., Steinberg, A.D., Yarboro, C.H., et al., 1992. Controlled trial of pulse methylprednisolone versus two regimens of pulse cyclophosphamide in severe lupus nephritis. *Lancet* 340 (8822), 741–745.
- Cade, R., Stein, G., Pickering, M., Schlein, E., Spooner, G., 1976. Low dose, long-term treatment of rheumatoid arthritis with azathioprine. *South Med. J.* 69 (4), 388–392.
- Calabrese, L.H., Rose-John, S., 2014. IL-6 biology: implications for clinical targeting in rheumatic disease. *Nat. Rev. Rheumatol.* 10 (12), 720–727. Available from: <https://doi.org/10.1038/nrrheum.2014.127>.
- Cao, W.W., Kao, P.N., Aoki, Y., Xu, J.C., Shorthouse, R.A., Morris, R.E., 1996. A novel mechanism of action of the immunomodulatory drug, leflunomide: augmentation of the immunosuppressive cytokine, TGF-beta 1, and suppression of the immunostimulatory cytokine, IL-2. *Transplant. Proc.* 28 (6), 3079–3080.
- Casciola-Rosen, L.A., Anhalt, G., Rosen, A., 1994. Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *J. Exp. Med.* 179 (4), 1317–1330.
- Chan, T.M., Li, F.K., Tang, C.S., Wong, R.W., Fang, G.X., Ji, Y.L., et al., 2000. Efficacy of mycophenolate mofetil in patients with diffuse proliferative lupus nephritis. Hong Kong-Guangzhou Nephrology Study Group. *N. Engl. J. Med.* 343 (16), 1156–1162. Available from: <https://doi.org/10.1056/NEJM200010193431604>.
- Cherin, P., Pelletier, S., Teixeira, A., Laforet, P., Genereau, T., Simon, A., et al., 2002. Results and long-term follow-up of intravenous immunoglobulin infusions in chronic, refractory polymyositis: an open study with thirty-five adult patients. *Arthritis Rheum.* 46 (2), 467–474.
- Choi, H.K., Hernan, M.A., Seeger, J.D., Robins, J.M., Wolfe, F., 2002. Methotrexate and mortality in patients with rheumatoid arthritis: a prospective study. *Lancet* 359 (9313), 1173–1177. Available from: [https://doi.org/10.1016/S0140-6736\(02\)08213-2](https://doi.org/10.1016/S0140-6736(02)08213-2).
- Cohen, S., Genovese, M.C., Choy, E., Perez-Ruiz, F., Matsumoto, A., Pavelka, K., et al., 2017. Efficacy and safety of the biosimilar ABP 501 compared with adalimumab in patients with moderate to severe rheumatoid arthritis: a randomised, double-blind, phase III equivalence study. *Ann. Rheum. Dis.* 76, 1679–1687.
- Contreras, G., Pardo, V., Leclercq, B., Lenz, O., Tozman, E., O'Nan, P., et al., 2004. Sequential therapies for proliferative lupus nephritis. *N. Engl. J. Med.* 350 (10), 971–980. Available from: <https://doi.org/10.1056/NEJMoa031855>.
- Cragg, M.S., Walshe, C.A., Ivanov, A.O., Glennie, M.J., 2005. The biology of CD20 and its potential as a target for mAb therapy. *Curr. Dir. Autoimmun.* 8, 140–174. Available from: <https://doi.org/10.1159/000082102>.
- Cronstein, B.N., 1996. Molecular therapeutics. Methotrexate and its mechanism of action. *Arthritis Rheum.* 39 (12), 1951–1960.
- Dalakas, M.C., Illa, I., Dambrosia, J.M., Soueidan, S.A., Stein, D.P., Otero, C., et al., 1993. A controlled trial of high-dose intravenous immune globulin infusions as treatment for dermatomyositis. *N. Engl. J. Med.* 329 (27), 1993–2000. Available from: <https://doi.org/10.1056/NEJM19931230292704>.
- Dawson, L.J., Caulfield, V.L., Stanbury, J.B., Field, A.E., Christmas, S.E., Smith, P.M., 2005. Hydroxychloroquine therapy in patients with primary Sjögren's syndrome may improve salivary gland hypofunction by inhibition of glandular cholinesterase. *Rheumatology (Oxford)* 44 (4), 449–455. Available from: <https://doi.org/10.1093/rheumatology/keh506>.
- De Groot, K., Rasmussen, N., Bacon, P.A., Tervaert, J.W., Feighery, C., Gregorini, G., et al., 2005. Randomized trial of cyclophosphamide versus methotrexate for induction of remission in early systemic antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum.* 52 (8), 2461–2469. Available from: <https://doi.org/10.1002/art.21142>.
- Dimitrijevic, M., Bartlett, R.R., 1996. Leflunomide, a novel immunomodulating drug, inhibits homotypic adhesion of mononuclear cells in rheumatoid arthritis. *Transplant. Proc.* 28 (6), 3086–3087.
- Dooley, M.A., Jayne, D., Ginzler, E.M., Isenberg, D., Olsen, N.J., Wofsy, D., et al., 2011. Mycophenolate versus azathioprine as maintenance therapy for lupus nephritis. *N. Engl. J. Med.* 365 (20), 1886–1895. Available from: <https://doi.org/10.1056/NEJMoa1014460>.
- Edwards, C.J., Blanco, F.J., Crowley, J., Birbara, C.A., Jaworski, J., Aelion, J., et al., 2016. Apremilast, an oral phosphodiesterase 4 inhibitor, in patients with psoriatic arthritis and current skin involvement: a phase III, randomised, controlled trial (PALACE 3). *Ann. Rheum. Dis.* 75 (6), 1065–1073. Available from: <https://doi.org/10.1136/annrheumdis-2015-207963>.
- Edwards, M.H., Pierangeli, S., Liu, X., Barker, J.H., Anderson, G., Harris, E.N., 1997. Hydroxychloroquine reverses thrombogenic properties of antiphospholipid antibodies in mice. *Circulation* 96 (12), 4380–4384.
- Emery, P., Breedveld, F.C., Lemmel, E.M., Kaltwasser, J.P., Dawes, P.T., Gomor, B., et al., 2000. A comparison of the efficacy and safety of leflunomide and methotrexate for the treatment of rheumatoid arthritis. *Rheumatology (Oxford)* 39 (6), 655–665.
- Ernst, E., Rose, M., Lee, R., 1984. Modification of transoperative changes in blood fluidity by hydroxychloroquine: a possible explanation for the drug's antithrombotic effect. *Pharmatherapeutic* 4 (1), 48–52.
- Feldman, S.R., 2017. Treatment of Psoriasis in Adults. UpToDateRetrieved from . Available from: <http://www.uptodate.com/home>.
- Fischer, A., Brown, K.K., Du Bois, R.M., Frankel, S.K., Cosgrove, G.P., Fernandez-Perez, E.R., et al., 2013. Mycophenolate mofetil improves lung function in connective tissue disease-associated interstitial lung disease. *J. Rheumatol.* 40 (5), 640–646. Available from: <https://doi.org/10.3899/jrheum.121043>.
- Fleischmann, R., Kremer, J., Cush, J., Schulze-Koops, H., Connell, C.A., Bradley, J.D., et al., 2012. Placebo-controlled trial of tofacitinib mono-therapy in rheumatoid arthritis. *N. Engl. J. Med.* 367 (6), 495–507. Available from: <https://doi.org/10.1056/NEJMoa1109071>.
- Fox, R.I., 1993. Mechanism of action of hydroxychloroquine as an antirheumatic drug. *Semin. Arthritis Rheum.* 23 (2 Suppl 1), 82–91.

- Fox, R.I., 1998. Mechanism of action of leflunomide in rheumatoid arthritis. *J. Rheumatol. Suppl.* 53, 20–26.
- Fox, D.A., McCune, W.J., 1994. Immunosuppressive drug therapy of systemic lupus erythematosus. *Rheum. Dis. Clin. N. Am.* 20 (1), 265–299.
- Fox, R.I., Kang, H.I., 1993. Mechanism of action of antimalarial drugs: inhibition of antigen processing and presentation. *Lupus (2 Suppl 1)*, S9–12.
- Furie, R., Petri, M., Zamani, O., Cervera, R., Wallace, D.J., Tegzova, D., et al., 2011. A phase III, randomized, placebo-controlled study of belimumab, a monoclonal antibody that inhibits B lymphocyte stimulator, in patients with systemic lupus erythematosus. *Arthritis Rheum.* 63 (12), 3918–3930. Available from: <https://doi.org/10.1002/art.30613>.
- Galindo-Rodriguez, G., Avina-Zubieta, J.A., Pizarro, S., Diaz de Leon, V., Saucedo, N., Fuentes, M., et al., 1999. Cyclophosphamide pulse therapy in optic neuritis due to systemic lupus erythematosus: an open trial. *Am. J. Med.* 106 (1), 65–69.
- Genestier, L., Paillot, R., Fournel, S., Ferraro, C., Miossec, P., Revillard, J.P., 1998. Immunosuppressive properties of methotrexate: apoptosis and clonal deletion of activated peripheral T cells. *J. Clin. Invest.* 102 (2), 322–328. Available from: <https://doi.org/10.1172/JCI2676>.
- Ghassy, N.O., Sibbitt Jr., W.L., Qualls, C.R., 2001. Cigarette smoking, alcohol consumption, and the risk of systemic lupus erythematosus: a case-control study. *J. Rheumatol.* 28 (11), 2449–2453.
- Ginzler, E.M., Dooley, M.A., Aranow, C., Kim, M.Y., Buyon, J., Merrill, J.T., et al., 2005. Mycophenolate mofetil or intravenous cyclophosphamide for lupus nephritis. *N. Engl. J. Med.* 353 (21), 2219–2228. Available from: <https://doi.org/10.1056/NEJMoa043731>.
- Ginzler, E.M., Wofsy, D., Isenberg, D., Gordon, C., Lisk, L., Dooley, M.A., et al., 2010. Nonrenal disease activity following mycophenolate mofetil or intravenous cyclophosphamide as induction treatment for lupus nephritis: findings in a multicenter, prospective, randomized, open-label, parallel-group clinical trial. *Arthritis Rheum.* 62 (1), 211–221. Available from: <https://doi.org/10.1002/art.25052>.
- Goekoop-Ruiterman, Y.P., de Vries-Bouwstra, J.K., Allaart, C.F., van Zeben, D., Kerstens, P.J., Hazes, J.M., et al., 2005. Clinical and radiographic outcomes of four different treatment strategies in patients with early rheumatoid arthritis (the BeSt study): a randomized, controlled trial. *Arthritis Rheum.* 52 (11), 3381–3390. Available from: <https://doi.org/10.1002/art.21405>.
- Goekoop-Ruiterman, Y.P., de Vries-Bouwstra, J.K., Allaart, C.F., van Zeben, D., Kerstens, P.J., Hazes, J.M., et al., 2007. Comparison of treatment strategies in early rheumatoid arthritis: a randomized trial. *Ann. Intern. Med.* 146 (6), 406–415.
- Gonzalez-Lopez, L., Gamez-Nava, J.I., Jhangri, G., Russell, A.S., Suarez-Almazor, M.E., 2000. Decreased progression to rheumatoid arthritis or other connective tissue diseases in patients with palindromic rheumatism treated with antimalarials. *J. Rheumatol.* 27 (1), 41–46.
- Gourley, M.F., Austin III, H.A., Scott, D., Yarboro, C.H., Vaughan, E.M., Muir, J., et al., 1996. Methylprednisolone and cyclophosphamide, alone or in combination, in patients with lupus nephritis. A randomized, controlled trial. *Ann. Intern. Med.* 125 (7), 549–557.
- Greco, C.M., Rudy, T.E., Manzi, S., 2004. Effects of a stress-reduction program on psychological function, pain, and physical function of systemic lupus erythematosus patients: a randomized controlled trial. *Arthritis Rheum.* 51 (4), 625–634. Available from: <https://doi.org/10.1002/art.20533>.
- Grigor, C., Capell, H., Stirling, A., McMahon, A.D., Lock, P., Vallance, R., et al., 2004. Effect of a treatment strategy of tight control for rheumatoid arthritis (the TICORA study): a single-blind randomised controlled trial. *Lancet* 364 (9430), 263–269. Available from: [https://doi.org/10.1016/S0140-6736\(04\)16676-2](https://doi.org/10.1016/S0140-6736(04)16676-2).
- Grisar, J., Aringer, M., Koller, M.D., Stummvoll, G.H., Eselböck, D., Zwölfer, B., et al., 2004. Leflunomide inhibits transendothelial migration of peripheral blood mononuclear cells. *Ann. Rheum. Dis.* 63 (12), 1632–1637. Available from: <https://doi.org/10.1136/ard.2003.018440>.
- Gul, A., Ozdogan, H., Erer, B., Ugurlu, S., Kasapcopur, O., Davis, N., et al., 2015. Efficacy and safety of canakinumab in adolescents and adults with colchicine-resistant familial Mediterranean fever. *Arthritis Res. Ther.* 17, 243. Available from: <https://doi.org/10.1186/s13075-015-0765-4>.
- Hahn, B.H., 2013. Belimumab for systemic lupus erythematosus. *N. Engl. J. Med.* 368 (16), 1528–1535. Available from: <https://doi.org/10.1056/NEJMct1207259>.
- Hahn, B.H., McMahon, M.A., Wilkinson, A., Wallace, W.D., Daikh, D.I., Fitzgerald, J.D., et al., 2012. American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis. *Arthritis Care Res. (Hoboken)* 64 (6), 797–808. Available from: <https://doi.org/10.1002/acr.21664>.
- Harris Jr., R.C., 2002. Cyclooxygenase-2 inhibition and renal physiology. *Am. J. Cardiol.* 89 (6A), 10D–17D.
- Hashkes, P.J., Spalding, S.J., Giannini, E.H., Huang, B., Johnson, A., Park, G., et al., 2012. Rilonacept for colchicine-resistant or -intolerant familial Mediterranean fever: a randomized trial. *Ann. Intern. Med.* 157 (8), 533–541. Available from: <https://doi.org/10.7326/0003-4819-157-8-201210160-00003>.
- Hatemi, G., Melikoglu, M., Tunc, R., Korkmaz, C., Turgut Ozturk, B., Mat, C., et al., 2015. Apremilast for Behcet's syndrome—a phase 2, placebo-controlled study. *N. Engl. J. Med.* 372 (16), 1510–1518. Available from: <https://doi.org/10.1056/NEJMoa1408684>.
- Hazlewood, G.S., Barnabe, C., Tomlinson, G., Marshall, D., Devoe, D., Bombardier, C., 2016. Methotrexate monotherapy and methotrexate combination therapy with traditional and biologic disease modifying antirheumatic drugs for rheumatoid arthritis: abridged Cochrane systematic review and network meta-analysis. *BMJ* 353, i1777. Available from: <https://doi.org/10.1136/bmj.i1777>.
- Hirohata, S., Ohshima, N., Yanagida, T., Aramaki, K., 2002. Regulation of human B cell function by sulfasalazine and its metabolites. *Int. Immunopharmacol.* 2 (5), 631–640.
- Hoffman, G.S., Cid, M.C., Hellmann, D.B., Guillemin, L., Stone, J.H., Schousboe, J., et al., 2002. A multicenter, randomized, double-blind, placebo-controlled trial of adjuvant methotrexate treatment for giant cell arteritis. *Arthritis Rheum.* 46 (5), 1309–1318. Available from: <https://doi.org/10.1002/art.10262>.
- Houssiau, F.A., 2016. Moving East: the Euro-Lupus Nephritis regimen in Asia. *Kidney Int.* 89 (1), 25–27. Available from: <https://doi.org/10.1016/j.kint.2015.11.003>.
- Houssiau, F.A., Vasconcelos, C., D'Cruz, D., Sebastiani, G.D., Garrido Ed Ede, R., Danieli, M.G., et al., 2002. Immunosuppressive therapy in lupus nephritis: the Euro-Lupus Nephritis Trial, a randomized trial of low-dose versus high-dose intravenous cyclophosphamide. *Arthritis Rheum.* 46 (8), 2121–2131. Available from: <https://doi.org/10.1002/art.10461>.
- Houssiau, F.A., D'Cruz, D., Sangle, S., Remy, P., Vasconcelos, C., Petrovic, R., et al., 2010a. Azathioprine versus mycophenolate mofetil for long-term immunosuppression in lupus nephritis: results from the MAINTAIN Nephritis Trial. *Ann. Rheum. Dis.* 69 (12), 2083–2089. Available from: <https://doi.org/10.1136/ard.2010.131995>.

- Houssiau, F.A., Vasconcelos, C., D'Cruz, D., Sebastiani, G.D., de Ramon Garrido, E., Danieli, M.G., et al., 2010b. The 10-year follow-up data of the Euro-Lupus Nephritis Trial comparing low-dose and high-dose intravenous cyclophosphamide. *Ann. Rheum. Dis.* 69 (1), 61–64. Available from: <https://doi.org/10.1136/ard.2008.102533>.
- Hu, W., Liu, Z., Chen, H., Tang, Z., Wang, Q., Shen, K., et al., 2002. Mycophenolate mofetil vs cyclophosphamide therapy for patients with diffuse proliferative lupus nephritis. *Chin. Med. J. (Engl.)* 115 (5), 705–709.
- Illei, G.G., Austin, H.A., Crane, M., Collins, L., Gourley, M.F., Yarboro, C.H., et al., 2001. Combination therapy with pulse cyclophosphamide plus pulse methylprednisolone improves long-term renal outcome without adding toxicity in patients with lupus nephritis. *Ann. Intern. Med.* 135 (4), 248–257.
- Isenberg, D., Appel, G.B., Contreras, G., Dooley, M.A., Ginzler, E.M., Jayne, D., et al., 2010. Influence of race/ethnicity on response to lupus nephritis treatment: the ALMS study. *Rheumatology (Oxford)* 49 (1), 128–140. Available from: <https://doi.org/10.1093/rheumatology/kep346>.
- Izmirly, P.M., Kim, M.Y., Llanos, C., Le, P.U., Guerra, M.M., Askanase, A.D., et al., 2010. Evaluation of the risk of anti-SSA/Ro-SSB/La antibody-associated cardiac manifestations of neonatal lupus in fetuses of mothers with systemic lupus erythematosus exposed to hydroxychloroquine. *Ann. Rheum. Dis.* 69 (10), 1827–1830. Available from: <https://doi.org/10.1136/ard.2009.119263>.
- Jancinova, V., Nosal, R., Petrikova, M., 1994. On the inhibitory effect of chloroquine on blood platelet aggregation. *Thromb. Res.* 74 (5), 495–504.
- Jones, S.K., 1992. Ultraviolet radiation (UVR) induces cell-surface Ro/SSA antigen expression by human keratinocytes in vitro: a possible mechanism for the UVR induction of cutaneous lupus lesions. *Br. J. Dermatol.* 126 (6), 546–553.
- Jones, G., Sebba, A., Gu, J., Lowenstein, M.B., Calvo, A., Gomez-Reino, J.J., et al., 2010a. Comparison of tocilizumab monotherapy versus methotrexate monotherapy in patients with moderate to severe rheumatoid arthritis: the AMBITION study. *Ann. Rheum. Dis.* 69 (1), 88–96. Available from: <https://doi.org/10.1136/ard.2008.105197>.
- Jones, R.B., Tervaert, J.W., Hauser, T., Luqmani, R., Morgan, M.D., Peh, C.A., et al., 2010. Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis. *N. Engl. J. Med.* 363 (3), 211–220. Available from: <https://doi.org/10.1056/NEJMoa0909169>.
- Jover, J.A., Hernandez-Garcia, C., Morado, I.C., Vargas, E., Banares, A., Fernandez-Gutierrez, B., 2001. Combined treatment of giant-cell arteritis with methotrexate and prednisone. a randomized, double-blind, placebo-controlled trial. *Ann. Intern. Med.* 134 (2), 106–114.
- King, J.K., Hahn, B.H., 2007. Systemic lupus erythematosus: modern strategies for management: a moving target. *Best Pract. Res. Clin. Rheumatol.* 21 (6), 971–987. Available from: <https://doi.org/10.1016/j.berh.2007.09.002>.
- Kirouka, B.D., 2007. Systemic glucocorticoid therapy in systemic lupus erythematosus. In: Wallace, D.J., Hahn, B.H. (Eds.), *Dubois' Lupus Erythematosus*, 7th ed. Lippincott Williams and Wilkins, Philadelphia, PA.
- Klareskog, L., Stolt, P., Lundberg, K., Kallberg, H., Bengtsson, C., Grunewald, J., et al., 2006. A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. *Arthritis Rheum.* 54 (1), 38–46. Available from: <https://doi.org/10.1002/art.21575>.
- Kovacs, B., Lafferty, T.L., Brent, L.H., DeHoratius, R.J., 2000. Transverse myopathy in systemic lupus erythematosus: an analysis of 14 cases and review of the literature. *Ann. Rheum. Dis.* 59 (2), 120–124.
- Kremer, J., Li, Z.G., Hall, S., Fleischmann, R., Genovese, M., Martin-Mola, E., et al., 2013. Tofacitinib in combination with nonbiologic disease-modifying antirheumatic drugs in patients with active rheumatoid arthritis: a randomized trial. *Ann. Intern. Med.* 159 (4), 253–261. Available from: <https://doi.org/10.7326/0003-4819-159-4-20130820-00006>.
- Kyburz, D., Brentano, F., Gay, S., 2006. Mode of action of hydroxychloroquine in RA-evidence of an inhibitory effect on toll-like receptor signaling. *Nat. Clin. Pract. Rheumatol.* 2 (9), 458–459. Available from: <https://doi.org/10.1038/ncprheum0292>.
- Lee, C.K., Lee, E.Y., Chung, S.M., Mun, S.H., Yoo, B., Moon, H.B., 2004. Effects of disease-modifying antirheumatic drugs and antiinflammatory cytokines on human osteoclastogenesis through interaction with receptor activator of nuclear factor kappaB, osteoprotegerin, and receptor activator of nuclear factor kappaB ligand. *Arthritis Rheum.* 50 (12), 3831–3843. Available from: <https://doi.org/10.1002/art.20637>.
- Lee, Y.H., Lee, H.S., Choi, S.J., Dai Ji, J., Song, G.G., 2011. Efficacy and safety of tacrolimus therapy for lupus nephritis: a systematic review of clinical trials. *Lupus* 20 (6), 636–640. Available from: <https://doi.org/10.1177/0961203310389486>.
- Lenschow, D.J., Walunas, T.L., Bluestone, J.A., 1996. CD28/B7 system of T cell costimulation. *Annu. Rev. Immunol.* 14, 233–258. Available from: <https://doi.org/10.1146/annurev.immunol.14.1.233>.
- Li, X., Ren, H., Zhang, Q., Zhang, W., Wu, X., Xu, Y., et al., 2012. Mycophenolate mofetil or tacrolimus compared with intravenous cyclophosphamide in the induction treatment for active lupus nephritis. *Nephrol. Dial. Transplant.* 27 (4), 1467–1472. Available from: <https://doi.org/10.1093/ndt/gfr484>.
- Liu, Z., Zhang, H., Liu, Z., Xing, C., Fu, P., Ni, Z., et al., 2015. Multitarget therapy for induction treatment of lupus nephritis: a randomized trial. *Ann. Intern. Med.* 162 (1), 18–26. Available from: <https://doi.org/10.7326/M14-1030>.
- Lu, X., Xie, W., Reed, D., Bradshaw, W.S., Simmons, D.L., 1995. Nonsteroidal antiinflammatory drugs cause apoptosis and induce cyclooxygenases in chicken embryo fibroblasts. *Proc. Natl. Acad. Sci. U.S.A.* 92 (17), 7961–7965.
- MacDermott, R.P., 2017. Treatment of Ulcerative Colitis. UpToDateRetrieved from . Available from: <http://www.uptodate.com/home>.
- Magee, C., 2012. In: Furst, D.E., Romain, P.L. (Eds.), *Pharmacology and Side Effects of Cyclosporine and Tacrolimus*. UpToDate, Retrieved from <http://www.uptodate.com/home>.
- Mahid, S.S., Minor, K.S., Soto, R.E., Hornung, C.A., Galandiuk, S., 2006. Smoking and inflammatory bowel disease: a meta-analysis. *Mayo Clin. Proc.* 81 (11), 1462–1471. Available from: <https://doi.org/10.4065/81.11.1462>.
- Maldonado-Ficco, H., Perez-Alamino, R., Maldonado-Cocco, J.A., 2016. Secukinumab: a promising therapeutic option in spondyloarthritis. *Clin. Rheumatol.* 35 (9), 2151–2161. Available from: <https://doi.org/10.1007/s10067-016-3350-6>.
- Manna, S.K., Aggarwal, B.B., 1999. Immunosuppressive leflunomide metabolite (A77 1726) blocks TNF-dependent nuclear factor-kappa B activation and gene expression. *J. Immunol.* 162 (4), 2095–2102.
- Marmor, M.F., Kellner, U., Lai, T.Y., Lyons, J.S., Mieler, W.F., American Academy of O, 2011. Revised recommendations on screening for chloroquine and hydroxychloroquine retinopathy. *Ophthalmology* 118 (2), 415–422. Available from: <https://doi.org/10.1016/j.ophtha.2010.11.017>.

- Maxwell, L.J., Singh, J.A., 2010. Abatacept for rheumatoid arthritis: a Cochrane systematic review. *J. Rheumatol.* 37 (2), 234–245. Available from: <https://doi.org/10.3899/jrheum.091066>.
- McCune, W.J., Fox, D., 1989. Intravenous cyclophosphamide therapy of severe SLE. *Rheum. Dis. Clin. N. Am.* 15 (3), 455–477.
- McInnes, I.B., Kavanaugh, A., Gottlieb, A.B., Puig, L., Rahman, P., Ritchlin, C., et al., 2013. Efficacy and safety of ustekinumab in patients with active psoriatic arthritis: 1 year results of the phase 3, multicentre, double-blind, placebo-controlled PSUMMIT 1 trial. *Lancet* 382 (9894), 780–789. Available from: [https://doi.org/10.1016/S0140-6736\(13\)60594-2](https://doi.org/10.1016/S0140-6736(13)60594-2).
- McInnes, I.B., Mease, P.J., Kirkham, B., Kavanaugh, A., Ritchlin, C.T., Rahman, P., et al., 2015. Secukinumab, a human anti-interleukin-17A monoclonal antibody, in patients with psoriatic arthritis (FUTURE 2): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 386 (9999), 1137–1146. Available from: [https://doi.org/10.1016/S0140-6736\(15\)61134-5](https://doi.org/10.1016/S0140-6736(15)61134-5).
- Mello, S.B., Barros, D.M., Silva, A.S., Laurindo, I.M., Novaes, G.S., 2000. Methotrexate as a preferential cyclooxygenase 2 inhibitor in whole blood of patients with rheumatoid arthritis. *Rheumatology (Oxford)* 39 (5), 533–536.
- Merrill, J.T., Neuwelt, C.M., Wallace, D.J., Shanahan, J.C., Latinis, K.M., Oates, J.C., et al., 2010. Efficacy and safety of rituximab in moderately-to-severely active systemic lupus erythematosus: the randomized, double-blind, phase II/III systemic lupus erythematosus evaluation of rituximab trial. *Arthritis Rheum.* 62 (1), 222–233. Available from: <https://doi.org/10.1002/art.27233>.
- Merrill, J.T., Ginzler, E.M., Wallace, D.J., McKay, J.D., Lisse, J.R., Aranow, C., et al., 2012. Long-term safety profile of belimumab plus standard therapy in patients with systemic lupus erythematosus. *Arthritis Rheum.* 64 (10), 3364–3373. Available from: <https://doi.org/10.1002/art.34564>.
- Morabito, L., Montesinos, M.C., Schreibman, D.M., Balter, L., Thompson, L.F., Resta, R., et al., 1998. Methotrexate and sulfasalazine promote adenosine release by a mechanism that requires ecto-5'-nucleotidase-mediated conversion of adenine nucleotides. *J. Clin. Invest.* 101 (2), 295–300. Available from: <https://doi.org/10.1172/JCI1554>.
- Morganroth, P.A., Kreider, M.E., Werth, V.P., 2010. Mycophenolate mofetil for interstitial lung disease in dermatomyositis. *Arthritis Care Res. (Hoboken)* 62 (10), 1496–1501. Available from: <https://doi.org/10.1002/acr.20212>.
- Mottonen, T., Hannonen, P., Leirisalo-Repo, M., Nissila, M., Kautiainen, H., Korppela, M., et al., 1999. Comparison of combination therapy with single-drug therapy in early rheumatoid arthritis: a randomised trial. FIN-RACo trial group. *Lancet* 353 (9164), 1568–1573.
- Mukhtyar, C., Guillemin, L., Cid, M.C., Dasgupta, B., de Groot, K., Gross, W., et al., 2009. EULAR recommendations for the management of primary small and medium vessel vasculitis. *Ann. Rheum. Dis.* 68 (3), 310–317. Available from: <https://doi.org/10.1136/ard.2008.088096>.
- Navarra, S.V., Guzman, R.M., Gallacher, A.E., Hall, S., Levy, R.A., Jimenez, R.E., et al., 2011. Efficacy and safety of belimumab in patients with active systemic lupus erythematosus: a randomised, placebo-controlled, phase 3 trial. *Lancet* 377 (9767), 721–731. Available from: [https://doi.org/10.1016/S0140-6736\(10\)61354-2](https://doi.org/10.1016/S0140-6736(10)61354-2).
- Navarro-Millan, I., Singh, J.A., Curtis, J.R., 2012. Systematic review of tocilizumab for rheumatoid arthritis: a new biologic agent targeting the interleukin-6 receptor. *Clin. Ther.* 34 (4), 788–802. Available from: <https://doi.org/10.1016/j.clinthera.2012.02.014>, e783.
- Neuwelt, C.M., Lacks, S., Kaye, B.R., Ellman, J.B., Borenstein, D.G., 1995. Role of intravenous cyclophosphamide in the treatment of severe neuropsychiatric systemic lupus erythematosus. *Am. J. Med.* 98 (1), 32–41. Available from: [https://doi.org/10.1016/S0002-9343\(99\)80078-3](https://doi.org/10.1016/S0002-9343(99)80078-3).
- Nigrovic, P.A., Mannion, M., Prince, F.H., Zefft, A., Rabinovich, C.E., van Rossum, M.A., et al., 2011. Anakinra as first-line disease-modifying therapy in systemic juvenile idiopathic arthritis: report of forty-six patients from an international multicenter series. *Arthritis Rheum.* 63 (2), 545–555. Available from: <https://doi.org/10.1002/art.30128>.
- O'Dell, J.R., Haire, C.E., Erikson, N., Drymalski, W., Palmer, W., Eckhoff, P.J., et al., 1996. Treatment of rheumatoid arthritis with methotrexate alone, sulfasalazine and hydroxychloroquine, or a combination of all three medications. *N. Engl. J. Med.* 334 (20), 1287–1291. Available from: <https://doi.org/10.1056/NEJM199605163342002>.
- Ostensen, M., 2017. Sexual and reproductive health in rheumatic disease. *Nat. Rev. Rheumatol.* 13 (8), 485–493. Available from: <https://doi.org/10.1038/nrrheum.2017.102>.
- Otto, R., Mackay, I.R., 1967. Psycho-social and emotional disturbance in systemic lupus erythematosus. *Med. J. Aust.* 2 (11), 488–493.
- Padyukov, L., Silva, C., Stolt, P., Alfredsson, L., Klareskog, L., 2004. A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of seropositive rheumatoid arthritis. *Arthritis Rheum.* 50 (10), 3085–3092. Available from: <https://doi.org/10.1002/art.20553>.
- Pagnoux, C., Mahr, A., Hamidou, M.A., Boffa, J.J., Ruivard, M., Ducroix, J.P., et al., 2008. Azathioprine or methotrexate maintenance for ANCA-associated vasculitis. *N. Engl. J. Med.* 359 (26), 2790–2803. Available from: <https://doi.org/10.1056/NEJMoa0802311>.
- Papp, K., Bissonnette, R., Rosoph, L., Wasel, N., Lynde, C.W., Searles, G., et al., 2008. Efficacy of ISA247 in plaque psoriasis: a randomised, multicentre, double-blind, placebo-controlled phase III study. *Lancet* 371 (9621), 1337–1342. Available from: [https://doi.org/10.1016/S0140-6736\(08\)60593-0](https://doi.org/10.1016/S0140-6736(08)60593-0).
- Pathan, E., Abraham, S., Van Rossen, E., Withrington, R., Keat, A., Charles, P.J., et al., 2013. Efficacy and safety of apremilast, an oral phosphodiesterase 4 inhibitor, in ankylosing spondylitis. *Ann. Rheum. Dis.* 72 (9), 1475–1480. Available from: <https://doi.org/10.1136/annrheumdis-2012-201915>.
- Prefontaine, E., Sutherland, L.R., Macdonald, J.K., Cepoiu, M., 2009. Azathioprine or 6-mercaptopurine for maintenance of remission in Crohn's disease. *Cochrane Database Syst. Rev.* (1), CD000067. Available from: <https://doi.org/10.1002/14651858.CD000067.pub2>.
- Rantalaiho, V., Korppela, M., Hannonen, P., Kautiainen, H., Jarvenpaa, S., Leirisalo-Repo, M., et al., 2009. The good initial response to therapy with a combination of traditional disease-modifying antirheumatic drugs is sustained over time: the eleven-year results of the Finnish rheumatoid arthritis combination therapy trial. *Arthritis Rheum.* 60 (5), 1222–1231. Available from: <https://doi.org/10.1002/art.24447>.
- Regius, O., Lengyel, E., Borzsonyi, L., Beregi, E., 1988. The effect of smoking on the presence of antinuclear antibodies and on the morphology of lymphocytes in aged subjects. *Z. Gerontol.* 21 (3), 161–163.
- Ribi, C., Cohen, P., Pagnoux, C., Mahr, A., Arene, J.P., Puechal, X., et al., 2010. Treatment of polyarteritis nodosa and microscopic polyangiitis without poor-prognosis factors: a prospective randomized study of one hundred twenty-four patients. *Arthritis Rheum.* 62 (4), 1186–1197. Available from: <https://doi.org/10.1002/art.27340>.
- Rich, E., Moreland, L.W., Alarcon, G.S., 1999. Paucity of radiographic progression in rheumatoid arthritis treated with methotrexate as the first disease modifying antirheumatic drug. *J. Rheumatol.* 26 (2), 259–261.

- Rodenburg, R.J., Ganga, A., van Lent, P.L., van de Putte, L.B., van Venrooij, W.J., 2000. The antiinflammatory drug sulfasalazine inhibits tumor necrosis factor alpha expression in macrophages by inducing apoptosis. *Arthritis Rheum.* 43 (9), 1941–1950. Available from: [https://doi.org/10.1002/1529-0131\(200009\)43:9<1941::AID-ANR4>3.0.CO;2-O](https://doi.org/10.1002/1529-0131(200009)43:9<1941::AID-ANR4>3.0.CO;2-O).
- Rovin, B.H., Furie, R., Latinis, K., Looney, R.J., Fervenza, F.C., Sanchez-Guerrero, J., et al., 2012. Efficacy and safety of rituximab in patients with active proliferative lupus nephritis: the Lupus Nephritis Assessment with Rituximab study. *Arthritis Rheum.* 64 (4), 1215–1226. Available from: <https://doi.org/10.1002/art.34359>.
- Saag, K., et al., 2011. Systemic glucocorticoid therapy in rheumatology. *Rheumatology*, 5th ed. Mosby Elsevier, Philadelphia, PA.
- Saag, K.G., Cerhan, J.R., Kolluri, S., Ohashi, K., Hunninghake, G.W., Schwartz, D.A., 1997. Cigarette smoking and rheumatoid arthritis severity. *Ann. Rheum. Dis.* 56 (8), 463–469.
- Salmi, M., Rajala, P., Jalkanen, S., 1997. Homing of mucosal leukocytes to joints. Distinct endothelial ligands in synovium mediate leukocyte-subtype specific adhesion. *J. Clin. Invest.* 99 (9), 2165–2172. Available from: <https://doi.org/10.1172/JCI119389>.
- Sands, K., Jansen, R., Zaslau, S., Greenwald, D., 2015. Review article: the safety of therapeutic drugs in male inflammatory bowel disease patients wishing to conceive. *Aliment. Pharmacol. Ther.* 41 (9), 821–834. Available from: <https://doi.org/10.1111/apt.13142>.
- Schafer, P.H., Parton, A., Gandhi, A.K., Capone, L., Adams, M., Wu, L., et al., 2010. Apremilast, a cAMP phosphodiesterase-4 inhibitor, demonstrates anti-inflammatory activity in vitro and in a model of psoriasis. *Br. J. Pharmacol.* 159 (4), 842–855. Available from: <https://doi.org/10.1111/j.1476-5381.2009.00559.x>.
- Schreiber, S.L., Crabtree, G.R., 1992. The mechanism of action of cyclosporin A and FK506. *Immunol. Today* 13 (4), 136–142. Available from: [https://doi.org/10.1016/0167-5699\(92\)90111-J](https://doi.org/10.1016/0167-5699(92)90111-J).
- Schultz, C., 2013. Voclosporin as a treatment for noninfectious uveitis. *Ophthalmol Eye Dis.* 5, 5–10. Available from: <https://doi.org/10.4137/OED.S7995>.
- Schur, P.H., 2017. Randomized Clinical Trials of Tumor Necrosis Factor Inhibitors in Rheumatoid Arthritis. UpToDate, Retrieved from <http://www.uptodate.com/home>.
- Scott, D.G., Bacon, P.A., 1984. Intravenous cyclophosphamide plus methylprednisolone in treatment of systemic rheumatoid vasculitis. *Am. J. Med.* 76 (3), 377–384.
- Seitz, M., Zwicker, M., Wider, B., 2001. Enhanced in vitro induced production of interleukin 10 by peripheral blood mononuclear cells in rheumatoid arthritis is associated with clinical response to methotrexate treatment. *J. Rheumatol.* 28 (3), 496–501.
- Siemasko, K., Chong, A.S., Jack, H.M., Gong, H., Williams, J.W., Finnegan, A., 1998. Inhibition of JAK3 and STAT6 tyrosine phosphorylation by the immunosuppressive drug leflunomide leads to a block in IgG1 production. *J. Immunol.* 160 (4), 1581–1588.
- Silvergleid, A.J., Berger, M., 2011. In: Schrier, S.L., Stiehm, E.R., Tirnauer, J.S., Feldweg, A.M. (Eds.), General Principles in the Use of Immune Globulin. UpToDate, Retrieved from <http://www.uptodate.com/home>.
- Singh, J.A., Christensen, R., Wells, G.A., Suarez-Almazor, M.E., Buchbinder, R., Lopez-Olivo, M.A., et al., 2009. A network meta-analysis of randomized controlled trials of biologics for rheumatoid arthritis: a Cochrane overview. *CMAJ* 181 (11), 787–796. Available from: <https://doi.org/10.1503/cmaj.091391>.
- Singh, J.A., Beg, S., Lopez-Olivo, M.A., 2011. Tocilizumab for rheumatoid arthritis: a Cochrane systematic review. *J. Rheumatol.* 38 (1), 10–20. Available from: <https://doi.org/10.3899/jrheum.100717>.
- Singh, J.A., Saag, K.G., Bridges Jr., S.L., Akl, E.A., Bannuru, R.R., Sullivan, M.C., et al., 2016. 2015 American College of Rheumatology guideline for the treatment of rheumatoid arthritis. *Arthritis Care Res. (Hoboken)* 68 (1), 1–25. Available from: <https://doi.org/10.1002/acr.22783>.
- Smolen, J.S., Kalden, J.R., Scott, D.L., Rozman, B., Kvien, T.K., Larsen, A., et al., 1999. Efficacy and safety of leflunomide compared with placebo and sulphasalazine in active rheumatoid arthritis: a double-blind, randomised, multicentre trial. European Leflunomide Study Group. *Lancet* 353 (9149), 259–266.
- So, A., De Smedt, T., Revaz, S., Tschopp, J., 2007. A pilot study of IL-1 inhibition by anakinra in acute gout. *Arthritis Res. Ther.* 9 (2), R28. Available from: <https://doi.org/10.1186/ar2143>.
- Solomon, D., 2012. In: Furst, D.E., Romain, P.L. (Eds.), Nonselective NSAIDs: Overview of Adverse Effects. UpToDate, Retrieved from <http://www.uptodate.com/home>.
- Stone, J.H., 2010. General principles of the use of cyclophosphamide in rheumatic and renal diseases. In: Furst, D.E., Ramirez, M.P. (Eds.), UpToDate, Retrieved from <http://www.uptodate.com/home>.
- Stone, J.H., Merkel, P.A., Spiera, R., Seo, P., Langford, C.A., Hoffman, G.S., et al., 2010. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. *N. Engl. J. Med.* 363 (3), 221–232. Available from: <https://doi.org/10.1056/NEJMoa0909905>.
- Stone, J.H., Tuckwell, K., Dimonaco, S., Klearman, M., Aringer, M., Blockmans, D., et al., 2017. Trial of tocilizumab in giant-cell arteritis. *N. Engl. J. Med.* 377 (4), 317–328. Available from: <https://doi.org/10.1056/NEJMoa1613849>.
- Tashkin, D.P., Elashoff, R., Clements, P.J., Goldin, J., Roth, M.D., Furst, D.E., et al., 2006. Cyclophosphamide versus placebo in scleroderma lung disease. *N. Engl. J. Med.* 354 (25), 2655–2666. Available from: <https://doi.org/10.1056/NEJMoa055120>.
- Tashkin, D.P., Roth, M.D., Clements, P.J., Furst, D.E., Khanna, D., Kleerup, E.C., et al., 2016. Mycophenolate mofetil versus oral cyclophosphamide in scleroderma-related interstitial lung disease (SLS II): a randomised controlled, double-blind, parallel group trial. *Lancet Respir. Med.* 4 (9), 708–719. Available from: [https://doi.org/10.1016/S2213-2600\(16\)30152-7](https://doi.org/10.1016/S2213-2600(16)30152-7).
- Taylor, P.C., 2010. Pharmacology of TNF blockade in rheumatoid arthritis and other chronic inflammatory diseases. *Curr. Opin. Pharmacol.* 10 (3), 308–315. Available from: <https://doi.org/10.1016/j.coph.2010.01.005>.
- Timmer, A., McDonald, J.W., Macdonald, J.K., 2007. Azathioprine and 6-mercaptopurine for maintenance of remission in ulcerative colitis. *Cochrane Database Syst. Rev.* (1), CD000478. Available from: <https://doi.org/10.1002/14651858.CD000478.pub2>.
- Timmerman, L.A., Clipstone, N.A., Ho, S.N., Northrop, J.P., Crabtree, G.R., 1996. Rapid shuttling of NF-AT in discrimination of  $\text{Ca}^{2+}$  signals and immunosuppression. *Nature* 383 (6603), 837–840. Available from: <https://doi.org/10.1038/383837a0>.
- Tobin, M.V., Logan, R.F., Langman, M.J., McConnell, R.B., Gilmore, I.T., 1987. Cigarette smoking and inflammatory bowel disease. *Gastroenterology* 93 (2), 316–321.

- Touma, Z., Gladman, D.D., Urowitz, M.B., Beyene, J., Uleryk, E.M., Shah, P.S., 2011. Mycophenolate mofetil for induction treatment of lupus nephritis: a systematic review and metaanalysis. *J. Rheumatol.* 38 (1), 69–78. Available from: <https://doi.org/10.3899/jrheum.100130>.
- Tsokos, G.C., 2004. B cells, be gone—B-cell depletion in the treatment of rheumatoid arthritis. *N. Engl. J. Med.* 350 (25), 2546–2548. Available from: <https://doi.org/10.1056/NEJMmp048114>.
- Urowitz, M.B., Gordon, D.A., Smythe, H.A., Pruzanski, W., Ogryzio, M.A., 1973. Azathioprine in rheumatoid arthritis. A double-blind, cross over study. *Arthritis Rheum.* 16 (3), 411–418.
- van der Hilst, J., Moutschen, M., Messiaen, P.E., Lauwers, B.R., Vanderschueren, S., 2016. Efficacy of anti-IL-1 treatment in familial Mediterranean fever: a systematic review of the literature. *Biologics* 10, 75–80. Available from: <https://doi.org/10.2147/BTT.S102954>.
- Vastert, S.J., de Jager, W., Noordman, B.J., Holzinger, D., Kuis, W., Prakken, B.J., et al., 2014. Effectiveness of first-line treatment with recombinant interleukin-1 receptor antagonist in steroid-naïve patients with new-onset systemic juvenile idiopathic arthritis: results of a prospective cohort study. *Arthritis Rheumatol.* 66 (4), 1034–1043. Available from: <https://doi.org/10.1002/art.38296>.
- Von Feldt, J.M., 1995. Systemic lupus erythematosus. Recognizing its various presentations. *Postgrad. Med.* 97 (4), 79, 83, 86 *passim*.
- Wahl, C., Liptay, S., Adler, G., Schmid, R.M., 1998. Sulfasalazine: a potent and specific inhibitor of nuclear factor kappa B. *J. Clin. Invest.* 101 (5), 1163–1174. Available from: <https://doi.org/10.1172/JCI992>.
- Wallace, D.J., 2007. Antimalarial therapies. In: Wallace, D.J., Hahn, B.H. (Eds.), *Dubois' Lupus Erythematosus*, 7th ed. Lippincot Williams and Wilkins, Philadelphia, PA.
- Wang, S., Li, X., Qu, L., Wang, R., Chen, Y., Li, Q., et al., 2012. Tacrolimus versus cyclophosphamide as treatment for diffuse proliferative or membranous lupus nephritis: a non-randomized prospective cohort study. *Lupus* 21 (9), 1025–1035. Available from: <https://doi.org/10.1177/0961203312448105>.
- Wasko, M.C., Hubert, H.B., Lingala, V.B., Elliott, J.R., Luggen, M.E., Fries, J.F., et al., 2007. Hydroxychloroquine and risk of diabetes in patients with rheumatoid arthritis. *JAMA* 298 (2), 187–193. Available from: <https://doi.org/10.1001/jama.298.2.187>.
- Weinblatt, M.E., 2013. Methotrexate in rheumatoid arthritis: a quarter century of development. *Trans. Am. Clin. Climatol. Assoc.* 124, 16–25.
- Weinblatt, M.E., Weissman, B.N., Holdsworth, D.E., Fraser, P.A., Maier, A.L., Falchuk, K.R., et al., 1992. Long-term prospective study of methotrexate in the treatment of rheumatoid arthritis. 84-month update. *Arthritis Rheum.* 35 (2), 129–137.
- Weinblatt, M.E., Kaplan, H., Germain, B.F., Block, S., Solomon, S.D., Merriman, R.C., et al., 1994. Methotrexate in rheumatoid arthritis. A five-year prospective multicenter study. *Arthritis Rheum.* 37 (10), 1492–1498.
- Weisman, M.H., Rinaldi, R.Z., 2017. Sulfasalazine in the Treatment of Rheumatoid Arthritis. UpToDate, Retrieved from <http://www.uptodate.com/home>.
- Wiederrecht, G., Lam, E., Hung, S., Martin, M., Sigal, N., 1993. The mechanism of action of FK-506 and cyclosporin A. *Ann. N.Y. Acad. Sci.* 696, 9–19.
- Wilke, W.S., 2010. In: Furst, D.E., Romain, P.L. (Eds.), *Pharmacology and Side Effects of Azathioprine When Used in Rheumatic Disease*. UpToDate, Retrieved from <http://www.uptodate.com/home>.
- Willrich, M.A., Murray, D.L., Snyder, M.R., 2015. Tumor necrosis factor inhibitors: clinical utility in autoimmune diseases. *Transl. Res.* 165 (2), 270–282. Available from: <https://doi.org/10.1016/j.trsl.2014.09.006>.
- Wolfe, F., 2000. The effect of smoking on clinical, laboratory, and radiographic status in rheumatoid arthritis. *J. Rheumatol.* 27 (3), 630–637.
- Woodland, J., Chaput de Saintonge, D.M., Evans, S.J., Sharman, V.L., Currey, H.L., 1981. Azathioprine in rheumatoid arthritis: double-blind study of full versus half doses versus placebo. *Ann. Rheum. Dis.* 40 (4), 355–359.
- Yeilding, N., Szapary, P., Brodmerkel, C., Benson, J., Plotnick, M., Zhou, H., et al., 2012. Development of the IL-12/23 antagonist ustekinumab in psoriasis: past, present, and future perspectives—an update. *Ann. N.Y. Acad. Sci.* 1263, 1–12. Available from: <https://doi.org/10.1111/j.1749-6632.2012.06670.x>.
- Zhang, X., Brunner, T., Carter, L., Dutton, R.W., Rogers, P., Bradley, L., et al., 1997. Unequal death in T helper cell (Th)1 and Th2 effectors: Th1, but not Th2, effectors undergo rapid Fas/FasL-mediated apoptosis. *J. Exp. Med.* 185 (10), 1837–1849.

# Emerging Biological and Molecular Therapies in Autoimmune Disease

*Lucienne Chatenoud*

INSERM U1151, CNRS UMR 8253, Institute Necker-Enfants Malades, University Paris Descartes,  
Sorbonne Paris Cité, Paris, France

## OUTLINE

<b>Introduction</b>	1437	<i>The Breakthrough in Autoimmune Diabetes</i>	1443
<b>Monoclonal Antibodies Used Clinically: Ways to Make Them More Efficient</b>	1438	<i>The Surprises and the Expectations of B Lymphocyte Targeting</i>	1445
<i>Engineering Fc Regions of Monoclonal Antibodies to Avoid Side Effects and Prolong Half-Life</i>	1439	<b>Bone-Marrow Transplantation</b>	1445
<i>Engineering Variable Regions of Monoclonal Antibodies to Increase Affinity</i>	1439	<b>Soluble Autoantigens</b>	1447
<i>Engineering Variable Regions of Monoclonal Antibodies to Decrease Immunogenicity</i>	1439	<b>Cell Therapy and Antigen Receptor</b>	
<b>The Adequation of the Antibody Specificity to the Target Disease: From Immunosuppression to Immune Tolerance</b>	1440	<b>Gene–Modified T Cells</b>	1449
<i>The Breakthrough in Rheumatoid Arthritis</i>	1440	<i>Cell Therapy Using Regulatory T Cells</i>	1449
<i>The Breakthrough in Multiple Sclerosis</i>	1441	<i>Cell Therapy Using Antigen Receptor</i>	
		<i>Gene–Modified T Cells</i>	1449
		<b>Perspectives and Conclusions</b>	1451
		<b>References</b>	1451

## INTRODUCTION

Autoimmune diseases represent a major therapeutic challenge. In many cases the disease is severe enough to significantly reduce longevity. In other cases the disease causes major handicaps and discomfort that justify the usage of aggressive treatments generating their own hazards. The past treatments were mainly palliative (substitutive), antiinflammatory, or immunosuppressive without any specificity for the pathogenic mechanisms of the disease.

Over the last decades, modern technologies have made new agents available, in particular monoclonal antibodies (MAbs) directed to key immune cell receptors or cytokines, which have created a true revolution and fostered major advancements in the treatment of rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus (SLE), psoriasis, and inflammatory bowel diseases. Many of these treatments that have been approved and are part of the regular armamentarium clinicians use to treat autoimmune patients have been described in previous chapters.

We will return to some of these treatments in a more holistic perspective to understand how the use strategies have been adapted and continue to adapt to achieve better efficacy and fewer side effects. We will also discuss strategies still in development that may, in a not too distant future, fill important gaps. In particular an ambitious goal is to induce or, in the case of established autoimmune diseases, to restore immune tolerance to target auto-antigens. This may be defined operationally as the possibility to harness the pathologic immune response following a short-term treatment while keeping intact the capacity of the host to respond normally to exogenous antigens. Restoration of self-tolerance has the major advantage of avoiding the side effects linked to chronic immunosuppression and, most importantly, to provide a real cure for the disease.

## MONOCLONAL ANTIBODIES USED CLINICALLY: WAYS TO MAKE THEM MORE EFFICIENT

Murine MAbs produced by mouse or rat hybridomas (Kohler and Milstein, 1975) specific for immune cell receptors were introduced in clinical practice close to 40 years ago and their use initially developed in the field of solid organ transplantation. Two major side effects, namely, the sensitization against the xenogeneic molecule and the cytokine-releasing potential observed with some particular specificities, explain why the use of rodent MAbs remained initially mostly confined to transplantation with only very few attempts in autoimmunity. The advent of humanized and human MAbs that are less immunogenic and better tolerated has completely changed the picture and allowed a more widespread use of these interesting therapeutic tools. At variance with conventional immunosuppressants, some MAbs specific for relevant lymphocytes receptors are unique in their capacity to induce, under adequate circumstances, immune tolerance to soluble proteins, foreign tissue alloantigens, and autoantigens (Cobbold et al., 2006; Chatenoud, 2003, 2010; Chatenoud and Bluestone, 2007).

MAbs display a wide spectrum of pharmacological and biological activities highly relevant to their capacity to “reprogram” the immune system. Thus depending on their fine specificity, MAbs will remove target cells, inhibit or block the functional capacity of the target without depleting it, neutralize major cytokines, and/or serve as receptor agonists triggering activation signals for specialized T-cell subsets and in particular regulatory T cells (Tregs) (Sakaguchi et al., 2008; Bach, 2003).

The repeated administration of murine MAbs invariably triggered a humoral immune response, a major clinical consequence of which was the neutralization of the antibody's therapeutic activity. Interestingly, this was not a global antimouse or antirat response; it was very restricted in its specificity with essentially antiisotypic and antiidiotypic antibodies being produced (Chatenoud et al., 1986a,b; Benjamin et al., 1986). Antiidiotypic antibodies that compete with the therapeutic antibody for antigen binding represent the neutralizing component of the response while antiisotypic antibodies are mostly nonneutralizing (Baudrihaye et al., 1984). Another peculiarity of this humoral response is its oligoclonality (Chatenoud et al., 1986a,b; Villemain et al., 1986), which explains that, at variance with what observed in patients immunized to polyclonal antilymphocyte globulins, serum sickness was a rare consequence of sensitization to MAbs since the amount of immune complexes formed would be insufficient to elicit a generalized reaction. Antimonoclonal IgE responses associated with symptoms of anaphylaxis were reported but remained a very uncommon observation (Abramowicz et al., 1992, 1996).

Until humanized MAbs or more recently fully human antibodies became available the only way to cope with the problem of sensitization was to associate adequate doses of chemical immunosuppressants (Hricik et al., 1990). Two types of humanized antibodies have been derived from molecular engineering. Chimeric MAbs express intact rodent variable regions linked to human immunoglobulin constant domains (Morrison et al., 1984). In fully reshaped or complementarity determining region (CDR)-grafted antibodies the rodent hypervariable regions interacting with the antigen (CDRs) are included within human heavy and light chain immunoglobulin frameworks (Riechmann et al., 1988). Fully human MAbs have been produced by different means. First, mice have been invalidated for the expression of endogenous (mouse) immunoglobulin genes and concurrently made transgenic for sufficient human constant and variable immunoglobulin-encoding sequences to provide for antibody diversity; B cells from these immunized mice produce human antibodies that can be used in conventional fusions to obtain hybridomas yielding high-affinity MAbs derived from *in vivo* antigen-driven selection (Lonberg et al., 1994). Second, there is a fully *in vitro* approach using cDNA libraries expressed on filamentous phages to derive high-affinity antibodies to a wide variety of antigens, including those for which the conventional hybridoma technology fails due to poor immunogenicity (Marks et al., 1991). Third, human–mouse chimeras can be established using normal mice irradiated and reconstituted with bone-marrow cells from severe

combined immunodeficiency (SCID) mice; after human lymphocytes from presensitized donors are inoculated, these mice are boosted with the antigen of interest, and sensitized human B cells recovered and used for conventional fusions (Lubin et al., 1994).

Humanized MAbs and fully human MAbs have significantly reduced though not totally avoided the risk of deleterious antiidiotypic responses. Both chimeric and reshaped humanized MAbs may be immunogenic when administered alone, without associated immunosuppressants (Herold et al., 2002; Keymeulen et al., 2005; Elliott et al., 1994). As for murine antibodies, combining low doses of chemical immunosuppressants, as is regularly done with anti-tumore necrosis factor (TNF) in rheumatoid arthritis, is a very efficient way to overcome sensitization (Feldmann, 2002; Nashan et al., 1997; Vincenti et al., 1998). Importantly, the frequency of immunization varies according to the fine specificity of the MAbs. For example, immunization is common for humanized CD3 and CD52 antibodies (Somerfield et al., 2010; Herold et al., 2002; Keymeulen et al., 2005). It is lower for anti-TNF antibodies, especially in rheumatoid arthritis because of the regular association with conventional immunosuppressants such as methotrexate (Feldmann, 2002). At variance, in inflammatory bowel diseases the problem is relevant because the immunization leads to the neutralization of the therapeutic effect, and the possibilities of switching to other immunotherapies are more limited than in rheumatoid arthritis, as soluble TNF receptors are ineffective in this indication.

### Engineering Fc Regions of Monoclonal Antibodies to Avoid Side Effects and Prolong Half-Life

Antibody engineering allows the design of tailor-made antibodies to fit at best therapeutic indications. Antibodies expressing human Fc portions have a significantly prolonged half-life. In addition, the choice of the human Fc portion will influence the antibody effector capacities, that is, its activity in terms of complement fixation, opsonization, and antibody-dependent cell cytotoxicity. In addition, in the case of CD3 antibodies, humanization can circumvent problems due to their intrinsic mitogenic and cytokine-releasing capacity that leads in vivo to a "flu-like" syndrome. This syndrome was regularly observed with murine CD3 MAbs such as OKT3, and although transient, it represented a major and troublesome side effect that totally precluded the use of CD3 MAbs for indications other than organ transplantation (Chatenoud et al., 1990; Abramowicz et al., 1989; Chatenoud, 2003). This mitogenic capacity is linked to the ability of the Fc portion of CD3 MAbs to interact with monocyte Fc receptors (Chatenoud, 2003). Thus "nonmitogenic," non-Fc binding, CD3 antibodies were obtained by inserting adequate mutations into the Fc domains to hamper Fc receptor binding (Alegre et al., 1994; Bolt et al., 1993; Chatenoud, 2003). Clinical use of two different Fc-mutated CD3 MAbs, teplizumab (OKT3 $\gamma$ 1Ala-Ala) and otelixizumab (ChAglyCD3), in autoimmunity and transplantation, confirmed that their use was free of major side effects (Friend et al., 1999; Herold et al., 2002; Sherry et al., 2011; Keymeulen et al., 2005; Woodle et al., 1999; Hering et al., 2004).

