People's Democratic Republic of Algeria
Ministry of Higher Education and Scientific Research
Ferhat Abbas University-Setif 1-
Faculty of Natural and Life Sciences

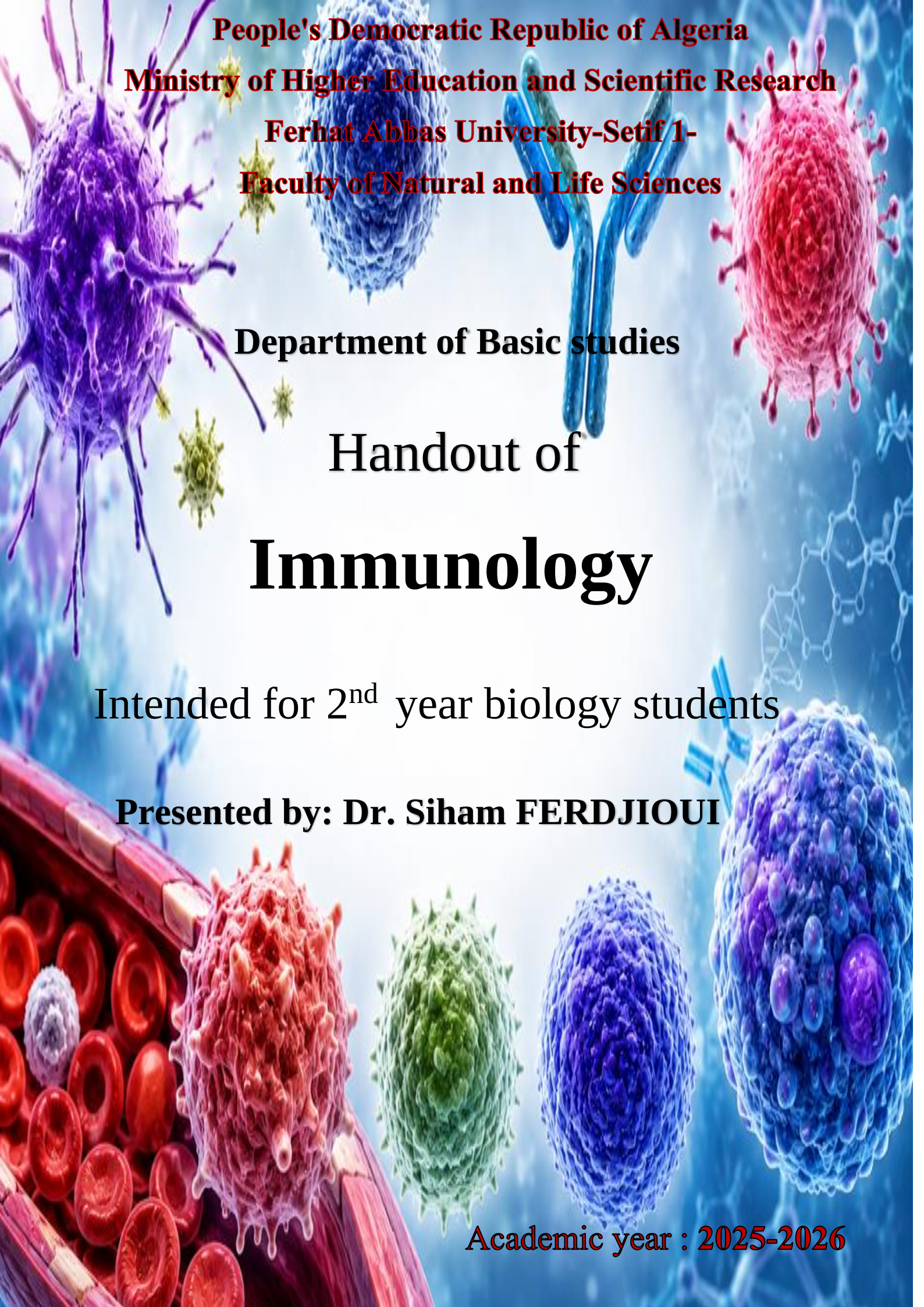
Department of Basic studies

Handout of

Immunology

Intended for 2nd year biology students

Presented by: Dr. Siham FERDJIOUI



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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Preface

Immunology is a fundamental branch of biological sciences that studies the structure, functions, and mechanisms of the immune system. Throughout evolution, the immune system has developed highly specialized defense strategies that enable the organism to distinguish self-components from foreign elements and to maintain physiological homeostasis. Its study encompasses the body's responses to infectious agents such as viruses, bacteria, fungi, and protozoa, as well as immune-related disorders including autoimmune diseases, hypersensitivity reactions, and transplant rejection. Immunology also investigates the cellular and molecular mechanisms underlying immune responses, including antibody production, complement activation, and the coordination of immune cells.

This course is designed for second-year undergraduate students enrolled in the Common Core Program of Biological Sciences. It provides a comprehensive introduction to the fundamental principles of immunology and presents the major cellular and molecular events involved in innate and adaptive immune responses. Particular emphasis is placed on the components of the immune system, their interactions, and the mechanisms that ensure effective protection against pathogens while preserving self-tolerance.

By the end of this course, students will have acquired the essential knowledge required to understand the organization and functioning of the immune system, the mechanisms involved in host defense against infectious agents, and the immunological basis of autoimmune diseases, hypersensitivity reactions, and other immune-mediated disorders.

Chapter I: Introduction to Immunology

I.1. Basic Concepts of Immunology

I.1.1. Immunology

Immunology is the scientific discipline dedicated to the study of immunity. It investigates the physiological and pathological functioning of the immune system, characterizing the properties of its cellular and molecular effectors alongside their respective targets both *in vivo* and *in vitro*. Furthermore, immunology explores the translational application of these effectors in biotechnology, as well as the therapeutic modalities designed to modulate—either via immunostimulation or immunosuppression—immune activity. The primary physiological function of the immune system is to prevent microbial colonization and to eradicate established infections.

I.1.2. Immunity

Immunity is fundamentally defined as the host's resistance to disease. It encompasses the physiological defense mechanisms deployed by a living organism to safeguard its homeostatic integrity and survival. These mechanisms are directed against exogenous threats, particularly pathogenic microorganisms, as well as endogenous aberrations, most notably oncogenic transformation (malignancy). Etymologically, the term "immunity" is derived from the Latin word *immunitas*, meaning "exempt from" or "free from"—historically denoting freedom from disease.

I.1.3. The Immune System

The immune system comprises the complex network of specialized organs, tissues, cells, and soluble molecules that collectively mediate the immune response.

I.1.4. The Immune Response

The immune response refers to the coordinated collective of cellular and molecular mechanisms mobilized by an organism to recognize, neutralize, and eliminate an immunogenic or pathogenic agent.

I.1.5. The Immunological "Self"

The immunological **self** is defined as the complete repertoire of autologous cellular structures, proteins, and macromolecules that arise from the physiological expression of an organism's own genome. These components constitute the unique biological blueprint of the individual. Under physiological conditions, these autologous molecules are recognized by the immune system without triggering an effector response, a state of profound unresponsiveness maintained through central and peripheral mechanisms collectively known as **immunological tolerance**.

I.1.6. The Immunological "Non-Self"

Conversely, the non-self encompasses the entire spectrum of molecular structures that lie outside the biological identity of the host organism. This category is broadly bifurcated into altered-self and foreign non-self:

- **Altered-Self Components:** These represent endogenous host molecules that have undergone abnormal structural modifications. They include senescent cells, necrotic debris, and malignantly transformed cells harboring mutated proteins that no longer reflect the normal genetic expression of the physiological genome.
- **Exogenous Non-Self Agents:** These comprise all foreign substances, xenobiotics, and pathogenic microorganisms (such as viruses, bacteria, fungi, and parasites) to which the organism is exposed.

I.1.7.Antigen

An antigen is any substance or molecule specifically recognized by components of the immune system, such as antibodies or T-cell receptors.

I.1.8. Pathogen

A pathogen is a microorganism or agent capable of causing disease, such as bacteria, viruses, fungi, or parasites

I.1.9.Immunogen

An immunogen is an antigen capable of inducing an immune response.

I.1.10. Antibody

An antibody is a protein produced by B lymphocytes and plasma cells that specifically recognizes and binds to antigens.

I.1.11. Antigenic Potential

Any molecule categorized as non-self—whether derived from an invading pathogen or an aberrant, mutated host cell—possesses the structural complexity to be recognized by specific immune receptors (B-cell receptors, T-cell receptors, or innate Pattern Recognition Receptors). Consequently, these non-self entities act as antigens, possessing the immunogenic capacity to disrupt immunological homeostasis, activate cellular and humoral defense cascades, and mount a targeted immune response aimed at their elimination.

I.2. Intersections with Daily Life (Relevance to Everyday Health)

The relationship between immunology and daily life is fundamentally rooted in the continuous, dynamic interactions between the host immune system and diverse environmental pathogens, directly influencing systemic health, homeostasis, and overall human well-being.

- **Disease Prevention:** The primary baseline function of the immune system is to shield the host organism from infectious diseases. It achieves this by continuously screening tissues, detecting pathogenic microorganisms, and executing targeted elimination cascades.Vaccinations serve as a primary, practical application of immunological principles in daily life. By safely introducing non-pathogenic antigenic structures to the host, vaccines prime and reinforce the adaptive immune response, establishing long-term immunological memory and preventing subsequent clinical disease upon pathogen exposure.
- **Management of infections :** Understanding how the immune system functions is essential for the treatment of infections. The use of antibiotics and other antimicrobial drugs is based on immunological knowledge to combat pathogens.
- **Personal hygiene :** Adopting good hygiene practices, such as regular hand washing, helps prevent infections by reducing the spread of pathogens. This highlights the importance of understanding immunology in order to take preventive measures.

- **Allergic responses:** Immunology is also related to allergic reactions. Understanding allergies and food intolerances, for example, makes it possible to adjust the diet in order to avoid undesirable immune system reactions.
- **Autoimmune diseases:** Advances in understanding the mechanisms of autoimmune diseases have led to new therapeutic approaches. These discoveries have a significant impact on the quality of life of people affected by these disorders.
- **Balanced diet:** A balanced and nutritious diet contributes to maintaining a strong immune system. Certain nutrients, such as vitamins and minerals, are essential for the optimal functioning of the immune system. Table 1 summarizes the core physiological and pathological functions of the immune system.

Table 1 : Clinical and Physiological Significance of the Immune System in Health and Disease

Immune Function	Physiological Homeostasis (Health)	Pathological Manifestation (Disease)
Defense Against Infections	Effective eradication of exogenous pathogens (viruses, bacteria, fungi, parasites); development of immunological memory.	**Deficiency:** Increased susceptibility to infections (immunodeficiency); opportunistic infections. **Excess:** Immunopathology and collateral tissue damage.
Immune Surveillance (Tumors)	Detection and clearance of senescent, damaged, or malignantly transformed host cells.	**Failure:** Tumor evasion, survival, and oncogenic progression (malignancy).
Self/Non-Self Discrimination	Maintenance of immunological tolerance toward autologous (self) tissues and harmless environmental antigens.	**Failure:** Autoimmune diseases (destruction of self-tissues) and hypersensitivity reactions (allergies, anaphylaxis).
Tissue Homeostasis & Repair	Resolution of inflammation, clearance of cellular debris, and promotion of tissue regeneration/wound healing.	**Dysregulation:** Chronic fibrosis, persistent inflammatory states, or impaired tissue healing.

I.3. Historical Background of Immunology

Immunology began as a branch of microbiology. The study of infectious disease and the body's response to them has a major role for the development of immunology. More over, the concept of germ theory of disease has contributed to the field of immunology.

It was Edward Jenner who first studied the response of the body to foreign substances. He observed that dairy maids who had naturally contracted a mild infection called cowpox seemed to be protected against smallpox, a horribly disfiguring disease and a major killer.

In 1796, Jenner inoculated an eight year-old boy with fluid from cowpox blisters on the hand of a dairymaid. The boy contracted cowpox. Then two month later Jenner inoculated him with fluid from a small pox blister, the boy only developed a small sore at the site of inoculation. His exposure to the mild disease cowpox had made him immune to the small pox infection. These were some of the vital events occurred in the history of immunology following Jenner's achievement.

In 1879, the first human pathogen, gonococcus, was isolated by Neisser. In 1883, Klebs and Loeffler isolated diphtheria bacilli which led to the production of the first defined antigen, diphtheria toxin, by Roux and Yersin in 1888. In the same year the first antibodies, serum bactericidins, were reported by Nuttal and Pasteur.

In 1890, von Behring and Kitasato discovered antitoxins that led to the development of toxoids for diphtheria and tetanus.

In 1900, Land Steiner discovered the blood group antigens and their corresponding antibodies. This led to the ability to give blood transfusion with out provoking reactions.

It was in 1916 that the first journal of immunology began publication in which many of new findings published on it. In general, immunology has always depended on and stimulated the application of technology, such as the use of microscopy, electrophoresis, immunoelectrofluorescence, etc.

Thus immunology has not become an inborn discipline but has maintained close associations with many other fields of medical sciences.

Chapter II: Immune System Ontogenesis

II.1. Introduction

The development of the various defense mechanisms of the immune system, both innate and adaptive, begins progressively during fetal life. At birth, immune cells are not yet fully mature, which explains the increased susceptibility of newborns, particularly premature infants, to bacterial and viral infections. Indeed, due to inadequate cytokine production:

- Phagocytic cells, although possessing bactericidal functions, remain unable to efficiently migrate to sites of infection.
- The cytotoxic activity of Natural Killer (NK) cells remains incomplete.

The maturation of the complement system, which is still immature at birth, as well as that of the innate immune system, occurs gradually during the first year of life.

Regarding the adaptive immune system, T and B lymphocytes and antigen-presenting cells (APCs), which are essential for specific immune responses, begin their differentiation early during gestation. At this stage of development, B and T lymphocytes are immunologically naïve and therefore exhibit a primary immune response that is delayed, slow, and relatively inefficient. Full maturation of the adaptive immune system is achieved through repeated antigenic stimulation and T–B cell cooperation during the first years of life.

II.2. Immune system Ontogeny definition

Immune system ontogeny refers to the entire process of development of the various immune cell populations, from their formation in the fetal liver (during fetal life) or in the bone marrow, to their maturation. This maturation involves the acquisition of their functional capacities, the expression of specific membrane receptors, and the establishment of self-tolerance toward self-antigens.

II.3. The Components of the Immune System

The immune system is composed of a complex network of specialized organs, tissues, and cells through which innate and adaptive immune cells continuously circulate and interact. It is organized into primary (central) lymphoid organs**,** including the bone marrow and thymus, and secondary (peripheral) lymphoid organs**,** including the lymph nodes**,** spleen**,** and mucosa-associated lymphoid tissues (MALT) (Fig.1)**.** These structures work together to ensure the

development, activation, and coordination of immune responses.This communication network provides the immune system with three essential properties:

➔ A significant capacity for information exchange, either through direct membrane contacts between cells or by the release of soluble mediators. These exchanges occur between different components of the immune system.

➔ A highly effective effector arm capable of protecting the body's integrity.

➔ A strong regulatory mechanism, which is crucial for maintaining immune system balance (homeostasis) at all times and in all locations, ensuring an appropriate immune response.

Disruptions in any of these systems can lead to pathological disorders, such as immune deficiencies, autoimmune diseases, or hypersensitivity conditions.

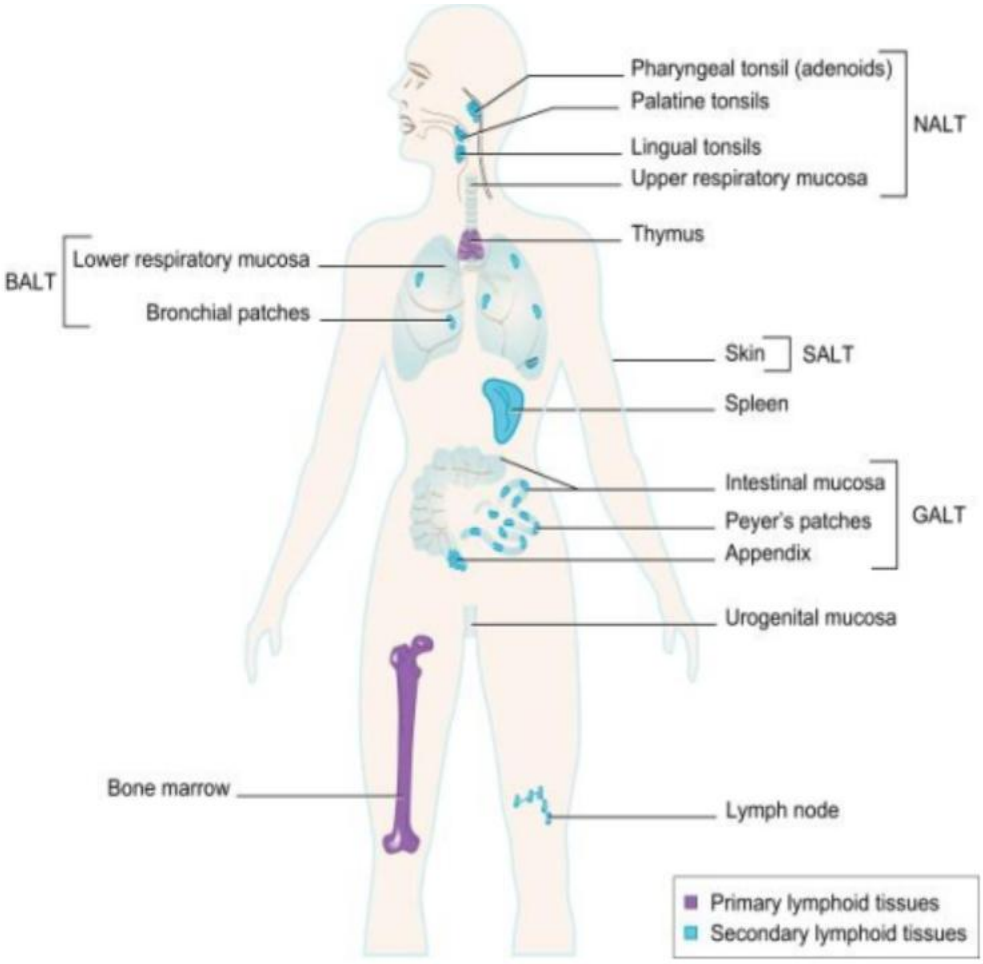


Figure 1 : Primary and secondary lymphoid organs and tissues

II.3.1. Primary (or Central) Lymphoid Organs

The thymus and bone marrow are referred to a primary lymphoid organ because they provide conducive microenvironments that are essential for initial production and maturation of lymphocytes from progenitor cells.

II.3.1.1. The Bone Marrow

Bone marrow is a soft tissue that occupies the cavities and free spaces inside both long bones (such as the femur and humerus) and flat or short bones (such as the skull, ribs, vertebrae, and pelvis). It is the primary lymphoid organ responsible for hematopoiesis and immune cell development. There are two types lof *bone marrow*, "red marrow," which is made up of hematopoietic tissue including cells of the immune system, and "yellow marrow," which consists of fat cells (Fig.2). The bone marrow's pluripotent stem cells can be differentiated into a variety of cell types, e.g., lymphoid stem cell. The bone marrow's stroma provides the hematopoietic microenvironment that facilitates hematopoiesis, in particular, B-cell lymphopoiesis, through the parenchymal cells that produce colony-stimulating factors (CSFs) and other cytokines. Nowadays, it is known that the bone marrow may perform a valve-like function to prevent the backflow of lymphatic fluid in the lymphatic system. Furthermore, the blood vessels of the bone marrow create a blood-marrow barrier, which inhibits immature cells from leaving the bone marrow.From a clinical viewpoint, the bone marrow is the most important object for transplantation in children with primary immunodeficiencies. Biopsies of the bone marrow widely use for diagnostic purposes in hematology.

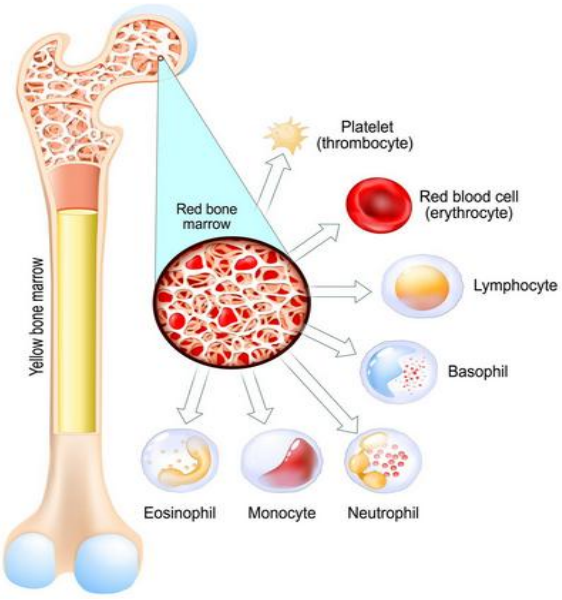


Figure 2 : Yellow bone marrow and Red bone marrow. Blood cells develop

Note: In birds, the bursa of Fabricius is the primary lymphoid organ responsible for B lymphocyte differentiation. Its equivalent in mammals is the bone marrow.

II.3.1.2. The Thymus

The thymus is a gland situated in front of the heart and behind the sternum and consists of capsule-coated lobules containing the strictly isolated parenchyma, and the future T cells, thymocytes, which may pass through the vessel endothelium here. There are three parenchymal zones of the thymus, subcapsular, cortical, and medullary (Fig. 3), that accord with major stages of T-cell lymphopoiesis. In the first and second zones, many thymic follicles are available, whereas in the third zone, thymic corpuscles (Hassall's) occur. The role of Hassall's corpuscles is not entirely determined. In the thymus, suspension thymocytes account for approximately 97%, namely, 85% in the cortex and 12% in the medulla. Giant epithelial "nurse" cells, macrophages, thymic dendritic cells, and NK cells make up less than 1% in total. These cells are constant representatives of the thymic microenvironment, "professors," which teach "students," the thymocytes.

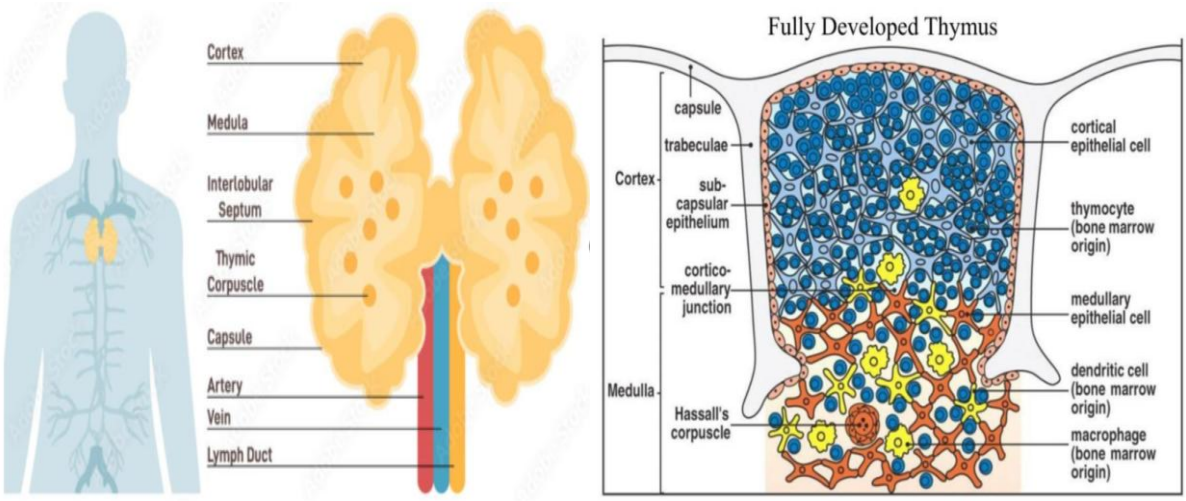


Figure 3 : thymus position and overall structure

The thymus produces numerous hormones, neurotransmitters, and molecules of the immune system. This includes thymosins; antidiuretic hormone (ADH) or vasopressin and oxytocin, which serve as local cryptic signals essential for T-cell education and immunotolerance; glucocorticoids, β-endorphins, and enkephalins, which regulate thymocyte proliferation and apoptosis thresholds; along with cytokines (IL1, IL2, IL3, IL6, IL7, IL9, GM-CSF, etc.) and chemokines that collectively establish the functional microenvironment for the thymocytes. The thymus progressively tends to atrophy starting almost from the birth. It is the so-called *age-*

dependent thymus involution, which corresponds to a prolonged decrease in its endocrine function and a substitution for lipid tissue. This process differs individually. It seems to be the programmed duration of the thymus's ability to perform its strategic homeostatic function that may affect the lifespan.

