



An Integrative Approach to Autoimmune Disease: Leaving the Era of Reaction and Entering the New Proactive Era of Prediction



David M. Brady, ND, DC, CCN, DACBN

Vice President for Health Sciences
Director, Human Nutrition Institute
Associate Professor of Clinical Sciences



UB Clinics



Integrative Healthcare
SYMPOSIUM



Dr. David M. Brady

- Naturopathic Doctor (ND)
Licensed in CT and VT
- Doctor of Chiropractic (DC)
- Certified Clinical Nutritionist (CCN)
- Diplomate of the American Clinical Board
of Nutrition (DACBN)
- Vice President for Health Sciences
Director, Human Nutrition Institute
Associate Professor of Clinical Sciences
University of Bridgeport, Conn.
- Chief Medical Officer,
Designs for Health, Inc.
Diagnostic Solutions Laboratory
- Private Practice (Fairfield, CT)
Whole Body Medicine



Disclosures

- Chief Medical Officer: Designs for Health, Inc. (DFH)
- Chief Medical Officer: Diagnostic Solutions Labs (DSL)
- Consultant: Cell Science Systems, Inc. (CSS-ALCAT)





Autoimmune Disease: A Modern Epidemic? Molecular Mimicry, the Hygiene Hypothesis, Stealth Infections, and Other Examples of Disconnect Between Medical Research and the Practice of Clinical Medicine

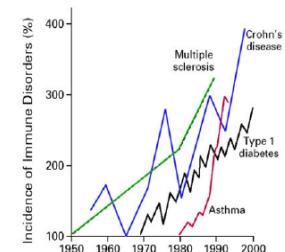
By David M. Brady, ND, DC, CCN, DACBN

The genesis of this article is as a follow-up to a presentation delivered at the 2011 American Association of Naturopathic Physicians (AANP) annual convention on the topic of autoimmune disease, which resulted in a substantial amount of inquiry and requests for further exploration of the topics presented. There is simply no doubt that the incidence of autoimmune disorders has been rising sharply over the past several decades in the Western industrialized countries, particularly the US (Figure 1).¹ A broad array of disorders considered immune-dysregulatory and autoimmune in nature, encompassing both those classically categorized as Th1- and Th2-dominant, are included in this phenomenon. The question is, why has there been such a sharp rise in the incidence of these disorders? The answers may very well be found in the current medical research, but you would probably never know it by visiting a doctor. This may be because this situation serves as an example of the giant chasm that often exists between Western medical research, which is often outstanding, and the practice of clinical medicine, which often leaves quite a bit to be desired when it comes to the management of chronic disorders with high morbidity but low mortality.

The typical allopathic clinical approach to autoimmune disorders focuses on the management of symptoms with various anti-inflammatory medications and often the use of chemotherapeutics, and very potent

immunosuppressive agents with nasty potential side effects such as leukemia and lymphoma.² While these approaches admittedly can provide substantial symptomatic relief to the patient, they do not get to the cause of these conditions, and some research suggests that these approaches may result in a furthering of the pathological process.

Figure 1: Rising Incidence of Autoimmune Disorders



From: Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med*. Sep 2002;347(12):911-920.

However, modern research into autoimmune phenomena suggests that radically different approaches may be required to reverse the above-cited trends, including a strong emphasis on very early detection with predictive auto-antibodies, a

Brady David M. Autoimmune Disease. *Townsend Letter*. June 2012;347:45-50.

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Molecular Mimicry, the Hygiene Hypothesis, Stealth Infections and Other Examples of Disconnect between Medical Research and the Practice of Clinical Medicine in Autoimmune Disease

David M. Brady

University of Bridgeport, Division of Health Sciences, Bridgeport, CT, USA
Email: dbrady@bridgeport.edu

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ABSTRACT

Autoimmune disorders have been on a steep rise in the industrialized countries over the past several decades and while research has been starting to develop a detailed understanding of pathophysiology and many of the underlying mechanisms, any meaningful incorporation of this information into clinical medicine has been painfully slow. Concepts of molecular mimicry, the hygiene hypothesis, intestinal hyper-permeability (leaky gut syndrome) and aggressive use of predictive antibody testing are explored in this article with examples given on how emerging information on these phenomena may aid the clinician in a new, more proactive, approach to management of these conditions.

Keywords: Autoimmunity; Inflammation; Arthritis; Mimicry; Hygiene

1. Introduction

There is simply no doubt that the incidence of autoimmune disorders has been rising sharply over the past several decades in the Western industrialized countries, particularly the United States (see Figure 1) [1]. A broad array of disorders considered immune-dysregulatory and autoimmune in nature, encompassing both those classically categorized as Th1 and Th2-dominant, are included in this phenomenon. The question is why has there been such a sharp rise in the incidence of these disorders? The answers may very well be found in the current medical research, but you would probably never know it by visiting a doctor. This may be because this situation serves as an example of the giant chasm that often exists between medical research, which is often outstanding, and the practice of clinical medicine, which often leaves quite a bit to be desired when it comes to the management of chronic disorders with high morbidity but low mortality.

The typical allopathic clinical approach to autoimmune disorders focuses on the management of symptoms with various anti-inflammatory medications and often the use of chemotherapeutics, and very potent immunosuppressive agents with nasty potential side-effects like leukemia and lymphoma [2]. While these approaches admittedly can provide substantial symptomatic relief to the patient, they do not really get to the cause of these condi-

tions and some research suggests that these approaches may result in a furthering of the pathological process. However, modern research into autoimmune phenomena suggest radically different approaches may be required to reverse the above cited trends, including a strong emphasis on very early detection with predictive auto-antibodies, a focus on optimizing gastrointestinal

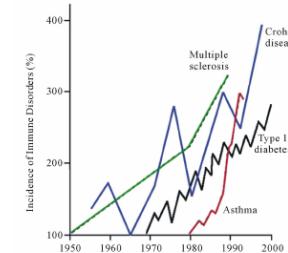
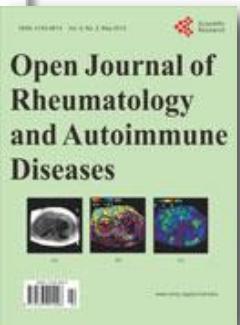
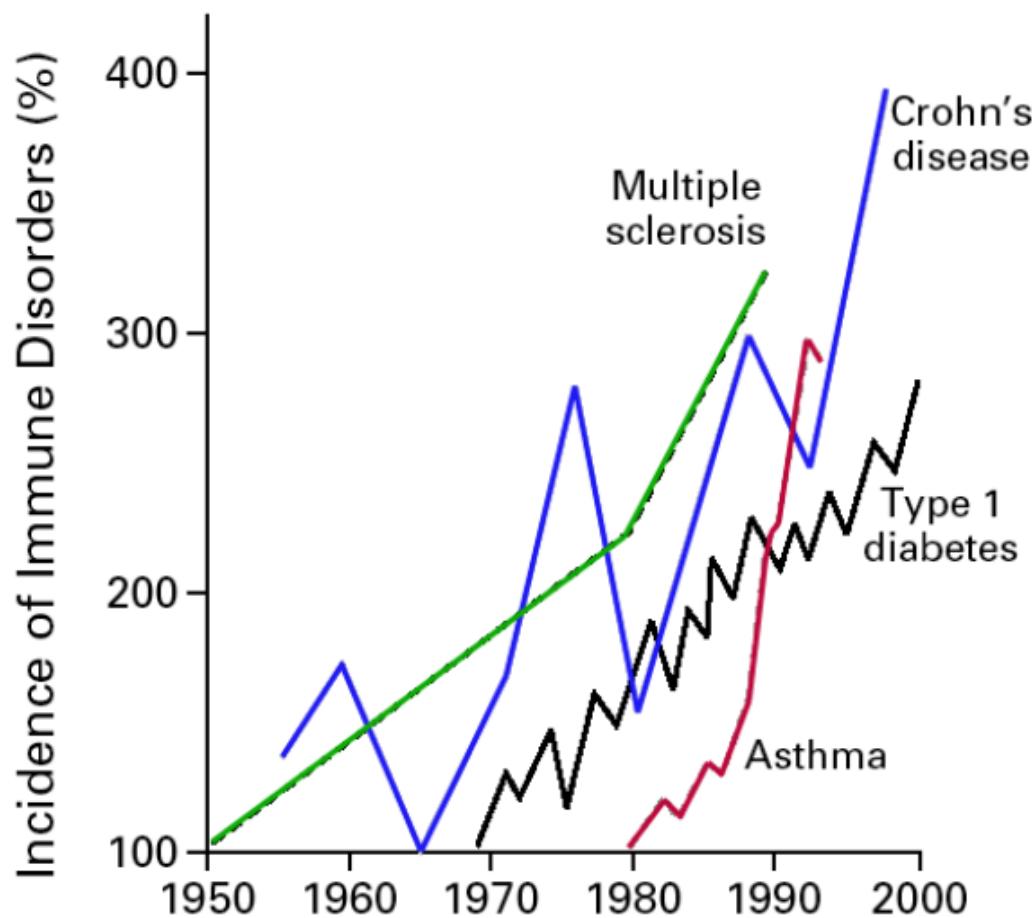


Figure 1. Rising incidence of autoimmune disorders [1].



Increasing Incidence of Immune Regulatory Disorders



How do we see illness?

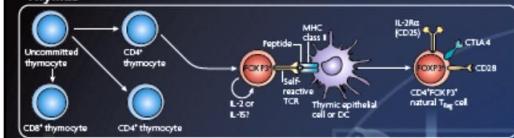


Autoimmune Disease

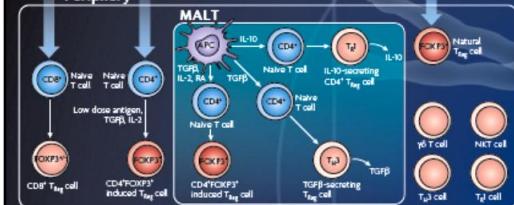
Development and phenotype of regulatory T cells

Many T cell types have immune regulatory function, but the two most important T_{Reg} cell subsets express the transcription factor FOXP3 and develop in the thymus or can be induced in peripheral sites including the mucose-associated lymphoid tissue (MALT). Although expression of FOXP3 is considered a useful marker for these cell subpopulations in mice, FOXP3 expression may also be induced in non-T cells that lack T cell function. A functional activated human FOXP3⁺ T cell population can be induced in a variety of cell types, including dendritic cells, and these cells can facilitate their proliferation. A third type of T cell, the FOXP3⁺ T cell, secretes the immunosuppressive cytokine interleukin-10 (IL-10) and may develop from conventional CD4⁺ T cells by activation in the presence of IL-10 or may develop from T helper 1 (T_H1) or T_H2 cell subsets. Other T cell subpopulations including natural killer T (NKT) cells, γδ T cells and CD8⁺ T cells can also exert potent suppressor functions in certain settings. Although both human and mouse CD8⁺ T cells can be induced to express FOXP3, a suppressive function for these cells *in vivo* has yet to be clarified.

Thymus



Periphery



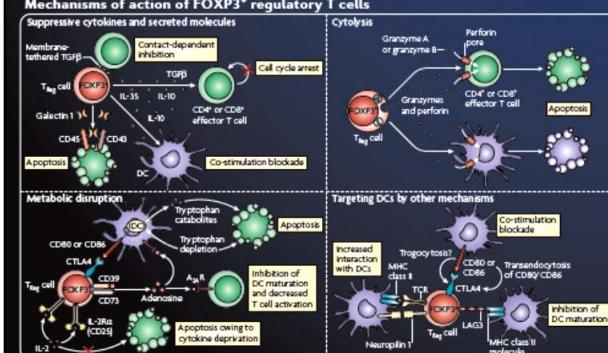
Plasticity in the periphery

Under certain conditions, FoxP3⁺ T_H1 cells can downregulate expression of FoxP3, lose suppressive functions and manifest some of the functions of conventional effector T_H1, T_H2, T_H17 and T_{M1} cell subsets. The key causes of this loss of FoxP3 expression include inflammatory environments with high levels of cytokines that are normally involved in the induction of effector T cells, such as IL-6 and interferon-γ (IFNγ). In addition, T_H1 cell-specific deletion of certain transcription factors that are shared between T_H1 cells and T_{Reg} cells (for example, the T_H17 cell-specific factor IRF4) results in impaired suppression of T_H2 cell responses by the T_H1 cells.

Phenotypic markers of FOXP3⁺ regulatory T cells

Markers shared by FOXP3 ⁺ T_{Reg} cells and conventional activated CD4 ⁺ T cells (mice and humans)	CD25 ^{hi} GITR CD45RB ^{hi} (mice only) CD45RA (humans only) Putative receptor 4 (mice only)
Markers preferentially expressed by activated mouse FOXP3 ⁺ T_{Reg} cells	CD27 ^{hi} CTLA4
Markers specifically expressed by activated human FOXP3 ⁺ T_{Reg} cells	TGFβ ^{hi} Latent TGFβ GARF CD103 (IL-19) CD108 (IL-10)

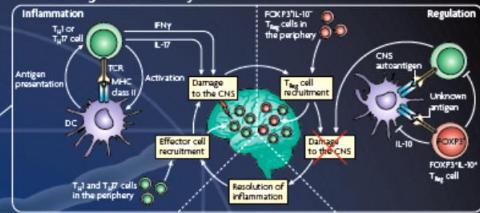
Mechanisms of action of FOXP3⁺ regulatory T cells



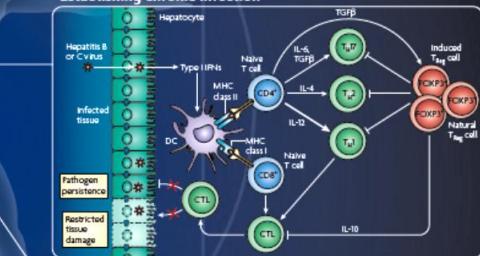
Function of FOXP3⁺ regulatory T cells

FOXP3⁺ T_{Reg} cells have been shown to influence the outcome of immune responses in several tissues. For example, in the intestine T_{Reg} cells have a key role in maintaining tissue homeostasis by inhibiting the overactivation of dendritic cells (DCs) and effector T cells. In the case of autoimmunity, such as that depicted in the central nervous system (CNS), T_{Reg} cells can have a beneficial effect by short circuiting the inflammatory cycle of T cells and antigen-presenting cells (APCs). This same general mechanism can also be seen in the prevention of chronic infection, where T_{Reg} cells contribute to the resolution of inflammation, thereby preventing tumour clearance. During T_{Reg} cell recruitment, damage to the CNS can occur, but this is balanced by the ability of T_{Reg} cells to prevent further damage, thus maintaining CNS homeostasis. In each of the examples shown, we focus on the role of FOXP3⁺ T_{Reg} cells, although interactions between multiple immune cell types and indeed different types of regulatory T cell are also likely to be important in the regulation of immune responses.

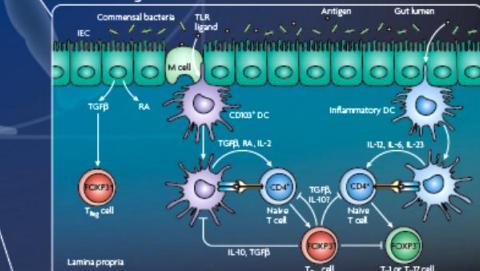
Preventing autoimmunity



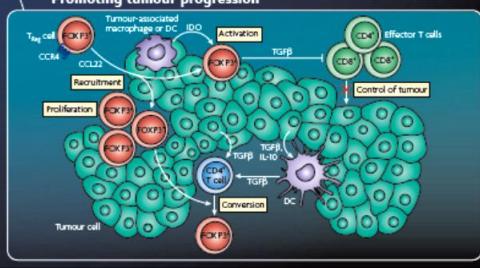
Establishing chronic infection



Maintaining intestinal homeostasis

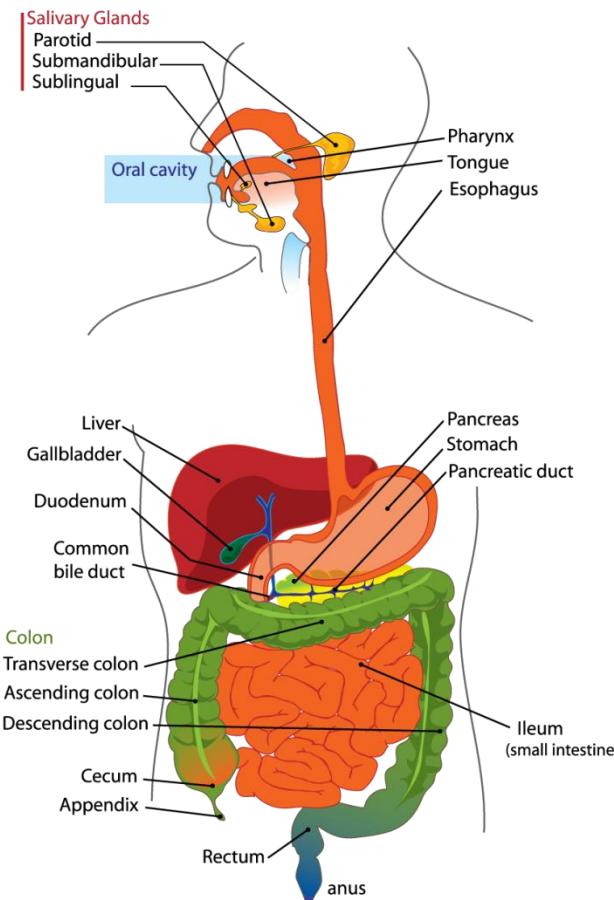


Promoting tumour progression



The Importance of Mucosal Immunity

"The dominating part of the immune defense, even if flora is excluded, is localized in the gut—no less than 75% of the immune cells of the body are suggested to be found in the GI tract."



Bengmark S. Acute and "chronic" phase reaction--a mother of disease, ClinNutr, Vol. 23, No. 6, pp. 1256-1266, December 2004

“Death begins in the colon”

- E. E. Metchnicoff
 - Russian Pathologist,
 - 6th Ever Nobel Laureate (1908)
 - Father of “orthobiosis” theory and probiotics





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The Human Microbiome: at the interface of health and disease

Ilseung Cho^{1,2} and Martin J. Blaser^{1,2,3,4}¹Department of Medicine, NYU Langone Medical Center, New York, NY 100²New York Harbor Department of Veterans Affairs Medical Center (Manhattan 10010, USA)³Department of Microbiology, NYU Langone Medical Center, New York, NY⁴Department of Biology, New York University, New York, NY 10003, USA

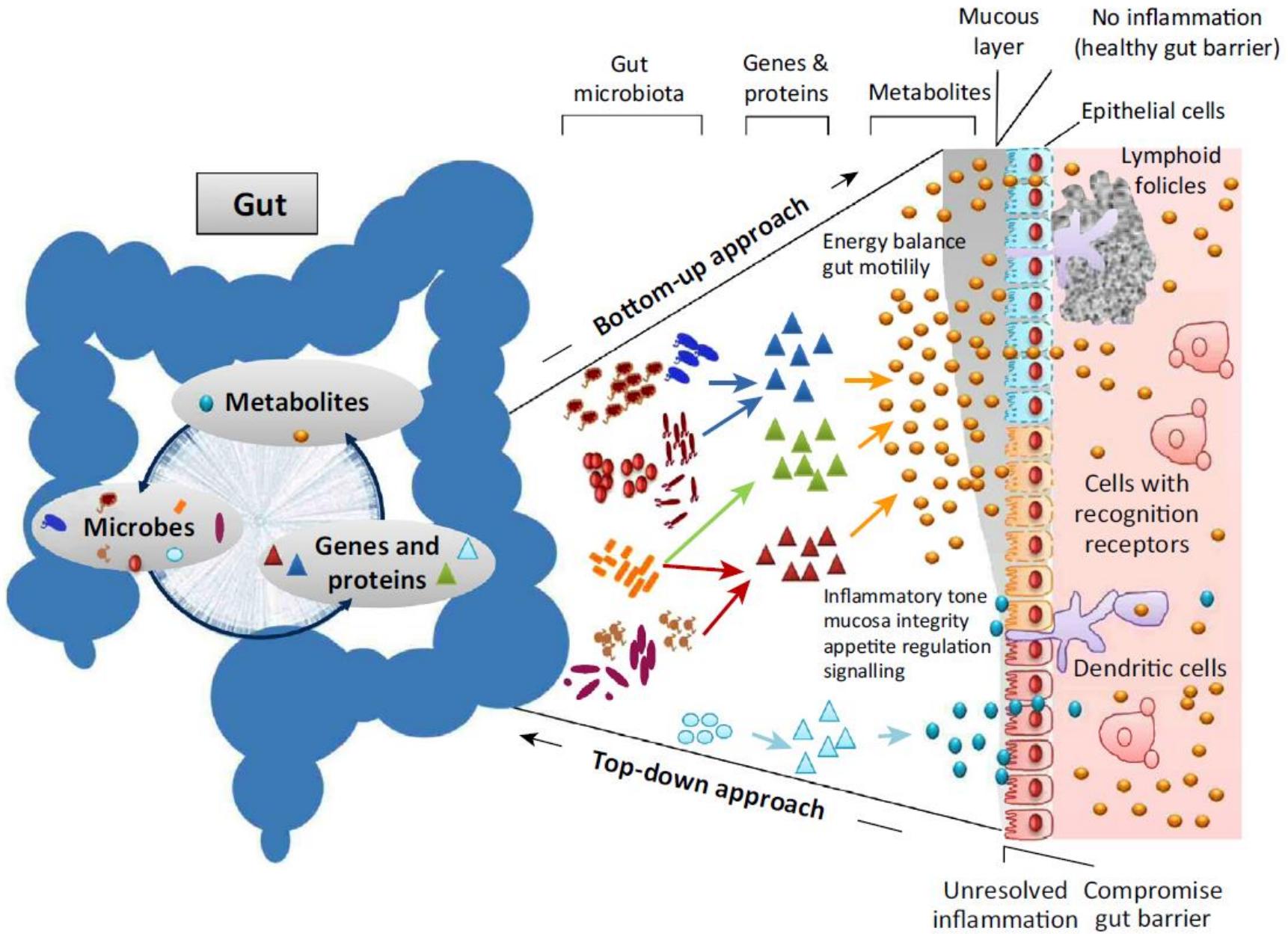
Abstract

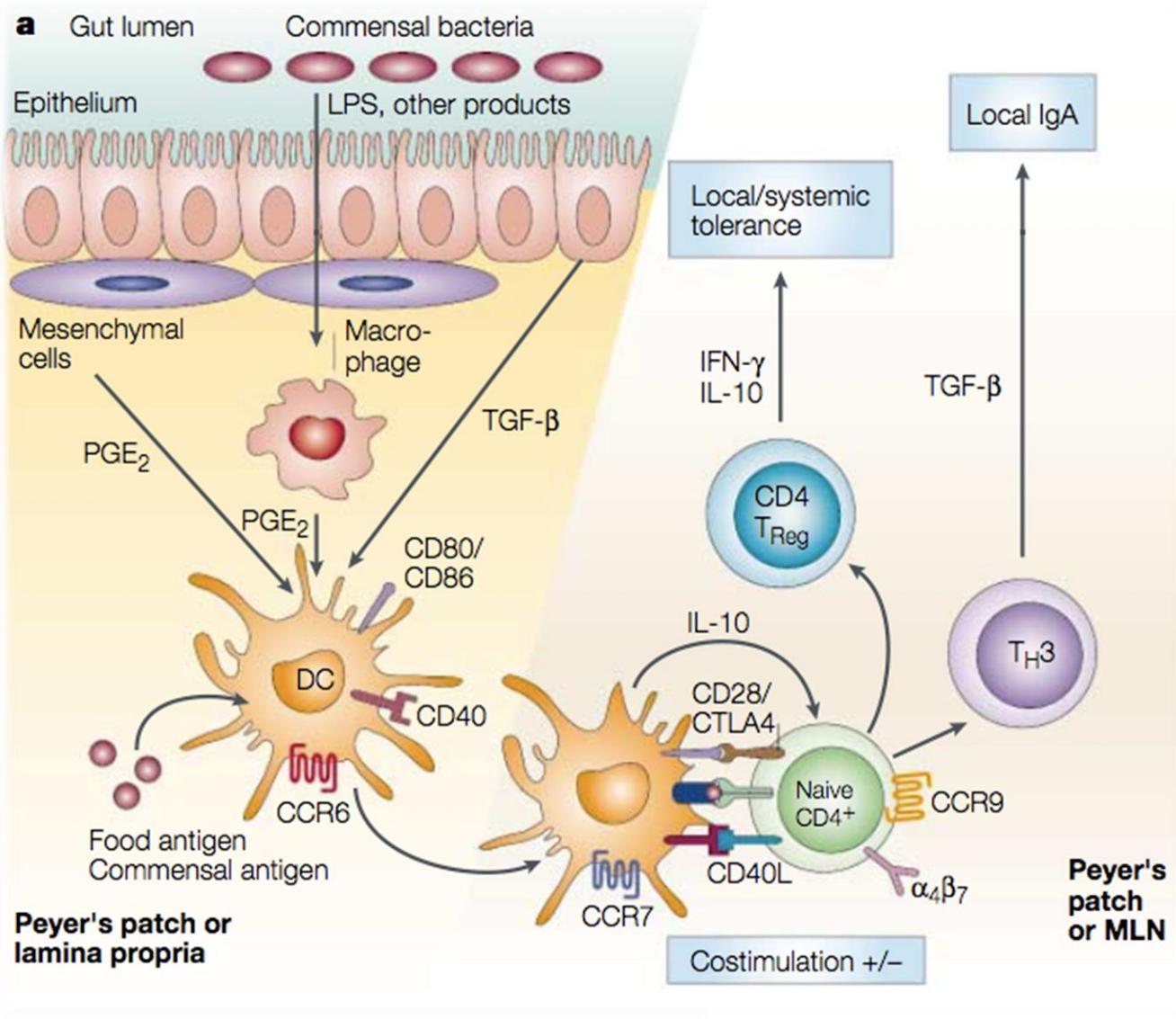
Interest in the role of the microbiome in human health has burgeoned over the past decade. The dynamics of the microbiome can be described by many of the tools and observational study of population ecology. Deciphering the metagenome and its aggregate genes also can be used to understand the functional properties of the microbial community. The microbiome and metagenome probably have important functions in health and disease. Further exploration is a frontier in human genetics.

Until recently, the properties of the *microbiota* of humans (formerly called the gut flora) were largely a black box. Cultivation *in vitro*, which has been the cornerstone of microbiology since the 19th century, cannot be applied to many of the populated microbial communities¹. However, DNA-based analyses have now reached a new horizon, by generating enormous new data sets that can be mined for the composition and functional properties of vastly greater numbers of microorganisms. For example, the Human Microbiome Project (HMP) by the NIH has generated terabyte 16S rRNA metagenomic datasets of over 35 billion reads taken from 300 U.S. subjects, across 15 body sites. Large-scale endeavors (e.g. the European project, Metahit²) provide a preliminary understanding of the significance of the human microbiome and its collective genes (the metagenome).

The aim of these projects, particularly the HMP, is to characterize the properties of the ‘normal’ microbiome of healthy individuals. Important questions concerning commonalities and differences between healthy individuals in both the microbiome and metagenome and functional pathways are being addressed. The presence of major clusters such as the vagina⁴ and the gastrointestinal tract⁵ provide new ways to study the microbiome.

- The human microbiome and its relationship to disease is a new and rapidly evolving field of study.
- Co-evolution of hosts and their microbiomes has led to cooperative interactions in metabolism and homeostasis.
- Concepts from community ecology such as resilience, community disturbances, and extinction are useful in understanding the microbiome.
- New computational and statistical tools are being actively developed to analyze the large sequence datasets generated by the increasingly powerful technologies.
- The taxonomic composition and functional characteristics of the microbiome may allow individuals to be categorized into different microbial patterns, called “enterotypes”, in the gastrointestinal tract. Although low-level taxonomy varies substantially among individuals, higher level taxonomy and functional characteristics appear largely preserved.
- Many factors affect the composition of the microbiome over the course of a human lifetime. These include inheritance, mode of infant delivery, diet, and age-related changes in adults.
- The relationships between the microbiome and several human diseases are being intensively studied for conditions that include colorectal cancer, inflammatory bowel disease, and immunologically-mediated skin diseases.
- Causal relationships for many of the associations between the microbiome and disease states have yet to be proven.
- Understanding the links between the microbiome and human disease may provide prophylactic or therapeutic tools to improve human health.





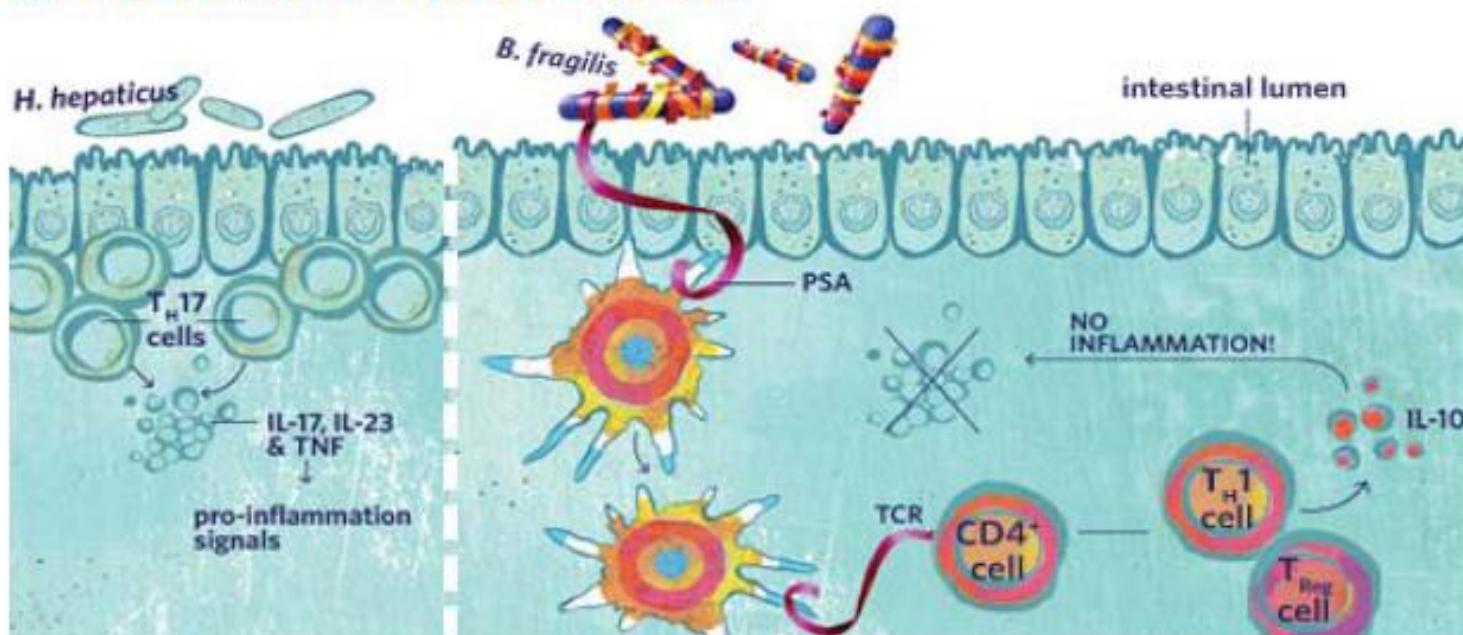
ARTICLES

A microbial symbiosis factor prevents intestinal inflammatory disease

Sarkis K. Mazmanian¹*, June L. Round¹* & Dennis L. Kasper^{2,3}

Humans are colonized by multitudes of commensal organisms representing members of five of the six kingdoms of life; however, our gastrointestinal tract provides residence to both beneficial and potentially pathogenic microorganisms. Imbalances in the composition of the bacterial microbiota, known as dysbiosis, are postulated to be a major factor in human disorders such as inflammatory bowel disease. We report here that the prominent human symbiont *Bacteroides fragilis* protects animals from experimental colitis induced by *Helicobacter hepaticus*, a commensal bacterium with pathogenic potential. This beneficial activity requires a single microbial molecule (polysaccharide A, PSA). In animals harbouring *B. fragilis* not expressing PSA, *H. hepaticus* colonization leads to disease and pro-inflammatory cytokine production in colonic tissues. Purified PSA administered to animals is required to suppress pro-inflammatory interleukin-17 production by intestinal immune cells and also inhibits *in vitro* reactions in cell cultures. Furthermore, PSA protects from inflammatory disease through a functional requirement for interleukin-10-producing CD4⁺ T cells. These results show that molecules of the bacterial microbiota can mediate the critical balance between health and disease. Harnessing the immunomodulatory capacity of symbiosis factors such as PSA might potentially provide therapeutics for human inflammatory disorders on the basis of entirely novel biological principles.

BACTERIAL BALANCE



We inoculated a wild-type mouse with the bacterium *H. hepaticus* to create an experimental mouse version of the autoimmune disorder inflammatory bowel disease (IBD). *H. hepaticus* activates Th17 cells which release cytokines associated with inflammation, like IL-17, causing symptoms of disease. But once *B. fragilis* expressing the polysaccharide A (PSA) is added to the gut, dendritic cells take up and present the PSA molecule on their surface, activating CD4 T cells and regulatory T cells (Tregs). The Tregs release IL-10 which suppresses the inflammatory action of IL-17, in effect alleviating IBD in mice.

The Scientist

Volume 23 | Issue 8 | Page 34

Disorders Associated with Dysbiosis and Intestinal Hyperpermeability

Inflammatory Bowel Disease	Dermatitis Herpetiformis
Irritable Bowel Syndrome	Autism
Celiac Disease	Childhood Hyperactivity
Infectious Enterocolitis	Spondyloarthropathies
Cystic Fibrosis	Pancreatic Insufficiency
Chronic Fatigue Immune Deficiency Syndrome	Weight Gain
Acne	Neoplasia Treated with Cytotoxic Drugs
Eczema	Hepatic Dysfunction
Psoriasis	Alcoholism
Urticaria	Environmental Illness

Unno N, Fink MP. Intestinal epithelial hyperpermeability. Mechanisms and relevance to disease. *Gastroenterol Clin North Am.* 1998;27(2):289-307.

Real-Time PCR Quantitation of Clostridia in Feces of Autistic Children

Yuli Song,^{1*} Chengxu Liu,¹ and Sydney M. Finegold^{2,3,4}

Research Service¹ and Infectious Diseases Section,² VA Medical Center West Los Angeles, and
Department of Medicine³ and Department of Microbiology, Immunology, and
Molecular Genetics,⁴ UCLA School of Medicine,
Los Angeles, California

Received 11 February 2004/Accepted 27 June 2004

Based on the hypothesis that intestinal clostridia play a role in late-onset autism, we have been characterizing clostridia from stools of autistic and control children. We applied the TaqMan real-time PCR procedure to detect and quantitate three *Clostridium* clusters and one *Clostridium* species, *C. bolteae*, in stool specimens. Group- and species-specific primers targeting the 16S rRNA genes were designed, and specificity of the primers was confirmed with DNA from related bacterial strains. In this procedure, a linear relationship exists between the threshold cycle (C_T) fluorescence value and the number of bacterial cells (CFU). The assay showed high sensitivity: as few as 2 cells of members of cluster I, 6 cells of cluster XI, 4 cells of cluster XIVab, and 0.6 cell of *C. bolteae* could be detected per PCR. Analysis of the real-time PCR data indicated that the cell count differences between autistic and control children for *C. bolteae* and the following *Clostridium* groups were statistically significant: mean counts of *C. bolteae* and clusters I and XI in autistic children were 46-fold ($P = 0.01$), 9.0-fold ($P = 0.014$), and 3.5-fold ($P = 0.004$) greater than those in control children, respectively, but not for cluster XIVab (2.6×10^8 CFU/g in autistic children and 4.8×10^8 CFU/g in controls; respectively). More subjects need to be studied. The assay is a rapid and reliable method, and it should have great potential for quantitation of other bacteria in the intestinal tract.

Autism is a complex disease with unclear causes. Many autistic subjects exhibit a range of gut disorders, which include constipation, diarrhea, retention of gas, and abdominal pain and discomfort. Abnormal gut microflora may play a role in these problems. Research into the characteristics of the gut flora in autism has been limited. In our initial studies that characterized the fecal bacterial composition by culturing, we noted abnormalities in the fecal bacterial composition of children with autism compared to age- and sex-matched controls. We found higher counts of clostridia overall and more species of clostridia in stools of autistic children than in healthy children (11). In particular, *Clostridium bolteae*, a novel species that we described previously (29; called *Clostridium clostridio-*

sensitive results as well as providing ease of use and speed (23, 24, 32). Most recently, real-time quantitative PCR has been used for the specific detection and quantitation of selected bacteria from fecal DNA (1, 2, 4, 9, 16, 18, 21, 22, 25, 33).

Few studies have reported on using real-time PCR for quantitation of clostridia in different environments. Belanger et al. (2) and Kimura et al. (17) reported on the successful quantitation of *Clostridium difficile* in feces and *Clostridium botulinum* type E in fish samples using specific primers and probes targeted to toxin genes, respectively. A very recent study investigated the feasibility of using 16S rRNA gene-targeted specific primers and probes for quantitation of major intestinal bacteria, including certain *Clostridium* species by real-time PCR (26).

