*The following text is an internal study material intended as a supplementary study resource for second-year students of General Medicine studying in the English language on the Department of Microbiology and Immunology, JFM in Martin, CU in Bratislava.*

**SOURCE: Martina Neuschlová, Elena Nováková, Jana Kompaníková: Imunológia - ako pracuje imunitný systém. Multimediálna podpora výučby klinických a zdravotníckych disciplín :: Portál Jesseniovej lekárskej fakulty Univerzity Komenského [online] , [cit. 24. 04. 2026]. Dostupný z WWW: https://portal.jfmed.uniba.sk//clanky.php?aid=372. ISSN 1337-7396.**

**Translation and text editing: J. Kompaniková , V. Sadloňová.**

# CHAPTER 1

## 1.1 The role of Immune system

The immune system is a “team sport” involving a wide range of different “players” who work together to ensure effective protection against an attacker. Thousands of components of the immune system have been, and continue to be, gradually identified, and thanks to advances in molecular biology and genetics, our understanding has progressed to the subcellular level. If we were to focus on only a single “player,” it would be very difficult to comprehend the entire game. Therefore, in this textbook we will view the game from the perspective of a spectator—a fan—so that we can objectively observe the mutual relationships and interactions and gain a comprehensive understanding of what the immune system is all about.

Immunity is defined as resistance to disease, particularly infectious disease. The group of molecules, cells, and tissues that provide resistance to infection is referred to as the immune system. The coordinated response of these cells and molecules against an infectious microorganism is known as the immune response. Immunology is the study of the immune system, including its responses to microbial pathogens and damaged tissues, as well as its role in health and disease. The most important physiological function of the immune system is to prevent the development of infection and to eliminate infections once they arise. The importance of the immune system for an individual’s health is underscored by the susceptibility to severe and potentially life-threatening infections observed in individuals with immune system disorders. On the other hand, stimulation of the immune system through vaccination is an effective method of protecting individuals against infection and its consequences.

The immune system is one of the fundamental systems that maintain homeostasis and primarily ensure the defense of the organism against adverse environmental factors. One of the basic mechanisms by which the organism recognizes its surrounding environment is the ability to present substances (antigens) to the immune system and the immune system’s capacity to distinguish foreign molecules from self.

The immune system is a complex, effective, and highly regulated system that protects against foreign structures (antigens), recognizes and discriminates between self and non-self, and regulates and directs defensive responses. It is composed of numerous components—proteins, cells, and organs—that participate in the defense of the individual, particularly in response to the threat of disease caused by infectious microorganisms.

- An infectious microorganism that causes disease is referred to as a **pathogen**.
- An individual who is infected by a pathogen is referred to as a **host**.

Not all microorganisms cause disease; on the contrary, some are beneficial. For example, bacteria living in the intestine assist in the breakdown of certain dietary components. Microorganisms that benefit the host are referred to as **commensal microorganisms** and constitute part of the **physiological microflora (PMF)**. Protective commensal microorganisms of the PMF inhibit the growth of pathogens. They interfere with pathogens during invasion and colonization, control pathogen colonization of mucosal surfaces (by competing for receptor sites and nutritional factors), immunize the host against pathogens through similar or cross-reactive antigens, and participate in the production of bacteriocins (proteinaceous antimicrobial substances that inhibit the growth of certain bacteria).

Although the immune system is primarily involved in defense against infectious microorganisms, it also plays a significant role in many other diseases. It provides defense against certain types of cancer, prevents the growth of some tumors, and several cancer treatment strategies are based on stimulating the immune response against developing tumor cells. The immune system also contributes to the removal of dead cells and to tissue repair processes. Products of immune cells have practical applications in the treatment of immunological diseases.

In contrast to these beneficial roles, abnormal functioning of the immune system is also responsible for numerous diseases. Proper functioning of the immune system is essential for maintaining good health. However, like other physiological systems of the body, the immune system can also malfunction, leading to the development of disease. The most common disorders resulting from improper immune system function are allergies. Another category of diseases caused by immune system dysfunction includes autoimmune diseases. When one or more components of the immune system are absent or when their function is impaired, immunodeficiencies develop. The immune response also represents a major obstacle to successful organ and tissue transplantation, as it is responsible for graft rejection.

### 1.2 The primary function of the immune system

The primary function of the immune system is to maintain the integrity of the organism.

1. It recognizes what is harmful and what is harmless to the organism, thereby protecting it against various harmful substances (antigens) originating from both the external and internal environment.
2. It provides the organism’s defense against pathogenic microorganisms and their products.

3. It induces mechanisms of tolerance to the body's own tissues – **self-tolerance (autotolerance)**.
4. It ensures immune surveillance by recognizing harmful agents from the internal environment and continuously removing old, damaged, or pathologically altered cells.

The immune system closely communicates with the nervous and endocrine systems, resulting in mutual interaction, regulation, and surveillance.

### 1.3 Types of Immune Mechanisms

Immune mechanisms can be classified into several basic categories:

- **Innate** – identical in all individuals; some are species-specific, pre-formed, evolutionarily older, and their responses are always the same.
- **Acquired** – develop during an individual's lifetime through activation of the individual's own immune system following stimulation; they are evolutionarily younger.
- **Non-specific / non-adaptive** – antigen-independent (also referred to as innate).
- **Specific / adaptive** – antigen-dependent (also referred to as acquired).
- **Cell-mediated** – carried out by immunocompetent cells.
- **Humoral** – mediated by soluble substances present in the serum and various body fluids.
- **Systemic** – act throughout the entire organism via the circulation.
- **Local** – act only at specific sites within the organism, for example at mucosal surfaces.

### QUESTIONS

**How can the immune system be characterized?**
**What are the main functions of the immune system?**
**List (name) the types of immune mechanisms.**

# CHAPTER 2

## INNATE AND ACQUIRED IMMUNITY

### 2.1 Definition of Innate and Acquired Immunity

The host defense mechanisms consist of three lines of defense. The first line of defense is provided by a set of mechanical, chemical, and biological barriers that protect the individual. However, if these barriers are overcome, the mechanisms of the second and third lines of defense—namely innate immunity and acquired (adaptive) immunity—are activated.

Both innate and acquired immunity use receptors to detect potential threats, primarily microorganisms. Cells and molecules of the innate immune system respond very rapidly to the entry of microorganisms and are often sufficient to ensure effective defense of the individual. However, if not only the barriers but also the mechanisms of innate immunity are overcome, the mechanisms of acquired immunity come into play. These form the third line of defense against potential threats to the human organism.

Although the acquired immune response develops more slowly—because it requires the expansion and differentiation of cells (lymphocytes) needed to respond to microorganisms—it subsequently provides highly specialized and highly effective protection against infection.

### 2.2 First Line of Defense – Barriers

The immune system therefore consists of three levels of defense. The first level is represented by mechanical, chemical, and biological barriers that prevent microorganisms from entering the tissue environment of the human body. These barriers can be imagined as solid fortress walls that protect the inhabitants from enemy attacks.

For protection of the organism against infection and for maintaining its overall integrity, an intact surface of the skin and mucous membranes is essential, as well as the presence of their natural non-immune defense mechanisms—mechanical, chemical, and biological barriers.

#### 2.2.1 Mechanical Barriers

Mechanical barriers prevent the entry of potentially pathogenic microorganisms into the body. The mechanical barriers of the skin and mucous membranes include the outer layer of the skin and the epithelial layer of mucosal surfaces.

**Skin**

The skin is a very effective barrier against the penetration of microorganisms into the body. Its thickness varies in different parts of the body. Most bacteria are unable to pass through intact skin. The outer layer of the skin—the epidermis—and its most superficial layer, the stratum corneum, consist of dead, keratinized cells that are continuously shed. This layer is water-resistant, prevents dehydration, and the dry environment on its surface is unfavorable for microorganisms. Even if some microorganisms manage to adhere to the skin, they are removed through the constant shedding of dead keratinized cells.

Intact skin therefore represents not only an effective mechanical barrier, but is also supported by chemical and biological barriers, such as sweat, secretions of sebaceous glands, unsaturated fatty acids, microbicidal substances, and the physiological skin microflora.

**Mucous Membranes**

The mucosal epithelium lines all body cavities that come into contact with a wide variety of particles from the external environment. It is composed of several types of cells, some of which have secretory functions, while others are absorptive or protective. The mucosal epithelium lines the respiratory, gastrointestinal, and urogenital tracts.

It produces mucus, which helps protect epithelial cells and adjacent tissues and contributes to the trapping of microorganisms. Mucus is slippery and coats the delicate mucosal surfaces, thereby protecting them from damage caused by food particles, digestive enzymes, or dust. At the same time, it is viscous and adhesive. Microorganisms become “trapped” in it and are therefore unable to bind to susceptible cells.

Secretory IgA antibodies also bind to mucus and contribute to the capture of microorganisms. In addition, mucus contains substances capable of destroying or at least slowing the multiplication of microorganisms, such as lysozyme, lactoferrin, and lactoperoxidase.

In the respiratory tract, mucus (together with trapped microorganisms) is moved by cilia and subsequently expelled by sneezing or coughing.

In the gastrointestinal tract (GIT), approximately 4 liters of mucus are produced daily, most of which is reabsorbed in the large intestine. Mucus with adherent microorganisms is propelled by peristalsis and eliminated from the body.

The uterine cavity is mechanically protected against the entry of microorganisms by the presence of a mucus plug in the cervical canal.

Cells of the mucosal epithelium are washed by fluids (saliva, tears, urine) and are continuously renewed in a manner similar to skin cells. This represents an important mechanism for the removal of microorganisms from many mucosal surfaces.

**Respiratory Tract**

The movement of fine hairs at the entrance to the nose mechanically traps particles larger than 10 µm during breathing. The ciliated epithelium of the airways then transports trapped particles outward from the body via nasal secretions. Mucus produced in the respiratory tract captures inhaled microorganisms and other particles.

Microorganisms and impurities trapped in mucus are moved by the ciliated epithelium and can be removed from the respiratory tract through sneezing or coughing. The movement of cilia is synchronized and organized to ensure the transport of mucus outward from the body.

However, the function of the ciliated epithelium can be significantly impaired (for example by smoking, chronic alcohol abuse, certain viral infections, or whooping cough), which negatively affects the clearance capacity of the respiratory tract. The importance of the ciliated epithelium and mucus in the respiratory tract is also demonstrated by recurrent respiratory infections caused by opportunistic pathogens (such as *Pseudomonas aeruginosa*) in patients with congenital defects in chloride ion permeability, as seen in cystic fibrosis. This defect leads to the production of extremely thick and viscous mucus, whose physiological removal is very difficult. The mucus often obstructs the airways, resulting in recurrent respiratory infections.

**Urinary Tract**

In the urinary tract, the primary mechanical barrier is urination, which mechanically prevents the ascent of microorganisms into the urinary tract through the outward flow of fluid. At the same time, urination serves to eliminate bacteria that may enter this region due to inadequate hygiene (most commonly from the anal area), therapeutic catheterization, or anatomical abnormalities that prevent regular and sufficiently strong urine flow (such as strictures or urinary stones).

As in other body sites, chemical and biological barriers also contribute to defense within the urogenital tract.

### 2.2.2 Chemical Barriers

Chemical barriers against microorganisms include the pH of certain body sites and microbicidal substances.

**Environmental pH**

Medically significant bacteria are **neutrophilic**, meaning that a pH below 6 is unfavorable for their growth. The skin contains sweat glands and sebaceous (oil) glands, some of

whose products are mildly acidic (e.g., lactic acid in sweat), resulting in an acidic skin pH of approximately 5.5. Excessive sebum production may lead to obstruction of sebaceous glands, causing alterations in skin pH that are less protective. In addition, various microorganisms may become trapped in blocked sebaceous glands. A high concentration of sodium chloride in sweat also inhibits the growth of many bacteria.

The stomach has a highly acidic environment (pH 1.0–3.0) due to the presence of gastric juice. As a result, only a few microorganisms can survive in the stomach. This chemical barrier prevents microorganisms from entering the small intestine. Its importance is demonstrated by the frequent occurrence of intestinal infections in patients with achlorhydria (deficiency of hydrochloric acid in gastric juice).

In the duodenum, the pH changes due to the admixture of alkaline pancreatic secretions with the intestinal contents. However, bile salts are present, which are toxic to non-intestinal bacteria. On the other hand, bile is harmless to some bacteria—normal intestinal bacteria tolerate bile salts, and in some cases bile may even promote their growth (e.g., *Salmonella* species and their persistence in the gallbladder).

In the vagina of healthy women, the pH ranges from approximately 4.4 to 4.6. This is due to the presence of lactic acid produced by *Lactobacillus acidophilus* (a component of the physiological microflora), which metabolizes glycogen from vaginal epithelial cells. Some strains of lactobacilli also produce hydrogen peroxide, and the production of microbicidal substances (bacteriocins—lacticins) is also significant.

**Microbicidal Substances**

Microbicidal substances inhibit the growth of microorganisms or destroy them by damaging their cell walls and membranes, leading to lysis. They attract cells of innate immune mechanisms and facilitate phagocytosis. These substances are found in the skin, sweat, tears, saliva, and secretions of the respiratory, gastrointestinal, and urogenital tracts.

The skin contains a wide range of microbicidal substances, including RNase, DNase, defensins, cathelicidin, and fatty acids, which are toxic to Gram-negative bacteria and some fungi. Sweat contains lysozyme, which degrades peptidoglycan, primarily in the cell walls of Gram-positive bacteria. The openings of hair follicles and sweat glands are protected by lactic acid, lysozyme, and unsaturated fatty acids.

In the respiratory system, microbicidal **β-defensins** are present; they bind to microorganisms and thereby facilitate their ingestion and destruction by phagocytosis.

In the gastrointestinal tract, microbicidal lysozyme, alpha-defensins (cryptdins), and digestive enzymes present in saliva, the stomach, and the small intestine are active.

Although these enzymes primarily function in digestive processes, they are also effective in killing and eliminating potential pathogens that enter the gastrointestinal tract.

Tears, which wash the ocular conjunctiva, contain lysozyme, lactoferrin, and secretory immunoglobulins IgA. Impaired tear secretion leads to severe damage to the eye.

### 2.2.3 Biological Barriers

The biological barrier is represented by the **physiological microflora (PMF)**, which comprises a spectrum of commensal microorganisms that exist in mutual symbiosis with the human organism. These microorganisms colonize the skin, the gastrointestinal tract, and other body sites, where they inhibit the growth of other potential pathogens.

Physiological colonization of the human body begins immediately after birth and depends on environmental factors and diet. Over the course of life, a delicate ecological balance is established between the bacteria that physiologically colonize certain body sites and the human organism. This state is referred to as **eumicrobia**, defined as the presence of bacteria on the skin and mucosal surfaces in optimal composition and quantity.

Disruption of eumicrobia, termed **dysmicosis (dysmicrobia)**, may occur as a result of changes in microbial composition or in the relative abundance of specific species within a tissue (e.g., overgrowth of a single component of the PMF), or due to alteration of the physiological localization of microorganisms (their spread to sites that should normally be sterile). Such disturbances may lead to disease.

The composition of the PMF is species-specific and has developed based on ecological interactions. It is relatively stable within a given species and individual, although it is influenced by lifestyle habits, age, and the environment in which the individual lives.

Colonization by pathogenic bacteria is regarded as a manifestation of dysmicrobia, even in the absence of clinical symptoms. Examples include encapsulated strains of *Haemophilus influenzae* and *Neisseria meningitidis* in the nasopharynx of individuals living in closed communities (such as military camps, youth training centers, or preschool facilities). Likewise, the presence of parasites, fungi, or viruses—even without clinical symptoms—is not considered part of the physiological microflora.

#### Importance of the Physiological Microflora

The significance of the PMF lies in the beneficial effects of commensal bacteria:

- They compete with pathogenic microorganisms for receptor sites and nutritional factors during colonization and invasion.

- They immunize the host against pathogens through similar or cross-reactive antigens.
- They create a favorable environment that inhibits the growth of pathogens (e.g., *Lactobacillus* species).
- They participate in the production of bacteriocins (proteinaceous antimicrobial substances that inhibit the growth of certain bacteria).
- They contribute to vitamin synthesis (e.g., *Escherichia coli*—vitamin K) and to toxin elimination (e.g., *Bacteroides* species).

Early exposure to appropriate non-pathogenic bacteria after birth stimulates Th1 lymphocytes, which inhibit the activity of Th2 lymphocytes and thereby suppress the release of cytokines that promote allergic reactions.

Bacteria of the physiological microflora (PMF) may sometimes be mistaken for etiological agents due to their presence on mucosal surfaces and their morphological similarity to pathogenic microorganisms. Some of these bacteria may contribute to the development of non-infectious diseases (e.g., *Streptococcus mutans*, *Streptococcus milleri* – dental caries). When nonspecific immune mechanisms fail, the physiological microflora may assume the role of pathogens (the PMF is essentially conditionally pathogenic). For example, in immunocompromised individuals, bacteria of the PMF may cause infections.

Bacteria of the PMF may spread from the skin to sites that are physiologically sterile or to body sites where the invading bacteria are not normally part of the PMF. This may occur, for example, during the insertion of medical or therapeutic devices (such as catheters) into the body, subsequently leading to infection.

The typical composition of the PMF can be disrupted, for instance, by the administration of broad-spectrum antibiotics, hormones, or antiseptics. This disruption may manifest clinically as loss of appetite, diarrhea, digestive disturbances, itching, and other symptoms. In some cases, it may even lead to life-threatening conditions. An example is the overgrowth of anaerobic bacteria in the intestine following administration of broad-spectrum antibiotics, which can result in pseudomembranous colitis. In pseudomembranous colitis, the pathogenic anaerobic bacterium *Clostridium difficile* produces toxins that damage the gastrointestinal tract and cause profuse watery diarrhea, fever, and abdominal cramps.

### 2.3 Second Line of Defense – Innate Immunity

If pathogens penetrate the first line of defense—barriers—the second line of defense is provided by innate immune mechanisms. Innate immunity is, however, the first

immunological line of defense. It is evolutionarily older and is pre-existing and ready for action within the organism.

Innate immunity (also referred to as nonspecific immunity) provides rapid defense against infection because it acts immediately. It is always present in healthy individuals and is constantly prepared to prevent the entry of microorganisms or to rapidly eliminate those that have succeeded in crossing the host's barriers. The innate immune system is already established before antigen exposure. Responses of innate immunity are always the same upon repeated contact with an antigen; they are antigen-independent and consistent in both timing and intensity. Innate immune responses always react as if encountering the antigen for the first time.

Innate immune mechanisms include the rapid destruction of infectious agents, activation of phagocytes, and a protective response known as inflammation. During inflammation, cells and molecules of innate immunity are stimulated to isolate and destroy pathogens and to initiate tissue repair processes.

## QUESTIONS:

**Which mechanisms does the host use for its defense?**
**Which immunological lines of defense are utilized by the host?**
**What is physiological microflora?**
**Define the term eumicrobia.**
**Define the term dysmicrobia.**
**The presence of which organisms is not considered physiological microflora?**

# CHAPTER 3

## MAIN COMPONENTS OF THE IMMUNE SYSTEM – LYMPHOID TISSUES AND ORGANS

### 3.1 Lymphoid Tissues and Organs

The main components of the immune system are cells and molecules that may be present individually in the circulation and tissues, or accumulated within lymphoid tissues and organs. Lymphoid organs are divided into **primary** and **secondary** lymphoid organs.

In the **primary lymphoid organs** (the thymus and bone marrow), lymphocytes develop. In the **secondary lymphoid organs** (the spleen, lymph nodes, and accumulations of lymphocytes within tissues), circulating immune cells are able to interact with one another, and antigens are captured and concentrated. Specific immune responses are initiated through interactions that take place within secondary lymphoid tissues.

Lymphoid tissues and organs are connected to other organs and tissues of the body by a dense network of lymphatic and blood vessels. Lymphatic vessels that deliver lymph to lymph nodes are referred to as **afferent lymphatic vessels**, while those that drain lymph into the bloodstream are called **efferent lymphatic vessels**. An exception is the spleen, as it has no direct connection to the lymphatic vessels.

#### 3.1.1 Primary Lymphoid Organs

Primary lymphoid organs are referred to as “primary” because they are the sites of origin, differentiation, and maturation of immunocompetent cells. In humans, the primary lymphoid organs are the **thymus** and **bone marrow**. All types of leukocytes develop in the bone marrow from a pluripotent hematopoietic stem cell, which differentiates into the myeloid and lymphoid cell lineages.

**Thymus**

Cells of the lymphoid lineage that are destined to become T lymphocytes migrate to the thymus at an early stage of development, where they undergo a form of “higher education.” This specialized maturation enables them to recognize self and non-self structures.

The thymus is a bilobed organ that enlarges during the fetal and neonatal periods and gradually involutes from puberty onward. Precursors of T lymphocytes enter the thymus as so-called **pro-thymocytes** and migrate to the thymic cortex, where they are referred to as **cortical thymocytes**. In this environment, they acquire a definitive **T-cell receptor**

**(TCR)** (an antigen-specific receptor on T lymphocytes) and the **CD3 complex**. In addition, they express other important surface molecules.

Of particular importance are the surface molecules **CD4** and **CD8**, which, together with CD3, are essential components associated with the T-cell antigen receptor.

The essence of education in the thymus is to select T cells that are capable of recognizing self molecules of the major histocompatibility complex (MHC) but do not react to self antigens. Those cells that are unable to recognize self MHC molecules at all (because they possess a non-functional TCR) undergo apoptosis. Only those cells whose receptors can recognize and bind to self MHC molecules are allowed to proliferate further—this process is referred to as **positive selection**.

On the other hand, some of these cells bind very strongly to the MHC protein complex (they possess receptors with high affinity) and thus become potentially dangerous because they are autoreactive. These cells also undergo apoptosis—this process is known as **negative selection**.

Positively selected thymocytes are therefore those that recognize MHC proteins in the thymus but possess receptors with low affinity for the MHC protein complex. Thymocytes that have undergone positive selection no longer express both molecules (CD4 and CD8) simultaneously, but only one of them. Cells whose TCR binds to MHC class I molecules retain expression of the CD8 coreceptor, whereas those whose TCR binds to MHC class II molecules retain expression of CD4. Mature T lymphocytes leave the thymus and populate secondary lymphoid organs.

A large number of pro-thymocytes enter the thymus; however, approximately 95–98% of them die, indicating that selection at this “university” is extremely stringent. The development of T lymphocytes in the thymus is regulated by stromal cells (see Chapter 4).

**Bone Marrow**

Lymphocyte lineages that do not migrate to the thymus but instead undergo “education at home,” that is, in the bone marrow, differentiate into **B lymphocytes**. In the bone marrow, these cells undergo early differentiation. Through rearrangement of DNA genes, they form the **B-cell receptor (BCR)** (the specialized receptor of B lymphocytes) and additional molecules such as **Igα and Igβ**, which are required to initiate intracellular signaling cascades. They also express surface IgM. Expression of surface IgM marks the cell as an **immature B lymphocyte**.

During further development, simultaneous expression of surface IgM and IgD occurs, resulting in a **mature B lymphocyte**.

In the bone marrow, some B cells may randomly generate BCRs capable of recognizing and binding self molecules. These cells are potentially dangerous because they react to the body's own structures. Therefore, apoptosis is initiated in these cells, eliminating autoreactive clones. As in T-cell development in the thymus, stromal cells in the bone marrow cooperate in regulating B-cell development.

### 3.1.2 Secondary Lymphoid Organs

The main secondary lymphoid organs include the **spleen** and **lymph nodes**. **Mucosa-associated lymphoid tissues** represent secondary accumulations of lymphoid tissue. Secondary lymphoid organs and tissues capture and concentrate antigens and serve as sites where large numbers of circulating immunocompetent cells come into contact. Secondary lymphoid tissue also has a filtration function, removing foreign material and dead cells from the circulation. These organs and tissues are the sites in which the major phases of antigen-specific immune responses take place.

#### Spleen

The spleen is the largest lymphoid organ. It concentrates antigens from the blood and removes unwanted particles from the circulation. It contains both B and T lymphocytes, antigen-presenting cells (APCs), other leukocytes, and a large number of plasma cells that secrete immunoglobulins into the bloodstream. The spleen has a smooth, shiny, dark-red surface and is histologically divided into **red pulp** and **white pulp**. The red pulp is rich in erythrocytes, whereas the white pulp is rich in lymphocytes. The spleen has no direct connection to the lymphatic vessel system.

From an immunological perspective, the spleen is particularly important in defense against pathogens present in the blood. All of these components reach the spleen via the bloodstream.

#### Mucosa-Associated Lymphoid Tissue

Mucosal lymphoid tissue is referred to as **MALT (Mucosa-Associated Lymphoid Tissue)**.

MALT is composed of clusters of lymphoid follicles (tonsils, adenoids, appendix, Peyer's patches in the small intestine) as well as diffuse lymphoid tissue. MALT therefore represents either well-organized or diffuse lymphoid tissue that protects mucosal surfaces and is localized at sites where microorganisms most frequently enter the body. It is found in the gastrointestinal, respiratory, and urogenital tracts. MALT contains lymphoid follicles similar to those found in the spleen and lymph nodes.

### 3.2 Lymphatic Circulatory System

Lymphocytes continuously migrate between the blood, lymphatic vessels, and lymphoid organs. The lymphatic circulatory system is an extensive capillary network that collects lymph from various organs and tissues. Lymphatic capillaries merge to form lymphatic vessels that transport lymph to the lymph nodes.

Lymph is a clear, watery fluid containing leukocytes and cellular debris. Before entering the bloodstream, it is filtered in the lymph nodes, where it is cleared of cellular debris and microorganisms. Lymphatic vessels in the villi of the small intestine contain a milky fluid called **chyle**, which is produced during digestion.

Lymphocytes circulate in the blood for approximately 30 minutes. About 45% of them enter the spleen, remain there for about 5 hours, and then return to the bloodstream.