### Engineering Variable Regions of Monoclonal Antibodies to Increase Affinity

In case the affinity of a given MAbs is too low for in vivo use, phage display technology is an interesting approach to generate an improved fully human reagent with no requirement for prior immunization or use of hybridoma technology. Phage display can be used to mimic artificially the processes used in vivo by the immune system to obtain high-affinity antibodies. This has been achieved by shuffling the heavy or light chains by random or directed mutagenesis of CDR (Barbas et al., 1994), as done by error prone polymerase chain reaction. Using such artificial affinity maturation of phage antibody repertoires, affinities of MAbs in the nanomolar to picomolar range have been generated that are perfectly suitable for therapeutic use (Foote and Eisen, 1995; Barbas, 1995).

### Engineering Variable Regions of Monoclonal Antibodies to Decrease Immunogenicity

Herman Waldmann's group made a very important observation in the mid-1980s, showing that immunization against idiotypic determinants of therapeutic MAbs, that as discussed earlier neutralizes the therapeutic effect, was essentially observed with MAbs recognizing cell-surface determinants but not with MAbs directed against soluble molecules (Benjamin et al., 1986). Building on these data, more recently, the working hypothesis they proposed is that a few mutations in key CDRs transforming a given cell-binding MAb into a nonbinder would generate a "tolerizing" form of the MAb. To directly test the hypothesis, humanized mice expressing the human

CD52 molecule as a transgene at the T-cell surface were first used. Injection in these mice of the human IgG1 version of humanized CD52 MAb ablated mouse T lymphocytes as expected and induced a humoral response to the foreign antibody (mouse anti-CD52 idiotype response). In sharp contrast, mice previously injected with nonbinding mutants of the human IgG1 CD52 MAb (the “tolerogens”) did not mount a humoral response when treated with the conventional therapeutic cell-binding CD52 Mab (Gilliland et al., 1999; Waldmann, 2019). Remarkably, the clinical proof-of-concept was obtained in patients presenting with multiple sclerosis and treated with the IgG1 CD52 antibody, alemtuzumab. A noncell binding MAb mutant, the “tolerogen,” injected prior to alemtuzumab treatment very significantly reduced the humoral response of the patients to both a first and a second course of alemtuzumab (Somerfield et al., 2010). Despite its effectiveness, it is clear that this two-step strategy is complicated to implement on a regular basis. In order to overcome the practical problem of consecutive administration of two different MAbs the authors engineered an antibody molecule they named “Stealth” with a covalently attached blocker antigen mimotope into the binding site leading to a transiently nonbinding MAb at the time of infusion that renders it “tolerogenic” (Waldmann, 2019). Within a few days the mimotope detaches itself from the antibody molecule, which thus “recovers” all its binding capacity and therefore its therapeutic potential (Waldmann, 2019). The Stealth version of the CD52 MAb is T-cell depleting in the humanized CD52 transgenic mouse but not immunogenic (Waldmann, 2019). It would be highly relevant for obvious reasons to further implement this technology on different MAbs specificities.

## THE ADEQUATION OF THE ANTIBODY SPECIFICITY TO THE TARGET DISEASE: FROM IMMUNOSUPPRESSION TO IMMUNE TOLERANCE

It is interesting to step back and observe that the biological products which made their way to approval were launched into a given autoimmune disease and then showed a more or less pronounced therapeutic efficacy in other autoimmune diseases. This is important because these results have strongly influenced our understanding of the pathophysiological heterogeneity within given autoimmune diseases. Indeed, for each pathology the response or not to a given treatment turned out a precious tool to define subgroups of patients, more commonly defined as patient’s endotypes. Defining subgroups of patients leads to the characterization of biological markers that make it possible to identify these different individual before any treatment, to better adapt the therapies and also the possible therapeutic combinations. This ultimately means moving toward a personalized treatment of autoimmunity to improve therapeutic efficacy, thereby reducing side effects.

Another important point that should guide our thinking concerns the type of therapeutic effect obtained. Is it immunosuppression, with the necessity of repeating the treatment at regular intervals and, as a result, ultimately exposing patients to particularly infectious risks? Do some of the available biologics show an effect that is closer to operational tolerance with short term treatment leading to an effect which may be more long-lasting in the absence of chronic immunosuppression? Is this effect of operational tolerance more easily observed in certain subgroups of patients at precise stages of different diseases? This is the kind of thinking that we can finally have nowadays after years of experience with these quite numerous therapeutic tools applied for the regular treatment of autoimmune diseases (Chapter 71, Treatment of Autoimmune Disease: Established Therapies).

To illustrate these points, we shall address three indications where biological therapies have changed patient management while opening up in-depth thinking on how to improve their use on the basis of a better understanding of the pathophysiology of the disease.

### The Breakthrough in Rheumatoid Arthritis

There is no doubt that the story of MAbs to TNF in rheumatoid arthritis consequent on the pioneering experimental and clinical work of Feldmann and Maini (Brennan et al., 1989; Feldmann, 2002) is a straightforward and beautiful one that constituted a real revolution in the field.

The seminal finding was that neutralizing antibodies to TNF significantly decreased the production of most proinflammatory cytokines, that is, interleukin (IL)-1, IL-6, IL-8, GM-CSF, normally produced in vitro cultures of cells that infiltrate synovial membranes in rheumatoid arthritis (Brennan and Feldmann, 1992; Feldmann, 2002). The relevance of this finding to events in vivo was validated in mice that expressed a human TNF transgene and developed a form of chronic arthritis fully preventable by MAbs to TNF (Brennan and Feldmann, 1992; Feldmann, 2002). In addition, in collagen-induced arthritis, neutralizing antibodies to murine

TNF given at onset of disease decreased the severity of objective and histopathological features (swollen joints and bone erosions) (Piguet et al., 1992; Williams et al., 1992, 1994). These data rapidly led to first clinical trials and to the approval of infliximab both in the United States and Europe. Other biologic agents against TNF were developed, in particular two fusion proteins linking the TNF receptor molecules p55 or p75 to a human IgG constant region (lenercept and etanercept/Enbrel) (Furst et al., 2003; Moreland et al., 1996, 1997). However, only etanercept was developed in the clinic and was approved.

It soon became apparent that while the therapeutic effect of TNF blockers was important, it was of limited duration and required regular treatment. Then rapidly the number of new candidates tested and retained has increased with abatacept, rituximab, tocilizumab, and also small molecules blocking the Janus kinase, signaling pathway such as tofacitinib coming into the scene.

Regardless of therapeutics *per se*, practice has evolved to treat patients at early stages of the disease, defined as early rheumatoid arthritis, in order to avoid progression to irreversible lesions, thus obtaining better long-term results. Presently, patients presenting early rheumatoid arthritis are mostly stratified according to their autoantibody patterns and, more recently, on very early joint lesions detected by sensitive imaging methods (de Brito Rocha et al., 2019; Ma et al., 2014; Seegobin et al., 2014). The absence or presence of anticitrullinated peptide antibodies (ACPA) and the seroconversion are important prognostic markers to monitor the effect of treatment. Furthermore, recent studies highlight that the effect of early antirheumatic therapy on ACPA seroconversions varies if responses against cyclic citrullinated peptides and citrullinated peptides derived from vimentin (cVim), fibrinogen, and  $\alpha$ -enolase (CEP-1) are distinguished. The disappearance of particular ACPA reactivities (anti-cVim) in early rheumatoid arthritis is linked to a better radiological outcome (Kastbom et al., 2016). This is the best demonstration that the characterization of robust biomarkers derives very quickly from the use of effective treatments.

It is now well established that anticitrullinated peptide antibodies (ACPA), rheumatoid factors, and some other specificities, for example, antibodies to carbamylated proteins, may be detected many years before onset of joint lesions (Rantapaa-Dahlqvist et al., 2003; Catrina et al., 2017); ACPAs are the first autoantibody to appear, rapidly followed by the occurrence of rheumatoid factors (van de Stadt et al., 2011). The group of Klareskog proposes that the distinct patterns of autoantibody development before disease onset might provide insight into disease pathogenesis, contemplating in particular that pathogenic local immunity toward citrullinated proteins is initiated at other sites than the joints and more particularly in the lungs (Catrina et al., 2017). Therapeutic tools targeting these very early disease-triggering immune events, therefore targeting “prerheumatoid arthritis,” before joint inflammation appears could lead to a real cure of the disease (Catrina et al., 2017).

It is interesting before concluding to go back to the experimental setting to mention an unexpected and interesting observation. In established collagen-induced arthritis, combination of a suboptimal dose of anti-TNF (which had no significant effect *per se*) with a short course with monoclonal anti-T-cell antibodies such as CD4 and CD3 MAbs greatly improved joint inflammation and helped heal paw-swelling and bone erosions in the long term (Williams et al., 1994; Depis et al., 2012). Thus neutralizing inflammation, as done with anti-TNF, effectively “sensitizes” the immune system in rheumatoid arthritis to T cell–directed immunointervention. Given the capacity of CD3 antibodies to restore tolerance, which will be described in more detail below, it is relevant to contemplate that combining agents targeting inflammation and T cells could be a novel and very powerful tool to achieve a long-term therapeutic effect in rheumatoid arthritis, even in established disease, thereby approaching operational tolerance.

## The Breakthrough in Multiple Sclerosis

The introduction of natalizumab (Tisabry), a humanized antibody specific for the  $\alpha 4$ -chain of  $\alpha 4\beta 1$  integrin (VLA-4) expressed by leukocytes, in 2004 was a decisive step in the use of biotherapies as major disease-modifying drugs in multiple sclerosis. Integrins are adhesion molecules of fundamental importance to the recruitment of leukocytes in inflammation. The alpha4beta1 integrin VLA-4 is a leukocyte ligand for endothelial vascular cell adhesion molecule-1 (VCAM-1), fibronectin, and osteopontin. The interaction between VLA-4 at the surface of activated lymphocytes and monocytes with its ligand VCAM-1 is essential for cell migration into inflamed parenchyma. Promising data in experimental models of blockade of VLA-4 prompted use of natalizumab in patients with relapsing–remitting or relapsing secondary progressive multiple sclerosis. A marked reduction in the number of new brain lesions [gadolinium-enhanced magnetic resonance imaging (MRI)] was observed in treated patients (Miller et al., 2003).

Unfortunately, natalizumab is highly immunosuppressive and discontinuation of treatment precipitates relapses. Patients therefore receive repeated treatments to maintain remission. Reports rapidly focused on the risk of opportunistic brain infection caused by the polyoma JC virus (JCV). The use of natalizumab was temporarily stopped. In 2006 a panel of experts assessed 3417 patients with multiple sclerosis, Crohn's disease, or rheumatoid arthritis, who had received the antibody for an average of 17 months, and did not identify additional cases of progressive multifocal leukoencephalopathy (PML). Given the beneficial effect of the antibody on the progression of multiple sclerosis, this reassuring assessment led to the antibody being reintroduced (Brandstädter and Katz Sand, 2017). The benefit/risk ratio of the drug being regarded as positive, natalizumab remains a therapeutic option of interest in multiple sclerosis, subject however to strict patient selection, for example, exclusion of patients with anti-JCV antibodies and limiting the duration of treatment [the risk is higher in patients treated for more than 2 years (Brandstädter and Katz Sand, 2017)].

It is interesting to discuss the case of the other major therapeutic tool for multiple sclerosis, the CD52 antibody Campath-1 (alemtuzumab), which is also immunosuppressive but whose long-term therapeutic efficacy does not require frequent treatment cycles such as natalizumab. We are here on a biological product which could, we will discuss it further, with a favorable combination, bring us closer to a situation of operational tolerance.

Antibodies to CD52 target a small (12 amino acids) glycosylphosphatidylinositol-anchored protein of undefined function expressed at the surface of human B and T cells and monocyte/macrophages. CD52 MAbs are highly depleting and have potent efficacy in long-term acceptance of organ allografts and maintaining remission in established and otherwise intractable autoimmune diseases, notably multiple sclerosis and vasculitis (Calne et al., 1998; Lockwood et al., 1993, 1996; Mathieson et al., 1990). The first rat MAb to CD52, Campath-1H, was characterized in 1983. A fully reshaped humanized version, Campath-1H (human IgG1), was derived by genetic engineering (Riechmann et al., 1988) and is marketed as alemtuzumab (Lemtrada). Its depleting capacity has led to its extensive use in vivo to treat CD52<sup>+</sup> hematologic malignancies and in vitro to purge bone-marrow transplants to prevent graft-versus-host disease.

Initial trials included patients with long-standing relapsing/remitting multiple sclerosis unresponsive to conventional treatments. Long-term follow-up showed a marked decrease in the appearance of new lesions in the central nervous system as assessed by MRI scanning that correlated with the persisting and significant depletion of peripheral CD4<sup>+</sup> T lymphocytes (Coles et al., 1999a,b, 2004). Phase III trials have been completed, and alemtuzumab has been approved for treatment of relapsing/remitting multiple sclerosis (Cohen et al., 2012; Coles et al., 2012).

Treatment with alemtuzumab elicits some side effects including a long-lasting lymphopenia not associated with increased rate of opportunistic infections, a transient cytokine release syndrome after the first injection (Coles et al., 1999a,b), and a neutralizing antiidiotypic immunization is observed in a significant proportion of patients (Somerfield et al., 2010). Another more unexpected side effect was the development of autoimmune disorders, particularly autoimmune thyroiditis (in up to 30% of patients with multiple sclerosis) or more rarely autoimmune cytopenias (Coles et al., 1999a,b; CAMMS223 Trial Investigators et al., 2008; Jones et al., 2009). In one of the trials in multiple sclerosis, several cases (2.8%) of idiopathic thrombocytopenic purpura have been reported (CAMMS223 Trial Investigators et al., 2008; Cuker et al., 2011). The occurrence of these complications is independent of the therapeutic effect of the antibody but appears to be related to treatment-induced lymphopenia. These autoimmune manifestations occur in patients in whom homeostatic cell proliferation following depletion induced by the antibody is more important. This phenomenon is dependent on IL-21, a cytokine for which circulating levels are increased before treatment in patients who will develop the posttreatment autoimmune manifestations. It has been therefore proposed to use IL-21 pretreatment levels may serve as a predictive parameter to identify patients at risk of this type of side effect (Jones et al., 2009).

Campath-1H also proved very effective in severe systemic small vessel vasculitis, in which the pathogenesis depends mainly on T cell-mediated mechanisms. The long-term remissions that were obtained when combining antibodies to CD52 and CD4 were particularly impressive (Lockwood et al., 1993, 1996; Mathieson et al., 1990).

In conclusion, alemtuzumab appears to be a treatment of choice in relapsing/remitting multiple sclerosis and also for severe autoimmune diseases such as vasculitis refractory to other treatment.

It is obvious that the lymphopenia induced by alemtuzumab, and especially the period of reconstitution post-lymphopenia, poses a clinical problem (i.e., thyroid disease). In the transgenic mouse model that expresses human CD52 on the surface of T lymphocytes, it has also been shown that lymphopenia prevents the induction of immune tolerance against organ allografts because reconstitution is accompanied by a significant homeostasis proliferation of T lymphocytes with emergence of pathogenic lymphocytes. This is a situation that can be effectively circumvented, at least in mice, by combining to alemtuzumab a MAb-neutralizing IL-7 that is mandatory to drive homeostatic proliferation (Piotti et al., 2014). This is a therapeutic combination that would be very interesting to test in clinic as soon as an antibody-neutralizing IL-7 becomes available.

## The Breakthrough in Autoimmune Diabetes

The story of CD3 MAbs is paradoxical and remarkable. They were the first therapeutic antibody introduced in clinical practice in 1981, about 4 years before the molecular complexities and the key functional role of the CD3 molecule were discovered (Clevers et al., 1988). OKT3, a mouse IgG2a MAb directed to human CD3 (Kung et al., 1979), was initially used to treat and prevent renal allograft rejection (Cosimi et al., 1981; Debure et al., 1988; Vigeral et al., 1986). This occurred without any in vivo preclinical data available due to the tight species-specificity of anti-T-cell MAbs in general and CD3 antibodies in particular. Chimpanzees are in fact the only nonhuman primates harboring T lymphocytes cross-reacting with MAbs to human CD3. In addition, antibodies to mouse CD3 were difficult to produce, the first one being characterized by Leo et al. (1987). Over the 1980s, controlled studies clearly demonstrated that MAb OKT3 was a potent immunosuppressant very efficient at reversing early acute renal allograft rejection episodes (Cosimi et al., 1981; Ortho, 1985), an indication for which this MAb was rapidly licensed both in the United States and Europe. Through the study of OKT3-treated patients an enormous knowledge was gained on the mode of action of murine anti-T-cell monoclonals and their side effects. These studies have been invaluable for the design of more refined approaches using humanized MAbs. As other immunosuppressants developed, the use of OKT3 was abandoned, essentially because of its cytokine-releasing potential (Chatenoud et al., 1989, 1990; Abramowicz et al., 1989).

CD3 MAbs used in vitro in functional studies and in vivo, both in the experimental and the clinical setting, are specific for the  $\epsilon$  chain of the CD3 complex. The experimental work conducted in different rat and mouse models suggested that more than simply depressing all immune responses, CD3 MAbs could also induce immune tolerance to both alloantigens and autoantigens (Nicolls et al., 1993; Plain et al., 1999; Goto et al., 2013; You et al., 2012). Perhaps more impressively, CD3 MAbs could restore self-tolerance in established autoimmunity (Belghith et al., 2003; Chatenoud et al., 1994, 1997; Chatenoud, 2003). Based on these data, CD3 MAbs have again entered the clinical arena but now as well-tolerated humanized nonmitogenic MAbs (Alegre et al., 1994; Bolt et al., 1993) used not only in transplantation, but also in autoimmunity in protocols aimed at antigen-specific long-term effects rather than just immunosuppression.

### **CD3 Monoclonal Antibodies and Autoimmune Diabetes**

Trials have been conducted using CD3 MAb to treat patients with recent-onset type 1 diabetes based on our data in diabetes-prone nonobese diabetic (NOD) mice that spontaneously develop autoimmune diabetes. Short-term (5 days) treatment of overtly diabetic NOD mice with low doses (5–20  $\mu$ g/day) of CD3 MAbs, in either their mitogenic (whole 145 2C11) or nonmitogenic version [F(ab')2 fragments of 145 2C11], induced disease remission by restoring self-tolerance (Belghith et al., 2003; Chatenoud et al., 1994, 1997; Chatenoud, 2003). The effect was long-lasting and specific to  $\beta$ -cell autoantigens (Belghith et al., 2003; Chatenoud et al., 1994, 1997; Chatenoud, 2003). Immune mechanisms mediating this tolerogenic capacity evolve in two distinct consecutive phases (Chatenoud, 2010; Chatenoud and Bluestone, 2007). The first, the induction phase, coincides with antibody administration and results in clearing of insulitis, explaining the rapid return to normoglycemia, with a transient Th2 polarization which is irrelevant to the long-term effect since there is prolonged remission of disease after anti-CD3 treatment of IL-4 deficient NOD mice (NOD IL-4 $^{-/-}$ ) (Belghith et al., 2003; Chatenoud, 2003). The second maintenance phase results in upregulation and/or appearance of specialized subsets of CD4 $^{+}$ CD25 $^{+}$ FoxP3 $^{+}$  and CD4 $^{+}$ CD62L $^{+}$ FoxP3 $^{+}$  Treg that mediate transferable active tolerance, and that effectively control pathogenic effector cells as shown by cotransfer experiments in immunodeficient NOD SCID mice (Belghith et al., 2003; Chatenoud et al., 1994, 2001). The proportions of CD4 $^{+}$ CD25 $^{+}$ FoxP3 $^{+}$  Treg increase in pancreatic and mesenteric lymph nodes of CD3 MAb-treated tolerant mice (Belghith et al., 2003). Interestingly, CD4 $^{+}$ CD25 $^{+}$ FoxP3 $^{+}$  Treg induced by CD3 MAb may not derive exclusively from conventional natural regulatory CD4 $^{+}$ CD25 $^{-}$ FoxP3 $^{+}$  T cells but also, and perhaps even essentially so, from CD4 $^{+}$ CD25 $^{-}$  precursors that differentiate in the periphery (Belghith et al., 2003; You et al., 2007). In fact, CD3-specific MAb treatment induces diabetes remission also in NOD mice deficient for the costimulation molecule CD28 (NOD CD28 $^{-/-}$ ) that are devoid of thymic CD4 $^{+}$ CD25 $^{+}$ FoxP3 $^{+}$  Treg (Belghith et al., 2003). The immunoregulatory cytokine TGF $\beta$  appears to be a key player in this T cell-mediated regulation. Thus CD4 $^{+}$  T cells from mice tolerant after CD3 MAb treatment consistently produce high levels of TGF $\beta$ , and in vivo neutralization of TGF $\beta$  after injection of specific MAbs fully prevents anti-CD3-specific-induced remission (Belghith et al., 2003; Kuhn et al., 2011).

Clinical trials have been conducted to explore modalities that would reproduce this remarkable effect. Results from an open trial using teplizumab (OKT3 $\gamma$ 1Ala-Ala) in patients that present recent-onset type 1 diabetes were very encouraging (Herold et al., 2002, 2005). Thus at 1 year after a short-term treatment, a significant preservation

of the  $\beta$ -cell mass was observed in treated patients compared with controls (Herold et al., 2002, 2005). The results of a European multicentric randomized placebo-controlled trial using the otelixizumab (ChAgyCD3) MAb, also in recent-onset autoimmune type 1 diabetes fully confirmed the expectations. Results obtained at 18 months of follow-up showed not only a significant preservation of the  $\beta$ -cell mass in otelixizumab versus placebo-treated patients but also an impressive decrease in the insulin needs that lasted for up to 4 years after the end of the short-course treatment (Keymeulen et al., 2005, 2010). Another important study also showing the efficacy and the good safety profile of teplizumab was the Autoimmunity-Blocking Antibody for Tolerance (AbATE) protocol, 2-year results of which were reported in 2013 (Herold et al., 2013). In AbATE the treatment group received a 14-day course of teplizumab at study entry and at 1 year. A post hoc analysis of the data identified metabolic and immunological variables that differentiated individuals who responded to treatment with teplizumab from the nonresponders; 45% of the drug-treated patients were classified as responders, and these individuals had lower HbA1c and lower insulin requirements prior to treatment, suggesting the presence of a functionally larger beta-cell mass at the time of treatment initiation (Herold et al., 2013). Recently, the long-term follow-up (7 years on average, range up to 9 years) of patients included in AbATE was reported (Perdigoto et al., 2018).

Phase III trials were launched by two biotech companies in association with large pharmaceutical companies using designs, which were quite different from that of the previous Phase II studies. The Phase III trial using otelixizumab (Tolerx/GlaxoSmithKline trial) used a reduced dose with the aim to reduce side effects. This dose, which has not been validated for efficacy in a proper Phase II placebo-controlled study, was 16 times lower than the one used in the successful Phase II academic trial (3.1 mg compared to 48 mg).

The Phase III study using teplizumab (Macrogenics/Eli Lilly trial) had a composite end point chosen arbitrarily (i.e., insulin requirement  $\leq 0.5$  U/kg/day and HbA1c  $\leq 6.5\%$ ) that had not been previously validated in a controlled trial and which seemed to be unfortunate choice (Bach, 2011).

Importantly, a post hoc analysis of the data from the teplizumab study was performed using the conventional end points validated by all previous trials in the field, namely, C-peptide production and insulin needs, which evidenced a significant therapeutic effect (Sherry et al., 2011). A better response was observed in patients presenting the highest stimulated C-peptide at inclusion (e.g., less than 6 weeks of insulin therapy since diagnosis) and in children and young adults (8–17 years). The response was dose-dependent, that is, only observed in patients receiving the higher dose tested of 17 mg (cumulated, equivalent for a 70 kg individual) (Sherry et al., 2011; Daifotis et al., 2013). A second Phase III trial using teplizumab is presently ongoing to extend the data of Protégé.

In a parallel effort, TrialNet conducted a prevention trial using teplizumab in young subjects “at risk of developing hyperglycemia” but screened as beta-cell autoantibody positive in families including at least one type 1 diabetic probant (Triolo et al., 2019). The rational here is to intervene earlier in disease progression to recruit patients with a higher number of functioning beta cells. The consensus is in fact that a large fraction of beta cells have been destroyed by the autoimmune process when hyperglycemia is first diagnosed: this is the case for patients enrolled in the Phases I, II, and III trials we described earlier. Estimates suggest that about 30% of the functional beta-cell mass is still present in such patients. Under these circumstances, it may be difficult to objectively evaluate any long-term efficacy due to an already reduced beta-cell mass at the beginning of treatment, hence the interest to perform trial in subjects/patients who have already an ongoing immunological disease but who are not yet hyperglycemic. The TrialNet study recruited 76 patients who were randomized to receive a single course of 14 days of teplizumab (17 mg; cumulated, equivalent for a 70 kg individual) or placebo. The end point will address whether teplizumab, compared to placebo, may significantly delay and/or prevent progression to hyperglycemia. The trial is completed, the data are being analyzed, and results will be available mid-2019.

As a whole, nearly 800 patients (166 young children aged 8–11 years, and 308 adolescents aged 12–17 years) included in the various trials have received teplizumab. This further points to the therapeutic efficacy of the drug and its favorable safety profile of teplizumab, confirmed by the results of the 7-year follow-up of AbATE (Perdigoto et al., 2018).

In conclusion, autoimmune diabetes is confronted to the dramatic increase in incidence in industrialized countries that is in addition affecting an increasingly younger patient population (Bach, 2002, 2018; Patterson et al., 2018). Presently, chronic insulin therapy, the only palliative treatment available, is far from satisfactory. There is yet no immunotherapy on the market. This lack of approved therapies to tackle the cause of the disease, namely, the autoimmune reaction, must be corrected as soon as possible. CD3 MAbs appear as very good candidates to start solving the issue. Major hopes for the future are also based on combination therapies. Potential candidates are numerous, including new biologicals, autoantigens, and also cell therapy (i.e., Treg). It is worth noting that a number of the proposed combination therapies include a CD3 MAb (Hu et al., 2013; Mamchak et al., 2012; You et al., 2013; Besancon et al., 2018).

Time will tell if the promising results obtained in autoimmune diabetes suggesting that CD3 MAb induces an “operational” restoration of immune tolerance can be extended to other autoimmune diseases.

## The Surprises and the Expectations of B Lymphocyte Targeting

Rituximab is a human–mouse chimeric MAb, specific for the CD20 B-cell antigen, which causes rapid depletion of B lymphocytes. Rituximab (MabThera) was approved to treat severe refractory CD20-positive non-Hodgkin’s B-cell lymphoma. The use of CD20 MAb has been extended to first-line therapy and maintenance therapy in lymphoma, for stem-cell transplantation procedures, and also for a variety of autoimmune disorders, including rheumatoid arthritis, immune thrombocytopenic purpura, autoimmune hemolytic anemia, SLE, vasculitis, dermatomyositis, multiple sclerosis, and autoimmune type 1 diabetes (De Vita et al., 2002; Leandro et al., 2002, 2005; Pescovitz et al., 2009; Rastetter et al., 2004; Silverman and Weisman, 2003; Gelfand et al., 2017; Greenfield and Hauser, 2018; Sabatino et al., 2019). Among these there are obvious indications since the pathophysiology of the disease involves pathogenic autoantibodies, and therefore it seems appropriate to destroy the B cells producing these autoantibodies. The surprise was that in some of these indications, such as SLE, the effectiveness of CD20 antibody was rather disappointing compared to the very encouraging results observed in multiple sclerosis a condition wherein pathogenic T cells more than autoantibodies are regarded as the main pathogenic actors. At a fundamental level, these results highlight the pathogenic role of B lymphocytes not only as antibody-producing cells but also as antigen-presenting cells, which hitherto had been considered of marginal importance.

In SLE a first report, further supported by a series of off-label trials, described that B-cell depletion was successfully obtained in patients using rituximab and that disease remission could be achieved (Leandro et al., 2005). In most studies, better therapeutic efficacy correlated with the degree of B-cell depletion achieved and a good clinical response was accompanied by improvement in laboratory parameters. However, two multicenter randomized placebo-controlled trials, one in patients with moderate-to-severe nonrenal disease and the other in proliferative lupus nephritis, did not confirm such benefit (Merrill et al., 2010; Rovin et al., 2012). Numerous reasons may explain this discrepancy that have been cogently reviewed by Furtado and Isenberg (2013). These include differences in the clinical profile of patients, their ethnicity, the cumulative dose of rituximab administered, the duration of follow-up, the degree of B-cell depletion achieved, and, last but not the least, the choice of end points such as criteria for response and flares as well as their assessment (Furtado and Isenberg, 2013). Selecting end point criteria is in fact a major problem since there is a variety of scores to grade disease severity, and a very good clinical experience is mandatory to apply them wisely (Furtado and Isenberg, 2013). A consensus still exists for not abandoning the track of B cell in SLE. It is considered vital however that clinical trials make the most of the controversial experience with rituximab.

The quest to find agents that will better and more efficaciously target B cells in SLE continues with agents targeting B-cell growth factors and with new-generation CD20 MAbs. One example, atacicept is a recombinant molecule (formerly referred to as transmembrane activator and CAML interactor (TACI)-Ig) coupling a human Fc fragment and soluble TACI that is the receptor for the cytokines BlyS/BAFF (B-lymphocyte stimulator/B-cell activating factor) and APRIL (a proliferation-inducing ligand). Results showing evidence of efficacy were recently reported in a Phase IIb study together with good safety (Merrill et al., 2018). New-generation CD20 MAbs include humanized antibodies, for example, ocrelizumab, veltuzumab, and obinutuzumab, and one fully human MAb ofatumumab. These CD20 antibodies have increased binding affinity to the Fc receptor on B cells and increased complement-dependent cytotoxicity and/or antibody-dependent cellular cytotoxicity ability. The next 4–5 years will be decisive for concluding.

In multiple sclerosis a Phase II trial showed that a single course of rituximab reduced inflammatory brain lesions and clinical relapses (Hauser et al., 2008). Ocrelizumab has recently been granted US Food and Drug Administration (FDA) approval for both relapsing/remitting and primary/progressive multiple sclerosis. Further studies are in progress to better define the utility of ocrelizumab over the other biological therapies available (Gelfand et al., 2017; Greenfield and Hauser, 2018; Sabatino et al., 2019). From a more fundamental point of view, these clinical data underscore the importance of advancing rapidly on the issue of the role of B cells in multiple sclerosis.

## BONE-MARROW TRANSPLANTATION

Autoimmune diseases include genetic components expressed in lymphoid and macrophage lineages qualify as stem-cell disorders. This explains that, in particular, before the availability of biological disease-modifying drugs

that patients with serious autoimmune diseases have been considered for high-dose immunosuppression followed by hemopoietic stem-cell transplantation (HSCT) (Ikehara et al., 1990; Ikehara, 1998; Tyndall and Gratwohl, 1997). In the clinic the strategy stemmed from observations in patients with malignancies and concurrent autoimmune diseases (McAllister et al., 1997). Experimental results also showed that whether allogeneic or autologous HSCT induced a high rate of disease remission provided adequate conditioning regimens were administered (van Bekkum, 1998; Karussis et al., 1992, 1993; Ikehara et al., 1985).

The First International Symposium on Haemopoietic Stem Cell Therapy in autoimmune diseases took place in 1996 (Tyndall and Gratwohl, 1997) and inaugurated collaborations Autoimmune Diseases Working Party (ADWP) of the European Society for Blood and Marrow Transplantation (EBMT); guidelines were proposed that participating centers reported their results in a dedicated registry. The EBMT ADWP database now includes over 2500 patients treated during the past 20 years (Alexander et al., 2018). Among 2606 HSCT procedures reported, 2417 patients have undergone autologous HSCT and 133 patients allogeneic HSCT. Main indications for autologous HSCT were multiple sclerosis, scleroderma, Crohn's disease, and SLE. A similar registry was created in North America (Center for International Blood and Marrow Transplant Research).

The source of the stem-cell transplant is mostly peripheral cells mobilized using cyclophosphamide in combination with hematopoietic growth factors, granulocyte-colony stimulating factor (G-CSF) alone, or combined with granulocyte macrophage-colony stimulating factor (GM-CSF). According to different studies, the grafts are either purged of mature T cells, which may contain autoreactive effectors, by selection of CD34<sup>+</sup> cells with or without additional MAb-dependent T-cell depletion, or are not manipulated. Pretransplant conditioning regimens, for which the aim is to ablate as completely as possible the diseased (autoreactive) component of the immune system, include total body irradiation, chemotherapy-based regimens such as BEAM (Carmustine (BCNU), etoposide, cytosine arabinoside, melphalan), cyclophosphamide with or without antilymphocyte globulins and with or without other drugs, or total body irradiation and busulfan (Daikeler et al., 2011).

Results from both pilot and large trials show benefit in patients with systemic sclerosis in favor of HSCT over cyclophosphamide (Alexander et al., 2015, 2018).

The first studies performed in patients with severe multiple sclerosis with significant functional disability allowed assessment of risk/safety ratio but not of efficacy. Subsequently, with the implementation of the procedure in patients with less severe disease, significant clinical benefit was observed in some groups of patients, including those with aggressive recurrent/relapsing forms of the disease. A recent long-term retrospective study that included unselectively any transplants performed between 1996 and 2005 ( $n = 281$ ) in both relapsing-remitting and progressive multiple sclerosis patients reported long-standing remissions (Muraro et al., 2017a,b). These data were confirmed by a meta-analysis (Sormani et al., 2017). These data and those from the retrospective analysis of the EBMT registry indicate that autologous bone-marrow transplantation is an option only in patients with severe and active multiple sclerosis unresponsive to other treatments (Daikeler et al., 2011; Mancardi et al., 2018). Trials comparing HSCT with the more recently introduced high-efficacy disease-modifying treatments such as alemtuzumab are being planned, although their development is hampered by difficulties in accessing financial support (Muraro et al., 2017a,b).

Patients with severe SLE received an autologous stem-cell transplantation. Two major studies reported a disease-free survival rate at 5 years of 50%. The transplant not only induced an improvement in serological markers of disease activity but also a prolonged remission (at least 5 years) of lung damage and of the associated anti-phospholipid syndrome. These data are fully confirmed by those of the EBMT registry (Alexander et al., 2018). Overall, when SLE is achieved, remissions last long term also in absence of chronic immunosuppression. However, these results must be considered side by side to the risk of mortality that ranges 4%–12%. One controlled trial is currently in progress in Germany, comparing HSCT with best available biologicals, including rituximab (NCT00750971) (Alexander et al., 2018).

Before the introduction of biological therapies (MAbs or fusion proteins blocking TNF), rheumatoid arthritis was one of the first indications for autologous stem-cell transplantation. The procedure was well tolerated and induced good clinical responses (Van Laar and Tyndall, 2003). Nowadays, given the progress with TNF blockers and other new biological therapies, autologous stem-cell transplants are rare (Alexander et al., 2018).

To be complete, one should quote the results of a trial in autoimmune insulin-dependent diabetes that included 23 patients aged 13–31 years (Couri et al., 2009; Voltarelli et al., 2007, 2008). Autologous hematopoietic stem cells mobilized with cyclophosphamide and G-CSF were injected intravenously after conditioning with cyclophosphamide and rabbit antithymocyte globulin. During the follow-up, 20/23 were weaned from insulin treatment; 12 patients were insulin independent for 14–52 months. Eventually all patients relapsed and return to insulin dependency. Concerning side effects, two patients developed bilateral nosocomial pneumonia, three developed late

endocrine dysfunction, and nine developed oligospermia (Couri et al., 2009; Voltarelli et al., 2007, 2008). Thus although this therapy afforded disease remission with insulin independency for 1–4 years, the conditioning regimen required was heavy, comparable to that used in life-threatening autoimmune diseases. Considering the risk/benefit ratio, it is difficult to recommend such a strategy for wide application in type 1 diabetes even limited to adolescent and adults. For obvious reasons, it is inappropriate for use in children.

In conclusion, despite the fact that this strategy showed spectacular results that approach operational tolerance in patients with severe autoimmune diseases, the important and often vital side effects explain that over recent years, it represented less and less an alternative. In addition, the possibilities now accessible that we will discuss below to generate, using genetic engineering, tools of cellular therapy “à la carte” further question the future use of bone-marrow transplantation in autoimmunity.

## SOLUBLE AUTOANTIGENS

This strategy has given and still gives rise to many studies and, at first glance, it may seem a paradox. In order to understand the rationale here, we have to go back to the early 1960s when challenging the prevailing dogma that clonal deletion during lymphocyte development was the main, if not the exclusive, mechanism explaining immune tolerance, Dresser described the first results showing that in the adult host, the immune system can be “inactivated” rather than “activated” upon challenge with an antigen, depending on the modalities of administration of the antigen in question (Dresser, 1962). The concept of tolerance was therefore extended to also encompass states of unresponsiveness termed “immune paralysis,” observed instead of the usual immunization, when an antigen was delivered according to particular protocols. The classical example is that of tolerance to human gammaglobulins (IgG) observed in adult mice upon intravenous injection of deaggregated IgGs. Not only the mice thus treated do not produce antibodies against human IgG but, remarkably, they become refractory to any immunization with this antigen, even when it is inoculated in the presence of complete Freund’s adjuvant, a condition that is otherwise very immunogenic leading to massive production of specific antibodies. These seminal experiments were confirmed by many other authors using different antigens. All reiterated in the various models that antigen-specific B cells in tolerant animals were still present but functionally inactive. Regarding the respective role of B and T lymphocytes in this tolerance, the data showed that both compartments were concerned: the tolerance was more rapidly induced and persisted longer in T cells than B cells (Chiller et al., 2013).

It may be important to recall here that reprogramming the immune system toward immune tolerance using antigen-specific therapy has been successfully applied for several decades now in allergy where “desensitization” is common practice.

Things turned out more complex in autoimmunity. Thus immunological tolerance to a wide spectrum of autoantigens can be induced upon administration of the autoantigen by different routes, for example, parenteral, nasal, or oral. The approach has proven very successful in several animal models of spontaneous or experimentally induced autoimmunity. Thus the onset of diabetes in NOD mice can be prevented by administration of insulin or glutamic acid decarboxylase (GAD) using various routes of administration s.c., i.v., nasal, or oral (Tisch et al., 1993, 1994; Charlton et al., 1994; Kaufman et al., 1993; Tian et al., 1996). Similarly, experimental autoimmune encephalomyelitis (EAE) can be effectively prevented by administration of soluble myelin antigens (Smilek et al., 1991, 1992; Weiner et al., 1995; Wraith, 1995; Wraith et al., 1989; Miller et al., 1992). Mechanistic studies confirmed the working hypotheses that soluble autoantigen treatment had a direct effect on pathogenic lymphocytes and on antigen-presenting cells involved in the autoreactive reaction. Depending on the experimental model, the route of delivery, the dose administered or the antigen-presenting cells involved a functional inhibition of autoreactive T lymphocytes was observed. This occurred notably through triggering of anergy and/or Th1/Th2 immune deviation (Tisch et al., 2001; Tian et al., 1997), and elimination of antigen specific autoreactive T cells (especially when high doses of autoantigen are used) (Bercovici et al., 2000) and stimulation of various subsets of specialized Treg either FoxP3+ or FoxP3- (this is particularly the case in oral tolerance protocols where TGFβ-dependent and producing FoxP3- Treg), formerly termed Th3, play a major role (Weiner et al., 1991, 1995). Much attention was focused on autoantigen-presenting cells as their functional capacities greatly vary depending on the anatomical sites and they are therefore important immune actors explaining the differences in effect depending on the route of administration of autoantigens (mucosal, subcutaneous, intradermal, intravenous, intraperitoneal).

Based on these data translation to the clinic was attempted using different autoantigens under various forms, for example, proteins, peptides or altered peptide ligands and was rapidly confronted to major problems which were predictable from the experimental data collected. These included limitation of the therapeutic window to

early disease stages with loss of effectiveness as disease progresses; a long lag time to achieve efficacy, which may represent a problem in the case of acute autoimmune responses; risk of disease acceleration by triggering rather than down-regulating the autoimmune response; the potential risks of sensitization (e.g., anaphylaxis and/or production of neutralizing antibodies leading to serious problems when the autoantigen molecule, for example, insulin is physiologically relevant).

It is undoubtedly in insulin-dependent diabetes and multiple sclerosis that the largest amount of clinical data has been accumulated to date.

In insulin-dependent diabetes Phases I, II and even Phase III protocols that included large number of patients were conducted with great methodological rigor. The trials included both patients presenting with overt hyperglycemia in whom, as previously discussed, the insulin-secreting beta cell is largely reduced and in "at risk individuals" (Chaillous et al., 2000; Ludvigsson et al., 2012; Nanto-Salonen et al., 2008; Pozzilli et al., 2000; Skyler, 2002; Walter et al., 2009; Krischer et al., 2017; Wherrett et al., 2011). These are subjects screened as beta-cell autoantibody positive in families including at least one type 1 diabetic patient; when two or more beta-cell autoantibodies are detected these high risk individuals have about a 70% risk to develop hyperglycemia within 5–7 years (Triolo et al., 2019). The problem is that results were disappointing. None of the autoantigens used in autoimmune diabetes gave, when reaching the stage of Phase III trials, any positive therapeutic effect in terms of decreasing disease progression even in at risk individuals whom, as detailed earlier, present at an earlier disease stage. No side effects were observed in these trials.

Better defining the stage of disease were soluble antigen therapy in autoimmune diabetes may lead to effectiveness is very carefully considered. Subjects at risk already presenting with beta-cell autoantibodies may be at a too late stage as active beta-cell destruction is already ongoing, autoantibodies being the markers of this destruction. Hence the aim of the Pre-POINT study conducted by the groups of Ziegler and Bonifacio, a double-blind, placebo-controlled, dose-escalation, Phase I/II international clinical pilot study enrolling 25 islet autoantibody-negative children aged 2–7 years with a family history of type 1 diabetes and susceptible human leukocyte antigen class II genotypes (Bonifacio et al., 2015). The children received a daily oral administration of 67.5 mg of insulin or placebo. The treatment was safe (no hypoglycemia), an immune response to insulin was observed (Bonifacio et al., 2015). These data pave the ground for a Phase III trial to determine whether oral insulin can prevent islet autoimmunity and diabetes in such children.

In multiple sclerosis early clinical use of an altered peptide ligand of myelin basic protein was hampered by serious side effects including an aggravation/relapse of the disease and hypersensitivity reactions which forced stopping the trials (Bielekova et al., 2000; Pedotti et al., 2001; Smith et al., 2005). A myelin basic protein peptide that exhibited a good safety profile did not meet the efficacy end points in a Phase III trial enrolling patients with secondary progressive disease (Freedman et al., 2011).

What conclusions should we draw? Should the strategy be abandoned? Should we somehow throw the baby with the bath water? The answer is of course no; the experimental data cannot be ignored and pursuing efforts to approach effective autoantigen-specific immunotherapeutic approaches are fully warranted.

The problem has probably been the one of an overwhelming enthusiasm for a strategy that was expected to deliver immune tolerance without exposing to the side effects of long-term immunosuppression. Such enthusiasm led to a too rapid clinical translation that ignored major elements of the equation, not the least being the chemical formulation of the autoantigen administered and also the question of selecting subgroups of patients who would be more sensitive to the effect of treatment. Finally, one should not discard the possibility of combining antigen-specific therapy with other immune-interventions which may potentiate efficacy (Manchak et al., 2012).

Concerning the chemical formulation of autoantigen, the results of Wraith's group are very interesting. Step by step, based on robust experimental data in humanized mice these authors have shown that complications linked to unwanted immune reactions are not seen when CD4+ T-cell epitopes (e.g., synthetic peptides) that mimic naturally processed T-cell epitopes, for example, apitope (antigen processing independent epitope) are used. These are peptides that bind directly to major histocompatibility complex class II on immature dendritic cells which prime tolerogenic T-cell circuits. In vivo these soluble peptides inhibit both Th1 and Th2 immune responses and enhance secretion of the immunoregulatory cytokine IL-10.

The authors identified a cocktail of 4 peptides (ATX-MS-1467), behaving as apitopes, that suppress EAE in a humanized mouse model (Streeter et al., 2015). Furthermore, a Phase I trial of antigen-specific immunotherapy with ATX-MS-1467 has shown that treatment with this apitope cocktail is safe and so far the data on the clinical efficacy in Phase II studies are promising (Streeter et al., 2015; Chataway et al., 2018; Wraith et al., 2015). Apitopes are in development for treatment of Grave's disease; presently experimental preclinical data are encouraging (Jansson et al., 2018).

Another completely different way to tackle the problem of autoantigen-specific therapies is to design strategies that through particular targeting of specific epitopes may trigger sustained expansion or reprogramming of pre-existing autoreactive regulatory immune cells. Autoantigen delivery using nanoparticles appears as a promising path toward this aim as demonstrated in experimental models (Neef and Miller, 2017; Pearson et al., 2018; Clemente-Casares et al., 2016; Newbigging et al., 2016; Serra and Santamaria, 2018; Verdaguer et al., 1997).

Many laboratories are rapidly making substantial progress in both the design of tools to apply at tolerogenic nanoparticles to mouse models of autoimmunity. The reader is referred to the work of the groups of Miller and Santamaria that are both contributing very extensively to this area (Neef and Miller, 2017; Pearson et al., 2018; Clemente-Casares et al., 2016; Newbigging et al., 2016; Serra and Santamaria, 2018; Verdaguer et al., 1997). Major efforts are also devoted to mechanistic studies to unravel the cellular and molecular key steps involved in the therapeutic effect. Autoantigen-specific therapies have many more things to reveal to us and still represent conceptually a fabulous approach, whether used alone or in combination, to approach immune tolerance in clinic.

## CELL THERAPY AND ANTIGEN RECEPTOR GENE-MODIFIED T CELLS

### Cell Therapy Using Regulatory T Cells

After a long “crossing of the desert” during the 1990s, the existence of T lymphocytes that were called suppressors and which we now call regulatory T lymphocytes is no longer questioned. It is the discovery in 2003, simultaneously by three independent groups, that the FoxP3 transcription factor is a lineage marker of the CD4 + CD25 + T cells in the thymus and that it has a fundamental role in the expression of the regulatory function also in the periphery which has unlocked the situation (Hori et al., 2003; Fontenot et al., 2003; Khattri et al., 2003). Tregs underpin a fundamental mechanism of peripheral tolerance. Suffice is to observe the major, often life-threatening, polyautoimmune syndrome IPEX (immunodysregulation, polyendocrinopathy, enteropathy, X-linked) that is caused by loss of function mutations of FoxP3 (Wildin et al., 2001).

The list of autoimmune diseases where the number or the functional capacity of Treg was shown to be abnormally reduced is long; this has sometimes been ascribed to a deficit in key Treg growth factors, in particular IL-2, or an excess of proinflammatory cytokine in the environment. Hence, the rationale for the attempts to use cell therapy with ex vivo expanded Tregs. Particular interest was focused in autoimmune diabetes. Experimental data in the NOD mouse showed the efficacy of expanded Treg, and in particular autoantigen-specific Treg, to treat established disease (Bluestone and Tang, 2004; Tang et al., 2004). Circulating Tregs were grown in culture in the presence of CD3 and CD28 antibodies and IL-2. Phase I studies have shown the safety of the inoculum and suggested of course preliminary clinical activity (Bluestone et al., 2015; Marek-Trzonkowska et al., 2013). The implementation of Phase II trials was, therefore, warranted, and the results from such a study are expected in 2019 (NCT02691247).

Even if it concerns a single case it is interesting to quote here a recent report of adoptive polyclonal Treg treatment of a patient presenting SLE with active skin disease (Dall'Era et al., 2018). Deuterium tracking of infused Tregs revealed the migration of highly activated Tregs into diseased skin expressing a shift from Th1 to Th17 responses (Dall'Era et al., 2018).

### Cell Therapy Using Antigen Receptor Gene–Modified T Cells

In recent years the field of cell therapy has entered a new era, given on the one hand, the advances in our understanding of the molecular mechanisms that underlie the function of different immune cell subpopulations (effector T cells and Tregs) and, secondly, the incredible amount of technical resources that cell bioengineering has developed. The culture *in vitro* of specialized subsets of immune cells, and of antigen receptor gene–modified T cells, that can be reinfused into patients with an autoimmune disease is becoming a reality.

It all started in the field of oncology when it became possible to express at the surface of effector T cells with killing abilities an antigen receptor [T-cell receptor (TCR)] or chimeric antigen-specific receptors (CARs) specific for the tumor antigen. These CAR T cells expressed at their surface a fusion protein including an extracellular antibody domain specific to the antigen of interest (in this case the CD19 B-cell antigen) linked by a transmembrane domain to an intracellular-signaling portion that activates the T cell upon antigen binding. Initial trials were performed in 2013, using CAR T cells targeting the CD19 receptor and treating patients with B-cell acute lymphoblastic leukemia and then non-Hodgkin's lymphoma and chronic

lymphocytic leukemia (Brentjens and Curran, 2012; Brentjens et al., 2013; Gill and June, 2015). Based on the impressive results obtained in 2017, the FDA approved this adoptive cell-based gene therapy tisagenlecleucel (Kymriah) for acute lymphoblastic leukemia and in 2018 for non-Hodgkin's lymphoma. This breakthrough also explains the great efforts made to extend this fascinating technology to solid tumors, infectious diseases, organ transplantation, bone-marrow transplantation, and autoimmunity (Maldini et al., 2018; Bluestone and Tang, 2018).

In autoimmunity, one possibility would be to take advantage of CAR T cells to kill autoimmune pathogenic effector lymphocytes as recently proposed in a very elegant paper by Ellebrecht et al. (2016). These authors focused on pemphigus vulgaris a dermatological autoimmune disease where autoantibodies directed to desmoglein 3 (Dsg3) are the pathogenic effectors in the disease. Dsg3 is expressed on keratinocytes in the basal lower levels of the epidermis. Anti-Dsg3 autoantibodies provoke the loss of keratinocyte cell–cell adhesion in the basal and immediate suprabasal layers of the deeper epidermis, leaving the superficial epidermis intact, thereby causing the blisters that are the hallmark of the disease. Ellebrecht et al. engineered T cells to express a chimeric autoantibody receptor, CAAR T cells, selectively destroy B lymphocytes producing anti-Dsg3 autoantibodies both in vitro and in vivo in humanized mouse models. As compared to the conventional CAR T cells discussed earlier in the case of hematological malignancies, CAAR T cells produced by Ellebrecht et al. expressed an extracellular domain that included Dsg3, the autoantigen which upon binding to the specific autoantibodies on the autoreactive B-cell surface triggered the signaling machinery of the T cell through the intracellular domains of the CAAR that in this case included molecules CD3 $\zeta$  and CD137 (Ellebrecht et al., 2016). One of the humanized models used was set up with NSG (NOD-SCID-gamma) immunodeficient mice engrafted with human skin xenografts; injection of Dsg3 CAAR T cells in these recipients and no direct toxicity to keratinocytes was observed, supporting the lack of “off-target” effect of the approach and confirming its safety. The case of pemphigus vulgaris was ideal proof-of-concept for the CAAR T-cell strategy as the autoantigen structure has been very well dissected together with the specific binding sites of the pathogenic autoantibodies which, in addition, are known to be oligoclonal (Hammers et al., 2015). As discussed in a commentary I wrote on this interesting and provocative piece of work, it would be very important to identify other autoimmune diseases whose treatment could be enriched by this new tool that are CAAR T cells. The obvious first candidates are autoimmune diseases where autoantibodies, with well-identified autoantigen epitopes, are pathogenic. This is certainly the case for myasthenia gravis. Another potential target is a disease affecting the fetus in autoimmune pregnant mothers presenting with SLE or Sjögren syndrome known as the autoantibody-associated neonatal lupus syndrome (e.g., congenital heart block) (Ambrosi et al., 2014; Skog et al., 2016). Here maternal autoantibodies to the Ro/SSA autoantigen, including the unrelated Ro52 and Ro60 proteins that cross the placenta are the pathogenic effectors (Ambrosi et al., 2014; Skog et al., 2016). Concerning the other autoimmune diseases in which autoantibodies have a clear pathogenic effect, it will be mandatory to attempt applying the CAAR T-cell technology to have better definition and molecular characterization of key autoantigen epitopes.

The CAAR T-cell technology may also be exploited to target autoreactive B cells which rather than producing pathogenic autoantibodies act as autoantigen-presenting cells. The prototypic situation is for instance that of insulin-dependent diabetes a disease where the pathogenic effectors are exclusively autoreactive CD4 $^{+}$  and CD8 $^{+}$  T lymphocytes that selectively destroy insulin-secreting beta cells within pancreatic islets of Langerhans. The autoantigens that are the targets of the autoreactive T cells are well characterized (e.g., insulin/proinsulin, GAD, the IA-2 phosphatase). Autoantibodies against these antigens are present, from early stages of the disease, and they are used to screen patients with genetic susceptibility who present early stages of beta-cell destruction and who are at risk to develop hyperglycemia within a relatively short time frame (Triolo et al., 2019). It is very well established that autoantibodies are not pathogenic but compelling experimental and clinical evidence has been accumulated to show that B cells play a key role in the disease as autoantigen-presenting cells. Thus CD20 antibodies, which eliminate B cells, have a transient yet clear therapeutic effect in NOD mice as well as in patients with recent-onset type 1 diabetes. Applying CAAR T-cell treatment at early disease stages when only few anti-beta-cell autoantibody specificities are detected one could expect to target B cells before they become fully committed at efficacious autoantigen presentation and at mediating autoantigen spreading and progression to massive beta-cell destruction.

Last but not least TCR or CAR gene transfer could be implemented to improve Treg therapy by generating antigen-specific Tregs which as discussed earlier have proved more effective in experimental models. Of course, a major obstacle will be that of defining very precisely the epitopes that are recognized by autoantigen-specific Treg. The task is not simple, but interesting data are emerging in autoimmune diabetes (Yeh et al., 2017).

## PERSPECTIVES AND CONCLUSIONS

As a conclusion, it is hoped if not anticipated that emergent therapies based on the progress of biotechnology, including gene and cell therapy, will progressively complement and in some cases even replace conventional treatments. The rapidity with which TNF blockers have become accessible to patients with rheumatoid arthritis and Crohn's disease is most encouraging. The multitude of these drugs and their potential clinical applications are remarkable. Nevertheless, there are still numerous problems concerning the development and evaluation of the various drugs being studied.

Major efforts should be made to identify the best applications and promote their development for the benefit of patients beyond commercial constraints.

Increasing efficacy without side effects is the major long-term goal. Well-designed combination therapy appears as a sensible approach. The list of candidates is longer every day going from conventional chemical immunosuppressants to other biological and in particular cases cell therapy (Treg).

Each disease has its own therapeutic profile, but manifestations differ between subgroups of patients as their response to treatment also do. An in-depth rejuvenation of the field of immunotherapy will come from our ability to characterize robust and reliable biomarkers to identify key subgroups of patients within a given disease entity and propose the most appropriate single or combination therapy. This is the essence of personalized medicine to offer to every patient the possibility of remission, as sustainable as possible, with the fewest side effects. The fact that our therapeutic arsenal was enriched with products such as CD3 antibodies that may achieve long-term remissions in the absence of chronic treatment is a major asset to go step by step and in well-identified subgroups of patients, toward clinical operational tolerance.

## References

- Abramowicz, D., Crusiaux, A., Goldman, M., 1992. Anaphylactic shock after retreatment with OKT3 monoclonal antibody. *N. Engl. J. Med.* 327 (10), 736.
- Abramowicz, D., Crusiaux, A., Niaudet, P., Kreis, H., Chatenoud, L., Goldman, M., 1996. The IgE humoral response in OKT3-treated patients—incidence and fine specificity. *Transplantation* 61 (4), 577–581.
- Abramowicz, D., Schandene, L., Goldman, M., Crusiaux, A., Vereerstraeten, P., De Pauw, L., et al., 1989. Release of tumor necrosis factor, interleukin-2, and gamma-interferon in serum after injection of OKT3 monoclonal antibody in kidney transplant recipients. *Transplantation* 47 (4), 606–608.
- Alegre, M.L., Peterson, L.J., Xu, D., Sattar, H.A., Jeyarajah, D.R., Kowalkowski, K., et al., 1994. A non-activating “humanized” anti-CD3 monoclonal antibody retains immunosuppressive properties in vivo. *Transplantation* 57 (11), 1537–1543.
- Alexander, T., Bondanza, A., Muraro, P.A., Greco, R., Saccardi, R., Daikeler, T., et al., 2015. SCT for severe autoimmune diseases: consensus guidelines of the European Society for Blood and Marrow Transplantation for immune monitoring and biobanking. *Bone Marrow Transplant* 50 (2), 173–180.
- Alexander, T., Farge, D., Badoglio, M., Lindsay, J.O., Muraro, P.A., Snowden, J.A., 2018. Hematopoietic stem cell therapy for autoimmune diseases—clinical experience and mechanisms. *J. Autoimmun.* 92, 35–46.
- Ambrosi, A., Sonesson, S.E., Wahren-Herlenius, M., 2014. Molecular mechanisms of congenital heart block. *Acta Obstet. Gynecol. Scand.* 93 (1), 2–9.
- Bach, J.F., 2002. The effect of infections on susceptibility to autoimmune and allergic diseases. *N. Engl. J. Med.* 347 (12), 911–920.
- Bach, J.F., 2003. Regulatory T cells under scrutiny. *Nat. Rev. Immunol.* 3 (3), 189–198.
- Bach, J.F., 2011. Anti-CD3 antibodies for type 1 diabetes: beyond expectations. *Lancet* 378 (9790), 459–460.
- Bach, J.F., 2018. The hygiene hypothesis in autoimmunity: the role of pathogens and commensals. *Nat. Rev. Immunol.* 18 (2), 105–120.
- Barbas III, C.F., 1995. Synthetic human antibodies. *Nat. Med.* 1 (8), 837–839.
- Barbas III, C.F., Hu, D., Dunlop, N., Sawyer, L., Cababa, D., Hendry, R.M., et al., 1994. In vitro evolution of a neutralizing human antibody to human immunodeficiency virus type 1 to enhance affinity and broaden strain cross-reactivity. *Proc. Natl. Acad. Sci. U.S.A.* 91 (9), 3809–3813.
- Baudrihaye, M.F., Chatenoud, L., Kreis, H., Goldstein, G., Bach, J.F., 1984. Unusually restricted anti-isotype human immune response to OKT3 monoclonal antibody. *Eur. J. Immunol.* 14 (8), 686–691.
- Belghith, M., Bluestone, J.A., Barriot, S., Megret, J., Bach, J.F., Chatenoud, L., 2003. TGF-beta-dependent mechanisms mediate restoration of self-tolerance induced by antibodies to CD3 in overt autoimmune diabetes. *Nat. Med.* 9 (9), 1202–1208.
- Benjamin, R.J., Cobbold, S.P., Clark, M.R., Waldmann, H., 1986. Tolerance to rat monoclonal antibodies. Implications for serotherapy. *J. Exp. Med.* 163 (6), 1539–1552.
- Bercovici, N., Heurtier, A., Vizler, C., Pardigon, N., Cambouris, C., Desreumaux, P., et al., 2000. Systemic administration of agonist peptide blocks the progression of spontaneous CD8-mediated autoimmune diabetes in transgenic mice without bystander damage. *J. Immunol.* 165 (1), 202–210.
- Besancon, A., Goncalves, T., Valette, F., Dahllof, M.S., Mandrup-Poulsen, T., Chatenoud, L., et al., 2018. Oral histone deacetylase inhibitor synergises with T cell targeted immunotherapy to preserve beta cell metabolic function and induce stable remission of new-onset autoimmune diabetes in NOD mice. *Diabetologia* 61 (2), 389–398.