From a clinical point of view, the prolonged abuse of drugs including exogenous opioids because of the endogenous β endorphin system's exhaustion and oxidative stress due to the accumulation of reactive oxygen species (ROS) may result in the accelerated thymic involution, progressive thymic atrophy, and early aging of the whole body. A thymectomy performed in adults does not lead to any immunodeficiency. On the other hand, in clinical practice, many thymic products are used for immune enhancement therapy.

II.3.2. Secondary (or Peripheral) Lymphoid Organs

Secondary lymphoid organs are the primary sites where antigens encounter immune cells involved in the adaptive immune response. They serve as specialized locations for the drainage and concentration of antigens originating from tissues, lymphatic fluid (lymph nodes), blood (spleen), and mucosal surfaces (mucosa-associated lymphoid tissue, MALT). These organs are highly vascularized, facilitating the continuous recirculation of naïve lymphocytes and promoting efficient antigen recognition and immune cell activation.

II.3.2.1. The Lymph Nodes

Some blood fluid from the bloodstream leaks out into tissues and because of the pressure gradient is absorbed into the lymphatic system becoming the *lymph*. In the course of lymph flow, the lymph picks up antigens, antigen-presenting cells, and lymphocytes throughout the body and carries them via lymphatics into the lymph nodes.

The lymph nodes are among the secondary organs of the immune system, widely present in many parts of the body, and have an artery, vein, and afferent and efferent lymphatic vessels. The afferent lymphatics are multiple and wider than efferent vessels, so cells and molecules can easily enter the lymph nodes. Conversely, the passage of large cells like macrophages through the efferent vessels is difficult, so that they remain to function within the lymph node.

Each lymph node is divided into lymph nodules, which contain a cortical zone of primary follicles with B cells, a paracortical zone of T cells, and a basal part of the nodule in the medulla. The primary follicles develop into secondary follicles in the course of B-cell-mediated immune

responses. Lymphocytes enter the lymph nodes through specialized high endothelial venules (HEV) found in the paracortical zone (Fig.4).

From a clinical point of view the lymph nodes have essential clinical significance. They may be enlarged, swollen, and inflamed in many infections, tumors, and even under some noninfectious conditions. The palpation of accessible lymph nodes serves as a suitable means of monitoring for any physician.

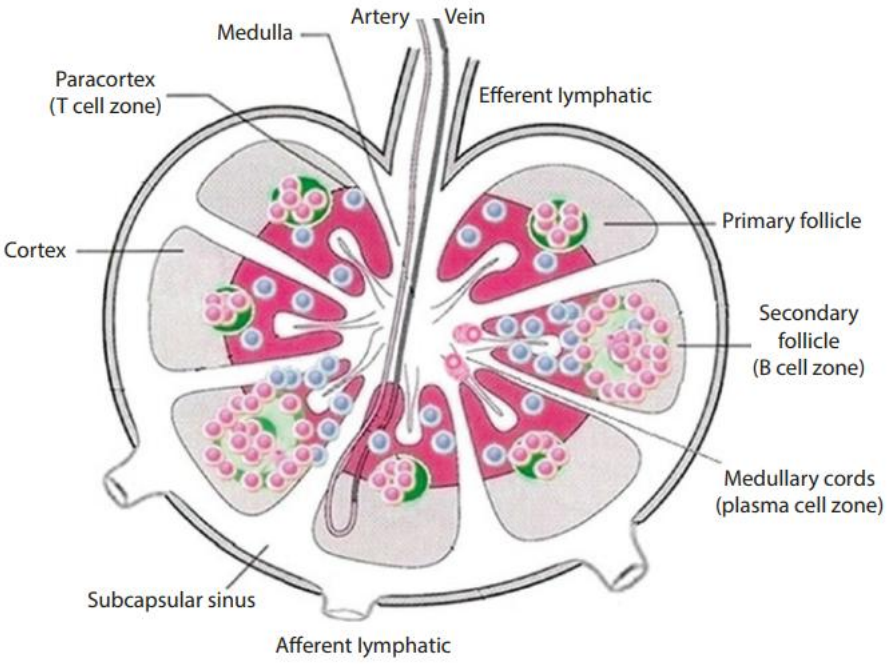


Figure 4 : Structure of the lymph node

II.3.2.2. The Spleen

The spleen is one of the secondary organs of the immune system but functions at the systemic level when antigens enter the blood. Thus, the spleen is of importance in fighting infections that have invaded the blood. It is similar in structure to a large lymph node located in the left upper quadrant of the abdomen. However, the spleen has the splenic artery, splenic vein, and only efferent lymphatic vessels. It consists of red pulp and white pulp. The red pulp plays a role in blood clearance, removing old erythrocytes, maintaining an additional reservoir of blood, and metabolizing hemoglobin.

The white pulp is composed of lymphoid follicles, rich in B cells*;* marginal zones *(*MZ*),* rich in MZ B cells; and periarteriolar lymphoid sheaths *(*PALS), rich in T cells (Fig. 5*).* The white pulp is vital for the immune processes including B-cell-mediated responses, synthesis of

antibodies, removal of antibody-coated microbes, production of properdin, and storage of monocytes.

From a clinical view point, a splenectomy leads to a significant increase in the usual death rate from pneumonia, an increase the death rate from ischemic heart disease, and a much diminished frequency of memory B cells. If splenectomy is planning, vaccination against *Pneumococci* must perform.

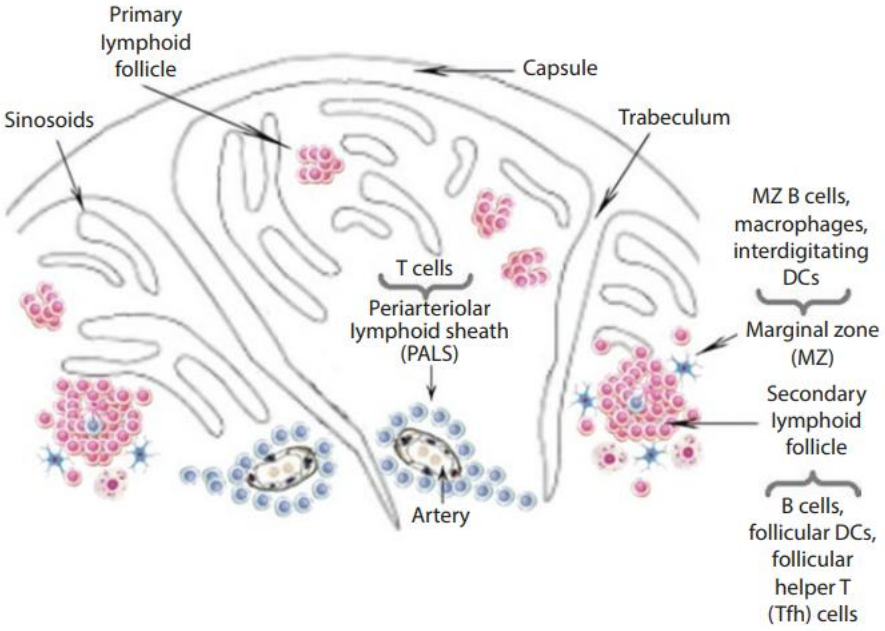


Figure 5 : Spleen's white pulp

II.3.3. Mucosa-Associated Lymphoid Tissues

Diffuse lymphoid tissue involved in mucosal immune defense is collectively known as MALT (Mucosa-Associated Lymphoid Tissue), which constitutes the largest component of the human immune system, containing more than 50% of the body's total lymphocytes. This specialized immune system forms an essential protective barrier at vulnerable mucosal linings particularly within the gastrointestinal, respiratory, and urogenital tracts which are continuously exposed to external pathogens, antigens, and environmental microorganisms. To preserve the integrity of these tissues and prevent infections, MALT contains various immune cells, including lymphocytes, macrophages, dendritic cells, and plasma cells, that cooperate through both innate and adaptive immune responses to recognize and eliminate invading pathogens while maintaining mucosal immune homeostasis. It protects these surfaces through distinct, shared mechanisms: specialized M cells (microfold cells) that sample antigens directly from the lumen, localized secretory IgA production, and a highly specialized homing network of lymphocytes

that ensures targeted immune traffic back to mucosal sites (Fig.6). According to their anatomical localization, MALT can be classified into the following major components :

- BALT (Bronchus-Associated Lymphoid Tissue),
- NALT (Nasopharynx-Associated Lymphoid Tissue), and
- GALT (Gut-Associated Lymphoid Tissue).

SALT (Skin-Associated Lymphoid Tissue) refers to the diffuse lymphoid tissue responsible for skin immune protection.

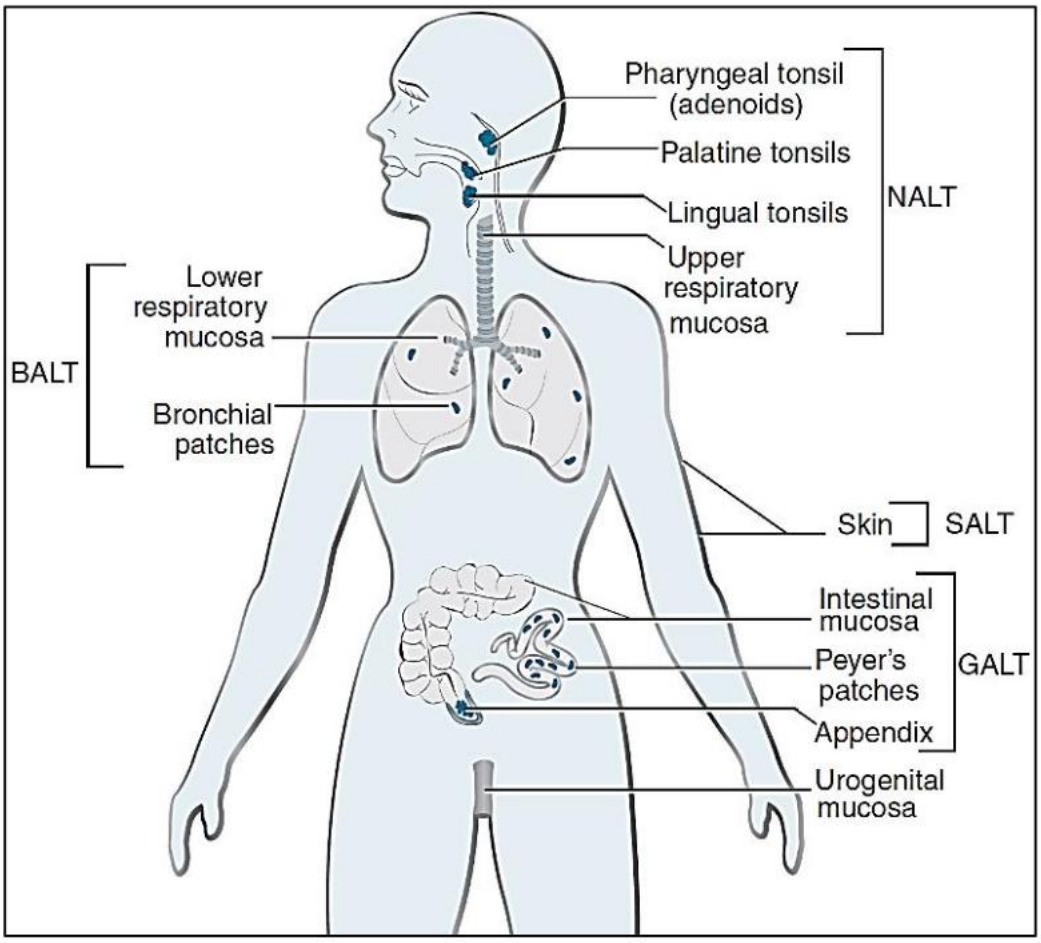


Figure 6 : Mucosa-Associated Lymphoid Tissues and Skin-Associated Lymphoid Tissue

II.3.3.1. GALT (Gut-Associated Lymphoid Tissue)

GALT (Gut-Associated Lymphoid Tissue) represents the largest and most extensively characterized component of the mucosa-associated lymphoid tissue (MALT) system. It plays a fundamental role in monitoring and regulating immune responses within the gastrointestinal tract, which is continuously exposed to a wide variety of dietary antigens and commensal

microorganisms constituting the gut microbiota. GALT is composed of several specialized lymphoid structures, including:

- **Peyer's patches:** organized aggregates of lymphoid follicles predominantly located in the lamina propria of the ileum.
- **Isolated lymphoid follicles (ILFs):** dynamic single-follicle lymphoid structures distributed throughout the small and large intestines.
- **Appendix and mesenteric lymph nodes:** structures that contribute to intestinal immune surveillance and coordinate immune responses associated with the gut.

A major function of GALT is to maintain a delicate balance between protective immunity against pathogenic microorganisms and oral tolerance toward harmless food antigens and beneficial commensal bacteria. Furthermore, GALT constitutes the principal site for the production of secretory dimeric IgA antibodies, which neutralize pathogens within the intestinal lumen while minimizing excessive inflammatory responses that could damage intestinal tissues

II.3.3.2. BALT (Bronchus-Associated Lymphoid Tissue)

Is a component of the mucosa-associated lymphoid tissue (MALT) that contributes to the immune protection of the lower respiratory tract and the alveolar surfaces involved in gas exchange. It participates in the detection and elimination of inhaled pathogens and environmental antigens. BALT consists of lymphoid aggregates located beneath the epithelium of the bronchi and bronchioles, particularly near airway bifurcations.

In humans, BALT is generally poorly developed under normal conditions but may appear or expand in response to respiratory infections or chronic inflammation; this form is known as inducible BALT (iBALT). BALT promotes local immune responses mediated by B and T lymphocytes against inhaled microorganisms before the establishment of systemic immunity.

II.3.3.3. NALT (Nasopharynx-Associated Lymphoid Tissue)

NALT guards the upper respiratory tract and entryways of the digestive system against inhaled or ingested pathogens. In humans, NALT is organized into Waldeyer's tonsillar ring, which includes:

- The palatine tonsils ("the tonsils")
- The nasopharyngeal tonsil (adenoid)

- The lingual tonsil (base of the tongue)
- Tubal tonsils (near the opening of the Eustachian tube)

Unlike GALT, NALT does not rely on M cells within a strict follicle-associated epithelium; instead, it utilizes deep invaginations called tonsillar crypts to capture and channel antigens down into the dense lymphoid follicles beneath. It serves as the primary induction site for intranasal immunity.

II.3.4. SALT (Skin-Associated Lymphoid Tissue)

SALT is the diffuse immune network responsible for protecting the skin, which constitutes the body's first physical barrier against environmental pathogens. SALT is composed of highly mobile immune cells distributed throughout the different layers of the skin. In the epidermis, Langerhans cells function as specialized dendritic cells that capture and process antigens, while intraepidermal lymphocytes provide rapid local immune defense. In the dermis, dermal dendritic cells, macrophages, mast cells, innate lymphoid cells, and resident memory T lymphocytes cooperate to initiate and maintain cutaneous immune responses. SALT plays a critical role in immune surveillance and protection against cutaneous infections.

Remark : A major distinction between MALT and SALT lies in their anatomical localization and mode of organization. MALT protects mucosal surfaces which are continuously exposed to external antigens and microorganisms. In contrast, SALT is specialized for the immune protection of the skin, a dry and keratinized physical barrier. Furthermore, MALT often contains organized lymphoid structures such as Peyer's patches and tonsils, whereas SALT mainly consists of a diffuse and highly mobile network of immune cells distributed throughout the epidermis and dermis.

II.4. Cells of the immune system

All blood cells, including leukocytes, are produced in the bone marrow through a process known as hematopoiesis (Fig.7). Hematopoietic cells derived from the bone marrow differentiate into two main lineages: the myeloid lineage and the lymphoid lineage. Depending on their lineage of origin, each of these cells will have one or more specific functions in the immune response.

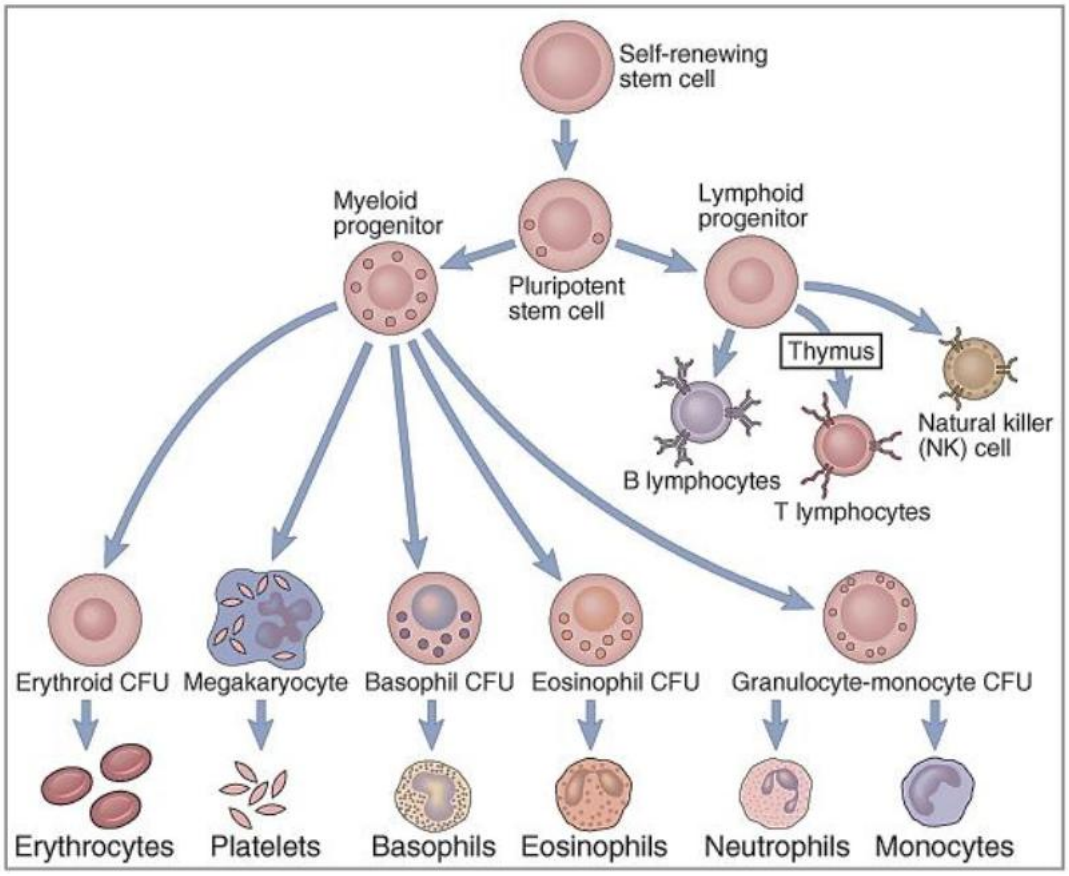


Figure 7 : Hematopoiesis: Differentiation of Hematopoietic Stem Cells into Myeloid and Lymphoid Lineages.

II.4.1. Innate immune system cells

The cells of the innate immune system have several functions that are essential for defense against pathogens. Some cells form physical barriers that impede infections. Several cell types express the various PRRs that recognize PAMPs and DAMPs, which respond by producing inflammatory cytokines to kill microbes or infected cells. These cells include nonmyeloid cells, myeloid cells, and some lymphoid cells.

II.4.1.1. Nonmyeloid cells

Nonmyeloid cells include **epithelial cells, fibroblasts**, etc., that basically form a barrier between the internal and external environment. These cells produce antimicrobial substances that hinder the entry of pathogens. These antimicrobial substances are called antimicrobial peptides (AMPs), and they are essential components of the innate immune response, which contribute to the first line of defense against infections. In humans, AMPs are classified into three main families: defensins (α and β), cathelicidin, and statins. AMPs have a wide spectrum of antimicrobial activity, exerting their functions through electrostatic interactions between

their positive charge and the negative charge that certain pathogens have on their cell wall. AMPs mediate the inflammatory response allowing cytokine release, cell proliferation, angiogenesis, wound healing, and chemotaxis. Currently, their synergistic activity with antibiotics used in the clinic has been demonstrated. Therefore, their study on potent adjuvants in the eradication of bacterial infections continues to be studied.

II.4.1.2. Myeloid lineage cells

Myeloid cells include monocytes, macrophages, dendritic cells (DCs), neutrophils, eosinophils, basophils, mast cells, and platelets. All these cells have specialized functions for defense against invading pathogens

A. Monocytes and Macrophages

Monocytes are related to WBC and have a bean-shaped nucleus. Monocytes are precursors of some macrophages, dermal DCs, and Langerhans DCs.

Macrophages are large mononuclear cells important for both innate and adaptive immunity. They are able to phagocyte large objects like protozoans and infected cells, secrete a lot of active substances like cytokines, fulfill the presentation of antigen/Class II HLA molecules complexes to CD4+ T lymphocytes, and take part in type IV hypersensitivity.

There are two distinct types of macrophages: (1) inflammatory (M1) and (2) anti-inflammatory (M2). Also, tumor-associated macrophages (TAMs) characterized by ambivalent patterns of activity are described.

Monocytes originate from hematopoietic stem cells in the bone marrow. Normally, monocytes make up about 6–8% of white blood cells (WBC). They may circulate in the blood for about 24 h and then differentiate into a type of macrophages, inflammatory macrophages (M1), which are important during pathogen invasions and tissue injury. They are activated by IFNγ and lipopolysaccharide (LPS). M1 macrophages are antigen-presenting cells for CD4+T cell-mediated immune response. Another type of macrophages is known, anti-inflammatory macrophages (M2), which are activated by IL4 and IL13 and secrete IL10 and TGFβ. They play a role in constructive processes such as wound healing and tissue repair but also in tumor growth.

Macrophages were first discovered by 1908 Nobel Laureate E.E. Metchnikoff who also first discovered phagocytosis. In the absence of infections, all tissues of the body contain *tissue-*

resident macrophages or tissue-specialized types of macrophages that are available throughout human life:

- Intestinal lamina propria macrophages (GI tract)
- Peritoneal macrophages (the peritoneal cavity)
- Kupffer's cells (the liver)
- Intraglomerular mesangial cells (the kidney)
- Alveolar macrophages (the lungs)
- Pleural macrophages (the pleural cavity)
- Dermal macrophages (the skin)
- Sinus histiocytes (the lymph nodes)
- Splenic macrophages (marginal zone, red and white pulp of the spleen)
- Osteoclasts (the bone)
- Microglial, perivascular, and meningeal macrophages (the CNS)

They play roles in tissue development, muscle regeneration, clearance of dead cells, and cellular debris (like scavenger cells) and may take part in antigen processing and presentation, immune inflammation, and interaction with tumors. It is the well-known plasticity of macrophages that allows them to change their behavior depending on distinct signals. They may be activated to kill intracellular pathogens (M1) and, alternatively, may limit inflammation and stimulate wound healing (M2).