Clostridia in Autism by Real Time PCR

	<i>C. bolteae</i>	<i>Clostridium cluster I</i>	<i>Clostridium cluster XI</i>	<i>Clostridium cluster XIVab</i>
Control (N=8)	(3.9 ± 0.3) 10^3	(4.1 ± 0.3) 10^5	(4.0 ± 0.4) 10^6	(2.6 ± 0.2) 10^8
Autistic (N=15)	(1.8 ± 0.1) 10^5	(3.7 ± 0.4) 10^6	(1.4 ± 0.1) 10^7	(4.8 ± 0.6) 10^8

- Group I (*Clostridium cluster I*)
 - Forward primer, CI-F1 TACCHRAGGAGGAAGCCAC 54.6
- Group II (*Clostridium cluster XI*)
 - Forward primer, CXI-F1 ACGCTACTTGAGGAGGA 46.5
- Group III (*Clostridium cluster XIVab*)
 - Forward primer, CXIV-F1 GAWGAAGTATYTCGGTATGT 46.2

Autism, Gut Bugs and Fecal Transplant

Kang et al. *Microbiome* (2017) 5:10
DOI 10.1186/s40168-016-0225-7

Microbiome

RESEARCH

Open Access



Microbiota Transfer Therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study

Dae-Wook Kang^{1†}, James B. Adams^{2‡}, Ann C. Gregory^{3,15†}, Thomas Borody⁴, Lauren Chittick^{5,15}, Alessio Fasano⁶, Alexander Khoruts^{7,8,9}, Elizabeth Geis², Juan Maldonado¹, Sharon McDonough-Means¹⁰, Elena L. Pollard², Simon Roux^{5,15}, Michael J. Sadowsky^{8,11}, Karen Schwarzberg Lipson¹², Matthew B. Sullivan^{3,5,15,16*}, J. Gregory Caporaso^{12,13*} and Rosa Krajmalnik-Brown^{1,14*}

Abstract

Background: Autism spectrum disorders (ASD) are complex neurobiological disorders that impair social interactions and communication and lead to restricted, repetitive, and stereotyped patterns of behavior, interests, and activities. The causes of these disorders remain poorly understood, but gut microbiota, the 10¹³ bacteria in the human intestines, have been implicated because children with ASD often suffer gastrointestinal (GI) problems that correlate with ASD severity. Several previous studies have reported abnormal gut bacteria in children with ASD. The gut microbiome-ASD connection has been tested in a mouse model of ASD, where the microbiome was mechanistically linked to abnormal metabolites and behavior. Similarly, a study of children with ASD found that oral non-absorbable antibiotic treatment improved GI and ASD symptoms, albeit temporarily. Here, a small open-label clinical trial evaluated the impact of Microbiota Transfer Therapy (MTT) on gut microbiota composition and GI and ASD symptoms of 18 ASD-diagnosed children.

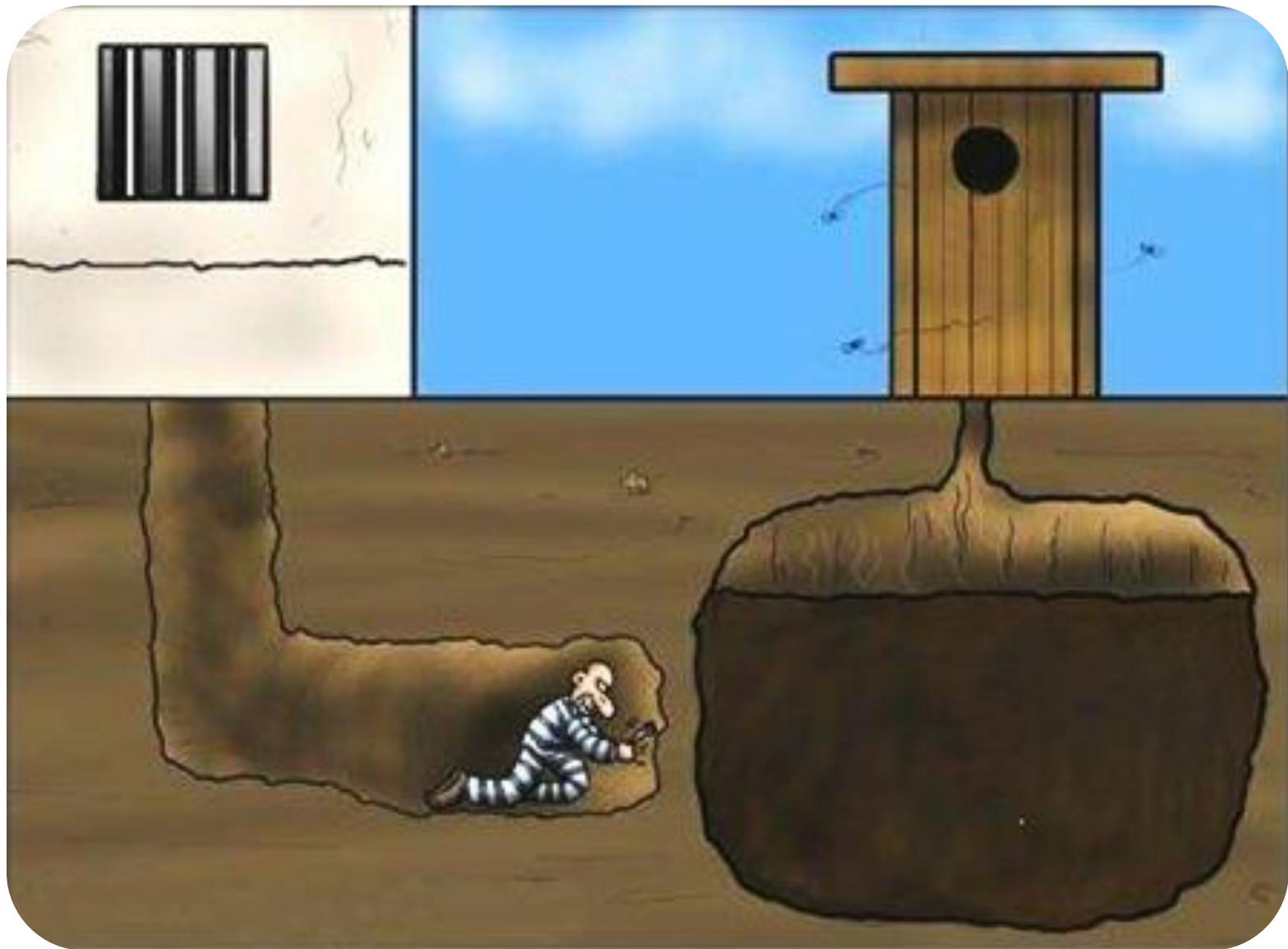
Results: MTT involved a 2-week antibiotic treatment, a bowel cleanse, and then an extended fecal microbiota transplant (FMT) using a high initial dose followed by daily and lower maintenance doses for 7–8 weeks. The Gastrointestinal Symptom Rating Scale revealed an approximately 80% reduction of GI symptoms at the end of treatment, including significant improvements in symptoms of constipation, diarrhea, indigestion, and abdominal pain. Improvements persisted 8 weeks after treatment. Similarly, clinical assessments showed that behavioral ASD symptoms improved significantly and remained improved 8 weeks after treatment ended. Bacterial and phage deep sequencing analyses revealed successful partial engraftment of donor microbiota and beneficial changes in the gut environment. Specifically, overall bacterial diversity and the abundance of *Bifidobacterium*, *Prevotella*, and *Desulfovibrio* among other taxa increased following MTT, and these changes persisted after treatment stopped (followed for 8 weeks).

Conclusions: This exploratory, extended-duration treatment protocol thus appears to be a promising approach to alter the gut microbiome and virome and improve GI and behavioral symptoms of ASD. Improvements in GI symptoms, ASD symptoms, and the microbiome all persisted for at least 8 weeks after treatment ended, suggesting a long-term impact.
(Continued on next page)

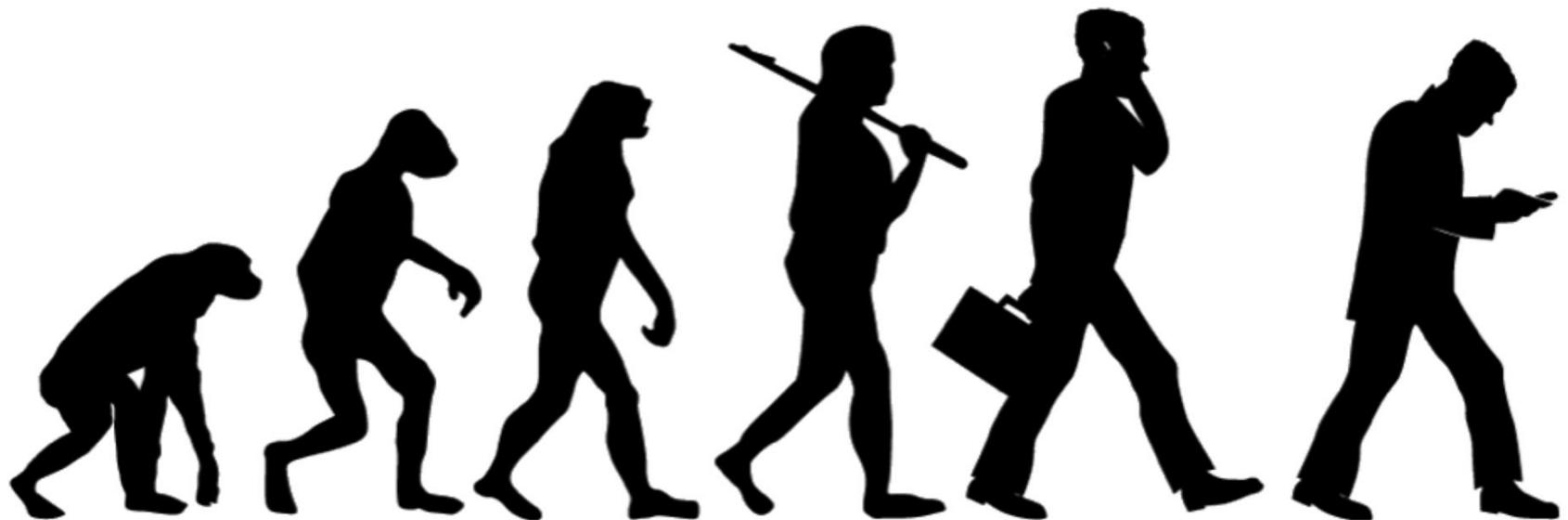
Conclusions

Together, these findings suggest that MTT is safe and well-tolerated in children with ASD ages 7–16 years. MTT led to significant improvements in both GI- and ASD-related symptoms, and the improvements were sustained at least 8 weeks after treatment. Coincident with these clinical improvements, both microbiota and phage from the donors appear to have engrafted, at least partially, in the recipients. This shifted gut microbiota of children with ASD toward that of neurotypical children is consistent with the hypothesis that gut microbiota may be at least partially responsible for GI and ASD symptoms. While this study was an open-label trial that is subject to placebo effects, these results are promising and provide a crucial step for understanding the connection between the microbiome and ASD. A randomized, double-blind, placebo-controlled study is the next step to investigate the value of MTT in treating children with ASD and GI problems.

Kang et al. *Microbiome* (2017) 5:10



The Evolution of Stool Analysis



LEADING ARTICLE

A molecular revolution in the study of intestinal microflora

E Furrie

Bacterial colonisers of the colon comprise several hundred bacterial species that live in a complex ecosystem. Study of this complex ecosystem has been carried out, until recently, by traditional culture techniques with biochemical methods to identify organisms. The development of molecular techniques to investigate ecological microbial communities has provided the microbiologist with a vast array of new techniques to investigate human intestinal microflora. Metagenomics, the science of biological diversity, combines the use of molecular biology and genetics to identify and characterise genetic material from complex microbial environments. The combination of metagenomics and subsequent quantitation of each identified species using molecular techniques allows the relatively rapid analysis of whole bacterial populations in human health and disease.

Bacteria permanently colonise the whole length of the gastrointestinal tract with by far the highest concentration of organisms

Gut 2006;55:141–143. doi: 10.1136/gut.2005.081695

disease (IBD) has focused on the search for a causative bacterial agent, with many and varied candidates being proposed.^{5–9} It has now been generally accepted that analysis of the microbial ecosystem and changes in the balance of organisms at initiation and during disease yields far more relevant information than hunting for the proverbial “needle in the haystack”. This change has partly been driven by the general ineffectiveness of targeted antibiotic therapy to treat IBD^{10–14} and the potential of probiotics as therapy for IBD, allowing re-establishment of homeostasis present in healthy gut.^{15–17}

In order to develop these alternative therapies it is essential to determine what comprises a healthy colonic ecosystem and how this balance of organisms is altered during various states and stages of IBD. As a large majority of bacterial species present in the colon are effectively unculturable,^{18, 19} it is impossible for detailed examination of the colonic microflora to be achieved using traditional culture techniques. The increased ease in which molecular analysis can be carried out by most microbiologists has led to an explosion in sequencing of ribosomal DNA (rDNA) from different bacterial species and strains from many different environments. This



311 Ferst St NW Ste 1355B
Atlanta GA 30332
877-485-5336

Patient: Jane Doe Accession: 20150727-0102
Collected: 07/24/2015 Received: 07/24/2015
DOB: 12/19/1971 Completed: 07/31/2015

Ordered by: Diane Farhi, MD

Pathogens

Bacterial Pathogens

	Result	Expected
Campylobacter	Negative	Neg
<i>C. difficile</i> Toxin A	Negative	Neg
<i>C. difficile</i> Toxin B	Negative	Neg
<i>E. coli</i> O157	Negative	Neg
Enterotoxigenic <i>E. coli</i> LT	Negative	Neg
Enterotoxigenic <i>E. coli</i> ST	Negative	Neg
Shiga-like Toxin <i>E. coli</i> stx1	Negative	Neg
Shiga-like Toxin <i>E. coli</i> stx2	Negative	Neg
<i>Salmonella</i>	Positive	Neg
<i>Shigella</i>	Negative	Neg
<i>Vibrio cholera</i>	Negative	Neg
<i>Yersinia enterocolitica</i>	Negative	Neg

Parasitic Pathogens

<i>Cryptosporidium</i>	Negative	Neg
<i>Entamoeba histolytica</i>	Negative	Neg
<i>Giardia</i>	Negative	Neg

Viral Pathogens

Adenovirus 40	Positive	Neg
Adenovirus 41	Positive	Neg
Norovirus GI	Negative	Neg
Norovirus GII	Negative	Neg
Rotavirus A	Negative	Neg

H. pylori

<i>Helicobacter pylori</i>	4.03 E7	High	<7.0 E3
Virulence Factor, cagA	Positive		Neg

Normal Bacterial Flora

<i>Bacteroides fragilis</i> grp	6.2 E7		5.0 E5 - 3.2 E9
<i>Bifidobacter</i>	9.5 E7	Low	>8.9 E9
<i>Enterococcus</i>	2.0 E7	High	1.2 E4 - 3.1 E6
<i>E. coli</i>	4.2 E8	High	1.0 E4 - 7.6 E7
<i>Lactobacillus</i>	4.4 E5		1.0 E6 - 5.8 E9

Patient: Jane Doe

Accession: 20150727-0102

Opportunistic Bacteria

Potential Autoimmune Triggers	Result	Range
<i>Citrobacter</i> spp.	8.1 E6	High
<i>Klebsiella pneumoniae</i>	4.2 E3	
<i>Proteus</i> spp.	1.2 E3	
<i>Proteus mirabilis</i>	1.7 E3	
<i>Yersinia enterocolytica</i> (from pg 1)	Negative	Neg

Additional Dysbiotic/Overgrowth Bacteria

<i>Morganella morganii</i>	8.0 E2	<1.0 E3
<i>Pseudomonas</i> spp.	1.2 E3	<2.5 E3
<i>Pseudomonas aeruginosa</i>	5.3 E8	High
<i>Staphylococcus</i> spp.	9.2 E3	<1.0 E4
<i>Streptococcus</i> spp.	4.2 E2	<1.0 E3

Parasites

<i>Blastocystis hominis</i>	9.1 E5	High	0.00
<i>Dientamoeba fragilis</i>	Negative		Neg
<i>Endolimax nana</i>	Negative		Neg
<i>Entamoeba coli</i>	Negative		Neg
<i>Chilomastix mesnelli</i>	Negative		Neg
<i>Pentatrichomonas hominis</i>	Negative		Neg
<i>Microsporidia</i> spp.	Negative		Neg

Fungi/Yeast

<i>Candida albicans</i>	8.7 E7	High	>5.0 E3
<i>Candida</i> spp.	Negative		Neg
<i>Cyclospora cayetanensis</i>	Negative		Neg
<i>Geotrichum</i> spp.	<1000 cfu/g	Low	Neg
<i>Trichosporon</i> spp.	Negative		Neg

Additional Tests

	Result	Range
SgA	458	High
Anti-gliadin	0.8	
Elastase 1	102	Low
Lactoferrin	3.8	
Occult blood	Negative	neg

Fungi/yeast; *Candida albicans* is reported as a quantitative value. All other fungi/yeast are reported semi-quantitative where Low = <1000, Mod = 1,000-100,000 and High = >100,000 cfu/g stool.

The assays were developed and the performance characteristics determined by Diagnostic Solutions Laboratory.

CLIA# 11D-2097795
Medical Director - Diane Farhi, MD

Patient: Jane Doe

Accession: 20150727-0102

Antibiotic Resistance Genes

	Phenotype	Genotype	Expected
Salmonella			
Sulfonamides	Negative	Positive	Neg
Trimethoprim	Negative	Negative	Neg
Fluoroquinolones	Negative	Negative	Neg
Macrolides	Negative	Negative	Neg
<hr/>			
Pseudomonas			
Sulfonamides	Negative	Positive	Neg
Trimethoprim	Negative	Negative	Neg

Phenotype; refers to resistance genes of the antibiotic/class that can be found on the genome of the positive organism.

Genotype; refers to resistance genes of the antibiotic/class that are not found on the genome of the positive organism but are found on genomes of bacteria of the microbiome.

Evaluation of the Luminex xTAG Gastrointestinal Pathogen Panel and the Savyon Diagnostics Gastrointestinal Infection Panel for the detection of enteric pathogens in clinical samples

Michael D. Perry, Sally A. Corden and Robin A. Howe

Correspondence

Michael D. Perry
michael.perry@wales.nhs.uk

Public Health Wales Microbiology Cardiff, University Hospital of Wales, Heath Park, Cardiff, UK

Table 1. Targets detected using conventional diagnostic methods, Luminex GPP and Savyon GIP assays

✓, Pathogen detectable; ×, pathogen not detectable.

Pathogen/target	Detection method		
	Conventional diagnostics	Luminex GPP	Savyon GIP
<i>Campylobacter</i>	✓	✓	✓
<i>Clostridium difficile</i> toxin A/B	✓*	✓	✓
<i>Escherichia coli</i> O157	✓	✓	✗
Enterotoxigenic <i>Escherichia coli</i> (ETEC)	✗	✓	✗
Shiga-like toxin-producing <i>Escherichia coli</i> (STEC)	✗	✓	✗
<i>Salmonella</i>	✓	✓	✓
<i>Shigella</i>	✓	✓	✓
<i>Vibrio cholerae</i>	✓*	✓	✗
<i>Yersinia enterocolitica</i>	✓*	✓	✗
<i>Cryptosporidium</i>	✓†	✓	✓
<i>Giardia lamblia</i>	✓†	✓	✓
<i>Entamoeba histolytica</i>	✓†	✓	✓
<i>Dientamoeba fragilis</i>	✗	✗	✓
Adenovirus 40/41	✗	✓	✗
Norovirus	✗	✓	✗
Rotavirus A	✗	✓	✗

Received 21 February

Accepted 5 August

INTRODUCTION

Infectious gastrointestinal disease (IID) is a major public health problem whose aetiology is often unknown. The clinical presentation of IID can range from mild, self-limiting diarrhoea to life-threatening haemorrhagic colitis or even death. The economic cost of IID to society has been demonstrated to be significant, both in the wider community and in the wider food chain. The rapidity of result generation is important for effective infection control efforts.

Abbreviations: ETEC, enterotoxigenic *Escherichia coli*; GIP, Gastrointestinal Infection Panel; GPP, Gastrointestinal Pathogen Panel; IID, infectious intestinal disease; MFI, median fluorescence intensity; NAAT, nucleic acid amplification test; STEC, shiga-like toxin-producing *Escherichia coli*.

that target groups of bacteria, parasites and viruses (Coupland *et al.*, 2013; Cunningham *et al.*, 2010; de Boer *et al.*, 2010; Higgins *et al.*, 2011; Koziel *et al.*, 2013; McAuliffe *et al.*, 2013; Schuurmann *et al.*, 2007; Stark *et al.*, 2011). Several publications show the feasibility and accuracy of identifying bacteria, parasites and viruses within a single

M. D. Perry, S. A. Corden and R. A. Howe

Luminex - Gastroenteritis: a serious medical and economic burden – a new approach

The advantages of multiplex molecular diagnostic technology seem clear: it unifies the NHS's fractured laboratory processes, and in doing so, brings a wealth of financial, medical and logistical benefits. As Anson concludes: "We're tantalisingly optimistic about this new technology."

Enteric Pathogen "Panels"

Luminex xTAG GPP

- FDA-cleared

- Targets

- ✓ Bacterial—*Salmonella*, *Shigella*, *Campylobacter*, *E. coli* O157, ETEC (LS/ST), *C. difficile**
- ✓ Viral—Norovirus (GI/II), Rotavirus A
- ✓ Parasites—*Giardia*, *Cryptosporidium*



- Comprehensive panel

- Requires nucleic acid extraction, PCR, hybridization/reading
 - ✓ Manual pipetting, setup, open transfer of amplicon, equipment
 - ✓ 5 h TAT

* Frequently tested separately due to incidence in patient populations

Evaluation of the Luminex xTAG Gastrointestinal Pathogen Panel and the Savyon Diagnostics Gastrointestinal Infection Panel for the detection of enteric pathogens in clinical samples

Michael D. Perry, Sally A. Corden and Robin A. Howe

Correspondence
Michael D. Perry
michael.perry@wales.nhs.uk

Public Health Wales Microbiology Cardiff, University Hospital of Wales, Heath Park, Cardiff, UK

Infectious gastrointestinal disease is caused by a diverse array of pathogens, and is a challenging syndrome to correctly diagnose and manage. Conventional laboratory diagnostic methods are

Received
Accepted

INTRO

Infectious gastrointestinal illness is a clinical syndrome whose aetiology is as varied as its presentation. Symptoms range from mild, self-limiting diarrhoea to potentially life-threatening haemolytic uraemic syndrome or pseudomembranous colitis. Attempts to estimate the human and economic cost of infectious intestinal diseases (IDs) have demonstrated their far-reaching effects within hospitals and in the wider community. Increased sensitivity and rapidity of result reporting have the potential to aid infection control efforts, reduce overall social and healthcare

Abbreviations: ETEC, enterotoxigenic *Escherichia coli*; GIP, Gastrointestinal Infection Panel; GPP, Gastrointestinal Pathogen Panel; IID, infectious intestinal disease; MFI, median fluorescence intensity; NAAT, nucleic acid amplification test; STEC, shiga-like toxin-producing *Escherichia coli*.

The Luminex GPP assay identified all intestinal pathogens encountered using conventional techniques in 472 liquid stool samples over a 2 month period when samples were tested concurrently with increased sample input volume (200 versus 100 µl). During this period the Luminex GPP assay also further identified additional pathogens as seen in the stored sample testing.

2012; Lopman *et al.*, 2004; Roberts *et al.*, 2000; STEC Workshop Reporting Group, 2012; Vonberg *et al.*, 2008).

Many conventional techniques for the microbiological diagnosis of IID (e.g. microscopy, culture and ELISA) are labour-intensive and time-consuming, and frequently result in low detection rates. A plethora of in-house and commercial nucleic acid amplification tests (NAATs) have been developed to detect a wide range of individual micro-organisms associated with diarrhoeal illness. Many authors have also reported the successful use of multiplexed assays that target groups of bacteria, parasites and viruses (Coupland *et al.*, 2013; Cunningham *et al.*, 2010; de Boer *et al.*, 2010; Higgins *et al.*, 2011; Koziel *et al.*, 2013; McAuliffe *et al.*, 2013; Schuermann *et al.*, 2007; Stark *et al.*, 2011). Several publications show the feasibility and accuracy of identifying bacteria, parasites and viruses within a single

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our stool
samples.



Gut Microbes and Systemic Pathology

- Examples of epidemiologic associations between GI microbes and systemic autoimmune pathology:
 - *Klebsiella*: Ankylosing Spondylitis
 - *Citrobacter, Klebsiella, Proteus, Prevotella*: Rheumatoid Arthritis
 - Bacteriodetes spp: Arthritis in general
 - *Fusobacterium*: Systemic sclerosis
 - Mycobacteria: Psoriasis
 - *Yersinia*: Grave's Disease & Hashimoto's Dz.
 - *Streptococcus*: PANDAS
 - Chlamydia, Salmonella, Shigella, Yesrsinia: Reactive arthritis (ReA)
 - *S. Pyogenes*: Rheumatic Fever
 - *Camphylobacter jejuni*: Gullian Barre Syndrome
 - *E. coli, Proteus*: Autoimmunity in general

Modified from: Mayes MD. Epidemioloic studies of environmental agents and systemic autoimmune diseases. *Environ Health Perspect* 1999;107(suppl. 5):743-748

Systemic sclerosis is associated with specific alterations in gastrointestinal microbiota in two independent cohorts

Elizabeth R Volkmann,¹ Anna-Maria Hoffmann-Vold,² Yu-Ling Chang,³ Jonathan P Jacobs,¹ Kirsten Tillisch,¹ Emeran A Mayer,¹ Philip J Clements,¹ Johannes R Hov,^{4,5,6} Martin Kummen,^{4,5} Øyvind Midtvedt,^{2,6} Venu Lagishetty,¹ Lin Chang,¹ Jennifer S Labus,¹ Øyvind Molberg,² Jonathan Braun³

specific alterations in gastrointestinal microbiota in two independent cohorts.
BMJ Open Gastro 2017;3:e000134. doi:10.1136/bmjgast-2017-000134

To compare faecal microbial composition with systemic sclerosis (SSc) from 2

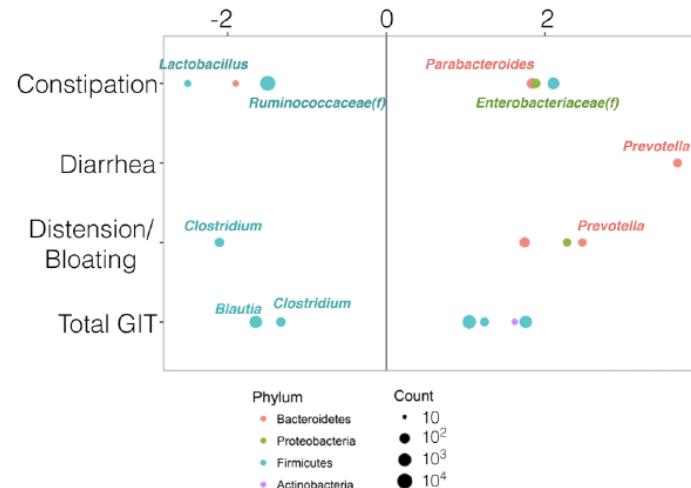
cohorts with controls and to determine whether certain genera are associated with SSc-gastrointestinal tract (GIT) symptoms.

Design: Adult patients with SSc from the University of California, Los Angeles (UCLA) and Oslo University Hospital (OUH) and healthy controls participated in this study (1:1). All participants provided stool specimens for 16S rRNA sequencing, linear discriminant analysis

Summary box

What is already known about this subject?

- Gastrointestinal tract dysfunction affects over 90% of patients with systemic sclerosis.
- The pathogenesis of lower gastrointestinal tract dysfunction in systemic sclerosis is largely unknown.
- Emerging evidence suggest that gastrointestinal tract dysbiosis may be a feature of the systemic



p=0.002). Patients with SSc had significantly lower levels of commensal genera deemed to protect against inflammation, such as *Bacteroides* (UCLA and OUH), *Faecalibacterium* (UCLA), *Clostridium* (OUH); and significantly higher levels of pathobiont genera, such as *Fusobacterium* (UCLA), compared with controls.

SSc from 2 geographically and clinically distinct cohorts. These findings suggest that GIT dysbiosis may be a pathological feature of the SSc disease state.

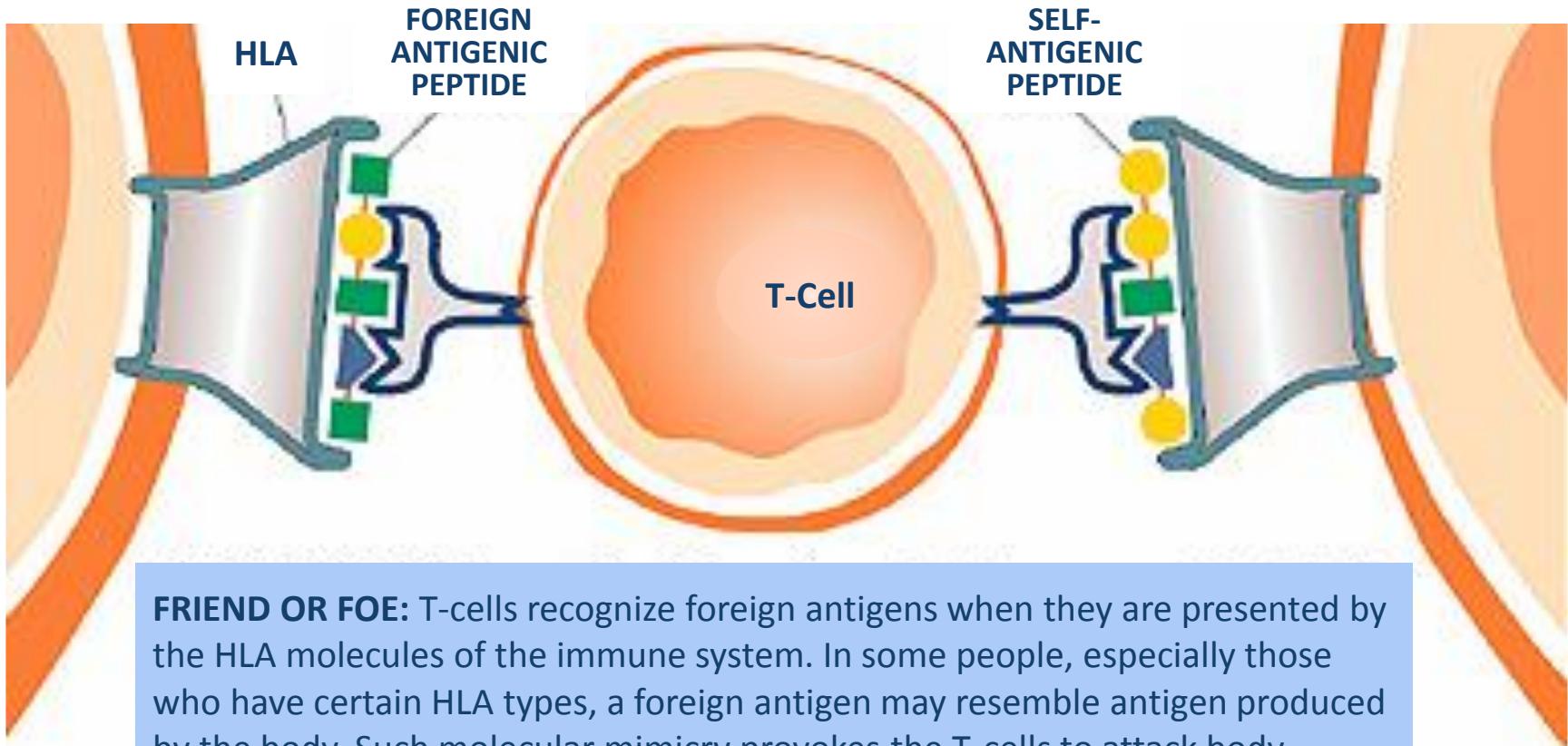
For numbered affiliations see end of article.

GIT involvement³ adversely affect quality of life and social functioning.^{4,5} Unfortunately, no effective treatment options exist for eliminating these disruptive symptoms, largely because the pathogenesis of this dimension of SSc is poorly understood.

GIT dysbiosis occurs in a number of chronic inflammatory conditions, including inflammatory bowel disease (IBD),⁶⁻⁹ and

INTRODUCTION
The majority of patients with systemic sclerosis (SSc) experience gastrointestinal tract (GIT) dysfunction.^{1,2} Symptoms of lower

Bugs/Foods: Friend or Foe?



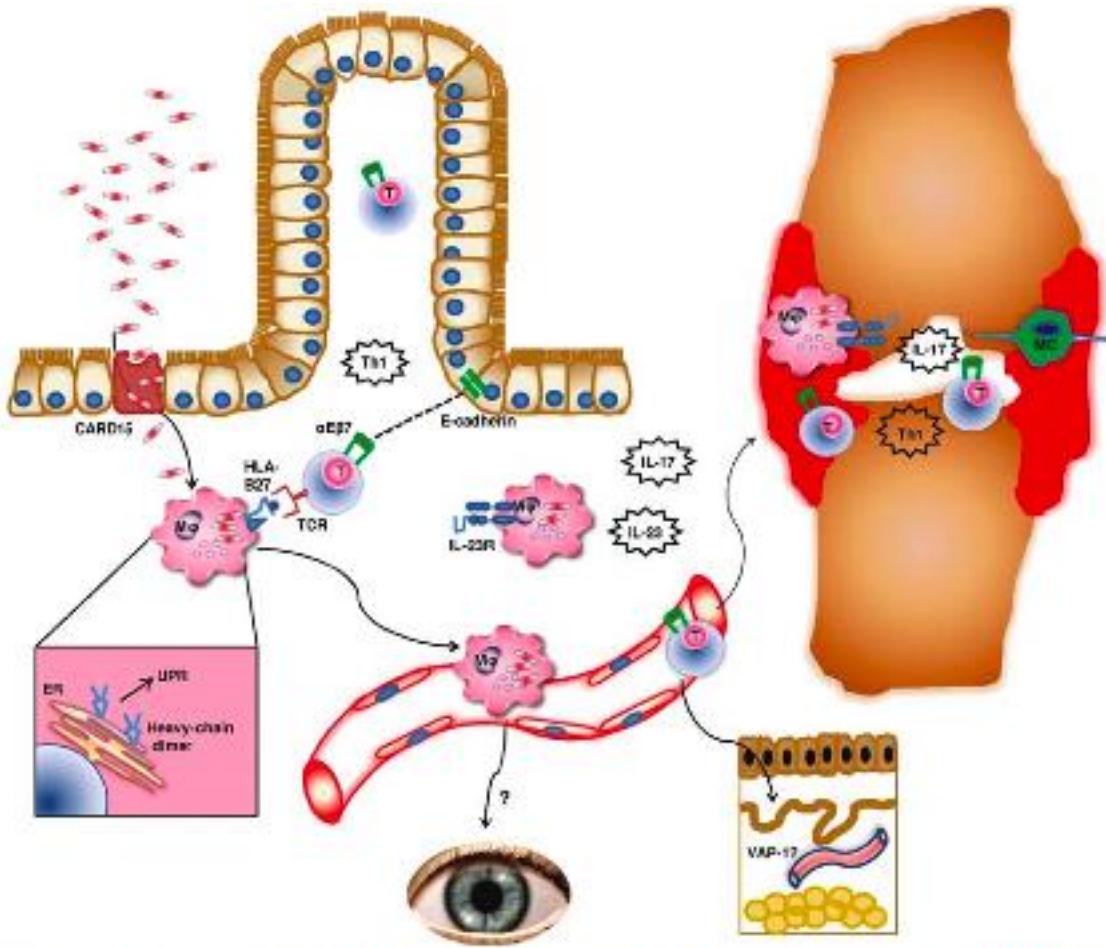
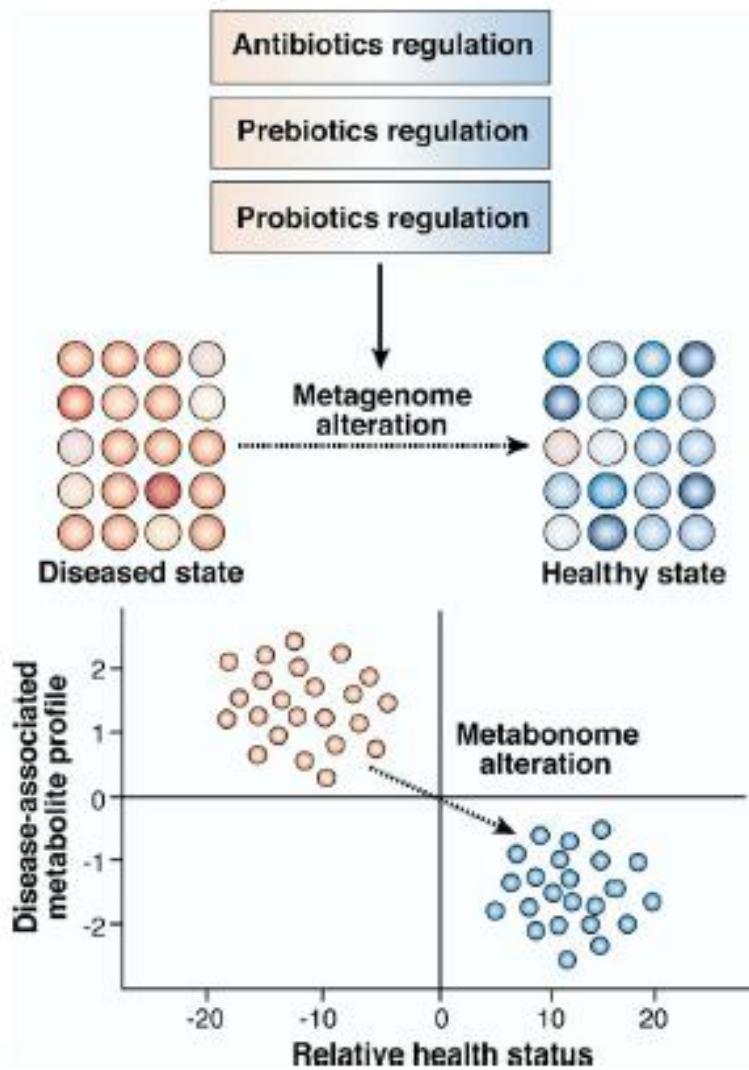


Figure 2 A model linking gut to joint inflammation in spondyloarthritis. (1) Bacteria attach to and invade the intestinal epithelium and the lamina propria. HLA-B27 or CARD15 polymorphisms can result in altered recognition and handling of bacterial antigens. (2) Invading bacteria infect, or are taken up by, macrophages (M) in the lamina propria and survive intracellularly. (3) HLA-B27 can present bacterial or autologous antigens to T cells (T). Furthermore, the heavy chain easily misfolds, leading to an unfolded protein response (UPR) and stress. (4) Bacterial infection induces Th1 and Th17 responses, and IL-23R susceptibility variants, expressed on macrophages and other antigen-presenting cell types, may modulate the Th17 response. (5) More T cells and other immune mediators are recruited, releasing proinflammatory cytokines. (6) Activated T cells and macrophages carrying bacterial components migrate via blood vessels to the target joint or eventually to other sites such as skin and eye. (7) In the target joint, gut-derived macrophages and T cells recruit other immune cells and result in the activation of mesenchymal cells (MCs), which further enhance and sustain inflammation. HLA-B27, human leukocyte antigen B27; IL-23R, IL-23 receptor.