Lymphocytes also have the unique ability to migrate directly from the bloodstream into lymph nodes by passing through specialized blood vessels with cuboidal endothelium. Approximately 40% of lymphocytes enter lymph nodes in this manner and remain there for about 12 hours. They subsequently leave the lymph nodes via efferent lymphatic vessels and are transported to the **thoracic duct (ductus thoracicus)**, which drains lymph into the bloodstream.

Approximately 15% of lymphocytes enter lymph nodes through afferent lymphatic vessels. The remaining small percentage of lymphocytes migrate into mucosal lymphoid tissue. This extensive circulation allows lymphocytes to migrate from the bloodstream to the spleen and lymph nodes one to two times per day.

## QUESTIONS:

**What are the two main functions of lymph nodes?**
**What is the function of B cells in the immune response?**
**Which three areas of the human body are characterized by a high concentration of lymph nodes?**
**What is lymph?**
**What is the name of the terminal duct that drains the body?**

# CHAPTER 4

**CELLS OF IMMUNE SYSTEM**

### 4.1 Hematopoiesis

Cells that provide an immune response are called immunocompetent cells. The cells of the immune system have different lifespans. Their loss is complemented by hematopoiesis. Hematopoiesis is the formation and development of red and white blood cells from a pluripotent hematopoietic stem cell. It is a process of creating and renewing blood cells, which go through the stages of proliferation, differentiation and maturation. The myeloid and lymphoid lineages of the multipotent cells are differentiated from the pluripotent stem cell. Lymphocytes develop from the lymphoid lineage, other blood cells (granulocytes, monocytes, erythrocytes and thrombocytes) from the myeloid lineage. Adult hematopoiesis is performed in the bone marrow and is regulated by numerous cytokines (blood growth factors GM CSF, M-CSF, G-CSF, erythropoietin, thrombopoietin, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9), also by some hormones and nerve effects. Vitamins and minerals are required for proper hematopoiesis.

### 4.2 Criteria for the classification of white blood cells

According to their lineage of origin, leukocytes are divided into **myeloid** and **lymphoid** cells. Based on the characteristic shape of their nuclei, they are classified as **polymorphonuclear** and **mononuclear leukocytes**. Depending on the presence and amount of cytoplasmic granules, they are further divided into **granulocytes** and **agranulocytes**.

#### 4.2.1 Cells of the myeloid lineage

Cells of the **myeloid lineage** are involved in mechanisms of **innate immunity**. Most of these cells possess the ability to phagocytose, produce cytokines, and release other soluble mediators. Some of them—**dendritic cells, monocytes, and macrophages**—act as **antigen-presenting cells (APCs)** and present antigens to T lymphocytes.

Monocytes and macrophages function as scavenger cells that remove cellular debris, foreign cells, and particles, which they subsequently degrade enzymatically, thereby contributing to the “clean-up” of the body. Dendritic cells actively phagocytose, particularly at sites of pathogen entry, and they may be of either myeloid or lymphoid origin.

### 4.2.2 Cells of the lymphoid lineage

Cells of the **lymphoid lineage** are primarily involved in mechanisms of **acquired (adaptive) immunity**. T lymphocytes, B lymphocytes, and natural killer (NK) cells differentiate from the lymphoid lineage.

B lymphocyte development takes place in the bone marrow. After encountering an antigen in secondary lymphoid organs, B lymphocytes differentiate into **plasma cells** that produce antibodies. **B lymphocytes and plasma cells are the only cells capable of producing immunoglobulins.**

After leaving the bone marrow, the development of **T lymphocytes** continues in the thymus, where they acquire essential membrane CD markers. Mature T lymphocytes leave the thymus and localize in secondary lymphoid organs. The surface molecules **CD4 and CD8**, together with CD3, are essential components associated with the T-cell antigen receptor.

A proportion of T and B lymphocytes differentiates into **memory cells** after antigen encounter. These cells are responsible for **immunological memory**, which enables a faster and more effective immune response upon repeated exposure to the same antigen (**secondary immune response**) compared to the response during the first encounter (**primary immune response**).

**NK cells** also develop from the lymphoid lineage but differ from T and B lymphocytes. They are large, non-phagocytic granular cells that destroy abnormal host cells, such as virus-infected cells or tumor cells.

### 4.2.3 Mononuclear and polymorphonuclear leukocytes

**Mononuclear cells** contain a single, non-lobulated nucleus in their cytoplasm. **Polymorphonuclear leukocytes** are characterized by a multilobed nucleus.

Mononuclear leukocytes include, from the **myeloid lineage**, **monocytes, macrophages, mast cells, and dendritic cells** (the latter may also originate from the lymphoid lineage). **NK cells, T lymphocytes, and B lymphocytes** belong to the mononuclear cells derived from the **lymphoid lineage**.

Polymorphonuclear leukocytes include **neutrophils, eosinophils, and basophils**, which originate from the **myeloid lineage** .

### 4.2.4 granular and agranular leukocytes

White blood cells can also be classified according to the presence of cytoplasmic granules. White blood cells that contain granules in their cytoplasm are referred to as

**granulocytes (granular leukocytes)**. From the **myeloid lineage**, these include **neutrophils, eosinophils, basophils, and mast cells**. From the **lymphoid lineage**, **NK cells** are classified as granular leukocytes.

White blood cells that lack cytoplasmic granules are referred to as **agranulocytes**. From the **myeloid lineage**, these include **monocytes, macrophages, and dendritic cells**. From the **lymphoid lineage**, **T lymphocytes and B lymphocytes** belong to the agranulocytes

### 4.3 Properties of cells of the immune system

#### 4.3.1 Neutrophils

Neutrophilic granulocytes (also referred to as **microphages**) constitute more than half of all leukocytes (approximately 60%). They belong to **polymorphonuclear leukocytes**. They possess a multilobed nucleus and cytoplasmic granules that do not stain with either basic or acidic dyes (Fig. 8). Healthy individuals have approximately 5,000 neutrophils per drop of blood.

Immature neutrophils (juvenile forms) have a rod-shaped nucleus, whereas mature neutrophils have a segmented nucleus. Two types of cytoplasmic granules containing antimicrobial substances are present:

- **Primary (azurophilic) granules** contain hydrolases and myeloperoxidase.
- **Secondary granules** contain lactoferrin, lysozyme, alkaline phosphatase, collagenase, and other substances.

Neutrophils are among the most active leukocytes with a highly developed capacity for phagocytosis and are classified as **professional phagocytes**. They represent the basic cellular component of the **first line of defense** against pathogens and foreign cells.

Neutrophils are highly effective immune cells (“workhorses” of the immune system) and play a key role in defense against infection by attacking bacteria. They are crucial in the inflammatory process because they are rapidly mobilized to the site of inflammation and are the first cells to arrive at sites of tissue damage. During inflammation, molecules appear on vascular endothelium that allow neutrophils to firmly adhere and migrate through the vessel wall by **diapedesis**. They then migrate toward the source of chemotactic signals.

Substances released from neutrophil granules have strong bactericidal effects and serve to destroy engulfed particles. Neutrophils phagocytose pathogens but **do not act as antigen-presenting cells**.

Neutrophils have a very short lifespan, less than one day, and lack mitochondria. They use cytoplasmic glycogen as an energy source, providing glucose for anaerobic glycolysis. This enables neutrophils to function effectively even at sites of bacterial infection in damaged tissue with low oxygen levels. Neutrophils are highly active in bacterial killing. An increased number of neutrophils in peripheral blood is often an indicator of acute infection. During infectious diseases, the proportion of juvenile neutrophil forms in circulation increases.

### 4.3.2 Eosinophils

Eosinophilic granulocytes belong to **polymorphonuclear leukocytes**. They have a multilobed nucleus and cytoplasmic granules that stain red with acidic dyes. They constitute approximately 4% of leukocytes. Healthy individuals have several hundred eosinophils per drop of blood.

Eosinophils contain approximately 200 cytoplasmic granules that include specialized proteins such as **major basic protein**, **eosinophilic cationic protein**, **eosinophil peroxidase**, and others that exert toxic effects on parasites (especially helminths) and tumor cells.

Eosinophils have only limited phagocytic capacity. However, after activation, active metabolites are released from their granules, and leukotrienes, prostaglandins, and cytokines are secreted, whereby they are the damaged surface of the parasite. The number of eosinophils is increased during parasitic infections, which represents one of the laboratory diagnostic indicators. Eosinophils express receptors for **immunoglobulin IgE** and for **complement components** on their cell membrane. They play an important role in **allergic reactions** as well as in **chronic inflammation**.

### 4.3.3 Basophils

Basophilic granulocytes belong to **polymorphonuclear leukocytes**. They have a bilobed nucleus and cytoplasmic granules that stain dark blue with basic dyes. Basophils constitute less than **0.2% of leukocytes**. They circulate in the blood and are morphologically similar to mast cells.

The cytoplasmic granules of basophils contain large amounts of **inflammatory mediators**, including **histamine, heparin**, and others. Basophils do **not contain peroxidase**.

They are activated primarily during **allergic reactions**, in which inflammation is a common component. **Histamine** causes vasodilation and contributes to the development of **urticaria (hives)**, which is frequently present during allergic reactions.

Receptors for **immunoglobulin IgE** are present on the cell membrane. **Basophils do not phagocytose**.

### 4.3.4 Mast Cells

Mast cells (also known as **mastocytes**) belong to **mononuclear leukocytes**. They possess a single, non-lobulated, relatively small nucleus and contain a large number of **metachromatically staining granules** in their cytoplasm. Mast cells are **tissue-resident cells**. They are found in connective tissue, particularly near **blood and lymphatic vessels** and **peripheral nerves**.

They are mainly localized in close proximity to epithelial surfaces of the **respiratory tract**, **gastrointestinal tract**, and **skin**, which are exposed to allergens from the external environment. Morphologically, mast cells resemble basophils that circulate in the blood.

Their granules contain numerous **inflammatory mediators**, such as **heparin** and **histamine**, among others. Mast cells **do not contain peroxidase**.

Mast cells are the most important cells involved in allergic reactions. Their cell membrane contains receptors for **immunoglobulin IgE (FcεRI)**. During the first contact with an allergen, the individual becomes sensitized, and allergen-specific **IgE antibodies** are produced. These antibodies bind via their Fc fragment to mast cells (or basophils), which express specific IgE receptors (FcεRI).

Upon subsequent exposure to the same antigen, the antigen binds to the IgE antibodies already attached to the mast cells (or basophils), which triggers **degranulation** and the release of mediators of the allergic reaction into the extracellular space. These mediators are of two types, **primary** and **secondary**:

- **Primary mediators** are pre-formed and stored in granules: histamine, proteoglycans (heparin, chondroitin sulfate), proteases, and some cytokines.
- **Secondary mediators** are synthesized de novo and include leukotrienes, prostaglandins, and platelet-activating factor (PAF).

The effects of these mediators include contraction of smooth muscle, bronchoconstriction, marked vasodilation, and increased vascular permeability with leakage of fluid into the extravascular space.

### 4.3.5 Monocytes

Monocytes are **mononuclear leukocytes** lacking cytoplasmic granules (**agranulocytes**). They account for approximately **6–8% of leukocytes**. Monocytes are characterized by strongly vacuolated cytoplasm that stains weakly basophilic gray-blue. They have a round

or irregular shape with a large eccentrically positioned nucleus, which typically has an irregular kidney-shaped appearance. Healthy individuals have approximately **500 monocytes per drop of blood**.

Monocytes are equipped with numerous receptors that enable them to recognize particles destined for phagocytosis and elimination. Mature monocytes migrate from the bone marrow into the bloodstream and, after about three days, leave the circulation by **diapedesis** and enter tissues. There, they differentiate into **tissue macrophages**, which can survive for several months (if not activated by inflammation), and into **dendritic cells**.

### 4.3.6 Macrophages

Macrophages are **mononuclear cells** with a strong capacity for **phagocytosis**. They do not contain cytoplasmic granules (they belong to **agranulocytes**). Macrophages are **professional phagocytes** and also function as **antigen-presenting cells (APCs)**. Their precursors are **monocytes**.

Tissue macrophages play an important role in the **first line of defense** against pathogens. **Fixed macrophages** are immobile and reside in connective tissues; when necessary, they can differentiate into **free macrophages**, which are capable of amoeboid movement and phagocytosis.

Macrophages are found in various tissues and are named according to their location. Most macrophages are located at strategic sites where invasion by microorganisms is likely or where accumulation of foreign particles can be expected. Macrophages were first described by **Metchnikoff in 1892**, based on their ability to phagocytose microorganisms.

Macrophages are referred to by different names depending on their tissue location:

- in **connective tissue** they are called **histiocytes**,
- in the **liver** they are **Kupffer cells**,
- in **bone** they are **osteoclasts**,
- in the **brain** they are **microglia**,
- in the **lungs** they are **alveolar macrophages**,
- in **serous cavities** they are **peritoneal and pleural macrophages**,
- in **submucosal tissue** they are part of **MALT (mucosa-associated lymphoid tissue)**,
- and others.

Macrophages have a long lifespan, surviving for several months unless activated by inflammation or tissue injury. In addition to phagocytosis, their functions include **antigen presentation to T lymphocytes**, regulation of inflammation, destruction of microorganisms, removal of dead cells ("housekeeping" of the body), destruction of tumor cells and cells infected with fungi and parasites, production of various signaling and antimicrobial proteins, promotion of wound healing, and tissue repair. During wound healing, macrophages first ingest and kill bacteria, then produce enzymes needed for degradation of damaged tissue, and finally synthesize growth factors required for tissue remodeling.

### 4.3.7 Dendritic Cells

**Dendritic cells (DCs)** are the most important **antigen-presenting cells**. They play a crucial role in initiating the **adaptive immune response**. They may originate from either the **myeloid or lymphoid lineage** and function as messengers between **innate and adaptive immunity**. Activated dendritic cells provide activation signals to **naive T lymphocytes**.

Dendritic cells are named for their characteristic appearance with numerous membrane projections ("dendrites"; *dendron* is the Greek word for tree). Their precursors are **monocytes**.

They are predominantly localized in **non-lymphoid peripheral tissues** at sites of contact with the external environment. They are found in tissues such as:

- the **skin** (where they are referred to as **Langerhans cells**),
- the **mucosa of the respiratory and gastrointestinal tracts** (**interstitial DCs**),
- **afferent lymphatic vessels** (**veiled DCs**),
- **peripheral blood circulation** (approximately **0.4%**),
- **secondary lymphoid tissues** (**interdigitating DCs**),
- the **medulla of the thymus** (**thymic DCs**).

Immature forms of dendritic cells are also present in the blood. Dendritic cells can be divided into **immature and mature** forms.

The result is increased expression of **costimulatory molecules** on dendritic cells, which interact with corresponding partner molecules on **Th lymphocytes**. These costimulatory interactions provide the **second signal** required for optimal activation of naïve Th lymphocytes into Th subpopulations capable of producing various cytokines and thereby regulating the immune response.

However, not all antigens enter cells through phagocytosis. Some antigens are bound on the surface of the target cell and are subsequently degraded by **proteasomes**; they are then presented in association with **HLA class I molecules** to **cytotoxic Tc lymphocytes**.

Mature dendritic cells therefore present antigens depending on whether the antigen is **extracellular** or **intracellular**. Extracellular antigens are processed via the **exogenous antigen presentation pathway**, in which antigens are presented on the dendritic cell surface in association with **HLA class II molecules**. Intracellular antigens—such as autologous, viral, or tumor antigens—are processed via the **endogenous antigen presentation pathway** and are presented by dendritic cells in association with **HLA class I molecules**.

Dendritic cells are also classified into **myeloid** and **plasmacytoid** dendritic cells. **Myeloid dendritic cells (mDCs)** are found in lymphoid organs and at sites with the highest likelihood of antigen entry, including the skin, respiratory tract, and gastrointestinal tract. Their primary biological function is antigen presentation and subsequent polarization of the immune response.

**Plasmacytoid dendritic cells (pDCs)** circulate in the blood and are also found in peripheral lymphoid organs. They produce large amounts of **interferons**, especially **interferon-α**, which leads to activation of immune cells, particularly **NK cells and macrophages**, and enhances phagocytosis. Interferons are known to play a crucial role in the development of **antiviral immune responses**.

Another group consists of **follicular dendritic cells (FDCs)**, which are named according to their localization within **lymphoid follicles**, where they form a microarchitecture (network). Although they possess long "dendritic" extensions, they are **not true dendritic cells**. They are **not of hematopoietic origin**, do **not express HLA class II molecules**, do **not phagocytose**, and do **not present antigen**.

Follicular dendritic cells bind antigens opsonized by **complement or antibodies** and highly concentrate these antigens on their surface, allowing recognition by **B lymphocytes**, particularly those generated through clonal expansion following primary antigen exposure. They also participate in the formation of **memory B cells**. **4.3.8**

**Lymphocytes**

Lymphocytes constitute **25–30% of all leukocytes**. They belong to **mononuclear cells** whose cytoplasm does not contain granules; therefore, they are classified as **agranulocytes**. They originate from the **lymphoid lineage** and are responsible for **specific immune responses**. They are found mainly in the **lymph** and in **all organs of the lymphatic system**.

The basis for their classification is the presence of **membrane markers** (CD markers, described below) and the **expression of antigen-recognition receptors** (TCR, BCR).

**T Lymphocytes**

T lymphocytes develop from a **pluripotent hematopoietic stem cell** in the **bone marrow** and migrate to the **thymus**, where they acquire their final **morphological and functional characteristics**.

They are responsible for **cell-mediated specific immunity (cellular immunity)**. The antigen receptors of T lymphocytes recognize **peptide fragments of protein antigens** that are bound to **HLA molecules** on the surface of **antigen-presenting cells (APCs)**. The T-lymphocyte receptor recognizes a small portion of the antigen that is associated with HLA on the surface of the APC.

Based on the expression of the **antigen receptor (TCR)**, T lymphocytes are divided into **two main populations**: those expressing the **TCRαβ receptor** and those expressing the **TCRγδ receptor**. T lymphocytes with the **TCRαβ receptor** predominate (**95%**) compared with T lymphocytes bearing the **TCRγδ receptor** (**5%**).

T lymphocytes expressing the TCRαβ receptor are further divided into **helper (Th)**, **cytotoxic (Tc)**, and **natural regulatory (nTreg)** lymphocytes. All of these cells express the **CD2 and CD3 membrane markers**. In addition, **helper T lymphocytes (Th)** are characterized by the **CD4 marker**, **cytotoxic T lymphocytes (Tc)** by the **CD8 marker**, and **regulatory nTreg lymphocytes** by the **CD25 marker**.

- **Helper T Lymphocytes (Th)** Helper Th lymphocytes assist **B lymphocytes in antibody production** and help **phagocytes destroy ingested microorganisms**.
- **Cytotoxic T Lymphocytes (Tc) -** Cytotoxic Tc lymphocytes **kill intracellularly surviving microorganisms**.
- **Natural Regulatory T Lymphocytes (nTreg) -** Natural regulatory nTreg lymphocytes represent another group of T lymphocytes. They **suppress immune responses** and participate in **maintaining tolerance to self-antigens** and in **preventing the development of autoimmune processes**. They regulate immune responses to **self-antigens** and to **harmless foreign antigens**. They produce the cytokine **IL-17**.

Data from studies conducted on **animal models** suggest that in different **microenvironments of secondary lymphoid tissues and organs**, specialized subsets of Treg cells with **distinct mechanisms of action** may occur.

**Activation and Differentiation of T Lymphocytes**

After activation by an antigen, T lymphocytes differentiate into **effector cells** and **memory cells**. For naïve T lymphocytes to be activated by an antigen, the antigen must be **presented to them by an activated antigen-presenting cell (APC)** that also expresses **costimulatory molecules**.

The **TCR receptor** recognizes the antigen in the form of a **peptide fragment bound to an HLA molecule** on the surface of the APC. The TCR can recognize an antigen **only when it is bound to HLA class I molecules** (endogenous antigen-presentation pathway) or **HLA class II molecules** (exogenous antigen-presentation pathway).

Activation of T lymphocytes occurs only if APCs simultaneously express the **costimulatory molecules required as a second signal** for the activation of naïve T lymphocytes.

**Effector and Memory T Lymphocytes**

Effector T lymphocytes **directly attack and eliminate virus-infected or tumor-transformed cells** and regulate the cooperation of various immune cells by synthesizing molecules that help other cells destroy pathogens.

Memory T lymphocytes **persist in the body for many years** and, upon repeated exposure to the antigen, ensure a **faster and more intense immune response**.

A characteristic feature of T lymphocytes is their **constant movement**. They leave the thymus and, via the bloodstream, enter **secondary lymphoid organs** (spleen, lymph nodes). From there, they migrate into tissues, return to the lymph nodes, and finally re-enter the bloodstream. This cycle lasts **approximately 24–48 hours**.

**B Lymphocytes**

B lymphocytes develop and mature in the **bone marrow**. Mature B lymphocytes carry **immunoglobulin molecules on their surface**, which function as **specific antigen receptors (BCR)**.

They are responsible for the **humoral component of specific immunity**. In the presence of an antigen—either **independently of T lymphocytes** (in the case of thymus-independent antigens) or **in cooperation with T lymphocytes** (in the case of thymus-dependent antigens)—B lymphocytes differentiate into **plasma cells**, which produce **large quantities of specific antibodies** (B lymphocytes themselves do not have the ability to produce antibodies).

A portion of activated B lymphocytes does not differentiate into plasma cells but remains in a relatively quiescent state in the body for a long time as **memory B lymphocytes**.

**Plasma Cells**

Plasma cells have an **eccentrically positioned nucleus** and a **large amount of cytoplasm** containing numerous **mitochondria**, an extensive **rough endoplasmic reticulum**, and a well-developed **Golgi apparatus**.

Plasma cells are **fully differentiated effector cells of the B-lymphocyte lineage**, specialized for the **efficient production of antibodies**. Their function is to produce antibodies with the **same specificity** as the original B lymphocyte from which they developed.

Plasma cells express **unique antigens** that are not present on their B-cell precursors. They **do not express surface immunoglobulin receptors** and carry the **CD38 membrane marker** on their surface. They survive for **only a few days**.

The antibody produced by a particular plasma cell (and its specific clone) may belong to **any immunoglobulin class** (IgM, IgG, IgA, IgE, IgD), but it will bind **only the antigen that induced its production**.

### 4.3.9 Natural Killer (NK) Cells

Natural killer (NK) cells, also referred to as **natural killers**, are **mononuclear cells** that contain **cytoplasmic granules**. They originate from the **lymphoid lineage** and are characterized as **non-B and non-T cells**, as they do not express **TCR or BCR**. They possess the ability to **non-specifically recognize and kill certain foreign cells**.

NK cells are components of **innate immunity**, specifically its **cellular arm**. They are capable of **rapidly attacking infected cells without prior sensitization**. NK cells play an important role in **antitumor and antiviral immunity** and participate in the **regulation of immune processes** through the production of numerous cytokines.

They are the **main effectors of antibody-dependent cellular cytotoxicity (ADCC)** and express **Fc receptors for antibodies**. The released cytokines **IL-2 and IFN-γ** increase the proliferation and cytotoxic activity of NK cells. Under the influence of **IL-2**, NK cells differentiate into a more aggressive population known as **LAK cells (lymphokine-activated killer cells)**.

**IFN-γ** activates macrophages, enhancing their ability to **phagocytose and destroy ingested microorganisms**. NK cells and macrophages represent an example of **effective cooperation between two immune cell types** in the elimination of intracellular pathogens. Macrophages engulf microorganisms and produce **IL-12**, which activates NK

cells to secrete **IFN-γ**; this cytokine subsequently activates macrophages to destroy the phagocytosed microorganisms

**NK cells and their effector functions depend on interactions with myeloid cells**, such as **macrophages** or **dendritic cells**. **IL-12** is released from these cells and stimulates NK cells, which subsequently begin to produce **IFN-γ**. This leads to a **positive feedback loop**, meaning that macrophages are stimulated to destroy ingested structures.

Interactions between NK cells and **target cells** (virus-infected, tumor, or stressed cells) and the decision whether to initiate the process of **cell death** depend on the **balance between activating and inhibitory receptors** on NK cells and their corresponding **ligands expressed on the surface of target cells**.

**NK cells play a key role in tumor rejection, immune surveillance, resistance to infections, and the regulation of immune responses.** The destruction of tumor cells and virus-infected cells first requires their recognition. NK cells then release the contents of their **cytoplasmic granules** into the extracellular space at the site of contact with the infected cell.

The released proteins, such as **perforin, granzymes, and serine esterases**, enter cancerous or infected cells and ultimately lead to their destruction. The NK cell is then released and can bind again to another cancerous or infected cell, allowing the entire process to be repeated.

### 4.3.10 NKT Cells

NKT cells represent a **small, distinct subpopulation of T lymphocytes** (approximately **0.01–1% in peripheral blood**). They mature in the **thymus** and are capable of recognizing **various types of antigens**.

They participate in the **modulation of immune responses**, as upon stimulation they can very rapidly produce large amounts of **cytokines**. NKT cells also have the ability to **lyse target cells** and, similarly to NK cells, they play a major role particularly in **antitumor and antimicrobial immunity**.

### 4.3.11 K Cells

K cells (**killer cells**) represent a **functional category of lymphocytes** that are capable of destroying a cell in association with an **antibody bound to its surface**. K cells participate in **antibody-dependent cellular cytotoxicity (ADCC)**. They are components of the **innate immune system**.

### 4.4 CD Markers of Immune System Cells

Cells of the immune system are characterized by the presence of **membrane markers** (glycoprotein antigens). These markers appear on the cell membrane during development and are referred to as **CD (Cluster of Designation) markers**.

A CD marker is a **unique molecule or molecular complex** expressed by a cell. These molecules help differentiate a cell's **type or subtype, stage of maturation, degree of activation**, and other characteristics.

The name consists of the letters **CD** followed by a number (e.g., CD1, CD2, etc.). Some CD markers are **specific to certain cell types**, which allows their use in the more precise identification of different cell populations. An overview of some of the most important CD markers and cell types is shown in table .

