Setif 1 University – Ferhat ABBAS

Immunology Handout

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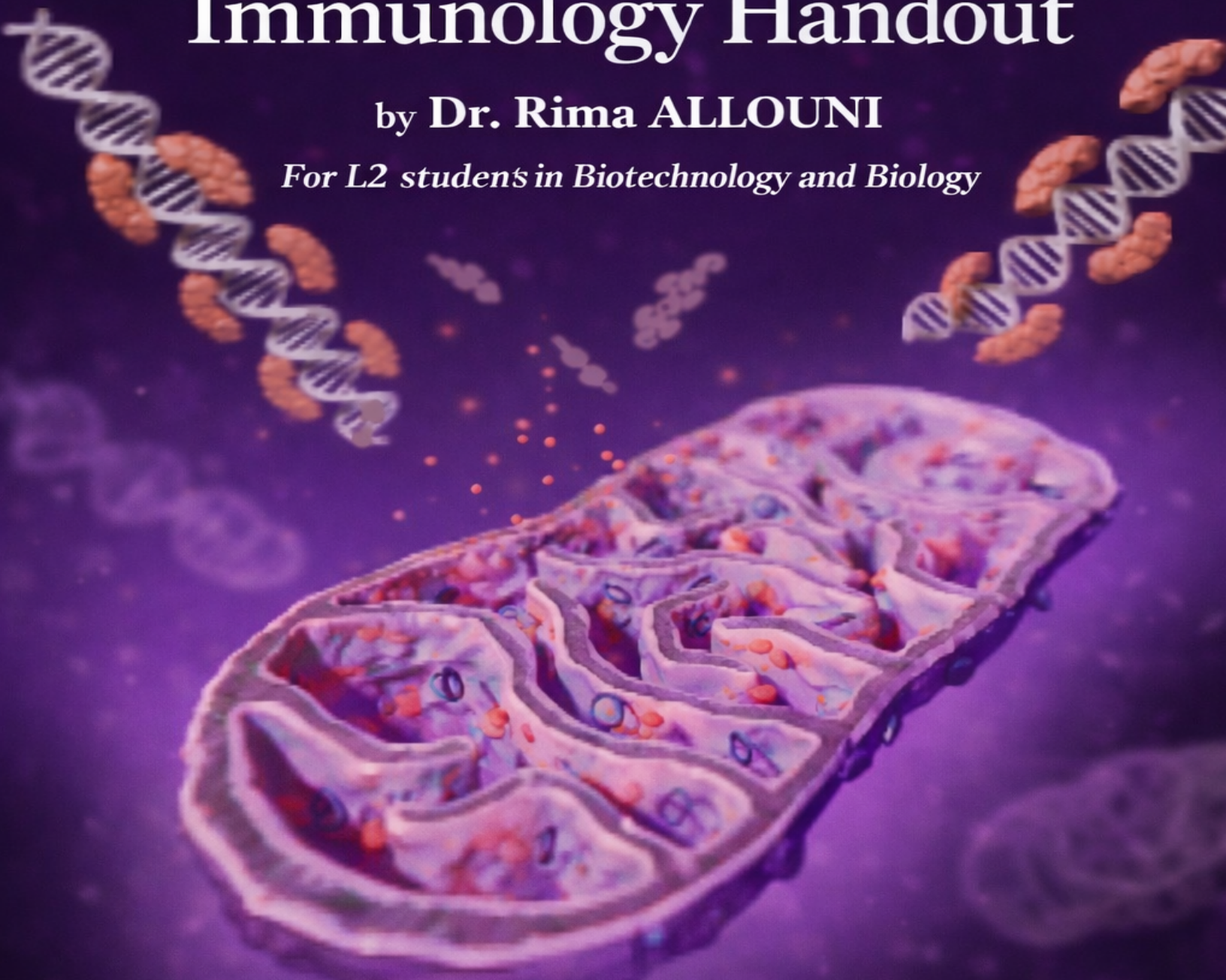
For L2 students in Biotechnology and Biology



Academic year 2025–2026

Semestre: 4ème Semestre

U.E: Unité d'Enseignement Fondamentale 2

Matière 2: Immunologie

Objectif de l'enseignement

L'objectif de cet enseignement est de faire connaître aux étudiants le rôle de l'immunité, les systèmes de défense immunitaire, les types de réponse immunitaire et les dysfonctionnements du système immunitaire.

Connaissances préalables recommandées (*descriptif succinct des connaissances requises pour pouvoir suivre cet enseignement – Maximum 2 lignes).*

L'étudiant doit avoir des notions élémentaires sur le système immunitaire.

Contenu de la Matière

1. Introduction à l'immunologie.

1.1. Rôle de l'immunité

1.2. Rapport avec la quotidienne et grande découverte

2. Ontogénèse du système immunitaire

2.1. Cellules B et organes lymphoïdes

2.2. Cellules T

2.3. Education des cellules B à l'intérieur de la moelle

2.4. Education des cellules T à l'intérieur du thymus

2.5. Autres cellules (Cellules myéloïdes)

3. CMH

4. La réponse immunitaire non spécifique

- Cellules intervenantes et complément

5. La réponse immunitaire spécifique

5.1. Cellulaire

5.2. Humorale

6. Cooperation cellulaire et humorale

6.1. Coopération entre les différentes cellules

6.2. Cytokines

7. Dysfonctionnement du système immunitaire

8. Les principaux tests en immunologie

8.1. Agglutination

8.2. Immuno-précipitation

8.3. Immunoélectrophorèse

8.4. Immunofluorescence

8.5. Elisa Techniques

Travaux Dirigés

TD N°1: Réaction Ag-Ac (précipitation : immunodiffusion, ELISA, RIA….)

TD N°2 : Préparation de lymphocytes de monocytes à partir de sang total

TD N°3 : Séparation de lymphocytes T et B

TD N°4 : Test de lymphomicrocytotoxicité

Mode d'évaluation

Contrôle continu et Examen semestriel

Références

1. Marie-Christine Bené, Yvon Lebranchu, François Lemoine et Estelle Seillès, 2013- Immunologie fondamentale et immunopathologie. Ed. Elsevier Masson, Paris, 260p.

2. Judy Owen, Jenni Punt et Sharon Stranford, 2014- Immunologie. Ed. Sciences de la vie, 832p.

3. Abul-K Abbas et Andrew-H Lichtman, 2013- Les bases de l'immunologie fondamentale et clinique. Ed. Elsevier Masson, Paris, 284p.

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Introduction to Immunology

1. History and major discoveries

- Around 6000 BC, practices of deliberate transmission of smallpox existed in China as a preventive measure. This technique, known as variolation, involved collecting pus from a mildly infected patient and inoculating it into a healthy individual using a needle.
- Variolation became known in England around 1722, notably through the wife of the British ambassador to Constantinople, who had her son inoculated using this method. It subsequently spread throughout Europe in the following years.
- On May 14, 1796, Edward Jenner collected material from a pustule of a young woman infected with cowpox and inoculated it into an eight-year-old boy. After the boy recovered from the mild infection, Jenner exposed him to smallpox. The boy did not develop severe symptoms.

  Edward Jenner is now considered the founder of immunology.

- Another major milestone in immunology was the development of a rabies vaccine by Louis Pasteur in 1885. On July 6, 1885, Pasteur vaccinated Joseph Meister, a nine-year-old boy who had been bitten by a rabid dog two days earlier. Joseph Meister became the first person to survive rabies. Within one year, 350 individuals were vaccinated, and none died from the disease.
- Robert Koch identified the causative agent of tuberculosis (*Mycobacterium tuberculosis*). His work laid the foundation for Calmette and Guérin, who developed the BCG vaccine against tuberculosis.
- In 1888, Émile Roux and Alexandre Yersin discovered the diphtheria toxin.
- At the beginning of the 20th century, immunology research followed two main directions:
  - ☞ **Humoral immunity**, led by Paul Ehrlich and Emil von Behring.
  - ☞ **Cellular immunity**, based on the work of George Nuttall and Ilya Metchnikov, particularly on phagocytosis.
- In 1901, Karl Landsteiner discovered the ABO blood groups.
- In 1906, Clemens von Pirquet introduced the term "allergy" to describe hypersensitivity reactions.
- The phenomenon of anaphylaxis was discovered by Charles Richet.

Modern Immunology

- Between 1959 and 1961, Rodney Porter and Gerald Edelman elucidated the structure of antibodies.
- In 1959, Joseph Murray performed the first successful organ transplantation (kidney allograft).

- In 1980, Jean Dausset, Baruj Benacerraf, and George Snell described the major histocompatibility complex (MHC), known in humans as the HLA system (Human Leukocyte Antigen).

2. Basic concepts in immunology

a. Immunity

Immunity, derived from the Latin "immunitas" *(im*, marking negation; *munus, charge, or tax; immunitas,* dispensation or exemption from burden), initially referred to the resistance of an organism to an infectious agent to which it is exposed. This definition was later extended to include all reactions aimed at eliminating foreign substances. Currently, immunology is defined as the study of the body's defenses against any situation that is potentially harmful to the host (harmful to health and can even lead to death): **i.** recognition and elimination of non-self, such as pathogenic microorganisms responsible for infections; and **ii**. Eliminate stressed, damaged, or pathogenic cells of the "self" (e.g., cancerous, or virus-infected cells).

b. Immune system

IS made up of a set of organs, tissues, cells, and molecules whose distribution covers the different points of the body and which cooperate in the development of immune responses capable of eliminating infectious agents. This system protects the organism against four major groups of pathogens defined according to the immunological mechanisms developed against them and according to their natural habitat (extra- or intracellular): **1.** Bacteria, parasites, and extracellular fungi; **2.** bacteria and intracellular parasites; **3.** viruses (intracellular); and **4.** extracellular parasitic worms.

c. Elements of the immune system

- *Immune System Organs*

The organs of the immune system are divided into two categories:

- Primary lymphoid organs: bone marrow and thymus
- Secondary lymphoid organs: lymph nodes, spleen, and associated lymphoid tissues

- *Cells*

The main cells of the immune system are lymphocytes, which are produced in the bone marrow and mature in peripheral tissues. These include:

- T lymphocytes, responsible for cell-mediated immunity
- B lymphocytes, responsible for humoral immunity and the production of immunoglobulins (antibodies)
- Natural killer (NK) cells, involved in innate immune responses.

A second category consists of antigen-presenting cells (APCs), including macrophages, dendritic cells, and B lymphocytes. Their function is to capture, process, and present antigens to lymphocytes.

A third category includes phagocytic cells, mainly macrophages and neutrophils, which are involved in pathogen elimination.

- *Antigen recognition molecules*

- Receptors on phagocytes:

  Toll-like receptors (TLRs) recognize conserved molecular patterns on microorganisms. In humans, there are approximately 10–12 types identified.

- Receptors on B lymphocytes (BCR):

  The B-cell receptor (BCR) is essentially a membrane-bound immunoglobulin. Immunoglobulins are proteins composed of two heavy chains and two light chains. They are classified into five major classes based on the heavy chain type: IgG (gamma), IgM (mu), IgA (alpha), IgE (epsilon), and IgD (delta).

- Receptors on T lymphocytes (TCR):

  The T-cell receptor (TCR) consists of two polypeptide chains and is associated with the CD3 complex, which is essential for signal transduction.

- *Cytokines*

Cytokines are signaling molecules secreted by various cells that mediate intercellular communication in the immune system. They can act:

- On the same cell → autocrine action
- On nearby cells → paracrine action
- At a distance → endocrine-like action

Cytokines include interleukins, chemokines, interferons, and tumor necrosis factors (TNF). They are generally low-molecular-weight proteins (< 30 kDa).

Cytokines exhibit several biological properties:

- Pleiotropy: one cytokine can act on different cell types

- Redundancy: different cytokines can have similar effects
- Synergy: combined effects are enhanced

- *Major Histocompatibility Complex (MHC)*

The major histocompatibility complex (MHC) is a group of genes encoding cell surface molecules involved in antigen presentation. These molecules are classified into three classes: Class I, Class II, and Class III.

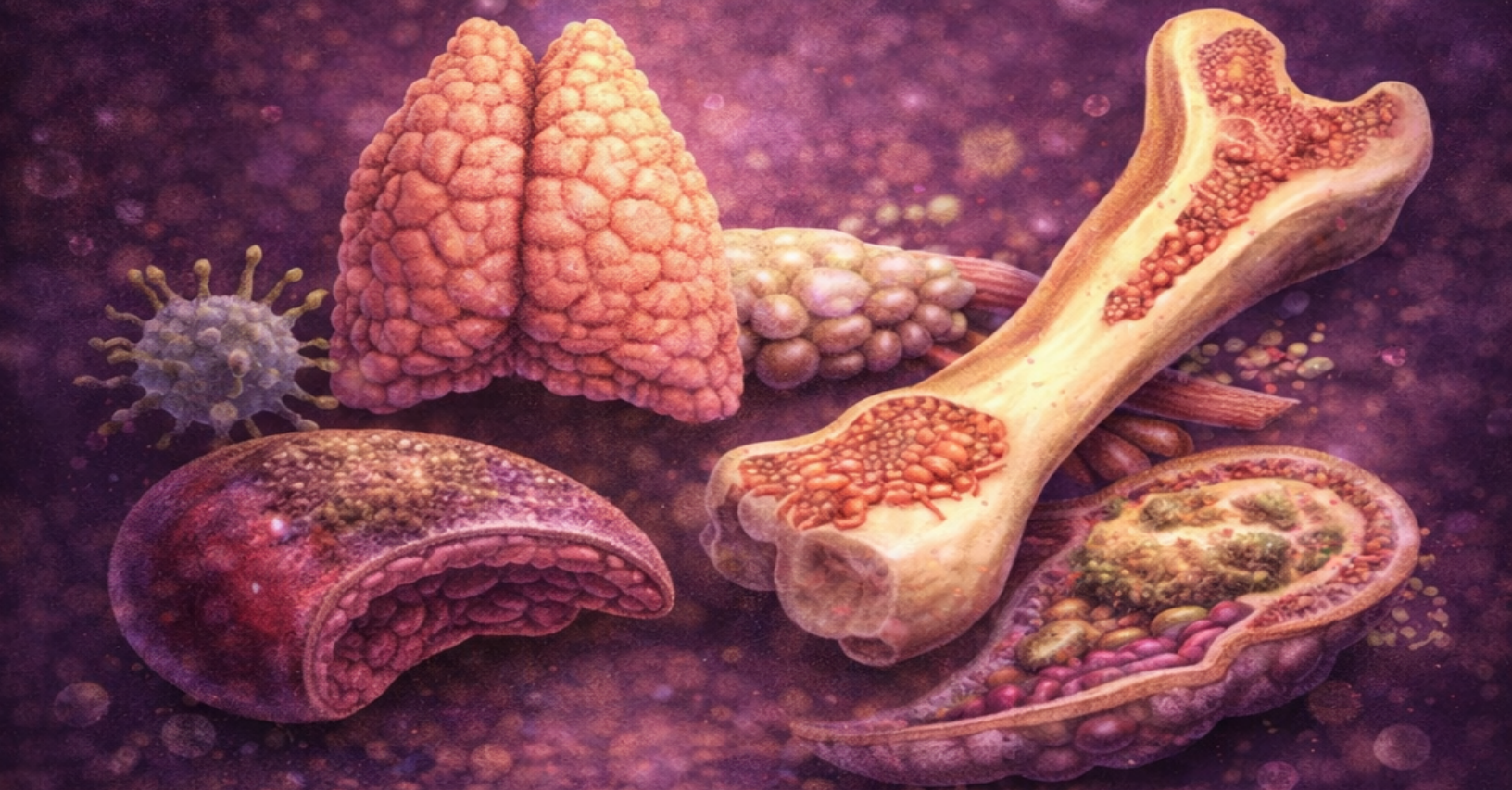
- *Complement system*

The complement system consists of a group of plasma proteins that circulate in an inactive form. Upon activation, they trigger a cascade reaction leading to various immune effector functions, including pathogen elimination and inflammation.

d. Natural immunity and specific immunity

Immunity involves two major processes that have evolved during species evolution: non-specific (innate) immunity and specific (adaptive) immunity. Non-specific immunity acts immediately and involves cells responsible for phagocytosis. It relies on the protective functions of mucocutaneous barriers and on coordinated interactions between plasma protein systems (such as complement and coagulation proteins) and immune cells, including neutrophils, macrophages, and natural killer (NK) cells. This form of immunity is responsible for the inflammatory response. In contrast, specific immunity develops over several days and depends on the specific recognition of antigens, leading to their elimination and the establishment of immunological memory. This immunity involves highly specific molecules known as antigen receptors, including antibodies produced by B lymphocytes, as well as T-cell receptors (TCRs) and B-cell receptors (BCRs) expressed on lymphocytes.

Chapter I.
Organs and Tissues of Immune System



I. Organs and Tissues of The Immune System

I.1. Immune system organs

The immune system (IS) is composed of specialized organs and tissues through which cells of innate and adaptive immunity, known as immunocompetent cells, continuously circulate. Through complex communication mechanisms involving both soluble and membrane-bound molecules, the immune system generates and regulates effector responses that ensure protection of the host.

The lymphoid system comprises two categories of organs (**Fig. 1.1**):

-Primary (central) lymphoid organs, namely the bone marrow and thymus, which are the principal sites of lymphocyte development and maturation, where cells acquire immunological competence, including the expression of antigen receptors (B-cell receptors, or BCR, and T-cell receptors, or TCR).

-Secondary (peripheral) lymphoid organs and tissues, which include encapsulated organs such as the lymph nodes and spleen, as well as diffuse lymphoid tissues collectively referred to as mucosa-associated lymphoid tissue (MALT). These sites are responsible for the capture, processing, and presentation of antigens to immune cells involved in the immune response.

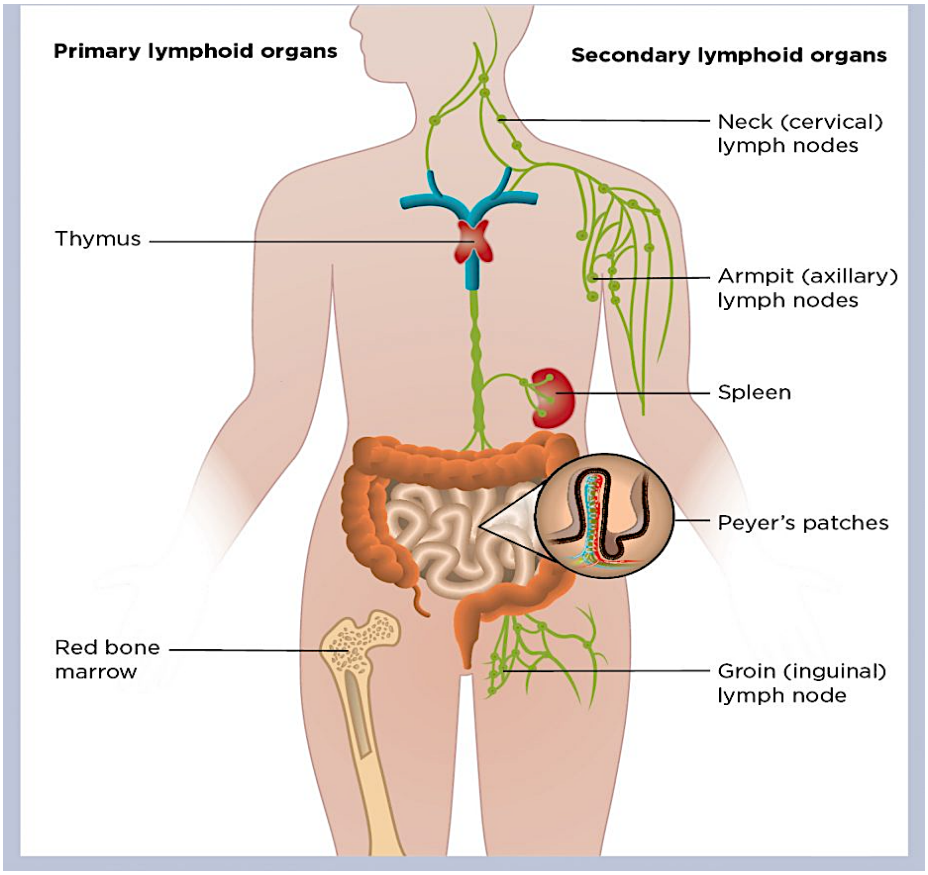


Figure 1.1. The lymphoid organs in adults.

I.1.1. Central (primary) lymphoid organs

a. Bone marrow

➢ **Definition**

Bone marrow is the soft tissue located within the medullary cavities of bones. In adults, hematopoietic activity (hematopoiesis) is primarily confined to the bone marrow of flat bones (sternum, ribs, vertebrae, pelvis, and skull) and the proximal ends of long bones such as the humerus and femur. These regions contain red bone marrow, which is rich in multipotent hematopoietic stem cells (HSCs). In contrast, yellow bone marrow, composed mainly of adipocytes, has little or no hematopoietic activity (**Fig. 1.2**). Hematopoietic stem cells possess the capacity for self-renewal and can differentiate into all blood cell lineages.

In addition to its role in blood cell production, the bone marrow is the primary site of B-lymphocyte maturation. It also contains stromal cells, which form a supportive microenvironment that regulates the proliferation and differentiation of hematopoietic stem cells.

Note: *In birds, B-lymphocyte development occurs in a specialized organ called the bursa of Fabricius, which is functionally analogous to the bone marrow in mammals.*

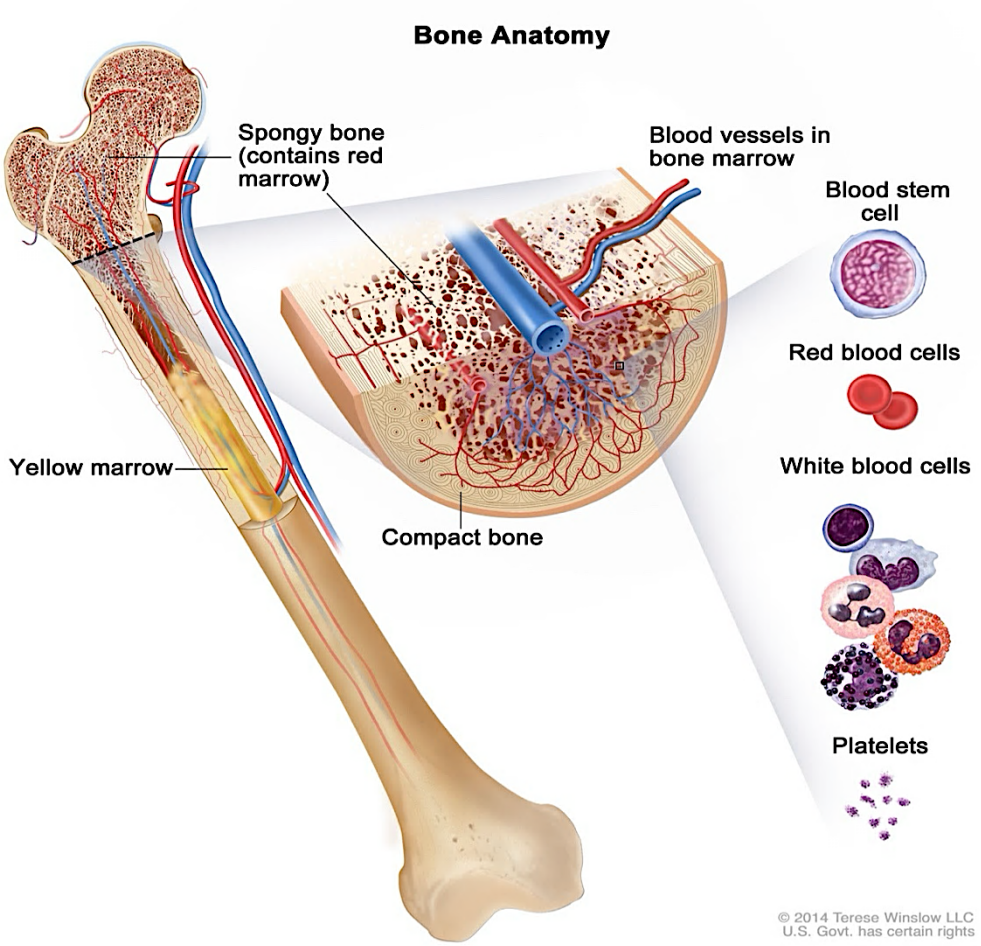


Figure 1.2. The bone marrow of the long bones.

➢ Hematopoiesis

Hematopoiesis is the physiological process responsible for the production, differentiation, and renewal of all blood cells, including immune cells, erythrocytes, and platelets, from multipotent hematopoietic stem cells (HSCs). This process is tightly regulated at both central and peripheral levels by cytokines and transcription factors, ensuring the homeostasis of blood cell populations. Cell proliferation and differentiation are balanced by programmed cell death (apoptosis).

Hematopoietic stem cells give rise to two major progenitor lineages. The myeloid lineage produces erythrocytes, platelets, monocytes/macrophages, neutrophils, eosinophils, basophils, mast cells, and dendritic cells. The lymphoid lineage gives rise to T lymphocytes, B lymphocytes, and natural killer (NK) cells (**Fig. 1.3**).

In addition, dendritic cells (DCs) may arise from myeloid progenitors (conventional DCs) or from distinct precursors giving rise to plasmacytoid DCs (pDCs).

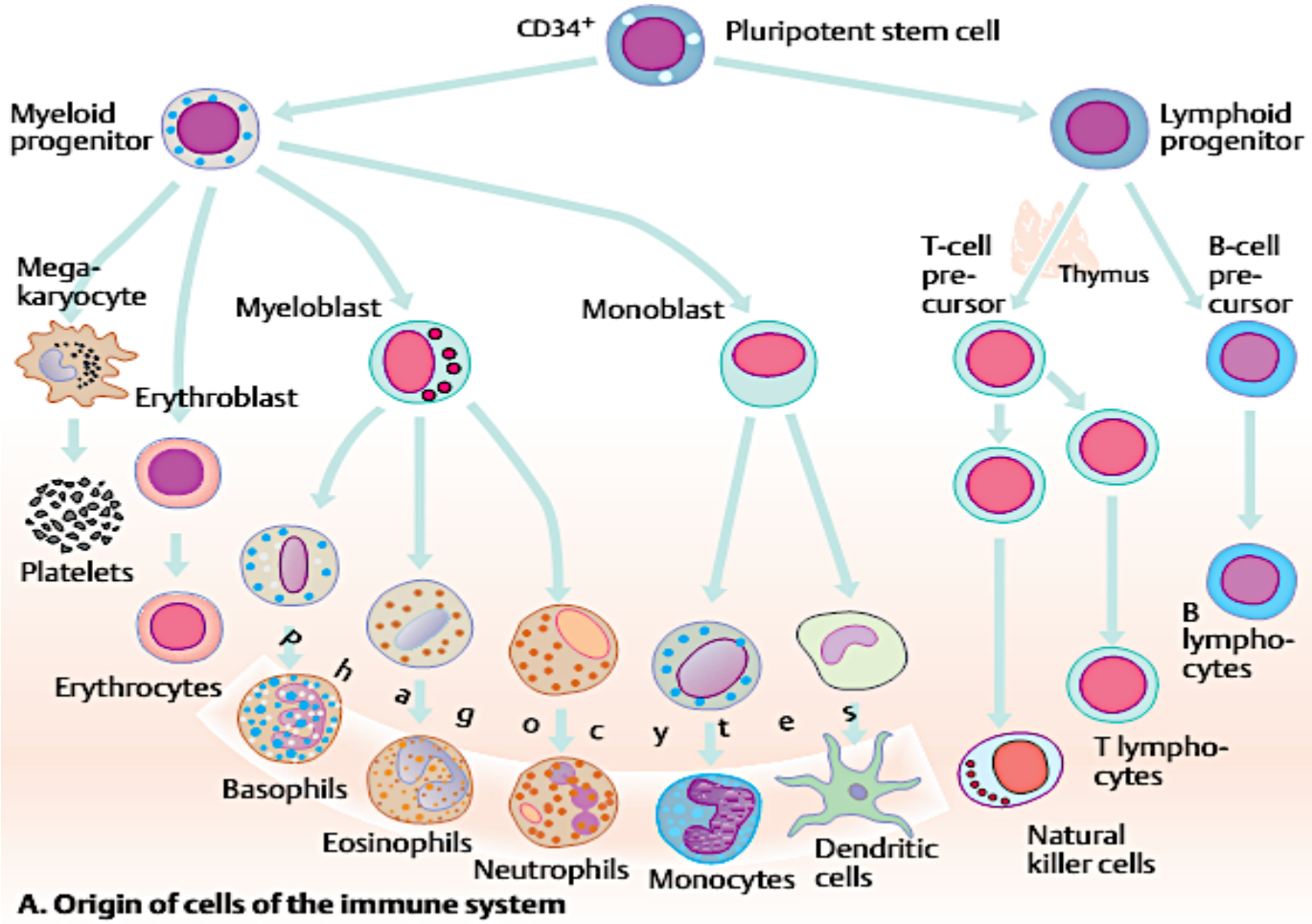


Figure 1.3. Origin of immune system cells.

b. Thymus

The thymus is a primary lymphoid organ of lymphoepithelial origin, located in the anterior superior mediastinum, above the heart. It increases in size until puberty, after which it undergoes involution, although it does not completely disappear. The thymus consists of two lobes enclosed by a capsule and divided into lobules by connective tissue septa. Its blood supply is provided by branches of the thoracic arteries.

Each lobule is organized into two distinct regions:

- ☞ The **cortex**, a peripheral zone densely populated by immature thymocytes derived from bone marrow progenitors.
- ☞ The **medulla**, a central zone containing fewer and more mature T lymphocytes (**Fig. 1.4**).

Both regions contain epithelial cells, dendritic cells, and macrophages. Thymic epithelial cells produce factors that are essential for the maturation and selection of thymocytes. In the medulla, epithelial cells form characteristic structures known as Hassall's corpuscles. Dendritic cells and macrophages function as antigen-presenting cells (APCs).

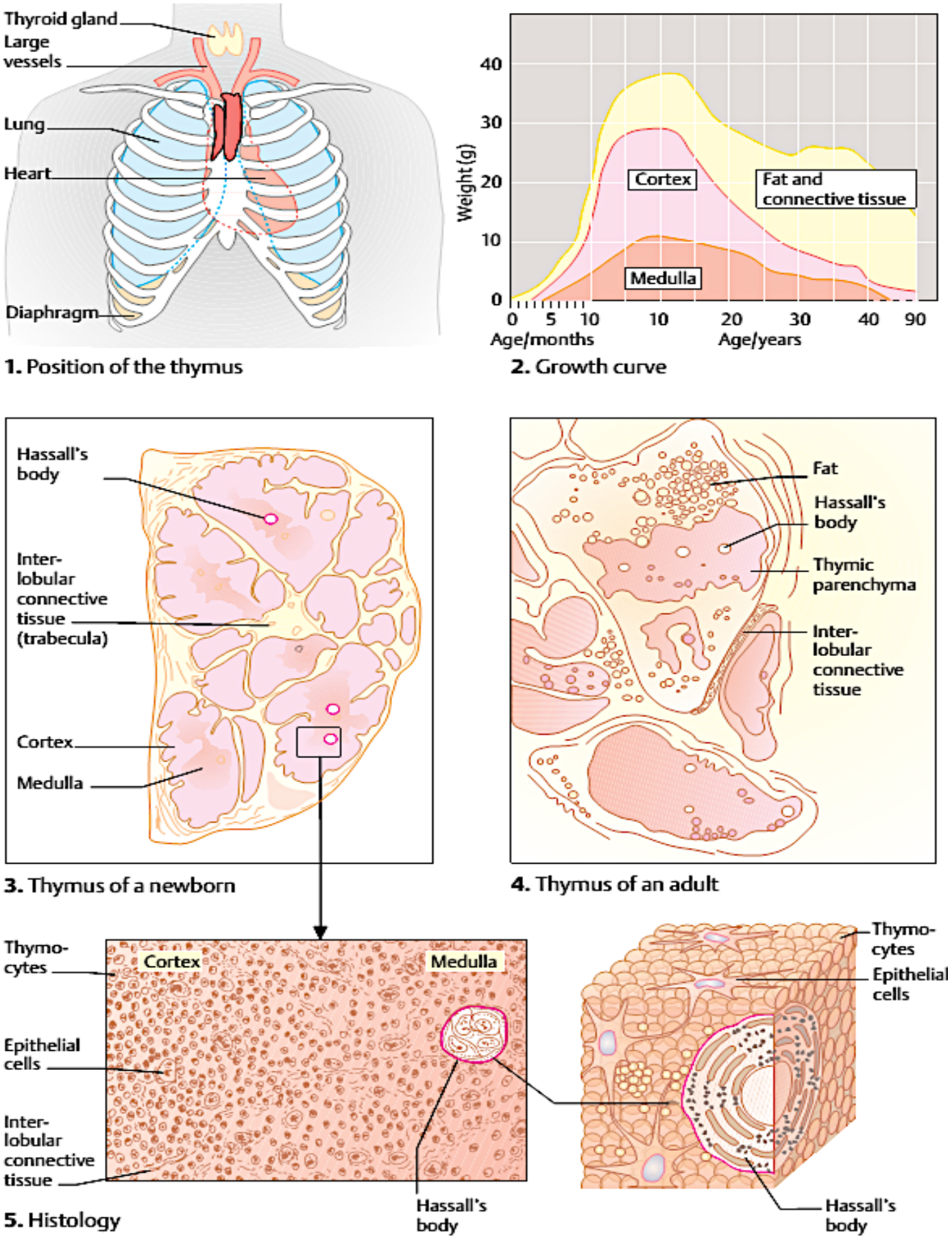


Figure 1.4. Anatomy and development of thymus.

I.1.2. Peripheral (secondary) lymphoid organs

a. Spleen

The spleen is an intraperitoneal organ located in the left hypochondrium. Unlike lymph nodes, it is directly connected to the blood circulation and functions primarily in the filtration of blood, including the removal of senescent erythrocytes and blood-borne pathogens. It is the largest lymphoid organ, measuring approximately 12 × 7 × 4 cm and weighing about 200 g.

The spleen is composed of two main compartments:

- ☞ The **red pulp** (approximately 80% of the splenic volume), which is responsible for the destruction of aged red blood cells and serves as a reservoir of erythrocytes.
- ☞ The **white pulp** (approximately 20%), which consists of organized lymphoid tissue. It includes periarteriolar lymphoid sheaths (PALS) rich in T lymphocytes. Surrounding these areas is the marginal zone, which contains B lymphocytes, dendritic cells, and macrophages and plays a key role in initiating immune responses (**Fig. 1.5A**).

b. Lymph nodes

The lymphatic system consists of lymphatic vessels and lymph nodes. Lymph is an interstitial fluid formed by the filtration of blood plasma through the walls of capillaries. It is collected by small lymphatic capillaries in the tissues and transported through progressively larger lymphatic vessels toward the lymph nodes and ultimately back into the bloodstream.

Lymph nodes are secondary lymphoid organs distributed throughout the body (approximately 1000 in humans). They are small, rounded or kidney-shaped structures (1 to 15 mm in diameter), surrounded by a capsule. Lymph enters the node through afferent lymphatic vessels, flows through the subcapsular and medullary sinuses, and exits via efferent lymphatic vessels at the hilum.

Lymph nodes perform a dual function: the elimination of pathogens through phagocytosis by macrophages and the initiation of adaptive immune responses, including the activation and proliferation of T and B lymphocytes. The lymph node parenchyma is organized into three regions (**Fig. 1.5B**):

- ☞ The **cortex**, a B-cell-dependent zone containing primary and secondary lymphoid follicles composed of B lymphocytes, macrophages, and follicular dendritic cells (FDCs).

- ☞ The **paracortex**, a T-cell-dependent region rich in T lymphocytes and interdigitating dendritic cells (IDCs). This is the site where lymphocytes enter from the blood and where key cellular interactions occur.
- ☞ The **medulla**, which contains a mixture of B and T lymphocytes, plasma cells, and macrophages.

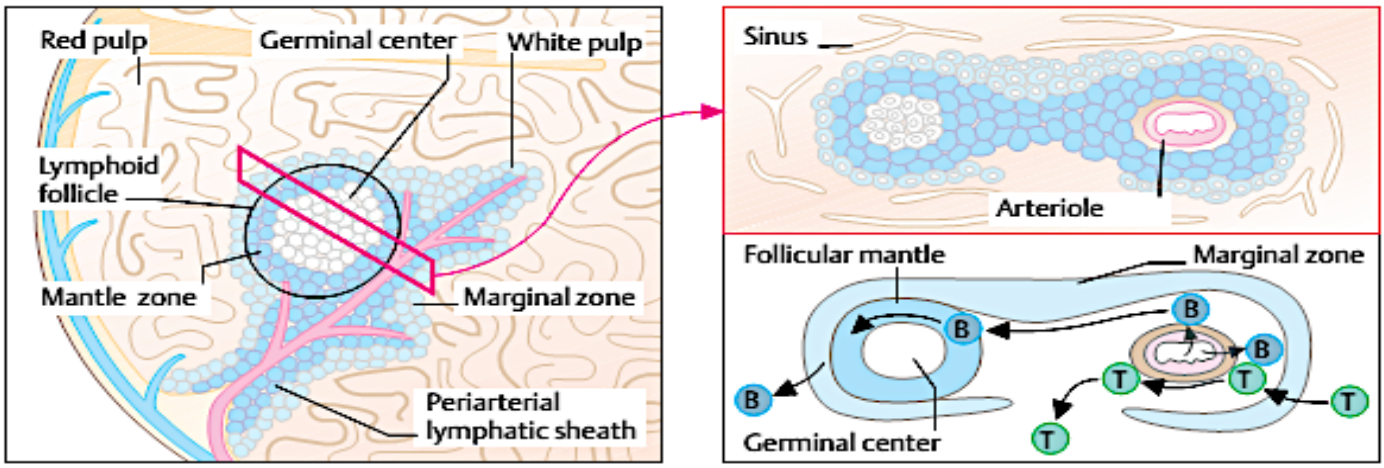
Antigens carried by the lymph are captured by antigen-presenting cells (APCs) and presented to T lymphocytes. In humoral immune responses, activated helper T cells interact with B lymphocytes in the cortical follicles, leading to their differentiation into plasma cells.

c. Mucosa-associated lymphoid tissues

Mucosa-associated lymphoid tissue (MALT) protects extensive mucosal surfaces, estimated at several hundred square meters, that are exposed to environmental agents, including the ocular, respiratory, digestive, and urogenital mucosa. At these sites, there is a predominance of the humoral immune response, characterized by the production of IgA antibodies. These antibodies are transported across epithelial cells by transcytosis, forming secretory IgA that contributes to mucosal protection (**Fig. 1.5C**).

In the digestive tract, MALT includes organized lymphoid structures such as Peyer's patches, which are part of the gut-associated lymphoid tissue (GALT). GALT contains a large proportion of the body's immune cells and includes specialized M cells, which facilitate the uptake and transcytosis of antigens and microorganisms from the intestinal lumen.

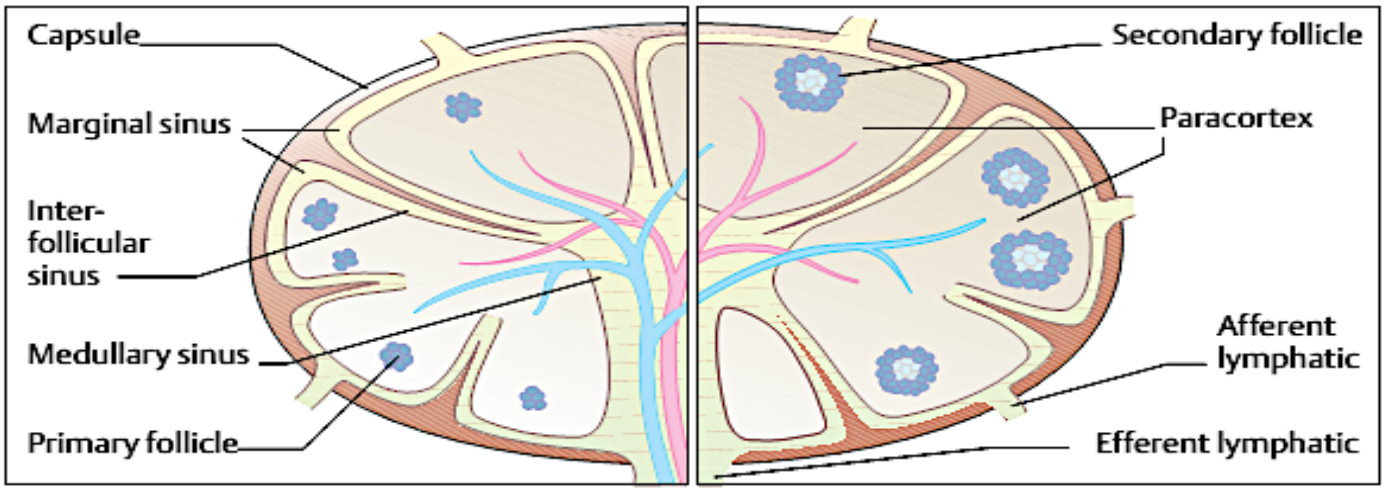
In the respiratory system, lymphoid tissues associated with the bronchi form the bronchus-associated lymphoid tissue (BALT). In contrast, lymphoid structures located at the entrance of the upper airways, such as the tonsils and adenoids, are part of Waldeyer's ring.