Jaques P, Elewaut D. Joint expedition: linking gut inflammation to arthritis. *Mucosal Immunology*. Vol 1, No 5, Sept. 2008

The Colonization Resistance of the Mucous Membrane of the Large Intestine in Patients with Rheumatoid Arthritis in a Period of Exacerbation

“The mucous membrane of healthy people is colonized by bifidobacteria, *lactobacilli*, *Bacteroides*, *Escherichia* and *enterococci*. The mucous membrane in RA subjects is mainly colonized by aerobic opportunistic conventionally pathogenic enterobacteria (*enteropathogenic Escherichia*, *Citrobacter*, *Enterobacter*, *Klebsiella*, etc.), *staphylococci*, *enterococci* and anaerobic bacteria (*Bacteroides*, *peptococci*, *peptostreptococci*, etc.). Taking into account significant changes of colonization resistance in the colon mucous membrane in remission period of RA, it is necessary to apply bacteriotherapy, using bacterial drugs containing bifidobacteria and lactobacteria.”



ORIGINAL ARTICLE

H. Tiwana · C. Wilson · R.S. Walmsley
A. J. Wakefield · M. S. N. Smith · N. L. Cox
M. J. Hudson · A. Ebringer

Antibody responses to gut bacteria in ankylosing spondylitis, rheumatoid arthritis, Crohn's disease and ulcerative colitis

Received: 25 September 1996 / Accepted: 18 December 1996

Abstract Specific immunoreactive anti-*Klebsiella* bodies are found in patients with ankylosing spondylitis (AS), a significant proportion of whom have inflammatory bowel disease. Molecular mimicry between *Klebsiella* or other bacterial antigens and HLA-B27 has been suggested in the pathogenesis of AS. The specific increased immunoreactivity against *Klebsiella pneumoniae* was assessed against the abundant anaerobic bacteria present either in healthy controls or in patients with active colitis (UC) and Crohn's disease (CD). Immunoglobulin (Ig; IgG, IgA, IgM) immunoreactivity measured by ELISA against *Klebsiella pneumoniae*, *Proteus mirabilis*, *Escherichia coli* and ten anaerobes of the predominant normal bowel flora in 35 patients with active AS, 60 patients with inflammatory bowel disease (30 CD, 30 UC), 60 patients with active rheumatoid arthritis (RA) and 60 healthy controls. Ig immunoreactivity

The data suggested an increased immune response to *Klebsiella* in patients with AS, UC, CD and to *Proteus* in patients with RA. The specificity of these responses in some patients supported a possible role for enteric *Klebsiella* in the pathogenesis of AS and *Proteus* in RA. The role of *Klebsiella* in inflammatory bowel disease requires further study.

spondylitis · Inflammatory bowel disease ·
Rheumatoid arthritis

Opportunistic Bacteria

Potential Autoimmune Triggers	Result		Range
<i>Citrobacter spp.</i>	5.0 E5	High	<1.0 E4
<i>Klebsiella pneumoniae</i>	8.8 E3	High	<7.2 E3
<i>Proteus spp.</i>	<dl		<6.2 E3
<i>Proteus mirabilis</i>	3.9 E3	High	<1.0 E3
<i>Yersinia enterocolitica</i> (from pg 1)	Negative		Neg

Additional Dysbiotic/Overgrowth Bacteria

<i>Morganella morganii</i>	<dl	<1.0 E3
<i>Pseudomonas spp.</i>	<dl	<2.5 E3
<i>Pseudomonas aeruginosa</i>	<dl	<1.0 E3
<i>Staphylococcus spp.</i>	3.3 E3	<1.0 E4
<i>Streptococcus spp.</i>	9.1 E2	<1.0 E3



Expansion of intestinal *Prevotella copri* correlates with enhanced susceptibility to arthritis

Jose U Scher^{1†}, Andrew Szesnak^{2,3†}, Randy S Longman^{2,4†}, Nicola Segata^{5,6}, Carles Ubeda^{7,8}, Craig Bielski⁶, Tim Rostron⁹, Vincenzo Cerundolo⁹, Eric G Pamer⁷, Steven B Abramson¹, Curtis Huttenhower⁴, Dan R Littman^{2,10*}

¹Department of Medicine, New York University School of Medicine and Hospital for

We identified the presence of *Prevotella copri* as strongly correlated with disease in new-onset untreated rheumatoid arthritis (NORA) patients. Increases in *Prevotella* abundance correlated with a reduction in *Bacteroides* and a loss of reportedly beneficial microbes in NORA subjects. We also identified unique *Prevotella* genes that correlated with disease. Further, colonization of mice revealed the ability of *P. copri* to dominate the intestinal microbiota and resulted in an increased sensitivity to chemically induced colitis. This work identifies a potential role for *P. copri* in the pathogenesis of RA.

*For correspondence: Dan Littman, d.littman@med.nyu.edu

†These authors contributed equally to this work

Competing interests: The authors declare that no competing interests exist.

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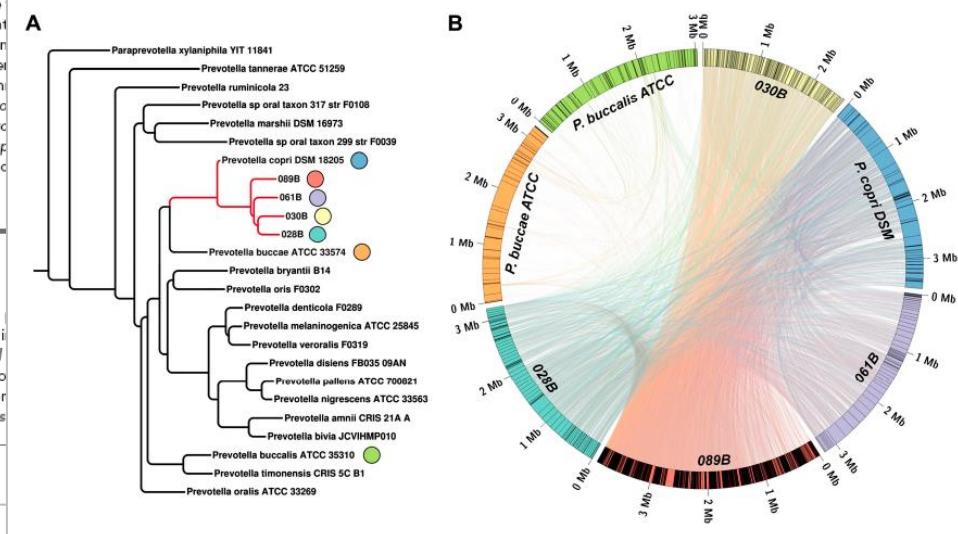
Abstract Rheumatoid arthritis (RA) is a prevalent systemic autoimmune disease, caused by a

combination of genetic and environmental factors. Animal models suggest a role of bacteria in supporting the systemic immune response required for joint inflammation. We performed 16S sequencing on 114 stool samples from rheumatoid arthritis patients and shotgun sequencing on a subset of 44 such samples. We identified the presence of *Prevotella copri* as strongly correlated with disease in new-onset untreated rheumatoid arthritis patients. Increases in *Prevotella* abundance correlated with a reduction in *Bacteroides* and a loss of reportedly beneficial microbes in NORA subjects. We also identified unique *Prevotella* genes that correlated with disease. Further, colonization of mice revealed the ability of *P. copri* to dominate the intestinal microbiota and resulted in an increased sensitivity to chemically induced colitis. This work identifies a potential role for *P. copri* in the pathogenesis of RA.

DOI: 10.7554/eLife.01202.001

Introduction

Rheumatoid arthritis (RA) is a highly prevalent systemic autoimmune disease with joints. If left untreated, RA can lead to chronic joint deformity, disability, and death. Despite recent advances towards understanding its pathogenesis (*McInnes and Abolhassani, 2010*) the etiology of RA remains elusive. Many genetic susceptibility risk alleles have been discovered (*Stahl et al., 2010*) but are insufficient to explain disease incidence. RA is therefore a complex (factorial) disease requiring both environmental and genetic factors for onset (*McInnes and Abolhassani, 2010*).





Infectious agents like *Porphyromonas gingivalis*, a bacterial strain driving periodontal disease, are able to produce peptidyl arginine deiminase 4 (PAD4), an enzyme that mediates the citrullination of proteins like vimentin, collagen and fibrinogen, which serve as autoantigens in RA.

REVIEW

The association between rheumatoid arthritis and periodontal disease

Jacqueline Detert¹, Nicole Pischon², Gerd R Burmester¹ and Frank Buttgereit^{*1}

Abstract

Chronic, plaque-associated inflammation of the gingiva and the periodontium are among the most common oral diseases. Periodontitis (PD) is characterized by the inflammatory destruction of the periodontal attachment and alveolar bone, and its clinical appearance can be influenced by congenital as well as acquired factors. The existence of a rheumatic or other inflammatory systemic disease may promote PD in both its emergence and progress. However, there is evidence that PD maintains systemic diseases. Nevertheless, many mechanisms in the pathogenesis have not yet been examined sufficiently, so that a final explanatory model is still under discussion, and we hereby present arguments in favor of this. In this review, we also discuss in detail the fact that oral bacterial infections and inflammation seem to be linked directly to the etiopathogenesis of rheumatoid arthritis (RA). There are findings that support the hypothesis that oral infections play a role in RA pathogenesis. Of special importance are the impact of periodontal pathogens, such as *Porphyromonas gingivalis* on citrullination, and the association of PD in RA patients with seropositivity toward rheumatoid factor and the anti-cyclic citrullinated peptide antibody.

Introduction

Periodontitis (PD), the most common oral disease, is a destructive inflammatory disease of the supporting tissues of the teeth and is caused by specific microorganisms [1]. As a rule, PD develops through gingivitis, an inflammation of the marginal periodontium. However, not every gingivitis develops further into PD. Both the amount and virulence of the microorganisms and the

resistance factors of the host (risk factors and immune status) are crucial for the progression of the periodontal destruction (Figure 1). PD has been proposed as having an etiologic or modulating role in cardiovascular and cerebrovascular disease, diabetes, and respiratory disease and adverse pregnancy outcome, and several mechanisms have been proposed to explain or support such theories. Moreover, oral lesions are indicators of disease progression, and the oral cavity can be a window to overall health and body systems. In recent years, remarkable epidemiological and pathological relationships between periodontal diseases and rheumatic diseases, especially rheumatoid arthritis (RA), have been presented.

Pathogenesis of periodontal diseases

There are findings that support the hypothesis that oral infections play a role in RA pathogenesis. Of special importance are the impact of periodontal pathogens, such as *Porphyromonas gingivalis* on citrullination, and the association of PD in RA patients with seropositivity toward rheumatoid factor and the anti-cyclic citrullinated peptide antibody.



Figure 1. Severe periodontitis with loss of periodontal attachment and alveolar bone.

*Correspondence: frank.buttgereit@charite.de

¹Department of Rheumatology and Clinical Immunology, Charité - Universitätsmedizin Berlin, Campus Mitte, Charitéplatz 1, 10117 Berlin, Germany
Full list of author information is available at the end of the article

Oral-Hematogenous Spread

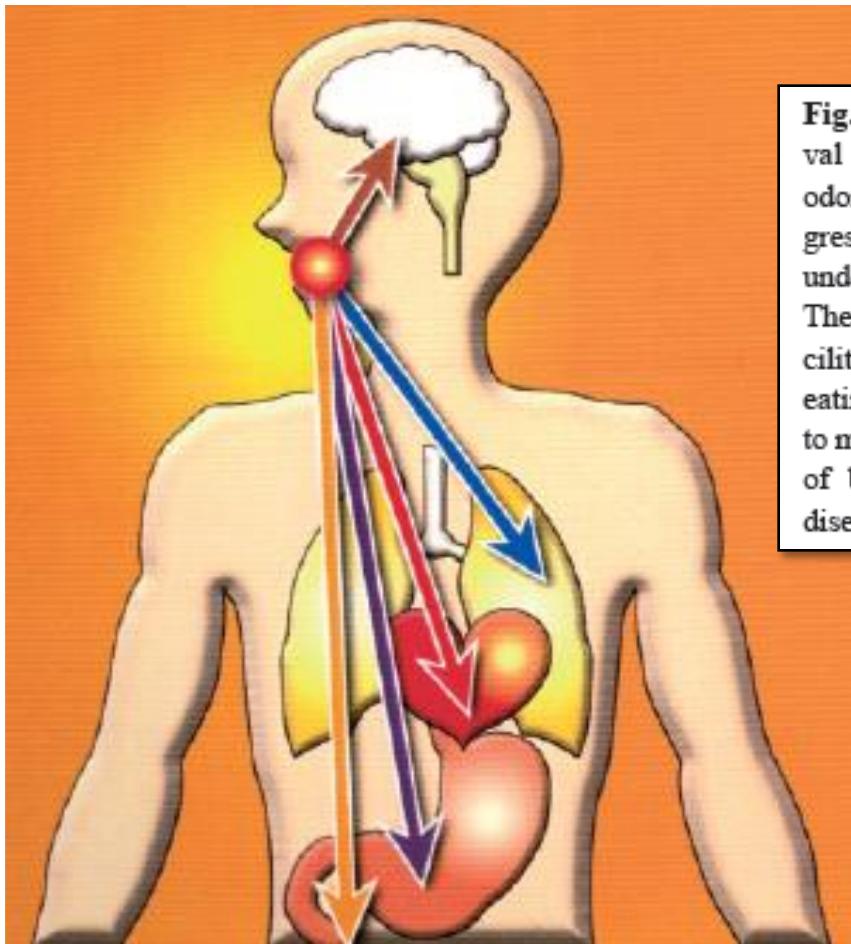


Fig. 2. Cause of periodontitis-related systemic diseases. The gingival epithelium functions as an innate physical barrier to protect periodontal tissues from bacterial invaders. However, with disease progression, local inflammation ulcerates the epithelium to expose the underlying connective tissues and blood capillaries to plaque biofilm. The exposed ulcerative area ($8 - 20 \text{ cm}^2$ in affected oral cavities) facilitates direct entry of biofilm pathogens into the circulation during eating and tooth brushing. Eventually, periodontal pathogens are able to migrate throughout the entire body. This oral-hematogenous spread of bacteria is a primary cause of periodontitis-derived systemic diseases.

Leaky Mouth?

Oral Bacterium and Colon Cancer?

Research

Genomic analysis identifies association of *Fusobacterium* with colorectal carcinoma

Aleksandar D. Muzica,¹ Daniel L. Miller,¹ Michael J. Karcher,¹ Fujiko Duke,¹ Joseph Tabernero,² Bruce W. Birren,¹ and Matthew Meyerson¹

¹ Broad Institute of Molecular Pathology, Harvard School of Medicine, Boston, Massachusetts, USA; ² Institut d'Investigacions Biomèdiques de Bellvitge, Barcelona, 08035 Barcino, Spain; Department of Medicine, Gainesville, Florida 32610, USA; ³ Department of Cell Biology, University of Texas Southwestern Medical School, Dallas, Texas 75390, USA

The tumor microenvironment contains nonneoplastic cells that contribute to carcinogenesis; however, the composition of the microbiota in cancerous tissue has not been well characterized. We used genome-wide DNA pairs, which revealed a significant role of *Fusobacterium* in the development of colorectal tumors.

Supplemental information is available online at www.genome.org.

Malignant tumors are composed of transformed cells with varying degrees of differentiation. These cells include normal epithelial cells, stem cells, and other nonneoplastic cells, including bacteria. Some bacteria can integrate into the human genome, such as the human papillomavirus (HPV) and Kaposi's sarcoma-associated herpesvirus (KSHV) (Chang et al. 1994). In addition, the risk of developing cancer through chronic infection by *Helicobacter pylori* has been implicated in the development of MALT lymphoma (Cawthon et al. 2006).

In the human digestive tract, the gut microbiome can impact both the host physiology and the development of cancer. The gut microbiome has been implicated in the development of colorectal cancer (Rowland et al. 2006; Rowland 2009), and the concept of the "altered gut microbiome" has been proposed as a key factor in the development of cancer (Rowland 2009). The gut microbiome, including *Fusobacterium*, has been implicated in the development of colorectal cancer (Rowland 2009).

Recent studies suggest that *Fusobacterium* predispose humans to colon cancer. *Fusobacterium* were known before this, of course, but were thought of as microbes that mostly live in the mouth — they are often in plaque and are associated with periodontal disease. But there are also recent reports associating them with ulcerative colitis and Crohn's disease. Both of these diseases, especially ulcerative colitis, increase the risk of colon cancer.

Dr. Robert A. Holt, a genomics researcher at the British Columbia Cancer Agency

*Corresponding author.
E-mail: matthew_meyer@broadinstitute.org
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DOI: 10.1101/1088-9051.11.042424; available online January 12, 2011.

Rheumatoid arthritis is an autoimmune disease triggered by *Proteus* urinary tract infection

ALAN EBRINGER & TAHAR RASHID

School of Biomedical and Health Sciences, Kings College London, London, UK

Abstract

Rheumatoid arthritis (RA) is a chronic and disabling polyarthritic disease, which affects mainly women in middle and old age. Extensive evidence based on the results of various microbial, immunological and molecular studies from different parts of the world, shows that a strong link exists between *Proteus mirabilis* microbes and RA. We propose that sub-clinical *Proteus* urinary tract infections are the main triggering factors and that the presence of molecular mimicry and cross-reactivity between these bacteria and RA-targeted tissue antigens assists in the perpetuation of the disease process through production of cytopathic auto-antibodies.

Patients with RA especially during the early stages of the disease could benefit from *Proteus* anti-bacterial measures involving the use of antibiotics, vegetarian diets and high intake of water and fruit juices such as cranberry juice in addition to the currently employed treatments.

Keywords: Humoral autoimmunity, *Proteus mirabilis*, rheumatoid arthritis, urinary tract infection

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease, which affects millions of people all around the world, with a prevalence rate ranging from 0.5 to 1% (Lawrence et al. 1998). The disease in the majority of patients takes a mild to moderate course, whilst in others it has a more disabling consequence, which might have a great effect on the socio-economic status of the patient (Cooper 2000).

RA affects individuals of middle age groups and occurs three times more frequently in women than in men.

Aetiopathogenesis

A general scientific consensus exists, which considers RA as an immune-mediated disease that could possibly be triggered by an environmental (microbial) factor in a genetically susceptible individual.

Extensive evidence supports the role of cellular and humoral autoimmunity in the development of RA, and some of these are listed as follows:

1. Predominant role of B lymphocytes in the pathogenesis of RA (Weyand et al. 2005) and signs of accumulations of immunoglobulins and other inflammatory products such as complements at the site of synovial pathological lesions in RA patients (Low and Moore 2005).
2. Detection of elevated levels of auto-antibodies in the serum and/or synovial fluid of patients with RA (Rantapaa-Dahlqvist 2005).
3. Significant improvements in RA disease parameters following B cell depletion therapy, e.g. with the use of anti-CD20 antibodies (Perosa et al. 2005).

Role of HLA genes in RA

The role of genetics in development of RA has been examined mainly through family, twin and molecular analytical studies. For instance, familial distribution of RA among first-degree relatives (Deighton et al. 1992a) and twins (MacGregor et al. 2000), indicates that RA runs in some families, basically supporting the

REVIEW

Rheumatoid arthritis: proposal for the use of anti-microbial therapy in early cases

Alan Ebringer^{1,2}, Taha Rashid¹, and Clyde Wilson¹

¹Division of Life Sciences, Infection and Immunity Group, King's College London, ²Department of Rheumatology, Middlesex Hospital, University College, London, UK

Rheumatoid arthritis (RA) is a chronic disease, affecting women more than men, especially in those possessing the "shared epitope" (EQK/RRAA) amino acid sequences present in HLA-DR1/4 molecules. *Proteus mirabilis* carries sequences showing molecular mimicry to the "shared epitope" and to type XI collagen of hyaline cartilage. Elevated levels of antibodies to *Pmirabilis* have been reported from 14 different countries involving 1375 RA patients and the microbe has been isolated from urine cultures of such patients. Our working hypothesis is that the disease develops as a result of repeated episodes of *Proteus* upper urinary tract infections. Prospective studies involving the trial of anti-*Proteus* measures in RA patients should be evaluated in the management of this disease. Antibiotics, high fluid intake, and fruit extracts, such as cranberry juice, have all been found to be effective in the treatment of urinary tract infections. Such measures could be used as possible additional adjuncts to the standard therapy with NSAIDs and DMARDs.

Key words: rheumatoid arthritis, *Proteus* urinary tract infections, diet

Rheumatoid arthritis (RA) is the most common inflammatory joint disease, affecting millions of people all around the world. It usually takes a progressive course with characteristic exacerbations and remissions. Virtually any joint can be involved in RA, but the disease predominantly affects the small joints of the hands and feet, and less frequently the large joints including the cervical spine. (1)

RA is three times more common in women than men, affecting mainly middle aged and elderly people. (2) Although the world-wide distribution of the disease ranges between 0.2% and 5.3%, (3) the incidence, however, is showing a noticeable decline during the last 4 decades, especially among Western World populations. (4)

Genetic backgrounds

Familial aggregation has been observed, but no strong pattern of inheritance has been established among relatives of patients with RA. (5) In a study on two groups of twins with RA from Finland and UK, it was shown that genetic factors have a substantial contribution to RA, the heritability in each group was observed to be 53% and 65%, respectively. (6)

Alan Ebringer, Division of Life Sciences, Infection and Immunity Group, King's College London, 150 Stamford Street, London SE1 8WA, UK. E-mail: alan.ebringer@kcl.ac.uk

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More than 25 years ago a significant discovery was made when the HLA-DR4/Dw4 genetic marker was found in 70% of the patients with RA, using both mixed lymphocyte culture assay (7) and serological methods. (8) Subsequently it was observed that only certain types and subtypes of HLA-DR molecules were associated with RA. The third hypervariable regions of all RA-associated HLA-DR β 1 alleles were found to share a hexamer amino acid sequence homology involving positions 69–74 and consisting of glutamic acid, glutamine, lysine or arginine, arginine, alanine and alanine "EQK/RRAA". This sequence similarity in RA, which has been given the name of "shared epitope" (SE) (9) motif, was not found in other HLA-DR molecules like HLA-DR4 subtypes (DR β 1*0402 and 0403), which are not associated with RA.

Regardless of the difference in the distribution of HLA genes among various ethnic groups, it has been reported that more than 95% of patients possess one or another of the RA-linked HLA-DR molecules (10) which contain the SE motif. These include HLA-DR4 subtypes (DR β 1*0401, *0404, 0405 and 0408), HLA-DR1 subtypes (DR β 1*0101 and 0102), HLA-DR6 (DR β 1*1402) and HLA-DR10 (DR β 1*1001), (11, 12).

The importance of SE amino acid sequences in conferring susceptibility to RA is not only confined to humans, but also to animals such as the canine species. (13) The mere association of the SE moiety with RA, however, is not necessarily sufficient to trigger the disease. In spite of a positive correlation between the presence of DR4 and SE homozygosity

Antibodies to Proteus in RA

Table I. Worldwide distribution of elevated *Proteus* antibodies in patients with RA.

No.	Location (country)	RA patients	Year	Reference
1.	London (England)	30	1985	17
2.	Winchester (England)	162	1988, 1997	104, 105
3.	Newcastle (England)	142	1992	106
4.	Epsom (England)	27	1999	107
5.	Stevenage (England)	140	1995, 1995, 1996	30, 108, 34
6.	Dundee (Scotland)	176	1995, 1999	109, 40
7.	Dublin (Ireland)	29	1988	110
8.	Toulouse (France)	15	1994	111
9.	Brest (France)	50	1995	112
10.	Hamilton (Bermuda)	34	1995	108
11.	Oslo (Norway)	53	1995	100
12.	Otsu (Japan)	80	1997	113
13.	Chandigarh (India)	70	1997	114
14.	Amsterdam (Holland)	25	1998	115
15.	Taichung (Taiwan)	39	1998	116
16.	Barcelona (Spain)	34	1999	107
17.	Moscow (Russia)	27	2000	117
18.	Bethesda & Philadelphia (USA), Montreal (Canada)	113	2000	19
19.	Tokyo (Japan)	30	Submitted	
20.	Helsinki (Finland)	99	Submitted	
		1375	Total numbers of RA patients	

Alan Ebringer, MD, King's College, London

Scand J Rheumatol 2003;32:2–11

REVIEW

Rheumatoid arthritis: proposal for the use of anti-microbial therapy in early cases

Alan Ebringer^{1,2}, Taha Rashid¹, and Clyde Wilson¹

Rheumatoid arthritis and microbial therapy

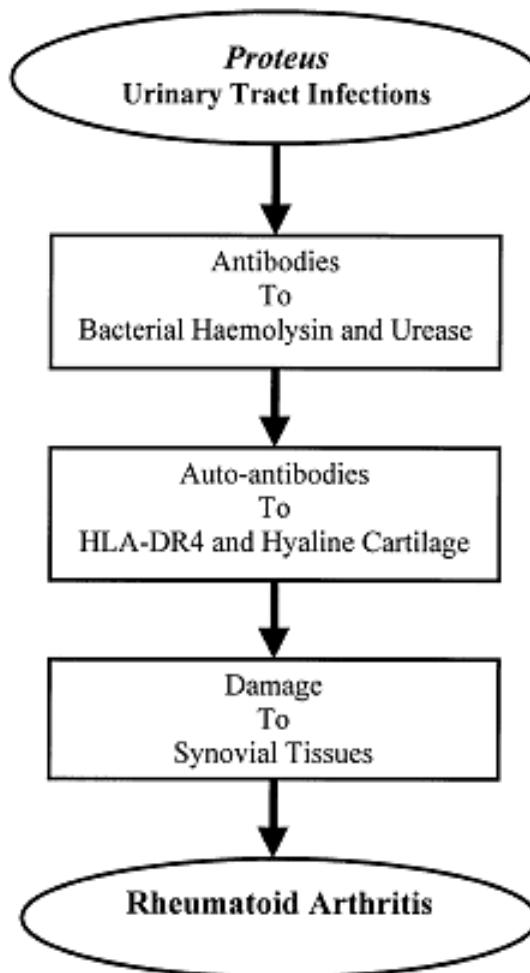
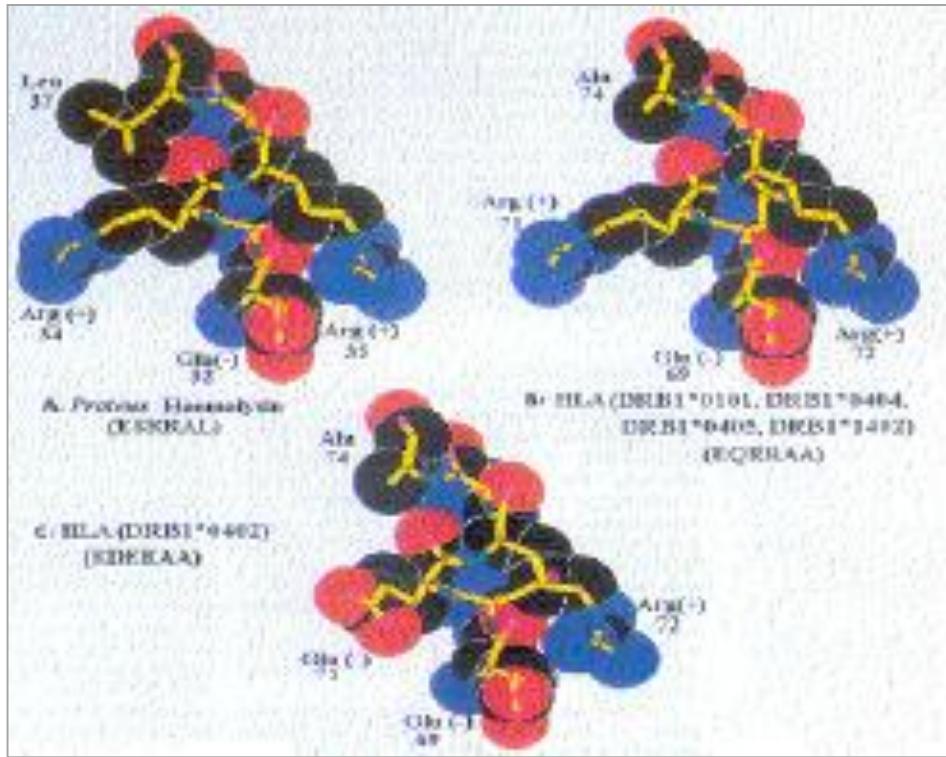


Fig. 1. Suggested etiopathogenetic sequence from *Proteus* urinary infections to RA

Molecular Mimicry: Proteus and HLA-DR1/DR4



Alan Ebringer, MD
King's College, London

- A: ESRRAL sequence of *Proteus mirabilis* haemolysin
- B: EQRRAA sequence within DRB1*0101 (HLA-DR1)
- C: EDERAA sequence of DRB1*0402 (HLA-DR4/Dw10)
(predicted from known crystallographic structure)

Table II. Possible explanation for some commonly encountered features in RA.

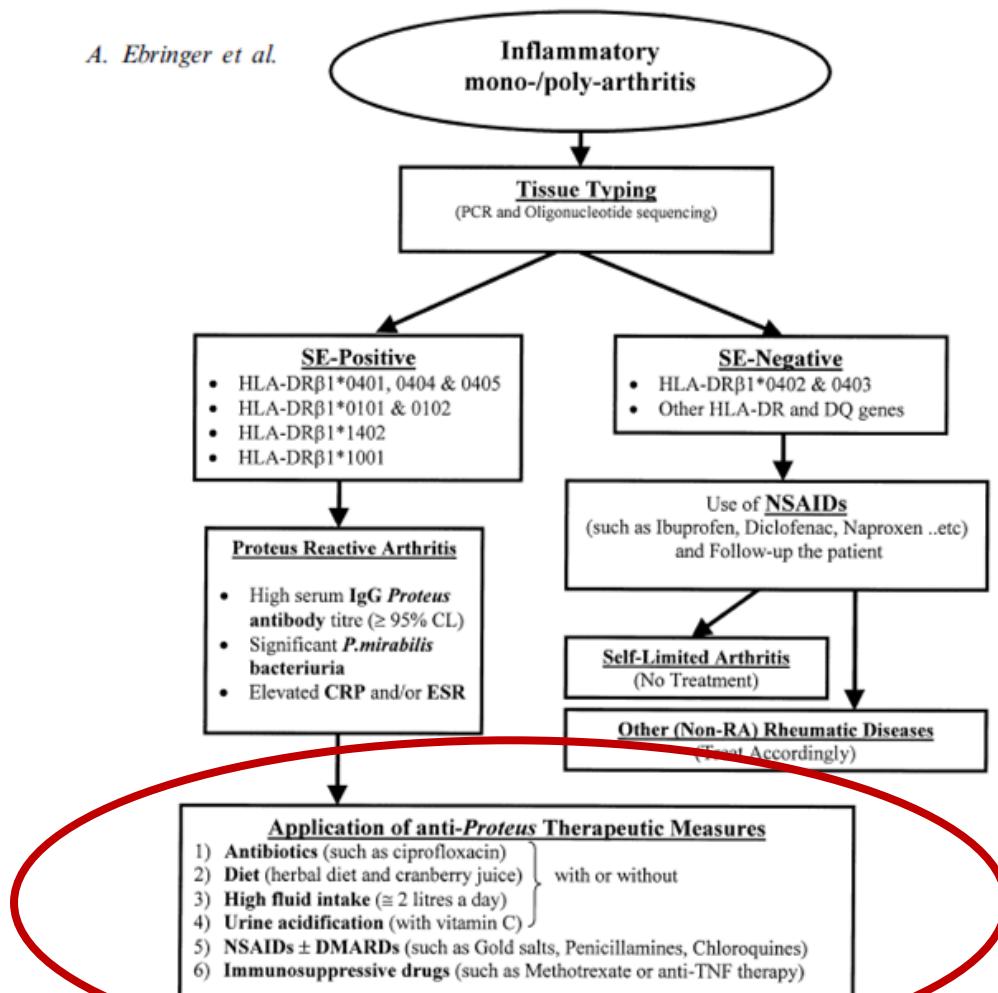
Associated RA Features	Suggested Explanations
Female preponderance 3:1	Increased incidence of UTIs in females
Disease onset in 30–50 years	Increased incidence of UTIs among middle and older age groups
Exacerbation after pregnancy	Increased incidence of UTIs in the puerperium
Low concordance rate in identical twins and fluctuating course of the disease	Involvement of non-genetic environmental factors in the pathogenesis of the disease
Presence of rheumatoid factors in high proportions of RA patients	A secondary phenomenon due to B cell stimulation and presence of antigen-antibody complexes
Presence of " EQRRAA " amino acid motif in over 95% of patients possessing the RA-associated HLA-DR molecules	Cross-reactivity with " ESRRAL " amino acid sequences present in the Proteus hemolysins
High proportion of small joints involvement, having hyaline cartilage which contains type XI collagen, possessing the " IRRET " amino acid sequence	Cross-reactivity with " LRREI " amino acid motif present in the Proteus urease enzyme

Scand J Rheumatol 2003;32:2–11

REVIEW

Rheumatoid arthritis: proposal for the use of anti-microbial therapy in early cases

Alan Ebringer^{1,2}, Taha Rashid¹, and Clyde Wilson¹



* CRP: C-Reactive Protein; ESR: Erythrocyte Sedimentation Rate; PCR: Polymerase Chain Reaction; SE: Shared epitope; CL: Confidence Limit; NSAIDs: Non-Steroidal Anti-inflammatory Drugs; DMARDs: Disease Modifying Anti-Rheumatic Drugs; TNF: Tumour Necrosis Factor

Fig. 2. Proposed schematic representation for the identification and treatment of patients with *Proteus* reactive arthritis and early RA

T Cell-Mediated Autoimmune Disease Due to Low-Affinity Crossreactivity to Common Microbial Peptides

Maria Harkiolaki,^{1,8} Samantha L. Holmes,^{1,2,8} Pia Svendsen,³ Jon W. Gregersen,³ Lise T. Jensen,³ Roisin McMahon,^{1,2} Manuel A. Friese,^{2,5} Gijs van Boxel,¹ Ruth Etzensperger,^{2,6} John S. Tzartos,⁵ Kamil Kranc,² Sarah Sainsbury,¹ Karl Harlos,¹ Elizabeth D. Mellins,⁴ Jackie Palace,⁵ Margaret M. Esiri,⁶ P. Anton van der Merwe,⁷ E. Yvonne Jones,^{1,*} and Lars Fugger^{2,3,5,*}

¹Division of Structural Biology, Henry Wellcome Building for Genomic Medicine, University of Oxford, Roosevelt Drive, Oxford OX3 7BN, UK

²MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, University of Oxford

“We show that a microbial peptide, common to several major classes of bacteria, can induce MS-like disease in humanized mice by cross-reacting with a T cell receptor (TCR) that also recognizes a peptide from myelin basic protein, a candidate MS autoantigen. Structural analysis demonstrates this crossreactivity is due to structural mimicry of a binding hotspot shared by self and microbial antigens, rather than to a degenerate TCR recognition. Thus, these data suggest a possible explanation for the difficulty in incriminating individual infections in the development of MS.”

Multiple sclerosis (MS) is an incurable inflammatory and degenerative disease of the central nervous system (CNS), affecting approximately 1–2 million people worldwide (Sospedra and Martin, 2005). Susceptibility to MS is jointly determined by genetic and environmental factors. Identifying these factors and their interplay is important in understanding the disease mechanisms, which can be targeted by drugs.

Progress has been made in understanding which genes confer risk of MS. In Northern European Caucasoid populations, MS is

one to two orders of magnitude more common in individuals homozygous for the DRB1*15 allele (Hakkinen et al., 2005). This association is explained by the presence of the DRB1*15 allele in the DRB1 locus, which encodes the DRB1 chain of the DR2b heterodimeric MHC class II molecule. DR2b binds the MBP 85–99 peptide with a low affinity (Hahn et al., 2005). DR2b is expressed on the surface of oligodendrocytes, the myelin-forming cells of the CNS. The DR2b allele is also associated with other diseases, such as type 1 diabetes and rheumatoid arthritis (Hawkins et al., 2005).

8

348 Immunity 30, 348–357, March 20, 2009 ©2009 Elsevier Inc.

Harkiolaki M, Holmes SL, Svendsen P, et al. T-Cell-Mediated Autoimmune Disease Due to Low-Affinity Crossreactivity to Common Microbial Peptides. *Immunity* 30, 348–357, March 20, 2009

Thyroid Disorders, Autoimmunity and The GI Environment

(“Microbes”)



Infection, Thyroid Disease, and Autoimmunity*

YARON TOMER AND TERRY F. DAVIES

Division of Endocrinology and Metabolism, Department of Medicine, Mount Sinai School of Medicine,
New York, New York 10029

- I. Introduction
- II. Infections of the Thyroid Associated with Autoimmune Phenomena

pathogenesis of a variety of autoimmune diatheses, namely, rheumatic fever, Reiter's syndrome, systemic lupus erythematosus (SLE), myasthenia gravis, insulin-dependent diabetes

“Molecular mimicry has long been implicated as a mechanism by which microbes can induce autoimmunity.”

- Graves' disease**
- B. Human autoimmune thyroiditis and infection
 - IV. Lessons from Infectious Triggers in Autoimmune Disease
 - A. Viral induced changes in self-antigen expression
 - B. Molecular mimicry
 - C. Superantigens
 - D. Alterations in the idiotypic network
 - E. Immune complex formation
 - F. Heat shock proteins and thyroid autoimmunity
 - G. Induction of MHC antigens on nonimmune cells
 - V. Conclusions
 - VI. Summary

I. Introduction

TRADITIONALLY, it was assumed that infectious agents induced disease by causing direct tissue damage (for example via secretion of exotoxins and endotoxins). However, we now know only too well that infectious agents play a role in the induction of noninfectious consequences, including malignancies (for example Ebstein Barr virus and Burkitt's lymphoma, HTLV-1, and adult T cell leukemia), acquired immunodeficiency syndrome (human immunodeficiency virus), peptic ulcer (*Helicobacter pylori*), and autoimmune diseases. Infectious agents have been implicated in the

A. Human subacute thyroiditis

First defined by De Quervain (1), subacute thyroiditis is a self-limited inflammatory disorder of the thyroid gland. The disease is most prevalent in females in a ratio of 3–6:1. The illness is usually characterized by a sudden onset of neck pain and tenderness, fever, malaise, and variable changes in thyroid function tests, and usually lasts several weeks to several months (2). However, painless subacute thyroiditis occurs regularly and recurrent subacute thyroiditis has been reported (3).