B. Dendritic cells

Dendritic cells (DCs) are a heterogeneous cell population characterized by outgrowth (dendrite) morphology. *Dendritic cells* (*DCs*) are distinguished from the human mononuclear phagocyte system by their outgrowth or dendrite morphology, high levels of Class I and Class II HLA molecules expression, and properties of superior migration, antigen presentation, and activation of naive lymphocytes. This cell type was discovered by 2011 Nobel Laureate R.M. Steinman, but one subset, Langerhans cells, was revealed in 1868 by P. Langerhans. All DCs are capable of pathogen engulfing, processing, and antigen/Class I or Class II HLA complex presenting to lymphocytes**.** Immature DCs function through the uptake and accumulation of any antigens at the skin and mucosal level, whereas mature DCs take part in antigen presentation and initial stages of adaptive immune responses in the secondary lymphoid organs. They also can initiate the induction of immunological tolerance. Most described DCs express TLR to recognize

"patterns" as well as receptors for cytokines and chemokines and upregulate reactions of the innate immunity (Fig.8).

There are sevral subtypes of DCs:

- **Langerhans cells** originate from the yolk sac and fetal liver's monocytes. These DCs can be identified by the presence of langerin/CD207-containing Birbeck granules as well as the expression of Class I and Class II HLA molecules and CD1ahi. Langerhans cells are located in the epidermis and mucosal epithelium, may uptake antigens, migrate to SALT and MALT-draining lymph nodes, and form **interdigitating cells** to initiate the T-cell-mediated immune responses.
- **Dermal DCs**, or **CD14+DCs**, originate from monocytes and are a transient population of monocyte-derived macrophages. Upon inflammation, CD14+ classical monocytes are also the putative precursors of **inflammatory DCs**, which display an activated and pro-inflammatory phenotype.
- **Type 1 myeloid** (**classical or conventional DCs**) (**mDCs/cDCs**) are myeloid progenitor-derived DCs. They are subdivided into two subtypes: (1) the major DC subset (mDC-1), CD1c+, which mainly expresses Class II HLA molecule and may activate naive CD4+T cells to clonal expansion, differentiation, and immune inflammation, and (2) a rarer DC subset (mDC-2), CD141+, which expresses Class I and Class II HLA molecules, is capable of cross-presentation, and may activate both naive CD4+ and CD8+T cells. Both subtypes possess characteristic long outgrowths called dendrites required for antigen presentation and secrete IL12 and other pro-inflammatory cytokines. Mature mDCs are described as **interdigitating DCs**, which are resident in the secondary lymphoid organs. If any mature DCs are located in the barrier organs, they are **interstitial DCs**. **Thymic DCs** in the thymus are closer to interdigitating DCs (mDCs).
- **Type 2 plasmacytoid DCs** (**pDCs**) are lymphoid progenitor-derived DCs that in the immature state are characterized by spherical morphological features similar to plasma cells. In the mature state, they acquire conventional DC-like morphology with dendrites, express Class II HLA molecules, CD123+ and CD303+, and secrete IFN**α** and IFN**β**. It is probable that pDCs are involved in the B-cell-mediated response and antiviral defense.

Most described DCs express TLR to recognize “patterns” as well as receptors for cytokines and chemokines and upregulate reactions of the innate immunity.

- **Follicular DCs (fDCs)**, a distinct subset, appear to arise from mesenchymal progenitor cells. They are located in lymphoid follicles of the lymph nodes, spleen, and MALT and have dendrites and high expression of complement receptors.

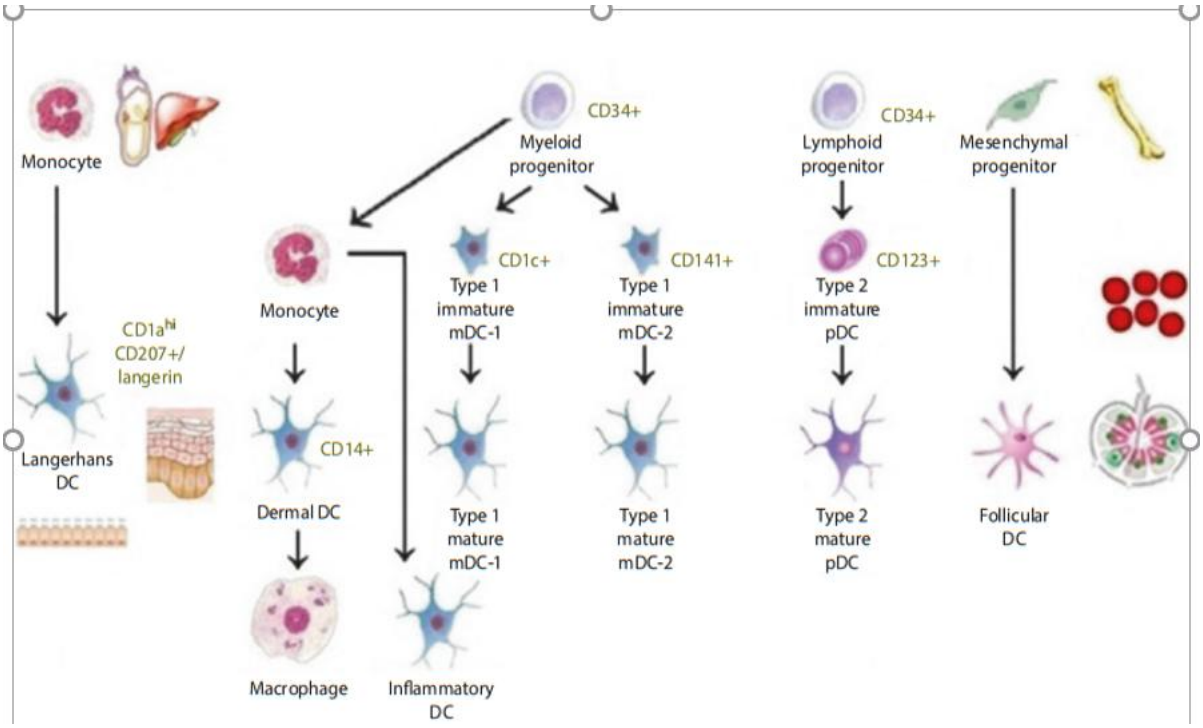


Figure 8 : Ontogeny of dendritic cells

C. **Neutrophils**

In humans, about 100 billion neutrophils enter the bloodstream each day. Neutrophils originate from hematopoietic stem cells in response to both extracellular stimuli and intracellular regulators. They come from the myeloid cell line in the formation of granulocytes. The granulopoyesis that occurs in the bone marrow is initiated when the neutrophils myeloblasts (MB) develop in promyelocytes (PM), characterized by a round nucleus and presence of azurophil granules. Subsequently, they mature into myelocytes with specific granules, maturing to metamyelocytes (MM), cells composed by a nucleus with kidney form. Metamielocitos mature to band cells (CB) and in segmented cells (CS) also known as polymorphonuclear cells (PMNs). Neutrophils play a major role in the resolution of microbial infections. After pathogens break into epithelial barriers, neutrophils are the first cell line of defense for the innate immune response, which are recruited from the bloodstream to the site of infection. Neutrophils cross

the blood vessels and migrate to the infection site with the help of chemotactic factors and cytokines, which are produced as inflammatory signals during the tissue damage caused by the invading pathogens. Neutrophils reach the infection site and initiate the phagocytosis process through recognition of PAMPs by their receptors such as TLRs. Neutrophils exert their antimicrobial actions through the release of reactive oxygen species and cytotoxic components contained in their granules such as AMPs. Likewise, neutrophils using a mechanism called extracellular traps (NETs) composed of DNA fibers, which are formed and released into the extracellular space, are used by the innate immune system to destroy and eliminate pathogens. However, studies have shown that neutrophils NETs are involved in the development of several pathologies. Finally, neutrophils can also regulate the adaptive immune response, as they mediate suppression of T cells proliferation as well as their activity. Neutrophils can also stimulate and activate splenic B lymphocytes.

D. **Eosinophils**

Eosinophils are produced in the bone marrow from pluripotent stem cells, which first differentiate into a precursor for basophils and eosinophils and then differentiate into an eosinophilic lineage. IL-3, IL-5, and GM-CSF are particularly important in regulating the eosinophils development. Of these three cytokines, IL-5 is the most specific for the eosinophilic lineage and is responsible for the selective differentiation and release of eosinophils from the bone marrow into the peripheral circulation. IL-5 plays a critical role in the eosinophils production, as the overproduction and neutralization of this cytokine are associated with a significant increase or decrease in eosinophilia, respectively.

Eosinophils are multifunctional leukocytes involved in the pathogenesis of numerous inflammatory processes, including parasitic helminths infections and allergic diseases. Under basal conditions, most eosinophils traffic into the gastrointestinal tract. Recruitment of gastrointestinal eosinophils is regulated by the constitutive expression of eotaxin-1, a chemokine involved in allergen-induced eosinophil responses.

In response to several stimuli, such as immunoglobulins, cytokines, and complement system, eosinophils are activated and recruited from the circulation to the site of inflammation. The trafficking of eosinophils into inflammatory sites involves various cytokines derived from a Th2 immune response such as IL-4, IL-5, and IL-13, adhesion molecules (e.g., β1, β2, and β7 integrins) and chemokines (e.g., eotaxins). Once at the site of inflammation, eosinophils can modulate the immune response through the secretion of several proinflammatory mediators

such as IL-2, IL-6. IL-8, TGF-α/β, GM-CSF, TNF-α, INF-γ, as well as chemokines and lipid mediators, such as platelet-activating factor (PAF) and leukotriene (LT)-C4, which exert proinflammatory effects as positive regulation of adhesion systems, modulation of cellular trafficking, activation and regulation of vascular permeability, mucus secretion, and smooth muscle constriction. In addition, eosinophils can serve as effector cells, which can induce tissue damage by releasing a diverse of cationic proteins from their cytotoxic granules, major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil peroxidase (EPO), and neurotoxin derived from eosinophils (EDN). These proteins are very important, because they are directly related to the effector functions of eosinophils.

E. Basophils

Basophils are cells derived from the myeloid hematopoietic progenitors in the bone marrow, and they are phenotypically and functionally distinct from other leukocytes, including mast cells, since mast cells reside in tissues while basophils reside in the circulation and can be recruited to the tissues. Basophils have the ability to bridge innate and adaptive immunity, including the capacity to induce and propagate Th2 immune responses. Basophils are important in all allergic diseases, including anaphylaxis, allergic rhinitis, asthma, urticaria, and food allergies. Basophils rapidly release histamine after that immunoglobulin (Ig)-E binds to their receptor FcεRI, causing the clinical symptoms of immediate hypersensitivity, also promoting delayed hypersensitivity reactions.

F. Mast cells

Mast cells are granulated tissue-resident cells from CD34+ hematopoietic progenitor cells. Mast cells circulate as immature cells and migrate to vascularized tissues, where they complete their differentiation. Mast cells represent, together with dendritic cells, the first immune cells that interact with environmental antigens, pathogens, and toxins. Therefore, they can be considered "sentinels" of the innate immune system. Mast cells are activated by danger stimuli, which they react by rapidly releasing a wide range of mediators, both preformed and newly produced. Some of these mediators (e.g., histamine, TNF-α, vascular endothelial growth factor, VEGF) contribute to local vascular permeability and edema at the site of inflammation [105], while chemokines (e.g., IL-8/CXCL8, eotaxin) induce the recruitment of other immune cells [106], such as neutrophils, natural killer (NK) cells, and eosinophils. It is important to note that mast cells may also be involved in the defense against pathogens by different mechanisms, such as phagocytosis, antimicrobial peptide release, or the production of extracellular traps similar to

those described in neutrophils. Mast cells detect these invading pathogens through PRRs, such as TLRs.

Mast cells are known primarily as effector cells for IgE-mediated (Th2-like) responses, an arm of the adaptive immune system against helminths infection, and as primary effector cells in hypersensitivity reactions. In addition to their functions as effector cells, recent evidence suggests that mast cells are capable to modulate both the innate and adaptive immune response, acting as immunomodulatory cells.

G. Platelets

Platelets are cytoplasmic fragments (1 to 4 μm in diameter) produced as a result of fragmentation from megakaryocytes that are cells from bone marrow. Platelets are non-nucleated organelles that have functional characteristics like complete cell, since they possess cytoskeleton, mitochondria, Golgi residues, and endoplasmic reticulum involved in the synthesis of enzymes, storage of calcium ions, as well as storage granules. These storage granules are δ-granules, α-granules, and lysosomal granules, which play an important role in homeostasis, inflammation, wound healing, and cell-matrix interactions. During the inflammatory response, platelets can be activated through their receptors, which act as adhesion molecules that interact with damaged endothelium, other platelets and leukocytes, playing an important role in the coagulation process for repairing the damaged blood vessel and restoring its integrity.

II.4.1.3. Lymphoid lineage cells: Natural killer cells

NK cells are derived from cellular lymphoid progenitors. However, they do not mediate the conventional adaptive immune response because they lack antigen-specific receptors such as T and B lymphocytes. Previously, it was believed that the development of NK cells in humans occurred exclusively in the bone marrow. However, recent studies have shown that NK cells also develop in secondary lymphoid organs. NK cells are important effector lymphoid cells of the innate immune system, since they represent a key element in the rapid recognition and death of both infected or tumorigenic cells, which can cause damage to the integrity of host tissues.

II.4.1.3.1. Ontogeny of Natural Killer (NK) Cells

A. Different stages of NK cell development

In humans, NK cell development occurs mainly in the bone marrow; however, secondary lymphoid organs may also support this process. NK cells develop from a common $CD34^+$ hematopoietic progenitor shared with T and B lymphocytes. This development occurs through six differentiation stages based on the sequential expression of surface molecules: CD34 (adhesion molecule expressed on stem cells), CD117 (c-kit receptor), CD94, CD16 (FcγRIII), and CD56.

- **Stage 1: Pro-NK Cells**

NK cell development begins with $CD34^+$ common lymphoid progenitors (CLPs). These early precursors express CD34, CD10, and CD45RA and retain the potential to differentiate into several lymphoid lineages. Their progression toward the NK lineage depends on acquiring responsiveness to interleukin-15 (IL-15), a process supported by the combined action of Flt3 ligand, IL-3, and IL-7.

- **Stage 2: Pre-NK Cells**

At this stage, NK precursors acquire CD117 (c-kit) expression and begin expressing CD122 (IL-2Rβ) at the mRNA level, marking an important step in NK cell differentiation. These cells become responsive to IL-15, the key cytokine driving NK cell development, survival, and maturation.

- **Stage 3: Immature NK Cells (iNK)**

The immature NK cell stage represents definitive commitment to the NK lineage, accompanied by the loss of the ability to differentiate into T cells or dendritic cells. These cells strongly express IL-1R1 and begin to acquire characteristic NK cell receptors, including NKG2D, CD335 (NKp46), CD337 (NKp30), and CD161 (NK1.1).

- **Stage 4: CD56bright NK Cells**

This stage marks the transition from immature to mature NK cells. CD56bright NK cells express high levels of CD56 and activating receptors such as NKG2D, NKp46, NKp30, and CD161. They display low expression of KIR receptors and CD16 and contain relatively few

cytotoxic granules, making them less cytotoxic but highly efficient producers of immunoregulatory cytokines.

➢ Stage 5: CD56dim NK Cells

As maturation progresses, NK cells become CD56dim, characterized by reduced CD56 expression and the acquisition of CD16 (FcγRIIIA). Many cells also express killer immunoglobulin-like receptors (KIRs/CD158). This population constitutes the majority of circulating NK cells in peripheral blood and exhibits potent cytotoxic activity against infected or transformed cells.

➢ Stage 6: Memory-Like (Adaptive) NK Cells

Following exposure to specific antigenic stimuli, a subset of NK cells can differentiate into adaptive or memory-like NK cells. These cells are characterized by increased expression of NKG2C and display enhanced functional responses upon subsequent stimulation, suggesting features analogous to immunological memory. Fig. 9 summarizes the different stages of NK cell ontogeny and the associated phenotypic modifications occurring during NK cell development.

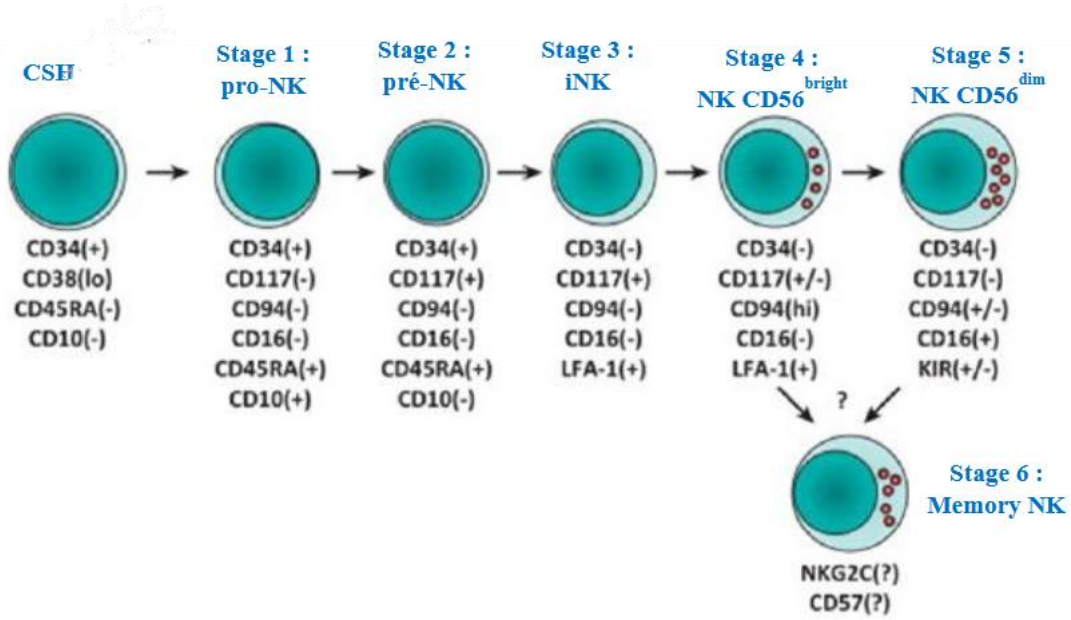


Figure 9 : NK Ontogeny.

B. NK Cell Subpopulations

NK cell are divided into two subpopulations: CD56bright and CD56dim.

- **CD56bright NK Cells**

CD56^bright NK cells represent approximately 10% of circulating NK cells and are mainly found in secondary lymphoid tissues. They exhibit limited cytotoxic activity but are potent producers of cytokines, particularly interferon-γ (IFN-γ)**,** TNF-α**,** and other immunoregulatory molecules. These cells play a crucial role in regulating immune responses and promoting communication between innate and adaptive immunity.

- **CD56dim NK Cells**

CD56^dim NK cells constitute about 90% of peripheral blood NK cells and are highly cytotoxic. They express elevated levels of CD16 (FcγRIII**)**, enabling them to mediate antibody-dependent cellular cytotoxicity (ADCC)**,** CD56^dim NK cells efficiently eliminate virus-infected and tumor cells, making them the primary effector subset responsible for NK cell-mediated cytotoxicity (Fig. 10).

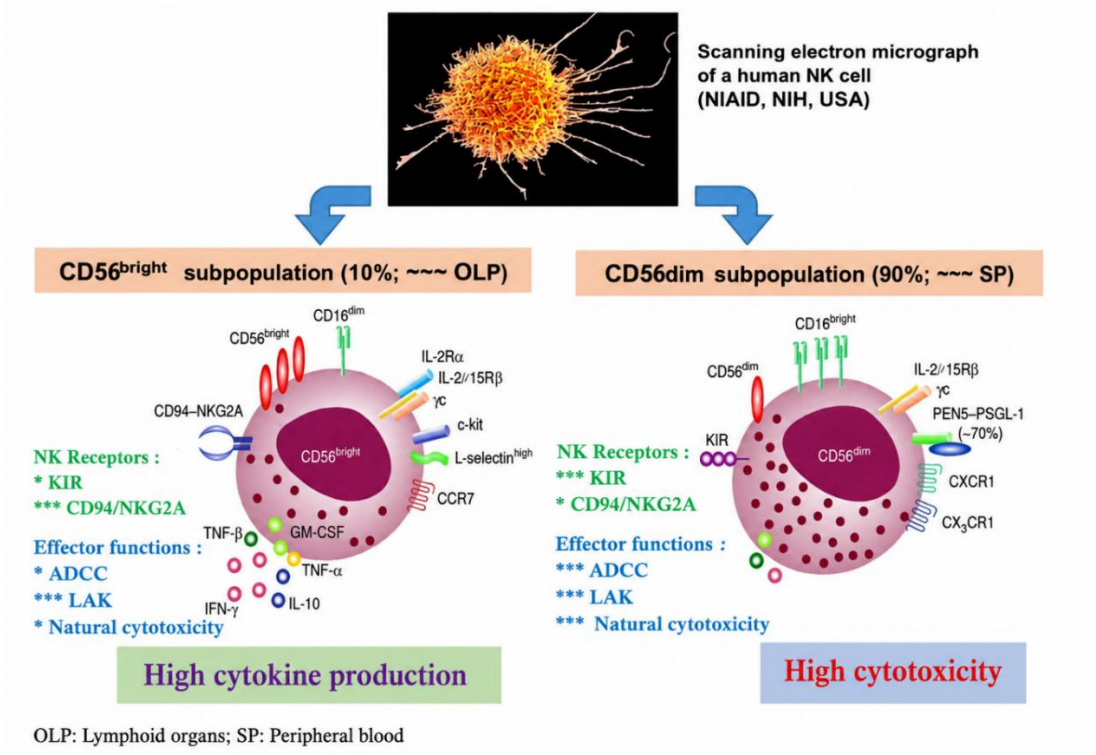


Figure 10 : Human NK Cell Subpopulations: Phenotypic and Functional Differences Between CD56bright and CD56dim Cells"

C.NK cell function

NK cells are widely distributed throughout the body. They actively patrol in large numbers in

the bloodstream and rapidly migrate to inflammatory sites. Overall, their functions can be summarized as follows:

➢ Elimination of infected cells (bacteria, viruses, parasites) during the early phase of infection.

➢ Immune surveillance and destruction of tumor cells that escape cytotoxic T lymphocytes due to the absence or reduced expression of HLA class I molecules recognized by these T cells.

➢ Regulation of specific immune responses through the production of multiple cytokines such as IFN-γ (activation of macrophages) and TNF-β (induction of apoptotic pathways in tumor cells).

D. Membrane Markers Expressed by NK Cells and Their Functions

Natural Killer (NK) cells express a variety of surface receptors that regulate their activation, cytotoxic activity, adhesion, and responsiveness to cytokines.

- **Inhibitory Receptors**

KIR (Killer-cell Immunoglobulin-like Receptors), LIR (Leukocyte Immunoglobulin-like Receptors), and CD94/NKG2A are inhibitory receptors that recognize MHC class I molecules expressed on healthy cells and prevent inappropriate NK cell activation, thereby maintaining self-tolerance and protecting normal tissues from NK cell-mediated lysis

- **Activating Receptors**

NKp30, NKp44, NKp46, and NKG2D are activating receptors that recognize stress-induced ligands expressed by infected, transformed, or damaged cells. Their engagement triggers NK cell activation, leading to cytotoxic activity and the secretion of pro-inflammatory cytokines

- **Fc Receptor**

CD16 (FcγRIII) is an Fc receptor for IgG antibodies that enables NK cells to mediate antibody-dependent cellular cytotoxicity (ADCC), allowing the selective elimination of antibody-coated target cells.

- **Adhesion Molecules**

LFA-1 (CD11a/CD18), CD18/CD11, and CD56 (NCAM) are adhesion molecules involved in NK cell migration, target-cell recognition, and the formation of the immunological synapse, facilitating efficient interactions between NK cells and their targets..