1. Anatomic structure

2. Cross-section through arteriole and follicle; lymphocyte circulation

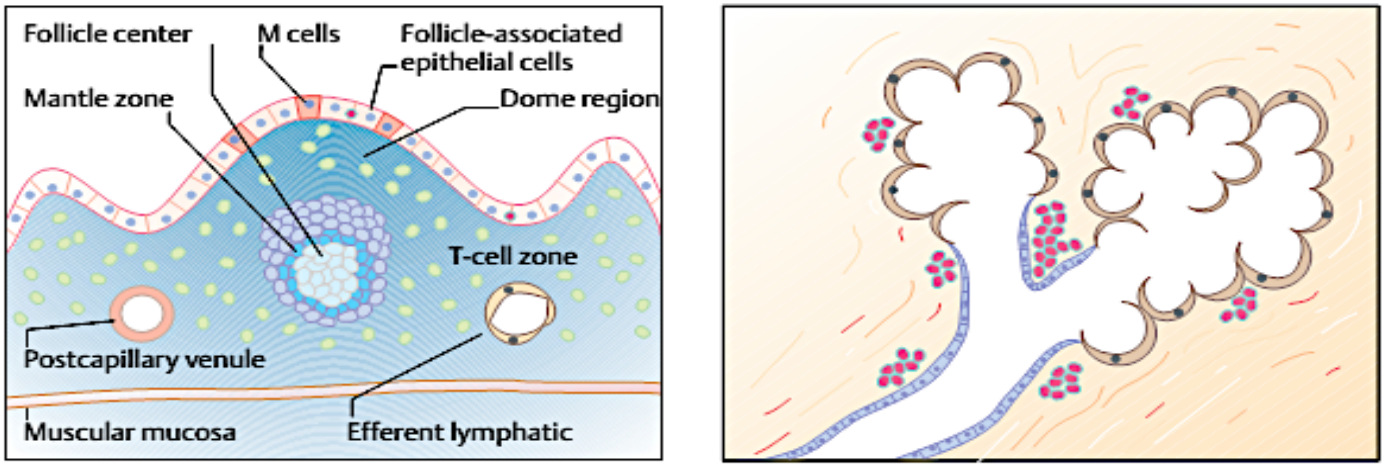
A. Structure of the spleen



1. Inactive lymph node

2. Active lymph node

B. Structure of the lymph node



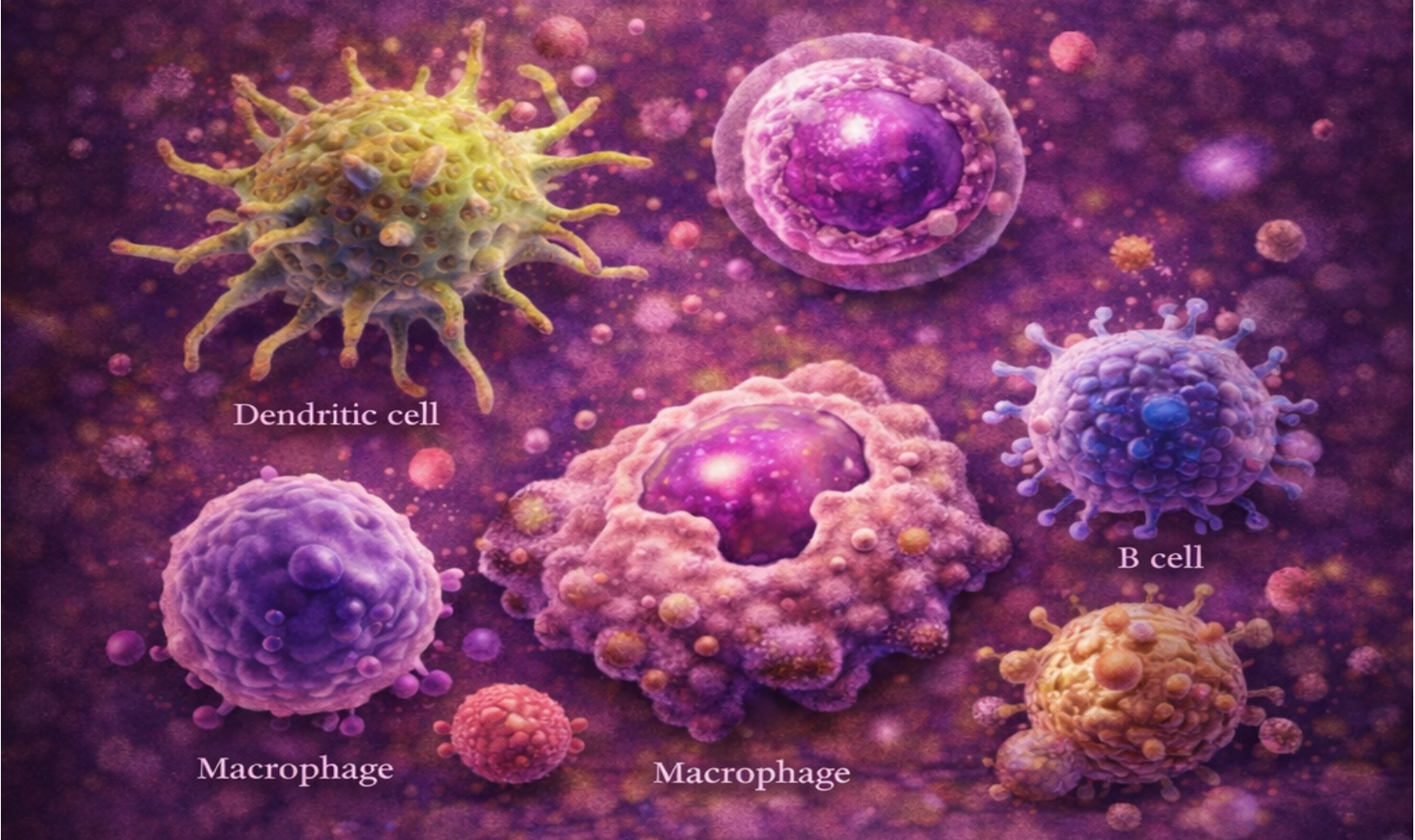
1. GALT: Gut-associated lymphoid tissue; Peyer's patch

2. BALT: Bronchus-associated lymphoid tissue

C. Mucosa-associated lymphoid tissue

Figure 1.5. Structure of secondary lymphoid organs: A: spleen, B: lymph nodes, and C: mucosa-associated lymphoid tissues (MALT).

Chapter II.
Cells of
The Immune System



Chapter II. Cells of the Immune System

II.1. CD system

Cluster of differentiation (CD) refers to a classification system of cell surface molecules identified by specific antibodies and designated by a number (e.g., CD4, CD8) (**Tab. 2.1**). These molecules serve as cell surface markers used to identify cell types, determine their stage of differentiation, and assess their functional state. CD molecules include a variety of proteins, such as receptors, glycoproteins, cell adhesion molecules (CAMs), and membrane-bound enzymes, while the functions of some CD markers remain unknown.

Table 2.1: Some examples of CD surface markers.

CD1 = a molecule of the major histocompatibility complex (MHC) that contains lipid molecules. CD3 characterizes T lymphocytes and TCR signal transduction. CD4 characterizes T helpers and monocyte lymphocytes. CD5 = unknown function. CD8 = characterizes cytotoxic T lymphocytes.
CD14 is an LPS receptor (surface antigen of gram-negative bacteria) that interacts with the Toll-like receptor 4 (TLR4), which causes the synthesis and migration into the nucleus of the transcription factor NFκB.
CD16 characterizes NK cells, a receptor with low affinity for the Fc region of IgG (FcγRIII). CD21 is CR2 of B cells, a receptor for C3d and the Epstein-Barr virus (mononucleosis and certain cancers). CD28 = receptor on T cells for the B7 molecule of co-stimulation of CPAs. CD40 = transduction of the B cell activation signal (CD40L = CD154 of TH). CD360 = interleukin-21, IL-21R receptor.

II.2. Natural immune cells

Innate immune cells express pattern recognition receptors (PRRs), which recognize conserved molecular structures and do not undergo genetic rearrangement, resulting in limited receptor diversity compared to adaptive immune receptors. PRRs detect two major types of molecular signals:

- ☞ Pathogen-associated molecular patterns (PAMPs), which are conserved structures present in microorganisms but absent from host cells. These patterns are shared among groups of pathogens and are often essential for their survival or infectivity (e.g., lipopolysaccharide (LPS) in Gram-negative bacteria and flagellin) **(Fig. 2.1, Tab. 2.2).**
- ☞ Damage-associated molecular patterns (DAMPs), which are released by stressed, damaged, or necrotic cells (e.g., HMGP-1) **(Fig. 2.2)**.

The interaction between PRRs and their ligands (PAMPs or DAMPs) triggers the activation of the innate immune response, leading to inflammation and contributing to the initiation of the adaptive immune response.

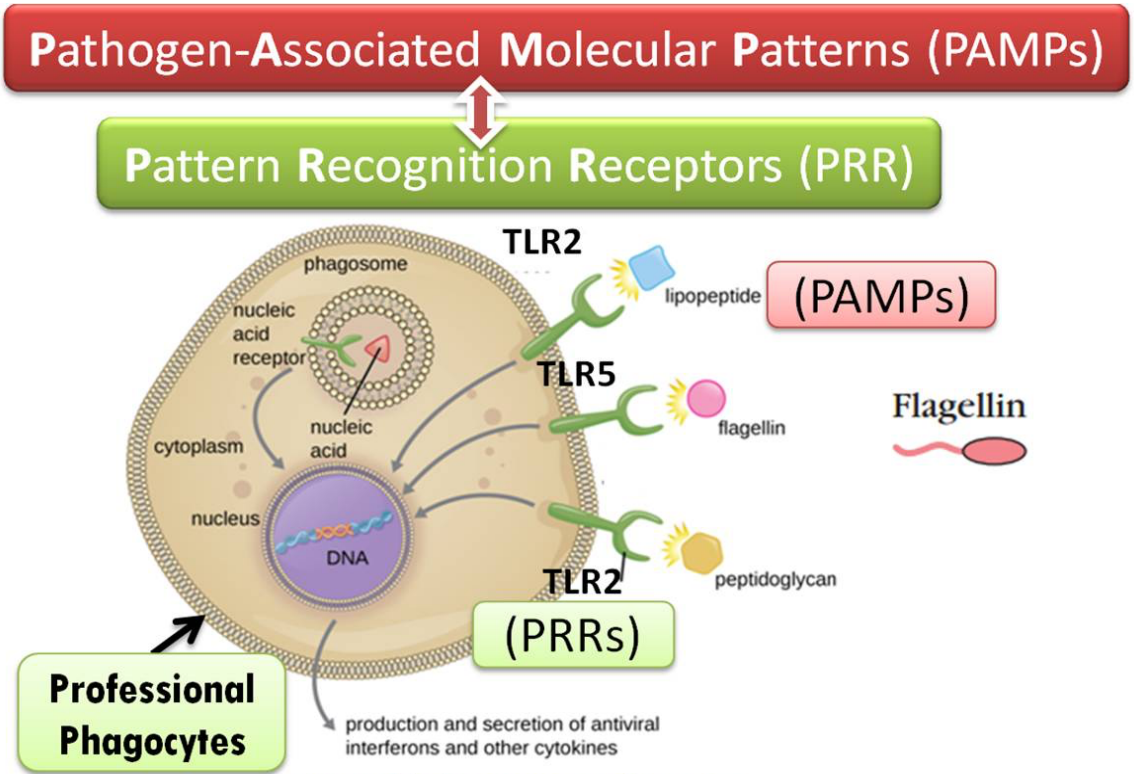


Figure 2.1. Pathogen-associated molecular patterns and PRRs.

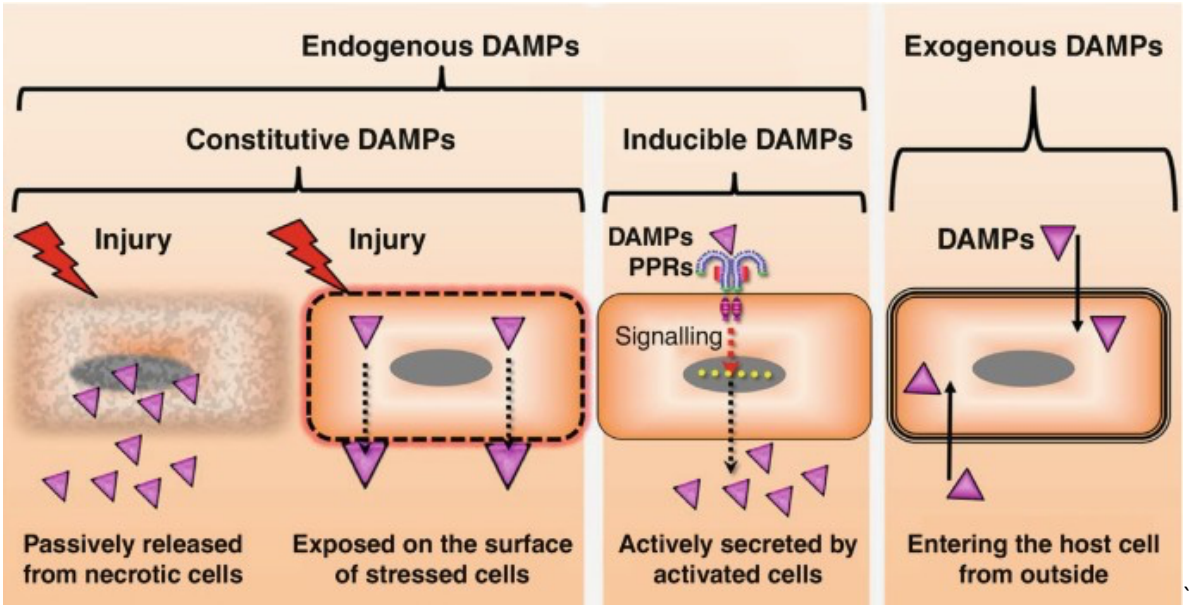


Figure 2.2. Damage-associated molecular patterns.

Depending on the location of the PRRs, a distinction is made between the following:

- **Soluble (secreted) PRRs** are present in body fluids (**Fig. 2.2**). These include:
  - Mannose-binding lectin (MBL), which binds to mannose residues on the surface of microorganisms and activates the complement system.
  - C-reactive protein (CRP), which acts as an opsonin by binding to pathogens and facilitating their clearance.
  - Lipopolysaccharide-binding protein (LBP), which binds to bacterial LPS and enhances its recognition by immune cells.

- **Membrane-bound PRRs,** including endocytic receptors, are more diverse and are involved in phagocytosis, activation of the inflammatory response, and initiation of antiviral responses. Major examples include:
  - Mannose receptors (MMR), lectin receptors, complement receptors, and scavenger receptors, which play key roles in phagocytosis.
  - Toll-like receptors (TLRs), which are involved in the activation of inflammatory and antiviral signaling pathways.

These receptors are primarily expressed on professional immune cells, such as macrophages and dendritic cells, but are also found on other cell types, including epithelial and endothelial cells.

As mentioned earlier, Toll-like receptors (TLRs) play a key role in the innate immune response by recognizing pathogen-associated molecular patterns (PAMPs) and activating signaling pathways that lead to inflammatory responses, antiviral defenses, and the enhancement of phagocytic activity. Some TLRs recognize surface components of microorganisms, whereas others detect nucleic acids derived from viruses or intracellular pathogens (**Fig. 2.3**).

- **Cytoplasmic PRRs (intracellular signaling receptors)**

In addition to Toll-like receptors (TLRs), which represent a major class of innate immune receptors, other families of cytoplasmic pattern recognition receptors (PRRs) are involved in the detection of intracellular pathogens. Among these, the most well-characterized are the NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs), which recognize bacterial and viral components within the cytoplasm (**Fig. 2.3**).

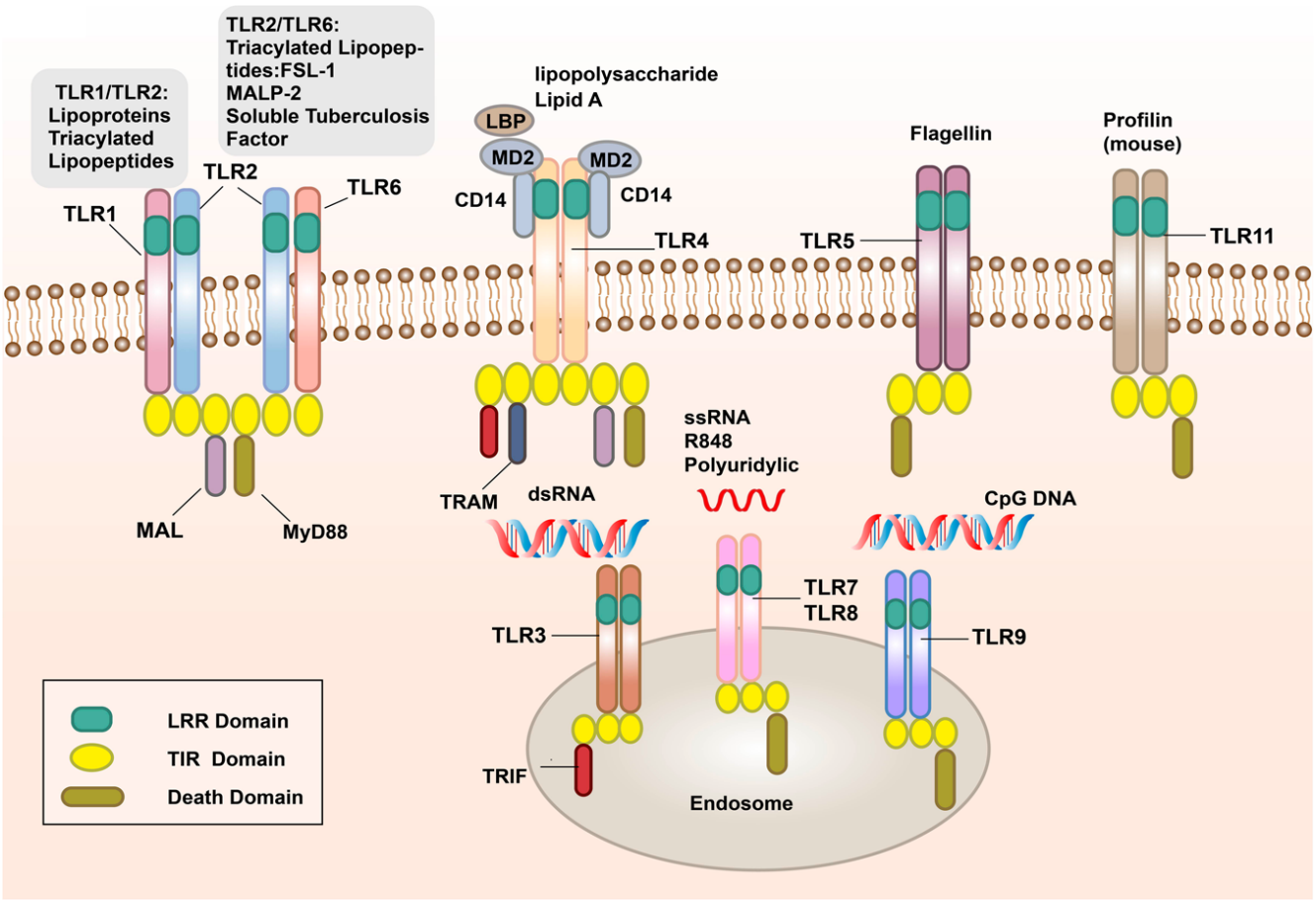


Figure 2.3. The different PRRs according to their location and their ligand.

Table. 2.2. Major types of PAMPs and major families of PRRs.

Major types of PAMPs	Major families of PRRs
1. Bacterial PAMPs • Lipopolysaccharide (LPS) → Gram-negative bacteria • Peptidoglycan → Gram-positive bacteria • Lipoteichoic acid → Gram-positive bacteria • Flagellin → bacterial flagella • Unmethylated CpG DNA → bacterial DNA	**1. Toll-like receptors (TLRs)** Located on: Cell surface and endosomal membranes. Examples: • TLR4 → recognizes LPS. • TLR3 → recognizes dsRNA. • TLR5 → recognizes flagellin.
2. Viral PAMPs • Double-stranded RNA (dsRNA) • Single-stranded RNA (ssRNA) • Viral DNA in the cytoplasm	**2. NOD-like receptors (NLRs)** • Located in the cytoplasm. • Detect intracellular bacterial components. • Activate the inflammasome, leading to: ☞ IL-1β production ☞ IL-18 production
3. Fungal PAMPs • β-glucans • Mannans • Chitin	**3. RIG-I-like receptors (RLRs)** • Cytoplasmic receptors • Detect viral RNA. • Trigger type I interferon production
4. Parasitic PAMPs • Glycosylphosphatidylinositol (GPI) anchors • Helminth glycans	**4. C-type lectin receptors (CLRs)** • Recognize fungal carbohydrates (e.g., β-glucans) • Important in antifungal immunity

II.2.1. Phagocytes

Phagocytes are specialized immune cells capable of engulfing and eliminating pathogens and cellular debris through the process of phagocytosis. Major phagocytic cells include macrophages, dendritic cells, and neutrophils.

a. Monocytes

Monocytes are circulating leukocytes that originate from the bone marrow and represent approximately 2-5% of white blood cells. They circulate in the bloodstream for 1 to 3 days before migrating into tissues, where they differentiate into macrophages or dendritic cells (**Fig. 2.4**).

- **Morphology:** Monocytes are large cells (10-20 μm) with an irregular, often horseshoe-shaped nucleus and contain azurophilic granules rich in enzymes such as esterases, lipases, and peroxidases.

After leaving the bloodstream, monocytes migrate into tissues by diapedesis (transendothelial migration) and undergo differentiation into macrophages.

b. Macrophage

Macrophages are professional phagocytes derived from monocytes and are widely distributed throughout tissues. Depending on their location, they receive specific names, such as Kupffer cells (liver), microglial cells (central nervous system), alveolar macrophages (lungs), histiocytes (connective tissue), and osteoclasts (bone).

- **Morphology:** During differentiation, macrophages increase in size and cellular complexity, with a higher number of organelles, including mitochondria, and an enhanced phagocytic capacity (**Fig. 2.4**). Their lifespan ranges from weeks to months.
- **Granules:** Macrophages contain enzymes such as lysozyme, proteases, collagenase, elastase, and acid hydrolases, which contribute to their degradative functions.
- **Receptors:** Macrophages express a wide range of surface receptors, including pattern recognition receptors (PRRs); MHC class I and II molecules, co-stimulatory molecules (e.g., B7), Fcγ receptors (FcγR), complement receptors (CR1, CR3); CD14 and mannose receptors, and adhesion molecules (e.g., LFA-1, ICAM-1).
- **Function:** Macrophages and monocytes perform three major functions:

- ☞ Phagocytosis of pathogens and debris
- ☞ Antigen presentation, involving the presentation of peptide fragments to T lymphocytes
- ☞ Secretion of cytokines and other mediators, which regulate the immune response

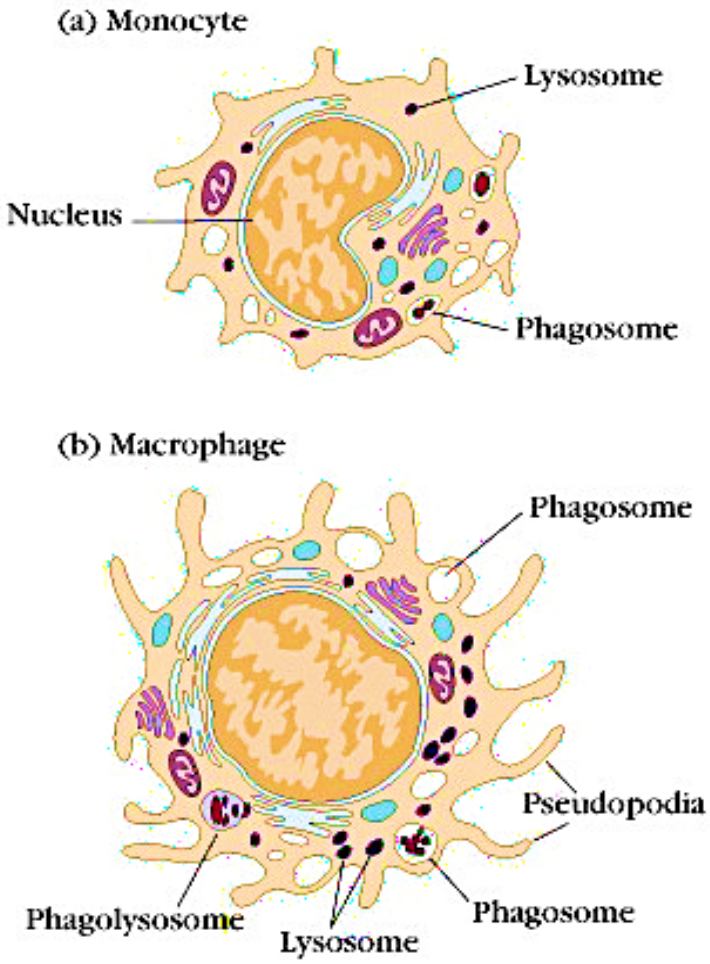


Figure 2.4. Mononuclear phagocytes.

c. Dendritic cells

Dendritic cells (DCs) are specialized immune cells characterized by cytoplasmic extensions known as dendrites. They are widely distributed throughout the body, particularly in barrier tissues such as the skin and mucosa, as well as in lymphoid organs (**Fig. 2.5**).

Dendritic cells arise from two main lineages:

- ☞ Myeloid dendritic cells, derived from hematopoietic progenitors (and, under certain conditions, from monocytes)
- ☞ Plasmacytoid dendritic cells, which represent a distinct lineage

- **Receptors:** Dendritic cells express a broad range of surface molecules, including pattern recognition receptors (PRRs) (both endocytic and signaling receptors), MHC class I and class II molecules, and co-stimulatory molecules (e.g., B7 family).
- **Functions:** Dendritic cells are specialized in the capture, processing, and presentation of antigens to T lymphocytes, thereby playing a central role in the initiation of adaptive immune responses.

In the thymus, dendritic cells contribute to the maintenance of self-tolerance by participating in the negative selection of T lymphocytes.

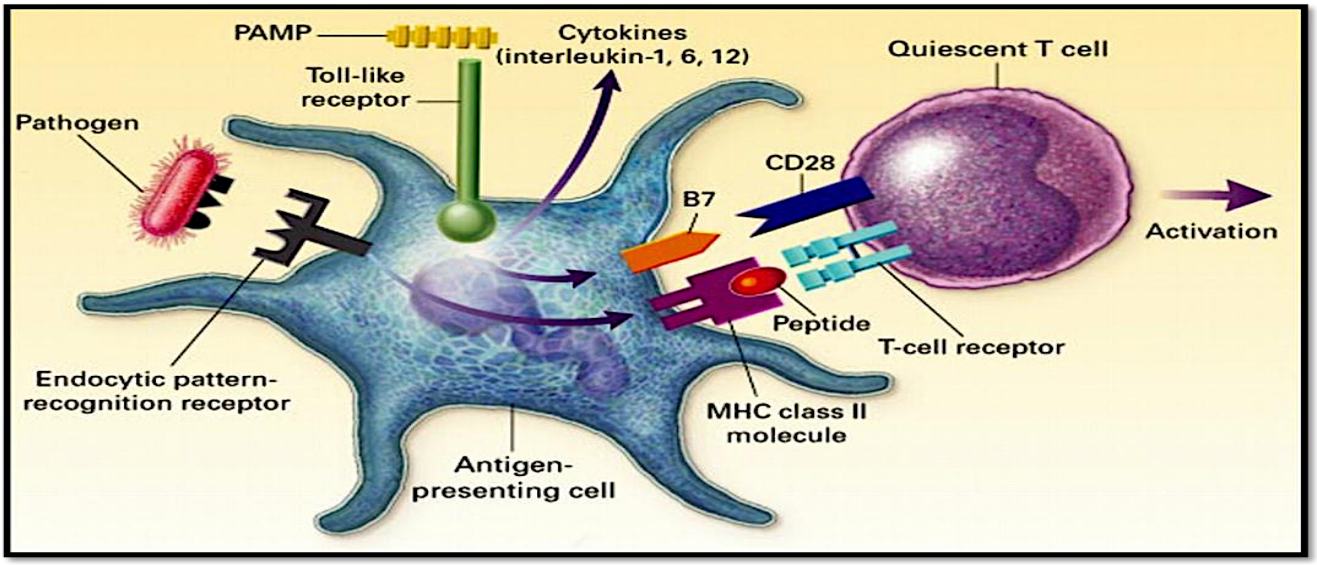


Figure 2.5. The dendritic cells.

- **Polymorphonuclear leukocytes (Granulocytes)**

Polymorphonuclear leukocytes (PMNs) are granulocytic leukocytes originating from the bone marrow. They include three main types: neutrophils, eosinophils, and basophils.

a. Neutrophils

Neutrophiles are the most abundant leukocytes in the blood (60–70%). They circulate for approximately 6-10 hours and survive in tissues for 1-2 days, during which they exert their biological functions (**Fig. 2.6**).

- **Morphology:** Neutrophils are approximately 10-15 µm in diameter, with a multilobed nucleus (3-5 lobes) and cytoplasmic granules that stain weakly with both acidic and basic dyes.
- **Granules:** are two types,

- ☞ Primary (azurophilic) granules: myeloperoxidase, defensins, lysozyme, acid hydrolases, proteases
- ☞ Secondary granules: lactoferrin, collagenase, gelatinase

- ➢ **Receptors:** Neutrophils express a wide range of receptors, including Fcγ receptors (FcγRII, FcγRIII), complement receptors (CR1, CR3), anaphylatoxin receptors (C3aR, C5aR), pattern recognition receptors (TLRs), and chemotactic receptors (e.g., CXCR1, CXCR2, FMLP receptor).
- ➢ **Functions:** Neutrophils are key cells of the first line of defense, performing the following:

  - ☞ Phagocytosis
  - ☞ Microbial killing (ROS, enzymes)
  - ☞ Cytokine secretion, contributing to the recruitment of other immune cells

After activation, neutrophils undergo apoptosis, contributing to the resolution of inflammation.

b. Eosinophils

Eosinophils play a major role in defense against parasites and contribute to allergic reactions. They circulate for a few hours in the blood and survive several days in tissues **(Fig. 2.6).**

- ➢ **Morphology:** Eosinophils are 12-15 µm cells with a bilobed nucleus and large granules that stain orange-red with eosin.
- ➢ **Granules:** Contain toxic proteins such as major basic protein (MBP), eosinophil cationic protein, and enzymes such as histaminase and arylsulfatase
- ➢ **Receptors:** Complement receptors (CR1, CR3), Fc receptors for IgG and IgE, and chemokine receptors (e.g., eotaxin receptor)
- ➢ **Functions:**

  - ☞ Antiparasitic activity via degranulation
  - ☞ Participation in allergic and inflammatory responses
  - ☞ Modulation of inflammation

Eosinophils have a limited phagocytic capacity compared to neutrophils.

c. Basophils

Basophils are the least abundant granulocytes (<1% of leukocytes) and have a short lifespan.

- **Granules:** Contain histamine, heparin, and other inflammatory mediators.
- **Functions:** Basophils are involved in immediate hypersensitivity reactions (Type I). Upon activation through IgE receptors, they release histamine, which increases vascular permeability and promotes inflammation. Heparin acts as an anticoagulant.

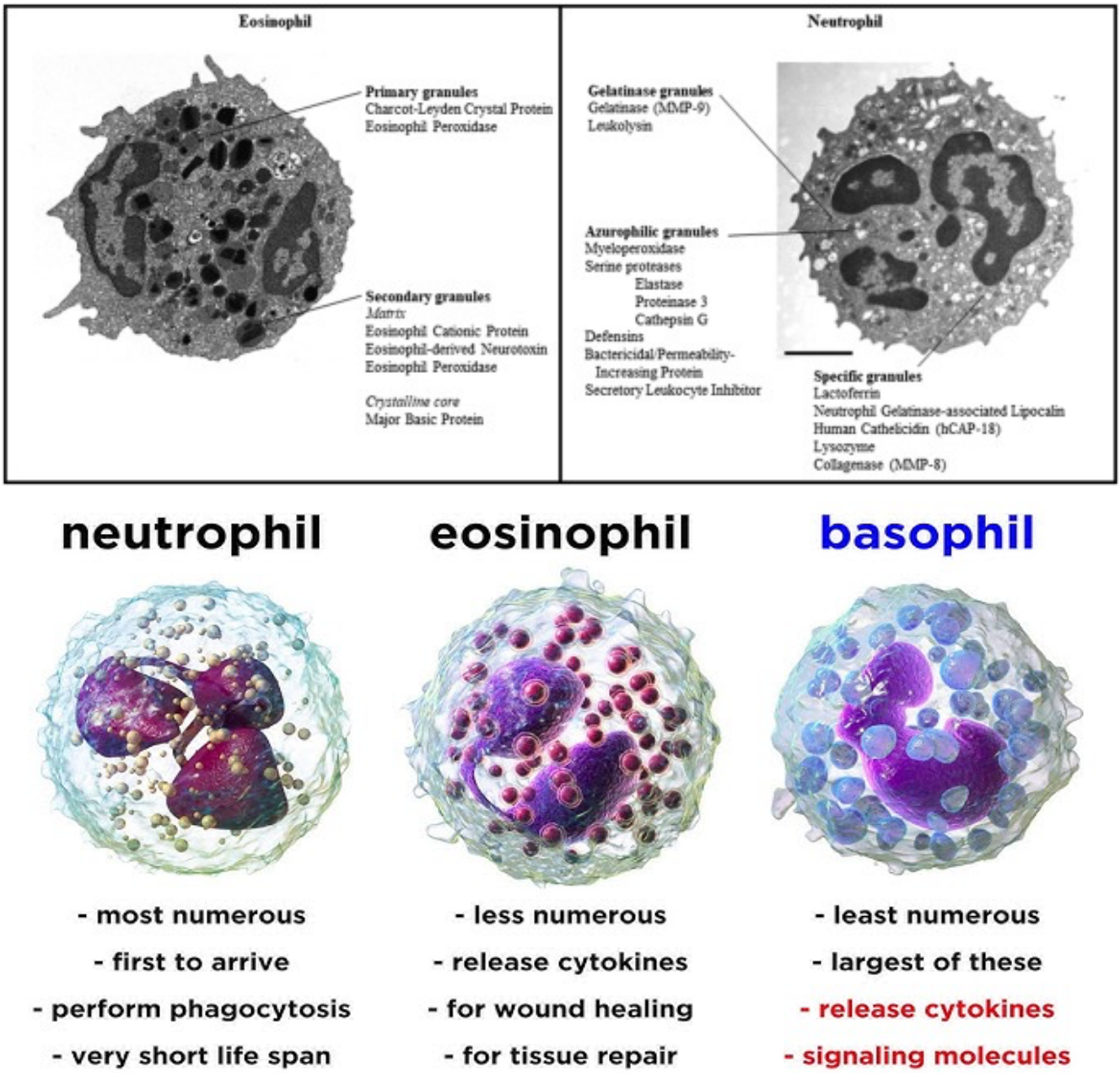


Figure 2.6: Polymorphonuclear leukocytes (granulocytes).

II.2.3. Mast cells

Mast cells are tissue-resident immune cells derived from hematopoietic precursors and play a central role in allergic and inflammatory responses. Although functionally similar to basophils, they represent a distinct cell population.

- **Granules:** Mast cells contain cytoplasmic granules rich in histamine, heparin, proteases, and other bioactive mediators **(see Fig 2.7)**.

Receptors: They express high-affinity FcεRI receptors, which bind the Fc region of IgE antibodies. Upon exposure to a specific allergen, cross-linking of IgE molecules triggers mast cell degranulation, leading to the release of inflammatory mediators.

Functions: Mast cells contribute to:

- ☞ Initiation and amplification of inflammatory responses
- ☞ Increased vascular permeability
- ☞ Recruitment of immune cells to sites of inflammation
- ☞ Immediate hypersensitivity reactions (Type I allergy)

Heparin released from mast cells also exerts anticoagulant effects, although this is not their primary function.

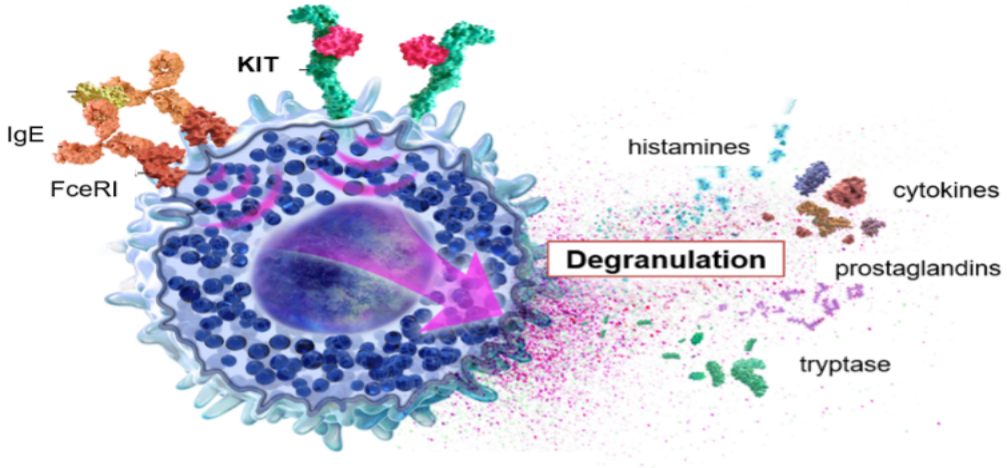


Figure 2.7. Mast cell.

II.2.4. Natural Killer

Natural killer (NK) cells represent a third population of lymphocytes derived from a common lymphoid progenitor, shared with T and B lymphocytes. Unlike these cells, NK cells do not express rearranged antigen-specific receptors, such as the T-cell receptor (TCR) or B-cell receptor (BCR), and they lack CD3 expression. They are therefore classified as innate lymphoid cells and are also known as large granular lymphocytes (LGLs).

NK cells constitute approximately 5-20% of peripheral blood mononuclear cells and are also found in the spleen, bone marrow, and peripheral tissues, including mucosal and epithelial sites. Unlike T

lymphocytes, NK cells do not require maturation in a specialized organ such as the thymus; instead, they develop primarily in the bone marrow and peripheral tissues. Their lifespan is estimated to be 7 to 10 days (**Fig. 2.8**).

- **Granules:** NK cells contain cytotoxic granules rich in perforin and granzymes, which are essential for their cytolytic activity.
- **Receptors:** NK cells express a variety of surface receptors, including CD16 (FcγRIII), which binds the Fc region of IgG and mediates antibody-dependent cellular cytotoxicity (ADCC), CD56, a characteristic NK cell marker, A balance of activating and inhibitory receptors, including killer immunoglobulin-like receptors (KIRs), which regulate NK cell activity
- **Cytokine :** NK cells produce cytokines such as interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and various chemokines, which contribute to immune regulation.
- **Functions:**
  - Cytotoxic activity: NK cells eliminate virus-infected cells and transformed (tumor) cells during the early phase of infection.
  - Tumor surveillance: NK cells recognize and destroy tumor cells that have downregulated MHC class I (HLA-I) molecules to evade cytotoxic T lymphocytes.
  - Immunoregulation: NK cells modulate immune responses through cytokine secretion and interaction with other immune cells.

NK cells operate at the interface between innate and adaptive immunity, contributing to early defense while influencing adaptive immune responses.

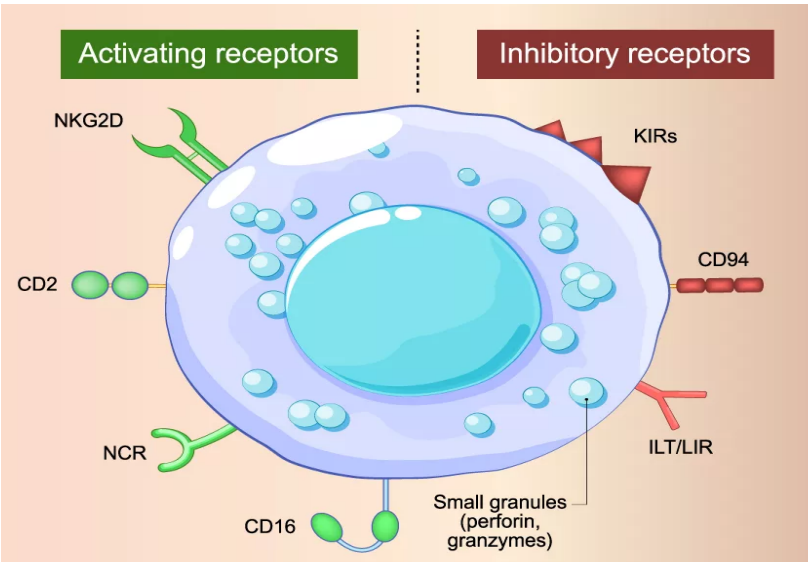


Figure 2.8. Natural killer cells.

II.3. Cells of the adaptive immune response

Lymphocytes are the principal cells of the adaptive immune response and belong to the leukocyte population. They are mainly divided into two major types: T lymphocytes and B lymphocytes.

II.3.1. T lymphocytes

T lymphocytes (T cells) derive their name from the thymus, where they undergo maturation. They are characterized by the expression of the T-cell receptor (TCR), which recognizes antigenic peptides presented by major histocompatibility complex (MHC) molecules.

Naive T lymphocytes are primarily found in secondary lymphoid organs, blood, and lymph. Upon activation, they migrate to sites of infection or tissue damage. T lymphocytes are responsible for cell-mediated immunity, which targets and eliminates infected or transformed (tumor) cells.

- **Receptors:** T cells express several surface molecules, including TCR, CD3, CD4 or CD8, CD28, and CD45 (**Fig. 2.9**).
- **Cytokines:** T lymphocytes produce a variety of cytokines that regulate immune responses.
- **Main types of T lymphocytes:**
  - $CD8^+$ T lymphocytes, which differentiate into cytotoxic T cells (CTLs) capable of killing infected or tumor cells
  - $CD4^+$ T lymphocytes, which differentiate into helper T cells (Th cells) that coordinate immune responses through cytokine secretion

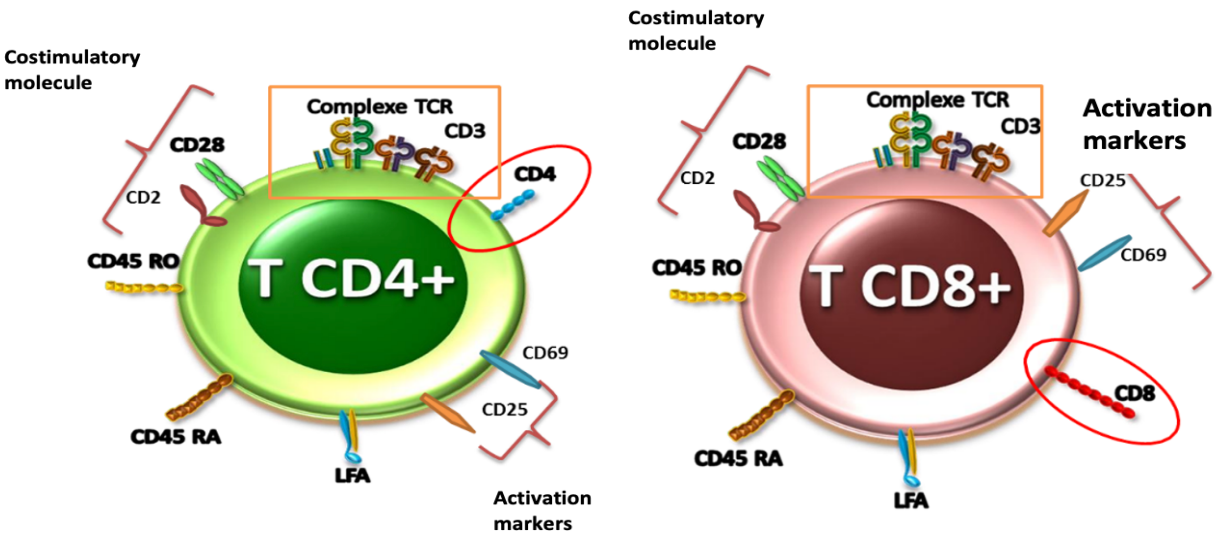


Figure 2.9. The membrane molecules of TCD4 and TCD8 lymphocytes.

❖ **Structure of the T-cell receptor (TCR)**

The T-cell receptor (TCR) is a highly diverse heterodimeric molecule composed of two polypeptide chains (αβ or γδ), linked by a disulfide bond. The diversity of TCRs arises from gene rearrangement, allowing the recognition of a wide range of antigens.