1. Viral infection and the etiology of subacute thyroiditis. Older literature first suggested a viral etiology for human subacute thyroiditis. Clinically the disease has several characteristics typical of viral infections including a typical viral prodrome with myalgias, malaise and fatigue, absence of leukocytosis, and usually a self-limited course. Additionally, clusters of the disease have been reported during outbreaks of viral infection (4). A higher prevalence of subacute thyroiditis has also been reported during the summer, coinciding with the seasonal distribution of the enteroviruses (5), and in Holland an epidemic of subacute thyroiditis affecting 23 individuals has been described (6). Eylan and colleagues (7) reported 11 patients with subacute thyroiditis diagnosed during a mumps epidemic. These 11 patients were found to have circulating anti-mumps antibodies without clinical evidence of mumps. In two patients the mumps virus was cultured from thyroid tissue obtained at biopsy. Others have also reported an association between mumps virus and subacute thyroiditis (8–10). Different viruses reportedly associated with subacute

Address requests for reprints to: T. F. Davies, M.D., Box 1055, Mount Sinai Medical Center, 1 Gustave L. Levy Place, New York, New York 10029.

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Review

Open Access

Viruses and thyroiditis: an update

Rachel Desailloud^{*1,2} and Didier Hober¹

Address: ¹Laboratoire de Virologie/UPRES EA3610 Faculté de Médecine, Université Lille 2, CHRU Lille, Centre de Biologie/Pathologie et Parc Eurasanté, 59037 Lille, France and ²Service d'Endocrinologie-Diabétologie-Nutrition, CHU Amiens, 80054 Amiens, France

Email: Rachel Desailloud* - desailloud.rachel@chu-amiens.fr; Didier Hober - dhober@chru-lille.fr

* Corresponding author

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This article is available from: <http://www.virologyj.com/content/6/1/5>

Abstract

Viral infections are frequently cited as a major environmental factor involved in subacute thyroiditis and autoimmune thyroid diseases. This review examines the data related to the role of viruses in the development of thyroiditis.

Our research has been focused on human data. We have reviewed virological data for each type of thyroiditis at different levels of evidence; epidemiological data, serological data or research on circulating viruses, direct evidence of thyroid tissue infection. Interpretation of epidemiological and serological data must be cautious as they don't prove that this pathogen is responsible for the disease. However, direct evidence of the presence of viruses or their components in the organ are available for retroviruses (HFV) and mumps in subacute thyroiditis, for retroviruses (HTLV-1, HFV, HIV and SV40) in Graves's disease and for HTLV-1, enterovirus, rubella, mumps virus, HSV, EBV and parvovirus in Hashimoto's thyroiditis. However, it remains to determine whether they are responsible for thyroid diseases or whether they are just innocent bystanders. Further studies are needed to clarify the relationship between viruses and thyroid diseases, in order to develop new strategies for prevention and/or treatment.

severe thyroid pain); iii/Autoimmune thyroid disease which includes Hashimoto's thyroiditis (and painless thyroiditis also known as silent thyroiditis or subacute lym-

phritis); iv/Infectious thyroiditis (caused by viruses such as mumps virus, rubella virus, enterovirus, parvovirus, cytomegalovirus, varicella-zoster virus, hepatitis C virus, HIV, HTLV-1, SV40, etc.).

5: *Acta Med Austriaca* 1987;14(1):11-4

[Antibodies to *Yersinia enterocolitica* in immunogenic thyroid diseases].

[Article in German]

Petru G, Stunzner D, Lind P, Eber O, Mose JR

“*Yersinia* shows on its surface saturable binding sites for TSH. TSH receptor antibodies could be produced in selected individuals having been infected with bacteria showing TSH receptors”

(19.6%). The antibody titres were mainly directed towards *Yersinia* subtypes 8 and 3. It may, therefore, be assumed that the gram-negative bacterium *Yersinia enterocolitica* may have an active part in triggering immunogenic thyroid diseases such as Graves' disease or Hashimoto thyroiditis.

PMID: 3618088, UI: 87294986

5: Acta Med Austriaca 1987;14(1):11-4

[Antibodies to Yersinia enterocolitica in immunogenic thyroid diseases].

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Petru G, Stunzner D, Lind P, Eber O, Mose JR

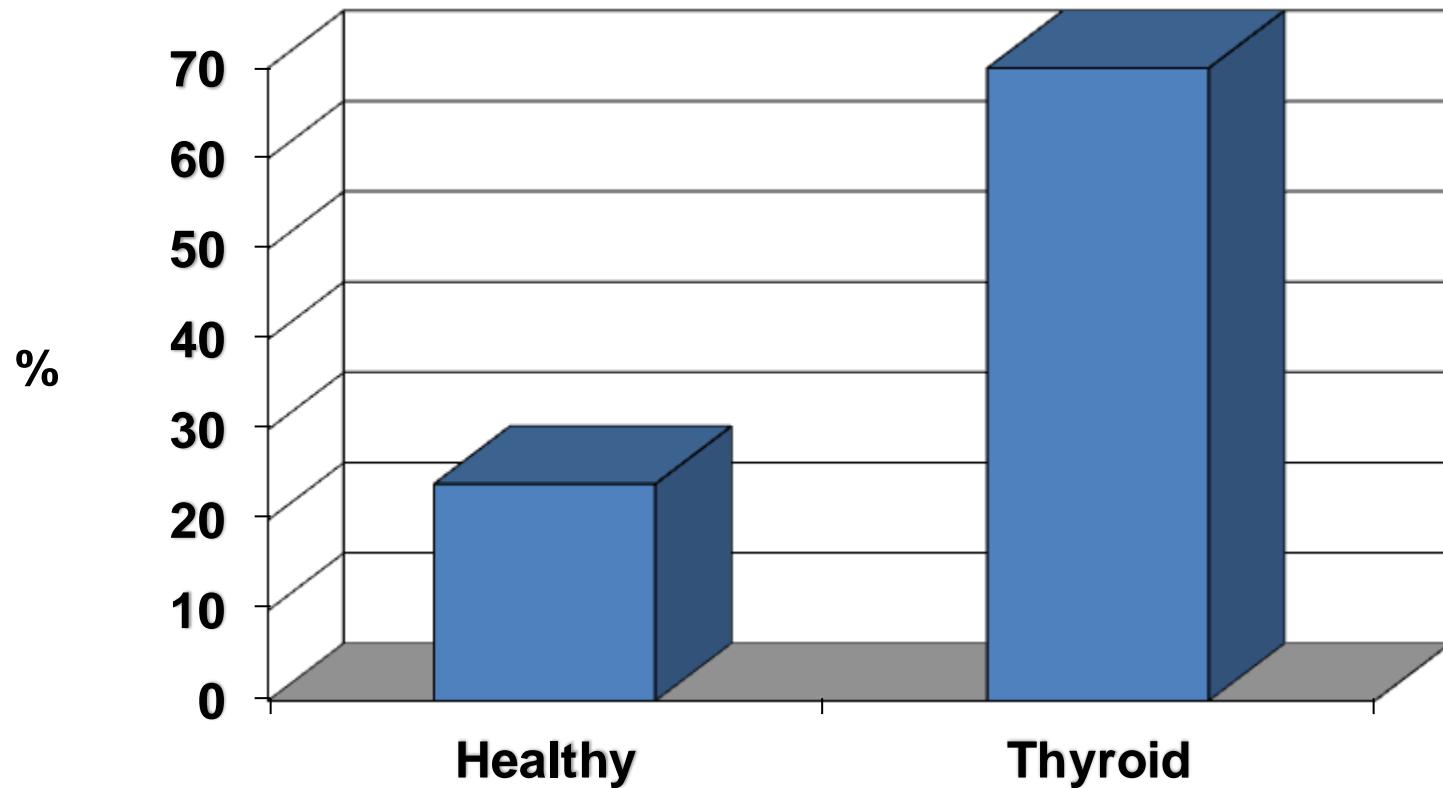
In 1976 Shenkman et al. revealed that in patients with thyroid disorders antibodies

“It may, therefore, be assumed that the gram-negative bacterium Yersinia enterocolitica may have an active part in triggering immunogenic thyroid diseases...”

demonstrated in a significantly higher percentage (58.5%) in patients suffering from immunogenic than in patients with non-immunogenic thyroid disorders (19.6%). The antibody titres were mainly directed towards Yersinia subtypes 8 and 3. It may, therefore, be assumed that the gram-negative bacterium Yersinia enterocolitica may have an active part in triggering immunogenic thyroid diseases such as Graves' disease or Hashimoto thyroiditis.

PMID: 3618088, UI: 87294986

Prevalence of Yersinia Antibodies in Thyroid Disorder Patients



Opportunistic Bacteria

Potential Autoimmune Triggers	Result	Range
<i>Citrobacter spp.</i>	1.1 E3	<1.0 E4
<i>Klebsiella pneumoniae</i>	3.4 E3	<7.2 E3
<i>Proteus spp.</i>	<dl	<6.2 E3
<i>Proteus mirabilis</i>	<dl	<1.0 E3
<i>Yersinia enterocolitica</i> (from pg 1)	Positive	Neg

Additional Dysbiotic/Overgrowth Bacteria

<i>Morganella morganii</i>	<dl	<1.0 E3
<i>Pseudomonas spp.</i>	<dl	<2.5 E3
<i>Pseudomonas aeruginosa</i>	<dl	<1.0 E3
<i>Staphylococcus spp.</i>	2.3 E2	<1.0 E4
<i>Streptococcus spp.</i>	<dl	<1.0 E3

Sample Natural GI Antimicrobial

Amounts per serving	
Serving size	2 caps
Number of servings per container	30
Number of capsules per container	60
Tribulus terrestris (standardized to 40% furostanolsaponins)	400 mg
Chinese Wormwood (Artemisia annua/apiacea) (standardized to >10% artemisinin)	300 mg
Berberine sulfate (from Berberus aquifolius)	200 mg
Barberry (Berberis vulgaris) (standardized to 6% berberine)	100 mg
Bearberry Arctostaphylos uva ursi)	100 mg
Grapefruit/Citrus Seed Extract	200 mg
Magnesium Caprylate *(Yielding 267 mg of Caprylic Acid)	300 mg
Black Walnut (Juglans nigra)	100 mg

Suggested Dose: Take 2 capsules, one to three times daily, in between meals as directed by your health care practitioner.

Probiotics as Inflammatory Modulators

Clinical Nutrition (2007) 26, 169–181



Available at www.sciencedirect.com
ScienceDirect
journal homepage: www.elsevierhealth.com/journals/clnu

REVIEW

Bioecological control of inflammatory bowel disease

Stig Bengmark^{*,*1}

UCL Department of Hepatology, University College, London, UK

Received 31 August 2006; accepted 4 October 2006

KEYWORDS
Ulcerative colitis;
Crohn's disease;
Probiotics;
Prebiotics;
Symbiotics;
Antioxidants

Summary
It is today generally accepted that pathogenesis of human unwanted or lack of extent important bacteria which are observed in ulcerative colitis and prevent close to the manifestations membrane function acid production. A association with the splanchnic metabolism plant fibres, antioxidants dramatic effects are tried. The general compositions of probiotics are reserved.

Contents

The role of flora
Immune system and dendritic cells (DCs)
Flora and immune response
Chronic intestinal inflammation

*185 Barrier Point Road, Royal Docks, London, E16 2SE, UK
E-mail address: s.bengmark@kcl.ac.uk.
¹Member Academia Europaea, Emeritus Professor Lund University (UCL), London University, affiliation to Departments of Hepatology and Gastroenterology, Lund University, Lund, Sweden.

0261-5614/\$ - see front matter © 2006 Elsevier Ltd and European Society for Clinical Nutrition and Metabolism. All rights reserved.
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“It is today generally accepted that intestinal flora is deeply involved in the pathogenesis of human inflammatory bowel diseases (IBDs). Although the exact presence of unwanted or lack of specific crucial bacteria are not yet known. Westerners lack to large extent important immunomodulatory and fibre-fermenting lactic acid bacteria (LAB), bacteria which are present in all with a more primitive rural lifestyle”.

Bengmark S. Bioecological control of inflammatory bowel disease. ClinNutr, 26, 169-181 (2007).

Probiotic foods

- kimchi
- sauerkraut
- yogurt
- kefir
- kombucha

Prebiotic foods

- jicama
- dandelion greens
- garlic
- chickory root
- Jerusalem artichoke

Favorable Manipulation of the Gut Microbiota



Use plant based foods to boost **Healthy Gut Bacteria**

Blueberries

Enhance the immune system and destroys harmful bacteria



Polenta

High in fiber with fermentable components



Beans

Releases SCFA and boosts vitamin absorption and satiety



Tempeh

Helps prevent unhealthy bacteria and boosts nutrient absorption



Jerusalem Artichokes

Rich in inulin fiber which serves as a strong prebiotic



Cruciferous Vegetables

Fights inflammation and cancer



Sauerkraut & Kimchee

Boosts immune system and improves the health of the intestinal walls



Bananas

Fights inflammation and helps to stabilize gut bacteria



Resistant Starch Regulates Gut Microbiota: Structure, Biochemistry and Cell Signalling

Xiaoping Yang^{a,b} Kwame Oteng Darko^a Yanjun Huang^{a,b} Caimei He^{a,b}
Huansheng Yang^b Shanping He^b Jianzhong Li^b Jian Li^c Berthold Hoyer^{c,d}
Yulong Yin^{b,e}

^aDepartment of Pharmacy, School of Medicine, Hunan Normal University, Changsha, China; ^bAnimal Nutrition and Human Health Laboratory, College of Life Sciences, Normal University, Changsha, China;
^cDepartment of Basic Medicine, School of Medicine, Hunan Normal University, Changsha, China;
^dInstitute of Nutritional Sciences, University of Potsdam, Potsdam, Germany; ^eChinese Academy of Science, Institute of Subtropical Agriculture, Research Center for Healthy Breeding of Livestock and Poultry, Hunan Engineering and Research Center of Animal and Poultry Science and Key Laboratory for Agroecological Processes in Subtropical Region, Scientific Observation and Experimental Station of Animal Nutrition and Feed Science in South-Central, Ministry of Agriculture, Changsha, China

Key Words

Resistant starch • Gut microbiota • Nutrition

Abstract

Starch is one of the most popular nutritional sources for both human and animals. Due to the

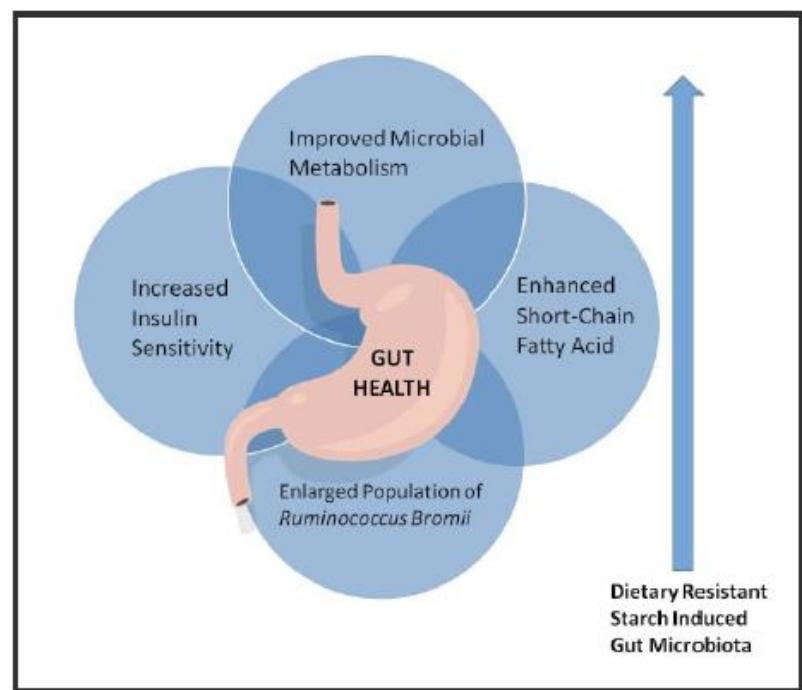
Raw potato starch dramatically induces changes in microbial composition and modulates the metabolic pathway of microbial metabolism in the gut. Resistant starch attenuated disease-induced intestinal fibrosis, inflammation, tubular damage, activation of NFkB, up-regulation of pro-inflammatory, pro-oxidant, and pro-fibrotic molecules; impaired Nrf2 activity, down-regulation of antioxidant enzymes, and disruption of colonic epithelial tight junctions, and enhanced production of beneficial short-chain fatty acids (SCFAs).

starch (SDS) is a starch fraction that is digested completely in the small intestine at a slower rate as compared with rapidly digestible starch. Resistant starch (RS) is the starch portion

Xiaoping Yang and Yulong Yin, Ph. D.

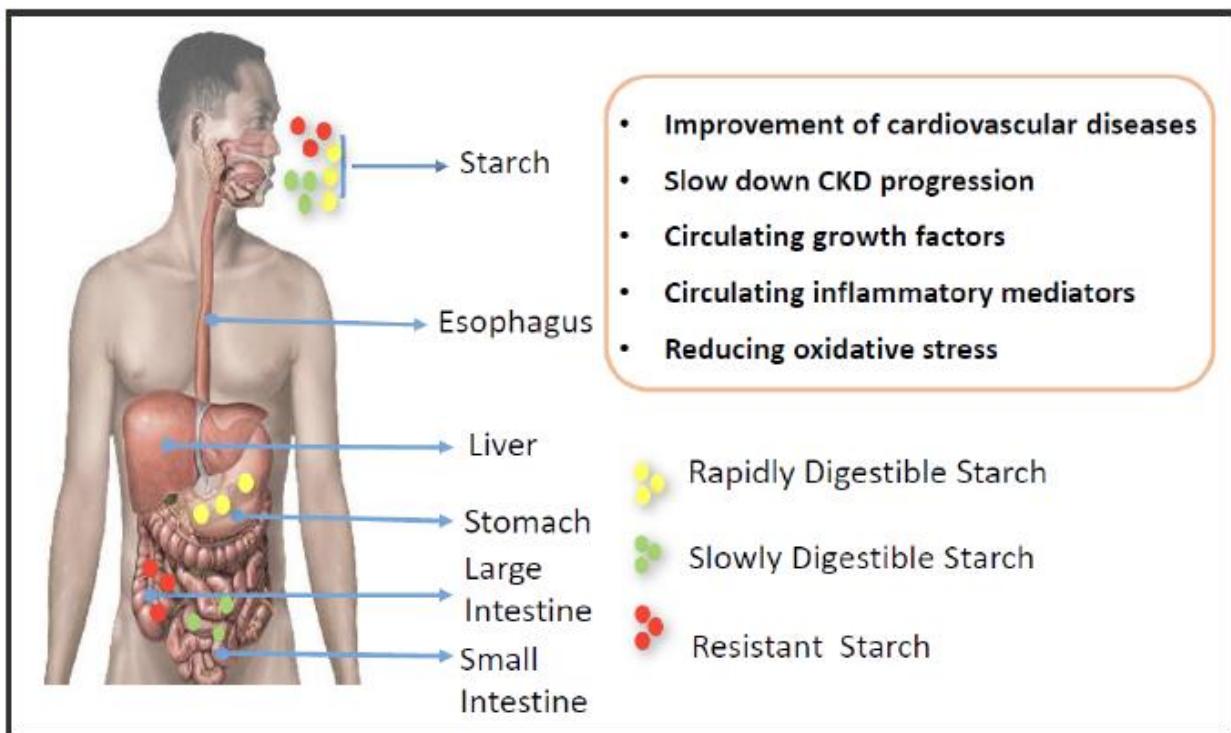
Department of Pharmacy, School of Medicine, Hunan Normal University, Changsha, Hunan, 410013 (China), and Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, Hunan, 410125 (China)
E-Mail: Xiaoping.Yang@hunnu.edu.cn / yinyulong@isa.ac.cn

KARGER



Resistant Starches

Fig. 1. Types of starch and parts of the digestive system where they are absorbed and functions of resistant starch for treating metabolic dysfunction.



Resistant Starches

The four different categories of resistant starch

RS Type 1 – Starch that is bound by fibrous cell walls and therefore resists digestion, such as beans, legumes and grains, and nuts/seeds.

RS Type 2 – Indigestible due to its high amylase content when in its raw form, such as found in potatoes, bananas (green), plantains. Heating or over-ripening these foods renders the starch to be no longer indigestible.

RS Type 3 – This type of resistant starch is the result of a process called retrogradation- when starches are cooked and then immediately cooled, which allows the digestible starch in some foods like rice, potatoes, and beans to be more resistant to digestion.

RS Type 4 – Industrial resistant starch that does not occur in nature. It is man made via a chemical process and should be avoided.



E. COLI VEGETABLES



EDDIE COLI COULDN'T UNDERSTAND
WHY BUSINESS WAS BAD.

Thyroid Disorders, Autoimmunity and The GI Environment

("Food Antigens")



Autoimmune Thyroid Disease and Celiac Disease

- Celiac patients have approximately 10 times the rate of auto-immune thyroid diseases, such as Hashimoto's thyroiditis and Grave's disease, as non-celiac individuals



Ansaldi N et al, Autoimmune thyroid disease and celiac disease in children (Abstract), *J Pediatr Gastroenterol Nutr*, Vol. 37, No. 1, pp. 63-6, July 2003.

Opportunistic Bacteria

Potential Autoimmune Triggers	Result	Range
<i>Citrobacter spp.</i>	1.1 E3	<1.0 E4
<i>Klebsiella pneumoniae</i>	3.4 E3	<7.2 E3
<i>Proteus spp.</i>	<dl	<6.2 E3
<i>Proteus mirabilis</i>	<dl	<1.0 E3
<i>Yersinia enterocolitica</i> (from pg 1)	Positive	Neg

Additional Dysbiotic/Overgrowth Bacteria

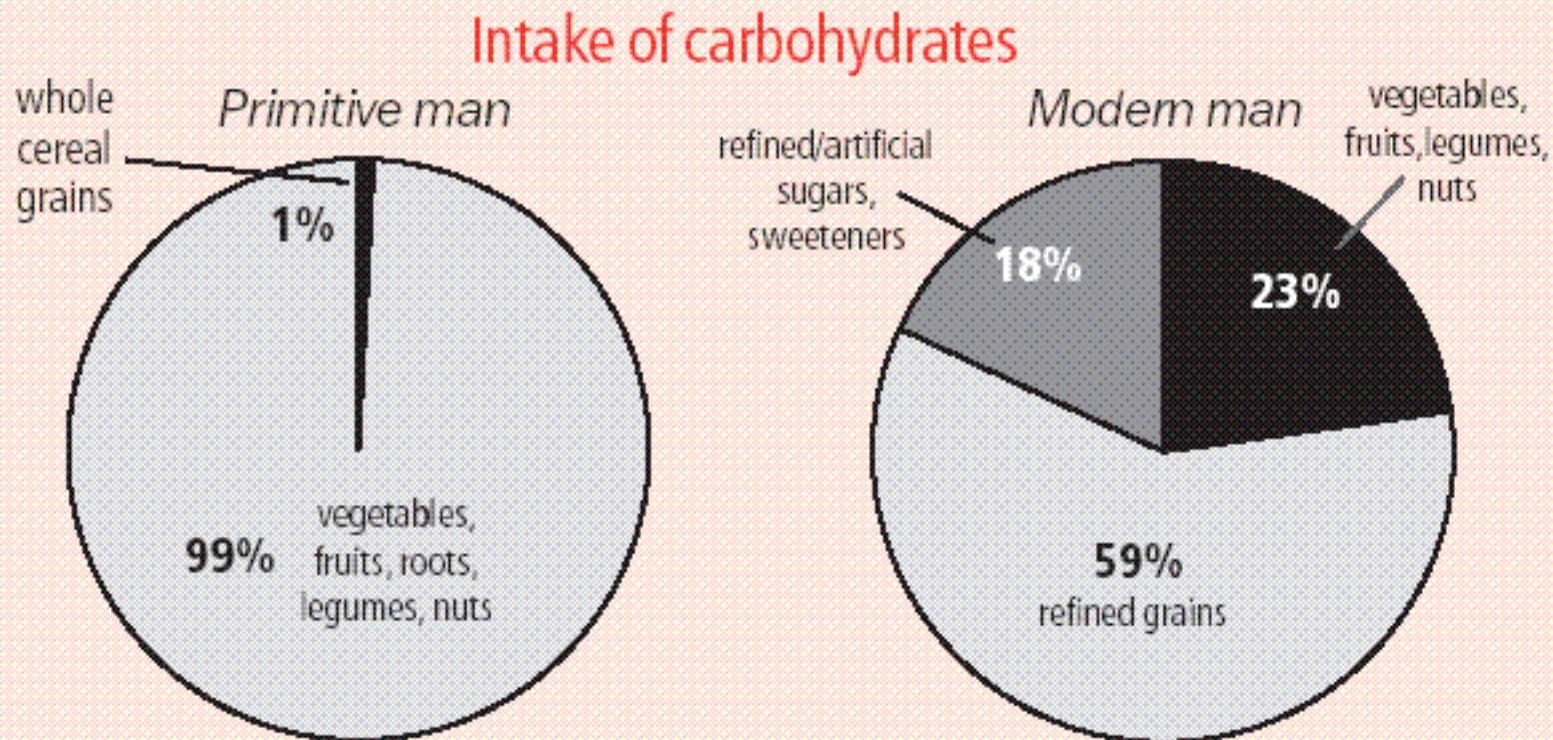
<i>Morganella morganii</i>	<dl	<1.0 E3
<i>Pseudomonas spp.</i>	<dl	<2.5 E3
<i>Pseudomonas aeruginosa</i>	<dl	<1.0 E3
<i>Staphylococcus spp.</i>	2.3 E2	<1.0 E4
<i>Streptococcus spp.</i>	<dl	<1.0 E3

Additional Tests

	Result		Range
SIgA	2293	High	510-2040 ug/mL
Anti-gliadin	69.2	High	0.0-6.4 ug/mL
Elastase 1	192		>175 mcg/g
Lactoferrin	3.8		0.0-7.2 ug/mL
Occult blood	Negative		neg

Figure 3: Changing carbohydrate intake patterns

The two pie charts compare the carbohydrate intake patterns of primitive and modern man. Note the extremely high intake of essential sugar-rich fruits, roots, nuts and legumes of the former⁽¹⁾.



Salt Intake and Autoimmunity



Sodium chloride drives autoimmune disease by the induction of pathogenic TH17 cells.

Kleinewietfeld M, Manzel A, Titze J, Kvakan H, Yosef N, Linker RA, Muller DN, Hafler DA.

1] Departments of Neurology and Immunobiology, Yale School of Medicine, 15 York Street, New Haven, Connecticut 06520, USA [2] Broad Institute of MIT and Harvard, 7 Cambridge Center, Cambridge, Massachusetts 02142, USA.

Abstract

Here we show that increased salt (sodium chloride, NaCl) concentrations found locally under physiological conditions *in vivo* markedly boost the induction of murine and human TH17 cells. The TH17 cells generated under high-salt conditions display a highly pathogenic and stable phenotype characterized by the upregulation of the pro-inflammatory cytokines GM-CSF, TNF- α and IL-2. Moreover, mice fed with a high-salt diet develop a more severe form of EAE, in line with augmented central nervous system infiltrating and peripherally induced antigen-specific TH17 cells. Thus, **increased dietary salt intake might represent an environmental risk factor for the development of autoimmune diseases through the induction of pathogenic TH17 cells.**

PMID: 23467095 [PubMed - as supplied by publisher]

Milk Proteins in the Etiology of Insulin-Dependent Diabetes Mellitus (IDDM)

Julio M. Martin, Barry Trink, Dennis Daneman, Hans-Michael Dosch and Brian Robinson

The etiology of insulin-dependent diabetes mellitus (IDDM) is multifactorial. The final cause of the disease, the specific destruction of the islet beta-cells, is the result of a cellular/humoral autoimmune process that operates in individuals with a particular genetic background in response to an external triggering factor(s). The most likely environmental triggers are virus infections and dietary factors. Among the latter

Abstract

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as the result of a cellular/humoral autoimmune process that operates in individuals with a particular genetic background in response to an external triggering factor (1, 2). Dietary factors have been consistently listed as possi-

the CDI mouse (4). There are reports indicating a reduced incidence of IDDM in children of countries with a low protein content in their diets, such as in the Polynesians of Western Samoa where no case of IDDM in children under 15 years of age ever occurred. The age specific prevalence of IDDM in Samoan children less than 15 years of age resident in Auckland is 0.65/1,000 compared with Auckland Caucasian children (1.01/1,000) and Auckland Maori children (0.52/1,000) (5). An epidemiological study done in Denmark (6) suggested that the

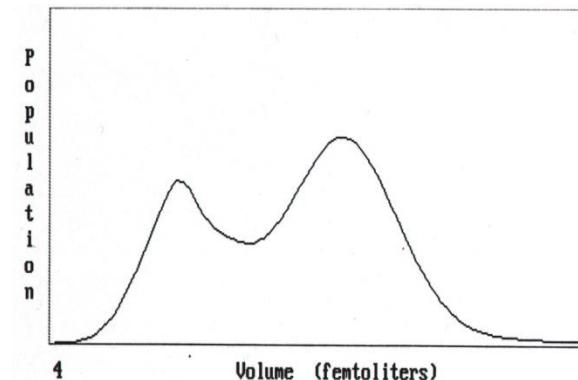
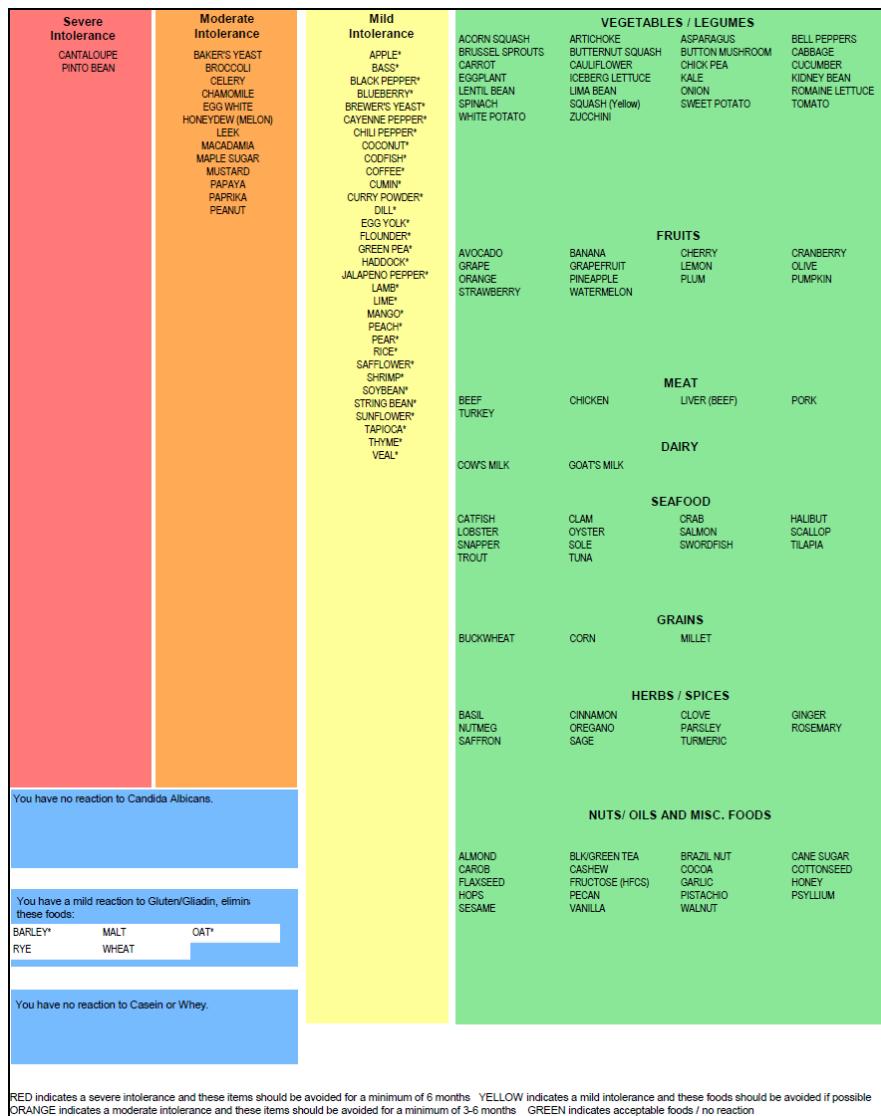
From the Hospital for Sick Children, Toronto, Ontario, Canada.

Address and reprint requests: J.M. Martin, M.D., The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, M5G 1X8 Canada.

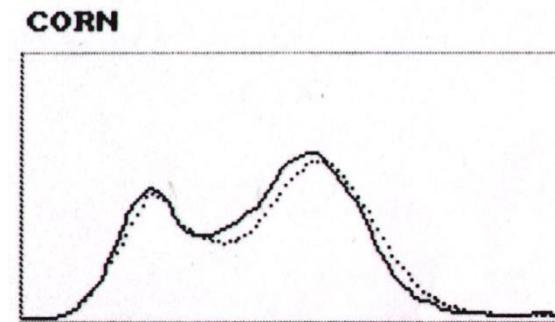
Food Sensitivity Testing

Bloodspot™ IgG Food Antibodies				Methodology: ELISA
Negative	Foods to Avoid			
	Mild +1 and +2	Moderate +3 and +4	Severe +5	
	Almond Aspergillus Beef Cantaloupe Cashew Chicken Corn Crab Garlic Lobster Milk Mustard Oat Orange Pea, Green Peanut Pinto Bean Pork Rice Salmon Shrimp Soybean Strawberry Sunflower Tomato Tuna Turkey Walnut Wheat			Egg, Whole

Food-Immune Activation Testing



Pre-antigen exposure



Control Count: 4878
Sample Count: 5015
Reaction % : 13

Post-antigen exposure



The Alcat Test Predicts the Release of DNA and Myeloperoxidase by Innate Immune Peripheral Blood Leukocytes Via a PKC Dependent Pathway¹

Yale School of Medicine

Authors: Irma Garcia-Martinez PhD,¹ Theresa R. Weiss MPH,² Ather Ali ND MPH mhs,² Wajahat Mehal MD phd

Departments of (1) Internal Medicine (Digestive Diseases), and (2) Pediatrics (General Pediatrics), Yale School of Medicine, New Haven, Connecticut

Purpose: The Alcat test identifies food items which stimulate a cellular response in peripheral blood leukocytes, and has been used to individualize the diets of patients with a diverse range of medical conditions. **We wished to assess any relationship between Alcat responses and immunological parameters.** Extracellular DNA results in an inflammatory response. We hypothesized that the immune responses initiated by Alcat positive food items are due to the release of DNA from blood immune cells.

Methods: Whole blood samples from 20 healthy volunteers underwent standard Alcat testing at Cell Science Systems, Corp. (Deerfield Beach, FL). Other analyses were performed at Yale University (New Haven, CT). Quantification of total DNA and myeloperoxidase (MPO) in the supernatant was performed in the presence and absence of specific inhibitors of key signaling pathways (Phosphoinositide 3-kinase, nuclear factor- κ B, c-Jun N-terminal kinase, mitogen-activated protein kinase P38, protein kinase C and calmodulin).

Results: Alcat positive foods gave a higher supernatant DNA content in 53 of 76 (70%), and a higher MPO in 18 of 28 (64%) (significant at $P < 0.05$). PKC inhibitors resulted in inhibition of Alcat positive food stimulated DNA release ($P < 0.05$). Activation of neutrophils, eosinophils, and basophils was identified by established cell surface markers and flow-cytometric analysis. Alcat positive samples resulted in CD63 levels greater than Alcat negative samples in eosinophils in 76% of tests ($p < 0.02$), but only 47% and 41% for neutrophils and basophils respectively (NS).

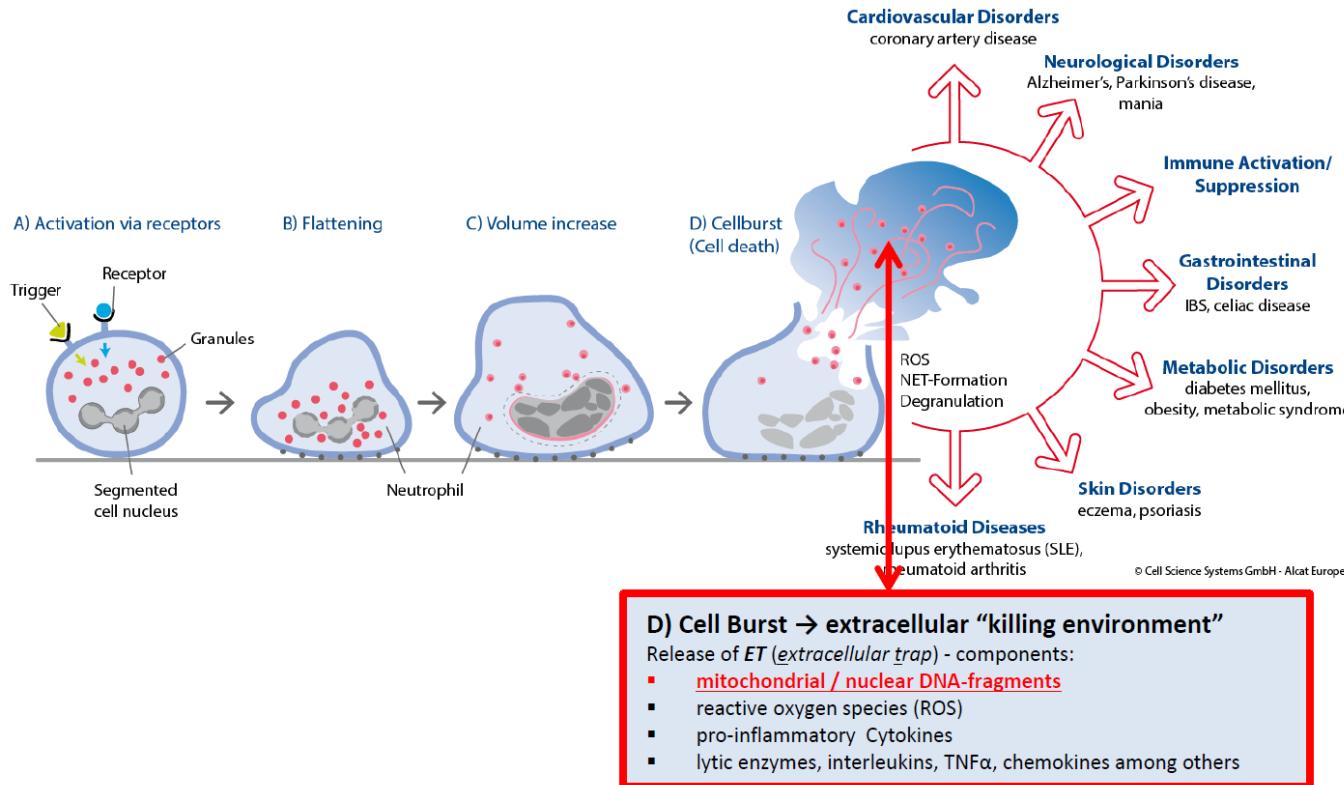
Conclusion: The Alcat test identifies food items that result in release of **DNA²** and **MPO³** and activation of peripheral blood innate immune cells in a **PKC** dependent manner.