By **2017**, **417 CD markers** were officially recognized, with additional markers continuing to be identified. A current overview of officially recognized CD markers is available at: **http://www.hcdm.org**, in the section *"Molecule Information"*.

| | |
|---|---|
| CD2 | immature T-lymphocytes |
| CD3 | all T-lymphocytes (part of TCR) |
| CD4 | T helper lymphocytes |
| CD8 | T cytotoxic lymphocytes |
| CD14 | Monocytes and macrophages |
| CD15 | Neutrophils and eosinophils |
| CD16 | NK cells, neutrophils |
| CD19 | B-lymphocytes |
| CD38 | Plasma cells |
| CD56 | NK cells |
| CD58 | Endotelial cells, antigen-presenting cells |
| CD68 | Dendritic cells, macrophages |
| CD80 a 86 | Antigen-presenting cells |
| CD203 | Basophils, mast cells |

### 4.5 Molecules of the Immune System

Immune reactions are mediated not only by numerous cells but also by various **molecules and their mutual interactions**. The basic molecules of the immune system include **receptors located on cell surfaces**, **MHC class I and class II molecules**, various **adhesion and costimulatory molecules**, **immunoglobulins**, **cytokines**, individual **components of the complement system**, and **receptors for microbial components**.

These molecules may be located on the **surface of host cells** or may occur in a **soluble form**. Further details about the molecules of the immune system are described in the individual chapters.

**QUESTIONS:**

**Which leukocytes are present in increased numbers during parasitic infections?**

**Which leukocytes belong to the most active phagocytes?**

**In which organ does further development of T lymphocytes take place?**

**In which organ does further development of B lymphocytes take place?**

**How would you characterize NK cells?**

**To which cell type do Kupffer cells in the liver belong?**

**What is a CD marker?**

**What is the difference between monocytes and macrophages?**

# CHAPTER 5

## ANTIGENS AND RECEPTORS

## DISCRIMINATION OF SELF AND NON-SELF

### 5.1 Antigens

Antigens are substances that are **recognized by the immune system and elicit an immune response**. Antigens may be organisms, molecules, or parts of molecules that can be recognized by the immune system. They may be **simple or complex**, including **proteins, carbohydrates, or synthetic substances**.

The smallest identifiable part of an antigen that can be recognized by receptors is called an **epitope**, also known as an **antigenic determinant**. It is the portion of the antigen **against which the immune response is directed**.

The immune system is stimulated to mount an immune response through **interactions between receptors and ligands**. A receptor may be located on the **surface of cells** (surface receptors) or may be present in a **soluble form** (e.g., products of leukocytes). Ligands may be found on the **surface of cells** (surface molecules of microorganisms) or may exist as **soluble molecules** (cell products).

For effective cooperation, the **shape and charge** of both the receptor and the ligand are important, as these affect the **efficiency and strength of binding**, known as **binding affinity**. Binding affinity represents the strength of interaction between **one binding site and one antigen**. The collective affinity is referred to as **avidity**, which depends both on the strength of individual binding sites and on the **number of binding interactions occurring simultaneously**, meaning it reflects the strength of multiple bonds.

The conditions under which a cell receives a signal can influence how the cell responds. A cell usually must integrate information from **multiple activated receptors**. Some of these signals may be **positive**, while others may be **negative**. Therefore, the cell must first integrate these signals and then decide **which activity will be initiated**.

Groups of ligands that can be recognized by immune system cells are referred to as **antigens**. Antigens may be **any chemical structures**, provided that they are recognized by the immune system as **macromolecules**, whether soluble or bound to the cell surface. The most important antigens are **protein antigens**, followed by **polysaccharide**, **lipid**, and **lipoprotein** antigens.

### 5.1.1 Classification of Antigens

Antigens can be classified according to several criteria.

**Classification by Origin:**

- **Natural antigens** – occur in nature and are components of various microorganisms
- **Synthetic antigens** – artificially prepared, with known structure

**Classification by Relationship to the Host:**

- **Exogenous antigens** – derived from the external environment
- **Endogenous antigens** – (autoantigens, autologous antigens) are components of the body's own cells and tissues (e.g., nuclear and cytoplasmic antigens)

**Classification by Solubility:**

- **Particulate (insoluble) antigens**
- **Colloidal (soluble) antigens**

✓ **Particulate antigens** include bacteria, viruses, yeasts, latex particles, erythrocytes, etc.
✓ **Colloidal antigens** include bacterial toxins, enzymes, microbial extracts, etc.

**Classification Relevant to Tissue and Organ Transplantation**

Based on the genetic relationship between donor and recipient, antigens are classified as:

- **Syngeneic antigens** – derived from a donor genetically identical to the recipient (e.g., identical twins)
- **Allogeneic antigens** – derived from a genetically non-identical donor of the same species (e.g., between two different humans)
- **Xenogeneic antigens** – derived from an individual of a different species (animal → human)
- **Autologous antigens** – derived from the same individual (transfer of tissue or cells within the same person)

**Classification by Mechanism of Immune Response Induction:**

- **T-independent antigens** – these antigens can directly stimulate **B lymphocytes to produce antibodies** without the assistance of T lymphocytes (e.g., polysaccharides and lipopolysaccharides)
- **T-dependent antigens** – these antigens stimulate **B lymphocytes to produce antibodies only with T-lymphocyte help**, following prior processing by **antigen-presenting cells (APCs)**
- **Superantigens** – foreign substances, usually of **exogenous origin**, though they may also be endogenous. They are capable of activating **large numbers of lymphocytes независимо of antigen specificity**. They nonspecifically stimulate many clones of T or B lymphocytes and **do not require processing by APCs** (e.g., staphylococcal enterotoxin, toxic shock syndrome toxin, streptococcal pyrogenic exotoxin, staphylococcal exfoliative toxins).

The consequences of superantigen activity include **massive cytokine production**, **vascular leakage**, and **systemic toxicity**, leading to **shock and death due to multiorgan failure**.

- **Allergens** – foreign substances from the external environment (**exoantigens**) capable of inducing a **pathological immune response** in sensitive individuals. Allergens primarily stimulate the production of **IgE antibodies**, leading to the development of **allergic reactions**.
  Common allergens include proteins or glycoproteins such as **pollen grains**, **animal dander**, **insect allergens**, **mites**, and **food allergens**.

### 5.1.2 Epitope (Antigenic Determinant) – the Basic Unit of Recognition

The smallest parts of an antigen that can be recognized by receptors on **T and B lymphocytes** and are capable of binding to these receptors are called **antigenic determinants** or **epitopes**.

Some receptors (e.g., **BCRs**) are able to recognize epitopes that may be parts of **free soluble molecules**, **surface molecules**, or **degraded proteolytic fragments of antigens**. Other receptors (e.g., **TCRs**) can recognize only epitopes that are bound to **small peptide fragments associated with specialized host cell surface molecules**, which present them to T cells.

Based on the characteristic immune response they induce, antigens are divided into **three types**: **immunogens**, **haptens**, and **tolerogens**.

### Immunogens

**Immunogens** contain epitopes that **induce an immune response**. This property is referred to as **immunogenicity**. Antigens that elicit an immune response are called immunogens and represent **complete functional antigens**.

They are characterized by two fundamental properties:

- **Immunogenicity** – the ability to induce an immune response
- **Antigenicity** – specificity of recognition

Not all antigens are immunogenic. Good immunogens are typically **protein molecules** (most often pure proteins, glycoproteins, or lipoproteins), while **polysaccharides** are less effective. **Lipids and nucleic acids** are very weak immunogens and usually become immunogenic only after binding to proteins.

### Haptens

Very small antigens, known as **haptens**, are **unable to induce an immune response on their own**. They are **incomplete antigens**. Their basic properties are **lack of immunogenicity** but retention of **antigenicity**.

Only after binding to another, larger immunogenic molecule—known as a **carrier**—do haptens become immunogenic and gain the ability to induce an immune response. In such cases, the **carrier** is responsible for immunogenicity, while the **hapten** determines the antigenic specificity of the immune response.

Although haptens are not immunogenic by themselves, they are capable of **reacting with antibodies**.

### Tolerogens

Antigens that **induce tolerance rather than an immune response** are referred to as **tolerogens**. During the development of the immune repertoire (i.e., the complete set of epitopes for which immune receptors have been generated), tolerance to **self cells and molecules**—so-called **natural (innate) tolerance**—is established first.

Throughout life, tolerance is also induced to certain antigens (e.g., **orally administered antigens**). This specific lack of immune responsiveness (**non-reactivity**) to a tolerogen results from its **chemical structure**, which allows it to be tolerated by the immune system.

If the chemical structure of a tolerogen changes, it may be transformed into an **immunogen** capable of inducing an immune response. Whether an antigen acts as a

tolerogen or an immunogen also depends on the **dose**, **route of administration**, **use of adjuvants**, as well as **genetic factors and age**.

Tolerogens induce **specific immune unresponsiveness**. Under healthy conditions, such immune non-reactivity to self antigens is **physiological**.

**Immunogenicity**

**Immunogenicity** is the ability of a substance to induce an immune response. Several factors influence immunogenicity:

- **Size** – although no precise size threshold exists, larger molecules are generally more immunogenic. Proteins larger than **10 kDa** are typically immunogenic.
- **Chemical composition** – more complex substances are usually more immunogenic. Proteins are excellent immunogens; purified polysaccharides and lipopolysaccharides are also immunogenic. Steroids, lipids, and nucleic acids are weaker immunogens and usually require binding to proteins. Individual amino acids and haptens are non-immunogenic.
- **Conformation** – antigenic determinants (epitopes) must be **accessible and recognizable** by the immune system.
- **Complexity** – complex proteins containing multiple different epitopes are more likely to induce an immune response than simple proteins with few or only one epitope.
- **Physical form** – particulate antigens are generally more immunogenic than soluble antigens. Denatured antigens are more immunogenic than native antigens.
- **Degradability** – antigens that are easily phagocytosed are usually more immunogenic, as immune responses to most antigens require **phagocytosis, processing, and presentation to T helper lymphocytes (Th cells)** via **antigen-presenting cells (APCs)**.
- **Foreignness** – the immune system distinguishes **non-self (foreign)** from **self**.

Immunogenicity is also influenced by **biological factors** such as **age, hormones, and genetic background**, as well as the **route of antigen administration**. The **dose of the immunogen**, **method of delivery**, and presence of **adjuvants** can significantly enhance the immune response.

### 5.2 Receptors

The immune system is stimulated to mount an immune response through **interactions between receptors and ligands**. Depending on the ligand and the receptor—according to the type of molecule or cell to which the receptor binds—**different cellular activities** are initiated.

Some receptors bind molecules and generate **signals for the cell**, which then produces signals used to communicate with other cells. Other receptors **monitor the environment** and capture components from the surroundings in order to detect the presence of invaders. Still other receptors **monitor the immediate cellular neighborhood**, checking whether neighboring cells belong to **self** and whether they are potentially dangerous.

#### 5.2.1 Preformed Receptors

**Preformed (ready-made) receptors** are part of the **innate immune system**. They enable a **rapid response**, providing protection during the time required for the mechanisms of **adaptive immunity** to develop.

**PREFORMED RECEPTORS INCLUDE:**

**PRRs – Pattern Recognition Receptors**

**PRRs (Pattern Recognition Receptors)** are receptors of the **innate immune system**. They are found **on the surface of host cells** or in a **soluble form** (e.g., complement proteins, which will be discussed later).

PRRs recognize a wide range of **molecular patterns present on microorganisms but not on host cells**. These receptors detect so-called **PAMPs (Pathogen-Associated Molecular Patterns)**—molecular patterns characteristic of pathogens.

PAMPs consist of various combinations of **carbohydrates, proteins, lipids, and nucleic acids** associated with microorganisms. The binding of PRRs to PAMPs subsequently triggers **various inflammatory responses** aimed at **eliminating the pathogen**.

**TLR – Toll-Like Receptors**

**TLRs** are **preformed receptors in humans**. They are also **pattern recognition receptors** (and therefore belong to **PRRs**). TLRs are located on **host cells**. After binding **PAMPs of microorganisms**, they trigger immune responses such as **macrophage activation** and **secretion of inflammatory cytokines**, which attract immune cells to the site of infection and promote inflammation.

**KAR – Killer Activating Receptors**

**KARs (Killer Activating Receptors)** are receptors located on **NK cells**. They are capable of detecting changes in host cells that have been infected, primarily by viruses, or that have been altered by **carcinogenic processes**.

KARs can recognize the presence of **stress-induced molecules** (so-called **MICA and MICB molecules**) on human host cells. These stress molecules are expressed on host cells that are **not healthy**. Binding of KARs to stress molecules leads to the **attachment of NK cells to the target host cell** (e.g., virus-infected or cancer-transformed) and to its **subsequent destruction**.

**KIR – Killer Inhibitory Receptors**

**KIRs (Killer Inhibitory Receptors)** are receptors located on **NK cells**. These receptors monitor **MHC class I molecules**, which are normally expressed on the surface of **all nucleated cells** in the human body. In this way, NK cells assess whether host cells are **normal**.

When host cells are infected with viruses or undergo malignant transformation, the **expression of MHC class I molecules on their surface is reduced**. If a target host cell binds to an NK cell via **KARs**, the NK cell then uses its **KIRs** to evaluate the level of **MHC class I expression** on the target cell.

If the NK cell determines that the level of MHC class I molecules is **below normal**, it proceeds with the **killing of the target cell**. However, if the expression level is **normal**, the cytotoxic process is terminated, and the target cell is **released intact without damage**.

**CR – Complement Receptors**

The **complement system** is a complex of approximately **30 serum glycoproteins** that form one of the fundamental effector systems of **innate (nonspecific) immunity**. Its individual components are activated in a **cascade**, and the final result of this activation is the **lysis of the target cell** (destruction of the pathogen).

Complement components bind to microorganisms. **Complement receptors (CRs)**, which are located on the surface of **phagocytic cells and B cells**, recognize these complement fragments bound to microbes and facilitate the **elimination of the opsonized microorganism**.

**FcR – Receptors for the Fc Fragment of Immunoglobulins**

**Fc receptors (FcRs)** are located on the surfaces of **phagocytic cells**. Immunoglobulins **IgA, IgM, and IgG** are able to bind epitopes through their **Fab fragments**, which leads to a **conformational change in the Fc fragment** of the immunoglobulin.

Fc receptors on phagocytic cells recognize this conformational change and bind to the **Fc fragment of the immunoglobulin** (which already has an epitope bound to its Fab fragment). The result is **phagocytosis**.

If an immunoglobulin does not have an epitope bound, it does not bind to FcRs and remains in circulation. An exception is **Fc receptors that bind IgE**, which can bind IgE even before it encounters its epitope (IgE can bind via its Fc fragment to **mast cells**).

### 5.2.2 Somatically Generated Receptors

While **preformed receptors** are important in the mechanisms of **innate immunity**, **somatically generated receptors** are used by **adaptive immunity**. These receptors are generated **de novo** in each individual through **random chromosomal rearrangements and mutations**.

As a result, each individual develops a vast number of receptors with specific molecular features, creating **enormous diversity** in the range of specificities that an individual can recognize. Thus, there is **individual variability** in generated receptors among different individuals.

**BCR – Receptors on B Cells**

**BCRs (B Cell Receptors)** are receptors expressed on **B cells**. They are composed of an **immunoglobulin monomer**, which is linked by **disulfide bonds** to transmembrane heterodimers known as **Igα and Igβ**.

Upon binding of an epitope to the BCR, the **cytoplasmic portions of Igα and Igβ** initiate **intracellular signal transduction cascades**, leading to **B-cell activation**. Subsequently, some activated B cells differentiate into **plasma cells**, which synthesize and secrete **immunoglobulins of the same specificity** as their BCR.

Thus, immunoglobulin monomers function as **epitope-specific receptors** on B cells (BCRs).

### TCR – Receptors on T Cells

**TCRs (T Cell Receptors)** are receptors expressed on **T cells**. Structurally, they are **similar to immunoglobulin molecules**. They are **heterodimers** composed of **α/β or γ/δ chain pairs**.

TCRs are always **membrane-bound** and are associated with the **CD3 complex**. TCRs recognize epitopes **in combination with MHC molecules**, with the cooperation of the **CD4 or CD8 coreceptors**, which function as **cofactors** in antigen recognition.

## 5.3 Discrimination of Self and Non-Self

The fundamental function of the immune system is to **acquire information from the external and internal environment**, process it, **distinguish between self and non-self**, and ultimately generate an **appropriate immune response**. The human body is constantly exposed to various harmful influences, particularly numerous **infectious or toxic agents** originating from the external environment, as well as threats arising from the internal environment (e.g. **aged, damaged, or tumor-transformed cells**).

The human immune system must be able to distinguish **foreign (non-self) molecules**, which are potentially harmful, from **self molecules**, which are harmless. To recognize harmful molecules, the systems of **innate and adaptive immunity** use receptors that may be located either on **cell surfaces** or present in a **soluble form**.

An important feature of the immune system is **immunological memory**—the ability to remember prior contact with a specific antigen for varying periods of time, sometimes even for a lifetime. Under normal conditions, the immune system **tolerates and does not react** to healthy self cells and tissues. However, it recognizes **foreign cells**, **damaged self cells**, **self cells infected by microorganisms**, as well as **tumor-altered cells**. It induces an appropriate immune response aimed at destroying what is foreign or damaged in the body and preventing the further spread of foreign structures.

The immune system closely cooperates with the **nervous and endocrine systems**, forming an integrated **neuro-endocrine-immune information system**.

### Recognition of Self

The ability to recognize what is **self** allows human cells to determine whether other cells they encounter belong to the same individual and **do not pose a threat**. These self structures are normally **absent from microorganisms**, may be missing on **damaged self cells**, and may also be absent on cells of **other individuals of the same species**, such as in the case of transplantation.

**Recognition of the Absence of Self**

If a self cell becomes **virus-infected** or **tumor-transformed**, the immune system uses receptors to determine that the cell does not possess a sufficient number of **surface molecules** normally present. In nucleated cells, infection or malignant transformation leads to a **decrease in the expression of MHC class I molecules**.

The immune system, particularly **NK cells**, ensures the destruction of these abnormal cells on which the **absence or reduced presence of self structures** has been recognized (evaluation is based on the presence of **MHC class I molecules**).

**Recognition of Non-Self**

Recognition of foreign structures is enabled by the presence of a wide variety of receptors of both **innate and adaptive immunity**.

The **innate immune system** uses **preformed PRR receptors**, which recognize molecules present on microbial cells but not on human cells. This represents a **genetically stable set of receptors** that is transmitted from generation to generation and is fundamentally identical in all individuals.

The **adaptive immune system** uses **somatically generated receptors (SGRs)** on **B and T lymphocytes**, which are created randomly and are **individual-specific** in each person. These receptors are **extremely diverse** and present in vast numbers. Each cell produces only **one type of receptor**, capable of recognizing **only one specific structure**. However, because the total number of cells undergoing receptor generation is enormous, the overall number of SGRs available to recognize diverse structures is also enormous (**more than $10^{10}$**).

**QUESTIONS:**

**By what other term is an epitope known?**

**Explain the concept of binding affinity.**

**Explain the concept of avidity.**

**Which receptors are part of innate immunity?**

**Which receptors are used by adaptive (acquired) immunity?**

**In what ways can foreign structures be recognized?**

# CHAPTER 6

## BASIC PHYSIOLOGICAL PRINCIPLES OF IMMUNE SYSTEM FUNCTIONING

### 6.1 Activation of Innate Immune Mechanisms

The individual components of the immune system are **interconnected and cooperate closely**. However, the **innate (nonspecific) immune mechanisms** are the first to become involved in the body's defensive responses. These mechanisms are primarily based on the **recognition of chemical structures originating from microorganisms (PAMPs)**.

After foreign structures are recognized, the **cellular mechanisms of innate immunity**—especially **phagocytes**—are activated, as well as the **humoral mechanisms of innate immunity**, particularly the **complement system**. The aim is to **eliminate the foreign microorganism**.

Some phagocytes also play an important role in the **activation of adaptive immune mechanisms**, as they serve as **antigen-presenting cells (APCs)** for T lymphocytes. In this context, **MHC molecules** are essential, as fragments of antigens (engulfed within APCs) are displayed on the APC surface in cooperation with MHC molecules, enabling recognition by **T lymphocytes**.

### 6.2 Activation of Adaptive Immune Mechanisms

The mechanisms of adaptive immunity utilize a **wide range of molecules**. Some of these are also used by innate immune mechanisms, while others are exclusive to adaptive immunity. Adaptive immune mechanisms are **antigen-specific**.

The body is pre-equipped with an enormous number of **T and B lymphocytes** (more than $\mathbf{10^{10}}$), each expressing **distinct antigen-specific surface receptors** (TCRs and BCRs). When an antigen enters the body, it will eventually encounter lymphocytes that possess **specific surface receptors** capable of binding it with sufficient affinity.

Under appropriate conditions—specifically in the presence of **helper (costimulatory) signals**—these lymphocytes undergo **clonal expansion**, giving rise to populations of cells with the same specificity. Corresponding **effector lymphocyte populations** are generated, which are then able to eliminate the antigen.

The immune system is thus prepared **anticipatorily**, in the sense that it can respond to virtually any antigen encountered during life by expanding antigen-specific lymphocyte clones. Critical to this process are **costimulatory signals** provided by other immune system cells, particularly certain innate immune cells.

When an antigen is recognized by adaptive immune cells (lymphocytes), this constitutes the **first signal**, but it is **insufficient for activation**. A **second signal** in the form of costimulation is also required. If only the first signal is present without the second, the lymphocyte will not be activated and may instead undergo **long-term functional suppression (anergy)** or even **apoptosis**.

The requirement for both signals is essential for immune regulation. It prevents overly easy and potentially dangerous lymphocyte activation, which would occur if the first signal alone were sufficient.

### 6.3 Cooperation Between Innate and Adaptive Immune Mechanisms

Under physiological conditions, immune responses occur through the **mutual cooperation and coordination** of both innate and adaptive immune mechanisms. Against some antigens, **innate immunity** is more effective; against others, **adaptive immunity** plays a dominant role. In some cases, **cell-mediated immunity** is predominant, whereas in others, **humoral immunity** is more important.

Against **extracellular bacteria**, the **humoral component of adaptive immunity**—B lymphocytes and helper Th lymphocytes—plays a major role, along with the **cellular component of innate immunity**, particularly **phagocytes**. Against **intracellular bacteria**, predominantly **cell-mediated immune responses** are employed.

Against **viruses**, **cytotoxic Tc lymphocytes** and **NK cells** are especially important, although other immune components also cooperate in viral elimination. **Fungal infections** are primarily controlled by **cellular mechanisms of innate immunity** and **humoral components of adaptive immunity**.

Against **intracellular parasites**, cell-mediated responses are more prominent, whereas **multicellular parasites** are mainly targeted by **humoral responses** in cooperation with innate immune mechanisms (phagocytes, complement, NK cells).

At all levels, numerous **regulatory mechanisms** are present to optimize the immune response and ensure elimination of the antigen. In some cases, however, the antigen is not eliminated; instead, **tolerance is induced** (e.g. to the body's own cells and molecules).

Thus, the immune system contributes to maintaining the **integrity of the organism** by ensuring tolerance toward self cells and molecules while retaining the ability to respond to dangerous stimuli. Immune reactions result from the cooperation of the **cellular and humoral components** of both innate and adaptive immunity.

This cooperation is itself regulated to ensure that the immune response is **proportionate to the degree of danger** posed by the antigen (e.g. a healthy self cell, a virus-infected self

cell, or a pathogenic microorganism). The course of immune responses also depends on whether the organism encounters a particular antigen **for the first time or repeatedly**.

Thanks to **immunological memory**—a hallmark of adaptive immunity—subsequent encounters with the same antigen result in **much faster and more intense immune responses** than during the initial exposure.

**QUESTIONS:**

**Which mechanisms are the first to be involved in the defense of the organism?**

**Which mechanisms are antigen-specific?**

**Which signals are required for lymphocyte activation?**

**Which component of immunity is involved in defense against extracellular bacteria?**

**Which component of immunity is involved in defense against intracellular bacteria?**

**Explain what enables immune mechanisms to be activated much faster and to proceed more intensely upon repeated contact with the same antigen.**

# CHAPTER 7

## INNATE IMMUNE MECHANISMS:

## RECOGNITION FUNCTIONS

### 7.1 Tools of Innate Nonspecific Immunity Within the Body

Innate immune mechanisms represent a **rapid means of defense against infection** once microorganisms have penetrated the **first line of defense**, namely mechanical, chemical, and biological barriers. They employ **genetically programmed receptors** capable of rapidly recognizing structures characteristic of microorganisms that **are not found in the host**.

If adaptive immunity is also engaged in the defense, the two systems **communicate and cooperate**. Innate immune mechanisms present in blood and tissues can be divided into **cellular components** (phagocytes, NK cells) and **humoral components** (complement system, interferons, etc.), and they involve the **recognition and rapid destruction of microbes**.

In this context, **elevated body temperature** and the **function of inflammation** should also be mentioned. During inflammation, cells and molecules are stimulated to **isolate and destroy infectious microorganisms** and to **activate repair processes** in damaged tissues.

### 7.2 Recognition Functions of Innate Immunity

To recognize pathogenic microorganisms, innate immunity uses **preformed (ready-made) receptors**. These receptors are referred to as **PRRs (pattern recognition receptors)**. They are located on the **surface of host cells** or exist in a **soluble form**.

The genes encoding PRRs are present in the genome and **do not require further modification**. PRRs recognize molecular patterns found **exclusively on pathogenic microorganisms (PAMPs)** and not on the body's own cells. Therefore, PRRs of innate immunity can **easily distinguish foreign from self structures**.

It has also been found that pattern recognition receptors can recognize **self molecules generated during pathological tissue damage**, such as **necrosis** (violent cell death). These molecules are referred to as **DAMPs (damage-associated molecular patterns)** or **alarmins**. They arise during cell death and activate inflammatory processes.