Each chain consists of several distinct regions:

- A variable (V) region, responsible for antigen recognition. This region contains complementarity-determining regions (CDRs) that interact specifically with the peptide-MHC complex.
- A constant (C) region
- A transmembrane region
- A short cytoplasmic tail

The TCR is non-covalently associated with the CD3 complex, which is essential for signal transduction. This complex is composed of several subunits organized into three dimers: γε, δε, and ζζ (**Fig. 2.10**).

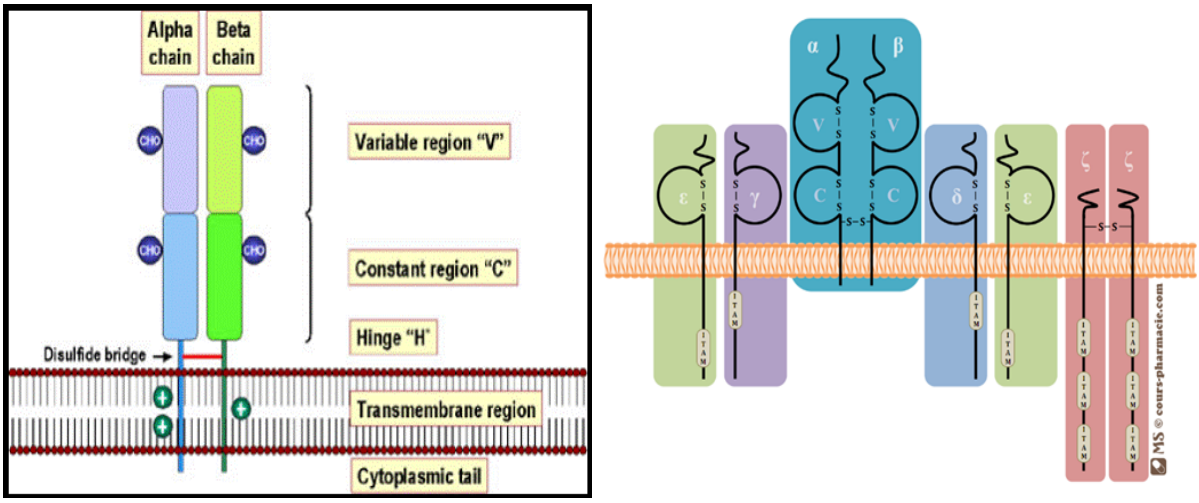


Figure 2.10. The structure of a TCR.

II.3.2. B lymphocytes

B lymphocytes (B cells) derive their name from the bursa of Fabricius, an organ in birds where they were first identified. In humans, B lymphocytes develop and mature in the bone marrow. They are characterized by the expression of the B-cell receptor (BCR), which allows them to recognize native antigens **(see Fig. 2.11)**.

B cells are responsible for humoral immunity, which involves the production of antigen-specific antibodies.

- **Receptors:**The B-cell receptor consists of a membrane-bound immunoglobulin (IgM or IgD) associated with the Igα/Igβ (CD79a/CD79b) signaling complex. B lymphocytes also express several surface molecules, including: Co-receptors like CD19, CD21, CD81, complement receptors as CD21, CD35, co-stimulatory molecules including CD80 (B7-1), CD86 (B7-2), adhesion molecules (LFA-1), and other markers: CD45.
- **Cytokine production:** B lymphocytes produce cytokines such as IL-6, IL-10, TNF-α, and TGF-β, which contribute to the regulation of immune responses.

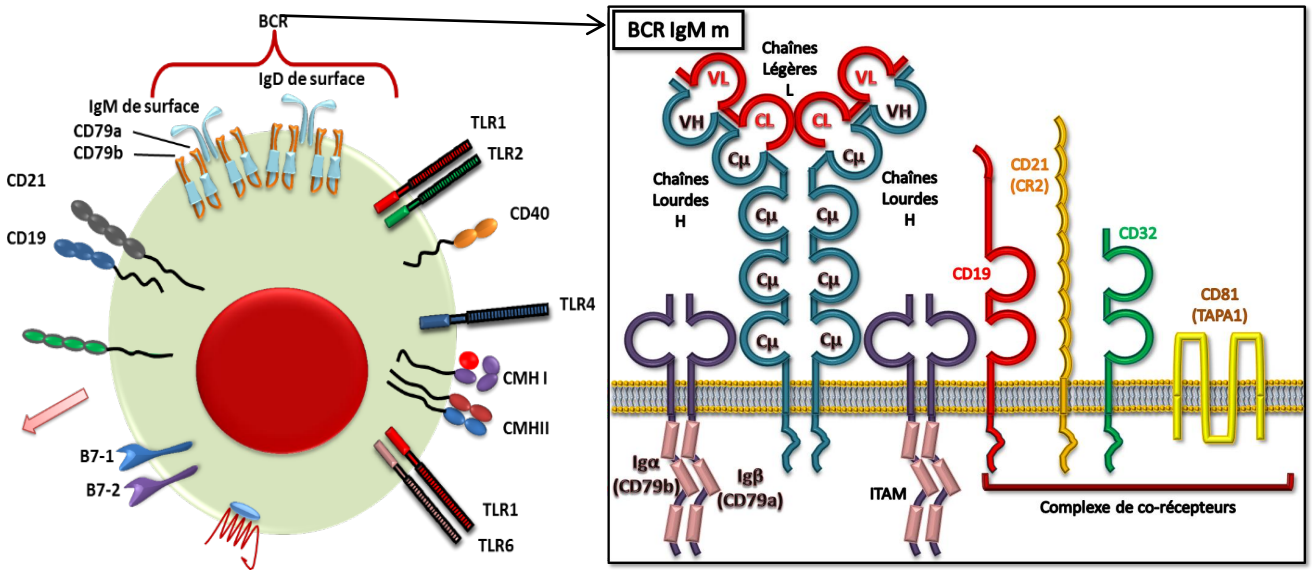


Figure 2.11. B lymphocyte and the structure of the BCR.

B lymphocytes can differentiate into two main cell types:

- **Plasma cells**, which secrete large amounts of soluble antibodies. These antibodies bind to antigens, leading to neutralization, opsonization, and activation of the complement system, thereby facilitating pathogen elimination. Plasma cells do not express membrane-bound immunoglobulins.
- **Memory B lymphocytes**, which persist long-term and express antigen-specific B-cell receptors (BCRs) on their surface. These cells enable a faster and more efficient immune response upon subsequent exposure to the same antigen.

In addition, B lymphocytes can function as antigen-presenting cells (APCs) by presenting antigenic peptides via MHC class II molecules to helper T lymphocytes.

❖ **Structure of the B-cell receptor (BCR)**

On the surface of B lymphocytes, the B-cell receptor (BCR) consists of a membrane-bound immunoglobulin associated with the Igα–Igβ (CD79a/CD79b) heterodimer, which is responsible for signal transduction. Each B lymphocyte expresses a single specificity of BCR in multiple copies, acquired during its development in the bone marrow.

BCRs and antibodies are highly diverse glycoproteins. They are composed of two identical heavy (H) chains and two identical light (L) chains, linked by disulfide bonds **(see Fig 2.11)**. Each chain contains:

- ☞ A **variable (V) region** at the N-terminus, which forms the antigen-binding site
- ☞ A **constant (C) region** at the C-terminus

II.4. Cells at the interface between innate and adaptive immunity

II.4.1. Natural killer T (NKT) cells

Natural killer T (NKT) cells are a specialized subset of lymphocytes that exhibit characteristics of both T lymphocytes and natural killer (NK) cells. They originate from a common lymphoid progenitor and develop in the thymus, where they acquire a T-cell receptor (TCR) and CD3 complex.

Unlike conventional αβ T lymphocytes, NKT cells express a semi-invariant TCR, particularly in the invariant NKT (iNKT) subset. These cells may express CD4, CD8, or neither (double-negative).

A distinctive feature of NKT cells is their ability to recognize lipid and glycolipid antigens presented by CD1d molecules, which are structurally similar to MHC class I molecules but are non-polymorphic. These antigens may be derived from microorganisms or from endogenous lipids produced during cellular stress or infection.

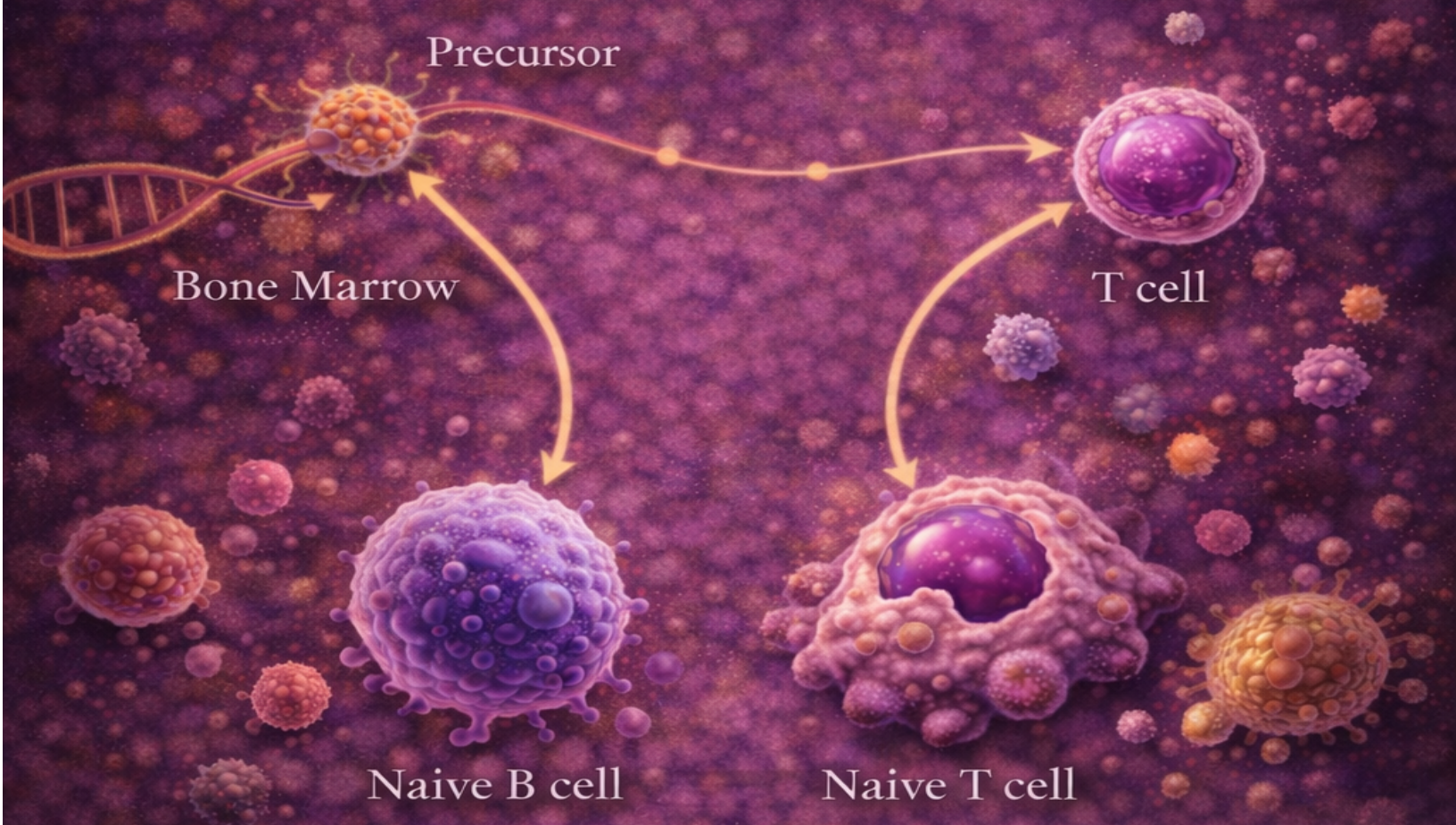
Upon activation, NKT cells rapidly produce a wide range of cytokines, such as IL-4, IL-13, and interferon-γ (IFN-γ), thereby modulating both innate and adaptive immune responses.

II.4.2. The γδ T lymphocytes

γδ T lymphocytes are a distinct subset of T cells characterized by the expression of a γδ T-cell receptor (TCR) associated with the CD3 complex. Unlike conventional αβ T cells, γδ T cells typically lack CD4 and CD8 co-receptors.

These cells are primarily found in epithelial and mucosal tissues and play a key role in early immune responses, particularly in the recognition of non-peptide antigens and stress-induced molecules. They contribute to immune surveillance, tissue homeostasis, and rapid defense against infections.

Chapter III.
Ontogeny of T and B lymphocytes



Chapter III. Ontogeny of T and B Lymphocytes

III.1 Ontogeny of T lymphocytes

The ontogeny of T lymphocytes refers to their development and maturation in the thymus. This process involves the acquisition of the T-cell receptor (TCR) associated with the CD3 complex, followed by the expression of both CD4 and CD8 co-receptors (double-positive stage), and ultimately the differentiation into single-positive $CD4^+$ or $CD8^+$ T cells. It also includes the establishment of self-tolerance through positive and negative selection processes, which ensure that T lymphocytes respond to foreign antigens while remaining tolerant to self-components.

III.1.1. From bone marrow to thymus

Hematopoietic progenitor cells originate in the bone marrow and migrate to the thymus via the bloodstream. They enter the thymic tissue by crossing the vascular endothelium (diapedesis) and localize within the thymic microenvironment, particularly in the cortex. At this stage, these progenitor cells still retain the potential to differentiate into T, B, or NK lymphocytes. However, upon entering the thymus, they receive signals that commit them to the T-cell lineage, after which they are referred to as thymocytes.

Within the thymus, thymocytes undergo intense proliferation, but the majority are eliminated by apoptosis during selection processes. These processes are essential for the establishment of self-tolerance, ensuring that T lymphocytes respond to foreign antigens (non-self) while remaining tolerant to self-components.

T-cell differentiation occurs through several stages and involves the rearrangement of genes encoding the T-cell receptor (TCR), allowing its expression and functional maturation.

III.1.2. Maturation of thymocytes and acquisition of self-tolerance

Progenitor cells entering the thymus first differentiate into double-negative (DN) thymocytes, characterized by the absence of CD4, CD8, CD3, and TCR expression. These cells represent approximately 5% of thymocytes.

a. Lineage commitment

During the double-negative stage, thymocytes undergo TCR gene rearrangement, which determines their differentiation into either γδ T cells or αβ T cells. A small proportion of thymocytes differentiate into γδ T cells, whereas the majority (approximately 90–95%) proceed toward the αβ T-cell lineage.

In αβ lineage cells, successful rearrangement of the β chain leads to its association with a pre-Tα chain, forming the pre-TCR complex. This complex delivers a survival and proliferation signal, allowing thymocytes to progress to the double-positive ($CD4^+CD8^+$) stage.

Subsequently, rearrangement of the α chain results in the formation of a complete αβ TCR associated with CD3.

b. Positive selection

Positive selection occurs in the thymic cortex and ensures that thymocytes are capable of recognizing self-MHC molecules **(Fig. 3.1)**.

- ☞ Thymocytes that recognize self-MHC with low to moderate affinity receive survival signals and are positively selected.
- ☞ Thymocytes that fail to recognize self-MHC undergo death by neglect (apoptosis).

This process ensures that only thymocytes capable of interacting with self-MHC survive.

c. CD4/CD8 lineage commitment

At the double-positive stage, thymocytes differentiate into either $CD4^+$ or $CD8^+$ T cells depending on the MHC molecule recognized:

- ☞ Recognition of MHC class I → differentiation into $CD8^+$ T cells
- ☞ Recognition of MHC class II → differentiation into $CD4^+$ T cells

d. Negative selection

Negative selection occurs in the thymic medulla and eliminates self-reactive thymocytes.

This process is mediated by medullary thymic epithelial cells (mTECs) and dendritic cells, which present self-antigens via MHC molecules **(see Fig. 3.1)**. The transcription factor AIRE (Autoimmune Regulator), expressed by mTECs, enables the presentation of tissue-specific antigens, then:

- ☞ Thymocytes that recognize self-antigens with high affinity undergo apoptosis (negative selection).
- ☞ Thymocytes with low affinity for self-antigens survive and exit the thymus as mature, self-tolerant T cells.

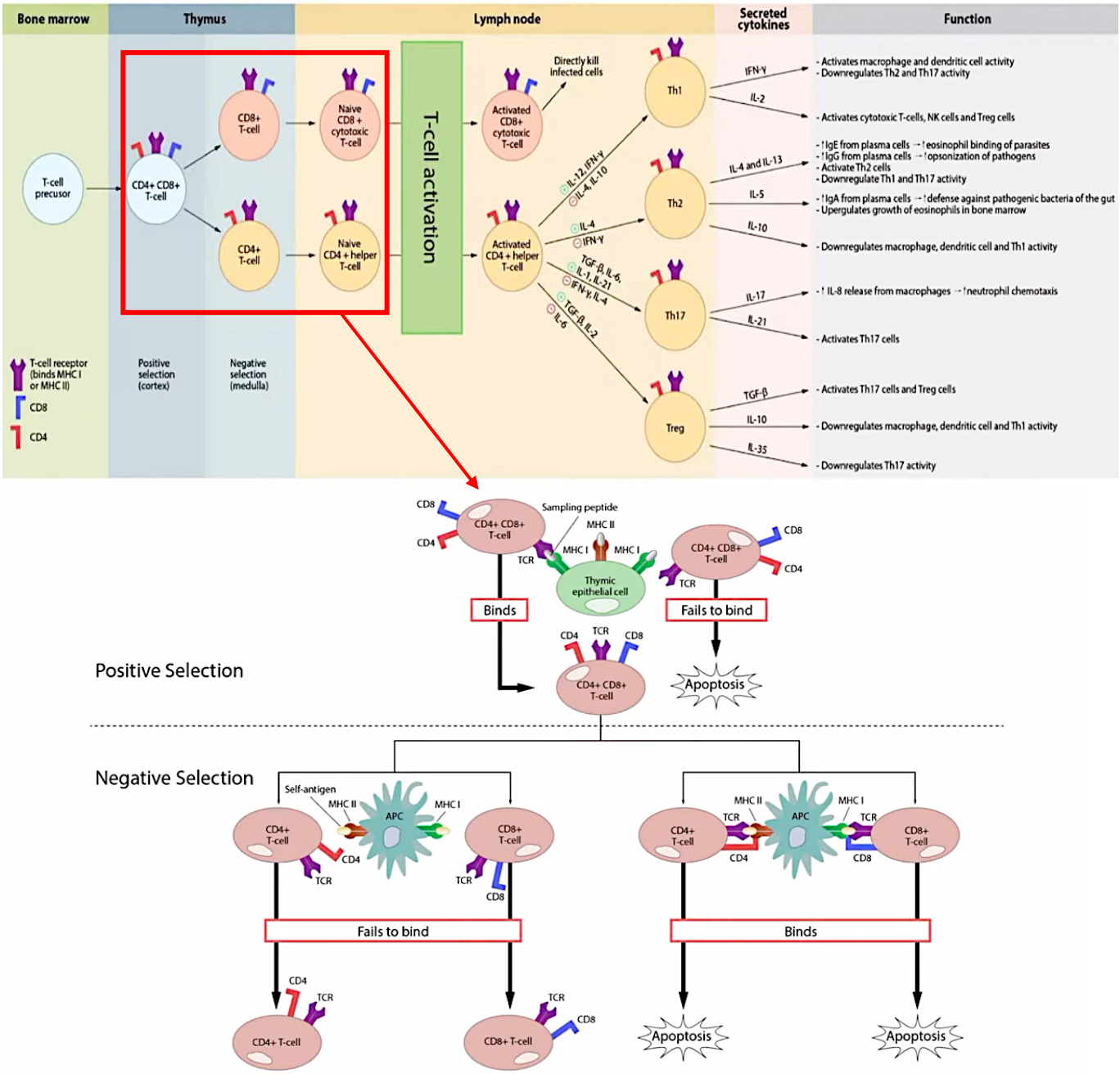


Figure 3.1. The positive and negative selection of thymocytes within the thymus.

III.2. Ontogeny of B lymphocytes

B lymphocyte ontogeny refers to their development and maturation, including the acquisition of the B-cell receptor (BCR) associated with the Igα–Igβ (CD79a/CD79b) signaling complex, as well as the establishment of self-tolerance. B lymphocytes develop in the bone marrow from hematopoietic stem cells. B-cell development occurs in two main stages:

- ☞ The first stage is antigen-independent and takes place in the bone marrow. During this phase, immature B lymphocytes are generated and express surface immunoglobulin (primarily IgM), enabling antigen recognition.

- ☞ The second stage occurs in secondary lymphoid organs and is antigen-dependent. Upon encountering foreign antigens, B lymphocytes become activated and differentiate into plasma cells and memory B cells specific to the antigen.

The large diversity of antigens encountered by the immune system requires the generation of a vast repertoire of B-cell receptors. This diversity arises from the recombination of gene segments encoding the heavy and light chains of the BCR. These include variable (V), diversity (D), and joining (J) segments for the heavy chain, while the constant region can later undergo class switching. The heavy chain gene locus is located on chromosome 14.

III.2.1. Different stages of B-cell development

B-cell development occurs in the bone marrow through a series of well-defined stages involving immunoglobulin gene rearrangement and selection processes **(see Fig. 3.2)**.

- **Early pro-B stage:** Rearrangement of the D and J segments of the immunoglobulin heavy chain occurs on both chromosomes.
- **Late pro-B stage:** V-DJ rearrangement of the heavy chain takes place. If the rearrangement is productive, development proceeds; otherwise, rearrangement occurs on the second chromosome **(see Fig. 3.3)**.
- **Large pre-B cell stage:** The rearranged heavy chain is expressed in association with a surrogate light chain, forming the pre-B-cell receptor (pre-BCR). Expression of a functional pre-BCR provides a survival and proliferation signal, allowing progression to the next stage.
- **Small pre-B cell stage:** Rearrangement of the light chain genes (κ first, then λ if necessary) occurs. Successful rearrangement leads to the expression of a complete B-cell receptor.
- **Immature B cell stage:** Cells express surface IgM. At this stage, central tolerance mechanisms eliminate or inactivate self-reactive B cells. These processes include:
  - ☞ Clonal deletion (apoptosis)
  - ☞ Anergy (functional inactivation)
  - ☞ Receptor editing (additional light chain rearrangement)

Selection is based on the recognition of self-antigens, not peptide-MHC complexes.

- **Mature B cell stage:** Mature naïve B cells co-express IgM and IgD through alternative splicing and migrate to secondary lymphoid organs.

Upon antigen encounter, activated B cells undergo clonal expansion, followed by somatic hypermutation and class-switch recombination in germinal centers, leading to the generation of plasma cells and memory B cells.

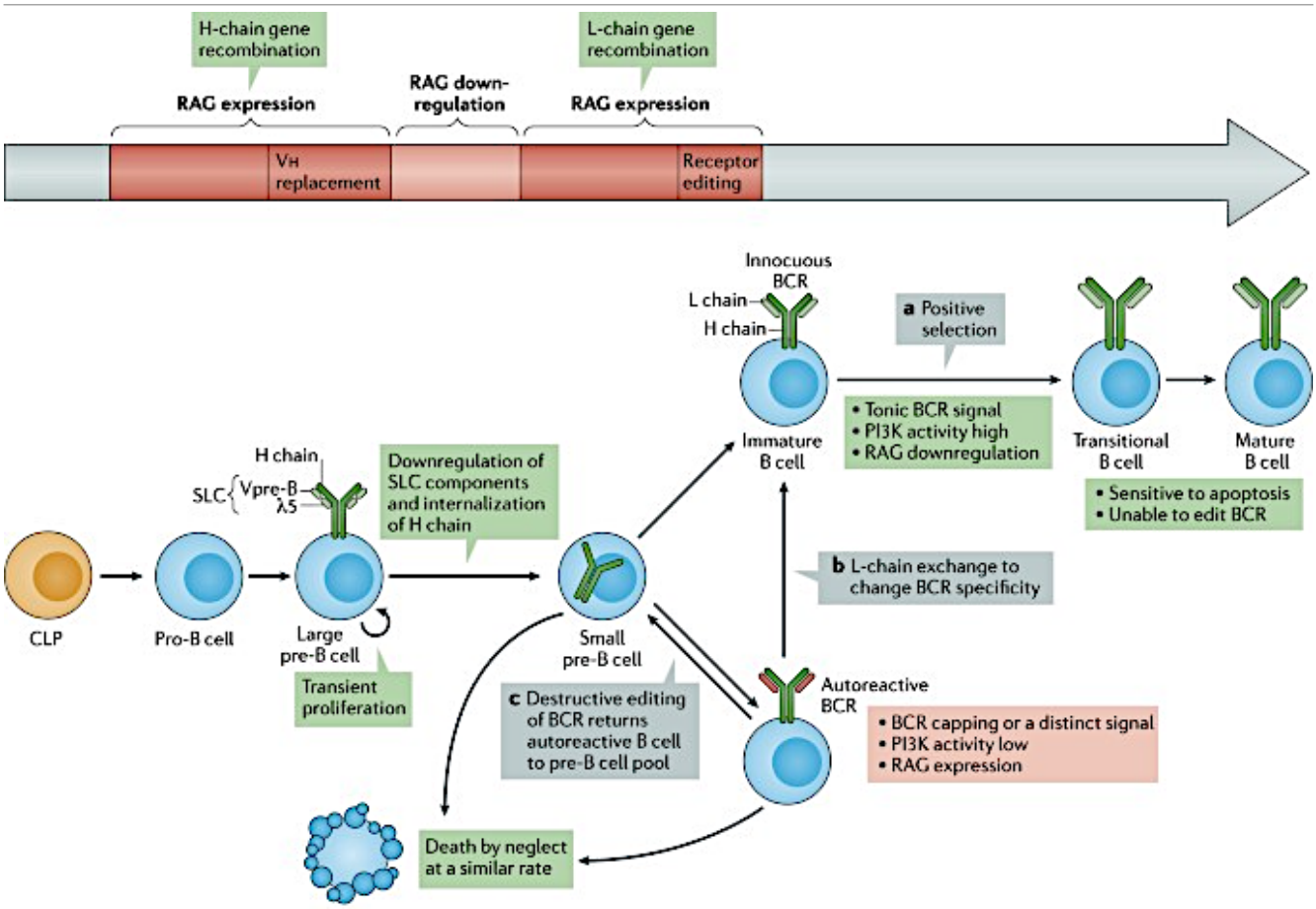


Figure 3.2. Ontogeny of B lymphocytes.

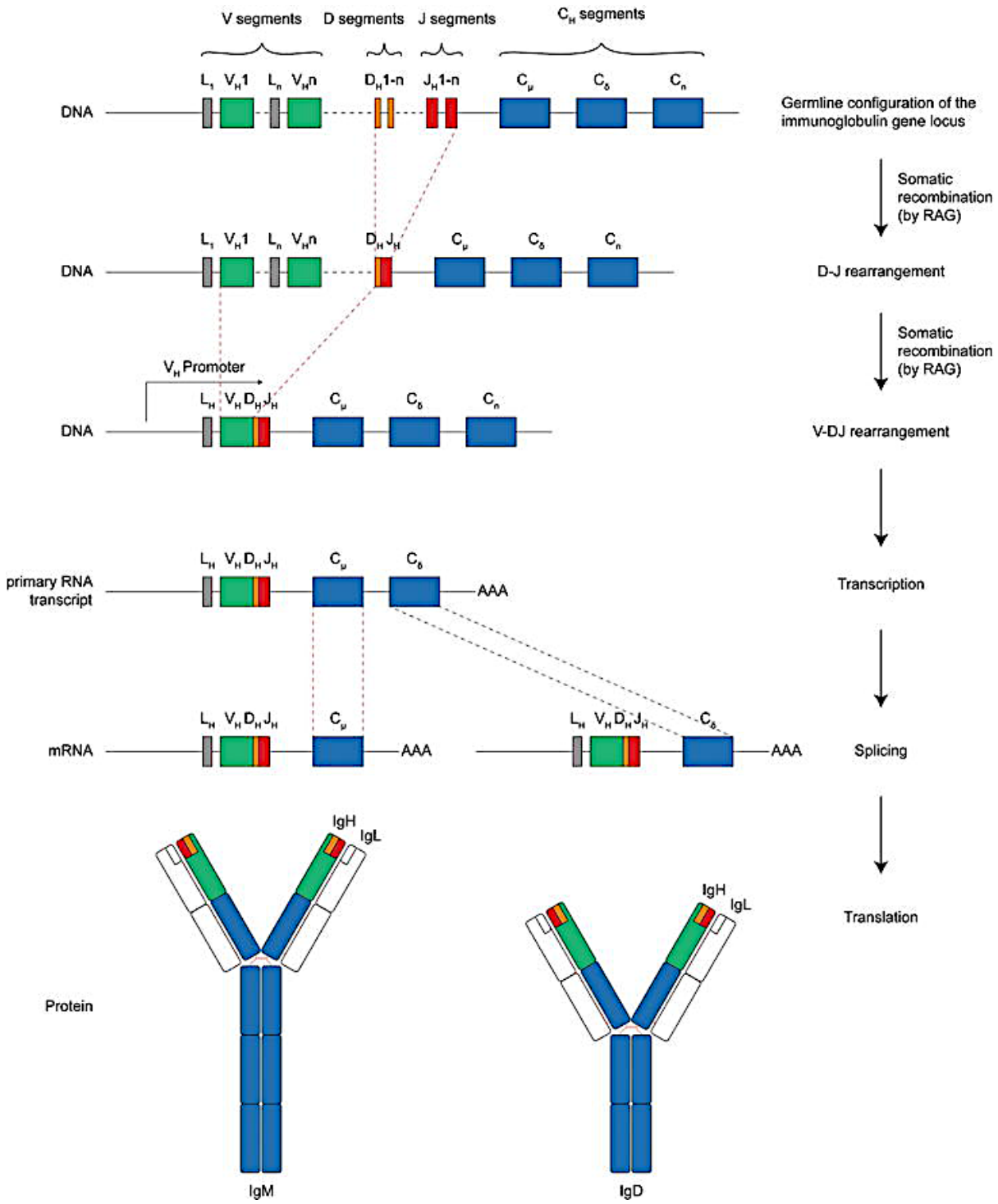
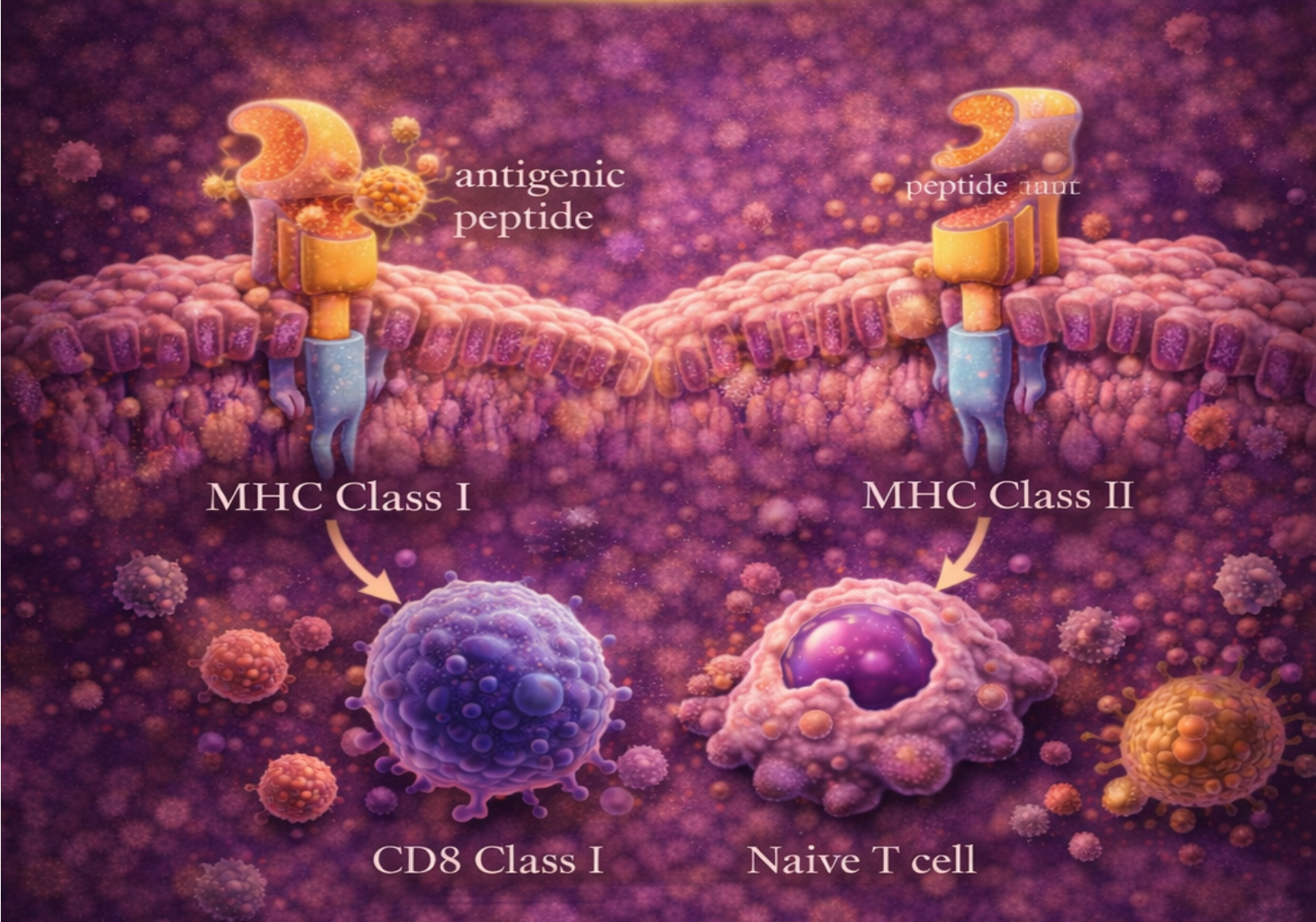


Figure 3.3. Organization of immunoglobulin genes and generation of Ig diversity.
Organization of gene families encoding lambda light chains on chromosome 22, for kappa light chains on chromosome 2 and for heavy chains on chromosome 14

Chapter IV.
Major Histocompatibility Complex



Chapter IV. Major Histocompatibility Complex

The major histocompatibility complex (MHC), also known in humans as the **Human Leukocyte Antigen (HLA) system**, is a group of closely linked genes located on chromosome 6. It was initially identified due to its critical role in graft rejection (histocompatibility).

MHC genes are classified into three main groups (**Fig. 4.1**):

- **Class I genes** encode MHC class I molecules, primarily HLA-A, HLA-B, and HLA-C. These molecules are highly polymorphic, meaning that multiple allelic forms exist within the population, making the MHC the most polymorphic genetic system in humans.
- **Class II genes** encode MHC class II molecules, including HLA-DP, HLA-DQ, and HLA-DR, located within the D region. Like class I genes, class II genes exhibit a high degree of polymorphism.
- **Class III genes**, located between class I and class II regions, encode various proteins involved in immune and inflammatory responses, such as complement components (e.g., C2, C4, factor B) and certain cytokines (e.g., TNF).

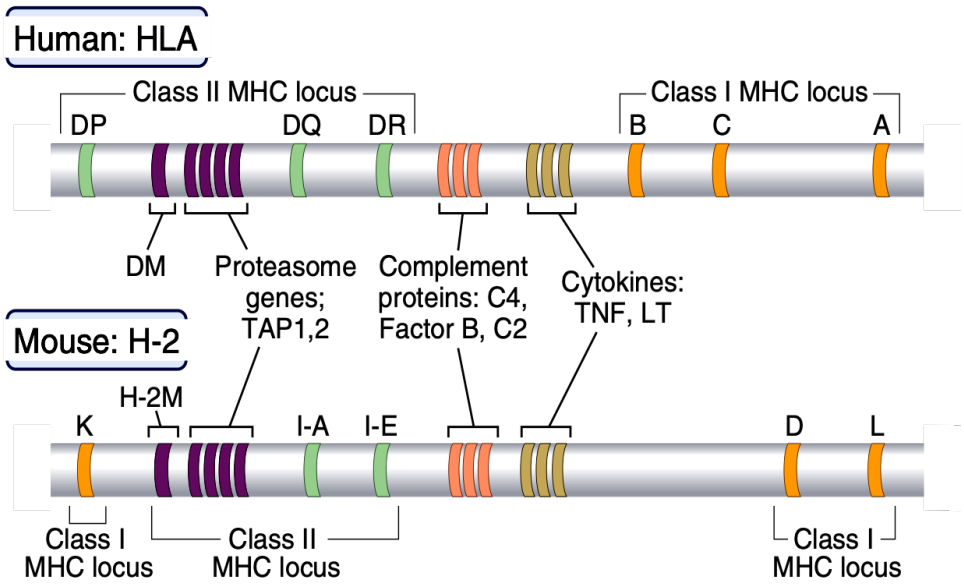


Figure 4.1. Genes of the major histocompatibility complex.

IV.1. Major histocompatibility complex class I (MHC-I)

MHC class I molecules are expressed on all nucleated cells of the body, with the exception of red blood cells, and their expression levels vary depending on the cell type. These molecules play a crucial role in the immune system by presenting endogenous peptide antigens to $CD8^+$ T lymphocytes.

Upon recognition of peptide-MHC class I complexes, $CD8^+$ T cells become activated and differentiate into cytotoxic T lymphocytes (CTLs), which are responsible for the elimination of infected or transformed cells.

IV.1.1. Structure of MHC class I molecules

MHC class I molecules are heterodimers composed of a heavy α chain associated non-covalently with **β2-microglobulin** (**Fig. 4.2**).

The **α chain** contains three extracellular domains (α1, α2, α3), a transmembrane region, and a short cytoplasmic tail. The α1 and α2 domains form the peptide-binding region (PBR), which creates a groove that accommodates endogenous peptide antigens. These domains are also the most polymorphic regions of the molecule.

The α chain is associated non-covalently with **β2-microglobulin**, a smaller, non-polymorphic protein that is entirely extracellular and is essential for the structural stability and surface expression of MHC-I molecules.

a. The α chain

The α chain is encoded by the HLA-A, HLA-B, and HLA-C genes. It has a molecular weight of approximately 45 kDa and consists of about 340 amino acids.

- **Extracellular region:** Composed of three domains (α1, α2, α3). The α1 and α2 domains form the peptide-binding groove, while the α3 domain interacts with the CD8 co-receptor.
- **Transmembrane region:** A hydrophobic segment of approximately 20 amino acids.
- **Cytoplasmic region:** A short intracellular tail of 30-40 amino acids.

b. β2-Microglobulin

β2-microglobulin is a light chain-like protein with a molecular weight of approximately 12 kDa. It is entirely extracellular and associates non-covalently with the α chain.

It consists of a single domain stabilized by a disulfide bond and is highly conserved across species. Unlike the α chain, β2-microglobulin is non-polymorphic and is encoded by a gene located outside the MHC locus. This molecule is essential for the proper folding, stability, and surface expression of MHC class I molecules.

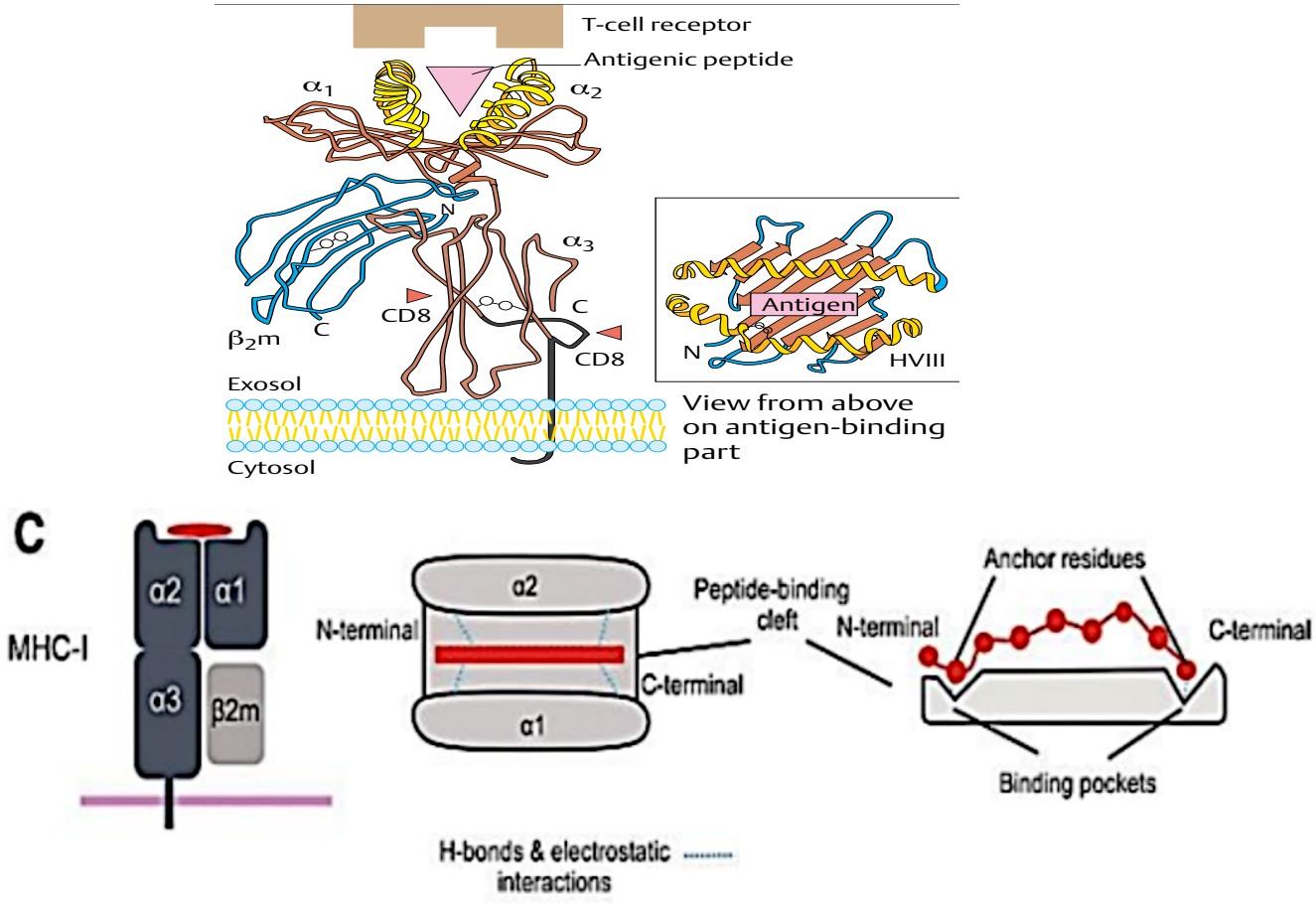


Figure 4.2. Structure of an HLA class I molecule.