This demonstrates that the Alcat test identifies food items that result in release of inflammatory markers and activation of innate immune cells.

Yale University | Study Results

Molecular Pathomechanisms of the Alcat Test (2016)

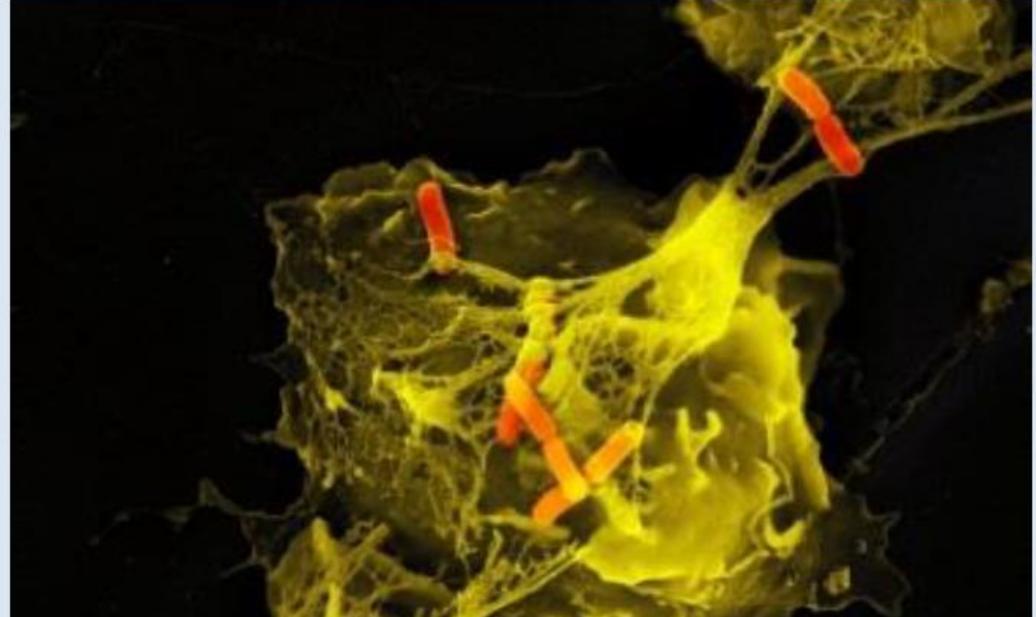
ALCAT



COMMENT: What does “Release of DNA and Myeloperoxidase by Innate Immune Peripheral Blood Leukocytes” mean?

Image (top of page): Cellular defense reaction of a neutrophil in different stages. Trigger may be ⁴exogenous factors, i.e. invading pathogens, microorganisms, foreign particles or microbial structures (PAMP; pathogen associated molecular patterns) or ⁵endogenous factors, i.e. internal molecules (DAMP; danger/damage associated molecular pattern): host-molecules, which initiate/maintain inflammatory cascades caused by DNA, ATP, stress, cytosolic enzymes, UV radiation, thermal damage, food molecules etc.

Thus, a “*killing environment*” is created outside the cell, in which the intracellular antimicrobial mediators, granules and reactive oxygen spe-

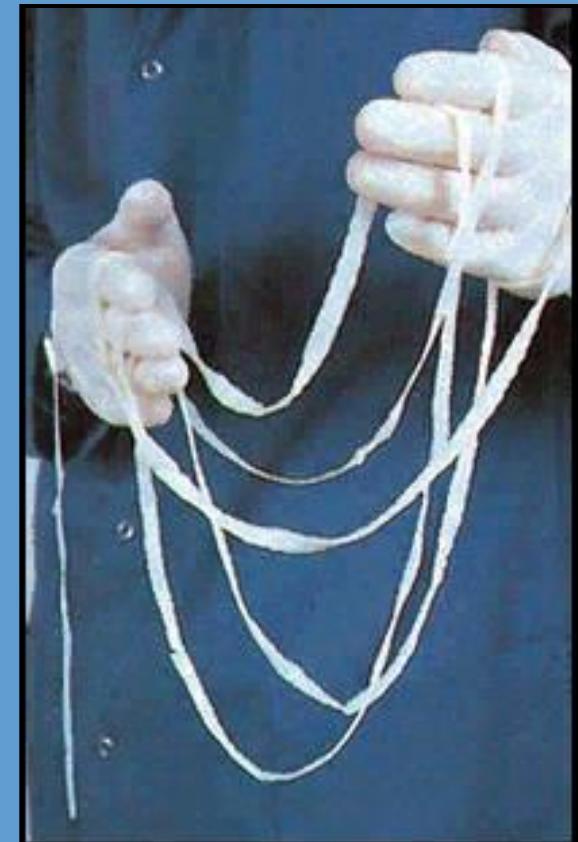


Unmethylated DNA is highly inflammatory!

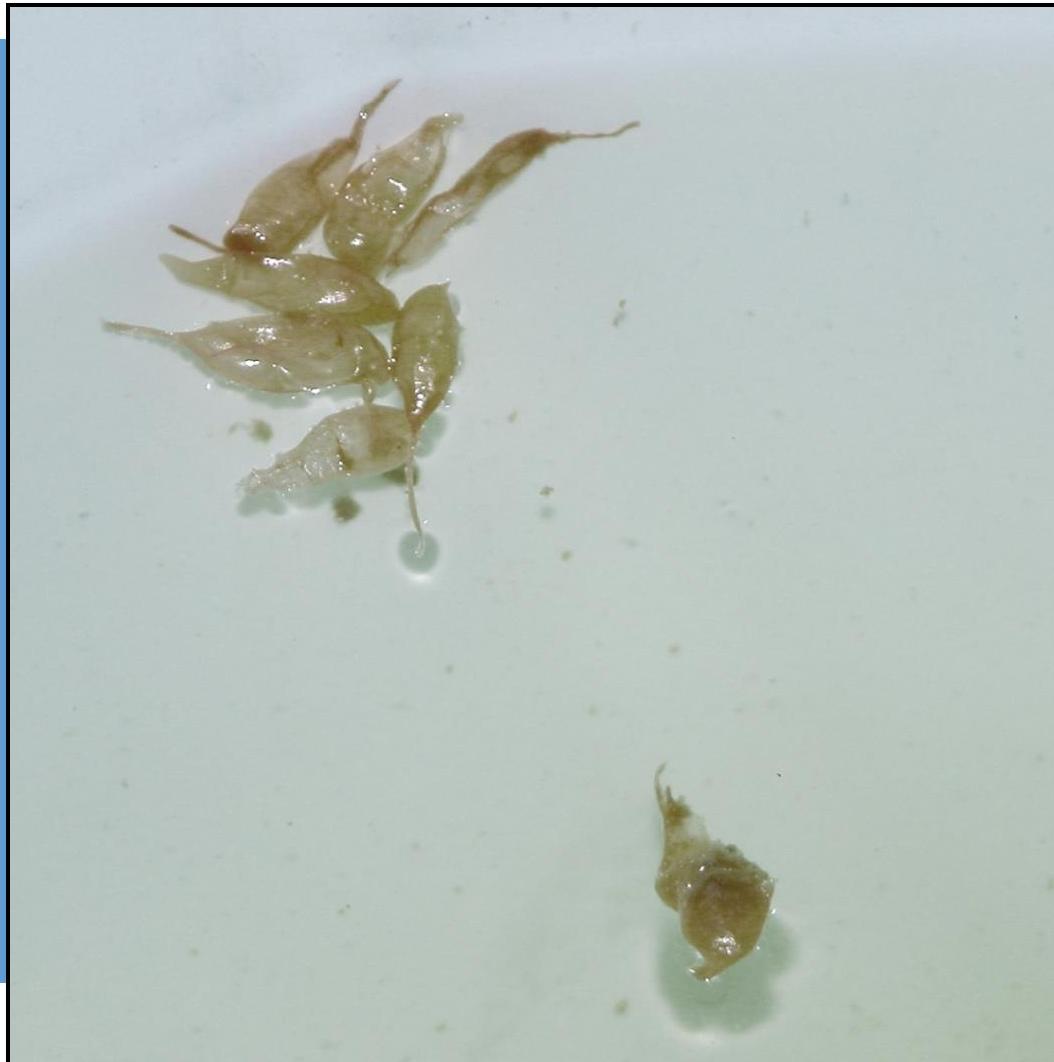
DNA release. *ETosis* is a novel cell death pathway including the full disintegration of the nuclear and cellular membranes (cell burst) and ***ET formation***. *ETs* are a special kind of "trap" composed of condensed chromatin fibers containing the antimicrobial mediators from the granules and pro-inflammatory acting DNA.



GI Parasites

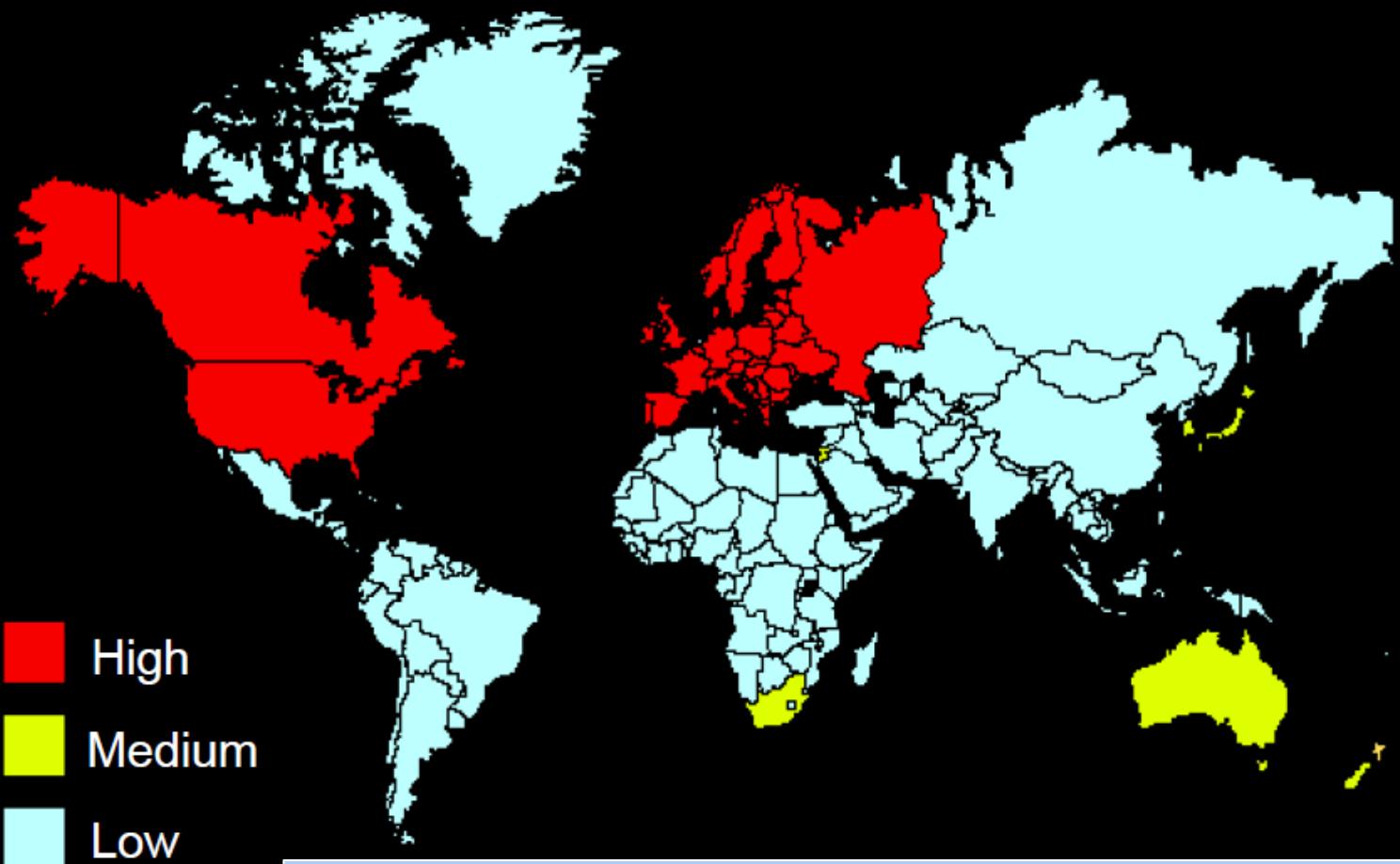


Patient Sample



Global Prevalence of IBD

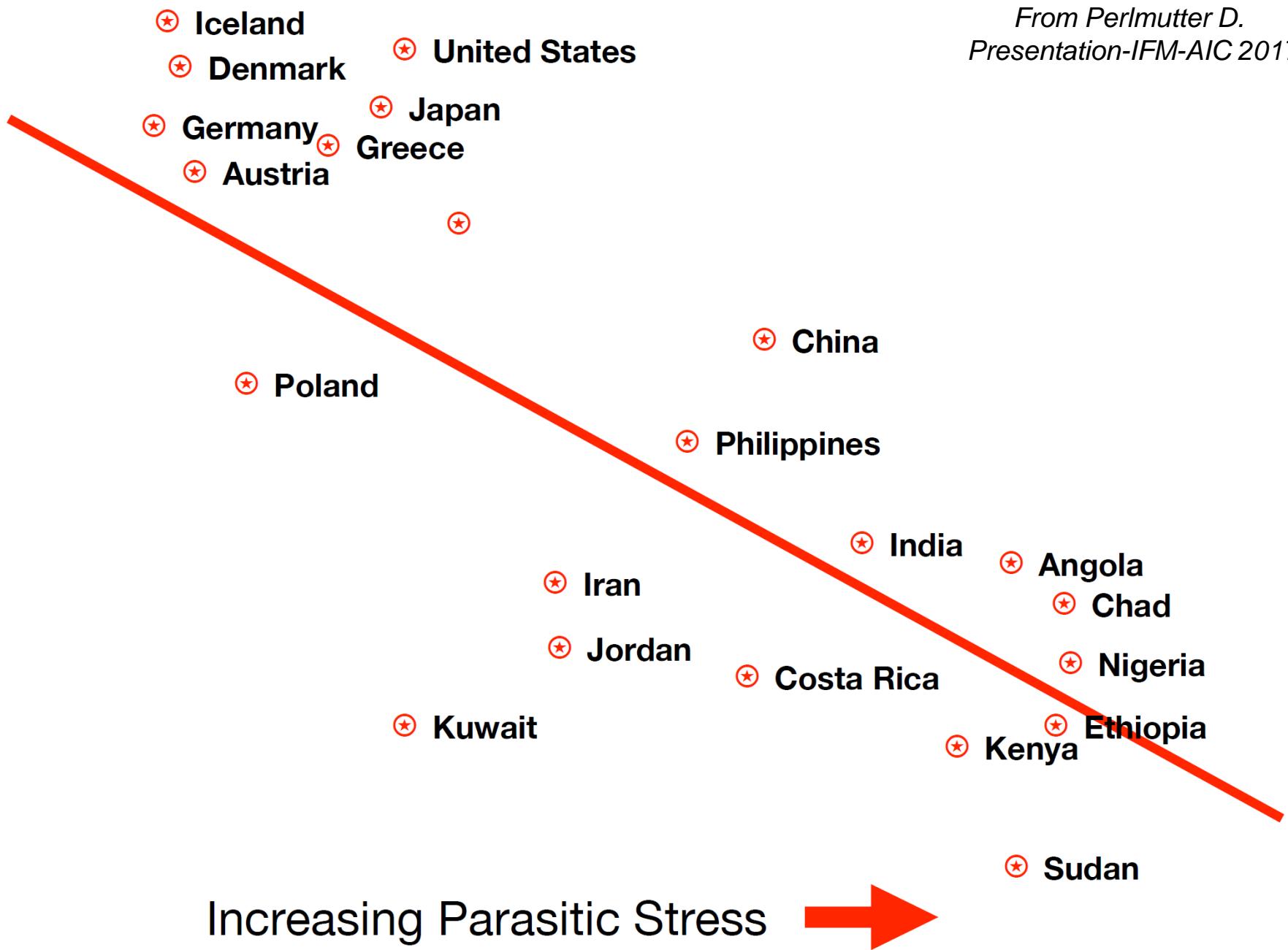
Weinstock J: IFM Annual Symposium (2011)

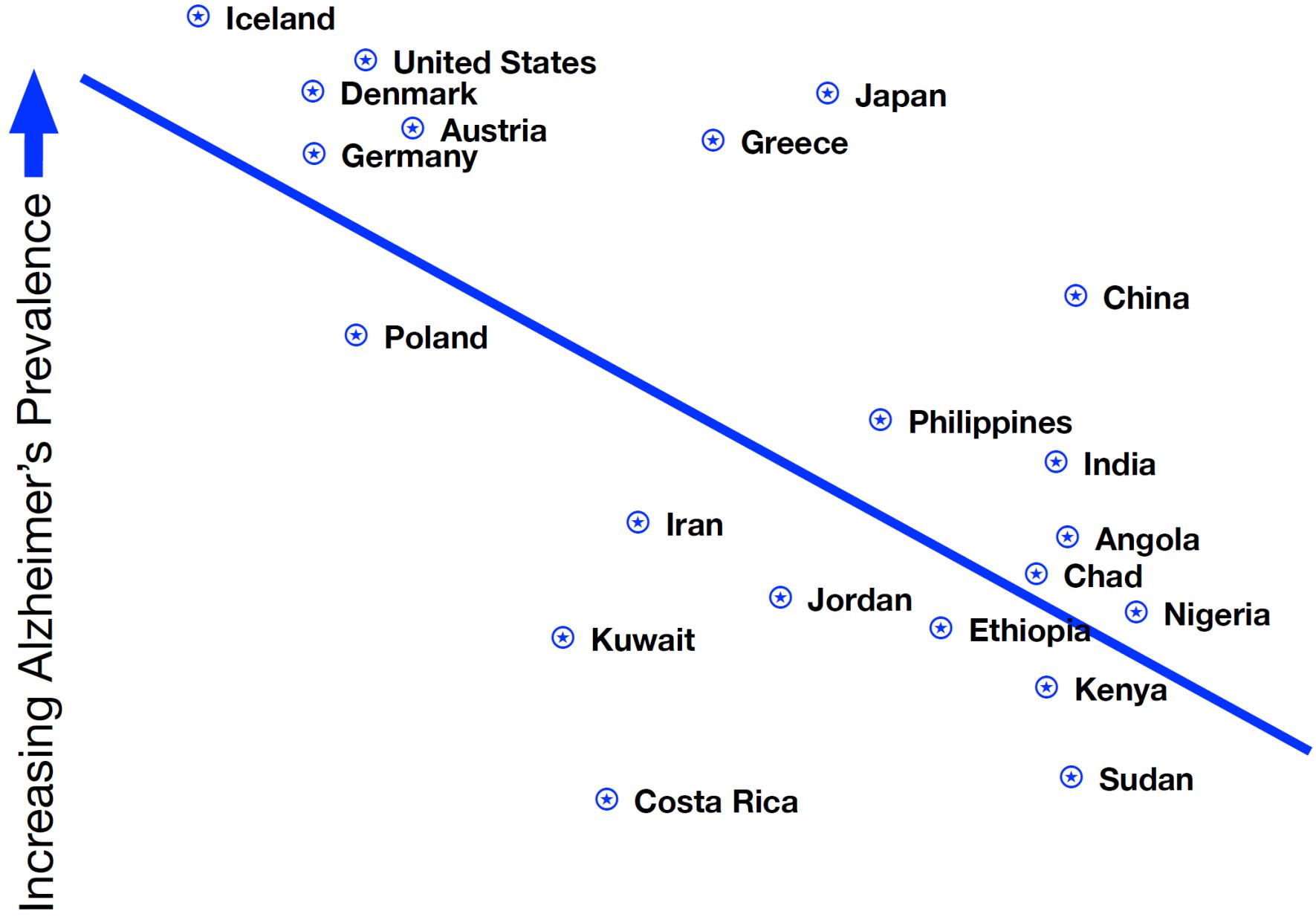


IBD was unheard of before the 20th century. Beginning of 20th century incidence thought to be about 1:10,000 and now 1:250 (Environmental factors at play!). Similar data exists with asthma, hay fever, DM, MS, etc.



Weinstock J: IFM Annual Symposium (2011)





Hygiene and the world distribution of Alzheimer's Disease

Conclusions: Variation in hygiene may partly explain global patterns in AD rates. Microorganism exposure may be inversely related to AD risk. These results may help predict AD burden in developing countries where microbial diversity is rapidly diminishing.

Evolution, Medicine, and Public Health, August 11, 2013

*Editorials***EAT DIRT — THE HYGIENE HYPOTHESIS AND ALLERGIC DISEASES**

THREE has been an epidemic of both autoimmune diseases (in which the immune response is dominated by type 1 helper T [Th1] cells, such as type 1 diabetes, Crohn's disease, and multiple sclerosis) and allergic diseases (in which the immune response is dominated by type 2 helper T [Th2] cells, such as asthma, allergic rhinitis, and atopic dermatitis), as documented in the article by Bach in this issue of the *Journal*.¹ The occurrence of these diseases is higher in more affluent, Western, industrialized countries. One theory proposed to explain this increase in the prevalence of autoimmune and allergic diseases is that it results from a decrease in the prevalence of childhood infection. Although this theory dates back to at least the mid-1960s in relation to Th1-mediated diseases, Strachan first proposed in 1989 that this theory might also explain the increase in Th2-mediated diseases,² and it has subsequently come to be called the hygiene hypothesis. A gradual change in the frequency of childhood infection has been occurring for a long time, affected by the introductions of indoor plumbing in the 19th century, antibiotics in the middle of the 20th century, and cleaner, more energy-efficient homes at the end of the 20th century.

Bach details a number of potential mechanisms by which the decrease in the frequency of childhood infections might influence the frequency of autoimmune diseases. In the light of the article by Braun-Fahrländer and coworkers,³ also in this issue of the *Journal*, two mechanisms deserve special attention. The first is that the decrease in antigenic stimulation related to the decrease in the frequency of childhood infections has resulted in a decrease in the levels of regulatory cytokines — specifically, interleukin-10 and possibly transforming growth factor β (TGF- β). CD25-positive T cells and other regulatory T cells produce interleukin-10 and TGF- β and act to down-regulate both Th1-mediated responses and Th2-mediated responses. It is unclear how interleukin-10 and TGF- β

Fahrländer et al. is that stimulation of the innate immune system by endotoxin may be important in the ontogeny of the normal immune system.

A series of epidemiologic reports suggests that there has been a decrease in the frequency of allergy and asthma among children of farmers in Western, industrialized countries.^{4,5} The current study by Braun-Fahrländer et al.³ is a cross-sectional study involving 812 children between 6 and 13 years of age from farming and nonfarming households in rural areas of central Europe. The investigators measured endotoxin levels in mattress dust and found a relation between higher levels of endotoxin in the dust and a decreased frequency of hay fever, allergic asthma, and allergic sensitization in these children.

Endotoxin is a lipopolysaccharide that forms the outer layer of the cell membrane of all gram-negative bacteria. Endotoxin levels vary widely but tend to be highest in environments where there are farm animals such as cows, horses, and pigs, because the fecal flora of larger mammals is a major source of endotoxin. Endotoxin is also found in the dust in houses and outdoors in dirt and can be measured in dust or air. In its airborne form, endotoxin can be inhaled or swallowed and acts as a potent immunostimulatory molecule through its lipid A moiety, which signals, through CD14 and toll-like receptor 4 (TLR4), other molecules (MyD88 and toll-like receptor 9 [TLR9]) of the innate immunity pathway.

How does endotoxin decrease Th2-mediated diseases such as allergies and allergic asthma? At low levels, lipopolysaccharide is a potent inducer of interleukin-12 and interferon- γ , which are cytokines that stimulate Th1-mediated immunity, and also decreases the production of Th2 inflammatory cytokines such as interleukin-4, interleukin-5, and interleukin-13. Finally, lipopolysaccharide increases defensins and collectins, such as surfactant protein A in the lungs, which enhance the developing immune response of a neonate. The effects of endotoxin are dose-dependent; at high doses, endotoxin produces hypersensitivity pneumonitis and stimulates the release of inflammatory mediators.⁶ Even at low doses, endotoxin is associated with wheezing during the first year of life.⁷ Given these potential opposing effects of endotoxin exposure, greater knowledge about what dose of endotoxin is protective and what dose is a risk factor is needed.

PERSPECTIVES

SCIENCE AND SOCIETY

Farm living: effects on childhood asthma and allergy

Erika von Mutius and Donata Vercelli

Abstract | Numerous epidemiological studies have shown that children who grow up on traditional farms are protected from asthma, hay fever and allergic sensitization. Early-life contact with livestock and their fodder, and consumption of unprocessed cow's milk have been identified as the most effective protective exposures. Studies of the immunobiology of farm living point to activation and modulation of innate and adaptive immune responses by intense microbial exposures and possibly xenogeneic signals delivered before or soon after birth.

The prevalence of asthma, hay fever, atopic dermatitis and allergic sensitization is higher in affluent, Western countries than in developing countries. A rise in the prevalence of these conditions has also occurred in the last decades of the twentieth century¹. From a global perspective, some comparisons seem particularly informative and studies of populations with comparable ethnic backgrounds but striking differences in environmental exposures may be especially revealing. In many developing countries, westernization accompanies urbanization and thus reflects a loss of rural living conditions.

In Europe, studies comparing rates of childhood asthma and hay fever in urban and rural areas have been inconclusive². However, large differences in the prevalence of childhood asthma, hay fever and atopic sensitization exist in rural areas. As we discuss here, children from rural areas who grow up on farms are at a significantly lower risk of developing these conditions than children who live in the same rural area but do not grow up on farms. This protective 'farm effect' is seen for both the atopic and non-atopic phenotype of childhood asthma^{3,4} and has been shown to be sustained into adult life. Many of the studies that primarily investigated childhood farm exposures (TABLE 1; Supplementary Information S1 (table)) were carried out in Switzerland, Austria and Germany^{5–8} where, traditionally, farming has been the main source of subsistence.

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will have been exposed continuously until adolescence and beyond.

In this article, we discuss three main aspects of the farm effect. First, protective environmental exposures that are inhaled and ingested by exposed individuals have been identified. Second, the study of farm populations has pointed to the time period in which these exposures are effective for mediating the farm effect. Third, immune response studies in farm children and experiments in animal models have identified components of the protective immunobiology of farm exposures.

Allergy-protective farm exposures

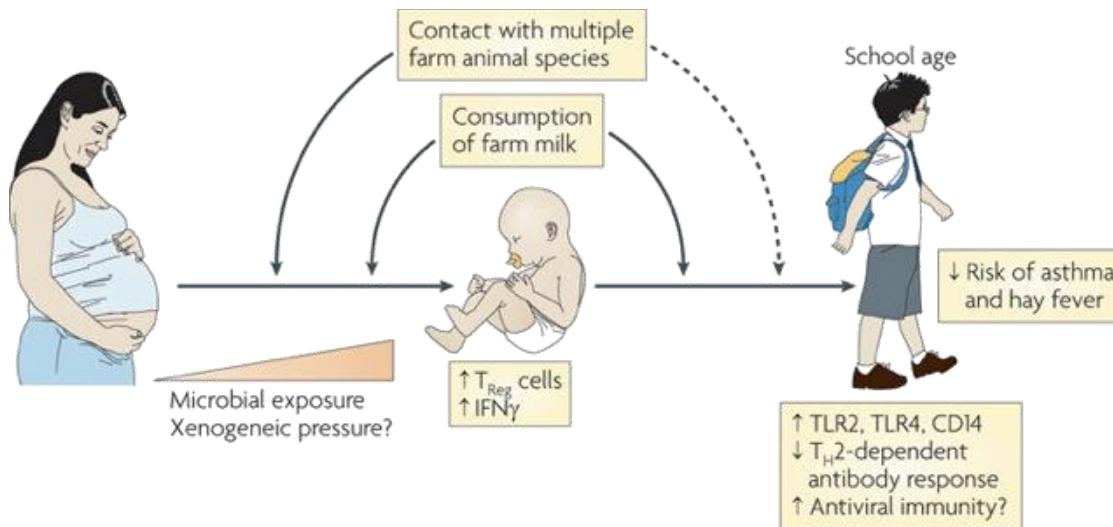
Several studies have identified some of the exposures associated with a farming lifestyle that contribute to the reduced risk of asthma and allergies in farm children, namely contact with livestock, mostly cattle, pigs and poultry; contact with animal feed such as hay, grain, straw and silage; and the consumption of unprocessed cow's milk^{4–9}. These exposures had an independent protective farm effect, which indicates that inhalation and ingestion are the two main routes of exposure. Other differences in lifestyle, such as duration of breast feeding, family and sibship size, day care, pet ownership, other dietary habits, parental education and a family history of asthma and allergies, did not account for the pro-

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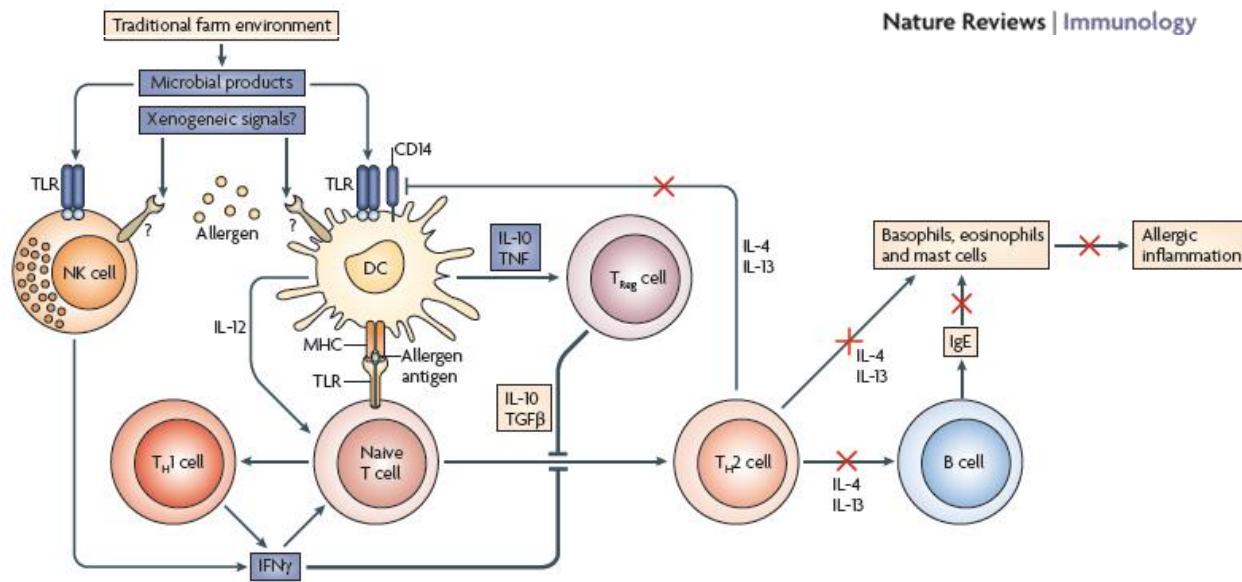
families still use unprocessed milk, even for pregnant women and infants. Five studies have shown a protective effect of unprocessed milk on the development of asthma, hay fever, allergic sensitization and atopic dermatitis^{4,7,12,14–16}. The cow's milk that is used for commercial purposes has been pasteurized and homogenized. In most countries, pasteurization is achieved by heating the milk for a short period (~72–75°C for up to 30 seconds) to significantly reduce the level of microorganisms in the milk. In the homogenization process, fat globules are broken up to produce

NATURE REVIEWS | IMMUNOLOGY

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Nature Reviews | Immunology



House dust exposure mediates gut microbiome *Lactobacillus* enrichment and airway immune defense against allergens and virus infection

Kei E. Fujimura^{a,1}, Tine Demoor^{b,1}, Marcus Rauch^a, Ali A. Faruqi^a, Sihyug Jang^b, Christine C. Johnson^c, Homer A. Boushey^d, Edward Zoratti^e, Dennis Ownby^f, Nicholas W. Lukacs^{b,2}, and Susan V. Lynch^{a,2}

^aDivisions of ^aGastroenterology and ^bPulmonary and Critical Care Medicine, University of California, San Francisco, CA 94143; ^bDepartment of Pathology, University of Michigan, Ann Arbor, MI 48109; ^cDepartment of Pathology, Detroit Medical System, Detroit, MI 48202; and ^dDepartment of Pediatrics, review June 6, 2013

Exposure to dogs in early infancy has been shown to reduce the risk of childhood allergic disease development, and this is associated with a distinct house dust microbial environment. We demonstrate, using murine models, that exposure to dog-associated house dust protects against ovalbumin (OVA)-mediated airway pathology. Protected animals exhibited significant reduction in the total number of airway T cells, down-regulation of Th2-related airway responses, as well as mucin secretion. Following dog-associated dust exposure, the gut microbiome of protected animals was extensively reconstituted with significant enrichment of, amongst others, *Lactobacillus johnsonii*. Supplementation of wild-type animals with *L. johnsonii* in their diet against both airway allergen challenge or influenza A virus (IAV) infection revealed that the gut microbiome composition of activated CD11c⁺/CD11b⁺ and CD11c⁺/CD64⁺ cells significantly reduced airway Th2 cytokine expression. These results reveal that exposure to dog-associated household dust may provide protection against airway allergen challenge and a distinct gut microbiome composition. Moreover, the presence of *L. johnsonii* as a pivotal species within the gastrointestinal tract is capable of influencing adaptive immunity at remote mucosal surfaces in a manner that is protective against a variety of respiratory insults.

house environment | airway adaptive immunity | gastrointestinal bacterial community | *Lactobacillaceae*

The emerging field of human microbiome research has demonstrated the key role microbial communities play in a variety of critical mammalian processes including ancillary mucosal barrier function (1) and metabolism (2, 3), as well as development and modulation of host immune responses (4, 5). This is particularly evident in the gastrointestinal (GI) tract where the composition of the microbiome in this niche and, specifically, the presence of particular bacterial species such as segmented filamentous bacteria and those belonging to *Clostridium* clades IV and XIV, have been shown to induce specific T-cell repertoires, i.e., Th17 and CD4⁺ FoxP3⁺ T-regulatory cells, respectively (4, 6). These studies demonstrate that despite the complexity of the GI microbiome, the presence or absence of specific bacterial species can dramatically alter the adaptive immune environment.

Human studies appear to support this concept. A large European birth cohort study demonstrated that a significant increase in the number of *Escherichia coli* or *Clostridium difficile* in fecal samples from 3-wk-old infants was associated with a greater risk of developing a spectrum of childhood allergic diseases (7), commonly characterized by overactive Th2 adaptive immune response. Early-life exposures, including those known to impact GI microbiome composition, e.g., antibiotic administration and caesarian section delivery, have also been associated with increased risk for childhood asthma (8, 9). Conversely, exposure

membership and the immune response of the human host. Because GI microbiome composition clearly impacts immune function, and specific GI colonization patterns are linked to allergic

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L. johnsonii as a pivotal species within the gastrointestinal tract capable of influencing adaptive immunity at remote mucosal surfaces in a manner that is protective against a variety of respiratory insults.

Moreover, the study identifies

Significance

Early-life exposure to dogs is protective against allergic disease development, and dog ownership is associated with a distinct milieu of house dust microbial exposures. Here, we show that mice exposed to dog-associated house dust are protected against airway allergen challenge. These animals exhibit reduced Th2 cytokine production, fewer activated T cells, and a distinct gut microbiome composition, highly enriched for *Lactobacillus johnsonii*, which itself can confer airway protection when orally supplemented as a single species. This study supports the possibility that host-environment interactions that govern allergic or infectious airway disease may be mediated, at least in part, by the impact of environmental exposures on the gastrointestinal microbiome composition and, by extension, its impact on the host immune response.

Author contributions: T.D., C.C.J., H.A.B., E.Z., D.O., N.W.L., and S.V.L. designed research; K.E.F., T.D., M.R., and S.J. performed research; K.E.F., A.A.F., and C.C.J. contributed new reagents/analytic tools; K.E.F., T.D., M.R., A.A.F., S.J., N.W.L., and S.V.L. analyzed data; and K.E.F., T.D., M.R., N.W.L., and S.V.L. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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Data deposition: The microbiome data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GSE52909).

¹K.E.F. and T.D. contributed equally to this work.

²To whom correspondence may be addressed. E-mail: susan.lynch@ucsf.edu or nlukacs@umich.edu.

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Fujimura et al.

www.pnas.org/cgi/doi/10.1073/pnas.1310750111

Causation of Crohn's disease by *Mycobacterium avium* subspecies *paratuberculosis*

John Hermon-Taylor MB MChir FRCS, Timothy John Bull BSc PhD, Joseph Michael Sheridan BSc PhD, Jun Cheng MD,
Michael Laurence Stellakis MB FRCS, Nasira Sumar BSc PhD

J Hermon-Taylor, TJ Bull, JM Sheridan, J Cheng, ML Stellakis, N Sumar. Causation of Crohn's disease by *Mycobacterium avium* subspecies *paratuberculosis*. *Can J Gastroenterol* 2000;14(6):521-539. *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is:

- differs genetically from IS900 and a single case surface carbohydrate of chronic inflammation including primates.
- cimicibial, with chronic disease causing clinical disease in Western Europe and up to 54%. These subclinical milk and onto pasture the risk that is common in domestic water supplies colon mucosa of the rectum in a high prevalence of Crohn's disease gut polymerase chain reaction in Crohn's disease is found, can evade immune system dysregulation. As with other MAC, MAP is resistant to most standard antituberculous drugs. Treatment of Crohn's disease with combinations of drugs more active against MAC such as rifabutin and clarithromycin can bring about a profound improvement and, in a few cases, apparent disease eradication. New drugs as well as effective MAP vaccines for animals and humans are needed. The problems caused by MAP constitute a public health issue of tragic proportions for which a range of remedial measures are urgently needed.

