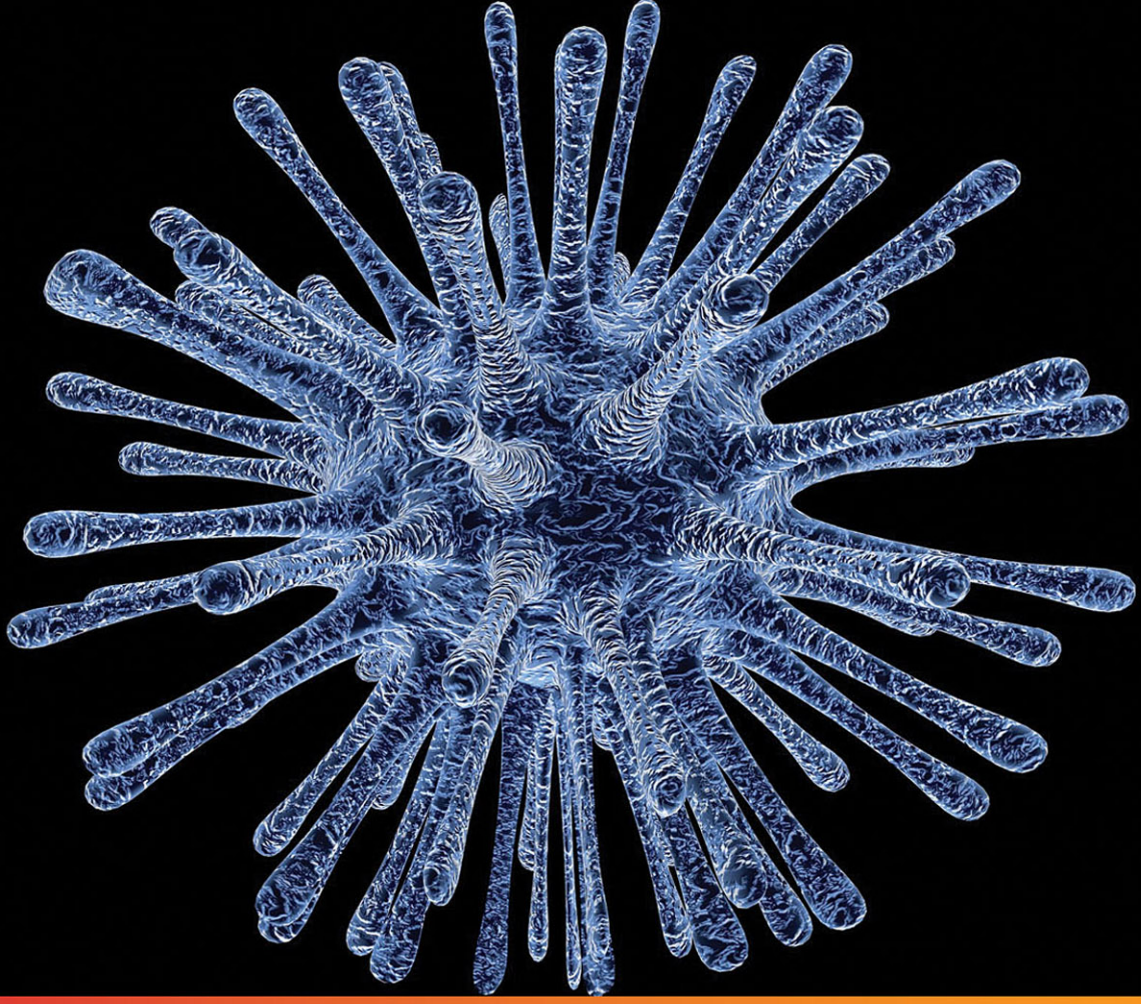

PÉTER GOGOLÁK – GÁBOR KONCZ

Elementary Immunology

Short textbook for BSc students

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UNIVERSITY OF DEBRECEN
FACULTY OF MEDICINE
DEPARTMENT OF IMMUNOLOGY

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Elementary Immunology

Short textbook for BSc students

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Preface

This textbook is primarily intended for BSc students, but it is useful for everyone who seeks fast basic knowledge in the field of immunology. The information provided also makes the understanding of the "full-bodied", advanced level immunology text books easier. The length of the text is kept limited and a significant part of it is constituted in the glossary, which contains the most important terms of the fields of biology and immunology. These terms are indicated in the text by italic typeface at their first appearances.

Introduction

The main function of the immune system is protection of the body against various *pathogens*, different biological *toxins* and even against altered, dangerous, abnormal cells inside the body. The two chief and clearly distinct functions of the immune system are recognition of the dangerous and/or pathogenic structures followed by the elimination/neutralization of these structures.

What can be recognized by the immune system?

Signs of biological danger

Microbes differ significantly from the cells of our own body. These differences are manifested in their building materials, physical and chemical structure. Most of these are completely absent from our cells, while they are characteristic of various taxonomic groups of microbes or other *pathogens* (viruses, bacteria, uni- and multicellular parasites). Typical examples of these small conserved molecular motifs are double stranded RNA of some viruses, or *lipopolysaccharides* (LPS) found in the cell wall of the Gram-negative bacteria. These materials are collectively referred to as *pathogen-associated molecular patterns*, or *PAMPs*. The immune system can effectively sense the presence of these chemical structures with relatively few receptors called pattern-recognition receptors, PRRs, and interprets them as "danger". Engagement of PRRs evokes strong activation signals. In addition to the above mentioned microbe-derived danger signals, injury or abnormal processes in the body may also act as danger signals resulting in the activation of PRRs. In the case of cell damage intracellular materials can appear in the extracellular space (outside of the cell). This presents a danger signal and alarms the immune system even in the absence of pathogens. These types of danger signaling molecules are called as damage-associated molecular patterns (DAMPs).

The antigen

The concept of antigen is easily understandable, yet antigen is one of the most ill-defined terms in immunology. As a general definition, the antigen is an entity recognized **specifically** by the B- and T-*lymphocytes*. The focus of this definition is on "specificity". This presumes the presence of highly specific receptors that can bind to a given material or structure, but fail to recognize others. These extreme specificities have been demonstrated by experiments in which some small chemical modifications of an antigen can render an antigen unrecognizable by the receptor which had been recognized in its original form. Or it may work the other way around; after chemical modification the altered antigen can be recognized with high specificity by another receptor which,

before the modification, was unresponsive to it. Though in most cases small changes do not cause such a drastic effect in antigen recognition: modifications usually result in small changes in the binding affinity of the receptor to its specific antigen. The immune system can produce a vast variety of highly specific *antigen receptors* (in the order of billions). This receptor diversity is achieved by an elegant and sophisticated molecular genetic mechanism. An individual cell produces only one type of antigen-specific receptor, so one lymphocyte is specific to only one type of antigen. Based on these we can create a simple but seemingly circular definition: The antigen is an entity recognized by the antigen receptors.

It is important to note, that not only pathogens can be considered as antigens. Antigen recognition could involve the recognition of the self-derived materials, but normally these don't provoke a destructive immune response. The immune system is normally tolerant to self-antigens. According to the response following antigen recognition, we can classify immune responses into immunogenic and tolerogenic immune responses, and antigens into immunogenic and tolerogenic antigens (as discussed later).

What terms should be used to describe immune recognition? Lipopolysaccharide, a bacterial cell wall component or a viral double stranded RNA should be considered as a PAMP, when it is recognized by pattern-recognition receptors on various cell types. However, these structures are referred to as antigens when discussed from the point of view of *lymphocytes* that recognize them with their antigen-specific receptors, potentially capable of recognizing minor chemical or structural differences in PAMPs. Generally the first recognition of the pathogens and other danger signals are mediated by PRRs, and the fine recognition and distinctions between self, non-self or modified self-molecules are mediated by lymphocytes with antigen receptors.

Destroying pathogens and tumour cells

The elimination of the pathogens, infected cells and dangerous tumour cells can be mediated by only a few mechanisms. Defense mechanisms against extracellular and intracellular pathogens are based on distinctly different mechanisms. Extracellular pathogens can be directly attacked by the immune system while elimination of the intracellular pathogens is almost always mediated by killing of the infected cells. Elimination of tumour cells is similar to that of cells infected with intracellular pathogens.

The structure and organization of the immune system

The cells of the immune system can be found almost everywhere throughout the body. Some of them operate “lonely” residing in various peripheral tissues. Immune cells can even be found in the epidermis. Others, together with several other immune cell types assemble into immune tissues with various levels of complexity. Lots of immune tissue ‘isles’ (follicles, folliculi) can be found in the mucosal membranes. At several places the immune tissues are organized into smaller or larger organs called lymphoid organs. The so called *primary lymphoid organs* are responsible for the production of the immune cells (interchangeably called ‘white blood cells’). The secondary lymphoid organs are sites where cells of the *adaptive immune system* (the B- and T-lymphocytes) can first encounter the antigens. The *secondary lymphoid organs* provide an appropriate environment for the activation, proliferation, and differentiation of the lymphocytes.

Primary lymphoid organs

The red bone marrow is located in the spongy bone tissue of various bones. All the blood cells, so almost all white blood cells (*leukocytes*) including the *lymphocytes* of the immune system derive from the red bone marrow. The development of these cells starts from *stem cells* (hematopoietic stem cells). The stem cells in the red bone marrow initially differentiate into some specialized immune cell type in response to various/specific cytokines, and other chemical and local environmental stimuli. Two large developmental lineages can be defined:

The myeloid lineage gives rise to the *macrophages*, *dendritic cells*, *mast cells*, and *granulocytes*. The other developmental pathway, the lymphoid lineage, provides the precursors of *lymphocytes*, which finally develop into B cells, T cells, or NK-cells. (Figure 1.)

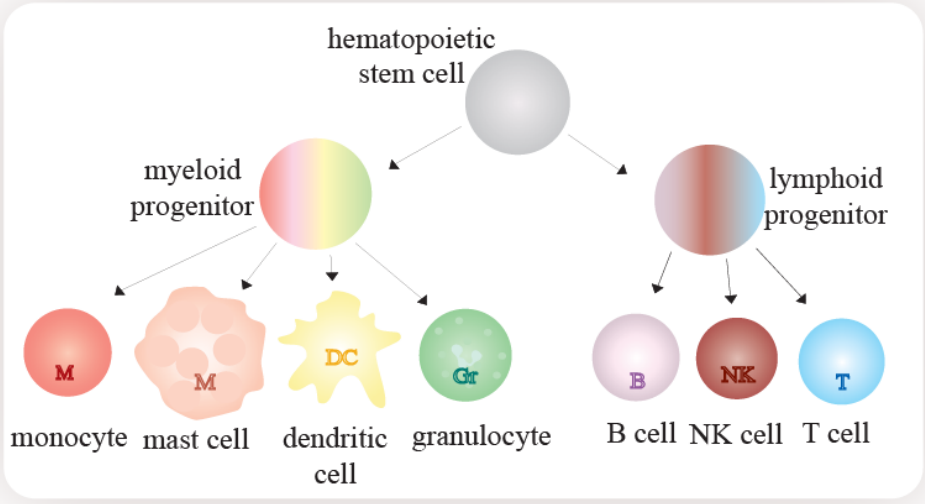


Figure 1. The development of immunocytes in the red bone marrow

The cells of the immune system develop from the haematopoietic stem cell derived myeloid- and lymphoid precursor cells

The red bone marrow and the thymus are the two *primary lymphoid organs* in the body as they are the sites of lymphocyte development. The progenitors of T-lymphocytes exit the bone marrow and enter the thymus where they complete their development. There is no active (immunogenic) immune response in the protected environment of the primary lymphoid organs. In healthy individuals pathogens are absent in the primary organs because this is a place where self-materials of the body (the self-antigens) are typically introduced to the developing lymphocytes. The cells of the *adaptive* arm of the immune system can meet the self-antigens here, and can 'learn' the antigens which should be considered as harmless, and thus, must be tolerated. Thanks to this 'education' the lymphocytes that leave the primary lymphoid organs will tolerate self-antigens present in both the primary lymphoid organs and the peripheral tissues of the body. You can find more information about these processes later in the 'Immunological tolerance' chapter.

Secondary lymphoid organs and the lymphatic system:

Secondary lymphoid organs are the activation sites of the *adaptive* arm of the immune response. Lymphocytes which successfully leave the primary lymphoid organs are called naïve lymphocytes, because they have not yet

encountered their specific antigen. naïve lymphocytes migrate into the secondary lymphoid organs, where they can meet and recognize microbial antigens. Antigen recognition induces activation of the naïve lymphocytes, which as a result, will proliferate and differentiate to become fully functional, ‘effector’ lymphocytes.

The blood transports nutrients and oxygen for the cells and tissues of the body. Some components of the blood plasma are filtered out from the capillaries into the tissues. This fluid, also known as lymph, contains various metabolic products of the tissue cells, or in case of an injury, microbes or other foreign materials from the environment.

Lymph is collected by the lymphatic capillaries. Lymphatic capillaries are glove-like structures that originate in the tissues with closed ends and unite to form small lymphatic vessels, and later the lymphatic system. Foreign antigens are transported by the lymph into a nearby lymph node (also called as the draining lymph node) which provides a convenient checkpoint for monitoring antigen content of the lymph. The lymph filtered through the lymph node will enter into a larger lymphatic vessel. The small, bean shaped lymph nodes are distributed along the lymphatic vessels. They can form small groups in some parts of the body, the neck, armpits, groin or the abdomen, in particular. The continuously migrating cells of the immune system can gather in special, well defined compartments of the lymph nodes to encounter the accessible antigens, and to meet and communicate with other cells of the immune system. (Figure 2.)

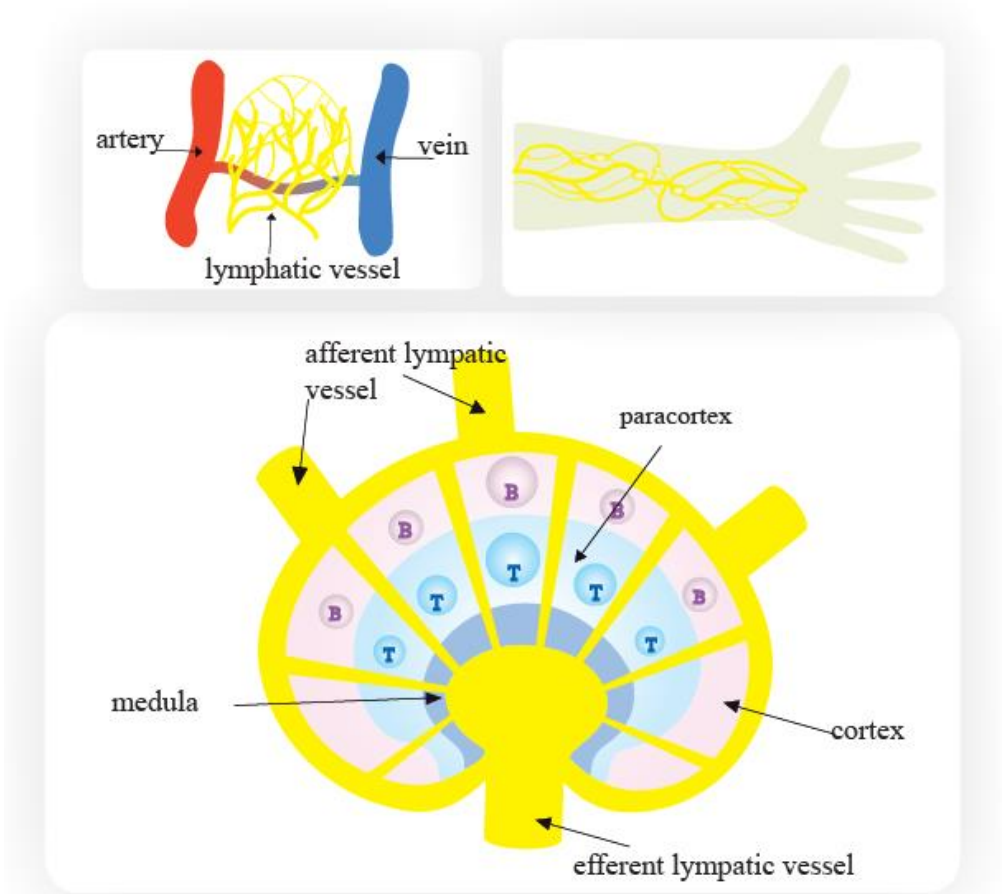


Figure 2. The scheme of the lymphatic system and the lymph node.

The lymphatic circulation is a network of lymphatic capillaries, small and larger lymphatic vessels that collects and transvers lymph into the venal blood. Lymph enters the regional lymph node via the afferent lymphatic vessels. Lymph exits the lymph node via a single, large efferent lymphatic vessel and continues its journey further in the lymphatic system. The lymphocytes can encounter the antigens within the B- and T cell zones of the lymph node.

The secondary lymphoid organs are the places where the lymphocytes meet their specific antigens the first time. If B- and T-lymphocytes "camping" in lymph nodes meet the antigen to which they possess a specific antigen receptor, they recognize it. After binding the antigen, provided other necessary activation signals are also received, they rapidly go through several cycles of cell division (proliferation). Thus, at the end of this process the number of antigen specific lymphocytes is increased many fold. The non-specific

lymphocytes, which fail to recognize any antigen will exit the secondary lymphoid organ sooner or later. They leave via the efferent lymphatic vessel, subsequently enter other secondary lymphoid organs/tissues and eventually, they will return into the blood circulation. They repeat these cycles until they find an appropriate antigen. If they fail to meet their specific antigen within a few days or for some cells for a few weeks they will die by *apoptosis*.

Antigens not only enter the lymph nodes passively carried by the lymph. They can also be transported by phagocytic cells patrolling the tissues e.g. tissue macrophages and dendritic cells. These cells can engulf different antigens and actively transport them into the local lymph nodes using the lymphatic vessels. Once they have arrived at the lymph node they present the engulfed and processed antigens to the assembled T-lymphocytes.

Lymphocytes can use both the lymphoid vessels and the blood vessels for traveling throughout the body. They generally enter the lymph nodes through the special kind of post-capillary veins called high endothelial venules, present in the lymph node. Lymphocytes exit the nodes through the efferent lymphatic vessel. They use the lymphatic system to travel back into the bloodstream. The lymphatic and the blood circulation is directly connected at the shoulder-neck region of the body. By the help of the left and right large main collecting lymphatic ducts the lymph, together with the travelling cells returns into the large veins. This way the lymphocytes can visit a large number of lymph nodes in all parts of the body in a relatively short time in order to find pathogen-derived antigens.

It is important to note that the naïve lymphocytes usually can't get access to the tissues where the pathogens enter the body (skin, mucosa). But after their efficient activation in the secondary lymphoid organs, they become fully functional effector lymphocytes which can reach the peripheral tissues.

The spleen is a long flat secondary lymphoid organ located at the upper left part of the abdomen. Similar to lymph nodes, the spleen has compartments, where the immune cells can communicate with each other, and can be activated. The spleen doesn't have direct (afferent) connection with the lymphatic vessels, instead it functions as a filter of blood. The assembled lymphocytes in the spleen can encounter the antigens present in the blood.

Lymphoid tissues (follicles) can be found at several locations in the body. Significant lymphoid tissues can be found in the wall of the digestive system and in the airways which are the main gateways of infection by various pathogens. Such lymphoid tissues are present in the tonsils and in the adenoid of the upper respiratory tract, in Peyer's patches found within the wall of the small intestine, or in the appendix of the coecum.

Multiple cell types may participate directly or indirectly in the immune response. In addition to cells various body fluids contain components essential to the coordinated operation of the immune system. Depending on whether the cellular or the soluble component plays a more prominent role in a particular response we call it a cellular- or humoral immune response, respectively.