IV.1.2. Complex formation and mechanism of action

MHC class I molecules present endogenous peptide antigens derived from proteins synthesized within the cell. These peptides may originate from normal cellular proteins, altered self-proteins (e.g., tumor antigens), or viral proteins produced during intracellular infection. Proteins are degraded in the cytoplasm by the proteasome into peptides typically 8-10 amino acids in length.

The α heavy chain of MHC-I is synthesized in the endoplasmic reticulum (ER), while β2-microglobulin is synthesized in the cytosol and subsequently associates with the α chain in the ER. Proper folding of the MHC-I molecule requires several chaperone proteins, including calnexin, calreticulin, ERp57, and tapasin, which together form the peptide-loading complex.

Peptides generated in the cytosol are transported into the ER lumen by the transporter associated with antigen processing (TAP), composed of TAP-1 and TAP-2 subunits. Within the ER, peptides bind to the peptide-binding groove of the MHC-I molecule. This binding stabilizes the complex, leading to the dissociation of chaperone proteins.

The stable peptide-MHC class I complex is then transported through the Golgi apparatus to the cell surface, where it is presented to $CD8^+$ T lymphocytes (**Fig. 4.3**).

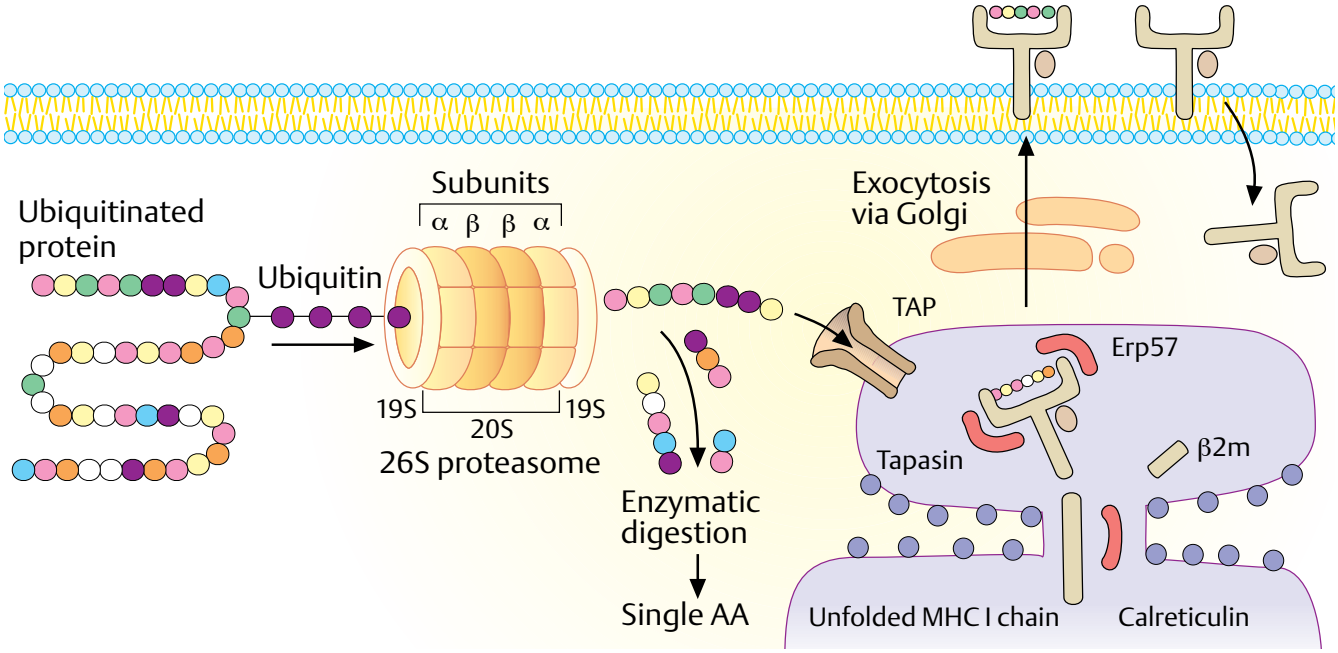


Figure 4.3. Processing and presentation of endogenous antigen.

IV.2. Major histocompatibility complex class II (MHC-II)

MHC class II molecules are expressed on a restricted subset of cells, including monocytes, macrophages, dendritic cells, B lymphocytes, and thymic epithelial cells. These cells are known as professional antigen-presenting cells (APCs).

MHC class II molecules present exogenous peptide antigens to $CD4^+$ T lymphocytes. After activation, these cells differentiate into helper T cells (Th cells), which play a central role in coordinating and regulating immune responses.

IV.2.1. Structure of MHC class II molecules

MHC class II molecules are composed of two non-covalently associated protein chains: an **α chain** (≈33 kDa) and a **β chain** (≈28 kDa), both encoded by MHC class II genes.

Each chain contains two extracellular domains (α1 and α2 for the α chain; β1 and β2 for the β chain), as well as a transmembrane segment and a short cytoplasmic region **(Fig. 4.4).** These extracellular domains are approximately 90 amino acids in length.

The α1 and β1 domains form the peptide-binding groove, which accommodates exogenous peptide antigens. This region is shared between the α and β chains and represents the most polymorphic part of the molecule.

The α2 and β2 domains exhibit an immunoglobulin-like structure, and each contains a disulfide bond. The β2 domain is involved in the interaction with the CD4 co-receptor.

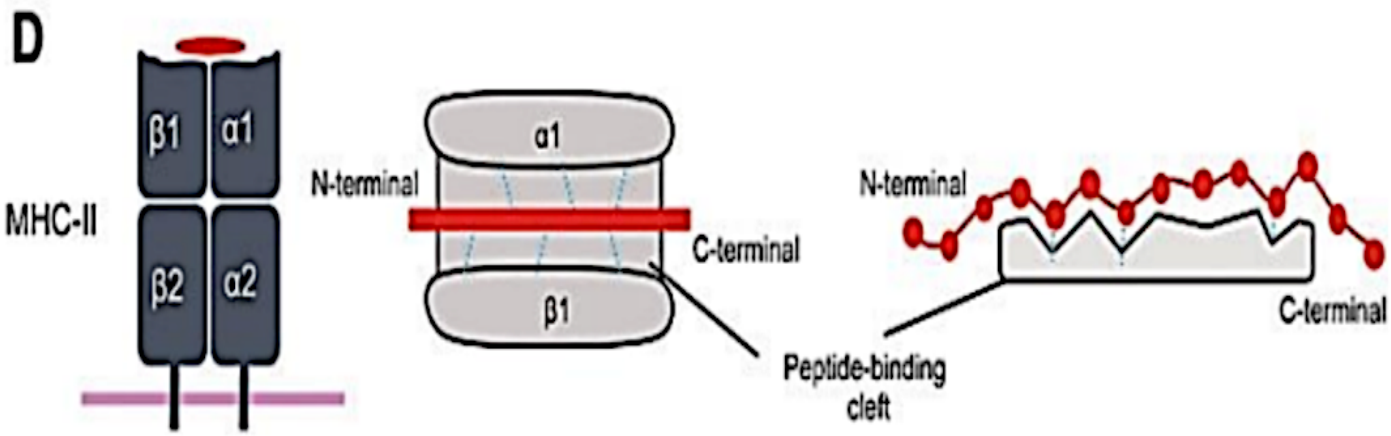


Figure 4.4. Structure of the major histocompatibility complex class II.

IV.2.2. Complex formation and mechanism of action

MHC class II molecules present exogenous peptide antigens derived from extracellular sources, such as pathogens or internalized material. These antigens are taken up by antigen-presenting cells (APCs) through endocytosis or phagocytosis and degraded within the endosomal-lysosomal system into peptides typically 12-25 amino acids in length.

The α and β chains of MHC class II molecules are synthesized in the endoplasmic reticulum (ER), where they associate with the invariant chain (Ii). This molecule stabilizes the MHC-II complex and prevents

premature peptide binding. A fragment of the invariant chain, known as CLIP (Class II-associated invariant chain peptide), occupies the peptide-binding groove.

The MHC-II–Ii complex is transported through the Golgi apparatus and directed to specialized endosomal compartments (MIIC), where the invariant chain is progressively degraded by proteolytic enzymes (cathepsins), leaving CLIP in the binding groove.

Within these compartments, HLA-DM facilitates the removal of CLIP and promotes the loading of antigenic peptides onto MHC class II molecules. The open structure of the peptide-binding groove allows binding of peptides of variable length.

The stable peptide-MHC class II complex is then transported to the cell surface, where it is presented to $CD4^+$ T lymphocytes (**Fig. 4.5**).

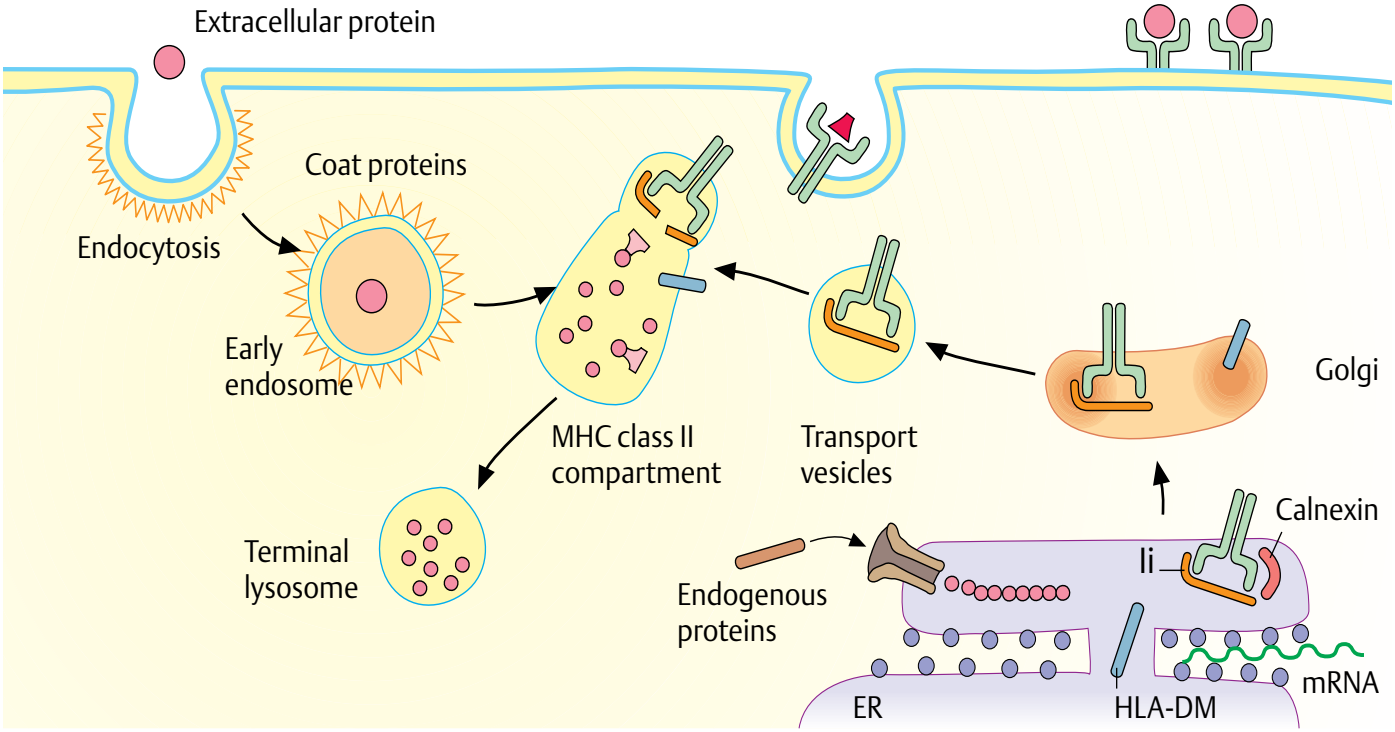
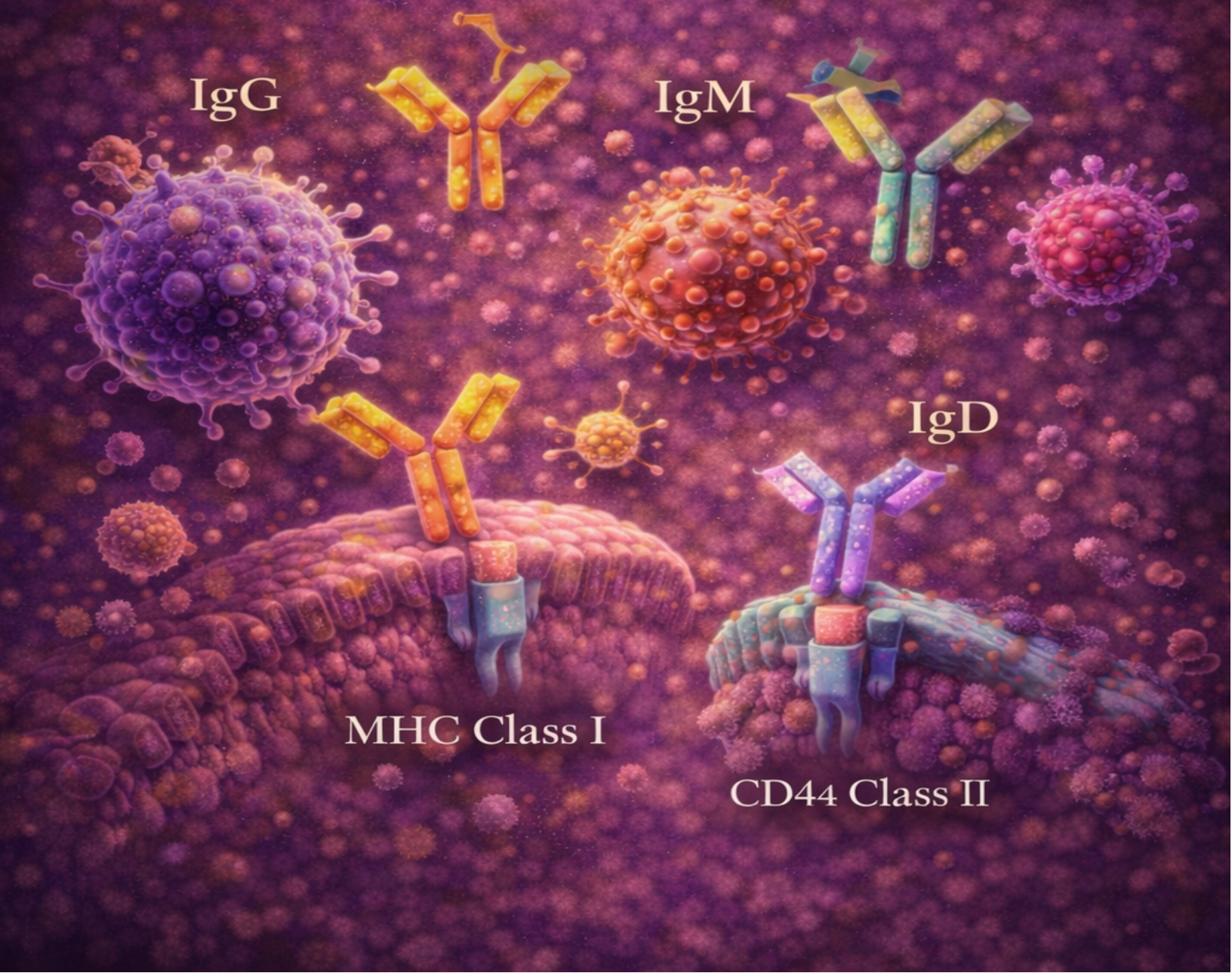


Figure 4.5. MHC Class II-dependent antigen processing.

Chapter V.
Immunoglobulins and Antigens



Chapter V. Immunoglobulins and Antigens

V.1. Immunoglobulins

Immunoglobulins (Ig), also known as antibodies, are glycoproteins produced by plasma cells in response to antigenic stimulation. They play a central role in the immune system, existing both as membrane-bound receptors on B lymphocytes (B-cell receptors, BCRs) and as soluble molecules in their secreted form, where they specifically bind to antigens.

Immunoglobulins constitute the basis of the adaptive humoral immune response. Their structural diversity has led to the classification of five major classes, based on the type of heavy chain: IgA, IgD, IgE, IgG, and IgM.

V.1.1. Basic structure

a. Heavy and light chains

Immunoglobulins exhibit a conserved structure composed of four polypeptide chains:

- **Two identical light chains (≈25 kDa):** There are two types of light chains, κ (kappa) and λ (lambda). In humans, approximately 60% of immunoglobulins contain κ chains and 40% contain λ chains. Each immunoglobulin molecule contains only one type of light chain, either κ or λ.
- **Two identical heavy chains (≈50-70 kDa):** The heavy chains determine the isotype (class) of the immunoglobulin. Amino acid sequence analysis has identified five types of heavy chains: α (alpha), δ (delta), ε (epsilon), γ (gamma), and μ (mu), corresponding to the five antibody classes: IgA, IgD, IgE, IgG, and IgM, respectively.

The heavy and light chains are linked by inter-chain disulfide bonds, as well as non-covalent interactions, which together ensure the structural stability of the immunoglobulin molecule (**Fig. 5.1**).

Note: *The number and positions of disulfide bonds vary depending on the class and subclass of the immunoglobulin.*

b. Variable and constant regions

The polypeptide chains of both light (L) and heavy (H) immunoglobulin chains are organized into two main types of regions:

- **Variable regions (N-terminal):** The N-terminal portions of both chains, consisting of approximately 110 amino acids, exhibit a high degree of variability. These regions are referred

to as VL (variable region of the light chain) and VH (variable region of the heavy chain) and are responsible for antigen recognition.

- **Constant regions:** Following the variable regions, the remaining portions of the chains show relatively conserved sequences. These are known as the constant regions, designated CL for the light chain and CH domains (CH1, CH2, CH3, and ± ±CH4) for the heavy chain (**Fig. 5.1**).

Note: *Immunoglobulins are glycoproteins, and carbohydrate moieties are primarily attached to the constant region of the heavy chain (Fc region), where they contribute to structural stability and biological function.*

V.1.2. Structural organization of immunoglobulins

The heavy and light chains of immunoglobulins (Ig) are organized into homologous structural units, known as domains, each consisting of approximately 110 amino acids. Each domain is stabilized by a characteristic intrachain disulfide bond.

- **Light chains** consist of one variable domain (VL) and one constant domain (CL).
- **Heavy chains** consist of one variable domain (VH) and three or four constant domains (CH1, CH2, CH3, and, in some classes, CH4), depending on the immunoglobulin isotype **(see Fig. 5.1).**

a. Variable region domains

The variable regions of the light (VL) and heavy (VH) chains exhibit variability primarily within specific regions known as complementarity-determining regions (CDRs). Each chain contains three CDRs, which form the antigen-binding site.

The remaining portions of the variable regions are more conserved and are referred to as framework regions (FRs). These regions provide structural support and maintain the proper conformation of the antigen-binding site (**Fig. 5.1**).

b. Constant region domains

The constant domains of immunoglobulins determine their structural stability and biological functions.

- The CL (light chain) and CH1 (heavy chain) domains contribute to the structural integrity of the antibody and are involved in inter-chain interactions between heavy and light chains.
- A proline-rich hinge region is present in IgG, IgA, and IgD, providing flexibility that allows the Fab arms to adopt different orientations during antigen binding. The number of disulfide bonds in the hinge region varies depending on the immunoglobulin class.

- The heavy chains of IgG, IgA, and IgD contain three constant domains (CH1-CH3) and a hinge region.
- In contrast, IgM and IgE contain four constant domains (CH1-CH4) and lack a hinge region. Their flexibility is conferred by the presence of the additional constant domain rather than a hinge.
- Immunoglobulins are glycoproteins, and glycosylation sites are primarily located in the constant regions of the heavy chains, particularly in the CH2 domain, although their distribution may vary among different classes.
- The C-terminal domain corresponds to CH3 in IgG, IgA, and IgD and to CH4 in IgM and IgE.

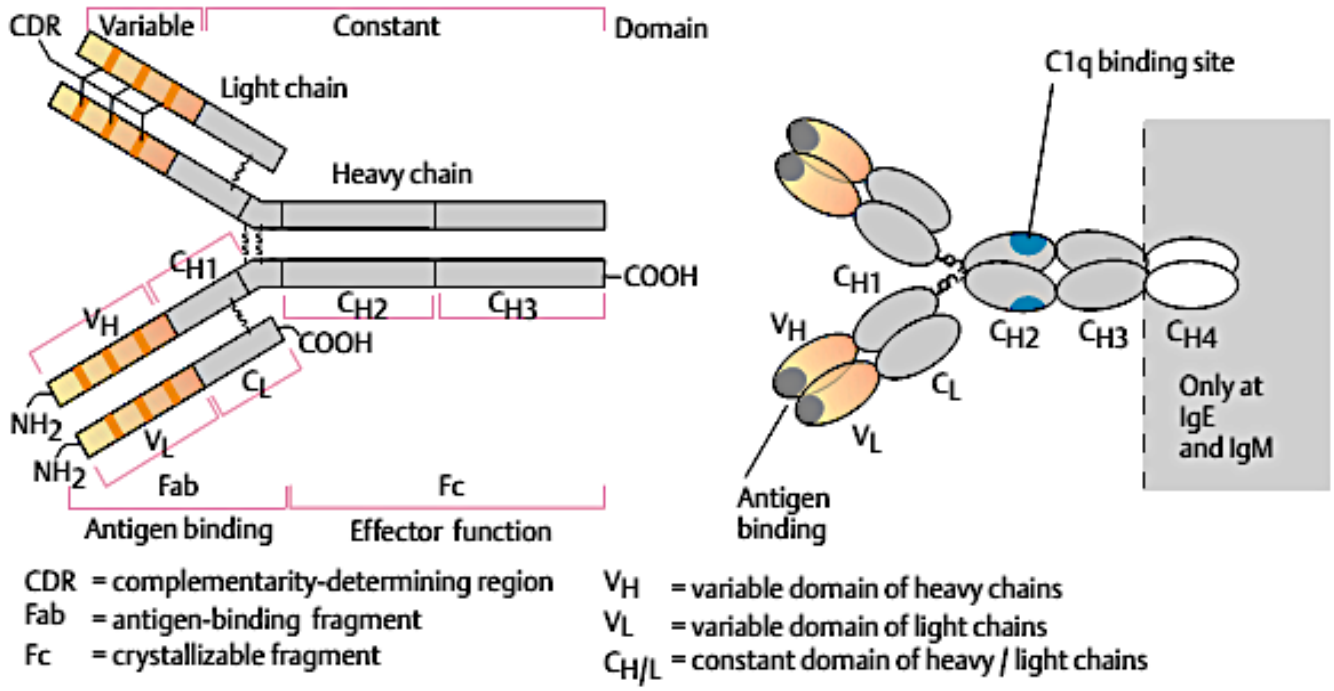


Figure 5.1. Immunoglobulin structure.

V.1.3. Fragmentation of immunoglobulins: Structure-function relationships

Proteolytic fragmentation of immunoglobulins has provided valuable insights into the relationship between their structure and biological functions.

a. Fab and Fc fragments

Digestion of immunoglobulins by **papain** cleaves the molecule at the hinge region (**Fig. 5.2**). This process generates:

- **Two identical Fab fragments (fragment antigen-binding):** Each Fab fragment consists of a complete light chain associated with the VH and CH1 domains of a heavy chain. These fragments contain the antigen-binding sites.
- **One Fc fragment (Fragment crystallizable):** This fragment contains the CH2 and CH3 domains of the two heavy chains. It is responsible for the effector functions of the antibody and was originally named for its ability to form crystals under specific experimental conditions.

b. F(ab')$_2$ fragment

Digestion with **pepsin** cleaves the immunoglobulin below the hinge region, preserving the disulfide bonds that link the two heavy chains. This results in the formation of a bivalent F(ab')$_2$ fragment, which contains both antigen-binding sites. The Fc portion is degraded into smaller peptides during pepsin digestion.

The F(ab')$_2$ fragment retains the ability to bind antigen and, due to its bivalency, can cross-link antigens and form immune complexes, but it lacks the effector functions associated with the Fc region.

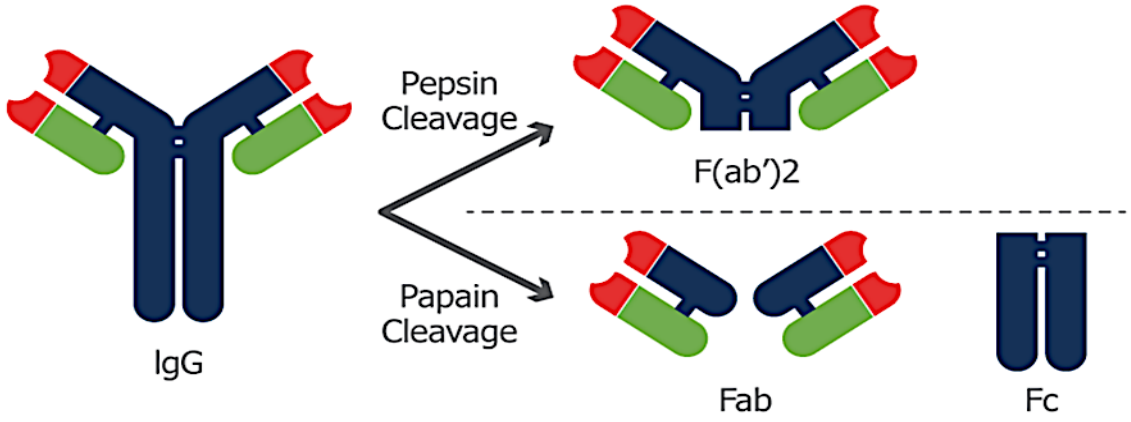


Figure 5.2. Immunoglobulin fragments generated by proteolysis.

c. Reduction by mercaptoethanol

Reducing agents, such as β-mercaptoethanol, cleave the disulfide bonds of immunoglobulins. Under appropriate conditions, this reduction leads to the separation of the molecule into two heavy chains (H) and two light chains (L).

V.1.4. Effector functions of immunoglobulins

The variable regions of immunoglobulins are responsible for antigen recognition, whereas the constant region (Fc) of the heavy chains mediates interactions with cells and proteins, giving rise to various effector functions.

a. Opsonization

Opsonization enhances the phagocytosis of antigens by phagocytic cells such as macrophages and neutrophils. This process is mediated by Fc receptors (FcγR), which recognize the Fc region of IgG antibodies, facilitating the engulfment and destruction of pathogens.

b. Complement activation

IgM and certain IgG subclasses activate the classical complement pathway, leading to a cascade of reactions that result in:

- ☞ Formation of the membrane attack complex (MAC), which can lyse target cells
- ☞ Deposition of C3b, an opsonin that enhances phagocytosis by cells expressing complement receptors

c. Antibody-dependent cell-mediated cytotoxicity (ADCC)

In ADCC, antibodies bound to target cells (e.g., virus-infected or tumor cells) are recognized by Fc receptors, particularly CD16 (FcγRIII) on natural killer (NK) cells. This interaction triggers the release of cytotoxic molecules, leading to the destruction of the target cell.

V.1.5. Classes of immunoglobulins

Immunoglobulins are classified into five major isotypes based on the structure of their heavy chains: IgM, IgD, IgG, IgE, and IgA (**Fig. 5.3 - Tab. 5.1**).

- **IgG** comprises four subclasses: IgG1, IgG2, IgG3, and IgG4
- **IgA** comprises two subclasses: IgA1 and IgA2

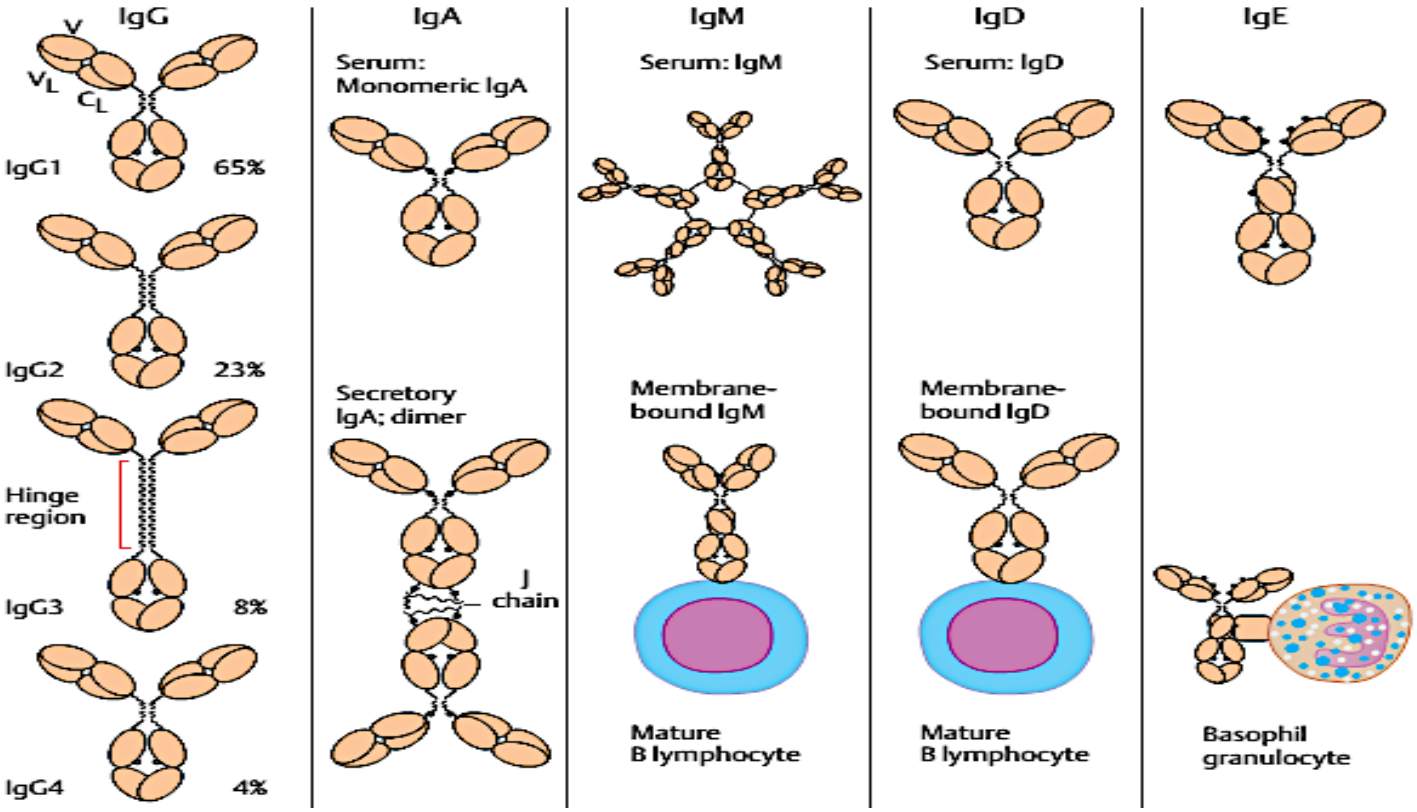


Figure 5.3. Classes of immunoglobulins.

Table 5.1 illustrates the main characteristics of the different classes of immunoglobulins.

Table 5.1. Main characteristics of the different classes of immunoglobulins.

Parameter	IgM	IgD	IgG	IgE	IgA
Structure	Pentamer	Monomer	Monomer	Monomer	Monomer / Dimer
Heavy chain	μ	δ	γ	ε	α
Molecular weight (kDa)	900-970	180-185	150-160	~188	160 (mono) / ~385 (dimer)
Localization	Serum, B cells	B cells	Serum, extracellular fluids	Bound to mast cells and basophils	Mucosal secretions
Half-life (days)	5-10	~3	7-23	~2	~6
Complement activation	Strong	No	Yes (except IgG4)	No	No
Placental transfer	No	No	Yes	No	No
Main functions	Primary response, agglutination, complement activation	BCR on naïve B cells	Opsonization, neutralization, ADCC	Allergy (type I hypersensitivity), antiparasitic	Mucosal immunity

V.2. Antigens

V.2.1. Immunogenicity and antigenicity

Immunogenicity and antigenicity are related but distinct immunological properties.

- **Immunogenicity** refers to the ability of a substance to induce an immune response, either humoral or cell-mediated.
- **Antigenicity** refers to the ability of a molecule to specifically bind to immune receptors, such as antibodies or T-cell receptors (TCRs).

Thus, all immunogens are antigens, but not all antigens are immunogenic.

V.2.2. Antigenic determinants (Epitopes)

Epitopes are the specific regions of an antigen that are recognized by the immune system. They interact with:

- B-cell receptors (BCRs) and antibodies (native antigen)
- T-cell receptors (TCRs) when presented by MHC molecules

Epitopes vary in size, with B-cell epitopes being variable, while T-cell epitopes are typically 8-10 amino acids when presented by MHC class I and 12-25 amino acids when presented by MHC class II.

Note: *Most antigens contain multiple epitopes, leading to a polyclonal immune response.*

V.2.3. Types of epitopes

- **Linear epitopes:** Composed of continuous amino acid sequences; recognized even after protein denaturation **(see Fig. 5.4)**.
- **Conformational epitopes:** Formed by amino acids brought together by the three-dimensional structure; recognition is lost upon denaturation.

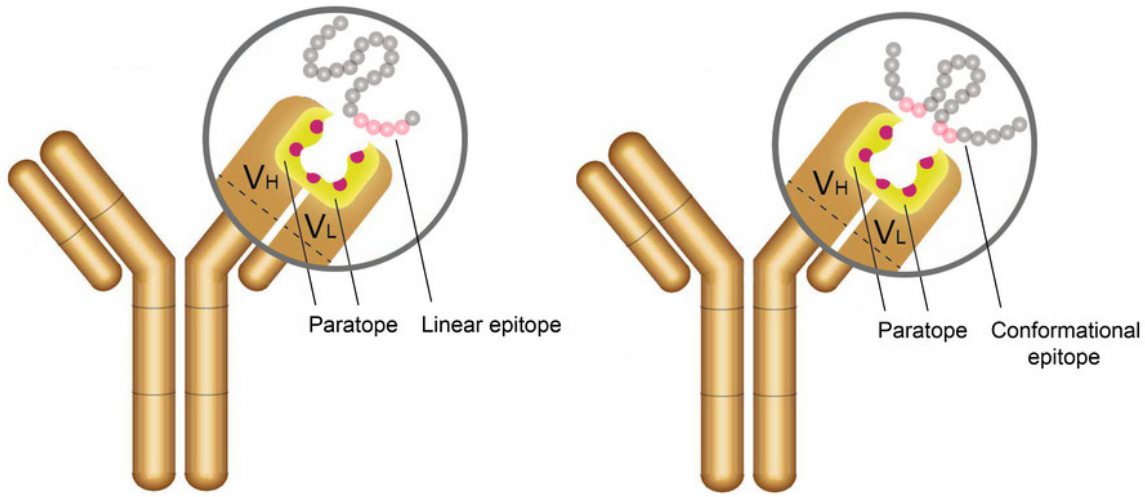


Figure 5.4. Linear and conformational epitopes.

V.2.4. Factors influencing immunogenicity

Immunogenicity depends on several key factors:

- **Foreignness:** The greater the difference between the antigen and host molecules, the stronger the immune response.
- **Molecular size:** Large molecules (typically >10 kDa) are more immunogenic.
- **Chemical complexity:** Structurally diverse molecules are more immunogenic than simple or repetitive structures.
- **Processability and presentation:** Antigens must be processed by APCs and presented via MHC molecules. Molecules resistant to degradation (e.g., D-amino acid polymers) are poorly immunogenic.

V.2.5. Chemical nature of antigens

Antigens are typically macromolecules capable of being recognized by the immune system and inducing an immune response.

1. Proteins are the most immunogenic antigens (enzymes**,** bacterial toxins**,** viral capsid proteins**,** cell surface receptors, etc.); they are highly immunogenic because they:

- Possess complex tertiary and quaternary structures.
- Contain multiple epitopes
- Are efficiently processed and presented by antigen-presenting cells via MHC molecules.

2. Polysaccharides, like bacterial capsules and lipopolysaccharides (LPS) (polysaccharides are typically T-independent antigens):

- ☞ Induce mainly an IgM response
- ☞ Generate weak or absent immunological memory.
- ☞ Do not efficiently activate T helper cells.

3. Lipids and nucleic acids are generally poor immunogens when administered alone, however:

- ☞ They can become immunogenic when conjugated to proteins (hapten-carrier effect)
- ☞ Some lipid antigens are presented by CD1 molecules instead of MHC.

The general order of immunogenicity is **Proteins > Polysaccharides > Lipids > Nucleic acids.**

V.2.6. Thymus-dependent and thymus-independent antigens

Antigens can also be classified based on their ability to **require T-cell help** for the activation of B lymphocytes.

a. Thymus-dependent antigens

Thymus-dependent antigens are typically protein antigens that require the cooperation of $CD4^+$ T helper cells to induce a full immune response.

After antigen uptake and processing by antigen-presenting cells (APCs), peptide fragments are presented via MHC class II molecules to T helper cells. Activated T cells then provide co-stimulatory signals and cytokines to B cells, leading to:

- ☞ B-cell activation and proliferation
- ☞ Class switching (isotype switching)
- ☞ Somatic hypermutation and affinity maturation
- ☞ Generation of memory B cells

These antigens induce a strong, long-lasting, and highly specific immune response.

b. Thymus-independent antigens

Thymus-independent antigens can activate B cells without T-cell help. These are usually polysaccharides, lipopolysaccharides (LPS), and repetitive molecular structures.

These antigens directly stimulate B cells by extensive cross-linking of B-cell receptors (BCRs). The immune response induced is characterized by:

- Predominant production of IgM antibodies
- Limited class switching
- Low-affinity antibodies
- Weak or absent immunological memory

These responses are generally rapid but less effective and short-duration.

V.2.7. Classification of antigens based on valency and epitope diversity

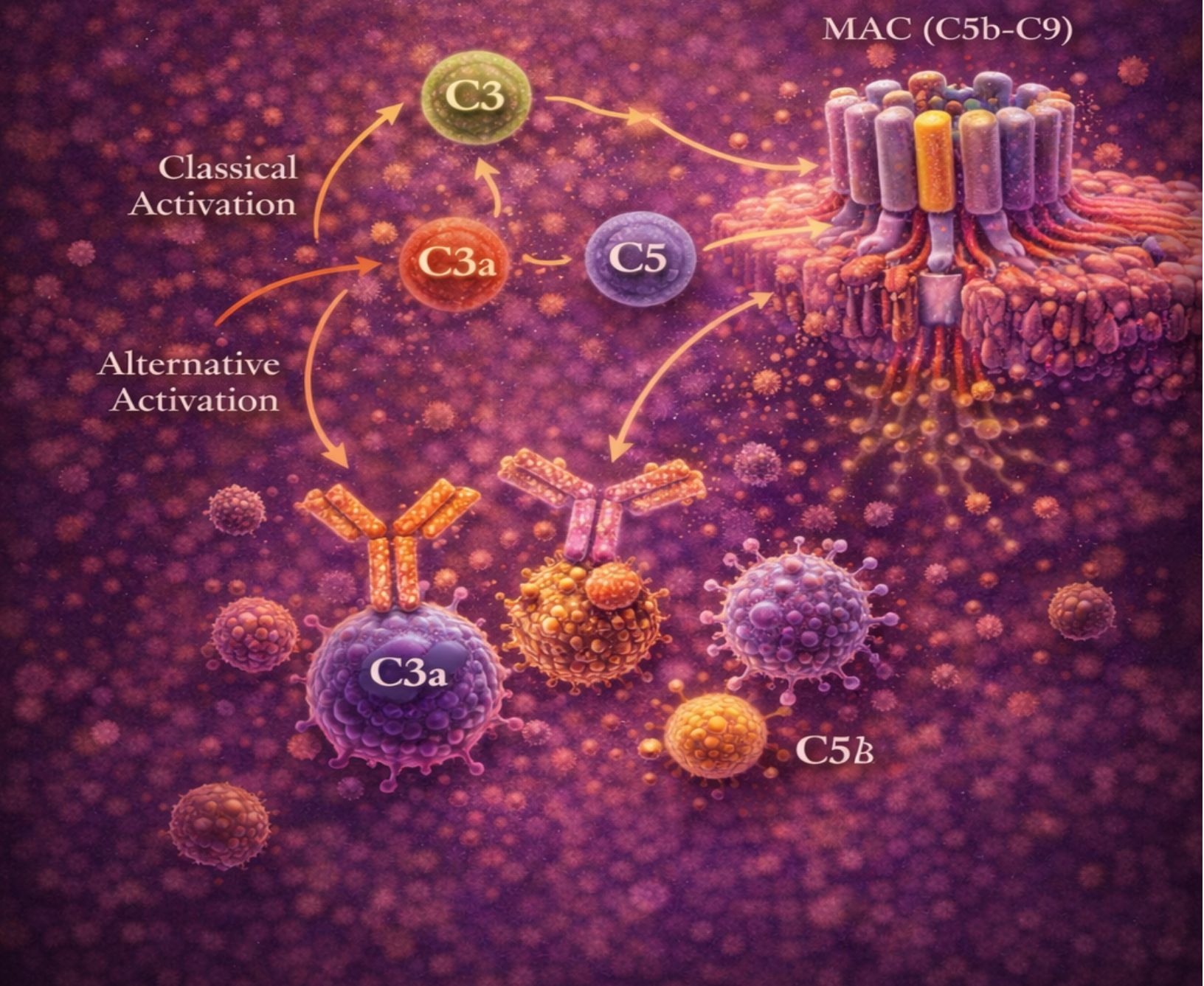
Antigens can be classified according to their **valency (number of identical epitopes)** and **epitope diversity (number of different epitopes)**:

a. **Monovalent and monoepitopic (unideterminant) antigen:** An antigen that possesses a single epitope, allowing binding to only one antibody molecule, for example, haptens.
b. **Multivalent and monoepitopic antigen:** An antigen that contains multiple identical epitopes, allowing simultaneous binding of several antibodies of the same specificity. This configuration enhances cross-linking of antibodies or BCRs.
c. **Monovalent and multiepitopic (multideterminant) antigen:** An antigen that contains different epitopes but only one copy of each epitope. This situation is rare in practice but theoretically possible.
d. **Multivalent and multiepitopic antigen:** An antigen that contains multiple different epitopes, each present in multiple copies. Most natural protein antigens, viruses, and cells fall into this category.

V.2.8. Categories of antigens

- **Allergens:** induce allergic reactions in sensitized individuals
- **Xenoantigens:** derived from a different species
- **Alloantigens:** differ between individuals of the same species
- **Autoantigens:** self-components targeted in autoimmune diseases
- **Complete antigens:** capable of inducing an immune response independently
- **Haptens:** small molecules that become immunogenic only when bound to a carrier

Chapter VI.
The Complement System



Chapter VI. The Complement System

VI.1. Definition

The complement system is a group of plasma proteins that function in a cascade of activation reactions, playing a crucial role in both innate immunity and the effector phase of adaptive immune responses. Most complement proteins are synthesized in the liver by hepatocytes and circulate in the blood as inactive precursors (zymogens). Additional production occurs in monocytes, macrophages, and epithelial cells.