Key Words: Antimicrobial chemotherapy; Crohn's disease; Food safety; *John's disease*; *Mycobacterium avium* subspecies *paratuberculosis*; Polymerase chain reaction; Potable water; vaccine

La maladie de Crohn causée par *Mycobacterium avium* sous-espèce *paratuberculosis*

As with other MAC, MAP is resistant to most standard antituberculous drugs. Treatment of Crohn's disease with combinations of drugs more active against MAC such as rifabutin and clarithromycin can bring about a profound improvement and, in a few cases, apparent disease eradication.

Dans la maladie de Crohn, MAP se présente sous une forme non bactérienne résistante aux protéases, peut évoquer une reconnaissance immunologique et cause probablement un dérèglement immunitaire. Tout comme les autres MAC, MAP est résistante à la plupart des traitements antituberculeux habituels. Le traitement de la maladie de Crohn avec des combinaisons de médicaments plus actifs contre MAC comme la rifabutine et la clarithromycine peut apporter une importante amélioration et, dans certains cas, entraîner une éradication de la maladie. Il est nécessaire de développer de nouveaux médicaments de même que des vaccins efficaces contre MAP destinés aux animaux et aux humains. Les problèmes causés par MAP constituent une question de santé publique d'une envergure dramatique pour laquelle un ensemble de mesures curatives s'imposent d'urgence.

This mini-review was prepared from a presentation made at the World Congress of Gastroenterology, Vienna, Austria, September 6 to 11, 1998
Department of Surgery, St George's Hospital Medical School, London, United Kingdom

Correspondence and reprints: John Hermon-Taylor, Department of Surgery, St George's Hospital Medical School, London, SW17 ORE, United Kingdom. Telephone +44-181-767-7631, fax +44-181-725-3594, e-mail jhermon@sghms.ac.uk

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Crohn's Disease May Be Differentiated Into 2 Distinct Biotypes Based on the Detection of Bacterial Genomic Sequences and Virulence Genes Within Submucosal Tissues.

Chiodini RJ, Dowd SE, Davis B, Galandiuk S, Chamberlin WM, Kuenstner JT, McCallum RW, Zhang J.

*Division of Gastroenterology #Division of Gastroenterology **Department of Internal Medicine ††Department of Anesthesiology ‡Department of Surgery, Paul L. Foster School of Medicine, Texas Tech University Health Sciences Center, El Paso †Molecular Research (Mr. DNA), Shallowater, TX §Department of Surgery, University of Louisville, Louisville, KY ¶St. Vincent Physician Network, Sisters of Charity of Leavenworth Health System, Gastroenterology Associates, Billings, MT ¶Clinical Laboratories, Charleston Area Medical Center, Charleston, WV.

Abstract

OBJECTIVE: To determine whether bacterial pathogens can be detected within the diseased submucosal tissues of patients with Crohn's disease by molecular techniques independent of cultural methods. **DESIGN:** We designed a quantitative polymerase chain reaction to detect 32 virulence genes and transposons within submucosal tissues of patients with Crohn's disease and controls and compared the microbiome of the submucosa with mucosal bacterial populations. **RESULTS:** Within submucosal tissues, the bacterial invasion/adherence genes *eaeA* and *invA* were detected in 43% of patients ($P=0.01$ and 0.008 vs. mucosa and controls, respectively) and the *Mycobacterium*-specific *IS900* and *251F* genes detected in 50% of patients ($P=0.03$ vs. mucosa and controls). These findings were mutually exclusive: invasion/adhesion genes and *Mycobacterium*-associated transposons were not detected in the same patient. Metagenomic sequencing and quantitative polymerase chain reaction results confirmed effective separation of the submucosal and mucosal microbiome and the existence of a submucosal bacterial population within diseased tissues. **CONCLUSIONS:** This study is the first to examine the microbial populations of submucosal tissues during intestinal disease and provide evidence of a distinct submucosal microbiome and biotypes within Crohn's disease. These data suggests that Crohn's disease may not be a single disease, but a spectrum that can be divided into distinct biotypes based on the presence of invasion/adherence genes or *Mycobacterium*-associated transposons. If corroborated by larger population studies, these findings could revolutionize the diagnosis, management, and treatment of Crohn's disease by the identification of patient biotypes and the application of targeted chemotherapeutic treatments that go beyond supportive in nature.

CONCLUSION: *These data suggests that Crohn's disease may not be a single disease, but a spectrum that can be divided into distinct biotypes based on the presence of invasion/adherence genes or Mycobacterium-associated transposons.*

Parasites in Your Gut Actually Help Protect You From Allergies

by David Gutierrez, staff writer

(NaturalNews) Humans and gastrointestinal parasites might have co-evolved in such a way that the parasites actually help regulate the human immune system to prevent against allergies, according to a study conducted by researchers from the University of Nottingham.

Researchers believe that over the course of millions of years, gastrointestinal parasites have evolved an ability to suppress the human immune system as a survival mechanism. Because parasitic infestation has been so common throughout human evolutionary history, the human immune system has in turn evolved to compensate for this effect.

This means that if the parasites are removed, the immune system may actually function too strongly, resulting in maladaptive immune responses such as asthma, eczema and other allergies.

To test this hypothesis, researchers used drugs to eliminate hookworm infection in a 1,500 children between the ages of six and 17 who were living in a rural village in central Vietnam. This region was selected for its very low rates of allergies and high parasitic infestation rate. Two-thirds of all children in the area are infested with hookworm or other gastrointestinal parasites.

The researchers found that once the children were no longer infected with parasites, their rates of dust mite allergies significantly increased. This supports the hypothesis that parasites help regulate immune responses.

"The next step is to understand exactly how and when gut parasites program the human immune system in a way that protects against allergies, and for such studies, follow-up from birth will be essential," said researcher Carsten Flohr.

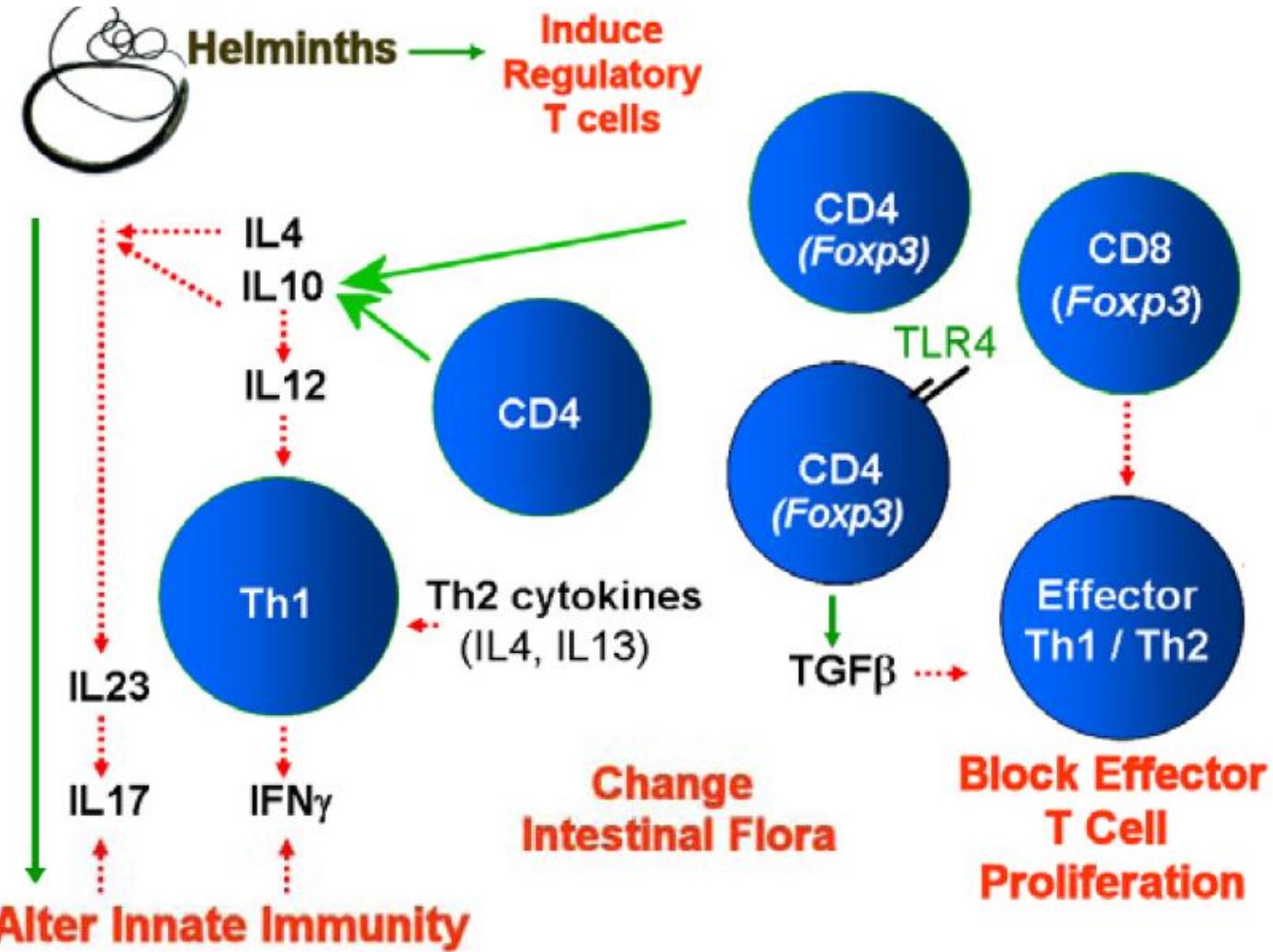
Researchers hope that understanding the relationship between parasites and the human immune system could lead to a better overall understanding of allergies.

"The prospects of further studies in this area are very exciting, as we could see groundbreaking treatments for asthma and other allergies developed as a result," said Elaine Vickers of Asthma UK, which funded the study.

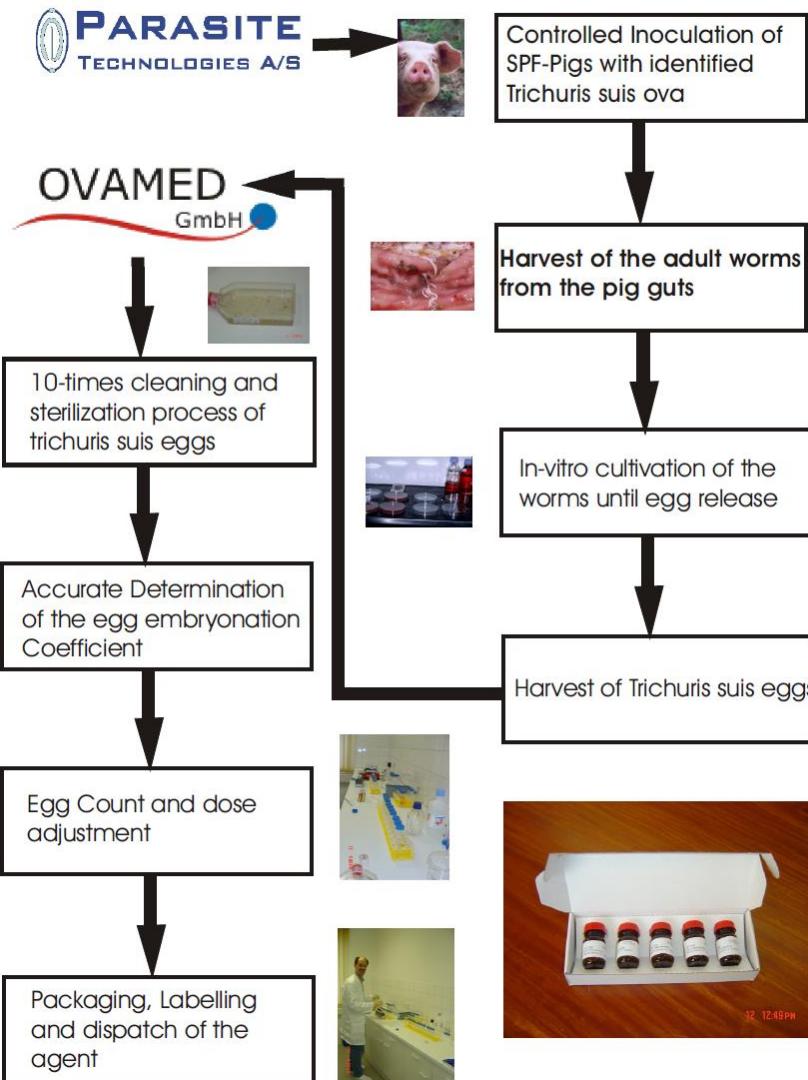
Trichuris suis
(Porcine whipworm)



Weinstock J: IFM Annual Symposium (2011)



Flowchart of TSO Production



Research Article

Infection with *Hymenolepis diminuta* Is More Effective than Daily Corticosteroids in Blocking Chemically Induced Colitis in Mice

Alexandra Melon, Arthur Wang, Van Phan, and Derek M. McKay

Gastrointestinal Research Group, Department of Physiology and Pharmacology, Calvine, Phoebe & Joan Snyder Institute of Infection, Immunity and Inflammation, University of Calgary, Calgary, AB, Canada T2N 4N1

Correspondence should be addressed to Derek M. McKay, dmckay@ucalgary.ca

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Purpose. To compare infection with the tapeworm, *Hymenolepis diminuta*, with steroid (dexamethasone) administration in the inhibition of dinitrobenzene sulfonic acid- (DNBS-) induced colitis in mice. **Procedures.** Mice were treated with DNBS ± infected daily dexamethasone (2 mg/Kg, ip.) and were assessed 72 hours post-DNBS by the calculation

between the groups. **Conclusions.** *H. diminuta* was superior to dexamethasone in the prevention of DNBS-induced colitis and did not result in additional side effects (i.e., collagen deposition). Comparisons with current therapeutics and long-term followup to studies are essential if “helminth therapy” is to become a viable treatment for specific inflammatory diseases in the gut or other tissues.

1. Introduction

During the last three decades there have been dramatic increases in autoimmune and inflammatory diseases, such as allergy/atopy, diabetes, and inflammatory bowel disease (IBD) that cannot be explained solely on the basis of genetics [1]. In the search for environmental triggers for these conditions, the hygiene hypothesis has arisen that suggests that reduced exposure to infectious agents (via increases in hygiene, sterile drinking water, and use of antibiotics) may result in the generation of greater numbers of autoreactive immune cells in humans, and hence the emergence of autoimmune and idiopathic inflammatory disease [2]. Compatible with this postulate is the geographical divergence in the occurrence of diseases such as IBD and areas of pandemic helminth infection [3]. Epidemiological data must be viewed cautiously when used in support of causation rather than association. They do nevertheless raise

the question: could infection with parasitic helminths protect against other concomitant disease?

Representatives of all classes of helminth parasite have been shown to modulate immunity in their hosts, both qualitatively and quantitatively [4–6]. Moreover, infection with helminth parasites evokes stereotypic immune responses in humans and mice that are dominated by T helper 2 (TH2) cytokines. Thus as putative modifiers of disease, the release of immunomodulatory or immunosuppressive molecules from helminths would be expected to impact on concurrent disorders in the host, and the stimulation of TH2 events has the potential to antagonize or inhibit diseases in which the immunopathology is driven by TH1 reactions. We have shown that infection with the rat tapeworm, *Hymenolepis diminuta*, protects mice from the colitic effects of direct instillation of dinitrobenzene sulfonic acid (DNBS) into the lumen of the colon [7]. A substantial amount of data has amassed showing that a variety of species of helminths



Helminth vs. Steroid Therapy in Mice with Colitis

Journal of Biomedicine and Biotechnology
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doi:10.1155/2010/384523

Gastroenterology

Worms Flop in Crohn's Disease

Published: Nov 8, 2013 (MedPageToday)

The German partner of Coronado Biosciences has terminated a clinical trial of *Trichuris suis ova* -- a whipworm parasite of pigs -- for Crohn's disease because of a lack of efficacy. This action was taken because of a recommendation of an independent data monitoring committee, which noted that no safety concerns had arisen during the study, known as TRUST-2. The committee conducted a second interim analysis of 240 patients who had been treated for 3 months in a phase II study conducted by Dr. Falk of Pharma GmbH. Coronado chief executive officer Harlan Weisman acknowledged that the company wasn't surprised at the disappointing results, because its own double-blind trial, TRUST-1, of helminth treatment in Crohn's also had shown inadequate efficacy. TRUST-1 had not met its primary endpoint of response, which was defined as a decrease of 100 points on the Crohn's Disease Activity Index, or a secondary endpoint of remission, or a score on the disease activity index of 150 or lower. "We believe [*Trichuris suis ova*] has therapeutic potential in other diseases and will continue to work diligently to advance its development for the treatment of autoimmune diseases," Weisman said in a statement. Parasitic helminths have evolved to live in their mammalian hosts, which respond with the release of several cytokines of the interleukin family and other immune cells such as eosinophils and mast cells. The overall response is similar to the Th2 component of the immune response. Epidemiologic studies have found that the prevalence of inflammatory bowel disease is highest in locales where helminthic infections no longer exist, and animal studies of initial clinical studies have suggested that induced infection might be protective against autoimmunity.

Mycobacterium avium Subspecies *paratuberculosis* Infection in Cases of Irritable Bowel Syndrome and Comparison with Crohn's Disease and Johne's Disease: Common Neural and Immune Pathogenicities[†]

Antonio M. Scana,¹ Tim J. Bull,⁴ Sara Cannas,² Jeremy D. Sanderson,³ Leonardo A. Sechi,² Giuseppe Dettori,¹ Stefania Zanetti,² and John Hermon-Taylor^{4*}

¹Instituto di Clinica Chirurgica Generale¹ and Dipartimento di Scienze Biomediche,² Sezione di Microbiologia Sperimentale e Clinica, Università degli Studi di Sassari, Sassari, Italy; Department of Gastroenterology, St Thomas' Hospital, Lambeth Palace Road, London SE1 7EH, United Kingdom³; and Department of Cardiovascular Sciences-Surgery, St. George's, University of London, Cranmer Terrace, London SW17 0RE, United Kingdom⁴

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Mycobacterium avium subsp. *paratuberculosis* causes Johne's disease, a systemic infection and chronic inflammation of the intestine that affects many species, including primates. Infection is widespread in livestock, and human populations are exposed. Johne's disease is associated with immune dysregulation, with involvement of the enteric nervous system overlapping with features of irritable bowel syndrome in humans. The present study was designed to look for an association between *Mycobacterium avium* subsp. *paratuberculosis* infection and irritable bowel syndrome. Mucosal biopsy specimens from the ileum and the ascending and descending colon were obtained from patients with irritable bowel syndrome attending the University of Sassari, Sassari, Sardinia, Italy. Crohn's disease and healthy control groups were also included. *Mycobacterium avium* subsp. *paratuberculosis* was detected by IS900 PCR with amplicon sequencing. Data on the potential risk factors for human exposure to these pathogens and on isolates from Sardinian dairy sheep were also obtained. *Mycobacterium avium* subsp. *paratuberculosis* was detected in 15 of 20 (75%) patients with irritable bowel syndrome, 3 of 20 (15%) healthy controls, and 20 of 23 (87%) people with Crohn's disease ($P = 0.0003$ for irritable bowel syndrome patients versus healthy controls and $P = 0.0000$ for Crohn's disease patients versus

($P = 0.0018$) between *Mycobacterium avium* subsp. *paratuberculosis* infection and the consumption of handmade cheese. *Mycobacterium avium* subsp. *paratuberculosis* is a candidate pathogen in the causation of a proportion of cases of irritable bowel syndrome as well as in Crohn's disease.

NO. INC_002944) is an established murine pathogen with the specific ability to cause Johne's disease, a systemic infection and chronic inflammation of the intestine of a range of histopathological types which can affect many animals, including primates (12, 14, 41). *Mycobacterium avium* subsp. *paratuberculosis* infection in cases of Johne's disease is associated with a chronic enteric neuritis (6, 29), together with immune activation and dysregulation (15, 16, 61, 65, 74, 75).

Subclinical *Mycobacterium avium* subsp. *paratuberculosis* infection is widespread in farm animals (38). Infected animals shed large numbers of *Mycobacterium avium* subsp. *paratuberculosis* cells into the environment, and there are wildlife reservoirs (1). These robust pathogens can survive for a long time in the environment and within environmental protists (43, 52). In some localities people are at risk of exposure from sources of environmental contamination (53, 71). People are also ex-

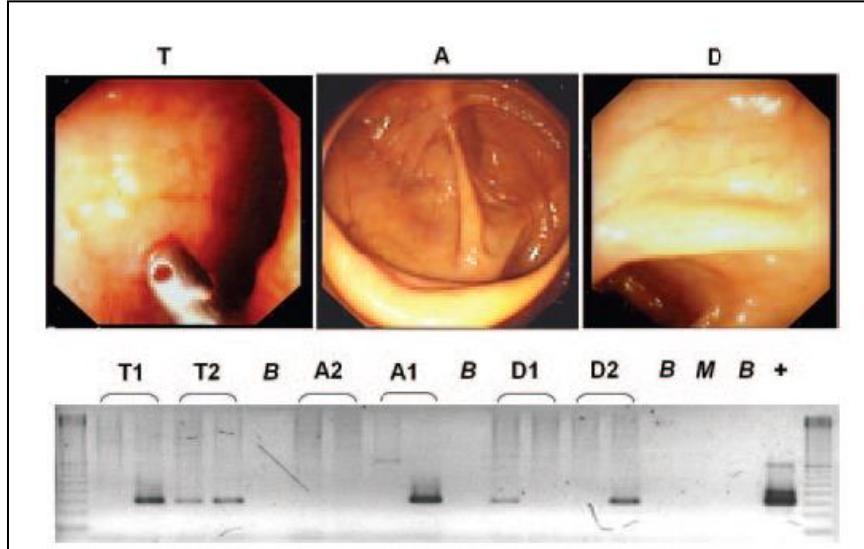
posed to specific association between *Mycobacterium avium* subsp. *paratuberculosis* infection and chronic inflammation of the intestine of the Crohn's disease type in humans (21).

Irritable bowel syndrome (IBS) (18) is a widespread abdominal condition that affects about 10 to 15% of people in the industrialized economies of Europe, North America, Australasia, and Japan, with a rising prevalence among the populations in the developing economies of Asia. The onset can be triggered by incidental enteric and systemic infections (66). IBS results in a substantial impairment of quality of life and has a major impact on health care costs and resource utilization (40, 50). The causes of IBS are unknown.

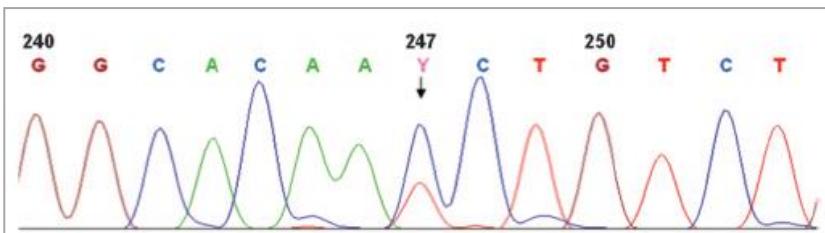
IBS is defined symptomatically by the persistence of abdominal discomfort or abdominal pain relieved by defecation, together with diarrhea, constipation, or a mixture of both, in the absence of detectable organic disease and with normal appearances at endoscopy. IBS is frequently accompanied by systemic symptoms, such as lethargy, back and muscle aches, headache, and urinary disorders. IBS overlaps symptomatically with microscopic colitis (37, 70). In recent years evidence of abnormalities affecting the enteric nervous system and its neurotransmitters in patients with IBS (2, 4, 11, 17, 19, 25, 69),

* Corresponding author. Mailing address: Department of Surgery, St. George's University of London, Cranmer Terrace, London SW17 0RE, United Kingdom. Phone: 44 (0)208 767 7631. Fax: 44 (0)208 725 2812. E-mail: jhermon@sug.ac.uk.

[†] Published ahead of print on 3 October 2007.



There was a significant association between *Mycobacterium avium* subsp. *paratuberculosis* infection and the consumption of handmade cheese. *Mycobacterium avium* subsp. *paratuberculosis* is a candidate pathogen in the causation of a proportion of cases of irritable bowel syndrome as well as in Crohn's disease.





OPEN ACCESS

ORIGINAL ARTICLE

A microbial signature for Crohn's disease

Victoria Pascal,¹ Marta Pozuelo,¹ Natalia Borruel,^{1,2} Francesc Casellas,^{1,2}
 David Campos,¹ Alba Santiago,¹ Xavier Martinez,¹ Encarna Varela,¹
 Guillaume Sarrabayrouse,¹ Kathleen Machiels,³ Severine Vermeire,³ Harry Sokol,⁴
 Francisco Guarner,^{1,2} Chaysavanh Manichanh^{1,2}

► Additional material is published online only. To view please visit the journal online (<http://dx.doi.org/10.1136/gutjnl-2016-313235>).

¹Department of Gastroenterology, Vall d'Hebron Research Institute, Barcelona, Spain
²CIBERehd, Instituto de Salud Carlos III, Madrid, Spain

³Department of Gastroenterology, University Hospital Gasthuisberg, Leuven, Belgium

⁴Department of Gastroenterology, AP-HP, Hôpital Saint-Antoine, Paris, France

Correspondence to
 Dr Chaysavanh Manichanh,
 Department of Gastroenterology, Vall d'Hebron Research Institute,
 Pg Vall d'Hebron, Barcelona 119-129, Spain;
 cmmanicha@gmail.com

VP and MP share co-first authorship

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ABSTRACT

Objective A decade of microbiome studies has linked IBD to an alteration in the gut microbial community of genetically predisposed subjects. However, existing profiles of gut microbiome dysbiosis in adult IBD patients are inconsistent among published studies, and did not allow the identification of microbial signatures for CD and UC. Here, we aimed to compare the faecal microbiome of CD with patients having UC and with non-IBD subjects in a longitudinal study.

Design We analysed a cohort of 2045 non-IBD and IBD faecal samples from four countries (Spain, Belgium, the UK and Germany), applied a 16S rRNA sequencing approach and analysed a total dataset of 115 million sequences.

Results In the Spanish cohort, dysbiosis was found significantly greater in patients with CD than with UC, as shown by a more reduced diversity, a less stable microbial community and eight microbial groups were proposed as a specific microbial signature for CD. Tested against the whole cohort, the signature achieved an overall sensitivity of 80% and a specificity of 94%, 94%, 89% and 91% for the detection of CD versus healthy controls, patients with anorexia, IBS and UC, respectively.

Conclusions Although UC and CD share many epidemiologic, immunologic, therapeutic and clinical features, our results showed that they are two distinct subtypes of IBD at the microbiome level. For the first time, we are proposing microbiomarkers to discriminate between CD and non-CD independently of geographical regions.

INTRODUCTION

CD and UC, the two main forms of IBD with a similar annual incidence (10–30 per 100 000 in Europe and North America), have both overlapping and distinct clinical pathological features.¹ Given that these conditions do not have a clear aetiology, diagnosis continues to be a challenge for physicians. Standard clinical testing to diagnose CD and UC includes blood tests and stool examination for biomarker quantification, endoscopy and biopsy. The diagnosis of IBD, particularly CD, can be missed or delayed due to the non-specific nature of both intestinal and extra-intestinal symptoms at presentation. In this regard, non-invasive, cost-effective, rapid and reproducible biomarkers would be helpful for patients and clinicians alike.

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How might it impact on clinical practice in the foreseeable future?

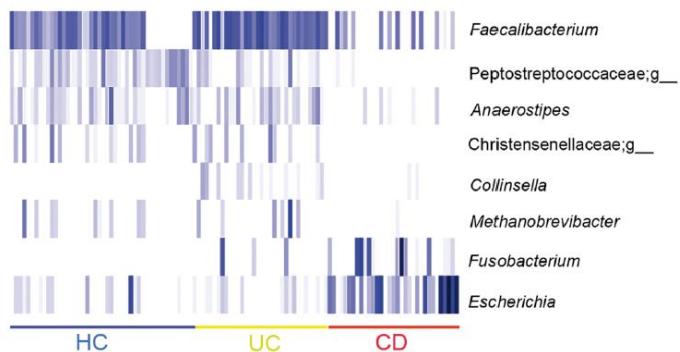
- Considering CD and UC as two distinct subtypes of IBD at the microbiome level could help designing specific therapeutic targets.
- The microbial signature specific to CD combined with either imaging techniques or calprotectin data could help decision-making when the diagnosis is initially uncertain among CD, UC and IBS.

the last two groups in higher relative abundance in CD.

How might it impact on clinical practice in the foreseeable future?

- Considering CD and UC as two distinct subtypes of IBD at the microbiome level could help designing specific therapeutic targets.
- The microbial signature specific to CD combined with either imaging techniques or calprotectin data could help decision-making when the diagnosis is initially uncertain among CD, UC and IBS.

Dysbiosis, which is an alteration of the gut microbial composition, has been reported in IBD over the last 10 years.^{2–5} Patients with IBD, in particular patients with CD, are associated with a



Healthy Villi of Small Intestine



Surprises from Celiac Disease

Study of a potentially fatal food-triggered disease has uncovered a process that may contribute to many autoimmune disorders • BY ALESSIO FASANO

KEY CONCEPTS

- Celiac disease (CD) is an autoimmune disorder triggered by ingestion of gluten, a major protein in wheat, or of related proteins in other grains.
- Research into the root causes indicates that the disorder develops when a person exposed to gluten also has a genetic susceptibility to CD and an unusually permeable intestinal wall.
- Surprisingly, essentially the same trio—an environmental trigger, a genetic susceptibility and a “leaky gut”—seems to underlie other autoimmune disorders as well. This finding raises the possibility that new treatments for CD may also ameliorate other conditions.

—The Editors

My vote for the most important scientific revolution of all time would trace back 10,000 years ago to the Middle East, where people first noticed that new plants arise from seeds falling to the ground from other plants—a realization that led to the birth of agriculture. Before that observation, the human race had based its diet on fruits, nuts, tubers and occasional meats. People had to move to where their food happened to be, putting them at the mercy of events and making long-term settlements impossible.

Once humans uncovered the secret of seeds, they quickly learned to domesticate crops, ultimately crossbreeding different grass plants to create such staple grains as wheat, rye and barley, which were nutritious, versatile, storable, and valuable for trade. For the first time, people were able to abandon the nomadic life and build cities. It is no coincidence that the first agricultural areas also became “cradles of civilization.”

This advancement, however, came at a dear price: the emergence of an illness now known as celiac disease (CD), which is triggered by ingesting a protein in wheat called gluten or eating similar proteins in rye and barley. Gluten and its relatives had previously been absent from the human diet. But once grains began fueling the

growth of stable communities, the proteins undoubtedly began killing people (often children) whose bodies reacted abnormally to them. Eating such proteins repeatedly would have eventually rendered sensitive individuals unable to properly absorb nutrients from food. Victims would also have come to suffer from recurrent abdominal pain and diarrhea and to display the emaciated bodies and swollen bellies of starving people. Impaired nutrition and a spectrum of other complications would have made their lives relatively short and miserable.

If these deaths were noticed at the time, the cause would have been a mystery. Over the past 20 years, however, scientists have pieced together a detailed understanding of CD. They now know that it is an autoimmune disorder, in which the immune system attacks the body's own tissues. And they know that the disease arises not only from exposure to gluten and its ilk but from a combination of factors, including predisposing genes and abnormalities in the structure of the small intestine.

What is more, CD provides an illuminating example of the way such a triad—an environmental trigger, susceptibility genes and a gut abnormality—may play a role in many autoimmune disorders. Research into CD has thus sug-

[THE AUTHOR]



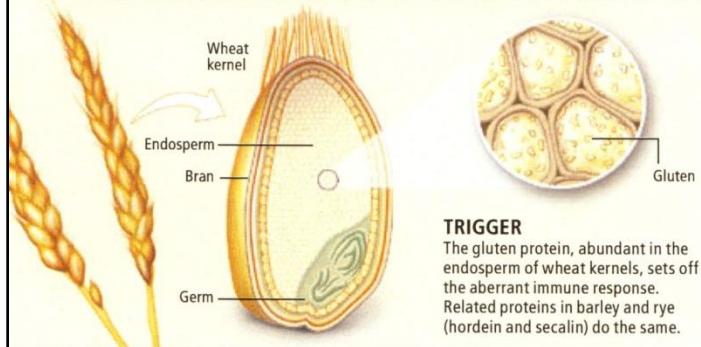
Alessio Fasano is professor of pediatrics, medicine and physiology and director of the Mucosal Biology Research Center and the Center for Celiac Research at the University of Maryland School of Medicine. Much of his basic and clinical research focuses on the role of intestinal permeability in the development of celiac disease and other autoimmune disorders.



The theory that a leaky gut contributes to CD and autoimmunity in general was initially greeted with skepticism.

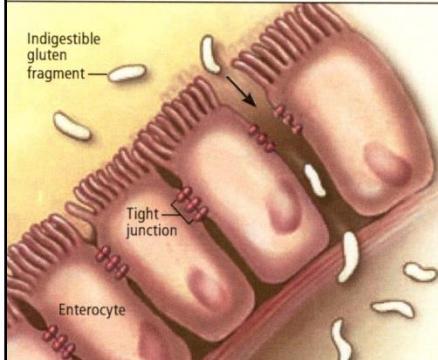
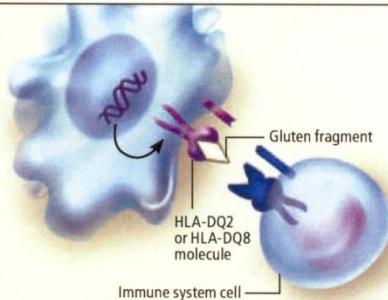
A TRIO OF CAUSES

Three factors underlie celiac disease: an environmental trigger, a genetic susceptibility and, according to the author's research, an unusually permeable gut (*below*). The author suspects that the same basic triad contributes to other autoimmune diseases, although each disorder will have its own triggers and genetic components.

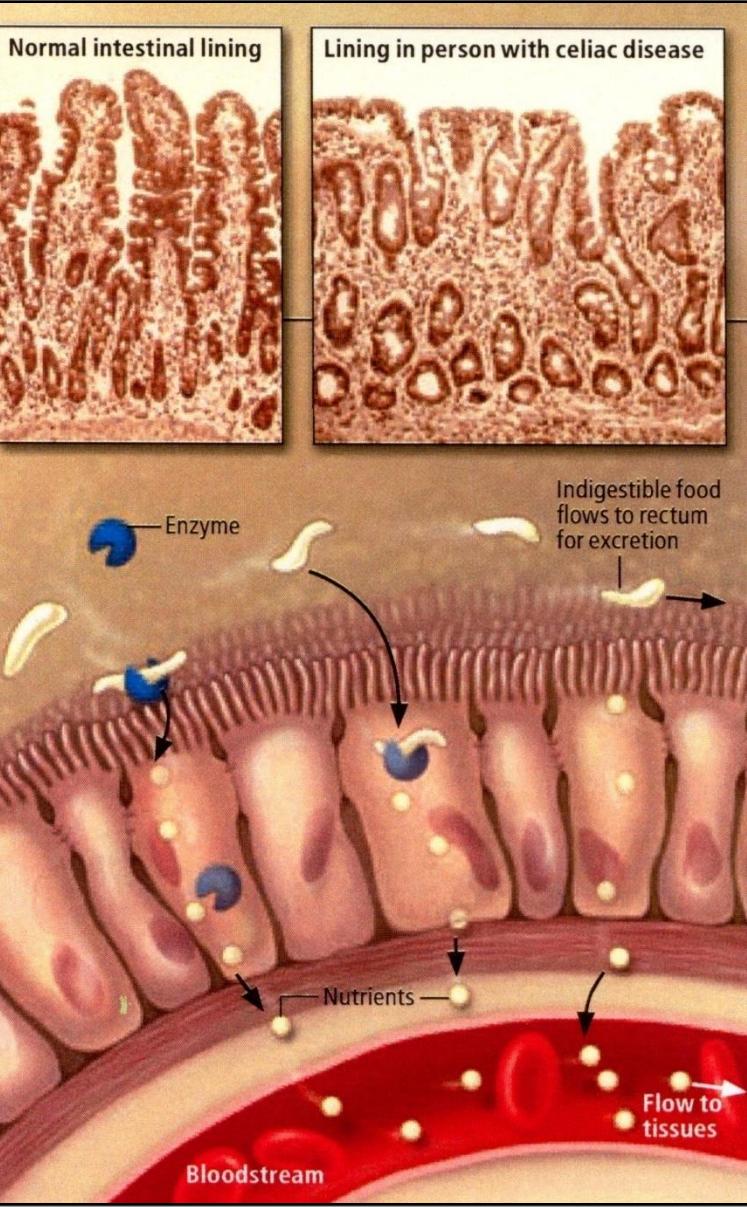


GENETIC PREDISPOSITION

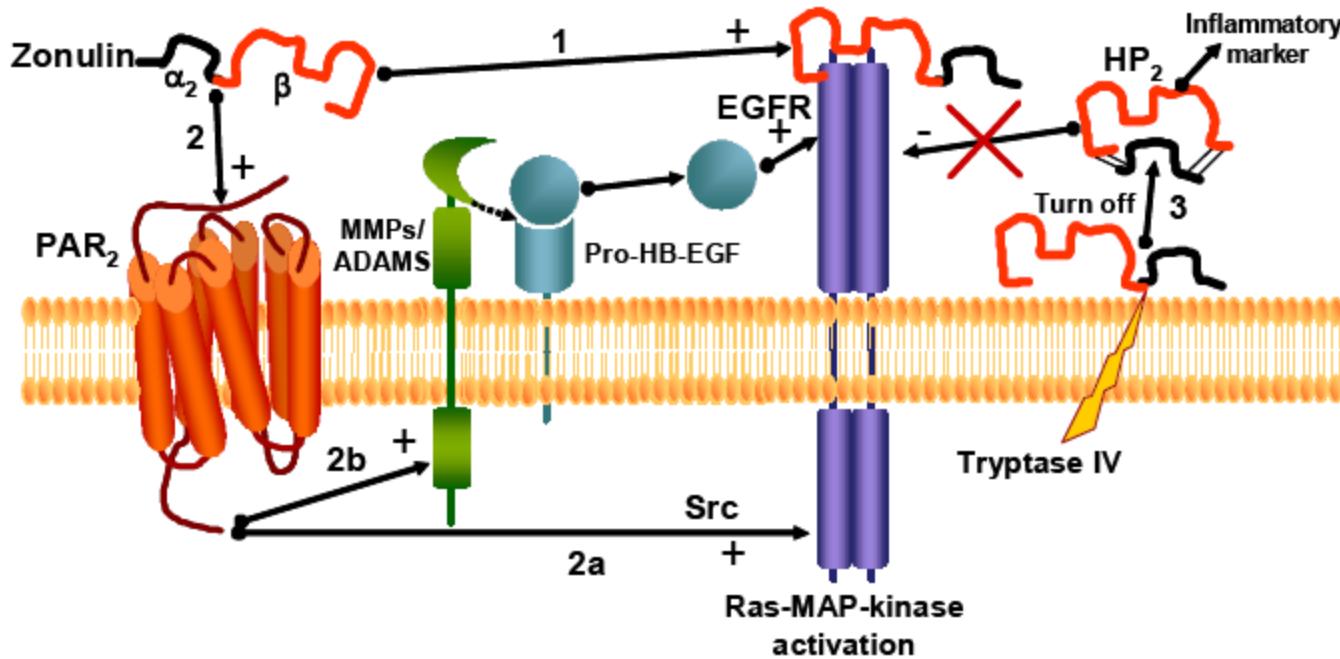
Almost all patients harbor a gene for either the HLA-DQ2 protein or the HLA-DQ8 protein, or both. These HLA molecules display gluten fragments to immune system cells, which then direct an attack on the intestinal lining. Other genes are likely to be involved as well, but these additional culprits may differ from person to person.



