Immunology

Code: SCI2031

2017/2018

Period 4

Contents

General information

The course

The focus of this course lies on the role of different humoral factors, cells and cell interactions of the immune system that are involved in the defence of the human body against intruders like microbes and toxins. In addition, attention will be paid to unwanted immune responses such as in allergy and transplant rejection. The course starts with a series of self-study assignments (SSA) in which students are introduced to the theoretical concepts and basics of immunology. The SSAs are post-discussed in the tutorial meetings. In the second part of the course, students are confronted with a series of case studies in which the knowledge acquired during the SSAs can and must be applied. The SSAs and the case studies are accompanied by lectures. A (mandatory) practical session is scheduled to allow students to practice what they studied. The course is rounded off by student presentations and a written exam.

Course objectives

Knowledge and insight

- Tissues, cells and humoral factors of the innate and adaptive immune system
- Cellular and molecular effector mechanisms of the innate and adaptive immunity during inflammation and infection
- The structure and function of primary and secondary lymphoid tissue
- The processes in the immune response after immunisation and vaccination
- Immune mechanisms in disease

Study instructions

For the self-study assignments the course reference book *Basic Immunology. Functions and disorders of the immune system* by Abbas, Lichtman, and Pillai can be used. The course manual provides guidelines for the parts that need to be studied in the book. The preparation for the self-study assignments also includes studying the figures, including the legends, referred to in the text. The topics of the self-study assignments and especially the remaining issues or questions are addressed and discussed in the tutorial meeting.

The case studies are discussed in a regular PBL set-up (pre- and post-discussion) in the second part of the course. Each case study is accompanied by a lecture, which discusses the topic in further detail and in a broader context with attention to recent developments. In addition, the lecture is the moment for students to direct remaining questions to an expert in the field.

Assessment

This course contains two elements of assessment:

1. a written exam consisting of open questions (70% of the overall course grade);
2. a presentation on an immunologic topic (30% of the overall course grade) in pairs – for more information, see below.

Presentation Assignment

The presentation assignment during the course gives you an opportunity to discuss, develop and elaborate on an immunological topic together with a fellow student. You are free to pick any topic within immunology that has your interest, but keep in mind that it has to be an academic presentation, including more than just a summation of facts as stated in the literature. Choose a topic that provokes some sort of controversy, or from which mechanisms or treatments are not completely clear. You are encouraged to form your opinion on the existing controversy or on a future direction of research in the field and to come up with your own approaches and solutions to an immunological problem. Please communicate the topic of your choice with your tutor to avoid overlapping topics within a session. In addition to that, you should move beyond the contents of the course.

Format of the presentation

Every duo has 15-20 minutes for the presentation, including questions and group discussion. A laptop and beamer will be available.

The presentation should contain at least the following items:

- Introduction
  - Introduction of research question/hypothesis
  - Explanation of context and relevance
  - Knowledge gap
- State of the art
  - Overview of current research and findings as presented in the literature
- Discussion
  - Possible solutions (as provided in the literature or thought of by the students)
- Conclusion(s)
- Group discussion (presenters lead the discussion)

Assessment of the presentation

The assessment of the presentation will be based on:

- The quality of the introduction (are you able to explain the context and relevance of your research question in such a way that the audience has enough insight to understand the context and reasons for your topic?)
- The quality of the presentation of the problem (are you able to explain the problem and capable of explaining why the solutions you provide are the best solutions?)
- The handling of questions/discussion (are you able to answer questions and discuss the problem?)
- The delivery of the presentation (quality of slides, speaking posture, etc.)

The presentation should be theoretically informed. You do not have to explain basic concepts discussed in the course, since you are presenting to students from your own discipline. However, don't take all background knowledge for granted!

The tutor will assess the presentations, supported by a second assessor. The presentation is the result of a cooperation of two students. There are no requirements as to who gives the presentation. You may present together, or decide to have one student present and have the other student lead the discussion and answer the questions. Either way, keep in mind that you will receive a group grade for this assignment. Your tutor will assess the final product; s/he cannot evaluate whether you divided the work equally. It is your own responsibility to assure each team member invests approximately the same amount of work.

Readings

In addition to the main course book other books may be helpful in studying learning goals that rise from the cases. Many of those books are available in the UCM Reading Room and/or in the library at the FHML (UNS50). Students are encouraged to use additional books or other sources of information.

Course reference book:

- Abbas, A.K., Lichtman, A.H., Pillai, S. (2014 or later). *Basic Immunology*. *Functions and disorders of the immune system*. (4th ed. or later). Philadelphia: Elsevier

Other immunology books:

- Nairn, R. & Helbert, M. (2007). *Immunology for Medical Students.* (2nd ed.). Philadelphia: Elsevier
- Abbas, A.K., Lichtman, A.H., Pillai, S. (2012 or later). *Cellular and Molecular Immunology*. (7th ed. or later). Philadelphia: Elsevier
- Gartner, L.P. & Hiatt, J.L. (2007). *Color Textbook of Histology*. (3rd ed.). Philadelphia: Elsevier
- Male, D., Brostoff, J., Roth, D., Roitt, I. (2013). *Immunology.* (8th ed.). Philadelphia: Elsevier
- …

Attendance requirements

The attendance requirement for the 11 tutorial meetings is 85%. You are allowed to miss two regular tutorials without further consequences. If you miss more meetings, you will have to apply for an additional assignment (request forms are available via the Student Portal). In order to qualify for an additional assignment you have to have valid reasons for all missed sessions. If you don't meet the attendance requirement, you are not eligible for a resit. You automatically fail the course if you miss over 30% of the scheduled meetings.