Complement components are designated by numerical names (C1 to C9) or by specific factors (e.g., factor B, factor D). Upon activation, these proteins are cleaved into fragments denoted by lowercase letters. In most cases, the smaller fragment is labeled "a" and the larger fragment "b" (e.g., C3a, C3b), with the exception of C2, where the larger fragment is termed C2a.

Activated complement fragments interact to form enzymatic complexes, such as C3 convertases (C4b2a, C3bBb), which amplify and propagate the complement cascade.

VI.2. Roles of the complement system

The complement system is a major component of innate immunity and contributes to host defense through several key mechanisms:

- **Defense against infection:** The complement system eliminates pathogens through three principal mechanisms:
  - **Direct lysis of target cells:** This occurs via the formation of the membrane attack complex (MAC; C5b-C9), which creates pores in the membrane of target cells, leading to their destruction.
  - **Opsonization:** Complement activation results in the deposition of C3b on the surface of pathogens, enhancing their recognition and phagocytosis by immune cells via complement receptors.
  - **Inflammation and cellular activation:** Complement activation generates small peptide fragments, particularly C3a and C5a (anaphylatoxins), which induce chemotaxis, increased vascular permeability, and activation of immune cells.

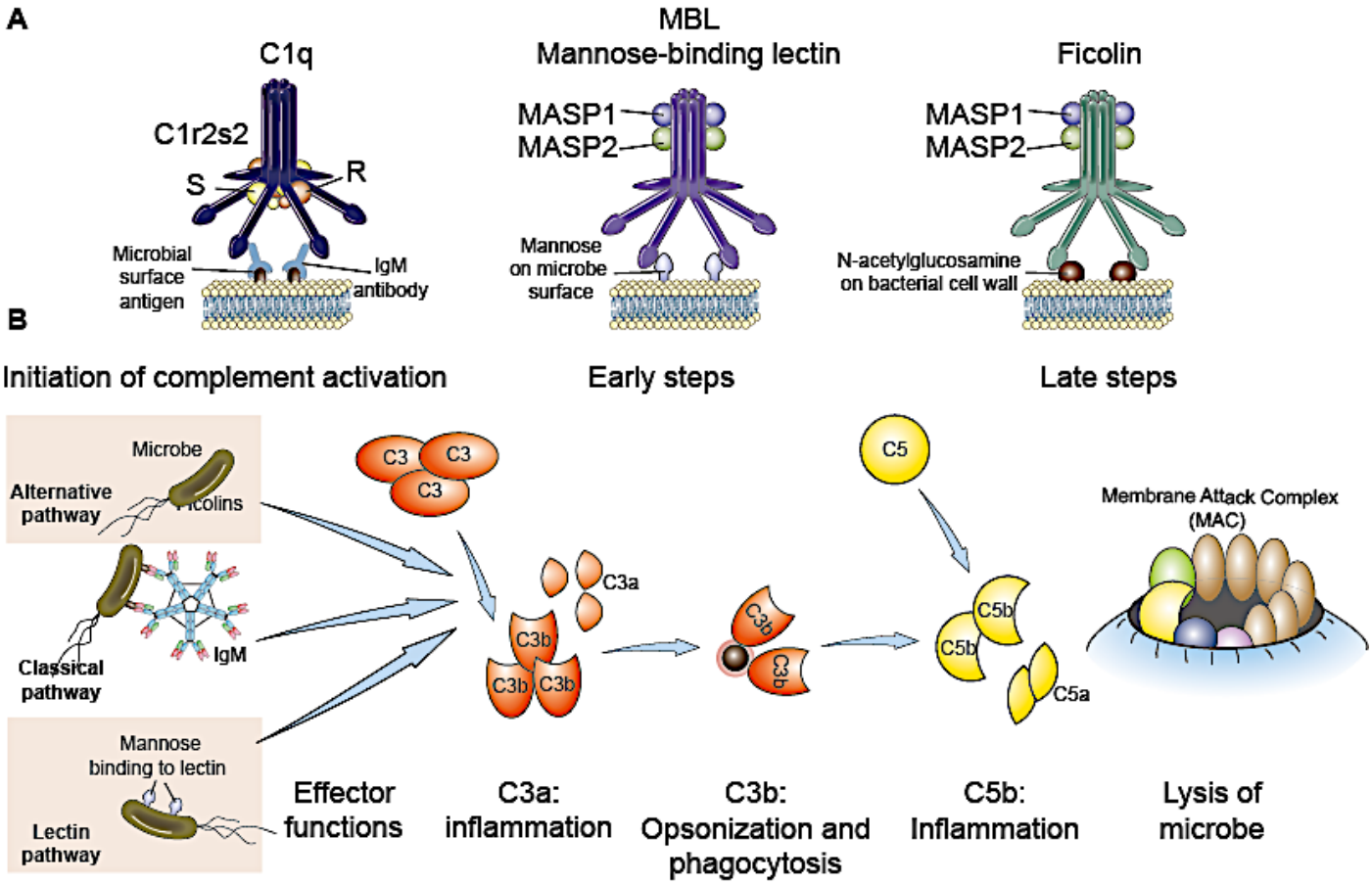


Figure 6.1. Overview of activation and functions of complement system.

- **Clearance of immune complexes**

The complement system facilitates the transport and elimination of antigen-antibody complexes. These complexes bind to complement receptors (CR1/CD35) on erythrocytes, which transport them to the liver and spleen for clearance, thereby preventing their deposition in tissues such as the kidneys.

- **Modulation of adaptive immunity**

The complement system acts as a bridge between innate and adaptive immunity, contributing to the regulation of B-cell activation and antibody responses.

VI.3. Complement activation

The activation of the complement system can be initiated through three distinct pathways: the classical, alternative, and lectin pathways. These pathways differ in their initial steps but converge at the formation of C3 convertase, leading to the generation of C5 convertase and the cleavage of C5 into C5b. The subsequent steps, known as the terminal pathway, are common to all three pathways and result in the formation of the **membrane attack complex (MAC)**.

VI.3.1. The classical pathway

Activation of the classical pathway is typically antibody-dependent and occurs via the formation of antigen-antibody (Ag-Ab) complexes. It can also be initiated by certain innate immune molecules such as C-reactive protein (CRP). Only specific immunoglobulin isotypes, namely IgM, IgG1, and IgG3, and to a lesser extent IgG2, have the capacity to activate the complement cascade, whereas IgG4 does not. The initial stages of activation involve the sequential activation of complement components C1, C4, C2, C3, and C5.

C1 is a macromolecular complex that serves as the recognition unit of the classical complement pathway. It consists of C1q, associated with two C1r molecules and two C1s molecules, forming a Ca^{2+}-dependent complex. C1q itself is composed of 18 polypeptide chains organized into six globular head domains. These globular heads bind to the Fc region of antibodies, enabling initiation of the complement cascade **(Fig. 6.2)**.

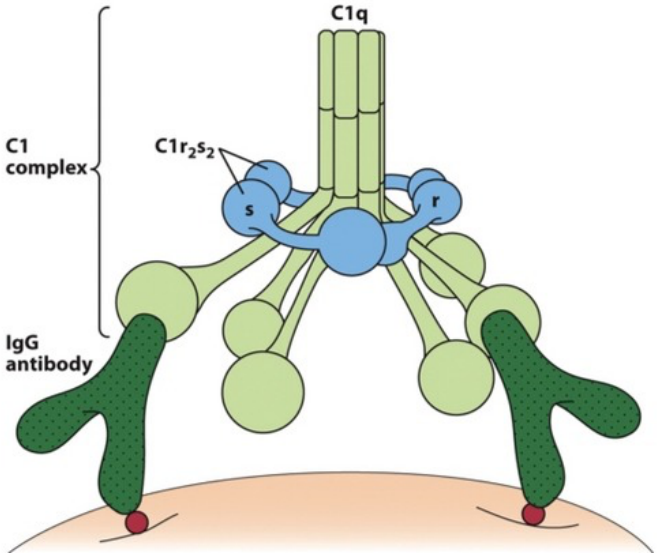


Figure 6.2. The C1 macromolecule complex.

Activation occurs when the globular heads of C1q bind to the Fc fragments of antibodies within immune complexes. This interaction induces a conformational change in C1r, which becomes enzymatically active and subsequently activates C1s by proteolytic cleavage. Activated C1s then acts on its substrates, C4 and C2.

- ☞ Each C1 complex must bind to at least two Fc regions for stable activation. Thus, two IgG molecules are required, whereas a single IgM molecule is sufficient due to its pentameric structure.
- ☞ C1s cleaves C4 into C4a and C4b. C4b binds to the target surface. C1s then cleaves C2 into C2a and C2b.
- ☞ C2a associates with C4b to form the C3 convertase (C4b2a).

- ☞ The C3 convertase cleaves C3 into C3a and C3b.
- ☞ C3b binds to the target surface or immune complexes and acts as an opsonin, enhancing phagocytosis. Some C3b molecules bind to C4b2a, forming the C5 convertase (C4b2a3b).
- ☞ The C5 convertase cleaves C5 into C5a and C5b.
- ☞ C5b initiates the formation of the membrane attack complex (MAC) by sequential binding to C6, C7, C8, and C9 **(Fig. 6.3).**
- ☞ Each cleavage step generates small peptide fragments, notably C3a and C5a, which act as anaphylatoxins, promoting inflammation, chemotaxis, and activation of immune cells. C4a has weak anaphylatoxin activity, whereas C2b does not function as an anaphylatoxin.

VI.3.2. The alternative pathway

The alternative pathway is initiated independently of antibodies and is triggered by the spontaneous hydrolysis of C3 or by the direct interaction of C3b with microbial surfaces.

C3b binds to factor B, which serves as a substrate for the serine protease factor D. Factor D cleaves factor B into Ba and Bb, leading to the formation of the C3bBb complex, which possesses C3 convertase activity (analogous to C4b2a in the classical pathway). The C3 convertase C3bBb has a short half-life of approximately 5 minutes. However, the binding of properdin (factor P) stabilizes this complex, extending its half-life to approximately 30 minutes.

C3bBb cleaves additional C3 molecules, generating more C3b, thereby amplifying the complement response. Some of the newly generated C3b binds to the C3bBb complex, forming the C5 convertase (C3bBb3b) (analogous to C4b2a3b in the classical pathway). This C5 convertase cleaves C5 into C5a and C5b. The fragment C5b subsequently binds to the target surface and initiates the formation of the membrane attack complex (MAC) **(Fig. 6.3).**

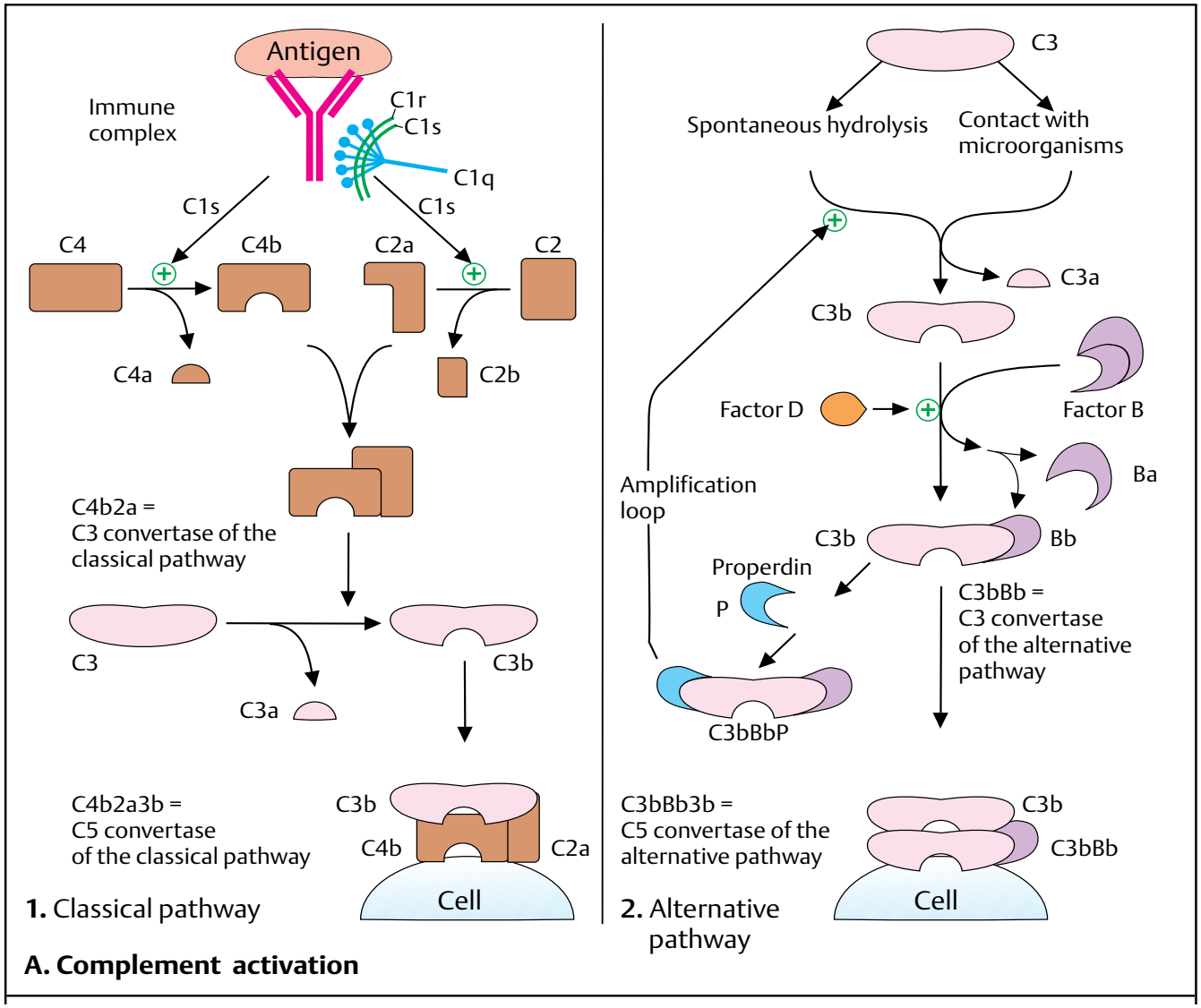


Figure 6.3. Complement activation via the classical and alternative pathways.

VI.3.3. The lectin pathway

Lectins are proteins that specifically bind to carbohydrates. Similar to the alternative pathway, the lectin pathway does not rely on antibodies for activation. However, its mechanism closely resembles that of the classical pathway, as both pathways involve the activation of C4 and C2, leading to the formation of the C3 convertase (C4b2a) and subsequently the C5 convertase.

The lectin pathway is initiated by mannose-binding lectin (MBL), which shares structural similarities with C1q. In this pathway, C1r and C1s are replaced by MASPs (MBL-associated serine proteases), mainly MASP-1 and MASP-2. MBL binds to mannose and other carbohydrate residues present on the surface of microorganisms. Upon binding, MASP-1 and MASP-2 become activated. These proteases then cleave C4 and C2, leading to the formation of the C3 convertase (C4b2a), which drives the complement cascade forward **(Fig. 6.4).**

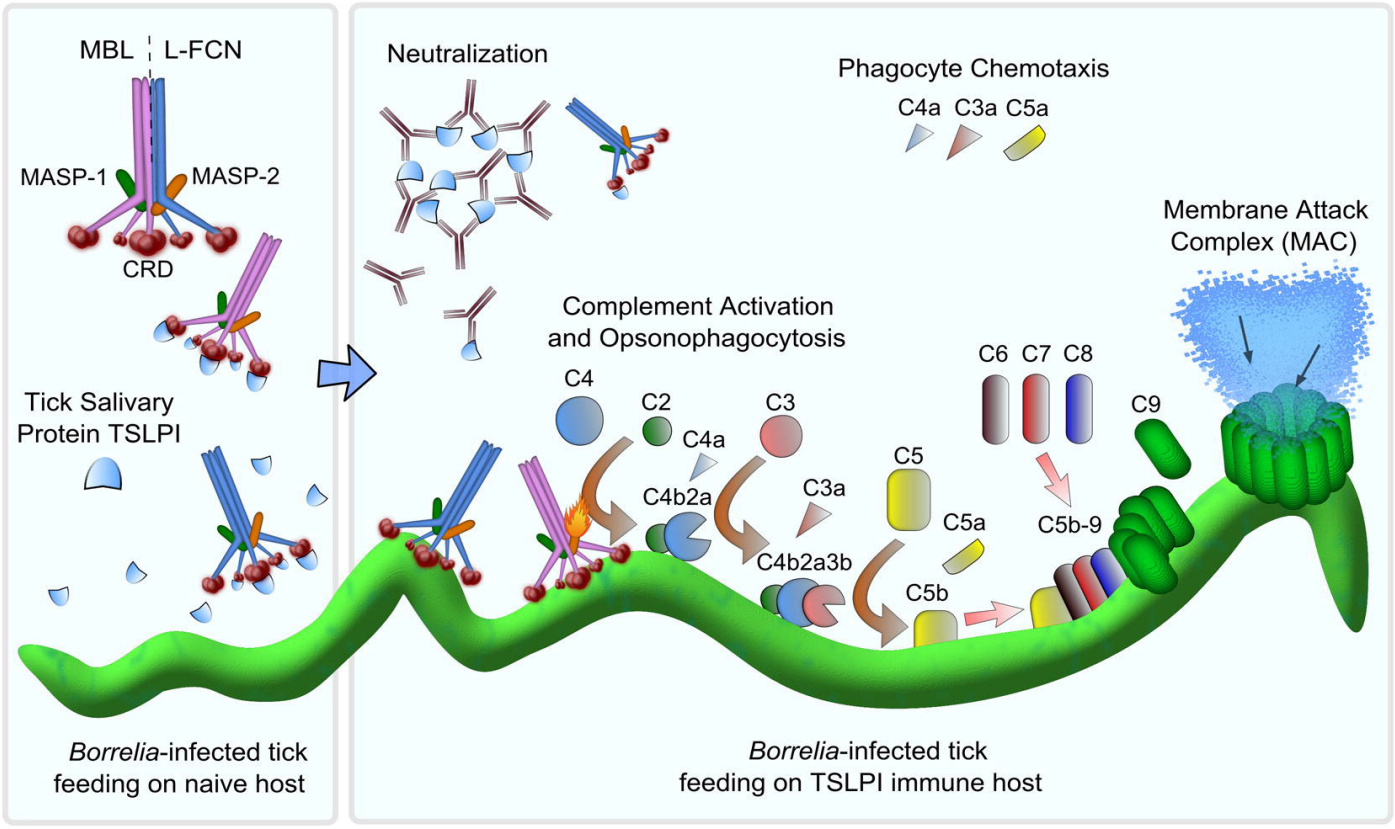


Figure 6.4. Complement activation via the lectin pathway.

VI.3.4. The lytic terminal sequence

The terminal phase of complement activation involves a series of interactions between C5b, C6, C7, C8, and C9, which sequentially assemble to form a large transmembrane pore known as the membrane attack complex (MAC). This complex disrupts the integrity of the target cell membrane.

C5b, generated by the activity of the C5 convertase, rapidly binds to C6, forming the stable C5b6 complex, which subsequently associates with C7 to form C5b67. This complex undergoes a conformational change that allows its insertion into the target cell membrane. The binding of C8 leads to the formation of the C5b678 complex, which further destabilizes the membrane. The final step in MAC formation involves the binding and polymerization of multiple C9 molecules (approximately 10-16) onto the C5b678 complex, forming a poly-C9 pore structure. The complete MAC consists of a C5b678 core surrounded by polymerized C9.

The formation of this transmembrane pore disrupts cellular homeostasis, leading to osmotic imbalance, influx of water and ions, and ultimately cell lysis **(Fig. 6.5)**.

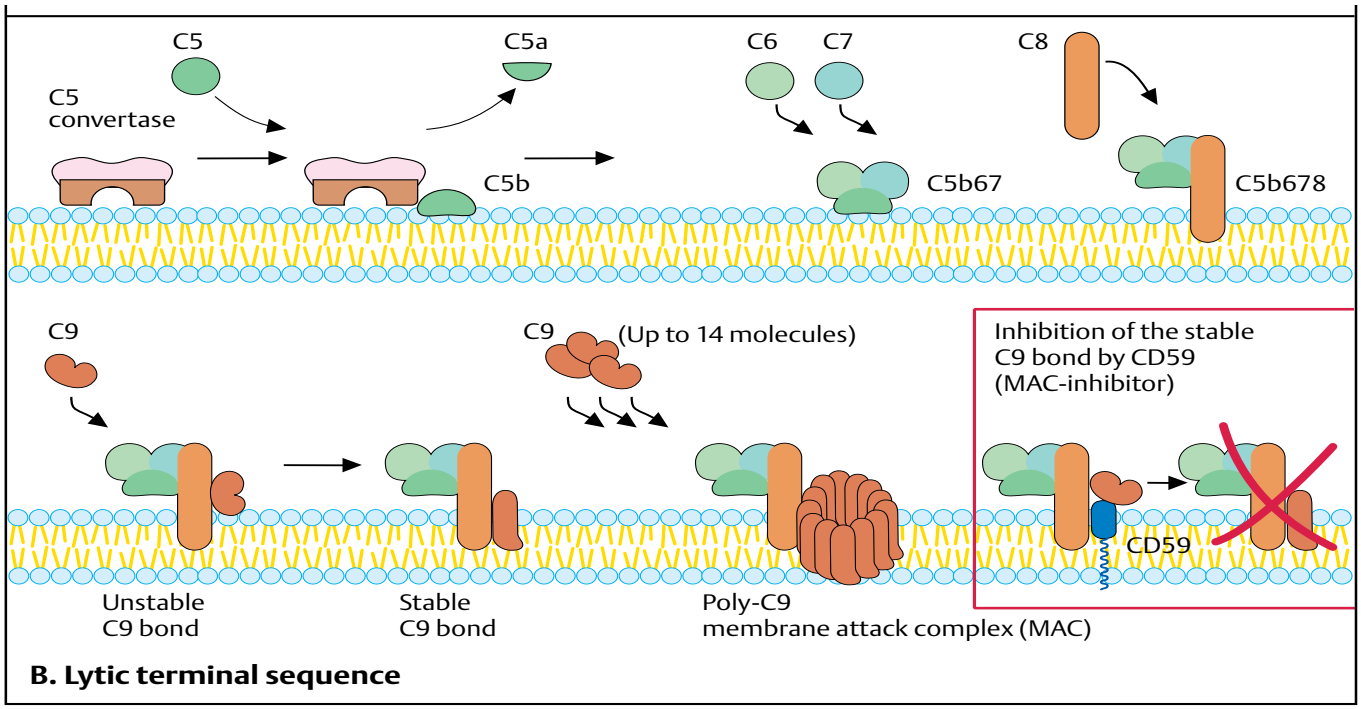


Figure 6.5. The lytic terminal sequence of complement activation (MAC formation).

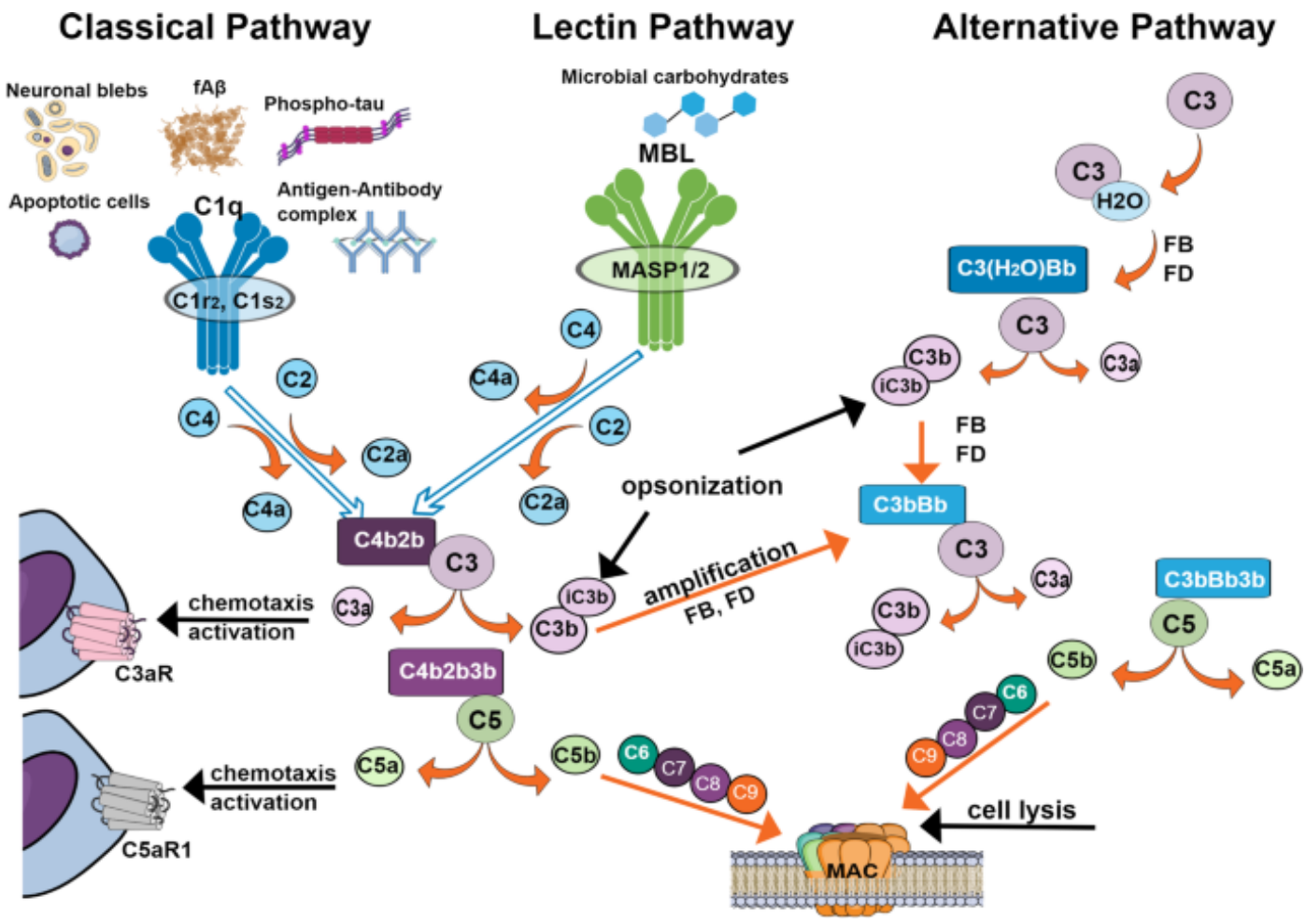


Figure 6.6. The three pathways of activation cascade of the complement system.

Chapter VII.
Cytokines

Chapter VII. Cytokines

VII.1 Definition

Cytokines represent a heterogeneous group of bioactive proteins that function as key mediators of intercellular communication. Initially identified as products of immune cells, cytokines play a central role in the regulation and coordination of immune responses. It is now well established that many cytokines are also synthesized by non-immune cells and can act on a wide variety of target cells, extending their functions beyond the immune system.

The term **"cytokine"** is used as a general designation for this class of signaling molecules, although more specific terms are used to describe certain subsets, including the following:

- **Monokines:** Cytokines secreted by mononuclear phagocytes, such as monocytes and macrophages.
- **Lymphokines:** Cytokines produced by activated lymphocytes, particularly T-helper (Th) cells.
- **Interleukins:** A broad group of cytokines originally described as mediators between leukocytes, but now known to act on diverse cell types.
- **Chemokines:** A specialized subset of small cytokines that regulate leukocyte migration and chemotaxis.

Cytokines exert their biological effects through several modes of action, including autocrine, paracrine, endocrine, and, in some cases, juxtacrine signaling.

VII.2. Characteristics

- Cytokines are low-molecular-weight, soluble glycoproteins (generally <30 kDa) that function as key mediators of intercellular communication.
- They are synthesized de novo in response to various stimuli, including antigens, mitogens, microbial components, and other cytokines.
- Cytokine production does not necessarily induce cellular proliferation. While it requires gene transcription and RNA synthesis, it does not require DNA replication.
- The production of a given cytokine is tightly regulated through positive and negative feedback mechanisms, involving multiple factors, particularly other cytokines, at different stages of its synthesis and activity.

VII.3. Mode of action

Three primary modes of cytokine action, endocrine, paracrine, and autocrine, are well documented in the literature. Cytokines exhibit a broad functional versatility, characterized by:

- **The producing cell:** A given cytokine can be secreted by multiple cell types, and a single cell can produce several different cytokines.
- **The target cell:** Cytokines can act on a wide variety of target cells, depending on the cellular context and the expression of specific receptors.

Key characteristics of cytokine actions include the following:

- **Pleiotropy:** A single cytokine can exert multiple biological effects on different cell types.
- **Redundancy:** Different cytokines can produce similar or overlapping biological effects.
- **Synergy:** Two or more cytokines can act together to amplify their biological effects.
- **Antagonism:** Certain cytokines can inhibit or counteract the effects of others.
- **Cascade:** Cytokines can act in a sequential manner, whereby one cytokine induces the production of others, leading to amplification of the immune response **(see Fig. 7.1)**.

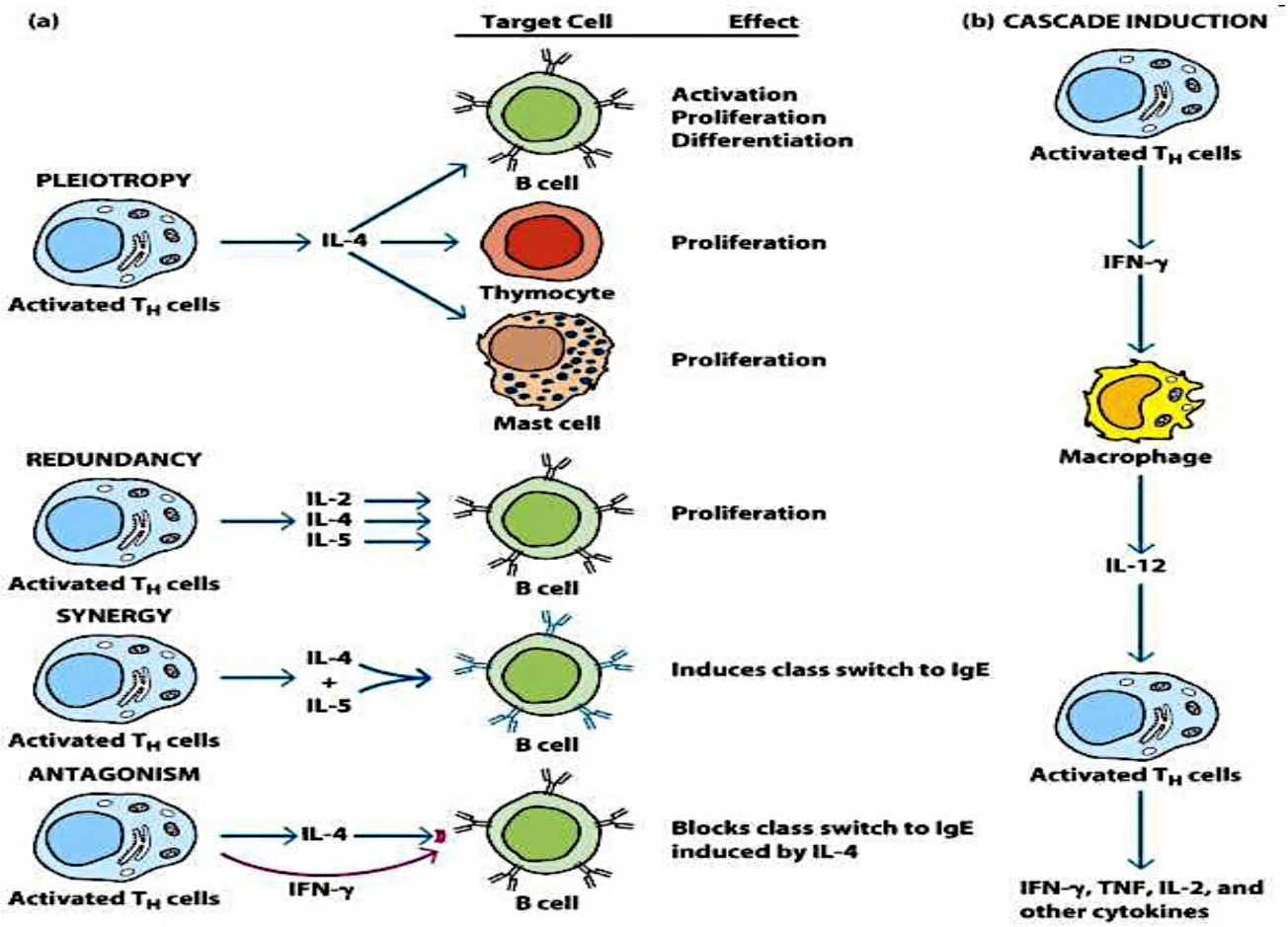


Figure 7.1. Mode of action of cytokines.

VII.4. Categories

VII.4.1. Cytokines of natural immunity

Cytokines that play pivotal roles in the innate immune response include tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-12 (IL-12), type I interferons (IFN-α and IFN-β), interferon-gamma (IFN-γ), and various chemokines.

- **Tumor Necrosis Factor-alpha (TNF-α)** is primarily produced by macrophages activated in response to microbial stimuli, particularly lipopolysaccharide (LPS) from Gram-negative bacteria. It is a key mediator of acute inflammation. TNF-α promotes the recruitment of neutrophils and macrophages by inducing the expression of adhesion molecules and chemokines in endothelial cells, facilitating leukocyte diapedesis. It also acts on the hypothalamus to induce fever.
- **Interleukin-1 (IL-1)** is a pro-inflammatory cytokine that acts on multiple organs. It induces fever via prostaglandin synthesis in the hypothalamus and stimulates the liver to produce acute-phase proteins.
- **Interleukin-6 (IL-6)** is a pro-inflammatory cytokine involved in the acute-phase response. It stimulates the liver to synthesize proteins such as C-reactive protein (CRP), fibrinogen, and serum amyloid A. IL-6 also promotes B-cell activation and T-cell differentiation.
- **Interleukin-10 (IL-10)** is produced by macrophages, regulatory T cells, and Th2 cells. It is an inhibitory cytokine that limits immune responses by suppressing cytokine production and reducing MHC class II and co-stimulatory molecule expression on antigen-presenting cells.
- **Interleukin-12 (IL-12)** is produced by macrophages and dendritic cells. It promotes IFN-γ production and induces differentiation of $CD4^+$ T cells into Th1 cells. It also enhances NK cell and cytotoxic T-cell activity.
- **Type I interferons (IFN-α and IFN-β)** are produced by infected cells in response to viral infections. They inhibit viral replication, increase MHC class I expression, and activate NK cells.
- **Interferon-gamma (IFN-γ)** is produced by NK cells and T lymphocytes. It plays a major role in macrophage activation, enhances antigen presentation by increasing MHC I and II expression, and promotes Th1-type immune responses.
- **Chemokines** are small cytokines involved in leukocyte migration. They bind to specific receptors (e.g., CCR, CXCR) and direct immune cells toward sites of infection. For example, IL-8 (CXCL8) recruits neutrophils during inflammation.

VII.4.2. Cytokines of adaptive immunity

Cytokines that play pivotal roles in adaptive immunity include IL-2, IL-4, IL-5, TGF-β, IL-10, and IFN-γ. These cytokines regulate lymphocyte activation, proliferation, and differentiation.

- **Interleukin-2 (IL-2)** is primarily produced by activated $CD4^+$ T cells and, to a lesser extent, $CD8^+$ T cells. It is the principal growth factor for T lymphocytes, promoting their proliferation and differentiation. IL-2 also supports B-cell growth and activates NK cells. Its receptor (IL-2R) is upregulated upon T-cell activation.
- **Interleukin-4 (IL-4)** is primarily produced by Th2 cells, mast cells, and basophils. It promotes differentiation of $CD4^+$ T cells into Th2 cells and stimulates B cells to undergo class switching, particularly toward IgE production.
- **Interleukin-5 (IL-5)** is produced mainly by Th2 cells. It promotes the growth, differentiation, and activation of eosinophils and supports B-cell function.
- **Transforming growth factor-beta (TGF-β)** is produced by T cells and other cell types. It is an inhibitory cytokine that suppresses T-cell proliferation and macrophage activation, while contributing to immune regulation and tolerance.

VII.4.3. Hematopoiesis stimulatory cytokines

Some cytokines play a critical role in stimulating the differentiation of hematopoietic cells **(see Fig. 7.2)**:

- **GM-CSF (Granulocyte-Macrophage Colony-Stimulating Factor):** stimulates the differentiation of progenitor cells into granulocytes and macrophages.
- **M-CSF (Macrophage Colony-Stimulating Factor):** promotes the differentiation of monocytes and their maturation into macrophages.
- **G-CSF (Granulocyte Colony-Stimulating Factor):** stimulates the production and differentiation of neutrophils in the bone marrow.

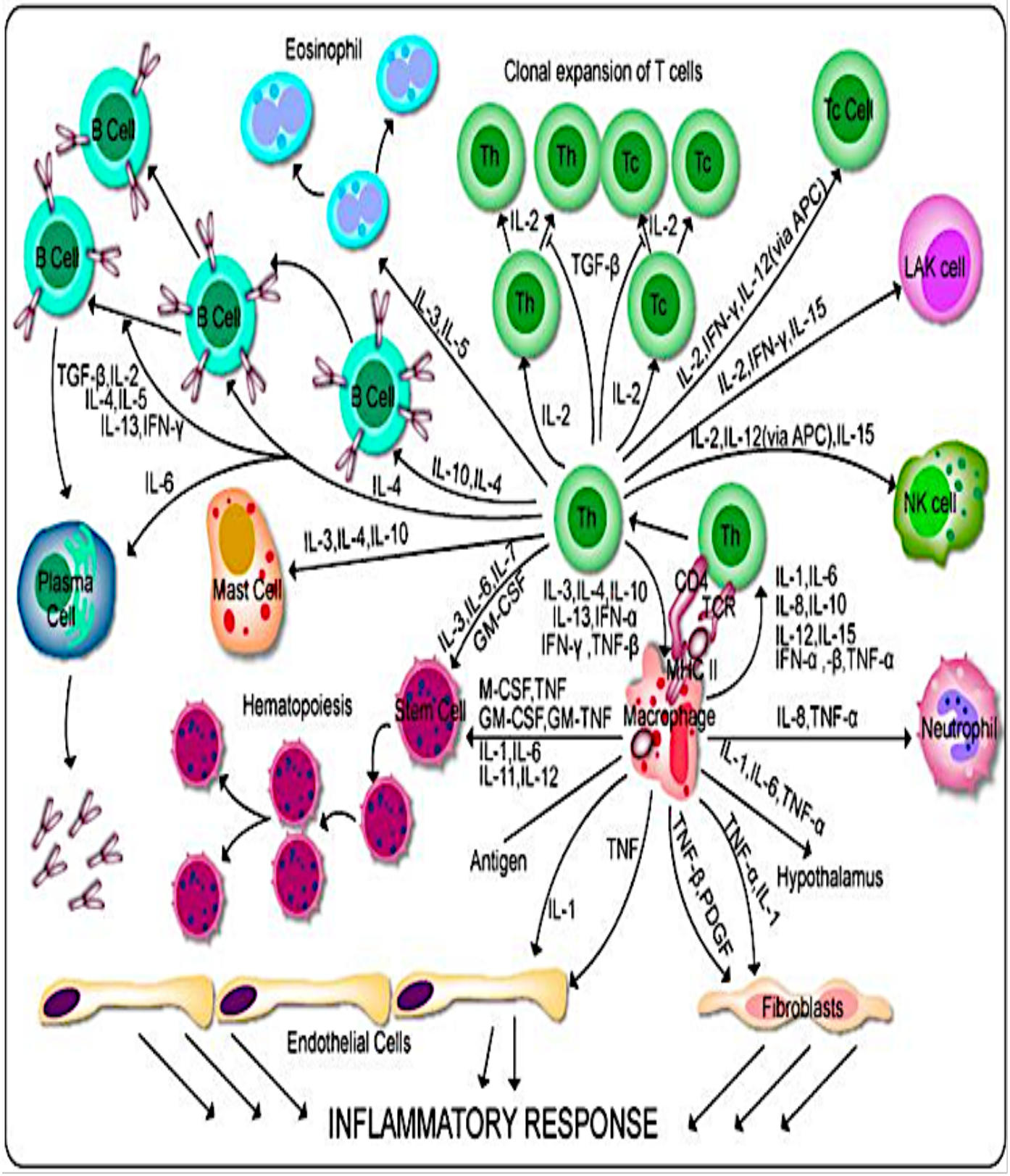
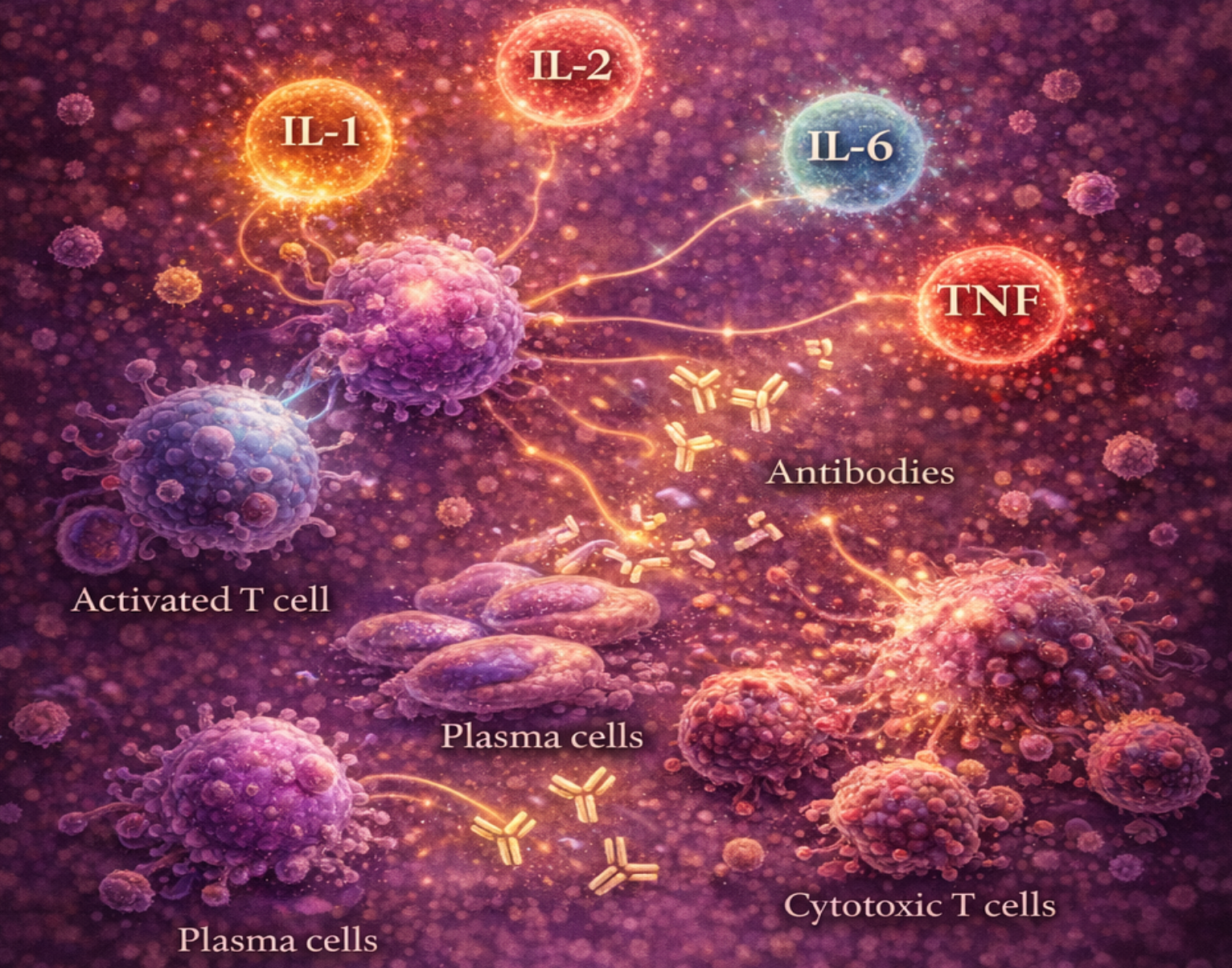


Figure 7.2. The cytokine networks.