- Bielekova, B., Goodwin, B., Richert, N., Cortese, I., Kondo, T., Afshar, G., et al., 2000. Encephalitogenic potential of the myelin basic protein peptide (amino acids 83–99) in multiple sclerosis: results of a phase II clinical trial with an altered peptide ligand. *Nat. Med.* 6 (10), 1167–1175.
- Bluestone, J.A., Buckner, J.H., Fitch, M., Gitelman, S.E., Gupta, S., Hellerstein, M.K., et al., 2015. Type 1 diabetes immunotherapy using polyclonal regulatory T cells. *Sci. Transl. Med.* 7 (315), 315ra189.
- Bluestone, J.A., Tang, Q., 2004. Therapeutic vaccination using CD4 + CD25 + antigen-specific regulatory T cells. *Proc. Natl. Acad. Sci. U.S.A.* 101 (Suppl. 2), 14622–14626.
- Bluestone, J.A., Tang, Q., 2018. Treg cells—the next frontier of cell therapy. *Science* 362 (6411), 154–155.
- Bolt, S., Routledge, E., Lloyd, I., Chatenoud, L., Pope, H., Gorman, S.D., et al., 1993. The generation of a humanized, non-mitogenic CD3 monoclonal antibody which retains in vitro immunosuppressive properties. *Eur. J. Immunol.* 23 (2), 403–411.
- Bonifacio, E., Ziegler, A.G., Klingensmith, G., Schober, E., Bingley, P.J., Rottenkolber, M., et al., 2015. Effects of high-dose oral insulin on immune responses in children at high risk for type 1 diabetes: the Pre-POINT randomized clinical trial. *JAMA* 313 (15), 1541–1549.
- Brandstädter, R., Katz Sand, I., 2017. The use of natalizumab for multiple sclerosis. *Neuropsychiatr. Dis. Treat.* 13, 1691–1702.
- Brennan, F.M., Chantry, D., Jackson, A., Maini, R., Feldmann, M., 1989. Inhibitory effect of TNF alpha antibodies on synovial cell interleukin-1 production in rheumatoid arthritis. *Lancet* 2 (8657), 244–247.
- Brennan, F.M., Feldmann, M., 1992. Cytokines in autoimmunity. *Curr. Opin. Immunol.* 4 (6), 754–759.
- Brentjens, R.J., Curran, K.J., 2012. Novel cellular therapies for leukemia: CAR-modified T cells targeted to the CD19 antigen. *Hematology (Am. Soc. Hematol. Educ. Program)* 2012, 143–151.
- Brentjens, R.J., Davila, M.L., Riviere, I., Park, J., Wang, X., Cowell, L.G., et al., 2013. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. *Sci. Transl. Med.* 5 (177), 177ra38.
- Calne, R., Friend, P., Moffatt, S., Bradley, A., Hale, G., Firth, J., et al., 1998. Prope tolerance, perioperative campath 1H, and low-dose cyclosporin monotherapy in renal allograft recipients [letter] [published erratum appears in Lancet 1998 Aug 1;352(9125):408] *Lancet* 351 (9117), 1701–1702.
- CAMMS223 Trial Investigators, Coles, A.J., Compston, D.A., Selmaj, K.W., Lake, S.L., Moran, S., et al., 2008. Alemtuzumab vs. interferon beta-1a in early multiple sclerosis. *N. Engl. J. Med.* 359 (17), 1786–1801.
- Catriona, A.I., Svensson, C.I., Malmstrom, V., Schett, G., Klareskog, L., 2017. Mechanisms leading from systemic autoimmunity to joint-specific disease in rheumatoid arthritis. *Nat. Rev. Rheumatol.* 13 (2), 79–86.
- Chaillous, L., Lefevre, H., Thivolet, C., Boitard, C., Lahoulou, N., Atlan-Gepner, C., et al., 2000. Oral insulin administration and residual beta-cell function in recent-onset type 1 diabetes: a multicentre randomised controlled trial. *Diabète Insuline Orale group. Lancet* 356 (9229), 545–549.
- Charlton, B., Taylor Edwards, C., Tisch, R., Fathman, C.G., 1994. Prevention of diabetes and insulitis by neonatal intrathymic islet administration in NOD mice. *J. Autoimmun.* 7 (5), 549–560.
- Chataway, J., Martin, K., Barrell, K., Sharrack, B., Stolt, P., Wraith, D.C., 2018. Effects of ATX-MS-1467 immunotherapy over 16 weeks in relapsing multiple sclerosis. *Neurology* 90 (11), e955–e962.
- Chatenoud, L., 2003. CD3-specific antibody-induced active tolerance: from bench to bedside. *Nat. Rev. Immunol.* 3 (2), 123–132.
- Chatenoud, L., 2010. Immune therapy for type 1 diabetes mellitus—what is unique about anti-CD3 antibodies? *Nat. Rev. Endocrinol.* 6 (3), 149–157.
- Chatenoud, L., Baudrihaye, M.F., Chkoff, N., Kreis, H., Goldstein, G., Bach, J.F., 1986a. Restriction of the human in vivo immune response against the mouse monoclonal antibody OKT3. *J. Immunol.* 137 (3), 830–838.
- Chatenoud, L., Bluestone, J.A., 2007. CD3-specific antibodies: a portal to the treatment of autoimmunity. *Nat. Rev. Immunol.* 7, 622–632.
- Chatenoud, L., Ferran, C., Bach, J.F., 1989. In-vivo anti-CD3 treatment of autoimmune patients. *Lancet* 2 (8655), 164.
- Chatenoud, L., Ferran, C., Legendre, C., Thouard, I., Merite, S., Reuter, A., et al., 1990. In vivo cell activation following OKT3 administration. Systemic cytokine release and modulation by corticosteroids. *Transplantation* 49 (4), 697–702.
- Chatenoud, L., Jonker, M., Villemain, F., Goldstein, G., Bach, J.F., 1986b. The human immune response to the OKT3 monoclonal antibody is oligoclonal. *Science* 232 (4756), 1406–1408.
- Chatenoud, L., Primo, J., Bach, J.F., 1997. CD3 antibody-induced dominant self tolerance in overtly diabetic NOD mice. *J. Immunol.* 158 (6), 2947–2954.
- Chatenoud, L., Salomon, B., Bluestone, J.A., 2001. Suppressor T cells—they're back and critical for regulation of autoimmunity!. *Immunol. Rev.* 182, 149–163.
- Chatenoud, L., Thervet, E., Primo, J., Bach, J.F., 1994. Anti-CD3 antibody induces long-term remission of overt autoimmunity in nonobese diabetic mice. *Proc. Natl. Acad. Sci. U.S.A.* 91 (1), 123–127.
- Chiller, J.M., Habicht, G.S., Weigle, W.O., 2013. Pillars article: kinetic differences in unresponsiveness of thymus and bone marrow cells. *Science*. 1971. 171: 813–815. *J. Immunol.* 191 (3), 989–991.
- Clemente-Casares, X., Blanco, J., Ambalavanan, P., Yamanouchi, J., Singha, S., Fandos, C., et al., 2016. Expanding antigen-specific regulatory networks to treat autoimmunity. *Nature* 530 (7591), 434–440.
- Clevers, H., Alarcon, B., Wileman, T., Terhorst, C., 1988. The T cell receptor/CD3 complex: a dynamic protein ensemble. *Annu. Rev. Immunol.* 6 (1), 629–662.
- Cobbold, S.P., Adams, E., Graca, L., Daley, S., Yates, S., Paterson, A., et al., 2006. Immune privilege induced by regulatory T cells in transplantation tolerance. *Immunol. Rev.* 213, 239–255.
- Cohen, J.A., Coles, A.J., Arnold, D.L., Confavreux, C., Fox, E.J., Hartung, H.P., et al., 2012. Alemtuzumab versus interferon beta 1a as first-line treatment for patients with relapsing-remitting multiple sclerosis: a randomised controlled phase 3 trial. *Lancet* 380 (9856), 1819–1828.
- Coles, A., Deans, J., Compston, A., 2004. Campath-1H treatment of multiple sclerosis: lessons from the bedside for the bench. *Clin. Neurol. Neurosurg.* 106 (3), 270–274.
- Coles, A.J., Twyman, C.L., Arnold, D.L., Cohen, J.A., Confavreux, C., Fox, E.J., et al., 2012. Alemtuzumab for patients with relapsing multiple sclerosis after disease-modifying therapy: a randomised controlled phase 3 trial. *Lancet* 380 (9856), 1829–1839.

- Coles, A.J., Wing, M.G., Molyneux, P., Paolillo, A., Davie, C.M., Hale, G., et al., 1999a. Monoclonal antibody treatment exposes three mechanisms underlying the clinical course of multiple sclerosis. *Ann. Neurol.* 46 (3), 296–304.
- Coles, A.J., Wing, M.G., Smith, S., Corradu, F., Greer, S., Taylor, C., et al., 1999b. Pulsed monoclonal antibody treatment and autoimmune thyroid disease in multiple sclerosis. *Lancet* 354, 1691–1695.
- Cosimi, A.B., Colvin, R.B., Burton, R.C., Rubin, R.H., Goldstein, G., Kung, P.C., et al., 1981. Use of monoclonal antibodies to T-cell subsets for immunologic monitoring and treatment in recipients of renal allografts. *N. Engl. J. Med.* 305 (6), 308–314.
- Couri, C.E., Oliveira, M.C., Stracieri, A.B., Moraes, D.A., Pieroni, F., Barros, G.M., et al., 2009. C-peptide levels and insulin independence following autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. *JAMA* 301 (15), 1573–1579.
- Cuker, A., Coles, A.J., Sullivan, H., Fox, E., Goldberg, M., Oyuela, P., et al., 2011. A distinctive form of immune thrombocytopenia in a phase 2 study of alemtuzumab for the treatment of relapsing-remitting multiple sclerosis. *Blood* 118 (24), 6299–6305.
- Daifotis, A.G., Koenig, S., Chatenoud, L., Herold, K.C., 2013. Anti-CD3 clinical trials in type 1 diabetes mellitus. *Clin. Immunol.* 149 (3), 268–278.
- Daikele, T., Labopin, M., Di Gioia, M., Abinun, M., Alexander, T., Miniati, I., et al., 2011. Secondary autoimmune diseases occurring after HSCT for an autoimmune disease: a retrospective study of the EBMT Autoimmune Disease Working Party. *Blood* 118 (6), 1693–1698.
- Dall'Era, M., Pauli, M.L., Remedios, K., Taravati, K., Sandova, P.M., Putnam, A.L., et al., 2018. Adoptive Treg cell therapy in a patient with systemic lupus erythematosus. *Arthritis Rheumatol.* 71 (3), 431–440.
- de Brito Rocha, S., Baldo, D.C., Andrade, L.E.C., 2019. Clinical and pathophysiologic relevance of autoantibodies in rheumatoid arthritis. *Adv. Rheumatol.* 59 (1), 2.
- De Vita, S., Zaja, F., Sacco, S., De Candia, A., Fanin, R., Ferraccioli, G., 2002. Efficacy of selective B cell blockade in the treatment of rheumatoid arthritis: evidence for a pathogenetic role of B cells. *Arthritis Rheum.* 46 (8), 2029–2033.
- Debure, A., Chkoff, N., Chatenoud, L., Lacombe, M., Campos, H., Noel, L.H., et al., 1988. One-month prophylactic use of OKT3 in cadaver kidney transplant recipients. *Transplantation* 45 (3), 546–553.
- Depis, F., Hatterer, E., Lamacchia, C., Waldburger, J.M., Gabay, C., Reith, W., et al., 2012. Long-term amelioration of established collagen-induced arthritis achieved with short-term therapy combining anti-CD3 and anti-tumor necrosis factor treatments. *Arthritis Rheum.* 64 (10), 3189–3198.
- Dresser, D.W., 1962. Specific inhibition of antibody production. II. Paralysis induced in adult mice by small quantities of protein antigen. *Immunology* 5, 378–388.
- Ellebrecht, C.T., Bhoj, V.G., Nace, A., Choi, E.J., Mao, X., Cho, M.J., et al., 2016. Reengineering chimeric antigen receptor T cells for targeted therapy of autoimmune disease. *Science* 353 (6295), 179–184.
- Elliott, M.J., Maini, R.N., Feldmann, M., Kalden, J.R., Antoni, C., Smolen, J.S., et al., 1994. Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. *Lancet* 344 (8930), 1105–1110.
- Feldmann, M., 2002. Development of anti-TNF therapy for rheumatoid arthritis. *Nat. Rev. Immunol.* 2 (5), 364–371.
- Fontenot, J.D., Gavin, M.A., Rudensky, A.Y., 2003. Foxp3 programs the development and function of CD4+ CD25+ regulatory T cells. *Nat. Immunol.* 4 (4), 330–336.
- Foote, J., Eisen, H.N., 1995. Kinetic and affinity limits on antibodies produced during immune responses. *Proc. Natl. Acad. Sci. U.S.A.* 92 (5), 1254–1256.
- Freedman, M.S., Bar-Or, A., Oger, J., Traboulsee, A., Patry, D., Young, C., et al., 2011. A phase III study evaluating the efficacy and safety of MBP8298 in secondary progressive MS. *Neurology* 77 (16), 1551–1560.
- Friend, P.J., Hale, G., Chatenoud, L., Rebello, P., Bradley, J., Thiru, S., et al., 1999. Phase I study of an engineered aglycosylated humanized CD3 antibody in renal transplant rejection. *Transplantation* 68, 1632–1637.
- Furst, D.E., Weisman, M., Paulus, H.E., Bulpitt, K., Weinblatt, M., Polisson, R., et al., 2003. Intravenous human recombinant tumor necrosis factor receptor p55-Fc IgG1 fusion protein, Ro 45-2081 (lenercept): results of a dose-finding study in rheumatoid arthritis. *J. Rheumatol.* 30 (10), 2123–2126.
- Furtado, J., Isenberg, D.A., 2013. B cell elimination in systemic lupus erythematosus. *Clin. Immunol.* 146 (2), 90–103.
- Gelfand, J.M., Cree, B.A.C., Hauser, S.L., 2017. Ocrelizumab and other CD20(+) B-cell-depleting therapies in multiple sclerosis. *Neurotherapeutics* 14 (4), 835–841.
- Gill, S., June, C.H., 2015. Going viral: chimeric antigen receptor T-cell therapy for hematological malignancies. *Immunol. Rev.* 263 (1), 68–89.
- Gilliland, L.K., Walsh, L.A., Frewin, M.R., Wise, M.P., Tone, M., Hale, G., et al., 1999. Elimination of the immunogenicity of therapeutic antibodies. *J. Immunol.* 162 (6), 3663–3671.
- Goto, R., You, S., Zaitsu, M., Chatenoud, L., Wood, K.J., 2013. Delayed anti-CD3 therapy results in depletion of alloreactive T cells and the dominance of Foxp3(+) CD4(+) graft infiltrating cells. *Am. J. Transplant.* 13 (7), 1655–1664.
- Greenfield, A.L., Hauser, S.L., 2018. B-cell therapy for multiple sclerosis: entering an era. *Ann. Neurol.* 83 (1), 13–26.
- Hammers, C.M., Chen, J., Lin, C., Kacir, S., Siegel, D.L., Payne, A.S., et al., 2015. Persistence of anti-desmoglein 3 IgG(+) B-cell clones in pemphigus patients over years. *J. Invest. Dermatol.* 135 (3), 742–749.
- Hauser, S.L., Waubant, E., Arnold, D.L., Vollmer, T., Antel, J., Fox, R.J., et al., 2008. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. *N. Engl. J. Med.* 358 (7), 676–688.
- Hering, B.J., Kandaswamy, R., Harmon, J.V., Ansrite, J.D., Clemmings, S.M., Sakai, T., et al., 2004. Transplantation of cultured islets from two-layer preserved pancreases in type 1 diabetes with anti-CD3 antibody. *Am. J. Transplant.* 4 (3), 390–401.
- Herold, K.C., Gitelman, S.E., Masharani, U., Hagopian, W., Bisikirska, B., Donaldson, D., et al., 2005. A single course of anti-CD3 monoclonal antibody hOKT3gamma1(Ala-Ala) results in improvement in C-peptide responses and clinical parameters for at least 2 years after onset of type 1 diabetes. *Diabetes* 54 (6), 1763–1769.
- Herold, K.C., Gitelman, S.E., Ehlers, M.R., Gottlieb, P.A., Greenbaum, C.J., Hagopian, W., et al., 2013. Teplizumab (anti-CD3 mAb) treatment preserves C-peptide responses in patients with new-onset type 1 diabetes in a randomized controlled trial: metabolic and immunologic features at baseline identify a subgroup of responders. *Diabetes* 62 (11), 3766–3774.

- Herold, K.C., Hagopian, W., Auger, J.A., Poumian Ruiz, E., Taylor, L., Donaldson, D., et al., 2002. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N. Engl. J. Med.* 346 (22), 1692–1698.
- Hori, S., Nomura, T., Sakaguchi, S., 2003. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 299 (5609), 1057–1061.
- Hricik, D.E., Mayes, J.T., Schulak, J.A., 1990. Inhibition of anti-OKT3 antibody generation by cyclosporine—results of a prospective randomized trial. *Transplantation* 50 (2), 237–240.
- Hu, C., Ding, H., Zhang, X., Wong, F.S., Wen, L., 2013. Combination treatment with anti-CD20 and oral anti-CD3 prevents and reverses autoimmune diabetes. *Diabetes* 62 (8), 2849–2858.
- Ikehara, S., 1998. Bone marrow transplantation for autoimmune diseases. *Acta Haematol.* 99 (3), 116–132.
- Ikehara, S., Kawamura, M., Takao, F., Inaba, M., Yasumizu, R., Than, S., et al., 1990. Organ-specific and systemic autoimmune diseases originate from defects in hematopoietic stem cells. *Proc. Natl. Acad. Sci. U.S.A.* 87 (21), 8341–8344.
- Ikehara, S., Ohtsuki, H., Good, R.A., Asamoto, H., Nakamura, T., Sekita, K., et al., 1985. Prevention of type I diabetes in nonobese diabetic mice by allogenic bone marrow transplantation. *Proc. Natl. Acad. Sci. U.S.A.* 82 (22), 7743–7747.
- Jansson, L., Vrolix, K., Jahraus, A., Martin, K.F., Wraith, D.C., 2018. Immunotherapy with apitopes blocks the immune response to TSH receptor in HLA-DR transgenic mice. *Endocrinology* 159 (9), 3446–3457.
- Jones, J.L., Phuah, C.L., Cox, A.L., Thompson, S.A., Ban, M., Shawcross, J., et al., 2009. IL-21 drives secondary autoimmunity in patients with multiple sclerosis, following therapeutic lymphocyte depletion with alemtuzumab (Campath-1H). *J. Clin. Invest.* 119 (7), 2052–2061.
- Karussis, D.M., Slavin, S., Lehmann, D., Mizrahi-koll, R., Abramsky, O., Ben-nun, A., 1992. Prevention of experimental autoimmune encephalomyelitis and induction of tolerance with acute immunosuppression followed by syngeneic bone marrow transplantation. *J. Immunol.* 148 (6), 1693–1698.
- Karussis, D.M., Vourka-karussis, U., Lehmann, D., Ovadia, H., Mizrahi-koll, R., Ben-nun, A., et al., 1993. Prevention and reversal of adoptively transferred, chronic relapsing experimental autoimmune encephalomyelitis with a single high dose cytoreductive treatment followed by syngeneic bone marrow transplantation. *J. Clin. Invest.* 92 (2), 765–772.
- Kastbom, A., Forslind, K., Ernestam, S., Geborek, P., Karlsson, J.A., Petersson, I.F., et al., 2016. Changes in the anticitrullinated peptide antibody response in relation to therapeutic outcome in early rheumatoid arthritis: results from the SWEFOT trial. *Ann. Rheum. Dis.* 75 (2), 356–361.
- Kaufman, D.L., Clare-Salzler, M., Tian, J., Forsthuber, T., Ting, G.S., Robinson, P., et al., 1993. Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. *Nature* 366 (6450), 69–72.
- Keymeulen, B., Vandemeulebroucke, E., Ziegler, A.G., Mathieu, C., Kaufman, L., Hale, G., et al., 2005. Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. *N. Engl. J. Med.* 352 (25), 2598–2608.
- Keymeulen, B., Walter, M., Mathieu, C., Kaufman, L., Gorus, F., Hilbrands, R., et al., 2010. Four-year metabolic outcome of a randomised controlled CD3-antibody trial in recent-onset type 1 diabetic patients depends on their age and baseline residual beta cell mass. *Diabetologia* 53 (4), 614–623.
- Khatri, R., Cox, T., Yasayko, S.A., Ramsdell, F., 2003. An essential role for Scurfin in CD4 + CD25 + T regulatory cells. *Nat. Immunol.* 4 (4), 337–342.
- Kohler, G., Milstein, C., 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 256 (5517), 495–497.
- Krischer, J.P., Schatz, D.A., Bundy, B., Skyler, J.S., Greenbaum, C.J., 2017. Effect of oral insulin on prevention of diabetes in relatives of patients with type 1 diabetes: a randomized clinical trial. *JAMA* 318 (19), 1891–1902.
- Kuhn, C., You, S., Valette, F., Hale, G., van Endert, P., Bach, J.F., et al., 2011. Human CD3 transgenic mice: preclinical testing of antibodies promoting immune tolerance. *Sci. Transl. Med.* 3 (68), 68ra10.
- Kung, P., Goldstein, G., Reinherz, E.L., Schlossman, S.F., 1979. Monoclonal antibodies defining distinctive human T cell surface antigens. *Science* 206 (4416), 347–349.
- Leandro, M.J., Cambridge, G., Edwards, J.C., Ehrenstein, M.R., Isenberg, D.A., 2005. B-cell depletion in the treatment of patients with systemic lupus erythematosus: a longitudinal analysis of 24 patients. *Rheumatology (Oxford)* 44 (12), 1542–1545.
- Leandro, M.J., Edwards, J.C., Cambridge, G., 2002. Clinical outcome in 22 patients with rheumatoid arthritis treated with B lymphocyte depletion. *Ann. Rheum. Dis.* 61 (10), 883–888.
- Leo, O., Foo, M., Sachs, D.H., Samelson, L.E., Bluestone, J.A., 1987. Identification of a monoclonal antibody specific for a murine T3 polypeptide. *Proc. Natl. Acad. Sci. U.S.A.* 84 (5), 1374–1378.
- Lockwood, C.M., Thiru, S., Isaacs, J.D., Hale, G., Waldmann, H., 1993. Long-term remission of intractable systemic vasculitis with monoclonal antibody therapy. *Lancet* 341 (8861), 1620–1622.
- Lockwood, C.M., Thiru, S., Stewart, S., Hale, G., Isaacs, J., Wright, P., et al., 1996. Treatment of refractory Wegener's granulomatosis with humanized monoclonal antibodies. *QJM* 89 (12), 903–912.
- Lonberg, N., Taylor, L.D., Harding, F.A., Trounstein, M., Higgins, K.M., Schramm, S.R., et al., 1994. Antigen-specific human antibodies from mice comprising four distinct genetic modifications. *Nature* 368 (6474), 856–859.
- Lubin, I., Segall, H., Marcus, H., David, M., Kulova, L., Steinitz, M., et al., 1994. Engraftment of human peripheral blood lymphocytes in normal strains of mice. *Blood* 83 (8), 2368–2381.
- Ludvigsson, J., Krisky, D., Casas, R., Battelino, T., Castano, L., Greening, J., et al., 2012. GAD65 antigen therapy in recently diagnosed type 1 diabetes mellitus. *N. Engl. J. Med.* 366 (5), 433–442.
- Ma, M.H., Scott, I.C., Dahanayake, C., Cope, A.P., Scott, D.L., 2014. Clinical and serological predictors of remission in rheumatoid arthritis are dependent on treatment regimen. *J. Rheumatol.* 41 (7), 1298–1303.
- Maldini, C.R., Ellis, G.I., Riley, J.L., 2018. CAR T cells for infection, autoimmunity and allotransplantation. *Nat. Rev. Immunol.* 18 (10), 605–616.
- Mamchak, A.A., Manenkova, Y., Leconet, W., Zheng, Y., Chan, J.R., Stokes, C.L., et al., 2012. Preexisting autoantibodies predict efficacy of oral insulin to cure autoimmune diabetes in combination with anti-CD3. *Diabetes* 61 (6), 1490–1499.

- Mancardi, G., Sormani, M.P., Muraro, P.A., Boffa, G., Saccardi, R., 2018. Intense immunosuppression followed by autologous haematopoietic stem cell transplantation as a therapeutic strategy in aggressive forms of multiple sclerosis. *Mult. Scler.* 24 (3), 245–255.
- Marek-Trzonkowska, N., Mysliwec, M., Siebert, J., Trzonkowski, P., 2013. Clinical application of regulatory T cells in type 1 diabetes. *Pediatr. Diabetes* 14 (5), 322–332.
- Marks, J.D., Hoogenboom, H.R., Bonnert, T.P., McCafferty, J., Griffiths, A.D., Winter, G., 1991. By-passing immunization. Human antibodies from V-gene libraries displayed on phage. *J. Mol. Biol.* 222 (3), 581–597.
- Mathieson, P.W., Cobbold, S.P., Hale, G., Clark, M.R., Oliveira, D.B., Lockwood, C.M., et al., 1990. Monoclonal antibody therapy in systemic vasculitis. *N. Engl. J. Med.* 323 (4), 250–254.
- McAllister, L.D., Beatty, P.G., Rose, J., 1997. Allogeneic bone marrow transplant for chronic myelogenous leukemia in a patient with multiple sclerosis. *Bone Marrow Transplant* 19 (4), 395–397.
- Merrill, J.T., Neuwelt, C.M., Wallace, D.J., Shanahan, J.C., Latinis, K.M., Oates, J.C., et al., 2010. Efficacy and safety of rituximab in moderately-to-severely active systemic lupus erythematosus: the randomized, double-blind, phase II/III systemic lupus erythematosus evaluation of rituximab trial. *Arthritis Rheum.* 62 (1), 222–233.
- Merrill, J.T., Wallace, D.J., Wax, S., Kao, A., Fraser, P.A., Chang, P., et al., 2018. Efficacy and safety of atacicept in patients with systemic lupus erythematosus: results of a twenty-four-week, multicenter, randomized, double-blind, placebo-controlled, parallel-arm, phase IIb study. *Arthritis Rheumatol.* 70 (2), 266–276.
- Miller, D.H., Khan, O.A., Sheremata, W.A., Blumhardt, L.D., Rice, G.P., Libonati, M.A., et al., 2003. A controlled trial of natalizumab for relapsing multiple sclerosis. *N. Engl. J. Med.* 348 (1), 15–23.
- Miller, S.D., Tan, L.J., Pope, L., McRae, B.L., Karpus, W.J., 1992. Antigen-specific tolerance as a therapy for experimental autoimmune encephalomyelitis. *Int. Rev. Immunol.* 9 (3), 203–222.
- Moreland, L.W., Baumgartner, S.W., Schiff, M.H., Tindall, E.A., Fleischmann, R.M., Weaver, A.L., et al., 1997. Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein. *N. Engl. J. Med.* 337 (3), 141–147.
- Moreland, L.W., Margolies, G., Heck Jr., L.W., Saway, A., Blosch, C., Hanna, R., et al., 1996. Recombinant soluble tumor necrosis factor receptor (p80) fusion protein: toxicity and dose finding trial in refractory rheumatoid arthritis. *J. Rheumatol.* 23 (11), 1849–1855.
- Morrison, S.L., Johnson, M.J., Herzenberg, L.A., Oi, V.T., 1984. Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains. *Proc. Natl. Acad. Sci. U.S.A.* 81 (21), 6851–6855.
- Muraro, P.A., Martin, R., Mancardi, G.L., Nicholas, R., Sormani, M.P., Saccardi, R., 2017a. Autologous haematopoietic stem cell transplantation for treatment of multiple sclerosis. *Nat. Rev. Neurol.* 13 (7), 391–405.
- Muraro, P.A., Pasquini, M., Atkins, H.L., Bowen, J.D., Farge, D., Fassas, A., et al., 2017b. Long-term outcomes after autologous hematopoietic stem cell transplantation for multiple sclerosis. *JAMA Neurol.* 74 (4), 459–469.
- Nanto-Salonen, K., Kupila, A., Simell, S., Siljander, H., Salonsaari, T., Hekkala, A., et al., 2008. Nasal insulin to prevent type 1 diabetes in children with HLA genotypes and autoantibodies conferring increased risk of disease: a double-blind, randomised controlled trial. *Lancet* 372 (9651), 1746–1755.
- Nashan, B., Moore, R., Amlot, P., Schmidt, A.G., Abeywickrama, K., Soulillou, J.P., 1997. Randomised trial of basiliximab versus placebo for control of acute cellular rejection in renal allograft recipients [published erratum appears in Lancet 1997 Nov 15;350(9089):1484]. *Lancet* 350 (9086), 1193–1198.
- Neef, T., Miller, S.D., 2017. Tolerogenic nanoparticles to treat islet autoimmunity. *Curr. Diab. Rep.* 17 (10), 84.
- Newbigging, S., Serra, P., Khadra, A., Chan, W.C.W., Santamaria, P., Clemente-Casares, X., et al., 2016. Expanding antigen-specific regulatory networks to treat autoimmunity. *Nat. Nanotechnol.* 530 (7591), 434–440.
- Nicolls, M.R., Aversa, G.G., Pearce, N.W., Spinelli, A., Berger, M.F., Gurley, K.E., et al., 1993. Induction of long-term specific tolerance to allo-grafts in rats by therapy with an anti-CD3-like monoclonal antibody. *Transplantation* 55 (3), 459–468.
- Ortho, X., 1985. A randomized clinical trial of OKT3 monoclonal antibody for acute rejection of cadaveric renal transplants. Ortho Multicenter Transplant Study Group. *N. Engl. J. Med.* 313 (6), 337–342.
- Patterson, C.C., Harjutsalo, V., Rosenbauer, J., Neu, A., Cinek, O., Skrivarhaug, T., et al., 2018. Trends and cyclical variation in the incidence of childhood type 1 diabetes in 26 European centres in the 25 year period 1989–2013: a multicentre prospective registration study. *Diabetologia* 62 (3), 408–417.
- Pearson, R.M., Podjil, J.R., Shea, L.D., King, N.J., Miller, S.D., Getts, D.R., 2018. Overcoming challenges in treating autoimmunity: development of tolerogenic immune-modifying nanoparticles. *Nanomedicine* 18, S1549–9634.
- Pedotti, R., Mitchell, D., Wedemeyer, J., Karpuk, M., Chabas, D., Hattab, E.M., et al., 2001. An unexpected version of horror autotoxicus: anaphylactic shock to a self-peptide. *Nat. Immunol.* 2 (3), 216–222.
- Perdigoto, A.L., Preston-Hurlburt, P., Clark, P., Long, S.A., Linsley, P.S., Harris, K.M., et al., 2018. Treatment of type 1 diabetes with teplizumab: clinical and immunological follow-up after 7 years from diagnosis. *Diabetologia* 62 (4), 655–664.
- Pescovitz, M.D., Greenbaum, C.J., Krause-Steinrauf, H., Becker, D.J., Gitelman, S.E., Goland, R., et al., 2009. Rituximab, B-lymphocyte depletion, and preservation of beta-cell function. *N. Engl. J. Med.* 361 (22), 2143–2152.
- Piguet, P.F., Grau, G.E., Vesin, C., Loetscher, H., Gentz, R., Lesslauer, W., 1992. Evolution of collagen arthritis in mice is arrested by treatment with anti-tumour necrosis factor (TNF) antibody or a recombinant soluble TNF receptor. *Immunology* 77 (4), 510–514.
- Piotti, G., Ma, J., Adams, E., Cobbold, S., Waldmann, H., 2014. Guiding postablative lymphocyte reconstitution as a route toward transplantation tolerance. *Am. J. Transplant.* 14 (7), 1678–1689.
- Plain, K.M., Chen, J., Merten, S., He, X.Y., Hall, B.M., 1999. Induction of specific tolerance to allografts in rats by therapy with non-mitogenic, non-depleting anti-CD3 monoclonal antibody: association with TH2 cytokines not anergy. *Transplantation* 67 (4), 605–613.
- Pozzilli, P., Pitocco, D., Visalli, N., Cavallo, M.G., Buzzetti, R., Crino, A., et al., 2000. No effect of oral insulin on residual beta-cell function in recent-onset type I diabetes (the IMDIAB VII). IMDIAB Group. *Diabetologia* 43 (8), 1000–1004.
- Rantapaa-Dahlqvist, S., de Jong, B.A., Berglin, E., Hallmans, G., Wadell, G., Stenlund, H., et al., 2003. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum.* 48 (10), 2741–2749.

- Rastetter, W., Molina, A., White, C.A., 2004. Rituximab: expanding role in therapy for lymphomas and autoimmune diseases. *Annu. Rev. Med.* 55, 477–503.
- Riechmann, L., Clark, M., Waldmann, H., Winter, G., 1988. Reshaping human antibodies for therapy. *Nature* 332 (6162), 323–327.
- Rovin, B.H., Furie, R., Latinis, K., Looney, R.J., Fervenza, F.C., Sanchez-Guerrero, J., et al., 2012. Efficacy and safety of rituximab in patients with active proliferative lupus nephritis: the Lupus Nephritis Assessment with Rituximab study. *Arthritis Rheum.* 64 (4), 1215–1226.
- Sabatino Jr., J.J., Zamvil, S.S., Hauser, S.L., 2019. B-cell therapies in multiple sclerosis. *Cold Spring Harb. Perspect. Med.* 9 (2).
- Sakaguchi, S., Yamaguchi, T., Nomura, T., Ono, M., 2008. Regulatory T cells and immune tolerance. *Cell* 133 (5), 775–787.
- Seegobin, S.D., Ma, M.H., Dahanayake, C., Cope, A.P., Scott, D.L., Lewis, C.M., et al., 2014. ACPA-positive and ACPA-negative rheumatoid arthritis differ in their requirements for combination DMARDs and corticosteroids: secondary analysis of a randomized controlled trial. *Arthritis Res. Ther.* 16 (1), R13.
- Serra, P., Santamaria, P., 2018. Nanoparticle-based approaches to immune tolerance for the treatment of autoimmune diseases. *Eur. J. Immunol.* 48 (5), 751–756.
- Sherry, N., Hagopian, W., Ludvigsson, J., Jain, S.M., Wahlen, J., Ferry Jr., R.J., et al., 2011. Teplizumab for treatment of type 1 diabetes (Protege study): 1-year results from a randomised, placebo-controlled trial. *Lancet* 378 (9790), 487–497.
- Silverman, G.J., Weisman, S., 2003. Rituximab therapy and autoimmune disorders: prospects for anti-B cell therapy. *Arthritis Rheum.* 48 (6), 1484–1492.
- Skog, A., Lagnefeldt, L., Conner, P., Wahren-Herlenius, M., Sonesson, S.E., 2016. Outcome in 212 anti-Ro/SSA-positive pregnancies and population-based incidence of congenital heart block. *Acta Obstet. Gynecol. Scand.* 95 (1), 98–105.
- Skyler, J., 2002. Effects of insulin in relatives of patients with type 1 diabetes mellitus Diabetes Prevention Trial-Type 1 Diabetes Study Group. *N. Engl. J. Med.* 346 (22), 1685–1691.
- Smilek, D.E., Gautam, A.M., Pearson, C., Steinman, L., McDevitt, H.O., 1992. EAE: a model for immune intervention with synthetic peptides. *Int. Rev. Immunol.* 9 (3), 223–230.
- Smilek, D.E., Wraith, D.C., Hodgkinson, S., Dwivedy, S., Steinman, L., McDevitt, H.O., 1991. A single amino acid change in a myelin basic protein peptide confers the capacity to prevent rather than induce experimental autoimmune encephalomyelitis. *Proc. Natl. Acad. Sci. U.S.A.* 88 (21), 9633–9637.
- Smith, C.E., Eagar, T.N., Strominger, J.L., Miller, S.D., 2005. Differential induction of IgE-mediated anaphylaxis after soluble vs. cell-bound tolerogenic peptide therapy of autoimmune encephalomyelitis. *Proc. Natl. Acad. Sci. U.S.A.* 102 (27), 9595–9600.
- Somerfield, J., Hill-Cawthorne, G.A., Lin, A., Zandi, M.S., McCarthy, C., Jones, J.L., et al., 2010. A novel strategy to reduce the immunogenicity of biological therapies. *J. Immunol.* 185 (1), 763–768.
- Sormani, M.P., Muraro, P.A., Schiavetti, I., Signori, A., Laroni, A., Saccardi, R., et al., 2017. Autologous hematopoietic stem cell transplantation in multiple sclerosis: a meta-analysis. *Neurology* 88 (22), 2115–2122.
- Streeter, H.B., Rigden, R., Martin, K.F., Scolding, N.J., Wraith, D.C., 2015. Preclinical development and first-in-human study of ATX-MS-1467 for immunotherapy of MS. *Neurology*. *Neuroimmunol. Neuroinflamm.* 2 (3), e93.
- Tang, Q., Henriksen, K.J., Bi, M., Finger, E.B., Szot, G., Ye, J., et al., 2004. In vitro-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. *J. Exp. Med.* 199 (11), 1455–1465.
- Tian, J., Atkinson, M.A., Clare Salzler, M., Herschenfeld, A., Forsthuber, T., Lehmann, P.V., et al., 1996. Nasal administration of glutamate decarboxylase (GAD65) peptides induces Th2 responses and prevents murine insulin-dependent diabetes. *J. Exp. Med.* 183 (4), 1561–1567.
- Tian, J., Lehmann, P.V., Kaufman, D.L., 1997. Determinant spreading of T helper cell 2 (Th2) responses to pancreatic islet autoantigens. *J. Exp. Med.* 186 (12), 2039–2043.
- Tisch, R., Wang, B., Atkinson, M.A., Serreze, D.V., Friedline, R., 2001. A glutamic acid decarboxylase 65-specific Th2 cell clone immunoregulates autoimmune diabetes in nonobese diabetic mice. *J. Immunol.* 166 (11), 6925–6936.
- Tisch, R., Yang, X.D., Liblau, R.S., McDevitt, H.O., 1994. Administering glutamic acid decarboxylase to NOD mice prevents diabetes. *J. Autoimmun.* 7 (6), 845–850.
- Tisch, R., Yang, X.D., Singer, S.M., Liblau, R.S., Fugger, L., McDevitt, H.O., 1993. Immune response to glutamic acid decarboxylase correlates with insulitis in non-obese diabetic mice. *Nature* 366 (6450), 72–75.
- Triolo, T.M., Fouts, A., Pyle, L., Yu, L., Gottlieb, P.A., Steck, A.K., 2019. Identical and nonidentical twins: risk and factors involved in development of islet autoimmunity and type 1 diabetes. *Diabetes Care* 42 (2), 192–199.
- Tyndall, A., Gratwohl, A., 1997. Blood and marrow stem cell transplants in autoimmune disease. A consensus report written on behalf of the European League Against Rheumatism (EULAR) and the European Group for Blood and Marrow Transplantation (EBMT). *Br. J. Rheumatol.* 36 (3), 390–392.
- van Bekkum, D.W., 1998. New opportunities for the treatment of severe autoimmune diseases: bone marrow transplantation. *Clin. Immunol. Immunopathol.* 89 (1), 1–10.
- van de Stadt, L.A., de Koning, M.H., van de Stadt, R.J., Wolbink, G., Dijkmans, B.A., Hamann, D., et al., 2011. Development of the anti-citrullinated protein antibody repertoire prior to the onset of rheumatoid arthritis. *Arthritis Rheum.* 63 (11), 3226–3233.
- Van Laar, J.M., Tyndall, A., 2003. Intense immunosuppression and stem-cell transplantation for patients with severe rheumatic autoimmune disease: a review. *Cancer Control* 10 (1), 57–65.
- Verdaguer, J., Schmidt, D., Amrani, A., Anderson, B., Averill, N., Santamaria, P., 1997. Spontaneous autoimmune diabetes in monoclonal T cell nonobese diabetic mice. *J. Exp. Med.* 186 (10), 1663–1676.
- Vigeral, P., Chkoff, N., Chatenoud, L., Campos, H., Lacombe, M., Droz, D., et al., 1986. Prophylactic use of OKT3 monoclonal antibody in cadaver kidney recipients. Utilization of OKT3 as the sole immunosuppressive agent. *Transplantation* 41 (6), 730–733.
- Villemain, F., Jonker, M., Bach, J.F., Chatenoud, L., 1986. Fine specificity of antibodies produced in rhesus monkeys following in vivo treatment with anti-T cell murine monoclonal antibodies. *Eur. J. Immunol.* 16 (8), 945–949.
- Vincenti, F., Kirkman, R., Light, S., Bumgardner, G., Pescovitz, M., Halloran, P., et al., 1998. Interleukin-2-receptor blockade with daclizumab to prevent acute rejection in renal transplantation. Daclizumab Triple Therapy Study Group. *N. Engl. J. Med.* 338 (3), 161–165.

- Voltarelli, J.C., Couri, C.E., Stracieri, A.B., Oliveira, M.C., Moraes, D.A., Pieroni, F., et al., 2007. Autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. *JAMA* 297 (14), 1568–1576.
- Voltarelli, J.C., Couri, C.E., Stracieri, A.B., Oliveira, M.C., Moraes, D.A., Pieroni, F., et al., 2008. Autologous hematopoietic stem cell transplantation for type 1 diabetes. *Ann. N.Y. Acad. Sci.* 1150, 220–229.
- Waldmann, H., 2019. Human monoclonal antibodies: the benefits of humanization. *Methods Mol. Biol.* 1904, 1–10.
- Walter, M., Philotheou, A., Bonnici, F., Ziegler, A.G., Jimenez, R., 2009. No effect of the altered-peptide ligand NBI-6024 on beta cell residual function and insulin needs in new-onset type 1 diabetes. *Diabetes Care* 32 (11), 2036–2040.
- Weiner, H.L., Miller, A., Khoury, S.J., Zhang, Z.J., Al-sabbagh, A., Brod, S.A., et al., 1995. Treatment of autoimmune diseases by oral tolerance to autoantigens. *Adv. Exp. Med. Biol.* 371B, 1217–1223.
- Weiner, H.L., Zhang, Z.J., Khoury, S.J., Miller, A., Al-sabbagh, A., Brod, S.A., et al., 1991. Antigen-driven peripheral immune tolerance. Suppression of organ-specific autoimmune diseases by oral administration of autoantigens. *Ann. N.Y. Acad. Sci.* 636, 227–232.
- Wherrett, D.K., Bundy, B., Becker, D.J., Dimeglio, L.A., Gitelman, S.E., Goland, R., et al., 2011. Antigen-based therapy with glutamic acid decarboxylase (GAD) vaccine in patients with recent-onset type 1 diabetes: a randomised double-blind trial. *Lancet* 378 (9788), 319–327.
- Wildin, R.S., Ramsdell, F., Peake, J., Faravelli, F., Casanova, J.L., Buist, N., et al., 2001. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat. Genet.* 27 (1), 18–20.
- Williams, R.O., Feldmann, M., Maini, R.N., 1992. Anti-tumor necrosis factor ameliorates joint disease in murine collagen-induced arthritis. *Proc. Natl. Acad. Sci. U.S.A.* 89 (20), 9784–9788.
- Williams, R.O., Mason, L.J., Feldmann, M., Maini, R.N., 1994. Synergy between anti-CD4 and anti-tumor necrosis factor in the amelioration of established collagen-induced arthritis. *Proc. Natl. Acad. Sci. U.S.A.* 91 (7), 2762–2766.
- Woodle, E.S., Xu, D., Zivin, R.A., Auger, J., Charette, J., O'Laughlin, R., et al., 1999. Phase I trial of a humanized, Fc receptor nonbinding OKT3 antibody, huOKT3gamma1(Ala-Ala) in the treatment of acute renal allograft rejection. *Transplantation* 68 (5), 608–616.
- Wraith, D.C., 1995. Induction of antigen-specific unresponsiveness with synthetic peptides: specific immunotherapy for treatment of allergic and autoimmune conditions. *Int. Arch. Allergy Immunol.* 108 (4), 355–359.
- Wraith, D.C., Smilek, D.E., Mitchell, D.J., Steinman, L., McDevitt, H.O., 1989. Antigen recognition in autoimmune encephalomyelitis and the potential for peptide-mediated immunotherapy. *Cell* 59 (2), 247–255.
- Wraith, D.C., Streeter, H.B.S.O.C., Molecular, M., School of Clinical Bristol, U. K., 2015. Preclinical development and first-in-human study of ATX-MS-1467 for immunotherapy of MS. *Neurology* 2 (3), e93.
- Yeh, W.I., Seay, H.R., Newby, B., Posgai, A.L., Moniz, F.B., Michels, A., et al., 2017. Avidity and bystander suppressive capacity of human regulatory T cells expressing de novo autoreactive T-cell receptors in type 1 diabetes. *Front. Immunol.* 8, 1313.
- You, S., Leforban, B., Garcia, C., Bach, J.F., Bluestone, J.A., Chatenoud, L., 2007. Adaptive TGF- $\beta$ -dependent regulatory T cells control autoimmune diabetes and are a privileged target of anti-CD3 antibody treatment. *Proc. Natl. Acad. Sci. U.S.A.* 104 (15), 6335–6340.
- You, S., Piali, L., Kuhn, C., Steiner, B., Sauvaget, V., Valette, F., et al., 2013. Therapeutic use of a selective S1P1 receptor modulator ponesimod in autoimmune diabetes. *PLoS One* 8 (10), e77296.
- You, S., Zuber, J., Kuhn, C., Baas, M., Valette, F., Sauvaget, V., et al., 2012. Induction of allograft tolerance by monoclonal CD3 antibodies: a matter of timing. *Am. J. Transplant.* 12 (11), 2909–2919.

# Cameos: Candidates and Curiosities

Ian R. Mackay

Department of Biochemistry and Molecular Biology, Monash University, Clayton, VIC, Australia

## OUTLINE

<b>Introduction</b>	<b>1461</b>	<i>Lymphocytic Mastitis</i>	1466
<b>Autonomic Neuropathy</b>	<b>1461</b>	<i>Metabolic-Genetic Storage Diseases</i>	1466
<b>Birdshot Retinopathy</b>	<b>1462</b>	<i>Movement Disorders</i>	1466
<b>Cystitis, Interstitial</b>	<b>1462</b>	<i>Narcolepsy</i>	1467
<i>Endometriosis</i>	1463	<i>Osteoarthritis</i>	1467
<i>Epilepsy</i>	1464	<i>Parathyroid Disease</i>	1468
<i>Fatigue Syndrome</i>	1464	<i>Polychondritis, Relapsing</i>	1469
<i>Folate Deficiency</i>	1465	<i>Prostatitis</i>	1470
<i>Lichen Sclerosus</i>	1465	<i>Sarcoidosis</i>	1470
		<b>References</b>	<b>1470</b>

## INTRODUCTION

Previous editions described various candidate autoimmune diseases or rare ill-defined conditions with autoimmune features—cameos. Since then, several have been developed as full chapters; others together with some new entities are retained as cameos because of their rarity, controversial evidence for an autoimmune causation, or autoimmunity being just one of several contributing elements to pathogenesis.

## AUTONOMIC NEUROPATHY

Unmyelinated autonomic nerve fibers that innervate cardiovascular, gastrointestinal, urogenital, thermoregulatory, sudomotor, and pupillary structures are subject to neuropathies of disparate cause affecting nerve fibers or ganglionic structures, resulting in autonomic failure (dysautonomia). It is well recognized that paraneoplastic dysautonomia occurs usually in association with other paraneoplastic expressions (Freeman, 2005). The accompanying autoantibody is usually to nicotinic acetylcholine receptors (AChR) in autonomic ganglia (Vernino et al., 2000) or to the Hu family autoantigen (ANNA-1) or peripherin, or less often to presynaptic voltage-gated calcium channels (Iorio and Lennon, 2012). Alternatively, an autoimmune autonomic neuropathy occurs spontaneously independently of neoplasia (Vernino et al., 2000). Some 50% of patients with autoimmune gangliopathy are reactive with autonomic ganglia that are similar structurally to AChR pentamers at the neuromuscular junction, and among the spontaneous cases, one or another clinical expression of dysautonomia may be the more prominent, often intestinal hypomotility causing gastric stasis or constipation. In a reported case of neuropathic gastric stasis with type 1 diabetes, histological examination revealed a complete and presumably autoimmune depletion of

interstitial cells of Cajal of the myenteric plexus (He et al., 2001). Experimentally, rabbits immunized with the a3 subunit of the AChR developed an autonomic neuropathy (Lennon et al., 2003) that was passively transferable to mice with IgG from serum of affected rabbits and, from limited data, with sera from diseased humans (Vernino et al., 2004).

## BIRDSHOT RETINOPATHY

The slender reasons, additional to the piquancy of the title, for considering this rare ocular disease among the cameos is the presumed dependency for pathogenesis on the retinal S antigen and the remarkably high disease association with the class I major histocompatibility complex (MHC) molecule, human leukocyte antigen (HLA) A29. Autoimmune inflammatory eye diseases (see Chapter 54: Ocular Disease) comprise responses to antigens in uvea or retina, but serological responses to a putative characterized autoantigenic molecule are not readily demonstrable as in other autoimmune diseases. Birdshot retinopathy affects predominantly individuals of northern European background and occurs usually in mid-adult life, and there is a slight female excess. It is rare, accounting for only about 1% of all cases presenting clinically as “uveitis.” Birdshot retinopathy is seen at retinoscopy as multiple separate cream-colored spots on the postequatorial fundus. Opportunity for pathologic examination is most infrequent; the retina is predominantly affected, and microscopy reveals a T lymphocytic and granulomatous infiltrate (Gasch et al., 1999). A claimed serologic reactivity in vitro to retinal S antigen is not well documented. The risk conferred by the class I MHC allele HLA A29 (50–224-fold) is higher than that for any other disease and is greater for the A29.1 than the A29.2 subtype of HLA29. There are only a few other human diseases wherein a demonstrable HLA association is with a class I rather than a class II HLA molecule, including spondyloarthropathies (B27, Chapter 36: Spondyloarthritides) and psoriasis (Cw6, Tillikainen et al., 1980). Birdshot retinopathy is attributed to specific reactivity of CD8<sup>+</sup> cytotoxic T cells against the retinal S antigen. The provocation for this disease is unknown.

## CYSTITIS, INTERSTITIAL

Hunner's (1914) report of chronic recurrent edematous ulceration of the bladder in eight young women is taken to be the first on putative autoimmune cystitis; *interstitial* was subsequently added to accommodate the accompanying diffuse fibrosis of the bladder wall. Hand (1949) described a larger case series, 204 women and 19 men, representing almost 5% of urologically investigated patients in his clinic. Oravisto et al. (1970) reported on 54 cases from a urology clinic over a 10-year period, all female aged 1680 years, mean 59, with symptoms of urinary frequency and urgency, suprapubic pain, and hematuria; cystoscopic appearances were included comprising a greatly reduced bladder capacity and a bladder wall with stellate scars, clusters of granulations, and punctuate hemorrhages likened to an “angry scratch.” Microscopically, the bladder wall reveals mucosal ulcerations, edema, lymphoid-plasma cell infiltrates and prominence of mast cells (Sant and Theoharides, 1994), and, in longstanding cases, fibrosis. The best evidence for autoimmunity in interstitial cystitis is the disease association with systemic lupus erythematosus (SLE) (Fister, 1938; Shipton, 1965; Boye et al., 1979), Sjögren's syndrome, and autoimmune thyroiditis (Oravisto, 1980). In 129 cases Peeker et al. (2003) supplemented these associations with cases of “hypersensitivity/allergic disorders,” rheumatoid arthritis (RA), and inflammatory bowel diseases. The high population frequency of interstitial cystitis (12 per 1000, overall, predominantly among women, ≈90%) has led in the United States to a patient support group being founded (Interstitial Cystitis Association). Subtypes of interstitial cystitis are sometimes cited for case classification into ulcerative (typical) or nonulcerative types, and into primary (sole disorder) or secondary (associated with SLE or other immune-mediated disease) (de la Serna and Alarcon-Segovia, 1981; Alarcon-Segovia et al., 1984). Biomarkers are needed to assess accuracy of diagnosis and efficacy of treatment, with formal criteria for diagnosis being specified by the National Institute of Diabetes and Digestive and Kidney Diseases of the NIH (United States). Serologic studies have not been helpful. Bladder-specific autoantibodies were reported by Silk (1970) but have not been confirmed. Frequencies of antinuclear antibody (ANA) were raised according to Oravisto et al. (1970) and Jokinen et al. (1972). However, in a more contemporary study Ochs et al. (1994) assembled 96 patients whose sera were tested for ANA by immunofluorescence on tissue sections and on HEp-2 cells, and additionally, a cultured bladder epithelial cell line was used for Western blotting. Autoantibodies specific to bladder cells were not demonstrable, but there was an increased frequency, 36% versus 8% in female controls, of non-tissue-specific autoantibodies (cutoff titer 1:40); the reactivities were mostly ANA with staining patterns usually speckled or nucleolar, unlike what pertains in SLE, and tests for

anti-mitochondrial antibodies (AMA) were positive in three cases. The findings of [Ochs et al. \(1994\)](#) were not seen as indicative of a primary autoimmune attack on the bladder wall but rather as a consequence of an undefined chronic inflammatory process, with autoantibodies secondarily augmenting this process.

Experimental models of autoimmune cystitis have been attempted in mice and rats, first by direct immunization with bladder wall extracts ([Bullock et al., 1992](#)), and later by introduction of transgenically encoded protein antigen ([Liu et al., 2007](#)). Immunization of Balb/c AN mice with bladder extract-induced cystitis is shown histologically by edema, fibrosis, and lymphocytic and mast cell infiltrations; specific reactivities of serum antibodies or T cells were not described, but disease was transferable adoptively by lymphoid cells from affected mice. Cystitis was similarly inducible in Lewis rats and was adoptively transferable with splenocytes ([Luber-Narod et al., 1996](#)). More recently [Liu et al. \(2007\)](#) developed mice that transgenically expressed on bladder epithelial cells the model “self” antigen ovalbumin (URO-OVA). Such mice are unresponsive to OVA stimulation. Adoptive transfer studies were performed where in naïve OVA-specific T cells showed proliferation and infiltration into the bladder mucosa but no cystitis, whereas transfer of activated OVA-specific T cells did induce an inflammatory cystitis. Their model was further developed to demonstrate that bladder epithelium was capable of presenting a self-antigen in association with MHC class I, that activation of T cells occurred in bladder regional lymph nodes, and that features of the ensuing cystitis simulated those of the human counterpart.

In conclusion, human interstitial cystitis remains on the fringe of autoimmunity, since neither pathogenic nor disease-specific marker autoantibodies are regularly demonstrable, immune-mediated mechanisms of bladder wall damage in humans remain obscure, and lacking are genetic analyses and well-controlled studies of efficacy of corticosteroid or immunosuppressive drugs—yet the experimental transgenic models are persuasive. Perhaps there exist as yet undefined cocontributory mechanisms of pathogenesis such as a harmful constituent of urine to which the bladder wall could be persistently exposed.

## Endometriosis

Endometriosis was so named to convey the idea that fragments of sloughed uterine endometrium become distributed in the pelvic peritoneum and sites further afield ([Sampson, 1921](#); [Ridley, 1968](#); [Giudice and Koo, 2004](#)). The estimated frequency of this disease is 12% of all women and one of five women of reproductive age. Clinical expressions include (1) gynecologic symptoms, pelvic pain, menorrhagia, dysmenorrhea, and dyspareunia; (2) dysfunction of the pelvic colon or bladder; (3) infertility; and (4) repeated pregnancy loss. Definitive diagnosis requires laparoscopic surgery. Unsurprisingly, dysfunctions of the immune system are implicated in pathogenesis at various levels ([Giudice and Koo, 2004](#)) and include compromised natural killer cell activity resulting in decreased surveillance/removal of ectopic tissue ([Wilson et al., 1994](#)), activation of peritoneal macrophages and upregulation of proinflammatory cytokines, and there are various pointers to autoimmune reactivity.