- **Cytokine Receptors**

IL-2R (Interleukin-2 Receptor), IL-12R (Interleukin-12 Receptor), and IL-15R (Interleukin-15 Receptor) are cytokine receptors that enable NK cells to respond to immune stimulation and play essential roles in their development, survival, proliferation, maturation, activation, and cytokine secretion. These receptors ensure the maintenance and functional competence of NK cells during innate immune responses.

NK cells identify target cells through complex combinations of signals from the activation or inhibition of receptors, which interact with ligands that are expressed on the surface of stressed or normal cells, respectively. The decision to eliminate or not eliminate these cells depends on the result of the balance between positive (activation) and negative (inhibition) signals. Also, the activation of NK cells is regulated through cooperation with other immune cells, including DCs, which allows that NK cells to acquire potent cytotoxic activity, the ability to produce cytokines such as IFN-γ and contribute to the adaptive immune response by triggering the T cell–mediated response.

II.4.1.4. Receptors of innate immune system cells

Pattern recognition receptors (PRRs) are molecules expressed by cells of the innate immune system that detect conserved molecular structures, known as patterns, and initiate innate immune responses such as inflammation. In addition to their role in innate immunity, PRRs contribute to the activation and regulation of adaptive immune responses.

Structurally, PRRs typically contain one or more C-terminal recognition or regulatory domains responsible for sensing molecular patterns, as well as N-terminal effector domains that interact with intracellular signaling molecules. Some PRRs also possess a central domain involved in signal transduction. To date, five major families of PRRs have been identified: Toll-like receptors (TLRs), C-type lectin receptors (CLRs), NOD-like receptors (NLRs), RIG-I-like receptors (RLRs), and AIM2-like receptors (ALRs) (Fig. 11).

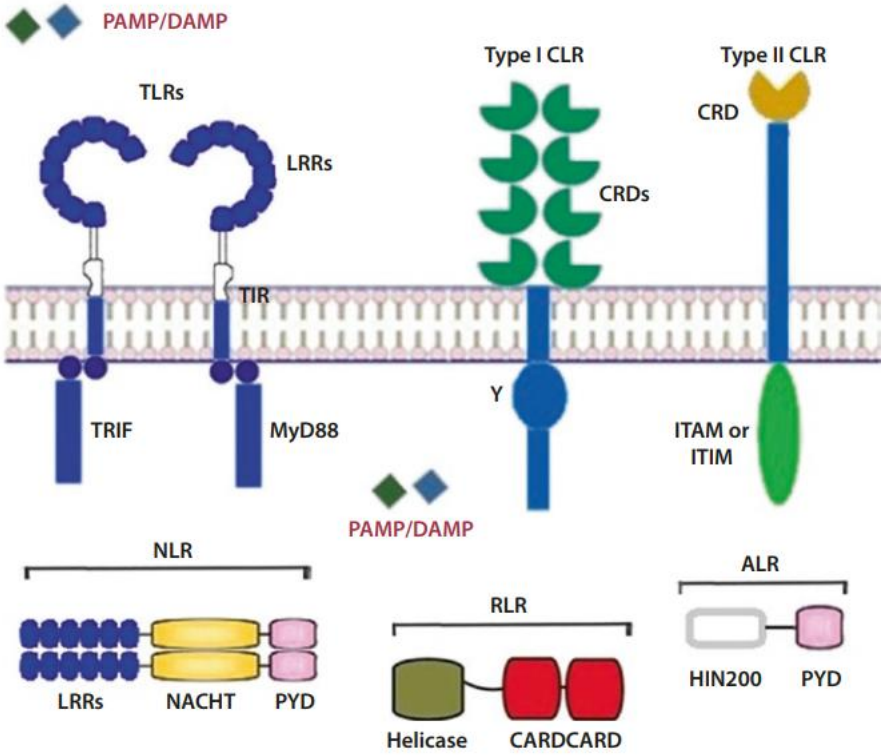


Figure 11 : Pattern recognition receptors (PRRs)

A. Toll-Like Receptors (TLRs)

Toll-like receptors (TLRs) are transmembrane PRRs expressed on the plasma membrane of various immune and non-immune cells, including macrophages, neutrophils, dendritic cells, lymphocytes, epithelial cells, platelets, splenocytes, and cells within atherosclerotic plaques. They are also found within intracellular endosomes. The term *Toll* originates from *Drosophila melanogaster*, in which these receptors were first identified.

TLRs contain extracellular leucine-rich repeat (LRR) domains responsible for ligand recognition and a cytoplasmic Toll/Interleukin-1 receptor (TIR) domain, which interacts with adaptor proteins such as MyD88 and TRIF.

Upon recognition of pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs), TLRs activate MyD88-dependent and TRIF-dependent signaling pathways. These pathways ultimately induce inflammatory responses through inflammasome activation and pyroptosis. TLRs also provide a crucial link between innate and adaptive immunity, as pathogens recognized by TLRs can be internalized, processed, and presented as antigens to lymphocytes. Table 2 shows the different types of TLRs and their ligands.

Table 2 : TLRs and their ligands

TLR	Ligands (PAMP, DAMP)
TLR1	Triacyl lipopeptides of *Mycobacterium tuberculosis*, DAMP
TLR2	Diacyl and triacyl lipopeptides of Gram-positive bacteria, lipoteichoic acid, peptido-glycan, yeast zymozan, DAMP
TLR3	Viral dsRNA
TLR4	Lipopolysaccharide (endotoxin, LPS) of Gram-negative bacteria, DAMP
TLR5	Flagellin of bacterial flagella
TLR6	Diacyl lipopeptides of Gram-positive bacteria
TLR7	Viral ssRNA
TLR8	Viral ssRNA
TLR9	Unmethylated CpG nucleotides of bacterial and viral DNA
TLR10	Unknown

B. C-Type Lectin Receptors (CLRs)

C-type lectin receptors (CLRs) are membrane-bound PRRs primarily involved in the recognition of fungi and in endocytic processes. Similar to TLRs, they recognize both molecular patterns and antigens, thereby serving as an important bridge between innate and adaptive immunity. Based on their structural organization, CLRs are classified into **Type I** and **Type II** receptors.

- **Type I CLRs**

Type I CLRs possess multiple **carbohydrate recognition domains (CRDs)** linked to cytoplasmic tails containing a tyrosine (Y) signaling motif. These receptors participate in carbohydrate recognition and intracellular signaling.

- **Type II CLRs**

Type II CLRs contain a single CRD and cytoplasmic domains bearing either ITIM (immunoreceptor tyrosine-based inhibitory motif)**,** ITAM (immunoreceptor tyrosine-based activating motif)**,** or other signaling sequences. They represent the predominant CLR family and are extensively studied due to their involvement in antifungal, antimycobacterial, and antitumor immunity, as well as allergy and tissue homeostasis.

For example, **Dectin-1** has significantly advanced the understanding of host-fungal interactions, whereas **Dectin-2** has been shown to recognize allergens such as house dust mite allergens and allergen-associated molecular patterns (AAMPs).

- **Soluble CLRs**

Some CLRs exist in soluble form and are associated with acute-phase proteins involved in inflammatory responses.

C. NOD-Like Receptors (NLRs)

NOD-like receptors (NLRs) are cytoplasmic PRRs involved in the detection of intracellular microbial components. The term *NOD* refers to the nucleotide-binding oligomerization domain**,** which binds nucleoside triphosphates. Among the best-characterized NLRs:

- **NOD1** recognizes peptidoglycan components from Gram-negative bacteria.
- **NOD2** recognizes intracellular muramyl dipeptide and peptidoglycan from both Gram-positive and Gram-negative bacteria.

Structurally, NLRs contain:

- Leucine-rich repeat (LRR) domains,
- A central **NACHT domain**,
- An effector domain, frequently a **PYD (pyrin) domain**.

The NACHT domain possesses NTPase activity and is essential for receptor oligomerization. The PYD domain contributes to the assembly of the **inflammasome**, a multiprotein complex responsible for activating caspase-1, processing the pro-inflammatory cytokines **IL-1β** and **IL-18**, inducing pyroptosis, and initiating inflammatory responses.

D. RIG-I-Like Receptors (RLRs)

RIG-I-like receptors (RLRs) are cytoplasmic sensors specialized in antiviral immunity. They contain an RNA helicase domain and CARD (caspase activation and recruitment) domains**.** Their principal ligands are viral double-stranded RNA (dsRNA) molecules. Following activation, RLRs trigger signaling pathways that lead to the production of type I interferons, which are essential for antiviral defense.

E. AIM2-Like Receptors (ALRs)

AIM2-like receptors (ALRs) are found in both the cytoplasm and nucleus. They contain one or more HIN200 domains and a PYD domain. The term *HIN200* refers to proteins characterized by hematopoietic expression, interferon inducibility, nuclear localization, and a conserved 200-amino-acid domain.

ALRs detect intracellular double-stranded DNA (dsDNA) derived from bacteria or viruses. Upon activation, AIM2 forms the AIM2 inflammasome, resulting in the production of pro-inflammatory cytokines and type I interferons.

- **Clinical Significance of PRRs**

Pattern recognition receptors play a central role in host defense by initiating inflammatory responses against pathogens. However, excessive or uncontrolled PRR activation may lead to tissue damage and excessive production of pro-inflammatory cytokines, potentially resulting in severe conditions such as toxic shock syndrome. Furthermore, dysregulation of PRR signaling pathways has been implicated in the pathogenesis of several autoinflammatory diseases, highlighting their clinical and therapeutic importance.

II.4.2. Adaptative immune system cells

Lymphoid cells (B cells, T cells, and NK cells) originate in the bone marrow or, during fetal development, in the fetal liver. They differentiate from common lymphoid progenitors (CLPs)**,** which are derived from primitive hematopoietic stem cells (HSCs) and are characterized by the expression of the $CD34^+$ surface marker**.**

B and T lymphocytes are part of the adaptive immune system. After antigen recognition, B lymphocytes differentiate into antibody-secreting plasma cells. Antibodies eliminate or neutralize pathogens through neutralization, phagocytosis, and complement activation.

Helper T lymphocytes secrete cytokines that stimulate various mechanisms of immunity and inflammation. Cytotoxic T lymphocytes kill infected cells.

Natural killer (NK) cells originate from lymphoid stem cells, like B and T lymphocytes. However, unlike B and T cells, NK cells belong to the innate immune system and eliminate infected or transformed cells without prior antigen sensitization (Fig.12).

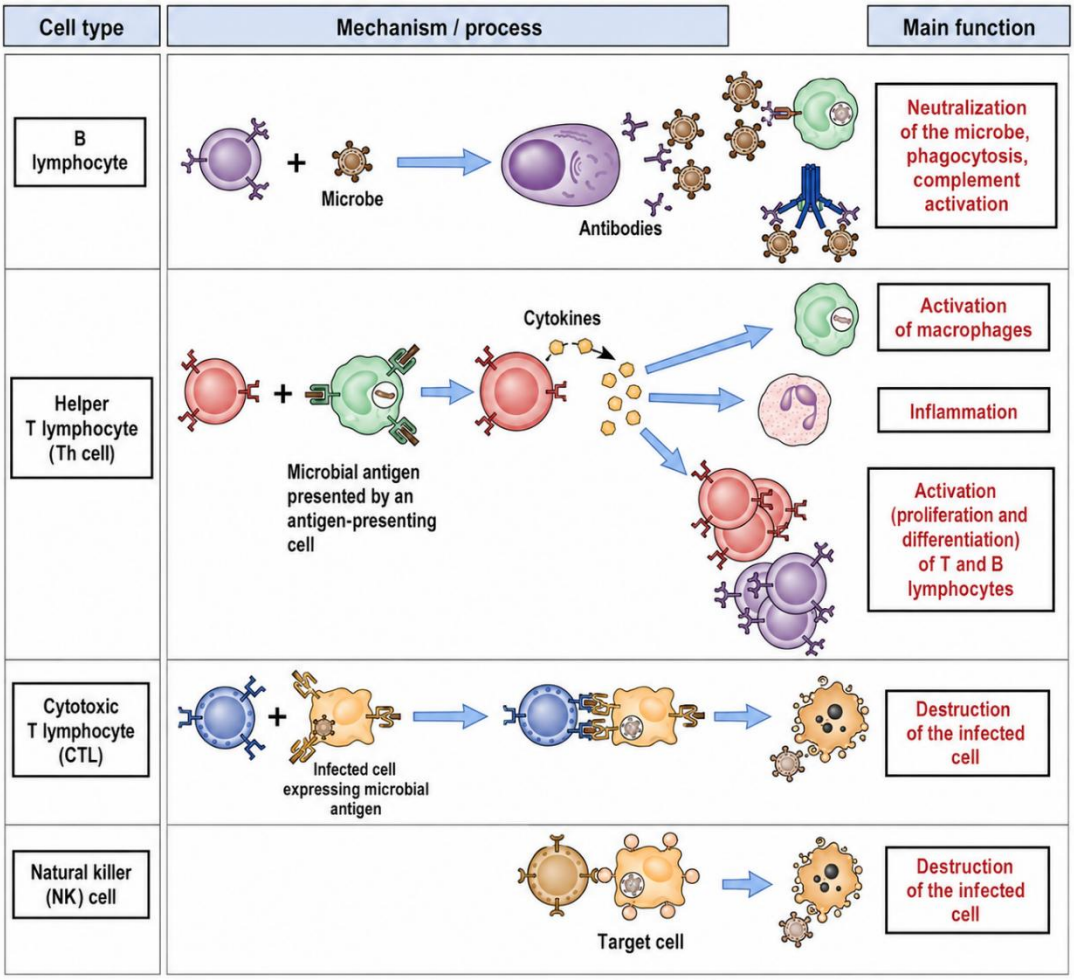


Figure 12 : Lymphoid Lineage Cells and Their Roles in the Immune Response.

II.4.2.1. T lymphocytes (T cells)

The letter "T" stands for the thymus, the organ in which T lymphocytes mature in humans. T cells are responsible for specific cell-mediated immunity, which aims to eliminate pathogenic cells, including cells infected by microorganisms as well as cancerous cells. T cells are oval, nucleated cells with a diameter of approximately 7 μm and a high nucleus-to-cytoplasm ratio**.** They constitute about 70–80% of circulating lymphocytes**.**

They are classified into several subsets according to the type of T-cell receptor (TCR) they express. The TCR is always associated with the CD3 complex**,** which is essential for signal transduction. T-cell subsets are further distinguished by specific surface markers, mainly CD4 and CD8.

- **$CD4^+$ T lymphocytes (Helper T cells)** coordinate immune responses through the secretion of cytokines.

- **$CD8^+$ T lymphocytes (Cytotoxic T cells)** directly destroy infected or abnormal cells.
- Other specialized subsets include **regulatory T cells (Tregs)** and **natural killer T (NKT) cells**, which contribute to immune regulation and defense

A. LT Lymphopoiesis

Some common lymphoid progenitors (CLPs)**,** derived from hematopoietic stem cells (HSCs) in the bone marrow **(**From the 12th week of fetal life), migrate to the thymus under the influence of signaling pathways such as Notch and its ligands. There, they differentiate into T lymphocytes (T cells). After interacting with cortical thymic epithelial cells (cTECs), the developing thymocytes begin expressing specific surface molecules, including CD25 and CD44, and progress to the double-negative (DN) stage, characterized by the absence of CD3, CD4, CD8, and T-cell receptor (TCR) expression. This developmental process is driven by Notch signaling and supported by interleukin-7 (IL-7)**.**

The differentiation and maturation of double-negative thymocytes occur through several sequential stages.

- ***Step 1: Lineage Commitment***

At the double-negative stage, pro-T cells express the recombination enzymes RAG1 and RAG2, which initiate the rearrangement of genes encoding either the β or γ chains of the TCR. Depending on the successful gene rearrangement, thymocytes develop either a TCRγδ (TCR-1), representing approximately 20% of T cells, or a TCRαβ (TCR-2)**,** representing approximately 80% of T cells. The latter population includes about 20% natural killer T (NKT) cells and 60% conventional $CD4^+$ and $CD8^+$ T lymphocytes**.**

Cells that fail to successfully rearrange these genes undergo **apoptosis**, whereas successful cells produce a functional β chain and lose CD25 expression. The β chain associates with a surrogate α chain to form the pre-TCR, which is expressed together with the CD3 complex**.** Signaling through the CD3/pre-TCR complex induces intense proliferation and the expression of both CD4 and CD8**,** generating double-positive (DP) thymocytes ($CD3^+CD4^+CD8^+$)**.**

These double-positive thymocytes subsequently rearrange the genes encoding the **α chain** of the TCR. The newly synthesized α chain pairs with the β chain to form a complete and functional TCRαβ**,** which is then expressed on the cell surface.

- *Step 2: Positive Selection and Commitment to the CD4 or CD8 Lineage*

Double-positive thymocytes are capable of interacting through their TCRs with the individual's major histocompatibility complex (MHC) molecules. They undergo positive selection in the deep thymic cortex**,** where cortical thymic epithelial cells present self-MHC class I and class II molecules**.** During this process, two outcomes are possible:

- If the interaction between the TCR and self-MHC is absent or too weak, the thymocyte fails to receive survival signals and dies by apoptosis**.**
- If the interaction is moderate**,** the thymocyte receives survival signals and is positively selected. Positive selection is followed by lineage commitment:

- Thymocytes whose TCR recognizes MHC class I increase CD8 expression and lose CD4, becoming immature $CD8^+$ T lymphocytes.
- Thymocytes whose TCR recognizes MHC class II increase CD4 expression and lose CD8, becoming immature $CD4^+$ T lymphocytes.

A small subset of thymocytes whose TCR recognizes **CD1d molecules** instead of conventional MHC molecules follows a distinct developmental pathway and differentiates into **NKT cells**.

- ***Step 3: Negative Selection in the Thymic Medulla***

The immature single-positive thymocytes migrate to the thymic medulla, where they undergo **negative selection**. The purpose of this process is to establish **self-tolerance** by eliminating autoreactive T cells.

Negative selection is based on the strength of interaction between the thymocyte TCR and **self-peptides presented by MHC class I or II molecules** on **dendritic cells and macrophages**.

- Thymocytes exhibiting a **high affinity** for self-peptide–MHC complexes receive apoptotic signals and are eliminated.
- Thymocytes that do not react strongly to self-antigens receive survival signals and mature into **naïve $CD4^+$ or $CD8^+$ T lymphocytes**.
- Some **$CD4^+$ thymocytes** displaying an intermediate affinity for self-antigens are selected to become **regulatory T cells (Tregs)**, which play a crucial role in maintaining immune tolerance (Fig.13).

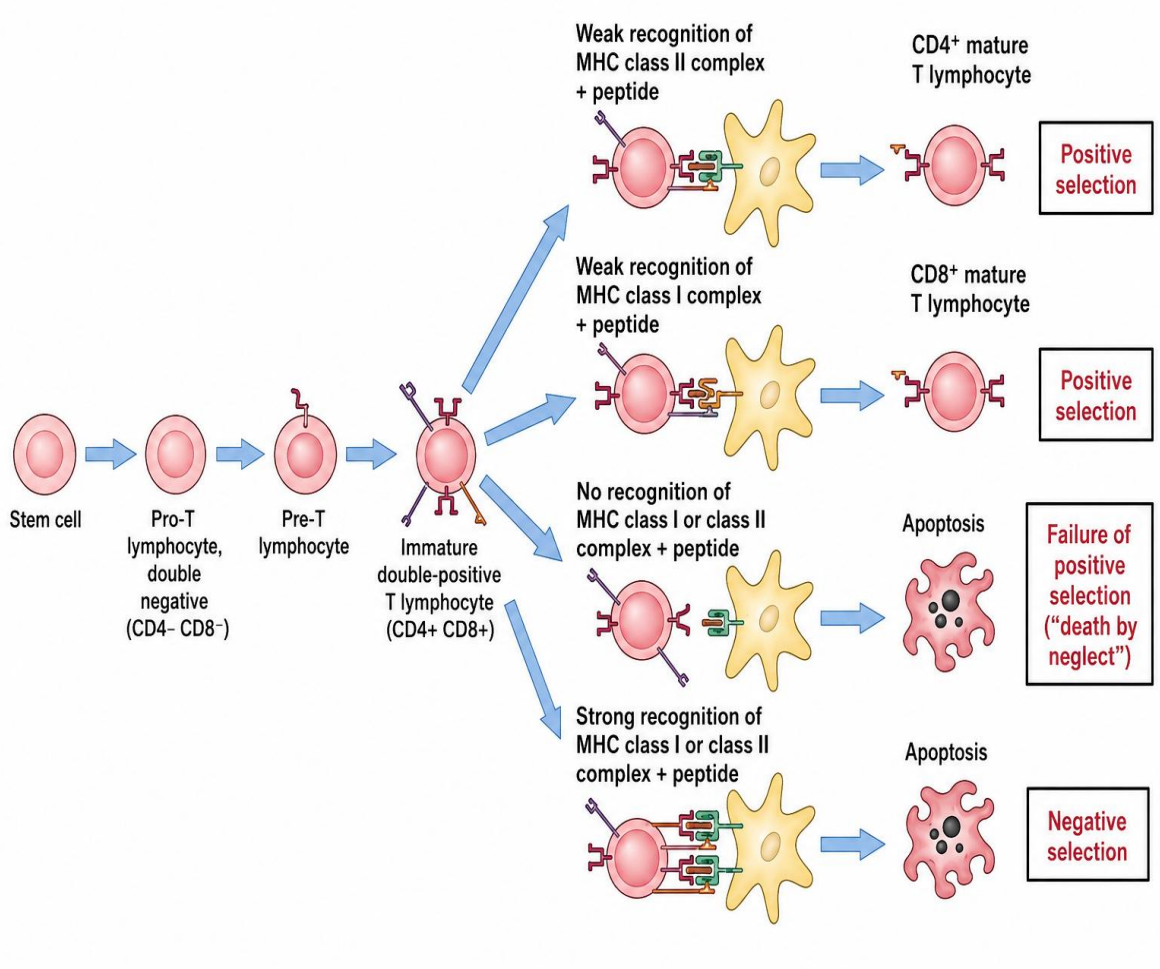


Figure 13 : Stages of T-Lymphocyte Development and Selection in the Thymus

Developing thymocytes progress from double-negative ($CD4^-CD8^-$) to double-positive ($CD4^+CD8^+$) stages. Cells that weakly recognize self-MHC molecules undergo positive selection and differentiate into mature $CD4^+$ or $CD8^+$ T cells. Thymocytes that fail to recognize self-MHC die by neglect, whereas those that strongly recognize self-antigens presented by self-MHC undergo negative selection and are eliminated by apoptosis, ensuring central tolerance and preventing autoimmunity.

Following successful positive and negative selection, mature naïve T lymphocytes leave the thymus and circulate through secondary lymphoid organs, where they can be activated upon encountering their specific antigen (Fig.14).