Primary lymphoid organs	Secondary lymphoid organs
• red bone marrow and thymus • production and initial differentiation of immunocytes • the main sites of the development of immunological tolerance	• spleen, lymph nodes, the different skin-, gut-, respiratory tract associated lymphoid tissues • induction sites of the adaptive immune response (not exclusive, mainly connected with the adaptive immune response) • site of lymphocyte activation and differentiation of naïve lymphocyte into effector cells

Properties and cells of the innate and adaptive immune responses

The elements of innate immunity appear long before birth, and are constitutively present in the body. Its components are generated continuously, their production can only be increased moderately, even when they are needed. Thus, certain elements of the innate system can be exhausted. Nevertheless, innate immunity can provide an immediate response because its components are always present in the body. The innate immune responses are not antigen specific. It senses various „danger" signals released from microbes or by damaged tissues. During an immune response the number of innate immune cells shows only moderate changes. Though infections can induce a small increase in cell numbers, rather the localization and activation state of the cells will be changed.

Cells of the innate immune system are constantly renewing to replace old dying cells ensuring continuous responsiveness of the system. The typical cellular elements of the innate immunity are granulocytes, dendritic cells, monocytes/macrophages and NK (natural killer) cells. Its humoral components

include the complement system (described later), antimicrobial proteins, enzymes or peptides.

It has been observed centuries ago that people who survived the ravages of an epidemic were untouched when faced with that same disease again – they had become immune to infection. The reason for this is that during the first (primary) immune response in addition to activated effector B and T cells memory B and T cells are also formed. During the subsequent exposure to the same pathogen, thanks to the presence of long-lived antigen specific memory cells, the immune system is ready to launch a faster, more intensive and thus much more effective immune response. This kind of antigen specific immune response – characterized by memory and improved response upon second exposure - is called *acquired or adaptive immune response*.

Active adaptive immunity is not yet present in the newborn. However, the adaptive immune system continuously produces new lymphocytes with diverse antigen specificity. Only a few of these (naïve) lymphocytes are able to recognize a particular pathogen, therefore, only a small number of lymphocytes are available to respond to any given pathogen. When an antigen enters the body, only a few cells will respond, but they will divide intensively to produce a large number of effector cells which can successfully fight even the fast-growing microbes. The immune system shapes its response according to its actual challenges. The number of effector cells gradually decreases after they have completed their functions, however, some long-lived memory cells will survive, providing protection should the same pathogen be detected again.

Activation, proliferation and differentiation of lymphocytes take time, therefore, relatively longer time (about one or two weeks) is necessary to achieve the maximal response following exposure to an antigen. However the immune system can react more quickly the next time it comes into contact with the same antigen because of the presence of memory cells that have gone through an initial proliferation and differentiation process. Only a few days (3-5 days) is sufficient to boost the immune response again.

The cellular components of the adaptive immunity are T and B cells bearing antigen-specific receptors. Its humoral elements include immunoglobulins or *antibodies* produced by plasma cells that differentiate from B cells. Antibodies can be found almost everywhere in our body.

The immune system consists of a wide range of different cell types including lymphocytes and also various types of phagocytes. The cells of innate immunity are able to fight different kinds of invading pathogens while a given cell of adaptive immunity is designed to recognize only one specific target by its antigen-specific receptor with high efficiency. For an effective response the

immune cells need to communicate with each other. Sometimes a direct cell-cell contact among the immune cells is required, while at other times cells use soluble messenger molecules for communication. The freshly generated, so-called „naïve" lymphocytes – which have not encountered their antigens yet – are unable to function without previous interaction with other immune cells. In this case they may become functionally unresponsive after antigen exposure. This state of the cells is referred to as *anergy*.

The components of innate immunity are essential for the activation of adaptive immunity, in return some elements of the adaptive immunity can facilitate some of the functions of natural/innate immunity. Thus, the two systems work in concert supporting each other's functions.

	Innate Immunity	**Adaptive Immunity**
properties	• immediate reaction • not antigen specific • no memory	• requires several days to develop • antigen-specific • has memory
cells	monocytes/macrophages dendritic cells granulocytes mast cells NK cells	B cells T cells

Phagocytes and other cells of innate immunity

The cells of the innate immune system include phagocytic cells such as monocytes/macrophages and the dendritic cells. These large cells are able to engulf, kill and digest microbes, but they are also involved in the phagocytosis of other materials, for example dead cells or cell debris. In the case of these cells phagocytosis is not only the function of the elimination of the engulfed material. The processing and „presentation" of pathogen-derived ingested materials to lymphocytes plays an important role in the activation of the adaptive immune response.

The following section provides an overview of innate immune cells:

Monocytes which circulate in the blood are the precursors of phagocytes. After entering the tissues they differentiate into **macrophages** or into **dendritic cells**. Specialized macrophages found in various tissues and organs often have different names. For example, macrophages are referred to as microglia in the central nervous system, Kupffer cells in the liver, osteoclast in the bones, alveolar macrophages in the lung and histiocytes in connective tissues.

The **macrophages** are perhaps the most ancient cells of the immune system, with numerous functions.

- During bacterial infections macrophages are one of the first cell types which recognize the pathogen.
- After detection of pathogens they may be able to destroy them alone.
- Upon activation, macrophages secrete messenger molecules (cytokines) which induce local inflammation and recruit other immune cells to the site of infection.
- As *professional antigen-presenting cells* (APC), macrophages participate in the induction of the adaptive immune response.
- In addition to engulfing and elimination of foreign antigens, dead cells or tissue debris of various sizes, macrophages also play an important role in the regeneration of damaged tissues, mainly by the secretion of growth factors.

The name macrophage – as discussed above – does not necessarily refer to a specific cell type, rather it is a collective name of cells with similar functions. The functions listed above are often divided among the different subtypes of the macrophages.

Granulocytes with typical morphological properties are the effector cells of innate immunity against bacteria and some eukaryotic parasites. Their cytoplasm contains a large number of preformed granules with powerful antimicrobial substances for the elimination of parasites or microbes.

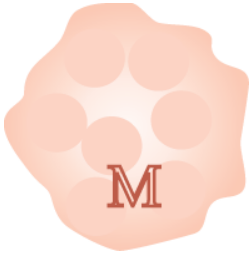
Neutrophil granulocytes are the most abundant members of the granulocyte family that also represent the most abundant population of circulating white

blood cells in the human body. These phagocytes are real „kamikaze cells" specialized only for the destruction of bacteria/pathogens. In healthy individuals neutrophils circulate in the blood stream and they are absent from healthy but not from inflamed tissues. In the case of an injury or pathogen exposure, a large number of neutrophil granulocytes migrate quickly from the blood to the sites of infection following chemical signals such as cytokines and chemokines produced by danger signal-sensing cells (e.g. macrophages). At the site of infection they engulf the bacteria or release the substances stored in their granules to eliminate the pathogens. Subsequently, neutrophils die by programmed cell death in a short time capturing the bacteria in their own apoptotic bodies. Later, the dead cells and other cell debris are cleaned up by macrophages.

The main functions of **eosinophil and basophil granulocytes** are similar to that of mast cells to be discussed later in this section. By special toxic substances stored in their granules they provide protection against unicellular and multicellular eukaryotic parasites that are too large to be taken up by phagocytosis. Eosinophils and basophils also play a pivotal role in the development of allergic reactions (described later).

Dendritic cells were named based on their branch-like projections, similar to the dendrites of neurons. Like macrophages, they also have various subtypes in different tissues and organs. During infection, as most innate cells, dendritic cells detect the pathogens immediately. However, the main function of dendritic cells is not the direct elimination of pathogens, rather to deliver pathogen-derived antigens into the lymph nodes and present them to various types of T cells. Because of this activity they are called **professional antigen-presenting cells**. Immature dendritic cells localize in the peripheral tissues mainly at the sites of pathogen entry (skin, gut, and lung). In these tissues their functions are the immediate recognition and uptake of pathogens. Activation by the recognition of harmful microbes induce characteristic changes in dendritic cells. They start to migrate into the draining lymph nodes through lymphatic vessels and they increase the expression of cell surface molecules essential for the activation of other immune cells. In the lymph nodes dendritic cells present the antigen in complex with MHC molecules for T cells (a more detailed description of the process will be provided in later sections). It is important to note that as the most effective professional antigen-presenting cells, dendritic cells are able to activate „naïve" T cells freshly released from the thymus.

The **natural killer cells (NK cells)** are a special class of lymphocytes. Similar to cytotoxic T cells they take part in the elimination of infected or malignant cells of the host. They store pre-formed toxic substances in their granules. They destroy the target cells by releasing the substances of these granules or by direct cell-cell interaction. In addition to the recognition of pathogen-associated molecular patterns and *opsonins* (described later), NK cells are specialized for sensing of typical stress-induced molecules on the surface of infected or tumour cells. However, the healthy host cells block the function of NK cells through inhibitory NK-cell receptors. Cells bearing „normal" self-markers are not attacked by NK cells. Furthermore NK cells are very aggressive toward cells that are missing „normal" self-markers. Overall, the activation of NK cells is regulated by the balance of inhibitory and activating signals. (a more detailed description of the process will be provided in later sections).



Mast cells provide protective immune responses against parasites. Mast cells unlike the functionally similar basophil granulocytes, are localized in various tissues, e.g. in the skin or in the wall of the bronchoalveolar or gastrointestinal tracts. The mediators of these cells released from their granules are responsible for the symptoms of allergic reactions; however, these mediators had originally evolved for the elimination of multicellular parasites.

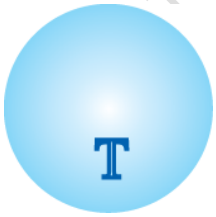
Platelets (thrombocytes) are blood circulating fragments derived from megakaryocytes, their large precursor cells present in the bone marrow. They also contain granules in their cytoplasm. Platelets play a role in the formation of thrombus during coagulation. When activated, they gather to form aggregates at the site of injured endothelium and then release substances of their granules containing coagulation factors, growth factors acting on various cell types involved in anti-microbial responses.

Phagocytes	Professional antigen-presenting cells
• Macrophages • Dendritic cells • Neutrophil granulocytes	• Macrophages • Dendritic cells • B-lymphocytes

Cells of the adaptive (acquired) immunity

The cells of adaptive immunity are the *B- and T-lymphocytes (or also called B and T cells*). These cells carry a unique receptor specialized for the recognition of only one type of antigen. Although the individual cells are able to recognize only one antigen, the billions of B and T cells together can detect billions of different structures.

B cells recognize the antigens by their cell surface antigen receptors. The antigen receptor of the B cell is a cell surface **immunoglobulin**. B cells express numerous membrane-bound cell surface immunoglobulins, so one antigen can be bound by more receptors at the same time. Following detection of antigens B cells get activated and differentiate into plasma cells. Plasma cells, the terminally differentiated form of B cells do not carry cell surface immunoglobulins, but they produce large amounts of these proteins in a soluble form which are commonly known as *antibodies*. Secreted immunoglobulins enter the surrounding tissue fluids and blood circulation. Secreted immunoglobulins or antibodies recognize the antigens of various pathogens and bind to them to mark (called *opsonization*) or to inactivate them (called *neutralization*).



Two main types of **T cells** are distinguished. The cytotoxic T cells specialized in the killing of infected or tumour cells and the helper T cells which play an important role in the facilitation and regulation of the immune responses. Helper T cells can enhance cytotoxic T cell responses, help macrophages to destroy the engulfed microbes and support antibody production of B cells. The two cell types can be easily distinguished based on their cell surface molecules. Cytotoxic T cells express *CD8* while helper T cells express *CD4* cell surface receptors. Briefly, they are CD8+ (CD8-positive) or CD4+ (CD4-positive) cells.

As has been repeatedly mentioned, please note that freshly generated so-called „naïve" T and B lymphocytes derived from the primary lymphoid organs are not fully functional! Further maturation/activation processes in the secondary

lymphoid organs or tissues are required for „naïve" lymphocytes to differentiate into functional effector cells.

Communication between immunocytes: cytokines and cell surface molecules

Immune cells communicate with each other in several ways. (Figure 3.)

- By cell surface molecules and receptors through direct cell-cell interaction. (The most important cell surface molecules are discussed in the appropriate chapters.)
- By soluble molecules, which play a pivotal role in the communication of immune cells. These chemical messengers can be different peptides, proteins, glycoproteins, which are collectively called ***cytokines.***

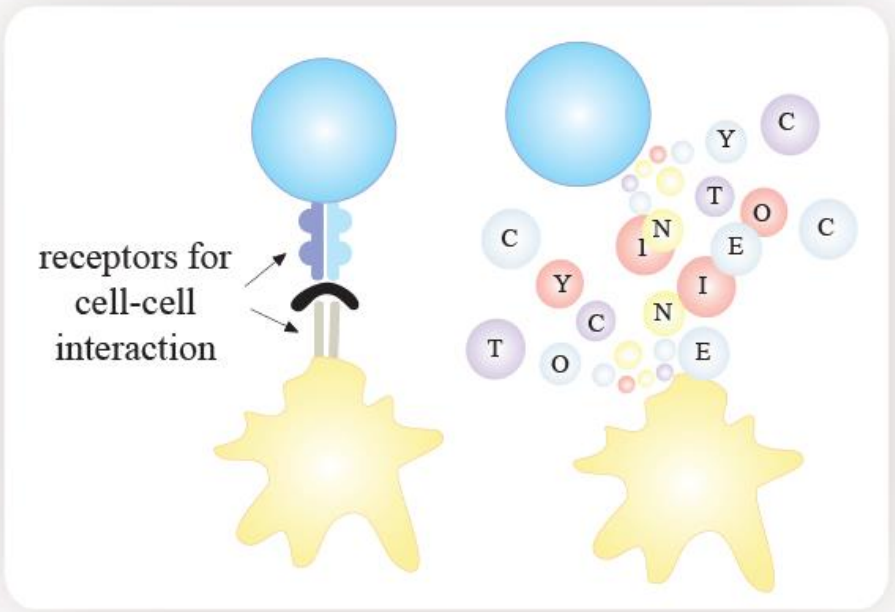


Figure 3. Communication in the immune system.

Cells of the immune system exchange information with each other through direct cell-cell contact or through soluble factors, primarily via secreted cytokines which are sensed by cytokine-specific receptors on the target cells. (Each cell can express a large number of different receptors. The number of cell surface cytokine receptors expressed by a given cell may vary considerably, reaching up to millions/ cell. In the figures of this lecture notes neither the number- nor the size of receptors are not presented on scale.)

Cytokines are hormone-like substances which are effective at very low concentration. Secreted cytokines can act in an *autocrine* manner on the cell that produced them or they can act locally on other cells in a *paracrine* manner. However, some cytokines can also have *endocrine* effects by influencing the function of distant cells or organs.

Responsiveness of a cells to a given cytokine is determined by the expression of the cell surface receptor specific to that particular cytokine. A single cell expresses various cytokine receptors so a given cell is usually affected by multiple cytokines which could modify (enhancing or inhibiting) each other's effect. Of course, a particular cytokine can affect the functions of many different cell types and can induce different responses in different types of cells. The functions of each cell will be influenced by the combined effects of the parallel signals (cytokines, other receptor signals, cell-cell interactions).

Cytokines are classified in many ways, and these groups often overlap with each other. For example, some cytokines belong to both the group of lymphokines and the group of monokines as they are produced by both lymphocytes and monocytes.

Cytokines involved in the communication between white blood cells (leukocytes) are often referred to as interleukins. Interleukins are distinguished from each other by numbers. To date, more than 30 kinds of interleukins have been described. One of them is interleukin-2 (IL-2) which induces the generation and cell division of T cells. Other cytokines regulate the activation and differentiation of leukocytes (e.g. IL-12, IL-4, IL-10). The so-called pro-inflammatory cytokines including for example TNF, IL-12 or IL-6, have pivotal roles during inflammatory processes.

There are some cytokines which can act as growth factors influencing maturation and differentiation of various cell types of the immune system. For example, the granulocyte-macrophage colony-stimulating factor (GM-CSF) induces the production of granulocytes and monocytes in the bone marrow.

Cytokines inhibiting (interfering with) viral infection are classified as a separate group called *interferons*.

Chemokines are cytokines that induce chemotaxis, attracting the appropriate chemokine receptor bearing cells towards the source of the chemokine. Such mediators facilitate for example the migration of blood-circulating lymphocytes and tissue-localized dendritic cells towards the lymph nodes. Large amounts of chemokines (e.g. CXCL8) and chemotactic factors (e.g. cleavage products of *complement proteins*) are produced in the infected or inflamed tissues which recruit neutrophil granulocytes, monocytes among other cells to the site of inflammation.

The innate immune system

The innate (or natural) immune cells are the monocytes/macrophages, the dendritic cells, the granulocytes and the NK cells. Humoral components of innate immunity include antimicrobial substances produced not only by immune cells: enzymes, antimicrobial peptides (e.g. defensins) can be produced by the liver or the epithelia. The proteins of the *complement system* and their cleaved fragments play a particularly important role.

The ancient mechanisms of innate immunity provide an immediate response. The most important phases of the response:

- detection of pathogen or danger signal,
- alarming and mobilization of other elements of immune system (including both the innate and the adaptive components),
- elimination of the pathogen as soon as possible.

Recognition of pathogens by the innate immune system: danger signal sensing receptors, pattern recognition, opsonins

The overall structure as well as composition of macromolecules present on the surface of microorganisms differ significantly from those found in higher order organisms. The „aim" of the innate immune cells is to detect characteristic danger signals and/or molecular structures present in microbial pathogens, but absent from human cells. These molecules are recognized by immune cells either in their soluble form or bound to the surface of a pathogen. For example the essential cell wall components of different bacteria (e.g. polysaccharides, peptidoglycans and lipopolysaccharides) or flagellin induce strong activation signals in phagocytes. Similarly, double-stranded RNA present in some viruses represents a danger signal for various cell types. These molecules indicating the presence of pathogens are called ***pathogen-associated molecular patterns*** (**PAMP**). Their presence is clearly a sign of danger, so their appearance has to be monitored by various types of pattern recognition receptors (**PRR**) expressed on different immune cells. The pattern recognition receptors appeared early on during evolution. They are expressed by all multicellular organisms from simple worms to the most sophisticated plants. PRRs are evolutionarily conserved structures as they recognize similarly conserved target molecules of pathogens.

The function of PRRs is not restricted to the recognition of extracellular pathogens. Intracellular pathogens living in the cytosol or in the vesicular system need to be recognized by immunocytes, thus some PRRs need to be localized in these compartments. Some of these receptors can readily bind to

particles in the endosomal compartment, another set of the receptors sense the pathogens in the cytoplasm. In addition to many immune cells, epithelial cells localized in various surfaces of the human body can also express pattern recognition receptors. Almost all types of these receptors are expressed on different macrophages and dendritic cells. (Figure 4.)

Sometimes self-derived molecules released by necrotic cell death or tissue damage called danger- or damage-associated molecular patterns (**DAMP**) can also serve as danger signals. This involves the "free" form of the genomic DNA itself, which is enclosed into the nucleus under normal circumstances. Its appearance in the cytoplasm may suggest that cells were infected by a DNA virus, or its extracellular localization may indicate damage-induced necrotic cell death which is an alarm signal for the immune system. Many other substances originally derived from nucleus or cytosol (e.g. extracellular ATP) are recognized extracellularly as DAMP by the immune system.

The recognition of pathogens and/or danger signals by the immune system is mediated by a group of a few dozen receptors only. These receptors are able to identify essential and conserved structures characteristic of various pathogen groups. Thus, innate immune cells can detect practically all pathogens by using these few pattern recognition receptors. However the recognition is not specific to each pathogen species, because specific (individual) recognition of tens of thousands of pathogens is impossible by only a few types of receptors. These receptors, however, provide sufficient information about the type of the infecting agent, about the site of the infection and about the type and localization (intra- or extracellular) of the pathogen allowing the induction of an appropriate anti-microbial immune response.