Thus [Weed and Arquembourg \(1980\)](#) surmised that “nonself” ectopic endometrial implants in the pelvis generated endometrial tissue-specific antibodies, with ensuing infertility. In general autoimmunity has not been held to explain the actual occurrence of displaced endometrium in the pelvis but rather particular consequences of this displacement. Yet despite considerable immunological investigation in the 1980s, results were relatively unimpressive ([Dmowski, 1987](#)). Even so, impetus was given to the autoimmune concept by [Grimes et al. \(1985\)](#), whose case-control study showed that endometriosis was associated with a twofold (but nonsignificantly) increased risk for developing SLE, and by increased frequencies of nontissue-specific autoantibodies, [Gleicher et al. \(1987\)](#) found that among 59 cases, 28.5% gave a positive test for ANA and 45.5% for lupus anticoagulant, and [Taylor et al. \(1991\)](#) compared 71 age-matched cases with 109 control women and reported that there was a very significantly increased frequency of autoantibodies to nuclei, ribonucleoproteins (Ro and La), to smooth muscle antigens, and to anticardiolipin and lupus anticoagulant.

While [Gleicher et al. \(1987\)](#) asserted that endometriosis “fulfils all the classic characteristics of an autoimmune disease,” [Taylor et al. \(1991\)](#) took a more reserved interpretation of the reported autoantibody responses. A serological study failed to show any disease-specific autoimmune serological reactivity ([Switchenko et al., 1991](#)). However, [Fernandez-Shaw et al. \(1993\)](#) reported sera of women with endometriosis specifically reacted with the cytoplasm of endometrial glands, whereas all control sera were negative, concluding that the demonstrable anti-endometrial antibodies could be reactive *in vivo* and impair fertility.

Interest in endometrium-specific autoimmune reactants over the last decade has faded. The most recent information on the immunopathology of endometriosis, obtained using a gene array procedure, is that of [Hever et al. \(2007\)](#). These authors compared endometriosis tissue with normal endometrium and found that the former was enriched in plasma cells and activated macrophages. The gene arrays did not point to the presence of mature B

or T cells but did show that there was activation of genes for the B-cell activating cytokine BAFF/BLyS, and the likely source of the various nontissue-specific autoantibodies described in endometriosis.

In conclusion endometriosis perhaps has less relevance to autoimmunity than to physiological immunology by indicating that autoantibodies may in fact serve to remove unwanted/ectopic tissue (Grabar, 1975), in this case displaced uterine endometrium.

## Epilepsy

Autoimmunity became implicated from the early 1990s in seizure disorders when a severe form of childhood encephalitis with seizures (Rasmussen disease, RD) was linked with antibodies to excitatory glutamate receptors in the CNS. Rabbits immunized simply to raise antibodies to glutamate receptor subunit 3 (GluR3) developed seizures and histological encephalitis, and this led to a search for autoantibodies to glutamate receptors in RD in children (Rogers et al., 1994). This is reminiscent of the discovery of the causation of myasthenia gravis by autoantibody to the AChR after the unexpected occurrence of myasthenia in rabbits immunized merely to raise an antiserum to AChR (Patrick and Lindstrom, 1973). However, at present, antiGluR3 figures much less prominently in autoimmune epilepsy in adults than do autoantibodies to various other neuronal antigens such as glutamic acid decarboxylase of 65 kDa (GAD65). Importantly, recognition in blood or cerebrospinal fluid of such autoantibodies is a distinct directive to immunotherapy.

It was the description of epilepsy-relevant autoantibodies during the 1990s that eventually led to the nomination in 2000 of certain epilepsies—particularly refractory and localization-related types—as “autoimmune” (Palace and Lang, 2000; Peltola et al., 2000), after which there have been various corroborative reports specifying associated autoimmune reactants notably anti-GAD65 (Saiz et al., 2008) (and others) in the context of the stiff person syndrome (Ali et al., 2011), voltage-gated potassium channels (VGKC) (Irani et al., 2011; Iorio and Lennon, 2012; Quek et al., 2012) in the context of chronic encephalitis syndromes, or N-methyl-D-aspartate receptor (NMDA-R) implicated mostly in neuropsychiatric dysfunctions other than epilepsy (Diamond et al., 2009; Dalmau et al., 2011). Quek et al. (2012) described 32 patients with an “exclusive or predominant seizure presentation” and suspected neuronal autoimmunity; among these were autoantibodies to VGKC complexes in 56%, GAD65 in 22%, collapsin response-mediator protein in 6%, and NMDA-R in just 3% (one case). “VGKC complexes” is used advisedly as this is a broad term that covers macromolecular assemblies that contain various potentially antigenic constituents (Iorio and Lennon, 2012). A further neuronal antibody species, often paraneoplastic, was characterized in cases of limbic encephalitis with early and prominent seizures being directed to the B1 subunit of the GABAB receptor; the assay used was a cell line transfected with rodent GABA receptor (Lancaster et al., 2010).

The two important points are, first, that “autoimmune epilepsy” should now be regarded as a definitive neurological entity for which there are valid serological diagnostic assays, and second, that immunotherapies can provide substantial relief from seizures in cases in which conventional antiepilepsy drugs have failed.

## Fatigue Syndrome

The “fatigue syndrome” was derived in 1955 from an outbreak of an unexplainable illness at the Royal Free Hospital in London that affected 292 staff members (Medical Staff of the Royal Free Hospital, 1957). The features were severe fatigue, loss of energy, poor exercise tolerance, muscle discomfort, fibromyalgia, and other nonspecific symptoms including malaise, neck stiffness, lymphadenopathy, and fever. A preceding viral illness was often implicated. The name “myalgic encephalomyelitis” (ME) was proposed by “an unusually uncritical Lancet editorialist” (Byrne, 1988). “ME” faded from use, but the fatigue syndrome attracted ongoing attention from patient support groups and various medical specialties: some argued for a functional basis and others for an unknown organic basis. By the 1980s, the syndrome had become accepted as an entity and in 1987 was formally designated as chronic fatigue syndrome (CFS), with recommended diagnostic criteria from the Centers for Disease Control (CDC) in Atlanta, GA (Holmes et al., 1988). The two major criteria for diagnosis were (1) a new onset of fatigue lasting 6 months and reducing activity to less than 50%, and (2) exclusion of any other condition usually producing fatigue, and 11 minor criteria (of which eight should be fulfilled) including eight symptomatic and three physical features, these being mild fever, nonexudative pharyngitis, and palpable cervical or axillary lymph nodes up to 2 cm in diameter. High among claimed causes of CFS are chronic viral infections, notably Epstein–Barr virus; the case for a murine retrovirus, xenotropic murine leukemia virus (XMRV), has been convincingly refuted. Other candidates are allergies, autoimmunity, hyperreactive responses to unusual environmental

exposures, and psychosomatic disorder. Despite evidence in CFS for immune activation and/or impaired indices of cell-mediated immunity (Lloyd et al., 1989, 1990; Buchwald and Komaroff, 1991), data from the conventional indications of inflammatory activity or tissue destruction (erythrocyte sedimentation rate (ESR), C-reactive protein) are non-supportive. Benefit from immunomodulatory treatment with intravenous immunoglobulin, of known efficacy in some well-defined antibody-mediated autoimmune diseases, is controversial, either endorsed (Lloyd et al., 1990) or refuted (Peterson et al., 1990).

Analysis of an autoimmune component in CFS encounters issues such as specification of a clear associated diagnosis and a scarcity of affirmative evidence, perhaps indicative of unpublished negative studies, opposed to which is the known occurrence of fatigue in certain well-accepted autoimmune disorders. The major question then is whether, among cases fulfilling CDC criteria for CFS, there can be regularly identified a highly raised level of any autoantibody specificity, particularly ANA. Positive studies include that of Behan et al. (1985) (50 cases) and another group (60 cases) (Konstantinov et al., 1996; von Mikecz et al., 1997) which cited a frequency of autoantibodies of 83% versus 17% in a control group, predominantly of ANA specificity, and directed particularly to nuclear envelope proteins, together with cytoplasmic staining patterns of intermediate filament-vimentin type thought to be indicative of viral infection. On the other hand, there are negative results for ANA in CFS (Skowera et al., 2002) or only modestly positive results (Vernon and Reeves, 2005).

It is not the purpose here to review all the varied suppositions on the basis of CFS, because none is sufficiently convincing. It can be said that autoimmunity does not provide an explanation for all cases of CFS that autoantibodies when present might reflect some other as yet unidentified cause, and that autoimmunity likely is but one among a conglomerate of causes for CFS.

## Folate Deficiency

Maldevelopment of the embryonic neural tube resulting in spina bifida, anencephaly, or other defects occurs in infants at a prevalence of about one in 1000. It has been widely ascertained that periconceptual folic acid supplements to mothers strikingly alleviate this, even though the mothers usually do not have evident folate deficiency (Rothenberg et al., 2004). The observation was made that antiserum to folate receptors in pregnant rats resulted in embryonic maldevelopment, and this prompted the search for autoantibodies to folate receptors in women in whom a pregnancy had resulted in an infant affected with a neural tube defect (Rothenberg et al., 2004). The procedure used was the specific blocking of ( $^3\text{H}$ ) folic acid to folate receptors on placental membranes and to indicator cell lines. The results were that nine of 12 women (vs two of 20 controls) with affected children had a receptor blocking antibody. The same authors (Ramaekers et al., 2005) in a further study investigated infantile-onset cerebral folate deficiency that develops 4–6 months after birth and is expressed as mental and psychomotor retardation, cerebellar ataxia, dyskinesis, seizures, visual disorder, and autism. There were low levels of 5-methyl tetrahydrofolate in the cerebrospinal fluid but normal levels in serum, and lack of evidence of extracerebral folate deficiency. Serum from 25 of 28 affected children, versus none of 28 controls, contained high-affinity blocking autoantibodies against membrane-bound folate receptors on the choroid plexus, indicating impediment to the passage of folic acid from serum to brain. This could be normalized by oral calcium folinate that led to clinical improvement. Notably, none of five tested mothers had autoantibodies. Perhaps the induction of the antifolate receptor antibodies in these affected children was due to soluble folate-binding proteins in milk, or to other unknown antigens (Ramaekers et al., 2005). This story is indeed a legitimate “cameo” in the world of autoimmunity, since antifolate receptor autoantibody could be generated either in the pregnant mother or the newborn child, causing neural developmental disorders. Indeed Schwartz (2005) was prompted to comment that “autoimmunization lurks behind every pillar.” And there is the added point that the catastrophic consequences in this particular example are remediable by a very simple therapy, folic acid.

## Lichen Sclerosus

“Lichen,” a compound plant (fungi in symbiotic union with algae) that spreads on rocks and trees, gives its name to chronic skin diseases seen mostly as thickened but sometimes atrophic inflammatory patches on skin or mucus membranes with, histologically, damage and cellular infiltration between the epidermis and dermis. There are many variants described of mucocutaneous lichenoid eruptions according to their location and visual appearances. The prototype is lichen planus for which no single specific cause has been identified, although one interesting association is infection with hepatitis C virus (Le Cleach and Chosidow, 2012). The lichenoid eruption

described as lichen sclerosus is, however, consistently linked to autoimmunity. Lichen sclerosus particularly affects the anogenital skin, and the vulval mucosa in women. The population prevalence is at least  $\approx 1$  per 1000 with a female:male ratio of at least 6:1; histologically, there is hydropic degeneration of basal keratinocytes with inflammatory changes (Dalziel and Shaw, 2010). The evidence for autoimmunity specified by Oyama et al. (2003) includes familial occurrence, association with the class II HLA allele DQ7 as seen in other mucus membrane/pemphigoid diseases, and cooccurrence with other autoimmune diseases and/or autoantibodies, mainly of the “thyrogastric” cluster, that is, thyroiditis, pernicious anemia, diabetes, vitiligo, and others, but better immunological data on these disease associations are called for.

Previous findings of “pemphigoid” autoantibodies in skin diseases with basement membrane pathology (see Chapter 60: Autoimmune Bullous Skin Diseases—Pemphigus and Pemphigoid) led a search for analogous autoantibodies to extracellular matrix protein 1 (ECM1) in lichen sclerosus (Oyama et al., 2003). The autoantigen ECM1 was chosen based on the observation that loss-of-function mutations of the gene encoding ECM1 result in a lichen sclerosus-like pathology (lipoid proteinosis, LP). The sera studied were from 86 patients with lichen sclerosus, 85 healthy controls, and 107 “other disease” contrast cases, and the technical procedures included Western immunoblotting (WB) on extracts from normal skin and LP skin that lacks ECM1, and indirect immunofluorescence (IIF) microscopy using affinity purified IgG. In brief, there was a specific signal for anti-ECM1 from lichen sclerosus sera by WB (to the low titer of 1:20), absence of signal using extracts from LP skin lacking ECM1, and specific reactivity by IIF of affinity-purified serum IgG with the basal keratinocyte layer of normal skin. Passive transfer of lichen sclerosus lesions to mice by serum or IgG was a contemplated next step.

## Lymphocytic Mastitis

The female breast is subject to a nonneoplastic multinodular fibrosing disease expressed clinically by recurrent mastalgia and histologically by periductular lymphocytic infiltrations. An early report described the case of a woman with a multisystem-like disease in whom there were dense collections of lymphoid cells within diseased breast tissue, with underlying autoimmunity thus implicated (Shelley and Hurley, 1960), but the idea of an “autoimmune mastitis” was not picked up. The disease (or diseases) characterized by lymphocytic mastitis has acquired several descriptors, granulomatous mastitis—although giant multinuclear cells are actually inconspicuous (Donn et al., 1994), diabetic mastopathy by reason of a frequent association with clinical diabetes (Tomaszewski et al., 1992; Camuto et al., 2000), fibrocystic mastitis, and some cases classed as IgG4 mastitis (Ogura et al., 2010). Lymphocytic mastitis may represent an immunological response to extruded breast milk, or to acinar or ductular breast tissue, but immunocytopathological examination of the characteristic lymphocytic infiltrates has received rather little attention, so the condition remains “idiopathic.”

## Metabolic-Genetic Storage Diseases

Batten disease is a rare, recessively inherited, and fatal neurodegenerative disease of children due to a mutation in both copies of a gene *CLN3*, so leading to accumulation of ceroid lipofuscin in neurons. A gene-disruption model in mice revealed altered expression of enzymes required for the synthesis of the neurotransmitter glutamate and circulating antibodies to brain proteins including GAD65. This prompted a search for anti-GAD65 in human Batten disease, with positive results (Chattopadhyay et al., 2002). As yet, the role (if any) of anti-GAD in the overall pathogenesis of Batten disease is uncertain.

Sandhoff disease is another metabolic autoimmune curiosity. It is a recessively inherited lysosomal storage disease of infancy in which neuronal cell death results from an enzyme deficiency that causes accumulation of gm2 gangliosides in lysosomes of brain cells. A murine equivalent has been created by knockout of the *hexb* gene that encodes the hexosaminidase enzyme. It is claimed that antiganglioside antibodies in Sandhoff disease accelerate premature neuronal death, perhaps via complexes of antibody and ganglioside that cause inflammatory activation of microglial cells. Similar events are postulated for the human counterpart (Yamaguchi et al., 2004).

## Movement Disorders

A miscellaneous group of movement disorders, observed particularly in children, and sharing the features of an antecedent streptococcal infection, are claimed to have in common the expression of autoantibodies to neurons of basal ganglia in the brain. Prototypic among these is Sydenham’s chorea, marked by frequent unintended jerky movements, and long known to occur occasionally in association with streptococcal-related rheumatic carditis.

Others include the Tourette syndrome of motor and vocal tics, and a pediatric autoimmune neuropsychiatric disorder associated with streptococcal infections (Shulman, 1999). Speculative extensions have brought in other entities including obsessive-compulsive and attention-deficit disorders. Autoimmunity has been implicated, but on rather slender evidence: (1) the claimed antecedent streptococcal infections that provide a reasoning for the occurrence of autoimmunity similar to that proposed for the onset of rheumatic carditis (see Chapter 63: Rheumatic Fever and Rheumatic Heart Disease), (2) positive tests for antineuronal autoantibodies particularly to autoantigens of basal ganglia neurons, and (3) a claimed capacity of patients' sera to transfer disease passively after direct injection into the striatum of rats. However, the autoantibody studies are in earlier literature and have not been convincingly confirmed (reviewed by Dale, 2005), and the validity of the data on passive transfer to animals by serum is likewise questionable (Giovannoni, 2005).

## Narcolepsy

Narcolepsy is a sleep–wake disorder with onset during adolescence in which there is irresistible daytime sleepiness. It is now confidently attributed to deficient neurotransmission dependent on a neurotransmitter that sustains wakefulness, known according to the discoverers as either hypocretin or orexin. Deficiency of this transmitter, synthesized by neurons in the hypothalamus, is the underlying fault—in canines with narcolepsy there is a profound loss of hypocretin-secreting neurons in the hypothalamus. *Prima facie*, narcolepsy would seem hardly likely as an autoimmune disease, yet there is a long-known extreme and puzzling coassociation with the class II MHC allele, HLA DQB1\*0602. Inconveniently for theorists, an autoantibody relevant to narcolepsy against hypocretin/orexin, or the cognate receptor, has not been demonstrated by conventional assays. However, some evidence for a serum factor was obtained by a bioassay based on passive transfer to mice of IgG from narcolepsy patients, by a readout of increased contractile responses of detrusor muscle strips (Smith et al., 2004), but these preliminary bioassay data would seem to require further work. There is a published personal account of a narcolepsy sufferer annotating the curious symptoms, together with comments on the occurrence of narcolepsy in inbred Doberman Pinscher dogs (although this is attributed to a genetically faulty cell receptor, see below), the extreme immunogenetic HLA D locus bias (DQ6//DQB1\*0602 and/or DR2/DRB11501) and seasonal (springtime) peak incidences suggesting an antecedent infection (Nicholls, 2012). Suspected infections include either H1N1 influenza or streptococcal throat infection substantiated by raised levels in childhood narcolepsy of anti-streptolysin O, as implicated also in movement disorders of childhood described above.

A possible disease pathway can now be specified based on the interaction of hypocretin/orexin and the cognate receptor. The first hints in the 1990s came from the identification of hitherto unknown hormone-like agents in the brain of rats and mice, hypocretins/orexins, and one group further identified the structure of the orexin receptors (Sakurai et al., 1998), although these authors directed their attention to the role of the orexin system in energy balance and eating behavior rather than sleep patterns. However, it was evident from other investigations that the hypothalamic cells that secrete hypocretin/orexin hormones provide also send “stay awake” messages to other cells in the CNS via the orexin-receptor pathway. Reliable detection of antibody or T cell reactivity to hypocretin/orexin or to the cognate receptor is still awaited. Meanwhile further immunogenetic and immunological anomalies have come to light (reviewed by Faraco and Mignot, 2011) and include polymorphism of the alpha chain of the T cell antigen receptor (Hallmayer et al., 2009), with three single-nucleotide polymorphisms within the TCRA locus showing significant association with narcolepsy syndromes across various ethnic groups; another susceptibility locus is the gene for the P2RY11 purinergic receptor. There is an autoantibody association too, depending on the tribbles2 homolog (trib2) protein, in 14%–26% of DQB1 (cataplexy-associated) cases, but trib2 is a ubiquitously expressed protein, and there are no indications that antibodies to it are related to narcolepsy. One wonders how the story will eventually pan out!

## Osteoarthritis

Osteoarthritis (OA) is prototypically a “degenerative” articular disease with usually a late-in-life onset and a relationship to articular stresses and previous trauma, but there can be an early inflammatory phase with an obviously acute onset. In tests for specificity of serological reactants used for the diagnosis of rheumatic diseases, that is, autoantibodies to citrullinated peptides or native collagen type II (CII) (nCII), a degree of reactivity is usually observed. In regard to anti-nCII, Xiang et al. (2004) reported that sera from patients with OA reacted more with denatured than with native CII, and Burkhardt et al. (2002) found that OA sera were reactive but, in contrast to RA, sera engaged different antibody epitopes of nCII, notably CII-F4 located at the C-terminal region of nCII,

rather than the characteristic RA-related epitopes, CII-C1 and CII-M2139. Interestingly, a murine anti-CII-F4 monoclonal antibody (mAb), in contrast to other mAbs reactive with specific epitopes of nCII, is not pathogenic on passive transfer in mice but actually inhibits the adverse effects (matrix disruption) of mAbs that do react with nCII epitopes other than F4 (Burkhardt et al., 2002). Xiang et al. (2004) further found that those OA sera, as well as reacting with CII-F4, reacted with an antigen of different provenance, triose phosphate isomerase. In regard to pathogenicity, the capacity of autoantibodies to nCII to perpetrate articular damage remains an open question.

T lymphocytes have not been neglected in OA, according to a recent review by Sakkas and Platsoukas (2007). T-cell infiltrates are demonstrable in the affected articular cartilage, and particularly in the synovial membrane, appearing as nodular aggregates suggestive of an antigen-driven process, although a provocative cartilaginous autoantigen has not so far been identified, despite much search and speculation. Curiously, erosive effects of cartilage/synovial inflammation associated with activated T lymphocytes infiltrating subchondral bone, as seen in RA, are not evident in OA. The T cells demonstrable in synovial membrane in OA are of the Th1 subtype and their activated phenotype is revealed by transcripts for cytokines such as IL-12, detectable in synovial fluid. Sakkas and Platsoukas (2007) discuss the utility of future procedures to assess the degree of clonality of T-cell infiltrates in joints in OA. However, while few of the conventional markers (particularly reactivity with an “accepted” autoantigenic structure) are fulfilled, it remains difficult to ascribe OA to an adaptive autoimmune response. In addition, results of a large (and latest) genome-wide association study (GWAS) that included stratified cases of OA defined some eight candidate loci for association with OA, although none could be aligned with those often associated with autoimmune disease (arcOGEN Consortium and arcOGEN Collaborators, 2012).

Hence it is of interest to consider a new slant on the immunological contribution to OA, namely an inflammatory association with an anomaly of the complement cascade. Wang et al. (2011) found in mice that injury to articular cartilage in the setting of dysregulation of complement gene expression in joint tissues led to formation of the membrane attack complex on chondrocytes, with either killing or production of matrix degrading enzymes, so linking OA to such age-related diseases as macular degeneration and Alzheimer’s disease. Possible applications of this concept to define subsets of human OA are awaited. This theme was developed in a recent review in which various components of innate immunity were implicated, without recourse to an adaptive response (Haseeb and Haqqi, 2013). So perhaps OA may be better placed in the category of autoinflammatory diseases.

## Parathyroid Disease

An autoimmune basis for hypoparathyroidism was proposed by Solomon and Blizzard (1963), and then by Seemann (1967), whose histological study revealed lymphocyte-plasma cell infiltrates—hence “lymphocytic parathyroiditis.” In our first edition of this text, Maclarens and Blizzard (1985) described autoimmune polyendocrine syndrome (APS) types 1 and 2. For type 1, the three major components appeared usually in a uniform sequential order: candidiasis, hypoparathyroidism, and Addison’s disease. Autoimmune hypoparathyroidism, despite being a characteristic feature of APS type 1, did not occur in any of 224 cases of APS type 2. However, serological reactivity with parathyroid tissue was insufficiently convincing to generate clinical laboratory assays, although accompanying autoantibodies were reported to bind to the cell surface of human parathyroid cells and to inhibit parathyroid cell secretion. Perhaps these autoantibodies were related to those revealed by a mAb raised by immunizing mice with parathyroid tissue and shown to react with a parathyroid cell surface antigen called parathyroid antigen (PTA) (Cance et al., 1986). An autoimmune basis was strengthened when Li et al. (1996) demonstrated, in both APS type 1–associated and sporadic cases of hypothyroidism, serological reactivity with the ubiquitous calcium-sensing receptor (Ca-SR) on the parathyroid cell surface; an immunoprecipitation assay showed that the extracellular domain of the Ca-SR contained the reactive epitopes. This autoimmune reactivity, anti-Ca-SR, was demonstrable in 56% of 25 cases and was far more frequent among females than males. Also, cases were recognized wherein antibodies to the parathyroid Ca-SR had an inactivating effect, so rendering the glands insensitive to ambient calcium, such that the ensuing oversecretion of parathyroid hormone had effects like those seen with parathyroid adenomas, causing hypocalciuric hypercalcemia (Pallais et al., 2004). These antibodies were functionally active but nondestructive were of the IgG4 subclass, and in one case were associated with autoimmune pancreatitis, perhaps illustrating another facet of “IgG4 disease” (see Chapter 64: Myocarditis and Dilated Cardiomyopathy). Thus the clinical expressions of autoimmune parathyroiditis with anti-Ca-SR reactivity may be either hypoparathyroidism if the antibodies are nonblocking as pertains when the glands are subject to lymphocytic-mediated destruction, or hyperparathyroidism if the antibodies are blocking and the glands retain functional activity.

In 2008 a novel autoimmune reactant was discovered by screening a cDNA expression library with serum samples from cases of APS type 1, identified as the NACHT leucine-rich-repeat protein 5 (NALP5) (Alimohammadi et al., 2008). These authors, in their Introduction, negated the role of other described autoantigens in parathyroiditis, although anti-Ca-SR reactivity in parathyroiditis had been well established in earlier studies (see Gavalas et al., 2007). The authors of the NALP5 study decisively showed that autoantibody was limited to cases of APS type 1 hypoparathyroidism with AIRE mutations, was not demonstrable in "isolated" (sporadic) hypoparathyroidism, and was detectable as a subcellular autoantigen only in the chief and not the oxyphilic cells of the parathyroid gland. Still to come are studies on patterns of T-cell reactivity to NALP5, and the relative contributions of defective thymic deletional tolerance versus Treg-mediated suppressive tolerance to this putative parathyroid autoantigen.

## Polychondritis, Relapsing

Relapsing polychondritis (RP) was so named by Pearson et al. (1960) to designate recurring inflammatory damage to, and degradation of, (mainly) type II collagenous cartilage throughout the body (Michet et al., 1986). More particularly vulnerable is cartilage of nasal, auricular, tracheobronchial, and audiovestibular sites than is articular cartilage. However, there is a very wide distribution of lesions to noncartilaginous sites, such as ocular sclera, heart valves, skin, and others, and therefore RP is called "an autoimmune disease with many faces" (Lahmer et al., 2010). There is clustering in some 30% of cases with one or another systemic autoimmune rheumatic disease, notably RA and SLE. Lymphoid cell accumulation is prominent at affected sites. There is a weak association with the MHC class II allele HLA DR4, and clear benefit accrues from prednisolone (McAdam et al., 1976). Thus polychondritis has most of the hallmarks of an autoimmune process.

Specific antibodies to cartilage were detected using IIF and cartilage substrate from mouse leg, or from human costochondral and tracheal cartilage, after preincubation with hyaluronidase to remove masking proteoglycan (Dolan et al., 1976; Foidart et al., 1978). Autoantibodies were more readily detected in the acute stages, were of IgG class and noncomplement-binding, and levels correlated with disease severity. Absorption exclusively with CII removed reactivity with cartilage from serum. Using a direct immunofluorescence procedure, and serum from in a single case, Bergfeld (1978) detected deposits in vivo of IgG, IgA, and C3 in affected cartilage. In a subsequent study positive results were reported in six of nine patients for antibodies to cartilage, and there was association with organ (thyroid)-specific autoantibodies as well (Ebringer et al., 1981). Assays for T cell-mediated immune responses to cartilage proved indecisive (Foidart et al., 1978; Ebringer et al., 1981).

Reactivity of serum with CII has been investigated in polychondritis. This was rather low by ELISA among Japanese patients, 202 with RA, 26 with polychondritis, and 92 with other rheumatic diseases, positivity being only 42%, 11%, and 0.3%, respectively (Terato et al., 1990). In tests using peptides derived by cyanogen bromide (CB) digestion of CII, polychondritis sera reacted preferentially with the CB 9.7 kDa peptide, unlike the anti-CII reactivity of sera from other diseases, and the species of anti-CII antibody detectable in polychondritis lacked the epitope specificity of sera from patients with erosive articular inflammation (Burkhardt et al., 2002). However, subsequent studies have indicated that the primary reactant in polychondritis might not be CII, since reactivity of serum antibodies and T cells was greater with collagens IX and XII (Yang et al., 1993). Moreover, in NOD mice carrying a human HLA DQ1 transgene and immunized with CII, there developed an auricular chondritis that simulated the abnormality seen in human polychondritis, and sera reacted to CIX as well as CII (Taneja et al., 2003). Perhaps, then, in polychondritis, anti-CII occurs secondarily by epitope spreading.

The cartilage protein matrilin is another suggested autoantigen in polychondritis (Hansson et al., 2004). Matrilin is a cartilage-specific protein abundant at sites affected in polychondritis (trachea, ears) but sparse in sites that are spared (joints). A model of polychondritis induced in mice by active immunization with matrilin I showed that functional B cells and complement factor V were required for disease expression and that passive transfer could be accomplished by a mAb to matrilin I suggesting that polychondritis, in this model at least, is essentially an antibody-mediated autoimmune disease (Hansson et al., 2004).

The disease itself, and in particular expressions of it, limits survival, for example, after 10 years to 55%. Therapists have relied mainly upon corticosteroid drugs that are of variable efficacy, supplemented with the conventional immunosuppressives. Successful use of TNF antagonists is reported but in very limited case studies (Lahmer et al., 2010) and a recent review on the utility of biological therapies identified some benefits with the authors deplored the limitations of the data available (Kempta Lekpa et al., 2012).

## Prostatitis

Aging men become afflicted by the urinary obstructive effects of benign prostatic hyperplasia due to prostatic cell proliferation. Often a component of inflammation is demonstrable histologically despite no evident infection. The question thus has arisen whether BPH is an “immune inflammatory disease” (Kremer et al., 2007), or even whether autoimmunity is implicated. From the earlier days, prostate-specific autoantigens were demonstrable by immunization of rabbits with a saline extract of prostate tissue that provoked an autoantibody response (Shulman et al., 1965) but histological data were not provided.

However, interest waned, as judged by a lapse in citations on inflammatory prostatitis even until the post-2000 era, according to Penna et al. (2009). These authors implicated stromal cells in the prostate as having the properties of immune accessory cells, that is, antigen-presenting cells (APCs), that induced expression of MHC class II molecules, costimulatory molecules, and demonstrability of high levels of the two interrelated cytokines, IL-12p75 and IL-23p40, as well as other cytokines and chemokines characteristic of immunoinflammatory reactivity (see Chapter 15: Cytokines, Their Receptors and Signals). Penna et al. (2009) also cited publications on the detection in semen from cases of chronic prostatitis of proinflammatory cytokines IL-6, IL-8, IL-1beta, and TNF-alpha, together with other data suggesting that products of immunoreactive cells in the prostate foster chronic inflammation and prostate cell growth. A credible sequence for an autoimmune prostatitis would require a candidate prostate-specific autoantigen; among those cited is the controversial cancer marker, prostate-specific antigen. There is clearly more work needed, but considerations such as the high frequency of prostatitis among aging males and its dependency on hormonal influences leave open the idea of an autoimmune component to prostate disease in men.

## Sarcoidosis

An early suspicion that sarcoidosis could have an autoimmune connection (Mackay and Burnet, 1963) proved not sustainable, although few other more credible pathogeneses have emerged. Epidemiological data implicate an environmental influence, with possible person-to-person transmission, or a shared response to a provocative transmissible agent (Newman et al., 1997).

The disease expressions depend on noncaseating granulomatous lesions in multiple sites—skin, lymph nodes, lung, liver or CNS, and pathogenicity depends either on inflammatory fibrosis as in the lungs, or pressure effects of lesions as in the CNS. The immune system is clearly implicated as judged by the Th1-directed granulomatous histopathology. Also, there is solid evidence from a comparative population study in Italy of coassociation with other autoimmune diseases, notably autoimmune thyroiditis, although the strength of this association is questionable (Antonelli et al., 2006).

Ho et al. (2005) drew on the known Th1-biased CD4<sup>+</sup> T-cell response in sarcoidosis, together with possible involvement of the natural killer T (NKT) cell system. NKT cells are activated by glycolipid antigens presented by CD1 molecules on APCs (Chapter 11: Dendritic Cells in Autoimmune Disease). There is a particular class of CD1 (CD1d) that, after interaction with NKT cells with an invariant receptor (Va24/JaQ, paired with Vb11), can exert regulatory effects. Also, in mice at least, the CD1-restricted repertoire includes autoreactive T cells (Park et al., 2001). Ho et al. (2005) ascertained in sarcoidosis a deficiency (for unknown reasons) of Va24 NKT cells, with ensuing loss of their normal regulatory effect on CD1d-dependent reactivity. However, for sarcoidosis to be confidently ascribed to autoimmunity, depletion of Va24 NKT cells would need to be associated with persistent stimulation of the Th1 T-cell pathway by some endogenous autoantigen, for which the evidence is meagre, at best.

## References

- Alarcon-Segovia, D., Abud-Mendoza, C., Reyes-Gutierrez, E., Iglesias-Gammara, A., Diaz-Jovanen, E., 1984. Involvement of the urinary bladder in systemic lupus erythematosus: a pathologic study. *J. Rheum.* 11, 208–210.
- Ali, F., Rowley, M.J., Jayakrishnan, B., Teuber, S., Gershwin, M.E., Mackay, I.R., 2011. Stiff-person syndrome (SPS) and anti-GAD-related CNS degenerations: protean additions to the autoimmune central neuropathies. *J. Autoimmun.* 37, 79–87.
- Alimohammadi, M., Bjorklund, P., Hallgren, A., Pontynen, N., Szinnai, G., Shikama, N., et al., 2008. Autoimmune polyendocrine syndrome type 1 and NALP5, a parathyroid autoantigen. *N. Engl. J. Med.* 358, 1018–1028.
- Antonelli, A., Fazzi, P., Fallah, P., Ferrani, S.M., Ferannini, E., 2006. Prevalence of hypothyroidism and Graves' disease in sarcoidosis. *Chest* 130, 526–532.
- arcOGEN Consortium and arcOGEN Collaborators, 2012. Identification of new susceptibility loci for osteoarthritis: a genome wide association study. *Lancet* 380, 815–823.
- Behan, P.O., Behan, W.M., Bell, E.J., 1985. The post-viral fatigue syndrome—an analysis of the findings in 50 cases. *J. Infect.* 10, 211–222.
- Bergfeld, W.F., 1978. Relapsing polychondritis with positive direct immunofluorescence (letter). *Arch. Dermatol.* 114, 127.

- Boye, E., Morse, M., Huttner, M., Erlanger, I., MacKinnon, K.J., Klassen, J., 1979. Immune complex-mediated interstitial cystitis as a major manifestation of systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* 13, 67–76.
- Buchwald, D., Komaroff, A.L., 1991. Review of laboratory findings for patients with chronic fatigue syndrome. *Rev. Infect. Dis.* 13, 512–518.
- Bullock, A.D., Becich, M.J., Klutke, C.G., Ratliff, T.L., 1992. Experimental autoimmune cystitis: a potential murine model for ulcerative interstitial cystitis. *J. Urol.* 148, 1951–1956.
- Burkhardt, H., Koller, T., Engstrom, A., Nandakumar, S.K., Turnay, J., Kraetsch, H.G., et al., 2002. Epitope-specific recognition of type II collagen by rheumatoid arthritis antibodies is shared with recognition by antibodies that are arthritogenic in collagen-induced arthritis in the mouse. *Arthritis Rheum.* 46, 2339–2348.
- Byrne, E., 1988. Idiopathic chronic fatigue and myalgia syndrome myalgic encephalomyelitis. Some thoughts on nomenclature and aetiology. *Med. J. Aust.* 148, 80–82.
- Camuto, P.M., Zetrenne, E., Ponn, T., 2000. Diabetic mastopathy. A report of 5 cases and a review of the literature. *Arch. Surg.* 135, 1190–1193.
- Cance, W.G., Wells Jr., S.A., Dilley, W.G., Welch, M.J., Otsuka, F.L., Davie, J.M., 1986. Human parathyroid antigen, characterization and localization with monoclonal antibodies. *Proc. Natl. Acad. Sci. U.S.A.* 83, 6112–6116.
- Chattopadhyay, S., Ito, M., Cooper, J.D., Brocks, A.L., Curran, T.M., Powers, J.M., et al., 2002. An autoantibody inhibitory to glutamic acid decarboxylase in the neurodegenerative disorder Batten disease. *Hum. Mol. Genet.* 11, 1421–1431.
- Dale, R.C., 2005. Post-streptococcal autoimmune disorders of the central nervous system. *Dev. Med. Child Neurol.* 47, 785–791.
- Dalmau, J., Lancaster, E., Martinez-Hernandez, E., Rosenfeld, M.R., Balice-Gordon, R., 2011. Clinical experience and laboratory investigations in patients with anti-NMDAR encephalitis. *Lancet Neurol.* 10, 63–74.
- Dalziel, K., Shaw, S., 2010. Lichen sclerosus. *BMJ* 340, 757–761.
- de la Serna, A.R., Alarcon-Segovia, D., 1981. Chronic interstitial cystitis as an initial major manifestation of systemic lupus erythematosus. *J. Rheumatol.* 8, 808–810.
- Diamond, B., Huerta, P.T., Mina-Osorio, P., Kowal, C., Volpe, B.T., 2009. Losing your nerves? Maybe it's the antibodies. *Nat. Rev. Immunol.* 9, 449–456.
- Dmowski, W.P., 1987. Immunologic aspects of endometriosis. *Contrib. Gynecol. Obstet.* 16, 48–55.
- Dolan, D.L., Lemmon, G.B., Teitelbaum, S.L., 1976. Relapsing polychondritis. Analytical literature review and studies on pathogenesis. *Am. J. Med.* 41, 285–297.
- Donn, W., Rebbeck, P., Wilson, C., Gilks, C.B., 1994. Idiopathic granulomatous mastitis. *Arch. Pathol. Lab. Med.* 118, 822–825.
- Ebringer, R., Rook, G., Swana, G.T., Bottazzo, G.F., Donaich, D., 1981. Autoantibodies to cartilage and type II collagen in relapsing polychondritis and other rheumatic diseases. *Ann. Rheum. Dis.* 40, 473–479.
- Faraco, J., Mignot, E., 2011. Immunological and genetic aspects of narcolepsy. *Sleep Med. Res.* 2, 1–9.
- Fernandez-Shaw, S., Hicks, B.R., Yudkin, P.L., Kennedy, S., Barlow, D.H., Starkey, P.M., 1993. Anti-endometrial and anti-endothelial autoantibodies in women with endometriosis. *Hum. Reprod.* 8, 310–315.
- Fister, G.M., 1938. Similarity of interstitial cystitis (Hunner's ulcer) to lupus erythematosus. *J. Urol.* 40, 37–51.
- Foidart, J.-M., Abe, S., Martin, G.R., Zizic, T.M., Barnett, E.V., Lawley, T.J., et al., 1978. Antibodies to type II collagen in relapsing polychondritis. *N. Engl. J. Med.* 299, 1203–1207.
- Freeman, R., 2005. Autonomic peripheral neuropathy. *Lancet* 365, 1259–1270.
- Gasch, A.T., Smith, J.A., Whitcup, S.M., 1999. Birdshot retinochoroidopathy. *Br. J. Ophthalmol.* 83, 241–249.
- Gavalas, N.G., Kemp, E.H., Krohn, K.J., Brown, E.M., Watson, P.F., Weetman, A.P., 2007. The calcium-sensing receptor is a target of autoantibodies in patients with autoimmune polyendocrine syndrome type 1. *J. Clin. Endocrinol. Metab.* 92, 2107–2114.
- Giovannoni, G., 2005. Anti-neuronal antibodies and movement disorders. *J. Neuroimmunol.* 163, 5–7.
- Giudice, L.C., Koo, L., 2004. Endometriosis. *Lancet* 364, 1789–1793.
- Gleicher, N., el-Roeiy, A., Confino, E., Friberg, J., 1987. Is endometriosis an autoimmune disease? *Obstet. Gynecol.* 70, 115–122.
- Grabar, P., 1975. Hypothesis. Autoantibodies and immunological theories. An analytic review. *Clin. Immunol. Immunopathol.* 4, 453–466.
- Grimes, D.A., Lebolt, S.C., Grimes, K.R., Wingo, P.A., 1985. Systemic lupus erythematosus and reproductive function. A case-control study. *Am. J. Obstet. Gynecol.* 153, 179–186.
- Hallmayer, J., Faraco, J., Lin, L., Hesselson, S., Winkelmann, J., Kawashima, M., et al., 2009. Narcolepsy is strongly associated with the T-cell receptor alpha locus. *Nat. Genet.* 41, 708–711.
- Hand, J.R., 1949. Interstitial cystitis: report of 223 cases 204 women and 19 men. *J. Urol.* 61, 291–310.
- Hansson, A.-S., Johannesson, M., Svensson, L., Nandakumar, K.S., Heinegård, D., Holmdahl, R., 2004. Relapsing polychondritis, induced in mice with Matrilin 1, is an antibody and complement-dependent disease. *Am. J. Pathol.* 164, 959–966.
- Haseeb, A., Haqqi, T.M., 2013. Immunopathogenesis of osteoarthritis. *J. Clin. Immunol.* 146, 185–196.
- He, C., Soffer, E.E., Ferris, C.D., Walsh, R.M., Szurszewski, J.H., Farrugia, G., 2001. Loss of interstitial cells of Cajal and inhibitory innervation in insulin-dependent diabetes. *Gastroenterology* 121, 427–434.
- Hever, A., Roth, R.B., Hevezi, P., Marin, M.E., Acosta, J.A., Acosta, H., et al., 2007. Human endometriosis is associated with plasma cells and overexpression of B lymphocyte stimulator. *Proc. Natl. Acad. Sci. U.S.A.* 104, 12451–12456.
- Ho, L.-P., Urban, B.C., Thickett, D.R., Davis, R.J.O., McMichael, A.J., 2005. Deficiency of a subset of T cells with immunoregulatory properties in sarcoidosis. *Lancet* 365, 1062–1072.
- Holmes, G.P., Kaplan, J.E., Gantz, N.M., Komaroff, A.L., Schonberger, L.B., Straus, S.E., et al., 1988. Chronic fatigue syndrome. A working case definition. *Ann. Intern. Med.* 108, 387–389.
- Hunner, G.L., 1914. A rare type of bladder ulcer in women. *Trans. South Surg. Gynecol. Assoc.* 27, 247–292.
- Iorio, R., Lennon, V.A., 2012. Neural antigen-specific autoimmune disorders. *Immunol. Rev.* 248, 104–121.
- Irani, S.R., Bien, C.G., Lang, B., 2011. Autoimmune epilepsies. *Curr. Opin. Neurol.* 24, 146–153.
- Jokinen, E.J., Alfthan, O.S., Oravisto, K.J., 1972. Anti-tissue antibodies in interstitial cystitis. *Clin. Exp. Immunol.* 11, 333–339.

- Kempta Lekpa, F., Kraus, V.B., Chevalier, X., 2012. Biologics in relapsing polychondritis; a literature review. *Semin. Arthritis Rheum.* 41, 712–719.
- Konstantinov, K., von Mikecz, A., Buchwald, D., Jones, J., Gerace, L., Tan, E.M., 1996. Autoantibodies to nuclear envelope antigens in chronic fatigue syndrome. *J. Clin. Invest.* 98, 1888–1996.
- Kremer, G.D., Mitteregger, D., Marberger, M., 2007. Is benign prostatic hyperplasia (BPH) an immune inflammatory disease? *Eur. Urol.* 51, 1202–1216.
- Lahmer, T., Treiber, M., von Verder, A., Foeger, F., von Knopf, A., Heemann, U., et al., 2010. Relapsing polychondritis: an autoimmune disease with many faces. *Autoimmun. Rev.* 9, 540–546.
- Lancaster, E., Lai, M., Peng, X., Hughes, E., Constantinescu, R., Raizer, J., et al., 2010. Antibodies to the GABA(B) receptor in limbic encephalitis with seizures, case series and characterization of the antigen. *Lancet Neurol.* 9, 67–76.
- Le Cleach, L., Chosidow, O., 2012. Lichen planus. *N. Engl. J. Med.* 366, 723–732.
- Lennon, V.A., Ermilov, L.G., Szurzewski, J.H., Vernino, S., 2003. Immunization with neuronal nicotinic acetylcholine receptor induces neurological autoimmune disease. *J. Clin. Invest.* 111, 907–913.
- Li, Y., Song, Y.-H., Rais, N., Connor, E., Schatz, D., Muir, A., et al., 1996. Autoantibodies to the extracellular domain of the calcium sensing receptor in patients with acquired hypoparathyroidism. *J. Clin. Invest.* 97, 910–914.
- Liu, W., Evanoff, D.P., Chen, X., Luo, Y., 2007. Urinary bladder epithelium antigen induces CD81 T cell tolerance, activation, and autoimmune response. *J. Immunol.* 178, 539–546.
- Lloyd, A.R., Wakefield, D., Boughton, C.R., Dwyer, J.M., 1989. Immunological abnormalities in the chronic fatigue syndrome. *Med. J. Aust.* 151, 122–124.
- Lloyd, A., Hickie, I., Wakefield, D., Boughton, C., Dwyer, J., 1990. A double-blind placebo-controlled trial of intravenous immunoglobulin therapy in patients with chronic fatigue syndrome. *Am. J. Med.* 89, 561–568.
- Luber-Narod, J., Austin-Ritchie, T., Banner, B., Hollins, C., Maramag, C., Price, H., et al., 1996. Experimental autoimmune cystitis in the Lewis rat, a potential animal model for interstitial cystitis. *Urol. Res.* 24, 367–373.
- Mackay, I.R., Burnet, F.M., 1963. The Autoimmune Diseases: Pathogenesis, Chemistry and Therapy. Charles C Thomas, Springfield, IL, pp. 242–243.
- Maclarens, N.K., Blizzard, R.N., 1985. Adrenal autoimmunity and autoimmune polyglandular syndromes. In: Rose, N.R., Mackay, I.R. (Eds.), The Autoimmune Diseases. Academic Press, Orlando, FL, pp. 201–225.
- McAdam, L.P., O'Hanlan, M.A., Bluestone, R., Pearson, C.M., 1976. Relapsing polychondritis. Prospective study of 23 patients and a review of the literature. *Medicine (Baltimore)* 55, 193–215.
- Medical Staff of the Royal Free Hospital, 1957. An outbreak of encephalomyelitis in the Royal Free Hospital Group, London, in 1955. *Br. Med. J.* 2, 1436–1437.
- Michet Jr., C.J., McKenna, C.H., Luthra, H.S., O'Fallon, W.M., 1986. Relapsing polychondritis. Survival and predictive role of early disease manifestations. *Ann. Intern. Med.* 104, 74–78.
- Newman, L.S., Rose, C.S., Maier, L.A., 1997. Sarcoidosis. *N. Engl. J. Med.* 336, 1224–1234.
- Nicholls, H., 2012. Eyes wide shut. *New Sci.* 24 (March), 48–51.
- Ochs, R.L., Stein Jr., T.W., Peebles, C.L., Gittes, R.F., Tan, E., 1994. Autoantibodies in interstitial cystitis. *J. Urol.* 151, 587–592.
- Ogura, K., Matsumoto, T., Aoki, Y., Kitabatake, T., Fujisawa, M., Kojima, K., 2010. IgG4-related tumour-forming mastitis with histological appearances of granulomatous lobular mastitis, comparison with other types of tumour-forming mastitis. *Histopathology* 57, 39–45.
- Oravisto, K.J., 1980. Interstitial cystitis as an autoimmune disease. A review. *Eur. Urol.* 6, 10–13.
- Oravisto, K.J., Alftahn, O.S., Jokinen, E.J., 1970. Interstitial cystitis: clinical and immunological findings. *Scand. J. Urol. Nephrol.* 4, 37–42.
- Oyama, N., Chan, I., Neill, S.M., Hamada, T., South, A.P., Wessagowitz, V., et al., 2003. Autoantibodies to extracellular matrix protein 1 in lichen sclerosus. *Lancet* 362, 118–123.
- Palace, J., Lang, B., 2000. Epilepsy, an autoimmune disease. *J. Neurol. Neurosurg. Psychiatr.* 69, 711–714.
- Pallais, J.C., Kifor, O., Chen, Y.-B., Slovik, D., Brown, E.M., 2004. Acquired hypocalciuric hypercalcemia due to autoantibodies against the calcium-sensing receptor. *N. Engl. J. Med.* 351, 362–364.
- Park, S.-H., Weiss, A., Benlagha, K., Kyin, T., Teyton, L., Bendelac, A., 2001. The mouse CD1d-restricted repertoire is dominated by a few auto-reactive T-cell families. *J. Exp. Med.* 193, 893–904.
- Patrick, J., Lindstrom, J., 1973. Autoimmune response to acetyl choline receptor. *Science* 180, 871–872.
- Pearson, C.M., Kline, H.M., Newcomer, V.D., 1960. Relapsing polychondritis. *N. Engl. J. Med.* 263, 51–58.
- Peeker, R., Atanasiu, L., Logadottir, Y., 2003. Intercurrent autoimmune conditions in classic and non-ulcer interstitial cystitis. *Scand. J. Urol. Nephrol.* 37, 60–63.
- Peltola, J., Kulmala, P., Isojärvi, J., Saiz, A., Latvala, K., Palmio, J., et al., 2000. Antibodies to glutamic acid decarboxylase in patients with therapy resistant epilepsy. *Neurology* 55, 46–50.
- Penna, G., Fibbi, B., Amuchastegui, S., Cossetti, C., Aquilano, F., Laverty, G., et al., 2009. Human benign prostatic hyperplasia stromal cells as inducers and targets of chronic immuno-mediated inflammation. *J. Immunol.* 182, 4056–4064.
- Peterson, P.K., Shepard, J., Macres, M., Schenck, C., Crosson, J., Rechtman, D., et al., 1990. A controlled trial of intravenous immunoglobulin G in chronic fatigue syndrome. *Am. J. Med.* 89, 554–560.
- Quek, A.M.L., Britton, J.W., McKeon, A., So, A., Lennon, V.A., Shin, C., et al., 2012. Autoimmune epilepsy. Clinical characteristics and response to immunotherapy. *Arch. Neurol.* 69, 582–593.
- Ramaekers, V.T., Rothenberg, S.P., Sequeira, J.M., Opladen, T., Blau, N., et al., 2005. Autoantibodies to folate receptors in the cerebral folate deficiency syndrome. *N. Engl. J. Med.* 352, 1985–1991.
- Ridley, J.H., 1968. The histogenesis of endometriosis: a review of facts and fancies. *Obstet. Gynecol. Surv.* 23, 1–35.
- Rogers, S.W., Andrews, P.I., Gahring, L.C., Whisenand, T., Cauley, K., Crain, B., et al., 1994. Autoantibodies to glutamate receptor GluR3 in Rasmussen's encephalitis. *Science* 265, 648–651. *J. Med.* 350, 134142.

- Rothenberg, S.P., da Costa, M.P., Sequeira, J.M., Cracco, J., Roberts, J.L., Weedon, J., et al., 2004. Autoantibodies against folate receptors in women with a pregnancy complicated by neural-tube defect. *N. Engl. J. Med.* 350, 134–142.
- Saiz, A., Blanco, Y., Sabater, L., Gonzalez, F., Bataller, L., Casamitjana, R., et al., 2008. Spectrum of neurological syndromes associated with glutamic acid decarboxylase antibodies, diagnostic clues for this association. *Brain* 131, 2553–2563.
- Sakkas, L.T., Platsoukas, C.D., 2007. The role of T cells in the pathogenesis of osteoarthritis. *Arthritis Rheum.* 56, 409–424.
- Sampson, J.A., 1921. Perforating haemorrhagic (chocolate) cysts of the ovary: their importance and especially their relation to pelvic Adenomas of the endometrial type ("adenomyoma" of the uterus, rectovaginal septum, sigmoid etc.). *Arch. Surg.* 3, 245–323.
- Sant, G.R., Theoharides, T.C., 1994. The role of the mast cell in interstitial cystitis. *Urol. Clin. N. Am.* 21, 41–53.
- Sarkurai, T., Amemiya, A., Ishii, M., Matzusaki, I., Chemelli, R.M., et al., 1998. Orexin and orexin receptors, a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behaviour. *Cell* 92, 573–585.
- Schwartz, R.S., 2005. Autoimmune folate deficiency and the rise and fall of "horror autotoxicus" [d.q.]. *N. Engl. J. Med.* 352, 1948–1950.
- Seemann, N., 1967. Untersuchungen zur Häufigkeit der lymphozytären Parathyreoiditis. *Dtsch. Med. Wschr.* 92, 106–108.
- Shelley, W.B., Hurley, H.J., 1960. An unusual autoimmune syndrome. Erythema annulare centrifugum, generalized pigmentation and breast hypertrophy. *Arch. Dermatol.* 81, 889–897.
- Shipton, E.A., 1965. Hunner's ulcer (chronic interstitial cystitis). A manifestation of collagen disease. *Br. J. Urol.* 37, 443–449.
- Shulman, S.T., 1999. Pediatric autoimmune neuropsychiatric disorders associated with streptococci (PANDAS). *Pediatr. Infect. Dis. J.* 18, 281–282.
- Shulman, S., Yantorno, C., Barnes, G.W., Conder, M.J., Soanes, W.A., Witebsky, E., 1965. Studies on autosensitization to prostatic tissue and related tissues. *Ann. N.Y. Acad. Sci.* 124, 279–291.
- Silk, M.R., 1970. Bladder antibodies in interstitial cystitis. *J. Urol.* 103, 307–309.
- Skowera, A., Stewart, E., Davis, E.T., Cleare, A.J., Unwin, C., Hull, L., et al., 2002. Antinuclear autoantibodies in Gulf War-related illness and chronic fatigue syndrome patients. *Clin. Exp. Immunol.* 129, 354–358.
- Smith, A.J., Jackson, M.W., Neufing, P., McEvoy, R.D., Gordon, T.P., 2004. A functional autoantibody in narcolepsy. *Lancet* 364, 2122–2124.
- Solomon, I.L., Blizzard, R.M., 1963. Autoimmune disorders of the endocrine glands. *J. Pediatr.* 63, 1021–1033.
- Switchenko, A.C., Kauffman, R.S., Becker, M., 1991. Are there antiendometrial antibodies in sera of women with endometriosis? *Fertil. Steril.* 56, 235–241.
- Taneja, V., Griffiths, M., Behrens, M., Luthra, H.S., David, C.S., 2003. Auricular chondritis in NOD.DQ8.A $\beta$ 0 (Ag7 2/2) transgenic mice resembles human relapsing polychondritis. *J. Clin. Invest.* 112, 1843–1850.
- Taylor, P.V., Maloney, M.D., Campbell, J.M., Skerrow, S.M., Nip, M.M.C., Parmar, A., et al., 1991. Autoreactivity in women with endometriosis. *Br. J. Obstet. Gynecol.* 98, 680–684.
- Terato, K., Shimozuru, Y., Katayama, K., Takemitsu, Y., Yamashita, I., Miyatsu, M., et al., 1990. Specificity of antibodies to type II collagen in rheumatoid arthritis. *Arthritis Rheum.* 33, 1493–1500.
- Tillikainen, A., Lassus, A., Karvonen, J., Vartiainen, P., Julin, M., 1980. Psoriasis and HLA-Cw6. *Br. J. Dermatol.* 102, 179–184.
- Tomaszewski, J.E., Brooks, J.S., Hicks, D., Livolsi, V.A., 1992. Diabetic mastopathy: a distinctive clinicopathologic entity. *Hum. Pathol.* 23, 780–786.
- Vernino, S., Low, P.A., Fealey, R.D., Stewart, J.D., Farrugia, G., Lennon, V.A., 2000. Autoantibodies to ganglionic acetylcholine receptors in autoimmune autonomic neuropathies. *N. Engl. J. Med.* 343, 847–855.
- Vernino, S., Ermilov, L.G., Sha, L., Szurszewski, J.H., Low, P.A., Lennon, V., 2004. Passive transfer of autoimmune autonomic neuropathy to mice. *J. Neurosci.* 24, 7037–7042.
- Vernon, S.D., Reeves, W.C., 2005. Evaluation of autoantibodies to common and neuronal cell antigens in chronic fatigue syndrome. *J. Autoimmune Dis.* 2, 5.
- von Mikecz, H., Konstantinov, K., Buchwald, D.S., Gerace, I., Tan, E.M., 1997. High frequency of autoantibodies to insoluble cellular antigens in patients with chronic fatigue syndrome. *Arthritis Rheum.* 40, 295–305.
- Wang, Q., Rozelle, A.L., Lepus, C.M., Scanzello, C.R., Song, S.S., Larsen, D.M., et al., 2011. Identification of a central role for complement in osteoarthritis. *Nat. Med.* 17, 1674–1679.
- Weed, J.C., Arquembourg, P.C., 1980. Endometriosis: can it produce an autoimmune response resulting in infertility? *Clin. Obstet. Gynecol.* 23, 885–893.
- Wilson, T.J., Hertzog, P.J., Angus, D., Munnery, L., Wood, E.C., Kola, I., 1994. Decreased natural killer cell activity in endometriosis patients: relationship to disease pathogenesis. *Fertil. Steril.* 62, 1086–1088.
- Xiang, Y., Sekine, T., Nakamura, H., Imajoh-Ohmi, S., Fukuda, H., Nishioka, K., et al., 2004. Proteomic surveillance of autoimmunity in osteoarthritis: identification of triosephosphate isomerase as an autoantigen in patients with osteoarthritis. *Arthritis Rheum.* 50, 1511–1521.
- Yamaguchi, A., Katsuyama, K., Nagahama, K., Takai, T., Aoki, I., Yamanaka, S., 2004. Possible role of autoantibodies in the pathophysiology of GM2 gangliosides. *J. Clin. Invest.* 113, 200–208.
- Yang, C.L., Brinkmann, J., Rui, H.F., Vehring, K.H., Lehmann, H., Kakow, J., et al., 1993. Autoantibodies to cartilage collagens in relapsing polychondritis. *Arch. Dermatol. Res.* 285, 245–249.

# Index

Note: Page numbers followed by “f,” “t,” and “b” refer to figures, tables, and boxes, respectively.