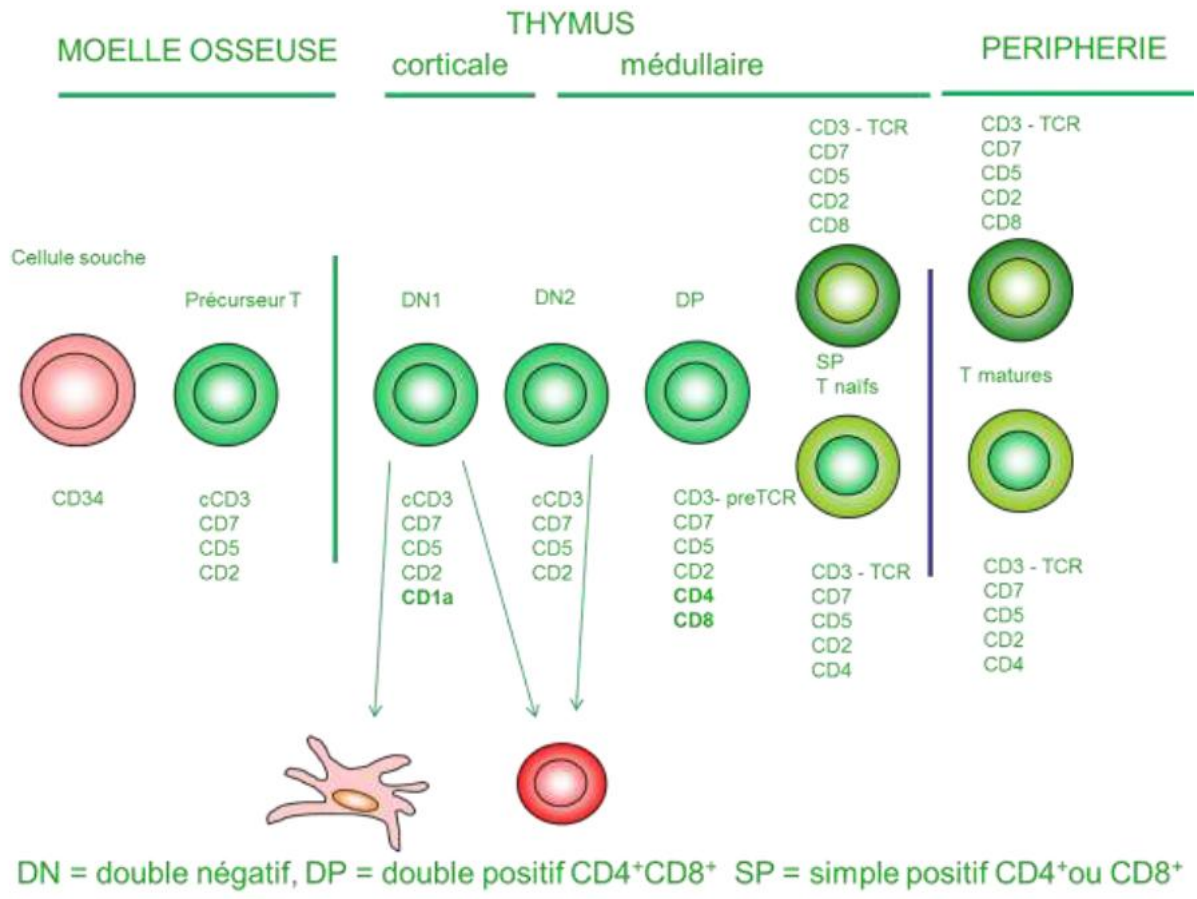


Figure 14 : T Lymphocyte Ontogeny.

B. T Lymphocyte Receptors

The antigen receptor of T cells, known as the T-cell receptor (TCR)**,** is a glycoprotein composed of either two α and β chains or two γ and δ chains, linked together by a disulfide bond. These chains contain a variable N-terminal (NH_2-terminal) region responsible for specific antigen recognition and binding. More than 95% of mature T cells express the αβ TCR on their surface, whereas the remaining T cells express the γδ TCR (Fig. 15)**.**

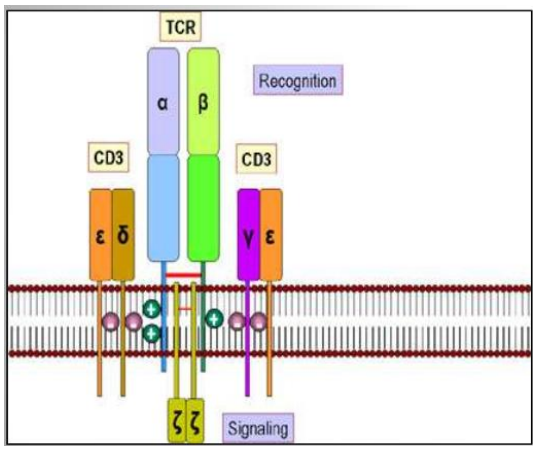


Figure 15 : Structure of the T-Cell Receptor (TCR) Complex

II.4.2.2. Characteristics and Functions of B Lymphocytes

The letter "B" originates from the Bursa of Fabricius, a primary lymphoid organ in birds where B cells are generated and mature. In contrast, in mammals and other vertebrates, including humans, B-cell production and maturation occur in the bone marrow**.**

B lymphocytes **(**B cells) are the principal mediators of adaptive humoral immunity, whose ultimate effector molecules are antibodies, also known as immunoglobulins (Ig)**.** Antibodies protect the host by specifically binding to foreign and pathogenic substances, thereby neutralizing them and facilitating their elimination by phagocytic cells and other immune mechanisms.

B lymphocytes account for approximately 5–15% of circulating lymphocytes, corresponding to about 200–400 cells/mm³ of blood**.** They are characterized by the expression of a highly specific surface receptor known as the B-cell receptor (BCR)**,** which enables the recognition of specific antigens.

Upon encountering their cognate antigen, B cells are generally activated and undergo clonal expansion, followed by differentiation into:

- **Plasma cells**, specialized in the production and secretion of antibodies.
- **Memory B cells**, which provide long-term immunological memory and ensure a faster and more effective response upon subsequent exposure to the same antigen.

A. Ontogeny of B Lymphocytes

From the 10th week of fetal life, B-cell precursors originating from the lymphoid stem cell start out to differentiate into B cells. Early B lymphopoiesis in humans is first induced in the fetus's liver and then within the bone marrow and MALT microenvironment

The development and maturation of **B lymphocytes (B cells)** occur through several sequential stages. Some of these stages take place in the **bone marrow** and are **antigen-independent** (*lymphopoiesis*), whereas others occur in peripheral lymphoid organs and are antigen-dependent (*immunopoiesis*).

Under the influence of signals provided by stromal cells, including soluble cytokines and direct cell-to-cell interactions, common lymphoid progenitor cells (CLPs)**,** characterized by the expression of the CD34 surface marker**,** commit to the B-cell lineage and remain within the bone marrow. The major stages of B-cell development are described below.

- **Pre-Pro-B Cell Stage**

Pre-pro-B cells represent the earliest and most immature B-cell precursors. At this stage, the cells are not yet fully committed to the B-cell lineage and have not initiated immunoglobulin gene rearrangement. They express very low levels of the recombination-activating genes **RAG1** and **RAG2**. However, expression of the gene encoding **Igα (CD79α)** can already be detected.

- **Pro-B Cell Stage**

During the pro-B cell stage, rearrangement of the V, D, and J gene segments of the immunoglobulin heavy-chain locus (located on chromosome **14**) takes place. Recombination occurs first between the D and J segments**,** followed by the joining of a V segment to the newly formed DJ complex**.** If the rearrangement is productive and generates a functional heavy-chain gene, development proceeds; otherwise, the cell undergoes apoptosis (Fig.16).

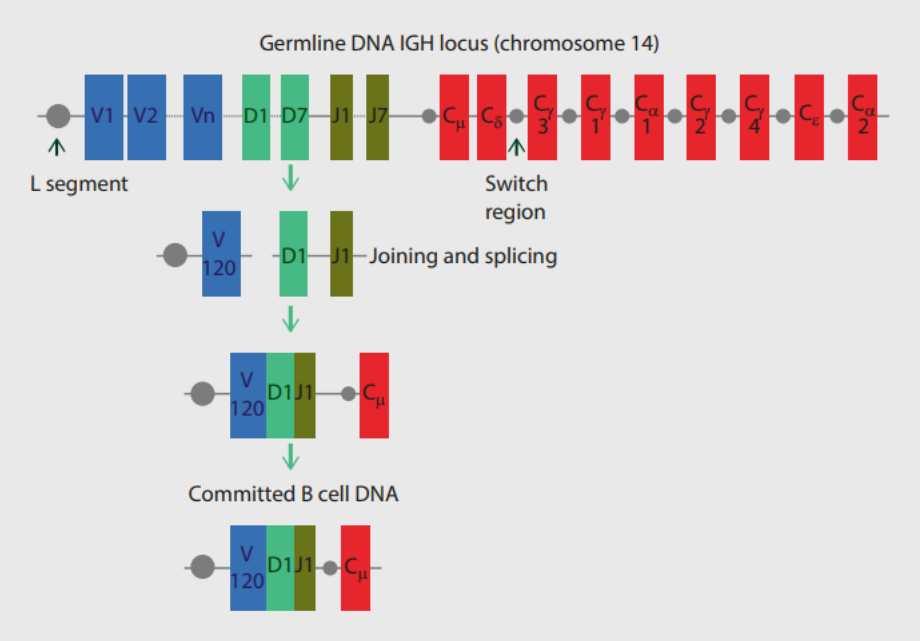


Figure 16 : Recombinations in immunoglobulin H chains genes (IGH locus)

- **Large Pre-B Cell Stage**

Once heavy-chain rearrangement has been successfully completed, a **μ heavy chain** is synthesized. This chain associates with a **surrogate light chain (SLC)**, composed of the proteins **VpreB** and **λ5**, as well as the signaling molecules **Igα** and **Igβ**, forming the **pre-B-cell receptor (pre-BCR)**.

Following expression of the pre-BCR at the cell surface, its functionality is evaluated through interactions with self-antigens expressed by stromal cells, a process known as positive selection**.** Two possible outcomes may occur:

- If the avidity of the pre-BCR for self-antigens is either too low or too high, the cell fails to receive survival signals and dies.
- If the avidity is moderate, the cell survives and progresses to the next developmental stage.

- **Small Pre-B Cell Stage**

Expression of the pre-BCR temporarily suppresses RAG gene expression**.** Subsequently, RAG1 and RAG2 are re-expressed to initiate rearrangement of the VL–JL gene segments encoding the immunoglobulin light chains.

Rearrangement first occurs at the Igκ locus on chromosome 2. If no productive rearrangement is achieved, recombination continues at the Igλ locus on chromosome 22.

- **Immature B Cell Stage**

Successful rearrangement of the light-chain genes leads to the synthesis of a conventional light chain, which replaces the surrogate light chain. This results in the formation of a complete surface **IgM molecule**, serving as the **B-cell receptor (BCR)** and conferring antigen specificity.

At this stage, immature B cells undergo a second checkpoint known as **negative selection**, which establishes **self-tolerance** by eliminating autoreactive B cells.

Stromal cells present self-peptides associated with **MHC molecules** to immature B cells, leading to several possible outcomes:

- *High avidity for self-antigen*

Immature B cells undergo **apoptosis** after attempts at **BCR editing**, a process involving additional rearrangements of light-chain genes to generate a new receptor specificity.

- *Intermediate avidity for self-antigen*

Cells become permanently unresponsive to antigen stimulation, a state known as **anergy**. Although these B cells migrate to secondary lymphoid organs, they eventually die because they cannot be activated.

- *Low avidity for self-antigen*

Cells are positively selected and develop into follicular B cells, which primarily respond to T-dependent peptide antigens**.** Their activation can generate long-lasting serological memory**.**

- *Absence of self-reactivity*

Cells are selected to become marginal zone B cells of the spleen. These cells respond mainly to T-independent non-peptide antigens, such as microbial polysaccharides and lipopolysaccharides. Their activation occurs through extensive BCR cross-linking or through

combined BCR and Toll-like receptor (TLR) signaling and generally does not produce long-term serological memory.

- **Mature Naïve B Cell Stage**

Immature B cells that successfully pass central tolerance checkpoints leave the bone marrow and migrate to secondary lymphoid organs, particularly the spleen, where they undergo additional peripheral selection processes.

Cells surviving this peripheral selection mature into naïve mature B cells, characterized by the co-expression of surface IgM and IgD.

These cells differentiate into either:

- Conventional follicular B lymphocytes**,** which participate in T-cell-dependent humoral immune responses**, or**
- Marginal zone B lymphocytes**,** which mediate T-cell-independent humoral responses**.**

Mature naïve B cells continuously recirculate among secondary lymphoid organs in search of their specific antigen. Upon encountering their cognate antigen, they enter the second phase of development, which is antigen-dependent and leads to B-cell activation, proliferation, and differentiation into effector and memory cells.

- **Note**

Stromal cells regulate the growth, maturation, and survival of B-cell precursors through two principal mechanisms**:**

1. **Secretion of soluble factors**, including: Interleukin-7 (IL-7), Stem Cell Factor (SCF), Stromal Cell-Derived Factor-1 (SDF-1/CXCL12).
2. **Direct cell-to-cell interactions** with developing B cells, mediated by:
    - Adhesion molecules, particularly VLA-4 expressed on pro-B cells and its ligand VCAM-1 expressed on stromal cells.
    - The interaction between the c-Kit receptor (CD117) on pro-B cells and Stem Cell Factor (SCF) presented by stromal cells.

These molecular interactions provide essential signals that support B-cell lineage commitment, proliferation, differentiation, survival, and retention within the bone marrow microenvironment (Fig.17).

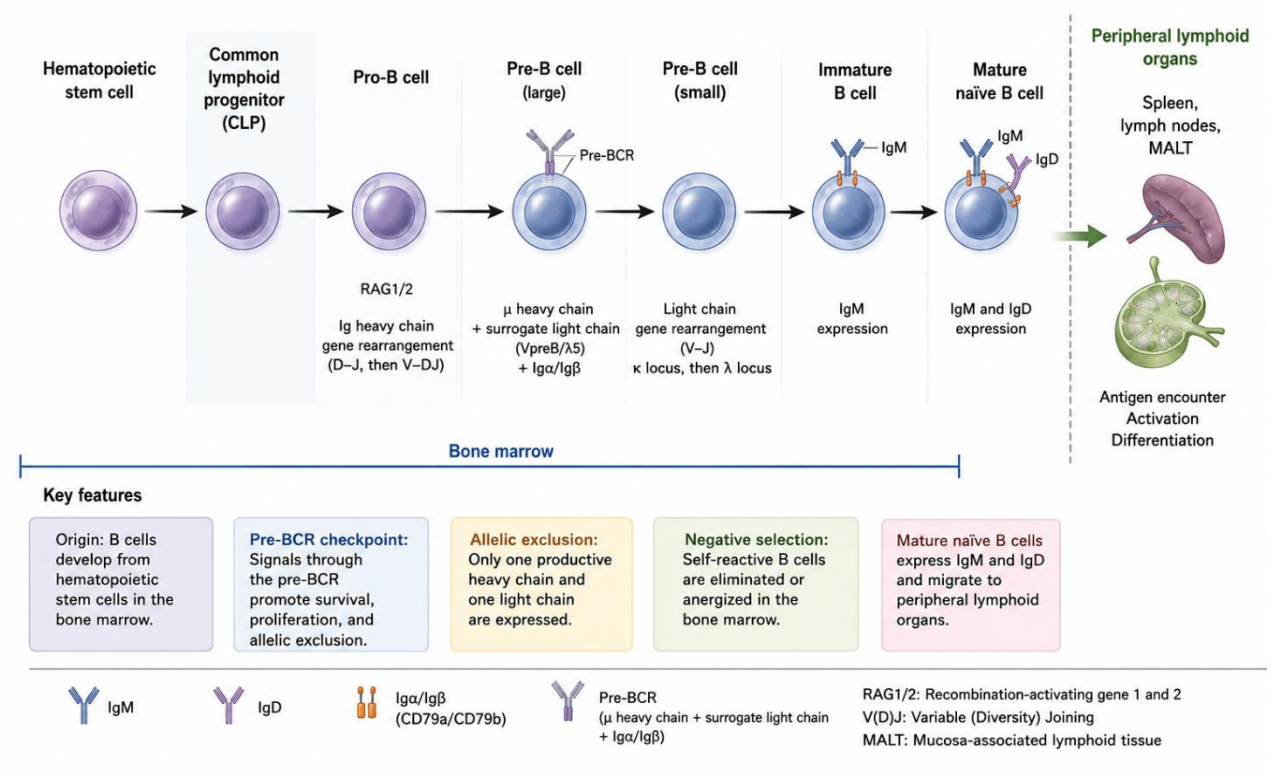


Figure 17 : LB cell developpement in bone morrow

This figure summarizes the sequential stages of B-cell development in the bone marrow, from hematopoietic stem cells to mature naïve B lymphocytes. During this process, progenitor cells undergo immunoglobulin gene rearrangement, expression of the pre-B-cell receptor, negative selection of autoreactive cells, and maturation into immunocompetent B cells ready to migrate to peripheral lymphoid organs.

C. B Lymphocyte Receptors

B lymphocytes are characterized by the presence of surface immunoglobulins (Igs)**,** which are responsible for antigen recognition and constitute the B-cell receptor (BCR)**.** Naïve B lymphocytes express monomeric **IgM** and **IgD** on their surface. Approximately 10^5 surface immunoglobulin molecules are present on a single B lymphocyte, all recognizing the same antigenic determinant. Immunoglobulins are protein heterodimers composed of two identical eavy chains H (for heavy), and two identical light chains L (for light). Each chain is composed of a constant region C and a variable region V. The spatial association of the variable domains

of the heavy and light chains defines the site of attachment to the antigen or paratope. Binding of an antigen to the BCR triggers B-cell activation and induces the synthesis of antibodies that possess the same antigen specificity (the same paratope, i.e., the same variable region) as the surface immunoglobulin (Fig. 18).

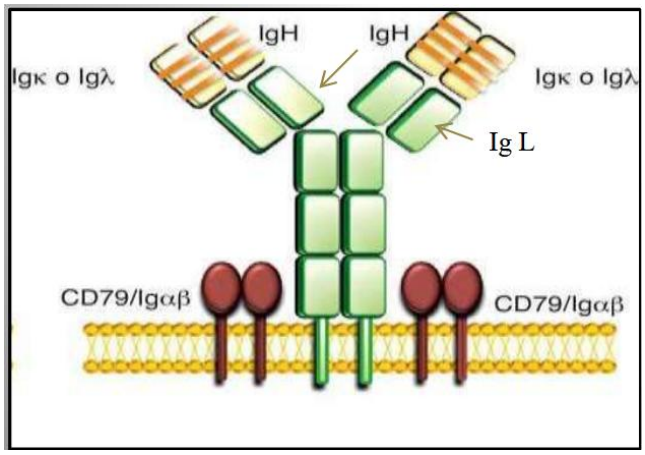


Figure 18 : Structure of the T-Cell Receptor (TCR) Complex

II.4.1.3. Cells at the interface between innate and acquired immunity

A. Natural killer T cells

NKT cells constitute a small subpopulation of lymphocytes that are characterized by the markers expression of the NK cell lineage, as well as receptors of the αβ T lineage. NKT cells develop in the thymus and have the same common lymphoid precursor of conventional T cells, but they have phenotypic and functional characteristics different of T cells. Four subpopulations of NKT cells $CD4^{+}$, $CD8^{\alpha\beta+}$, $CD8^{\alpha\alpha+}$, and double negatives ($CD4^{-}CD8^{-}$) were identified in human peripheral blood, which differ in the cytokine secretion profile and the expression of chemokines receptors, integrins, and NK receptors. In addition, NKT cells recognize glycolipid antigens that are presented through CD1d molecules, MHC-like molecules that are constitutively expressed by antigen presenting cells such as DCs, B cells, and macrophages. NKT cells also have the ability to respond to cells participating in innate immunity with minimal involvement of the T cell receptor (TCR), and memory cells through a portion of the TCR, which makes them capable to be a bridge between the innate and adaptive immune response.

A. γδ T Lymphocytes and MAIT Cells

γδ T lymphocytes (γδ T cells) 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 T lymphocytes, they generally do not express either CD4 or CD8 coreceptors. These cells represent a small proportion of circulating T lymphocytes (approximately 0.5–5%) compared with the more abundant αβ T cells. A unique feature of γδ T cells is their ability to recognize antigens in their native form without the need for presentation by major histocompatibility complex (MHC) molecules. They are predominantly located in epithelial tissues, particularly in the skin and mucosal surfaces, where they contribute to early immune defense and tissue surveillance.

B. **Mucosal-Associated Invariant T (MAIT) cells**

MAIT constitute another population of unconventional T lymphocytes. They are $CD3^+$ T cells that express a semi-invariant TCR and are found mainly in mucosal tissues. MAIT cells possess potent antimicrobial functions and are activated by cells infected with a wide range of bacteria and yeasts, but not typically by virus-infected cells. Unlike conventional T cells that recognize peptide antigens presented by classical MHC molecules, MAIT cells recognize non-peptide microbial metabolites presented by the MR1 molecule, a non-classical MHC class I-related molecule. Through this mechanism, MAIT cells play an important role in the rapid immune response against microbial infections at mucosal sites.

II.4.1.4. Cellular Cooperation

A. Definition

The immune response results from interactions and cooperation between different immune cells. These interactions occur:

- Through physical contacts known as the immunological synapse; for example, the cellular cooperation between an antigen-presenting cell (APC) (magrophage, dendritic cell and LB) and a T lymphocyte (T cell).
- Through soluble factors such as cytokines; for example, the cellular cooperation between helper T lymphocytes ($CD4^+$ Th cells) and B lymphocytes (B cells) and/or cytotoxic T lymphocytes ($CD8^+$ Tc cells).

B. *Cytokines*

Cytokines are a means of communication between immune cells. They are small soluble proteins produced by cells of the immune system and have a regulatory role in the immune response. They are known by different names, including interleukins, interferons, and chemokines**.** Their main characteristics include:

- High biological activity at very low concentrations**,** typically ranging from 10^{-10} to 10^{-15} mol/L**.**
- Pleiotropic effects**,** as they can induce a variety of biological responses, including cell activation, differentiation, proliferation, and apoptosis**.**
- Different modes of action depending on the target cell**:**
    - **Autocrine action:** cytokines act on the same cells that produce them.
    - **Paracrine action:** cytokines act on neighboring cells within the local microenvironment.
    - **Endocrine action:** cytokines enter the circulation and exert effects on distant cells and tissues throughout the body, similarly to hormones.

C. Functional Properties of Cytokines

- *Redundancy*

Redundancy refers to the ability of different cytokines to exert similar biological effects. For example, IL-2, IL-4, and IL-5 can all stimulate B-lymphocyte proliferation**,** as they activate common signaling pathways in B cells.

- *Synergy*

Synergy occurs when two or more cytokines act together to produce a stronger effect than either cytokine alone. For instance, the combined action of IL-4 and IL-5 on B lymphocytes enhances IgE production.

- *Antagonism*

Antagonism describes the ability of one cytokine to inhibit or counteract the effect of another. For example, IL-4 promotes IgE synthesis, whereas IFN-γ (Interferon-gamma) inhibits this effect, thereby regulating the immune response.

CHAPTER III: Major histocompatibility complex (MHC)

III.1. Background

The major histocompatibility complex (MHC) were initially identified because of their strong influence on immune responses during tissue transplantation ; therefore, this genetic region was termed the “major histocompatibility complex.” MHC genes, known as the H-2 complex in mice, were first recognized in 1937 as major barrier to transplantation in experimental mouse models. In humans, these molecules are commonly referred to as human leukocyte antigens (HLA), since they were first discovered through antigenic differences observed between leukocytes of different individuals.

The MHC participates in the development of both humoral and cell mediated immune responses. While antibodies may react with antigens alone, most T cellsantibodies may react with antigens alone, most T cells recognize antigen only when it is combined with an MHC molecule.

Furthermore, because MHC molecules act as antigen- presenting structures, the particular set of MHC molecules expressed by an individual influences the repertoire of antigens to which that individual’s LTh and LTc cells can respond.

III.2. Definition of the MHC

MHC is a set of cell surface proteins expressed on the surface of all nucleated cells and encoded by a large gene family which controls a major part of the immune system in all vertebrates.

The MHC region is located on the short arm of chromosome 6 at position 6p21.31 in humans and on chromosome 17 in mice. In humans, this genomic region spans approximately 5–6 megabases on chromosome 6p21.3 and contains more than 260 genes involved in immune regulation and antigen presentation and are divided into three categories or classes (Fig.19).