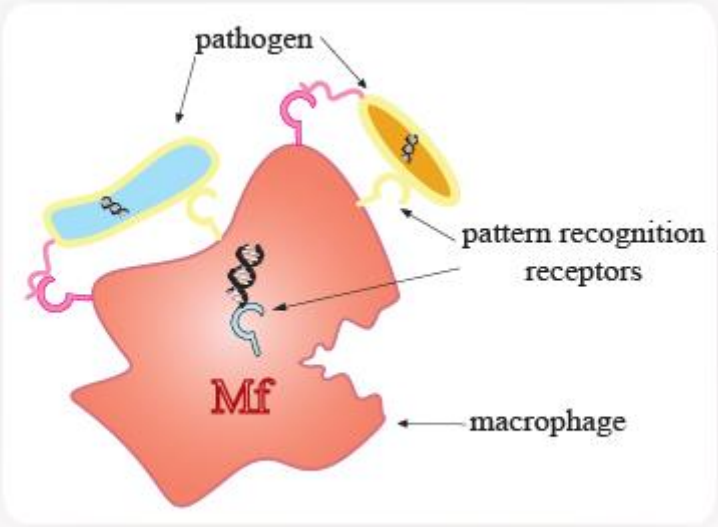


Figure 4. Detection of the pathogens by pattern recognition receptors.

The pattern recognition receptors of the innate immune system recognize structures that are characteristic of pathogens but not of human cells (e.g. bacterial cell wall components, double-stranded viral RNA). The pattern recognition receptors localize either on cell surface or inside the cells allowing the recognition of both extracellular and intracellular pathogens. They do not distinguish between individual species of microbes, they rather indicate the appearance of pathogens in the body.

Although innate immune cells are capable of recognizing pathogens in itself, the response is accelerated and enhanced in the presence of opsonins. Opsonins produced by the human body bind to pathogens to mark or target them for destruction. ***Opsonization*** facilitates the recognition and phagocytosis of pathogens by the innate immune cells. In addition to many cell surface receptors detecting pathogens directly, there are different opsonin receptors specialized for the recognition of opsonins bound to the surface of pathogens. The opsonin is forming a bridge between the pathogen and the opsonin receptor of the phagocytic cell (e.g. *Fc receptors or complement receptors*). Various molecules can act as opsonin during the immune response. Such molecules include antibodies, some complement fragments and the so-called acute phase proteins synthesized by the liver in response to pro-inflammatory cytokines. (Figure 5.).

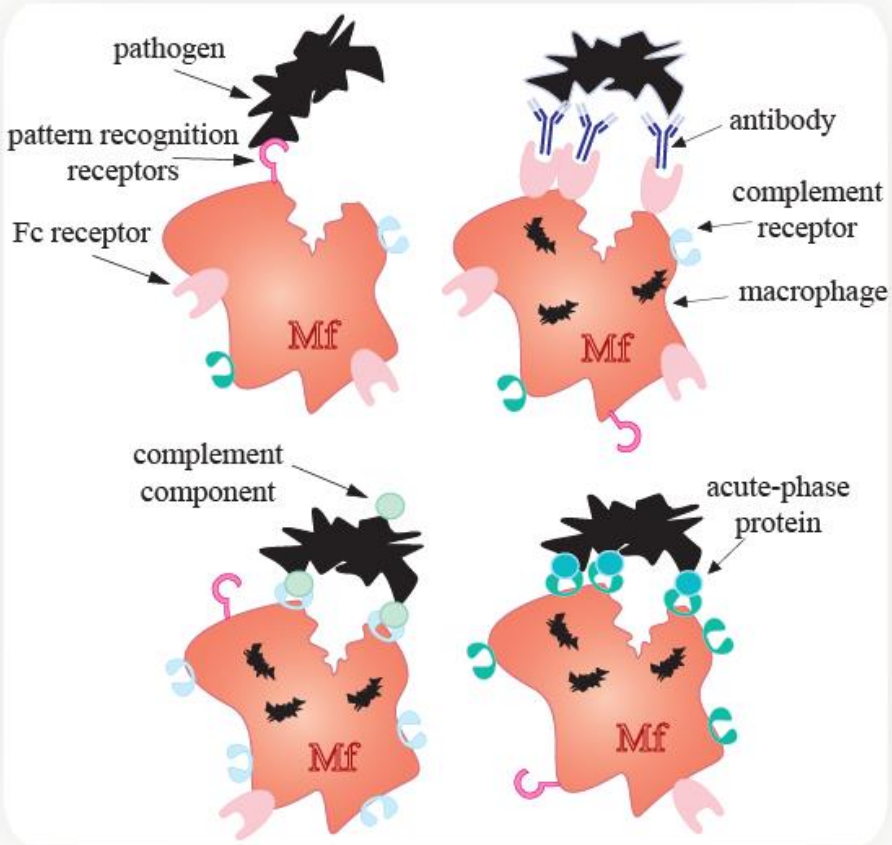


Figure 5. Opsonization and pathogen recognition

Opsonization facilitates detection and accelerates phagocytosis of pathogens. The phagocytes can recognize the microbes by pattern recognition receptors. If the pathogen is coated by opsonins, the opsonin receptors are sufficient for the indirect detection of pathogens.

Elimination of pathogens by the innate immune system

The extremely fast reproduction rate of pathogens far exceeds that of the human populations. The number of some bacteria can increase by a hundred fold within a few hours in ideal circumstances. Therefore, both the immediate recognition and the early elimination of the invaders are crucial for protecting the host. The innate immune system represents the first line of defence. It is responsible both for the quick recognition and the rapid elimination of the pathogens. In lots of cases a few simple mechanisms can ensure the effective eradication of the highly variable pathogens:

- Phagocytic cells, mainly macrophages and neutrophil granulocytes engulf the pathogens and subsequently kill them, even at the cost of the death of the phagocytes themselves.
- Granulocytes and macrophages secrete toxic mediators to kill microbes, including reactive oxygen species, nitric oxide, or different degrading enzymes. Multicellular parasites are relatively complex organisms, often protected by capsule. Mast cells, basophil- and eosinophil granulocytes are specialized to eliminate these parasites with their specific destructive enzymes.
- Activation of the complement system may cause the direct lysis of some pathogens. Alternatively, complement fragments act as opsonins.
- The elimination of intracellular pathogens requires a different strategy. In this case, microbes are not attacked directly, the infected human cells need to be killed instead. Among the cells of innate immune system, NK cells are capable of rapid killing of the infected cells. (Beside NK cell, the cytotoxic T cells, as part of the adaptive immunity play a major role in this process, as we will discuss it later.)

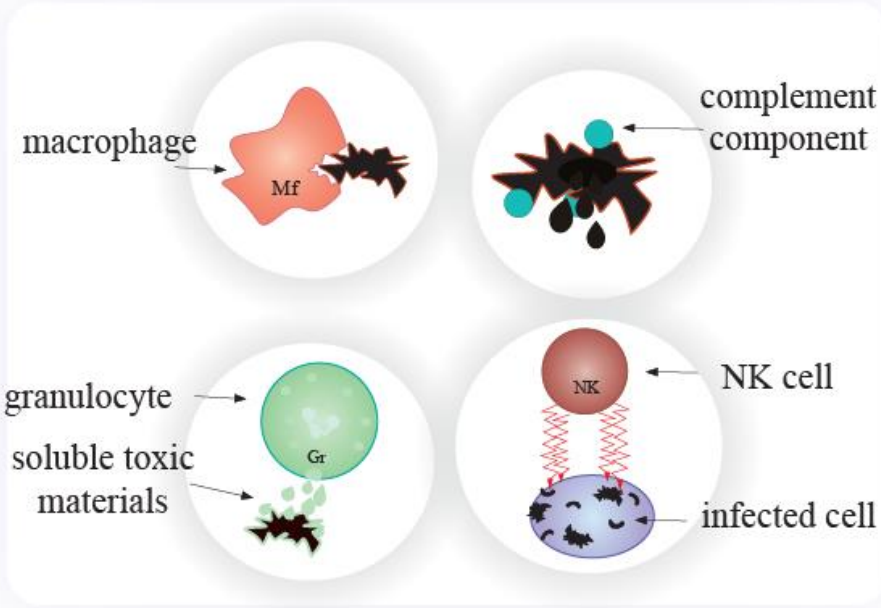


Figure 6. The elimination of microbes by the cells of innate immune system

Following pathogen recognition, phagocytic cells engulf, kill and intracellularly digest the microbes. Granulocytes and macrophages secrete toxic mediators. Complement components lyse pathogens by binding to their surface. NK cells kill infected cells in order to eliminate intracellular pathogens.

The alarm system of the body: the acute inflammation

Interactions among few cells can be sufficient to initiate the immune response. Macrophages and dendritic cells play a critical role in this process. These two cell types are present all over in the human body, especially around the external and internal surfaces, where pathogens can enter into the tissues. Besides dendritic cells and macrophages, the cells of the epithelia also contribute to the alarming process. These few cell types express the entire panel of pattern recognition receptors. However, their effector functions are markedly different following the recognition of pathogens. The primary role of dendritic cells is not the elimination of the pathogens, but to transport the antigens to the lymph nodes, to present them for T lymphocytes, and thus, alarm and activate the adaptive immune system. Macrophages on the other hand initiate effector functions straight away for immediate elimination of the pathogens. Small number of pathogens can be eliminated by macrophages lining around the pathogens' entry site. When a large number of pathogens enter our body

macrophages alone cannot cope with the infection. Upon activation of their pathogen or opsonin sensors they alarm other actors of the innate immune system, and recruit them into the site of infection by initiating local inflammation (Figure 7). The extremely fast proliferation of pathogens demands instant responses. The innate immune system delivers an immediate immune response, so it plays the main role in the rapid and short-lived (acute) inflammation. In addition to this „gate-keeper" function dendritic cells and macrophages are capable and necessary in the activation of the adaptive immune response. Local inflammation developing around the entry site of pathogens is absolutely necessary in immune defense against microbes. The recognition of incoming pathogens by opsonin- or pattern recognition receptors, activates macrophages resulting in the production of hormone like molecules –cytokines and chemokines- which recruit and activate other immune cells. Epithelial cells and mast cells are also able to initiate similar alarm mechanisms.

The volume of circulating blood increases in inflamed tissues due to vasodilation (an increase in the diameter of the vessels) and in the meantime, vessels become permeable (leaky). The chemokines and cytokines produced by macrophages also activate the cells of the vessel walls, the endothelial layer. As a result, the expression of several adhesion molecules are induced on the surface of the endothelial cells around the infected area. Secreted cytokines also assist the extravasation when phagocytes, such as granulocytes, monocytes and other cells (e.g. NK cells) migrate into the site of inflammation. Leaky vessels will allow diffusion of more and more humoral factors, including those of the complement system and even antibody molecules from the blood to the infected tissue. Some cytokine-like mediators lower the sensory-threshold of the nociceptors. As a result, a painful swelling, so-called oedema develops at the site of infection. Reactions in the inflamed tissue increase the intensity of lymph flow and thus facilitate the transport of antigens towards the surrounding lymph nodes initiating the activation of antigen specific lymphocytes.

The clotting system is also activated to cope with the collateral damage of the blood vessels and possibly to isolate the pathogens or at least slow down their spreading. The last phase of inflammation is tissue regeneration. During this process, special cytokines acting as growth factors potentiate the proliferation of fibroblasts and/or the process of angiogenesis.

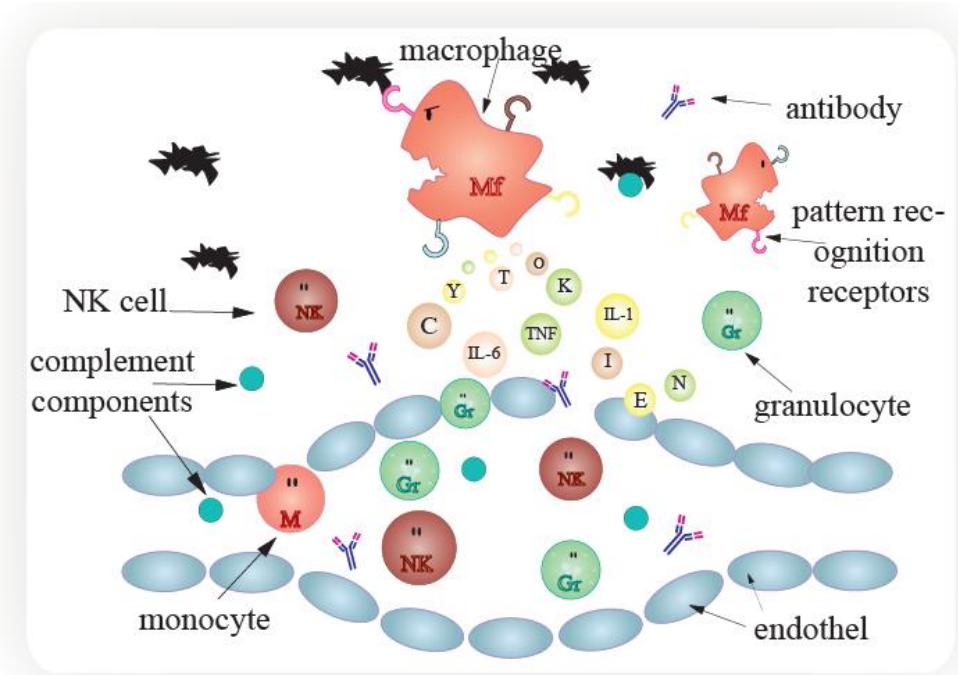


Figure 7. Acute inflammation

At the site of infection macrophages recognize the pathogens and subsequently produce inflammatory cytokines, inducing vasodilation and increased vascular permeability. Granulocytes, Monocytes, NK cells, complement components can exit from the circulation at these sites and eliminate the pathogens in the infected tissues.

Inflammatory cytokines (especially TNF, IL-1, IL-6) are produced by the activated cells, chiefly by macrophages. These cytokines are important at the site of inflammation, however they also have endocrine effects, acting throughout the entire human body:

- Induction of fever by acting on the hypothalamus,
- Increased production of acute phase proteins and antimicrobial molecules by the liver.
- Enhanced production of leukocytes in the bone marrow, to replenish cells lost during the immune reactions

Various mechanisms of the innate and adaptive immune response create the anti-viral immune response. Besides cellular components, Type I interferons play a critical role in innate anti-viral immune responses. Almost all kinds of cells, (not only the cells of the immune system) are able to produce these

cytokines upon viral infection. Infected cells secrete interferons following recognition of viruses by their pattern recognition receptors. Interferons transmit autocrine and paracrine effects, inducing anti-viral state inside the cells, which block the propagation of the viral replication by blocking protein synthesis or activating the degradation of viral RNA.

The function of the complement system

The complement system itself is able to recognize and eliminate many pathogens and in the meantime alert further components of the immune system. It consists of around 30 proteins. Cooperative actions of these proteins assist and complement the function of antibody molecules. Most of the complement proteins are present in the blood plasma as inactive proenzymes. Activation of the first element leads to the activation of the proteolytic cascade system by a domino effect. Activated complement components process subsequent proteins of the cascade by limited proteolysis resulting in the activation of the next component. The different complement components are cleaved in a regulated, determined sequence. Before the inactivation of an activated complement factor (enzyme), multiple copies of the next member of the proteolytic cascade are usually cleaved, which leads to the amplification of the signal.

The surface of pathogens is able to activate the complement system (either directly or mediated by the opsonization by antibodies). Certain types of antibody molecules, related to the adaptive arm of the immune system, are highly effective opsonins initiating the complement activation.

Several specialized inhibitors block spontaneous, pathogen independent activation of the complement system in the human body. Some of these inhibitors are expressed constitutively in the human serum while others on the surface of cells. The absence of complement inhibitors on the pathogens may result in complement activation without the specific recognition of pathogens. The main effector functions of the complement system:

- Mark different microbes (**opsonization**) and thus help the phagocytic cells of the innate immune system to recognize, engulf and destroy opsonized pathogens. In addition, complement factors can bind to antibody molecules attached to the pathogen surface (antigen-antibody complexes or *immune complexes*), consequently further facilitate phagocytosis.
- Complement driven opsonization is necessary for efficient **removal of soluble immune complexes** from the human body (discussed later).

- Following activation, the last elements of the complement cascade creates a pore across the bacterial or cellular membrane. This process leads to the loss of the transmembrane ion balance, which finally **results in the death of the attacked cells.**
- Some of the activated complement components function as a chemotactic factor and recruit different cells of the immune system to the site of complement activation. These complement components – sometimes called as **anaphylatoxins –** contribute to the process of inflammation by enhancing vasodilation and increasing the permeability of blood vessels.

MHC molecules, antigen presentation

T cells and B cells recognize antigens in a very different way. T cells cannot see the antigen in its native form, only the fragments of the antigens after partial degradation and processing.

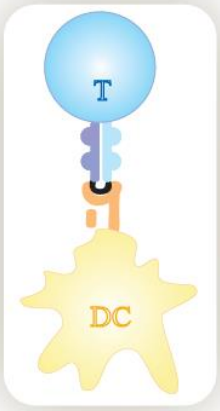


Figure 8. Antigen presentation

T cells recognize peptides in complex with MHC molecules on the surface of antigen presenting cells. The peptide and the MHC molecule, as a complex, is recognized simultaneously by the T cell receptor.

Most of the T cells recognize *peptide* fragments of protein antigens. Small peptides in their native form cannot achieve stable organised conformation. Peptides are therefore displayed by cell surface molecules of the host cells which stabilize the structure of peptides by limiting conformational changes.

These 'peptide display molecules' are called MHC (Major Histocompatibility Complex) proteins, and cells that express them are called antigen-presenting cells. Thus, the main task of the MHC molecules on the surface of an antigen presenting cell is to present peptides derived from various proteins to T cells. The antigen-specific receptors of T cells recognize the complex of a peptide displayed by an appropriate MHC molecule. (Figure 8)

The MHC molecules

The MHC molecules (in human also called HLAs – Human Leukocyte Antigens)**,** are encoded in the Major Histocompatibility Complex gene region. The MHC gene region encodes most of the proteins that are involved in the process of antigen presentation.

This region contains the most polymorphic genes (with the highest number of allelic variations). As a result these genes encode proteins with the greatest diversity in the human population. Thus, most individuals of the population possess MHC molecules with slightly different structures but these molecules have identical function. The different MHC molecules encoded by different alleles can be recognised as "foreign" when some tissue or organ is transplanted into an other person, and the immune system will attack, reject the transpklant. This is why the name of this gene region refers to histocompatibility.

On the cell surface, a vast number – even millions – of MHC molecules can be found simultaneously. One MHC molecule has affinity to various peptide sequences, but one MHC molecule binds only one peptide. All the MHC molecules on a cell surface together are able to present many different peptides from various different proteins at the same time to the T cells.

Under normal circumstances only common self-protein derived peptides are presented by the help of MHC molecules. However, in the case of an infection microbial peptides can also appear on the cell surface presented by several MHC molecules among the normal self-peptide presenting ones. Tumour cell-specific peptide fragments (from altered proteins) can be also presented among the normal self-peptides this way.

By checking tens of thousands of different peptides bound to MHC molecules on the surface of the antigen presenting cell, T cells are able to find a few specific MHC-peptide complexes, which activate them.

MHC I and MHC II

Two main types of antigen presenting molecules exist. Almost all cells of the body express MHC class I molecules (red blood cells can be mentioned as an exception). MHC I molecules bind peptides derived from proteins which are sliced in the cytosol. This is called as **endogenous** antigen presentation. Either the self-proteins of the cell or the proteins of intracellular bacteria, viruses or the abnormal proteins of the tumour cells can be presented this way to CD8+ cytotoxic T cells.

MHC class II molecules show a much more restricted pattern of expression, being expressed mainly on the surface of the so-called professional antigen presenting cells. Using MHC II molecules, macrophages and dendritic cells present extracellular (**exogenous**) peptides derived from antigens engulfed from the extracellular space. Self-peptides of the body or foreign microbial peptides can be presented in complex with MHC II molecules, which can be recognized by CD4+ helper T cells.

Thus cytotoxic T cells and helper T cells recognize antigens presented by different types of MHC molecules and the peptides presented by this different type of MHC molecules are derived from different cellular or tissue compartments.