These include various **antimicrobial peptides**, **heat-shock molecules**, cytokines such as **IL-33, IL-1α**, and others. They attract inflammatory cells, activate cytokine synthesis,

and stimulate **antigen-presenting cells (APCs)**, allowing adaptive immune mechanisms to become involved through APC-mediated responses.

### 7.2.1 PAMPs – Pathogen-Associated Molecular Patterns

**PAMPs (Pathogen-Associated Molecular Patterns)** are molecular structures found **only on pathogenic microorganisms**, not on host cells and not on non-pathogenic microorganisms. They are components of structures essential for **microbial survival or pathogenicity** and are common across entire groups of pathogenic microorganisms.

PAMPs may consist of **carbohydrates, proteins, lipids, nucleic acids**, or combinations of these molecules. Important structures containing PAMPs include **lipopolysaccharides of Gram-negative bacterial cell walls** and **peptidoglycan of Gram-positive bacterial cell walls**.

Binding of a PRR (receptor) to a PAMP (ligand) leads to **immobilization of the microorganism**, its **phagocytosis and degradation**, **activation of macrophages**, **release of cytokines**, and the initiation of various forms of **inflammation** aimed at eliminating the pathogen.

### 7.2.2 PRRs – Pattern Recognition Receptors

Once PRRs recognize a pathogen-associated molecular pattern, they **immediately activate the cell**. In contrast, in adaptive immunity, cells with the appropriate receptor must first proliferate and form a clone. Therefore, innate immune mechanisms can respond to foreign microbial structures **almost immediately**.

During PAMP recognition, **multiple PRRs are typically involved simultaneously**, mediating phagocytosis and microbial destruction, as well as the production of inflammatory cytokines and chemokines. If some PRRs fail to function, **others can compensate**, reducing the vulnerability of the immune system.

This ability of immune components to substitute for one another is known as **redundancy**, an important property of the immune system.

#### Functional Groups of PRRs

From a functional perspective, PRRs are divided into several groups. The best-known are **Toll-like receptors (TLRs)**, which recognize a wide range of pathogenic microorganisms. Based on their ligands, they are currently classified into **TLR1 through TLR10**.

Upon PAMP recognition, intracellular signaling pathways are activated, including the activation of **nuclear factor NF-κB**, which enters the nucleus and activates genes encoding **antimicrobial factors, cytokines, chemokines, adhesion molecules, and costimulatory molecules**. These drive inflammation, leukocyte recruitment to infection sites, and the activation of innate and later adaptive immune responses.

**Opsonins and Opsonization**

A major group of PRRs includes molecules that function as **opsonins**. These bind to the surface of microorganisms and **label ("coat") them**, making them more attractive to phagocytes and the complement system. An example is **mannose-binding lectin (MBL)**, which activates the **lectin pathway of complement activation**.

Receptors for opsonins are found on phagocytes and enhance phagocytosis. The process of increasing the efficiency of phagocytosis of a foreign particle is known as **opsonization**.

**Endocytic and Scavenger Receptors**

Other PRRs include **endocytic receptors**, such as the **mannose receptor (MR)**, which recognizes carbohydrates rich in mannose residues—structures typical of microorganisms. MR is primarily expressed on **macrophages**. Binding leads to phagocytosis, and antigenic determinants are presented on the macrophage surface via **MHC class I or II molecules** to **T lymphocytes**, initiating adaptive immune responses.

Another example is **scavenger receptors**, which participate in the phagocytosis of bacteria and host cells undergoing **apoptosis** (programmed cell death). Apoptosis removes old or damaged cells without causing tissue damage or inflammation. Scavenger receptors, mainly expressed on macrophages, recognize modified low-density lipoproteins and apoptotic cell markers. These cells are engulfed and degraded **without inducing pro-inflammatory cytokine production**; instead, anti-inflammatory cytokines are produced.

**PRRs and Recognition of Altered Host Cells**

Some PRRs detect changes on **virus-infected or tumor-transformed host cells**, such as the appearance of **heat-shock proteins and stress-induced molecules (MICA and MICB)**. These signals alert the immune system to danger.

Stress molecules are recognized by **NK cell activating receptors (KARs)**, leading to destruction of the affected cell. Additionally, virus-infected nucleated host cells exhibit

**reduced expression of MHC class I molecules**, which is detected by **NK cell inhibitory receptors (KIRs)**, triggering the elimination of the abnormal host cell.

**QUESTIONS**

**Which mechanisms does innate immunity use to recognize pathogens?**

**What structures are recognized by PRRs?**

**Define PAMP.**

**Which pattern recognition receptors are the best known?**

**What role do scavenger receptors play?**

# CHAPTER 8

## INNATE IMMUNE MECHANISMS:

## HUMORAL COMPONENT

Like the adaptive immune system, the **innate immune system** consists of both **cellular and humoral components**. Among the most important humoral components of innate immunity is the **complement system**. Other components include **lysozyme, basic polypeptides, interferons, pro-inflammatory cytokines, acute-phase proteins**, and other soluble substances that influence **nonspecific immunity**.

### 8.1 Complement

The **complement system** is a complex composed of approximately **30 serum glycoproteins**. It represents one of the **fundamental effector mechanisms of innate immunity**. The synthesis of individual complement components occurs predominantly in the **liver**, and to a lesser extent in **mononuclear phagocytes, the spleen, and bone marrow**. Complement is present in **plasma**, where it functions as a **plasma protein system**.

The main components of the complement system are serum proteins designated **C1 through C9**. Individual complement components are activated in a **cascade**, through conversion from an inactive form into **active enzymes** that act on subsequent components of the cascade. Proper function of the complement system depends on **precise coordination of all its components**.

### Principles of Complement Activation

The activation of complement components involves the conversion of an inactive component into an **active proteolytic enzyme**, which cleaves the next component into **two fragments**:

- a **larger fragment (b)**
- a **smaller fragment (a)**

*(An exception is component **C2**, where the nomenclature is reversed.)*

The **larger "b" fragment** usually has proteolytic activity and remains bound to the **antigen surface**. The **smaller "a" fragment** has other biological functions. Small fragments such as **C3a, C4a, and C5a** act as **anaphylatoxins**, meaning they attract and activate various leukocytes and other cells at the site of infection, thereby aiding in the elimination of microorganisms.

**Complement receptors (CRs)** are present on the surface of **phagocytic cells and B lymphocytes**. These receptors recognize complement fragments bound to microbes and facilitate the **destruction of opsonized microorganisms**.

A **horizontal line above a complement component** indicates its **enzymatic activity**. Some complement components are not numbered but are designated as **factors**, such as **factor B, D, H, I**, and **P (properdin)**. Fragments that have lost biological activity are indicated by the prefix **"i"** (e.g. **iC3b**). Some components are named according to their function, such as **MASP** and **MBL**.

**Central Role of C3**

The **central component of the complement system** is the glycoprotein **C3**, whose fragments exhibit numerous biological effects. Its fragment **C3b** is the **most important opsonin** of the complement system and binds to **microbial surfaces**.

Complement component **C3**, as well as components **C5–C9**, participates in **all complement activation pathways**. The **final outcome** of complement activation is the formation of the **membrane attack complex (MAC)** and **lysis of the target cell**.

### 8.1.1 Classical Pathway of Complement Activation

The main components of the **classical complement activation pathway** are glycoproteins **C1–C9**. They are numbered in the order in which they were discovered and, fortunately, they also participate in the complement activation cascade in the same numerical order (with one exception, namely **component C4**). The classical pathway begins with the **activation of C1** .

**Binding of C1 to the Antigen–Antibody Complex Initiates the Classical Pathway**

When an antibody binds to an antigen, a **conformational change** occurs in the immunoglobulin molecule, exposing the **binding site for the first complement component, C1**. This binding site is located in the **Fc region** of the immunoglobulin molecule. This process is also known as **complement fixation**.

Not all immunoglobulins are capable of binding C1. **IgM** is the most effective, followed by **IgG1, IgG2, and IgG3**, which are also able to bind C1 (**IgG4 cannot bind C1**).

**Structure of C1**

C1 consists of **three components: C1q, C1r, and C1s**. The entire C1 complex resembles a **bouquet of tulips**, with globular heads and six stalks formed by the **C1q component**, to which **two C1r and two C1s molecules** are attached, resembling a decorative ribbon.

After C1q binds, the conformation of C1r and C1s changes. Each C1q molecule has a "flower" at the end of its stalk, which binds to the Fc region of IgM or IgG immunoglobulin molecules.

For activation of the entire C1 component, at least two "flowers" of the C1q fragment must bind to Fc binding sites. This means that, in the case of IgG molecules, which have only one Fc region, at least two IgG molecules must be bound close together on the bacterial surface for C1 activation to occur **.**

In contrast, a single IgM molecule, which contains five Fc regions, is sufficient for C1 activation if it is bound to the bacterial surface.

After successful binding of C1q to these immunoglobulin molecules, a conformational change of C1r occurs, followed by C1s, which then become serine proteases. Through their proteolytic activity, they cleave subsequent components of the complement system. The substrates for the activated C1s fragment are complement components C4 and C2.

**Activated C1 Generates Products from C4 and C2**

Activation of **C1** leads to the **cascade activation of components C4 and C2**. Component **C4** is cleaved by fragment **C1s** into a small fragment **C4a** and a large fragment **C4b**. Component **C2** is cleaved by C1s into a **larger fragment C2a** and a **smaller fragment C2b**. Fragment **C2a binds to C4b**, forming the **classical C3 convertase**.

**C4b2a Is the C3 Convertase**

The **C3 convertase** cleaves inactive **C3** into fragments **C3a** and **C3b**. A single molecule of C3 convertase can generate **up to 200 molecules of C3b**.

Most **C3b fragments function as opsonins**, thereby enhancing **phagocytosis**. The ability of C3b to act as an opsonin represents **one of the most important functions of the complement system**. The importance of C3 is further demonstrated by the fact that individuals with **C3 deficiency** are more susceptible to **recurrent and potentially life-threatening bacterial infections**.

Some C3b fragments bind to **C4b2a**, forming the **classical C5 convertase**.

**C4b2a3b Is the C5 Convertase**

The **C5 convertase** cleaves inactive **C5** into fragments **C5a** and **C5b**. Cleavage of C5 represents the **final enzymatic step** of the complement cascade.

**Terminal Phase of the Complement Cascade**

This is followed by formation of a **C5b complex with additional complement components (C6, C7, and C8)**. The resulting **C5b678 complex** inserts into the membrane of the attacked cell, creating **small pores**, which may lead to lysis of some microorganisms.

However, most microorganisms are **resistant to the lytic activity** of the C5b678 complex alone and require the **final complement component C9**. Only after **C9 binds** do **large pores** form in the membrane of the attacked cell, resulting in the formation of the **membrane attack complex (MAC)**.

The pores formed by the MAC are large, allowing the **leakage of cytoplasmic contents**, ions, and small molecules, thereby disrupting **osmotic balance**. The attacked cell begins to **absorb water through these pores**, ultimately leading to **cell lysis and rupture**.

### 8.1.2 Lectin Pathway of Complement Activation

The **lectin pathway of complement activation** is very similar to the **classical pathway**, except for the initial steps of the pathway. A key role is played by the **mannose-binding protein (MBP)**, also known as **mannose-binding lectin (MBL)**. MBL binds to **carbohydrates** (e.g., mannose, fucose, glucose) present on the **surface of pathogens**, but not on the surface of **eukaryotic cells**; therefore, the body's own cells are not endangered.

MBL belongs to the **acute-phase proteins**, and its levels increase during inflammation. After MBL binds to the surface of a pathogen, **serine proteases known as MASPs (MBL-associated serine proteases)** bind to it, forming a complex analogous to the **C1qrs complex** of the classical pathway.

The **MBL–MASP complex** acts on **complement component C4**, which it cleaves into **C4b and C4a**, and on **component C2**, which it cleaves into **C2a and C2b**. The subsequent steps of the lectin pathway are **identical to those of the classical pathway**.

The significance of the lectin pathway lies in the fact that it can respond **immediately** and **does not require the formation of specific antibodies**, unlike the classical pathway.

### 8.1.3 Alternative Pathway of Complement Activation

Like the lectin pathway, the **alternative pathway** is activated **immediately** and **does not depend on the presence of specific antibodies**. Both pathways are activated primarily during the **early stages of infection**, when adaptive immunity has not yet been engaged in defense mechanisms.

The most notable difference compared with the classical and lectin pathways is that the **alternative pathway does not involve complement components C1, C4, or C2**.

However, **C3 and components C5–C9** are also part of this pathway, as they participate in **all complement activation pathways**.

Under normal conditions, **spontaneous slow activation of complement component C3** occurs in plasma, because C3 is highly labile and undergoes spontaneous hydrolysis into **C3a and C3b**. Most of the newly generated **C3b fragment** is hydrolyzed to an inactive form within a few seconds of its formation.

Some C3b fragments bind to **self cells**, but on these cells they are **inactivated by specific regulatory proteins**, which either cleave C3b directly or assist other proteolytic enzymes in degrading it. **Microorganisms**, however, **lack these regulatory proteins**, and therefore C3b bound to microbial membranes is **not inactivated**.

This system provides an elegant mechanism by which the **innate immune system protects self cells and distinguishes them from foreign ones**. Self cells are able to inactivate C3b fragments through regulatory proteins, so complement activation normally does not proceed. Microorganisms cannot do this because they lack regulatory proteins, and thus **complement activation via the alternative pathway continues**.

Another serum protein, **factor B**, binds to C3b and is then cleaved by the **serine protease factor D** into fragments **Ba and Bb**. Fragment **Bb** remains bound to C3b, forming the **C3bBb complex**, which functions as the **alternative C3 convertase** (the equivalent of the classical C3 convertase, C4b2a). The alternative C3 convertase is **stabilized by properdin (factor P)**.

Through a **positive feedback mechanism**, this pathway triggers a **self-amplifying cascade**, very efficiently cleaving additional C3 molecules into further C3b and C3a fragments. The **C3b fragment** thus serves two major functions:

- on one hand, it acts as an **opsonin**, playing a crucial role in the elimination of microorganisms by facilitating **phagocytosis**, particularly in the classical pathway;
- on the other hand, it participates in the formation of the **membrane attack complex (MAC)**, as it is also a component of the **alternative pathway C5 convertase (C3bBb3b)**, thereby initiating the **terminal lytic phase of the complement cascade**.

### 8.1.5 Regulation of the Complement System

Activation of the **complement cascade is tightly regulated**. Complement regulation is ensured by several mechanisms. Individual complement components are synthesized in an **inactive form**, and once activated they have a **very short half-life**.

On the surface of **host (self) cells**, there are **specific regulatory proteins** that allow complement components to be **inactivated**, thereby protecting host cells from damage.

The **classical pathway** is regulated at its very beginning by the **C1 inhibitor**, which blocks activation of the **C1 component**. Formation of the **alternative pathway C3 convertase** is inhibited by **factors H and I**, which cleave **C3b**, as well as by several other regulatory proteins that induce the **dissociation of the C3 convertase**.

The **terminal lytic phase** of the complement cascade is inhibited by the protein **CD59**, which binds between the final complement components and **prevents the formation of the membrane attack complex (MAC)**.

### 8.2 Microbicidal Molecules

Microbicidal molecules inhibit microbial growth. Examples include **RNase, DNase, defensins, cathelicidins** (skin), **β-defensins, α-defensins, cryptidins** (epithelium).

#### 8.2.1 Defensins

**Defensins** are natural **antimicrobial peptides**. They belong to **basic polypeptides**. In addition to their antimicrobial activity, they also possess **chemotactic** and **immunostimulatory properties**. They attract immune cells to sites of inflammation by chemotaxis and induce the synthesis of **cytokines and chemokines**.

It has been shown that, for example, the skin of individuals with **atopic dermatitis** has markedly reduced levels of defensins. In combination with impairment of the skin barrier, this leads to an **increased susceptibility to bacterial and viral skin infections** in atopic individuals.

#### 8.2.2 Lysozyme

Lysozyme is a protein with a very low molecular weight (14.6 kDa) composed of a single polypeptide chain consisting of 129 amino acids. Its internal stability is ensured by disulfide bonds. Lysozyme is an important hydrolytic enzyme with antibacterial activity, which destroys the cell wall of Gram-positive bacteria by cleaving the β-1,4-D-glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine in the murein layer of the bacterial cell wall.

Murein (peptidoglycan) is the basic structural component of the bacterial cell wall. Its polysaccharide chains consist of alternating residues of N-acetylglucosamine and N-acetylmuramic acid. Attached to the carboxyl group of muramic acid is a chain of four amino acids in the sequence: L-alanine, D-glutamic acid, a variable amino acid, and D-alanine. These chains are cross-linked. The action of lysozyme cleaves these bonds, resulting in lysis of the cell wall from the outside. Since the cell wall is essential for bacterial survival, its damage leads to functional failure.

Lysozyme was discovered in 1922 by Alexander Fleming. It occurs as a component of specialized cellular organelles—lysosomes—and is also found in leukocyte granules.

Lysozyme participates in degradative and microbicidal processes and kills many Gram-positive bacteria. However, pathogens may exhibit resistance to lysozyme.

In Gram-negative bacteria, the murein layer lies beneath an outer lipid membrane; therefore, lysozyme requires cooperation with the complement system to exert its effect. Complement disrupts the outer membrane by forming pores through the membrane attack complex (MAC). Lysozyme thus acts synergistically with complement and antibodies in bacterial lysis.

Lysozyme is present in relatively high concentrations in neutrophil granules, blood serum, and in most body secretions, including nasal mucus, saliva, tears, and breast milk, where it can reach high levels. In general, this nonspecific humoral factor is found in both invertebrates and vertebrates. It is also released from the tail of bacteriophages, causing perforation of the host cell and facilitating the release of newly formed bacteriophages.

A particularly high concentration of lysozyme is found in egg white. In infectious processes occurring in the oral cavity or pharynx, lysozyme may be used as a local antiseptic.

### 8.2.3 Interferons

Interferons have numerous immunoregulatory functions. They suppress viral replication in infected cells. They may inhibit viral replication directly within the cells in which they are produced, or they may be released from the cell and bind to receptors on other cells, which subsequently become resistant to viral infection by inducing the production of certain enzymes that suppress viral replication.

Interferons activate macrophages and NK cells, thereby enhancing innate immune responses.

Type I interferons include IFN-α and IFN-β. They are produced by many cell types, including macrophages, fibroblasts, lymphocytes, endothelial cells, and epithelial cells. Large amounts of type I interferons are produced by dendritic cells after contact with viruses.

Another interferon, IFN-γ, differs from type I interferons in both structure and function. IFN-γ is produced by activated Th1 lymphocytes and NK cells.

The production of interferons can be induced not only by viruses but also by other pathogens.

### 8.2.4 Cytokines and Chemokines

Cytokines are protein signaling molecules that transmit information between cells. They act in an autocrine manner—directly on the cell that produced them, paracrine manner—on nearby cells, or endocrine manner—after transport through the bloodstream to cells in distant tissues.

Cytokines are produced by cells of the immune system (such as lymphocytes, macrophages, and others) and regulate the activity of other cells. They influence inflammatory processes and play roles in both innate immunity and adaptive immunity. Cytokines exert their effects only on cells that express specific receptors for them.

In the body, cytokines are found in plasma, tissue fluid, or bound to cell membranes.

**Chemokines and Interleukins**

Chemokines are a subgroup of cytokines and have a low molecular weight. They exert a chemotactic effect and stimulate the movement of leukocytes.

Another subgroup of cytokines are interleukins, which have diverse biological effects. They influence one another, affect other cells of the immune system, as well as cells of other physiological systems. Their actions are complex, comprising not only primary effects but also secondary and regulatory effects.

Only a few interleukins are mentioned as examples. **Pro-inflammatory interleukins** include **IL-1, IL-6, IL-8, IL-12**, and **TNF**. For example:

- **IL-1** is responsible for the induction of **fever**.
- **IL-6** increases the production of **acute-phase proteins** in the liver.
- **IL-8** has strong **chemotactic activity for neutrophils** and contributes to **pus formation** at the site of inflammation.
- **IL-12** stimulates the production of **IFN-γ** in NK cells.
- **Tumor necrosis factor (TNF)** is capable of destroying tumor cells and, during infection and cancer progression, induces **cachexia and fever**.

### 8.2.5 Acute-Phase Proteins

Acute-phase proteins are proteins whose concentrations increase during the acute phase of inflammation in the body. For this reason, their measurement can be used for diagnostic purposes. They are predominantly produced in the liver, and their synthesis is increased under the influence of inflammatory cytokines (IL-1, IL-6, TNF-α).

Frequently measured acute-phase proteins include:

- **α-1-antitrypsin (A1AT)**
- **α-2-macroglobulin (A2M)**
- **Orosomucoid (orosomucoid, Orso)**

- **Ceruloplasmin (Cpl)**
- **Transferrin**
- **Fibrinogen**
- **C-reactive protein (CRP)**
- **Procalcitonin**

In healthy individuals, acute-phase proteins are present in serum at baseline (low) levels. At the onset of inflammation, the concentrations of some acute-phase proteins increase only 1.5- to 5-fold, whereas others increase 100- to 1,000-fold. Elevated concentrations lead to their accumulation at the site of inflammation, increased blood flow, and increased vascular permeability.

All acute-phase proteins have specific functions in the body. They directly participate in defensive responses, opsonize bacteria (e.g. CRP), regulate inflammatory responses by blocking proteases released from granulocytes—thus protecting tissues from excessive damage (e.g. α-1-antitrypsin). They may also function as ion transporters (transferrin, ceruloplasmin) and participate in blood coagulation (fibrinogen).

The most commonly measured acute-phase proteins are CRP, procalcitonin, and orosomucoid.

**C-Reactive Protein (CRP)**

CRP is a sensitive and highly dynamic marker of the acute phase of inflammation. Its kinetics are faster than those of the erythrocyte sedimentation rate (ESR). An increase in CRP levels within hours is an important indicator of early inflammation. CRP reflects the severity of inflammation, but as a nonspecific parameter, it does not determine the underlying cause of disease by itself.

CRP is named after its ability to bind polysaccharide C (phosphorylcholine) in the capsule of *Streptococcus pneumoniae*. It also binds to many other bacteria, yeasts, fungi, parasites, as well as to damaged and dying cells, thereby contributing to repair processes in damaged tissues. CRP assists phagocytes in recognizing bacteria and damaged cells.

CRP levels rise significantly in bacterial infections, in some non-infectious inflammatory conditions (e.g. myocardial infarction, postoperative states, and certain rheumatic diseases).

**Orosomucoid**

Orosomucoid, also known as α-1-acid glycoprotein, is synthesized mainly in the liver. Its concentration increases during inflammation with kinetics similar to CRP, but the onset is slower and the elevation persists longer.

**Procalcitonin**

Procalcitonin is a precursor of calcitonin, a hormone produced by the thyroid gland that regulates calcium levels. Under physiological conditions, procalcitonin is undetectable; its levels increase only during inflammation, particularly of bacterial or fungal origin.

In patients with suspected severe infections, procalcitonin levels are measured because they increase mainly in bacterial infections, but not in viral infections. For this reason, elevated procalcitonin levels are used to differentiate bacterial inflammation from inflammation of other etiologies.

**QUESTIONS:**

**Where does the synthesis of the individual components of the complement system take place?**

**What is the basic principle of complement component activation?**

**By which mechanism is the classical pathway of complement activation initiated?**

**How does the terminal phase of the complement cascade proceed?**

**Which components are not involved in the alternative pathway of complement activation?**

**Which components are part of all complement activation pathways?**

**What are cytokines, and how can they act on other cells?**

**Which acute-phase proteins are among the most frequently examined?**

# CHAPTER 9

# PHAGOCYTOSIS

In addition to the humoral component, the innate immune system also uses cellular components in the fight against infection. Among the most important are the elimination of microorganisms and abnormal body cells by phagocytes and NK cells.

### 9.1 Phagocytosis

Within the mechanisms of innate immunity, the most important barrier to the spread of microorganisms is their elimination through phagocytes. Moreover, phagocytes are important for cleaning processes in the body, as they remove cell debris and molecules that also arise during normal physiological processes.

Phagocytosis is the ability of professional phagocytes to recognize, engulf, kill, and degrade killed foreign material. Two types of phagocytes are classified as professional phagocytes:

- on the one hand, polymorphonuclear leukocytes (PMNLs) – also called microphages,
- on the other hand, macrophages and their precursors, monocytes. All of them develop from hematopoietic stem cells of the bone marrow.

A special type of phagocyte is the dendritic cell, which plays a role in the processing and presentation of antigens (they express MHC molecules). The function of phagocytes is, on the one hand, effector in nature – phagocytosis and elimination of microorganisms with the participation of bactericidal substances and hydrolytic enzymes. On the other hand, they also have a regulatory function – mediated by the production of cytokines, prostaglandins, leukotrienes, etc.

Microphages mainly include neutrophils and eosinophils. They circulate in the blood and are ready to rapidly move to the site of inflammation. The advantage of these granulocytes is that they are immediately prepared to perform their effector functions.

#### 9.1.1 Phagocytes

**Neutrophils**

Neutrophils have a very short lifespan, approximately 24 hours. They are the most numerous group of leukocytes in humans. They represent up to 60–70% of leukocytes (4.3–11 × $10^9$ per liter of blood), are very active, and play an important role in acute inflammation. Their advantage is rapid mobilization and the release of inflammatory mediators. Their main task is the phagocytosis and destruction of pathogens. They are the

basic cells of the first line of defense against pathogens and foreign cells. Because of these abilities, they are often referred to as “foot soldiers.” However, neutrophils do not belong to antigen-presenting cells (they do not express MHC class II molecules).

Inactive neutrophils remain in circulation for approximately 6–7 hours. After activation and migration into tissues, they survive for 1–2 days (pus contains live or dead neutrophils and killed bacteria). Immature young forms of neutrophils have a rod-shaped nucleus and have a lower phagocytic capacity than mature forms. Mature neutrophils have a segmented nucleus containing 4–5 segments. Approximately $10^{10}$ neutrophils mature daily. A portion of them adheres to the vascular endothelium; since this binding is reversible and not very strong, they slowly roll along it (“rolling”).