- Class I MHC genes encode glycoproteins expressed on the surface of nearly all nucleated cells; the major function of the class I gene products is presentation of peptide antigens to TC cells.cells.
- Class II MHC genes encode glycoproteins expressed primarily on antigen-presenting cells (macrophages, dendritic cells, and B cells), where they present processed antigenic peptides to LT4 cells.

- Class III MHC genes encode, in addition to other products, various secreted proteins that have immune functions, including components of the complement system and molecules involved in inflammation.

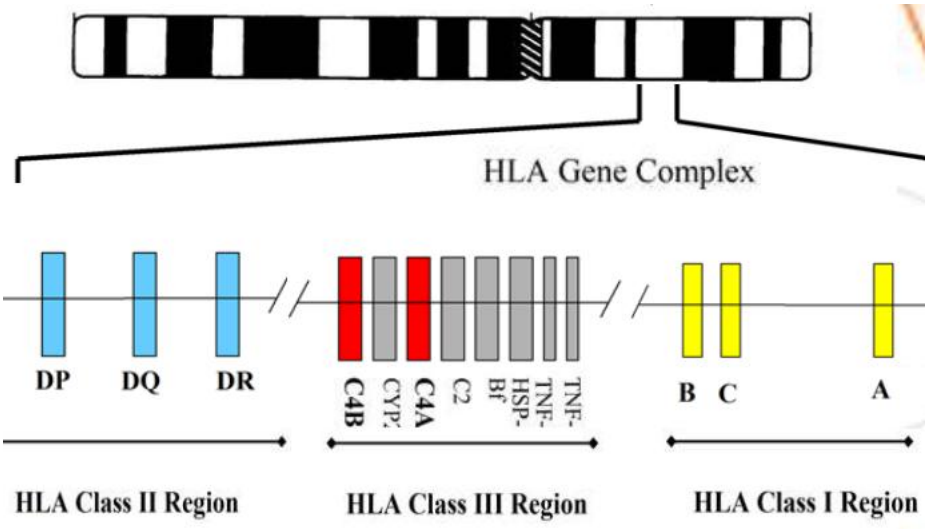


Figure 19 : HLA gene complexe

III.2.1. Class I MHC molecules

Class I MHC molecules contain a 45-kilodalton (kDa) chain associated noncovalently with a 12-kDa 2-microglobulin molecule. The chain is a transmembrane glycoprotein encoded by polymorphic genes within the A, B, and C regions of the human HLA complex and within the K and D/L regions of the mouse H-2 complex.

Beta 2-Microglobulin is a protein encoded by a highly conserved gene located on a different chromosome. Association of the chain with 2-microglobulin is required for expression of class I molecules on cell membranes.

The chain is anchored in the plasma membrane by its hydrophobic transmembrane segment and hydrophilic cytoplasmic tail. Structural analyses have revealed that the chain of class I MHC molecules is organized into three external domains (1, 2, and 3), each containing approximately 90 amino acids; a transmembrane domain of about 25 hydrophobic amino acids followed by a short stretch of charged (hydrophilic) amino acids; and a cytoplasmic segment of 30 amino acids. The beta 2-microglobulin is similar in size and organization to the 3 domain; it does not contain a transmembrane region and is noncovalently bound to the class I glycoprotein (Fig.20).

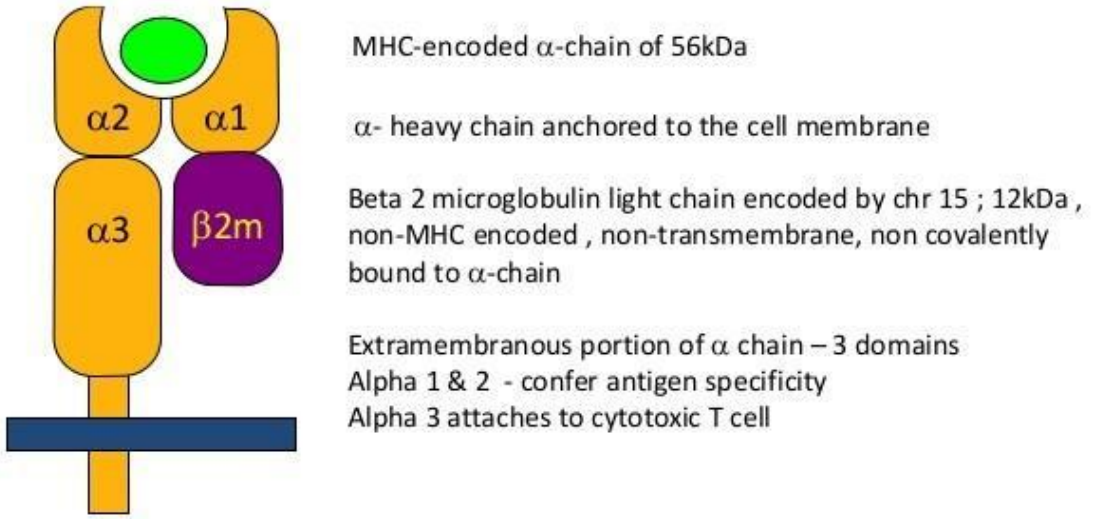


Figure 20 : Structure of Class I MHC molecules

III.2. 2. Class II MHC molecule

Class II MHC molecules contain two different polypeptide chains, a 33-Kd alpha chain and a 28-Kd beta chain, which associate by non covalent interactions. Class II MHC molecules are membrane-bound glycoproteins that contain external domains, a transmembrane segment, and a cytoplasmic anchor segment. Each chain in a class II molecule contains two external domains:

a) 1 and 2 domains in one chain

b)1 and 2 domains in the other. The membrane-proximal 2 and 2 domains, like the membrane-

proximal 3/2-microglobulin domains of class I MHC molecules, bear sequence similarity to the immunoglobulin-fold structure (Fig.21).

The membrane-distal portion of a class II molecule is composed of the 1 and 1 domains and forms the antigen binding cleft for processed antigen.

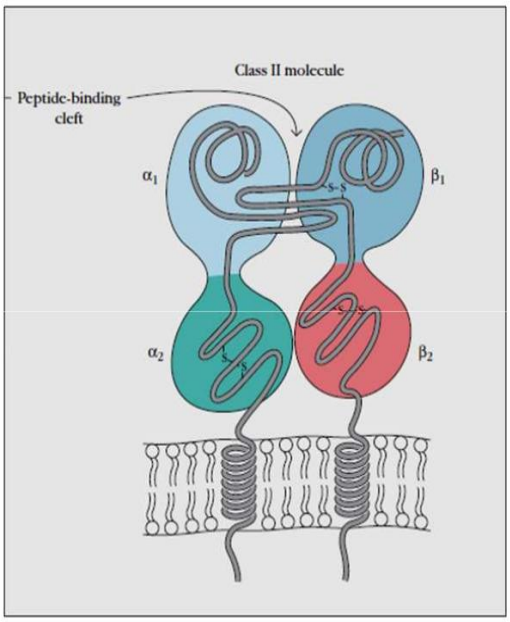


Figure 21 : Class II MHC molecule

III.3. Main characteristics of the MHC

The principal function of the MHC is to present antigen to T cells to discriminate between self (our cells and tissues) and nonself (the invaders or modified self). Two main characteristics of the MHC make it difficult for pathogens to evade immune responses:

- *The First* : the MHC is polygenic. It contains several different MHC-I and MHC-II genes so that every individual possesses a set of MHC molecules with different ranges of peptide-binding specificities.
- *The Second* : the MHC is extremely polymorphic. The MHC genes display the greatest degree of polymorphism in the human genome. There are multiple variants of each gene within the population as a whole. The different variants that are inherited by an individual from a parent are known as alleles. For example, HLA-B alone has more than 7000 known alleles (as of 2020). This high level of polymorphism, particularly in the classical HLA genes, is thought to be driven by positive selection for the ability to display a diverse spectra of peptides to the adaptive immune system.

III.4. Presentations of immunogenic peptides by MHC molecules

APCs take up antigen, either by surface receptors or by phagocytosis and then present it to immunologically competent lymphocytes. MHC class II expression is to a large extent confined to APCs, which are: Mononuclear Phagocytes (macrophages), Dendritic Cells, B Lymphocytes.

Complex antigens (e.g. cells, proteins) are degraded or processed into small antigenic fragments that are recognizable by T lymphocytes. These fragments are peptides that associate with either MHC class I or class II molecules . The actual association of the antigenic fragments takes place following cytoplasmic production (Class I) of the antigen or alternatively following phagocytosis or endocytosis (Class II) of the antigen.

III.4.1. MHC CLASS II ANTIGEN PROCESSING

MHC class II associated peptides are derived from antigens captured and internalized by specialized APCs. These antigens are degraded enzymatically, in endosomes and lysosomes, into peptides that bind MHC class II molecules.

Class II MHC molecules are synthesized in the endoplasmic reticulum and are transported to endosomes with an associated protein, invariant chain (Ii), which occupies the peptide binding cleft of the newly synthesized MHC class II molecule. Within the endosome, acidification cleaves Ii leaving a short peptide fragment, CLIP (class II-associated invariant chain peptide), bound to the peptide binding groove of the MHC class II molecule. Onse such endosomes fuse with a vesicle containing foreign antigen, CLIP is removed by a peptide unloader/loader, DM, which then places foreign peptides in the groove of the MHC class II molecule. (Degradation of Ii increases the mobility of these antigen presenting cells.) The peptide MHC complex then transits to the cell surface . The process of peptide loading and transport of MHC class I and class II molecules to the cell surface is summarized in Fig.22.

It is worth noting that MHC class I molecules under normal conditions (in the absence of foreign antigen) are loaded with self peptides derived from the normal degradation of self cellular proteins. MHC class II molecules, under normal conditions, are thought to contain only CLIP in their peptide binding groove.

III.4.2. MHC CLASS I ANTIGEN PROCESSING

Antigenic peptides that bind to MHC class I molecules are typically derived from viruses that take over the biosynthetic machinery of the cell, resulting in the production of viral proteins (foreign antigens). These viral proteins are degraded by the host cell's proteasomes [long cylindrical structures, comprised of subunits LMP2 and LMP7 that contain multicatalytic

proteases] into small peptide fragments.

Peptides generated in the cytoplasm are transported into the endoplasmic reticulum by TAP-1and TAP-2 (Transporters Associated with Antigen Processing-1, -2).

Newly synthesized MHC lass I molecules assemble in the endoplasmic reticulum with the help of several chaperones (calnexin, Erp57, calreticulin). These MHC class I molecules associate with TAP-1,-2 and when peptides are transported into the endplasmic reticulum they are trimmed by ERAAP (endoplasmic reticulum aminopeptidase associated with antigen processing) and the peptides bind to the MHC molecule, the peptide-MHC complex leaves the endoplasmic reticulum and is transported through the Golgi apparatus to the cell surface (Fig. 22).

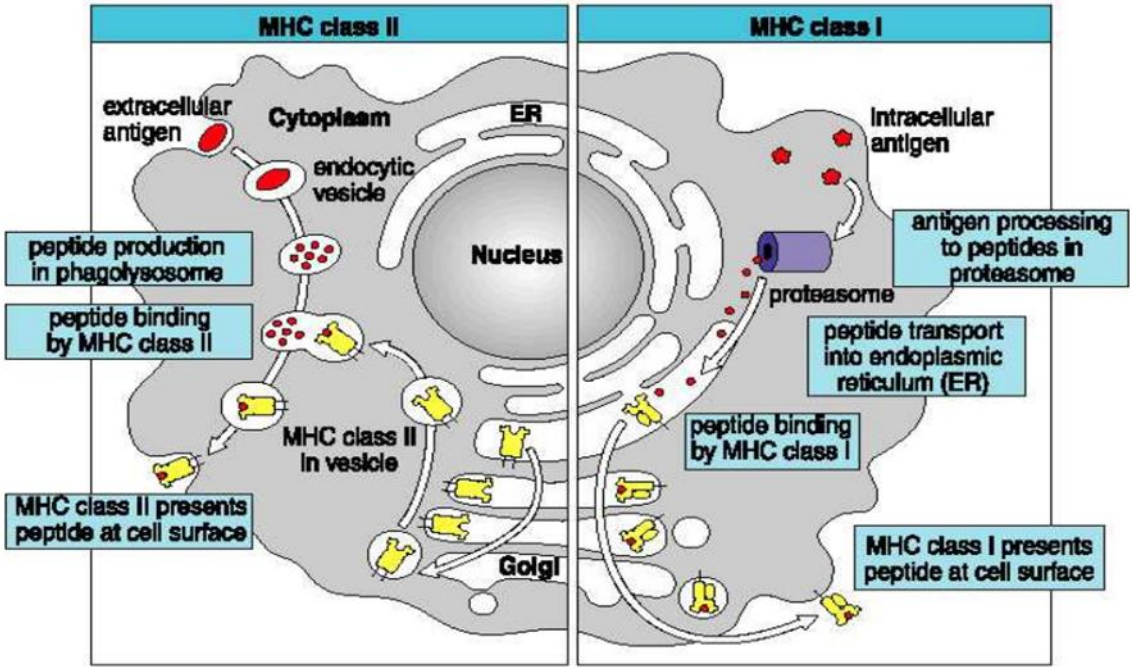


Figure 22 : Summary of peptide loading and transport of MHC Class I and II molecules to the cell surface

Chapter IV: Innate immunity

IV.1. Definition

Non-specific immunity, also called natural or innate immunity, is the first line of defense against any infectious agent. Non specific host responses provide an effective barrier that revents the microorganisms from penetrating, inhibit or destroy the invader if it gains access to the tissues, and eliminate or neutralize any toxic substance elaborated by infectious agent. Several mechanisms are available in the immunocompetent host. These include physical or mechanical barrier, biochemical factors, role of normal flora & inflammatory reactions.

IV.2. Natural Barriers

IV.2.1. Physical and Mechanical Barriers

The skin and mucous membranes constitute the body's first line of defense against infectious agents by forming effective physical and mechanical barriers. Intact skin prevents microbial entry and inhibits the growth of most microorganisms due to its low moisture content, acidic pH, and the presence of inhibitory substances, although some microorganisms may penetrate through hair follicles, sebaceous glands, or sweat glands. Mucous membranes, which line the digestive, respiratory, urinary, and reproductive tracts, consist of an epithelial layer supported by connective tissue and provide additional protection. In the respiratory tract, mucus traps microorganisms, while ciliated epithelial cells continuously move the mucus toward the mouth, where it is swallowed and eliminated. Coughing further helps remove mucus containing trapped pathogens. Similarly, the rapid flow of urine flushes microorganisms from the urethra, and tears continuously wash the conjunctiva, helping to prevent microbial colonization.

IV.2.2. Chemical and Biochemical Barriers

Various body secretions and biochemical substances contribute to innate immunity by inhibiting microbial growth. Keratin, a protein produced by the outermost skin cells, creates a dry environment unfavorable to microbial survival. In the gastrointestinal tract, hydrochloric acid secreted by the stomach and bile salts produced by the liver inhibit the growth of many microorganisms. Lysozyme, an enzyme present in tears, saliva, and other body fluids, destroys certain bacteria by hydrolyzing the peptidoglycan layer of their cell walls. In addition,

secretions originating from mucosal epithelial surfaces including tears, nasal mucus, bronchial mucus, saliva, and gastric juices contain antimicrobial substances that are toxic or inhibitory to microorganisms, thereby reinforcing the body's first line of defense against infection (table 3) .

Table 3 : Antimicrobial secretions and their sources

Site	Source	Secreted Substances
Eye	Lacrimal glands (**tears**)	Lysozyme
Ear	Sebaceous glands	Earwax (cerumen) secretion
Mouth	Salivary glands (**saliva**)	Digestive enzymes, lysozyme, lactoferrin
Skin	Sweat glands (**sweat**)	Lysozyme, NaCl, medium-chain fatty acids
Stomach	**Gastric juice**	Acidic digestive enzymes (**pepsin, rennin**), low pH (**1–2**)

IV.2.3. Normal flora

The human body is colonized by a vast number of microorganisms, mainly bacteria, collectively known as the normal flora or commensal microbiota. These microorganisms are generally harmless and are found on the skin, nasal cavities, mouth, throat, gastrointestinal tract, and genitourinary tract. The normal flora plays a crucial role in host defense by preventing the colonization and growth of pathogenic microorganisms through several mechanisms, including occupying attachment sites on mucosal surfaces, competing for essential nutrients, and producing antimicrobial substances that inhibit pathogen growth. The gastrointestinal microbiota, which consists of billions of bacteria, is particularly important in maintaining this protective barrier. In addition, vaginal Lactobacilli contribute to innate defense by maintaining an acidic environment (pH 4.0–4.5), which suppresses the growth of many pathogenic microorganisms and helps protect against infections.

IV.3. Inflammation

Inflammation is the rapid and early response of vascularized tissues to injury caused by biological, physical, chemical, or immunological agents. As a key component of the innate immune system, it functions to eliminate harmful stimuli, remove damaged cells and tissues, and initiate the repair process. It develops within minutes or hours after tissue injury and is typically short in duration, lasting from a few hours to a few days. It is characterized by vascular alterations, increased vascular permeability, and the migration of leukocytes to the site of injury, all of which contribute to the restoration of tissue homeostasis.

IV.3.1. Causes of Inflammation

Inflammation can be triggered by a variety of harmful stimuli. The most common causes are microbial infections, including bacterial, viral, fungal, and parasitic infections. It may also result from hypersensitivity reactions directed against environmental antigens or self-tissues. Physical and chemical agents, such as burns, frostbite, radiation, and toxic chemicals, can damage tissues and induce an inflammatory response. In addition, tissue necrosis resulting from ischemia, trauma, or chemical injury is a potent inflammatory stimulus.

IV.3.2. Major Characteristics of Inflammation

Regardless of the initiating cause, inflammation follows a similar sequence of events, although its intensity and duration may vary. It appears rapidly after tissue injury and generally lasts for up to 48 hours. Failure to eliminate the causative agent or resolve the inflammatory response may lead to chronic inflammation, which can persist for months or years. It is characterized by four cardinal signs: redness (*rubor*), heat (*calor*), swelling (*tumor*), and pain (*dolor*). Redness and heat result from vasodilation and increased blood flow to the affected area. Swelling (edema) is caused by the leakage of plasma and proteins from blood vessels into the surrounding tissues due to increased vascular permeability. Pain arises from the action of inflammatory mediators and the pressure exerted by accumulated fluid on local nerve endings. These manifestations reflect the body's protective efforts to contain injury, eliminate harmful agents, and promote tissue repair (Fig. 23).

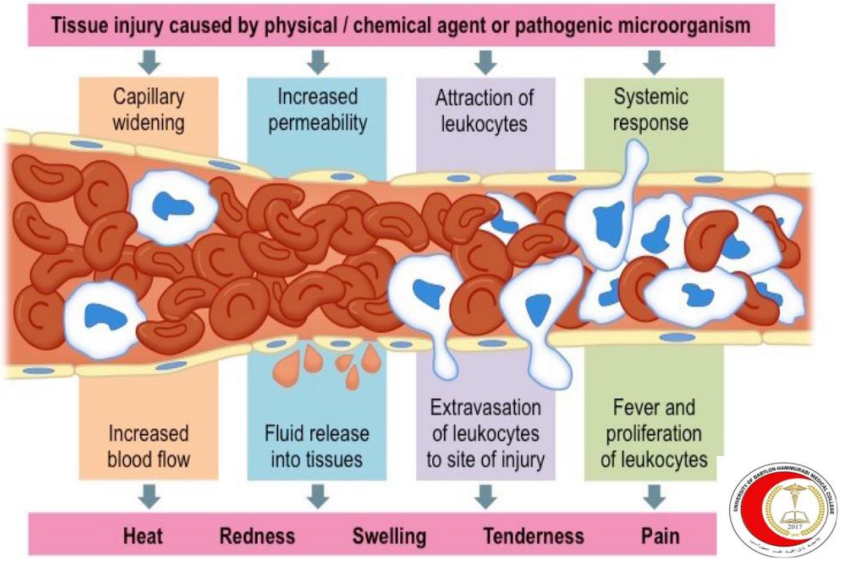


Figure 23 : The cardinal signs of inflammation and their underlying causes

IV.3.3. Components of Inflammation

Inflammation results from the coordinated interaction of two major components: a large variety of cells and numerous biochemical mediators.

IV.3.3.1. Cells Involved in Inflammation

Several cell types participate in the inflammatory response, including both immune and non-hematopoietic cells. The inflammatory process involves circulating blood cells as well as resident tissue cells that work together to initiate, regulate, and resolve inflammation. Circulating cells, such as neutrophils, monocytes, eosinophils, basophils, platelets, lymphocytes, and plasma cells, are recruited from the bloodstream to sites of injury or infection, where they contribute to pathogen elimination and tissue defense. Resident tissue cells, including macrophages, mast cells, endothelial cells, and fibroblasts, are already present within tissues and play essential roles in recognizing harmful stimuli, producing inflammatory mediators, coordinating leukocyte recruitment, and promoting tissue repair. Through their combined actions, these cells ensure the effective detection and elimination of damaging agents while facilitating the healing of injured tissues. The different cell types involved in the inflammatory response are summarized in the following table (Table 4)

Table 4 : Cells Involved in the Inflammatory Response.

Circulating Blood Cells	**Resident Tissue Cells**
Neutrophils	Macrophages
Monocytes	Mast cells
Eosinophils	Endothelial cells
Basophils	Fibroblasts
Platelets	
Lymphocytes	
Plasma cells	

IV.3.3.2. Mediators and Molecules Involved in Inflammation

The inflammatory response is regulated by a wide range of biochemical mediators that coordinate communication between immune and tissue cells. These mediators may be pro-inflammatory, promoting the initiation and amplification of inflammation, or anti-inflammatory, limiting the response and facilitating its resolution. Produced by plasma proteins, leukocytes, and resident tissue cells, they play essential roles in regulating vascular changes,

leukocyte recruitment and activation, pathogen elimination and tissue repair. The balance between pro-inflammatory and anti-inflammatory mediators is critical for an effective response; excessive or prolonged pro-inflammatory activity can lead to tissue damage and chronic inflammation, whereas anti-inflammatory mediators contribute to the termination of the inflammatory process and the recovery of normal tissue function. The major mediators and molecules involved in inflammation, together with their sources and biological functions, are summarized in Table 5**.**

Table 5 : Major Mediators and Molecules Involved in Inflammation.

Inflammatory Mediator	Member	Source	Characteristics	Effects
Serum proteins	**C-reactive protein (CRP)**	Secreted by the liver during the acute phase of inflammation.	Recognizes and binds to specific polysaccharides present on the surface of certain bacteria and fungi.	Activates the complement system and promotes pathogen elimination through phagocytosis.
Vasoactive amines	**Histamine**	Released during mast cell degranulation.	Binds to receptors located on capillaries and venules.	Causes vasodilation and increases vascular permeability.
Lipid mediators	**Leukotrienes and Prostanoids**	Produced through the COX-1/COX-2 pathways and the lipoxygenase pathway from arachidonic acid.	Lipid-derived inflammatory mediators.	Possess chemotactic properties for eosinophils and neutrophils and stimulate the secretion of cytokines such as IL-2 and IFN-γ.
	Platelet-Activating Factor (PAF)	Produced by several inflammatory cells.	Potent phospholipid mediator.	Increases vascular permeability, stimulates platelet aggregation, and activates leukocytes.
Reactive oxygen and nitrogen derivatives	**Nitric Oxide (NO)**	Produced by eosinophils, neutrophils, and macrophages.	Short half-life (approximately 5–50 seconds).	Contributes to apoptosis and extracellular matrix proteolysis, participates in NF-κB activation through IL-1β signaling, and exerts both pro-inflammatory and anti-inflammatory effects.
Enzymes	**Myeloperoxidase (MPO)**	Neutrophils.	Heme-containing enzyme present in neutrophil granules.	Exhibits peroxidase activity and contributes to microbial killing.
Cytokines	**IL-1, IL-6, TNF-α, IFN-α**	Produced by numerous cell types.	Soluble proteins with low molecular weight that act through specific receptors.	Enable intercellular communication and regulate cell proliferation, activation, differentiation, survival, and death.
Chemokines	Various chemokines	Produced by immune and tissue cells.	Specialized cytokines with chemotactic activity.	Promote the migration and recruitment of cells to sites of inflammation.