Chapter VIII.
Immune Response and Cellular-Humoral Cooperation



Chapter VIII. Immune Response and Cellular-Humoral Cooperation

Living organisms are constantly exposed to environmental threats, particularly infectious microorganisms. The immune system functions as a surveillance network that protects the host by detecting and eliminating harmful agents. This defense includes both innate immunity, which provides a rapid, non-specific first line of protection, and adaptive immunity, which is highly specific and capable of memory.

Innate immunity is present from birth and involves physical and chemical barriers, as well as cellular and molecular components that act quickly against pathogens. In addition to its direct protective role, innate immunity plays a crucial part in shaping and regulating adaptive immune responses **(Fig. 8.1).**

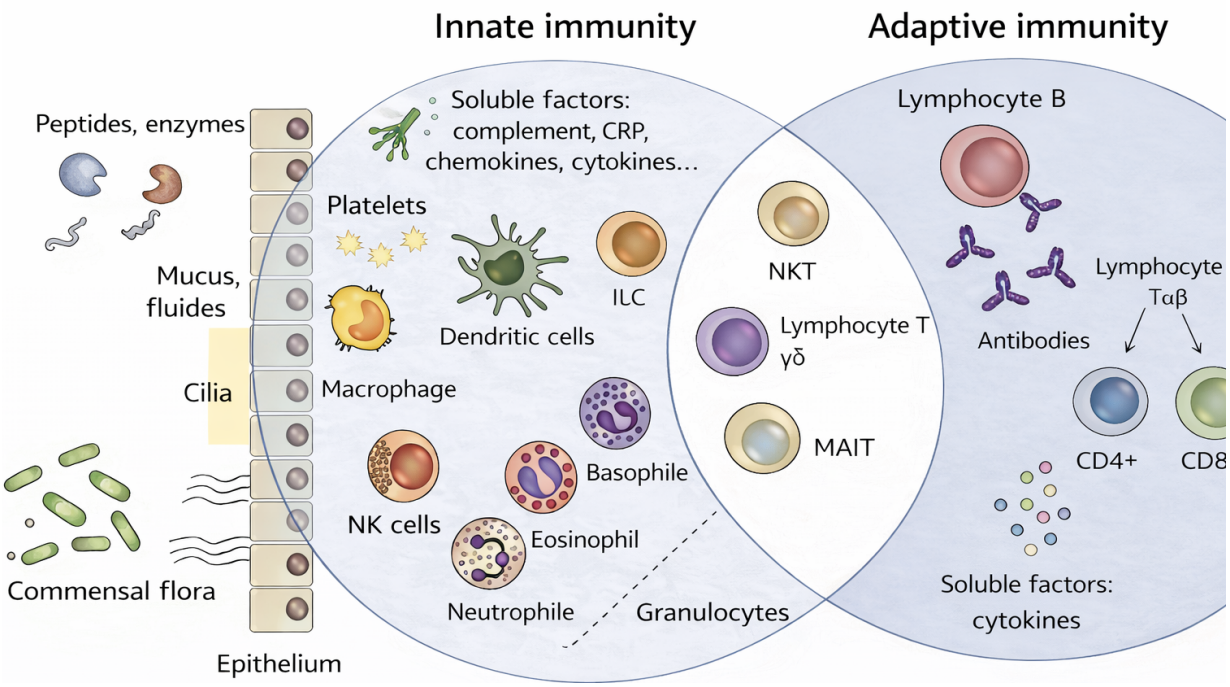


Figure 8.1. The organization of the immune responses.

VIII.1. Physical and chemical barriers of innate immunity

The skin constitutes a major **physical barrier** that prevents the entry of most pathogens. Although they may enter the body when the skin is damaged or through structures such as hair follicles and sebaceous glands. However, the acidic pH of sweat and sebaceous secretions, along with the presence of fatty acids and hydrolytic enzymes (e.g., lysozyme), exerts antimicrobial effects, thereby limiting this route of infection.

In addition, **soluble proteins,** including interferons and components of the complement system, contribute to non-specific immunity. Interferons are produced in response to viral infections and induce an antiviral state in neighboring cells. The activation of complement proteins triggers a cascade of enzymatic reactions that target microbial membranes and lead to their destruction.

Mucosal surfaces, particularly in the respiratory and gastrointestinal tracts, are protected by **mucus,** which traps microorganisms. These trapped pathogens are then removed by ciliary movement, preventing their colonization.

VIII.2. Innate immunity

Following the recognition of infectious agents by pattern recognition receptors (PRRs) through their interaction with pathogen-associated molecular patterns (PAMPs), pathogens are internalized by professional phagocytic cells. This process leads to the generation of damage-associated molecular patterns (DAMPs) and the activation of intracellular signaling pathways, which initiate the inflammatory response at the site of infection.

This inflammatory cascade is primarily mediated by cytokines, which act as signaling molecules to recruit and activate additional immune cells, thereby amplifying the immune response.

VIII.2.1. Cells of innate immunity

a. Phagocytes

The innate immune response, particularly during inflammation, relies on the recruitment and activation of phagocytic cells, notably neutrophils and monocytes/macrophages. These cells migrate from the bloodstream to sites of infection in response to chemotactic mediators, including chemokines (e.g., IL-8/CXCL8), complement fragments (C5a), and lipid mediators.

During this process, leukocytes interact with endothelial adhesion molecules, involving selectins, which mediate initial rolling, and integrins, which enable firm adhesion. This process, known as leukocyte extravasation, involves a coordinated sequence of events **(see Fig. 8.2):**

1. **Tethering and rolling (low-affinity interactions):** Leukocytes establish transient, low-affinity interactions with the endothelial surface mediated by selectins (E-selectin and P-selectin on endothelial cells and L-selectin on leukocytes). These interactions allow leukocytes to tether and roll along the vessel wall.
2. **Activation:** Chemokines presented on the endothelial surface bind to receptors on leukocytes, triggering intracellular signaling pathways that induce a conformational change in integrins, increasing their affinity.
3. **Firm adhesion (high-affinity binding):** Activated leukocyte integrins (e.g., LFA-1, Mac-1) bind strongly to endothelial adhesion molecules of the immunoglobulin superfamily, such as ICAM-1 and VCAM-1, resulting in firm adhesion and arrest of leukocyte movement.
4. **Transendothelial migration (diapedesis):** Leukocytes migrate across the endothelium, primarily through intercellular junctions, in a process mediated by molecules such as PECAM-1 (CD31). They then enter the surrounding tissue and migrate toward the site of infection along a chemotactic gradient.

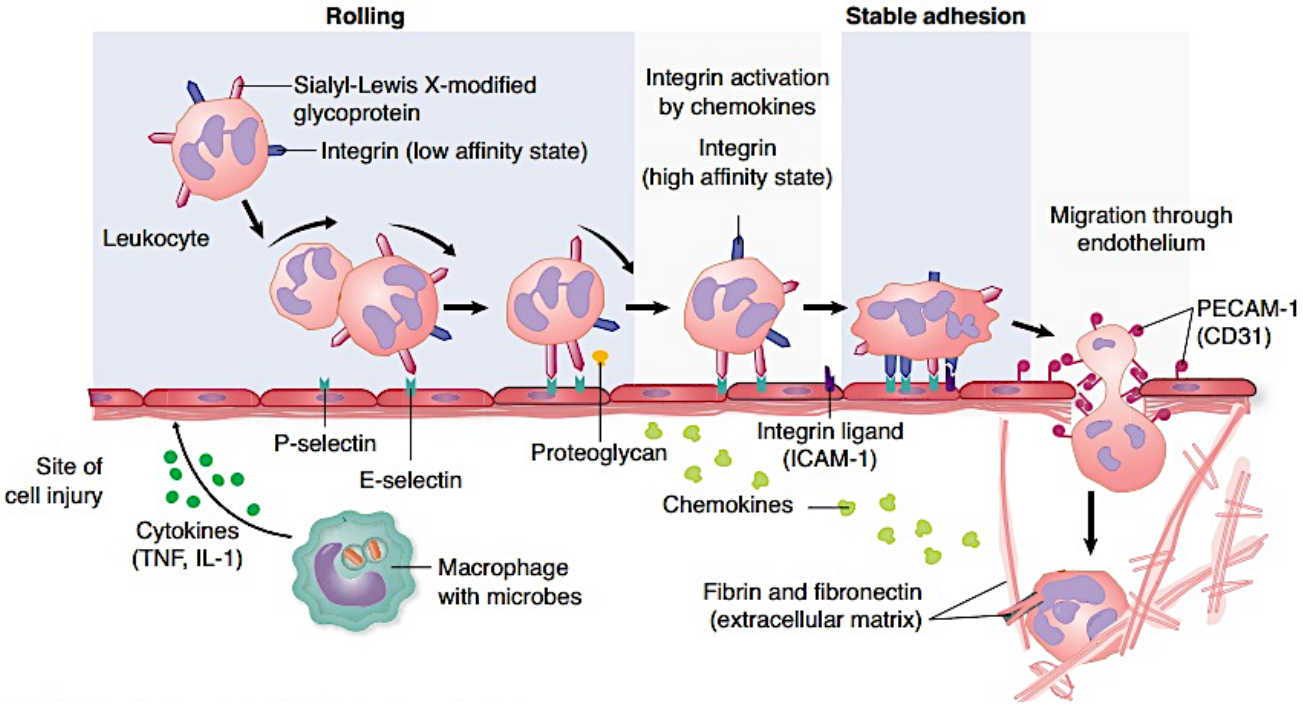


Figure 8.2. Leucocyte extravasation steps.

Macrophages and polymorphonuclear neutrophils, collectively referred to as professional phagocytes, play a central role in innate immunity. These cells act as primary scavengers, capable of internalizing pathogenic microorganisms and apoptotic cells through phagocytosis, a specialized form of receptor-mediated endocytosis.

More broadly, innate immune cells can sample their environment through two related processes:

- **Endocytosis**, which involves the uptake of soluble macromolecules
- **Phagocytosis**, which involves the engulfment of large particles or entire cells

-Endocytosis can occur via:

- **Pinocytosis**, a non-specific mechanism involving membrane invagination
- **Receptor-mediated endocytosis**, which depends on the selective binding of ligands to specific membrane receptors

Following internalization, the ingested material is enclosed within intracellular vesicles (endosomes or phagosomes), which undergo maturation and fusion with lysosomes, forming degradative compartments rich in hydrolytic enzymes such as nucleases, lipases, and proteases.

-Phagocytosis can proceed via two distinct pathways depending on the nature of the pathogen:

1. **Non-opsonic phagocytosis:** In the absence of opsonins, phagocytosis is mediated by the direct interaction between pattern recognition receptors (PRRs) on the phagocyte membrane and microbial antigens. These receptors, including Toll-like receptors (TLRs), mannose receptors, and scavenger receptors, recognize conserved microbial motifs known as pathogen-associated molecular patterns (PAMPs).
2. **Opsonin-dependent phagocytosis:** In this pathway, opsonins act as molecular bridges between the pathogen and the phagocyte. These molecules enhance recognition and uptake by binding both the microbial surface and specific phagocyte receptors. Major opsonins include immunoglobulin G (IgG), which binds to Fcγ receptors; complement component C3b, which binds to complement receptors (CR1, CR3); and mannose-binding lectin (MBL).

-Phagocytosis classically occurs in three sequential phases **(Fig. 8.3):**

1. **Chemotaxis and migration:** Phagocytes are recruited to sites of infection or tissue injury by chemotactic signals, including microbial products (e.g., formyl peptides), host-derived mediators (e.g., prostaglandins and leukotrienes), cytokines and chemokines (notably IL-8/CXCL8), and complement fragments such as C5a.
2. **Recognition and internalization:** The target particle is recognized and engulfed through receptor-mediated interactions, leading to the formation of an intracellular vesicle known as a phagosome, generated by the extension of pseudopodia.
3. **Intracellular killing and degradation:** The phagosome fuses with lysosomes, forming a phagolysosome, where the ingested material is destroyed through enzymatic degradation and oxidative mechanisms. These include the action of lysosomal enzymes, the respiratory

burst mediated by NADPH oxidase, and the production of reactive oxygen and nitrogen species (ROS/RNS).

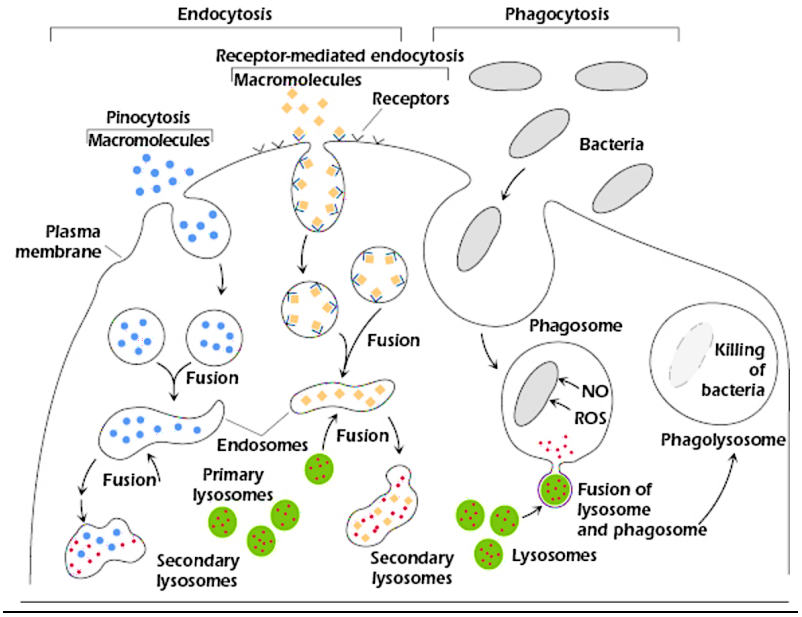


Figure 8.3. Endocytosis and phagocytosis by phagocytes.

b. Natural killer cells

A defining feature of NK cells is their ability to recognize and eliminate abnormal cells through non-antigen-specific mechanisms. This recognition is based on detecting alterations in the surface properties of target cells, including changes in membrane composition and reduced expression of major histocompatibility complex class I (MHC-I) molecules. NK cell activity is tightly regulated by a balance between activating and inhibitory receptors. Among the inhibitory receptors, **killer-cell immunoglobulin-like receptors (KIRs)** play a central role by binding to MHC-I molecules expressed on healthy cells. Engagement of these receptors generates inhibitory signals that prevent NK cell activation and cytotoxicity.

-NK cells exert their cytotoxic function through two main mechanisms that enable them to eliminate abnormal cells:

- ☞ Through an activation-inhibition balance, following the **"missing self" theory**

- Through antibody recognition, via the **ADCC mechanism (Antibody-Dependent Cellular Cytotoxicity)**, which confers antibody-dependent cytotoxic activity.

➔ The "missing self" theory

NK cell activation is regulated according to the "missing self" hypothesis, which is based on the expression of MHC class I molecules on target cells. Two situations can be distinguished:

- **Normal conditions**

  The target cell expresses both activating ligands and MHC class I molecules on its surface. Activating receptors on NK cells may be engaged; however, inhibitory receptors (e.g., KIRs) recognize MHC-I molecules and deliver dominant inhibitory signals, preventing NK cell activation. As a result, cytotoxicity is inhibited **(Fig. 8.4).**

- **Abnormal conditions (infected or tumor cells)**

  In many cases, viral infection or malignant transformation leads to a downregulation or alteration of MHC class I expression, allowing these cells to evade recognition by cytotoxic T lymphocytes. The absence or reduction of MHC-I prevents engagement of inhibitory receptors, thereby lifting the inhibition. Consequently, activating signals prevail, leading to NK cell activation and target cell lysis.

It is important to note that some normal cells in the body express low levels or lack MHC-I molecules. However, NK cell tolerance toward these cells is ensured through education (licensing) processes and tissue-specific regulatory mechanisms that prevent inappropriate NK cell activation.

➔ ADCC Mechanism (Antibody-Dependent Cellular Cytotoxicity)

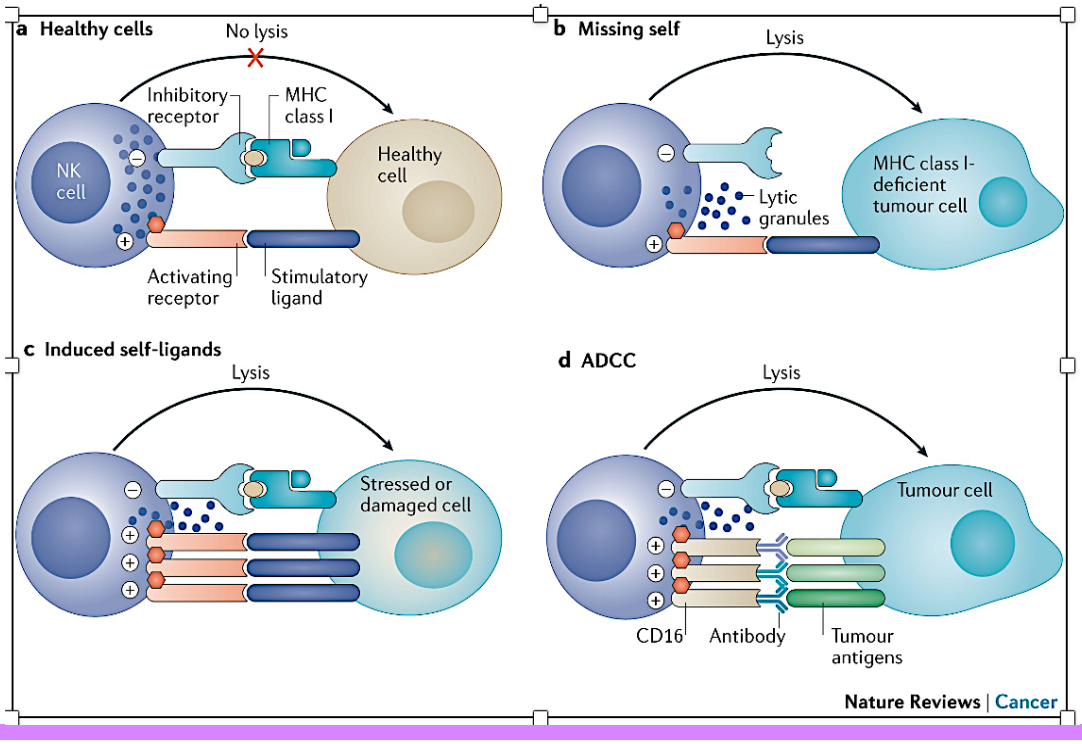
In addition to missing-self recognition, NK cells can eliminate target cells through ADCC. In this mechanism, IgG antibodies bind to antigens expressed on the surface of target cells (e.g., tumor or virus-infected cells).

NK cells express Fc receptors (CD16, FcγRIII), which recognize the Fc region of IgG antibodies. The binding of CD16 to antibody-coated target cells induces NK cell activation, leading to the release of cytotoxic granules and subsequent lysis of the target cell **(see Fig. 8.4).**

This mechanism highlights the role of NK cells as a bridge between innate and adaptive immunity, as they utilize antibodies produced by B lymphocytes to mediate cytotoxicity.

The cytotoxic function of NK cells is executed through direct cell-cell contact at a specialized interface known as the immunological synapse. Upon recognition of a target cell, NK cells release cytotoxic granules containing perforin and granzymes **(see Fig. 8.5)**. Perforin forms pores in the target cell membrane, facilitating the entry of granzymes into the cytoplasm. These enzymes activate apoptotic pathways, leading to DNA fragmentation, nuclear condensation, and programmed cell death (apoptosis). While perforin may contribute to membrane damage, the predominant mechanism of NK-mediated cytotoxicity is apoptosis rather than uncontrolled lysis, ensuring a controlled elimination of abnormal cells.

Beyond their direct cytotoxic activity, NK cells also play an important immunoregulatory role through the secretion of cytokines, particularly interferon-gamma (IFN-γ). This cytokine enhances



macrophage activation and promotes the development of Th1-type adaptive immune responses, thereby linking innate and adaptive immunity.

Figure 8.4. Mechanism of action of NK cells.

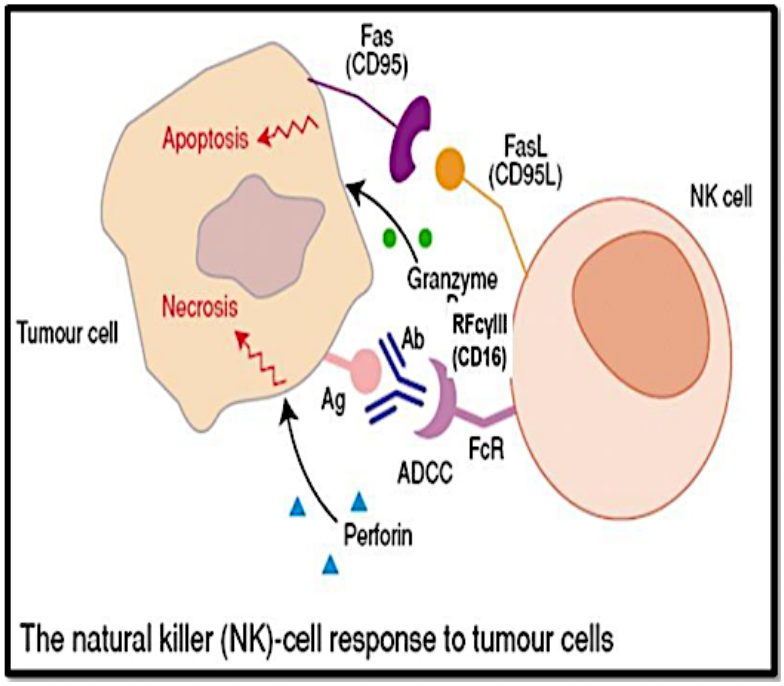


Figure 8.5. The natural killer cell response to tumor cells.

VIII.2.2. Humoral factors of innate immunity

a. Complement

The complement system enhances innate immunity through multiple mechanisms, including opsonization (via C3b), induction of inflammation (via anaphylatoxins such as C3a and C5a), and direct lysis of pathogens. The formation of the membrane attack complex (MAC), initiated by C5b and involving C6, C7, C8, and C9, leads to pore formation in target cell membranes, resulting in osmotic lysis, particularly in Gram-negative bacteria.

b. Interleukins-1 and 6 (IL-1 and IL-6)

IL-1 and IL-6 are pro-inflammatory, pleiotropic cytokines that play key roles in innate immunity. They induce fever by acting on the hypothalamus and stimulate hepatocytes to produce acute-

phase proteins, including C-reactive protein (CRP) and mannose-binding lectin (MBL). These proteins enhance pathogen recognition, opsonization, and clearance. IL-6 is particularly important in driving the acute-phase response.

c. Tumor necrosis factor-α (TNF-α)

TNF-α is a major pro-inflammatory cytokine produced primarily by activated macrophages. It promotes the expression of adhesion molecules on endothelial cells, increases vascular permeability, and facilitates the recruitment of leukocytes to sites of infection. TNF-α also contributes to the acute-phase response and can induce apoptosis in certain tumor or infected cells. Systemically, it plays a role in fever and, in excessive amounts, may contribute to septic shock.

d. Interferons (IFN-α and INF-β)

IFN-α and IFN-β are type I interferons with potent antiviral activity. They bind to receptors on neighboring uninfected cells and induce the expression of antiviral proteins that inhibit viral replication. These interferons also enhance MHC class I expression, improve antigen presentation, and stimulate the cytotoxic activity of natural killer (NK) cells, thereby reinforcing both innate and adaptive immune responses.

e. C-reactive protein (CRP)

CRP is an acute-phase protein synthesized by the liver in response to inflammation, primarily under the influence of IL-6. It binds to microbial components, such as phosphocholine residues present on bacterial surfaces (e.g., pneumococci), thereby promoting opsonization and phagocytosis. CRP can also activate the classical complement pathway, leading to enhanced deposition of C3b and improved clearance of pathogens.

VIII.2.3. Inflammatory response

Inflammation is a fundamental component of the innate immune response, representing a coordinated physiological reaction to tissue injury and infection. Derived from the Latin *inflammare* ("to set on fire"), inflammation is a protective process aimed at eliminating harmful stimuli, removing damaged cells, and restoring tissue homeostasis. Under normal conditions, inflammation is self-limiting; however, when the underlying cause persists, it may progress to chronic inflammation, leading to tissue damage and disease.

Inflammatory responses are triggered by a wide range of endogenous and exogenous factors. Endogenous causes include tissue necrosis and physical trauma (e.g., fractures), whereas exogenous causes encompass mechanical injury (cuts), physical damage (burns), chemical exposure, immunological reactions (hypersensitivity), and infections caused by microorganisms. Regardless of the initiating factor, the cellular and molecular mechanisms of inflammation are largely similar, involving activation of innate immune cells and the release of inflammatory mediators **(see Fig. 8.6)**.

a. Initiation of the inflammatory response

The inflammatory process begins rapidly, often within minutes following tissue injury or infection. Activation of innate immune cells, particularly macrophages and dendritic cells, occurs through recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs). This leads to the release of pro-inflammatory cytokines, notably IL-1, IL-6, and tumor necrosis factor-α (TNF-α).

These cytokines act locally and systemically to:

- ☞ Induce vasodilation and increased vascular permeability
- ☞ Promote leukocyte recruitment
- ☞ Stimulate the acute-phase response in the liver

During the acute-phase response, hepatocytes produce proteins such as C-reactive protein (CRP), which function as soluble pattern recognition molecules and enhance pathogen clearance.

b. Vascular and cellular changes

Inflammation is characterized by a series of vascular and cellular events that facilitate immune defense. The classical clinical signs of inflammation include:

-Redness (rubor). -Heat (calor) -Pain (dolor)

These manifestations result from vasodilation, increased blood flow, elevated metabolic activity, and the release of inflammatory mediators. Increased vascular permeability allows plasma proteins and fluids to leak into surrounding tissues, contributing to swelling and facilitating immune cell migration.

Simultaneously, endothelial cells express adhesion molecules that enable leukocyte extravasation, allowing neutrophils and monocytes to migrate into the affected tissue. These cells play a key role in pathogen elimination through phagocytosis and the release of antimicrobial mediators.

c. Role of soluble mediators

Several soluble systems contribute to the inflammatory response:

- ***Cytokines (IL-1, IL-6, TNF-α):*** regulate immune cell activation and systemic responses such as fever
- ***Kinins:*** increase vascular permeability, stimulate pain receptors, and contribute to smooth muscle contraction
- ***Coagulation system:*** forms clots that limit the spread of pathogens
- ***Complement system:*** enhances inflammation, opsonization, and pathogen lysis

Kinins are particularly important mediators of pain and vascular changes, while coagulation provides a physical barrier that restricts microbial dissemination.

d. Cellular response and link to adaptive immunity

As inflammation progresses, neutrophils are rapidly recruited, followed by monocytes/macrophages, which enhance phagocytosis and tissue cleanup. Macrophages also function as antigen-presenting cells, initiating the activation of T lymphocytes. Activated T cells produce cytokines that stimulate B lymphocytes, leading to the production of antigen-specific antibodies. These antibodies typically appear within 5-7 days, marking the transition from innate to adaptive immunity.

e. Acute-phase response and clinical markers

The acute-phase response is a systemic reaction characterized by:

- Increased production of acute-phase proteins (e.g., CRP)
- Elevated white blood cell counts
- Hormonal changes (e.g., increased corticosteroids)

Clinically, inflammation is assessed using markers such as the following:

- C-reactive protein (CRP)
- Erythrocyte sedimentation rate (ESR)

CRP is a non-specific but sensitive marker of inflammation and is widely used to monitor acute infections and chronic inflammatory diseases.

f. Chronic inflammation

When the causative agent cannot be eliminated, inflammation may become chronic. This occurs in conditions such as the following:

- Persistent infections (e.g., tuberculosis)
- Autoimmune diseases (e.g., rheumatoid arthritis, lupus)

Chronic inflammation is characterized by:

- ☞ Continuous immune activation
- ☞ Tissue destruction
- ☞ Attempts at repair, including fibrosis or scar formation

Although anti-inflammatory drugs (e.g., NSAIDs, corticosteroids) can alleviate symptoms, they do not eliminate the underlying cause.

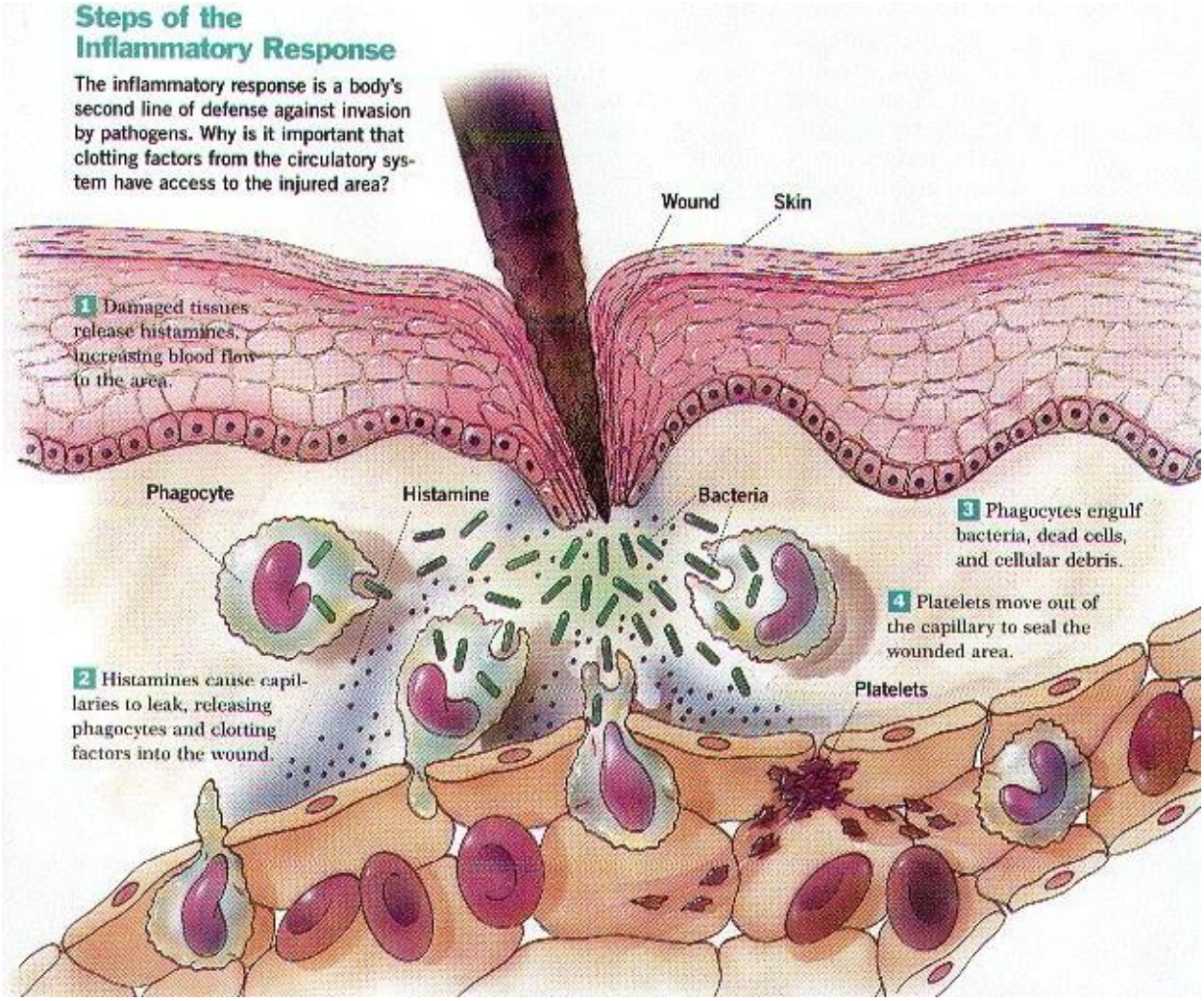


Figure 8.6. Inflammatory response.

g. Fever as a systemic manifestation

Fever is a common systemic feature of inflammation and infection. It is induced by endogenous pyrogens, including IL-1 and interferons, released by activated immune cells. These cytokines act on the hypothalamus to increase body temperature **(see Fig. 8.7)**.

Additionally, microbial products such as lipopolysaccharide (LPS) from Gram-negative bacteria stimulate cytokine production, further contributing to fever. Elevated temperature can inhibit microbial growth and enhance immune efficiency.

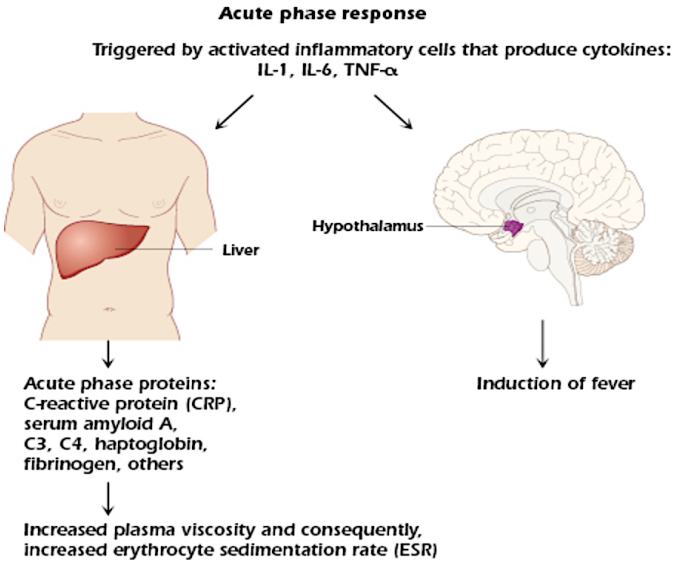


Figure 8.7. The acute phase response stimulated by cytokines produced by innate immune cells.

VIII.3. Adaptive immune response

The initiation and development of the adaptive immune response require that antigens be captured, processed, and presented to lymphocytes within secondary lymphoid organs (lymph nodes and spleen). This function is performed by antigen-presenting cells (APCs), including dendritic cells, macrophages, and B lymphocytes.

A diverse repertoire of lymphocytes, each bearing receptors specific for a given antigen, is generated prior to antigen exposure in primary lymphoid organs. These naïve lymphocytes migrate to secondary lymphoid organs, where they encounter antigens. When an antigen is presented by APCs, it is recognized by lymphocytes bearing the corresponding receptor. This fundamental principle is known as clonal selection, leading to lymphocyte activation, proliferation, and differentiation into effector and memory cells **(Fig. 8.8)**.

Adaptive immunity is divided into two major functional arms:

- **Humoral immunity**, mediated by B lymphocytes and antibodies
- **Cell-mediated immunity**, mediated primarily by T lymphocytes

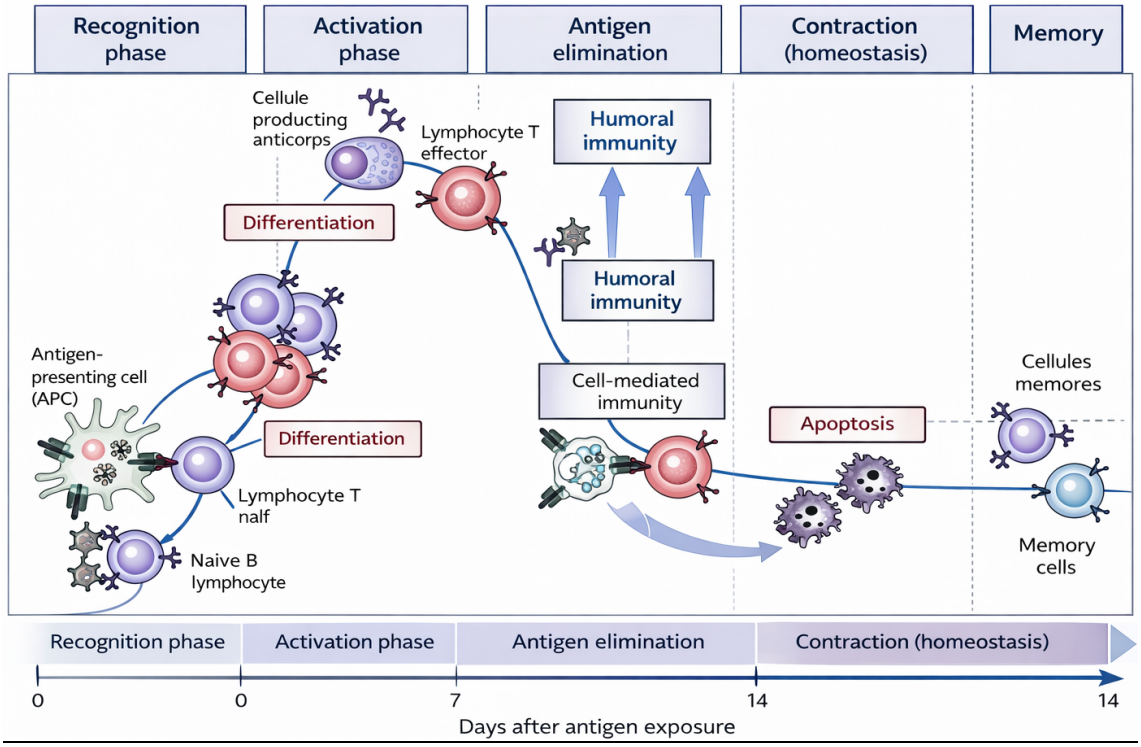


Figure 8.8. The adaptive immune response.

VIII.3.1. Humoral immunity

Humoral immunity involves the production of antibodies by B lymphocytes, which are essential for the elimination of extracellular pathogens and toxins.

a. B lymphocyte activation

a.1. T-dependent B cell activation

This mechanism occurs mainly with protein antigens **(see Fig. 8.9)**

- The antigen binds to the B-cell receptor (BCR).
- The antigen is internalized and processed.
- Peptides are presented via MHC class II to $CD4^+$ T helper cells.

Activation requires:

- CD40-CD40L interaction (essential co-stimulatory signal).
- Cytokine signaling, including **IL-4, IL-5**, and **IL-21.**

These signals lead to:

- Class switch recombination
- Somatic hypermutation
- Affinity maturation
- Formation of memory B cells

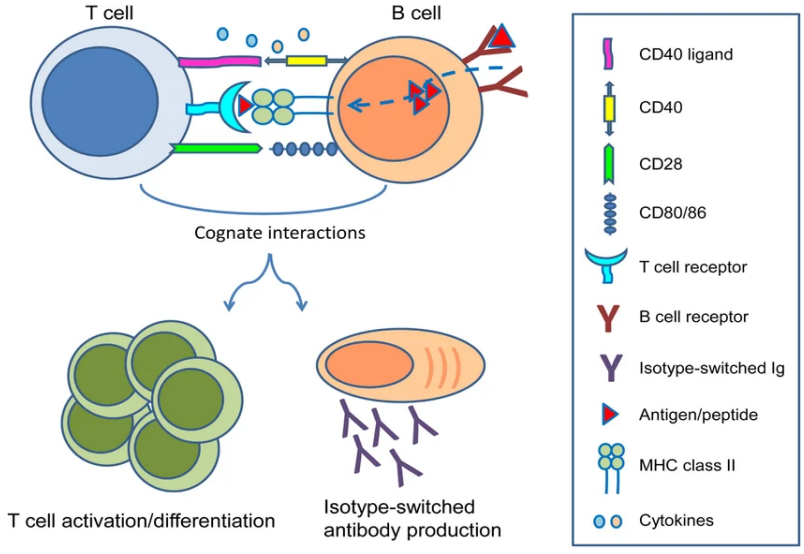


Figure 8.9. T-dependent B cell activation.

a.2.T-independent B cell activation

This occurs with non-protein antigens, such as polysaccharides and repetitive microbial structures **(Fig. 8.10)**.

These antigens induce direct cross-linking of BCRs, leading to:

- Rapid activation of B cells
- Predominantly IgM production
- Limited or absent memory formation

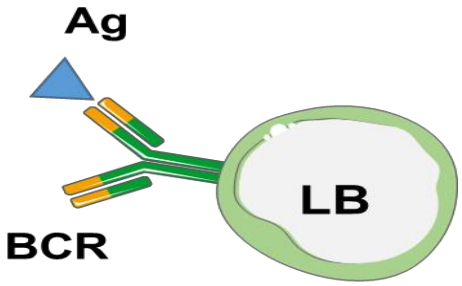


Figure 8.10. T-independent B cell activation.

b. Functions of antibodies

Antibodies contribute to immune defense through several mechanisms:

- ***Neutralization:*** antibodies bind toxins or viruses and block their interaction with host cells
- ***Opsonization:*** IgG enhances phagocytosis via binding to Fc receptors on phagocytes
- ***Complement activation:*** The classical pathway is triggered by antigen–antibody complexes
- ***Antibody-dependent cellular cytotoxicity (ADCC):*** NK cells recognize the Fc portion of IgG via CD16 (FcγRIII) and kill target cells

c. Immunoglobulin isotypes and functions

Different antibody classes have distinct roles:

- **IgM** → primary immune response
- **IgG** → secondary response, opsonization, complement activation
- **IgA** → mucosal immunity
- **IgE** → allergy and defense against parasites
- **IgD** → functions mainly as a B-cell receptor

d. Germinal center reaction

The germinal center reaction occurs in secondary lymphoid organs (lymph nodes and spleen) and is essential for antibody maturation. It involves:

- Proliferation of activated B cells
- Somatic hypermutation mediated by the enzyme AID (activation-induced cytidine deaminase)
- Affinity maturation
- Class switch recombination

This process results in the production of high-affinity antibodies and the generation of long-lived plasma cells and memory B cells.

VIII.3.2. Cellular immunity

Cytotoxic T lymphocytes, derived from $CD8^+$ T cells, recognize target cells presenting antigenic peptides via MHC class I molecules. Upon recognition, CTLs induce apoptosis of target cells through the perforin-granzyme pathway and the death receptor pathway.