Antigen presentation

Based on what we have learned so far, antigen presentation seems to affect the function of T cells only. In fact, during antigen presentation significant changes occur in the antigen presenting cells as well. The antigen presenting cell and the T cell mutually affect each other.

MHC I

With some exceptions (e.g. red blood cells) MHC I molecules are expressed on all human cells. They display mainly endogenous peptides on the cell surface for CD8+ cytotoxic effector T cells (Figure 9). Through the presented peptides, T cells can monitor what kinds of proteins are present inside the cells. Due to this process T cells could theoretically detect any intracellular pathogens, thus, antigen presentation by MHC I renders the intracellular space a subject for immunological monitoring or immunosurveillance. The antigen presentation process is

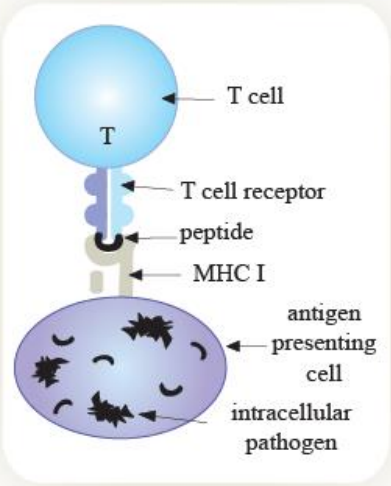


Figure 9. ***Presentation of intracellular pathogens on MHC I***

MHC I molecules display peptide fragments which derive from proteins degraded in the cytoplasm. In case of an infection microbial peptides derived from intracellular pathogens are (also) bound to MHC I. Cytotoxic T cells recognize the MHC-I – peptide complex and may kill the infected antigen presenting cell.

not selective. It presents peptides from any protein located in the cytosol (self/non-self), regardless whether it is derived from a pathogen or not. MHC I molecules can present peptides of the cell's own proteins, but they can display peptides derived from intracellular bacteria or in case of a viral infection viral peptides can be also displayed. Based on the displayed peptides the T cells will decide whether there is any dangerous modification or infection of the antigen presenting cell.

MHC I bound peptides are generated in the cytoplasm (cytosol). The large multi-subunit housekeeping enzyme complex called the *proteasome* cleaves proteins into peptide fragments of the correct size to allow complex formation with MHC I. The degradation of proteins in the cell is a natural process, as all kinds of cellular proteins are degraded by proteasomes. The generated peptides are delivered into the endoplasmic reticulum (ER) by a transporter protein complex. In the ER the freshly synthesized MHC molecules stabilised in peptide receptive conformation by chaperon proteins, so the transported peptides can bind to them. Usually 8-10 amino acids long peptides bind to the peptide-binding groove of MHC I molecules. Once an appropriate peptide has been bound to MHC I in the ER and the chaperons are dissociated, a conformational change occurs. The MHC molecule becomes „closed", unable to exchange the bound peptide.

In this peptide-bound state, the MHC I molecules can leave the endoplasmic reticulum and pass through the Golgi-apparatus, finally they appear on the cell surface. Under normal circumstances „empty" MHC I molecules, without a bound peptide, cannot be found on the cell surface.

The significance of this strict process is to prevent the binding of extracellular peptides to MHC I molecules on the cell surface, otherwise healthy cells near the site of infection could become the targets of cytotoxic effector cells.

When the CD8+ cytotoxic T cells recognize peptides of foreign or harmful cellular proteins in complex with MHC I molecules, they kill the target cell to prevent further spreading of the infection or the development of a tumour. MHC I is expressed on the surface of all nucleated cells, so if any cell becomes infected, following antigen presentation, they become a target for cytotoxic effector T cells.

In contrast to cytotoxic T cells, natural killer cells (NK cells) isn't activated by MHC I bound peptides, but rather the lack of MHC I molecules, which unleash their activation.

This mechanism can be effective in killing those cells, which lack MHC I molecules avoiding recognition by the adaptive immune system. For example certain virus-infected cells express only a very limited number of MHC

molecules, thus they become „invisible” to cytotoxic T cells. However NK cells detect the lack of MHC I and destroy the abnormal cells.

MHC II

The MHC II molecules are expressed by the professional antigen presenting cells such as dendritic cells, macrophages and B cells. These cells are able to engulf extracellular antigens and to present them on MHC II molecules to CD4+ helper T cells. MHC II molecules (unlike MHC I) are specialized to display antigens derived mainly from exogenous origin (Figure 10).

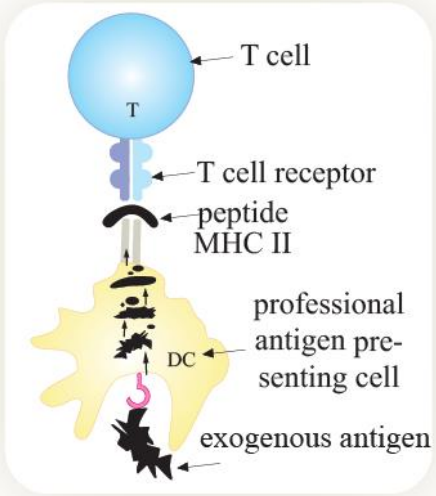


Figure 10. ***Peptides derived from extracellular pathogens are presented on MHC II molecules***

Following engulfment, peptides of the pathogen appear on the cell surface in association with MHCII molecules. The MHCII-peptide complex is recognized by helper T cells.

Following phagocytosis or endocytosis exogenous antigens get into the endosome. Later the endosome fuses with lysosomes to become endolysosomes containing proteolytic enzymes. Peptides generated here by proteolysis –from exogenous proteins or from engulfed or parasitic microbes– can form a complex with MHC II molecules.

Similar to MHC I, MHC II molecules are synthesized in the endoplasmic reticulum. However here they associate with a different set of chaperone proteins, most importantly with the so-called invariant chain (Ii). The main functions of the invariant chain is to form a complex with MHC II thus prevent binding of endogenous peptides into the peptide binding groove of MHC II and transport of the complex through the Golgi-apparatus in this ‘blocked’ state. In the endolysosome, proteolytic enzymes degrade the invariant chain, making the binding site available for peptides derived from extracellular antigens. Usually 10-20 amino acids long peptides bind to MHC II molecules, however, since its binding site is ”open at both ends”, longer, overhanging peptides are also able to fit. After peptide-binding, the MHC II-peptide complex appears on the cell surface to present antigens to CD4+ helper T cells. In return, activated

helper T cells are able to influence the immunological functions of the antigen presenting cells in several ways:

- Activated effector T-helper cells facilitate the activation and subsequent antibody production of the antigen presenting B cells, thus they support the humoral immune response.
- In case of antigen presenting macrophages the effector T-helper cells further activate them, increasing their efficiency in killing phagocytosed bacteria "settled in" in their endosomes.

Features of the MHCI and MHC II molecules

		MHC I	MHC II
Cells that express MHC		All nucleated cells	Professional antigen presenting cells
Bound peptide	**source**	self or foreign proteins	self or foreign proteins
	size	8-10 amino acids	10-20 amino acids
	origin	cytoplasmic and nuclear proteins	cell-surface and extracellular proteins
Site of peptide generation		cytoplasm	endolysosome
MHC transport		remains in the ER till the complex formation	Invariant chain directs it to specialized vesicles
Site of peptide loading of MHC		ER	specialized vesicles
MHC-peptide complexes on the cell surface		reflect the intracellular environment	reflect the extracellular environment

Taken together, activated CD4+ helper T cells increase the activation and facilitate the function of the antigen presenting cells, while activated CD8+ cytotoxic T cells induce death of the antigen presenting cells.

So far, little has been discussed about the antigen recognition of naïve T cells. Naïve T cells are activated in the secondary lymphoid organs. T cells that have not met their specific antigen can be most efficiently activated by dendritic cells. These professional antigen presenting cells in addition to the MHC molecules express those cell surface molecules, which provide effective stimulation for naïve T cells.

Activated naïve T cells proliferate and differentiate into effector T cells, which later will be able to carry out the above mentioned functions of cytotoxic and helper effector T cells.

B- and T cell receptors

The human population is threatened by a plethora of different microbial species. Moreover, even a single species can have altered variants with different pathogenicity. The specific recognition of this enormous variability requires (at least) an equal number of receptors efficiently recognizing a given variant of a particular microbe. The B cell receptor, its soluble form the antibody and the T cell receptor are glycoproteins produced in humans with a diversity that far exceeds that of the pathogens. One receptor is responsible for the recognition of a single pathogen, however the billions of receptor variations together are able to specifically recognize every pathogen.

The **antibody** (immunoglobulin) is a symmetrical molecule that consists of 4 polypeptide chains in humans, 2 identical shorter so-called light chains and 2 identical longer heavy chains (Figure 11). Each chain contains constant and variable domains (protein subunits). The light chains consist of a variable and a constant domain while the heavy chains have one variable domain and – depending on their type– 3 or 4 constant domains. The heavy and light chains as well as the two heavy chains are linked by covalent disulphide bonds (disulphide bridges between the cysteine amino acids). Additional intramolecular disulphide bridges stabilize the globular structure of each domain. As a result, a symmetrical Y-shaped structure is created, with 2 arms containing a light chain and the variable– and the first constant domain of a heavy chain. The variable domains at the end of the arms form the antigen binding site. The stem of the Y-shaped structure is built up from the 2 or 3 constant domains of the 2 heavy chains. The constant domains provide stability for the molecule and they are also responsible for certain effector functions (Figure 11).

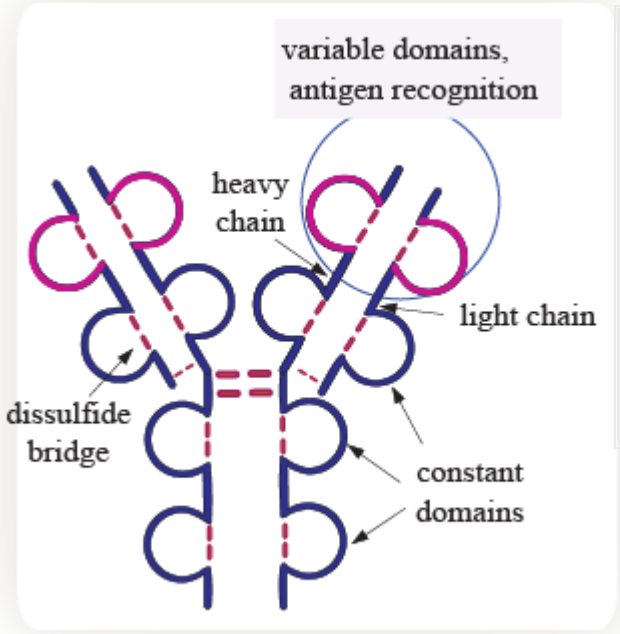


Figure *11 Structure of the antibody*

The antibody molecule consists of 2 identical heavy chains and 2 identical light chains. The terminal variable domains are responsible for antigen recognition, while the constant domains stabilize the structure of the molecule and are responsible for the induction of some effector functions.

Diversity is characteristic of the variable domains instead of the whole molecule. Variable domains are responsible for the recognition of the vast number of different antigens. While the number of variable domains with different sequences is extremely high, based on the variety of the constant domains the heavy chains can be divided into 5 major types (IgM, IgD, IgG, IgA, IgE) and the light chains into 2 types (κ and λ). These types are also known as isotypes.

The antigen binding site of the human immunoglobulin is formed by the variable domains of a light- and a heavy chain together. Therefore, the heavy and light chains together and not alone, are able to recognize a given antigen with high specificity. Since the basic unit of antibody is a symmetrical molecule (consisting of 2 identical heavy- and 2 identical light chains), it is capable of creating a dual, two pronged, so-called bivalent interaction with the specific antigen or bridge together two specific antigens.

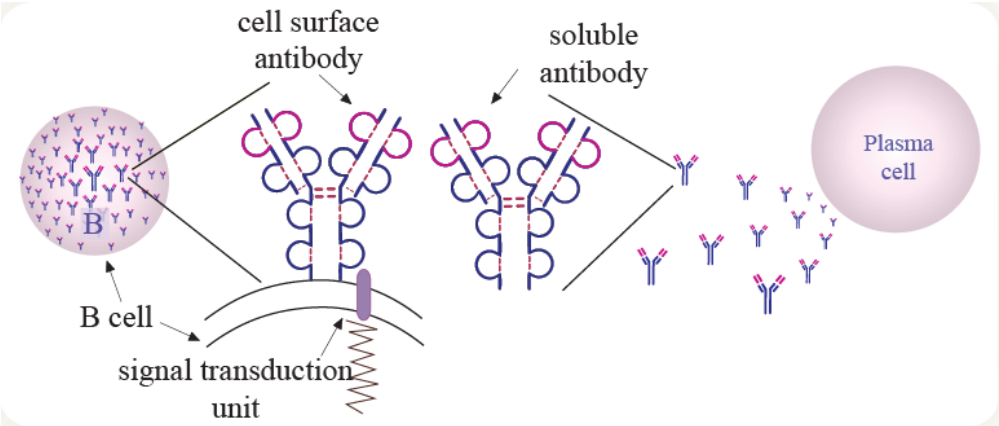


Figure 12. The cell surface- and soluble forms of the immunoglobulin

The immunoglobulin molecule is expressed on the surface of B cells where it functions as their antigen recognition receptor. Following antigen binding it transmits activating signals into the cell through associated signalling molecules. The immunoglobulin is expressed in a soluble form as well, secreted by plasma cells which had differentiated from activated B cells. The roles of soluble immunoglobulins are to inactivate pathogens and to facilitate their elimination.

The immunoglobulin molecules are produced in 2 forms during the immune response:

1. It is highly expressed on the surface of B cells. In this case the heavy chains contain an additional transmembrane region which anchors the molecule to the cell membrane. The cell surface-bound immunoglobulin interacts with other membrane-anchored molecules which are responsible for signal transduction. The cell surface-bound immunoglobulin and the associated signaling molecules together are called **B cell receptor (BCR)** (Figure 12).
2. The immunoglobulin/antibody can have soluble form as well. Antibodies are produced by plasma cells differentiated from B cells. Plasma cells do not express cell surface-bound immunoglobulin anymore, instead, they produce soluble immunoglobulins continuously and secrete them into the extracellular space. Structurally, the secreted antibody molecule is almost identical to the cell surface immunoglobulin. It recognizes the same antigen as the BCR, only the transmembrane region is absent, without which it fails to attach to the cell membrane, or associate with signal transduction molecules. (Figure 12)

The BCR is responsible for both the recognition of antigens and the activation of antigen specific B cells.

Soluble antibodies facilitate the recognition and contribute to the elimination of pathogens using other components of the immune system. (described in more detail at antibody effector functions)

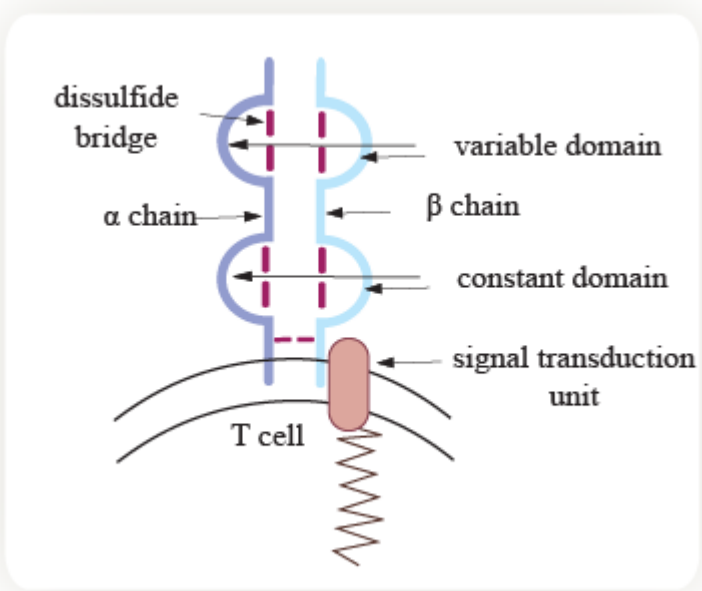


Figure 13. The structure of the T cell receptor (TCR)

The T cell receptor consists of 2 polypeptide chains. The variable domains of the TCR recognize the MHC-peptide complexes, while the constant domains ensure the stability of the molecule. Only membrane-bound forms exist.

The **T cell receptor (TCR)** is the antigen recognition receptor of the T cells. Its structure is similar to the immunoglobulin. Its antigen recognition site is formed by 2 polypeptide chains (the α and β chain in the case of the so-called αβ T cells or the γ and δ chains in the case of the γδ T cells). Each chain consists of a constant and a variable domain. Similar to the immunoglobulin, the chains are linked to each other by covalent bonds and further disulphide bridges within each chain stabilize the globular structure. The 2 variable domains together are responsible for the recognition of the antigen in a form of an MHC-peptide complex, while the constant domains stabilize the structure of the receptor. The chains are anchored to the cell surface by transmembrane domains. As part of the receptor complex non-covalently associated signaling molecules are present. These signaling chains are collectively called the CD3-complex (Figure 13).

From the point of the structure and function, there are important differences between the BCR and the TCR. TCR unlike BCR has only 1 antigen binding site and it does not exist in a soluble form, so the sole function of the TCR is antigen recognition followed by the activation of the T cell.

Recognition of microbes by receptors of the immune system		
cell	**receptor**	**recognized structure**
Innate cells (e.g. macrophages, dendritic cells)	Pattern recognition receptors (PRR)	Pathogen associated molecular patterns (PAMPs), Danger associated molecular patterns (DAMPs)
B cell	B cell receptor (BCR)	almost any structure
T cell	T cell receptor (TCR)	MHC-peptide complex
Various cell types of the immune system (especially the innate immune cells)	Fc- and complement receptors	Opsonins bound to antigens, foreign structures (antibodies, complement components, acute phase proteins)

Generation of Lymphocyte diversity

One of the major findings of immunology was the clarification of how the enormous diversity of antigen receptors is produced, using the relatively low number of genes present in the human genome. In fact, the immune system has developed highly efficient molecular genetic mechanisms for the generation of an extremely diverse set of antigen receptors from a limited number of inherited genes. Without this unique mechanism, recognition of many millions of different antigens would not be possible. The variable domains of the BCR and TCR are encoded by gene segments which are often located far away from each other in the DNA. While conventional genes encode the constant domains of immunoglobulin chains (heavy- and light) and the TCR chains (α and β), the variable domains are encoded by gene sequences which are created by the joining of multiple small gene segments by a random somatic recombination process. The variable domains of the receptors are combined from 2 or 3 gene segments. These gene segments are present in many (10-100) variations in the DNA. During their development lymphocytes start rearranging their original DNA (so-called germline DNA) to create unique "novel" DNA sequences originally not present in the germline of the host.

The variable domains of the heavy chain of the BCR and the β-chain of the TCR are randomly assembled from three gene segments, while the light chain and the α-chain variable regions are assembled from 2 segments. Random recombination of gene segments provides the basis of the so-called combinatorial diversity, which allows generation of over a million different receptor specificities for both T- and B cells. The diversity is further increased by several orders of magnitude due to inaccurate joining of the gene segments during gene rearrangement (some nucleotides are deleted and additional nucleotides are randomly added at the junctions points using appropriate DNA modification/repair enzymes). As a result the base sequence is altered, new sequences appear which again, were not present in the germline sequence. The junctional and combinatorial diversity together are able to generate around 10^{14} different B cell receptors and 10^{18} different T cell receptors.

Taken together, these recombination and repair processes create a new randomly generated gene sequence, which encodes the variable domain of the antigen recognition receptor in that particular lymphocyte. Importantly, the constant domains are ***not*** subject to the above assembly mechanisms, therefore not polymorphic among individual lymphocytes of the host.

Once the recombination process has been completed expression of the newly assembled gene is initiated. Only the rearranged gene sequence can be transcribed and translated into a protein from the mature mRNA. Following a productive rearrangement (that leads to the synthesis of structurally intact chains of the receptor), further rearrangements are blocked in that particular cell.