IV.3.4. Stages of the Inflammatory Response

The inflammatory response develops through a series of coordinated events that begin with the recognition of danger signals and progress through vascular and cellular changes designed to eliminate harmful stimuli and promote tissue repair.

IV.3.4.1. Recognition of Danger Signals

The inflammatory response is initiated by the recognition of danger signals originating either from damaged host cells or from invading microorganisms. Endogenous signals released by injured, stressed, or necrotic cells are known as Damage-Associated Molecular Patterns (DAMPs)**,** whereas exogenous microbial components are referred to as Pathogen-Associated Molecular Patterns (PAMPs)**.** These molecules are detected by Pattern Recognition Receptors (PRRs) expressed on immune and tissue cells. The interaction between PAMPs or DAMPs and PRRs activates intracellular signaling pathways that stimulate transcription factors, leading to the synthesis and release of inflammatory mediators such as cytokines, chemokines, and lipid mediators. These mediators initiate and coordinate the subsequent phases of the inflammatory response (Fig.24).

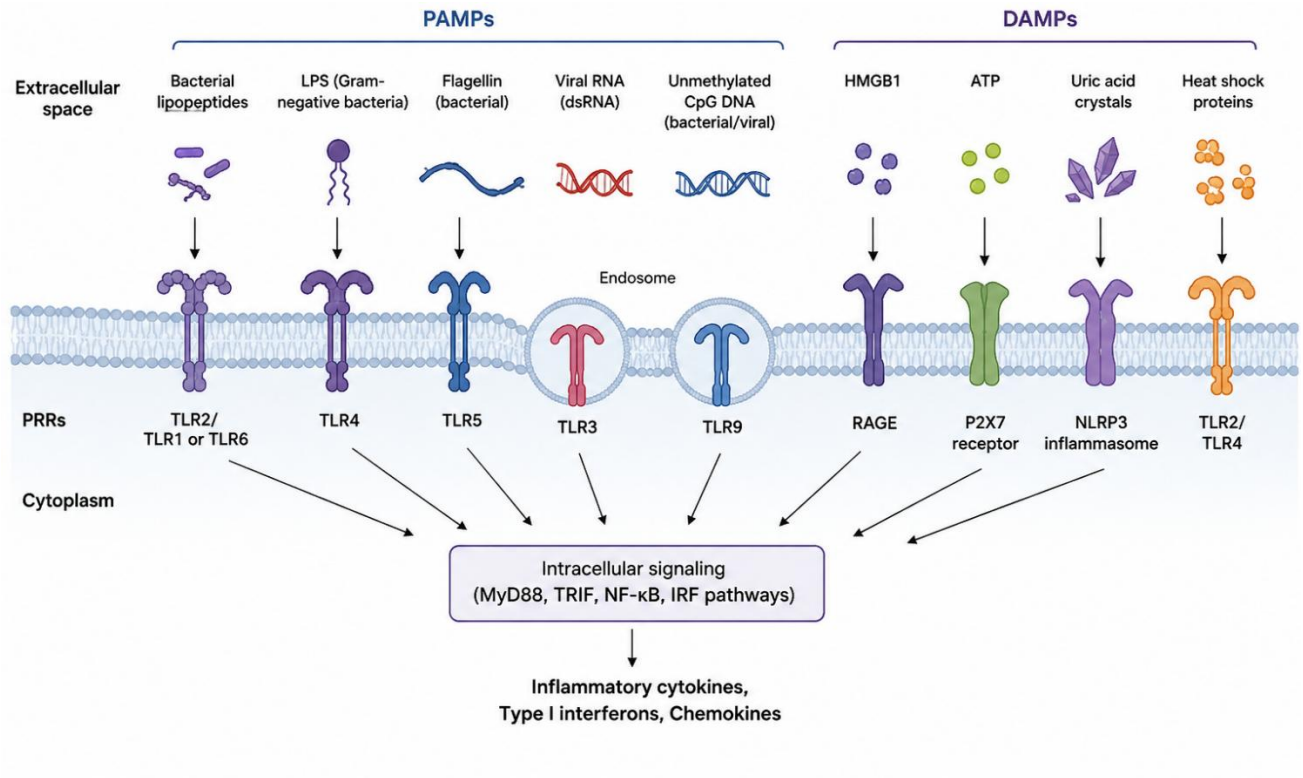


Figure 24 : PRR–PAMP/DAMP Interaction

IV.3.4.2. Vascular Phase

The vascular phase comprises the early vascular alterations that occur following the recognition of danger signals and the release of inflammatory mediators. These changes are essential for delivering plasma proteins and immune cells to the site of injury. Initially, vasodilation increases blood flow and blood volume within the affected area, producing the characteristic signs of redness and heat. Simultaneously, endothelial cells become more permeable through the opening of intercellular junctions, allowing plasma proteins and fluid to leave the circulation and enter the surrounding tissues.

The extravasation of plasma results in the formation of an inflammatory exudate and contributes to tissue edema. Among the plasma proteins that reach the inflammatory site is fibrinogen, which is converted into fibrin and participates in the formation of a provisional extracellular matrix. This fibrin network helps localize the inflammatory focus and limits the spread of the injurious agent. As fluid leaves the blood vessels, blood viscosity increases and blood flow slows down, a phenomenon known as **stasis** (**Table 6)**. These vascular changes facilitate the movement of water, plasma proteins, and subsequently leukocytes from the bloodstream into the damaged tissue, thereby preparing the site for the cellular phase of inflammation.

Table 6 : Mediators Produced That Initiate the Vascular Phase of Inflammation.

Function	Mediators	Sources / Cells
Increased capillary permeability	Histamine; Bradykinins; Leukotrienes C4, D4, E4; Platelet-activating factor; Substance P	Mast cells; Plasma; Leukocytes and endothelium; Nerve endings
Vasodilation	Histamine; Nitric oxide (NO); Prostaglandins; Bradykinins; CGRP	Mast cells and macrophages; Plasma; Nerve endings
Diapedesis	LPS; IL-1β; TNF-α; Thrombin	Bacteria; Macrophages and mast cells; Plasma
Pain	Bradykinins; Prostaglandins; Nitric oxide (NO)	Plasma; Mast cells and macrophages

IV.3.4.3. Cellular Phase: Formation of the Inflammatory Focus

The cellular phase of inflammation is characterized by the recruitment and accumulation of leukocytes at the site of injury or infection, resulting in the formation of an inflammatory focus. Following the vascular changes that promote stasis and increased vascular permeability, leukocytes are attracted to the affected tissue and migrate from the bloodstream into the extravascular space. Leukocyte recruitment to the site of inflammation is a multistep process involving margination, rolling, firm adhesion, diapedesis (transmigration), chemotaxis, and

ultimately phagocytosis. Diapedesis refers specifically to the passage of leukocytes through the vascular endothelium into the surrounding tissues (Fig. 25).

A. Margination and Rolling

As blood flow slows during stasis, red blood cells remain in the central axial column of the vessel, whereas leukocytes are displaced toward the endothelial surface, a process known as **margination**. In response to cytokines and other inflammatory mediators, endothelial cells express adhesion molecules that allow leukocytes to establish weak and transient interactions with the vessel wall. Consequently, leukocytes repeatedly attach and detach from the endothelium, rolling along its surface. This process, known as **rolling**, is mediated primarily by the selectin family of adhesion molecules, including **E-selectin** on endothelial cells, **P-selectin** on endothelial cells and platelets, and **L-selectin** on leukocytes.

B. Firm Adhesion

During rolling, leukocytes detect chemotactic signals and become activated, leading to the expression of high-affinity integrins on their surface. These integrins interact strongly with their ligands expressed on activated endothelial cells, resulting in **firm adhesion**. This stable attachment arrests leukocyte movement and prepares the cells for migration through the vessel wall.

C. Transmigration (Diapedesis)

Following firm adhesion, leukocytes pass through the vascular wall by squeezing between adjacent endothelial cells at intercellular junctions. This process, known as diapedesis or transmigration**,** occurs mainly in postcapillary venules and allows leukocytes to enter the surrounding tissues where the inflammatory reaction is taking place.

D. Migration (Chemotaxis) and Phagocytosis

Once in the extravascular tissues, leukocytes migrate toward the site of injury or infection by following gradients of chemotactic substances, a process known as chemotaxis. Chemotactic factors may originate from both exogenous and endogenous sources and include bacterial products, cytokines, complement components, and metabolites generated through the lipoxygenase pathway of arachidonic acid metabolism. Upon reaching the inflammatory focus,

leukocytes recognize, engulf, and destroy microorganisms, cellular debris, and other foreign materials through phagocytosis, thereby contributing to pathogen elimination and tissue repair.

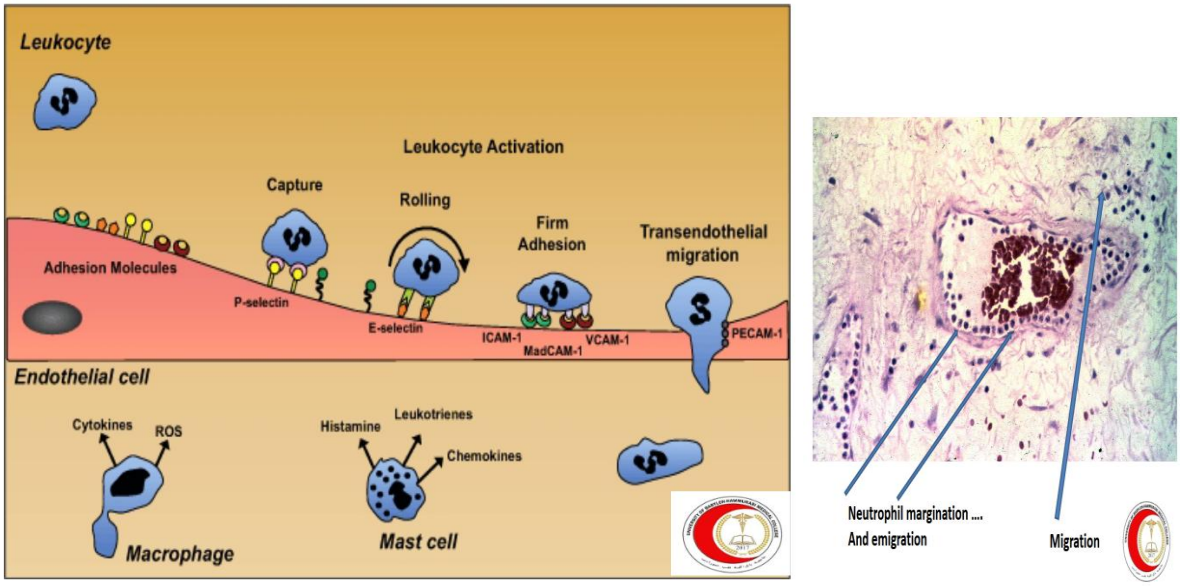


Figure 25 : Steps of Leukocyte Recruitment: Margination, Rolling, Adhesion, Transmigration, Chemotaxis, and Phagocytosis.

IV.3.5. Elimination of the Pathogen

Once leukocytes have accumulated at the inflammatory site, the elimination of the invading pathogen is achieved through several innate defense mechanisms. These include phagocytosis by neutrophils and macrophages, activation of the complement system, the action of lytic enzymes, Natural Killer (NK) cell-mediated cytotoxicity, and other innate immune responses. Together, these mechanisms contribute to the destruction and removal of microorganisms, thereby limiting the spread of infection and promoting tissue recovery.

IV.3.5.1. Phagosytosis

Phagocytosis is mainly carried out by neutrophils and macrophages and occurs in four principales phases (Fig. 26):

A. Directed migration (chemotaxis)

Phagocytes are attracted to the site of infection by substances produced by bacteria, degradation products from damaged tissues, chemokines, and other mediators such as complement activation products (C5a and C3a). The production of reactive oxygen species (ROS) is a common function of most phagocytic cells.

B. Adhesion of the particle to the phagocyte membrane

Phagocytic cells migrate by extending pseudopodia and come into contact with the target to be engulfed. Some bacteria possess surface components that inhibit their adhesion to phagocytes, such as the capsule of pneumococcus and the M protein of streptococcal cell walls.

C. Ingestion

Phagocytes engulf the particle into a phagocytic vacuole (phagosome). This vesicle detaches from the cell membrane and moves toward the interior of the cell.

D. Effector phase (intracellular killing)

- **Production of reactive oxygen species (ROS):**
  Binding and internalization of the microorganism stimulate the phagocyte membrane, leading to a rapid increase in oxygen consumption and the generation of ROS, including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen (O_2), and hydroxyl radicals (OH•).
- **Degranulation and phagolysosome formation:**
  Phagosomes fuse with lysosomes, forming phagolysosomes. The pH becomes acidic (around pH 4), which optimizes lysosomal enzyme activity.
- **Destruction of microorganisms:**
  Pathogens are destroyed by oxygen-derived toxic products (ROS and myeloperoxidase activity), nitric oxide (NO) produced from L-arginine via nitric oxide synthase, and lysosomal enzymes. NO can also generate other reactive nitrogen species such as NO_2 or combine with superoxide to form peroxynitrite ($ONOO^-$), a highly potent oxidant. Additional antimicrobial molecules include cationic proteins (which damage bacterial membranes), lactoferrin (which sequesters iron), and lysozyme (which degrades bacterial cell wall peptidoglycan).

Neutrophils and macrophages can therefore directly phagocytose pathogens through pattern recognition receptors (PRRs), or indirectly via receptors for complement and other opsonins such as CRP and MBL. These innate immune responses may either eliminate the infection completely or limit its progression while the adaptive immune response develops.

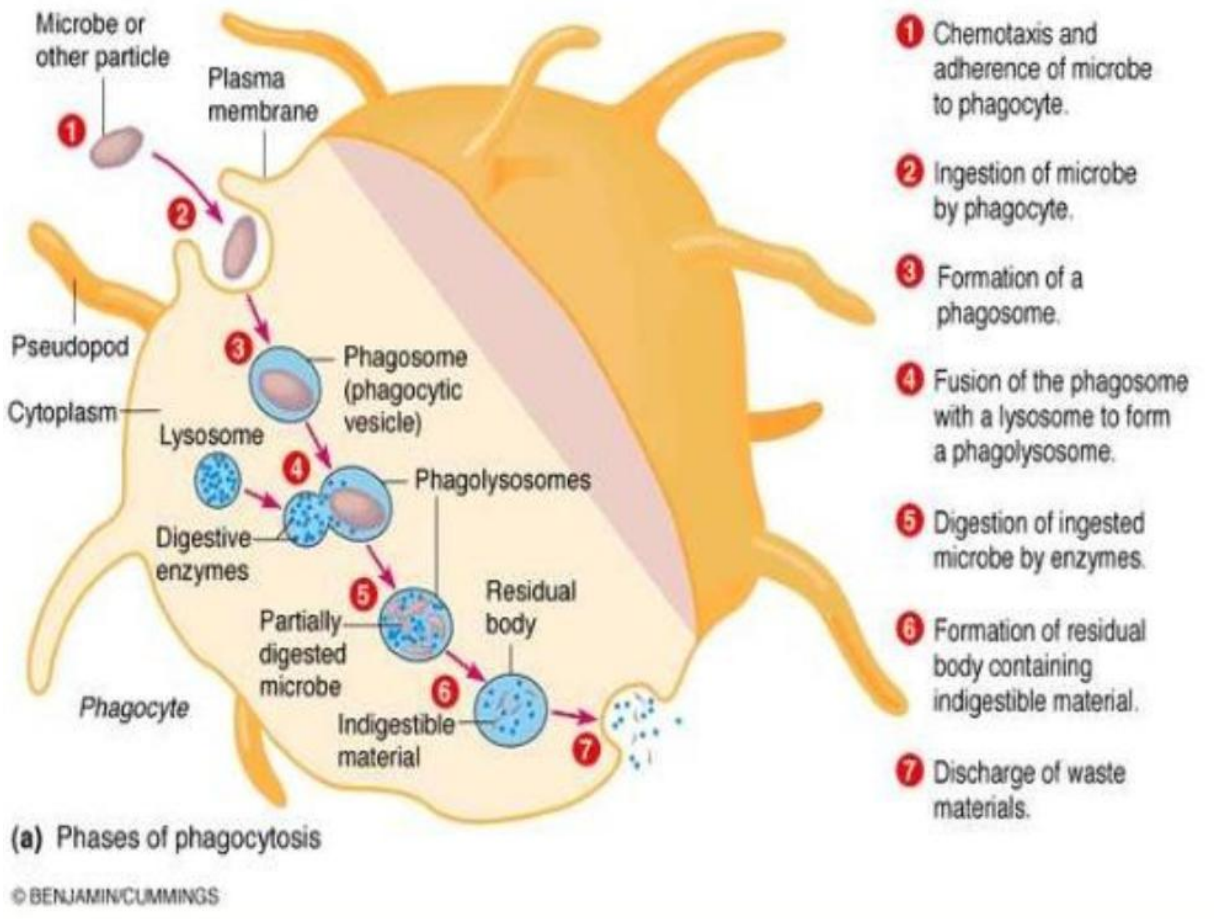


Figure 26 : phases of phagocytosis

IV.3.5.2. Natural Killer (NK) cells

Natural Killer (NK) cells are a type of cytotoxic lymphocyte critical to the innate systems that play a key role in the first line of defense against transformed and virus-infected cells. NK cells are now recognized as a separated lymphocyte lineage with both cytotoxicity and cytokine-producing effector functions. NK cells sense their target through a whole array of receptors, both activating and inhibitory. NK cells are unique, as they can discriminate target cell from other healthy 'self' cells and activate to kill cells that are 'missing self' markers of major histocompatibility complex (MHC) class I. This role is especially important because harmful cells that are MHC I marker cannot be detected and destroyed by other immune cells, such as T lymphocyte cells.

Once the balance shifts in favor of activation, an immunological synapse forms between the NK cell and the target cell. NK cells directly kill target cells through several mechanisms (Fig. 27):

- ✓ **Perforin–Granzyme Pathway**

NK cells kill target cells by releasing cytoplasmic granules containing perforin and granzymes. Perforin forms pores in the membrane of the target cell, allowing granzymes to enter the cytoplasm. Once inside, granzymes trigger apoptosis by activating caspase-dependent pathways and by inducing caspase-independent mechanisms, such as mitochondrial damage and DNA fragmentation. This results in the rapid and efficient elimination of infected or tumor cells.

- ✓ **Death Receptor-Mediated Apoptosis**

Activated NK cells can induce apoptosis of target cells through death receptor-mediated pathways. They express members of the tumor necrosis factor (TNF) superfamily, including Fas ligand (FasL, CD178) and TNF-related apoptosis-inducing ligand (TRAIL). FasL binds to the Fas receptor (CD95), whereas TRAIL interacts with TRAIL receptors (TRAIL-Rs) expressed on infected, transformed, or tumor cells. These receptor–ligand interactions trigger intracellular signaling cascades that activate initiator and effector caspases, ultimately leading to programmed cell death (apoptosis) of the target cell. This mechanism enables NK cells to eliminate infected and malignant cells without releasing cytotoxic granules.

- ✓ **Cytokine-Mediated Antitumor Activity**

NK cells also contribute to antitumor immunity by secreting effector cytokines, particularly interferon-gamma (IFN-γ). IFN-γ exerts antitumor effects by inhibiting tumor angiogenesis, enhancing antigen presentation, and stimulating adaptive immune responses through the activation of macrophages and T lymphocytes, thereby promoting the elimination of tumor cells.;

- ✓ **Antibody-Dependent Cellular Cytotoxicity (ADCC)**

During antibody-dependent cellular cytotoxicity (ADCC), NK cells recognize antibody-coated target cells through CD16 (FcγRIII). This interaction primarily triggers the release of perforin and granzymes, leading to target-cell apoptosis. Activated NK cells may also contribute to cell death through FasL- and TRAIL-mediated apoptotic pathways and by secreting immunomodulatory cytokines such as IFN-γ.

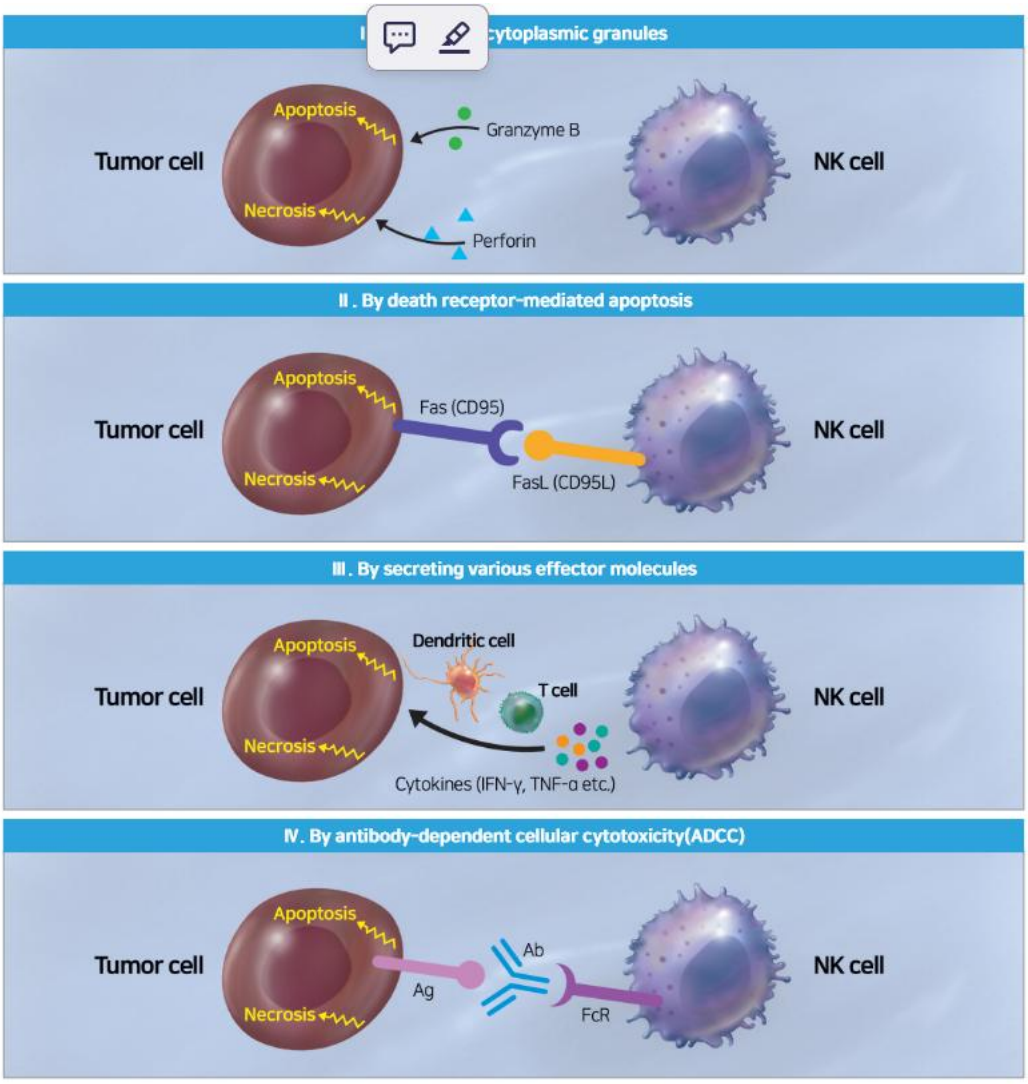


Figure 27 : Major Effector Mechanisms of NK Cell-Mediated Cytotoxicity Against Infected and Transformed Target Cells

IV.3.5.3. The Complement System

The complement system is a major effector component of innate immunity and consists of more than 30 circulating and membrane-associated proteins that interact in a highly regulated cascade. Many complement proteins are proteolytic enzymes that become activated sequentially, generating effector molecules involved in host defense. In addition to its antimicrobial functions, the complement system contributes to inflammation by increasing vascular permeability, promoting chemotaxis, enhancing phagocytosis, and facilitating the clearance of pathogens and damaged cells. Most complement components are synthesized by monocytes/macrophages and hepatocytes. Some complement proteins are also produced by epithelial cells of the thymus and the small intestine.