LEAKY SMALL INTESTINE
In most people, links known as tight junctions "glue" intestinal cells together. In those with celiac disease, the junctions come apart, allowing a large amount of indigestible gluten fragments to seep into the underlying tissue and incite immune system cells. Treatments that reduced leakiness could potentially ease not only celiac disease but also other autoimmune disorders involving unusually permeable intestines.



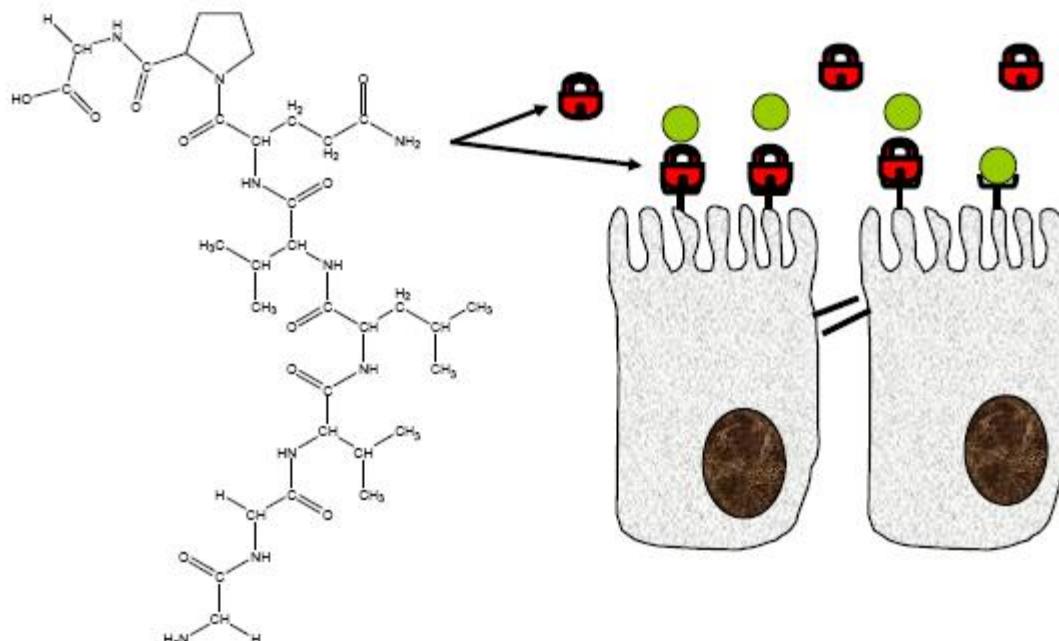
Zonulin Signaling Working Hypothesis



Proposed mechanisms through which zonulin activates EGFR. Zonulin can activate EGFR through direct binding (1) and/or through PAR2 transactivation (2). This second mechanism can be mediated by either Src signaling (2a) or by the release of MMPs and/or ADAMS that in turn will activate Pro-HB-EGF. When cell tryptase IV cleaves zonulin in its two subunits (so eliminating one of the three required disulfide bridges necessary for EGF activity), the molecule is not able to bind to EGFR (3), while will acquire a different function (Hb binding) and becomes an inflammatory marker.

Tripathi et al, PNAS 2009;106:16799-804.

AT1001, the Zonulin Inhibitor



$$\text{C}_{22}\text{H}_{34}\text{N}_6\text{O}_{10}$$

Exact Mass: 725.41

Mol. Wt.: 725.8

m/e: 725.41 (100.0%), 726.41 (35.6%), 727.41 (9.2%), 726.40 (3.3%)
 C, 52.95; H, 7.64; N, 17.37; O, 22.04

Alba Clinical Trial Summary in Celiac Disease with Larazotide Acetate – Tight Junction Regulator

- Phase Ib - Single Dose (CLIN1001-002)
 - 21 Celiac disease subjects
 - Double blind, placebo controlled
 - 3 days QD, single gluten challenge on day 2
 - In-patient study
 - Completed March 2006
 - Phase IIA - Multiple Dose (CLIN1001-004)
 - 86 celiac disease subjects
 - Double blind, placebo controlled
 - 2 weeks TID dosing and gluten challenge
 - Dose ranging - 7 arms
 - Multi-center Outpatient Study
 - Completed March 2007
 - Phase IIB - Multiple Dose (CLIN1001-006)
 - 184 celiac disease subjects
 - Double blind, placebo controlled
 - 6 weeks TID dosing and gluten challenge
 - Dose ranging - 4 arms
 - Multi-center Outpatient Study
- 
- 0% Bioavailability**
- No Adverse Safety Trends**
- Larazotide acetate acts locally in the gastrointestinal tract
 - No systemic exposure, no measurable plasma drug levels in any clinical study
 - No immunogenicity, no antibody development in any clinical study
 - No toxicity observed to date in 24 completed animal toxicology studies
 - No safety signals in ~500 celiac subjects exposed to larazotide acetate up to 8 weeks
 - To date, safety comparable to placebo

	Result		Range
Immune Response			
Secretory IgA	598		510-2040 ug/g
Anti-gliadin sIgA	191	High	0-100 U/g
Inflammation			
Calprotectin	78	High	<50 ug/g
Digestion			
Elastase 1	441		>200 ug/g
Steatocrit	13		<15%
Additional tests			
b-Glucuronidase	1955		<1123 U/ml
Occult blood	Negative		Negative
Zonulin	228	High	15-107 ng/g

Zolulin and Gut Bugs

Host-Dependent Zonulin Secretion Causes the Impairment of the Small Intestine Barrier Function After Bacterial Exposure

RAHZI EL ASMAR,^{*,†} PINAKI PANIGRAHI,^{*} PENELOPE BAMPFORD,^{*} IRENE BERTI,[§] TARCISIO NOT,[§] GIOVANNI V. COPPA,[†] CARLO CATASSI,[†] and ALESSIO FASANO^{*}

^{*}Department of Pediatrics and Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland; [†]Istituto Clinico Pediatrica, Università di Ancona, Ancona, Italy; and [§]Istituto Burlo Garofalo, Trieste, Italy

Background & Aims: Enteric infections have been implicated in the pathogenesis of both food intolerance and autoimmune diseases secondary to the impairment of the intestinal barrier. On the basis of our recent discovery of zonulin, a modulator of small-intestinal tight junctions, we asked whether microorganisms might induce zonulin secretion and increased small-intestinal permeability. **Methods:** Both ex vivo mammalian small intestines and intestinal cell monolayers were exposed to either pathogenic or nonpathogenic enterobacteria. Zonulin production and changes in paracellular permeability were monitored in Ussing chambers and micro-snapwells. Zonula occludens 1 protein redistribution after bacteria colonization was evaluated on cell monolayers. **Results:** Small intestines exposed to enteric bacteria secreted zonulin. This secretion was independent of either the species of the small intestines or the virulence of the microorganisms tested, occurred only on the luminal aspect of the bacteria-exposed small-intestinal mucosa, and was followed by a decrease in small-intestinal tissue resistance (transepithelial electrical resistance). The transepithelial electrical resistance decrement was secondary to the zonulin-induced tight junction disassembly, as also shown by the disengagement of the protein zonula occludens 1 protein from the tight junctional complex. **Conclusions:** This zonulin-driven opening of the paracellular pathway may represent a defensive mechanism, which flushes out microorganisms and contributes to the host response against bacterial colonization of the small intestine.

The intestinal epithelium is the largest epithelial mucosal layer and provides an interface between the external environment and the mammalian host.¹ Healthy, mature small-intestinal mucosa, with its intact tight junctions, serves as the main barrier to the passage of macromolecules. Further, it functions as the major organ of defense against foreign antigens, toxins, and macromolecules entering the host via the oral/enteric route.² Under physiological conditions, small but immunologically active antigens transverse the intestinal bar-

rier,³ resulting in antigen-specific immune responses.⁴ This passage occurs through the paracellular pathway and involves a subtle but sophisticated regulation of intercellular tight junctions that leads to antigen tolerance.^{5,6} When the integrity of the tight junctions system is compromised, such as in preterm babies or after exposure to radiation, chemotherapy, and/or toxins,⁷ an immune response to environmental antigens may develop. Whereas tight-junction structure and function have been thoroughly investigated, their physiological regulation has been only partially elucidated.⁸ We have recently described a novel protein, zonulin,⁹ a eukaryotic analogue of the *Vibrio cholerae*-derived zonula occludens toxin (Zot),¹⁰ which modulates small-intestinal tight-junction permeability through a protein kinase C α -mediated actin polymerization.¹¹

In the absence of enteric infections, the mammalian proximal small intestine is virtually sterile. The colonization of the small intestine by enteric microorganisms (even without apparent mucosal damage or elaboration of specific toxins) typically leads to a more permeable intestine that permits the passage of macromolecules and antigens that may cause immune-mediated pathologic conditions.¹² To date, there is no clear explanation for the disturbed physiological regulation of the intestinal permeability secondary to proximal bacterial contamination. In this study, we tested both nonpathogenic and pathogenic bacteria for their ability to induce zonulin secretion by the mammalian gut (small intestine and colon). In an attempt to address species specificity, we used nonpathogenic bacteria as potential inducers of zonulin release in ex vivo experiments by use of intestinal segments from multiple mammalian species, including nonhuman pri-

Abbreviations used in this paper: DMEM, Dulbecco's modified Eagle medium; ELISA, enzyme-linked immunosorbent assay; TEER, transepithelial electrical resistance; TNF, tumor necrosis factor; ZO1, zonula occludens 1 protein; Zot, zonula occludens toxin.

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0016-5085/02/\$35.00
doi:10.1053/gast.2002.36578

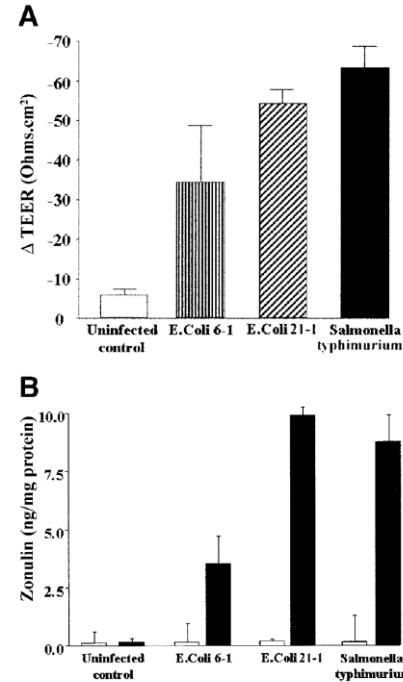


Figure 1. (A) TEER changes of unstripped rabbit small intestine mounted in the micro-snapwell system and exposed for 3 hours to nonpathogenic *Escherichia coli* 6-1, pathogenic *E. coli* 21-1, or *Salmonella typhimurium*. Uninfected controls are shown for comparison. The average baseline TEER was $120 \Omega/\text{cm}^2$; $n = 4$. (B) Zonulin concentration in the media collected from the lower chamber (serosal side, open bars) or upper chamber (mucosal side, closed bars) of rabbit small-intestinal tissues mounted in the micro-snapwell system and incubated for 3 hours with *E. coli* 6-1, pathogenic *E. coli* 21-1, or *S. typhimurium* added to the mucosal aspect of the intestine. Uninfected tissues are shown for comparison; $n = 4$.

Zolulin and Gut Bugs

- “Dr. Fasano’s group has also published a study showing that bacteria such as *E. coli* and *Salmonella* stimulate zonulin production in isolated intestinal tissue, and another recent study showed that short-term inoculation of rats with *E. coli* and *Shigella* enhanced the ability of gluten to cause intestinal damage while inoculation with *Bifidus* bacteria virtually eliminated gluten’s ability to cause damage. Neither of these studies show that dysbiosis contributes to celiac, or that it is responsible for the persistence of zonulin production on a gluten-free diet, but they offer strong support to the plausibility of these hypotheses.”(Chris Masterjohn, Ph.D.)

Changes in intestinal microbiota composition and metabolism coincide with increased intestinal permeability in young adults under prolonged physiologic stress.

Karl JP¹, Margolis LM², Madslien EH³, Murphy NE², Castellani JW⁴, Gundersen Y⁵, Hoke AV⁶, Levangie MW⁶, Kumar R⁷, Chakraborty N, Gautam A⁶, Hammamieh R⁸, Martini S³, Montain SJ⁴, Pasiakos SM².

Author information

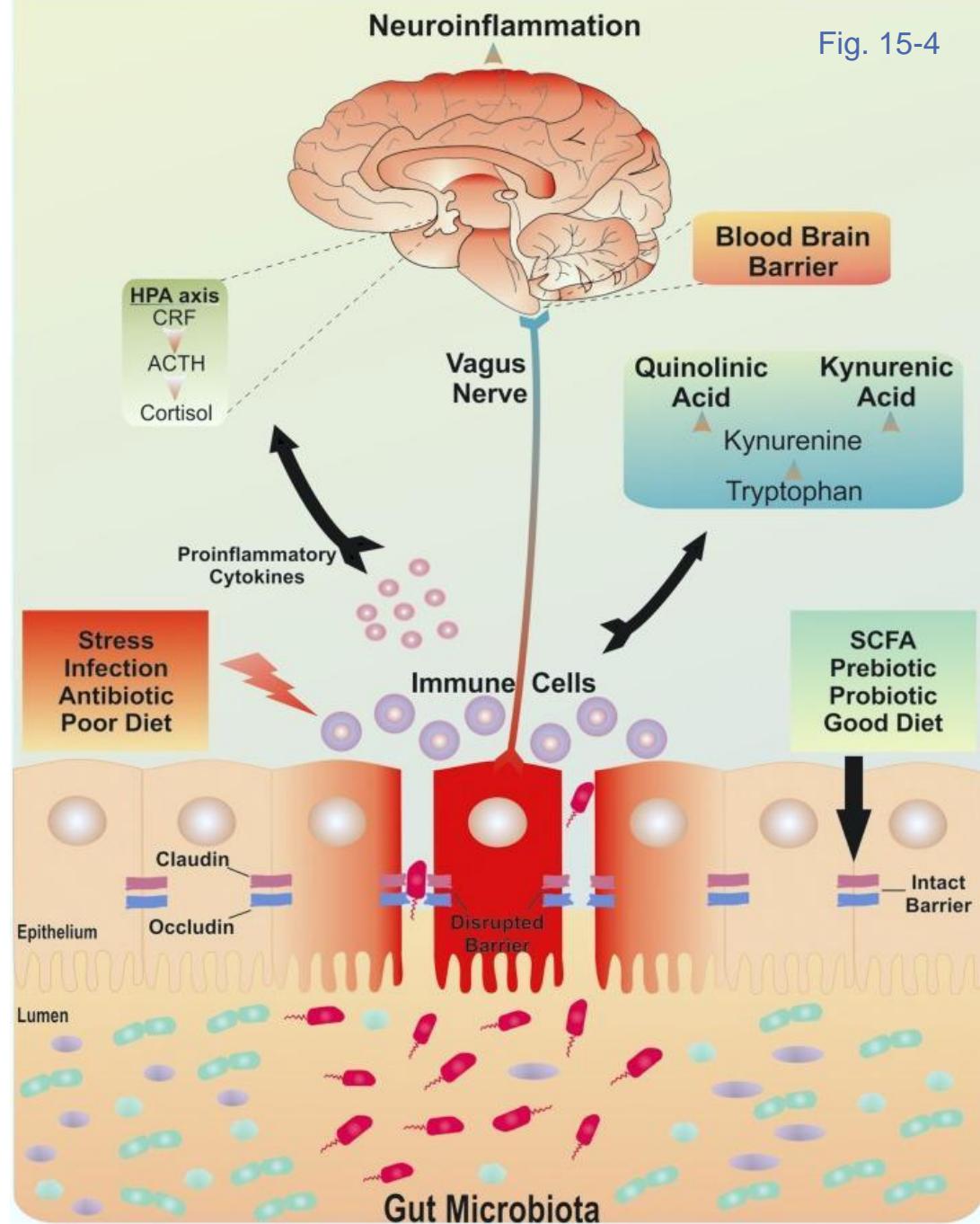
Abstract

The magnitude, temporal dynamics, and **physiologic effects of intestinal microbiome responses to physiologic stress** are poorly characterized. This study used a systems biology approach and multiple-stressor military training environment to determine the effects of **physiologic stress** on **intestinal microbiota composition** and metabolic activity, and **intestinal permeability (IP)**. 73 Soldiers were provided three rations/d with or without protein- or carbohydrate-based supplements during a four day cross-country ski march (**STRESS**). IP was measured before and during **STRESS**. Blood and stool samples were collected before and after **STRESS** to measure inflammation, stool **microbiota**, and stool and plasma global metabolite profiles. IP **increased** $62\% \pm 57\%$ (mean \pm SD, $P<0.001$) during **STRESS** independent of diet group, and was associated with **increased** inflammation. **Intestinal microbiota** responses were characterized by **increased** α -diversity, and **changes** in the relative abundance of >50% of identified genera, including **increased** abundances of less dominant taxa at the expense of more dominant taxa such as *Bacteroides*. **Changes in intestinal microbiota composition** were linked to 23% of metabolites that were significantly altered in stool after **STRESS**. Pre-**STRESS** *Actinobacteria* relative abundance, and **changes** in serum IL-6 and stool cysteine concentrations, collectively, accounted for 84% of the variability in the change in IP. Findings demonstrate that a multiple-stressor military training environment induced increases in IP that were associated with alterations in markers of inflammation, and with **intestinal microbiota composition and metabolism**. Observed associations between IP, the pre-stress **microbiota**, and **microbiota** metabolites suggest targeting the **intestinal microbiota** could provide novel strategies for preserving IP during **physiologic stress**.

Stress Induced Increase in GI Permeability

Kelly JR, Kennedy PJ, Cryan JF, Dinan TG, Clarke G, Hyland NP. Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders. *Front Cell Neurosci.* 2015 Oct 14;9:392. doi: 10.3389/fncel.2015.00392. Review. PubMed PMID: 26528128; PubMed Central PMCID: PMC4604320.
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4604320/>

Fig. 15-4



Example Natural GI Mucosal Repair Formulation

	Amounts per serving
Serving size	1 tsp. (6 g)
Number of servings per container	40
L-Glutamine	1500 mg
N-Acetyl Glucosamine	1000 mg
PepZin GI (Zinc-Carnosine)	75 mg
Deglycyrrhizinated Licorice (DGL)(Glycyrrhiza glabra)	400 mg.
Aloe vera (Aloe barbadensis)	300 mg
Slippery Elm (Ulmus fulva)	200 mg
Marshmallow (Althea officinalis)	100 mg
Chamomile (Matricaria chamomilus)	100 mg
Okra (Hibiscus esculentus)	100 mg
Cat's Claw (Uncaria tomentosa-TOA free)	100 mg
Mucin	200 mg
MSM	100 mg
Quercitin	100 mg
Prunus (concentrate)	100 mg
Citrus pectin	1000 mg
Stevia	
Natural Flavors	

Suggested Dose: Take 1/2-1 tsp., one to two times daily or as directed by your health care practitioner.

1: [Aliment Pharmacol Ther.](#) 2000 Dec;14(12):1567-79.

A pilot study of N-acetyl glucosamine, a nutritional substrate for glycosaminoglycan synthesis, in paediatric chronic inflammatory bowel disease.

Salvatore S, Heuschkel R, Tomlin S, Davies SE, Edwards S, Walker-Smith JA, French I, Murch SH.

University Department of Paediatric Gastroenterology, Royal Free, London, UK.

BACKGROUND: The breakdown of glycosaminoglycans is an important consequence of inflammation at mucosal surfaces, and inhibition of metalloprotease activity may be effective in treating chronic inflammation. **AIM:** To report an alternative approach, using the nutriceutical agent N-acetyl glucosamine (GlcNAc), an amino-sugar directly incorporated into glycosaminoglycans and glycoproteins, as a substrate for tissue repair mechanisms. **METHODS:** GlcNAc (total daily dose 3-6 g) was administered orally as adjunct therapy to 12 children with severe treatment-resistant inflammatory bowel disease (10 Crohn's disease, 2 ulcerative colitis). Seven of these children suffered from symptomatic strictures. In addition, similar doses were administered rectally as sole therapy in nine children with distal ulcerative colitis or proctitis resistant to steroids and antibiotics. Where pre- and post-treatment biopsies were available (nine cases), histochemical assessment of epithelial and matrix glycosaminoglycans and GlcNAc residues was made. **FINDINGS:** Eight of the children given oral GlcNAc showed clear improvement, while four required resection. Of the children with symptomatic Crohn's stricture, only 3 of 7 have required surgery over 1 year. Rectal administration induced improvement in all children. There was evidence of histological improvement in all children receiving GlcNAc. **CONCLUSIONS:** GlcNAc shows promise as an inexpensive and nontoxic treatment in chronic inflammatory bowel disease, with a mode of action which is distinct from conventional treatments. It may have the potential to be helpful in stricturing disease.

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Sugar supplement may treat immune disease

07 June 2007 by Aria Pearson

Magazine issue 2607. [Subscribe](#) and get 4 free issues.

A sugar supplement may sweeten the overactive immune cells responsible for autoimmune diseases such as multiple sclerosis (MS) and type 1 diabetes and stop them attacking the body's tissues.

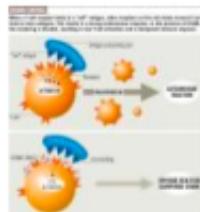
Autoimmune diseases are triggered when receptors on the outside of immune cells called T-helper 1 (Th1) cells start binding "self" antigens rather than pieces of foreign invaders. Anything that decreases the amount of binding should suppress the autoimmune response.

Previous studies suggested that glucosamine, a dietary supplement commonly taken by people with osteoarthritis, has some immunosuppressive effects. This led Michael Demetriou and colleagues at the University of California, Irvine, to investigate a similar but more potent compound called N-acetylglucosamine (GlcNAc).

A large number of proteins in the body are modified by the attachment of sugar molecules to their surface through a process called glycosylation, and altered glycosylation has been implicated in some autoimmune diseases. Demetriou's team found that naturally occurring GlcNAc molecules attach to T-cell receptors and these GlcNAc "branches" form a lattice on the cell surface that prevents the receptors from clustering near where the antigens are located (see Diagram). Less clustering means less antigen binding, and less activation of Th1 cells, reducing the autoimmune reaction.

Mice given oral GlcNAc supplements had twice as much GlcNAc branching on their T-cell receptors as untreated mice. The researchers also found that T-cells engineered to cause the mouse equivalent of MS failed to do so if they had been incubated in GlcNAc first. A daily oral dose of GlcNAc also prevented type 1 diabetes in mice genetically engineered to develop the disease (*The Journal of Biological Chemistry*, DOI: 10.1074/jbc.M701890200).

T-cells engineered to cause the mouse equivalent of multiple sclerosis failed to do so if they had been incubated in GlcNAc



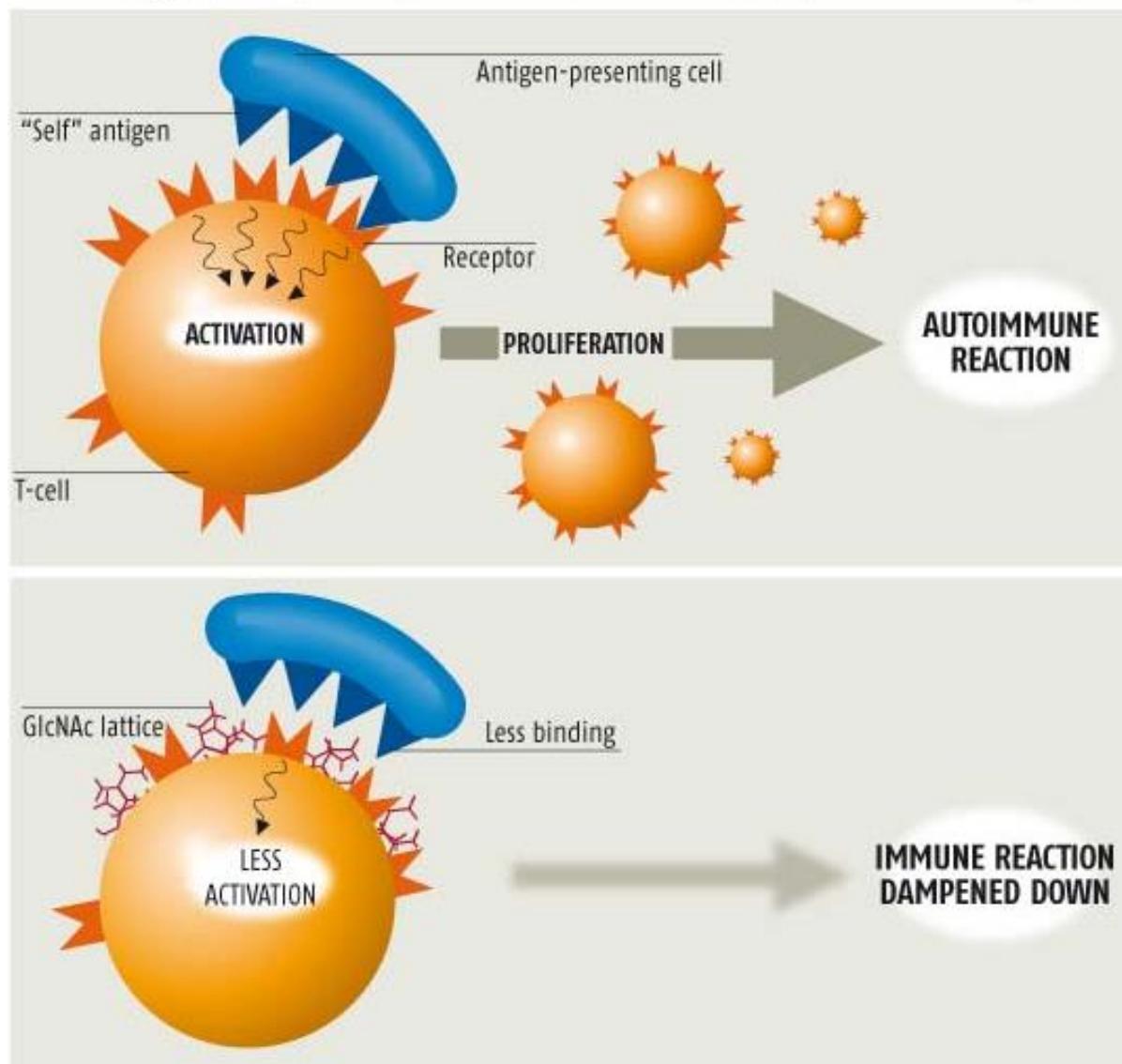
Crowd control
[Enlarge image](#)

The researchers found that naturally occurring GlcNAc molecules attach to T-cell receptors and these GlcNAc "branches" form a lattice on the cell surface that prevents the receptors from clustering near where the antigens are located... less clustering means less antigen binding, and less activation of Th1 cells, reducing the autoimmune reaction.

The Journal of Biological Chemistry, DOI: 10.1074/jbc.M701890200).

CROWD CONTROL

When a T-cell receptor binds to a "self" antigen, other receptors on the cell cluster around it and bind to more antigens. This results in a strong autoimmune response. In the presence of GlcNAc the clustering is blocked, resulting in less T-cell activation and a damped immune response



Invited Review

A Review of Complementary and Alternative Approaches to Immunomodulation

John O. Clarke, MD; and Gerard E. Mullin, MD

Division of Gastroenterology, The Johns Hopkins University School of Medicine, Baltimore, Maryland

ABSTRACT: Current Western therapies for inflammatory diseases are suboptimal; increasingly, patients are turning to complementary and alternative medicine for symptom relief and improved quality of life. There is emerging evidence that many of these therapies have the ability to modulate the immune system and disrupt the proinflammatory cascade through a variety of mechanisms, including antioxidant effects, alterations in cell signaling (in particular the nuclear factor (NF)- κ B pathway), cytokines, proinflammatory mediators, and disruption of bacterial flora. Using inflammatory bowel disease (IBD) as a model of inflammation, we explore the principal complementary and alternative medicine treatments that show promise in this regard, namely, resveratrol, green tea, curcumin, boswellia, fish oil, vitamin D, and probiotics. With each agent, we detail the mechanisms that have been described with regard to immune modulation, discuss the medical conditions for which it has been evaluated, and explore the data to date for the prevention or treatment of IBD.

The majority of reimbursed care in the United States today is via Western medicine, a tradition that harkens back, in a primitive form, only to the Renaissance. Complementary and alternative medicine (CAM) refers to medical practices that are not currently considered to be part of conventional medicine. However, these "alternative" and "natural" approaches have significant time-proven history, just not in Western literature. Traditional Chinese medicine stretches back 5000 years, and traditional Indian (Ayurvedic) medicine can trace its history for over 2000 years. At the start of the 20th century, in

fact, there were already 30,000–40,000 books regarding these practices already in existence.

With all the focus on drug development and marketing, it is easy to forget that nutrition represents the world's earliest medicinal therapy. In the words of Hippocrates (obviously translated) "He who does not know food—how can he cure the disease of man?" Many of the medicinal agents used for therapy today are directly derived from food sources. The role of functional foods in health and disease prevention is a rapidly growing field.¹ Who knows how many other agents are present in everyday foods that have not yet been tapped?

This article will aim to clarify what is known about alternative and nutrition therapies for immunomodulation. Obviously, this is a broad therapy, and so discussion will be restricted to a few key categories: polyphenols (including resveratrol, epigallocatechin, curcumin, and boswellia), ω -3 essential fatty acids (EFA; fish oil), vitamin D, and probiotics. Although many diseases can be examined as a model for inflammation (including inflammatory bowel disease [IBD], rheumatoid arthritis, and multiple sclerosis, to name a few), we have elected to focus on IBD exclusively because: (a) we are gastroenterologists and this is our bias, and (b) to dwell on every inflammatory condition would make this paper too unwieldy to be readable without coercion.

In the words of Hippocrates: "Let food be thy medicine."

Polyphenols

Polyphenols are phytochemicals that are found in food substances produced from plants. Polyphenols are separated from essential micronutrients in that a deficiency state has not been identified; nevertheless, these chemicals are believed to play a biologically active role and have been shown to be potentially immunomodulating.² Although numerous polyphenols have been identified, 4 in particular have a preponderance of evidence in the role of immune modulation and will be addressed in this review: resveratrol, epigallocatechin, curcumin, and boswellia. The findings of polyphenols to prevent and treat animal models of IBD are summarized in Table 1.^{3–22}

Correspondence: Gerard E. Mullin, MD, The Johns Hopkins Hospital, Division of Gastroenterology, 600 North Wolfe Street, Carnegie Building, Room 464, Baltimore, MD 21287. Electronic mail may be sent to gmullin1@jhu.edu.

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Nutrition in Clinical Practice 25:49–62, February 2008
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Using inflammatory bowel disease (IBD) as a model of inflammation, we explore the principal complementary and alternative medicine treatments that show promise in this regard, namely, resveratrol, green tea, curcumin, boswellia, fish oil, vitamin D, and probiotics.

Novel role of the vitamin D receptor in maintaining the integrity of the intestinal mucosal barrier

Juan Kong,¹ Zhongyi Zhang,¹ Mark W. Musch,¹ Gang Ning,² Jun Sun,³ John Hart,⁴ Marc Bissonnette,¹ and Yan Chun Li¹

Departments of ¹Medicine and ⁴Pathology, The University of Chicago, Chicago, Illinois; ²The Huck Institutes for Life Sciences, The Pennsylvania State University, University Park, Pennsylvania; and ³Gastroenterology and Hepatology Division, Department of Medicine, University of Rochester Medical Center, Rochester, New York

Submitted 31 August 2007; accepted in final form 23 October 2007

Kong J, Zhang Z, Musch MW, Ning G, Sun J, Hart J, Bissonnette M, Li YC. Novel role of the vitamin D receptor in maintaining the integrity of the intestinal mucosal barrier. *Am J Physiol Gastrointest Liver Physiol* 294: G208–G216, 2008. First published October 25, 2007; doi:10.1152/ajpgi.00398.2007.—Emerging evidence supports a pathological link between vitamin D deficiency and the risk of inflammatory bowel disease (IBD). To explore the mechanisms we used the dextran sulfate sodium (DSS)-induced colitis model to investigate the role of the vitamin D receptor (VDR) in mucosal barrier homeostasis. While VDR^{+/+} mice were mostly resistant to 2.5% DSS, VDR^{-/-} mice developed severe diarrhea, rectal bleeding, and marked body weight loss, leading to death in 2 wk. Histological examination revealed extensive ulceration and impaired wound healing in the colonic epithelium of DSS-treated VDR^{-/-} mice. Severe ulceration in VDR^{-/-} mice was preceded by a greater loss of intestinal transepithelial electric resistance (TER) compared with VDR^{+/+} mice. Confocal and electron microscopy (EM) revealed severe disruption in epithelial junctions in VDR^{-/-} mice after 3-day DSS treatment. Therefore, VDR^{-/-} mice were much more susceptible to DSS-induced mucosal injury than VDR^{+/+} mice. In cell cultures, 1,25-dihydroxy-vitamin D₃ [1,25(OH)₂D₃] markedly enhanced tight junctions formed by Caco-2 monolayers by increasing junction protein expression and TER and preserved the structural integrity of tight junctions in the presence of DSS. VDR knockdown with small interfering (si)RNA reduced the junction proteins and TER in Caco-2 monolayers. 1,25(OH)₂D₃ can also stimulate epithelial cell migration *in vitro*. These observations suggest that VDR plays a critical role in mucosal barrier homeostasis by preserving the integrity of junction complexes and the healing capacity of the colonic epithelium. Therefore, vitamin D deficiency may compromise the mucosal barrier, leading to increased susceptibility to mucosal damage and increased risk of IBD.

tight junction; inflammatory bowel disease; dextran sulfate sodium

THE INTESTINAL EPITHELIAL barrier consists of epithelial cells and the intercellular junctions. The barrier regulates macromolecule trafficking between the lumen and the internal milieu and protects the host by preventing harmful solutes, microorganisms, toxins, and luminal antigens from entering the body (40). Compromise or disruption of the intestinal barrier function causes deleterious effects and results in exposure of the host to luminal antigens and bacteria, leading to inflammation. Impaired barrier functions have been described in a number of common gastrointestinal disorders, including inflammatory bowel disease (IBD) (7).

Vitamin D and the Gut

In vitro experiments demonstrate that VDR mediates the activity of 1,25(OH)2D3 that induces junction protein expression and strengthens the tight junction complex. These data are consistent with, and explain at least in part, the observation reported in the literature that vitamin D deficiency is linked to increased incidence of IBD in human population.

The integrity of the intestinal mucosal barrier is preserved by the enormous regenerating capacity of the mucosal epithelium. The intestinal stem cells, located at the base of the crypt, are responsible for replenishing the epithelium through cell division and differentiation. After extensive destruction, rapid resealing of the surface epithelium is accomplished by epithelial cell restitution, proliferation, and differentiation (6). Another important component of the mucosal barrier is the apical and subapical intercellular junctions between the epithelial cells, namely tight junctions and adherens junctions (18). These junction structures seal the paracellular space and regulate the permeability of the mucosal barrier.

IBD, including Crohn's disease and ulcerative colitis, is a major chronic disorder affecting the gastrointestinal tract in humans. Although the etiopathogenesis of IBD has not been clearly elucidated, it is thought to involve a complex interplay among genetic, environmental, microbial, and immune factors (33). One potential pathogenic factor is impaired mucosal barrier function, and intestinal hyperpermeability is common in IBD patients (11). A relatively high number of first degree relatives of patients with Crohn's disease have increased intestinal permeability in the absence of clinical symptoms (32, 42), suggesting barrier dysfunction precedes, or is at least a very early defect, in the disease process that might require genetic predisposition and environmental triggers. Indeed, previous studies have demonstrated decreased expression and differential localization of junction complex proteins in the mucosa of patients with IBD (10, 16, 29). Therefore, dysregulation of junction proteins is an important pathogenic mechanism underlying the increased permeability seen in the intestinal epithelium of IBD patients.