The diversity of the antigen receptors randomly assembled by the above described mechanism is further increased by the fact that both the TCR and the BCR are formed as heterodimers randomly chosen from the pool of available diverse polypeptides: from the heavy and light chains in B cells and from the α and β (or γ and δ) chains in T cells. The binding site of the receptors is formed by both polypeptide chains which together determine receptor specificity.

Clonal selection of T- and B lymphocytes and development of tolerance

10 million-1 billion B and T cells are generated and mature daily in the bone marrow and in the thymus, replacing the dying cells in the peripheral tissues.

Lymphocytes recognize the antigen with their cell surface-expressed B cell receptors or T cell receptors. The antigen recognition receptors (BCR or TCR) are unique with different specificity and each lymphocyte expresses only one type of antigen recognition receptor of the same specificity. Thus one cell is

specialized for the recognition of only one antigen. Overall, lymphocytes produced on a daily basis represent ~10^7 – 10^9 different antigen specificities. As a result, our lymphocytes together are able to recognize millions of different antigens at any time. (In contrast with this, the innate immune system uses only a few dozen different receptors for recognition of highly conserved pathogen-associated structures.)

Lymphocytes develop in the primary lymphoid organs in the absence of foreign antigens. As the specificity of the antigen recognition receptors is generated by a completely random process, in addition to pathogen-specific receptors, many self-reactive receptors are also produced. In the special environment of the primary lymphoid organs, those lymphocytes that recognize self-structures with high "intensity" (have high affinity receptors for self-proteins) die or become inactivated in the early stage of their development. This process, called the **development of central tolerance**, ensures that the vast majority of strongly self-reactive, potentially autoreactive lymphocytes are not allowed to leave the primary lymphoid organs.

Mature lymphocytes that have completed their developmental program leave the primary lymphoid organs and through the blood circulation, regularly enter the secondary lymphoid organs in search for their specific antigen from the periphery. If they do not encounter their specific antigen, they continue their circulation in the blood and lymph to monitor the antigen repertoire of other secondary lymphoid organs.

Since each lymphocyte is specialized for one particular antigen, the majority of them will never find their specific antigen.

Those newly developed lymphocytes, for which specific antigen is not present in the body, spend only a few weeks in the circulation, then die by apoptosis, because in the absence of their specific antigen they are not needed. This also provides space for newly developing lymphocytes with different specificity.

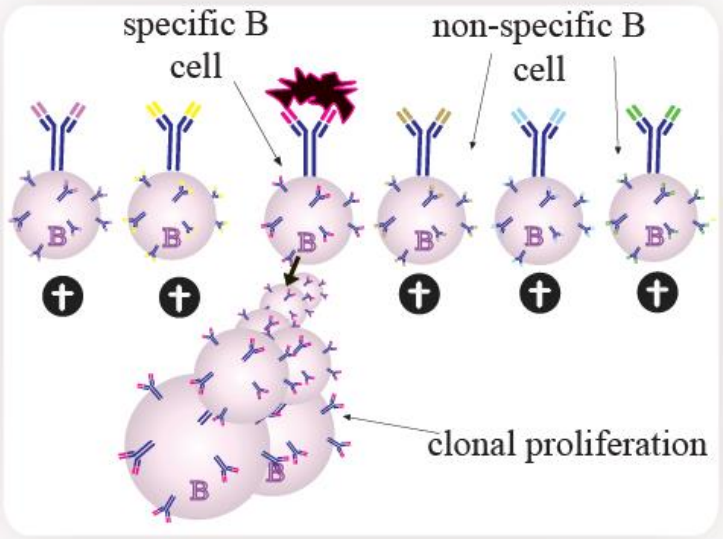


Figure 14 Clonal expansion of lymphocytes

From the large amount of different B cells those recognizing the pathogen start to proliferate and form a clone (clonal proliferation/clonal expansion). Non-specific B cells re-enter the circulation to "find" their specific antigen in the body. Without activation signals generated by antigen binding B cells die by apoptosis in a few weeks. Different pathogens activate a different set of specific B cells. Clonal proliferation is characteristic of B- and T cells only.

However, in the presence of pathogens, pathogen-specific lymphocytes present in the regional secondary lymphoid tissues become activated. Their frequency can range from 1:1000 to 1:100000.

Remarkably, it is the collection of pathogen-derived antigens that "selects" the antigen-specific lymphocytes from the available repertoire. The recognition of antigen leads to the activation and proliferation of the specific lymphocyte. The resulting progeny cells have identical specificity providing this way the large number of antigen-specific cells required to control even a massive infection. (Figure 14).

After elimination of the pathogen, its antigens disappear from the body, thus a large number of pathogen specific effector lymphocytes is not needed anymore. At the final stage of the immune response these unnecessary effector cells die by apoptosis.

However some of the specific B and T cells differentiate into memory cells or long-lived effector cells, to ensure a quicker and more efficient immune response in case of reinfection.

In the case of B cells the strength/ affinity of the antigen binding usually improves during the clonal proliferation due to point mutations introduced into the coding region of the antigen recognition receptor. Cells expressing these slightly modified receptors must bind the antigen to survive. Lymphocyte clones with BCR mutations resulting in binding to the antigen with higher affinity will win the competition for the available antigen, thus survive and proliferate. As a result, at the end of this selection process B cells recognizing the same pathogen, with higher affinity will be produced. This process called **"affinity maturation"** requires the contribution of helper T cells.

Functions of B cells and effector functions mediated by antibodies

Antigen presentation by B cells

Similar to dendritic cells and macrophages B cells are professional antigen presenting cells. The recognition of antigen by BCR induces not only activation, division and differentiation of the B cell, it also induces endocytosis of the bound antigen. The BCR-bound antigens can be efficiently internalized by B cells by the process of receptor-mediated endocytosis. Internalized antigens are processed and presented on the surface of B cells as peptide-MHC II complexes (as previously described in the concerning MHC II molecules). By this mechanism the protein antigens recognized by B cells can "be seen" by T cells as well. Under these circumstances, chances are good that the B cell and the T cell will recognize the same antigen, but not necessarily the same part (epitope) of the antigen. Antigen presentation by MHC II leads to the activation of helper T cells. The activated T cell can produce cytokines that facilitate activation and differentiation of B cells. During cellular contact T- and B cells mutually activate each other via cell surface receptors. Please note that in this process a positive feedback loop is created in which two lymphocytes (a T-and a B cell) with the same antigen specificity mutually support (as well as control) each other's function (Figure 15.).

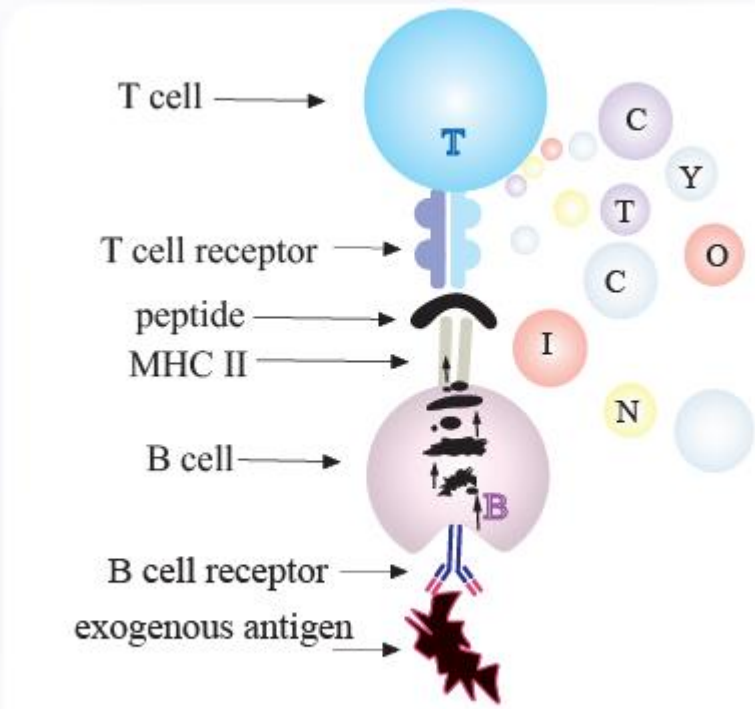


Figure 15. B and T cells may respond to the same pathogen by amplifying each other's response.

Recognition by the BCR triggers internalization of the antigen by receptor –mediated endocytosis. Following processing antigen-derived peptides are presented to helper T cells. In turn, helper T cells produce cytokines that help activation and differentiation of B cells.

B cell effector functions: the function of antibodies

Antibody molecules can protect against microbes or other dangerous materials in two ways. First, they may ***directly*** block the binding of the pathogen or toxin to their cell surface receptors (neutralization) and second, ***indirectly***, by marking them for destruction through mechanisms to be discussed later in detail.

The B cells' characteristic molecules are the immunoglobulins. The immunoglobulin expressed on the cell surface (BCR) is responsible for recognition of the antigen, initiation of B cell activation, however the

immunoglobulin produced by plasma cells (antibody) mediates the humoral part of the B cell immune response.

B cells usually encounter antigens in the secondary lymphoid organs, which provide suitable environment for B cell proliferation. The antigens to be recognized by B cells enter the lymphoid tissues via the blood- or lymphatic vessels or even bound to cell surface proteins. B cells do not require the antigens to be presented by MHC molecules. Antigens can be recognized by B cells in their original form bound to the surface of cells.

In response to activation by their specific antigen B cells undergo clonal expansion. A majority of the numerous identical daughter cells of the original B cell will differentiate into plasma cells, specialized to produce large amounts of antigen-specific antibody. It should be noted that although plasma cells do not express the BCR, the specificity of antibodies secreted by the plasma cells is identical to that of the original B cell. By clonal expansion, a few activated B cells may generate thousands of plasma cells, each of which is capable of producing billions of antibody molecules. As a result the number of antibody molecules produced in response to an infection far exceeds the number of pathogens. Different B cells recognize different parts (epitopes) of the same antigen. In case of complex pathogens (e.g. bacteria) several B cells with diverse but pathogen-specific BCRs are activated. The antibodies produced by plasma cells can be carried by blood to all tissues and recognize pathogens far away from the site of production in any tissues of the host (Figure 16.).

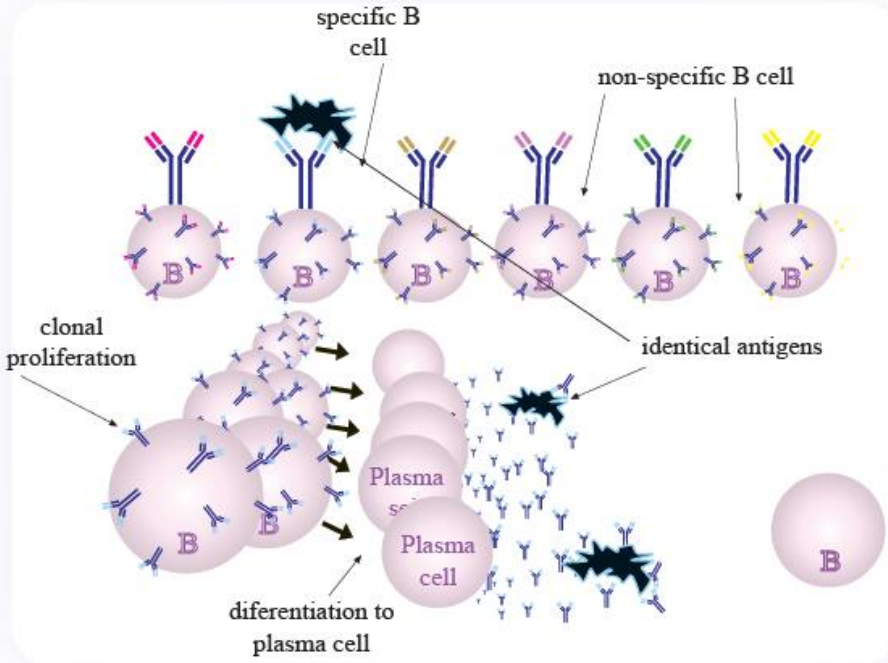


Figure 16. B cell differentiation into plasma cells.

Upon meeting with antigen, the antigen specific B cells go through clonal expansion, differentiate into plasma cells (or into memory cells). The plasma cells due to lacking BCR cannot recognize antigens, however they produce large amounts of antibodies. The antibodies produced by plasma cells recognize the same antigen as their progenitor B cell initially activated by the antigen.

Recognition of antigen, clonal expansion, differentiation and antibody production are time consuming, thus an efficient B cell response requires 7-14 days to develop following the first exposure to the antigen.

Every B cell has a unique BCR with a single specificity, thus, protection of the host is provided by a set of B cell clones selected from a vast repertoire of unique B cells by the actual pathogen.

Neutralization

Plasma cells in the body continuously produce surprisingly large amounts, approximately 10^{18} antibody molecules per day. Their antibody production increases in response to infection. Antibodies produced by plasma cells can exert their effect in several ways:

The variable domains are responsible for binding of the antigen with high affinity. In case of an infection pathogens become covered with specific antibodies, some of which physically block pathogen cell surface molecules required for binding to cell surface receptors of the host. Similarly, antibodies binding to the active parts of various animal or microbial toxins (venomous snakes, spiders, tetanus), inhibit their toxicity.

This kind of steric inhibitory effect is called **neutralization**, and antibodies delivering this effect, are called neutralizing antibodies (Figure 17.).

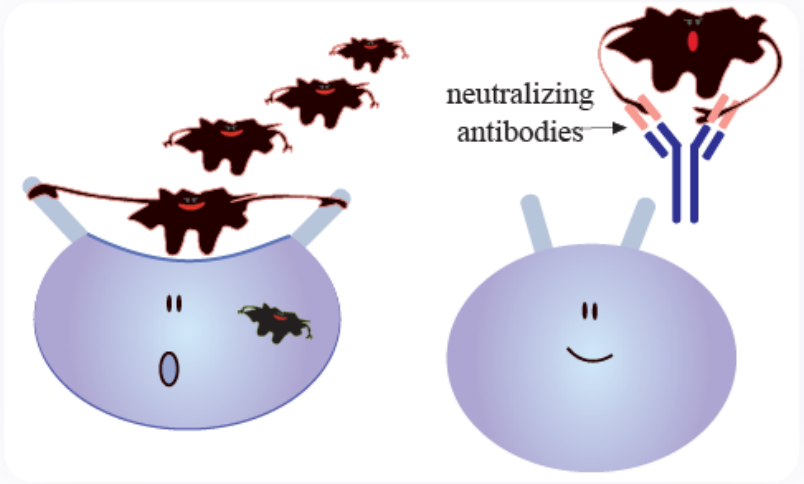


Figure 17. Neutralization of pathogens by neutralizing antibodies

Immunoglobulin molecules, produced by plasma cells, bound to pathogens or different toxins may inhibit their effects. They can block binding of pathogens (e.g. viruses) to the cell surface, thus preventing infection of the cell.

Antibodies produced by the mother's immune system can be transported through the placenta or into the breast milk. These antibodies protect the new born from infections for several months after birth, until the child's own B cell repertoire is fully developed. This maternal protection mechanism is also based mainly on neutralizing antibodies. Large amounts of neutralizing antibodies are produced on a daily basis by the immune system of the intestines, these antibodies are transported into the lumen, where they neutralize the dangerous materials taken up together with food. Some life-saving medical treatments utilize the direct neutralizing effects of antibodies. For example: upon a snake bite, antibodies neutralizing different toxins of the snake venom are given to the patient. (See the part about passive immunization!)

While neutralization is based primarily on the variable region of the antibody, other effector functions are dependent on the constant regions of the antibody molecule, more precisely on the Fc region.

The Fc region of the antibody (the "stem" of the Y- or fork-shaped antibody formed by the constant domains of the heavy chains) is recognized by various cell surface receptors, called Fc-receptors, expressed on several types of immunocytes. With these receptors, immune cells can recognize antibody molecules. Antibodies form a bridge between the opsonized pathogen and the immunocyte. The variable domain of the antibody binds to the pathogen, while the Fc region binds to the Fc receptor expressed on the immunocyte.

Unlike the variable domains of the antibodies the constant domains are more or less identical. (The different *isotypes* are recognized by distinct Fc receptors). Thus phagocytes and NK cells don't need to produce a diverse set of unique receptors for the recognition of millions of pathogens, they "smartly" recognize the Fc region of opsonizing antibodies attached to any pathogen. Importantly, most Fc-receptors are not activated by free antibodies. Efficient activation of phagocytes or NK cells via their Fc-receptors requires *immune complexes*. Thus we can say that these receptors are responsible for the recognition of opsonized antigens.

Effector mechanisms mediated by the Fc part of the antibodies

- Opsonization by immunoglobulins enhances receptor mediated phagocytosis of pathogens via cell surface expressed Fc-receptors. (Figure 18.)
- NK cells also express Fc receptors. With these receptors, they are able to bind cells opsonized by immunoglobulins. This interaction may trigger their cytotoxic function. This process is called antibody dependent cellular cytotoxicity (ADCC). (Figure 18.)
- The Fc region of many immunoglobulin isotypes contains complement binding sites. Such antibodies bound to the pathogen can activate the complement system. (Figure 18.).
- Fc-receptors recognizing the heavy chains of IgE, play a key role in the induction of allergic reactions, by activating mast cells and basophil granulocytes. (As described later!)

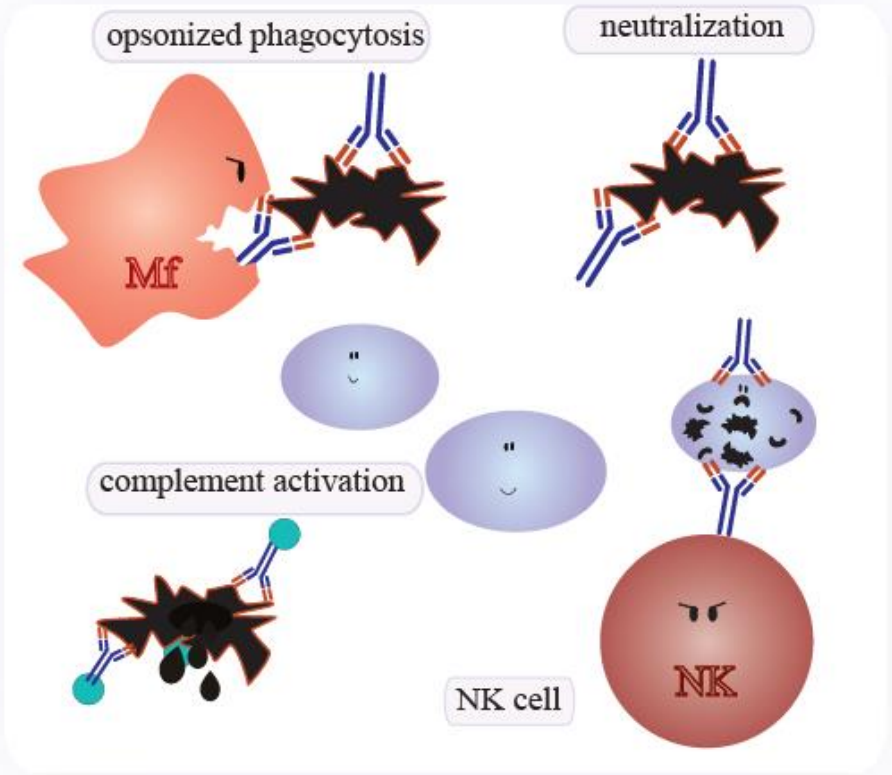


Figure 18. Antibody mediated effector functions

In addition to neutralization, binding of antibodies to pathogens (opsonization) may facilitate phagocytosis of the pathogen, may activate the complement system and the NK cells as well.