The presentation session and the practical session in week 6 of the course are mandatory meetings and are therefore not considered one of the regular tutorial meetings to which the 85% attendance requirement applies. The attendance requirement for these sessions is 100%.

Although the attendance for lectures is not mandatory, the content adds to the pre- and post-discussions in the tutorial group meetings. Therefore, the content of the lectures is considered part of the examination.

An inspection hour for the exam will be scheduled within 6 weeks after the end of the course. The inspection hour is not mandatory.

Resit

Students who initially fail the course, but who have complied with the compulsory attendance requirement and took part in all of the assessment during the course, are eligible for one resit.

The overall grade for this course consists of a grade for the written exam (70%) and a grade for the presentation (30%). The resit serves the purpose of lifting your overall grade to sufficient/above 5.5. It does not replace the overall grade. Hence, the resit can be either on the exam OR the presentation. In case of a resit, you will have to redo that part of the assessment for which you received the lowest grade. In case of redoing the presentation, you can either redo the original presentation, incorporating the feedback you received, or choose a new topic and prepare a totally new presentation. Beware that a resit for the presentation automatically changes this form of assessment into an individual assessment, even if both members of the presentation-team have to redo the presentation.

In order to receive a grade for the exam you will have to do a serious attempt at passing the exam. If it is not deemed a serious attempt you will not receive a grade and you will not qualify for a resit. The same thing holds for the presentation. If it is not deemed a serious attempt you will not receive a grade and you will not qualify for a resit.

Course coordinators

Questions or issues regarding the course content and e.g. assessment should first be discussed with the tutorial group and tutors. Those questions can also be discussed with the coordinators, but preferably during or directly after the lectures. Please note that the coordinators will not reply to individual e-mails that could have been dealt with during tutorial group meetings or during lectures.

Kristiaan Wouters, PhD

FHML, Department of Internal Medicine

UNS50, room 5.334B

Phone: 043 3884233

e-mail: kristiaan.wouters@maastrichtuniversity.nl

Bram Schmitz, MSc *(corresponding coordinator)*

FHS, University College Maastricht

SSK32 room 0.039C

Phone: 043 3885148

e-mail: bram.schmitz@maastrichtuniversity.nl

Overview of meetings, lectures and deadlines

week	meeting	topic
1	Tutorial meeting 1	*Introduction* Pre-discussion SSA-1 – *The innate immune system*
	Tutorial meeting 2	Post-discussion SSA-1 – *The innate immune system*
	Lecture	*Intro to immunology & Inflammation*
		Carnival break
2	Tutorial meeting 3	Post-discussion SSA-2 – *The adaptive immune system: T-cells*
	Tutorial meeting 4	Post-discussion SSA-3 – *The adaptive immune system: B-cells* Pre-discussion Case study 1 – *The immune system*
	Lecture	*Innate immunity*
3	Tutorial meeting 5	Post-discussion Case study 1 – *The immune system* Pre-discussion Case study 2 – *The innate immune system*
	Tutorial meeting 6	Post-discussion Case study 2 – *The innate immune system* Pre-discussion Case study 3 – *Vaccination*
	Lecture	*Adaptive immunity and Vaccination*
4	Tutorial meeting 7	Post-discussion Case study 3 – *Vaccination* Pre-discussion Case study 4 – *Lymphocyte diversity*
	Practical session - *mandatory*	Practical session (Randwyck, UNS40 and UNS50)
5	Tutorial meeting 8	Post-discussion Case study 4 – *Lymphocyte diversity* Pre-discussion Case study 5 – *Allergy*
	Tutorial meeting 9	Post-discussion Case study 5 – *Allergy* Pre-discussion Case study 6 – *Transplantation*
	Lecture	*Mucosal immunity & Lymphocyte diversity*
6	Tutorial meeting 10	Post-discussion Case study 6 - *Transplantation*
	Tutorial meeting 11 - *mandatory*	Student presentations
	Lecture	*Allergy and Transplantation*
7	Tutorial meeting 12	Concept Map
	Exam	Written exam

Note that this is a preliminary schedule. Please check your schedule on MyUM and the announcements on the Student Portal on a regular basis.

Introduction

Introduction to the immune system and the immune response

The immune system of vertebrates exists of a collection of cells, tissues and molecules that mediate resistance to foreign intruders. These intruders (e.g. pathogenic micro-organisms) can be looked upon as the enemy and the immune system as the defence system.

Micro-organisms can enter the body through the airways, the gastro-intestinal tract, the urogenital tract, and through damaged skin. The invaders usually have the capacity to multiply fast and overgrow the host in a short period of time. Fortunately, the immune system is able to destroy or restrain the intruders, or to neutralise the toxic substances the intruders produce.