After activation by appropriate chemotactic signals, neutrophils leave the bloodstream and migrate to sites of infection or damaged tissue after trauma, where they engulf and destroy bacteria.

**Chapter: 9.1 Phagocytosis**

They come into contact with microorganisms earlier than macrophages. However, they are quickly followed by blood monocytes, which subsequently differentiate at the site of invasion into macrophages that phagocytose and kill bacteria. The adhesion of neutrophils is enabled by receptors on neutrophils and ligands on endothelial cells (Fig. 25). During this process, under the influence of inflammatory cytokines, adhesion molecules are expressed on the surface of the endothelium of inflamed tissues. Interaction between endothelial adhesion molecules (selectins) and oligosaccharides on the surface of neutrophils slows down rolling.

In the presence of inflammatory cytokines or chemokines, binding then occurs between neutrophil surface molecules – leukocyte integrins – and surface molecules of endothelial cells – ICAM-1 (intercellular adhesion molecule-1). This binding is already irreversible, and neutrophils firmly adhere to the endothelium of capillaries in the affected area. Under the influence of chemotactic substances (for neutrophils, the main chemotactic factor is interleukin-8) released at the site of damage, neutrophils pass between endothelial cells of blood vessels. This process is called diapedesis (extravasation), and their subsequent movement is guided by chemotactic substances released at the site of tissue damage (inflammation).

In the cytoplasm of neutrophils, primary and secondary granules are present:

- **Primary (azurophilic) granules** are larger and contain myeloperoxidase, hydrolases, elastase, lysozyme, cathepsin G, and other substances.
- **Secondary (specific) granules** are smaller and contain lactoferrin, lysozyme, alkaline phosphatase, collagenase, and others.

Neutrophils release enzymes from their granules into the extracellular space, which enables them to actively move to the site of inflammation. If they fail to encounter bacteria within an appropriate time, they gradually die. If a larger amount of enzymes is released into the extracellular space, tissue liquefaction and the death of some neutrophils occur, resulting in the formation of pus. Neutrophils that have fulfilled their function also die and are removed by other phagocytes (especially macrophages). A large number of dead neutrophils is a component of pus.

**Eosinophils**

Eosinophils represent 1–3% of leukocytes. Their cytoplasm contains a large number of small acidophilic granules. Eosinophils express receptors for complement components (C3b) and receptors for IgE antibodies on their surface. They persist in circulation for 8–12 hours and in tissues for 8–12 days. Eosinophils phagocytose only very weakly. For eosinophils (as well as monocytes), it is characteristic that they adhere to the vascular wall via a different type of adhesion molecules than neutrophils, namely β1 integrins, whose endothelial partner molecule is VCAM-1 (vascular cell adhesion molecule-1).

After activation, however, they produce large amounts of metabolites. They produce leukotrienes, prostaglandins, and a number of cytokines. They play an important role in allergic reactions, in defense against parasitic infections caused by multicellular worms (especially helminths in the gastrointestinal tract), and in defense against tumor cells. Eosinophilia is present in parasitic diseases, allergies, and in some types of bone marrow and lymph node tumors.

**Monocytes**

Monocytes are activated shortly after neutrophils. At the site of microbial invasion in tissues, monocytes differentiate into macrophages that phagocytose bacteria. Monocytes represent 5–10% of leukocytes. They are large mononuclear cells with a large eccentrically located kidney-shaped nucleus and vacuolated cytoplasm. They circulate in the bloodstream for approximately 1–3 days. They then adhere to the vascular endothelium and leave the bloodstream by the process of diapedesis. Monocytes adhere to the vascular wall via β1 integrins, with VCAM-1 as their partner molecule on the endothelium. After migrating into tissues, monocytes transform into macrophages (Fig. 26). Monocytes mature into different types of macrophages depending on their anatomical location.

**Macrophages**

Macrophages are large mononuclear cells derived from monocytes after their transition from the bloodstream into tissues. Based on their anatomical location, tissue macrophages have different names. For example, alveolar macrophages are found in the lungs; Kupffer cells in the liver; histiocytes in connective tissue; microglia in the central nervous system; osteoclasts in bones; Langerhans cells in the skin; peritoneal and

pleural macrophages in the serous cavities; and macrophages in the submucosal tissue are part of MALT.

Tissue macrophages are important in the first line of host defense. They are professional phagocytes and are also classified as antigen-presenting cells. They express MHC class II molecules. Macrophages express receptors for complement components (C3b) and Fc receptors on their surface. They are capable of repeated phagocytosis and can ingest more than 100 bacteria during their lifetime. Macrophages are therefore also referred to as “large eaters.” They are able to recognize, engulf, process, and present antigens to T cells.

They can be activated, for example, by the complement fragment C3b, interferon IFN-γ, or endotoxins of Gram-negative bacteria, which subsequently increases their phagocytic capacity. In addition, they produce various immunoregulatory substances, signaling molecules, and antimicrobial proteins. Some of these molecules participate in triggering specific immune responses, resulting in a specific cellular immune response. Macrophages thus represent a link between nonspecific and specific immunity.

### 9.1.2 The Process of Phagocytosis

Phagocytosis is the process of engulfment, killing, and degradation of microorganisms (foreign material). It is a fundamental mechanism of innate immunity. It begins when a phagocytic cell binds to a cell or molecule via receptors (various PRRs) on the surface of phagocytes. Chemotaxis precedes the actual process of phagocytosis.

#### Chemotaxis

During chemotaxis, phagocytes migrate to the site of inflammation or tissue damage. They are attracted to this site by molecules called chemotaxins. Among the most effective chemotaxins are certain complement components, leukotrienes, interleukin IL-8, and especially bacterial components. Phagocytes move toward the inflammatory site along a chemotactic gradient. The main chemotaxin for neutrophils is interleukin IL-8. For monocytes and eosinophils, the main chemotaxins are MIP-1α, MIP-1β, MCP-1, and RANTES. Chemotaxins common to all phagocytes include complement components C3a and C5a, leukotriene LTB4, PAF (platelet-activating factor), and fMLP (formyl-methionyl-leucyl-phenylalanine), a peptide derived from bacterial proteins. Phagocytes express specific receptors for all of these chemotaxins on their surface. Stimulation of these receptors leads to chemotaxis and subsequent activation of phagocytosis.

**Adherence**

Adherence is the attachment of foreign material to the surface of a phagocyte. Phagocytes express PRR receptors on their surface that recognize foreign structures present on the surface of microorganisms, known as PAMPs. In this case, phagocytosis is independent of opsonins.

**Opsonization**

Another way by which phagocytes recognize and phagocytose foreign particles is opsonization. In this case, phagocytosis is opsonin-dependent. Opsonization is the "coating" of particles to make them more attractive to phagocytes. Opsonins include, for example, the complement fragment C3b, which adheres to microbial surfaces and is recognized by complement receptors (CR) on phagocytes. Important opsonins also include immunoglobulins IgG (especially IgG1 and IgG3), which bind to microbial surfaces and are recognized by Fc receptors. Other significant opsonins are MBL (mannose-binding lectin) and acute-phase proteins. Since phagocytes are able to eliminate not only pathogens but also the body's own apoptotic or damaged cells, they can recognize them by unusual structures on their surface. These structures (modified lipoproteins) are normally located on the inner side of the cell membrane but become exposed on the surface of apoptotic cells and are recognized by scavenger receptors. Apoptotic or damaged cells can also be recognized after opsonization with antibodies or complement components.

**Ingestion**

After recognition and attachment of a foreign particle, engulfment occurs – the process of ingestion. This involves remodeling of the membrane and cytoskeleton, dependent on cellular actin and myosin. The phagocyte extends projections called pseudopodia, which surround the foreign particle and internalize it via endocytosis. Dendritic cells also use another mechanism known as macropinocytosis, during which inward-directed "pits" form in the plasma membrane. These pits engulf extracellular fluid containing a large number of soluble molecules and enclose it within a cytoplasmic vesicle.

After ingestion, the phagocyte becomes highly active, increases in size, and enhances the production of certain molecules that contribute to the destruction of engulfed particles or act as chemotactic factors. Interleukins IL-1 and IL-6, TNF, MIP (macrophage inflammatory protein), and IFN-α and IFN-β act as endogenous pyrogens and lead to an increase in body temperature. Vascular permeability increases, allowing molecules and cells to pass from blood vessels into tissues. Additional leukocytes are attracted and activated.

**Phagosome, Phagolysosome, and Destruction of Engulfed Material**

Once the particle is completely enclosed, it is contained within a vacuole called a phagosome. The phagosome then fuses with a lysosome to form a phagolysosome. Bactericidal substances and numerous hydrolytic enzymes from lysosomes attack and destroy the engulfed particles. Optimal activity of hydrolases is supported by the acidic environment (pH 4–5) within phagolysosomes. In addition to the acidic environment and acidic hydrolases, lysosomes employ other mechanisms to destroy and degrade engulfed material.

Following particle uptake, phagocytes undergo an oxidative burst, during which reactive oxygen species with bactericidal activity are produced (superoxide radical, hydrogen peroxide, hydroxyl radicals, hypochlorite ion). This entire process is catalyzed by NADPH oxidase and myeloperoxidase (unlike neutrophils, macrophages do not synthesize myeloperoxidase but bind it from their surroundings). Deficiency or absence of certain enzymes leads to functional impairments or defects in the ability to eliminate bacteria.

Another very important microbicidal mechanism of phagocytes is nitric oxide (NO), which is produced following activation of the enzyme system nitric oxide synthase. NO synthase is activated in macrophages under the influence of cytokines produced by Th1 lymphocytes (interferon-γ, TNF). The NADPH oxidase system is important for the elimination of engulfed extracellular pathogens, while the NO system is crucial for the elimination of intracellular pathogens.

**Elimination of Waste Material**

After destruction of the engulfed material, indigestible or poorly digestible residues remain in residual bodies and are subsequently expelled as waste material.

## 9.2 NK Cells in the System of Innate Immunity

NK cells originating from the lymphoid lineage are specialized in recognizing and destroying altered host cells. They are capable of rapidly killing certain tumor-transformed and virus-infected cells. They are large granular cells that contain perforins and granzymes in their granules, which are the main cytotoxic tools of NK cells. NK cells play an important role in defense against intracellular viral, bacterial, and parasitic infections (for example, individuals with NK cell deficiency show increased susceptibility to herpesviruses).

NK cells enter sites of inflammation, where they can be stimulated by the cytokine IL-12 produced by activated macrophages. They begin to produce interferon IFN-γ, which in turn is a very strong activator of macrophages. In this way, activation of NK cells and macrophages is mutually enhanced (this is one example of effective cooperation between two cell types in eliminating pathogens or altered host cells). NK cells are also

activated by type I interferons (IFN-α and IFN-β), which are produced by cells during viral infection. By producing numerous cytokines, NK cells also participate in the regulation of immune processes; through TNF-α and IFN-γ they influence the differentiation of Th cells into the Th1 subpopulation, which leads to the development of a predominantly cellular type of immune response (stimulation of macrophages, which increase their ability to phagocytose pathogens as well as debris from damaged tissues).

### 9.2.1 NK Cell Receptors

For NK cells to function in the innate immune system, they are equipped with surface receptors.

**Activating Receptors – KAR**

Activating receptors (KARs) are able to recognize the presence of stress molecules MICA and MICB on host cells. These stress molecules are expressed on cells that are not healthy. Binding of KAR receptors to these stress molecules leads to the attachment of NK cells to the target cell and its subsequent destruction (Fig. 28).

**Inhibitory Receptors – KIR**

Before the actual destruction of a marked target cell, inhibitory receptors (KIRs) also come into play. These receptors monitor MHC class I molecules, which are normally present on all nucleated cells of the human body. If KIRs detect a low level of MHC class I expression, the NK cell proceeds with killing the target cell. However, if the level of MHC class I expression is normal, the signal from activating KAR receptors is blocked (the inhibitory KIR signal prevails), and the target cell is not killed but is released from the NK cell and remains viable (Fig. 28).

**Antibody Receptors – Fc**

NK cells are also important effectors of antibody-dependent cellular cytotoxicity (ADCC). They possess antibody receptors (Fc receptors) on their surface, which are capable of binding the Fc portion of antibodies. When an NK cell encounters a pathogen that has been opsonized by antibodies, it binds to it via the Fc receptor and activates cytotoxic mechanisms that lead to the destruction of the infected cell. It should be noted that ADCC is not the same as phagocytosis, where pathogens are engulfed and killed inside phagocytes. In ADCC, pathogens are too large to be engulfed and are killed extracellularly.

### 9.2.2 Destruction of Target Cells by NK Cells

The destruction of tumor cells or virus-infected cells (target cells) occurs after recognition by NK cells, which release the contents of their cytoplasmic granules into the

extracellular space at the site of contact with the target cell. Perforins create pores in the membrane of the target cell, while granzymes and other enzymes enter through these pores and disrupt the target cell's intracellular enzymes. The target cell is then destroyed by apoptosis and subsequently eliminated by macrophages.

**QUESTIONS**

**Define what phagocytosis is.**

**Which types of phagocytes are classified as professional phagocytes?**

**What is the term used for the process by which neutrophils pass between endothelial cells of blood vessels?**

**Which immune cells are important in acute inflammation, whose role is to phagocytose and kill pathogens, but which do not present antigens and, after fulfilling their function, die and become part of pus?**

**List the basic steps of phagocytosis.**

**What is the significance of NK cells in the body?**

# CHAPTER 10

# FEVER AND INFLAMMATION

The normal body temperature measured in the axilla is **37 °C**, while when measured rectally it is **37.5 °C**. Values above these are considered an elevated body temperature. If the temperature exceeds **38 °C in the axilla** or **38.5 °C in the rectum**, it is considered **fever**.

## 10.1 Fever

Fever represents a physiological response of the body, usually to the presence of an infectious agent, although non-infectious causes of fever also exist (e.g., tumors). On the other hand, there are situations in which even serious infections may occur without fever (for example in newborns, elderly people, etc.). Fever is a reaction of the body to various substances known as **pyrogens**.

### 10.1.1 Pyrogens

Pyrogens may originate from the external environment (**exogenous pyrogens**) or from within the body (**endogenous pyrogens**). The most common exogenous pyrogens are pathogens and their microbial products, such as components of bacterial cell walls, exotoxins, endotoxins, and antigen–antibody complexes. The action of these substances on immune cells leads to the release of various inflammatory mediators, which can act as endogenous pyrogens—primarily cytokines (IL-1, IL-6, TNF-α).

Endogenous pyrogens (IL-1) reach the thermoregulatory center in the brain via the circulation, where prostaglandin E2 production is increased and the temperature set point is shifted higher. This is followed by shivering and vasoconstriction in the skin, which increases heat production and reduces heat loss, ultimately resulting in elevated body temperature. Endogenous pyrogens (IL-6) also reach the liver, where they stimulate the production of acute-phase proteins.

### 10.1.2 Associated Effects of Fever

Negative accompanying effects of fever include tachycardia, tachypnea, increased metabolism and oxygen consumption, confusion or delirium, and in cases of high fever, febrile seizures, especially in small children. Positive effects of fever include mobilization of the immune response, faster reactions of immune cells, enhancement of phagocytosis, and intracellular destruction of pathogens. Fever is particularly important in viral infections, as viruses replicate less efficiently and are more easily inactivated at elevated body temperatures.

True fever must be distinguished from **hyperthermia**, which results from physical overheating of the body (overheating, heat stroke) and can only be influenced by physical cooling methods.

## 10.2 Inflammation

Inflammation is a reaction of the body to chemical, mechanical, or infectious damage and involves both innate and acquired immune mechanisms. The inflammatory response may be local or systemic, acute or chronic. The basic signs of acute inflammation are pain (**dolor**), heat (**calor**), redness (**rubor**), swelling (**tumor**), and loss of function (**functio laesa**). The main goal of the inflammatory response is to attract cells and soluble molecules to the site of inflammation to help defend against pathogens and harmful agents.

If the organism cannot eliminate the harmful agent through acute inflammation, the process becomes chronic. Acute inflammation usually resolves without consequences and the affected tissue heals completely. In chronic inflammation, however, tissue destruction often occurs, frequently leading to scarring and permanent damage. The most important components of inflammation include inflammatory cells (especially neutrophils, macrophages, mast cells, and others), humoral components (complement, lysozyme, transferrin, acute-phase proteins, components of coagulation and the kinin system, etc.), and the local environment at the site of inflammation (low pH).

### 10.2.1 Inflammatory Response

During inflammation, four key processes occur:

- **Vasodilation** widens capillaries, leading to redness and increased temperature at the site of inflammation, and increases the influx of immune cells and molecules.
- **Increased vascular permeability** allows cells and molecules to pass more easily from the blood into inflamed tissues. Leakage of plasma fluid and leukocyte infiltration contribute to swelling.
- **Activation of endothelial cells** in affected capillaries leads to increased production of mediators that make capillaries more permeable. Under the influence of inflammatory cytokines, adhesion molecules are expressed on the surface of endothelial cells, allowing leukocytes to adhere.
- **Chemotactic substances** released at the site of damage cause leukocytes to pass between endothelial cells of capillaries into tissues. This process is called **diapedesis (extravasation)**, after which further movement is guided by chemotactic agents released at the inflamed site.

### 10.2.2 Inflammatory Cells

During inflammation, inflammatory cells—especially neutrophils and tissue macrophages—are activated. They phagocytose and destroy pathogens and produce numerous cytokines and inflammatory mediators that increase vascular permeability, activate endothelial cells, and perform many other functions. Mast cells, after

degranulation, release histamine, heparin, and proteolytic enzymes, contributing to vasodilation, increased vascular permeability, and chemotactic attraction of neutrophils.

Cells recruited to the inflammatory site produce enzymes that may also damage healthy cells. If large amounts of enzymes are released into the extracellular space, tissue liquefaction occurs and some neutrophils die. Neutrophils that have fulfilled their function also die and are removed by other phagocytes, mainly macrophages. Dead cells and liquefied tissue fluid constitute pus.

Microbial antigens are captured at the site of inflammation by antigen-presenting cells and transported to regional lymph nodes, where they stimulate T and B lymphocytes. Effector T-cell subsets subsequently stimulate macrophages, increasing phagocytosis and cytotoxic activity, thereby helping eliminate microorganisms and tissue debris. Plasma cells derived from B lymphocytes contribute to opsonization and complement activation. Thus, both innate and adaptive immune mechanisms participate in inflammation.

### 10.2.3 Humoral Components of the Inflammatory Response

Mediators released from phagocytes stimulate local nerve endings and cause pain. Some mediators act as endogenous pyrogens and contribute to increased body temperature. The coagulation system is activated; fibrinogen is important not only in repair processes but also in preventing pathogen spread and attracting phagocytes. Bradykinin causes pain, vasodilation, and increased vascular permeability. Complement components are also important due to their opsonizing, chemotactic, degranulating, and lytic effects.

In extensive inflammation, systemic responses may occur. Massive entry of microorganisms into the bloodstream can result in septic shock, with extensive mediator release causing severe vasodilation, hypotension, and circulatory failure. Fever is a characteristic systemic manifestation of inflammation, caused by stimulation of the thermoregulatory center by prostaglandin E2. Cytokine stimulation of hepatocytes leads to production of acute-phase proteins, which assist in pathogen elimination, for example by acting as opsonins. During major inflammation, cytokines stimulate increased leukocyte production in the bone marrow, leading to **leukocytosis**.

### 10.2.3 Environment at the Site of Inflammation

The pH at the site of inflammation decreases due to accumulation of lactic acid produced by inflammatory cells. Low pH has antimicrobial effects. The partial pressure of oxygen initially increases due to improved blood supply but later decreases because of swelling and necrosis, influencing microbial growth.

### 10.2.4 Repair Processes

Finally, repair processes occur, involving a wide range of cytokines, enzymes, and hormones. Damaged phagocytes are removed, and fibroblastic mechanisms,

regeneration, and tissue remodeling are activated. In chronic inflammation, however, fibrotic processes prevail, often leading to tissue destruction, scarring, and permanent damage.

**QUESTIONS**

What are pyrogens?

What are the negative effects of fever?

What are the positive effects of fever?

Define inflammation.

What is the main goal of the inflammatory response?

Characterize the environment at the site of inflammation.

# CHAPTER 11

# ACQUIRED IMMUNE MECHANISMS: IMMUNOGLOBULIN MOLECULES

### 11.1 Basic Characteristics of Specific Immunity

Acquired specific (also called adaptive) immune mechanisms use some molecules that are common to both acquired and innate immunity. Some molecules, however, are characteristic only of acquired immunity processes (such as receptors on T and B cells). Specific and nonspecific immune mechanisms are intricately interconnected. The key cells of the specific immune system are T cells derived from the thymus and B cells derived from the bone marrow. Both immune mechanisms are linked by antigen-presenting cells and many molecular mediators.

### 11.2 Immunoglobulins

Specific immunity is directed against a particular agent, is not inherited, is acquired during life, and is unique for each individual. The main characteristics of specific immunity are **memory** and **specificity**. Thanks to immunological memory, the specific immune response during repeated contact with the same antigen is faster and more

intense than during the first encounter. Specific immunity allows very fine discrimination between antigens.

Like the innate immune system, acquired immunity has both a **humoral** and a **cellular** component. The humoral component of acquired immunity is represented by antibodies—**immunoglobulins**—which are produced by B cells. They are particularly effective in defense against pathogens present in the extracellular environment. The cellular component of acquired immunity (cell-mediated immunity) is represented by T cells and is especially important in defense against intracellular pathogens.

### 11.2 Immunoglobulins

Immunoglobulins (antibodies) are glycoproteins. They are produced by plasma cells, which arise from B lymphocytes after contact with an antigen. The produced immunoglobulins are then able to react specifically with the antigen that induced their formation. In addition to specific antigen binding, immunoglobulins participate in antigen neutralization. The binding of antigen to antibody triggers further reactions that lead to antigen elimination (e.g., opsonization, complement activation, macrophage activation).

#### 11.2.1 Structure of Immunoglobulins

The basic structure of an immunoglobulin consists of two identical long heavy (H) polypeptide chains and two identical short light (L) polypeptide chains, interconnected by disulfide bonds (Fig. 30). The immunoglobulin molecule has a Y-shaped structure. The hinge region of the immunoglobulin molecule is highly flexible and allows opening and closing of the antibody arms, enabling better binding to antigenic determinants on the antigen surface.

Light chains can be of the **kappa (κ)** or **lambda (λ)** type. Only one type (either κ or λ) is present in a single immunoglobulin molecule. Heavy chains can be of the **mu (μ)**, **gamma (γ)**, **alpha (α)**, **epsilon (ε)**, or **delta (δ)** type. Based on the type of heavy chain, five classes (isotypes) of immunoglobulins are distinguished: **IgM, IgG, IgA, IgE, and IgD**.

#### Immunoglobulin Domains

Both heavy and light chains are composed of smaller units called **domains**, each containing approximately 110 amino acids and stabilized by disulfide bonds. Heavy chains contain four to five domains, whereas light chains contain two domains. All chains contain **variable** and **constant** domains.

The domains located at the N-terminus of both heavy and light chains are called **variable domains** (VH and VL). These domains differ among immunoglobulins produced by different plasma cell clones. Together, the VH and VL domains form the **antigen-binding**

**site**. The remaining domains are constant. Constant domains of light chains are designated **CL**, and constant domains of heavy chains are designated **CH** (CH1, CH2, CH3, and sometimes CH4).

### Immunoglobulin Fragments

Immunoglobulin molecules can be proteolytically cleaved. Digestion with papain produces two identical **Fab fragments** and one **Fc fragment**. The Fab fragment contains the variable regions of both heavy and light chains and one constant domain from each chain, and it includes one antigen-binding site. The Fc fragment consists of the constant regions of the heavy chains only and does not bind antigen, but it binds to **Fc receptors (FcR)** on immune cells such as macrophages, neutrophils, mast cells, B lymphocytes, dendritic cells, and NK cells.

### Affinity and Avidity

The forces binding antigen and antibody are relatively weak (van der Waals, ionic, hydrogen, and hydrophobic interactions). The strength of binding between one epitope and one antigen-binding site is called **affinity**. **Avidity** refers to the overall binding strength between a multivalent antibody and a multivalent antigen. For example, **IgM**, which is a pentamer with ten binding sites, has very high avidity even if the affinity of individual binding sites is low. Antigen–antibody complexes are called **immune complexes**.

## 11.2.2 Classes of Immunoglobulins (Isotypes)

There are five immunoglobulin isotypes based on heavy chain type: **IgM, IgG, IgA, IgE, and IgD**. IgG has four subclasses (IgG1–IgG4), and IgA has two subclasses (IgA1 and IgA2). Immunoglobulins may occur in different structural forms, predominantly as monomers.

- **IgM** and **IgD** are present as monomers on the surface of B cells, forming the **B-cell receptor (BCR)**.
- In soluble form, **IgG** and **IgE** are monomers, **IgM** is a pentamer, and **IgA** occurs as a monomer or dimer.

### Immunoglobulin IgM

IgM is found either as a monomer on the surface of B cells or as a pentamer in soluble form. The pentamer consists of five monomers linked by disulfide bonds and a **J chain**.

IgM has ten antigen-binding sites and is the first antibody produced after antigen exposure. It is effective in antigen immobilization, opsonization, and activation of the complement system via the classical pathway. It plays a key role in the **primary immune response**.

**Immunoglobulin IgG**

IgG is the most abundant immunoglobulin in serum and exists as a monomer. It has four subclasses (IgG1–IgG4), with IgG1 being the most common. IgG activates the classical complement pathway, binds Fc receptors on phagocytes, efficiently opsonizes and neutralizes microorganisms, participates in ADCC, and crosses the placenta. Transplacental IgG provides **natural passive immunity** to the newborn and is important in the **secondary immune response**.

**Immunoglobulin IgA**

IgA occurs as a serum or mucosal immunoglobulin. Serum IgA is mainly monomeric or dimeric, while mucosal IgA is exclusively dimeric and present in secretions of the gastrointestinal and respiratory tracts, saliva, sweat, breast milk, and colostrum. Secretory IgA contains a **secretory component** that protects it from proteolytic degradation. IgA does not activate complement and provides mucosal defense by preventing microbial adhesion and neutralizing viruses.