## A

- A20-deficient mice, 540–541
- Abatacept, 536–539, 686, 724, 1416t, 1427–1428
- Aberrant antibodies, 9–10
- Aberrant SUMOylation, 437
- Abnormal liver tests, 1119–1120
- Absent in melanoma 2 (AIM2), 202
- Acanthamoeba castellanii*-induced EAE, 366
- Accelerated atherosclerosis, 1074
- Acetylation
  - of histones, 338
  - marks in rheumatoid arthritis immune cells, 435
- Acetylation of lysine 27 of H3 (H3K27ac), 432–433
- Acetylcholine (ACh), 1012–1013
- Acetylcholine receptor (AChR), 270, 1013–1014
  - antibodies, 1018–1019
  - pathogenicity, 1018
  - cellular immunology of AChR MG, 1022
- Acquired aplastic anemia, 923
- Acquired FVII deficiency, 942
- Acquired FX deficiency, 946
- Acquired hemophilia A (AHA), 943
- Acquired vWD (avWD), 948
- ACTH test, 798–799, 799t
- Activated B-cell type (ABC), 641
  - of PBC, 1152–1153
- Activated partial thromboplastin time (aPTT), 939
- Activated phosphoinositide-3-kinase δ syndrome (APDS), 518
- Activated protein C (APC), 623
- Activated prothrombin complex concentrate (aPCC), 941
- Activated thromboplastin time (aPTT), 621
- Activation-induced cell death (AICD), 77, 541
- Activation-induced cytidine deaminase (AID), 175, 516–517
- Active demethylation, 431
- Active immunization, 1042, 1054
- Acute adrenal failure, 806
- Acute anterior uveitis (AAU), 1044
- Acute disseminated encephalomyelitis, 965–966
- Acute hepatic episodes, 1119
- Acute human hantavirus infection, 237
- Acute inflammatory demyelinating polyradiculoneuropathy (AIDP), 988–989

- Acute inflammatory neuropathies, 987, 997
- Acute interstitial nephritis (AIN), 1360
- Acute kidney injury model (AKI model), 1355, 1358–1359
- Acute malar rash, 562
- Acute motor and sensory axonal neuropathy (AMAN), 988
- Acute motor and sensory neuropathy, 989
- Acute motor axonal neuropathy (AMAN), 988–989, 990f
- Acute neuropathies, 988–997
- Acute respiratory distress syndrome (ARDS), 613–614, 1336
- Adalimumab, 686, 886, 1045, 1059, 1416t, 1425
- Adaptive immune responses, 51–59
  - antibodies, 56–57, 56t
  - antigen receptors, diversity of, 52f
  - B-cell development and functions, 55–56
  - functional activities of T cells, 53–55
  - secondary lymphoid tissues, 57–59
  - T-cell development, 51–53
- Adaptive immune system, 558–559, 1052
  - cells, 19–20
- Adaptive immunity, 32, 559. *See also* Cellular immunity
  - AIP, 1179–1181
  - B cells, 559
  - T cells, 559
- Adaptive response, 45–46
- Addison, Thomas, 732, 789–790
- Addison's disease (AD), 500–501, 741, 789–792
  - autoantibodies identification to 21-hydroxylase, 797–798
  - autoantibodies identification to steroidogenic enzymes, 798
  - autoantigen identification, 797
  - autoimmune, 792–793, 802–803
    - diagnosis, 800–802
    - different clinical presentations, 802–808, 803t
    - family history and genetic predisposition, 793–794
    - natural history, 798–800
  - cellular immunity, 794–795
  - epidemiology, 791–792
  - histopathology
    - diffuse lymphocytic adrenalitis, 793
    - focal lymphocytic adrenalitis, 792
  - humoral immunity, 795–796
  - imaging, 801f, 802

- mortality, 807
- osteoporosis, 807–808
- therapy, 804–806
- Addressable laser bead immunoassays (ALBIA), 1370
- Adenoids, 57
- Adenosine deaminase (ADA), 515
- Adenylyl uridine-rich element (ARE), 1155–1156
  - del<sup>−/−</sup> mice, 1155–1156
- Adipocyte, 26
- Adipose tissue-derived mesenchymal stem cells, 1058
- Adjuvants to T-cell anergy, 69
- Adaptive immunity, 878–879
- Adoptive transfer-EAN (AT-EAN), 997
- Adrenal androgens, 790
- Adrenal cortex, 790
- Adrenal cortex autoantibodies (ACAs), 793, 798–799
- Adrenal crisis. *See* Acute adrenal failure
- Adrenalin, 793
- Adrenals, 789–790
  - anatomy and physiology of, 790, 791f
- Adult mice immunization, AOD in, 1243–1244
- Adult-onset generalized MG, 1018
- Adventitia, 1318
- Adventitial macrophages, 1322
- Adverse effects (AEs), 1419
- Affinity maturation, 175
- Age-related macular degeneration (AMD), 269
- Aggressive immunosuppression, 1052, 1059
- Agonist selection of self-reactive T cells, 70–72
- Agouti alleles, 430
- Agrin, 1019–1020
- Aicardi–Goutières disease, 298–299
- AIDS, 470
- Air pollution, 355
- Aire-deficient mouse as model for APS-1, 739
- AIRE<sup>−/−</sup> mice, 100
- Akita mouse, 500–501
- Alanine (A), 978
- Alanine aminotransferase (ALT), 1157
- Alarms, 287
- Alemtuzumab, 927–928, 974, 976, 1442
- Alexine, 10
- Alkaline phosphatase (ALP), 1156–1157
- Alkylating agents, 1045

- Allelic exclusion, 155  
 Allergic granulomatosis, 1072–1073  
 Allergy, 12–13  
 Alloantibodies, 945  
 Alloxan, 500–501  
 Alopecia areata (AA), 503, 1211–1215  
     autoantibodies as potential immunologic markers, 1215  
     autoimmune features, 1212–1213  
     clinical, pathologic, and epidemiologic features, 1211–1212  
     genetic features, 1213–1214  
     inflammatory response, 1215†  
     major susceptibility genes, 1214†  
     pathologic effector mechanisms, 1215  
     in vivo and in vitro models, 1214  
 Alopecia totalis (AT), 1211  
 Alopecia universalis (AU), 1211  
 $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), 1083, 1093–1094  
 encephalitis, 1087  
 $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), 1083  
 Alpha-enolase, 823  
 $\alpha$ -galactosylceramide ( $\alpha$ GalCer), 119, 128–129  
 17 $\alpha$ -hydroxylase (17 $\alpha$ -OH), 795–796  
 $\alpha$ -ketoglutarate, 194  
 $\alpha$ -methyldopa, 904  
 Alpha-myosin heavy chain, 1275  
 $\alpha$ 1 integrin, 584  
 Alternative treatments, 1134–1135  
 Alzheimer's disease (AD), 270  
 Amaurosis fugax, 1316–1317  
 Amboceptors, 263  
 Megakaryocytic thrombocytopenia, 923  
 American Association for Study of Liver Diseases (AASLD), 1133  
 American College of Rheumatology (ACR), 635–636  
 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR), 716  
 American Rheumatism Association (ARA), 577  
 American Society of Hematology (ASH), 913  
 American Thoracic Society (ATS), 1336  
 Aminosalicylates (5-ASA), 876, 1420–1421  
 5-Aminosalicylic acid. *See* Aminosalicylates (5-ASA)  
 Amphiphysin, 1095–1096  
 Amphotericin B, 741  
 Amyotrophic lateral sclerosis, 1020  
 Anagen, 1212  
 Anakinra (Kineret), 683, 1416t, 1426  
 Anaphylatoxins, 267–268  
 ANCA-associated vasculitides (AAVs), 247–248, 557, 1071, 1358  
     NET and AAVs, 247–248  
     neutrophil-induced vasculitic organ damage, 247  
 ANCA-associated vasculitis. *See* ANCA-associated vasculitides (AAVs)  
 Androgens, 422  
 Anemia, 833, 903  
 Anergy, 177, 179  
 Angiotensin-converting enzyme inhibitors (ACE inhibitors), 1272–1273  
 Anifrolumab, 568  
 Animal models, 378, 497b, 533, 1001–1002, 1042, 1057–1058, 1131–1132  
     for AD, 793  
     advantages and disadvantages, 497–498  
     of AIHA, 898  
     AIP, 1182  
     ANCA, 1298  
     of aplastic anemia, 926  
     APS, 739  
     of autoimmune disease, 20  
         in kidney, 1358–1359  
     of autoimmune hypophysitis, 824–825  
     of celiac disease, 482–483, 857  
     of colitis, 878  
     CV, 1302  
     DCM, 1277–1279  
     of disease, 993–995  
     for IBD, 880–881  
     IgA vasculitis, 1304  
     IgG4-RD, 720  
     KD, 1292  
     of MS, 93  
     of multiple sclerosis, 481  
     myocarditis, 1277–1279  
     of organ-specific autoimmunity, 493–505  
     PAN, 1290  
     of PBC, 1154–1156  
     with relevance for pathogenesis of SpA, 697–698  
     SLE, 560  
     spontaneous, 739  
     systemic autoimmunity  
         genetically manipulated models, 536–544  
         induced models, 544–545  
         spontaneous models, 534–536  
         thymectomy, 739  
         for type 1 diabetes, 483  
 Anionic phospholipid phosphatidylserine, 296  
 Anitschkow cells, 1262  
 Ank/ank mice, 535  
 Ankylosing spondylitis (AS), 469, 691, 1421  
 Anterior uveitis, 1036–1037  
 Anti-ADAMTS13 autoantibodies, 938  
 Anti-b2-glycoprotein-I antibody, 615–616  
 Anti-B2GPI antibodies, 616  
 Anti-CD20-targeted therapy, 724  
 Anti-CTLA4 therapy, 27  
 Anti-DNA antibodies, 1357–1358  
 Anti-dsDNA antibodies, 321  
 Anti-*E. coli* outer membrane porin (OmpC), 882  
 Anti-four and half LIM domains protein (FHL1), 706  
 Anti-GBM nephritis model, 1358  
 Anti-HSP70 antibodies, 1053, 1055  
 Anti-hydroxymethylglutaryl coenzyme A reductase (HMGCR), 704–705, 708  
 Anti-IL-6, 280  
 Anti-IL12/13 therapies, 887  
 Anti-La antibodies, 558  
 Anti-MAG paraproteinemic demyelinating peripheral neuropathy (anti-MAG PDPN), 1000  
 Anti-N-methyl-D-aspartate receptor encephalitis, 1084–1085  
 Anti-RA33, 662  
 Anti-RNA polymerase III (RNAP), 589  
 Anti-Ro antibodies, 558, 643, 645  
 Anti-TG2 antibodies, 853  
 Anti-TNF agents, 496  
     therapy, 885  
 Anti-x cell receptors, 11  
 Anti- $\beta$ 2-glycoprotein I ( $\beta$ 2GPI), 249  
 Antiannexin A5 antibodies, 619  
 Anti-asialoglycoprotein receptor (ASGPR), 1127  
 Antibodies (Abs), 56–57, 56t, 1015–1016, 1016t, 1054, 1055f, 1067  
     detection of, 1081  
     functional effects of, 991–993  
     to neuronal surface proteins, 1086–1088  
     nomenclature, 1081  
     production, 5, 976  
     receptor, 9  
     specificity adequation to target disease, 1440–1445  
     targeting intracellular and cell surface antigens, 1078–1081, 1079f  
 Antibodies to citrullinated peptide antigens (ACPA), 478  
 Antibodies to disintegrin and metalloproteinase with thrombospondin type 1 motif 13 (ADAMTS13), 269, 937–939  
 Antibody-associated clinical syndromes, 1083–1093  
     AE and limbic encephalitis, 1083–1086  
     encephalitis associated with antibodies to neuronal surface proteins, 1086–1088  
     isolated myopathies and visual loss, 1090  
     neuromyelitis optica and further disorders, 1091–1093  
     OMS, 1090  
     SCD, 1088–1089  
     SPSD, 1089–1090  
 Antibody-associated diseases of CNS, 1067, 1078–1099  
     antibodies nomenclature, 1081  
     antibodies targeting intracellular and cell surface antigens, 1078–1081  
     antibody-associated clinical syndromes, 1083–1093  
     diagnostical considerations, 1081  
     principles of treatment, 1081–1083  
         principles of immunotherapy, 1082–1083  
         tumor detection and management, 1082  
         target antigens, 1093–1099  
 Antibody-coated target cells, 48  
 Antibody-dependent cellular cytotoxicity (ADCC), 48, 270, 320, 323, 724, 902

Antibody-independent activity of B cells in tolerance, 181–183  
 antigen presentation by B cells, 181–182  
 cytokine production by B cells, 182  
 regulatory B cells, 182–183  
 Antibody-mediated autoimmune disease, 319–320  
 Antibody-mediated injury, 755–756  
 Anticandidal drugs, 741  
 Anticardiolipin (aCL), 615, 1074  
 Anticitrullinated peptide antibodies (ACPAs), 475, 661–662, 1441  
 Anticoagulant disorders, 939  
 autoimmune inhibitors  
   to factor IX, 945  
   to factor V, 941–942  
   to factor VII, 942  
   to factor VIII, 942–945  
   to factor X, 946  
   to factor XI, 946  
   to factor XII, 947  
   to factor XIII, 947–948  
   to fibrinogen and fibrin, 939, 940f  
   to proteins, 949  
   to prothrombin and thrombin, 940–941  
   to vWF, 948  
 Anti-cyclic citrullinated peptide (Anti-CCP), 1418–1419  
 Antidouble-stranded DNA antibodies (Anti-dsDNA antibodies), 555, 558  
 Antifibrillarin, 594–595  
 Antifibrotic agents, 1349  
 Antigaelectin-3 autoantibodies, 719  
 Antiganglioside antibodies, 988–989  
 in GBS variants, 991  
 Antigen-independent B-cell development.  
*See also* B-cell development  
 cellular environments, 159–160  
 early commitments to, 160–161  
 Antigen-presenting cells (APCs), 19, 69, 93, 120–121, 264, 277, 307–308, 399–400, 773–774, 860, 904, 970, 1128, 1258, 1415–1416  
 in GCA, 1320–1322  
 Antigenic determinants, 6  
 Antigens (Ags), 213, 540  
 antigen-driven B-cell activation, 172  
 antigen-experienced effector T cells, 75  
 antigen-selection model, 5  
 antigen-specific  
   immunotherapy, 1402  
   therapies, 496  
   Tregs, 1136  
 antigen–antibody complexes, 558  
 autophagy pathways during antigen presentation, 310–312  
 cell therapy using antigen receptor gene, 1449–1450  
 depots, 174–175  
 processing following B-cell activation, 173  
 recognition by lymphocytes, 46f  
 sequestering, 74–75  
 specificity, 5  
 Antiglial antibodies  
 neurological syndromes

associated with GFAP-Abs, 1093  
 associated with MOG-Abs, 1092–1093  
 neuromyelitis optica and further disorders  
   associated with, 1091–1093  
 NMOSD, 1091–1092  
 Antiglobulin, 897–898  
 Antiglomerular basement disease, 1356  
 Antiinflammatory cytokines, 978  
 Antiinflammatory gut microbiome, 1401  
 Anti–liver  
   cytosol type 1 antibodies, 1125f  
   kidney microsomal type 1 antibodies, 1125f  
 Antimalarials, 1420  
   drugs, 1416t  
 Antimetabolites, 1045  
 Anti–mitochondrial autoantibody (AMA), 1124–1125, 1149–1150  
   AMA-negative PBC, 1157–1158  
   epitopes, 1151–1152  
 Antimyelin antibody-mediated mechanism, 966–967  
 Antimyeloperoxidase (MPO-ANCA), 1288  
 Antineuronal antibodies, 1090  
 Antineuronal nuclear antibodies (ANNA)  
   ANNA-1, 1067  
   ANNA-3-Abs, 1096  
   HU (antineuronal nuclear antibodies 1), 1096  
   MA (paraneoplastic MA antigens), 1096–1097  
   RI (antineuronal nuclear antibodies 2), 1096  
 Antineutrophil antibodies  
   negative vasculitides, 1073–1076  
   positive vasculitides, 1071–1073  
 Antineutrophil cytoplasmic autoantibodies (ANCA), 195, 247, 253, 1054, 1287, 1293–1300, 1355  
 ANCA-induced GN model, 1358  
 ANCA–associated vasculitis, 346–347, 448–449  
 animal models, 1298  
 autoimmune features, 1296–1297  
 clinical features and disease associations, 1293–1294  
 diagnostic procedures, 1299  
 epidemiology, 1293  
 genetic features and environmental influences, 1297–1298  
 pathogenesis, 1295  
 pathological features, 1294–1295  
 treatment, 1299–1300  
 Antinuclear antibodies (ANAs), 479–480, 534–535, 555, 581, 678, 1052, 1118, 1124f, 1158, 1341, 1370, 1462–1463  
 Antiphosphatidylethanolamine (aPE), 617–618  
 Antiphosphatidylserine (aPS), 618  
 Antiphosphatidylserine/prothrombin antibodies (aPS/PT), 618–619  
 Antiphospholipid antibodies (aPL), 249, 607–608  
   aPL-associated nephropathy, 614  
   syndrome, 249  
 associated with GFAP-Abs, 1093  
 associated with MOG-Abs, 1092–1093  
 neuromyelitis optica and further disorders  
   associated with, 1091–1093  
 NMOSD, 1091–1092  
 Antiglobulin, 897–898  
 Antiglomerular basement disease, 1356  
 Antiinflammatory cytokines, 978  
 Antiinflammatory gut microbiome, 1401  
 Anti–liver  
   cytosol type 1 antibodies, 1125f  
   kidney microsomal type 1 antibodies, 1125f  
 Antimalarials, 1420  
   drugs, 1416t  
 Antimetabolites, 1045  
 Anti–mitochondrial autoantibody (AMA), 1124–1125, 1149–1150  
   AMA-negative PBC, 1157–1158  
   epitopes, 1151–1152  
 Antimyelin antibody-mediated mechanism, 966–967  
 Antimyeloperoxidase (MPO-ANCA), 1288  
 Antineuronal antibodies, 1090  
 Antineuronal nuclear antibodies (ANNA)  
   ANNA-1, 1067  
   ANNA-3-Abs, 1096  
   HU (antineuronal nuclear antibodies 1), 1096  
   MA (paraneoplastic MA antigens), 1096–1097  
   RI (antineuronal nuclear antibodies 2), 1096  
 Antineutrophil antibodies  
   negative vasculitides, 1073–1076  
   positive vasculitides, 1071–1073  
 Antineutrophil cytoplasmic autoantibodies (ANCA), 195, 247, 253, 1054, 1287, 1293–1300, 1355  
 ANCA-induced GN model, 1358  
 ANCA–associated vasculitis, 346–347, 448–449  
 animal models, 1298  
 autoimmune features, 1296–1297  
 clinical features and disease associations, 1293–1294  
 diagnostic procedures, 1299  
 epidemiology, 1293  
 genetic features and environmental influences, 1297–1298  
 pathogenesis, 1295  
 pathological features, 1294–1295  
 treatment, 1299–1300  
 Antinuclear antibodies (ANAs), 479–480, 534–535, 555, 581, 678, 1052, 1118, 1124f, 1158, 1341, 1370, 1462–1463  
 Antiphosphatidylethanolamine (aPE), 617–618  
 Antiphosphatidylserine (aPS), 618  
 Antiphosphatidylserine/prothrombin antibodies (aPS/PT), 618–619  
 Antiphospholipid antibodies (aPL), 249, 607–608  
   aPL-associated nephropathy, 614  
   syndrome, 249  
 associated with GFAP-Abs, 1093  
 associated with MOG-Abs, 1092–1093  
 neuromyelitis optica and further disorders  
   associated with, 1091–1093  
 NMOSD, 1091–1092  
 Antiglobulin, 897–898  
 Antiglomerular basement disease, 1356  
 Antiinflammatory cytokines, 978  
 Antiinflammatory gut microbiome, 1401  
 Anti–liver  
   cytosol type 1 antibodies, 1125f  
   kidney microsomal type 1 antibodies, 1125f  
 Antimalarials, 1420  
   drugs, 1416t  
 Antimetabolites, 1045  
 Anti–mitochondrial autoantibody (AMA), 1124–1125, 1149–1150  
   AMA-negative PBC, 1157–1158  
   epitopes, 1151–1152  
 Antimyelin antibody-mediated mechanism, 966–967  
 Antimyeloperoxidase (MPO-ANCA), 1288  
 Antineuronal antibodies, 1090  
 Antineuronal nuclear antibodies (ANNA)  
   ANNA-1, 1067  
   ANNA-3-Abs, 1096  
   HU (antineuronal nuclear antibodies 1), 1096  
   MA (paraneoplastic MA antigens), 1096–1097  
   RI (antineuronal nuclear antibodies 2), 1096  
 Antineutrophil antibodies  
   negative vasculitides, 1073–1076  
   positive vasculitides, 1071–1073  
 Antineutrophil cytoplasmic autoantibodies (ANCA), 195, 247, 253, 1054, 1287, 1293–1300, 1355  
 ANCA-induced GN model, 1358  
 ANCA–associated vasculitis, 346–347, 448–449  
 animal models, 1298  
 autoimmune features, 1296–1297  
 clinical features and disease associations, 1293–1294  
 diagnostic procedures, 1299  
 epidemiology, 1293  
 genetic features and environmental influences, 1297–1298  
 pathogenesis, 1295  
 pathological features, 1294–1295  
 treatment, 1299–1300  
 Antiphospholipid syndrome (APS), 445, 937, 1074  
   aPL, 607–608, 615–619  
    criteria-relevant, 615–616  
   CAPS, 614  
   classical clinical criterion, 607–608  
    Sydney Consensus Statement, 608t  
   classification vs. diagnostic criteria, 621  
   clinical features and disease associations, 609–614  
    OAPS, 610–611  
    thrombotic antiphospholipid syndrome, 611  
   complement system in, 625–626  
   diagnostic procedures, 621  
   epidemiology, 608  
   genetics, 620  
   historical aspects, 607–608  
   mechanisms, 621–626  
   mortality in, 626  
   noncriteria aPL antibodies, 616–619  
    antiannexin A5 antibodies, 619  
    aPE, 617–618  
    aPS, 618  
    aPS/PT, 618–619  
    aPT, 618  
    autoantibodies to domain 1 of b2-glycoprotein-I antibody, 617  
    IgA isotype, 616  
    low level antiphospholipid antibodies, 617  
    metaanalysis, 619  
    noncriteria APS manifestations, 611–614  
    obstetric manifestations, 624–625  
    risk assessment, 620  
    seronegative, 619–620  
    thrombotic manifestations, 623–624  
    treatment, 626–627  
 Antiphospholipid Syndrome Alliance For Clinical Trials and International Networking, 608  
 Antiproteinase 3 (Anti-PR3), 1288  
 Antiprothrombin antibodies (aPT), 618  
 “Antiself” immunocytes, 10  
 Anti–smooth-muscle antibodies, 1124f  
 Antisperm antibodies (ASA), 1241  
 Antisynthetase syndrome, 1343  
 Antithymocyte globulin (ATG), 926  
 Antiviral treatment, 1273  
 Antivoltage-gated potassium channels  
   antibody encephalitis, 1085–1086  
   contactin-associated protein-like-2 encephalitis, 1086  
   leucine-rich glioma inactivated-1 encephalitis, 1085–1086  
 ANTXR2, 697  
 Aortic regurgitation (AR), 1255–1256  
 Aortitis, 1317  
 Apheresis therapies, 1089–1090, 1092  
 Aphthous ulcers, 875  
 Apitopes, 1448  
 Aplastic anemia, 923, 1420. *See also* Autoimmune hemolytic anemia (AIHA)  
   animal models, 926

- Aplastic anemia (*Continued*)  
 aplastic anemia and clonality, 931  
 autoimmune features, 925–926  
 clinical, pathologic, and epidemiologic features, 924–925  
 diagnosis, 925t  
*eltrombopag*, 928–929  
 environmental features, 926  
 genetic features, 924  
 high-dose cyclophosphamide without BMT, 929  
 historic background, 923–924  
 HLA haploidentical BMT, 929–930  
 immunosuppressive therapy, 927–928  
 pathogenic mechanisms, 925–926  
 therapy, 926–927  
 ATG, 926  
 BMT, 926–927  
 BMT from unrelated donors, 927  
 CSA, 926  
 cyclophosphamide, 926–927
- Apolipoprotein B messenger RNA editing enzyme component 1 (AID/APOBEC), 431
- Apoptosis, 52–53, 291–292  
 apoptotic cells, 292  
   antiinflammatory effects of, 298  
   as sources of autoantigen, 293  
 apotopes, 293–294  
 in autoimmunity, 292–294  
 clearance of dead cells, 295–297  
   eat-me signals and receptors, 296–297  
   find-me signals, 295–296  
   phosphatidylserine, 296  
 defective, 292–293  
 excessive, 293
- Apoptosis-inducing factor (AIF), 292
- Apoptotic cells, 292, 295  
 clearance by macrophages, 204–205
- Apoptotic lymphocytes, 292
- Apoptotic neutrophils, 196
- Apoptoses, 293–294
- Apremilast, 1416t
- Aquaporin-4 (AQP4), 320, 1067, 1098
- Aquaporins, 1057
- Archetypal autoimmune disease, 1012
- Areflexia, 1025
- Arginine methylation, 433
- Arginine methyltransferase, 433
- Arrestin, 1035
- Arthritis with IBD, 692
- Arthrogryposis multiplex congenital, 1017
- Aryl hydrocarbon receptor ligands (AHR ligands), 94
- ASCEND trial, 1348
- Asparagine (Asn), 478
- Aspartate aminotransferase (AST), 1157
- Asthma, 124t, 126–127
- Asymptomatic islet autoimmunity, 770–771
- Atacicept, 568
- Atezolizumab, 826–827
- Atherosclerosis, 124t, 125–126
- Atherosclerotic cardiovascular disease, 640
- ATP-binding cassette subfamily F 1 (ABCF1), 1181
- Atrophic thyroiditis, 749–750
- Atypical form of HUS (aHUS), 269
- AU-rich elements (ARE), 698
- Aubagio. *See* Teriflunomide
- Autoantibodies (aab), 758, 836–838, 852–854, 914, 1052–1054, 1056, 1179, 1212, 1369  
 bullous pemphigoid, 1200–1201  
 effector mechanism in, 319–320, 320t  
*horror autotoxicus*, 10  
 identification  
   to 21-Hydroxylase, 797–798  
     in patients with AAD, 798  
     to steroidogenic enzymes, 798  
 as immunologic markers, 861–862  
 nature of Ehrlich's "contrivances", 11  
 passive transfer model, 1195  
 pathogenic role of, 1197  
 PF, 1197–1198  
 as potential immunologic markers, 841–842, 1197–1198, 1202  
 PV, 1194–1195  
 in SLE, 557–558
- Autoantibodies to citrullinated protein antigen (ACPA), 248
- Autoantibodies to insulin (IAA), 1402
- Autoantibodies to interferon types (IFNAbs), 798–799
- Autoantibodies to NALP5 (NALP5Abs), 798
- Autoantibodies to potassium channel regulator (KCNRGAbs), 798
- Autoantibodies to SOX9 (SOX9Abs), 798
- Autoantibody assays  
 advantages and disadvantages of EMR, 1380b  
 approaches to and standardizing  
   autoantibody testing, 1374–1376  
 clinical interpretation  
   and application of autoantibody testing, 1377–1378  
   of autoantibody testing, 1376b  
 contemporary and emerging technologies, 1371t  
 cost analysis, 1379–1381  
 CPG, 1378–1379  
 electronic medical records, 1379–1381  
 international efforts to standardize  
   autoantibody testing, 1381b  
 laboratory reports, 1379–1381  
 and relating technology platform, 1372t  
 spectrum of autoantibodies, 1373–1374  
 standardization and quality assurance, 1381–1382
- Autoantibody Standardization  
 Committee/International Union of Immunology Societies (ASSC/IUIS), 1381
- Autoantibody testing  
 approaches to and standardizing, 1374–1376, 1381b  
 by IIF patterns, 1375t  
 screening autoantibody tests for  
   systemic autoimmune rheumatic, 1375t
- Autoantigens, 779, 973, 996  
 autoantigen-based immunotherapy, 1403  
 autoantigen-sensitive phase, 161–162
- identification  
 of adrenal cortex autoantibodies, 797  
 of steroid-producing cells  
   autoantibodies, 797  
 soluble, 1447–1449
- Autocytotoxins, 12
- Autoimmune, 967  
 adrenalitis, 789–792  
 aggression, 1052–1053  
 antibodies, 270  
 autoimmune-susceptibility loci, 534  
 autonomic neuropathy, 1461–1462  
 avWS, 948  
 beta-cell disorder, 1392  
 cholangitis, 1157–1158  
 CNS, 1067  
 diabetes, 8  
 encephalitis, 1084, 1088  
 epilepsy, 1464  
 inflammatory eye diseases, 1462  
 inhibitors, 939  
 inner ear disease, 1051–1052, 1058  
 MN, 1360  
 neuropathies, 995, 995f  
 pathogenesis, 269, 1021, 1391–1392  
 polyendocrinopathies, 731  
 process, 1051  
 SNHL, 1051–1052  
 thrombocytopenia, 641, 904
- Autoimmune (type 1) diabetes, 769  
 classification, 776t  
 diagnosis, 770t  
   and classification, 771–772  
 epidemiology  
   asymptomatic islet autoimmunity, 770–771  
   symptomatic autoimmune diabetes, 771  
 etiology  
   environmental factors associated with first appearing autoantibodies, 774–775  
   genetic etiology of islet autoimmunity, 772–774
- intervention, 781
- pathogenesis, 775–780  
 cellular mechanisms, 779  
 environmental factors, 778–779  
 genetic factors, 777–778  
 humoral biomarkers, 779  
 pathology, 779–780  
 pathophysiology, 776–777  
 staging of autoimmune type 1 diabetes, 775f
- primary prevention, 780–781
- secondary prevention, 781
- in vivo* and *in vitro* models, 780
- Autoimmune Addison's disease (AAD), 791–793
- autoantibodies detection in, 798
- diagnosis  
   clinical manifestations, 800  
   general biochemical indices, 800  
   hormonal tests, 801–802
- different clinical presentations  
   of, 802–808
- hypothetical pathogenesis of, 796f
- natural history of, 798–800

- Autoimmune bullous skin diseases  
autoimmune blistering diseases of skin, 1193t  
bullous pemphigoid, 1200–1202  
PF, 1197–1199  
PV, 1193–1197  
subepidermal bullous diseases, 1202–1204  
treatment, 1204  
types of pemphigus, 1199–1200
- Autoimmune Diseases Working Party (ADWP), 1446
- Autoimmune diseases/disorders, 4–6, 8, 12, 94–95, 191–192, 235, 278, 286, 331, 345–346, 939, 968, 1015, 1395, 1437
- Apremilast, 1428
- bacterial metabolites influence immune response, 336
- basophils and IgE antibodies in bullous pemphigoid, 252
- IgE antibodies in other autoimmune disorders, 252
- in systemic lupus erythematosus, 251 therapeutic implications, 252
- biologic agents, 1424–1428
- B-cell-targeted therapies, 1427
- belimumab, 1427
- cytokine-targeted therapies, 1424
- IL-1 antagonists, 1426
- rituximab, 1427
- secukinumab, 1426
- T-cell-targeted therapies, 1427–1428
- tocilizumab, 1425–1426
- tumor necrosis factor inhibitors, 1425
- ustekinumab, 1426–1427
- breakthrough in, 1443–1445
- CD3 MAbs and autoimmune diabetes, 1443–1445
- cells of adaptive immune system, 19–20
- costs of therapies, 1429
- DCs and, 219–221 targeting, 220–221
- defective downregulation of immune response, 28–29
- defining, 20
- dietary metabolites influence immune response, 336
- environmental agents role in, 346–348, 347b
- environmental factors, 473
- eosinophils in  
in EGPA, 253–254  
in other vasculitis, 254
- evidence for diet with, 332–333
- flares and remissions during, 31
- general considerations, 1418
- genetic and epigenetic interactions in, 451–452
- genetic factors, gut microbiome in, 475–476
- goals for future, 33
- gut microbiome  
evidence for, 332–333  
major products of, 334–336
- HLA class II association with, 471–472, 472t
- identifying and defining, 348–349, 348t
- immune homeostasis metabolite-sensing GPCRs, 336–338
- immune suppression, 1418
- immune system activation, 26–27
- infectious agents, 473–474
- innate immune activation, 18
- IVIG, 1428–1429
- in kidney, 1355
- animal models, 1358–1359
- autoimmune features, 1357–1358
- clinical features and disease associations, 1361–1363
- environmental factors, 1356
- epidemiology, 1356–1357
- genetics, 1357
- history, 1355–1356
- pathological features, 1359–1360
- transplantation, 1360
- microbiome-mediated gut, 336–338
- noninfectious agents associated with, 349–356
- nonspecific antiinflammatory drugs, 1419–1420
- ω-3 fatty acids, 338–339
- regulatory lymphocytes, 29–30
- therapeutic advances, 32–33
- tissue damage mechanisms, 31–32
- transcriptional and epigenetic effects, 338
- treatments of rheumatic diseases
- antimalarials, 1420
  - AZA, 1423–1424
  - calcineurin inhibitors, 1424
  - cyclophosphamide, 1422
  - leflunomide, 1421
  - MMF, 1423
  - MTX, 1421–1422
  - sulfasalazine, 1420–1421
  - tryptophan catabolites, 338–339
  - vitamin D in, 477
- Autoimmune encephalomyelitis (AE), 1083–1086
- anti-N-methyl-D-aspartate receptor encephalitis, 1084–1085
- antivoltage-gated potassium channels antibody encephalitis, 1085–1086
- Autoimmune features, 999–1000, 1042–1043, 1124–1127
- AIP, 1179–1181
- adaptive immunity, 1179–1181
  - complement activation system, 1179
  - innate immunity, 1179
- antiganglioside antibodies in GBS variants, 991
- anti-liver cytosol type 1 antibodies, 1125f
- anti-liver kidney microsomal type 1 antibodies, 1125f
- antinuclear antibodies, 1124f
- anti-smooth-muscle antibodies, 1124f
- bullous pemphigoid
- autoantibodies, 1200–1201
  - T-cell activation, 1201
- functional effects of antibodies, 991–993
- gangliosides in peripheral nerve, 991
- molecular mimicry, 989–990
- PF
- autoantibodies, 1197–1198
  - T-cell activation, 1198
- PV
- autoantibodies, 1194–1195
  - T-cell activation, 1195
- Autoimmune gastritis. *See also* Pernicious anemia
- Autoimmune hemolytic anemia (AIHA), 268, 516, 612, 897–898. *See also* Aplastic anemia
- animal models of, 898
  - classification, 898, 898t
  - clinical signs of, 902
  - drugs, 904
  - etiology, 903–904
  - gender and age, 904
  - genetic predisposition, 903
  - infectious agents, 904
  - laboratory diagnosis, 902–903
  - neoplasia, 904
  - predisposing factors, 903–904
  - RBC autoantigens, 902
  - RBC destruction mechanisms, 899–902
  - cold reactive antibodies, 899
  - hemolysis by warm antibodies, 901–902
  - pathogenicity of warm reactive IgG antibodies, 899–901
  - warm reactive antibodies, 899
- treatment of, 903
- Autoimmune hepatitis (AIH), 500–503, 1117–1118, 1157, 1161, 1177
- animal models, 1131–1132
  - autoimmune features, 1124–1127
  - clinical features, 1119–1123
  - criteria for diagnosis, 1121t
  - diagnostic procedures, 1119–1123
  - disease associations, 1119–1123
  - epidemiology, 1118–1119
  - genetics, 1127–1128
  - historical aspects, 1117–1118
  - pathogenic mechanisms, 1128–1131
  - pathological features, 1123–1124
  - PBC with features with, 1161
  - perspectives, 1137
  - treatment, 1132–1137
  - alternative, 1134–1135
  - duration, 1135–1136
  - future treatment approaches, 1136–1137
  - liver transplantation, 1136
  - standard, 1132–1134
- Autoimmune hypophysitis, 820
- animal models, 824–825
  - of anterior lobe, 816
  - autoimmune features, 823
  - body of literature, 816–817
  - classification, 815
  - classification of sellar masses, 821t
  - clinical features, 818–822
  - definition, 815
  - diagnosis, 825
  - epidemiology, 816–817
  - genetic and environmental influences, 824
  - historical background, 816

- Autoimmune hypophysitis (*Continued*)  
 hypophysitis secondary to CTLA-4 blockade, 826–827  
 outcome, 826  
 pathological features, 822–823  
 treatment, 825–826
- Autoimmune lymphoproliferative syndrome (ALPS), 21, 284, 523
- Autoimmune myopathies  
 association of malignancy with myositis, 709–710  
 association of myositis and cancer, 705  
 autoantibodies, association of, 705–709  
 hydroxymethylglutaryl coenzyme A reductase autoantibodies, 707–709  
 myositis-specific, 706–707, 707<sup>t</sup>  
 autoantigens, expression of, 710  
 GrB-mediated cleavage, 710  
 MDA5 and Ro52, 710  
 characteristic pathology, 704–705  
 clinical and pathological descriptions, 704  
 defining, 703  
 dermatomyositis, 703  
 enhancing expression of myositis autoantigens, 710  
 epidemiology, 705  
 immune effector pathways, 710  
 immune-mediated necrotizing myopathy, 703  
 inclusion body myositis, 703  
 mechanisms, 709–710  
 polymyositis, 703  
 skin manifestations, 704  
 therapy, 711
- Autoimmune oophoritis, 1242–1245  
 experimental autoimmune ovarian disease, 1243–1245  
 AOD in adult mice immunization, 1243–1244  
 AOD in d3tx, 1243  
 clinical autoimmune disease of ovary, 1245  
 neonatal AOD induction, 1244  
 spontaneous autoimmune ovarian disease, 1243  
 tolerance mechanism for ovary autoantigens, 1242–1243
- Autoimmune orchitis, 1236–1242  
 associated with bacterial and viral infections, 1240–1241  
 clinical autoimmune disease of testis, 1241–1242  
 antibody response in vasectomy and cystic fibrosis, 1242  
 idiopathic male infertility, 1241  
 infertility and antisperm antibodies, 1242  
 orchitis associated with bacterial and virus infections, 1242  
 in dark mink, 1240  
 experimental autoimmune disease of testis, 1237–1241  
 classical experimental autoimmune orchitis, 1237–1240  
 in Lewis rat with transgenic human HLA B27/β2m, 1240
- post-vasectomy, 1240  
 tolerance mechanism for testis autoantigens, 1236–1237
- Autoimmune ovarian disease (AOD), 1242–1243  
 in adult mice immunization, 1243–1244  
 in d3tx, 1243
- Autoimmune pancreatitis (AIP), 715, 1173–1178  
 animal models, 1182  
 autoimmune features, 1179–1181  
 clinical features, 1175–1178  
 diagnostic procedures, 1182–1183  
 disease associations, 1174  
 endoscopic retrograde cholangiopancreatography, 1176<sup>f</sup>  
 epidemiology, 1174  
 gateway from autoimmune pancreatitis to IgG4-RD, 1173–1174  
 genetics, 1181–1182  
 historical progression from, 1174  
 IgG4-SC, 1177
- IgG4-related  
 hypertrophic pachymeningitis, 1176  
 kidney disease, 1177  
 lacrimal and salivary gland lesions, 1176  
 liver disease, 1177  
 lung disease, 1177  
 ophthalmic disease, 1176  
 periaortitis/periarteritis, 1178  
 pituitary and stalk lesions, 1176  
 prostate disease, 1178  
 retroperitoneal fibrosis, 1177  
 thyroid disease, 1177  
 pathological features, 1178–1179  
 perspectives, 1183  
 treatment, 1183
- Autoimmune polyendocrine syndrome (APS), 731, 1237, 1468  
 animal models, 739  
 spontaneous animal models, 739  
 thymectomy animal model, 739  
 APS-1, 731–734  
 Aire-deficient mouse as model, 739  
 manifestations and organ-specific autoantibodies, 733<sup>t</sup>, 734<sup>f</sup>  
 APS-2, 731, 734–735  
 HLA alleles and non-HLA genes, 737<sup>t</sup>  
 autoimmune features, 735–736  
 clinical, pathologic, and epidemiologic features, 732–735  
 environmental features, 738  
 genetic features, 736–738  
 historic background, 732  
 immunologic markers in diagnosis, 741  
 pathogenic mechanisms, 740–741  
 treatment and outcome, 741–742
- Autoimmune polyendocrinopathy, candidiasis, ectodermal dysplasia (APECED), 522, 732, 1128
- Autoimmune polyendocrinopathy syndrome type 1. *See Autoimmune polyendocrinopathy, candidiasis, ectodermal dysplasia (APECED)*
- Autoimmune polyglandular syndromes (APS), 793–794, 802, 823  
 clinical classification, 802<sup>b</sup>
- Autoimmune regulator gene (AIRE gene), 21, 522, 731, 736, 738, 793–794, 1023  
 deficiency, 1235
- Autoimmune regulator-positive (AIRE<sup>+</sup>), 516
- Autoimmune responses  
 activating factor in iNKT cells, 134–135  
 iNKT cells influence, 135–136
- Autoimmune SC (ASC). *See Sclerosing cholangitis (SC)*
- Autoimmune symphony  
 autoimmunity and autoimmune disease, 4–5  
 clonal balance and regulation, 5–6  
 epidemiology and prediction, 7–8  
 genetics and exposures, 6–7
- Autoimmune thyroid diseases (AITD), 438  
 DNA methylation, 438  
 histone tail modifications in, 438  
 noncoding RNAs in, 438
- Autoimmune thyroiditis (AT), 749–757  
 autoantibodies, 750–751  
 functional studies, 752  
 as potential immunological markers, 756  
 T-cell responses, 751–752  
 thyroglobulin antibodies, 750  
 TPO, 750–751  
 autoimmune features, 750–752  
 clinical, pathologic, and epidemiologic features, 749–750  
 environmental influences, 753–754  
 genetic features, 752–753  
 historic background, 749  
 pathologic effector mechanisms, 755–756  
 antibody-mediated injury, 755–756  
 T-cell mediated injury, 756  
 treatment and outcome, 756  
 types, 750<sup>t</sup>  
 in vivo models, 754–755  
 experimental autoimmune thyroiditis, 754  
 immunization-induced thyroiditis, 754  
 spontaneous autoimmune thyroiditis, 754–755
- Autoimmunity, 4–5, 310–311, 935, 987, 1056, 1118, 1466–1467  
 antigen role as driver, 27–28  
 apoptosis in, 292–294  
 autophagy in tolerance and, 312–313  
 BAFF, tonic signaling and, 180–181  
 and central tolerance, 24  
 challenges to Ehrlich thesis, 11–12  
 “Dark Ages” of, 14<sup>t</sup>  
 development, 773  
 epigenetics changes associated with environment triggers in, 452–454  
 genetic linkage studies, 390–394  
 genetics and epigenetics  
 contribution of epigenetic modifications and transcriptional regulation, 23  
 monogenic disease, 21  
 polygenic disease, 21–22  
 shared risk alleles, 22–23  
 genome-wide association studies, 394–406  
 gut microbiota role in, 30  
 hormones and, 23  
 humanized animal models, 479  
 macrophages and, 206<sup>f</sup>

- mechanisms of complement and Fc associations with, 389
- MHC and, 468–469
- necroptosis in, 294–295
- nonrheumatoid arthritis-associated human leukocyte antigen alleles, 480–481
- parthanatos in, 295
- pathogenesis, 245
- and peripheral tolerance, 24–25
- posttranslational modifications in, 477–479
- prevalence of, 20–21
- return of immunobiology, 13–14
- search for autoantibodies, 10–11
- shift to immunochemistry, 12–13
- smoking and, 474–475
- triggers of, 26–27
- A**
- Autoinflammatory periodic fever syndromes, 285
- Autoinflammatory syndromes, 1057
- Autologous serum skin test (ASST), 1227
- Autonomic neuropathy, 1461–1462
- Autophagosome, 305–306, 310–311
- Autophagy, 203, 880
- in B-cell development and activation, 308–309
  - HLA-B27 and, 470
  - in innate immunity, 309–310
  - pathways, 305–308
    - during antigen presentation, 310–312
    - fusing with endolysosomal compartment, 306f
    - noncanonical, 307–308
  - in T-cell development and activation, 308–309
  - in tolerance and autoimmunity, 312–313
- Autophagy-related 16-like 1 gene (ATG16L1 gene), 880
- Autophagy-related proteins (ATGs), 305–306, 926–928
- ATG5-deficient DCs process, 311
  - Atg7-deficient HPCs, 308–309
- Autoreactive cells, 969
- bystander activation of, 367–368
- Autoreactive intraepithelial lymphocytes, 854
- Autoreactive T cells, 473, 973, 1056
- infectious triggering of, 364
- Autoreactivity, 20–21
- Autosomal-recessive disorder, 524
- Autotoxins, 10
- Avelumab, 826–827
- Avidity model of thymocytes, 70–71, 70f
- Axial spondyloarthritis (Axial SpA), 691–692
- Axonal loss, 964
- 5-Aza-2'-deoxycytidine (5-AZA), 436
- 5-Azacytidine, 455
- Azanucleosides, 455
- Azathioprine (AZA), 566, 825–826, 885, 903, 918, 1002, 1024, 1045, 1073, 1077, 1133, 1183, 1416t, 1423–1424
- Azulfidine. *See* Sulfasalazine
- B**
- B cell–activating factor (BAFF), 180, 559, 568, 914, 1179, 1428–1429
- in B-cell development and survival, 180
- tonic signaling, and autoimmunity, 180–181
- of tumor necrosis factor family (BAFF), 517
- B lymphocytes, 65, 155, 914
- targeting, 1445
- B lymphopoiesis, 158
- B regulatory cells (Bregs), 1043–1044
- B-cell activating factor (BAFF), 243–244, 284–285, 1025, 1428–1429
- B-cell activation, 171–173
- amplification and modulation of B-cell activation signals, 173
  - antigen processing following, 173
  - antigen-driven B-cell activation, 172
  - germinal center, 176–177
  - and germinal center response, 175
  - location, 174–175
  - requiring interaction with T helper cells, 173–174
- T cell–independent antibody responses, 177
- B-cell development
- autoantigen-sensitive phase, 161–162
  - B lymphopoiesis before IG repertoire generation, 158
  - checkpoint for emerging B-cell repertoire, 162–163
  - expression of IgL chains, 163–164
  - follicular B cells, 156
  - and functions, 55–56
  - generation, 155–156
  - intraepithelial B cells, 156–157
  - memory B cells, 157–158
  - sites and mechanisms of selection of  $\text{IgM}^+$  B cells, 164–165
  - waves, development in, 158–159
- B-cell maturation antigen (BCMA), 180
- B-cell receptors (BCR), 18, 24–25, 45–46, 155, 162–163, 172, 540
- activation, 182
  - features, 172b
- B-cell scaffold protein with ankyrin repeats 1 (BANK1), 403–404
- B-cell tolerance, 177–179
- antibody-independent activity, 181–183
  - B-cell anergy, 179
    - characteristics, 179
  - B-cell central tolerance, mechanisms of, 177
- B-cell development and survival
- BAFF in, 180
  - tonic signaling in, 180
- BAFF, tonic signaling, and autoimmunity, 180–181
- clonal deletion, 178
- defective, 178–179
- future directions, 166–167, 183
- receptor editing, 178
- defective, 178–179
- regulatory T cells, 181
- B-cells, 5, 24–25, 29–30, 55, 172–173, 559, 718, 973–974
- activation, 26
- antigen presentation by, 181–182
- apoptosis, 525
- autophagy in, 308–309
- B cell–targeted treatments, 724, 1427
- cytokine production by, 182
- function, 976
- maturation and activation in lymphoid organs, 174–177
- migration, 174–175
- peripheral tolerance, 181
- signature, 685
- and tolerance, 905
- B-cell–T-cell interactions
- cytokines involvement in, 174
  - surface molecules in, 174
- B-lymphocyte, 779
- B-lymphocyte stimulator (BlyS). *See* B cell–activating factor (BAFF)
- B-lymphocyte-induced maturation protein 1 (BLIMP1), 404–405, 644
- B-lymphoid tyrosine kinase (BLK), 404, 581
- B-regulatory cells (Bregs), 914
- B-type natriuretic peptide (BNP), 1274
- B1 lymphocytes, 156–157
- B2 B cells, 155–156
- B2GPI protein, 615, 616f
- B6/lpr mouse, 542
- B7 class I MHC molecules, 21–22
- B7-CD28/CTLA-4 costimulatory pathway, 536–539
- B8-DR3-DQ2, 468–469
- BACH2, 753
- Bacillus anthracis*, 202
- Back pain, 692
- Bacterial diversity, 333–334
- Bacterial metabolites influence immune response, 336
- Bacteroides fragilis*, 476
- Bacteroides thetaiotaomicron*, 334
- BAFF-receptor (BAFF-R), 180
- Balb/C strain, 739
- Bare lymphocyte syndrome, 517
- Barium enema X-ray, 882
- Basal ganglia encephalitis, 1088
- Basal lamina, 996
- Basement membrane zone (BMZ), 1199
- Basic leucine zipper ATF-like 3 transcription factor (BATF3), 216
- Basophils, 45
- biological principles and role in immunity, 250–251
  - and IgE antibodies in autoimmune diseases, 251–252
- Batten disease, 1466
- Bechterew–Strümpell–Marie disease*. *See* *Spondylitis ankylosans*
- Behçet's disease, 1040, 1040f, 1076–1077
- Belimumab, 568, 648, 1025, 1075, 1416t, 1427
- Benzene, 926
- β-Arrestin2, 338
- β-Interferon therapy, 978
- Beta-myosin heavy chain, 1275
- β-tectorin, 1056
- β-tubulin, 1056
- β<sub>1</sub>-Autoantibody effects (β<sub>1</sub>-AABs), 1275

- $\beta$ 1,6 N-acetylgalactosaminyltransferase V deficiency (Mgat5 deficiency), 540  
 $\beta$ c utilizing subset, 279–280  
BG-12, 977  
*Bifidobacterium longum*, 335  
BILAG score, 565  
Bilateral intracavernous carotid artery occlusion, 818  
Bilateral symmetrical skin thickening, 588  
Biliary epithelial cells (BECs), 1149–1150, 1152–1153  
Biobreeding rats (BB rats), 499–500, 739  
Biological agents, 1059  
Biologics, 1024–1025  
Biomarkers, 222  
Biopsy, 1069  
specimens, 1123  
Biosimilars, 1429  
Birdshot retinochoroidopathy (BCR), 1039  
Birdshot retinopathy, 1462  
Bleeding, 939  
acute, 945  
CHA and, 943  
phenotype, 941, 946  
Blind biopsies, 1069  
Blood cells, 320  
Blood–brain barrier (BBB), 964–965  
Blood–labyrinthine barrier, 1051  
Blood–nerve barrier (BNB), 988–989  
Blood–testis barrier (BTB), 1236  
Bone destruction, 666  
Bone marrow transplantation (BMT), 268–269, 923–924, 926–927  
BMT from unrelated donors, 927  
high-dose cyclophosphamide without, 929  
Bone mineral density (BMD), 807–808  
Bone morphogenic protein 6 (BMP6), 645–646  
Bone-marrow transplantation, 1445–1447  
Bortezomib, 568–569, 938–939  
Bovine collagen implants, 355  
Bovine serum albumin (BSA), 1155  
BP180 antigen, 1200–1201  
BP230 gene promoter, 446  
Brain-resident immune cells, 967–968  
Branched-chain 2-OADC (BCOADC-E2), 1151–1152  
Bronchiolitis fibrosa obliterans, 1336  
Bronchiolitis obliterans, 1336, 1345–1346  
syndrome, 1341  
Bronchiolitis obliterans organizing pneumonia (BOOP), 1336  
Bronchoalveolar lavage (BAL), 1336  
Bronchoalveolar lavage fluid (BALF), 126  
Bronchoscopic lung cryobiopsy, 1339  
Bruton's tyrosine kinase (BTK), 520  
BTB domain and CNC homolog 2 (BACH2), 397  
Budesonide, 885  
Bullous pemphigoid (BP), 252, 1192–1193  
autoantibodies as potential immunologic markers, 1202  
autoimmune features, 1200–1201  
basophils and IgE antibodies in, 252  
clinical feature, 1200  
epidemiologic feature, 1200  
genetic features, 1201  
pathologic effector mechanisms, 1201–1202  
pathologic feature, 1200  
in vivo and in vitro models, 1201  
Burnet, MacFarlane, 5, 13–14  
Busacca nodules, 1036–1037  
Busulfan, 926  
Butyrate, 338  
BXSB mice, 535  
Bystander activation of autoreactive cells and epitope spreading, 367–368
- C**
- C-C motif chemokine receptor 4 (CCR4), 402  
C-C motif ligand 11 (CCL11), 253  
C-Glycoside, 134  
c-Maf, 96  
C-reactive protein (CRP), 595, 881–882, 1316  
C-type lectin domain containing 16A (CLEC16A), 396  
C-type lectin receptors (CLRs), 200–201, 214–215  
C-type lectins, 297  
C-X-C motif ligand 2 (CXCL2), 249  
*C. elegans* death (CED), 291  
C1 inhibitor (C1 inh), 266  
C1-esterase inhibitor, 268  
C1q deficiency, 268–269, 297  
C1Q gene, 264, 388  
C2 gene, 388  
C3 glomerulopathies (C3GN), 269  
C3 glomerulopathy (C3G), 1360  
C3H/HeJ mouse model, 1213–1214  
C4-binding protein (C4BP), 266–267  
C4A gene, 388  
C4B gene, 388  
C57BL/6 mice, 645–646  
C5a, 267–268, 625–626  
Ca<sup>2+</sup> release-activated Ca<sup>2+</sup> channelopathy (CRAC channelopathy), 520  
CAAR T-cell technology, 1450  
*Caenorhabditis elegans*, 291  
Calcineurin, 886  
inhibitors, 567, 1134, 1424  
Calcium modulator, 180  
Calcium-dependent disruption, 992  
Calcium-sensing receptor (Ca-SR), 1468  
Cameos, 1461–1462  
Campath-1. *See* Alemtuzumab  
Campath-1H, 1442  
*Campylobacter jejuni*, 993  
infection, 989  
Canakinumab (Ilaris), 1416t, 1426  
Cancer, 345–346, 594, 876  
drugs, 826–827  
*Candida albicans*, 195  
infection, 95–96  
Candidate genes, 385–390  
Candle wax drippings, 1039–1040  
Canine autoimmune diabetes, 500–501  
CAPACITY studies, 1348  
Carbohydrates, 200  
Carboxypeptidase N (CPN), 267  
Cardiac  
antiphospholipid syndrome, 613  
autoantibodies, 1274–1275  
myosin, 1264  
SLE, 563  
valve pathology, 613  
Cardiac magnetic resonance imaging (cMRI), 593  
Cardiovascular magnetic resonance, 613  
Carditis, 1255–1256  
Carter effect, 424  
Cartilage damage, 666  
Case–control association studies, 394  
Casein kinase 1a (CK1a), 448  
Casitas B-lineage lymphoma-b (Cbl-b), 541  
Caspase 1, 202  
Caspase and recruitment domain (CARD), 201  
Catastrophic APS (CAPS), 608  
Caterpillar cells, 1262  
Cathelicidin antibacterial peptide, 246  
Cathepsin G (CTSG), 245  
Causal Pie Model, 477  
CBA/J mice, 1344–1345  
Cbl adaptor proteins, 541  
CC-chemokine ligand 5 (CCL5), 216–217  
CCR7, 175  
CD154, 174  
CD19 molecule, 173  
CD1d/ $\alpha$ GalCer tetramers, 121–123  
CD21 molecule, 173  
Cd224-deficiency, 237–238  
CD3 CD56<sup>1</sup> lymphocytes, 230  
CD3 MAbs, 1443–1445  
CD4 T cells, 559  
CD4<sup>+</sup> cytotoxic T lymphocytes, 724  
CD4<sup>+</sup> infiltrating T cells, 1263  
CD4<sup>+</sup> T cells, 53–54, 718, 857, 1321, 1356  
epitopes, 1152  
CD4<sup>+</sup> Tregs, 29  
CD4SP T cells, 72  
CD4SP thymocyte, 70–71  
CD5, 401–402  
CD6, 400–401  
CD8 T cells, 559, 824  
CD8<sup>+</sup> cytotoxic T lymphocytes (CTL), 45–46  
CD8<sup>+</sup> T cells, 53–54, 312, 696–698  
epitopes, 1152  
CD8SP thymocyte, 70–71  
CD8T cells, 217–218  
CD8 $\alpha$ , 216  
CD23, 174–175  
CD32, 174–175  
CD35. *See* Complement receptor 1 (CR1)  
CD40, 174, 399–400  
CD40 ligand (CD40L), 516–517  
CD40LG gene, 444  
CD41 T cell, 277–278  
CD46. *See* Membrane cofactor protein (MCP)  
CD55. *See* Decay-accelerating factor (DAF)  
CD56 NK cells, 231, 680  
CD58, 399  
CD59 receptor, 267, 321  
CD69, 398

CD70 ligand, 285  
 CD81 molecule, 173  
 CD86, 401, 1001  
 Celiac disease (CD), 134, 353, 471–472, 479, 501, 849–850, 1122  
 animal model of, 482–483  
 autoantibodies, 852–854  
     as immunologic markers, 861–862  
 autoantibodies as immunologic markers, 861–862  
 autoreactive intraepithelial lymphocytes, 854  
 clinical features and disease associations, 851  
 effector mechanisms, 861  
 environmental factors, 856  
     gluten proteins, 856  
     T-cell epitopes of gluten, 858f  
 epidemiology, 851  
 genetics factor, 854–856  
 historical achievements in study of, 850, 850b  
 HLA genes, 855, 855f  
 intestinal lesion stages in, 852f, 853f  
 non-HLA genes, 855–856  
 pathogenic mechanisms, 857–861  
     disease associated HLA-DQ molecules, 859–860  
     gluten-specific CD4<sup>+</sup> T cells, 857–858  
     macrophages and dendritic cells, 860  
     plasma cells, 861  
     TG2, 859  
 pathological features, 852  
 serology, 862  
 staining of immune complexes, 862  
 treatment and outcome  
     current treatment, 862  
     novel treatments, 863  
 in vivo and in vitro disease models  
     animal models, 857  
     organ culture assays, 857  
 Cell death, 291  
 Cell density-enhanced protein tyrosine phosphatase-1 (DEP-1/CD148), 1054  
 Cell infiltration, 1178  
 Cell surface  
     antigens, 1078–1081, 1080f  
     immunoglobulin, 56  
     resident receptors, 263–264  
 Cell therapy  
     using antigen receptor gene-modified T cells, 1449–1450  
     using regulatory T cells, 1449  
 Cell-based assays (CBAs), 1081, 1373  
 Cell-released vesicles, 624  
 Cellular and humoral immune elements, 997  
 Cellular components, 47–51, 967–968  
 Cellular immune components, 987  
 Cellular immunity, 584–585, 794–795, 1180–1181. *See also* Adaptive immunity  
 in MG, 1021–1024  
     cellular immunology of AChR MG, 1022  
     T lymphocytes, 1021–1022