It is essential that the immune system can distinguish between foreign and own structures, cells and tissues. The immune system must be tolerant towards structures belonging to the body. One can state that the immune system has specificity, since it can distinguish between own ("self") and foreign ("non-self").

The reaction of the immune system against an intruder is referred to as the immune response. Every structure that can initiate an immune response is called an antigen (comes from 'antibody generating' structure). Typical antigens are usually components of micro-organisms existing of proteins, carbohydrates, nucleic acids, lipids or a combination. However, every structure found in nature or made by mankind, and even made by your own body is a potential antigen.

When a micro-organism infects the body the antigenic structures will first encounter the *innate immune system* (also called the native immune system) and induce an immune response. If however this immune response is not able to quickly and effectively eliminate the invader, the latter can proliferate and initiate a pathological process, e.g. disease. Our body needs 5 to 10 days to build up a highly specific defence against the intruder. The immune system part that generates this micro-organism-specific response is called the *adaptive immune system* (also called specific or acquired immune system). The adaptive immune response that occurs after the first exposure of an individual to a foreign antigen is called the "primary immune response". The primary response usually eliminates the invader allowing the body to recover. The adaptive immune system triggered during this first encounter develops immunological memory and is vigilant to prevent for a second infection by that micro-organism. Upon a second encounter, the created memory will drive a quicker, more intense and precise immune response as compared to the primary response generated upon the first exposure. The adaptive immune response that occurs on second exposure to an antigen is referred to as the "secondary immune response". One could state that the adaptive immune system learns from a first infection, it adapts and creates memory for antigenic structures, by which it is more effective against these antigens during a second encounter.

The relation between the immune system and potential pathogens or harmful foreign structures is an ongoing battle. During each fight, the immune system has two objectives: identification of the invader and selective elimination of the invader without harming the body. The immune system has the capacity to produce several different recognition structures and is therefore able to identify almost every antigen. The innate immune system, which can be considered the 'first line of defence' is equipped with a limited repertoire of antigen-recognition molecules, whereas for the adaptive immune system, considered as the second line of defence, the repertoire of recognition molecules is unlimited, by which the adaptive immune system can operate more antigen-specific.

The innate immune system

The innate immune system is capable of rapid responses to microbes and informs the adaptive immune system about an infection. The innate immune system provides a first line of defence against infectious micro-organisms. It includes natural barriers, such as the skin as a physical barrier, stomach-acid as a chemical barrier, etcetera. In addition, the innate immune system produces several compounds that suppress or kill potential invaders.

An evolutionary old but effective part of the innate immune system is the complement system. It consists of about 20 serum and cell surface proteins that upon activation results in a cascade of enzyme activities in the serum. The cascade is characterised by a rapid, enhanced reaction steps in which each product acts as a catalyst for a subsequent reaction in the cascade. The activated and formed products in this cascade have different functions in the defence against micro-organisms. The most important factor, playing a central role in the complement system, is C3.

Phagocytes form the most important cell type of the innate immune system. They play an important role in the first line of defence by removal of intruders by phagocytosis. They are activated upon recognition of micro-organism-specific structures. Neutrophils form the most dominant phagocyte type in the blood, upon infection they act directly by migrating to the site of infection. A secondary population of phagocytic cells that arrive at the site of infection is formed by macrophages. They are derived from blood-monocytes, have a much longer lifespan than neutrophils and therefore play an important role in further defence processes and wound healing.

The adaptive immune system

The adaptive immune system consists of lymphoid tissues and T- and B-lymphocytes. Lymphocytes are produced and educated in the primary lymphoid tissues (bone marrow and thymus). Mature lymphocytes recirculate between the various secondary lymphoid tissues in the body where they encounter antigens form infectious micro-organisms. T-lymphocytes need the antigens to be presented by MHC molecules on so-called dendritic cells that are part of the innate immune system. The repertoire of T- and B-cell receptors for antigen (TCR and BCR) is, unlike for cells of the innate immune system, unlimited, every antigenic structure can be recognized, including self-antigens. To avoid self-reactivity (autoimmunity), T- and B-cells during production are selected for non-self reactivity. In addition, a back-up system of regulatory T-cells controls for unwanted immune responses. TCRs and BCRs recognize small fragments (epitopes) on antigens. Antigen activated T- and B-cells need to expand (proliferation) and to differentiate to develop a large population of effector cells to adequately combat infectious micro-organisms. It is therefore that it takes time before the adaptive immune system is operative. Effector B-cells produce antibodies against antigenic structures on infectious micro-organisms, where as effector T-cells produce immune-supporting cytokines and can function as cytotoxic T-cells that can kill infected cells.

A most important phenomenon of the adaptive immune system is the formation of immunological ‘memory’ (immunity) out of an executed immune response against a micro-organism. Upon a second infection, the memory system can respond more rapidly, with a greater magnitude and efficacy, thereby preventing development of disease.

Self-study assignments

Three self-study assignments (SSA) are scheduled in this course. Each SSA is discussed in a tutorial meeting where students can discuss remaining issues and questions.