Immunoglobulins – based on the structure of their heavy chain – are classified into five major classes, called *isotypes*: IgM, IgD, IgG, IgE and IgA. However, in response to adequate stimuli (with the contribution of helper T cells) the B cell can change the isotype of the produced antibody by a process called **isotype switching** or **class switching**. The cells of the immune system have different Fc-receptors on their surface that recognize and bind specific antibody isotypes. Importantly, different types of Fc receptors deliver distinct effector functions. As a result, by changing the isotype (but not the specificity) of the produced antibodies, the formed immune complexes or the opsonized pathogens will bind to a different set of Fc-receptors, thus the effector functions induced by them usually change.

There are some special Fc receptors in the body, which are responsible for the transport of antibody molecules. Such Fc-receptors transport IgG from the mother to the fetus, or IgA across epithelial cells or into the breast milk.

It's important to emphasize that during isotype switching, not the specificity, but the effector functions of the antibody are altered. As binding specificity remains the same, neutralizing function of an antibody is not influenced by isotype switching.

Effector functions of the main antibody isotypes:

- **Immunoglobulin G**, **or IgG** is the „swiss army knife" of antibodies. This isotype can efficiently opsonize pathogens and facilitate the process of phagocytosis. Some subtypes effectively activate the complement system as well as the killing function/capacity of NK cells. This isotype is present at the highest concentration in plasma, and has the longest half-life. Special Fc receptors transport IgG, from the mother's circulation into the fetus across the placenta.
- **IgM** Many species of bacteria opsonized by IgM can be destroyed efficiently by the complement system. This type of immunoglobulin can be found on the surface of naïve B cells where they function as antigen binding receptors. Unlike IgG, this immunoglobulin is not able to facilitate the phagocytosis of bacteria directly (only by activating the complement system). IgM can be transported by Fc receptors, but not from the mother to the fetus.
- **IgA** It appears in body fluids including tear, saliva, intestinal fluids, mainly to protect cells of the mucosal epithelium. Transport of IgA mediated by specific Fc receptors is considered the most efficient among antibodies.
- **IgE** its natural function is protection against parasites, however this immunoglobulin isotype is responsible for the symptoms of allergic reactions.
- **IgD** This isotype is present primarily as an antigen-specific receptor on the surface of newly formed B cells. Similar to IgM, IgD plays a role in B cell activation.

T cells

T-lymphocytes express a cell surface antigen receptor called T Cell Receptor (TCR) which resembles an antigen-binding arm of an antibody.

The antigen-specific activation of T cells requires direct contact between the T cell and an antigen presenting cell as it requires the interaction of the TCR and MHC molecules. In contrast to B cells, the majority of T cells exclusively recognize protein-derived peptide epitopes in complex with the histocompatibility complex (MHC) proteins.

Differences in antigen recognition by B and T cells.

	B cell	**T cell**
recognized substances	proteins carbohydrates lipids DNA steroids artificial substances etc.	peptide derived from proteins (8-20 amino-acids)
type of recognition	native/intact antigen tissue derived or humoral	processed and presented by an APC as MHC peptide complexes

The two main populations of T cells contribute to the immune response differently. Helper T-lymphocytes have more of a regulatory role in the immune response while cytotoxic T-lymphocytes are professional killer cells designed for killing of infected- or tumour-cells directly.

Completing their development in the thymus both naïve CD4+ ("helper") and naïve CD8+ T ("cytotoxic") cells enter the circulation. Leaving the blood, they migrate into secondary lymphoid organs and screen the local antigen-repertoire on the surface of dendritic cells available at the time of their surveillance. A continuous recirculation of T cells between various secondary lymphoid organs is maintained until circulating T cells meet their specific antigen that induces their activation, followed by clonal expansion and differentiation into effector T cells. Clonal proliferation of T cells, like that of B cells rapidly generates a large population of cells (a T cell clone) with antigen specificity identical to that of their progenitor cell. Those T cells that fail to find their specific antigen are destined to die by apoptosis within a few weeks.

In most cases antigens for naïve T cells are transported into the lymph node by dendritic cells. Dendritic cells dispersed in tissues sense pathogens using their

pattern recognition receptors. Upon recognition they engulf microbes and migrate into the regional lymph node. Meanwhile, processing of the pathogen-derived antigen is completed and peptide fragments are presented on MHC. Thus, initial activation of T cells occurs in a special environment, the lymph node, where clonal expansion of antigen-specific T cells occurs. Those T cells that have gone through clonal expansion and differentiation exit the lymph nodes. Once in the periphery, they are able to deliver a proper response upon the second activation by the same antigen present at the site of infection.

In the periphery, cytotoxic T cells (Tc) recognize their specific peptide fragment (antigen) presented by any nucleated cell expressing MHC I molecules. Notably, these antigen presenting cells recognized by Tc are usually infected or tumour cells.

Helper T-lymphocytes (Th cells) can only be activated by professional antigen presenting cells expressing cell surface MHC II molecules in peripheral tissues and also in peripheral lymphoid tissues.

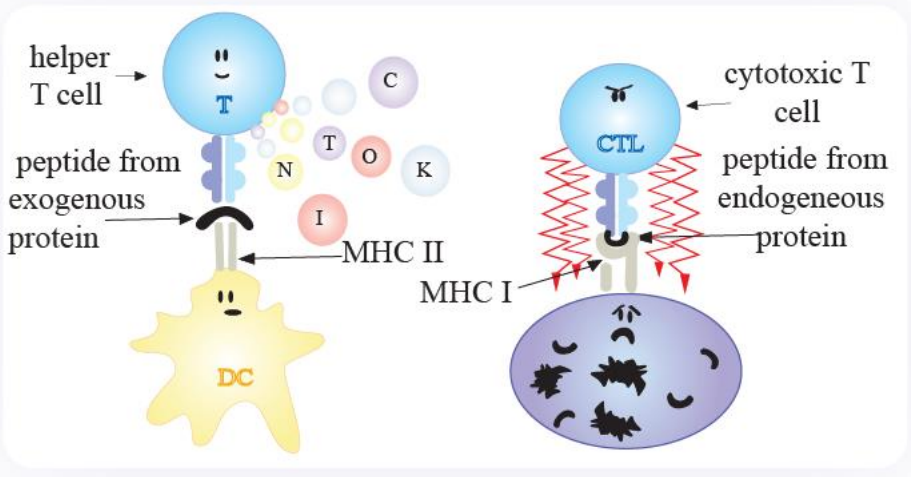


Figure 19. The main populations of T cells: helper and cytotoxic T-lymphocytes

T cell subsets and their functions

Helper T cells or **Th cells** are not directly involved killing of pathogens, they rather coordinate the immune response by communicating with other immunocytes. Exogenous proteins processed to peptides by professional APCs and presented to CD4 co-receptor expressing Th cells via cell surface MHC II molecules. Antigen-specific activation of Th-cells via the TCR induces

cytokine production and expression of novel cell surface molecules on the T-lymphocyte by which they coordinate the immune response. (Figure 19.)

We can distinguish some major helper T cell types, type 1 (**Th1**), type 2 (**Th2**) type 17 (**Th17**) and **follicular helper T cells**. **Th1 cells** are essential components of the immune response against intracellular pathogens. Cytokines secreted by Th1 cells are involved in the recruitment if phagocytes to the site of infection and enhance the antimicrobial (killing) activity of macrophages, the killing functions of NK- and cytotoxic T cells.

Th2 cells on the other hand help the immune response essential for the elimination of parasites and helmiths. By their cytokine secretion they facilitate the control of parasites by mast cells, basophil- and eosinophil granulocytes. In addition, macrophages activated by the same cytokines play an essential role in tissue re-building (repairing tissue damage) once the pathogens had been cleared.

Another, important subpopulation of Th cells is the **Th17** subset, partaking in immune responses against extracellular pathogens, bacteria or fungi in particular. They potentiate inflammatory responses by recruiting neutrophils and monocytes to the site of infection. Th17-cells can be found in large numbers near the epithelial barriers.

While Th1, Th2 and Th17 cells migrate and function to the peripheral tissues, **follicular helper T cells** facilitate the activation and differentiation of B cells in the secondary lymphatic organs. Their function is essential for the process of isotype switching of antibody molecules. This cell type was identified recently, before that, their function was assigned to classical Th1 or Th2 cells.

T-helper subsets can develop from the common naïve Th precursor (a Th0 cell) in secondary lymphoid organs. Their differentiation is predominantly regulated by the APC of the local secondary lymphoid tissues, however, cytokines in their environment have also profound regulatory functions. For example, secreted cytokines by the Th1 and Th2 subsets mutually inhibit each other's differentiation (Figure 20.).

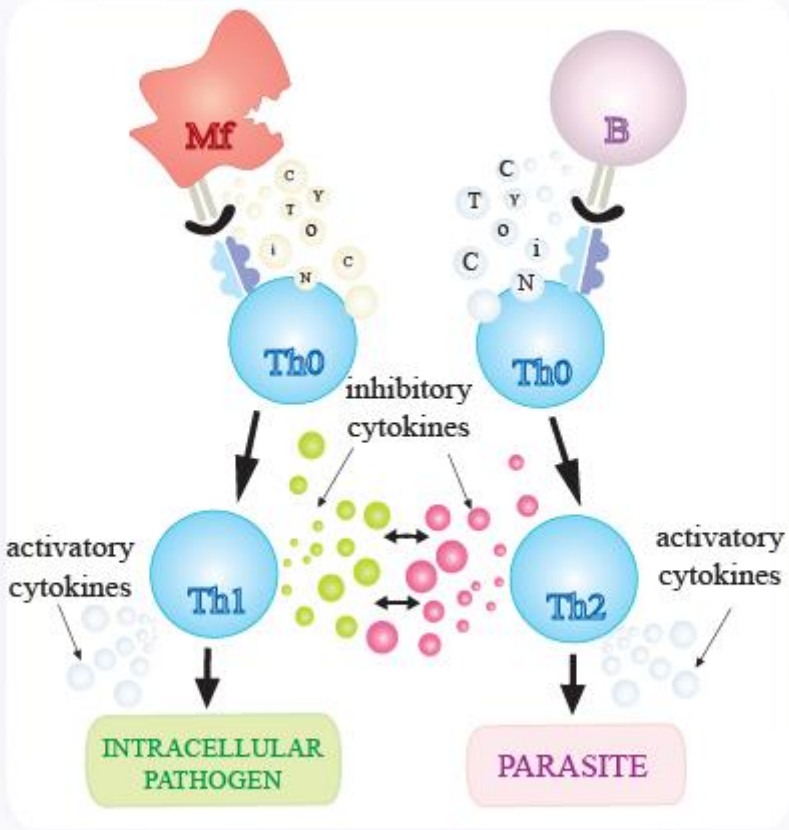


Figure 20. Differentiation of naïve helper T cells

Interaction with the APC, together with their cytokine secretion will determine the "fate" of the differentiating naïve T-helper cell. Various T cell subsets differentiating from the activated Th0 cells coordinate distinct immune cell functions. Th1 cells secrete cytokines mainly to enhance the immune response against intracellular pathogens, while Th2 support anti-parasite immunity. Cytokines produced by different T-helper subpopulations inhibit each other's function.

Cytotoxic T cells (killer cells, **CTL, CD8+ T cell**) recognize and kill "estranged", virus-infected or tumour cells present in our body. Under physiological conditions our cells continuously synthesize and degrade cellular proteins. Peptides derived from these degraded proteins are transported to the cell surface and displayed in complex with MHC I molecules. The same mechanism operates for the presentation of viral- and tumour-associated proteins.

Unlike Th cells, CTLs recognize antigen fragments in complex with MHC I molecules expressed by all nucleated cells, thus synthesis of foreign or mutant-self proteins in any cells can be readily detected and eliminated by CTLs. (Figure 19.)

Although the mechanism of recognition and activation of NK cells and CTLs are different, the mechanisms of killing the target cells are similar. Both cell types make contact with the infected- or tumour-cell directly and releases the content of their intracellular granules containing cytotoxic substances to the site of cell-cell contact. Some of these cytotoxic substances like *perforin* molecules will form pores within the target cell membrane leading to disruption of the transmembrane ion balance which alone may be sufficient to cause the death of the target cell.

Granzymes also released by these granules enter the target cell via perforin-induced pores and trigger apoptosis. Additionally, effector CTL expresses molecules on the surface which induce apoptosis in the target cell.

Various subsets of **regulatory T cells** produce inhibitory cytokines that suppress immune responses against self-antigens.

The immunological memory, passive and active immunization

The appearance of the adaptive immune system during evolution allowed the specific recognition of unique antigens which is essential for the development of the immunological memory. In case of repeated exposure to the same pathogen the organism responds faster and more effectively compared to the first exposure. Memory response is characteristic only of B and T cells. The mechanism of pathogen recognition by innate cells does not allow the development of an effective, highly-specific memory response.

During clonal proliferation and differentiation, some antigen-specific B and T lymphocytes in the secondary lymphoid tissues differentiate into memory cells. The memory cells and their precursors have the same antigen receptor specificity therefore they recognize the same antigen. Following elimination of the pathogen, the majority of antigen specific B and T cells becomes unnecessary and die by apoptosis. However, a relatively large number of lymphocytes survive and become memory cells. After elimination of the pathogen, memory cells may survive and preserve their antigen specificity for decades, even in the absence of their specific antigen. B cells can differentiate into long-lived plasma cells and may produce antibodies throughout the entire lifetime of an individual.

Characteristics of the memory response

- Upon second exposure (with the same pathogen), antibodies produced by long-lived plasma cells act immediately to neutralize and opsonize the pathogen
- The frequency of specific lymphocytes upon antigen re-exposure within the long-lived memory T or B cell repertoire is much higher than in the repertoire of naïve lymphocytes.
- Activation of B as well as T memory cells is simpler, thus faster than that of naïve cells.

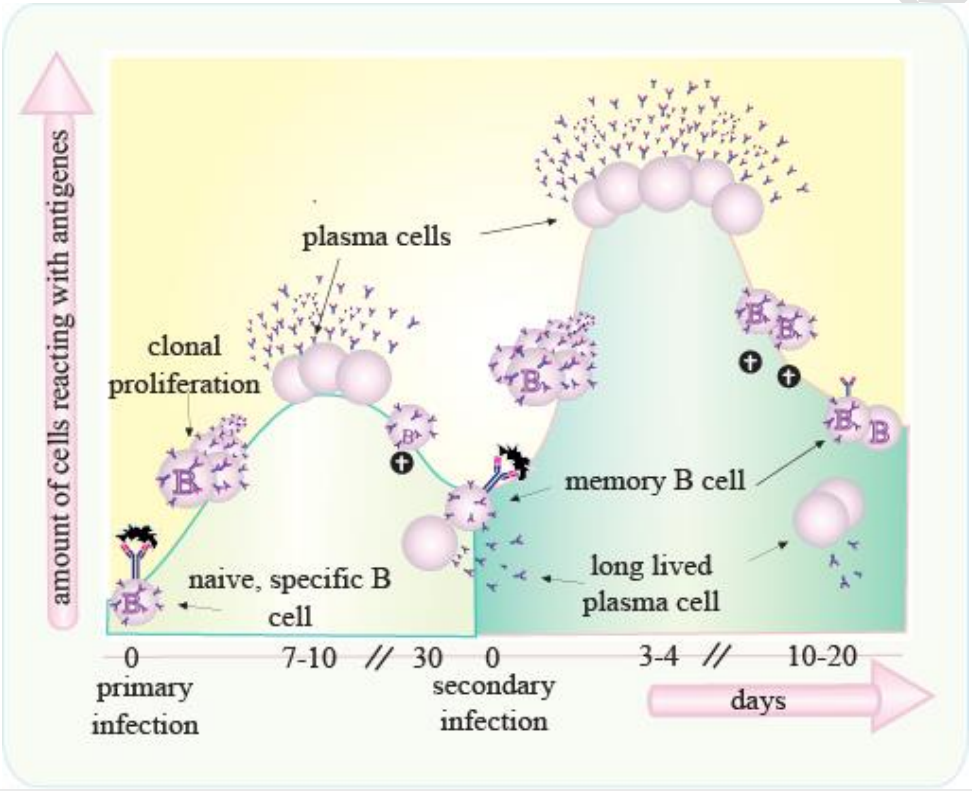


Figure 21. The response of memory B cells.

After the specific recognition of the pathogen B cells go through clonal expansion and differentiate to plasma cells or memory B cells. Following successful eradication of a pathogen the majority of the plasma cells die, however, some of the antigen specific long-lived plasma cells and memory B cells survive. Re-infection with the same pathogen induces the activation of already existing memory cells. In this population, frequency of antigen-specific B cells is higher than in naïve B cell populations. In addition, they are already differentiated B cells that have gone through a germinal center reaction, which warrants a faster, more robust and more effective B cell response. The amount, the specificity and the affinity of the secreted antibodies are also higher.

Antigen-specific effector cells, which develop from memory cells in peripheral tissues or in secondary lymphoid organs complete their proliferation and differentiation more rapidly.

Compared to the first encounter *(primary immune response)*, the *memory response* reaches its peak three-times faster (within 3-5 days).

- During a memory response not only the number of responding B cells but the amount of antibodies produced is higher compared to the primary immune response (Figure 21.)
- Both the affinity and the specificity of antibodies produced during the memory response are enhanced (affinity maturation) and often their *isotype* is switched. In a primary response IgM-type antibodies are dominant, while repeated infections with the same pathogen increases the amount of other isotypes (IgG, IgA, IgE)

The differentiation of long-lived plasma cells, affinity maturation as well as isotype switching of B cells requires the contribution of Th cells.

With ageing the production of naïve lymphocytes decreases. Thus, in elderly people the immune response is based on memory lymphocytes. Senior people may be sensitive to infections with pathogens yet unknown to them, however they can readily maintain protection against microbes they have encountered long time ago.

The B cell mediated primary and secondary immune response

	primary response	**memory response**
Intensity	lower	high
Antibody isotype	mainly IgM	(IgM) IgG, IgA, IgE
Antibody affinity	low affinity	high affinity
Time interval of a max. response	7-14 days	3-5 days

Immunization

The beneficial features of the memory immune response are utilized when artificial immunization is used to develop protection against pathogens. Using passive immunization, antigen-specific antibodies are injected or transfused into to body. By active immunization both cellular and humoral antigen-

specific memory response are induced artificially that usually prevent the appearance of the disease following exposure to pathogen.

Passive immunization

Immunity can be transferred from one person to another. Antigen specific antibodies or antibody-enriched serum (antiserum) can be transfused to a person we aim to protect. These antibodies **act immediately,** neutralize and opsonize the antigen.

The application of antiserum or purified antibody is warranted in all cases when fast and efficient protection is needed immediately (when the antigen has entered the body). For example, serum containing neutralizing antibodies are used in cases when venom of poisonous snakes or a toxin of arthropods enter the victim's body.

The protection time of passive immunization is limited by the half-life of the antibody (usually some days to some weeks depending on the subtype). Newborns are protected from many pathogens by antibodies produced by their mother. Until birth, IgG antibodies are transcytozed from the maternal to the fetal blood via the placenta by special Fc receptors. After birth these antibodies provide temporary protection distributed in the body of the newborn. The baby's digestive and respiratory tract are protected by the maternal IgA supplied by breast feeding.

Active immunization and vaccines

An immune response can be induced by either infection or **active immunization**. Vaccines may contain live attenuated or inactivated pathogenic microbes, purified or artificially synthetized immunogenic components/subunits of microbes. Attenuated pathogens lose some of their virulence factors essential for fast proliferation and dissemination. This blocks their ability to induce serious disease, but allows the development of protective immunity by the host. Vaccines containing small parts or purified recombinant proteins of microbes called subunit vaccines are usually less efficient and require the use of *adjuvants.* The pathogen-derived antigens are recognized by the adaptive immune system and a memory response is generated, but most vaccines will induce transient, mild symptoms only, if any.