Cellular immunology, 319  
     of AChR MG, 1022  
     pathway, 780  
 Cellular mechanisms, 779, 995–997  
 Cellular players in SLE, 558–560  
 Centers for Disease Control (CDC), 967, 1382, 1464–1465  
 Central nervous system (CNS), 132, 268–269, 384, 639, 961–962, 1012, 1067  
 antibody-associated diseases, 1078–1099  
     antibodies nomenclature, 1081  
     antibodies targeting intracellular and cell surface antigens, 1078–1081  
     antibody-associated clinical syndromes, 1083–1093  
     diagnostical considerations, 1081  
     principles of treatment, 1081–1083  
     target antigens, 1093–1099  
     autoimmunity, 1093–1099  
     imaging, 1068–1069  
 Central tolerance, 68, 91, 177, 218, 774  
     autoimmunity and, 24  
     checkpoints, 1022  
     fetal tolerance to, 66–68  
     mechanisms, 65–66  
 Centroblasts, 176–177  
 Cerebral vasculitis, 1069  
 Cerebrospinal fluid (CSF), 962, 988, 1073–1074  
 Certolizumab, 886, 1416t, 1425  
 Cevimeline, 648  
 cGAS, 203  
 Chagas disease, 1269–1270  
 Chaperone-mediated autophagy (CMA), 305–306, 308–309  
 CHARGE syndrome, 515  
 Chemical leukoderma, 1218  
 Chemiluminescence (CLA), 1370  
 Chemokine fractalkine, 296  
 Chemokine ligands (CCL), 1043  
 Chemokine motif ligand 5 (CCL5), 445  
 Chemokines, 235, 275–276, 281, 287, 483–484, 1238  
     CCL3, 229–230  
     receptors, 584, 970–971  
 Chemotherapy, 1087. *See also* Cancer  
 Chenodeoxycholic acid (CDCA), 1159  
 Chest radiography, 590, 1338–1339  
 Chimeric antigen-specific receptors (CARs), 1449–1450  
 Chimeric MAbs, 1438–1439  
 Chlorambucil, 1045  
 Chloroquine, 524–525, 566, 1420  
 Cholangitis, cirrhosis to, 1150  
 Cholera toxin B-subunit (CTB), 1403  
 Choline transporter-like protein 2 (CTL2), 1056  
 Choroid plexus, 971  
 Chromatin remodeling in type-1 diabetes, 440  
 Chronic anterior uveitis, 1040  
 Chronic ataxic neuropathy with ophthalmoplegia, M-protein, and antidisialosyl antibodies (CANOMAD), 999  
 Chronic cholestasis, 1162  
 Chronic fatigue syndrome (CFS), 1464–1465  
 Chronic granulomatous disease (CGD), 526–527  
 Chronic inflammation, 1418  
 Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP), 987, 998–1002  
 epidemiology and clinical features, 998–999  
     animal models, 1001–1002  
     autoimmune features, 999–1000  
     environmental influences, 1001  
     immunogenetic features, 999–1000  
     MMNCB, 998  
     multifocal-acquired demyelinating sensory and motor neuropathy, 998  
     multifocal-acquired sensory and motor neuropathy, 998–999  
     paraproteinemic demyelinating peripheral neuropathy, 999  
     pathogenic mechanisms, 1002  
     treatment and outcome, 1002  
     history, 998  
 Chronic insulin therapy, 1444  
 Chronic kidney disease (CKD), 1355  
 Chronic lymphocytic leukemia (CLL), 899  
 Chronic mucocutaneous candidiasis (CMC), 522, 732  
 Chronic neuropathies, 998–1002  
 Chronic nonsuppurative destructive cholangitis (CNSDC), 1149–1150  
 Chronic pancreatitis, 1174  
 Chronic periaortitis, 722  
 Chronic rejection, 1360  
 Chronic spontaneous urticaria, 1226  
 Chronic TIN, 1360  
 Chronic urticaria (CU), 1226–1229  
     autoantibodies as potential immunologic markers, 1228  
     autoimmune features, 1227  
     clinical, pathologic, and epidemiologic features, 1226  
     genetic features, 1227  
     index, 1227  
     pathologic effector mechanisms, 1228  
     in vivo and in vitro models, 1227  
 Churg–Strauss syndrome. *See* Eosinophilic granulomatosis with polyangiitis (EGPA)  
 Cicatricial pemphigoid (CP), 1192–1193, 1202–1203  
 Ciclosporin, 1002  
 Ciclosporin A, 567  
 Cigarette smoking, 474–475  
 Cirrhosis to cholangitis, 1150  
 Citrate accumulation, 194  
*Citrobacter rodentium*, 283, 333–334  
 Citrullinated/citrullination, 478, 662  
     antigens, 668  
     autoantigens, 248  
     peptides, 661–662  
 Citrulline, 661–662  
 Class 1-restricted Tc-associated molecule-1 (CRTAM-1), 1213

Class II cytokine receptor family, 282–283  
 Class-switch recombination (CSR), 174–175  
 Classic autoantibodies, 1274–1275  
 Classical affinity model, 70  
 Classical arthritogenic peptide theory, 696  
 Classical polyarteritis nodosa (cPAN), 1070–1071  
 Clec9a receptor, 216  
 Clinical practice guidelines (CPG), 1378–1379  
 Clinically isolated syndromes (CISs), 962–963  
 Clonal balance and regulation, 5–6  
 Clonal deletion, 177–178, 779  
     defective, 178–179  
 Clonal expansion and/or reactivation, 77–78  
 Clonality, aplastic anemia and, 931  
*Clostridiales*, 881  
 “Clustered AChR” Abs, 1018–1019  
 Clusterin, 267  
 Coactivator-associated arginine methyltransferase-1 (CARM1), 433  
 Coagulation  
     cascade, 935  
     system, 936f  
     test, 941  
 Coagulation factor C homology gene (COCH gene), 1056  
 Cochlear implantation, 1059  
 Cochlin, 1056  
 Cogan’s syndrome, 1054–1055  
     antibodies against Cogan peptide bind human cochlea, 1055f  
     DEP-1/CD148, 1054  
     grand average of auditory brainstem responses, 1055f  
     inner ear pathology, 1054  
     morbidity, 1054  
 Cold agglutinin syndrome (CAS), 899  
 Cold reactive antibodies, 899  
 Colitis, 124t, 127  
 Collagen antibody–induced arthritis model (CAIA model), 545  
 Collagen type I matrix, 693  
 Collagen-induced arthritis (CIA), 94, 281–282, 479–480, 495, 545  
 Collagen-like tail (ColQ), 1013  
 Collapsing response mediator protein 5 (CRMP5), 1083, 1097  
 Collectin kidney 1 (CL-K1), 264–265  
 Collectin-11 (CL-11), 264–265  
*Collinsella aerofaciens*, 476–477  
 Colonoscopy, 882, 889  
 Combined immune deficiencies (CIDs), 516–518  
     with syndromic features, 518–520  
 Common DC precursors (CDPs), 216  
 Common lymphoid progenitors (CLP), 158–159  
 Common variable immunodeficiency (CVID), 515–516, 913  
 Comorbidities management, 566  
 Complement, 297  
     activation system, 1179  
     and complement receptor proteins, 542–543

complement-mediated cytotoxicity, 270  
 effector mechanism in complement cascades, 321  
 inhibitors, 997  
 Complement 3 (C3), 1355  
 Complement 5 (C5), 1025  
 Complement factor H-related 5 (CFHR5), 269  
 Complement receptor 1 (CR1), 264, 266–268, 321, 899  
 Complement receptor 1-related protein y (Crry), 270  
 Complement receptor 2. *See* CD21 molecule  
 Complement system, 50–51, 50f, 263–266  
     in antiphospholipid syndrome, 625–626  
     biological effects, 267–268  
     complement activation pathways, 263–266, 265f  
     alternative pathway, 266  
     classical pathway, 264  
     LP, 264–265  
     membrane attack complex, 266  
     control of activation, 266–267  
     fluid phase regulators, 266–267  
     membrane-bound regulators, 267  
     involvement in autoimmune diseases, 268–271  
 Complementarity determining regions (CDRs), 56, 161, 925–926, 1438–1439  
 Complete blood count (CBC), 913  
 Complete Freund’s adjuvant (CFA), 1237–1238  
 Compound muscle action potential (CMAP), 988  
 Compressive neuropathies, 1073–1074  
 Computed tomographic angiography (CTA), 1070  
 Computed tomography (CT), 802, 882, 1174  
 Computer simulation models, 1219  
 Congenic mouse strains, 839  
 Congenital adrenal hyperplasia (CAH), 804–805  
 Congenital aplastic anemia, 924  
 Congenital bleeding disorders, 935–937  
 Congenital hemophilia A (CHA), 943  
 Connexin-26, 1054  
 Constructed mouse models, 1224  
 Contact hypersensitivity (CHS), 129  
 Contactin-associated protein-1 (Caspr), 1000  
 Contactin-associated protein-like-2 (CASPR2), 1080–1081, 1095  
     encephalitis, 1086  
 Continuous subcutaneous hydrocortisone infusion (CSHI), 805  
 Conventional dendritic cells (cDCs), 214  
     cDC1, 214, 216  
     cDC2, 214, 216–217  
 Conventional immunosuppressive treatment, 1017  
 Conventional therapy, 798–800  
 Coombs’ test, 897–898  
 Copaxone. *See* Glatiramer acetate (GA)  
 Core histones, 432  
 Cortical thymic epithelial cells (cTEC), 70  
 Corticobasal degeneration, 1088  
 Corticosteroids, 648, 698–699, 884, 901, 903, 1024, 1044–1045, 1074, 1324, 1348  
     dependency, 884  
     treatment, 1183  
 Corticotropin deficiency, 818–819  
 Cost analysis, 1379–1381  
 Costimulation, 134–135, 173–174  
 Costimulatory blockade, 724  
 Costimulatory molecule B7–2, 1001  
 Costimulatory molecules, 978  
 Coxsackie B1 virus infections, 774  
 CpG islands, 430  
 CR3 receptor, 298  
 Cranial arteritis, 1316  
 Cranial GCA, 1316–1317  
 Cranial nerve, 962, 998, 1077  
 Creatine kinase (CK), 704  
 Crescentic GN, 1359, 1361  
 CREST syndrome, 577  
 Crohn’s disease (CD), 130t, 132, 219–220, 494–495, 502, 871–874  
     extraintestinal manifestations, 875–876  
 Crossfire, 377–378  
 Cryoglobulinemic vasculitis (CV), 1288, 1300–1302  
     animal models, 1302  
     autoimmune features, 1301  
     clinical features and disease associations, 1300  
     diagnostic procedures, 1302  
     epidemiology, 1300  
     genetic features and environmental influences, 1302  
     pathogenesis, 1301  
     pathological features, 1301  
     treatment, 1302  
 Cryoglobulins, 1300–1301  
 Cryopyrin-associated periodic syndromes (CAPS), 1426  
 Cryptogenic cirrhosis, 1119  
 Cryptogenic fibrosing alveolitis.  
     *See* Idiopathic pulmonary fibrosis (IPF)  
 Cryptogenic organizing pneumonia (COP), 1335  
     autoimmune features, 1340–1341  
     clinical, pathological and epidemiological features, 1337  
     genetic features, 1343  
     histologic pattern, 1337f  
     history, 1336  
     pathologic effector mechanisms, 1345–1346  
     treatment and outcome, 1348  
     *in vivo* and *in vitro* models, 1344–1345  
 Cryptogenic organizing pneumonitis, 1336  
 CT enterography (CTE), 882  
 CTLA4 haploinsufficiency with autoimmune infiltration (CHAI), 525  
 CTLA4-Ig. *See* Abatacept  
 Cuboidalization, 1339  
 Curdlan-induced SKG mouse model, 698  
 Curli, 476  
 Currie, Alastair, 291  
 Cutaneous and mucosal disease, 562–565

- CV2. *See* Collapsing response mediator protein 5 (CRMP5)
- CVB3-induced myocarditis, 1277
- CVID, 520–521
- CXC-chemokine ligand 8 (CXCL8), 217
- CXCL9, 1220
- CXCL10, 1220
- CXCR5, 175
- Cyclic GMP–AMP (cGAMP), 203
- Cyclooxygenase, 1419
- Cyclooxygenase-2 (COX-2), 440, 1361
- Cyclophilin-ligand interactor (TACI), 180
- Cyclophosphamide (CYC), 567, 903, 918, 926–927, 929, 974, 1045, 1074–1075, 1077, 1084–1085, 1183, 1349, 1416*t*, 1422
- Cyclosporine, 780, 825–826, 886, 1045, 1134, 1416*t*
- Cyclosporine A (CSA), 926–928, 1424
- CYP27B1, 399
- Cysteine residues at position 67 (Cys67), 696
- Cystitis, interstitial, 1462–1470
- Cytochrome *c* (CYTC), 292
- Cytochrome P450, 790, 797
- Cytochrome P450 side-chain cleavage enzyme (P450sc), 797
- Cytochrome P4502D6 (CYP2D6), 1126
- Cytokine-targeted therapies, 1424
- Cytokine(s), 45, 135, 174, 229–230, 275–276, 322, 483–485, 999–1000, 1021, 1130, 1238, 1264
- bias, 135
- class II cytokine receptor family, 282–283
- noninterferon members, 283
  - type I interferons  $\alpha$  and  $\beta$ , 282
  - type II interferon gamma, 282
  - type III interferon lambda, 283
- effector and targets, 325–326
- gain-of-function disorders of cytokine signaling, 525–526
- IL-1/TLR family of receptors, 285–286
- and immunity, 277–278
- immunosuppressive cytokines/growth factors, 286
- milieu, 901
- production, 235
- receptor subsets
- $\beta$ c utilizing subset, 279–280
  - common  $\gamma$ c chain subset, 278–279
  - cytokines sharing p35 or p40 ligand chain, 280–281
  - gp130 utilizing subset, 280
  - Th17 cytokines and receptors, 281–282
- and receptors, 541–542
- storm, 276
- tumor necrosis factor receptor family, 284–285
- Cytolytic granule exocytosis, NK cell, 234–235
- Cytoplasmatic antigens, 1097–1098
- collapsing response mediator protein 5 (CV2), 1097
- GAD, 1098
- MAP1B, 1097
- YO (purkinje cell antigen-1), 1097
- Cytoplasmic against proteinase 3 (c-ANCA-PR3), 1071
- Cytoplasmic ANCA (c-ANCA), 1071
- Cytoplasmic Y RNAs, 293
- Cytosine-guanosine dinucleotides (CG dinucleotides), 430
- Cytosine-phosphate-guanine dinucleotides (CpG dinucleotides), 430
- Cytosolic
- agents, 1349
  - drugs, 903
  - innate receptors, 18
  - pattern recognition receptors, 202–203
- Cytotoxic T cells (CTLs), 232, 322–323
- Cytotoxic T lymphocyte (CTL), 54*f*, 793–794
- Cytotoxic T lymphocyte antigen-4 (CTLA-4), 27, 77–78, 276, 524–525, 738, 815, 827, 1128, 1181–1182
- CTLA-4-deficient mice, 536–539
  - CTLA-4-targeted therapy, 496
  - gene, 390–391, 1261
  - polymorphism in, 753
  - promoter, 436
- Cytotoxicity, 232
- D**
- Daclizumab, 1045
- Dacryoadenitis, 720, 722*f*
- Dallas criteria, 1270–1271
- Damage-associated molecular patterns (DAMPs), 25, 28, 45–46, 196, 264–265, 294–295
- Danazol, 918
- “Danger” signal, 76
- Dapsone, 918, 1204
- “Dark Ages” of autoimmunity, 14*t*
- Darwin, Charles, 419–420
- ddY mouse, 1358
- “De novo AIH”, 1136
- Deacetylation, 432–433, 432*f*
- of histones, 338
- Dead cell clearance, 295–297, 543
- Deamidation, 478–479, 859
- “Death by neglect”, 52–53
- Death receptors (DRs), 322
- DR3, 285
- Death-inducing signaling complex (DISC), 292
- Decay-accelerating factor (DAF), 267–268
- Decidual vasculopathy, 587
- Dectin-1, 201
- Deep venous thrombosis (DVT), 608
- Defective DNA methylation, 436
- Defective pituitary hormones replacement, 826
- Deficiency of adenosine deaminase 2 (DADA2), 1289
- Deficient phagocytosis, 295
- DEFINE trial, 977
- Dehydroepiandrosterone (DHEA), 801–802, 804
- Deimination, 478
- Delta/notch-like epidermal growth factor-related receptor (DNER), 1081, 1096
- Demyelination, 961, 963–964, 966, 988
- Dendritic cell-specific ICAM-3 grabbing nonintegrin (DC-SIGN), 200–201
- Dendritic cells (DCs), 18–19, 45, 46*f*, 47–50, 71*f*, 75–76, 119–120, 191–192, 213, 229–230, 243–244, 277, 295–296, 307–308, 336, 421, 442, 559–560, 878–879, 1044, 1128, 1238–1239
- activation, 1321
- Ags uptake, processing, and presentation by, 214
- and autoimmune disease, 219–221
- in celiac disease, 860
- DC-based immunotherapy, 220–221
- in IBD, 219–220
- migration, 214
- PRR and activation, 214–215
- psoriasis and psoriatic arthritis, 220
- in SLE, 219
- subsets
- development, 216
  - human, 215
  - mouse, 215
  - phenotypes, 216–218
  - and tolerance, 218–219
- in T1D, 220–221
- targeting in autoimmune disease, 220–221
- tissue-specific, 217–218
- Dense fine speckles (DFS), 1380
- Density-enhanced protein tyrosine phosphatase-1 (DEP-1), 1054
- Deoxynucleotidyl transferase (TdT), 159–160
- Dermal cDCs, 217–218
- Dermal sclerosis, 588
- Dermatitis, 124*t*, 524
- Dermatitis herpetiformis (DH), 482, 500–501, 504, 857, 1192–1193, 1203–1204
- Dermatologic antiphospholipid syndrome, 612–613
- Dermatomyositis (DM), 250, 703
- Desmocollins (Dscs), 1192
- Desmogleins (Dsgs), 1192
- Dsg3, 1450
- Desmopressin, 948
- Desmosomes, 1191–1192, 1192*f*
- Desquamative interstitial pneumonia (DIP), 1336
- Dextran sodium sulfate (DSS), 881
- DHEA sulfate (DHEAS), 804
- Diabetes insipidus, 819–820, 827
- Diabetes mellitus, 130*t*
- Diabetes Prevention Trial (DPT), 1404
- 3,4-Diaminopyridine (3–4-DAP), 1026
- Diapedesis, 996
- Diet(ary), 333–334, 353, 1401–1402
- AGE, 200
  - components, 453–454
  - exposure, 774
  - fiber, 333–334, 335*f*
  - gluten, 1402
  - with human autoimmune diseases, 332–333
  - and immune system, 332*f*
  - metabolites influence immune response, 336

- Differentially methylated positions (DMPs), 446
- Diffuse alveolar hemorrhage, 614
- Diffuse cutaneous SSc (dcSSc), 577, 578<sup>t</sup>
- Diffuse lymphocytic adrenalitis, 793
- Diffuse lymphoplasmacytic infiltrates, 1374
- DiGeorge syndrome (DGS), 515, 518–519
- Digital subtraction angiography, 1069
- Digoxin, 1274
- Dihydrolipoamide dehydrogenase-binding protein (E3BP), 1151–1152
- Dihydroorotate dehydrogenase (DHODH), 1421
- Dilated cardiomyopathy (DCM), 1269  
animal models, 1277–1279  
autoimmune features, 1274–1276  
clinical, pathologic, and epidemiologic features, 1273–1274  
genetic features, 1276  
perspectives, 1279  
treatment, 1274
- Dilute Russell venom time (dRVVT), 621
- Dimethyl fumarate, 977
- Dipeptidyl-peptidase-like protein 6 (DPPX), 1083, 1095  
encephalitis, 1086–1087
- Direct agglutination test (DAT), 899–900
- Direct antibody-mediated disease, 320
- Direct antiglobulin test (DAT), 268, 913
- Direct Coombs' test, 268
- Direct or antigen-dependent activation, 119–120
- Disease activity scores (DAS), 1418
- Disease modifying drugs, 566–569
- Disease-causing IgG fraction, 719
- Disease-inducing bacteria, 30
- Disease-modifying rheumatoid drugs (DMARD), 1415–1416, 1418
- Disease-modifying therapy (DMT), 974
- Disease-Specific Quality of Life Questionnaire of AD (AddiQoL), 807
- Disorders associated with PBC  
HCC, 1162–1163  
hyperlipidemia and metabolic syndrome, 1162  
osteopenia and osteoporosis, 1162  
PBC with features with autoimmune hepatitis, 1161  
sicca syndrome, 1162
- Dissemination in space (DIS), 964
- Dissociation model, 172
- Diversity of Guillain–Barré variants, 988<sup>b</sup>
- Dizygotic twins (DZ twins), 439
- DKC1* gene, 924
- DNA methylation, 430–431, 431<sup>f</sup>  
in autoimmune thyroid diseases, 438  
and MS, 441  
profiling in type-1 diabetes, 439–440  
in rheumatoid arthritis immune cells, 436  
in scleroderma, 447  
in Sjögren's syndrome, 446  
in systemic lupus erythematosus, 443–444  
targeting, 455  
in Treg development and function, 450
- DNA methyltransferases (DNMTs), 430
- DNA repair defects, 923
- DNase I activity, 250, 543
- DNGR-1, 297
- DNMT1, 431
- DNMT3A, 431, 447–448
- DNMT3B, 431
- Docking protein 7 (DOK7), 1013
- Donath–Landsteiner hemolysins (DL hemolysins), 899
- Dopamine-2 receptor (D2R), 1095  
encephalitis, 1088
- Doppler parameters, 1274
- Double positive thymocytes (DP thymocytes), 70
- “Double positive” T cells, 52–53
- Double-stranded DNA (dsDNA), 244–245, 321, 1074
- Down-stream effects in iNKT cells, 120–121
- DQ molecule in predisposition to multiple sclerosis, 481–482
- DQA1* gene, 1261
- DQA1\**0501, 21–22
- DQB1* gene, 1261
- DQB1\**0302, 21–22
- DQB1\**0601, 480–481
- DQB1\**0604, 480–481
- DRB1* gene, 1261
- DRB1\**04, 483–484
- DRB1\**0401 mice, 474–475
- DRB3* gene, 1261
- DRB5\**0101, 390
- Drug-induced autoimmune-like hepatitis (DILI-AIH), 1122
- Drug-induced liver injury (DILI), 1122
- Drug-induced pemphigus, 1200
- Dry eye disease, 637
- Durvalumab, 826–827
- Dysautonomia, 1461–1462
- Dysbiosis, 475–476
- Dyserythropoiesis, 924
- Dyskeratosis congenita (DKC), 923–924
- Dyslipidemia, 1075
- Dysregulated neutrophil phenotype, 245
- Dysruption of B cell tolerance, 449
- Dystrophic calcifications, 589
- E**
- E2 component of pyruvate dehydrogenase complex (PDC-E2), 293
- E3 ubiquitin ligase, 536
- Ear/nose/throat, 720–721
- Early B-cell factor (EBF), 160
- Early-onset acetylcholine receptor-antibody positive MG, 1014<sup>f</sup>, 1016
- Early-onset AChR antibody-positive MG, 1023–1024
- EBV nuclear antigen (EBNA), 473–474  
EBNA1, 367
- Echocardiography, 1270
- Eculizumab, 268, 993, 997, 1025
- Edema, 962, 964
- Effector cells, 1225
- Effector mechanism, 319  
in celiac disease, 861
- Efferocytosis, 204–205, 295
- Eggerthella lenta*, 476–477
- Ehrlich, Paul, 4–5, 10–11, 17, 263
- Ehrlich thesis  
lens autoantibodies, 11  
PKH, 11–12  
sympathetic ophthalmia, 12  
Wassermann antibody, 12
- Ehrlich's “contrivances”, 11
- Ehrlich's dictum, 13
- Ehrlich's side-chain theory, 10
- Electric sigmoidoscope, 872
- Electrocardiogram (ECG), 1270
- Electromyography, 1026
- Electron microscopy (EM), 793, 1359–1360
- Electronic medical records (EMR), 1370, 1379–1381, 1380<sup>b</sup>
- Electrophysiological studies, 988, 998
- Elemental disorder hypothesis, 356
- Elf-1, 540
- Eltrombopag, 917, 928–929
- Embryonic lethal abnormal visual (ELAV), 1096
- Emergency card, 807
- Encephalitis associated with antibodies to neuronal surface proteins, 1086–1088
- α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor encephalitis, 1087
- D2R encephalitis, 1088
- Dipeptidyl-peptidase-like protein 6 encephalitis, 1086–1087
- GABA-B receptor encephalitis, 1087
- γ-aminobutyric acid type-A receptor encephalitis, 1087
- IgLON5 encephalitis, 1088
- ophelia syndrome, 1088
- Encephalomyelitis (EAE), 961–962
- Encephalopathy, 1134
- End-stage renal failure (ESRF), 555
- Endocarditis, 1255–1256
- Endogenous “autoantigenic” insulin, 1406
- Endogenous uveitis, 1040–1041
- Endometriosis, 1463–1464
- Endomyocardial biopsy (EBM), 1269
- Endoneurium, 999–1000
- Endonuclease G (ENDOG), 292
- Endoplasmic reticulum (ER), 234–235
- Endoplasmic reticulum aminopeptidase 1 (ERAP1), 470, 697
- Endoscopic/endoscopy, 882  
biopsies, 876  
retrograde cholangiopancreatography, 1176<sup>f</sup>  
transphenoidal approach, 826
- Endosomal TLRs, 200
- Endothelial cells (ECs), 245, 1238
- Endplate potential (EPP), 1013
- Enhancer of zeste homolog 2 (EZH2), 448–449
- Entameba histolytica*, 879
- Entamoeba histolytica*, 1297
- Enterohepatic circulation, 1160
- Enteroviruses, 1277
- Enthesitis-related arthritis, 677–678

Environmental deficiencies, 968  
effects, 993  
factors, 968–969, 1056  
involving in FS, 1199  
influences, 1001  
toxins, 97–98, 579  
triggering factors, 1153–1154

Environmental agents role in autoimmunity  
evidence for, 346–348, 347b  
identifying and defining, 348–349, 348t  
mechanisms for developing autoimmune diseases, 356, 356t  
noninfectious agents associated with autoimmune diseases, 352–356, 352t  
air pollution, 355  
drugs, 349–350, 350t  
exercise, 355  
foods, 353  
heavy metals, 354  
implants, 355  
microbiome, 355–356  
microchimerism, 354  
occupational exposures, 351–352, 351t  
stress, 355  
tobacco smoke, 354  
vaccines, 354–355  
vitamin D, 353–354

Enzyme-linked immunosorbent assays (ELISA), 558, 1018, 1081, 1275–1276, 1296, 1370

EOMES, 118–119, 401

Eosinophilia–myalgia syndrome (EMS), 353

Eosinophilic granulomatosis with polyangiitis (EGPA), 247, 1072–1073, 1287  
eosinophils in, 253–255

Eosinophils, 45, 47–48, 1072  
in autoimmune diseases, 253–254  
biological principles and role in immunity, 253  
in EGPA, 254–255

Eotaxins, 253

Epidemiology of MS, 967–969

Epidermal growth factor (EGF), 942

Epidermolysis bullosa acquisita (EBA), 1192–1193, 1203

Epididymal sperm granuloma, 1242

Epigenetic drift, 451–452

Epigenetic regulators of tolerant T cells, 449–450

Epigenetics, 430  
AITD, 438  
arginine methylation, 433  
changes associated with environment triggers in autoimmunity, 452–454  
cross talk between epigenetic regulations, 434

DNA methylation, 430–431  
epigenetic code, 430  
genetic and epigenetic interactions in autoimmune diseases, 451–452

HATs, 432–433  
histone acetylation and deacetylation, 432–433, 432f

histone methylation, 433  
histone posttranslational modifications, 431–434  
of immune tolerance, 449–451  
modifications, 430–435  
MS, 441–443  
ncRNAs, 434  
RA, 435–437  
regulatory T cells  
epigenetic modulation, 451  
generation, 457  
histone acetylation on development and function of, 450

SLE, 443–446  
SSc, 447–448  
stability, 435  
T1D, 439–440  
therapy, 454–457  
translational applications, 454–457  
ubiquitination, 433

Epilepsy, 1464  
epilepsy-relevant autoantibodies, 1464

Epileptiform activity, 1087

Epiligrin. *See* Laminin 5

Episcleritis, 1036

Epitopes, 45–46  
bystander activation of, 367–368

Epratuzumab, 568

Epsilon subunit epitope, 1021

Epstein–Barr virus (EBV), 367, 386, 473–474  
infection, 969

Epstein–Barr virus-induced gene 2 (EBI2), 175

Erasers of histone modifications, 433–434

ERBB3 gene, 396

Erectile dysfunction, 593–594

Erythema nodosum (EN), 875

Erythematous papules, 1200

Erythrocyte sedimentation rate (ESR), 881–882, 1292, 1317–1318

Erythrocytes, 45

Erythropoietin (EPO), 993

*Escherichia coli*, 244, 269, 1356

Estrogen receptors (ERs), 420

Estrogens, 421–422, 478

Etanercept, 1045, 1059, 1416t

Ethylnitrosourea (ENU), 541

European Association for Study of Liver (EASL), 1133, 1150

European Autoantibody Standardization Initiative (EASI), 1381

European Consensus Finding Study Group on Autoantibodies (ECFSGA), 1381

European League Against Rheumatism (EULAR), 635–636

European Medicines Agency, 686

European Respiratory Society (ERS), 1336

European Society for Blood and Marrow Transplantation (EBMT), 1446

Evidence-based guidelines, 1377

Ex vivo culture system, 857

Ex vivo phrenic nerve-diaphragm preparation, 992

“ex-Tregs”, 99

Excitatory amino acid transporter (EAAT2), 1091

Excitatory receptors  
AMPAR, 1093–1094  
mGluR1 and mGluR5, 1094  
NMDAR, 1093

Executioner, 292

“Exhausted” autoimmunity, 26–27

Exogenous cytokines, 232

Exosomes, 624

Exotoxins, 12

Experimental allergic neuritis (EAN), 987

Experimental autoimmune adrenalitis (EAA), 501, 793

Experimental autoimmune encephalomyelitis (EAE), 93, 132–133, 270, 279, 366, 378, 423, 441–442, 481, 504–505  
DR transgenic mice with, 481

Experimental autoimmune myasthenia gravis (EAMG), 270, 1018

Experimental autoimmune orchitis (EOA), 1237

Experimental autoimmune thyroiditis (EAT), 499–500, 752  
resulting from immune modulation, 754

Experimental autoimmune uveitis (EAU), 134, 279, 1035

Experimental studies, 1235

Exosome, 1393

Exposures, 6–7

Extracellular matrix (ECM), 299, 446

Extracellular matrix protein 1 (ECM1), 1466

Extracellular NETs, 195

Extracellular portion, 1014

Extracellular traps, 560

Extracranial atherosclerotic lesions, 1069

Extrahepatic manifestations, management of symptoms and, 1160–1161  
fatigue, 1160–1161  
pruritus, 1161

Extraintestinal manifestations (EIM), 875  
cancer, 876  
diseases with link to inflammatory bowel disease, 876

in UC and CD, 875–876  
eyes, 875  
hepatobiliary, 875  
joints, 875  
mouth, 875  
skin, 875

Extranuclear effects, 422

Extravascular GCA, 1323–1325

Extrinsic apoptosis, 292

Eye  
harbors autoimmune-inducing materials, 1035  
unique immune system of, 1042

F

18F-fluorodeoxyglucose (FDG), 1070

Fab-arm exchange, 1086

Faciobrachial dystonic seizures (FBDS), 1081

*Faecalibacterium prausnitzii*, 81, 476–477

Familial hemophagocytic lymphohistiocytosis (FHL), 236–237  
 Familial HLH (FHLH), 683  
 Fanconi anemia, 924  
 Farnesoid X receptor (FXR), 1149–1150  
 FAS ligand (FASL), 523, 534–535  
 Fas protein, 21  
 FAS-associated protein with DD (FADD), 292  
 Fatal anemia, 833  
 Fate-mapping techniques, 193  
 Fatigue syndrome, 640–641, 1160–1161, 1464–1465  
 Fc receptor-like 3 (FCRL), 1181  
 Fc receptors (FcRs), 191–192, 196–198, 245  
*FCGR2A* gene, 388–389  
*FCGR3A* gene, 388–389  
*FCGR3B* gene, 388–389  
 FcR- $\gamma$  chain, 536  
*Fc $\gamma$ RIIA* gene, 1260  
*Fc $\gamma$ RIIb*, 181, 540, 724  
*Fc $\gamma$ Rs*, 197  
 Fecal biomarkers, 882  
 Fecal calprotectin, 882  
 Fecal incontinence, 592  
 Fecal lactoferrin, 882  
 Feedback loops, 1225  
 Feedforward cycle, 708  
 Fetal tolerance to central tolerance, 66–68  
 Fibrates, 1160  
 Fibrillin-1 mutation, 583  
 Fibrin  
     autoimmune inhibitors to, 939  
     clot, 939  
     degradation, 623  
 Fibrinogen, 939, 1059  
     autoimmune inhibitors to, 939  
 Fibroblast–like synoviocytes (FLS), 435  
 Fibroblasts, 585  
     growth factor, 1323  
 Fibrosis, 32, 822, 1178, 1273–1274  
 Fibrous astrocytes, 966–967  
 Ficolin A (Fcna), 264–265  
*Ficolin* gene, 1260  
 Filamentous form of actin (F actin), 216  
 Fingolimod, 976–977  
 First-degree relative (FDR), 769  
 First-line therapies in ITP, 916  
 First-phase insulin response (FPIR), 1402  
 Fisher syndrome (FS), 987  
 Five factor score (FFS), 1290–1291  
 Flagellar antigen, 4  
 Fleck, Ludwik, 9  
 Flow cytometric assay (FCA), 619  
*Flt3*, 216  
 Fluconazole, 741  
 Fludarabine, 926–927  
 Fludrocortisone, 741  
 Fluorescein-human gamma globulin (Flu-HGG), 179  
 Fluorescence-based assays, 1219  
 Fluorescent in situ hybridization (FISH), 924  
 Fluorodeoxyglucose position emission tomography, 1174  
 Focal lymphocytic adrenalitis, 792

Focal orchitis, 1242  
 Focus score, 642  
 Fogo selvagem (FS), 1197  
     environmental factors involving in, 1199  
 Folate deficiency, 1465  
 Follicular B cells, 156  
 Follicular dendritic cells (FDC), 50, 174–175  
 Follicular helper T cell (Tfh cell), 541  
 Follicular regulatory T cells (Tfr cells), 1022  
 Follicular T helper cells (TFH cells), 102–104  
 Foods, 353  
 Forbidden clone, 5  
 Forced vital capacity (FVC), 581  
 Forkhead box P3 protein (Foxp3), 423, 450, 457, 523, 1180  
     CD4 T cells, 103–104  
     *iNKT* cells, 121  
     regulatory T cells, 81  
     Treg cells, 685  
 Formerly Churg–Strauss syndrome, 1072–1073  
 Formerly Wegener’s granulomatosis, 1071–1072  
 Formiminotransferase cyclodeaminase (FTCD), 1126  
 Foxp3+ cells to infiltrating mononuclear cells (Foxp3/Mono), 1180  
 FOXP3-regulatory T cells, 81  
 Fragment apoptosis stimulating (Fas), 284  
 Fragment crystallizable receptors (Fc receptors), 307–308  
 Freeze fracture electron microscope studies, 1026  
 Fresh frozen plasma (FFP), 941  
 Freund’s adjuvant, 14  
 Function-neutralizing monoclonal antibody, 996  
 Functional allelic variants, 1057  
 Functional autoantibodies, 1274–1275  
 Functional effects of antibodies, 991–993  
 Functional fluorescence energy transfer, 1275–1276

**G**

G-protein coupled receptors (GPCRs), 334, 976, 1274–1275  
     signaling, 335  
*G6PI*. *See* Glucose-6-phosphate isomerase (GPI)  
 GABA type-a receptors (GABAaR), 1083  
 GABA-B receptor encephalitis (GABAaBR encephalitis), 1087  
*GAD2* gene, 391  
 Gadolinium enhancement, 965  
 Gadolinium-enhancing lesions, 964–965, 978  
 Gain-of-function (GOF), 516  
     disorders of cytokine signaling, 525–526  
*Galectin-3*, 719  
*Galectin-9*, 201  
 Gallium scans, 1346  
 Gamma-aminobutyric acid type B receptors (GABAaBR), 1094  
     encephalitis, 1087  
 Gamma-aminobutyric acid type-a receptors (GABAaRs), 1083, 1094  
 Gamma/delta T cells, 1130  
 $\gamma$ c chain subset, 278–279  
 Gammaglobulins (IgG), 1447  
 Ganglioside  
     antigens, 989  
     in peripheral nerve, 991, 992f  
*Gas-6*, 297  
 Gastric antral vascular ectasia (GAVE), 591  
 Gastric atrophy, 833–836  
 Gastric mucosa, 836  
 Gastritis, 840  
 Gastrointestinal reflux disease (GERD), 587–588  
 Gastrointestinal tract (GI), 282  
 Gastroparesis, 591  
*GATA-3*, 104  
 Gateway from autoimmune pancreatitis, 1173–1174  
 GD1b immunization, 994  
 Gene  
     gene-disruption model, 1466  
     gene-function relationships, 495  
     testing, 880  
 Generalized vitiligo (GV), 1216, 1217f  
 Genetic risk scores (GRSs), 777–778, 780  
 Genetic studies of autoimmunity  
     genetic linkage studies, 390–394  
     genome-wide association studies, 394–406  
*HLA*, 385–390  
     *HLA-B*, 385  
     *HLA-B\*27*, 385  
     MS with, 386–387  
     type 1 diabetes, 385–386  
*HLA* association with autoimmunity, 389–390  
 mechanisms of complement and Fc associations, 389  
 MS, 384  
 SLE, 384–385  
     type 1 diabetes, 384  
 Genetic(s), 6–7, 1127–1128  
     aberrations, 1024  
 AIP, 1181–1182  
     genome-wide polymorphic markers, 1182  
     polymorphic markers in candidate genes, 1181–1182  
     aspects of GBS, 997  
     and associated studies, 556–557  
     association studies, 237–238  
     etiology of islet autoimmunity, 772–774  
     factors, 967–968, 1040–1042, 1056  
     features, 1195  
         bullous pemphigoid, 1201  
         PF, 1198  
 linkage studies of autoimmunity, 390–394  
     of type 1 diabetes, 390–392  
 polymorphisms, 282  
 predisposition, 1015, 1153, 1257–1258  
 regulation, 7  
 susceptibility, 1056–1057  
 Genetically engineered mouse models, 1224  
 Genetically manipulated models of systemic autoimmunity, 536–544  
 clearance of dead cells, 543

- complement and complement receptor proteins, 542–543
- cytokines and receptors, 541–542
- innate immune cell signaling, 543–544
- lymphocyte activation molecules, 536–540
- ubiquitination-related enzymes, 540–541
- Genome-wide association studies (GWAS), 21–22, 220–221, 473, 495, 557, 581, 643–644, 855–856, 967, 971, 1151, 1292, 1357
- of autoimmunity, 394–406
  - of multiple sclerosis, 398–402
  - of systemic lupus erythematosus, 403–406
  - of type 1 diabetes, 395–398
- Genome-wide epigenetic analysis, 1154
- Genome-wide polymorphic markers, 1182
- Germinal centers (GCs), 57, 58f, 102–103, 176–177
- response, 175
- GFAP-Abs, neurological syndromes associated with, 1093
- Gi protein–coupled receptors (GPCR), 287
- Giant cells, 1318
- Giant-cell arteritis (GCA), 505, 1069–1070, 1313–1320, 1318f
- clinical, pathologic, and epidemiologic features, 1316–1318
  - clinical profile in, 1317t
  - epidemiology, 1319
  - extravascular, 1323–1325
  - treatment, monitoring, and outcome, 1324–1325
  - genetic features, 1319
  - historic background, 1316
  - immuno-stromal interactions in vasculitis, 1323
  - macrophages in, 1322–1323, 1322t
  - neoangiogenesis of microvascular networks and intimal hyperplasia, 1323
  - pathogenic mechanisms, 1319–1320
  - principal features, 1314t
  - T cells and antigen-presenting cells in, 1320–1322
  - TLR, 1320
  - vascular lesion, 1318
- Gilenya. *See* Fingolimod
- Glatiramer acetate (GA), 978
- Gliadin proteins, 856
- Glia antigens, 1098–1099
- AQP4, 1098
  - GFAP, 1099
  - MOG, 1098
- Glia fibrillary acidic protein (GFAP), 1091, 1099
- Gliosis, 962, 966
- Global APS score (GAPSS), 620
- Global DNA methylation mapping, 324
- Glomerular basement membrane (GBM), 269, 1355
- Glomerular disease, 638
- Glomerular filtration rate (GFR), 1361
- Glomeruli (G), 1126
- Glomerulonephritis (GN), 534, 1287, 1355–1356
- Glucksman, Alfred, 291
- Glucocorticoids (GC), 723–724, 790, 804, 825–826, 884–885, 1290–1291, 1415–1416, 1419–1420
- GC-induced TNF-related ligand, 285
  - treatment, 1069
- Glucose 6-phosphate dehydrogenase (G6PD), 1204
- Glucose-6-phosphate isomerase (GPI), 535–536, 664–665
- Glutamate (E), 978
- Glutamate receptor subunit 3 (GluR3), 1464
- Glutamic acid decarboxylase (GAD), 1078, 1447
- GAD65, 391, 735, 1098, 1464
- Glutamine (Gln), 478
- Gluten-free diet, 853–854
- Gluten-sensitive enteropathy. *See* Celiac disease (CD)
- Gluten-specific CD4<sup>+</sup> T cells, 857–858
- Glycine receptor (GlyR), 1083, 1094
- Glycoprotein (GP), 914
- Glycoprotein fibrinogen, 939
- Glycosphingolipids, 119
- Glycosylated recombinant beta-interferon, 977–978
- Glycosylphosphatidylinositol (GPI), 197–198, 924
- Golimumab, 886, 1416t, 1425
- Gonadal insufficiency, 732–734
- Gonadotropin, 818–819
- Goodpasture's disease, 1356
- Gottron's papules, 704
- gp130 utilizing subset, 280
- GPCR-autoantibodies (GPCR-AABs), 1274–1275
- GPR35 receptor, 337–339
- Graft versus host disease (GvHD), 100–101, 515
- Graft-versus-host reaction–induced autoimmunity, 544
- Gram-negative bacteria, 177
- Granule polarization, 234
- Granulocyte-macrophage-CSF (GM-CSF), 160, 195, 279–280
- Granulocytes
- basophils, 250–252
  - eosinophils, 253–255
  - neutrophils, 243–250
- Granulomatosis with polyangiitis (GPA), 247, 1071–1072, 1287, 1422
- Granulomatous arteritis, 1316
- Granulomatous hypophysitis, 815, 822
- Granulomatous inflammation, 1039–1040
- Granulomatous lesions, 1319–1320
- Granulomatous panarteritis, 1069
- Granzyme B (GrB), 706–707
- Granzymes, 1391–1392
- Graves' disease (GD), 270–271, 438, 499–500, 757–763
- autoantibodies as potential immunological markers, 761
  - autoimmune features, 758–759
  - autoantibodies, 758
  - T-cell responses, 758–759
- clinical, pathologic, and epidemiologic features, 757–758
- environmental influences, 759
- genetic features, 759
- historic background, 757
- pathologic effector mechanisms, 761
- thyroid-associated ophthalmopathy and dermopathy, 762
- treatment and outcome, 761
- in vivo* models, 760–761
- Group A streptococci, 1256
- Group A streptococcus (GAS), 1262
- Growth impairment, 682
- Guillain Barré syndrome (GBS), 7, 270, 369, 376, 987–997
- animal models of disease, 993–995
  - autoimmune features
  - antiganglioside antibodies in GBS variants, 991
  - functional effects of antibodies, 991–993
  - gangliosides in peripheral nerve, 991
  - molecular mimicry, 989–990
- cellular and humoral immune elements, 997
- cellular mechanisms, 995–997
- clinical features and subtypes
- acute motor and sensory neuropathy, 989
  - AIIDP, 988–989
  - AMAN, 989
  - Miller Fisher syndrome, 989
- diversity of Guillain–Barré variants, 988b
- environmental effects, 993
- epidemiology, 988
- genetic aspects of GBS, 997
- historical background, 988
- treatment and outcomes, 997
- Gut bacterial microbiome diversity, 1394
- Gut biopsy, 861
- Gut epithelial homeostasis, 1394
- Gut immune system, 877
- "Gut leakiness", 1394
- Gut microbiome
- with human autoimmune diseases, 332–333
  - major products of, 334–336
  - modification, 1401–1402
- Gut microbiota, 333–334, 475–476
- role in autoimmunity, 30
- H**
- H2-A<sup>g7</sup> molecule, 483
- H3R17 code, 433
- Hair follicle bulb (HF bulb), 1212
- Hashimoto's thyroiditis (HT), 270–271, 438, 499–500, 749–750, 816, 823
- Hashitoxicosis, 750
- HDAC inhibitors (HDACis), 435, 440
- Health Protection Act and Freedom of Information Acts, 1380–1381
- Health-care expenditures (HCE), 1378
- Healthy immune system, 319
- Heat shock proteins (HSPs), 686
- HSP70, 1052

- Heavy metals, 354  
*Helicobacter pylori*, 323, 913, 1179  
 Helper T cells, 26–27  
 Hematologic antiphospholipid syndrome, 612  
 Hematologic changes, 565  
 Hematopoietic stem-cell transplantation (HSCT), 568–569  
 Hematopoietic cell transplantation (HCT), 515  
 Hematopoietic cell types, 822  
 Hematopoietic precursor cells (HPCs), 230, 308–309  
 Hematopoietic stem cells (HSCs), 45, 193  
 Hemidesmosome (HD), 446, 1191–1192, 1192f  
 Hemolysis, 901–902  
 Hemolytic anemia, 641  
 Hemolytic uremic syndrome (HUS), 269, 1356  
 Hemophagocytic lymphohistiocytosis (HLH), 677, 683  
 Hemopoietic stem-cell transplantation (HSCT), 1445–1446  
 Hemostasis, 935  
 Hen egg lysozyme (HEL), 179  
 Henoch–Schönlein purpura. *See IgA vasculitis*  
 HEp-2 cells, 558  
 Hepatitis B virus–associated PAN (HBV-PAN), 1289  
 Hepatitis C virus (HCV), 1290  
 hepatitis C-associated vasculitis, 100–101  
 Hepatitis-aplastic anemia syndrome, 925  
 Hepatobiliary, 875  
 Hepatocellular carcinoma (HCC), 1134, 1162–1163, 1162t  
 Hepatocytes, 1123  
 Hereditary DCM, 1273  
 Herpes gestationis (HG), 1192–1193, 1202  
 Herpes simplex virus (HSV), 366  
 Heterogeneity of tissue macrophages, 192–193  
 Heterogeneous nuclear ribonucleoprotein (hnRNP), 662  
 hnRNP-A2, 664  
 High endothelial venules (HEV), 58  
 High-dose steroid pulse, 1092  
 High-resolution computed tomography (HRCT), 589, 1337, 1340  
 Histamine-releasing IgG autoantibodies, 1228  
 Histidine decarboxylase (HDAbs), 798  
 Histone 3 lysine 27 acetylation (H3K27ac), 438  
 Histone 3 lysine 4 trimethylation (H3K4me3), 438  
 Histone acetylation, 432–433, 432f  
 Histone acetylation in type-1 diabetes, 440  
 Histone acetyltransferases (HATs), 338, 432–433  
 Histone code, 431  
 Histone deacetylase (HDAC), 335–336, 338, 431  
 inhibitors, 455–456  
 in T1D preclinical studies, 440  
 Histone demethylases (HDMs), 433  
 Histone methylation, 433  
 Histone methyltransferases (HMTs), 433  
 Histone modifications  
   in multiple sclerosis, 441–442  
   in RASFs, 435–436  
   in scleroderma, 447–448  
   in SLE, 445  
   writers, readers, and erasers of, 433–434  
 Histone posttranslational modifications, 431–434  
 Histone side chains, 432–433  
 Histone tail modifications in autoimmune thyroid diseases, 438  
 Histopathology, 1158–1159  
 Homotrimeric FAS receptor, 523  
 Homozygous mutations, 1132  
 Honeymoon phase, 1404  
 Hordeins, 856  
 Hormonal tests, 801–802  
 Hormones, 276, 790  
   and autoimmunity, 23  
*Horror autotoxicus*, 10, 66, 1369  
 Horse ATG (hATG), 927–928  
 Horton’s disease, 1316  
 Horvitz, Robert, 291  
*hTERC* gene, 924  
 HU (antineuronal nuclear antibodies 1), 1096  
 Human  
   human-derived cell lines, 494–495  
   islet autoantigen-specific vaccination trials in, 1404–1406  
   PBC, 1154  
   SLE T cells, 536  
   tissue culture cells, 1369  
 Human antigen D-related leukocyte (HLA-DR), 790  
 Human autoimmunity, NK cells and, 235–238, 236f  
   defective control of immune cells, 236–237  
   genetic association studies, 237–238  
 Human embryonic kidney (HEK), 1018  
 Human immunodeficiency virus (HIV), 913, 998, 1242, 1290  
 Human intrahepatic BECs (HIBECs), 1152–1153  
 Human leucocyte antigen-DRw4 (HLA-DRw4), 662–663  
 Human leukocyte antigen (HLA), 385–390, 468, 476–477, 636, 708, 769, 773, 773t, 777, 778f, 903, 924, 1036–1037, 1041–1042, 1041t, 1124, 1152, 1258, 1270–1271  
 alleles, 468–469  
 class II association with autoimmune diseases, 471–472, 472t  
 class II molecule  
   regulate autoimmunity by antigen-specific T regulatory cells, 484–485  
   regulating infection through modulation of cytokine networks, 483–484  
 genes, 855  
 haploidentical BMT with posttransplant cyclophosphamide, 929–930  
 haplotypes, 191–192  
*HLA-A\*03:01* allele, 387  
 HLA-B, 385  
 HLA-B\*27, 385  
 HLA-B27, 469–471, 691, 694  
   AIDS and, 470  
   and autophagy, 470  
   and evolution, 471  
   HLA-B27-associated uveitides, 1036–1037  
   and NK cells, 471  
   in pathogenesis of spondyloarthritis, 695–696  
   and peptide binding, 470–471  
   transgenic mice, 469–470  
 HLA-DQ8 transgenic mice, 483  
 HLA-DQB\*0601 allele, 482  
 HLA-DQB1, 440  
 HLA-DR4, 1319  
 HLA-DRB1\*03, 387  
 HLA-DRB1\*0401, 468–469  
 HLA-DRB1, 440  
 mechanisms association with autoimmunity, 389–390  
 MS with, 386–387  
 nonrheumatoid arthritis-associated human leukocyte antigen alleles, 480–481  
 onset, 473  
 predisposition, 472–473  
 type 1 diabetes, 385–386  
 Human monoclonal antibody, 1025  
 Human papillomavirus vaccine (HPV), 375  
 Human umbilical vein endothelial cells (HUVECs), 623–624  
 Humanized animal models of autoimmunity, 479  
 Humanized HLA DRB1\*0402 transgenic mice, 1196  
 Humanized MAbs, 1439  
 Humoral autoimmunity, 585  
 Humoral biomarkers, 779  
 Humoral immune elements, 997  
 Humoral immunity, 795–796  
*Hya* gene, 423  
 Hydrogen peroxide, 243  
 Hydroxychloroquine (HCQ), 566, 627, 1420  
   for musculoskeletal disease, 648  
 5-Hydroxymethylcytosine (5-hmC), 431  
 Hydroxymethylglutaryl coenzyme A reductase autoantibodies, 707–709  
 Hygiene hypothesis, 347–348, 753–754  
 Hyper-IgM syndrome (HIGM syndrome), 516–517  
   to B-cell defects, 520  
 Hyperacetylation, 435  
 Hyperacute rejection, 1360  
 Hyperbaric oxygen therapy, 1059  
 Hypercoagulant situation, 941  
 Hyperglycemia, 500–501  
 Hyperlipidemia and metabolic syndrome, 1162  
 Hyperprolactinemia, 820  
 Hypertrophic astrocytes, 966–967  
 Hypertrophy, 1273–1274  
 Hypoacetylation, 435  
 Hypocellular bone marrow, 924

Hypochlorous acid, 243  
 Hypocomplementemia, 625–626, 1302  
 Hypocretin receptor, 376  
 Hypomorphic mice, 1224  
 Hypoparathyroidism, 732–734, 741  
 Hypophysitis, 825  
 Hypopituitarism, 822  
 Hypopyon, 1036–1037  
 Hyposplenism, 734  
 Hypothalamus, 1077–1078  
 Hypothyroidism, 593–594

**I**

I-TRAF. *See* TANK receptor  
 Idiopathic AD, 793, 795  
 Idiopathic colitis, 872  
 Idiopathic interstitial lung diseases, 1336  
 Idiopathic interstitial pneumonia.  
     *See* Idiopathic pulmonary fibrosis (IPF)  
 Idiopathic interstitial pneumonitis.  
     *See* Idiopathic pulmonary fibrosis (IPF)  
 Idiopathic male infertility, 1241  
 Idiopathic pulmonary fibrosis (IPF), 1335, 1339  
     antifibrotic agents, 1349  
     autoantibodies associated with, 1342t  
     autoimmune features, 1341–1343  
     clinical, pathological and epidemiological features, 1337–1340  
     genetic features, 1344  
     histologic pattern, 1340f  
     history, 1336  
     pathologic effector mechanisms, 1346–1348  
     relationship to other idiopathic interstitial disorders, 1338f  
     treatment and outcome, 1348–1349  
         corticosteroids, 1348  
         cytotoxic agents, 1349  
     in vivo and in vitro models, 1345  
 Idiopathic SNHL, 1051–1052  
 IFN Induced with Helicase C domain 1 (IFIH1), 395  
 IgG4-related diseases (IgG4-RD), 1374  
 IgG4-related sclerosing cholangitis (IgG4-SC), 1177  
 Ikaros deficiency, 521  
 IL-1 receptor (IL1R), 387, 1426  
 IL-1 receptor antagonist (IL-1RA), 285, 325, 665  
 IL-1 receptor-associated kinase (IRAK1), 437  
 IL-12 receptor (IL-12R), 92–93  
 IL-18 binding protein (IL-18BP), 286  
 IL-2 receptor  $\alpha$  chain (IL2RA), 391–392  
     linkage analyses of combined datasets and limits of linkage analyses, 391–392  
     other loci, 391  
 IL-6 soluble receptor (sIL-6R), 682  
 Ileum bile acid transporter inhibitors (IBAT inhibitors), 1160  
 Imiquimod (IMQ), 1224  
 Immature B cells, 177  
 Immature NK cells (iNK cells), 230–231

Immune cells, 325  
     in autoimmune type 1 diabetes, 779–780  
 Immune checkpoint inhibitors, 826–827  
 Immune complexes (ICs), 244, 1175  
     effector mechanism in, 321  
 Immune dysregulation, 914, 971–973, 972f  
 Immune functions, 420  
 Immune hemolysis, 12  
 Immune homeostasis metabolite-sensing GPCRs, 336–338  
 Immune mechanisms, 1069–1071  
 Immune paralysis, 1447  
 Immune pathogenesis, 969–974  
     autoantigens, 973  
     B cells, 973–974  
         immune dysregulation, 971–973  
         T-cell pathogenesis, 970–971  
 Immune privileged organ, 1042  
 Immune response, 9, 1083  
     defective downregulation, 28–29  
 Immune system, 6, 9–10, 91, 295, 332, 332f, 969  
     activation, 26–27  
     cells, 46t  
         hormones effects on, 421–422  
 Immune thrombocytopenia (ITP), 911  
     causes of secondary ITP, 912t  
     diagnosis, 913  
     epidemiology, 912–913  
     first-line therapies, 916  
     megakaryopoiesis, 915–916  
     pathogenesis, 914  
     phases, 913t  
     platelet autoantibodies, 914  
     rituximab, 917  
     second-line therapies, 916  
     splenectomy, 916–917  
     T-cell involvement, 914–915  
     TPO-RAs, 917–918  
     treatment, 916  
 Immune thrombocytopenia purpura (ITP), 505, 516  
 Immune tolerance, 334–336, 1440–1445  
 Immune-based assay, 964  
 Immune-mediated destruction, 969  
 Immune-mediated hemolytic anemias, 904  
 Immune-mediated inner ear disease (IMIED), 1051, 1057t  
     animal models, 1057–1058  
     associated with primary vasculitides, 1053–1055  
     associated with systemic autoimmune diseases, 1053  
     clinical features, 1052–1055  
     evidence of autoimmunity, 1056  
     genetic susceptibility, 1056–1057  
     treatment, 1058–1059  
 Immune-mediated necrotizing myopathy (IMNM), 703  
 Immune-mediated response, 1036  
 Immune-mediated thrombotic–thrombocytopenic purpura (ITTP), 937  
 Immune-mediated vascular disorders, 1068  
 Immune-related adverse events, 827