SSA-1: The innate immune system - inflammatory response - antigen presentation

Background

In this self-study assignment, you will study the innate immune system in more detail. The most important molecules (cytokines, complement factors) and cells (phagocytes) of the innate immune system will be discussed. The different mechanisms used by the immune system to eliminate a target structure and the cooperation between the innate and adaptive immune system will be discussed.

Goal

After the self-study you should be able to explain and discuss the following topics:

- Name the general characteristics of the innate immune response
- Summarize the components and functions of the innate immune system
- What do cells of the innate immune system recognize on micro-organisms? In what way does this differ from receptors of the adaptive immune system?
- What are Toll-like receptors and what do they recognize?
- Describe the activation and function of the inflammasome.
- What are the physical barriers of the innate immune system?
- Name the cells of the innate immune system and their major function
- What are phagocytes, what is their origin and their role?
- How do phagocytes ingest and kill micro-organisms?
- Name cytokines of the innate immune system and mention their role
- Describe the step in the inflammatory response
- What are the steps in the migration process of leukocytes to the site of infection?
- Describe the role of the innate immune system in stimulating the adaptive immune response
- How do micro-organisms evade from the immune response?

Reading material

Abbas, A.K., Lichtman, A.H., Pillai, S. (2014 or later). *Basic Immunology*.

- Chapter 1

- Chapter 2

If necessary, other relevant sources may be consulted.

SSA-2: The adaptive immune system: antigen recognition - T-cell mediated cellular immune responses - T-cell effector mechanisms

Background

In this self-study assignment, the adaptive cell-mediated immune response will be studied. Antigen uptake and presentation to T-lymphocytes is one of the topics. In addition the genetic process behind the development of an unlimited repertoire of T- and B-cell antigen receptors is studied. The assignment ends with discussing the generation and different possible pathways in T-cell mediated immunity and the effector mechanisms.

Goal

After the self-study you should be able to explain and discuss the following topics:

- Explain what humoral and cell-mediated immunity is and in what type of microbial infection they operate
- Name and explain the most important properties of the adaptive immune system. Compare with innate immunity
- Name the classes of lymphocytes and their functions
- Describe maturation and life stages of B- and T-lymphocytes
- Name the types of lymphoid tissue , describe their morphology and function
- How and why do lymphocytes migrate in the body
- Mention the most important antigen-presenting cells of the immune system. What is their role?
- Describe in general the functions of MHC-class I and class II presentation to T-cells
- What is cross-presentation and by which cells can it be executed?
- What is the difference in what T- and B-cells recognize?
- What are primary and secondary immune responses?
- Describe the phases of CD4 and CD8 T-cell-mediated immunity
- Mention the differences in migration of naïve and effector T-cells and how this is regulated
- Describe the mechanism of killing infected cells by CD8 $T_{cytotoxic}$ cells
- How do some micro-organisms circumvent attack by the immune system?

Reading material

Abbas, A.K., Lichtman, A.H., Pillai, S. (2014 or later). *Basic Immunology*.

- Chapter 1, chapter 3, chapter 5, chapter 6

If necessary, other relevant sources may be consulted.

SSA-3: The adaptive immune system: B-cell mediated humoral immune responses - effector mechanisms of antibodies

Background

In this self-study assignment, the adaptive humoral immune response will be studied. The activation and differentiation of B-cells into antibody producing plasma cells is addressed. In addition, attention is paid to the types and effector functions of antibodies and the role of T_{helper} cells in class switching from primary to secondary antibodies. The assignment gives insight in how antibodies as an effector mechanism of the adaptive immune system can combat infectious micro-organisms and toxins.

Goal

After the self-study you should be able to explain and discuss the following topics:

- Describe the phases and types of the humoral immune response
- What are the signals and molecules involved in B-cell activation?
- Describe the characteristics of the primary and secondary antibody response
- What are primary and secondary antibodies?
- Describe the function of T_{helper} cells in the T_{helper} cell-dependent B-cell response
- Where and how do T_{helper} cells and B-cells interact?
- What is affinity maturation and how does it work?
- Describe the mechanism of antibody feedback in the humoral immune response
- Explain the effector functions of the different types of antibodies
- What is antibody-dependent cellular cytotoxicity?
- How do antibodies activate effector mechanisms of the innate immune system?
- Describe how some micro-organisms circumvent destruction by antibodies

Reading material

Abbas, A.K., Lichtman, A.H., Pillai, S. (2014 or later). *Basic Immunology*.

- Chapter 7

- Chapter 8

If necessary, other relevant sources may be consulted.

Case Studies

Case study 1. Bellyache

After a course class in Biomedicine, Ann and Marian decide to have a bite before they start studying. When they stand in the eatery, discussing what to choose, Marian says she is not feeling very hungry. She is nauseous and has mild abdominal pain. She only has a cup of tea, hoping the symptoms will soon disappear. However, the pain does not get any less and she does not feel well at all. They go to the ladies' room together, where Marian wants to freshen up a little. It does not help and she suddenly has to vomit, feels feverish and now has severe pain in her lower abdomen. It frightens Ann and she does not hesitate and takes her friend to the Accident & Emergency department. "It wouldn't surprise me if you had appendicitis," she says.