Vaccines prepare the immune system for a future infection. During vaccination the immune system completes all the steps of the relatively slow primary response, therefore a future response to an infection with the pathogenic microbe will show the characteristic of a rapid and robust secondary (memory) response that clears the infection quickly. Vaccination may completely block or dampen the symptoms of infections. Taken together, **vaccination** against a

microbe mimics the infection with the pathogen, induces a memory response which enables the host to eliminate the same pathogen and prevent a potentially fatal disease.

Active immunization with most vaccines is a slow process. It takes weeks or sometimes months to develop effective protection and sometimes repeated vaccination is required. Thus, an immediate response is not provided, but depending on the type of the vaccine it may protect the body against the targeted microbe for decades or even for life. Good examples of this are vaccines given to children at an early age.

The efficacy of a vaccine, just like the imunogenicity of antigens in general, may differ significantly from one person to another. Immunodeficient individuals may remain unprotected to some or to all pathogens despite the most sophisticated vaccination schemes. However, when the majority of individuals within a large human population are vaccinated, these unprotected individuals of the group are also protected. This phenomenon is called the "herd immunity", a kind of "social immunity".

Active and passive immunization.

	Active	**Passive**
Type of substance	inactivated/attenuated pathogen, or pathogen subunit	immune serum or purified antibody
Mechanism	the host develops an immune response memory cells produced	exogenous, neutralizing antibody is provided
Development of protection	slow	immediate
Duration of protection	long: years or lifetime	some weeks or months

Immunological tolerance

B and T cells of a healthy individual will fail to launch a destructive immune response to antigens present in self-tissues. This is a result of an active, antigen specific unresponsiveness as a result of two main mechanisms called **central- and peripheral tolerance.**

Central tolerance is established during lymphocyte development in the primary lymphoid organs, for B cells in the bonne marrow and in the thymus for T cells. Antigen receptors of lymphocyte progenitors are exposed to self-antigens during their development. Those cells carrying receptors that bind to self-antigens with high affinity are considered potentially autoreactive and die by apoptosis. Thus, most self-reactive, potentially dangerous autoimmune cells prone to cause tissue damage in various organs are eliminated at an early stage of lymphocyte development. This process generating central tolerance is referred to as **negative selection** or **clonal deletion** of autoimmune lymphocytes. Even though there are mechanisms inducing the ectopic expression of self-antigens in the primary lymphoid organs, some developing lymphocytes fail "to get acquainted" with all self-antigens. Thus central tolerance is considered an efficient but "leaky" mechanism.

Autoimmunity caused by the relatively few self–reactive cells can be controlled by various **peripheral tolerance** mechanisms.

1. As described above, *naïve* T-lymphocytes acquire activation signals exclusively from activated, mature dendritic cells (DCs) immigrating from the site of infection to the lymph node. DCs exposed to danger signals or pathogen associated molecular patterns elevate the cell surface expression of co-stimulatory molecules which are required for the proper activation of naïve T cells. The "maverick" autoreactive T-lymphocytes escaping clonal deletion in the primary lymphoid organs, will first encounter their specific self-antigen presented by non-professional APC, or non-activated APC in the absence of co-stimulation. In the absence of co-stimulation these T-lymphocytes enter into a state functional unresponsiveness called *anergy*.
2. Peripheral tolerance of B cells is maintained mainly by the absence of autoreactive T cells. B-lymphocytes recognizing and presenting self-antigens fail to find helper T-lymphocytes which are specific for the same autoantigen. In the absence of T cell mediated costimulatory signals activation of autoreactive B cells is inhibited.
3. Peripheral tolerance is maintained in part by the aforementioned regulator T-lymphocytes (Treg) which keep autoreactive lymphocytes supressed.

Communication between the innate and adaptive immune system

While in invertebrate species only the elements of the innate immune system are present, the innate and adaptive immune systems not only co-exist in

humans, rather, they co-operate in total harmony. The innate immune system, especially macrophages and dendritic cells are essential for initiation of the adaptive immune response. T cells are unable to function without these antigen presenting cells. With few exceptions B cells also require the signals coming from innate immune cells and / or T helper cells.

The cells of the innate immune system do not simply trigger the activation of T cells. Through their secreted cytokines, costimulatory and adhesion molecules, they orchestrate the adaptive response. Although activation of the adaptive immune system comes much later, it is able to augment significantly the function of innate immune cells.

Cytokines produced by T cells help the activation of macrophages, elimination of the ingested microbes, maturation of dendritic cells, antigen presentation and the cytotoxicity of NK cells. Lymphocyte-derived cytokines contribute to regulation of the action, the maturation and the localization of innate cells. At the same time cytokines also play a key role in limiting the innate immune response.

We have already discussed the effect of antibodies produced by B cells. The antibody molecules opsonize pathogens, facilitate their recognition and phagocytosis. Opsonization by antibodies also increases the activity of the complement system and enhances NK cell-mediated killing. Similarly, antibodies regulate the function of mast cells and granulocytes.

Both the innate and the adaptive immune system have self-control mechanisms. Cytokines and chemokines produced by macrophages have autocrine effects regulating macrophage functions. These cytokines are the main positive and negative regulators of neutrophils in the inflammatory process, and also of the functional activity of NK cells. Furthermore, they can increase the production and facilitate the exit of complement proteins from the blood vessels.

In the case of the adaptive immune system only those B cells can get help from Th cells which can present appropriate antigen to them. In optimal situation the B cell and the interacting Th cell recognize the same antigen. In this case, cytokines produced by the T cells contribute to the activation, affinity maturation of B cells as well as to generation of memory cells. Antigen presentation by B cells may facilitate the survival of effector / memory T cells.

The process of immune response

Pathogens may cause mild diseases, like rhinitis induced by rhinoviruses or debilitating, even fatal diseases, for example the HIV or HBV virus. Our

immune system consists of multiple, co-operating systems which defend the body against thousands of pathogens present in our environment. The skin or the mucosal epithelium lining the airways and gut are the first defense line against invading pathogens, forming a physical and chemical barrier against infection. The dry and hard outer layer of the skin is a formidable barrier when unbroken. Infections occur only when a pathogen crosses these barriers, as in the case of an injury (e.g. wounds by cuts and burns). Although the gastrointestinal and respiratory tracts are exposed to a plethora of pathogens they are protected effectively by multiple mechanisms. Pathogens colonizing mucosal surfaces enhance secretion of a viscous fluid called mucus. Foreign agents irritate the respiratory tract triggering cough and sneezing, which effectively remove these irritants. The acidic environment of the stomach destroys many pathogens ingested with meals.

The gastrointestinal, respiratory and urogenital tracts are covered by **mucosal epithelium** which consists of epithelial cells held together by tight junctions. These epithelial cells are covered with mucus, containing various oligo- and polysaccharides. Mucus has a number of protective functions, for example it may prevent microorganisms from adhering to the epithelium.

Epithelial cells can transport certain antibody isotypes (especially IgA) across the epithelial cell to the surface of mucosal epithelium using active transport mechanisms. Therefore, microbes are exposed to antibodies as soon as they enter our respiratory or digestive system. IgA-mediated protection against microbes is based primarily on its neutralizing effect.

Invading pathogens are encountered by various immune cells ready for combat. Macrophages, dendritic cells, effector T cells and B cell are all present in the subcutaneous or intraepithelial tissues. The invading pathogens are recognized first by the cells of the innate immune system (eg. macrophages and dendritic cells), expressing pattern recognition receptors. They immediately trigger the well-known reactions of the innate immune system (elimination of pathogens, release of cytokines, recruitment of other immunocytes) leading to induction of inflammation in the infected tissue.

The increased production and flow of lymph will carry some of the pathogens into the regional (draining) lymph nodes. Activated dendritic cells also reach the lymph nodes through the lymph, where they present processed phagocytosed antigens to the T lymphocytes. Thus B and T lymphocytes, which recognize the pathogen specific antigens, get activated, proliferate and differentiate into effector or memory cells.

Depending on the amount and the growth rate of the invading pathogen we can distinguish between three potential courses of the immune response.

Small amounts of pathogens are immediately recognized at the site of infection and are eliminated by macrophages found almost everywhere in our body.

If this mechanism is insufficient for clearing the pathogen more participants of the innate immune system will be recruited to help macrophages.

If the pathogen survives for an extended period, the adaptive immune system will also be activated, which finally eliminates the pathogens either by its own mechanisms, or more often by enhancing the innate response.

If the organism has met the pathogen in the course of an earlier infection, the effector mechanisms of adaptive immune response can provide immediate protection. Pathogen-specific antibodies produced by the long-lived plasma cells neutralize the pathogens and the effector T cells eliminate the infected cells in a few days, or produce cytokines to enhance the efficiency of the other participants of immune system. In parallel, memory cells in the lymph nodes get activated and begin to proliferate rapidly.

Bacteria, viruses and parasites

Hundreds or thousands of viruses, bacteria, fungi, unicellular or multicellular eukaryotic parasites are able to infect humans. Pathogens use diverse strategies for infection and to escape immune surveillance. Accordingly, the immune system uses different strategies to override these mechanisms.

The main strategies of immune system for elimination/neutralization of extracellular or intracellular pathogens:

	innate immune system	**adaptive immune system**
extracellular pathogens	• phagocytosis • production of soluble toxic agents • complement system	• antibody production, neutralization
intracellular pathogens	• destroying infected cells (NK cells) • intracellular killing and degradation • release of type I interferons, intracellular antiviral mechanisms	• destroying infected cells (cytotoxic T cells)

Most pathogenic bacteria and fungi entering the body live in the interstitial space between cells. These pathogens are called **extracellular pathogens**. These are the main targets of antibodies and complement proteins released from the blood plasma during inflammation. Pathogens opsonized by antibodies are efficiently recognized by phagocytes, mainly by macrophages and neutrophil granulocytes, and by the complement system. The complement system can itself lyse some microbes. As a result, via complement receptors and Fc-receptors phagocytes are induced to ingest and kill opsonized pathogens.

Dendritic cells activated by microbes induce high-level expression of costimulatory receptors and migrate along lymphatic vessels to draining lymph nodes where they present the pathogen-derived peptides to naïve T lymphocytes. Pathogens phagocytosed by macrophages are usually killed in the phagolysosomes. A fraction of microbial peptides not degraded in the phagolysosomes can be presented on MHC II to the helper T cells. In turn, effector T-helper cells activated by macrophages using cell-cell contact and secretion of cytokines (e.g. IFN-γ) will help macrophages to kill the ingested pathogens or at least prevent their spread (Figure 22).

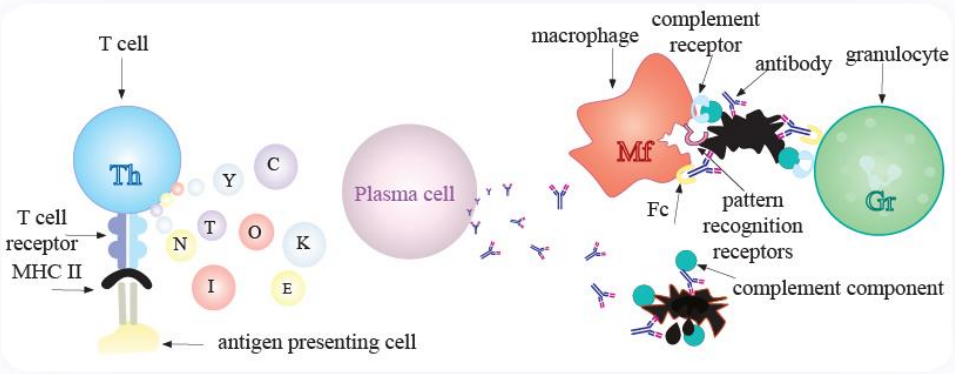


Figure 22 Elimination of extracellular pathogens

Phagocytes (Mf) recognize pathogens via PRR or pathogens opsonized with antibodies and/or complement via Fc- and/or complement-receptors. Pathogens bound by these receptors get ingested and killed in the phagolysosomes by multiple mechanisms (right). Antibodies produced by plasma cells may neutralize the pathogen and/or activate antibody-mediated effector functions (middle). (Complement components may directly eliminate pathogens.) Type 2 cytokine-producing T helper cells augment immune response against extracellular pathogens primarily (left).

Viruses and some bacteria are able to invade cells and generate intracellular infection, therefore their elimination requires an entirely different recognition and elimination process.

The epitopes of intracellular proteins synthetized in infected cells are presented at the cell surface through MHC class I molecules. These infected cells are recognized and eliminated by another group of effector T cells, the cytotoxic T cells. If intracellular pathogens downregulate expression of MHC class I molecules, infected cells are recognized and eliminated by NK cells, since the lack of MHC class I mediated inhibitory signals greatly enhance cytotoxic activity of NK cells.

Thus, effector functions against intracellular pathogens are not directed to the pathogen, they are rather focused on destruction of the infected host cells (Figure 23).

The same effector mechanisms are also characteristic of anti- tumour responses, as tumour cells as well are aberrant host cells that need to be eliminated.

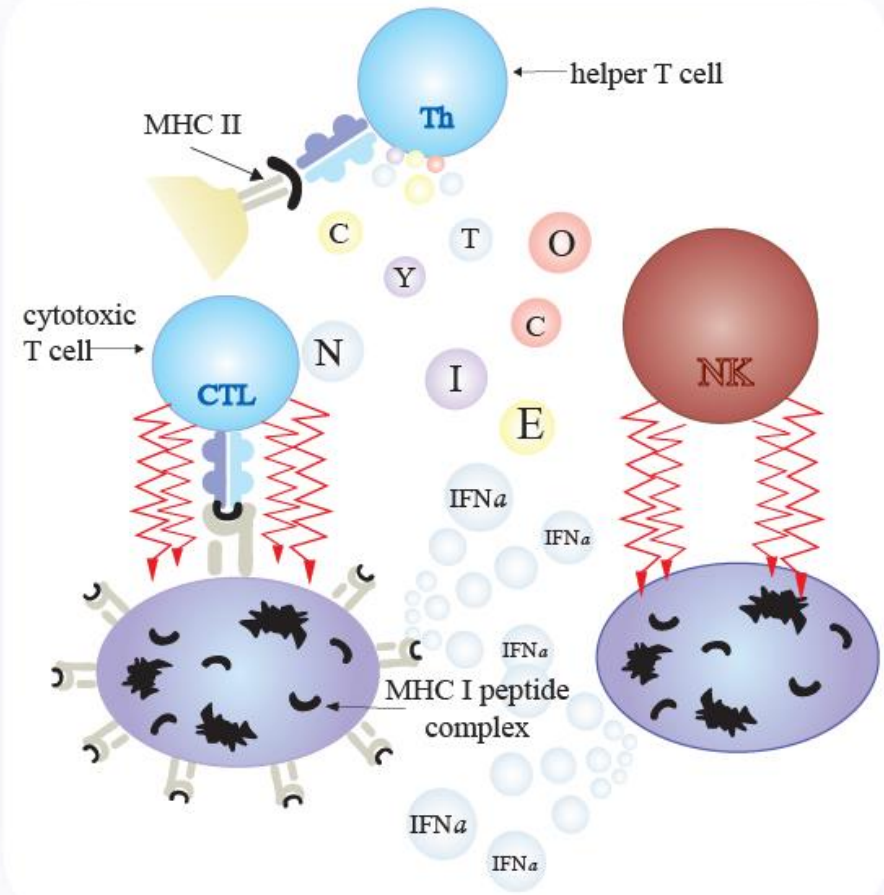


Figure 23 Elimination of intracellular pathogens

Virus-infected cells are recognized via intracellular pattern recognition receptors followed by production of type I interferons. Concomitantly, MHC class I molecules present antigens of intracellular pathogens on the cell surface and activate cytotoxic T lymphocytes, which kill the infected cells (bottom left). In the absence of cell surface MHC I (indicating an aberrant cellular function) cells become targets for NK cells, which again leads to the elimination of the cell (right).

Interferons play a key role in anti-viral immunity. IFN-γ is produced mainly by T helper cells and cytotoxic cells (NK, Tc), and besides their direct antiviral effect their main function is to promote macrophage functions. In the presence of IFN-γ macrophages kill engulfed microorganisms more efficiently. In addition, IFN-γ enhances the expression of MHC class I and II molecules on the surface of professional antigen presenting cells.

IFN-α, IFN-β can be produced by almost any cell type following viral infection. Interferons often have autocrine effects on the cells producing them, or paracrine effects on neighbouring cells. They interfere with viral replication by various mechanisms eg. through inhibition of protein synthesis, or increased expression of MHC class I molecules.

Antibodies may also partake in the defense against viruses. The neutralizing effect of antibodies prevents viral adhesion to host cells and thereby interferes with infection.

Viral proteins may also be integrated in the cell membrane of infected cells. Antibodies recognizing these antigens will bind to them, thus marking the cell for antibody-dependent cellular cytotoxicity (ADCC). NK cells can kill infected cells also by ADCC via activation by their Fc receptors.

Some small, unicellular eukaryotic **parasite,** which exist intracellularly in some part of their life cycle, such as Plasmodium species, can induce similar cytotoxic T cell responses. Their extracellular form can be recognized by antibodies

Elimination of large multicellular extracellular parasites (e.g. flat- and roundworms) often requires much broader spectrum of immune response delivered by specialized cells. Mast cells, basophil and eosinophil granulocytes are involved in inflammation triggered by parasites. These cells release special toxic agents and degrading enzymes from their vacuoles to destroy the parasites at the area of infection. In addition to toxic agents many pro-inflammatory cytokines are also secreted. Some of these increase peristaltic activity in case of intestinal infection or trigger coughing, sneezing and induce mucus production in case of a respiratory infection to help removing the parasites from the body. The parasite-specific IgE antibodies bound to high affinity Fc receptors of mast cells and basophil granulocytes play important roles in the activation of these cells and thereby in the immune response against parasites.

Disorders of immune system

“Too much” as well as “too little” reactivity of the immune system may cause diseases. The former may lead to the development of the so-called hypersensitivity reactions while the latter one is called immunodeficiency.

Hyper-reactivity can develop against self-antigens causing autoimmune diseases. Hypersensitivity reactions specific to either foreign or self-antigens may cause significant tissue damage to the host.

Allergic diseases

In some people immune response is triggered by innocuous (harmless) agents (allergens), such as plant pollen, certain food products (eg. peanut proteins), animal hair (animal-derived proteins) or house dust (which contains the excrement components of dust mite). These processes are connected with abnormal production of allergen-specific IgE antibodies by plasma cells.

The characteristic symptoms of the disease are itching, sneezing, rashes, or in more serious cases, **anaphylactic shock**.

This process is triggered by stable, compact, often enzymatically active antigens. Such antigens are able to cross the epithelium of the respiratory system or the gastrointestinal tract. T cells contribute to the development of the disease by promoting the development of antigen-specific, IgE producing B cells. Antigen-specific IgE antibodies gaining access to all peripheral tissues bind to the high affinity Fcε receptors (IgE-specific, Fc-epsilon receptors) on mast cells. From that point, the body is „sensitized" to the antigen. These sensitized mast cells survive for years.

At the second exposure the allergen will activate many Fcε-receptors via crosslinking the antigen-specific IgE bound to them. Consequently, mast cells become activated and release their stored bioactive agents (histamine, serotonin, various enzymes) which rapidly induce the typical symptoms of allergy (immediate reaction). This rapid, usually immediate reaction to the antigen (allergen) is called **Type I hypersensitivity** reaction (Figure 24).

Later, in the second phase of the reaction, (also called the late response) mast cells and basophil granulocytes produce additional inflammatory mediators (late lipid mediators and cytokines).