Immunity  
     basophils role in, 250–251  
     biological principles and role in, 243–244  
         and neutrophil extracellular traps, 244–245, 244f  
         systemic autoimmune diseases, 245–250  
         cytokines and, 277–278  
         helper T cell “differentiation” and master regulators, 277f  
         eosinophils role in, 253  
         neutrophils role in, 243–250  
 Immunization, 263, 1057–1058  
     immunization-induced thyroiditis, 754  
 Immuno-stromal interactions in vasculitis, 1323  
 Immunoabsorption, 1276  
 Immunobiological revolution, 13–14  
 Immunobiology, return of, 13–14  
 Immunoblotting (IB), 1370  
 Immunoochemistry, shift to, 12–13  
 ImmunoChip study, 684–685  
 Immunocompetence, 420  
 Immunocyte, 5  
 Immunocytochemical techniques, 790  
 Immunodiffusion (ID), 1369  
 Immunodominant epitopes, 859  
 Immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX syndrome), 423, 524  
 Immunofluorescence microscopy (IF microscopy), 1194, 1288, 1356  
 Immunogenetic features, 999–1000  
     repertoire, 1001  
 Immunoglobulin (Ig)  
     antibody molecules, 56  
     autoantibodies, 937  
     deposition, 998  
     glomerulopathies, 1355  
     IgA, 321, 334  
         animal models, 1304  
         antibodies, 836–838  
         autoantibodies, 901  
         autoimmune features, 1303  
         clinical features and disease associations, 1303  
         diagnostic procedures, 1304  
         epidemiology, 1302–1303  
         genetic features and environmental influences, 1303–1304  
         nephropathy, 1304, 1358  
         pathogenesis, 1303  
         pathological features, 1303  
         pemphigus, 1200  
         treatment, 1304  
     vasculitis, 1302–1304  
 IgE antibodies  
     in autoimmune diseases, 251–252  
     in BP, 252  
 IgG, 321, 661, 914, 1015, 1053, 1117  
     antiganglioside antibodies, 993–994  
     autoantibodies, 269, 937  
     IgG1, 949  
     IgG4, 1180  
     IgG4 plasmacytic hypophysitis, 822–823  
     IgG + /IgG + plasma cell ratio, 717

- Immunoglobulin (Ig) (*Continued*)
- IgLON5
    - encephalitis, 1088
    - receptors, 1095
  - IgM, 297, 321
  - isotypes, 172
  - Immunoglobulin G4-related disease (IgG4-RD), 715–716, 1173, 1175f, 1357
    - animal model, 720
    - autoimmune features, 719
    - autoimmune pancreatitis to, 1174
    - classification criteria, 723
    - clinical features and disease associations, 720–723
    - epidemiology, 716
    - evidence for autoimmunity in, 719
    - gateway from autoimmune pancreatitis to, 1173–1174
    - genetics, 720
    - historical aspects, 716
    - IgG4-SC, 1177
    - IgG4-related
      - hypertrophic pachymeningitis, 1176
      - kidney disease, 1177
      - lacrimal and salivary gland lesions, 1176
      - liver disease, 1177
      - lung disease, 1177
      - ophthalmic disease, 1176
      - periaortitis/periarteritis, 1178
      - pituitary and stalk lesions, 1176
      - prostate disease, 1178
      - retroperitoneal fibrosis, 1177
      - thyroid disease, 1177
    - immunoglobulin G4-related sclerosing
      - cholangitis and cholecystitis, 721
      - in kidney, 721–722
      - lesions, 723
      - in lung, 721
      - in nervous system, 722–723
      - pathology, 716–717
        - histological features, 716
        - immunostaining, 717
      - pathophysiology, 717–718
        - B cells, 718
        - T cells, 718
      - perspectives, 725
      - serum immunoglobulin G4 concentrations, 718
      - treatment, 723–724
    - Immunoglobulin heavy chain locus (IgH chain locus), 155
    - Immunoglobulin light chain loci (IgL chain loci), 155
      - expression of IgL chains, 163–164
    - Immunohistochemistry (IHC), 1081
    - Immunologic markers, autoantibodies as, 861–862
      - “Immunological homunculus”, 10
    - Immunological markers, 1044
      - in diagnosis, 965–966
    - Immunological tolerance, 65–66
    - Immunology, 3, 9, 1415–1416
    - Immunomodulation, 1024
      - drugs, 815
      - effect, 968
    - Immunopathology, 10
    - Immunopositivity, 790
    - Immunoproteasome, 54
    - Immunoreceptor tyrosine-based activation motifs (ITAM), 172
    - Immunoreceptor tyrosine-based inhibitory motif (ITIM), 173, 197–198, 232, 539–540
    - Immunoregulatory cytokines, 229–230
    - Immunosenescence, 420
    - Immunostaining, 717
    - Immunosuppressants, 1074
    - Immunosuppressive/immunosuppression, 938–939, 1082, 1122, 1440–1445
      - B lymphocytes, 1043–1044
      - cytokines, 29, 275
        - cytokines/growth factors, 286
      - drugs, 974, 1002, 1218
      - functions, 1022
      - therapy, 923–924, 927–928, 945, 1328
      - treatment, 1016, 1069, 1137
        - for autoimmune rheumatic diseases, 1416t
    - Immunotherapies, 974, 1082–1083, 1464
    - Implants, 355
    - Imuran. *See* Azathioprine (AZA)
    - In vitro
      - bullous pemphigoid, 1201
      - in C2C12 myotubes, 1019
      - models, 269
      - PF, 1198
      - PV, 1195
        - autoantibody passive transfer model, 1195
        - murine models of pemphigus vulgaris, 1196
        - studies, 364
      - In vivo models, 754–755
        - bullous pemphigoid, 1201
        - experimental autoimmune thyroiditis, 754
        - immunization-induced thyroiditis, 754
        - PF, 1198
        - PV, 1195–1196
          - spontaneous autoimmune thyroiditis, 754–755
      - Inclusion body myositis, 703
      - “Indeterminate” colitis, 877
      - Indirect immunofluorescence (IIF), 795–796, 1296, 1369
      - Indirect immunofluorescence assay (IIFA), 558
      - Indirect or antigen-independent activation, 119–120
      - Individual parenchymal lesions, 1068
      - Indoleamine 2,3-dioxygenase (IDO), 217, 338–339
      - Induced immunity, 793
      - Induced models of systemic autoimmunity, 544–545
        - CAIA model, 545
        - CIA model, 545
          - graft-versus-host reaction–induced autoimmunity, 544
          - pristane-induced lupus model, 544
      - Inducible costimulator (ICOS), 102–103
        - deficiency, 518
        - molecule, 1180
      - Induction T cells (iTregs), 1403
      - Infantile polyarteritis nodosa, 1287
      - Infections tolerance, 281
      - Infectious agents, 473–474, 904, 1356
      - Infectious triggering of autoreactive T cells, 364
      - Infectious triggers of autoimmunity
        - autoimmune CNS demyelinating disease, 370
        - bystander activation of autoreactive cells and epitope spreading, 367–368
        - emerging mechanisms, 369
        - infectious triggering of autoreactive T cells, 364
        - mechanisms, 364–370
          - infection-induced autoimmunity, 365f
        - pathogen-induced murine models of human autoimmune disease, 366t
        - reciprocal relationships of pathogen-derived mechanisms, 369–370
        - virus pathogens in human autoimmune diseases, 368t
      - Inflamasomes, 47, 47f
        - spreading, 203
      - Inflammation, 4, 17, 232–233, 962, 964, 966, 1036–1039, 1038f, 1053–1054
        - inflammation-inducing cells, 1043
        - macrophage heterogeneity during, 193–196
      - Inflammatory bowel disease (IBD), 18, 93, 219–220, 281, 453, 495, 691, 871–872, 1420–1421
        - animal models, 880–881
        - clinical features and disease associations, 873–876, 873t
        - colitis, 124t, 127
        - Crohn’s disease, 130t, 132
        - diagnostic procedures, 881–883
          - serologic markers, 881–882
          - stool markers, 882
        - epidemiology, 872–873
        - etiology including autoimmune features, 877–879
          - innate and adoptive immunity, 878–879
          - intestinal epithelium and barrier function, 878
          - microbiota, 877–878
        - genetics, 879–880
        - historical aspects of, 872
        - imaging, 882–883
        - pathological features, 876–877
        - treatment, 883–888, 883t
          - adalimumab, 886
          - aminosalicylates, 884–888
          - anti-IL12/13 therapies, 887
          - anti-TNF therapies, 886–887
          - certolizumab, 886
          - cyclosporine, 886
          - glucocorticoids, 884–885
          - golimumab, 886
          - infliximab, 886
          - inhibitors of leukocyte infiltration, 887
          - Janus kinase inhibitors, 888
          - medical, 883

- methotrexate, 886  
 nutritional support, 888  
 surgery, 888  
 thiopurines, 885–886
- Inflammatory cells, 961, 1036–1037  
 Inflammatory cellular infiltration, 998  
 Inflammatory cytokines, 248  
 Inflammatory DCs, 215  
 Inflammatory DNA, 298–299  
 Inflammatory pathology, 321  
 Inflammatory process, 4, 1044  
 Inflammatory response, 971  
 Inflammatory synovitis, 685  
*Infliximab*, 886, 1059, 1416<sup>t</sup>  
 Influenza nucleoprotein, 376  
 Infundibulum, 816  
 Infusion-based therapies, 974–976  
     alemtuzumab, 976  
     mitoxantrone, 976  
     natalizumab, 974–975  
     ocrelizumab, 976  
 Inhalational injuries, 1345–1346  
 Inhibitor eradication, 946  
 Inhibitor of DNA binding 2 (Id2), 216  
 Inhibitory receptors  
     GABAaRs, 1094  
     GABAbR, 1094  
     GlyR, 1094  
 Injection-based therapies, 977–978  
     glatiramer acetate, 978  
     interferons, 977–978  
 Innate immune  
     activation, 18  
     cell signaling, 543–544  
     defects, 526–527  
     responses, 277  
 Innate immunity, 28, 32, 559–560, 878–879, 1179  
     autophagy in, 309–310  
     dendritic cells, 559–560  
 Innate lymphoid cells (ILCs), 230, 278, 324  
 Innate response, 45–46  
     cellular components, 47–51  
     resolution, 59  
     soluble mediators, 50–51  
 Inner ear  
     antigens, 1056  
     pathology, 1054  
 Innocuous antigens, 9–10  
*INPP5D* gene, 394  
 Insulin (*INS*), 385–386  
 Insulin gene susceptibility locus (IDDM2), 1404–1405  
 Insulin-dependent diabetes, 1448  
     loci, 220–221  
 Insulin-dependent diabetes mellitus.  
     See Type 1 diabetes (T1D)  
 Insulin-like growth factor 1 (IGF-1), 682  
 Insulinoma-associated protein 2 (IA-2A), 775–776, 1392  
 Insulitis, 769, 776, 779–780  
 Integrin alpha M (ITGAM), 403  
 Intercellular adhesion molecule 1 (ICAM-1), 584, 648  
 Intercellular spaces (ICS), 1194  
 Interface hepatitis, 1123, 1123f  
 Interferon (IFN), 92–93, 214–215, 282, 387, 581, 962, 977–978, 1043, 1153  
     IFN- $\alpha$ , 237, 282, 1073, 1077  
     IFN- $\beta$ , 102  
         IFN- $\beta$ 1, 276  
         interferon- $\beta$ 1a, 1002  
     IFN- $\gamma$ , 92–93, 229–230, 275–276, 282, 420–421, 483–484, 693–694, 717, 840, 861, 925–926, 1056, 1277–1279, 1315–1316  
     IRF5, 644  
     IRF7, 403  
     IRF8, 216, 400  
 Interleukin (IL), 92–93, 229–230, 246–247, 355, 995–996, 1041–1042, 1419, 1440–1441  
     IL-1, 267–268, 275–276  
         antagonists, 1426  
         IL-1/TLR family of receptors, 285–286  
     IL-1R2, 697  
     IL-1 $\beta$ , 47, 717  
     IL-2, 216, 275–276, 278, 386, 397, 677  
     IL2RA, 399  
     IL-2R $\gamma$  chain, 423  
     IL-3, 280  
     IL-5, 253, 280  
     IL-6, 280, 325, 435, 584, 790  
     IL-6R $\alpha$ , 280  
     IL-7, 279  
     IL7R, 398–399  
     IL-9, 279  
     IL-10, 194, 283, 398, 405, 878  
     IL-12, 1021  
         cytokine family, 280  
         IL12A, 406  
         IL12B, 401  
     IL-15, 279, 861  
     IL-17, 278, 840, 880  
         IL-17 + T-cell numbers, 685–686  
         IL-17A, 1278–1279, 1426  
         pathway, 277–278  
         receptors, 325–326  
     IL-18, 47, 286, 1328  
     IL-19, 283  
     IL-20, 283  
     IL-21, 279  
     IL-22, 278, 283  
     IL-23, 281, 694–695, 698  
     IL-24, 283, 1228  
     IL-25, 253, 281–282  
     IL-26, 283  
     IL-27, 281  
     IL-33 functions, 286  
     IL-35, 281  
     IL-37, 286  
     IL-38, 286  
 Interleukin-1 receptor-associated kinase-1 (IRAK1), 577–579  
 Intermediate uveitis, 1037–1039  
 International Autoimmune Hepatitis Group  
     (HAIHG), 1118, 1120<sup>t</sup>
- International Federation of Clinical Chemistry Committee on Harmonization of Autoimmune Tests (IFCC C-HAT), 1381  
 International League of Associations for Rheumatology (ILAR), 675  
 International MS Genetics Consortium (IMSGC), 398  
 International Multiple Sclerosis Genetics Consortium (IMSGC), 387  
 International Society for Nephrology/Renal Pathology Society (ISN/RPS), 563  
 International Society of Thrombosis and Haemostasis (ISTH), 621  
 International Union of Immunology Societies/World Health Organization/Arthritis Foundation (IUIS/WHO/AF), 1370  
 International Working Group (IWG), 912  
 Interphotoreceptor retinoid-binding protein (IRBP), 1035  
 Interstitial keratitis, 1054  
 Interstitial lung disease (ILD), 577, 589–590, 704, 1335  
 Interstitial macrophages, 1239  
 Interstitial pneumonitis, 638  
 Intestinal dendritic cells, 878  
 Intestinal epithelium, 878  
 Intestinal lumen, 877  
 Intestinal microbiome, 1131  
 Intestine, environmental cues and Th17 cell regulation in, 97–98  
 Intraarticular steroid injections, 686  
 Intracellular adhesion molecule-1 (ICAM-1), 645  
 Intracellular DNA, 298  
 Intracellular molecules, 1067  
 Intracellular neuronal antigens  
     antineuronal nuclear antibodies, 1096–1097  
     cytoplasmatic antigens, 1097–1098  
     glial antigens, 1098–1099  
 Intracellular pathogens, 92–93, 281  
 Intracellular RNA, 298  
 Intracranial hemorrhage (ICH), 912  
 Intraepidermal neutrophilic (IEN), 1200  
 Intraepithelial B cells, 156–157  
 Intraepithelial lymphocytes (IELs), 852  
 Intranasal Insulin Trial II (INIT II), 1406  
 Intraneural injection, 991  
 Intraocular inflammatory reaction, 1036  
 Intradiscal  
     immunoglobulin production, 973  
     synthesis, 965  
 Intravenous immunoglobulin (ivIg), 913, 916, 997, 1002, 1015, 1416<sup>t</sup>, 1428–1429  
 Intravenous steroids, 567  
 Intravenous therapy (IV therapy), 1419  
 Intrinsic apoptosis, 292  
 Intrinsic factor (IF), 833  
 Invariant natural killer T cells (*i*NKT cells), 118, 309  
     activating factor in, 134–135  
     actors role, 133–134  
     beneficial roles, 130–133, 130<sup>t</sup>

Invariant natural killer T cells (*iNKT* cells)  
 (Continued)  
 detrimental roles, 125–129  
 distribution, 119  
 effector functions  
   activation, 119–120  
   cytokine production, 120  
   down-stream effects, 120–121  
 features of CD1d reactive NKT cell subsets, 122t  
 influencing autoimmune responses, 135–136  
 Janus-like character, 123–134  
 kinds, 121  
 phenotype, 118–119  
 species divide, 121–123  
 technical problems, 121–123  
 Invariant TCR (*iTCR*), 118  
*Ipilimumab*, 826–827  
*IRF5* transcription factor, 389  
*IRF8*, 405–406  
 Irradiation, 926  
 Ischemia of nervous system, 1077  
 Ischemia/reperfusion injury (IRI), 271  
 Ischemic heart disease, 1326–1327  
 Ischemic optic neuropathy (ION), 1315–1316  
 Islet antigen-2 (IA-2), 735, 770  
 Islet autoantibodies (IAA), 775–776  
 Islet autoantigen-specific vaccination trials in humans, 1404–1406  
 Islet autoimmunity, 1393  
 genetic etiology of, 772–774  
 Islet cell antibodies (ICAs), 1394–1395  
 Islet cell autoantibodies, 776–777  
 Isoimmunization, 793  
 Isolated myelopathies and visual loss, associated with antineuronal antibodies, 1090  
 Itraconazole, 741  
 IV anti-D treatment, 916

**J**  
 Janus kinase (JAK), 96, 325–326, 525, 878–879, 1428  
 inhibitors, 888, 1220  
 JAK1, 862  
 GOF, 526  
 JAK2/signal transducer, 993  
 Japanese Eculizumab Trial, 997  
 Jumonji C domain-containing protein 3 (JMJD3), 448–449  
 Juvenile hypophysitis, 820  
 Juvenile idiopathic arthritis (JIA), 518–519, 675, 1036  
 B cells, role of, 685  
 clinical features, 676–679  
   age at onset, 676t  
   sex ratio, 676t  
   systemic arthritis, 676–677  
 enthesitis-related arthritis, 677–678  
 epidemiology, 676  
 etiology, 680–682  
 FoxP3 + Treg cells, role of, 685  
 IL-17 + T cells, role of, 685

ILAR classification, 675, 676t  
 inflammatory synovitis, 685  
 life-threatening complications, 677  
 oligoarthritis, 678  
 oligoarticular juvenile idiopathic arthritis, 684–686  
 pathogenesis, 680–682  
 perspectives, 679  
 psoriatic arthritis, 679  
 rheumatoid factor negative polyarthritides, 678–679  
 rheumatoid factor-positive polyarthritis, 677  
 SF CD4:CD8 ratio, 685–686  
 systemic, 676–677, 680  
 Th1/Th17 phenotype, role of, 685  
 treatment, 686–687  
   anti-TNF agents, 686  
   cyclosporine, 686–687  
 undifferentiated arthritis, 679  
 Juvenile inflammatory arthritis (JIA), 1426

**K**  
 K/BxN mice, 535–536  
 Kawasaki's disease (KD), 1291–1292  
   animal models, 1292  
   autoimmune features, 1292  
   clinical features and disease associations, 1291  
   diagnostic procedures, 1292  
   epidemiology, 1291  
   genetic features and environmental influences, 1292  
   pathogenesis, 1291  
   pathological features, 1291  
   treatment, 1292  
 790 kDa C1 complex, 264  
 Keratinocyte adhesion molecules, 1191–1192  
 Keratoconjunctivitis sicca, 636  
 Ketoconazole, 741  
 Killer cell immunoglobulin-like receptors (KIRs), 230–231, 471  
 KIR2DS4-FL, 1127  
 Kinase-dead mutant of Mer (MerKD), 543  
 Kinsbourne syndrome, 1090  
 KIR receptor genes, 237  
 KLF13, 445  
 Klinefelter's syndrome, 423  
 Knockout mice, 1224  
 Koebner phenomenon, 1216  
 Krebs cycle, 194  
 Krebs von den Lungen-6 (KL-6), 595  
 Kresge Hearing Research Institute-3 (KHRI-3), 1056  
 Kupffer cells, 1123

**L**  
 L-23, 94–95  
 La or Sjögren syndrome antigen B (La/SSB), 249  
 Labial salivary glands (LSGs), 446  
 Laboratory reports, 1379–1381  
 Labyrinthine artery, 1053–1054  
 Lacrimal glands, 720

Lactate dehydrogenase 3 (LDH3), 1237  
 Lake Louise criteria, 1270  
 Lambert–Eaton myasthenic syndrome (LEMS), 1025–1026, 1089  
 clinical features, 1025  
 epidemiology and etiology, 1025  
 investigation and treatment, 1026  
 pathophysiology, 1026  
 Laminin 332. *See* Laminin 5  
 Laminin 5, 1202–1203  
 Large B-cell lymphoma (LBCL), 641  
 Large-vessel vasculitis (LVV), 1069–1070, 1313  
 giant cell arteritis, 1069–1070  
 Late-onset acetylcholine receptor-antibody MG, 1016  
 "Law of immunity research", 11  
 LC3-associated phagocytosis (LAP), 307–308, 543  
 LCMV glycoprotein (LCMV-GP), 500–501  
 Learned immunity. *See* Trained immunity  
 Lectin pathway (LP), 50–51, 264–265  
 Lectin receptors, 200–202  
 Leflunomide (LF), 1416t, 1421  
 Left ventricle (LV), 1270  
 Lemtrada. *See* Alemtuzumab  
 Lens autoantibodies, 11  
 Lens epithelium-derived growth factor (LEDGF), 1380  
 Lesion patterns, 1068  
 Leucine-rich glioma inactivated-1 (LGI1), 1080–1081, 1085–1086, 1095  
 Leucine-rich repeat containing receptor (NLR receptor), 214–215  
 Leukocyte functional antigen (LFA), 232  
 LFA-1, 234  
 Leukocyte immunoglobulin-like receptor (LILR), 696  
 Leukocyte infiltration inhibitors, 887  
 Leukocytoclastic vasculitis, 517  
 Leukopenia, 641  
 Libman–Sacks endocarditis, 563, 613  
 Licensing process, 232  
 Lichen sclerosus, 1465–1466  
 Ligand  
   binding, 422  
   ligand-regulated nuclear transcription factors, 421  
 Light chain 3 (LC3), 306–307, 543  
 Limbic encephalitis (LE), 1083–1086  
   anti-N-methyl-D-aspartate receptor encephalitis, 1084–1085  
   antivoltage-gated potassium channels antibody encephalitis, 1085–1086  
 Limited cutaneous SSc (lcSSc), 577, 578t  
 Line blots (LBs), 1081  
 Line immunoassays (LIAs), 621, 1370  
 Linear IgA dermatosis (LAD), 1192–1193  
 Linear IgA disease (LAD), 1203  
 Linkage studies  
   in multiple sclerosis, 392–393  
   in systemic lupus erythematosus, 393–394  
   of type 1 diabetes, 390–392  
   CTLA4 gene, 390–391  
   GAD2 gene, 391

Linked recognition, 173–174  
 Linker histones, 432  
 Lipid-laden macrophages, 966–967  
 Lipoic acid (LA), 1151–1152  
 Lipooligosaccharide (LOS), 993  
 Lipopolysaccharide (LPS), 126, 177, 280,  
   615–616, 993, 1043  
 Lipopolysaccharide-responsive beige-like  
   anchor (LRBA), 521  
 Lipoproteins, 200  
*Listeria monocytogenes*, 92–93, 218–219  
 Lithotripsy-associated kidney trauma, 1356  
 Live cell imaging of NK cells, 233–234  
 Live vaccines, 379–380  
 Livedo reticularis, 612–613  
 Liver biopsy, 1158  
 Liver transplantation (LT), 1136, 1149–1150  
 Liver-specific protein (LSP), 1127  
 Lobular fibrosis, 1131–1132  
 Local plasmacytoid dendritic cells, 642  
 Long ncRNAs (lncRNAs), 434  
 Long-acting thyroid stimulator (LATS), 757  
 Long-lived plasma cells, 157–158  
 Long-term steroid therapy, 1045  
 Longitudinal extensive transfer myelitis  
   (LETM), 1091  
 Loss of tolerance, 536  
 Loss-of-function (LOF), 515  
 Low level antiphospholipid antibodies, 617  
 Low molecular weight heparin (LMWH),  
   626–627  
 Low-density granulocytes (LDGs), 245–246  
 Low-density lipoprotein receptor related  
   protein 4 (LRP4), 1013  
 Low-density lipoproteins (LDLs), 200  
 Low-molecular-weight heparins, 1059  
 LOX-1, 297  
*LPP* gene, 753  
 LRBA protein deficiency with autoantibodies  
   (LATAIE), 524–525  
 LRP4 Abs, 1020–1021  
 LT repopulating hematopoietic stem cells  
   (LT-pHSCs), 157–158  
*LTBR* gene, 393–394  
 LUMINA trial, 1420  
 Lupoid hepatitis, 1117–1118  
 Lupus anticoagulants (LAC), 615, 937, 1074  
 Lupus autoimmunity, 294  
 Lupus erythematosus cells (LE cells), 1067,  
   1117–1118, 1369  
 Lupus low disease activity state (LLDAS),  
   565  
 Lupus nephritis (LN), 454, 555, 563,  
   1356–1359  
 Ly108, 534  
 LY49I inhibitory receptor, 1244  
 Lyme disease, 1037–1039  
 Lymph node stromal cell  
   (LNSC), 81–82  
 Lymph nodes (LNs), 157–158, 1239  
 Lymphadenopathy, 720  
 Lymphochoriomeningitis virus (LCMV),  
   66–68  
 Lymphocyte function-associated antigen-1  
   (LFA-1), 648

Lymphocyte-specific protein tyrosine  
   phosphatase (LYP), 386  
 Lymphocytes, 5, 45, 58, 236–237, 966  
   activation molecules, 536–540  
   antigen recognition by, 46/  
   development, 158  
   lymphocyte-derived factors, 275–276  
   lymphocyte-derived Th17 type of  
   cytokines, 277  
   lymphocyte-mediated destruction of  
   melanocytes, 1218  
   subsets, 277–278  
 Lymphocytic adenohypophysitis (LAH),  
   816–817  
 Lymphocytic choriomeningitis virus  
   (LCMV), 496, 898, 1403  
 Lymphocytic hypophysitis, 815, 822, 822f  
 Lymphocytic infiltrates, 1274  
 Lymphocytic infundibuloneurohypophysitis  
   (LINH), 816  
 Lymphocytic interstitial pneumonia (LIP),  
   637–638  
 Lymphocytic mastitis, 1466  
 Lymphodrek, 276  
 Lymphoid  
   DCs, 215  
   follicles, 57  
 Lymphokines, 275–276  
 Lymphoma, 756  
 Lymphomonocyte infiltration, 1241  
 Lymphoplasmacytic infiltration, 716, 717f,  
   1178  
 Lymphotoxin (LT), 642  
   LT $\alpha$ , 284  
 Lyn, 172, 540  
 Lysine (K), 978  
 Lysophosphatidic acid (LPA), 1161  
 Lysophosphatidylcholine (LPC), 295–296,  
   313

**M**

M<sub>2</sub>-autoantibody effects (M<sub>2</sub>-AABs),  
   1275–1276  
 Macroautophagy (MA), 305–307  
 Macrophage activation syndrome (MAS),  
   236–237, 677, 683–686  
 Macrophage colony-stimulating factor (M-  
   CSF), 160, 193  
 Macrophage inhibitory factor (MIF), 1260  
 Macrophage receptor with collagenous  
   structure (MARCO structure), 200  
 Macrophage(s), 32, 45, 47–48, 119–120, 191,  
   219–220, 295–296, 966, 996, 1044  
   apoptotic cell clearance by, 204–205  
   in celiac disease, 860  
   characteristics of, 191  
   cytosolic pattern recognition receptors,  
   202–203  
   effector mechanism in, 322  
   FcR, 196–198  
   in giant-cell arteritis, 1322–1323, 1322f  
   heterogeneity of tissue, 192–193  
   lectin receptors, 200–202  
   macrophage heterogeneity during  
   inflammation, 193–196

nonopsonic receptors in, 196–198  
 opsonic receptors in, 196–198  
 pattern recognition receptors, 198–203  
 phagocytic process, 203–204  
 polarization, 193–194  
 scavenger receptors, 200  
 toll-like receptors, 199–200  
*MAGI3*, 753  
 “Magic bullet” monotherapy, 1406–1407  
 Magnetic resonance (MR), 1068  
 Magnetic resonance imaging (MRI), 647,  
   722–723, 882, 962, 1068, 1174, 1270,  
   1274, 1441  
 Main pancreatic duct (MPD), 1174  
 Major histocompatibility complex (MHC), 6,  
   19, 68, 117–118, 191–192, 213,  
   229–230, 345–346, 385, 436, 468, 498,  
   708, 752, 769, 790, 967–968, 997,  
   1040–1041, 1127, 1213–1214, 1276  
 autoimmunity and, 468–469  
 class I, 19  
   deficiency, 517  
   molecules, 45–46, 54, 214  
 class II, 19  
   gene, 662–663  
   molecules, 45–46, 54, 214, 312–313,  
   471–472  
 Mammalian erythrocytes, 10  
 Mannose binding lectin (MBL), 264–265,  
   1260  
 Mannose receptor (MR), 193–194  
 Mannose-binding lectin pathway, 1179  
 Mannose-binding protein (MBP), 50–51  
 Marchiafava–Micheli syndrome, 268  
 Marginal zone (MZ), 155–157  
 MASP-1, 265  
*Masp2* gene, 1260  
 Mass spectrometry analysis, 1263  
 Mast cells, 45, 322  
 “Master regulators”, 277–278  
 Maternal engraftment, SCID with, 516  
 Maternal–fetal transfer, 1018  
 Matrilin, 1469  
 Matrix destruction, 964  
 Matrix metalloproteinases (MMPs), 243, 996,  
   1322–1323  
   MMP-3, 437  
   MMP-9, 642  
 Mature NK cells, 231  
 MBL-associated serine protease (Masp), 1260  
*MBL2* gene, 1260  
 Measles vaccination, 376  
 Medial macrophages, 1322  
 Mediterranean diet, 332–333  
 Medium vessel vasculitides, 1070–1071  
 Medium-sized ncRNAs, 434  
 Medullary thymic epithelial cells (mTECs),  
   68, 71f, 73, 516, 1001, 1023  
 Megakaryocytes (MKs), 45, 911  
 Megakaryopoiesis, 915–916  
 Megaloblastic anemia, 834  
 Meiotic germ-cell antigens, systemic  
   tolerance to exposing, 1237  
 Melanin, 1219–1220  
 Melanocyte(s), 1219–1220

- Melanocyte(s) (*Continued*)  
loss, 1217
- Melanoma differentiation associated factor 5 (MDA-5), 203
- Melasma suprarenale, 789–790
- Membrane  
hyperpolarization, 335  
loss of membrane symmetry, 48  
membrane-associated complement regulators, 270–271  
membrane-bound regulators, 267
- Membrane attack complex (MAC), 264, 266, 899
- Membrane bound receptor (mIL-6R), 1425–1426
- Membrane cofactor protein (MCP), 266–267, 321
- Membrane outer membrane polarization (MOMP), 292
- Membranoproliferative glomerulonephritis (MPGN), 269  
MPGN1, 269
- Membranous glomerulonephropathy, 722
- Membranous nephropathy (MN), 1355
- Memory B cells, 157–158, 176–177
- Memory cells, 171
- Menière's syndrome, 1052
- Meningococcal vaccine, 1051
- 6-Mercaptopurine (6-MP), 885, 1133, 1183, 1423–1424
- Mesalamine. *See* Aminosalicylates (5-ASA)
- Messenger RNA (mRNA), 431
- Metabolic disorders, 641
- Metabolic-genetic storage diseases, 1466
- Metabolite-sensing GPCRs, 335–338
- Metabotropic glutamate receptor (mGluR), 1083  
mGluR1, 1094  
mGluR5, 1088, 1094
- Methotrexate (MTX), 680, 825–826, 886, 1045, 1058–1059, 1073, 1077, 1183, 1416t, 1420–1422
- Methyl-CpG-binding domain protein (MBD protein), 430
- Methyl-CpG-binding protein 2 (MECP2), 431, 577–579
- 5-Methylcytosine (5-mC), 431
- MG thymus, 1023, 1023f
- MG with AChR antibodies (AChR-MG), 1014–1015
- MHC class II transactivator (MHC2TA), 793–794
- MHC class II-containing compartments (MIICs), 310–311
- MICL, 201
- Microarray technology, 1344
- Microautophagy (MI), 305–306
- Microbial colonization, 1394
- Microbiome, 355–356, 476–477, 659–660, 969  
dysbiosis, 1401  
epigenetics, 453  
mechanisms for microbiome-mediated gut, 336–338, 337f
- Microchimerism, 354, 774
- MicroRNA (miRNA), 434, 453  
microRNAs-targeting therapeutics, 456–457  
miRNA-124, 437  
miRNA-146a, 437  
miRNA-155, 437  
in multiple sclerosis, 443  
in scleroderma, 448  
in Sjögren's syndrome, 447  
in systemic lupus erythematosus, 445–446
- Microscopic form of periarteritis (MPA), 1287
- Microscopic polyangiitis (MPA), 247, 1071–1072, 1287
- Microspheres, 222
- Microtubule-associated protein 1B (MAP1B), 1097
- Microtubule-associated protein light chain 3, 543
- Microtubule-organizing center (MTOC), 234
- Microvascular disease in systemic sclerosis, 583–584
- Microvascular endothelial cells (mvEC), 1319–1320
- Migration, 214
- Migration inhibitory factors (MIF), 275–276, 1260
- Migratory DCs, 218
- Mikulicz' disease, 720
- Milk fat globule EGF factor 8 (MFG-E8), 296–297, 543
- Miller Fisher syndrome, 989
- Mincle, 201
- Mineralocorticoids, 790
- Miniature endplate potential (MEPP), 1013
- miR-21, 448
- miR-29, 448
- miR-126, 445–446
- miR-146a, 437
- miR-150, 448
- miR-154\*, 438
- miR-155, 437
- miR-376b, 438
- miR-431\*, 438
- "Misdirected" immunity, 9–10
- "Missing-self" recognition, 229–230
- Mitochondria, 243
- Mitochondrial DNA (mtDNA), 298–299
- Mitogen-activated protein kinase (MAPK), 325, 622
- Mitogenic factors for lymphocytes, 275–276
- Mitoxantrone, 976
- Mitral regurgitation, 1255–1256
- Mitral stenosis (MS), 1260
- MN-Heymann nephritis rat model, 1359
- Model for end-stage liver disease (MELD), 1133–1134
- Molecular mimicry, 364–367, 376, 989–990, 1015, 1128, 1263
- Monoclonal antibodies (mABs), 1058, 1425, 1437–1440  
engineering Fc regions to avoid side effects and prolong half-life, 1439  
engineering variable regions
- to decreasing immunogenicity, 1439–1440  
to increasing affinity, 1439
- Monoclonal cryoglobulins, 1301
- Monoclonal gammopathies of undetermined significance (MGUS), 999
- Monocytes, 45, 47, 195, 322, 966  
monocyte-derived DCs, 216  
monocyte-derived tissue macrophages, 193
- Monogenic disease, 21
- Mononuclear cell, 584
- Monozygotic twins (MZ twins), 384, 759 studies, 879
- Morbidity, 1054
- Morus Bechterew disease. See Spondylitis ankylosans*
- Morvan's syndrome, 1086
- Mosquito-borne RNA flavivirus, 993
- Motor nerve terminals (MNTs), 991
- Motor neuron disease (MND), 270
- Mouse models, 1017  
of autoimmune gastritis, 841f  
in pernicious anemia, 839
- Movement disorders, 1466–1467
- MR enterography (MRE), 882
- MR angiography (MRA), 1068
- MRL  
MRL/lpr, 644  
and gld mice, 534–535  
strain, 560
- Mucin (MUC)  
MUC-2 gene, 475–476  
MUC5B, 1344
- Mucosa-mediated antigen-specific tolerance, 1403–1404
- Mucous membrane pemphigoid (MMP), 1201–1202
- Multicentric fibrosclerosis, 723
- Multifocal inflammatory disease, 961–962
- Multifocal lymphocytic infiltration, 995–996
- Multifocal motor neuropathy with conduction block (MMNCB), 998
- Multifocal perivascular T-cell infiltration, 988–989
- Multifocal-acquired demyelinating sensory and motor neuropathy, 998
- Multifocal-acquired sensory and motor neuropathy, 998–999
- Multiorgan autoimmunity, 101–102
- Multiple neuritis, 998
- Multiple nuclear dots (MNDs), 1158
- Multiple sclerosis (MS), 93, 130t, 132–133, 270, 278, 367, 384, 441–443, 473–474, 495, 504–505, 961, 975t, 1037–1039, 1441–1442, 1445, 1448
- animal model, 481  
clinical features, 962–963  
CIS, 963  
progressive multiple sclerosis, 963  
RIS, 962  
RRMS, 963
- diagnostic criteria, 964
- DNA methylation and, 441
- DQ molecule in predisposition to, 481–482
- environmental factors, 968–969

- epidemiology of MS, 967–969  
generation of neo-epitopes, 442–443  
genetic factors, 967–968  
genome-wide association studies, 398–402  
histone modifications in, 441–442  
with HLA and other candidate genes, 386–387  
imaging, 964–965  
immune pathogenesis, 969–974  
  autoantigens, 973  
  B cells, 973–974  
  immune dysregulation, 971–973  
  T-cell pathogenesis, 970–971  
immunological markers in diagnosis, 965–966  
linkage studies in, 392–393  
microRNAs in, 443  
pathology, 966–967  
treatment, 974–978  
  infusion-based therapies, 974–976  
  injection-based therapies, 977–978  
  oral therapies, 976–977
- Multipotent myeloid/lymphoid progenitors (MPP), 158–159
- Murine autoimmune gastritis, 839
- Murine cytomegalovirus, 645
- Murine IgG anti-GD1a secreting hybridoma, 994
- Murine models of pemphigus vulgaris, 1196,  
  1196f
- Muscle-specific kinase (MuSK), 1013–1014  
  Abs, 1019  
    positive MG, 1017  
  in neuromuscular junction development  
    and maintenance, 1019–1020  
  pathogenicity of MuSK antibodies, 1018
- Musculoskeletal manifestations, 563
- “Mutton-fat” keratic precipitates, 1039–1040
- Myalgias, 704
- Myalgic encephalomyelitis (ME), 1464–1465
- Myasthenia gravis (MG), 270, 422, 1012  
  Abs, 1015–1016  
    AChR Abs, 1018–1019  
    characteristics and mechanisms, 1019  
    LRP4 Abs, 1020–1021  
    MuSK Abs, 1019  
  MuSK in neuromuscular junction  
    development and maintenance, 1019–1020  
  novel targets, 1021  
  pathogenicity of AChR and MuSK  
    antibodies, 1018  
  clinical aspects, 1015  
  clinical heterogeneity, 1015–1017  
  early-onset acetylcholine receptor-antibody  
    positive, 1016  
  epidemiology, 1014–1015  
  etiology, 1015  
  history, 1012t  
  late-onset acetylcholine receptor-antibody,  
    1016  
  LEMS, 1025–1026  
  muscle-specific kinase antibody positive,  
    1017  
  neonatal MG, 1017
- thymic pathology, 1015–1016  
thymoma associated MG, 1017  
thymus and cellular immunity, 1021–1024  
treatments  
  biologics, 1024–1025  
  general approach, 1024
- Mycobacterium tuberculosis*, 92–93, 194
- Mycophenolate, 566–567, 1002, 1024
- Mycophenolate mofetil (MMF), 566, 648, 918,  
  1002, 1045, 1135, 1183, 1416t, 1423
- Myelin, 999, 999f  
  myelin-reactive T cells, 969  
  myelin-specific autoreactive T cells, 971
- Myelin basic protein (MBP), 481, 965
- Myelin oligodendrocyte glycoprotein (MOG), 481, 965–966, 1091, 1098
- Myelin-associated glycoprotein (MAG), 987
- Myelin-oligodendrocyte glycoprotein-  
  antibodies (MOG-Abs), 1092–1093
- Myelodysplastic syndromes (MDS), 924, 931
- Myeloid dendritic cells (mDC), 559
- Myeloid dermal dendritic cells, 1225
- Myeloid differentiation factor 88 (MyD88),  
  309–310, 475–476, 542, 544
- Myeloid-related proteins (MRPs), 682–683
- Myeloperoxidase (MPO), 243, 448–449, 1070
- Myocardial infarction (MI), 608
- Myocardial ischemia, 613
- Myocarditis  
  animal models, 1277–1279  
  clinical, pathologic, and epidemiologic  
    features, 1270–1272  
    clinical diagnosis, 1269  
    Dallas criteria, 1270–1271  
  genetic features, 1276  
  perspectives, 1279  
  treatment, 1272–1273  
    immunosuppressive therapy, 1273  
  in vivo model of, 1262
- Myofibroblasts, 585
- Myointimal cells, 584
- Myosin, 1271–1272, 1275  
  IIA protein, 234–235
- Myosin light chain 4 (MLC4), 706
- Myositis, 563  
  autoantigens, 707–708  
  myositis-specific autoantibodies, 706–707,  
    707t
- N**
- N-acetylcysteine, 1059
- N-acetylglucosaminyl-phosphatidol, 268
- N-glycosylation of T-cell membrane proteins,  
  540
- N-methyl-D-aspartate (NMDA), 1074
- N-methyl-D-aspartate receptors (NMDAR),  
  1074, 1093, 1464
- N-terminal BNP, 1274
- N-terminal probrain natriuretic peptide (NT  
  proBNP), 590
- NACHT, LRR, and PYD domain-containing  
  protein (NLRP), 309–310
- NACHT leucine-rich-repeat protein 1  
  (NLRP1), 793–794
- NADPH oxidase (NOX), 307–308
- NOX1, 294
- NOX2, 543, 622, 1321–1322
- Nailfold capillaroscopy, 588
- Naïve antigen, 172
- Naïve T cells, 66
- NALP5, 735
- Narcolepsy, 376, 505, 1467
- Narcolepsy type 1 (NT1), 505
- Nasal ulceration, 562–563
- Naso-respiratory insulin, 1403–1404
- Natalizumab, 974–975, 1442
- National Childhood Vaccine Injury Act* (1986),  
  376
- National Institute for Biological Standards  
  and Control/World Health  
  Organization (NIBSC/WHO), 1381
- Native collagen type II (nCII), 1467–1468
- Natural antibodies, 55
- Natural killer cells (NK cells), 45, 49f,  
  229–230, 278, 297, 320, 322–323, 515,  
  1043–1044, 1212
- chemokine and cytokine production, 235  
contact and adhesion to target cells,  
  233–234
- cytolytic granule exocytosis, 234–235
- development and differentiation, 230–231
- functional responses by, 232
- HLA-B27 and, 471
- and human autoimmunity, 235–238
- lytic granule polarization and maturation,  
  234
- phenotype and tissue localization,  
  231–232
- receptor signaling and effector functions,  
  232–235
- receptors, 117–118, 237–238
- specificity and signaling of human  
  receptors, 233t
- Natural killer T cells (NKT cells), 117–118,  
  297, 1470
- NKT1 cells, 121
- NKT10 cells, 121
- NKT17 cells, 121
- NKT2 cells, 121
- NKT<sub>FH</sub> cells, 121
- NCF2, 405
- Necroptosis, 294
- Necrosis, 291, 294–295, 822  
  necroptosis in autoimmunity, 294–295  
  necrotic cells  
    immunostimulatory effects of, 298–299  
    receptors for, 297
- Necrotizing arteritis, 1286–1287
- Necrotizing crescentic GN (NCGN), 1358
- Necrotizing glomerulonephritis, 1072
- Necrotizing hypophysitis, 823
- Negative selection, 68, 155–156  
  of self-reactive T cells, 70–72
- Neo-autoantigens, 498
- Neo-epitopes generation, 442–443
- Neoangiogenesis of microvascular networks  
  and intimal hyperplasia, 1323
- Neomycin phosphotransferase II (NeoR), 311
- Neonatal AOD induction, 1244
- Neonatal lupus (NL), 293

Neonatal MG, 1017  
 Neonatal thymectomy, 839–840  
     to tregs, 68–69  
 Neoplasia, 904  
 Nephritic factor (NeF), 269  
 Nephrotoxic models, 1359  
 Nervous system, 991, 1067, 1076  
 NETosis, 18, 219, 244, 294, 322, 560  
     in rheumatoid arthritis, 248  
     SLE and, 246–247  
 Network for Pancreatic Organ Donors with  
     Diabetes (nPOD), 494  
 Neurodegeneration, 961–962  
 Neuroendocrine interactions, 59  
 Neurofascins, 1000  
 Neurologic manifestations, 1070–1071  
 Neurological antiphospholipid syndrome,  
     612  
 Neurological diseases, 1078  
 Neurological manifestations, 1068–1069,  
     1073–1074  
 Neurological symptoms, 1071–1072  
     associated with GFAP-Abs, 1093  
     associated with MOG-Abs, 1092–1093  
 Neuromuscular blockade, 992  
 Neuromuscular junction (NMJ), 1012–1013,  
     1013f  
     development and maintenance, 1019–1020  
 Neuromuscular transmission, 1013–1014  
 Neuromyelitis optica (NMO), 270, 1020  
     and disorders associated with antiglial  
         Abs, 1091–1093  
 Neuromyelitis optica immunoglobulin  
     (NMO-IgG), 965–966  
 Neuromyelitis optical spectrum disease  
     (NMOSD), 1081, 1091–1092  
 Neuronal cell surface antigens  
     amphiphysin, 1095–1096  
     CASPR2, 1095  
     D2R, 1095  
     DNER, 1096  
     DPPX, 1095  
     excitatory receptors, 1093–1094  
     IgLON5 receptors, 1095  
     inhibitory receptors, 1094  
     LGI1, 1095  
 Neuronal surface proteins, antibodies to,  
     1086–1088  
 Neuropsychiatric lupus, 564  
 Neutral endopeptidase (NEP), 1357  
 Neutralizing anti-IL-6 antibodies, 280  
 Neutropenia, 249  
 Neutrophil cytosolic factor 2 (NCF2), 405  
 Neutrophil elastase (NE), 243  
 Neutrophil extracellular traps (NET),  
     244–246, 244f, 250, 294, 322, 624, 1071  
     and AAVs, 247  
     NET-associated proteins, 244–245  
     in rheumatoid arthritis, 248  
 Neutrophil(s), 45, 47, 48f, 249, 560,  
     1294–1295  
     effector mechanism in, 322  
     neutrophil-induced vasculitic organ  
         damage, 247  
     priming, 1295

proteases, 245  
     proinflammatory effects in, 248  
 New Zealand black mice (NZB mice), 534,  
     560, 898, 1356  
 New Zealand mixed (NZM)  
     mouse models, 393  
     NZM2410 strain, 560  
     strains, 560  
 New Zealand White mice (NZW mice), 560,  
     1356  
 NFATC1 gene, 793–794  
 Niches, 157–159  
 Nicotinamide adenine dinucleotide  
     phosphate (NADPH), 243, 526–527  
 Nintedanib, 1348  
 Nitrogen oxide (NO), 193–194, 1225  
 Nitrotyrosine, 1322  
 Nivolumab, 826–827  
 NKG2D ligands, 237–238  
 NLR-family CARD domain-containing  
     protein 4 (NLRC4), 202, 684  
 NOD-like receptors (NLRs), 69, 198  
 Non-HLA genes, 855–856  
 Non-SAA, 925  
 Non-SET domain lysine methyltransferase,  
     433  
 Nonbulloous skin diseases  
     AA, 1211–1215  
     CU, 1226–1229  
     psoriasis, 1221–1226  
     vitiligo, 1215–1221  
 Noncanonical autophagy pathways,  
     307–308, 312  
 Noncanonical PRRs, 198  
 Noncoding RNAs (ncRNAs), 434  
     in autoimmune thyroid diseases, 438  
 Noncollagen (NC), 1200–1201  
 Noncompressive neuropathies, 1073–1074  
 Noncriteria antiphospholipid syndrome  
     manifestations, 611–614  
     cardiac antiphospholipid  
         syndrome, 613  
     dermatologic antiphospholipid syndrome,  
         612–613  
     hematologic antiphospholipid syndrome,  
         612  
     neurological antiphospholipid syndrome,  
         612  
     pulmonary antiphospholipid syndrome,  
         613–614  
     renal antiphospholipid syndrome, 614  
 Nonerosive polyarthritis, 517  
 Nongenetic factors, 346–347  
 Nongenomic effects, 422  
 Noninfectious purpura, 1288  
 Noninflammatory “antigen sink”, 719  
 Noninterferon members, 283  
 Noninvasive diagnosis, 1069  
 Noninvasive imaging techniques, 817  
 Nonobese diabetic mice (NOD mice),  
     131–132, 496–497, 739, 754–755, 780,  
     793, 1001, 1443  
     model, 367–368, 1391  
     NOD2, 219–220, 880  
     NOD-like receptor, 475–476

Nonobese diabetic-like receptor family  
     pyrin domain containing 6 (NLRP6),  
     475–476  
 Nonopsonic receptors in macrophages,  
     196–198  
 Nonpersistent hypointense lesions, 964–965  
 Nonrheumatoid arthritis-associated human  
     leukocyte antigen alleles, 480–481  
 Nonsegmental vitiligo (NSV), 1215–1216  
 Nonspecific antiinflammatory drugs,  
     1419–1420  
     GC, 1419–1420  
     NSAIDs, 1419  
 Nonspecific immunosuppressive agents,  
     1415–1416  
 Nonspecific interstitial pneumonia (NSIP),  
     586, 1336, 1339–1340  
 Nonsteroidal antiinflammatory drugs  
     (NSAIDs), 698–699, 1419  
 Normal-appearing white matter (NAWM),  
     441  
 Novantrone. *See* Mitoxantrone  
 Novel assay techniques, 621  
 Novel PBC model, 1155–1156  
*Novosphingobium aromaticivorans*, 128  
 Nuclear antigen 1 of Epstein–Barr virus  
     (EBNA1), 311  
 Nuclear factor E2-related factor-2 pathway,  
     977  
 Nuclear factor kappa B (NFκB), 325,  
     516–517, 577–579, 1419  
 Nuclear factor-κB essential modulator  
     syndrome (NEMO syndrome),  
     516–517, 519–520  
 Nuclear magnetic resonance, 802  
 Nuclear pore complex (NPC), 1158  
 Nucleoporin 62 (p62), 309–310  
 Nucleotide binding and oligomerization  
     domain (NOD), 198  
     NOD-like receptors, 364  
     NOD2, 277, 878  
 Nucleotide-binding domain, 214–215  
 Nucleotide-binding domain leucine-rich  
     repeat-containing receptor P3  
     (NLRP3), 246–247  
 Nucleotide-binding oligomerization-like  
     receptors (NLRs), 475–476, 1179  
 Nutri-epigenomics, 453–454  
 Nutrient-rich foods, 333  
 Nutritional support, 888  
 (NZB/NZW)F1 mouse model, 133, 560, 644

**O**  
 Obesity, 1393–1394  
 “Obesogenic” diet, 333  
 Obeticholic acid (OCA), 1149–1150, 1159  
 Obliterative phlebitis, 1178  
 Obliterative arteritis, 716  
 Obliterative microangiopathy, 585–586  
 Obliterative phlebitis, 716  
 Obstetric APS (OAPS), 610–611  
 Occlusive thromboarthritis.  
     *See* Takayasu’s arteritis (TA)  
 Occupational exposures, 351–352, 351t

Occupational silica exposure, 579  
 Ocrelizumab, 974, 976, 1445  
*Ocrevus. See Ocrelizumab*  
 2-Octynoic acid (2-OA), 1153–1154  
 Ocular disease  
     animal models, 1042  
     autoimmune features, 1042–1043  
     clinical features, 1036–1039  
         key anatomic features of eye, 1036f  
         manifestations of inflammation, 1037f, 1038f, 1039f  
         necrotizing scleritis with thinning of sclera, 1037f  
     epidemiologic features, 1040  
     genetic factors, 1040–1042  
     historical background, 1035–1036  
     immunological markers, 1044  
     pathogenic mechanisms, 1043–1044  
     pathologic features, 1039–1040  
     treatment and outcomes, 1044–1046  
         unique immune system of eye, 1042  
 Ocular-specific antigens, 1042  
 Odds ratio (OR), 662–663, 680  
 Ofatumumab, 1072  
 21-OH enzyme, 797  
     techniques for autoantibodies  
         identification to, 797–798  
 Oligoarthritis, 676, 678  
 Oligoarticular juvenile idiopathic arthritis, 684–686  
 Oligoclonal bands (OCBs), 962  
 Oligoclonal T cells, 966–967  
 Oligoclonality, 496  
 Oligodendrocyte, 965  
 Oligodendroglia-specific enzyme transaldolase, 973  
 Omalizumab, 252  
 $\omega$ -3 fatty acids, 335–336, 338–339  
 Omenn syndrome (OS), 515  
 Ophelia syndrome, 1088  
 Ophiasis, 1211–1212  
 Opsoclonus–myoclonus syndrome (OMS), 1083, 1090  
 Opsonic receptors in macrophages, 196–198  
 Opsonization, 321  
     of pathogens, 267–268  
 ORAI1 deficiency, 234–235, 520  
 Oral autoantigen trials, 1405  
 Oral glucose tolerance test (OGTT), 771–772  
 Oral therapies, 976–977  
     dimethyl fumarate, 977  
      fingolimod, 976–977  
      teriflunomide, 977  
 Oral tolerance, 1401  
 Orbita, 720  
 Orexin system, 1467  
 Organ culture assays, 857  
 Organ-specific autoimmune disease, 6, 839, 1124  
 Osteitis, 693  
 Osteoarthritis (OA), 435, 1467–1468  
 Osteoblasts, 693  
 Osteopenia, 1162  
 Osteoporosis, 807–808, 1162  
 Otelixizumab, 1439, 1443–1444

Otezla. *See Abatacept*  
 Other organ involvements (OOIs), 1173–1174  
 Ovalbumin (OVA), 1237  
 Owen, Ray, 5  
 OX40 ligand, 581  
 Oxidative stress, 1057  
 Oxidizing molecules, 1044  
 2-Oxo-acid dehydrogenase complex (2-OADC), 1151–1152  
 2-Oxo-glutaric acid dehydrogenase complex (OGDC-E2), 1151–1152  
 Ozanimod, 887

**P**

*p*-antineutrophil Abs (p-ANCA), 1070  
 p35 ligand chain, cytokines sharing, 280–281  
 p40 ligand chain, cytokines sharing, 280–281  
 Painful mononeuritis multiplex, 1068  
 Palatine tonsils, 57  
 Palmar papules, 704  
 Palmerston North mice (PN mice), 535  
 Pan-hypopituitarism, 818–819  
 Pancreas, 721  
 Pancreaticoduodenectomy, 1175  
 Pandemrix, 774  
 Panuveitis, 1039  
 Paracrine fibrotic mediators, 585  
 Paraneoplastic Ma antigens (PNMA), 1096–1097  
 Paraneoplastic neurological syndromes, 1067  
 Paraneoplastic pemphigus (PNP), 1192, 1199  
 Paraneoplastic syndromes (PNS), 1078, 1082t  
 Paraproteinemic demyelinating peripheral neuropathy, 999  
 Parathyroid disease, 1468–1469  
 Parenchyma, 10  
 Parenchymal tissues, 68  
 Parotid gland, 637  
 Paroxysmal cold hemoglobinuria (PCH), 9, 11–12, 899  
 Paroxysmal nocturnal hemoglobinuria (PNH), 268, 924  
 PARP-1, 295  
*Pars planitis*, 1037–1039  
 Parthanatos, 294–295  
     in autoimmunity, 295  
 Partial lipidodystrophy (PLD), 269  
 Passive antibody transfer, 994  
 Passive demethylation, 431  
 Passive ITP model, 505  
 Passive transfer animal model, 994–995  
 Pathogen-associated molecular patterns (PAMPs), 45–46, 264–265, 277, 544  
 Patients with Sjögren's syndrome (pSS), 446  
 Pattern recognition receptors (PRRs), 45–46, 48–49, 191–192, 198–203, 214–215, 309–310, 364, 1179  
     and DCs activation, 214–215  
 Pax5-deficient progenitors, 160  
 Pediatric rule, 686  
 Pembrolizumab, 826–827  
 Pemphigoid gestationis. *See Herpes gestationis (HG)*

Pemphigus, 1199–1200  
     drug-induced pemphigus, 1200  
     IgA pemphigus, 1200  
     PNP, 1199  
 Pemphigus foliaceus (PF), 1192  
     autoantibodies as potential immunologic markers, 1198  
     autoimmune features, 1197–1198  
     clinical feature, 1197  
     environmental factors involving in FS, 1199  
     epidemiologic feature, 1197  
     genetic features, 1198  
     pathologic effector mechanisms, 1198  
     pathologic feature, 1197  
     in vivo and in vitro models, 1198  
 Pemphigus vulgaris (PV), 1192  
     autoimmune features, 1194–1195  
     clinical features, 1193–1194  
     epidemiologic features, 1193–1194, 1194f  
     genetic features, 1195  
     pathologic effector mechanisms, 1197  
     pathologic features, 1193–1194, 1194f  
     in vivo and in vitro models, 1195–1196  
 Penicillamine, 1356  
 Peptide, 1054  
     HLA-B27 and peptide binding, 470–471  
     peptide-specific autoantibodies, 1054  
     therapy, 627  
 Peptide from mouse ZP 3 (pZP3), 1243–1244  
 Peptide-MHC (pMHC), 70  
 Peptidyl-arginine deiminase (PAD1), 311–312, 474–475, 659–660  
 Perforin, 1391–1392  
 Periarteritis nodosa (PAN), 1286–1287  
 Pericardial effusion, 563  
 Pericarditis, 563  
 Perinuclear antinuclear neutrophil antibodies (pANNA), 1126–1127  
 Perinuclear neutrophil cytoplasm antibodies (pANCA), 1126  
 Peripheral blood  
     flow cytometry, 924  
     T cells in SSc, 584  
 Peripheral blood mononuclear cells (PBMCs), 119, 438, 557, 680, 1214  
 Peripheral lymph node addressins, 58  
 Peripheral nerve diseases, 987  
 Peripheral nervous system (PNS), 639, 991, 1012, 1068  
 Peripheral neuropathies  
     acute neuropathies, 988–997  
     chronic neuropathies, 998–1002  
 Peripheral systemic tolerance, 1236  
 Peripheral tolerance, 65–66, 68, 91, 177  
     autoimmunity and, 24–25  
 Perivascular CIDP-like inflammation, 998  
 Perivascular distribution, 961  
 Perivascular infiltration, 966–967  
 Perivascular tissue, 976  
 Pernicious anemia (PA), 270, 501–502, 833  
     autoantibodies as potential immunologic markers, 841–842  
     autoimmune features  
         autoantibodies, 836–838  
         gastric parietal cell H<sup>+</sup>/K<sup>+</sup> ATPase, 837f