Marian is being examined at the A&E. Her temperature is taken and measures 38°C, i.e. she has a fever. Blood samples are taken for some blood tests. The pain in her lower abdomen is increasing. "I suspect an acute appendicitis," says the doctor attending her. Marian is taken to the surgery department where they perform a laparoscopy. This reveals a swollen, red, highly inflamed appendix, which is removed immediately (appendectomy).

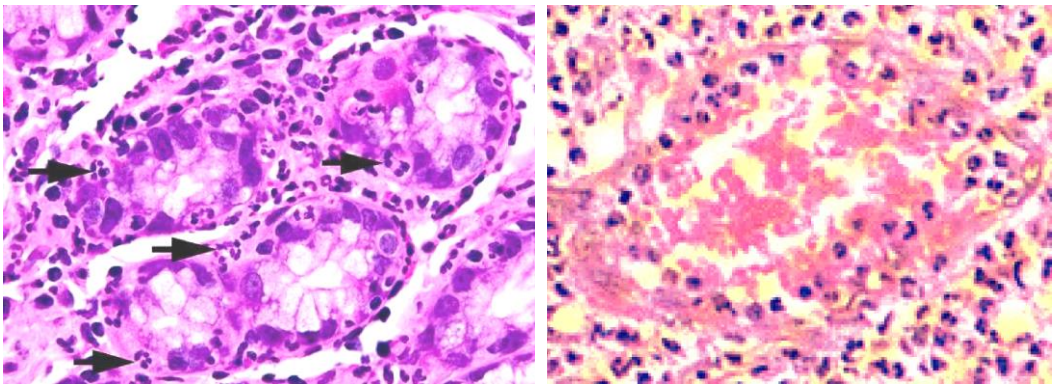
When Marian has recovered, she asks to see the examination results. In addition to the blood test results, the histology of the removed appendix is also available:

Blood tests results

		Normal value
- CRP*:	**150 mg/L**	*2-9 mg/L*
- Sedimentation:	**45 mm in 1st hour**	*< 20 mm in 1st hour*
- Leukocytes:	**12.5 x 10^{9}/L**	*3.4-8.9 x 10^{9}/L*
- Leukocyte differentiation:		
. band neutrophils:	**12%**	*< 4 %*
. segmented neutrophils:	**61%**	*20-68%*
. lymphocytes:	**21%**	*7-30 %*
. monocytes:	**5%**	*1-7 %*
. eosinophil granulocytes:	**1%**	*0-4 %*
. basophil granulocytes:	**0%**	*0-2 %*

*: CRP = C-reactive protein

Histology of the appendix



Marian and Ann are studying the test results. “Your blood values were pretty weird when you had appendicitis,” says Ann. “What does CRP mean?” asks Marian. “I’m not sure,” Ann replies. She picks up the histological images and points at the infiltrated leukocytes in the inflamed appendix (see black arrows in the left image). “These are neutrophils. You see them in the blood vessels as well, in contact with the endothelium,” she remarks (right image). “This is probably where the inflammatory cells enter the tissue. Isn’t it remarkable that the cells know where they should enter?” wonders Marian. “What about the fever you had?” asks Ann, “I guess that was related to the appendicitis as well?” Their discussion now focuses on how with the information from the blood test you know a lot about what happens in the inflamed appendix.

Case study 2. What goes wrong?

A) 'Infection without pus'

A toddler is hospitalised for a severe lung inflammation. There is some recovery two days after antibiotics were administered.

The child's medical history is as follows:

1. Recurrent necrotising wounds on the skin.
2. Chronically inflamed gingiva (gums) from the moment that the milk teeth erupted.

No pus was forming in the wounds on the skin or mucous membranes. At first, it was therefore assumed that the wounds could not have been caused by a bacterial infection. A blood sample was taken for further testing and skin biopsies were taken from the wounds.

Result of the histopathology of the skin biopsies

- Central necrotic areas with bacterial material and peripheral fibrotic areas.
- Absence of leukocytic infiltrate in the tissue biopsies.

Result of the blood test

All types of white blood cells present; neutrophilic granulocytes elevated (81%).

Additional tests by an interested scientist gave the following information:

Skin biopsies

- NADPH oxidase and myeloperoxidase (enzymes of macrophages and neutrophils): not shown to be present.
- Lysozyme (a proteolytic enzyme of macrophages): not shown to be present.
- TNF-α (an inflammatory cytokine): not shown to be present.

Tests on the blood cells and skin biopsies

- Absence of β2-integrins (a particular type of adhesion molecules) on any of the leukocytes.
- Selective binding of neutrophilic granulocytes and monocytes to the bacterial areas in the biopsies.

"Functional phagocytes are present in the blood, but not in the tissues; I can imagine that things go wrong upon infection," concludes the scientist.

B) 'Meningitis too often'

A 27-year-old man has been diagnosed with meningitis for the third time in 5 years. Bacteriological tests show that the bacterium Neisseria meningitidis has again caused the condition this time. Antibiotic treatment is successful, just like the previous times. However, having meningitis three times in such a relatively short period of time could be indicative of immune system problems. Additional blood tests are therefore carried out to check the man's immune status. The leukocyte picture is normal, and so is the level of immunoglobulins (antibodies).