-First: Target recognition

CTLs recognize target cells through peptide-MHC class I complexes presented on infected or malignant cells. This recognition requires:

- Binding of the T-cell receptor (TCR) to the peptide-MHC I complex
- Stabilization by the CD8 co-receptor

☞ Contribution of adhesion molecules, particularly LFA-1 (on T cells) and ICAM-1 (on target cells)

These interactions lead to the formation of a specialized structure known as the immunological synapse, which organizes intracellular signaling and directs the cytotoxic machinery toward the target cell **(Fig. 8.11)**.

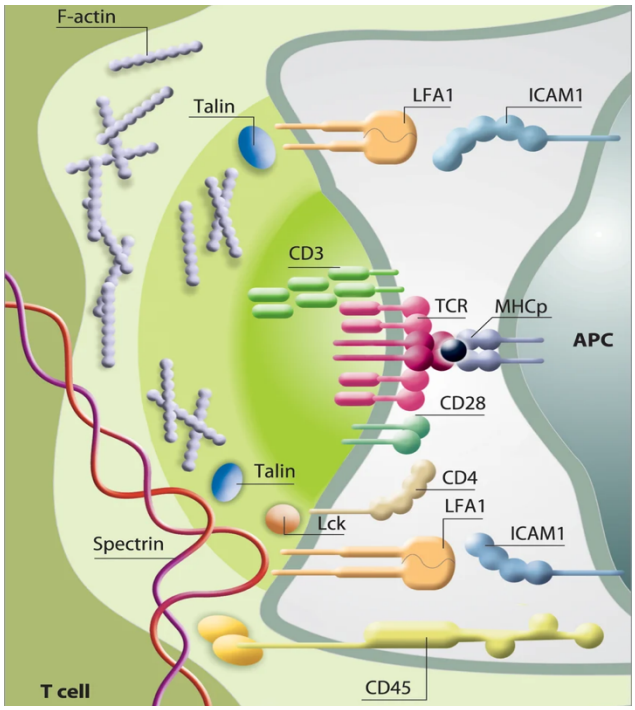


Figure 8.11. Immunological synapse.

-Second: Steps in CTL-mediated killing

➔ *Step 1: Immunological synapse formation and polarization*

Following recognition, the CTL undergoes cytoskeletal reorganization. The microtubule-organizing center (MTOC) and cytotoxic granules polarize toward the immunological synapse, ensuring precise and directed delivery of cytotoxic molecules to the target cell.

➔ *Step 2: Granule exocytosis (Perforin-granzyme pathway)*

The CTL releases cytotoxic granules containing:

- ☞ Perforin
- ☞ Granzymes (especially granzyme B)

Perforin inserts into the target cell membrane and facilitates the entry of granzymes into the cytoplasm. Once inside:

- Granzyme B activates caspases (notably caspase-3)
- It also promotes mitochondrial cytochrome c release, triggering the intrinsic apoptotic pathway

Outcome: programmed cell death (apoptosis) **(see Fig. 8.12, 13).**

➔ *Step 3: Death receptor pathway (Fas-FasL)*

As an alternative or complementary mechanism, CTLs express Fas ligand (FasL), which binds to Fas (CD95) on the target cell. This interaction leads to:

- ☞ Formation of the death-inducing signaling complex (DISC)
- ☞ Activation of caspase-8
- ☞ Induction of apoptosis

This pathway is particularly important in chronic immune stimulation and immune regulation.

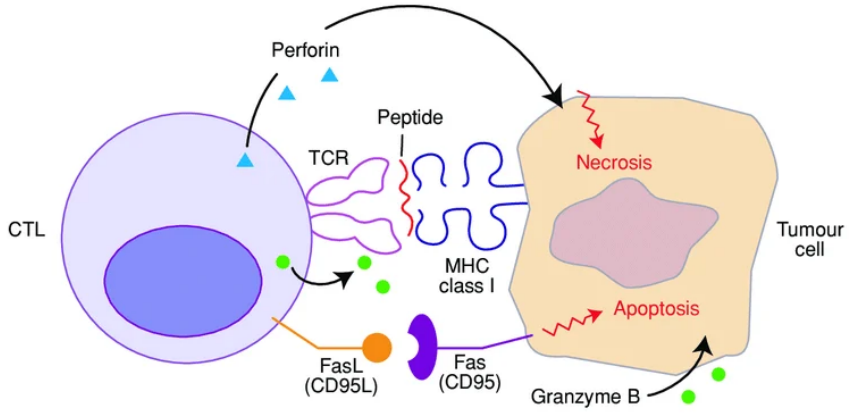


Figure 8.12. The cytotoxic (CTL) response to tumor cells.

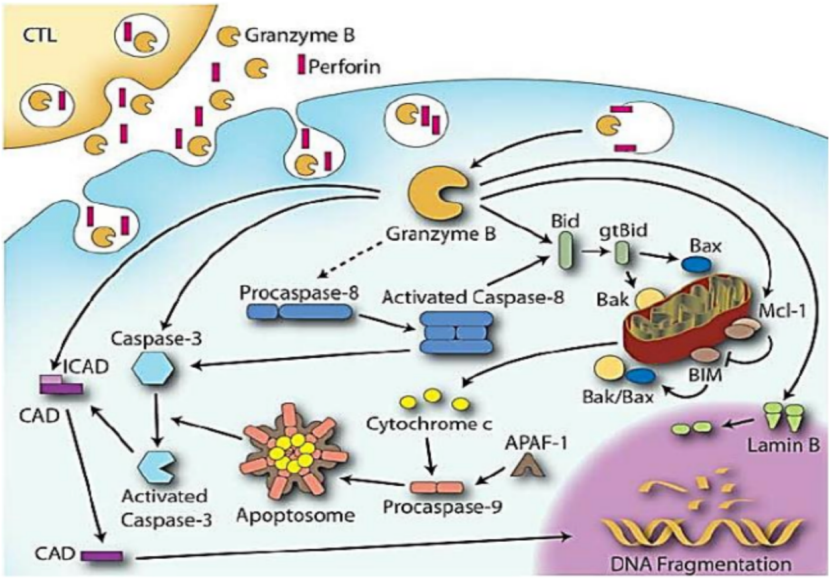


Figure 8.13. CTL-induced apoptosis mechanisms.

-Third: Key features

CTL-mediated killing is characterized by several essential properties:

- Antigen specificity → recognition of a specific peptide
- MHC class I restriction
- Induction of non-inflammatory apoptosis (no cell lysis with inflammation)
- Serial killing capacity: one CTL can kill multiple target cells
- High precision and efficiency

VIII.4. Humoral-cellular cooperation

The adaptive immune response is a highly coordinated system in which humoral and cellular components act synergistically to ensure effective protection against a wide range of pathogens. This cooperation is primarily mediated by $CD4^+$ T helper (Th) lymphocytes, which play a central regulatory role.

VIII.4.1. Central role of CD4⁺ T helper cells

CD4⁺ T helper cells are activated following antigen presentation by antigen-presenting cells (APCs) through the interaction of the TCR/CD3/CD4 complex with peptide-MHC class II molecules **(Fig. 8.14)**, along with co-stimulatory signals (B7-CD28) **(Fig. 8.15)**. Once activated, T helper cells proliferate and differentiate into distinct subsets (e.g., Th1 and Th2), each characterized by specific cytokine profiles that orchestrate immune responses **(Fig. 8.16)**.

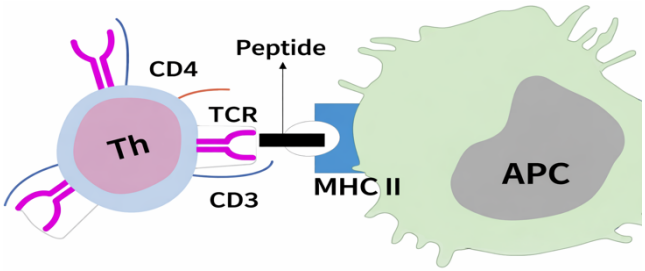


Figure 8.14. First signal by the interaction of MHC II-peptide/TCR/CD3/CD4.

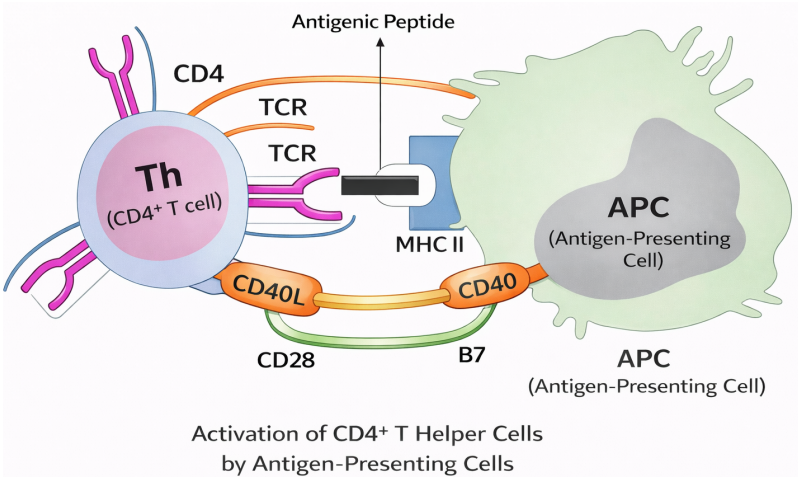


Figure 8.15. The second signal.

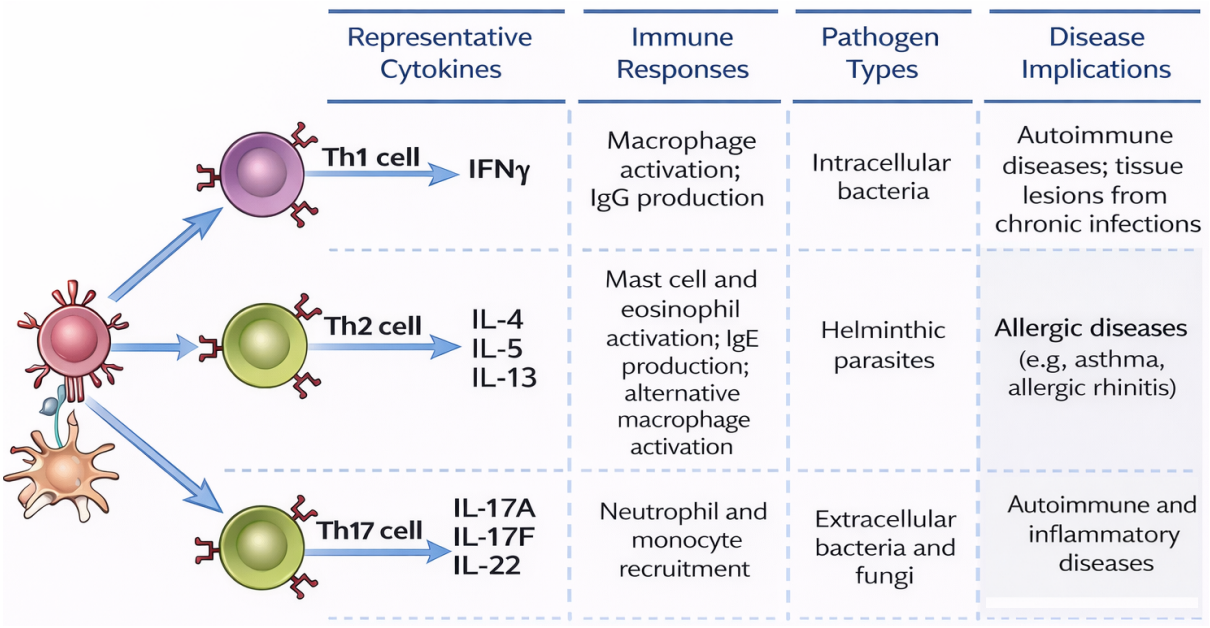


Figure 8.16. Different subsets of T lymphocytes and their characteristics.

VIII.4.2. Cooperation between APCs, T helper cells, and B lymphocytes

Following activation, Th cells interact with B lymphocytes that have recognized the same antigen. This cooperation involves:

- CD40 (B cell) - CD40L (Th cell) interaction, which is essential for B-cell activation
- Secretion of cytokines such as IL-4, IL-5, and IL-21, which promote B-cell proliferation and differentiation

These signals induce:

- Differentiation of B cells into plasma cells producing antibodies
- Class-switch recombination (IgM to IgG, IgA, or IgE)
- Affinity maturation and formation of memory B cells

This mechanism ensures the efficiency of the humoral immune response.

VIII.4.3. Cooperation between T helper cells and cytotoxic T lymphocytes

$CD4^+$ T cells also contribute to the activation of $CD8^+$ cytotoxic T lymphocytes (CTLs). This cooperation occurs through:

- Secretion of IL-2, which promotes proliferation and differentiation of $CD8^+$ T cells
- Enhancement of APC activation, improving antigen presentation to $CD8^+$ T cells

As a result, naïve $CD8^+$ T cells differentiate into effector CTLs, capable of inducing apoptosis in infected or malignant cells through:

- The perforin-granzyme pathway
- The Fas-FasL pathway

This interaction strengthens the cell-mediated immune response.

VIII.4.4. Cytokine-mediated regulation

Cytokines act as key mediators in the coordination of humoral and cellular responses. Different Th subsets produce specific cytokines that determine the nature of the immune response:

- ☞ Th1 cytokines (e.g., IFN-γ) → promote cellular immunity
- ☞ Th2 cytokines (e.g., IL-4, IL-5) → promote humoral immunity
- ☞ IL-2 → essential for T-cell proliferation

This cytokine network ensures a balanced and regulated immune response.

VIII.4.5. Functional complementarity

Humoral and cellular immunity exhibit complementary roles:

- ☞ Humoral immunity is effective against extracellular pathogens (via antibodies)
- ☞ Cell-mediated immunity targets intracellular pathogens (via CTLs) **(see Fig. 8.17)**.

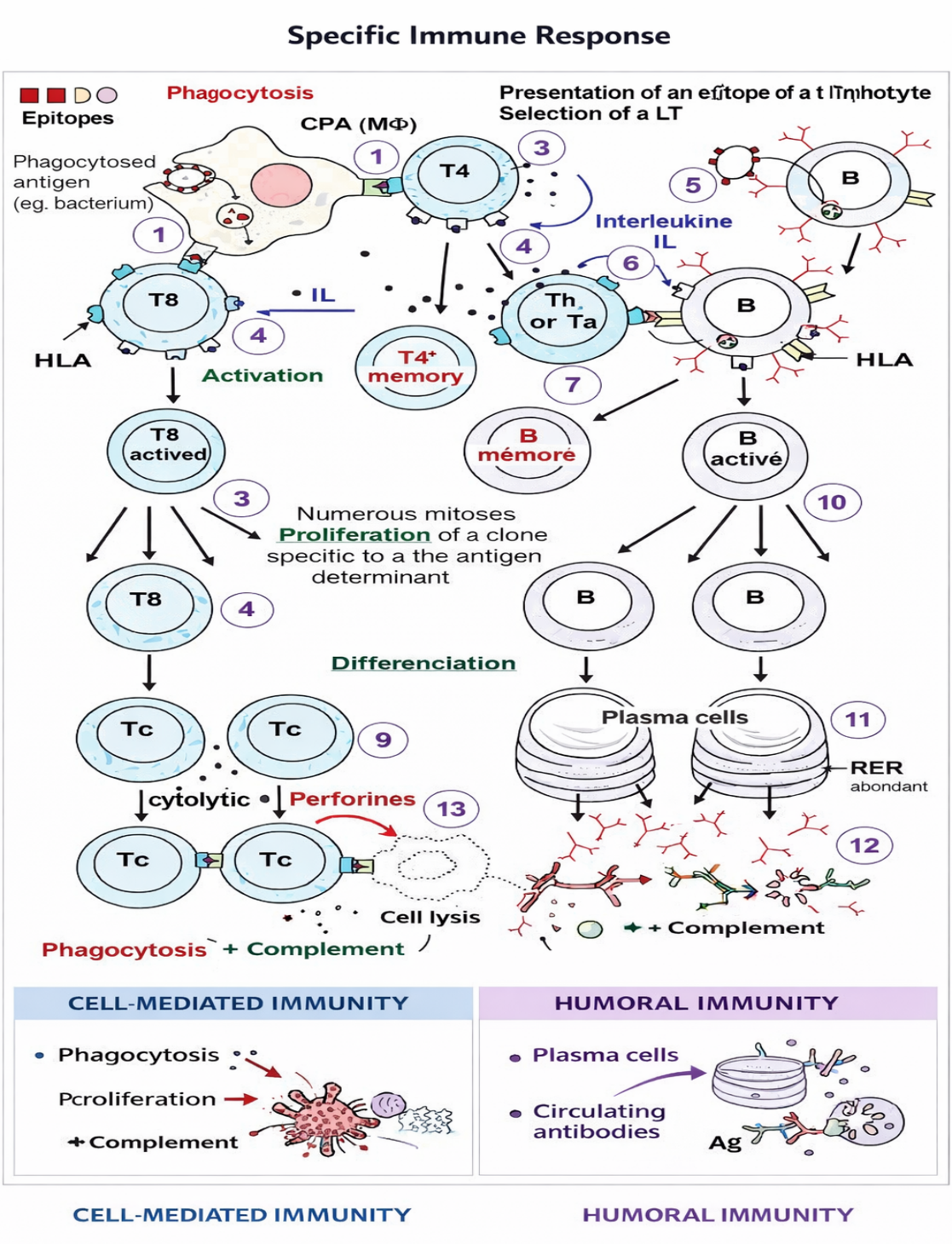
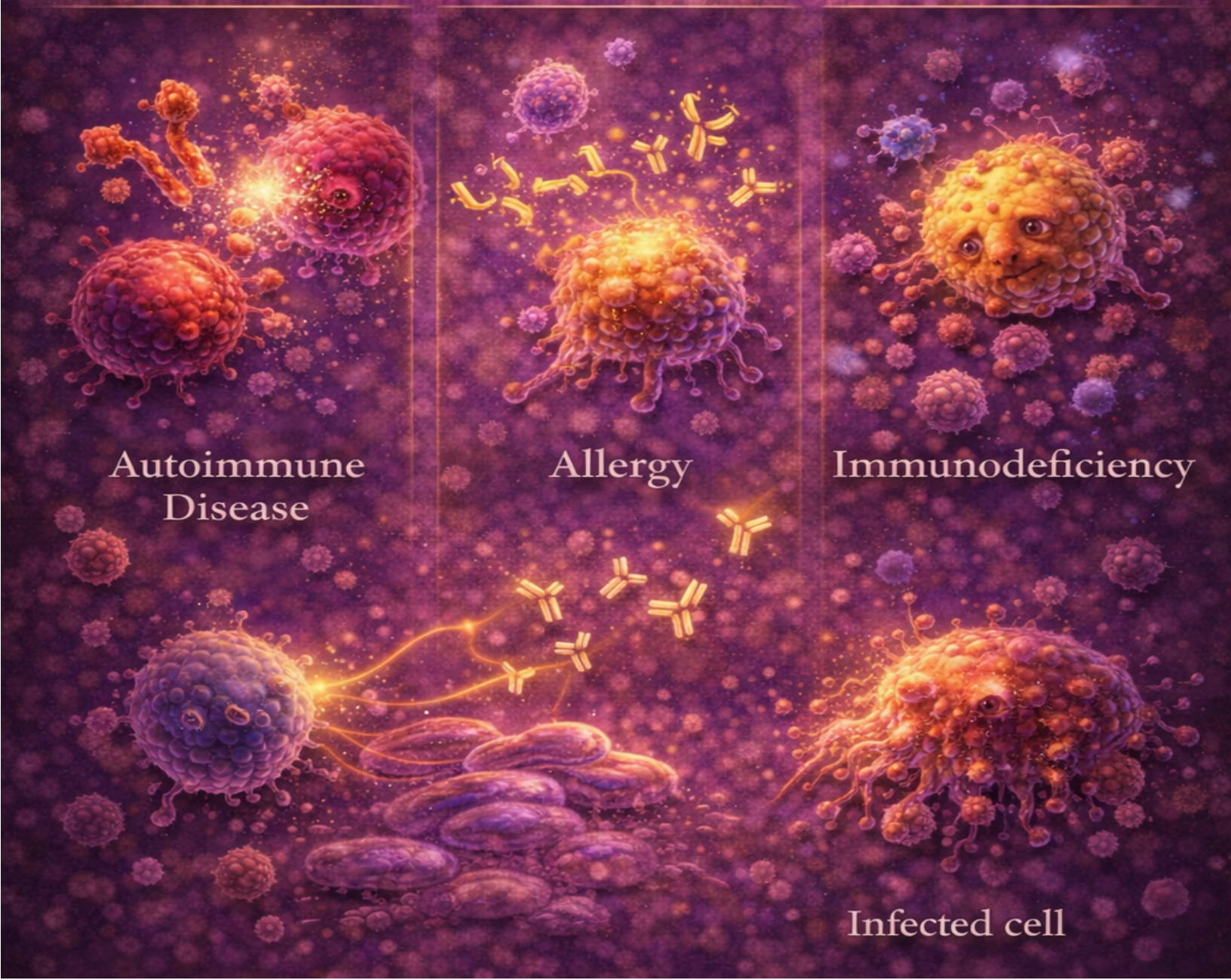


Figure 8.17. Summary of adaptive immune response.

Chapter IX.
Immune System Disorders



Chapter IX. Immune System Disorders

Immune system disorders refer to conditions in which immune responses are dysregulated, resulting in pathological consequences. These disorders arise either from excessive immune activity or from insufficient immune function. Accordingly, they can be broadly classified into:

- **Disorders due to immune hyperactivity**, including hypersensitivity reactions and autoimmune diseases
- **Disorders due to immune deficiency**, encompassing primary and secondary immunodeficiencies

IX.1. Disorders associated with immune hyperactivity

IX.1.1. Hypersensitivity

Hypersensitivity is an immune response that occurs in an exaggerated or inappropriate form. Responses may occur against innocuous external antigens, such as pollen in hayfever. In other cases, responses against pathogens are out of proportion to the damage caused by them. The different kinds of tissue damage seen in autoimmune diseases are also inappropriate responses. The hypersensitivity reactions were classified by Gell and Coombs according to the speed of the reaction and the immune mechanisms involved. Although they are classified separately, they do not necessarily occur in isolation, and several mechanistically different reactions may be included in one type. Hence, type I, II, and III reactions are now grouped as antibody-mediated, while type IV includes a number of different reactions due to cellular reactions **(Fig. 9.1).**

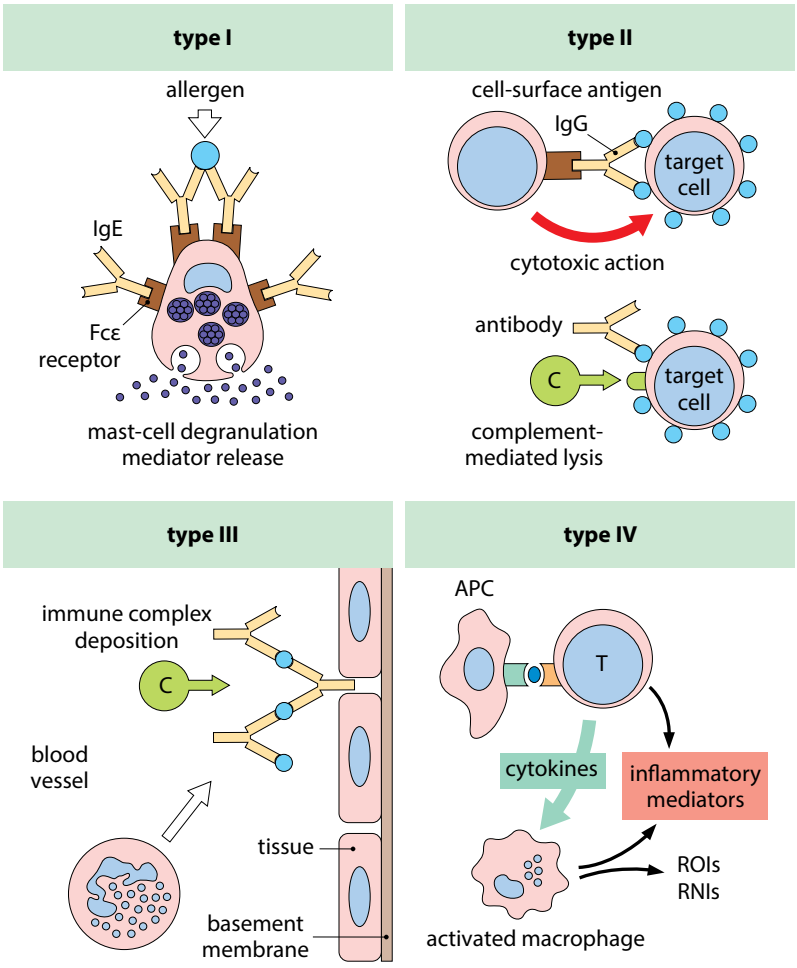


Figure 9.1. Four types of hypersensitivity reactions.

a. Type I (Immediate) hypersensitivity

Allergy, originally defined as an altered reactivity upon secondary exposure to an antigen, is now used to describe type I hypersensitivity reactions, which are mediated by immunoglobulin E (IgE) and reflect a T helper 2 (Th2)-dominated immune response.

➔ Allergens are antigens capable of inducing type I hypersensitivity reactions. Most allergens are proteins of environmental origin, including pollens, house dust mite feces, fungal spores, and animal dander (skin flakes).

These particles, typically measuring 3-30 µm, are inhaled in small quantities and deposited on the mucosal surfaces of the respiratory tract. Certain food proteins, such as those found in eggs, peanuts, tree nuts, and shellfish, may also act as allergens.

➔ Sensitization refers to the process by which a genetically predisposed individual develops an allergen-specific IgE response. The produced IgE binds to high-affinity FcεRI receptors on mast cells and basophils, thereby priming these cells for activation upon subsequent exposure to the same allergen.

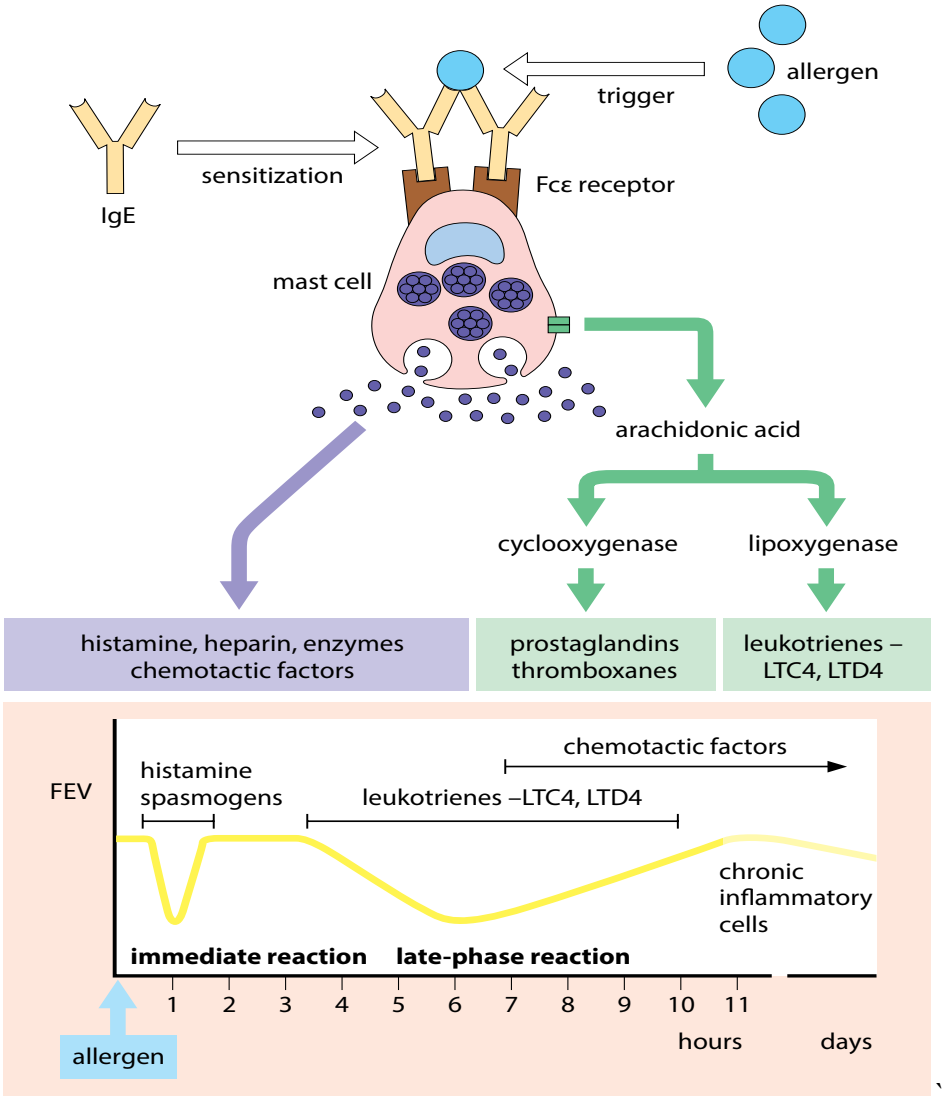


Figure 9.2. Hypersensitiviry type I.

➔ Upon re-exposure, allergens bind and cross-link IgE molecules on the surface of mast cells, triggering:

- ☞ An influx of Ca^{2+} ions
- ☞ Degranulation of mast cells
- ☞ Activation of phospholipase A_2, leading to the release of arachidonic acid **(see Fig. 9.2).**

Arachidonic acid is subsequently metabolized via:

- ☞ Lipoxygenase pathway → production of leukotrienes
- ☞ Cyclooxygenase pathway → production of prostaglandins and thromboxanes

Additionally, mast cells can be activated by anaphylatoxins (C3a, C5a) and certain drugs (e.g., opiates, vancomycin).

➔ Atopy refers to a genetic predisposition to develop type I hypersensitivity reactions. Common atopic conditions include: Asthma, allergic rhinitis (hay fever) and eczema.

These conditions often cluster in families. Genetic susceptibility has been associated with: HLA genes, cytokines: IL-4, IL-5, IL-10, IL-13 and receptors for leukotrienes and chemokines (e.g., CCR3).

➔ Following allergen exposure, two distinct phases of the allergic response are observed:

-*Immediate phase:* Occurs within minutes and is mediated by histamine, prostaglandins, kinins, and platelet-activating factor (PAF).

This leads to bronchoconstriction, increased vascular permeability and reduced airway patency.

-*Late phase:* Develops several hours later and is driven primarily by leukotrienes and chemokines.

This phase involves recruitment of inflammatory cells, including eosinophils, macrophages and basophils.

Eosinophil-derived granule proteins contribute to tissue damage, particularly in the airway epithelium.

➔ Anaphylaxis is a severe systemic type I hypersensitivity reaction occurring in sensitized individuals upon exposure to an allergen. It is characterized by:

- ☞ Massive release of vasoactive mediators
- ☞ Smooth muscle contraction
- ☞ Increased vascular permeability
- ☞ Hypotension

This may lead to respiratory distress or circulatory collapse, and can be triggered by agents such as insect venom or certain drugs/vaccines.

➔ Desensitization is a therapeutic approach aimed at reducing allergic responses by administering gradually increasing doses of the allergen over time. This treatment:

- ☞ Reduces allergen-specific IgE levels
- ☞ Promotes a shift from Th2 to a more regulatory/Th1 response
- ☞ Increases production of IgG (blocking antibodies)

b. Type II hypersensitivity (Antibody-mediated)

Type II hypersensitivity is mediated by IgG or IgM antibodies directed against cell surface or extracellular matrix antigens. These antibodies induce tissue damage through:

- ☞ Complement activation → cell lysis (MAC)
- ☞ Opsonization and phagocytosis (via Fcγ and C3 receptors)
- ☞ ADCC (antibody-dependent cellular cytotoxicity)

Main clinical examples:

1. ***Transfusion reactions:*** Incompatible blood transfusion leads to antibody binding to donor erythrocytes, causing complement-mediated lysis and extravascular destruction in spleen and liver.

2. ***Hemolytic disease of the newborn (HDN):*** Maternal IgG antibodies against fetal RBCs cross the placenta and destroy them. Most commonly due to Rh incompatibility.

Prevented by anti-RhD immunoglobulin (Rhesus prophylaxis)

3. ***Autoimmune hemolytic anemia :*** Autoantibodies against RBCs cause :

   - ☞ Warm type (IgG) → extravascular hemolysis
   - ☞ Cold type (IgM) → complement-mediated lysis

4. ***Drug-induced cytotoxicity:*** Drugs bind to blood cells, leading to antibody formation and hemolytic anemia and thrombocytopenia.

5. ***Receptor-targeting diseases***

   - ☞ Myasthenia gravis → antibodies against acetylcholine receptors
   - ☞ Lambert–Eaton syndrome → antibodies against calcium channels

6. ***Tissue-specific disorders***

   - ☞ Pemphigus → antibodies against desmosomes → blistering
   - ☞ Goodpasture's syndrome → antibodies against type IV collagen → kidney and lung damage

c. Type III hypersensitivity (Immune complex-mediated)

Type III hypersensitivity is caused by the formation and deposition of antigen-antibody (immune) complexes, often associated with complement components.

➔ Immune complexes deposit in tissues, particularly in areas of:

- ☞ High blood pressure

- ☞ Filtration (e.g., kidneys)
- ☞ Turbulent blood flow (e.g., vessels)

These complexes induce inflammation through:

- ☞ Complement activation → release of C3a and C5a
- ☞ Recruitment of neutrophils (via C5a)
- ☞ Activation of basophils and platelets → release of vasoactive mediators

Phagocytes attempting to eliminate deposited complexes release lysosomal enzymes and reactive oxygen intermediates (ROIs). This results in local tissue damage and inflammation.

➔ Normally, immune complexes are cleared by:

- ☞ Binding to erythrocytes
- ☞ Transport to the liver and spleen
- ☞ Removal by phagocytic cells

Clearance depends on the size of complexes, antibody class and affinity, antigen valency, and quantity of immune complexes. Excess formation or defective clearance leads to disease.

➔ Clinical manifestations are systemic type III reactions caused by antibodies against foreign proteins and lead to fever, arthritis, and nephritis.

d. Type IV (Delayed) hypersensitivity

Delayed hypersensitivity includes several reactions that are maximal more than 12 hours after challenge with antigen and are dependent on T cells rather than on antibody. The cells responsible are primarily $CD4^+$ T cells, and the reactions are of four main types **(Tab. 9.1)**.

Table 9.1. Subtypes of type IV hypersensitivity and their immunological mechanisms.

Subtype	Effector Cells	Target / Effect	Main Mediators
Type IVa	Th1 cells	Macrophage activation	IFN-γ, TNF-α
Type IVb	Th2 cells	Eosinophil activation	IL-4, IL-5, IL-13
Type IVc	Cytotoxic T cells ($CD8^+$)	Direct tissue cell killing	Perforin, granzymes, FasL
Type IVd	T cells	Neutrophil recruitment/activation	IL-17, CXCL8 (IL-8)

Contact hypersensitivity is a type IV hypersensitivity reaction that produces an eczematous skin lesion peaking about 48 hours after allergen exposure **(see Fig. 9.3)**. Allergens, often small haptens like nickel, bind to host proteins and are captured by Langerhans cells, which activate T lymphocytes in lymph nodes. Upon re-exposure, sensitized T cells migrate to the skin and induce inflammation characterized by cellular infiltration, edema, and microvesicle formation. Keratinocytes amplify the response by releasing pro-inflammatory cytokines and later contribute to resolution through regulatory cytokines such as IL-10 and TGF-β.

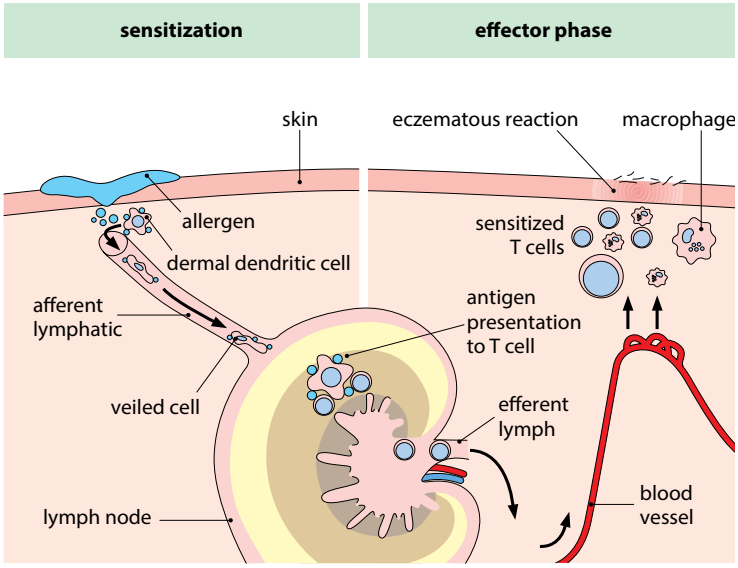


Figure 9.3. Sensitization and effector phases of contact hypersensitivity.

➔ Pathophysiological comparison of hypersensitivity reactions

Table 9.2. Pathophysiological comparison of hypersensitivity reactions

	Type I	Type II	Type III	Type IV
Main mediator	IgE	IgG / IgM	Immune complexes (Ag-Ab)	T cells
Complement involvement	No	Yes	Yes	No
Time of onset	Immediate (minutes)	Hours	Hours to days	Delayed (24–72 h)
Main effector cells	Mast cells, basophils	Phagocytes, NK cells	Neutrophils	T lymphocytes, macrophages
Pathophysiological mechanism	Allergic (IgE-mediated)	Cytotoxic (antibody-mediated)	Immune complex deposition	Cell-mediated (delayed-type)

IX.1.2. Autoimmune diseases

Autoimmune disease occurs when the immune system recognizes and reacts against the body's own cells or tissue. The antigens may be recognized by T cells or B cells and are referred to as autoantigens. Autoantibodies against these self-antigens are often valuable for diagnosis. There is a wide range of autoimmune diseases, but broadly they fall into two categories.

- ➔ **Organ-nonspecific autoimmune diseases** are directed to widely distributed autoantigens, such as anti-DNA antibodies in systemic lupus erythematosus or antibodies against antibodies (rheumatoid factors) in rheumatoid arthritis. These conditions often produce type III, immune complex-mediated, hypersensitivity reactions.
- ➔ **Organ-specific autoimmune diseases** are directed primarily at tissues, for example, antibodies against pancreatic β cells in diabetes. Organ-specific autoantibodies and disease tend to occur together in individuals, and they cluster in families, because of genetic predisposition. Examples of these conditions are given in **table 9.3.**

Table 9.3. Organ-specific autoimmune diseases.

Disease	Target cell/antigen	Pathology
Hashimoto's thyroiditis	Thyroid peroxidase	Destruction of thyroid follicles
Graves' disease	Thyroid-stimulating hormone receptor	Stimulation of the TSH receptor with chronic overactivity of the thyroid
Addison's disease	Adrenal 21-hydroxylase	Adrenal damage and lack of corticosteroids and/or aldosterone
Autoimmune parathyroid disease	Calcium-sensing receptor	Low serum calcium affecting nerve activity and producing muscle cramps
Goodpasture's syndrome	Kidney and lung basement membranes (type IV collagen)	Damage to kidney glomerulus and/or lung alveoli
Pernicious anemia	Intrinsic factor (stomach)	Failure to absorb vitamin B12
Pemphigus	Desmoglein in desmosomes	Separation of layers of the epidermis and cells in mucosal epithelium-blistering
Myasthenia gravis	Acetylcholine receptor on skeletal muscle cells	Damage to the motor endplate and impaired neuromuscular transmission
Guillain-Barré syndrome	Gangliosides in peripheral nerves	Inflammation and loss of myelin and nerve conduction
Type-1 diabetes	Insulin and GAD (enzyme)	Loss of pancreatic β-cells and inflammation

a. Mechanisms of self-tolerance

Autoimmunity is normally prevented by several tolerance mechanisms **(see Fig. 9.4)**:

- ☞ Sequestration of autoantigens
- ☞ Deletion of autoreactive lymphocytes (thymus and bone marrow)
- ☞ Limited processing and presentation of self-antigens
- ☞ Induction of T-cell anergy (lack of co-stimulation)
- ☞ Regulation by T regulatory cells (Tregs)
- ☞ Suppressive cytokines and hormones

b. Activation of autoreactive lymphocytes

Autoimmune responses may develop when these controls are bypassed:

- ☞ T-cell bypass → autoreactive B cells activated via foreign antigen-specific T cells
- ☞ Polyclonal activation (e.g., EBV) directly stimulates B cells
- ☞ Microbial triggers (e.g., LPS) induce co-stimulatory signals on APCs
- ☞ Molecular mimicry → cross-reactive microbial antigens activate autoreactive T cells
- ☞ Viral processing may lead to presentation of self-antigens in an immunogenic context

c. Failure of immune regulation

Breakdown of central or peripheral tolerance leads to:

- ☞ Reduced number or function of Tregs
- ☞ Loss of control of T-cell activation
- ☞ Sustained immune response against self-antigens → autoimmune disease

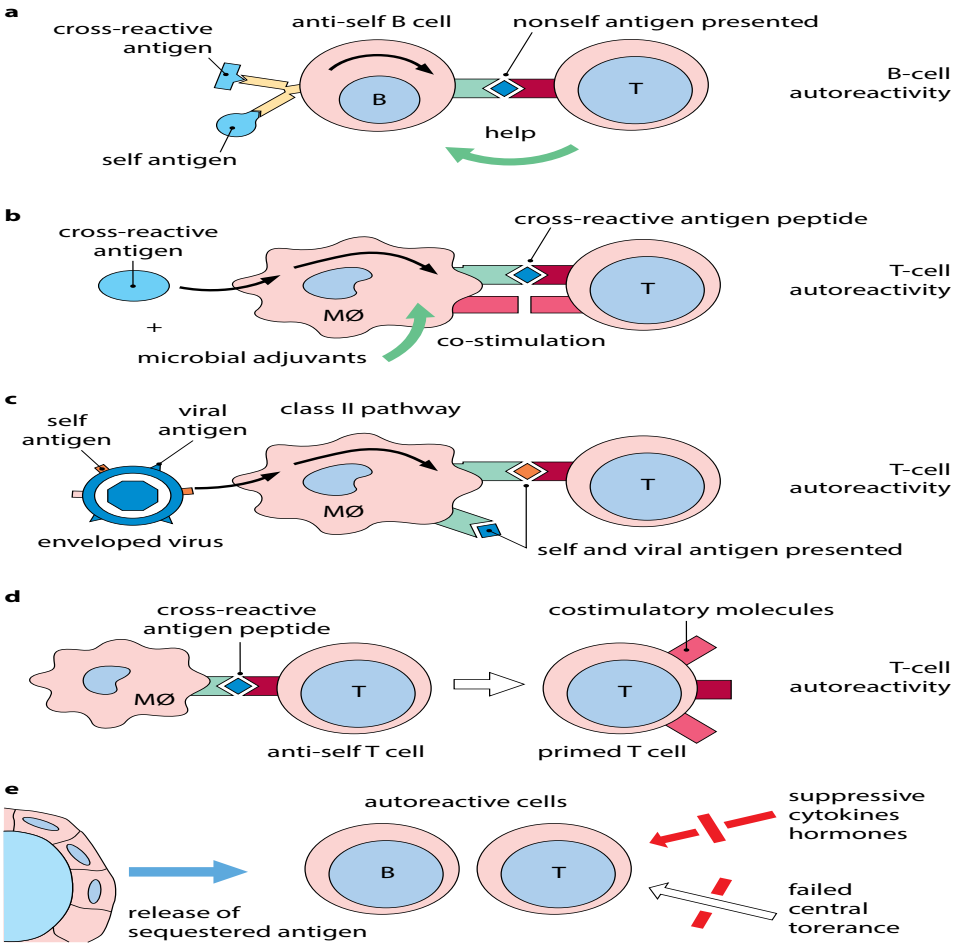


Figure 9.4. Mechanisms of breakdown of self-tolerance.