**Immunoglobulin IgE**

IgE is present in very low concentrations in serum. It plays a key role in defense against multicellular parasites at mucosal surfaces and in **type I hypersensitivity (allergic) reactions**. IgE binds to Fc receptors on mast cells, basophils, eosinophils, and monocytes. Antigen binding triggers degranulation and release of biologically active mediators such as histamine, prostaglandins, leukotrienes, and cytokines, leading to allergic inflammation.

**Immunoglobulin IgD**

IgD is a monomer found in unstable form in serum. It is bound to the surface of B cells, where it functions as a receptor together with IgM. It is also found in saliva, tears, nasal mucus, and tonsils—sites frequently exposed to microorganisms.

### 11.2.3 Allotypes, Idiotopes, Idiotypes, and the Idiotypic Network

**Allotypes** are genetic variations in immunoglobulin constant regions that differ slightly between individuals of the same species and are inherited. **Idiotopes** are unique structures in the antigen-binding regions of immunoglobulins. The sum of idiotopes forms the **idiotype**. Antibodies against idiotypes are called **anti-idiotypic antibodies**, effectively antibodies against antibodies. Interactions

between antibodies and anti-idiotypic antibodies form an **idiotypic network**, which may regulate antibody responses.

**QUESTIONS**

What forms the basic structure of immunoglobulins?

Which immunoglobulin has the highest number of antigen-binding sites, and how many does it have?

What are the functions of IgM?

Which immunoglobulins cross the placenta?

Which immunoglobulin is found in mucosal secretions, tears, saliva, breast milk, and colostrum?

Explain the role of IgE.

Which immunoglobulins serve as B-cell receptors (BCRs) on B lymphocytes?

# CHAPTER 12

# MOLECULES OF THE MAJOR HISTOCOMPATIBILITY SYSTEM – MHC

### 12.1 MHC – Major Histocompatibility Complex

The major histocompatibility complex (MHC) is a set of genes located on the short arm of chromosome 6, in the region **6p21.31**. The genes in this region encode numerous enzymes and molecules required for the activation and function of T and B lymphocytes. In humans, this complex is also known as the **HLA complex (human leukocyte antigen complex)**. These molecules are primarily responsible for **antigen presentation**.

On the surface of MHC molecules there is a groove into which a peptide fragment of an antigen can bind. MHC molecules are unique for each individual. They are divided into **three classes: class I, class II, and class III**.

### 12.1.1 MHC Class I Molecules

MHC class I molecules are glycoproteins expressed on the surface of **all nucleated cells**. They consist of two protein chains. The longer chain, designated **α**, has three domains (α1, α2, α3). This chain is transmembrane and is non-covalently associated with **β2-microglobulin**, which is structurally similar to immunoglobulin domains and is located exclusively on the extracellular side of the membrane (it has neither a transmembrane nor an intracellular domain).

The α1 and α2 domains form the peptide-binding site, which resembles a groove (or cleft) on the surface of the MHC molecule; the sides of this groove are formed by the α1 and α2 domains. MHC class I molecules bind peptides **8–10 amino acids** in length.

In humans, three classical MHC class I molecules are distinguished: **HLA-A, HLA-B, and HLA-C**. These molecules are highly polymorphic, with more than 100 alleles at each locus. In addition, there are less important **non-classical MHC class I molecules** (e.g., HLA-E, HLA-F, HLA-G, HLA-H), which are expressed only on certain cell types and show limited variability. These may play a role in presenting carbohydrate, lipid, and peptide fragments.

### 12.1.2 MHC Class II Molecules

MHC class II molecules are glycoproteins that under normal conditions are expressed on **antigen-presenting cells**, such as dendritic cells, monocytes, macrophages, and B lymphocytes, and also on some activated T lymphocytes. They consist of **two transmembrane protein chains**.
The α chain has two domains (α1 and α2), and the β chain also has two domains (β1 and β2). The α1 and β1 domains form the peptide-binding groove, similarly to MHC class I molecules. MHC class II molecules bind peptides **15–35 amino acids** long.

In humans, three types of MHC class II molecules are distinguished: **HLA-DP, HLA-DQ, and HLA-DR**.

### 12.1.3 MHC Class III Molecules

MHC class III molecules are neither structurally nor functionally similar to MHC class I and II molecules. The genomic region encoding these molecules contains genes for certain complement components (**C2, C4, factor B**), genes for **TNF-α and TNF-β**, **heat shock proteins (Hsp70)**, and others.

### 12.2 Function of MHC Molecules

The fundamental function of MHC molecules is to bind peptide fragments and present them on the surface of cells so that they can be recognized by **T lymphocytes**. T lymphocytes possess antigen-specific, somatically generated receptors known as **T-cell receptors (TCRs)**.

#### 12.2.1 Antigen Processing and Presentation by MHC Molecules

Peptides that bind to MHC molecules must have a limited length (8–10 or 15–35 amino acids). However, antigens are much larger and may contain up to 1000 amino acids. Therefore, antigens must first be **processed into smaller peptides inside cells**. MHC molecules bound to peptides are then transported and expressed on the cell surface.

The process differs for MHC class I and class II molecules and also depends on whether the antigen originates **inside the cell (endogenous, intracellular)** or **outside the cell (exogenous, extracellular)**.

- **Endogenous antigens** originate within cells and are processed and presented by **MHC class I molecules**.
- **Exogenous antigens** originate outside the cell and are processed and presented by **MHC class II molecules**.

**Presentation of Endogenous Antigens by MHC Class I**

When a virus infects a cell, viral proteins are synthesized in the cytoplasm. Peptides derived from these proteins are presented by MHC class I molecules. Viral proteins are tagged with **ubiquitin** and degraded by **proteasomes** into peptide fragments 5–15 amino acids long (optimally 8–10 amino acids).

These peptides are transported into the endoplasmic reticulum by the peptide pump via **TAP-1 and TAP-2 transporters**. In the ER, the α chain and β2-microglobulin assemble, a peptide binds into the groove, and the complex is transported via the Golgi apparatus to the cell surface as **pMHC I**, where it can be recognized by **CD8+ T cells**.

MHC class I molecules do not distinguish between self and non-self peptides. Self peptides exposed on the surface are usually not recognized because most autoreactive CD8+ T cells are eliminated during thymic development.

Non-classical MHC class I molecules can present unusual antigens such as lipids or glycolipids to **NKT cells**.

**Presentation of Exogenous Antigens by MHC Class II**

MHC class II molecules present peptide fragments derived from extracellular antigens. These antigens enter antigen-presenting cells via **endocytosis** (phagocytosis or macropinocytosis). Inside endosomes, which fuse with lysosomes, proteins are degraded into peptides.

In the endoplasmic reticulum, α and β chains of MHC class II associate with an **invariant chain**, which blocks the peptide-binding groove. After transport through the Golgi, vesicles fuse with endolysosomes, the invariant chain is removed, and peptide fragments bind to MHC class II molecules. The resulting **pMHC II** complex is transported to the cell surface, where it can be recognized by **CD4+ T cells**.

Self peptides are usually not recognized because autoreactive CD4+ T cells are eliminated in the thymus.

**Cross-Presentation**

In certain cases, **exogenous antigens** are presented by **MHC class I molecules**. This process is called **cross-presentation**, and activation of CD8+ T cells in this way is termed **cross-activation**.

Cross-presentation may occur when exogenous antigens are transferred into the cytoplasm, degraded in proteasomes, and loaded onto MHC class I molecules in the ER, or when peptides generated in endolysosomes bind directly to MHC class I molecules.

**QUESTIONS**

Which molecules are distinguished in humans within **MHC class I**?

Which molecules are distinguished in humans within **MHC class II**?

What is the basic function of MHC molecules?

Which MHC molecules participate in the presentation of **endogenous antigens**?

Which MHC molecules participate in the presentation of **exogenous antigens**?

Explain what **cross-presentation** means.

# CHAPTER 13

# SPECIFIC IMMUNE RESPONSE: T AND B CELLS

## 13.1 Basic Characteristics of Cells of Specific Immunity

The specific immune response is carried out by **T and B lymphocytes**. Unlike cells of the innate immune system, lymphocytes cannot be distinguished morphologically because they appear similar. They can be differentiated only by their size, by the presence of antigen-specific receptors, and by the organ in which they develop. Small lymphocytes measure approximately **4–7 μm**, medium-sized **7–11 μm**, and large **11–15 μm**.

Lymphocytes express different **antigen-specific receptors** on their surface. These are **somatically generated receptors**, created by random gene recombination on each individual lymphocyte before encountering antigen. They are referred to as **TCR** on T lymphocytes and **BCR** on B lymphocytes. Because these receptors are generated randomly, some may react with self antigens. However, due to selection mechanisms, such cells are eliminated before becoming fully mature, functional, and capable of attacking the body's own tissues.

Lymphocyte development occurs in the **thymus** or **bone marrow**, i.e. in the **primary lymphoid organs**. From there, they migrate to **secondary lymphoid tissues and organs** (spleen, lymph nodes, mucosa-associated lymphoid tissue), where they further differentiate into mature forms capable of responding to antigen.

T lymphocytes are responsible for **cell-mediated immunity**, while B lymphocytes mediate the **antibody (humoral) immune response**, as after antigen binding they differentiate into plasma cells capable of producing antibodies. In addition to these two main lymphocyte types, there are **NK cells** (large granular lymphocytes without antigen-specific somatically generated receptors—no TCR or BCR) and **NKT cells**, which share some properties with NK cells and some with T lymphocytes.

## 13.2 T Lymphocytes

T lymphocytes arise from hematopoietic stem cells in the bone marrow. They are considered the most important players in most specific immune mechanisms. They participate directly in immune reactions and regulate the activity of other cells. They are essential for antigen-specific cell-mediated and antibody immune responses. They play a key role in eliminating cells infected with intracellular parasites and in regulating the differentiation of B lymphocytes into plasma cells.

### 13.2.1 Development of T Lymphocytes

T cells can develop along three possible pathways: **αβ T cells**, **γδ T cells**, or **NKT cells**. In the bone marrow, an immature T cell called a **prothymocyte** is formed. The prothymocyte migrates to the thymus and enters the thymic cortex, where it is called a **cortical thymocyte** and initially does not express surface molecules. Shortly thereafter, it rapidly acquires them and undergoes demanding selection processes. If successful, it migrates to the thymic medulla and becomes a **medullary thymocyte**.

Only about **1–5%** of all thymocytes successfully pass selection and become mature T cells that populate secondary lymphoid organs. The remaining **95–99%** either leave the thymus earlier (γδ T cells) or fail selection and undergo apoptosis (programmed cell death).

#### T-Cell Receptors

The antigen-specific T-cell receptor (**TCR**) is a somatically generated receptor (Fig. 35). It is a membrane-bound heterodimer composed of either **αβ** or **γδ** polypeptide chains and is associated with the **CD3 complex** and either **CD4 or CD8**. The CD3 complex consists of CD3δ, CD3γ, two CD3ε chains, and CD247 (ζ-ζ homodimer).

TCRs do not bind soluble epitopes. They recognize only peptide fragments presented in association with **MHC class I or class II molecules**. The variable regions of the αβ (or γδ) chains form unique specificities that bind the peptide–MHC (pMHC) complex. CD4 or CD8 molecules stabilize this interaction. Because the TCR has only a short cytoplasmic tail, signal transduction into the cell is mediated by the CD3 complex. It is estimated that **1–5 million different TCR specificities** can be generated.

#### Development of αβ T Lymphocytes

αβ T lymphocytes are the predominant T-cell population in humans. Prothymocytes migrate to the thymus, proliferate, and enter the cortex as **double-negative cells** ($CD4^-$ $CD8^-$, no TCR or CD3). They soon begin expressing TCR, CD3, CD4, and CD8, becoming **double-positive cells**.

As thymocytes migrate from cortex to medulla, selection occurs:

- **Positive selection**: thymocytes capable of recognizing self-MHC molecules survive. Cells that fail to recognize MHC undergo apoptosis.
- Following this, double-positive cells committing to MHC class I become **$CD8^+$**, while those recognizing MHC class II become **$CD4^+$** (**single-positive cells**).

Cells surviving positive selection then undergo **negative selection**. Thymocytes that strongly bind self peptides presented by APCs are potentially autoreactive and are eliminated by apoptosis. Some autoreactive cells differentiate into **regulatory T cells (Treg)**, which maintain self-tolerance.

Cells that pass both selections leave the thymus as mature T lymphocytes and populate secondary lymphoid organs.

### Development of γδ T Lymphocytes

γδ T lymphocytes are a minority T-cell population. During development, thymocytes begin expressing γδ TCR and CD3 but do not express CD4 or CD8 and do not undergo positive or negative selection. They leave the thymus shortly after TCR formation. They recognize antigens independently of pMHC, have a limited recognition repertoire, respond rapidly, but do not form immunological memory. They play roles in skin and mucosal immunity.

### Development of NKT Cells

NKT cells share features of NK cells and T lymphocytes. They develop in the thymus and express TCRs with a limited recognition repertoire. They primarily recognize lipids, glycolipids, and some peptides presented by **non-classical MHC class I molecules**.

## 13.3 B Lymphocytes

B lymphocytes arise from hematopoietic stem cells in the **bone marrow**, where they also develop. They mediate antibody immune responses and are precursors of immunoglobulin-producing plasma cells. B lymphocytes synthesize immunoglobulins that form the **BCR**.

### B-Cell Receptors

The B-cell receptor (**BCR**) is a somatically generated receptor composed of an immunoglobulin molecule with two identical heavy and two identical light chains. It is associated with transmembrane heterodimers **Igα and Igβ**, which mediate intracellular signal transduction.

Gene rearrangement for immunoglobulin chains occurs early in B-cell development, independent of antigen exposure. Each B cell produces an immunoglobulin of **single epitope specificity**, determined by random recombination of variable regions. It is estimated that **over 26 million** potential BCR specificities can theoretically arise.

### 13.3.1 Development of B Lymphocytes

B lymphocytes derive from two lineages: **B-1** and **B-2**.

**B-1 Lineage**

- Arise during fetal development
- Self-renewing population
- Found mainly in tissues exposed to microorganisms (respiratory tract, peritoneal cavity)
- T-cell-independent activation
- Limited epitope repertoire (mainly polysaccharides)
- Poor immunological memory and limited class switching
- Important in innate immunity and autoimmunity

**B-2 Lineage**

- Found in lymphoid organs throughout the body
- Arise peri- and postnatally
- Continuously replenished from bone marrow
- Require T-cell help for activation
- Recognize primarily proteins
- Large repertoire and strong immunological memory
- Faster, more effective responses upon re-exposure, including isotype switching

**Mature B Lymphocytes**

Mature B lymphocytes express both **IgM and IgD** on their surface (IgM predominates). Their great diversity results from gene segment recombination, junctional variability, and somatic mutations. Mature B cells migrate from bone marrow to peripheral lymphoid tissues.

Upon antigen encounter in follicles, B cells with the **highest-affinity BCRs** are selected for survival.

Autoreactive B cells are eliminated in bone marrow by apoptosis. Some may persist but usually remain inactive due to the requirement for T-cell help. Control of autoreactivity is stricter in T cells than in B cells.

**Differentiation into Plasma Cells**

Naive B lymphocytes express only IgM and IgD and cannot secrete antibodies. Upon antigen stimulation, they differentiate into **plasma cells**, which are highly metabolically active antibody-producing cells.

B-cell differentiation involves two specialized processes:

- **Affinity maturation** – somatic hypermutations increase antibody affinity.
- **Isotype (class) switching** – constant regions change while antigen specificity remains the same, resulting in IgG, IgA, or IgE.

Primary immune responses are dominated by **IgM**, while secondary responses are dominated by **IgG, IgA, or IgE**. These processes occur **only in B lymphocytes**, not in T lymphocytes.

**QUESTIONS**

Which cells ensure the specific immune response?

What are the somatically generated receptors on T and B lymphocytes called, and how are they formed?

Which type of TCR is the predominant type in T lymphocytes?

What characterizes double-negative T lymphocytes?

What characterizes double-positive T lymphocytes?

What is positive selection?

What is typical of single-positive T lymphocytes?

What is negative selection?

Describe the B-1 lineage of B lymphocytes.

Describe the B-2 lineage of B lymphocytes.

What characterizes mature B lymphocytes?

What does isotype switching mean?

# CHAPTER 14

# ACTIVATION OF T LYMPHOCYTES

## 14.1 Interaction of T Lymphocytes with Antigen-Presenting Cells

The relationship between an antigen-presenting cell (APC) and a T lymphocyte is called the **immunological synapse**. T-cell receptors (TCRs) cannot recognize soluble molecules. They are able to recognize only peptides bound to MHC molecules and presented by antigen-presenting cells. How does antigen presentation occur?

### 14.1.1 Antigen Presentation by MHC Class II Molecules

Antigens are taken up from the surrounding environment by APCs through **phagocytosis** or **macropinocytosis**. The most important APCs are **dendritic cells** located at sites where microorganisms are most likely to enter the body. During phagocytosis, the phagocyte first contacts a small part of the antigen via various surface receptors. **Clathrin-coated pits** form on dendritic cells, enclosing the phagocytosed antigen and generating a **phagosome**. During macropinocytosis, antigens present in extracellular fluid are surrounded by cytoplasmic extensions (pseudopodia), which enclose the antigen into a vesicle, forming an **endosome**. This process is clathrin-independent.

The phagosome fuses with a lysosome to form a **phagolysosome**, where enzymes degrade the engulfed antigen into smaller peptide fragments. When an immature dendritic cell detects danger in the form of antigen, it rapidly matures and migrates to regional lymph nodes. At the same time, it decreases its phagocytic and macropinocytic activity and increases synthesis of **MHC class II molecules** in the endoplasmic reticulum. The α and β chains associate with an **invariant chain**, which prevents binding of peptides destined for MHC class I. MHC II molecules then pass through the Golgi apparatus, and vesicles fuse with phagolysosomes. The invariant chain is cleaved, freeing the peptide-binding groove.

The resulting **pMHC II complex** moves to the cell surface, where it is presented for recognition by **CD4+ T lymphocytes**. MHC II molecules cannot distinguish self from non-self peptides, but self-peptides usually remain unrecognized because most autoreactive CD4+ T cells were eliminated during thymic development.

### 14.1.2 Antigen Presentation by MHC Class I Molecules

Not all antigens enter APCs via phagocytosis or macropinocytosis. Some antigens, such as intracellular microorganisms and viruses, enter directly into the cytoplasm. These

pathogens are tagged with **ubiquitin** and degraded by **proteasomes**—tubular structures that proteolytically cleave proteins into small peptide fragments. These peptides are transported by a peptide pump into the endoplasmic reticulum, where they bind into the peptide-binding groove of **MHC class I molecules**, formed by the α chain and β2-microglobulin.

The resulting **pMHC I complex** passes through the Golgi apparatus and is transported in vesicles to the cell surface, where it can be recognized by **CD8+ T lymphocytes**. MHC I molecules also do not distinguish self from non-self peptides, but self pMHC I complexes are usually ignored because autoreactive CD8+ T cells are largely eliminated during thymic development.

### 14.1.3 Immunological Synapse

After antigen processing, APCs display various peptide fragments as **pMHC complexes** on their surface. If a TCR on a T lymphocyte recognizes such a complex, T-cell activation occurs. Recognition involves cooperation between TCR and co-receptors **CD4** (binding pMHC II) or **CD8** (binding pMHC I). A memory aid is the "rule of eight":

- CD4 with pMHC II (4 × 2 = 8)
- CD8 with pMHC I (8 × 1 = 8)

These co-receptors stabilize the otherwise weak TCR-pMHC interaction. The complex **TCR:pMHC:CD4 or CD8** constitutes the **first signal** of the immunological synapse, transmitted into the T cell via the **CD3 complex**. CD3 contains intracellular immunoreceptor tyrosine-based activation motifs (ITAMs) that initiate signal transduction.

However, a **second signal** is required for full activation: costimulatory interactions between APCs and T cells, such as **CD80/86–CD28**, **ICAM-1–LFA-1**, and **LFA-3–CD2**. Without costimulation, T lymphocytes become anergic or undergo apoptosis.

## 14.2 Proliferation and Differentiation of T Lymphocytes

Following activation, T lymphocytes undergo maturation. Their further development requires **interleukin-2 (IL-2)**, which acts as a growth factor.

### 14.2.1 Proliferation and Differentiation of CD4+ T Lymphocytes

After the initial antigen encounter (**priming**), CD4+ T lymphocytes differentiate into **T helper (Th) cells**, which assist other immune cells. Th precursors produce IL-2 and

express IL-2 receptors, becoming **Th0 cells**. Depending on cytokines in the microenvironment, Th0 cells differentiate into:

- **Th1 cells** (presence of IL-12)
- **Th2 cells** (presence of IL-4)
- **Th17 cells** (presence of IL-1 or IL-6)

**Th1 cells** collaborate with macrophages, stimulate CD8+ T-cell differentiation, and are essential against intracellular pathogens. They mainly produce **IFN-γ** and IL-2, activate macrophages, enhance ADCC, and are involved in type IV hypersensitivity and autoimmune diseases.

**Th2 cells** are important in defense against extracellular parasites and helminths and in allergy. They produce IL-4, IL-5, IL-10, and IL-13, stimulate B cells via direct interaction (CD154–CD40) and cytokines, and support humoral immunity.

**Th17 cells** promote pro-inflammatory responses, producing IL-17, which induces chemokine production and neutrophil recruitment, contributing to antibacterial defense.

#### Mutual Regulation of Th1 and Th2 Responses

The presence of IL-12 favors Th1 development, while IL-4 promotes Th2 differentiation. Th1-derived IFN-γ inhibits Th2 differentiation, while Th2-derived IL-4 and IL-10 suppress Th1 responses, ensuring regulatory balance.

### 14.2.2 Proliferation and Differentiation of CD8+ T Lymphocytes

**Cytotoxic T lymphocytes (Tc)** recognize cells infected with intracellular organisms, tumor cells, and stressed cells. Naive CD8+ T cells recognize pMHC I via TCR (signal 1) and express IL-2 receptors. IL-2, mainly produced by Th1 cells, is required for their proliferation.

With appropriate costimulation (CD28–CD80/86), CD8+ T cells differentiate into effector Tc cells. Mature Tc cells destroy target cells through **perforin-mediated pore formation** and **granzyme-induced apoptosis**, or via **Fas–FasL interactions**.

### 14.2.3 Generation of Memory T Lymphocytes

A hallmark of specific immunity is **memory**. During primary responses, most effector T cells die by apoptosis (90–95%), while 5–10% differentiate into memory T cells. These

cells express higher levels of adhesion molecules, migrate efficiently to infection sites, respond rapidly to APCs, and ensure faster secondary immune responses.

### 14.2.4 Activation of T Lymphocytes by Superantigens

**Superantigens** bypass antigen processing and presentation. They bind directly to MHC II on APCs and to TCRs on T cells, leading to massive, nonspecific T-cell activation and cytokine release, potentially resulting in shock. Examples include **toxic shock syndrome toxin**, **staphylococcal enterotoxins**, and **streptococcal pyrogenic exotoxins**.

## 14.3 Mechanisms of Cell-Mediated Specific Immunity in the Body

Intracellular hiding of microbes evades humoral immunity, necessitating cellular responses:

- **Delayed-type hypersensitivity (DTH)**: Mediated by Th1 cells, promoting macrophage activation and granuloma formation in chronic responses (e.g., tuberculosis).
- **Cytotoxic T-cell reactions**: CD8+ T cells recognize pMHC I on infected cells and induce apoptosis via perforins, granzymes, or Fas–FasL interactions.

## QUESTIONS

What is the relationship between antigen-presenting cells and T lymphocytes called?

Which cells are considered the most important antigen-presenting cells?

What are proteasomes?

Which cells recognize pMHC class I complexes?

Which cells recognize pMHC class II complexes?

What is the function of the CD3 complex?

What is required for T-lymphocyte activation?

What influences the development of Th1 and Th2 clones?

How are T lymphocytes activated by superantigens?

# CHAPTER 15

# ACTIVATION OF B LYMPHOCYTES

## 15.1 Antibody-Mediated Immune Response

### 15.1.1 Interaction of B Lymphocytes with Antigen

The antibody-mediated immune response is associated with antigen recognition through **BCR receptors** on B lymphocytes. Unlike TCRs, **BCRs can recognize and bind not only cell-bound epitopes but also epitopes on soluble molecules**. The BCR receptor consists of **IgM and IgD with identical specificity** and is responsible for antigen recognition. Signal transduction is mediated by a transmembrane heterodimer composed of **Igα and Igβ chains**. These accessory chains contain a cytoplasmic motif called **ITAM**.

When an antigen binds to two or more BCRs, **BCR cross-linking** occurs. The antigen must therefore have **at least two identical epitopes**. Cross-linking leads to conformational changes and activation of tyrosine kinases, resulting in **phosphorylation of the ITAM motifs** of Igα and Igβ. This initiates a signal-transduction cascade, activation of transcription factors, and gene activation.

The antigen is subsequently internalized by endocytosis and degraded into small peptide fragments. These peptides bind to **MHC class II molecules**, costimulatory molecules are generated, and the resulting **pMHC II complex** is transported to the cell surface so that it can be presented to **CD4+ T lymphocytes**. Thus, a B lymphocyte can function as an **antigen-presenting cell (APC)**. Because B lymphocytes can recognize antigens specifically via their receptors, they can present antigens even when present in very small amounts. When antigen concentration is high, dendritic cells and macrophages act primarily as APCs; when antigen concentration decreases, **B lymphocytes become the predominant APCs**.

### 15.1.2 Antibody Immune Response and Its Relationship to T Lymphocytes

Most antigens induce an antibody immune response that **depends on cooperation between Th and B lymphocytes**. These are **T-dependent antigens**, mainly proteins. Some antigens can activate B lymphocytes, induce their differentiation into plasma cells, and stimulate antibody secretion **without T lymphocyte help**. These are **T-independent antigens**, mainly polysaccharides.