An initial exploratory investigation into the status of the complement system does not show abnormal values for complement factors C3 (0.89 g/L) or C4 (0.15 g/L).

The haemolytic complement activity (lysis of erythrocytes by complement) was determined by incubation of the man's serum at 37˚C with:

- immunoglobulin-coated erythrocytes
- mannose-coated erythrocytes

In both cases there was binding observed of serum C3 to the erythrocytes, but no haemolysis was seen. The other complement factors in the man's serum were then determined. This showed a complete deficiency of complement factor C7.

Case study 3. Adequate vaccination?

Mrs van Loon, 60 years old, has received a letter from her general practitioner (GP) in which she is invited to come for a flu vaccination. She realises that her age means that she has become part of a risk group.

Her son Marcel, a sixth-year medical student, is enthusiastic when he hears her talking about it. He is doing a research elective at the immunology department and thinks that it may be possible to study his mother's vaccination in detail. His supervisor agrees, provided that Marcel personally carries out the experiment. He does not need to be told twice and he starts working diligently. His proposal is approved and he is told he can start working on it.

When Mrs van Loon goes to get her flu vaccination, Marcel has two blood samples taken from his mother beforehand.

- 1 coagulation tube to make serum
- 1 heparin tube to obtain blood cells

He immediately starts working with the samples and carries out his experiment. After two weeks, he asks for another two tubes of blood to be taken from his mother and he carries out the experiment once again. He puts the results of the two experiments in a table next to each other:

EXPERIMENT		RESULT	
		Before vaccination	*2 weeks after vaccination*
Antigen-specific antibodies present in the serum		- (negative)	++ (strong positive)
Proliferation of mononuclear white blood cells *in vitro* after adding the antigen*		-	++
Cytokine production by mononuclear white blood cells *in vitro* after adding the antigen	IL-17	-	-
	IL-2	-	++
	IL-4	-	-
	IL-12	+/-	++
	IFNγ	-	+
Proliferation of purified blood T-cells *in vitro* after adding the antigen		-	-
Proliferation of purified blood T-cells *in vitro* after adding the antigen + blood monocytes.		-	++

*antigen = the flu vaccine

Marcel is enthusiastic. He rings his mother and tells her that the vaccination has worked. “That's great,” she responds, “So that means that I'm immune to flu from now on.” “Afraid not, Mum,” says Marcel, “the flu virus is a deceptive microbe, you'll have to get another flu vaccination every year.”

Case study 4. Omenn Syndrome

After birth, little James seemed to be a normal healthy baby. However, 20 days after birth, he developed a dry cough that lasted for over a week. Moreover, he had purulent conjunctivitis (yellow discharge from his eyes). His condition did not improve. After developing pus accumulations in the skin behind the ear he was admitted to the hospital. At the hospital, pus-containing lesions were drained and *Staphylococcus aureus* and *Candida albicans* could be cultured from the drainage fluid. He also had an opportunistic *C. albicans* infection in his mouth (oral thrush).

Blood was drawn for lab tests (see below).

Lab. Clinical Immunology

University Medical Centre Maastricht

P. Debyelaan 25, P.O. Box 5800, 6202 AZ Maastricht

Lab. Results:

			normal
Total IgG	0.55	g/L	(6.90-19.10)
Total IgA	<0.06	g/L	(0.66-4.30)
Total IgM	<0.52	g/L	(0.52-3.50)
Total IgE	72	g/L	(< 0.5)

Leukocytes:	**8 x 10^9/L**	*3.4-8.9 x 10^9/L*
. neutrophils:	**20%**	*20-68%*
. lymphocytes:	**6%**	*7-30 %*
. monocytes:	**23%**	*1-7 %*
. eosinophil granulocytes:	**51%**	*0-4 %*

Due to these results, an immunologist was assigned to the case. She argued that antigen detection might be compromised in poor little James. In order to confirm her suspicion, flow cytometry analysis was performed on the blood sample. Surprisingly, virtually no B-cells could be detected. Moreover, testing various Vβ families on T-cells, showed that only few of them were expressed, indicating an oligoclonal, rather than polyclonal, T-cell receptor repertoire. Furthermore, James' blood cells responded very poorly to phytohemagglutinin stimulation. A chest X-ray showed clear lungs but there was no thymic shadow.

The suspicion of the immunologist was confirmed as DNA sequencing showed homozygosity for the Arg229Gln mutation in the *RAG2* gene. James was diagnosed with Omenn syndrome and could only be rescued by a bone marrow transplantation.

Case study 5. Allergy, can it be cured?

Laurent, a 17-year old boy, is having a hard time in high school. First of all, he is dyslectic and in addition to that, he suffers from hay fever resulting in often being fatigued; the combination of both turns doing homework into hell. His dyslexia cannot be cured, but he still hopes to get rid of his allergy one day.