- Pernicious anemia (PA) (*Continued*)  
 clinical, pathologic, and epidemiologic features, 834–836  
 genetic features, 838–839  
 $H^+/K^+$  ATPase activity, 837f, 840  
 laboratory diagnosis, 843  
 mouse model of autoimmune gastritis, 841f  
 pathologic effector mechanisms, 840, 842f  
 T-cell immunity, 838  
 in vivo and in vitro models, 839–840
- Pernicious anemia, 834, 836–838, 840
- Peroxisomes, 243
- “Personalized” approach, 1395
- PEST domain phosphatase (PEP), 540
- Peyer’s patches, 641
- Phacoanaphylaxis, 11
- Phagocytes, 203–204, 264  
 synapse, 296
- Phagocytosis, 47–48, 203–204
- Phagolysosomes, 204
- Phagophore, 306–307
- Phagosome  
 acidification, 204  
 maturation, 203–204  
 resolution, 204
- Phosphatase and tensin homolog (PTEN), 173
- Phosphatidylinositol 3-kinase (PI3K), 306–307
- Phosphatidylinositol (4,5)-bisphosphate (PIP2), 234
- Phosphatidylinositol glycan class A (PIG-A), 268
- Phosphatidylserine (PS), 204–205, 296, 307–308, 543
- Phosphodiesterase (PDE)  
 inhibitors, 593–594  
 PDE4, 1428
- Phosphoinositide-3-kinase (PI3K), 518, 645–646
- Phospholipase A2 receptor (PLA2R1), 1357
- Phosphorylated VAV1, 233–234
- pHSCs, 158–159
- Physiological inflammation, 877
- Pirfenidone, 1348
- Pituitary  
 antibodies to antigens, 823  
 autoimmunity, 817  
 gland, 1077–1078  
 inflammation, 815
- PIWI-interacting RNA, 434
- Plant homeodomain (PHD), 403, 736–737
- Plaques, 442–443
- Plasma cells, 19, 171, 176–177, 642, 861  
 tumors, 280
- Plasma exchange (PEx), 997, 1002, 1015, 1017–1018
- Plasmablasts, 175, 718
- Plasmacytoid dendritic cells (pDCs), 214–215, 217, 245, 309–310, 559–560
- Plasmapheresis, 1059
- Plasmatic coagulation, 935
- Plasminogen, 623
- Platelet-derived growth factor (PDGF), 584, 1323
- Platelet(s), 911  
 autoantibodies, 914
- Pleiotropic cytokine EPO, 993
- Pleocytosis, 1077, 1086–1087
- Pneumocystis jiroveci*, 1421
- Poliomyelitis, 988
- POLR3A* gene mutations, 709
- Polyangiitis, granulomatosis with, 1071–1072
- Polyarteritis nodosa (PAN), 1070, 1288–1291  
 animal models, 1290  
 autoimmune features, 1290  
 clinical features and disease association, 1289  
 diagnostic procedures, 1290  
 environmental influences and genetic features, 1290  
 epidemiology, 1288  
 pathogenesis, 1289  
 pathological features, 1289  
 treatment, 1290–1291
- Polyarthritis, 1255–1256
- Polychondritis, relapsing, 1469
- Polyclonal antibody, 949
- Polyclonal elevations, 1374
- Polygenic disease, 21–22
- Polyglandular autoimmune syndrome type 1, 732
- Polymerase chain reaction, 1274
- Polymerized flagellin, 59
- Polymorphic markers in candidate genes, 1181–1182
- Polymorphisms, 944, 1000, 1024  
 in autoantigens, 28
- Polymorphonuclear (PMN), 1043  
 leukocyte infiltration, 1073
- Polymyalgia arteritis, 1316
- Polymyositis (PM), 250, 703
- Polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes syndrome (POEMS syndrome), 999
- Polysaccharides (PSA), 59, 476
- Population heterogeneity, 1395
- Porphyromonas gingivalis*, 475, 659–660
- Positive selection, 68  
 of immunocompetent T cells, 70
- Positron emission tomography (PET), 1325
- Posterior pituitary bright spot, 825
- Posterior uveitis*, 1039
- Postinfectious autoimmune disorders, 989
- Postinfectious GN (PIGN), 1359–1360
- Postsynaptic motor “endplate”, 1012–1013
- Posttranslational modifications (PTMs), 431  
 in autoimmunity, 477–479  
 deamidation, 478–479  
 deimination, 478
- Posttransplant cyclophosphamide, 929–930
- Postvasectomy ASA, 1242
- Potential disease biomarkers, 454
- Potential immunologic markers, 1197–1198, 1202
- PRDM1, 404–405
- Pre-BII cells, 162–163
- Prednisolone, 903
- Predominantly antibody deficiencies, 520–522
- Pregnancy loss, 622
- Premature ovarian failure (POF), 795–796
- Premature ovarian insufficiency (POI), 1245
- Prevotella copri*, 659–660
- Primary antiphospholipid syndrome, 613, 1053
- Primary autoinflammatory disorders, 513–514
- Primary biliary cholangitis. *See Primary biliary cirrhosis (PBC)*
- Primary biliary cirrhosis (PBC), 124t, 127–128, 292, 500–501, 503, 1122, 1149–1150  
 animal models, 1154–1156  
 changing nomenclature for, 1150  
 chronic nonsuppurative destructive cholangitis, 1154f  
 diagnosis, 1156–1159, 1157f  
 histopathology, 1158–1159  
 mitochondrial and nuclear autoantibodies and frequencies, 1158t  
 molecular mimicry between lipoamide and 2-nonynamide, 1156f  
 serological testing, 1157–1158  
 serum biochemistry and imaging studies, 1156–1157
- disorders associated with PBC, 1161–1163
- epidemiology, 1150–1151
- etiology, 1151–1154  
 AMA epitopes, 1151–1152  
 biliary epithelial cells, 1152–1153  
 CD4+ and CD8+ T-cell epitopes, 1152  
 environmental triggering factors, 1153–1154  
 genetic predisposition, 1153  
 incidence and prevalence, 1151t  
 management of symptoms and extrahepatic manifestations, 1160–1161  
 molecular mimicry and immunodominant epitopes, 1152t  
 mouse models, 1155t  
 Paris criteria for, 1161t  
 perspectives, 1164  
 stratification of risk for progression, 1163–1164
- treatment  
 fibrates, 1160  
 IBAT inhibitors, 1160  
 OCD, 1159  
 UDCA, 1159
- Primary chronic ITP, 505
- Primary hemostasis, 935
- Primary hypophysitis, 815, 818t, 820
- Primary immune response, 1022
- Primary immunodeficiency disorders, 513–514  
 autoimmune clinical manifestations, 514t  
 gain-of-function disorders of cytokine signaling, 525–526

immunodeficiencies affecting cellular and humoral immunity, 515–520  
innate immune defects, 526–527  
T-cell tolerance, 522–525  
Primary intraocular lymphoma, 1039  
Primary ITP, 914  
Primary myxedema, 749–750  
Primary Oral Insulin Trial, 780–781  
Primary prevention, 1400–1402  
antigen-specific immunotherapy, 1402  
diet and gut microbiome modification, 1401–1402  
virus vaccination, 1402  
Primary progressive MS (PPMS), 962  
Primary Raynaud's phenomenon, 588  
Primary sclerosing cholangitis (PSC), 875  
Primary Sjögren syndrome, 249  
Primary thromboprophylaxis in aPL carriers, 626  
Primary vasculitides, 1053–1055  
  Cogan's syndrome, 1054–1055  
Primary–progressive MS (PPMS), 384  
Primitive hematopoiesis, 158  
Pristane. *See* Tetramethylpentadecane (TMPD)  
Pristane-induced lupus model, 544  
Proallergic cytokines, 279  
Procainamide, 452–453  
Progesterone, 422  
Programmed cell death, 204  
Programmed death ligand 1 (PD-L1), 793–794  
Programmed death-1 (PD-1), 539, 1127  
Progressive cerebellar syndrome, 963  
Progressive multiple sclerosis, 963  
Progressive supranuclear palsy, 1088  
Progressive systemic sclerosis, 1053  
Proinflammatory cytokine(s), 193–194, 281–282, 969  
  interferon gamma, 1131  
  production, 973–974  
  TNF- $\alpha$ , 970–971  
Proinsulin, 779  
Prolactin deficiency, 826–827  
Proliferating cell nuclear antigen (PCNA), 1370  
Prolylendopeptidases, 863  
Promiscuous expression of self-antigens in thymus, 73–74  
Properdin, 267  
Proptosis, 720  
Prostaglandins (PGD), 1419  
  PGD2, 135  
Prostate-specific protein, 72  
Prostatitis, 1470  
Protectin, 268  
Protein phosphatase 2A (PP2A), 541  
Protein tyrosine phosphatase, non-receptor Type 2 (PTPN2), 396–397  
Protein tyrosine phosphatase nonreceptor 22 (PTPN22), 22–23, 540, 663  
Protein tyrosine phosphatases (PTPase), 386, 539–540  
Protein(s)  
  autoimmune inhibitors to, 949

glycosylation, 194  
kinase C-delta expression, 645–646  
protein S, 297, 937  
protein Z, 937  
protein–protein interactions, 1020  
Proteinas (PR), 1044  
  PR3, 245, 247, 448–449  
Proteoglycan-induced arthritis model (PGIA), 545  
Proteolipid protein (PLP), 367, 481, 965–966  
Proteolytic FVIII antibodies, 944  
Prothrombin, autoimmune inhibitors to, 940–941  
Prothrombin time (PT), 939  
Prothrombotic disorders, 937  
  ADAMTS13, 937  
  autoantibodies  
    IgG and IgM autoantibodies, 937  
    protein S, 937  
Prototypical autoimmune disease, 11–12  
Pruritus, 588, 1161  
Pseudo-obstruction, 591  
Pseudotumors, 720  
Psoralen and ultraviolet radiation (PUVA), 1220  
Psoriasis, 124*t*, 129, 220, 278, 1221–1226  
  autoantibodies as potential immunologic markers, 1225  
  autoimmune basis for, 1222–1223  
  autoimmune features, 1222–1223  
  classification, 1221*t*  
  clinical, pathologic, and epidemiologic features, 1221–1222  
  genetic features, 1224  
  guttate psoriasis of trunk, 1223*f*  
  pathogenic mechanism, 1225  
  plaque, 1222*f*  
  pustular psoriasis of neck and upper chest, 1223*f*  
  in vitro models, 1225  
  in vivo models, 1224–1225  
Psoriatic arthritis (PsA), 32, 220, 679, 692, 1421  
Psoriatic keratinocytes, 1225  
Ptd-L-Ser. *See* Phosphatidylserine (PS)  
Pulmonary  
  APS, 613–614  
  capillaritis, 1072  
  features, 589–590  
  fibrosis, 1347  
  hypertension, 613  
Pulmonary alveolar proteinosis (PAP), 280  
Pulmonary arterial hypertension (PAH), 577, 590  
Pulmonary embolism (PE), 563, 611  
Pulmonary function testing (PFT), 589–590, 1338  
Pulmorenal syndrome, 1072  
Pulseless disease. *See* Takayasu's arteritis (TA)  
Purine nucleoside phosphorylase (PNP), 515  
Purkinje cell antigen (PCA), 1081  
  Purkinje cell antigen-2, 1097  
Purpura, 1288  
  *Purpura lapillus*, 1288

Putative autoantigens, 1058  
Putative immunogenetic modifiers, 1000  
Pyoderma gangrenosum (PG), 875  
Pyrin (PY), 202  
Pyruvate dehydrogenase complex E2 subunit (PDC-E2), 1151–1152  
**Q**  
Quality assurance, 1381–1382  
Quality of life (QoL), 804, 807  
Quantitative immunocytochemistry, 779–780  
Quantitative myasthenia gravis (QMg), 1024  
Quantitative susceptibility mapping (QSM), 965  
Quantitative trait loci (QTLs), 534  
Quasi-autoantigens, 156–157  
Quinacrine, 1420  
Quorum sensing, 193  
**R**  
R139X mutation, 736  
R402Q polymorphism, 1218  
RA synovial fibroblasts (RASFs), 435  
  genomic DNA hypomethylation and activated phenotype, 436  
  histone modifications in, 435–436  
  miRNA and destructive potential of, 437  
Rab27a proteins, 234–235  
Rabbit ATG (rATG), 927–928  
Racial heterogeneity in SLE, 394  
Radical oxygen species (ROS), 193–194  
Radiographic erosion, 563  
Radioimmunoprecipitation assay (RIA), 797, 1018  
Radiologically isolated syndromes (RISs), 962  
Randomized controlled trials, 1070  
Rapamycin, 1045  
Rapid progressive GN (RPGN), 1361  
Rapsyn, 1021  
Rasmussen disease (RD), 1464  
Rat insulin promoter (RIP), 483  
Raynaud's phenomenon, 563, 583, 587–588  
Reactive arthritis (ReA), 691–693  
Reactive oxygen species (ROS), 243, 245, 292  
  proinflammatory effects in, 248  
Readers of histone modifications, 433–434  
Receptor editing, 57, 177–178  
  defective, 178–179  
Receptor phosphorylation, 203–204  
Receptor-interacting protein (RIP), 292  
Reciprocal relationships of pathogen-derived mechanisms of autoimmunity, 369–370  
Recombinant myelin oligodendrocyte glycoprotein (rMOG), 481  
Recombinant porcine (rp), 944–945  
Recombination activating gene (RAG), 100, 160–161, 178  
  RAG1 and 2, 515  
Red blood cells (RBC), 897–898  
  autoantigens, 902  
  destruction mechanisms in AIHA, 899–902

- Red blood cells (RBC) (*Continued*)  
 cold reactive antibodies, 899  
 hemolysis by warm antibodies, 901–902  
 pathogenicity of warm reactive IgG antibodies, 899–901  
 warm reactive antibodies, 899
- Refractory ITP, 913
- Regenerative therapy, 805
- Regulator of complement activation (RCA), 266
- Regulatory B cells (Bregs), 182–183, 1022, 1180
- Regulatory CD4<sup>+</sup> T cells, 99–102
- Regulatory cells, 24, 29–30, 32–33
- Regulatory lymphocytes, 29–30
- Regulatory T cells (Tregs), 45–46, 100, 181, 286, 308–309, 334–335, 339, 420, 436, 731, 773–774, 838, 905–906, 914, 1155, 1180, 1358, 1403  
 at barrier sites, 81  
 cell therapy using, 1449  
 disorders, 524–525  
 diversity and role in self-tolerance, 80–81  
 epigenetic generation, 457  
 lymphocytes, 1022  
 mechanisms to maintaining tolerance, 78–80, 79f  
 neonatal thymectomy to, 68–69
- Relapsing polychondritis (RP), 1469
- Relapsing–remitting MS (RRMS), 384, 962–963
- Renal  
 disease, 648  
 function, 563–564
- Renal antiphospholipid syndrome, 614
- Reovirus, 856
- Repertoire diversity, 65
- Repetitive polysaccharides, 177
- Replacement, reduction, and refinement (3Rs), 493–494
- Reticuloendothelial system (RES), 264, 899
- Retinal  
 drusen, 269  
 inflammation, 1037–1039  
 S-antigen, 1035  
 vasculitis, 1040
- Retinal pigment epithelium (RPE), 269
- Retinoic acid receptor related orphan nuclear receptor  $\gamma\tau$  (ROR $\gamma\tau$ ), 96, 698
- Retinoic acid-inducible gene I (RIG-I), 202
- Retroperitoneal fibrosis (RPF), 715, 722
- Revascularization, 584
- Rh-specific Treg cells, 905–906
- Rheumatic disease treatment establishment, 1420–1424  
 antimalarials, 1420  
 AZA, 1423–1424  
 calcineurin inhibitors, 1424  
 cyclophosphamide, 1422  
 leflunomide, 1421  
 MMF, 1423  
 MTX, 1421–1422  
 sulfasalazine, 1420–1421
- Rheumatic fever (RF), 1255  
 adaptive immune response, 1260–1261
- autoantibodies as potential immunologic markers, 1264–1265  
 cardiac myosin, 1264  
*N*-acetyl- $\beta$ -D-glucosamine, 1265
- autoimmune features, 1257–1258  
 clinical, pathological, and epidemiologic features, 1255–1256  
 genetic features, 1258–1261  
 genetic polymorphism, 1259t  
 genetic predisposition  
*CTLA4* gene, 1261  
*DQA1* gene, 1261  
*DQB1* gene, 1261  
*DRB1* gene, 1261  
*DRB3* gene, 1261  
*Fc $\gamma$ RIIA* gene, 1260  
*Ficolin* gene, 1260  
*Masp2* gene, 1260  
*MBL2* gene, 1260  
*MIF* gene, 1260  
*TLR-2* gene, 1260  
 innate immune response, 1260–1261  
 pathologic effector mechanisms, 1263–1264  
 in vivo and in vitro models, 1262
- Rheumatic heart disease (RHD), 347–348, 1255–1256  
 autoantibodies as potential immunologic markers, 1264–1265  
 autoimmune features, 1257–1258  
 clinical, pathological, and epidemiologic features, 1255–1256  
 genetic features, 1258–1261  
 genetic polymorphism associated with, 1259t  
 pathologic effector mechanisms, 1263–1264  
 in vitro model, 1262
- Rheumatoid arthritis (RA), 93, 124t, 128–129, 163–164, 248, 270, 279, 299, 435–437, 471–472, 476–478, 495, 533, 556, 659, 677, 1036, 1067, 1073–1074, 1336, 1418, 1439  
 affected joints, 660–661  
 autoantibodies  
*ACPA*, 661–662  
 citrullinated antigens, 668  
 as potential immunologic markers, 668  
 autoimmune features, 661–662  
 citrullinated peptides, 661–662  
 RF, 661  
 breakthrough in, 1440–1441  
 causes, 659  
 clinical features, 659–661  
 environmental factors, role of, 664  
 epidemiologic features, 659–661  
 genetic characteristics, 662–664  
*HLA-DRB1* alleles, 662–663  
*HLA-DRW4*, 662–663  
*MHC* class II gene, 662–663  
*PTPN22* gene, 663  
 SNP, 663  
 STATs, 663  
 NET and netosis in, 248  
 neurological manifestations, 1073–1074
- pathologic effector mechanisms, 666–668  
 antigen presenting cells, role of, 666  
 bone destruction, 666  
 cartilage damage, 666  
 fibroblast-like synovial cells, role of, 666  
 osteoclast differentiation and activation, 666  
 T or B cell activation, 666  
 Th1 and Th17 cells, role of, 666  
 pathologic features, 659–661  
*Porphyromonas gingivalis*, 475  
 proinflammatory effects of neutrophil proteases and ROS, 248  
 in vivo models, 664–665  
 adjuvants, 664  
 cellular immune responses, 664  
 collagen-induced arthritis, 664  
 IL-1 receptor antagonist, 665  
 KRN mouse model, 664–665  
 SKG model, 665
- Rheumatoid factor (RF), 321, 478, 661, 1073, 1180  
 negative polyarthritides, 678–679  
 RF-positive polyarthritides, 677
- Rho-associated coil-containing protein kinase 1 (ROCK1), 294
- RI (antineuronal nuclear antibodies 2), 1096
- Ribonucleoprotein (RNP), 245, 1074
- Ribosomopathies, 923
- Riedel's thyroiditis, 723, 1177
- RIG-I-like receptors (RLRs), 202–203, 216–217
- Rig-like helicases (RLH), 214–215, 277
- Rilonacept (Arcalyst), 1426
- Ring Finger Domains 1, 403
- Risk assessment in antiphospholipid syndrome, 620
- Rituxan. *See* Rituximab (RTX)
- Rituximab (RTX), 567–568, 627, 648, 805, 825–826, 917, 948, 1002, 1015, 1024–1025, 1045, 1084–1085, 1416t, 1427, 1445
- Romiplostim, 917
- Roquin (Rc3h1), 541
- Ross River virus, 1297
- Rotavirus, 1394–1395
- Royal College of Pathologists of Australasia—Quality Assurance Program (RCPAQAP), 1381
- rs1990760T allele, 395
- Ryanodine receptor (RyR), 1015–1016
- S**
- S protein, 267
- S-adenosylmethionine (SAM), 430
- Saccharomyces cerevisiae*, 305–306
- Salivary gland epithelial cells (SGEC), 642
- Salivary glands, 720
- Salmonella* infection, 120  
*S. typhimurium*, 194, 309–310
- SAND domain, 736–737
- Sandhoff disease, 1466

- Sarcoidosis, 1036–1039, 1067, 1077–1078, 1470
- Scalp, 1211
- Scavenger receptors, 200
- Schilling test, 841–842
- Schimke immune-osseous dysplasia (SIOD), 520
- Scleritis, 1036
- Scleroderma renal crisis, 592
- Sclérose en plaques disseminées, 961
- Sclerosing cholangitis (SC), 1121–1122
- Screening colonoscopy, 876
- Second-line therapies in ITP, 916
- Secondary APS, 608
- Secondary hemostasis, 935
- Secondary hypophysitis, 815, 818<sup>t</sup>
- "Secondary ITP" models, 505
- Secondary lymphoid tissues (SLT), 57–59, 230
- Secondary prevention, 1402–1406
- mucosa-mediated antigen-specific tolerance, 1403–1404
  - trials of islet autoantigen-specific vaccination in humans, 1404–1406
- Secondary progressive MS (SPMS), 443, 962
- Secondary Raynaud's phenomenon, 588
- Secondary–progressive MS (SPMS), 384
- Secukinumab, 1416<sup>t</sup>, 1426
- L-Selectin, 58
- Selective IgA deficiency (sIgAD), 521–522
- Self-antigens
- peptides, 1128
  - promiscuous expression in thymus, 73–74
  - Self-derived endogenous ligands, 214–215
  - Self-MHC molecules, 472
  - Self-proteins, 504–505
  - Self-reactive CD4<sup>+</sup> T helper cells, 91–92
  - Self-tolerance
    - establishment in thymus, 70–74
    - negative *vs.* agonist selection of self-reactive T cells, 70–72
    - positive selection of immunocompetent T cells, 70
    - promiscuous expression of self-antigens in thymus, 73–74
    - Treg diversity and role in, 80–81
- Sensing systems, 193
- Sensorineural hearing loss (SNHL), 1051
- Sensory ataxic neuropathy, 994
- Sep (*O*-phosphoserine) tRNA:Sec (selenocysteine) tRNA synthetase (SEPSECS), 1126
- "Sequestered antigen" concept, 11
- Sequestosome (SQSTM), 309–310
- Serine protease (SP), 623
- Serine-threonine protein kinase deficiency (STK4 deficiency), 517–518
- Serologic(al)
- confirmation, 1015
  - markers, 881–882
  - studies, 991
  - testing, 1157–1158
- Serology, 862
- Seronegative antiphospholipid syndrome, 619–620
- Serpin G1. *See* C1 inhibitor (C1 inh)
- Serum, 263
- Abs, 1017
  - biochemistry and imaging studies, 1156–1157
  - immunoglobulin G4 concentrations, 718
  - insulin antibody concentration, 1406
- Serum amyloid P (SAP), 297
- Serum of anti-Ro (SS-A), 1076
- SET domain lysine methyltransferase, 433
- Severe aplastic anemia (SAA), 923–924
- Severe combined immunodeficiency (SCID), 515–516
- with maternal engraftment, 516
- Sex hormones, 421
- Sexual dimorphism
- consequences for autoimmunity of, 424–425
  - defined, 419
  - effects of hormones on immune system, 421–422
  - environmental effects, 424
  - in immune system, 420–421
  - sex chromosome, relation with, 419–420, 423–424
  - X chromosome, 423
  - Y chromosome, 423–424
- Sexual dysfunction, 962
- Sexually transmitted diseases, 3
- SH2 domain-containing inositol 5'-phosphatase 1 (SHIP-1), 173, 232–233
- SH2 domain-containing phosphatase 1 (SHP-1), 173, 232–233
- SH2B Adaptor Protein 3 (SH2B30), 396
- Shared epitope, 21–22, 1418–1419
- Shared risk alleles, 22–23
- Shiga toxin-producing *Escherichia coli* strains (STEC-HUS), 269
- Short-chain fatty acids (SCFAs), 333–336, 335<sup>f</sup>, 339, 453
- Shrinkage necrosis, 291
- Sialic acid binding Ig-like lectins (Siglecs), 200–202
- SIGLEC8, 253
- Sicca syndrome, 1162
- Sifalimumab, 568
- sIgM<sup>+</sup> immature B cells
- second phase B-cell development, 161–162
  - sites and mechanisms of selection of newly generated sIgM<sup>+</sup> B cells, 164–165
- Signal transducers and activators of transcription (STATs), 663
- STAT1 GOF, 525
  - STAT3, 96, 283, 396–397, 402, 526, 862
  - GOF syndrome, 526
  - STAT4, 394, 402
  - STAT5B, 231, 524
- Signaling lymphocytic activation molecule (SLAM), 534
- Signaling lymphocytic activation molecule family (SLAMF), 534
- SLAMF7, 717–718
- Silicone implants, 355
- Single nucleotide polymorphisms (SNPs), 21, 237–238, 451–452, 577–579, 663, 774, 1257–1258
- Single-cell PCR technology, 31
- Singular sclerosis, 963
- Sjögren's syndrome (SS), 133–134, 446–447, 635–636, 1074–1075
- animal models, 644–646
  - autoantibodies, 639
  - autoimmune features, 643
  - BAFF gene, 642
  - clinical features and disease associations, 637–641
  - eyes, 637
  - gastrointestinal tract, 638–639
  - genitourinary tract, 639–640
  - kidney, 638
  - lungs, 637–638
  - lymphoma and hematological manifestations, 641
  - musculoskeletal and constitutional symptoms, 640–641
  - nervous system, 639
  - oral cavity, 637
  - Raynaud's phenomenon, 640
  - vascular system, 640
  - vasculitis, 640
- diagnostic procedures, 646–647
- DNA methylation in, 446
- epidemiology, 636
- genetics, 643–644
- historical aspects, 636
- lymphocytic infiltration of exocrine in, 645
- miRNAs in, 447
- pathological features, 641–642
- perspectives, 649–650
- secondary, 635–636
- treatment, 647–649
- cevimeline, 648
  - corticosteroids, 648
  - dental treatment, 648
  - eye lubricants, 648
  - hydroxychloroquine, 648
- SKG mice, 536
- Skin, 217
- disorders, 129
  - features, 588–589
  - induration, 577, 578<sup>t</sup>
  - involvement, 704
  - skin-infiltrating Tcs, 1217
  - ulcers, 704
- Sle1*, 542
- Sle1b*, 534
- SLEDAI-2K instrument, 565
- Smad7, 448, 888
- Small bowel disease, 873
- Small cell lung carcinoma (SCLC), 1025
- Small ncRNAs, 434
- Small ubiquitin-like modifiers (SUMOs), 437
- Small vessel vasculitides, 1071–1076
- Behçet's disease, 1076–1077
  - EGPA, 1072–1073
  - granulomatosis with polyangiitis, 1071–1072
  - MPA, 1072

- Small vessel vasculitides (*Continued*)  
 RA, 1073–1074  
 sarcoidosis, 1077–1078  
 SLE, 1074–1075  
 SS, 1074–1075  
 Small-bowel follow-through (SBFT), 882  
 Small-cell lung cancer (SCLC), 1087  
 Small-vessel vasculitis (SVV), 1286, 1288  
 Smoking, 354, 873, 1418–1419  
   and autoimmunity, 474–475  
 Sneddon's syndrome, 612–613  
 Snowballs, 1039–1040  
 Sodium butyrate (NaB), 440  
 Sodium phenylacetate (SPA), 441–442  
 Sodium valproate (VPA), 440  
 Soluble autoantigens, 1447–1449  
 Soluble mediators, 50–51  
 Soluble pyrexins, 275  
 Soluble receptor for IL-6 (sIL-6R), 1425–1426  
 Somatic hypermutation (SHM), 175  
 SP-1 (transcription factor), 541  
 Spared sural nerve, 988  
 Spectrum of autoantibodies, 1373–1374  
 Sphingosine-1-phosphate receptor-1 (S1P1), 398, 887  
 Spinal cord, 961  
 Spirochetes, 4  
 Splenectomy, 916–917, 938–939  
 Splenic atrophy, 734  
*Spondylitis ankylosans*, 691  
 Spondyloarthritis (SpA), 691  
   animal models, 697–698  
   arthritis with IBD, 692  
   autoimmune features, 693–695  
   clinical features and disease associations, 692  
     back pain, 692  
   cytokines, role of, 693–694  
   epidemiology, 692  
   historical aspects, 691–692  
   HLA-B27, role of, 695–696  
     arthritogenic peptide hypothesis, 695  
   male-to-female ratio, 692  
   non-MHC genes *ERAP1* and *IL23R*, role of, 696–697  
   pathological features, 693  
   prevalence, 692  
   psoriatic arthritis, 692  
   reactive arthritis, 691  
   treatment, 698–699  
     corticosteroids, 698–699  
     NSAIDs, 698–699  
 Spondyloarthropathies, 32, 469–471, 1462  
 Spontaneous animal models, 739  
 Spontaneous autoimmune polyneuropathy models (SAP models), 1001  
 Spontaneous autoimmune thyroiditis, 754–755  
 Spontaneous gastritis, 839  
 Spontaneous models of systemic autoimmunity, 534–536  
 Ank/ank mice, 535  
 autoimmune-susceptibility loci, 534  
 BXSB mice, 535  
 K/BxN mice, 535–536  
 MRL/lpr and gld mice, 534–535  
 NZB mice, 534  
 PN mice, 535  
 SKG mice, 536  
 Spontaneous mouse models, 1224  
 Spontaneous murine models, 1155  
 Src homology 2 (SH2), 536  
 Src homology 2-containing inositol phosphatase-1 (SHIP1), 448  
 Src homology 2-containing phosphatase 1 (SHP1), 539–540  
*Sry* gene, 423  
 Standardization, 1381–1382  
*Staphylococcus aureus*, 95–96, 244, 1297  
 Statins, 704  
 Steady-state DCs, 218–219  
 Stereotactic radiotherapy, 826  
 Steroid-producing cell antibodies (StCA), 795–796  
 Steroid(s), 884–885, 916, 1002, 1059  
   replacement, 805–806  
   responsive polyneuropathy, 998  
   steroid-sparing agents, 1045  
 Stiff-person spectrum disorder (SPSD), 1089–1090, 1095–1096  
 Stimulator of interferon genes (STING), 246–247, 298  
 Stool markers, 882  
 Store-operated Ca21 entry (SOCE), 234–235  
 "Storiform" fibrosis, 716  
 Stratification  
   at baseline, 1163  
   of risk for progression, 1163–1164  
   during treatment, 1163–1164  
*Streptococcal pharyngitis* infections, 1256  
*Streptococcus pyogenes*, 1256  
   distribution, 1257  
   throat colonization, 1256f  
 Streptozotocin (STZ), 500–501  
 Stress, 355  
   stress-induced self-ligands, 229–230  
 Stroke (ST), 608  
 Stromal interaction molecule (STIM1), 520  
 Subacute cerebellar degeneration (SCD), 1083, 1088–1089  
 Subacute lupus rashes, 562  
 Subcorneal pustular dermatosis (SPD), 1200  
 Subepidermal bullous diseases, 1202–1204  
   cicatricial pemphigoid, 1202–1203  
   EBA, 1203  
   HG, 1202  
   LAD, 1203  
 Subepithelial "fibroblastic focus", 1339  
 Suberoylanilide hydroxamic acid (SAHA), 449, 456  
 Sublingual gland, 637  
 Submandibular gland, 637  
 Subpleural parenchyma, 1339  
 Subunits phosphorylation and receptor trafficking, 1093–1094  
 Succinate accumulation, 194  
 Suicidal NETosis, 244, 248  
 Sulfasalazine, 1416t, 1420–1421  
 Sulfated glucuronyl paragloboside (SGPG), 991  
 Sulston, John, 291  
 Superoxide anions, 243  
 "Suppressor T cells", 68–69  
 Surrogate light chain (SLC), 161  
 SWI/SNF-related matrix-associated actin-dependent regulator of chromatin, subfamily-a-like-1 gene (SMARCLA1 gene), 520  
 Swine influenza vaccine, 376  
 Sydenham's chorea, 1255–1256  
 Syk, 172  
 Sympathetic ophthalmia, 12, 1035–1036  
 Symptomatic autoimmune diabetes, 771  
 Synovial tissue ICs, 248  
 Synovitis, 563, 593  
 Synthetase syndrome, 706  
 Systemic arthritis, 676–677  
 Systemic autoimmune diseases, 1051, 1053  
   pathogenic role, 245–250  
   ANCA-AAVs, 247–248  
   antiphospholipid antibody syndrome, 249  
   polymyositis and dermatomyositis, 250  
   primary Sjögren syndrome, 249  
   rheumatoid arthritis, 248  
   SLE, 245–247  
   systemic sclerosis, 249  
   therapeutic implications, 250  
 Systemic autoimmune rheumatic diseases (SARD), 1369  
 Systemic autoimmunity  
   genetically manipulated models, 536–544  
   induced models, 544–545  
   spontaneous models of, 534–536  
 Systemic corticosteroids, 1204, 1218  
 Systemic diseases with CNS manifestations, 1067  
 Systemic disorder, 1051–1052  
 Systemic immunopathic disorders with  
   encephalitis and myelitis, 1068–1078  
   large vessel vasculitides, 1069–1070  
   medium vessel vasculitides, 1070–1071  
   small vessel vasculitides, 1071–1076  
   systemic vasculitides, 1068–1069  
 Systemic inflammatory processes, 1073–1074  
 Systemic juvenile idiopathic arthritis (sJIA), 236–237, 676–677, 680  
   genetics, 680–682, 681t  
   interleukin-1, role of, 682–683  
   interleukin-18, role of, 683  
   interleukin-6, role of, 682  
   MAS, 683–686  
   proinflammatory mediators, 682–683  
 Systemic lupus erythematosus (SLE), 100–101, 130t, 133, 163–164, 217–219, 245, 268, 292, 313, 384–385, 443–446, 533, 555, 608, 635–636, 899, 937, 1036, 1053, 1072, 1074–1075, 1117–1118, 1290, 1355, 1371–1373, 1418, 1437, 1445  
   animal models, 560  
   basophils and IgE Antibodies in, 251  
   biologic agents and small molecule inhibitors in, 567–569  
   disease features, 560–565

- disease modifying drugs, 566–569  
 DNA methylation in, 443–444  
 dysregulated neutrophil phenotype, 245  
 epidemiology, 555–556  
 function and proinflammatory role of neutrophil proteases, 245  
 future perspectives, 569  
 genome-wide association studies, 403–406  
 histone modifications in, 445  
 with HLA and other candidate genes, 387–389  
 linkage studies in, 393–394  
 measurement of disease activity, 565–566  
 microRNA in, 445–446  
 and netosis, 246–247  
 pathogenesis of disease, 556–560  
 racial heterogeneity in, 394  
 therapeutics in, 565–569
- Systemic Lupus International Collaborating Clinics (SLICC),** 560–561
- Systemic sclerosis (SSc),** 124<sup>t</sup>, 249, 447–448, 575–577, 1371–1373  
 autoantibodies in, 594–595  
 biomarkers in, 594–595  
 cardiac involvement, 592–593  
 classification criteria for diagnosis, 576<sup>t</sup>  
 clinical features, 576<sup>f</sup>, 587–588  
 clinically significant organ involvement IN, 587<sup>t</sup>  
 initial clinical presentation, 587–588  
 DNA methylation, 447  
 epidemiology, 577–580  
 fibrosis, 585  
 collagens involved, 585  
 TGF- $\beta$ , role of, 585  
 upregulation of ECM production, 585  
 gastrointestinal involvement, 590–592  
 lower gastrointestinal tract and  
 anorectal involvement, 591–592  
 upper gastrointestinal tract involvement, 591  
 gastrointestinal tract, 586  
 genetics, 580–583, 582<sup>t</sup>  
 heart, 586  
 histone modifications in, 447–448  
 inflammation and autoimmunity, 584–585  
 kidneys, 586  
 less recognized disease manifestations, 593–594  
 lungs, 586  
 management, 596<sup>b</sup>  
 microRNAs in, 448  
 microvascular disease in, 583–584  
 musculoskeletal complications, 593  
 natural history and prognosis, 598  
 organ involvement, 588–590  
 pathogenesis, 580  
 pathology, 585–587  
 preclinical disease models, 583  
 renal involvement, 592  
 screening and follow-up evaluation, 595–598  
 skin, 586  
 Ssc-related autoantibodies and demographic, 591<sup>t</sup>
- T**  
 T cell–dependent activation (TD activation), 171–172  
 T cells, 5, 14, 24–25, 559, 718, 773, 1356  
 activation, 26, 49<sup>f</sup>, 1198  
 bullous pemphigoid, 1201  
 PV, 1195  
 $\alpha\beta$ , 55  
 anergy, 69, 76  
 autophagy in, 308–309  
 dendritic cells, 75–76  
 development, 51–53  
 diversity, 925–926  
 DNA hypomethylation, 443–444  
 dominant tolerance through Treg-mediated immunosuppression, 78–81  
 exhaustion, 779  
 functional activities, 53–55  
 $\gamma\delta$ , 55  
 in GCA, 1320–1322, 1321<sup>t</sup>  
 hybridomas, 482  
 ignorance and antigen sequestering, 74–75  
 immunological tolerance, 65–66  
 induction and maintenance, 74–82  
 self-tolerance establishment in thymus, 70–74  
 inflammation, 988  
 intrinsic mechanisms suppressing clonal expansion and/or reactivation, 77–78  
 involvement  
 in GBS, 995  
 in ITP, 914–915  
 mediated injury, 756  
 pathogenesis, 970–971  
 responses, 751–752, 758–759  
 subpopulations, 55<sup>f</sup>  
 T cell–independent antibody responses, 177  
 T cell–mediated  
 autoimmunity in RA, 662  
 disease, 769  
 T-cell-targeted therapies, 1427–1428  
 Abatacept, 1427–1428  
 tofacitinib, 1428  
 in thymus, 53<sup>f</sup>  
 tolerance, 522–525  
 from adjuvants to T-cell anergy, 69  
 disorders of regulatory T cells, 524–525  
 from fetal tolerance to central tolerance, 66–68  
 monogenic defects affecting T-cell selection and homeostasis, 522–524  
 from neonatal thymectomy to tregs, 68–69  
 principles for induction, 67<sup>f</sup>  
 tolerogenic cells in periphery, 81–82  
 T follicular helper cells (Tfh cells), 279, 559, 1130
- T helper cells (Th cells), 420, 905  
 effector T-helper cell–mediated autoimmune disease, 323–324  
 optimal B-cell activation requiring interaction with, 173–174  
 regulatory CD4<sup>+</sup> T cells, 99–102  
 TFH cells, 102–104  
 Th1 cells, 92–93, 693–694, 914, 1069  
 Th1-type Treg effector phenotype, 973  
 Th2 cells, 92–93, 104–105, 250, 323–324, 914  
 lymphocyte-derived cytokines, 277  
 Th9 cells, 105  
 Th17 cells, 94–98, 324, 685, 1069, 1264  
 cytokines and receptors, 281–282  
 differentiation, 94  
 environmental cues and regulation in intestine, 97–98  
 function, 98  
 identification, 94  
 pathogenicity, 94–96  
 responses, 195–196  
 transcriptional regulation, 96  
 Th22 cells, 98–99  
 and tolerance, 905–906  
 Tr1 cells, 102  
 T lymphocytes, 51–52, 65, 779, 1468  
 cytotoxic, 54<sup>f</sup>  
 in MG, 1021–1022  
 T-cell immunoglobulin and mucin (TIM), 296  
 Tim-4, 543  
 T-cell independent response (TI response), 177  
 T-cell receptors (TCR), 18–19, 24–25, 45–46, 68, 117–118, 134, 364, 386–387, 473, 496, 515, 663, 696, 840, 978, 1000, 1043, 1258, 1449–1450  
 on T cells, 51–52  
 TCR- $\beta$  chain protein sequences, 1213  
 TCR- $\zeta$  chain, 536  
 T-independent antigen, 59  
 T-lymphocyte, 779  
 T1D-associated methylation variable positions (T1D–MVPs), 439  
 TACI gene, 521  
 Tacrolimus, 567, 1045, 1134, 1183, 1416<sup>t</sup>, 1424  
 Takayasu’s arteritis (TA), 1313–1314, 1325–1329  
 clinical, pathologic, and epidemiologic features, 1326–1327  
 clinical spectrum in, 1326<sup>t</sup>  
 genetic features, 1327–1328  
 historic background, 1325  
 pathogenic mechanisms, 1328  
 principal features, 1314<sup>t</sup>  
 treatment and outcome, 1328–1329  
 TANK receptor, 544  
 Target antigens, 1093–1099, 1376  
 intracellular neuronal antigens, 1096–1098  
 neuronal cell surface antigens, 1093–1096  
 Target of rapamycin complex 1 (TORC1), 305–306  
 Target protein, 1086–1087  
 Target-cell recognition, 235  
 TBX21 (T-bet), 231

- TCR-transgenic T cells (TCRtg T cells), 498  
 Tecfidera. *See* BG-12  
 Technetium 99m Tc-sodium pertechnetate scintigraphy, 647  
 Temporal arteritis, 1316  
 biopsies, 1317  
 Tendon rubs, 593  
 Ten-eleven translocation (TET), 431  
 Teplizumab, 1439, 1443–1444  
 Teratomas, 1084  
 Teriflunomide, 977  
 Terminal deoxynucleotidyl transferase (TdT), 51–52  
 Testicular  
 landscape of testicular autoantigen expression, 1236–1237  
 tumors, 1082  
 Testis, local regulation in, 1237  
 Tetanus–diphtheria, 1051  
 Tetramethylpentadecane (TMPD), 453  
 The Environmental Determinants of Diabetes in Young study (TEDDY study), 770, 774  
 Theiler's Murine Encephalomyelitis Virus (TMEV), 364  
 Therapeutic plasma exchange (TPE), 1276  
 Thin layer chromatography (TLC), 619  
 Thiopurines methyltransferase (TPMT), 885, 1133  
 Thoracic aorta and coronary lesions, 722  
 Three-dimensional skin models, 1219  
 Thrombin, autoimmune inhibitors to, 940–941  
 Thrombin time (TT), 939  
 Thrombocyte transfusions, 938–939  
 Thrombocytopenia, 612  
 Thrombopoietin (TPO), 911  
 Thrombopoietin receptor agonists (TPO-RAs), 917–918  
 Thrombosis, 248, 1289  
 Thrombotic antiphospholipid syndrome, 611  
 Thrombotic microangiopathy (TMA), 564, 586  
 Thrombotic thrombocytopenic purpura (TTP), 269, 586  
 Thymectomy, 1015, 1023  
 animal model, 739  
 Thymectomy on day 3 of life (d3tx), 1242–1243  
 AOD in, 1243  
 Thymic dendritic cells (tDCs), 70–71  
 Thymic epithelial cells (TECs), 312–313  
 Thymic pathology, 1015–1016, 1016t  
 Thymic stromal-derived lymphopoietin (TSLP), 278–279, 584  
 Thymic T CD4+ lymphocytes, 1022  
 Thymoma, 1023–1024  
 associated MG, 1017  
 Thymus, 1014f, 1017  
 in MG, 1021–1024  
 promiscuous expression of self-antigens in, 73–74  
 self-tolerance establishment in, 70–74  
 thymoma, 1024  
 Thymus follicular hyperplasia (TFH), 1023  
 Thyocytes, 270–271  
 Thyroglobulin (TG), 749–750  
 Thyroid  
 disease  
 AT, 749–757  
 Graves' disease, 757–763  
 follicles, 750  
 thyroid-specific autoimmunity, 499–500  
 Thyroid follicular cells (TFC), 750  
 Thyroid peroxidase (TPO), 750–751, 756  
 Thyroid stimulating hormone (TSH), 270–271  
 Thyroid-associated ophthalmopathy (TAO), 757, 762  
 Thyroid-stimulating hormone (TSH), 749–750  
 Thyroid-stimulating hormone receptor (TSHR), 499–500  
 Thyroxine4 (T4), 749–750  
 Tight-skin mice, 583  
 Tissue  
 adaptation, 192  
 damage, 17, 28  
 mechanisms, 31–32  
 heterogeneity of tissue macrophages, 192–193  
 tissue-specific dendritic cells, 217–218  
 transcriptome studies, 584–585  
 Tissue factor (TF), 445, 622, 935  
 Tissue transglutaminase (tTg), 478–479  
 Tissue-specific antigens (TSAs), 68  
 Tissue-type plasminogen activator (tPA), 623  
 TL1A receptor, 285  
 TNF alpha-induced protein 3 (TNFAIP3), 404, 582–583  
 TNF Alpha-induced protein 3 interacting protein 1 (TNIP1), 404  
 TNF receptor superfamily member (TNFRSF)  
 TNFRSF1A, 400, 582–583  
 TNFSF15, 697  
 TNFSF4 ligand, 387–388, 581  
 TNF receptor-associated factor 1 (TRAF1), 663  
 TNF receptor–associated factor–binding protein (TRAF-binding protein), 544  
 TNF-related apoptosis–inducing ligand (TRAIL), 284, 292  
 Tobacco smoke, 354  
 Tocilizumab, 1416t, 1425–1426  
 Tofacitinib, 568, 888, 1213, 1416t, 1428  
 Tolerogenic cells in periphery, 81–82  
 Toll-like receptors (TLR), 69, 177, 197–200, 214–215, 277, 293, 307–308, 364, 375–376, 422, 475–476, 556–557, 582–583, 1179, 1260, 1320, 1420  
 ligands, 18, 21  
 TLR-2, 244, 624, 1260  
 TLR-4, 624, 680  
 TLR-7, 423–424, 535, 543–544  
 Tonic signaling, 203–204  
 in B-cell development and survival, 180  
 BAFF, and autoimmunity, 180–181  
 Topical corticosteroids, 1218  
 Toxic  
 chemicals, 453  
 oil syndrome, 579  
 TPO receptor agonists (TPO-RAs), 912, 916  
 TRADD gene, 697  
 Trained immunity, 45–46  
 Tranexamic acid, 942  
 Transbronchial biopsies, 1339  
 Transcriptional analysis in SLE, 557  
 Transforming growth factor (TGF), 29, 996, 1042, 1155  
 TGF $\beta$ , 31, 276, 286, 326, 448, 582–583, 717, 878  
 Transgenic  
 mice, 1224  
 models, 500–501  
 Transglutaminase 2 (TG2), 478–479, 849–850, 859  
 Transglutaminase 3 (TG3), 854  
 Transglutaminase-4 (TGM-4), 735  
 Transient AA, 1211  
 Transient ischemic attack (TIA), 611  
 Translational applications of epigenetics, 454–457  
 Transmembrane  
 activator, 180  
 glycoproteins, 1192  
 Transmitter exocytosis, 1026  
 Transporter-associated proteins (TAPs), 312, 469, 517  
 Transverse myelitis, 1075  
*Treponema pallidum*, 3, 12  
 Treponemal immobilization test (TPI), 4  
 TrialNet, 1444  
 Trichloroethylene (TCE), 453  
 Trichostatin A (TSA), 449  
*Trichuris suis*, 104–105  
 Triiodothyronine (T3), 750  
 Trimethylation of histone H3 at lysine 4 (H3K4me3), 433  
 Trinitrobenzene sulfonic acid colitis (TNBS colitis), 502  
 Triple methylation of residues 9 or 27 (H3K27me3), 433  
 Troponin, 1271–1272  
*Trypanosoma cruzi*, 420–421  
 Tryptophan catabolites, 338–339  
 Tschopp, Jurg, 202–203, 214–215, 1276  
 TSH-R-stimulating antibodies (TSAb), 757  
 Tubular basement membranes (TBMs), 1359  
 Tubules (T), 1126  
 Tubulointerstitial nephritis (TIN), 720, 1357, 1360, 1362  
 Tumor  
 detection and management, 1082  
 tumor-associated antigen CA19–9, 1175  
 Tumor necrosis factor (TNF), 180, 245, 267–268, 276, 325, 335–336, 355, 379–380, 387, 496, 639, 659–660, 925–926, 996, 1043  
 inhibitors, 1425  
 TNF- $\alpha$ , 229–230, 540–541, 644, 693–694, 1057, 1131, 1322, 1420–1421  
 TNF $^{\Delta ARE}$  mice, 698

Tumor necrosis factor receptor (TNFR), 284–285, 542  
 TNFR1 gene, 284, 393–394, 697  
 TNFR2 gene, 284, 393–394  
 TWEAK/Fn14 pathway, 1052  
 Type 1 AIP, 721  
 Type 1 diabetes (T1D), 131–132, 219–221, 331–332, 346–347, 367–368, 384, 439–440, 494, 500–501, 643, 769, 1394  
 animal model for, 483  
 autoimmune pathology, 1391–1392  
 chromatin remodeling and histone acetylation in type-1 diabetes, 440  
 DNA methylation profiling in, 439–440  
 epilogue, 1406–1407  
 genome-wide association studies, 395–398  
 histone deacetylase inhibitors in type-1 diabetes preclinical studies, 440  
 with HLA and other candidate genes, 385–390  
 human type 1 diabetes with NOD mouse model, 1401<sup>t</sup>  
 linkage studies, 390–392  
 markers of risk for diabetes in islet autoantibody-positive relatives, 1392<sup>t</sup>  
 nasal insulin vaccination suppresses insulin antibody, 1406<sup>f</sup>  
 nature and nurture, 1393–1395  
 oral insulin vaccination delays development of diabetes, 1404<sup>f</sup>  
 prevention, 1395–1400  
 primary prevention, 1400–1402  
 antigen-specific immunotherapy, 1402  
 diet and gut microbiome modification, 1401–1402  
 virus vaccination, 1402  
 secondary prevention, 1402–1406  
 stages in natural history, 1392<sup>f</sup>  
 trials for prevention, 1396<sup>t</sup>  
 Type 1 Diabetes Genetics Consortium (T1DGC), 392  
 Type 1 diabetes mellitus (IDDM), 93  
 Type 1 interferons, 199, 642, 736  
 type I interferons  $\alpha$  and  $\beta$ , 282  
 Type 1 regulatory T cells (Tr1 cells), 97, 102  
 Type A gastritis, 834–835  
 Type B gastritis, 835  
 Type I Fc $\gamma$ Rs, 197–198  
 Type I interferon (IFN-I), 217–219, 245–247, 294, 309–310, 560  
 Type I NKT cells, 118  
 Type II collagen (CII), 536  
 Type II hypersensitivity reactions, 320  
 Type II interferon gamma, 282  
 Type III interferon lambda (Type III IFN- $\lambda$ ), 283  
 Type III secretory system (T3SS), 202  
 Type VII collagen (C-VII), 1203  
 Type-3 muscarinic receptor, 1076  
 Tyrosine (Y), 978  
 Tyrosine hydroxylase (THAbs), 798  
 Tyrosine kinases, 297  
 Tyrosine-based activation motifs (ITAMs), 53  
 Tyrosine-protein phosphatase nonreceptor type 22 (PTPN22), 793–794  
 Tysabri. *See* Natalizumab

**U**

*UBASH3A* gene, 392  
 Ubiquitin Conjugating Enzyme E2 L3 (UBE2L3), 406  
 Ubiquitination, 433  
 ubiquitination-related enzymes, 540–541  
 Uhlenhuth, Paul, 11, 1035  
 Ulcerative colitis (UC), 104–105, 127, 219–220, 502, 871–874, 1418–1419  
 extraintestinal manifestations, 875–876  
 Ultraviolet light (UV light), 452  
 exposures to, 452  
 Unc 51-like kinase (ULK), 306–307  
 Undifferentiated arthritis, 679  
 Unfolded protein response (UPR), 694–695  
 Unique immune system of eye, 1042  
 Unmyelinated autonomic nerve fibers, 1461–1462  
 Upadacitinib, 888  
 Upper limit of normal (ULN), 1159  
 Upper respiratory tract infection, 993  
 Uracil-DNA glycosylase (UNG), 516–517  
 Urokinase-type plasminogen activator (uPA), 448, 623  
 Uropathogenic *Escherichia coli* (UPEC), 1240–1241  
 Ursodeoxycholic acid (UDCA), 1149–1150, 1159, 1164<sup>t</sup>  
 Urticular plaques, 1200  
 US Food and Drug Administration (FDA), 686  
 US Food and Drug Administration (FDA), 686, 917, 1421, 1445  
 Ustekinumab, 568, 887, 1416<sup>t</sup>, 1426–1427  
 Usual interstitial pneumonia (UIP), 586  
 Uveal tract, 1036  
 Uveitis, 678, 1036–1037  
 Uveitogenic antigens, 1035

**V**

Vaccination, 7, 775  
 Vaccines, 354–355, 375, 379  
 animal models, 378  
 certainty and uncertainty about, 379–380  
 crossfire and coincidence, 377–378  
 human papillomavirus vaccine, 375  
 practical approach, 379  
 theoretical concerns, 376  
 Vacuolar protein sorting protein (Vps), 307  
 Valvulitis, in vivo model of, 1262  
 Variable number tandem repeat (VNTR), 385–386  
 Variant NKT cells (*v*NKT cells), 118  
 Vascular disease, 563  
 endothelium, 996  
 GCA, 1315–1316  
 growth endothelial factor, 999  
 injury, 583  
 neuro-Behçet, 1077  
 thrombosis, 622  
 Vascular cell adhesion molecule (VCAM), 974  
 VCAM-1, 1263, 1441  
 Vascular endothelial growth factor (VEGF), 1323

Vasculitides, 1068, 1287<sup>b</sup>  
 of large and medium-sized blood vessels, 1313–1315  
 Vasculitis, 640, 1054, 1286  
 eosinophils in, 254  
 immuno-stromal interactions in, 1323  
 Vasculitogenic T cells, 1321  
 Vasculogenesis, 584  
 Vasectomy, 1240  
 VAV1 phosphorylation, 233–234  
 Vedolizumab, 887  
 Ventricular dysfunction in APS, 613  
 Vertebrates, 336–337  
 Vesicle exocytosis, 234–235  
 Vessels (V), 1126  
 Vestibular dysfunction, 1058–1059  
 Vestibulo-auditory dysfunction, 1054  
 manifestations, 1069  
 VHDHJH-rearranged IgH loci, 161–162  
 Video capsule endoscopy (VCE), 883  
 Vincristine, 918  
 Violaceous eruption, 704  
 Viral exposures, 579–580  
 Virus pathogens in human autoimmune diseases, 368<sup>t</sup>  
 vaccination, 1402  
 Vitamin B12 deficiency, 834  
 Vitamin D, 353–354, 968  
 in autoimmune diseases, 477  
 deficiency, 353–354  
 Vitamin D response element (VDRE), 386  
 Vitiligo, 503, 1215–1221  
 atypical cases, 1216  
 autoantibodies as potential immunologic markers, 1220  
 autoimmune features, 1216–1218  
 classification, 1216<sup>t</sup>  
 clinical, pathologic, and epidemiologic features, 1215–1216  
 genetic features, 1218  
 immunosuppressive drugs, 1218  
 lymphocyte-mediated destruction of melanocytes, 1218  
 pathogenetic mechanism, 1219–1220  
 races and geographic areas, 1216  
 vitiligo-like depigmentation, 1218  
 vitiligo-like hypopigmentation, 588  
 in vivo and in vitro models, 1218–1219  
 Vitreous inflammatory cells, 1039–1040  
 Vitronectin, 1263  
 Voclosporin, 1416<sup>t</sup>, 1424  
 Vogt–Koyanagi–Harada syndrome (VKH syndrome), 1039–1040  
 Voltage-gated calcium channels (VGCCs), 1012–1013, 1089  
 Voltage-gated potassium channels (VGKCs), 1012–1013, 1083, 1085–1086, 1464  
 Voltage-gated sodium channels (VGSC), 1013  
 von Willebrand disease (vWD), 948  
 von Willebrand factor (vWF), 938  
 autoimmune inhibitors to, 948  
 Vo14i NKT cells, 119  
 Vo24-specific antibodies, 121–123

**W**

Warm AIHA, 904  
 immune mechanisms in, 904–906  
 B cells and tolerance, 905  
 T-helper cell and tolerance, 905–906  
 Warm reactive antibodies, 899  
 Warm reactive pathogenicity of warm reactive IgG antibodies, 899–901  
 WAS protein (WASP), 519  
 WASP interacting protein (WIP), 234  
 Wassermann antibody, 12  
 Wassermann antigen, 12  
 Wassermann test, 12–13  
 WD repeat domain phosphoinositide-interacting protein (WIPI), 306–307  
 Wegener's granulomatosis, 876, 1053–1054  
 Wellcome Trust Case Control Consortium (WTCCC), 395  
 WTCCC2, 696–697  
 Western diets, 333, 1393–1394  
 Western immunoblotting (WB), 1466  
 "Western lifestyle" diseases, 331

**White-dot syndromes**, 1039

Wild bank voles (*Myodes glareolus*), 780  
 Wiskott–Aldrich syndrome (WAS), 519  
 Witebsky, Ernest, 13, 499–500, 824, 989  
 Writers of histone modifications, 433–434  
 Wyllie, Andrew, 291

**X**

X chromosome, 23, 423  
 X isochromosome, 423  
 X monosomy, 423  
 X-linked agammaglobulinemia (XLA), 520  
 X-linked CD40L deficiency, 517  
 X-linked thrombocytopenia (XLT), 519  
 Xanthoma cells, 822  
 Xanthomatous hypophysitis, 822  
 XC-chemokine receptor 1 (XCR1), 214, 216  
 Xenobiotic(s)  
   induction, 1154  
   xenobiotics-triggered murine models, 1155  
 Xenograft mouse model, 1214, 1225

**XmAb5871**, 724

XXY phenotype, 23

**Y**

Y chromosome, 423–424  
 Y-linked autoimmune acceleration locus mice (*Yaa* mice), 251, 423–424, 535  
 YO (purkinje cell antigen-1), 1097  
 YTS, 234

**Z**

Zc3h12a, 544  
 Zeta chain–associated protein kinase 70 gene (ZAP70 gene), 515–516, 536, 665  
 Zika virus (ZIKV), 369  
 Zinc transporter 8 (ZnT8A), 770  
*Zona fasciculata*, 790  
*Zona glomerulosa*, 790  
*Zona pellucida* (ZP), 1243–1244  
*Zona reticularis*, 790  
 Zonadhesin (ZAN), 1237