**Antibody Response Induced by T-Independent Antigens**

T-independent antigens (TI) activate B lymphocytes without T-cell help and are divided into **TI-1 and TI-2** groups.

- **TI-1 antigens** are mainly bacterial lipopolysaccharides. They do not bind to BCRs but to other receptors (e.g., TLRs), causing activation of a large number of B lymphocytes and antibody production independent of antigen specificity. At high concentrations, they function as **polyclonal activators (B-cell mitogens)**. At low concentrations, they can activate antigen-specific lymphocytes.
- **TI-2 antigens** are mainly polysaccharide (capsular) or protein (flagellar) antigens forming **repetitive polymers**. These polymeric antigens strongly cross-link many BCRs simultaneously, leading to intense B-cell activation and differentiation into plasma cells without T-cell help. Only **mature B lymphocytes** can be activated this way. Immature B lymphocytes become anergic, explaining why children under about two years of age cannot produce antibodies against polysaccharides.

The antibody response to T-independent antigens results **only in IgM production**. **No isotype switching or affinity maturation occurs**.

**Antibody Response Induced by T-Dependent Antigens**

T-dependent antigens (mainly proteins) activate B lymphocytes **only with T-cell help**. The response occurs in **primary and secondary phases**.

The primary phase begins with the first antigen encounter in secondary lymphoid organs. Optimal stimulation of B lymphocytes requires cooperation among **B lymphocytes, APCs (mainly dendritic cells), and Th lymphocytes**. If a B lymphocyte becomes an efficient APC (expressing CD80/86), direct B–Th interaction is sufficient.

The **first signal** is antigen recognition by the BCR. The B lymphocyte must receive help from a Th lymphocyte that recognizes the **same antigen**. After internalization and processing, antigen peptides bind MHC II and are presented to CD4+ T lymphocytes, leading to Th cell activation (usually **Th2**). The Th2 cells provide the **second signal** via cytokines and cell–cell contact.

Signal transduction in B lymphocytes involves BCR signaling (via Igα/Igβ), costimulatory molecules (CD40, CD80/86), and interaction of pMHC II with the TCR. This induces expression of **IL-4 receptors** on B lymphocytes, allowing IL-4 from Th2 cells to promote differentiation into **plasma cells or memory B cells**.

Plasma cells are terminally differentiated antibody-producing cells. Memory B cells persist and provide long-term protection.

### 15.2 Primary and Secondary Phases of the Antibody Response

Antibody responses dependent on Th lymphocytes occur in **two phases**:

#### 15.2.1 Primary Phase

At first antigen exposure, B lymphocytes are stimulated via BCRs and Th lymphocytes are activated via APCs. In lymph nodes, B and T cells migrate to border zones of follicles to interact. Plasma cells emerge and secrete antibodies, predominantly **IgM**, which have lower affinity but effectively limit infection spread.

Some B and Th lymphocytes enter follicles and form **germinal centers**.

**Germinal Centers**

Processes occurring in germinal centers include:

- **Isotype (class) switching** (IgM/IgD → IgG, IgA, IgE)
- **Affinity maturation**
- **Generation of memory B lymphocytes**
- **Generation of plasma cells**

Affinity maturation and memory B-cell formation occur **exclusively in germinal centers**. Follicular dendritic cells store immune complexes and present antigens to B cells.

#### 15.2.2 Secondary Phase

Upon repeated exposure, memory cells respond rapidly and robustly, producing antibodies with **higher affinity** and varied effector functions. The dominant isotype in secondary responses is **IgG**, with IgA and IgE also present. The response is faster and stronger than the primary response.

### 15.3 Mechanisms of Antibody Action in the Body

Antibodies function through:

- **Neutralization** – mainly IgG and IgA bind microbes or toxins and prevent cell entry.
- **Opsonization** – IgG1 and IgG3 tag microbes for phagocytosis via Fc receptors and complement receptors.
- **Complement activation** – antibody–antigen complexes trigger the classical pathway.

- **ADCC** – NK cells and eosinophils recognize antibody-coated targets via Fc receptors.
- **Immediate hypersensitivity** – IgE bound to mast cells and basophils triggers degranulation upon cross-linking (allergy, asthma).

### 15.4 Immunological Memory

Immunological memory enables faster and stronger responses upon re-exposure to the same antigen and is a hallmark of **adaptive immunity**. Memory T and B cells generated during primary responses ensure rapid activation, high-affinity antibodies, and efficient pathogen elimination during secondary responses.

This principle underlies **vaccination**, which exposes individuals to non-pathogenic forms of antigens, inducing memory cells and protective immunity without causing disease.

### QUESTIONS

With what is the antibody-mediated immune response associated?

Characterize **T-independent antigens**.

Characterize **T-dependent antigens**.

List typical features of Th-dependent antibody responses.

Which antibody isotype predominates in the **primary phase** of the antibody response?

Where do **affinity maturation and memory B-cell formation** occur?

Which antibody isotype predominates in the **secondary phase** of the antibody response?

What does **toxoid (anatoxin)** mean?

# CHAPTER 16

# REGULATION OF SPECIFIC IMMUNE RESPONSES

### 16.1 Regulation of Immune Responses

The immune response is regulated by many mechanisms that ensure its optimal course. When the immune system functions correctly, it recognizes and attacks foreign molecules while sparing the body's own structures. Under normal conditions, the resulting immune response should be proportional to the triggering stimulus so that the harmful agent is eliminated without damaging self tissues. Regulation takes place at several levels.

It begins with the presence of the antigen itself, as its properties and quantity influence the quality and extent of the immune response. Disappearance of the antigen leads to a reduction and termination of the immune response. Conversely, a very large amount of antigen can lead to exhaustion of the immune system.

The immune system is capable of recognizing antigens of its own tissues and reacting in a way that maintains homeostasis. It tolerates self tissues and removes old or damaged cells of the organism.

### 16.2 Tolerance

The ability to tolerate self antigens is established during the maturation of T and B lymphocytes. Lymphocytes are educated to recognize and distinguish self from non-self. If mechanisms of tolerance to self tissues fail, autoimmune diseases develop. Tolerance occurs at the **central** and **peripheral** levels.

#### 16.2.1 Central Tolerance

Central tolerance develops in the **thymus for T lymphocytes** and in the **bone marrow for B lymphocytes**.

In the thymic cortex, **positive selection** occurs first. Only T lymphocytes capable of recognizing self MHC molecules presenting self peptides via their TCR survive; those that cannot recognize self MHC undergo apoptosis. Positive selection thus preserves T cells that recognize self MHC.

In the thymic medulla, **negative selection** follows. T lymphocytes that recognize self peptide–MHC complexes with high affinity are potentially dangerous (autoreactive) and are eliminated by apoptosis. Through negative selection, potentially autoreactive T

lymphocytes are removed. In this way, up to **95–98%** of thymocytes entering the thymus are eliminated during T-cell education.

In the bone marrow, **negative selection of B lymphocytes** takes place. B lymphocytes whose BCR can recognize and bind self molecules are potentially autoreactive and are eliminated by apoptosis.

Thus, tolerance to self antigens is established. Despite positive and negative selection, some T and B cells may escape tolerance and reach secondary lymphoid organs. Upon activation, these cells may initiate autoimmune processes. Moreover, some self antigens are located in anatomical sites inaccessible to the immune system, allowing autoreactive lymphocytes to escape selection.

### 16.2.2 Peripheral Tolerance

Mechanisms of central tolerance are significantly supplemented by **peripheral tolerance**, which occurs directly in tissues or regional lymph nodes. The outcomes of peripheral tolerance include:

- **Clonal anergy** – a state of lymphocyte unresponsiveness that occurs when lymphocytes receive a signal through the TCR or BCR but lack appropriate additional signals from APCs or T lymphocytes. The lymphocyte recognizes the autoantigen, but missing costimulatory signals prevent full activation. Anergy is a regulatory mechanism for naïve T and B lymphocytes. Anergic cells are not destroyed; they remain in circulation but normally cannot be reactivated.
- **Clonal deletion** – destruction of autoreactive lymphocyte clones that recognize autoantigens and become activated. These lymphocytes are eliminated by apoptosis.
- **Clonal ignorance** – autoreactive lymphocytes do not respond because the autoantigen is present in very low amounts, insufficient to generate a signal strong enough to activate lymphocyte receptors.
- **Suppression** – inhibition of autoreactive lymphocytes by other immune cells or their products, especially **regulatory T lymphocytes (Treg)**. Treg cells contribute to tolerance by inhibiting autoreactive lymphocytes. CD4+ regulatory T cells suppress immune responses by producing inhibitory cytokines and through other mechanisms. They are important in preventing certain autoimmune diseases (e.g., autoimmune gastritis, chronic colitis). Similar suppressive properties are exhibited by **CD8+ suppressor Ts lymphocytes**, whose presence has been associated with inhibition of certain autoimmune diseases (e.g., experimental autoimmune encephalomyelitis).

### 16.3 Th1 / Th2 Paradigm

This mechanism is considered an important element in immune regulation. Immune reactions often represent a balance between different response mechanisms. For example, helper T lymphocytes can produce many cytokines (TGF-β, IFN-γ, IL-4, IL-5, IL-6, IL-10, IL-12, IL-17, IL-21), which they use to communicate with other immune cells. However, a single cell does not produce the entire spectrum of cytokines. Th lymphocytes tend to produce specific cytokine subsets appropriate for immune responses against particular invaders, forming characteristic **cytokine profiles**.

Once Th lymphocytes begin producing a specific cytokine profile, they secrete growth factors specific to that Th subset, creating **positive feedback** and promoting proliferation of that subset. At the same time, **negative feedback** occurs. For example, IFN-γ produced by Th1 cells supports Th1 development but inhibits Th2 proliferation. Conversely, IL-4 and IL-10 produced by Th2 cells promote Th2 responses but inhibit Th1 proliferation.

As a result of these interactions, a dominant Th subtype emerges. Activation of a Th1 response stabilizes itself while suppressing Th2 responses, and vice versa. Similar principles apply to **Th17 regulation**: Th1 and Th2 cytokines (IFN-γ, IL-4) inhibit Th17 cells, while Th17 cytokines (e.g., TGF-β) inhibit Th1 and Th2 cells.

### 16.4 Regulatory Cytokines

Regulation of adaptive immune responses is largely mediated by **cytokines**, which are key regulatory molecules of the immune system. Cytokines transmit important information between cells and can act over short or long distances.

They act:

- **Autocrinely** – on the cell that produced them,
- **Paracrinely** – on nearby cells,
- **Endocrinely** – after transport through the bloodstream to distant tissues.

Cytokines include interleukins, interferons, chemokines, colony-stimulating factors, tumor necrosis factors, transforming growth factors, and other growth factors.

They regulate innate immunity, especially defense against bacteria and viruses, and adaptive immunity, particularly through cytokines produced by T lymphocytes. T-cell-derived cytokines are essential for B-cell activation and isotype switching, while B lymphocytes can also influence T-cell activation through cytokines. Different T-cell subsets produce different cytokines that regulate other T-cell populations (e.g., Th1 vs. Th2).

Each cytokine may have specific effects, some cytokines have overlapping functions, and others act antagonistically. Cytokines often affect multiple cell types. The final effect depends on cytokine concentration, target cell type, and the presence of other cytokines.

**QUESTIONS**

In which organs or tissues does **central tolerance** develop?

Where does **peripheral tolerance** develop?

Explain the concept of **clonal anergy**.

What is the difference between **clonal deletion** and **clonal ignorance**?

Characterize **cytokines**.

What does **autocrine action** of cytokines mean?

What does **paracrine action** mean?

What does **endocrine action** of cytokines mean?

# CHAPTER 17

# ANTI-INFECTIOUS IMMUNITY

### 17.1 Immunity in Bacterial Infections

The immune system is capable of responding to an enormous variety of foreign molecules. There are **three basic defensive lines** of the body against infectious agents:

- **Natural barriers** that prevent microorganisms from entering the body (skin, mucous membranes, ciliated epithelium, gastric acid, etc.).
- **Innate, antigen-nonspecific immune mechanisms** providing a rapid local response to the presence of microorganisms (fever, neutrophils, macrophages, NK cells, etc.).
- **Acquired, antigen-specific immune mechanisms** directed against a particular antigen that attack and eliminate it if it has overcome the first two defense lines (T and B lymphocytes and antibodies).

Although there is usually a dominant type of immune response to a particular group of pathogens (intracellular bacteria, extracellular bacteria, viruses, etc.), in practice multiple components of both nonspecific and specific immunity contribute to the response to some extent. This is very important because many microorganisms possess mechanisms that allow them to evade immune defenses. Activation of multiple immune response types against a single invader increases the likelihood that the infection will be eliminated or better controlled.

Based on their capacity to cause disease, microorganisms can be classified into several groups. **Commensal (saprophytic) microorganisms** live in mutual symbiosis with the host and may even provide beneficial functions (maintenance of appropriate pH, competition with pathogens for nutrients and energy, etc.). **Potentially pathogenic microorganisms** can cause infectious disease in individuals with weakened immune systems. **Pathogenic microorganisms** are capable of causing infectious diseases even in individuals with fully functional immunity.

Intact skin and mucous membranes mechanically prevent the entry of many microorganisms. Various additional natural defense mechanisms effectively block bacterial invasion (physiological microbiota, suitable pH, microbicidal molecules). If bacteria penetrate into tissues, the effectiveness of other lines of immune defense determines the extent of tissue damage and bacterial elimination.

Most bacteria present in the body remain in the **extracellular space** and are termed **extracellular bacteria**. Others reside most of the time inside host cells and are called

**intracellular bacteria**, although they may briefly appear extracellularly during infection initiation or spread.

### 17.1.1 Defense Against Extracellular Bacteria

Extracellular bacteria include many pathogenic species, such as **staphylococci, streptococci, neisseriae, bordetellae, haemophili, enteric bacteria**, and others. These bacteria are killed after being engulfed by phagocytes. **Phagocytosis** represents the most important barrier preventing further bacterial penetration.

Activated phagocytes produce cytokines that influence systemic responses, promote fever, stimulate synthesis of acute-phase proteins (which enhance opsonization), and activate inflammation. Another key mechanism is the **complement system**, which exerts cytolytic effects via formation of the **membrane attack complex (MAC)**. Many complement fragments also function as opsonins, chemoattractants, and mediators with various biological effects.

In later stages of infection, **antigen-specific immunity** is stimulated. Specific immunity against extracellular bacteria is primarily **antibody-mediated**. Initially, B lymphocytes are stimulated and produce **IgM antibodies**. With the help of Th lymphocytes, B cells proliferate, differentiate, undergo isotype switching, and produce antibodies of various classes with higher affinity.

Antibodies play a crucial role in defense against extracellular bacteria by:

- opsonizing bacteria,
- activating the classical complement pathway,
- neutralizing bacterial toxins,
- secretory IgA preventing bacterial adherence to epithelium.

Encapsulated bacteria possess polysaccharide capsules that act as **T-independent antigens**, capable of cross-linking many BCRs. This results in intense B-cell activation and antibody production without T-cell help. This type of response develops fully only after approximately **two years of age** and is impaired in individuals without a functional spleen. Children under two years and asplenic individuals are particularly susceptible to infections with encapsulated extracellular bacteria.

### 17.1.2 Defense Against Intracellular Bacteria

Intracellular bacteria include **mycobacteria, chlamydiae, rickettsiae, listeriae, salmonellae, brucellae, yersiniae**, as well as yeasts and fungi. These pathogens often cause **chronic infections** and generally do not produce strong toxins. They survive and replicate within host cells such as macrophages, epithelial, or endothelial cells.

Many intracellular bacteria evade intracellular killing mechanisms in phagocytes. Therefore, **T lymphocytes** are the primary defense component. **Delayed-type hypersensitivity (DTH)** responses are involved. After phagocytosis, bacteria survive inside macrophages and multiply. Cytokines such as **IL-12** induce differentiation of Th lymphocytes into **Th1 cells**, which produce **IFN-γ**. IFN-γ activates macrophages and stimulates nitric oxide (NO) production via nitric oxide synthase, which is crucial for intracellular bacterial killing.

Activated macrophages accumulate at infection sites, forming **granulomas** that prevent bacterial dissemination. **Cytotoxic T lymphocytes (Tc)** also contribute by recognizing pMHC I complexes on infected cells and inducing apoptosis. Individuals with impaired cellular immunity are more susceptible to intracellular bacterial infections.

### 17.2 Immunity in Viral Infections

Defense against viruses involves several mechanisms. In the **first line of defense**, nonspecific mechanisms dominate, particularly **interferons**, **NK cells**, and **NKT cells**. Virus-infected cells produce interferons that inhibit viral replication and induce antiviral protein synthesis in neighboring cells.

NK cells play a vital role early in infection before specific immunity develops. They are activated by IL-12 and eliminate virus-infected cells.

In the **second line of defense**, specific immune mechanisms—both humoral and cellular—are activated. **Secretory IgA** on mucosal surfaces prevents viral attachment, while **neutralizing IgG antibodies** block viral entry into host cells. Antibodies also promote opsonization and activate complement.

Once viruses enter cells, **cell-mediated immunity** becomes essential. **Cytotoxic T lymphocytes (CTLs)** recognize viral peptides bound to MHC I and destroy infected cells. Some viruses evade immune responses and persist latently, reactivating when immunity is weakened (e.g., varicella-zoster virus).

Individuals with T-cell or NK-cell deficiencies, combined immunodeficiencies, or antibody deficiencies are at increased risk of viral infections. Very young infants and elderly individuals are also more vulnerable.

### 17.3 Immunity in Parasitic Infections

Parasitic diseases include infections caused by **protozoa, helminths**, and **arthropod-borne parasites**. Parasites possess complex life cycles and numerous antigens, making immune responses stage-dependent and often chronic.

For **protozoa**, immune mechanisms resemble those against bacteria: cellular immunity is more effective against intracellular protozoa, and antibodies against extracellular forms.

For **helminths**, which are large multicellular parasites, immunity is dominated by **Th2 responses** with production of **IgE**, activation of **eosinophils, mast cells, and basophils**, and cytokines **IL-4, IL-5, IL-9, and IL-13**. IgE-mediated degranulation releases mediators that damage or expel parasites through smooth muscle contraction, increased vascular permeability, and enhanced peristalsis.

**Eosinophils** participate in **ADCC**, releasing cytotoxic granule contents that damage helminths but may also injure host tissues, necessitating concurrent tissue repair.

**QUESTIONS**

Which defense lines does the human body use against infectious microorganisms?

Which mechanisms are most important in defense against **extracellular bacteria**?

What is the role of antibodies in protection against extracellular bacterial infections?

Which mechanisms play a major role in defense against **intracellular bacteria**?

Which mechanisms are effective in defense against **viruses**?

Describe the **specific features of immunity in parasitic infections**.

# CHAPTER 18

# HYPERSENSITIVITY REACTIONS

### 18.1 Classification of Hypersensitivity Reactions

Hypersensitivity reactions cause tissue damage through the release of chemical mediators that attract and activate cells and molecules, leading to inflammation. According to **Coombs and Gell**, these reactions are divided into **four types**, based on the mechanisms by which they damage tissues:

- **Type I reactions (immediate hypersensitivity)** – the most common type; associated with the production of **IgE antibodies**, degranulation of mast cells and basophils; reactions develop very rapidly, within minutes after antigen exposure.
- **Type II reactions (cytotoxic type)** – triggered by binding of **IgG or IgM antibodies** to antigens present on the membrane of target cells or bound to their surface; these reactions are associated with **complement activation** or **ADCC (antibody-dependent cellular cytotoxicity)**.
- **Type III reactions (immune-complex type)** – involve interactions between antibodies and **soluble antigens**, leading to formation of antigen–antibody complexes (**immune complexes**) that deposit in various tissues and cause damage; they develop several days after antigen exposure.
- **Type IV reactions (delayed-type hypersensitivity)** – immune cells attack host cells **without antibody involvement**; occur physiologically in the tuberculin skin reaction, pathologically in allergic contact dermatitis, granuloma formation, and in some cytotoxic T-cell-mediated responses.

The first three types of hypersensitivity reactions are **antibody-dependent**. Type IV is **antibody-independent** and involves **cell-mediated immunity**.

### 18.2 Type I Hypersensitivity

Type I hypersensitivity is commonly referred to as an **allergic reaction** or **immediate hypersensitivity**. It develops very rapidly after antigen exposure. In some individuals, it can occur in response to otherwise harmless environmental antigens (allergens).

IgE antibodies bind to **Fcε receptors (FcεR)** on mast cells and basophils. These receptors can bind IgE even in the absence of antigen and thus function as antigen-specific surface receptors. Upon re-exposure to the allergen, interaction with IgE leads to **degranulation** of mast cells and basophils.

Released mediators—especially **histamine**—are highly potent and cause allergic inflammation with characteristic clinical symptoms. In the **early phase**, preformed mediators stored in granules are released (histamine, heparin, chondroitin sulfate, proteases). This is followed by a **late phase**, in which secondary mediators derived from arachidonic acid metabolism are synthesized (prostaglandins, leukotrienes, thromboxanes).

Mediator effects include:

- contraction of smooth muscle (bronchi, gastrointestinal tract),
- vasodilation and increased vascular permeability,
- fluid leakage into extravascular spaces (edema formation),
- decreased blood pressure.

Histamine also induces arterial smooth muscle contraction and accelerates redistribution of blood from central to peripheral tissues.

### 18.2.1 Local Reactions

Type I hypersensitivity reactions may be **local** or **systemic**. Mast cells are predominantly located in the respiratory tract, intestinal wall, and skin—common sites of allergen exposure. Examples of local reactions include **allergic rhinitis, conjunctivitis, bronchial asthma, and atopic dermatitis**.

### 18.2.2 Systemic Reactions

If allergens disseminate through the bloodstream (e.g., after intravenous administration), **systemic reactions** may occur, potentially leading to **life-threatening anaphylactic shock** (e.g., after insect stings, certain drugs, or foods).

Anaphylaxis involves massive mast cell and basophil degranulation and mediator release, leading to:

- vascular smooth muscle contraction,
- endothelial disruption with fluid leakage,
- severe loss of intravascular volume,
- hypotension and tachycardia.

Anaphylaxis can result in acute circulatory failure and death within minutes and requires immediate treatment.

### 18.3 Type II Hypersensitivity

Also known as **cytotoxic hypersensitivity**, Type II reactions are triggered by **IgG or IgM antibodies** binding to antigens on cell membranes or bound to cells. The resulting antigen–antibody complexes activate the **classical complement pathway**, leading to **cell lysis**.

Complement fragments act as opsonins, enhancing phagocytosis and destruction of target cells. Another mechanism involved is **ADCC**, mediated by cells expressing Fc receptors (monocytes, neutrophils, eosinophils, and especially NK cells).

Type II hypersensitivity includes **transfusion reactions**. IgM antibodies binding to erythrocyte antigens cause complement-mediated hemolysis. IgG antibodies (e.g., against Rh antigen) mainly cause erythrocyte destruction via phagocytosis.

A classic example is **hemolytic disease of the newborn** caused by anti-RhD antibodies. Preventive administration of anti-RhD antibodies to the mother after the first delivery prevents sensitization.

Some Type II reactions involve antibodies that do not cause cell destruction but instead **stimulate or block receptors**:

- **Stimulating antibodies**, e.g. anti-TSH receptor antibodies in **Graves' disease**, cause hyperthyroidism.
- **Blocking antibodies**, e.g. anti-TSH receptor antibodies, prevent ligand binding and lead to hypothyroidism.
  Similar mechanisms occur in **myasthenia gravis** and **type 1 diabetes mellitus**.

### 18.4 Type III Hypersensitivity

Type III hypersensitivity reactions are **immune-complex-mediated**. Circulating antigen–antibody complexes (small to medium size) deposit in tissues such as:

- blood vessel walls → **vasculitis**,
- renal glomeruli → **glomerulonephritis**,
- joints → **arthritis**.

A local example is the **Arthus reaction**, which develops 4–6 hours after intradermal antigen injection and leads to local inflammation, necrosis, and ulceration.

A systemic example is **serum sickness**, caused by widespread deposition of immune complexes after exposure to exogenous antigens (heterologous sera, certain drugs). Symptoms develop after **7–10 days** and include fever, myalgia, arthralgia, urticaria, and lymphadenopathy. The condition is self-limiting once the antigen is cleared.

Type III reactions also contribute to post-infectious complications (e.g., post-streptococcal glomerulonephritis, rheumatic fever) and autoimmune diseases such as **systemic lupus erythematosus (SLE)**.

### 18.5 Type IV Hypersensitivity

Type IV hypersensitivity, also called **delayed-type hypersensitivity (DTH)**, is mediated by **Th1 lymphocytes and macrophages** and does not involve antibodies.

A classic physiological example is the **tuberculin skin test**, which becomes positive 24–72 hours after intradermal antigen application due to recruitment and activation of Th1 cells and macrophages.

A pathological example is **allergic contact dermatitis**, caused by small reactive chemicals (**haptens**) that bind host proteins and form immunogenic neoantigens. Sensitization is initially asymptomatic; re-exposure leads to dermatitis within 24–72 hours.

Type IV hypersensitivity is also involved in **granuloma formation**, tissue damage in chronic infections (tuberculosis, leprosy, sarcoidosis), **hypersensitivity pneumonitis**, and **cytotoxic T-cell-mediated disorders** (viral exanthems, autoimmune diabetes, transplant rejection, hepatitis).

### QUESTIONS

Which type of hypersensitivity reaction is the most common?
What is Type II hypersensitivity called ?
What characterizes Type III hypersensitivity reactions?
What is delayed-type hypersensitivity?

# CHAPTER 19

# IMMUNODEFICIENCY

### 19.1 Basic Characteristics of Immunodeficiency States

Immunodeficiencies are caused by defects in various components of the immune system, leading to a reduced ability to resist infections. They occur in two forms.