He has heard rumours about desensitization when it is known to which allergens you are allergic. He has been checked for this. His blood IgE values turn out to be increased (1200 IU). A skin-prick test shows that he is hypersensitive to grass pollen, but not to pollen from trees or other inhalation allergens like house dust mite and cat's hair. Despite the fact that a desensitization programme is a long-lasting process, Laurent decides to go for it. The chances for success are between 60 and 80%. Since there are hardly any natural allergens in autumn and winter, desensitization programmes against these allergens are started in the fall period. According to a fixed protocol (see below), Laurent is injected subcutaneously with grass extract (a mix of 5 species of grass), alternately in his right and his left upper arm.

Immunization schedule

First series		Second series	
Week	Dose in units	Week	Dose in units
1	20	17	100000
2	40	20	100000
3	80	24	100000
4	200	Subsequently a monthly maintenance dose of 100000 units is injected for a period of 3-5 years	
5	400		
6	800		
7	2000		
8	4000		
9	8000		
10	10000		
11	20000		
12	40000		
13	60000		
14	80000		
15	100000		

In week 24 of the desensibilization programme, blood is drawn and the following variables are tested:

	Result
Total serum IgE:	400 IU
Specific IgE against grass extract (RAST-measurement):	negative
Specific IgG against grass extract (RAST-measurement):	positive
Cytokine production by allergen-activated mononuclear blood cells:	*IL-4*: <10 pg/mL
	IFNγ: 1.3 ng/mL
	IL-10 : 1.5 ng/ml

That summer, Laurent feels much better. He can leave his antihistamines at home for most of the time!

Case study 6. Organ transplantation: unwanted reactivity

Peter is 23-year old and an only child. His parents are both 49 years old. Five months ago, Peter was registered for a kidney transplant. He suffered from a kidney disease 2 years ago and as a result, his kidneys are no longer functioning properly. About 6 months ago, his situation deteriorated and he had to undergo dialysis. Currently, he visits the hospital for dialysis twice a week. Before registration on the transplantation list, Peter's blood was tested for HLA type and cytomegalovirus (CMV); his blood group was already known.

Result	
HLA type:	HLA-A3, -A28; -B7, -B12; -Cw4, Cw6; DR2, DR10
Blood group:	AB; rhesus positive
CMV:	positive

Peter's parents want to help and both offered to donate a kidney. In order to see whether they would be a good match, HLA type and blood group of mum and dad are determined.

Result	
Father:	
HLA type:	HLA-A10, -A28; -B7, -B52; -Cw2, Cw6; DR2, DR10
Blood group:	A; rhesus negative
Mother:	
HLA type:	HLA-A3, -A19; -B12, -B41; -Cw4, Cw6; DR2, DR7
Blood group:	AB; rhesus positive

The results of the HLA typing are positive. As expected, each of Peter's HLA-A, B, C and DR molecules match either his father or his mother. In addition, Peter has two HLA-DR matches with his father. The latter is preferred in donor selection. Since the dad's blood group can also be matched with Peter's, his kidney would be the best option.

Dad donates his kidney to Peter. The actual transplantation is successful. The transplant functions and dialysis is no longer required, but Peter needs immune suppression (Tacrolimus and Sirolimus/Rapamycine). In the long run, the dose can be diminished, but immune suppression will always be necessary.

In the second week after the transplant, Peter gets ill. He suffers from pneumonitis with fever and fatigue. Blood tests reveal a relapsed CMV infection. The transplant-nephrologist in attendance lowers the dose of the immune suppression and administers Ganciclovir.

Practical 'White blood cells'

White blood cells or leukocytes play an important role in the course of the immune response. Therefore it is important to have a good picture of the properties and morphology of the different cells that are involved. Therefore, in this practical you will get familiar with the different leukocyte populations in a practical approach. Fresh blood smear preparations will be made. For this purpose blood smear preparations will be prepared by means of a blood droplet from a finger prick. These preparations will be stained and examined under the microscope for typing and counting leukocytes. To get familiar with the morphology of the different cell types in the stained blood, the computer programme *HemoSurf* will be used.

Practical schedule

1. Microscope instruction
2. Students form pairs
3. Two blood smear preparations are prepared per student pair; one student will be finger pricked for a few drops of blood to make the smears.
4. During drying of the preparations, students receive a light microscope instruction.
5. Dried preparations are stained.
6. During drying of the stained preparations, students get introduced to the computer programme *HemoSurf*.
7. Students examine the blood smear preparations under the microscope and count the different leukocyte types. Each student counts one of the two preparations and calculates the % of each leukocyte type in the preparation.
8. The calculations of the examinations will be used to calculate the mean % of each leukocyte type in the blood.
9. The individual and mean percentages will be discussed with the supervisors for endorsement of the practical.