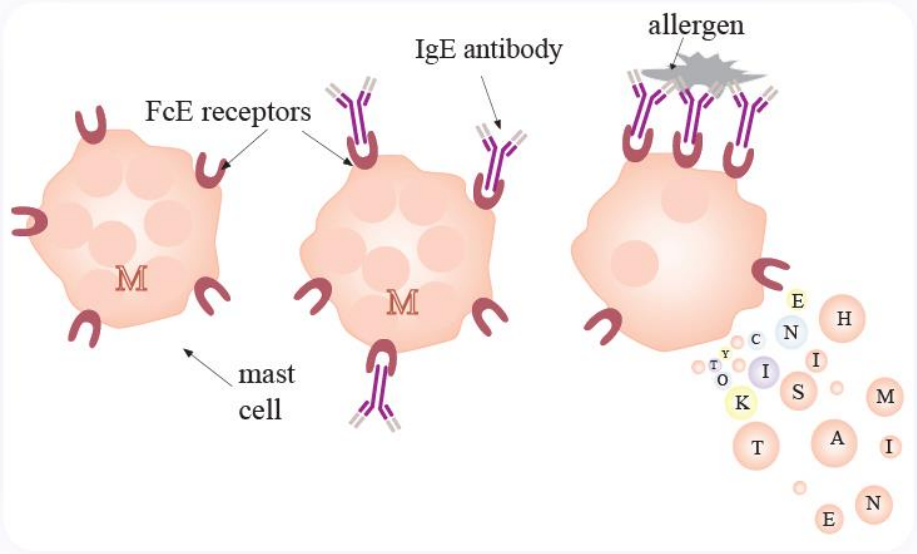


Figure 24 Mechanism of allergic response

Expression of high affinity Fcε receptors on mast cells is antigen independent. After appearance of an allergen specific IgE-type antibodies are produced by plasma cells which bind to these Fcε receptors whit high affinity. Crosslinking of Fcε receptor-linked antibodies by the allergen induces activation of the mast cell: the release of granular content and cytokine production.

The main role of the mast cells, basophil granulocytes and molecules (IgE) are well known in immune response against parasites. Thus most of the symptoms of allergic reactions results from effector mechanisms designed to kill or expel parasites.

Autoimmune diseases

Sometimes regulatory mechanisms maintaining self-tolerance fail, resulting in increased production of self-reactive antibodies and lymphocytes. As a result, healthy cells and tissues become targets of the effector mechanisms of immune response.

Antibodies can be produced against various cell surface receptors this way. Some of these auto-antibodies may interfere with the normal function of the receptor by inhibiting the binding of its natural ligand (blocking or antagonistic antibodies). This is the case in myasthenia gravis, an auto-immune disease, in which antibodies specific to acetylcholine receptors at the postsynaptic neuro-muscular junctions inhibit the excitatory effects of the neurotransmitter acetylcholine causing muscle weakness. Other auto-antibodies can have

stimulating effects on receptors (agonistic antibodies). In Graves' disease (also known as Basedow-syndrome) auto-antibodies continuously stimulate the *t*hyroid-*s*timulating *h*ormone (TSH) receptor, resulting in hyperthyroidism. In other cases, binding of the auto-antibody to the target cell does not change cellular functions, but triggers antibody-dependent effector functions described earlier. In penicillin-sensitive persons antibodies recognize penicillin covalently coupled to cell surface proteins of red blood cells. Similar to any other antigens or pathogens, red blood cells opsonized by penicillin-specific antibodies become targets of phagocytes and the complement system.

Those reactions when antibodies bind to common or modified cell surface antigens and trigger effector functions causing damage to self-tissues are called **Type II hypersensitivity reactions**.

T cells may also cause hypersensitivity or autoimmune disease by a mechanism called **Type IV hypersensitivity**. In patients with Type I diabetes mellitus cytotoxic T lymphocytes kill the insulin producing beta cells of the pancreas. In gluten-sensitive individuals the modified gliadin peptide derived from the wheat protein gluten is presented via MHC class II molecules to T cells leading to their activation that triggers the production of pro-inflammatory cytokines and thereby inflammation.

Some people are sensitive to chemicals produced by plants (e.g. poison ivy), others to nickel-containing jewellery. When these materials come into contact with skin they modify skin proteins. The modified protein presenting cells become targets of Tc- or Th cells that induce inflammation (contact dermatitis). These processes –with the antigen presentation– require 48-72 hours to develop, consequently, this type of reaction is often called **delayed-type hypersensitivity**.

Immune complex diseases

Immune complexes are antigens opsonized with antibodies (Figure 25). Antibodies may form large, cross-linked complexes with antigens, which under normal circumstances are rapidly eliminated from the circulation by phagocytic cells of the spleen.

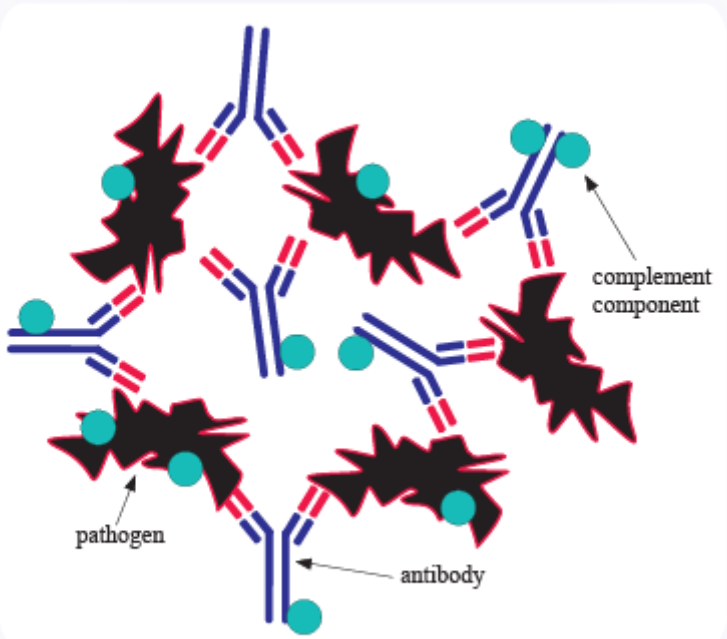


Figure 25 Immune complexes

Immune complexes consist of an antigen, an antigen-specific antibody and usually complement components as well.

In the presence of large quantities of auto-antigens and their specific auto-antibodies small immune complexes are formed, which remain in circulation for an extended period of time. In addition, small immune complexes have access even to the smallest blood vessels including capillaries of the skin, the glomeruli of the kidney or they can accumulate in the joints as well. Immune complexes stuck in small vessels activate the complement system via the classical pathway at the site of deposition. Also phagocytic cells get activated, since both the complement fragments and the antibody molecules in immune complexes act as opsonins. This leads to inflammation and destruction of local self-tissues. These immune complex-mediated autoimmune diseases include vasculitis, glomerulonephritis or arthritis. Many pathogens (viral hepatitis, malaria) or environmental agents may trigger similar diseases (eg. „farmer's lung" caused by a mold present on hay). Hypersensitivity reactions induced by immune complexes are called **type III hypersensitivity** reactions as well.

The mechanisms leading to the development of autoimmune diseases are often not clear. Multiple environmental and genetic factors may be responsible. Environmental factors may include pathogens, chemicals or even strong sunlight. Hormones may also play a role since most autoimmune diseases are more common in women than in men. The presence of certain MHC allotypes is also associated with a higher risk of autoimmune disease development.

Immunodeficiencies

Immunodeficiency is a state when the immune response to all or to a selected set of pathogens is insufficient, causing severe, recurrent infections or death. The so-called **primary immunodeficiencies** are caused by inherited genetic defects deleting essential components (cells or regulatory mechanisms) of the immune system. **Acquired immunodeficiencies** on the other hand are caused by environmental factors including toxic chemicals, chemotherapy or **immunosuppressive drugs** used at organ transplantations to impede transplant rejection.

Transient immunodeficiency may develop after viral infections, for example measles, influenza, Epstein-Barr virus-induced mononucleosis. Some surgical procedures, malnutrition, smoking and stress, even transfusion with white blood cell-containing blood may cause temporary immunosuppression.

There are over 200 primary immunodeficiency syndromes. Some babies are born without functional B cells and thus produce minimal or no antibodies, or when they have a defect in class switching, they produce only IgM antibodies. The lack of T cells causes an even more severe immune defect, as in the absence of T cells most B cell functions are also hampered. Therefore, in the absence of T cells, a combined, fatal immunodeficiency called *s*evere *c*ombined *i*mmuno*d*eficiency or **SCID** develops.

The acquired immune deficiency syndrome, or **AIDS** is an immunodeficiency caused by the human immunodeficiency virus **HIV.** This retrovirus infects the T-helper cells and monocyte-derived macrophages bearing CD4 surface molecules. The virus is integrated, in the form of DNA into the human genome, where it may remain silent and hidden from the immune system for a long time. However, sooner or later the virus destroys the infected cell. As the number of the helper T cells in HIV-positive individuals is reduced, the patients gradually loose immune competence leading to the appearance of opportunistic infections (infections with organisms that are normally not pathogenic in immunocompetent individuals) or to development of cancer. This stage of the disease is called AIDS, which is fatal if left untreated.

Glossary

adaptive immunity — Acquired / learnt immunity. A highly specific and efficient immune response, emerging after birth that uses antigen-specific receptors and antibody molecules. Capable of developing long-lasting immunological memory.

adjuvant — a substance mixed up with an immunogen in order to generate a more intense immune response

anaphylatoxin — Small cleavage products of some complement components, that can cause intense inflammatory reactions (even anaphylactic reaction).

anaphylaxis — anaphylactic shock/ systemic anaphylaxis Serious, systemic allergic reaction.

anergy — Functional unresponsiveness. Generally, naïve lymphocytes become anergic, if they react with an antigen without costimulation.

antibody — Glycoproteins produced by B cells. They bind appropriate antigens with high affinity and specificity.

antigen — Those molecules that can be recognized by antigen receptors and thus any material, that triggers a specific response from the immune system, either tolerance or a destructive immune response.

antigenic determinant — See epitope!

antigen presenting cell or APC. — A cell that processes extracellular or intracellular material and displays it in a form "visible" /detectable by T-lymphocytes. T cells can only recognize antigens that are processed and presented by antigen presenting cells as a peptide in complex with an MHC molecule. Nearly every cell in the body expresses MHC I molecules, thus can be regarded as APC. The professional antigen presenting cells express both MHC I, and MHC II molecules.

antigen receptor — A receptor expressed by B and T lymphocytes that recognizes native or processed antigens with high specificity.

apoptosis — Programmed or induced cell death. The „suicide" of the cell.

autocrine effect An effect of a hormone or other chemical substance acting on the cell by which the substance was produced.

autoimmunity A self-reactive state of the adaptive immune system, when adaptive immune cells attack self-structures (self-antigens).

BCR B cell receptor. The antigen receptor complex of the B cell.

B cell See B lymphocyte

B lymphocyte A cell type expressing antigen receptors that resemble an antibody. Precursors of plasma cells which produce large amounts of antibody.

CD, CD nomenclature, CD molecules, CD antigens Cluster of Differentiation. Each cell type or the differentiation state of a lineage can be identified by the set of these cell-surface expressed molecules. These molecules were found and identified using specific antibodies. They are numbered by an international committee, usually following the order of their discovery. For example: functionally distinct T cell populations are the CD8 bearing cytotoxic and the CD4 bearing helper T cells.

cellular immune response Immune response mediated by different immunocytes (e.g.: cellular cytotoxicity mediated by cytotoxic T or NK cells, or phagocytosis by macrophages)

chaperon A helper protein that facilitates other protein's folding, transport etc.

chemokine Cytokine that induces guided migration (chemotaxis) of cells.

chemotactic factor Any soluble factor with a chemotactic effect. They may not be produced directly by cells. For example complement protein's cleavage products are like this.

complement receptors Receptors that can recognize some cleaved, activated, molecules of the complement system.

complement system	Approximately 30 proteins found in the plasma, classified as part of the innate humoral immune response. Many of these proteins are proenzymes, others are able to recognize PAMPs. During their activation these proteins are able to activate each other in a proteolytic cascade reaction. Some cleavage products of the complement molecules can function as opsonins. They can induce inflammation others are able to destroy the activating pathogens directly.
cross presentation	Presentation of exogenous antigen on MHC I molecules. Dendritic cells are capable of this process.
CTL	Cytotoxic T Lymphocyte
cytokine	A soluble, hormone like messenger molecule produced by cells. It can influence the function of cells that express the cytokine-specific receptor.
cytotoxicity	Ability to kill cells. Many cells and humoral elements of the immune system are able to perform this function. It can be directed against free or engulfed pathogens. In a diseased state, it may be used against the organism's own cells.
DAMP	Danger (damage) associated molecular pattern. A molecular pattern that signals danger or damage.
dendritic cells	A group of a phagocytic cells with typical dendritic structure. They act as professional antigen presenting cells. These cells have a critical role in activation of naïve T cells.
domain	Structural and/ or functional unit of protein molecules. Domains are frequently encoded by independent exons.
endocrine effect	An effect induced far away from the effector cell (usually delivered by blood circulation to anywhere in the body).
endogenous	Originates from within (of an organism, tissue, cell)
epithelial cells	Cells separating the body from the environment, linening and protecting internal, external surfaces of our body (skin, intestines, respiratory system, urinary tract)
epitope	or antigen determinant. The part of the antigen that is in physical contact with the antigen receptor, or the antibody.
exogenous	Originates from outside (of an organism, tissue, cell)
extracellular	Something that happens or exists outside the cell.

Fc region	The region of the antibody, that is similar to a fragment formed during limited enzymatic cleavage of an antibody. It consists of disulphide linked constant domains of the heavy chains. Intact immunoglobulins have different effector functions, thanks to this region, e.g. by Fc receptor binding.
Fc receptor	A receptor, that is able to recognize the Fc region of an antibody. Fc receptors cannot recognize the variable region of the antibody, only the heavy-chains' constant domains.
glycoprotein	Protein post-translationally modified by covalent attachment of oligosaccharide chains to the polypeptide chain.
granulocytes	A group of polymorphonuclear myeloid cells, with similar appearance under the light microscope: They have granules in their cytoplasm and a lobed nucleus. Neutrophil, eosinophil and basophil granulocytes belong in this group, based on their granular content.
granzyme	A protease produced by cytotoxic cells. In the target cell it induces cell death by apoptosis. .
hematopoietic stem cell	Undifferentiated cell type, with the capacity of self-renewal. Precursors of all cellular components of the blood.
HLA	Human Leukocyte Antigen, an alternative name for the human MHC-encoded genes. There are 3 classical human MHC I molecule coding genes, the HLA-A, HLA-B and HLA-C genes. The HLA-DP, HLA, DQ and HLA-DR gene regions are responsible for the coding of the classical human MHC II molecules.
humoral immune response	Immune response delivered by soluble component of the immune system (e.g. antibodies, antimicrobial peptides or complement proteins).
immune complex	The complex of antigens with bound antigen specific antibodies which usually includes bound complement proteins
innate immunity	The immunity one is born with. Its activation is based on recognition of PAMPs and DAMPs, (pathogen- or danger associated molecular patterns). Also called natural immunity.

interleukin — Cytokines produced by leukocytes

interferon — Cytokines with antiviral activity. They are important in antiviral immune responses

intracellular — Something existing or happening inside the cell.

invariant chain (Ii) — The invariant chain participates in the maturation of the MHC II molecules. Binding to the MHC II molecule in the endoplasmic reticulum (ER), its main role is to inhibit binding of endogenous peptides in the ER to the MHC II

isotype (of an antibody) — Antibodies can be classified into isotypes or classes based on differences in their constant domains. Human antibodies based on their heavy chain's constant region can be classified into 5 main groups (IgM, IgD, IgG, IgA, IgE). Based on their light chains, they are divided into two groups (kappa and lambda). Different antibody (heavy chain) isotypes can induce distinct effector functions with different efficiency. Subclasses are called isotypes as well.

leukocyte — White blood cells. Granulocytes, monocytes, macrophages, dendritic cells, NK cells, mast cells, T and B cells belong here.

lymphocyte — White blood cell group based on simple microscopic similarities: cells of this group have relatively large nucleus, and minimal cytoplasm. Leukocytes with different functions that belong to the lymphoid lineage. The adaptive B-, T lymphocytes and the innate NK cells belong in this group.

LPS, lipopolysaccharide (also called endotoxin) — Oligo- or polysaccharides covalently coupled to lipid molecules, taking part in the formation of the outer membrane of gram negative bacteria.

macrophage — Monocyte derived large cells, very effective in phagocytosis of larger particles. Different types of macrophages play a role in inflammatory processes and in tissue regeneration. They can also function as professional antigen presenting cells.

mast cell — A cell type specialized in the destruction of single cells or multicellular parasites. Compounds stored in its granules are effective against eukaryotic parasites. They play a key role in some inflammatory and allergic reactions.

MHC	Major Histocompatibility Complex. A chromosomal region encoding proteins with important role in antigen presentation. Some proteins expressed by the MHC locus – also called shortly MHC molecules– are expressed on the surface of APCs where they present peptides to the T cells.
monocyte	A leucocyte that belongs to the myeloid lineage. They are larger than lymphocytes with a typical „bean„ shaped nucleus circulating in blood. They are the precursors of macrophages.
NK cell	Natural Killer cell. An innate lymphocyte that can kill tumour cells or cells infected with pathogens.
opsonin	Molecules used for opsonization. These can be antibodies, cleaved complement proteins or other antimicrobial proteins for example acute phase proteins produced by the liver.
opsonization	The marking of a pathogen or antigen, so effector mechanisms of the immune system's can be delivered more efficiently. Opsonized structures can be engulfed quicker or they can activate the complement system.
PAMP	Pathogen-associated molecular pattern, for example: bacterial lipopolysaccharide, flagellin, viral double stranded RNA.
paracrine effect	an effect, influencing cellular functions in the vicinity of the producing cell.
pathogen	Any agent causing a disease. It can be a single-cell or multicellular organism. Prokaryotic, eukaryotic, or virus.
peptide	A polymer of a few amino acids.
perforin	A cytotoxic protein produced by killer lymphocytes (cytotoxic T lymphocytes, NK cells). Its polymerized product forms a pore within the membrane of the target cell.
plasma cell	Antibody producing cells that differentiate from B cells.
primary immune response	The adaptive immune system's that is generated at the first encounter with an antigen or a pathogen. The process that leads to activation of naïve lymphocytes.

primary lymphoid organ Organs where the immune cells develop from undifferentiated precursors. Two organs should be mentioned as primary lymphoid organ: the red bone marrow and the thymus.

proenzyme Or zymogen. An inactive enzyme or enzyme precursor that must be activated by a biochemical process to perform its function. In case of some complement, limited proteolysis of zymogens is required for the activation of the subsequent component of the cascade.

proliferation serial cell divisions

professional antigen presenting cell. Professional APC. Antigen presenting cells are able to activate naïve T cells. Unlike other cells, these cells express MHC II molecules, by which they can present antigen derived peptides to helper T cells. Professional APCs are dendritic cells, macrophages and B cells.

proteasome Large enzyme complex in the cell, with a cylindrical shape. It's present in each eukaryotic cell and in some bacteria. It has role in the breakdown of unnecessary proteins. It produces peptides those are presented by MHC I molecules.

protein A polymer molecule made of amino acids, many of these polymers have stable secondary and tertiary structure.

PRR Pattern recognition receptor

secondary lymphoid organ The sites where adaptive immune responses are initiated. The encounter with antigen, the activation and differentiation of the naïve cells of adaptive immunity occurs here.

soluble not solid, or cell surface bound (for example: the antibody is the soluble form of B cell receptor)

stem cell Undifferentiated cell type that has self- renewing capacity. It can differentiate into a wide variety of cell types.

T cell See T lymphocyte.

T lymphocyte Lymphocyte that bares TCR antigen receptor. It has multiple functionally distinct types. Some can destroy infected cells, others influence the outcome of the immune response, or makes it more efficient

TCR	T cell receptor. T cell's antigen receptor complex
TNF	tumour necrosis factor. Cytokine, produced by many cell types of the immune system. It has wide range of effects. This cytokine has more types, however, unless indicated otherwise, TNF means TNF-α. It can be produced during inflammatory processes. It can have cell-activating, or cell-killing effect
Toxin	Poison. Toxins of immunologically distant organisms. Bacteria, produce toxins that behave as antigens. They can induce antibody production.
zymogen	See proenzyme