**Primary immunodeficiencies** are caused by congenital defects—gene mutations affecting individual components of the immune system. More than 150 primary immunodeficiencies are known, and for many of them a specific defective gene has been identified. Primary immunodeficiencies are rarer but more severe. Most are detected around **6 months of age**, when maternal antibodies disappear and the child becomes dependent on its own immunity. They manifest with a wide spectrum of clinical symptoms, from mild to very severe infections that can lead to death, often already in early childhood.

**Secondary immunodeficiencies** are caused by external factors such as infections, underlying diseases, malnutrition, stress, therapy, and others. They may arise at any time during life and are much more frequent than primary immunodeficiencies. Affected patients are more susceptible to infections and various other diseases.

Common manifestations present in many immunodeficiencies include **recurrent or chronic infections**, inability to eliminate infections with standard antibiotic therapy, and infections caused by unusual pathogens. In some cases, failure to thrive, hepatosplenomegaly, skin rashes, diarrhea, and abscesses are observed. Certain immunodeficiencies also show non-immunological features, such as thrombocytopenia in **Wiskott–Aldrich syndrome**, loss of balance due to cerebellar involvement with capillary dilation in **ataxia-telangiectasia**, or albinism in **Chediak–Higashi syndrome**.

### 19.2 Primary Immunodeficiencies

Primary immunodeficiencies may affect different immune lineages: T or B cells alone or in combination, antibody production, phagocytosis, or complement. A defect in one lineage can impair the function of others (e.g., T-cell defects affecting B-cell activation). They affect both sexes equally if autosomal, but X-linked defects affect males more often.

**Warning signs** include recurrent otitis, sinusitis, pneumonias, bronchitis, deep tissue infections or abscesses, sepsis or meningitis, persistent diarrhea, malabsorption, failure to thrive or growth delay in children, recurrent atypical infections, chronic infections, need for prolonged or intravenous antibiotics, and a family history of primary

immunodeficiency. In congenital immunodeficiencies, manifestations often appear in the **upper aerodigestive tract**, the most common portal of pathogen entry.

### 19.2.1 Antibody (Humoral) Immunodeficiencies

They are the most common, accounting for about **70%** of primary immunodeficiencies. Symptoms usually appear from **6 months of age** to adulthood; T-cell function is preserved. Patients mainly suffer from respiratory infections, otitis, and arthritis, often caused by **encapsulated bacteria** and **echoviruses**. There is also increased susceptibility to allergic, autoimmune, and malignant diseases. Survival into adulthood is enabled by **immunoglobulin replacement therapy** and antibiotics.

Examples include **X-linked agammaglobulinemia (Bruton disease)**, **selective IgA deficiency** (the most common primary immunodeficiency), **common variable immunodeficiency (CVID)**, **transient hypogammaglobulinemia of infancy**, and others.

- **Bruton agammaglobulinemia** results from a defect in **Bruton tyrosine kinase (BTK)**, blocking B-cell maturation and causing absence of B cells, plasma cells, and all immunoglobulin isotypes.
- **Selective IgA deficiency** features very low IgA levels; often asymptomatic, but when present causes recurrent respiratory infections and increased autoimmunity/malignancy risk.
- **CVID** presents later in life with low IgG, IgA, and IgM, recurrent infections, autoimmunity, malignancies, and sometimes malabsorption (e.g., *Giardia lamblia*).
- **Transient hypogammaglobulinemia of infancy** reflects delayed maturation of IgG production and often resolves spontaneously.

### 19.2.2 Cellular and Combined Immunodeficiencies

These involve defects of T cells or combined lymphocyte defects (~**20%** of primary immunodeficiencies). Symptoms appear at birth to ~2 years: failure to thrive, respiratory infections, diarrhea, dermatitis; pathogens include viruses, *Pneumocystis*, fungi, and mycobacteria. Without treatment, death often occurs within the first year. Live vaccines are dangerous. Autoimmune diseases and malignancies may develop.

The most severe form is **severe combined immunodeficiency (SCID)**. Others include **ADA deficiency**, **DiGeorge syndrome**, **Wiskott–Aldrich syndrome**, **Chediak–Higashi syndrome**, **ataxia-telangiectasia**, and **bare lymphocyte syndrome**.

- **SCID** comprises various genetic defects affecting DNA recombination, cytokine receptors, or lymphocyte interactions, leading to profound T and B cell dysfunction.
- **DiGeorge syndrome** involves thymic aplasia/hypoplasia with T-cell deficiency, hypoparathyroidism, congenital heart defects, and poor prognosis.
- **Wiskott–Aldrich syndrome** features thrombocytopenia, eczema, recurrent infections, and malignancies.
- **Ataxia-telangiectasia** includes cerebellar ataxia, telangiectasias, recurrent infections, and cancer risk.
- **Bare lymphocyte syndrome** involves defective MHC I/II expression with CD4+ and CD8+ T-cell deficiency.

### 19.2.3 Phagocytic Defects

These affect microbial killing and cooperation with other immune cells (~**9%**). Present with purulent, often localized infections (e.g., pyodermas, omphalitis, abscesses), commonly due to **staphylococci**, fungi, and enterobacteria.

Examples include **chronic granulomatous disease (CGD)**, **leukocyte adhesion deficiency (LAD)**, **Chediak–Higashi syndrome**, **Kostmann syndrome**, and **cyclic neutropenia**.

- **CGD**: NADPH oxidase defect → reduced reactive oxygen species and bactericidal activity; granuloma formation.
- **LAD**: defective leukocyte integrins → impaired adhesion and migration to infection sites.
- **Kostmann syndrome**: severe congenital neutropenia.

### 19.2.4 Complement Deficiency

These are rare (~**1%**). Manifest with pyogenic infections and edema, often due to **Neisseria**. Defects may involve complement components, inhibitors, or receptors.

- **C1–C4 deficiencies** impair immune complex clearance → immune-complex diseases plus infections.
- **C6–C9 deficiencies** predispose to neisserial infections.

- **Hereditary angioedema (HAE)** results from **C1 inhibitor deficiency**, causing uncontrolled classical pathway activation and recurrent edema—life-threatening when airways or vessels are involved.

### 19.3 Secondary Immunodeficiencies

Secondary immunodeficiencies are caused by **external factors** and may arise at any time: underlying diseases, therapies, malnutrition, infections, malignancies, stress, etc.

**Physiological factors:** chronic stress, malnutrition, deficiencies of iron, zinc, selenium, and vitamins D, A, B6, C, and E.

**Therapeutic factors:** immunosuppressive therapy (e.g., transplantation, autoimmune disease treatment), ionizing radiation, cytotoxic agents, corticosteroids.

**Cancer:** tumor-derived immunosuppressive molecules and malignant lymphocytes disrupt cytokine networks and immune interactions.

**Other causes:** metabolic disorders (uremia, diabetes, hypothyroidism), chronic infections, liver failure, alcoholism, splenectomy, trauma, burns, surgery, anesthesia, age extremes, chemical exposures. Globally, **HIV infection** and **malnutrition** dominate.

Temporary immunodeficiencies may follow infections with **herpesviruses**, **measles**, **rubella**, and other lymphocytotropic viruses. The most severe secondary immunodeficiency is **HIV infection**, leading to **AIDS**, with loss of CD4+ T cells and high susceptibility to opportunistic infections and cancers.

Many pathogens also evade immunity (e.g., antibody/complement degradation, blocking phagolysosome fusion, hiding in erythrocytes lacking MHC).

### 19.4 Treatment of Immunodeficiencies

Management includes temporary measures and potentially curative approaches, depending on type and severity.

- **Antibody deficiencies:** immunoglobulin replacement (IVIG) and prophylactic antibiotics.
- **Cellular/combined defects and severe phagocytic disorders: hematopoietic stem cell transplantation**, with success depending on donor match and early diagnosis (exceptions include non-hematopoietic defects like DiGeorge).
- **Supportive therapy:** antibiotics, cytokines (e.g., growth factors for neutrophils), vitamins.

- **Secondary immunodeficiencies:** treat or remove the underlying cause; immunomodulation as appropriate.
- **Gene therapy** holds promise; used experimentally (e.g., ADA deficiency) with partial success.

**QUESTIONS**

Characterize **primary immunodeficiencies**.

How do **immunodeficiencies manifest clinically**?

List some **warning signs** suggestive of a primary immunodeficiency.

Which **primary B-cell immunodeficiency** is the most well known?

Which **primary antibody immunodeficiency** is the most common?

Which immunodeficiency represents the **most severe primary immunodeficiency**?

What defect occurs in **chronic granulomatous disease**?

Which **system is affected in hereditary angioedema**, and how do **complement deficiencies** present?

What is typical for **secondary immunodeficiencies**?

Which **infections** can lead to **temporary immunodeficiencies**?

# CHAPTER 20

# AUTOIMMUNITY

## 20.1 Autoimmune reactions

Autoimmune diseases arise as a result of immune responses directed against one or more self antigens, usually against proteins that are components of the body. Self antigens to which the immune system responds by the development of an autoimmune disease are called **autoantigens**, and the immune response against autoantigens is referred to as an **autoimmune reaction**.

Autoimmunity is a complex problem and may arise through many mechanisms. The risk of its development is influenced by multiple environmental and genetic factors, many of which have not yet been identified. Together, they lead to a breakdown of the mechanisms responsible for tolerance to self antigens, resulting in an immune response directed against components of one's own body.

### 20.1.1 Autoreactivity

For the normal functioning of the human body, a certain degree of autoreactivity is necessary and occurs in every healthy individual. The immune system recognizes self antigens and reacts with them physiologically in order to maintain homeostasis. Old, damaged, or apoptotic self cells are recognized and removed. Antibodies directed against the body's own antigens are called **autoantibodies**.

Physiological (natural) autoantibodies have low affinity, are usually of the IgM isotype, occur at low concentrations, and are often not detectable by commonly available laboratory methods. Their frequency increases with age, and their detection is common in older individuals, but their role in autoimmunity is insignificant. In children, however, detection of autoantibodies requires increased attention.

A problem arises when the extent of autoimmune processes exceeds the regulatory capacity of the organism and autoimmune reactions begin to cause tissue damage. It is assumed that autoantibodies are not the immediate cause of autoimmune disease but are rather a marker. In autoimmune diseases, autoantibodies are present at high concentrations, predominantly as IgG and IgA isotypes with high affinity. Their detection may precede clinical manifestations of the autoimmune process, accompany it, or persist even after the disease. They may also be present in the body without disease. Therefore, the presence of autoantibodies must be evaluated in relation to the individual's clinical condition and the results of other diagnostic methods.

### 20.1.2 Humoral and cellular types of autoimmune diseases

Effector mechanisms in the development of autoimmune diseases may involve both antibodies and T lymphocytes; thus, autoimmune diseases may be of **humoral** or **cellular** type.

- In the **humoral type**, autoantibodies (especially IgG with high affinity) are produced and damage tissues by cytotoxic or immune-complex mechanisms, corresponding to hypersensitivity reactions of type II and III.
- In the **cellular type**, tissue damage is mediated by Th1 lymphocytes, cytotoxic Tc lymphocytes, and macrophages, corresponding to type IV hypersensitivity.

There is a wide range of autoimmune diseases, and virtually any tissue or organ may be affected. Some diseases are **organ-specific**, where the immune response is directed against antigens associated with a specific target organ, and pathology affects that organ. If the immune response is directed against organ-nonspecific antigens, multiple organs and tissues are involved, and these are referred to as **systemic (organ-nonspecific) autoimmune diseases**. However, the boundary between these types is sometimes unclear. Similarly, the contribution of humoral or cellular immunity may not always be clearly defined—sometimes one predominates, while in other cases both contribute equally. The influence of humoral or cellular immunity is sometimes inferred only from experimental models.

**Autoimmune diseases mediated by humoral immunity**

Autoreactive antibodies trigger hypersensitivity reactions of type II or III. These are usually IgG isotypes; IgM may also contribute partially. Complement activation and opsonization of damaged cells stimulate inflammatory responses, increasing tissue damage. Although autoreactive T lymphocytes are present, their role is not direct attack on host cells but activation of autoreactive B lymphocytes. Examples of humoral autoimmune diseases include autoimmune hemolytic anemia, Hashimoto thyroiditis, rheumatic fever, rheumatoid arthritis, systemic lupus erythematosus (SLE), and Goodpasture syndrome.

**Cell-mediated autoimmune diseases**

Cell-mediated damage leading to autoimmune disease involves type IV hypersensitivity. This includes delayed-type hypersensitivity mediated by CD4+ T lymphocytes (Th1) and macrophages, as well as cytotoxic reactions mediated by CD8+ T lymphocytes. In some diseases, specific antibodies may be present, but they do not contribute to the pathological process. Examples of cell-mediated autoimmune diseases include multiple sclerosis, type 1 diabetes mellitus (juvenile diabetes), psoriasis, and autoimmune uveitis. Rheumatoid arthritis is an example of an autoimmune disease involving both humoral and cellular mechanisms.

Autoimmune diseases are also characterized by imbalance in cytokine production. The predominant cytokine profile determines the differentiation of helper Th lymphocytes. It has been observed that in some diseases Th1 activity predominates, promoting cell-mediated responses, whereas Th2 predominance is observed, for example, in SLE and Sjögren's disease. Imbalance between Th1 and Th2 responses, and factors influencing this imbalance, may affect individual disease risk. For example, intestinal epithelial cells produce cytokines (IL-4, IL-10) that favor Th2 activity, enhance humoral responses, increase IgA production, and suppress Th1 responses. Changes promoting Th1 differentiation (e.g., due to pathogenic bacteria) may underlie the development of inflammatory bowel diseases such as Crohn's disease or ulcerative colitis.

## 20.2 Tolerance to self

Mechanisms maintaining immune responses to self antigens within physiological limits are overcome in autoimmune diseases, leading to a breakdown of self tolerance. Tolerance arises through inactivation or destruction of lymphocytes bearing BCR or TCR receptors capable of recognizing self antigens. This occurs during early development (**central tolerance**) or later when lymphocytes are already circulating (**peripheral tolerance**).

### 20.2.1 Central tolerance

Central tolerance develops in the thymus for T lymphocytes and in the bone marrow for B lymphocytes. In the thymus, positive selection preserves T cells whose TCR can recognize MHC molecules with self peptides. This is followed by negative selection, eliminating those with excessively high affinity for pMHC complexes. In the bone marrow, negative selection eliminates B lymphocytes whose BCR recognizes self antigens. Thus, potentially autoreactive T and B lymphocytes are removed.

A limitation of central tolerance is that not all antigens are present in the thymus or bone marrow, particularly those arising during puberty. Therefore, additional mechanisms—collectively termed **peripheral tolerance**—are required to prevent autoreactive cells from damaging self tissues.

### 20.2.2 Peripheral tolerance

Peripheral tolerance controls autoreactive T and B lymphocytes after they leave the thymus or bone marrow and occurs in tissues or regional lymph nodes. Lymphocytes may recognize antigens without responding. One mechanism is **anergy**, a state of unresponsiveness where antigen recognition occurs without costimulatory signals. Another mechanism is **suppression**, in which regulatory immune cells and their products inhibit autoreactive lymphocyte activity.

### 20.3 Loss of self tolerance

Despite mechanisms preventing autoreactivity, situations may arise in which autoreactive cells break tolerance and attack self tissues.

#### 20.3.1 Cross-reactions and molecular mimicry

Molecular mimicry involves cross-reactions between microbial antigens and host self antigens. When antigens are structurally similar, immune responses initially directed at microbes may cross-react with host tissues, causing damage. A classic example is rheumatic fever following *Streptococcus pyogenes* infection, where antibodies against bacterial M protein cross-react with heart tissues, leading to cardiac damage.

#### 20.3.2 Epitope spreading

Epitope spreading occurs when an immune response initially directed against a specific epitope leads to tissue damage and exposure of additional self epitopes, triggering broader autoimmune responses. This contributes to relapsing disease courses, as seen in multiple sclerosis, SLE, and inflammatory bowel diseases.

#### 20.3.3 Loss of suppression

Suppression involves regulation of autoreactive lymphocytes by other immune cells, particularly regulatory T cells. With aging, the number of suppressive cells decreases, increasing the risk of autoimmune diseases such as SLE.

#### 20.3.4 Sequestered antigens

Some self antigens are sequestered and normally inaccessible to the immune system, such as those in the brain (blood–brain barrier), eye, testes, or fetus. These **immunologically privileged sites** prevent immune responses. Hidden epitopes exposed upon tissue damage are called **cryptoepitopes**, illustrated by rheumatoid factor formation.

#### 20.3.5 Neoantigens

Neoantigens are self antigens modified by external factors (e.g., chemicals or drugs) and recognized as foreign. Responses against neoantigens cease once the modifying factor is removed, unlike true autoantigens.

### 20.4 Genetic predisposition

The risk of autoimmune disease is linked to genetic factors, as shown by familial clustering and higher concordance in monozygotic twins. Genetic predisposition is influenced by multiple factors, including immune regulation, environment, medications, and infections. The greatest association is with genes of the **human leukocyte antigen (HLA) complex**. The strength of association is assessed using **relative risk (RR)**, calculated for specific populations.

**QUESTIONS**

How is the immune response to an autoantigen called?

By which mechanisms can tissues be damaged in the humoral type of autoimmune diseases?

By which mechanisms does damage occur in the cellular type of autoimmune diseases?

What is the name of the process where infection is associated with subsequent autoimmune disease development?

Explain the concept of epitope spreading.

Which sites are referred to as immunologically privileged sites?

What are neoantigens?

# CHAPTER 21

# METHODS USED IN LABORATORY DIAGNOSTICS.

***A more detailed description of laboratory diagnostic methods used in immunology can be found in the publication Neuschlová M, Nováková E, Kompaníková J. Instructions for Practical Exercises in Immunology. Martin: Jessenius Faculty of Medicine, Comenius University, 2016; p. 114 (available at: https://portal.jfmed.uniba.sk/clanky.php?aid=348).***

## 21.1 Diagnostics in Immunology

An important point in immunological laboratory diagnostics is the correct collection of material. The most frequently collected material is blood; less often lymph nodes, tissue biopsy samples, bone marrow, bronchoalveolar lavage, cerebrospinal fluid, and saliva. When examining the status of humoral immunity, the concentration of immunoglobulins, acute-phase proteins, complement components, and the presence of autoantibodies are mainly measured. When examining the status of cellular immunity, the number and function of immune cells are determined. The basic examination in assessing a patient's immunity is the blood count, namely the number of individual cells in the blood and the differential blood count. Further examinations then follow.

For serological examination, venous blood is collected into a sterile test tube without an anticoagulant. The blood is allowed to clot, then centrifuged, and the serum separated from the blood clot is transferred into another tube. It is best to process the serum immediately. It may be stored at 4 °C for 2–3 days; otherwise, the serum must be frozen at −20 °C or, for longer-term storage, at −80 °C or in liquid nitrogen. For some tests, serum inactivation is required. Serum inactivation is performed by heating the serum to 56 °C for 30 minutes, thereby inactivating the function of the complement system.

When examining the status of cellular immunity, unclotted blood is used. It is collected into sterile tubes containing an anticoagulant. Heparin and EDTA (ethylenediaminetetraacetic acid) are most commonly used as anticoagulants.

The numbers or functions of various groups of white blood cells may be examined. The examination should be performed as soon as possible after collection (for most tests within 2 hours).

## 21.2 Methods Used for Examination of the State of Cellular Immunity

For analysis of cellular immunity, unclotted blood is required. Methods used to examine cellular immunity include isolation of polymorphonuclear leukocytes and lymphocytes and assessment of their functionality. Dextran is used for isolation of PMNL; it is mixed with blood, and after erythrocyte sedimentation, the plasma layer containing PMNL is aspirated. The obtained leukocytes are used for functional tests: determination of phagocytic activity and phagocytic index; oxidative burst tests by the NBT test (nitroblue

tetrazolium), INT test (iodonitrotetrazolium), or by chemiluminescence. Chemotactic activity of leukocytes is examined in agarose gel, where a chemotactic substance is added to leukocytes at a certain distance.

The standard method for analysis of individual cell populations (including lymphocyte subpopulations) is flow cytometry, in which the cell suspension is most often labeled using monoclonal antibodies conjugated with a fluorochrome.

For isolation of lymphocytes, separation solutions and centrifugation are used. After centrifugation, individual blood components separate into layers, and the mononuclear cell layer is aspirated. These cells may then be further processed, for example by rosette tests, which determine the numbers of T and B lymphocytes, active and total rosettes. To examine functional properties of lymphocytes, blast transformation tests are most commonly used, in which lymphocytes are stimulated by mitogens (concanavalin A, phytohemagglutinin), which has clinical significance mainly in the diagnosis of severe immunodeficiencies. To examine T-cell responses in vivo, skin tests are also used (the most well-known is the tuberculin skin test). Using ELISPOT in vitro, secretion of various lymphokines is determined spontaneously without stimulation and after stimulation of T lymphocytes with antigen.

### 21.3 Methods Used for Examination of the State of Humoral Immunity

Many methods are used to analyze humoral immunity. The complement system and its components (especially C3 and C4) are examined by simple radial immunodiffusion. If a disorder of a component of the classical complement activation pathway is suspected, the CH50 test is performed (or AH50 if a disorder of the alternative pathway is suspected). If hereditary angioedema is suspected, the serum concentration of C1-INH (C1 complement inhibitor) is examined. Lysozyme can be examined in most body secretions. The method for determining lysozyme uses its ability to lyse the cell wall of gram-positive microorganisms (Micrococcus). Acute-phase proteins are very often determined; their concentration increases during the acute phase of inflammation in the body. Among the most frequently measured acute-phase proteins are CRP (C-reactive protein), procalcitonin, and orosomucoid (also referred to as acidic α-1-antitrypsin). They may be determined by simple radial immunodiffusion, but more often by nephelometry or turbidimetry.

Among the basic methods for examining humoral immunity are serological reactions. They use reactions between antigen and antibody that occur in a specific environment. A property of antigens and antibodies is their specific binding. This means that based on one component of the reaction, the other component can be identified. They are used in both direct and indirect diagnostics. In direct diagnostics, they serve for direct detection of antigen in the examined material using known antibodies. An example is serotyping, in

which the antigenic structure of bacteria is determined using polyvalent and monovalent diagnostic sera. More often, however, serological reactions are used in indirect diagnostics. They serve to detect specific antibodies in biological material from the patient as evidence of the antibody response of the organism, using a known antigen.

Agglutination reactions use reactions of particulate antigens with specific antibodies and are used especially for detection of antibodies in various infectious diseases (e.g., Widal reaction, Weil–Felix reaction). If the antigen is soluble and bound to the surface of a suitable macromolecular particle (suitable carriers are latex particles or erythrocytes), this is referred to as latex agglutination or hemagglutination. Precipitation reactions are based on reactions of soluble antigens with specific antibodies. They may occur in liquid medium or in gel (then referred to as immunodiffusion). Simple radial immunodiffusion and double immunodiffusion are most commonly used. In simple immunodiffusion, only one component diffuses into the gel, while the other component is distributed in the gel. In double immunodiffusion, both components—antigen and antibody—diffuse into the gel.

For detection of antibodies in various infectious diseases, complement fixation reactions are also used, based on binding of complement to the antigen–antibody complex. This method is not suitable for determining individual immunoglobulin classes, because total immunoglobulins are detected.

Electrophoresis is also used in examination of humoral immunity; it separates proteins based on their different mobility in a gel medium placed in an electric field. For immunology, the gamma fraction is particularly important because it contains immunoglobulins. Orientational changes such as hypergammaglobulinemia, hypogammaglobulinemia, or monoclonal gammopathies can be detected. A technique combining electrophoresis and immunodiffusion is referred to as immunoelectrophoresis. It combines electrophoretic protein separation with immunodiffusion, which involves antigen–antibody reactions. Classical, rocket, counter-current immunoelectrophoresis and immunofixation are used. Immunofixation is used in the diagnosis of monoclonal gammopathies, especially to determine the isotype of a monoclonal immunoglobulin (paraprotein).

Immunofluorescence techniques are also used to detect antibodies, with formed immune complexes visualized using antibodies labeled with fluorescent dyes.

In modern bioanalytics, immunoanalytical methods are used that allow determination of even low concentrations of antibodies (or antigens) that usually cannot be detected by classical serological methods. Small amounts of various hormones, cytokines, tumor markers, etc., can also be determined. To visualize the reaction between antigen and antibody, a so-called secondary antibody must be used, labeled for example with an enzyme (enzyme immunoassay), radioisotope (radioimmunoassay), luminophore

(chemiluminescent immunoassay), or fluorochrome (fluorescent immunoassay). ELISA is very commonly used in practice.

Blotting techniques are currently widely used because of their high sensitivity and specificity. Blotting techniques combine electrophoresis and immunoanalysis. They are performed in various modifications, but the best known are Western blot and immunodot. They allow simultaneous detection of several specific antibodies against individual antigens from a sample. For determination of specific antibodies against infectious agents, these tests are used as confirmatory tests, as an additional option for specific detection in cases where other methods did not provide a clear result.

In examination of humoral immunity, levels of immunoglobulins and their subclasses are most often determined using several methods: radial immunodiffusion, turbidimetry, nephelometry, enzyme immunoassay ELISA, or radioimmunoassay RIA. For detection of autoantibodies, immunofluorescence, ELISA, immunoblots, immunodots, possibly RIA, and also flow cytometry are used. Newer trends allow detection of autoantibodies using biochips. The gold standard in autoantibody testing, however, remains indirect immunofluorescence. It allows detection of autoantibodies reacting with antigens present in substrate cells. The substrate may be a microtome section of tissue or organ, or a cell suspension, to which a serum sample from the examined patient is added. Each autoantibody bound to its target antigen exhibits characteristic fluorescence depending on antigen localization.

**QUESTIONS**

Which material is most commonly collected for laboratory diagnostics in immunology?

How is serum inactivated?

What type of blood is used for examination of cellular immunity status?

What type of blood is used for serological examination?

Which methods are the basic methods for examination of the state of humoral immunity?

What is the principle of complement fixation reactions?

What does electrophoresis mean?

What are serological reactions?