Previous studies have suggested a link between vitamin D deficiency and IBD risk (23). The prevalence of IBD exhibits a north-south gradient (24), paralleling sunlight exposure, an important source of vitamin D. Populations near the equator are at relatively lower risk for developing IBD. Seasonal variations in the onset and exacerbation of IBD have also been reported (27, 36) with high incidence in the winter. Early studies have reported a high prevalence of vitamin D deficiency in patients with established Crohn's disease (12, 38). Decreased vitamin D levels have also been detected in patients with newly diagnosed IBD (17, 19, 35). In the IL-10^{-/-} mouse model of intestinal inflammation, vitamin D deficiency or vitamin D receptor (VDR) deficiency exacerbates the symp-

Vitamin D: An Antimicrobial?

www.medscape.com

From Future Microbiology

The Vitamin D-antimicrobial Peptide Pathway and Its Role in Protection against Infection

Adrian F Gombart

Posted: 12/11/2009; Future Microbiology. 2009;4(9):1151-1165. © 2009

Abstract and Introduction

Abstract

Vitamin D deficiency has been correlated with increased rates of infection. Since the early 19th century, both environmental (i.e., sunlight) and dietary sources (cod liver) of vitamin D have been identified as treatments for TB. The recent discovery that vitamin D induces antimicrobial peptide gene expression explains, in part, the 'antibiotic' effect of vitamin D and has greatly renewed interest in the ability of vitamin D to improve immune function. Subsequent work indicates that this regulation is biologically important for the response of the innate immune system to wounds and infection and that deficiency may lead to suboptimal responses toward bacterial and viral infections. The regulation of the cathelicidin antimicrobial peptide gene is a human/primate-specific adaptation and is not conserv

low-affinity receptor selection to place the xenobiotic response regulation and lead

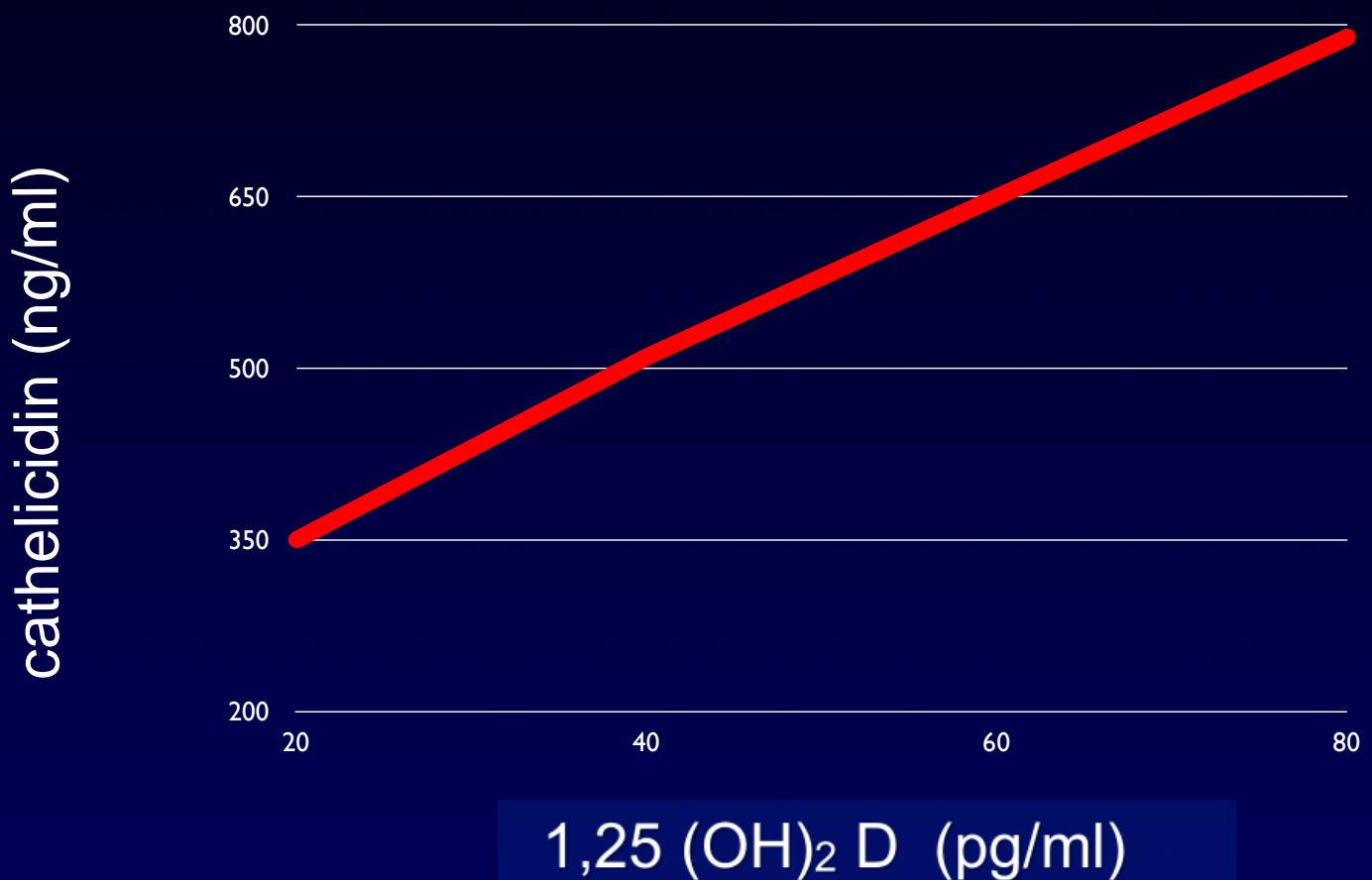
Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D₃



Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D₃

Adrian F. Gombart,^{*,†} Niels Borregaard,[†] and H. Phillip Koeffler^{*}

^{*}Department of Medicine, Division of Hematology/Oncology, Cedars-Sinai Medical Center, David Geffen School of Medicine at UCLA, Los Angeles, California, USA; and [†]The Granulocyte Research Laboratory, Department of Hematology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark



J Immunol. April 1, 2009; 182(7) 4289-95

CME

CSF oligoclonal bands in MS include antibodies against *Chlamydophila* antigens

CME

CSF oligoclonal bands in MS include antibodies against *Chlamydophila* antigens

Song-Yi Yao, MD; Charles W. Stratton, MD; William M. Mitchell, MD, PhD; and Subramaniam Sriram, MBBS

of *C pneumoniae*. None of the control subjects showed a prominent reactivity to elementary body antigens of *C pneumoniae*. In 14 of 17 patients with MS examined, oligoclonal bands were adsorbed either partially or completely from the CSF by elementary body antigens of *C pneumoniae*, but not by myelin basic protein, heat shock protein-60, or bacterial or viral antigens. In three patients with subacute sclerosing panencephalitis, adsorption of oligoclonal bands was seen with measles virus antigens but not with elementary body antigens of *C pneumoniae*. Conclusions: Oligoclonal bands in CSF of patients with MS include antibodies against *Chlamydophila* antigens.

NEUROLOGY 2001;56:1168–1176

Although the etiology of MS is not known, indirect and circumstantial evidence suggests the role of an infectious agent in the disease process.¹ We chose to examine a possible link between chronic CNS infection with *Chlamydophila pneumoniae* and MS because of our initial observation of CNS infection with *C pneumoniae* in a patient with rapidly progressive MS.² In extending these observations to a larger number of patients with established relapsing-remitting and progressive (primary and secondary) MS, we noted the presence of *C pneumoniae* in a majority of patients with MS.³

Chlamydophila belongs to a genus of intracellular pathogens. This family includes at least five species: *C pneumoniae*, *Chlamydophila psittaci*, *Chlamydophila abortus*, *Chlamydophila pecorum*, and *Chlamydophila felis*. Of these, *C pneumoniae* is infectious to humans, and is recognized as causing chronic persistent diseases, including those that affect the central nervous system.^{4–11} We and others have noted the presence of *C pneumoniae* in CSF

from patients with MS.^{12–14} Furthermore, we observed antibody responses to *C pneumoniae* antigens in the CSF of patients with MS, suggesting that chronic infection with *C pneumoniae* may be occurring in these patients.

To further examine the association between the development of MS and the presence of *C pneumoniae* infection in the CNS, we analyzed the reactivity of oligoclonal bands from patients with relapsing-remitting and progressive MS against *C pneumoniae* antigens.^{15,16} In virtually every chronic CNS infection, increased levels of immunoglobulins that recognize the pathogen are synthesized exclusively within the CNS compartment and are seen as oligoclonal bands by isoelectric focusing (IEF) methods.^{17–20} In MS, oligoclonal bands are a hallmark of the disease, although the antigenic specificity(s) of these bands remains unknown.^{20–22} Our present study examined the pattern and reactivity of oligoclonal bands (representing intrathecal antibody synthesis) to *C pneumoniae* antigens,

Infectious causes of multiple sclerosis

Donald H Gilden

Multiple sclerosis (MS) is a serious chronic neurological disorder in which demyelination and inflammation occur in the white matter of the CNS. The findings of many epidemiological studies and a discordance of MS in monozygotic twins suggest that the disorder is acquired. The most likely cause is a virus because more than 90% of patients with MS have high concentrations of IgG, manifest as oligoclonal bands, in the brain and CSF. Most chronic inflammatory CNS disorders are infectious. More indirect evidence that MS is caused by a virus is the association of several viruses with demyelinating encephalomyelitis in human beings, and the induction of demyelination in animals infected with viruses in research. Nevertheless, no virus has been isolated from the brains of patients who had MS. Molecular analysis of IgG gene specificity in the brain and CSF of those with MS has shown features of an antigen-driven response: clonal amplification and extensive somatic mutations. A viral antigen against which the IgG in MS brain and CSF is directed might be identified.

infiltrates concentrated in perivascular spaces. The infiltratory infiltrates have features consistent with an active infection: T lymphocytes, B lymphocytes, plasma cells, and macrophages or microglia. IgG is found primarily at the periphery of plaques.¹ Although inflammation is generally believed to be a primary feature of demyelination in MS, myelin destruction has recently been reported to occur before inflammation.² Thus, endogenous glia, such as microglia or astrocytes, might be a source of injury mediators.³

Infection and chronic neurological diseases

Various studies in the 1960s found that persistent virus infections caused chronic neurological disease. For example, paramyxovirus nucleocapsids were found in brains of patients with subacute sclerosing panencephalitis, a chronic inflammatory disease of both grey and white matter.⁴ Shortly after this study, high concentrations of antibody to measles virus were found in the serum and CSF of patients with subacute sclerosing panencephalitis.⁵ Within a few years, measles virus was isolated in tissue culture from subacute sclerosing panencephalitis brain explants.⁶

Another important discovery was that progressive multifocal leucoencephalopathy (PML), a fatal human demyelinating disease characterised by rapidly progressive dementia and motor deficit, was also caused by a virus. Human papovavirus (JCV virus) was found in

strains of Theiler's murine encephalomyelitis virus, coronaviruses, and lentiviruses.⁸

Antibody in brain and CSF

The most important evidence to support infection as the cause of MS is that the brain and CSF of more than 90% of patients with the disorder have high concentrations

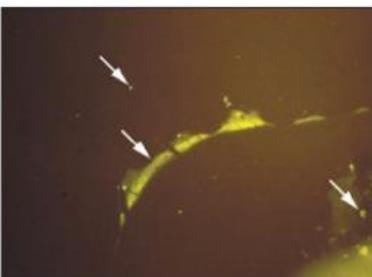


Figure 1: MS brain plaque-periplaque white matter
Direct immunofluorescence with a 1 to 20 dilution of antibody to human IgG conjugated to fluorescein isothiocyanate (green fluorescence) shows IgG deposition at the junction of plaque-periplaque white matter (middle arrow), in mononuclear cells (bottom arrow), and in normal white matter (top arrow). The antigen against which the IgG in MS brain and CSF is directed is unknown.

Lancet Neurol 2005; 4: 195–202

Departments of Neurology and Microbiology, University of Colorado Health Sciences Center, Denver, CO, USA (D H Gilden MD)

Correspondence to:
Dr Donald H Gilden, Department of Neurology, University of Colorado Health Sciences Center, 4200 E 9th Avenue, Mail Stop B182, Denver, CO 80262, USA
don.gilden@uchsc.edu

Autoimmune Disease

Where are we going?



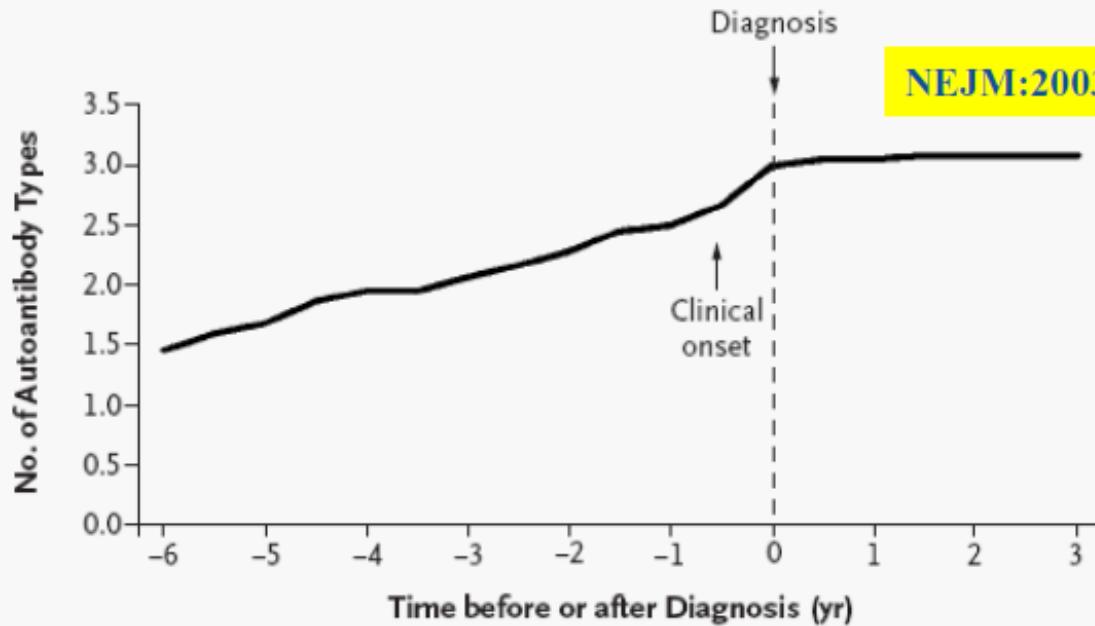
NEW PREDICTORS of DISEASE

Molecules called predictive autoantibodies appear in the blood years before people show symptoms of various disorders. Tests that detected these molecules could warn of the need to take preventive action.

Abner Louis Notkins, Scientific American, 296(3):72-79, 2007

Leslie D. Lipsky P, Notkins AL.
Autoantibodies as predictors of disease.
J Clin Invest 2001 ; 108 : 1417 -22





NEJM:2003;349:1526-1533

Figure 2. Accumulation of Systemic Lupus Erythematosus Autoantibodies.

The curve shows the average number of types of autoantibody in relation to the time of diagnosis of systemic lupus erythematosus. Seven autoantibodies were evaluated, which bind cellular constituents (anticellular antibodies), Ro, La, double-stranded DNA, Sm, phospholipid, and nuclear ribonucleoprotein. The time of diagnosis and the median time of the first appearance of any clinical criterion useful for the classification of systemic lupus erythematosus (clinical onset) are indicated by arrows.

Conc
(Units/mL)

AFP – Autoimmune Fatigue Panel

Ro60 - SSA	0.0 NEG (<9.0)
Ro52 - SSA (Trim21)	9.2 NEG (<10.0)
La - SS-B	27.4 POS (>15.0)
Sm – Smith	27.9 NEG (>22.0)
Ribonucleoprotein (U1-snRNP A/C/68kDA)	5.1 NEG (<13.0)
PmScl100 - Polymyositis/Scleroderma	0.0 NEG (<12.0)
dsDNA - Double-stranded DNA	67.3 POS (>16.0)
Histone	0.0 NEG (<15.0)
Ribosomal P0	44.5 POS (>21.0)
Ku - p70/p80 protein	0.0 NEG (<10.0)
PCNA - Proliferating Cell Nuclear Antigen	0.0 NEG (<10.0)
Cenp B - Centromere Protein B	0.0 NEG (<10.0)
Jo_1 - Histidyl-tRNA-synthetase	0.0 NEG (<10.0)
Scl70 - Scleroderma	6.6 NEG (<9.0)
MI-2 Chromatin Remodeling Enzyme	0.0 NEG (<10.0)
PL7/PL12 - (threonyl)/(alanyl)-tRNA synthetase	0.0 NEG (<8.0)
SRP - Signal Recognition Particle	2.2 NEG (<7.0)

AAP - Autoimmune Abdominal Panel

IgA Status - Immune System Producing Antibodies	NORMAL
ASCA - Saccharomyces Cerevisiae IgA & IgG	0.0 NEG (<10.0)
DGP - Deamidated Gliadin	0.0 NEG (<12.0)
TPO - Thyroid Peroxidase	0.0 NEG (<10.0)
tTG - Tissue Transglutaminase	0.9 NEG (<10.0)
AGPC - Gastric Parietal Cells	49.5 POS (>20.0)
IFAB - Intrinsic Factor	0.0 NEG (<10.0)
PR3 - Proteinase 3 (cANCA)	0.0 NEG (<9.0)
MPO - Myeloperoxidase (pANCA)	0.0 NEG (<7.0)
BPI - Bactericidal Permeability Increasing Protein	0.0 NEG (<25.0)
LC1 - Liver Cytosol type 1	0.0 NEG (<9.0)
Mitochondrial (M2)	0.0 NEG (<10.0)
Liver Kidney Microsomal (LKM1)	0.0 NEG (<10.0)
GBM - Glomerular Basement Membrane	0.0 NEG (<8.0)
sp100 Nuclear Antigen Protein	0.0 NEG (<8.0)
gp210 Glycoprotein 210	1.5 NEG (<15.0)
SLA - Soluble Liver Antigen	0.0 NEG (<10.0)

ARTICLE

doi:10.1038/nature16962

Expanding antigen-specific regulatory networks to treat autoimmunity

Xavier Clemente-Casares¹, Jesus Blanco^{2,3*}, Poornima Ambalavanan^{1*}, Jun Yamanouchi^{1*}, Santiswarup Singha^{1*}, Cesar Fandos², Sue Tsai¹, Jinguo Wang¹, Nahir Garabatos⁴, Cristina Izquierdo⁴, Smriti Agrawal⁵, Michael B. Keough⁵, V. Wee Yong⁵, Eddie James⁶, Anna Moore⁷, Yang Yang^{1,8}, Thomas Stratmann⁴, Pau Serra² & Pere Santamaria^{1,2}

Regulatory T cells hold promise as targets for therapeutic intervention in autoimmunity, but approaches capable of expanding antigen-specific regulatory T cells *in vivo* are currently not available. Here we show that systemic delivery of nanoparticles coated with autoimmune-disease-relevant peptides bound to major histocompatibility complex class II (pMHCII) molecules triggers the generation and expansion of antigen-specific regulatory CD4⁺ T cell type I (T_{R1})-like cells in different mouse models, including mice humanized with lymphocytes from patients, leading to resolution of established autoimmune phenomena. Ten pMHCII-based nanomedicines show similar biological effects, regardless of genetic background, prevalence of the cognate T-cell population or MHC restriction. These nanomedicines promote the differentiation of disease-primed autoreactive T cells into T_{R1} -like cells, which in turn suppress autoantigen-loaded antigen-presenting cells and drive the differentiation of cognate B cells into disease-suppressing regulatory B cells, without compromising systemic immunity. pMHCII-based nanomedicines thus represent a new class of drugs, potentially useful for treating a broad spectrum of autoimmune conditions in a disease-specific manner.

Autoimmune diseases such as type 1 diabetes (T1D), multiple sclerosis and rheumatoid arthritis result from chronic autoimmune responses involving T cells and B cells recognizing numerous antigenic epitopes on incompletely defined lists of autoantigens^{1–3}. Eliminating or suppressing all polyclonal autoreactive T-cell specificities (known and unknown) in each individual autoimmune disorder without compro-

complexes might be able to expand disease-specific regulatory CD4⁺ T cells *in vivo*.

Expansion of disease-specific T_{R1} cells

We treated non-obese diabetic (NOD) and NOD *Foxp3-eGFP* mice (expressing enhanced green fluorescent protein (eGFP) under the con-

Researchers at the university's Cumming School of Medicine have discovered a novel mechanism that stops the immune attack, and have developed a new class of drugs that harnesses this mechanism to treat various autoimmune diseases without compromising the entire immune system.

T cells, which produce the cytokines IL-10 and IL-21, and express the surface markers CD49b and LAG-3 and the transcription factor

CD49b and LAG-3 (Fig. 1c, d). A similar outcome was observed in mice treated with 2.5mi/IA^{g7}-NPs upon depletion of CD4⁺CD25⁺ T cells

differentiation of disease-primed autoreactive T cells into T_{R1} -like cells, which in turn suppress autoantigen-loaded antigen-presenting cells and drive the differentiation of cognate B cells into disease-suppressing regulatory B cells, without compromising systemic immunity. pMHCII-based nanomedicines thus represent a new class of drugs, potentially useful for treating a broad spectrum of autoimmune conditions in a disease-specific manner.

exposure (and exposure to pMHC-NPs) would trigger the differentiation of autoreactive T cells into regulatory T-cell progeny. By this reasoning, we predicted that NPs coated with disease-relevant pMHCII

in the *Il10* locus¹⁴ in the presence of 2.5mi/IA^{g7}-NPs, 2.5mi peptide or 2.5mi/IA^{g7} monomer. Naive T cells expressed neither CD49b nor LAG-3, even after incubation with 2.5mi/IA^{g7}-NPs, 2.5mi/IA^{g7} monomer



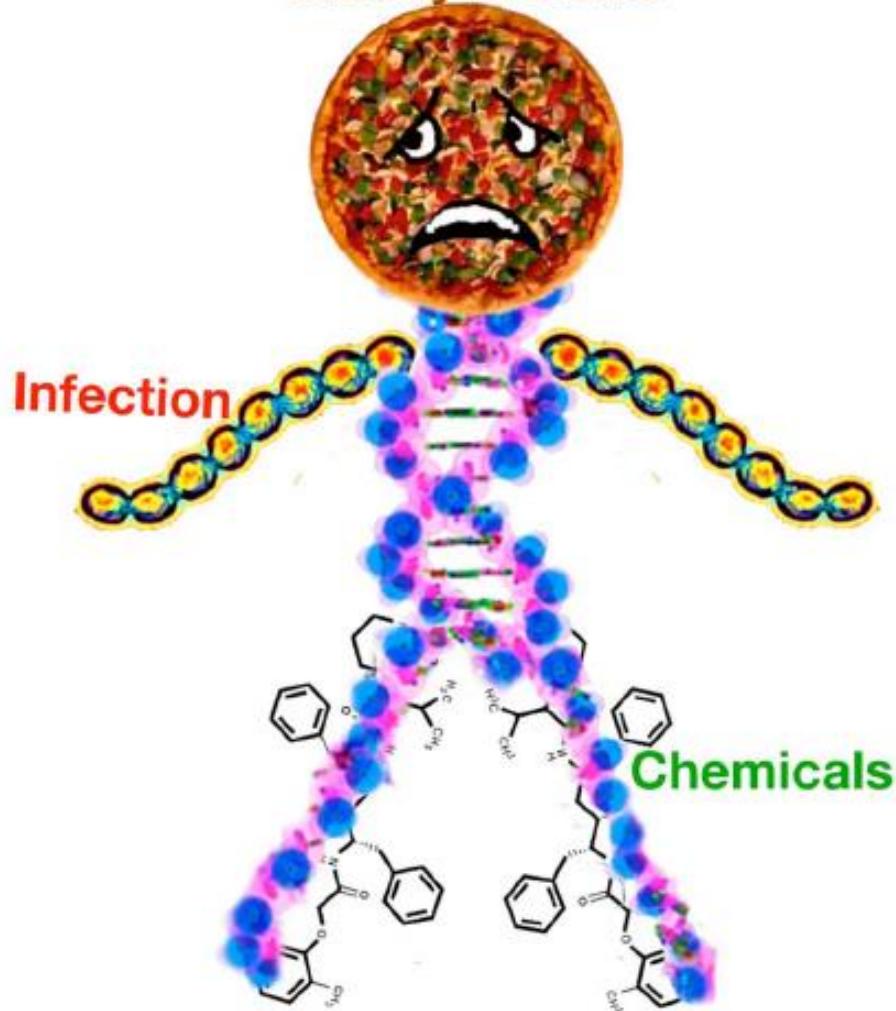
These nanomedicines promote the

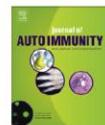
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*Julia McFarlane Diabetes Research Centre (JMDRC), and Department of Microbiology, Immunology and Infectious Diseases, Snyder Institute for Chronic Diseases and Hotchkiss Brain Institute, Cumming School of Medicine, University of Calgary, Calgary, Alberta T2N 4N1, Canada. ¹Institut D'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, 08036, Spain. ²Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Instituto de Salud Carlos III, Madrid 28029, Spain. ³Department of Physiology and Immunology, Faculty of Biology, University of Barcelona, Barcelona 08028, Spain. ⁴Hotchkiss Brain Institute and Department of Clinical Neurosciences, Faculty of Medicine, University of Calgary, Calgary, Alberta T2N 4N1, Canada. ⁵Benaroya Research Institute at Virginia Mason, Seattle, Washington 98101-2795, USA. ⁶Molecular Imaging Laboratory, MGIV/MITHMS Athinoula A. Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital, Charlestown, Massachusetts 02129, USA. ⁷Department of Biochemistry and Molecular Biology, Cumming School of Medicine, University of Calgary, Calgary, Alberta T2N 4N1, Canada.
*These authors contributed equally to this work.

Dietary Proteins





Review

'ASIA' – Autoimmune/inflammatory syndrome induced by adjuvantsYehuda Shoenfeld ^{a,b,*}, Nancy Agmon-Levin ^a^aThe Zabludowicz Center for Autoimmune Diseases, Department of Medicine B' Sheba Medical Center, Tel-Hashomer, Israel^bIncumbent of the Laura Schwarz-Kipp Chair for Research of Autoimmune Diseases, Sackler Faculty of Medicine, Tel-Aviv University, Israel

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ABSTRACT

The role of various environmental factors in the pathogenesis of immune mediated diseases is well established. Of which, factors entailing an immune adjuvant activity such as infectious agents, silicone, aluminium salts and others were associated with defined and non-defined immune mediated diseases both in animal models and in humans. In recent years, four conditions: siliconosis, the Gulf war syndrome (GWS), the macrophagic myofasciitis syndrome (MMF) and post-vaccination phenomena were linked with previous exposure to an adjuvant. Furthermore, these four diseases share a similar complex of signs and symptoms which further support a common denominator. Thus, we review herein the current data regarding the role of adjuvants in the pathogenesis of immune mediated diseases as well as the amassed data regarding each of these four conditions. Relating to the current knowledge we would like to suggest to include these comparable conditions under a common syndrome entitled **ASIA**, "Autoimmune (Auto-inflammatory) Syndrome Induced by Adjuvants".

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1. Introduction

In recent years four enigmatic medical conditions, defined by hyperactive immune responses were described. These conditions, namely siliconosis, the Gulf war syndrome (GWS), the macrophagic myofasciitis syndrome (MMF) and post-vaccination phenomena share a similar complex of signs and symptoms which suggest a common denominator to each one. Immune mediated conditions (i.e. autoimmune and auto-inflammatory diseases) are a leading cause of morbidity and mortality worldwide and their prevalence is rising in different geographical areas [1–3]. These geo-epidemiological changes can be explained by a complex of genetic and environmental factors [4,5]. Thus, a genetically susceptible subject may develop an autoimmune or auto-inflammatory disease (AI/AIFD) following exposure to a certain environmental factor [5–8]. Noteworthy, infections, toxins, and drugs were linked not only with the occurrence of immune mediated conditions but also with their clinical manifestations [7,8]. Environmental factors that comprise an immune **adjuvant** effect have been recognized for several decades. These adjuvants (i.e. silicone, alum, pristane, infectious components) were found to induce autoimmunity by themselves in different animal models and may possibly provoke AI/AIFD in

humans [9–13]. Exposure to these substances were documented in the four medical conditions conversed herein, suggesting that the common denominator to these syndromes is a trigger entailing adjuvant activity. Therefore, in this review we suggest to include these conditions under a common syndrome entitled **ASIA**, "Autoimmune (Auto-inflammatory) Syndrome Induced by Adjuvants".

1.1. *Adjuvancy – the mechanisms*

The term "adjuvant" derives from the Latin word *adjuvare*, meaning to aid. An immunologic adjuvant is a substance that enhances antigen-specific immune response preferably without triggering one on its own [13]. Adjuvants are commonly used in medicine to boost an immune response to treatments such as vaccination. The adjuvant effect is accomplished via several mechanisms that impinge on both the innate and adaptive immune systems [13–15]. Adjuvants increase innate immune responses by mimicking evolutionarily conserved molecules (e.g. bacterial cell walls, LPS, unmethylated CpG-DNA) and binding to Toll-like receptors (TLRs). Additionally, they augment the activities of dendritic cells (DCs), lymphocytes, macrophages and activate the intracellular Nalp3 inflammasome system [13]. Thus, adjuvants increase the local reaction to antigens (e.g. at the site of infection) and subsequently the release of chemokines and cytokines from T-helper and mast cells [13,16–18]. Currently the most widely used adjuvant in medicine is aluminium. Following an injection of aluminium salts (i.e. vaccination) danger-associated molecular patterns such as uric acid are released. High concentrations of uric acid form monosodium urate crystals

* Corresponding author at: Department of Medicine 'B' and Center for Autoimmune Diseases, Chaim Sheba Medical Center, Tel-Hashomer 52621, Israel.
 Tel.: +972 3 5302652; fax: +972 3 5352855.
 E-mail address: shoenfel@post.tau.ac.il (Y. Shoenfeld).

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Table 1

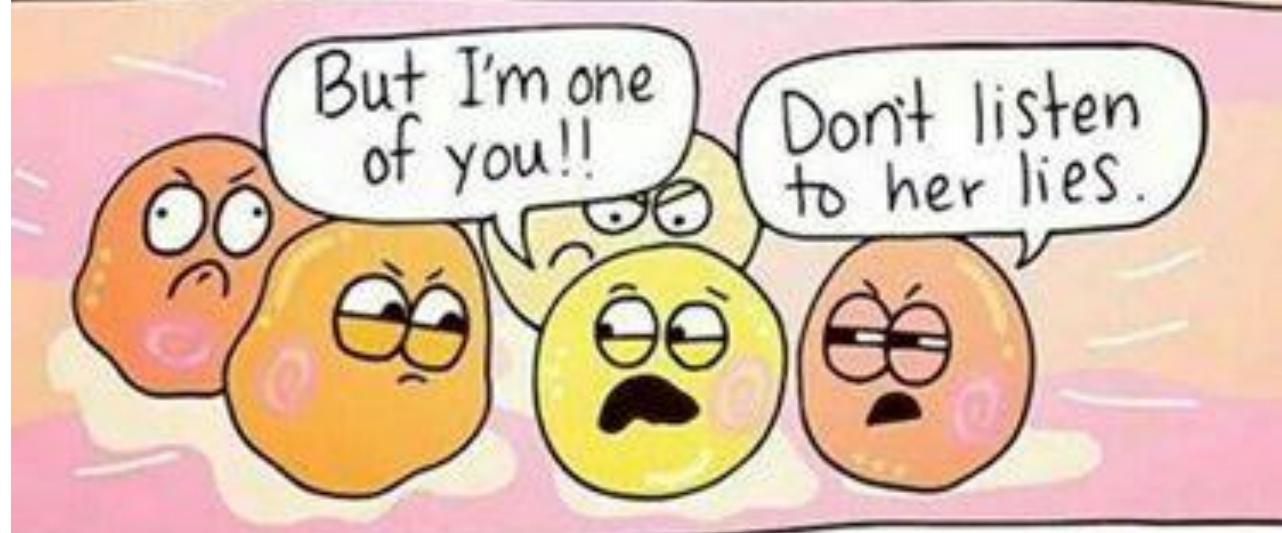
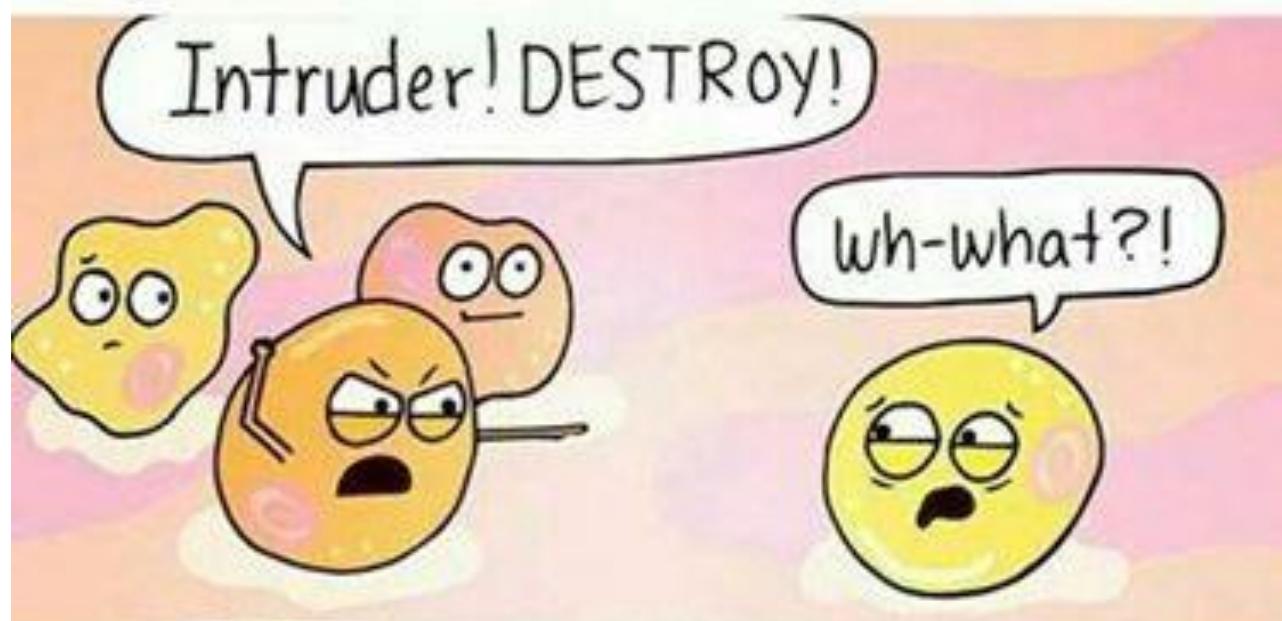
The prevalence of clinical manifestations: MMF, Silicone related disease; GWS and post-vaccination events.

Symptoms	MMF N = 250	Silicone N = 100	GWS N = 4600	Post Vaccines N = 30000
Myalgias/myopathy/muscle weakness	+++	+++	+	+
Arthralgias/arthritis	+++	+++	++	+
Chronic fatigue/sleep disturbances	+++	+++	+++	+
Neurological/cognitive impairments	+	++	++	+
Fever	+	NR	NR	+
Gastrointestinal	+	NR	+	+
Respiratory	NR	NR	+	+
Skin		+	+	+
Diagnosis of defined autoimmune disease	+	+	NR	+/-
	33% MS			
Antibodies	NR	+	+	NR
Increased ESR	++	NR	NR	+
References	8, 21	22	11, 23	24

The prevalence of signs and symptoms was defined as (+) if reported in <30% of subjects, (++) in 30–60% and (+++) if present in more than >60% of subjects. MS – multiple sclerosis; NR – not reported.

Assessments & Interventions for Autoimmune Disease

- Detect and remove opportunistic and pathogenic GI bugs
- Detect and eliminate food-inflammasome activations
- Predictive autoantibody testing
- Optimize estrogen metabolism
- Check for toxins & support detoxification
- Vitamin D status optimization
- Quench excess inflammation & oxidative stress
- Nutritional interventions (anti-inflammatory diet, Low AA)
- Gastrointestinal restoration (4R program)
- Stress Reduction

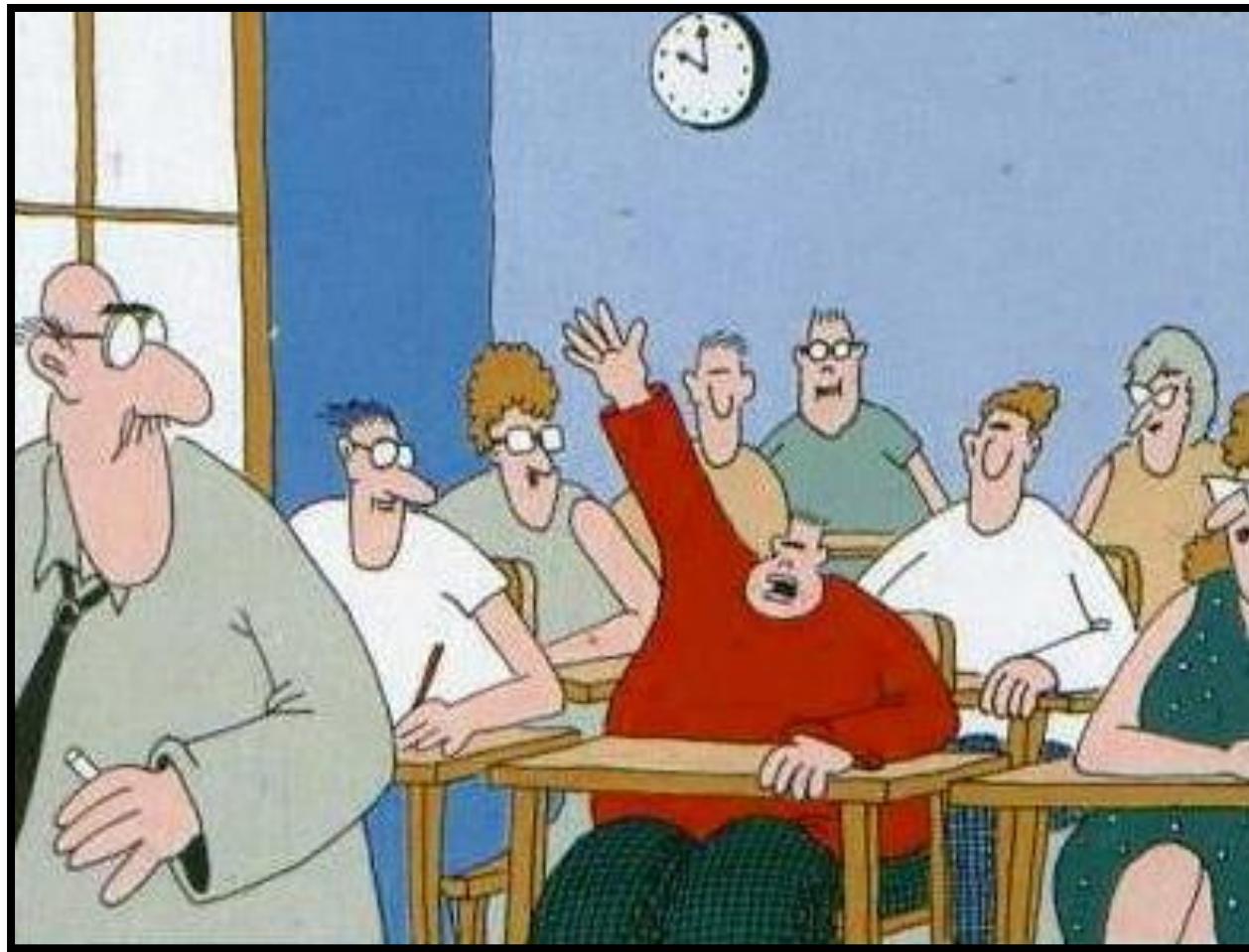


Autoimmune disorders in a nutshell.

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“Dr. Brady, may I be excused? My brain is full.”