IX.2. Immunodeficiency

Immunodeficiency refers to a condition characterized by the deficiency or dysfunction of one or more components of the immune system, leading to an increased susceptibility to infections and other pathological manifestations. Immunodeficiencies are categorized into two main types:

- **Congenital (Primary) immunodeficiencies**
- **Acquired (Secondary) immunodeficiencies**

IX.2.1. Congenital or primary deficiencies

Primary immunodeficiencies are genetic (inherited) disorders characterized by defects in one or more components of the immune system, leading to impaired immune function. Affected individuals have an increased susceptibility to infections, particularly recurrent or severe infections caused by opportunistic pathogens. These conditions may involve humoral immunity (B cells and antibodies), cellular immunity (T cells), or both (combined immunodeficiencies) **(Fig. 9.5)**. The severity varies widely: while some forms are mild and compatible with normal life, severe formssuch as severe combined immunodeficiency (SCID) can be life-threatening in early infancy if not treated, sometimes requiring protective isolation (e.g., "bubble" environments) and early medical intervention.

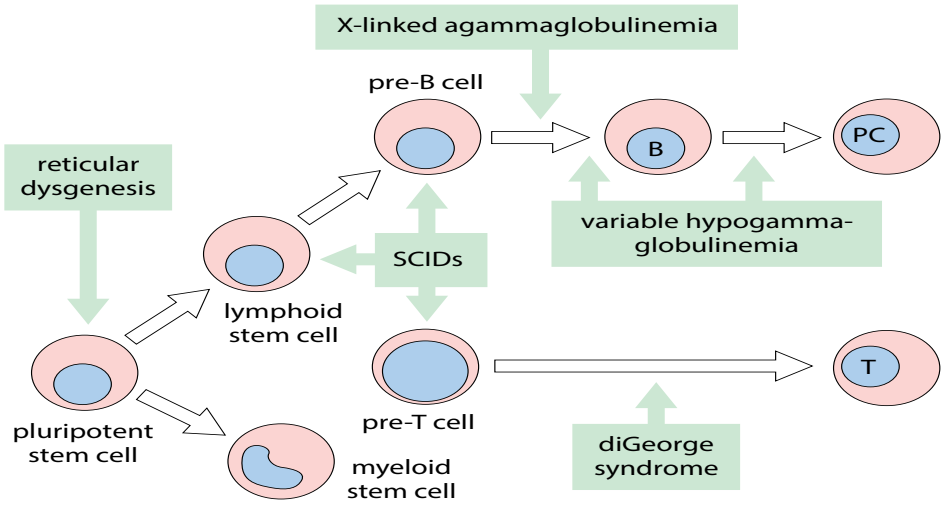


Figure 9.5. Immunodeficiencies.

➔ **Deficiency of humoral immunity**

This type is characterized by a reduced number or impaired function of B lymphocytes and plasma cells, leading to decreased antibody production. As a result, the body's ability to defend against extracellular pathogens, particularly bacteria, is compromised.

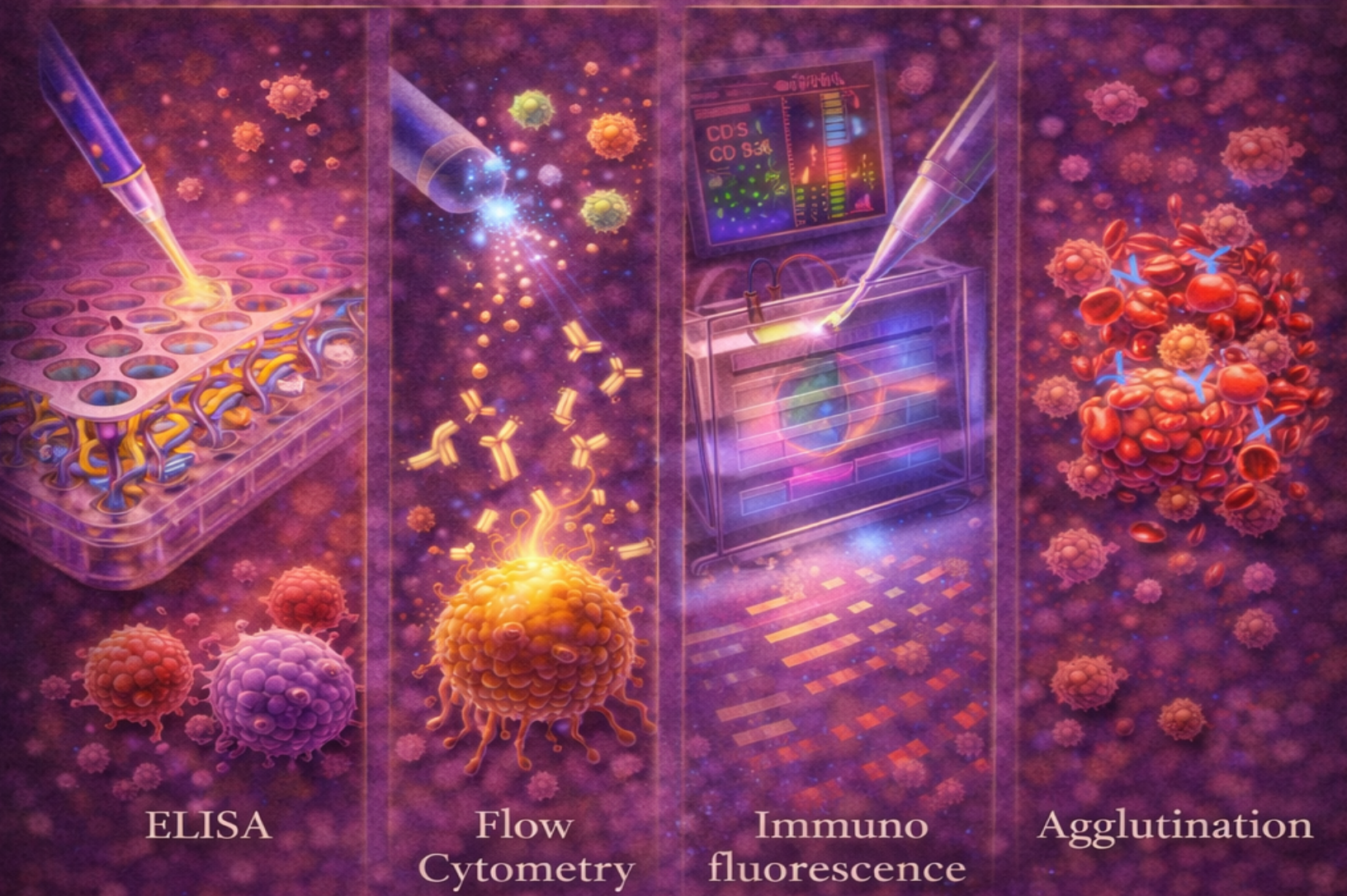
➔ **Deficiency of cellular immunity**

This deficiency is characterized by a reduction or dysfunction of T lymphocytes, which impairs the body's ability to respond to intracellular pathogens (such as viruses and some bacteria) and disrupts the regulation of immune responses.

IX.2.2. Acquired (secondary) deficiencies

Secondary immunodeficiencies arise as a consequence of external factors such as infections, malnutrition, medical treatments (e.g., chemotherapy, immunosuppressive therapy), or chronic diseases. These conditions lead to a functional decline of the immune system, increasing susceptibility to infections. A prominent example is Acquired Immunodeficiency Syndrome (AIDS), caused by the human immunodeficiency virus (HIV), which progressively destroys $CD4^+$ T lymphocytes and severely impairs both cellular and humoral immune responses, rendering the individual vulnerable to opportunistic infections.

Chapter X.
Immunological Techniques



Chapter X. Immunological Techniques

X.1. Antigen-antibody reaction

The antigen-antibody reaction (Ag-Ab) is due to the interaction between antigen epitopes and antibody paratopes. It involves four types of non-covalent interactions (hydrogen bonds, electrostatic interactions, hydrophobic interactions, and Van der Waals forces). Antibodies are highly specific molecular probes that recognize antigenic substances. The Ag-Ab reaction has two main types of applications: the detection and determination of antigens (by methods whose specificity, reproducibility, and sensitivity must be verified) and the detection and titration of antibodies (against an antigen or a mixture of antigens).

X.2. Agglutination reactions

Agglutination is the visible clumping of particulate antigens (such as red blood cells, bacteria, or latex particles) resulting from their specific interaction with antibodies, known as agglutinins. For agglutination to occur, the antigen must be multivalent (possessing multiple identical epitopes), and the antibody must be bivalent or multivalent, allowing cross-linking. This interaction leads to the formation of a lattice network of antigen–antibody complexes that becomes visible to the naked eye or under a microscope.

X.2.1. Principle

Antibodies possess at least two antigen-binding sites (Fab regions). When mixed with particulate antigens, they cross-link adjacent particles, forming a three-dimensional lattice. This lattice results in granular clumping (agglutination).

The efficiency of the reaction depends on several factors:

- Zeta potential (surface charge of particles)
- Optimal antigen-antibody ratio (zone of equivalence)
- Reaction conditions (pH, ionic strength, temperature)

X.2.2. Types of agglutination reactions

a. Direct agglutination

Occurs when antibodies directly agglutinate naturally occurring antigens on cells or microorganisms **(Fig. 10.1)**.

Examples:

- ABO blood grouping (RBC surface antigens)
- Widal test (typhoid fever-Salmonella)
- Brucellosis testing

b. Indirect (Passive) agglutination

Soluble antigens are artificially attached to carrier particles (e.g., latex beads, red blood cells, bentonite). Antibodies in the sample then cause visible agglutination.

Examples:

- Rheumatoid factor detection
- Latex agglutination tests
- Detection of microbial or viral antigens

c. Reverse passive agglutination

In this method, antibodies (instead of antigens) are bound to carrier particles. It is used to detect soluble antigens in patient samples.

Examples:

- Detection of microbial antigens (*Neisseria meningitidis*)
- Cryptococcal antigen test

d. Hemagglutination

A specific type of agglutination involving red blood cells (RBCs).

- ☞ Direct hemagglutination: natural RBC antigens interact with antibodies (e.g., ABO and Rh blood grouping)
- ☞ Indirect (passive) hemagglutination: antigens are coated onto RBCs to detect antibodies in serum
- ☞ Hemagglutination inhibition is used in viral diagnostics (e.g., influenza, measles), where inhibition of agglutination indicates the presence of specific antibodies

e. Coagglutination

In coagglutination, antibodies are bound to carrier bacteria (commonly *Staphylococcus aureus*) via protein A, which binds the Fc region of IgG. This leaves the Fab regions free to bind antigens, enabling rapid detection of pathogens.

X.2.3. Applications of agglutination reactions

a. Clinical diagnosis

- ☞ Blood typing in transfusion medicine (ABO and Rh systems)
- ☞ Detection of red cell antibodies (Coombs test)

b. Infectious disease diagnosis

- Widal test (typhoid fever)
- Weil–Felix test
- Brucellosis agglutination tests
- Rapid antigen detection (e.g., streptococcal infections)

c. Viral diagnosis

- Hemagglutination inhibition tests (e.g., influenza, rubella)

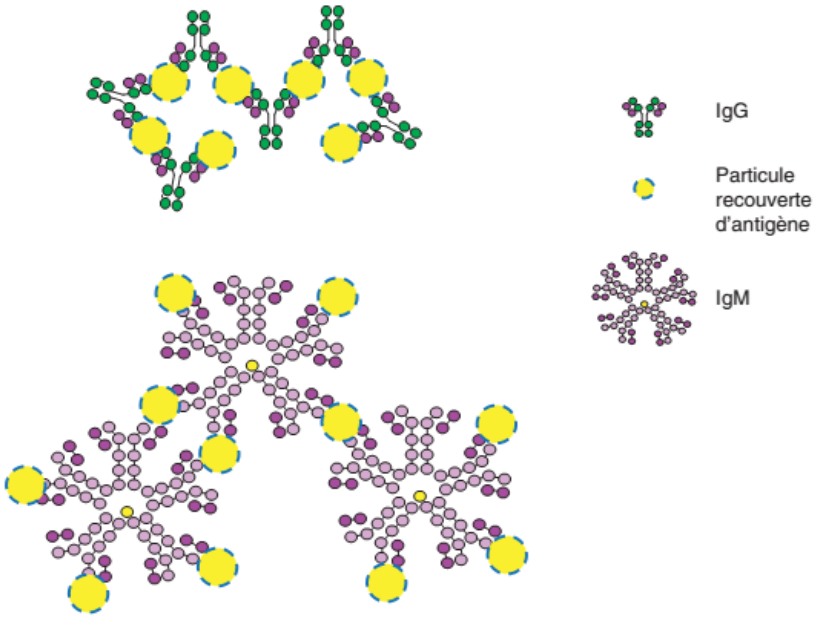


Figure 10.1. Example of direct agglutination with agglutinating antibodies IgG or IgM.

X.3. Precipitation reactions

A precipitation reaction is a type of antigen-antibody interaction in which a soluble antigen combines with its specific antibody (precipitin) in a liquid or gel medium to form a visible insoluble complex (precipitate). This reaction occurs only when antigen and antibody are present in optimal proportions, known as the zone of equivalence.

X.3.1. Principles

The antigen must be soluble (commonly proteins or polysaccharides; nucleic acids are less commonly involved), and the antibody, typically IgG (mainly) or IgM, acts as a precipitin.

➔ **Mechanism**

Antibody molecules cross-link multiple antigen molecules, forming a **lattice network**. When this network reaches a sufficient size, it becomes insoluble and precipitates out of solution.

➔ **Zone phenomenon**

In the prozone (antibody excess), no visible precipitation occurs because antigens are saturated individually and lattice formation is impaired. In the zone of equivalence, optimal antigen-antibody proportions allow maximal lattice formation and visible precipitation. In the postzone (antigen excess), precipitation is again reduced because insufficient antibodies are available to form a cross-linked network **(see Fig. 10.2)**.

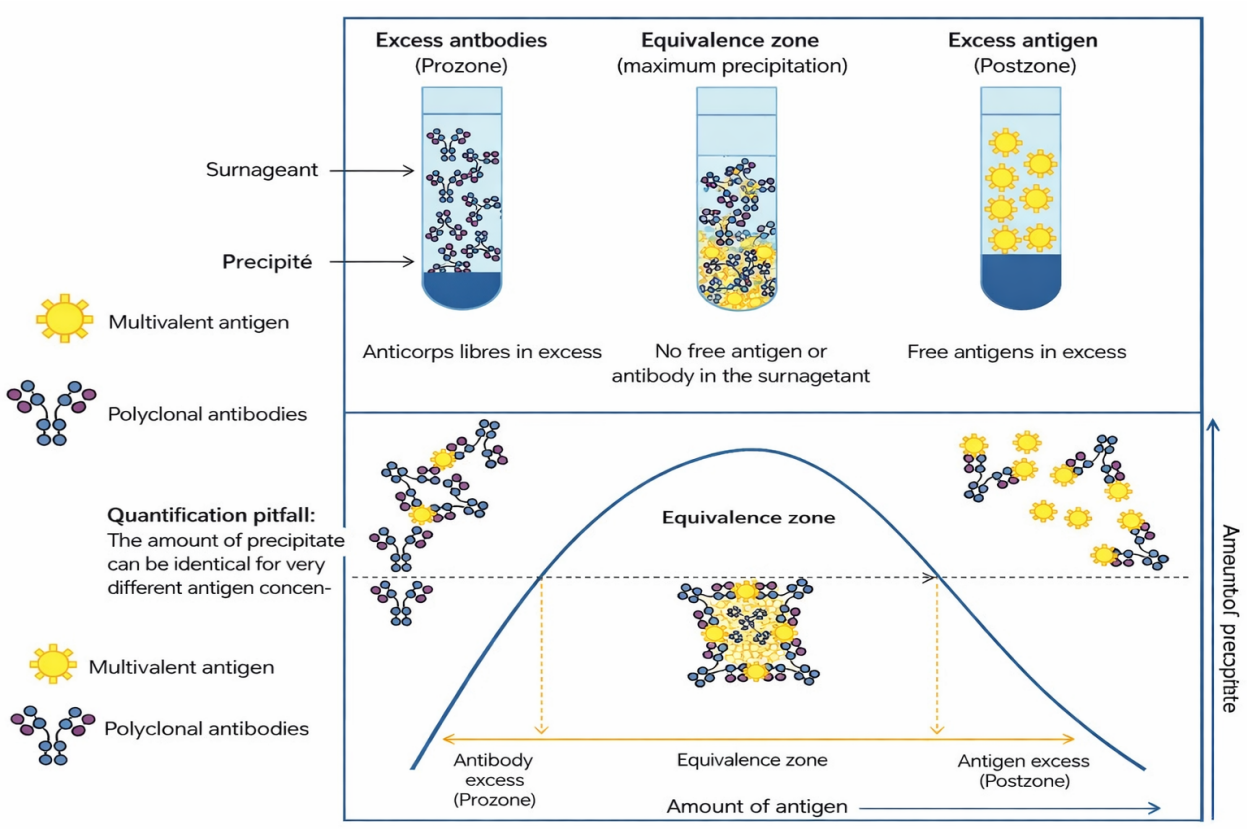


Figure 10.2. Heidelberger precipitation curve.

X.3.2. Types of precipitation reactions

Precipitation reactions can be broadly divided into two main categories:

➔ Precipitation in solution

➔ Precipitation in gel (immunodiffusion techniques)

a. Precipitation in solution

This is the simplest form of precipitation reaction. When a soluble antigen is mixed with its specific antibody in a liquid medium, antigen-antibody complexes form a visible precipitate when both are present in optimal proportions (zone of equivalence).

Examples:

- **Ring test (precipitin ring test):** Antibody-containing serum is layered over an antigen solution in a test tube. A white precipitin ring forms at the interface if the reaction occurs (e.g., **Ascoli's test for anthrax**) **(Fig. 10.3)**.
- **Slide test:** Antigen and antibody solutions are mixed on a glass slide and observed for precipitation.
- **Tube test:** Antigen and antibody solutions are incubated together until a precipitate forms.

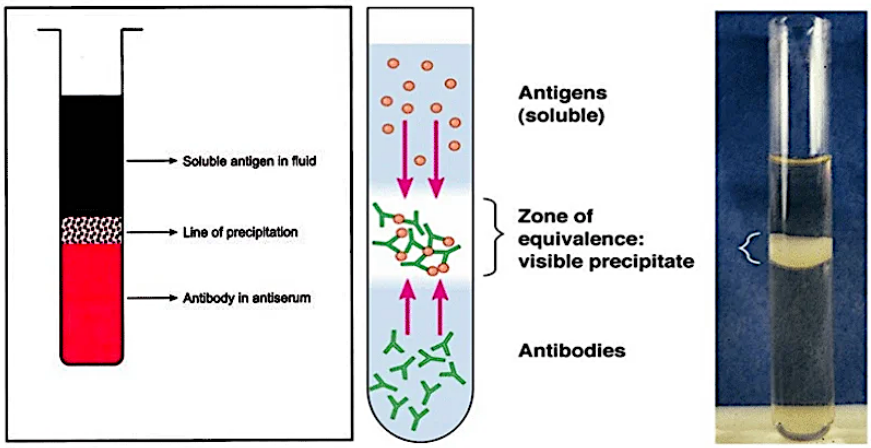


Figure 10.3. Ring test.

b. Precipitation in gel (Immunodiffusion techniques)

In these methods, antigen and antibody diffuse through a semisolid medium (agar or agarose). A visible precipitin line or band forms where they meet at the zone of equivalence. These techniques allow not only detection but also analysis of antigenic relationships and quantification.

b.1. Single diffusion in one dimension (Oudin method)

-Principle: Antibody is incorporated uniformly in the gel, and soluble antigen is layered on top. The antigen diffuses downward, forming a precipitin band where it meets the antibody at optimal proportions **(Fig. 10.4)**.

-Application: Rarely used today; historically used for antigen detection.

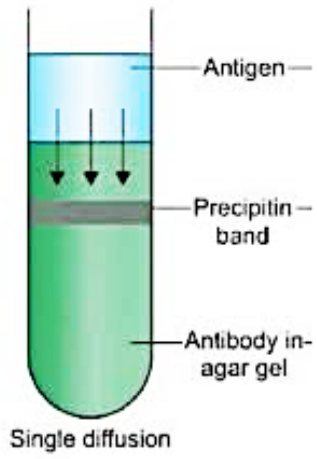


Figure 10.4. Oudin method.

b.2. Single diffusion in two dimensions (Mancini method - radial immunodiffusion, RID)

-Principle: Antibody is uniformly distributed in the agar gel, and antigen is placed in wells. The antigen diffuses radially, forming a circular precipitin ring. The diameter of the ring is proportional to antigen concentration **(Fig. 10.5)**.

***-Application*:** Quantification of serum proteins such as immunoglobulins (IgG, IgA, IgM) in clinical laboratories.

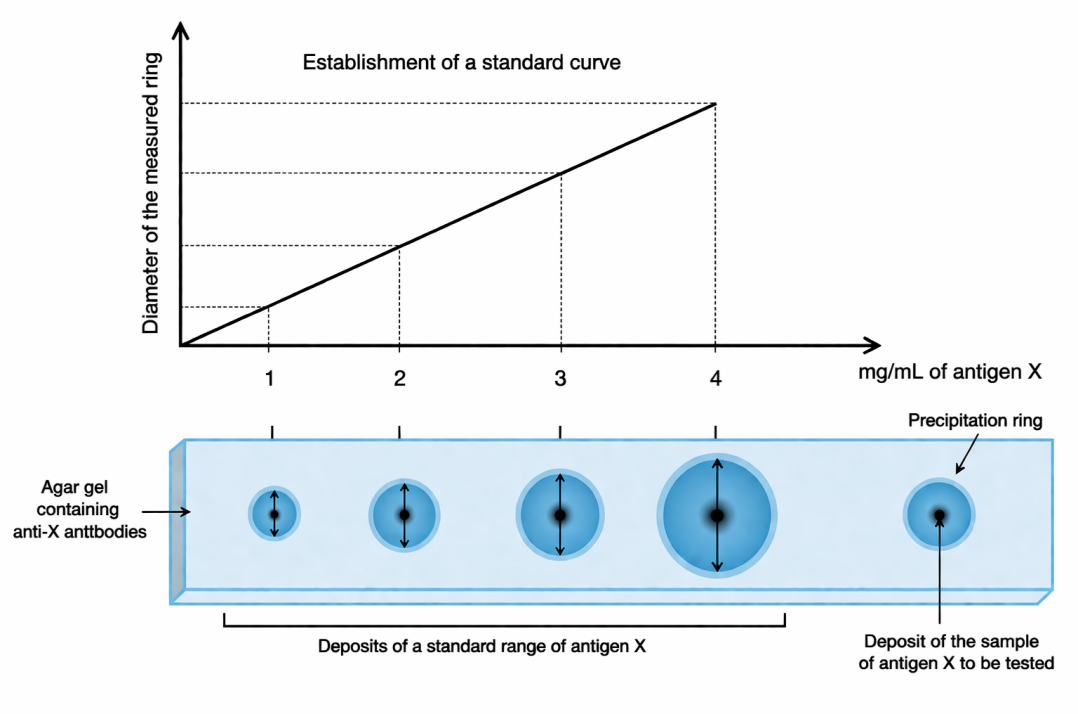


Figure 10.5. Mancini method.

b.3. Double diffusion in one dimension (Ouchterlony variant / Linear diffusion)

-Principle: Antigen and antibody diffuse toward each other in one dimension within layered gel systems, forming precipitin lines at equivalence.

-Application: Mainly used for demonstration and teaching of antigen-antibody reactions.

b.4. Double diffusion in two dimensions (Ouchterlony technique)

-Principle: Antigen and antibody are placed in separate wells in an agar gel and diffuse radially toward each other. A precipitin line forms where they meet at equivalence **(Fig. 10.6)**.

-Types of reactions observed:

- **Identity:** continuous fused line → antigens are identical
- **Non-identity:** crossing lines → antigens are unrelated
- **Partial identity:** spur formation → antigens share some epitopes

-Applications: Comparison of antigenic relatedness, detection of microbial or fungal antigens, and evaluation of antigenic components in vaccines.

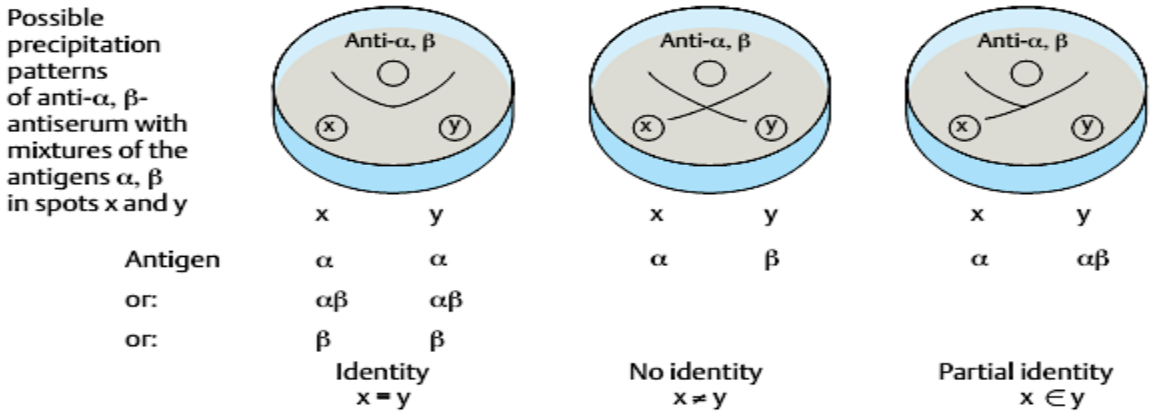


Figure 10.6. Ouchterlony technique.

X.4. Immunoelectrophoresis

-Principle: Immunoelectrophoresis combines electrophoresis and immunodiffusion. First, a mixture of antigens is separated by electrophoresis in an agar or agarose gel. Then, antibodies diffuse from adjacent troughs into the gel. Precipitin arcs form where specific antigens and antibodies meet at the zone of equivalence.

Applications: This technique is used in the clinical diagnosis of multiple myeloma and immunodeficiencies, particularly for identifying abnormal immunoglobulins. It is also useful for the analysis of complex antigenic mixtures, such as serum proteins and bacterial extracts.

X.4.1. Countercurrent immunoelectrophoresis

-Principle: In this method, antigen and antibody are placed in separate wells in an agar gel, and an electric field is applied. The antigen migrates toward the anode (positive electrode), while the antibody migrates toward the cathode (negative electrode). They rapidly meet in the gel and form a precipitin line **(Fig. 10.7)**.

-Advantages: This method is faster than passive diffusion techniques.

-Applications: It is used for the rapid diagnosis of bacterial and viral infections, such as *Streptococcus pneumoniae* and *Haemophilus influenzae*.

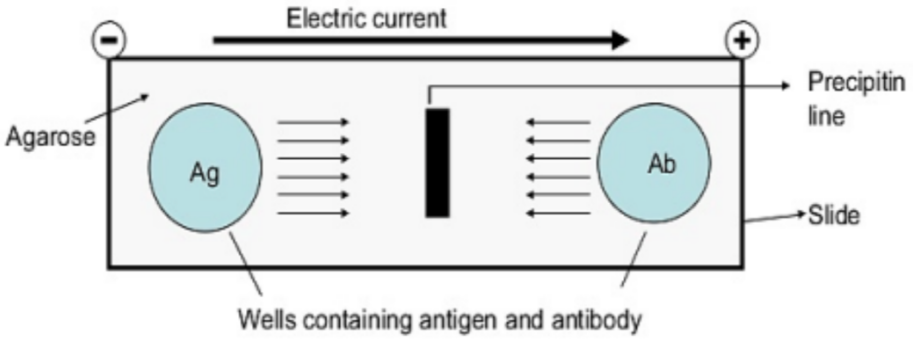


Figure 10.7. Countercurrent immunoelectrophoresis.

X.4.2. Rocket immunoelectrophoresis (Laurell method)

-Principle: In this technique, the antibody is uniformly incorporated into the agar gel, while the antigen is placed in wells. Upon application of an electric field, the antigen migrates through the gel and forms a rocket-shaped precipitin peak. The height of the rocket is directly proportional to the antigen concentration **(Fig. 10.8)**.

-Applications: This method is used for the quantification of proteins and antigens in serum, including complement components, immunoglobulins, and other serum proteins.

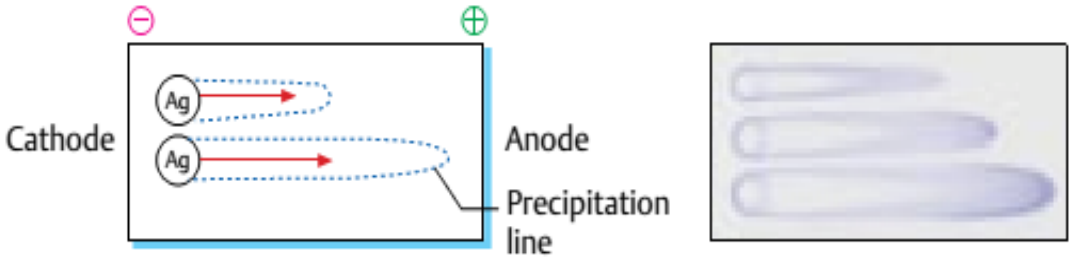


Figure 10.8. Rocket immunoelectrophoresis.

X.5. Immunofluorescence

These are techniques that visualize Ag-Ac complexes using fluorescent markers excited by

ultraviolet light. These markers, fluorochromes, under the effect of UV rays, emit light:
-Fluorescein isothiocyanate (or FITC) absorbs at λ=490nm and emits at λ=517nm (green).
-Rhodamine isothiocyanate absorbs at λ=550nm and emits at λ=580nm (red, orange).
Usually, they marked at the level of the Fc fragment. Fluorescent antibody labeling of cell membrane molecules or tissue sections can be direct or indirect. Fluorescent preparations are examined using a microscope with a UV lamp.

X.5.1. Direct immunofluorescence

Direct immunofluorescence allows the detection of antigens in tissues or cells using fluorescently labeled antibodies. In the case of tissues, sections are placed on a slide and incubated with the labeled antibody. In the case of isolated cells, they are incubated in solution with the fluorescent antibody. In both cases, after incubation, the preparations are washed to remove unbound antibodies and then examined using a fluorescence microscope (**Fig. 10.9).**

X.5.2. Indirect immunofluorescence

Indirect immunofluorescence is mainly used to detect antibodies in biological fluids (e.g., serum). The antigen (often tissue sections or cells used as substrates) is first incubated with the sample containing the antibodies to be detected. If present, these antibodies bind to the antigen, forming an Ag-Ab complex. This complex is then revealed by a secondary anti-immunoglobulin antibody labeled with a fluorochrome. This method is more sensitive than direct immunofluorescence due to signal amplification, although it requires additional steps **(Fig. 10.9).**

-Applications: Immunofluorescence allows the detection of both antigens (direct IF) and antibodies (indirect IF).

- In bacteriology, it is used to identify microorganisms such as *Streptococcus*, *Neisseria meningitidis*, and *Salmonella*.
- In immunology and hematology, it is used to characterize normal cells (e.g., lymphocytes, monocytes) and pathological cells.
- In pathology, direct immunofluorescence is used to detect tumor markers and immune deposits in tissues.

☞ Indirect immunofluorescence is widely used in serology, including the detection of antibodies against bacterial and viral antigens, as well as autoantibodies (e.g., ANA tests).

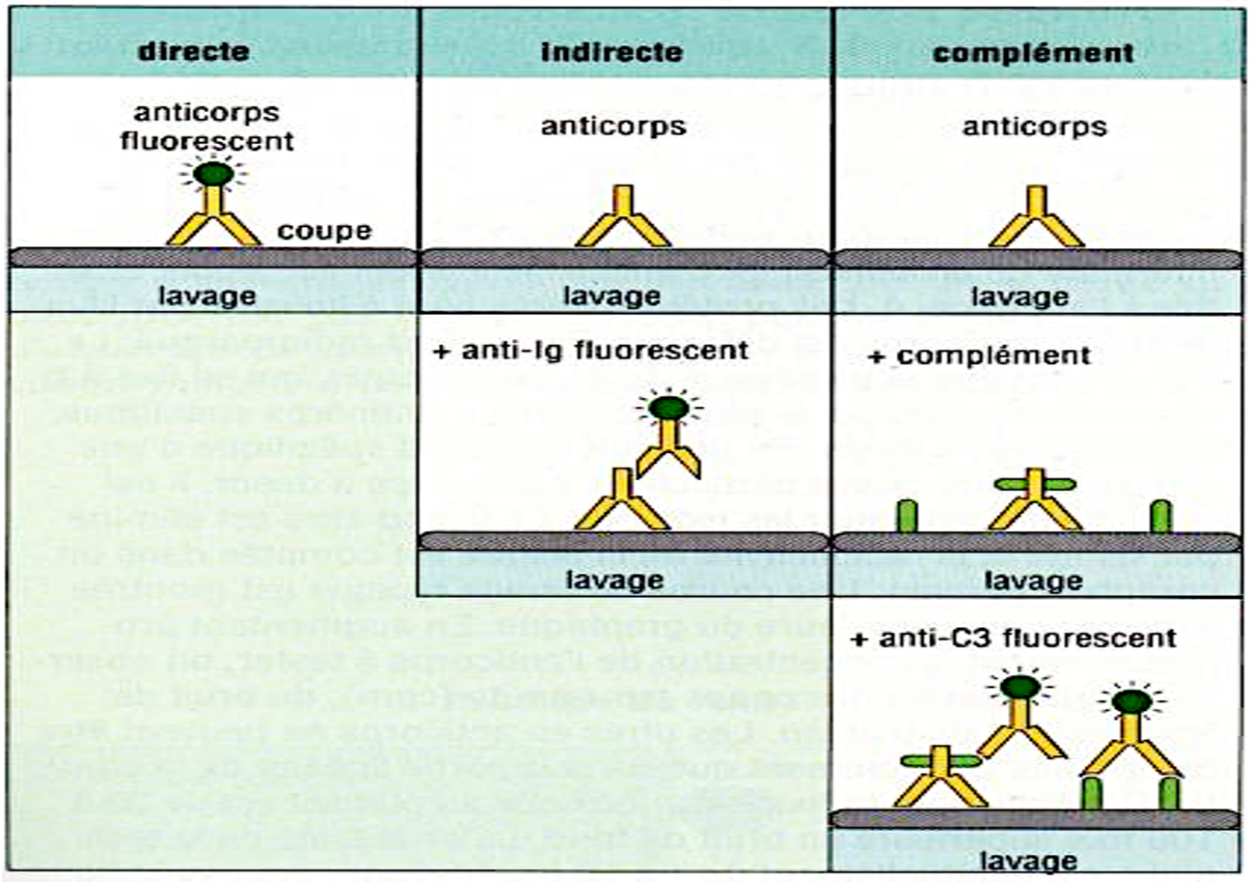


Figure 10.9. Direct and indirect immunofluorescence.

X.6. Enzyme-linked immunosorbent assay (ELISA)

ELISA is an immunological assay that uses antigen-antibody interactions combined with an enzyme-linked detection system to detect and quantify antigens or antibodies in biological samples such as serum, plasma, urine, or cell culture supernatants.

X.6.1. Principle

ELISA is based on three main principles:

1. Specific binding between an antigen and its corresponding antibody
2. Detection using an enzyme-labeled antibody (or antigen)

3. Substrate conversion, in which the enzyme catalyzes the transformation of a colorless substrate (chromogen) into a colored product, measurable by a spectrophotometer (ELISA reader) **(see Fig. 10.10)**.

The intensity of the color is proportional (or inversely proportional, depending on the format) to the amount of antigen or antibody present in the sample.

-Enzymes used in ELISA: Enzymes are essential in ELISA because they generate a detectable signal by acting on specific substrates, producing a colorimetric, fluorescent, or chemiluminescent signal.

The choice of enzyme depends on required sensitivity, type of substrate, and detection system (spectrophotometric, fluorescent, or chemiluminescent).

Major enzymes used: Horseradish peroxidase (HRP), alkaline phosphatase (AP), β-galactosidase (more common than β-glucosidase), and glucose oxidase (less commonly used).

X.6.2. Types of ELISA

a. Direct ELISA

- ☞ The antigen is immobilized on the plate
- ☞ The enzyme-labeled antibody binds directly to the antigen
- ☞ Substrate is added → color develops

-Advantages: simple, rapid, fewer steps

-Disadvantages: lower sensitivity, no signal amplification, higher background

b. Indirect ELISA

- ☞ The antigen is immobilized on the plate
- ☞ The primary antibody (from patient sample) binds to antigen
- ☞ The enzyme-labeled secondary antibody binds to the primary antibody
- ☞ Substrate added → color develops

-Advantages: higher sensitivity, signal amplification, versatility

-Disadvantages: possible cross-reactivity of secondary antibody

c. Sandwich ELISA

- ☞ Capture antibody is coated on the plate
- ☞ Sample antigen binds to the capture antibody
- ☞ Detection antibody (enzyme-labeled or followed by a secondary antibody) binds to another epitope

***-Advantages*:** very high specificity and sensitivity

-Disadvantages: requires well-matched antibody pairs

d. Competitive ELISA

- ☞ The sample antigen competes with labeled antigen for limited antibody binding sites
- ☞ Inverse relationship: higher antigen concentration → lower signal

-Advantages: suitable for small molecules (hormones, drugs)

-Disadvantages: more complex interpretation

X.6.3. Applications of ELISA

a. Disease diagnosis

- ☞ HIV antibody detection
- ☞ Hepatitis B and C antigen/antibody detection
- ☞ COVID-19 serology
- ☞ Dengue and malaria diagnosis

b. Hormone and cytokine measurement

- ☞ Insulin, hCG, thyroid hormones, interleukins, interferons

c. Allergy testing

- ☞ Detection of allergen-specific IgE antibodies

d. Drug monitoring

☞ Detection of therapeutic drugs and drugs of abuse

e. Research applications

☞ Quantification of proteins in cell culture supernatants or tissue extracts

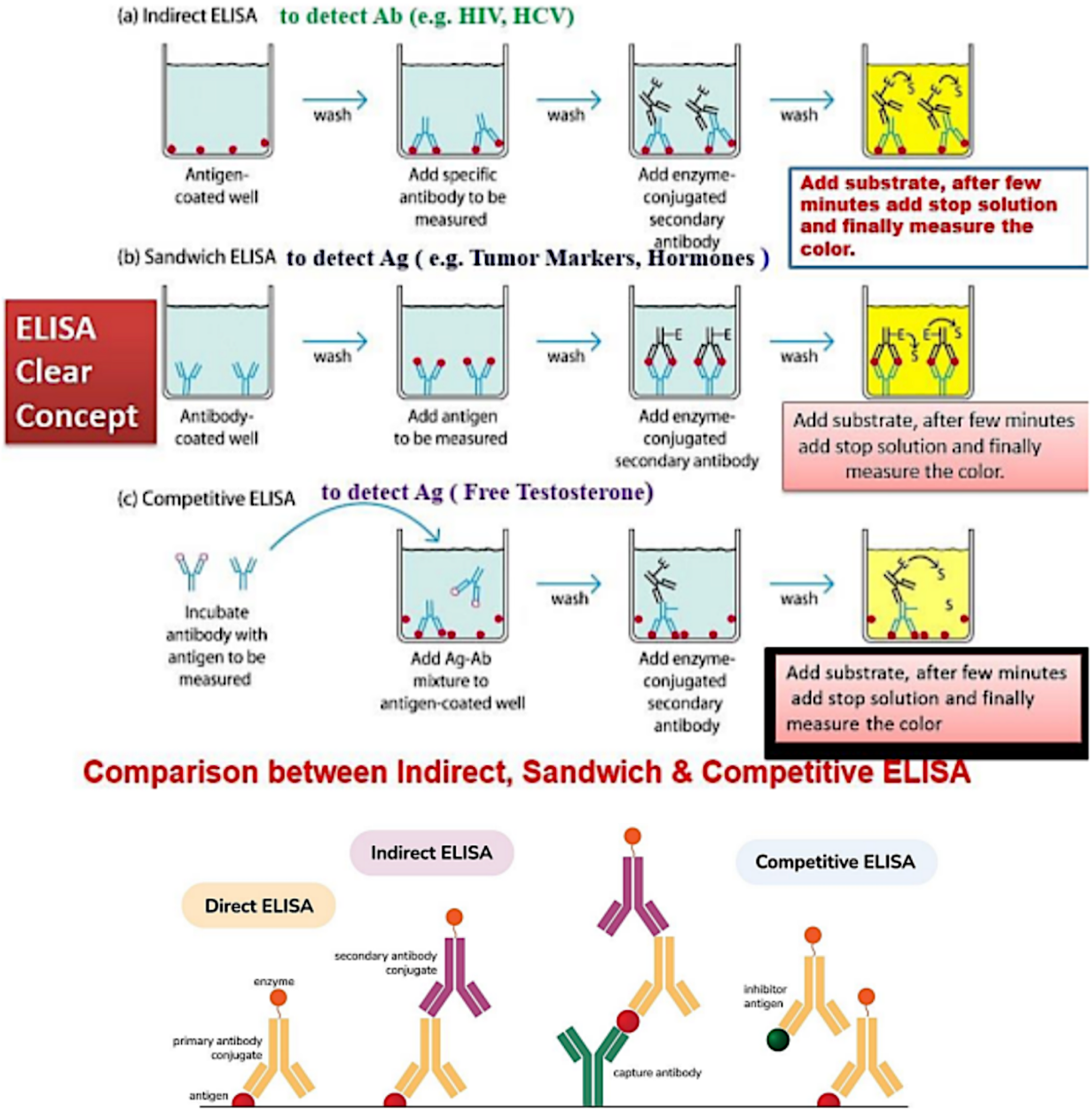


Figure 10.10. Types of ELISA.

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