PART 1: Instructions using the light-microscope

- Turn on the illumination of the microscope using the switch on the back
- Open the field diaphragm completely
- Adjust the light intensity with the knob at the right side
- Place the object stage in a lower position by turning the macro focus knob
- Take a low magnification objective
- Place the slide with the preparation clip on the object stage
- Set de illumination to the right intensity
- Adjust the distance between the eyepieces and start viewing the image from a distance of about 5 cm from the eyepieces
- Try to see one circle and the image by searching for the good distance to the eyepieces
- Close your right eye and focus the image with your left eye
- Close your left eye and focus the image with your right eye
- Choose a low objective magnification
- Focus your eyes on the specimen on the slide with the macro focusing knob
- Make with the micro focusing knob a precisely focused specimen
- When you change the objective you have to focus again with the micro focusing knob
- Change the objective by rotating the revolver to a higher magnification
- Move the object stage position to examine your specimen on the slide

PART 2: Blood smear preparation and differentiation

Blood smears are used for counting white blood cell types. It is not the absolute numbers that will be counted but the relative proportions in percentages of total. Of the various types of white blood cells, the normal values are known. In clinical situations, abnormal values give information on possible disease processes such as inflammation or acute leukaemia (blood cancer).

The finger prick

Instructions how to operate and how use the *glycolet* will be given. The preparation of a blood smear preparation from a fresh blood droplet will be demonstrated.

Methods for making a blood smear

- Write name on frosted end with pencil (to make difference between upper- and under side of the object glass)
- Decide who will donate the blood drop and who will make the blood smear
- Transfer a small drop of blood from the finger (don't take the first drop) onto the glass slide in the middle, near the marked, frosted end
- Don't let the drop clot
- Make a smear on the slide by moving a second slide, under a 45° angle, towards the drop
- Allow the drop to flow along the edge
- Then move the second slide on the first slide; the blood is spread in a thin film
- Allow the specimen to air dry

See also https://www.uvm.edu/~jschall/pdfs/techniques/bloodsmears.pdf

Blood smear staining with May-Grünwald-Giemsa

Materials

- Glass slides
- Pipets (200 - 1000 µl) and tips
- May-Grünwald-solution
- Giemsa-solution (prepare fresh, see below)
- Stainingbox
- Rinsing box
- Aqua bidest
- Gloves
- Tissues
- Alcohol 70%
- Glycolet
- Lancets

Composition Giemsa-solution

6 ml Giemsa

180 ml H_2O

20 ml Phosphate buffer pH=6,9

Composition phosphate buffer pH=6,9

3,265 g $Na_2HPO_4.2H_2O$

2,042 g KH_2PO_4

500 ml H_2O

Protocol for staining a blood smear

- Put the slides in the staining box
- Bring May-Grünwald-solution on the blood smear; cover the whole smear
- Incubate for 5 minutes; be aware the blood smear doesn't become dry
- Rinse the slides with aqua bidest 3 or 4 times in rinsing box; the rinsing solution has to be clear
- Put the slides back in the staining box
- Bring Giemsa-solution on the blood smear; cover the whole smear
- Incubate for 10 minutes; be aware the blood smear doesn't become dry
- Rinse the slides with aqua bidest 3 or 4 times in rinsing box; the rinsing solution has to be clear
- Let the slides dry in open air

Microscopy and counting of blood the smear

- Check the quality of the stained preparations first by eye.
- Each student counts one of the 2 preparations.
- Search under the microscope by low magnification (10x objective) for a thin area in the preparation where the red blood cells are lying just free from each other.
- Switch to the 40x objective to differentiate the leukocyte types in the preparation. Count the leukocyte types per view and move to a next view in horizontal and/or vertical direction. Using the typing table below, count a total of about 100 cells.
- Calculate the percentage of each leukocyte type in the smear.
- Calculate for each leukocyte type the mean % from the 2 counted preparations.
- When ready, ask for a supervisor for discussion of the results.

Every leukocyte will be identified as one of the types listed below and registered in the counting table (a haemogram).

Counting table (haemogram)

	Total	%	ref. value (to be filled out during the discussion with the supervisor)
Band neutrophils	:	:	
Segmented neutrophils	:	:	
Eosinophils	:	:	
Basophils	:	:	
Monocytes	:	:	
Lymphocytes	:	:	

PART 3: *HemoSurf*: A self-training haematology programme

URL: http://fhmloisrv1501.unimaas.nl/hemosurf/

This program will introduce the students to several types of leucocytes. It is linked to case 1 and the practical session. The students can work on this programme independently by using the URL link on a computer.

Contents and setup

HemoSurf gives students and doctors the chance to learn about morphological haematology using over 3000 pictures of blood and bone marrow films. The students gain confidence in their abilities to interpret blood and bone marrow films by going through modules of increasing difficulty and by receiving various forms of feedback. Fundamental theoretical knowledge is provided on demand while examining a blood film or a case. For more in-depth knowledge, learners should consult textbooks and journal articles.

Instructions

Only some parts of the programme are related to the content of this course. You use them as follows: When you open the programme, click on This way to surf the cells and then To the program! Make sure rule of filament has been ticked.

Read the text at A: Learning modules. Subsequently, click in learning module A on the module White Blood Cells.

The following parts are relevant for this course: A: Leukocytes that normally appear in the peripheral blood. Execute Step 1, Step 2, and Step 3. After completing Step 3 successfully, in Step 4 choose a patient presenting clinical picture that interests you and carry out a leukocyte differentiation, after which a specialist will comment on it and give a diagnosis.