A. Complement Activation Pathways

Complement activation can be initiated through three major pathways: the **classical pathway**, the **lectin pathway**, and the **alternative pathway**. Although they differ in their mechanisms of initiation, all three pathways converge at the activation of C3 and ultimately lead to the formation of the membrane attack complex (MAC), which can lyse target cells **(**Fig.28**).**

- **Classical Pathway**

The classical pathway is initiated by antigen–antibody complexes, primarily involving IgM or IgG antibodies. It represents an important link between innate and adaptive immunity. Activation begins when the C1 complex, composed of C1q, C1r, and C1s, binds to the Fc region of antibodies attached to an antigen. C1q is responsible for antigen-antibody recognition, whereas C1r and C1s function as proteolytic enzymes that activate subsequent complement components.

Activated C1 cleaves C4 and C2, generating the fragments that combine to form the **C3 convertase (C4b2a)**. This enzyme cleaves C3 into C3a and C3b. C3b associates with the C3 convertase to generate **C5 convertase**, which cleaves C5 into C5a and C5b. C5b subsequently binds C6, C7, C8, and multiple C9 molecules to form the **membrane attack complex (MAC)**, which creates pores in the target cell membrane and induces cell lysis.

The classical pathway can be divided into three stages:

1. Recognition.
2. Enzymatic activation.
3. Membrane attack and target cell destruction.

- **Lectin Pathway**

The lectin pathway is activated by specific carbohydrate residues present on the surface of microorganisms or apoptotic cells. It is initiated by soluble pattern-recognition molecules, principally **mannose-binding lectin (MBL)** and **ficolins**, which recognize microbial carbohydrates. These recognition molecules are associated with serine proteases known as **MASP-1, MASP-2, and MASP-3**.

Upon activation, MASPs cleave C4 and C2, resulting in the formation of the same **C3 convertase (C4b2a)** generated by the classical pathway. Consequently, the lectin pathway follows the same downstream sequence of complement activation, leading to C3 cleavage, C5 activation, and ultimately MAC formation.

C. Alternative Pathway

The alternative pathway is activated independently of antibodies and serves as an important mechanism of innate immune defense. It can be triggered directly by microbial surfaces and other foreign structures. Unlike the classical and lectin pathways, it does not require the participation of C1, C2, or C4.

Activation involves complement proteins **factor B**, **factor D**, and **properdin**. C3b deposited on a microbial surface binds factor B, which is then cleaved by factor D to form the **C3 convertase (C3bBb)**. Properdin stabilizes this complex, increasing its half-life and enhancing complement activation. The alternative pathway amplifies the generation of C3b, leading to the formation of a C5 convertase and the subsequent activation of the terminal complement components. As in the other pathways, the cascade culminates in the formation of the membrane attack complex and target cell lysis.

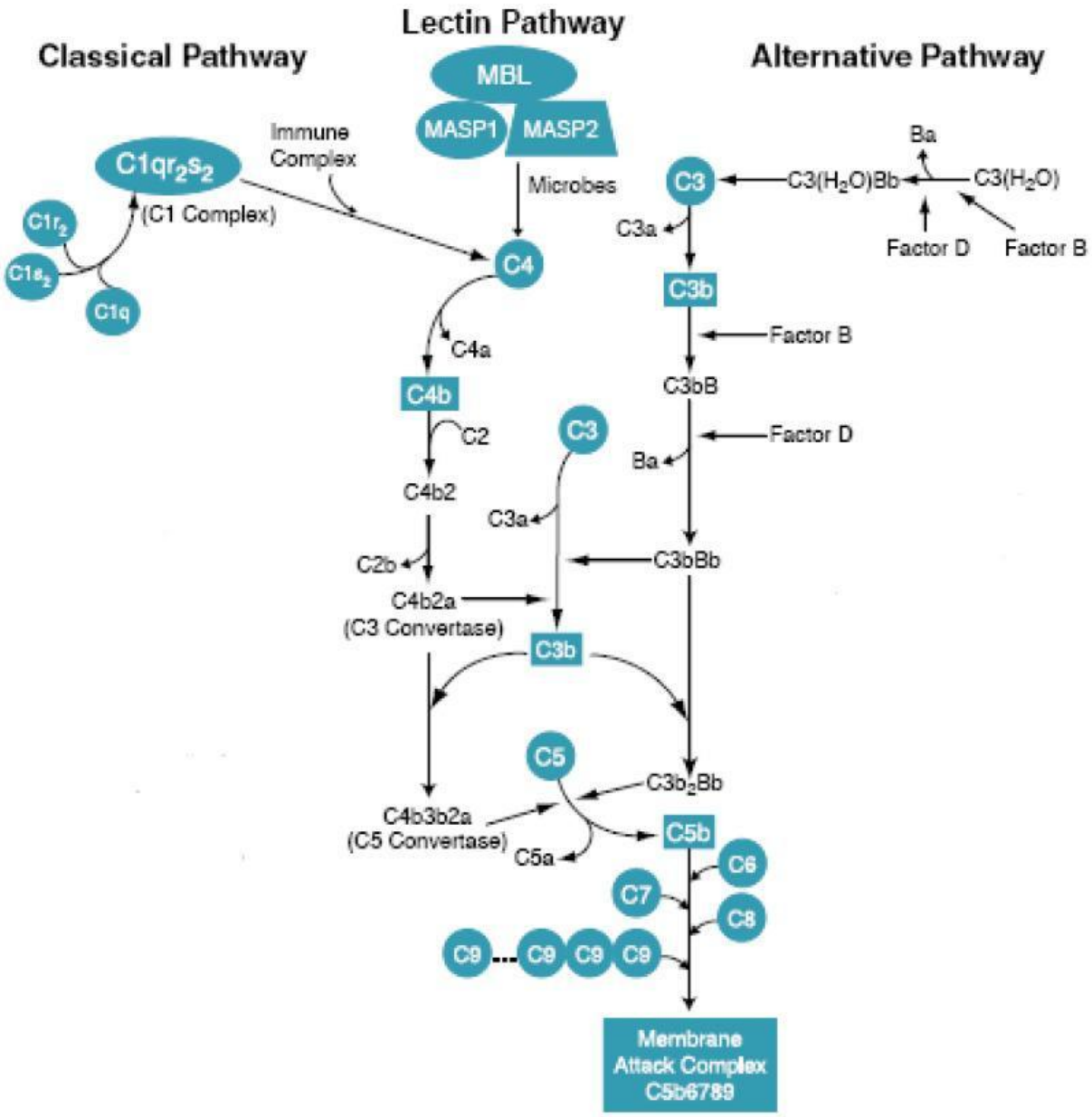


Figure 28 : Complement Activation Pathways (Classical, Lectin, and Alternative) and Formation of the Membrane Attack Complex.

B. Role of Activated Complement in the Immune Response

Activation of the complement system generates biologically active fragments that play essential roles in both immunity and inflammation. These effector molecules promote **opsonization**, facilitating the recognition and phagocytosis of pathogens; stimulate **chemotaxis**, attracting leukocytes to sites of infection; increase **vascular permeability**, thereby enhancing inflammatory responses; and induce **direct lysis** of susceptible microorganisms through the membrane attack complex. Through these coordinated activities, the complement system contributes significantly to pathogen elimination and the regulation of inflammatory reactions.

IV.3.6. Resolution of Inflammation, Return to Homeostasis, and Tissue Repair

Following the elimination of pathogens and the clearance of cellular debris, the inflammatory response enters a resolution phase that restores tissue homeostasis and promotes healing. This process is mediated by anti-inflammatory cytokines and growth factors that suppress inflammation and initiate tissue repair. Fibroblasts proliferate and synthesize collagen, proteoglycans, and other extracellular matrix (ECM) components, forming a structural framework that supports tissue regeneration. Through these coordinated events, damaged tissues are repaired and normal structure and function are progressively restored.

IV.3.7. Regulation of the Inflammatory Response

To prevent excessive tissue damage and the development of chronic inflammation, the inflammatory response must be tightly regulated once the harmful stimulus has been eliminated. This regulation involves the production of anti-inflammatory cytokines, particularly IL-4 and IL-10**,** by macrophages and regulatory T lymphocytes (Treg cells). Growth factors are also released to promote tissue repair and regeneration. At the same time, the synthesis of pro-inflammatory mediators is suppressed, reducing leukocyte activation and inflammatory signaling. These mechanisms ensure the effective resolution of inflammation, the restoration of tissue homeostasis, and the prevention of chronic inflammatory processes.

IV.3.8. Type of inflammation

Depending on the time course and spatial evolution of the inflammatory response, inflammation can be classified into two main types:

- **Acute inflammation**

Acute inflammation is the rapid and early response of the body to a harmful stimulus such as infection, injury, or toxic agents. It develops quickly, usually within minutes or hours, and is of short duration, lasting from a few days to a few weeks. It is characterized by marked vascular and exudative changes, including increased blood flow, vascular permeability, and migration of immune cells (mainly neutrophils) to the affected tissue. Acute inflammation may resolve completely with restoration of normal tissue structure, especially after removal of the causative agent, or it may be controlled by treatment. However, in cases of severe tissue damage or persistent stimuli, it can lead to complications such as tissue necrosis, abscess formation, or progression to chronic inflammation with possible residual tissue scarring.

- **Chronic inflammation**

Chronic inflammation is a prolonged inflammatory response that does not resolve spontaneously and may persist for months or even years. It is characterized by continuous tissue injury and repair occurring simultaneously, leading to progressive tissue destruction and remodeling. This type of inflammation often results from persistent infections, prolonged exposure to irritants, autoimmune reactions, or failure of the acute inflammatory response to eliminate the causative agent. Unlike acute inflammation, chronic inflammation is dominated by the presence of mononuclear cells such as macrophages, lymphocytes, and plasma cells, and it is frequently associated with fibrosis and loss of normal tissue architecture over time.

IV.3.9. Role of Cytokines in Inflammatory Diseases

Pro-inflammatory cytokines, particularly TNF-α, IL-1β, and IL-6, play central roles in the pathogenesis of numerous inflammatory disorders. Their excessive production contributes to the development and persistence of diseases such as asthma**,** rheumatoid arthritis, and other chronic inflammatory conditions. As a result, these cytokines have become important targets for modern therapeutic interventions.

IV.3.10. Anti-Inflammatory Therapies and Mechanisms of Action

In certain pathological conditions, the inflammatory response fails to resolve and becomes chronic, leading to progressive tissue and organ damage. Chronic inflammation may result from excessive production of pro-inflammatory mediators, insufficient anti-inflammatory regulation, or persistent exposure to inflammatory stimuli. Consequently, inflammatory mediators and the cells that produce them represent important therapeutic targets for controlling inflammation and limiting tissue injury.

A. Targets of Anti-Inflammatory Therapies

Current anti-inflammatory therapies primarily target key mediators involved in the inflammatory process. Major therapeutic targets include the cyclooxygenase enzymes (COX-1 and COX-2), which are responsible for prostaglandin synthesis, as well as pro-inflammatory cytokines such as Tumor Necrosis Factor-alpha (TNF-α)**,** Interleukin-1 beta (IL-1β)**,** and Interleukin-6 (IL-6)**.** By inhibiting these molecules, anti-inflammatory treatments reduce inflammatory signaling and tissue damage.

B. Major Classes of Anti-Inflammatory Drugs

Two major classes of drugs are commonly used to control inflammation:

- **Corticosteroids**

Corticosteroids exert powerful anti-inflammatory and immunosuppressive effects by inhibiting the production of inflammatory cytokines and other mediators. They also reduce leukocyte activation, migration, and accumulation at sites of inflammation, thereby limiting tissue damage and suppressing excessive immune responses.

- **Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)**

NSAIDs act primarily by inhibiting COX-1 and/or COX-2 enzymes, leading to a reduction in prostaglandin synthesis. Because prostaglandins are major mediators of pain, fever, and inflammation, their inhibition produces significant anti-inflammatory, analgesic, and antipyretic effects. Common examples of NSAIDs include aspirin**,** ibuprofen**,** and diclofenac**.**

Together, these therapeutic approaches help control excessive inflammation, prevent tissue damage, and improve clinical outcomes in chronic inflammatory diseases.

V. Adaptive Immunity

V.1. Definition

Adaptive immunity, also known as acquired immunity, constitutes the second line of defense and is activated when the innate immune response fails to eliminate an invading pathogen. It is characterized by its high specificity toward antigens and its capacity to generate immunological memory, allowing a faster and more effective response upon subsequent exposure to the same microorganism. This system is mediated by lymphocytes, which specifically recognize foreign antigens and mount targeted immune responses.

Adaptive immunity comprises two complementary components: humoral immunity and cell-mediated immunity.

V.2. Specific Cell-mediated immunity

The specific cell-mediated immune response is mediated by **$CD4^+$ and $CD8^+$ T lymphocytes**. Unlike B lymphocytes, T cells are unable to recognize antigens in their native form. Instead, they recognize antigenic peptides that have been processed by antigen-presenting cells and displayed on the cell surface in association with **major histocompatibility complex (MHC)** molecules. $CD4^+$ T lymphocytes recognize peptides presented by **MHC class II** molecules, whereas $CD8^+$ T lymphocytes recognize peptides presented by **MHC class I** molecules. This antigen-specific recognition initiates T-cell activation and the development of an effective cellular immune response.

V.2.1. Antigen Presentation

At the onset of an immune response, the presence of an antigen triggers local inflammation. In response to inflammatory cytokines such as **IL-1** and **TNF-α**, produced by activated macrophages, local cells including epithelial cells, endothelial cells, and fibroblasts secrete chemokines. These chemokines recruit immune cells, particularly **monocytes** and **dendritic cells**, to the site of infection. Once recruited, these cells amplify the inflammatory response by producing additional chemokines that attract more immune cells.

Dendritic cells are professional **antigen-presenting cells (APCs)**. Initially immature, they capture and process antigens at the site of inflammation. During maturation, they increase in size, develop numerous dendritic extensions, and modify their pattern of chemokine receptor

expression. In particular, they acquire **CCR7**, enabling their migration through afferent lymphatic vessels toward regional lymph nodes in response to **CCL21**. Mature dendritic cells also exhibit increased and stable surface expression of **MHC class II molecules**, as well as **MHC class I molecules**, enhancing their antigen-presenting capacity. In addition, they express high levels of the co-stimulatory molecules **CD80 and CD86**, which are essential for the full activation of naïve T lymphocytes. They also transiently secrete a variety of cytokines, the nature of which depends on the initial stimulus encountered, The Table 7 summarizes the differences between mature and immature dendritic cells.

Upon reaching the lymph node via afferent lymphatic vessels, dendritic cells localize in the **paracortical region** around the **high endothelial venules (HEVs)**. This strategic positioning greatly increases the likelihood of interaction with naïve T lymphocytes entering the lymph node through the HEVs. Through antigen presentation, co-stimulation, and cytokine secretion, dendritic cells initiate the primary adaptive immune response and drive T-cell activation and differentiation.

Table 7 : Immature and mature dendritic cells

Criterion	Immature	Mature
HLA	(+)	+++ (except follicular DCs)
Costimulatory molecules: CD80, CD86, CD40, etc.	(+)	+++
CD83+ marker	–	++
CCL19/CCR7 CCL20/CCR6 CCL21/CCR6, CCR7	++	–
CCL3/CCR1, CCR5 CCL4/CCR5 CCL5/CCR1, CCR3, CCR5 CXCL1/CXCR1	–	++
Antigen endocytosis	+++	(+)
Antigen presentation	+	+++
Number of dendrites	+	+++
Presence in the blood	0.1–0.5%	–

V.2.2. Recognition and Activation of $CD4^+$ T Cells

Recognition and binding of the antigenic peptide presented by an antigen-presenting cell (APC) through the T-cell receptor (TCR) activates the $CD4^+$ T lymphocyte via the CD3 complex, which transduces an activation signal (Signal 1) to the $CD4^+$ T cell (Th0 cell).

The interaction between the co-stimulatory molecules CD80 (B7-1) or CD86 (B7-2) expressed on the APC and the CD28 receptor expressed on the $CD4^+$ T cell provides the second signal (Signal 2) required for complete T-cell activation. If a T lymphocyte receives Signal 1 (TCR binding to the antigen) but does not receive Signal 2 (co-stimulation), the T cell becomes anergic. In this state, it is unable to respond to any subsequent stimulation by the same antigen.

Following the reception of these two signals, the activated $CD4^+$ T lymphocyte produces interleukin-2 (IL-2) and expresses high-affinity IL-2 receptors (IL-2R). IL-2 induces the transformation of the activated T cell into a lymphoblast, which subsequently undergoes approximately ten rounds of cell division, resulting in clonal expansion.

At the same time, the APC is stimulated through the interaction between its surface molecule CD40 and CD40 ligand (CD40L, CD154) expressed by the activated T lymphocyte. This interaction induces the APC to produce interleukin-12 (IL-12). Under the influence of IL-12 (Signal 3), the daughter cells generated during clonal expansion differentiate into Th1 effector cells. A small proportion of these Th1 cells become memory T cells, retaining information about the initial encounter with the antigen.

Approximately 48 hours after activation, naïve T cells begin to express **CTLA-4 (Cytotoxic T-Lymphocyte Antigen 4)** on their surface. The interaction of CTLA-4 with **CD80** or **CD86** on APCs delivers an inhibitory signal that limits T-cell activation and prevents an excessive immune response (Figure 29).

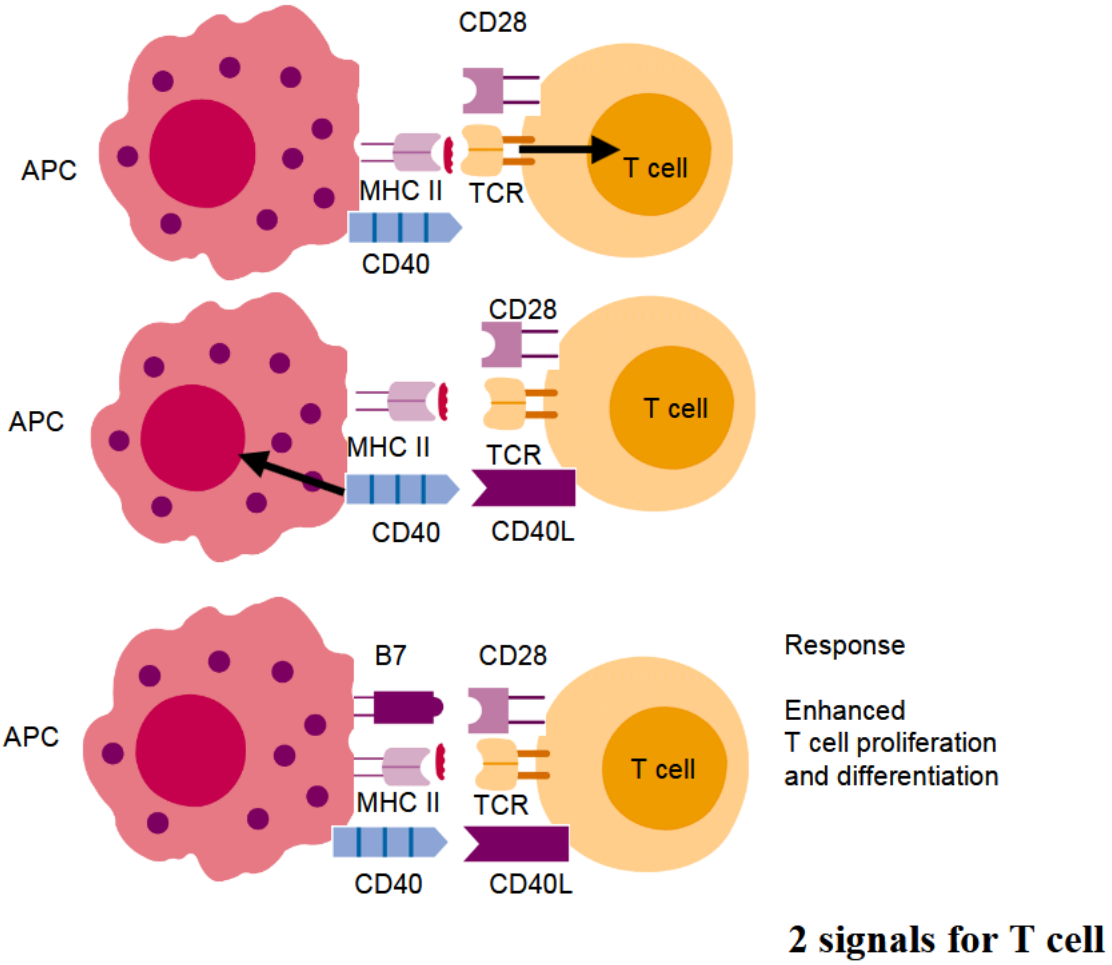


Figure 29 : T cell activation requires a co-stimulatory signal provided by antigen presenting cells (APCs).

- **Effector Functions of Th1 Lymphocytes**

Th1 lymphocytes (effector CD4⁺ T cells) leave the lymph node through the **efferent lymphatic vessel** and enter the bloodstream. They then migrate to the tissue where the pathogen initially invaded the body. At the site of infection, these cells coordinate the immune response by secreting cytokines that activate other immune cells, particularly macrophages, thereby enhancing the elimination of intracellular pathogens. At the site of infection, Th1 lymphocytes recognize antigenic peptides presented on the surface of **macrophages** and become reactivated. Upon activation, they express **CD40 ligand (CD40L, CD154)** on their surface and secrete several cytokines, the most important being **interferon-gamma (IFN-γ)** and **tumor necrosis factor-alpha (TNF-α).** Through the combined action of CD40L-CD40 interaction and these cytokines, Th1 cells activate macrophages and enhance their microbicidal functions.

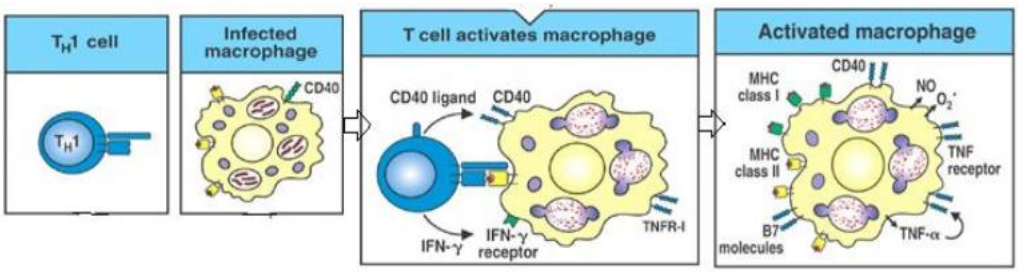


Figure 30 : Macrophage Activation.

- Activated macrophages exhibit:

- Enhanced microbicidal activity, characterized by increased production of reactive oxygen species (ROS) and nitric oxide (NO), as well as increased synthesis of enzymes such as hydrolases, proteases, and other lysosomal enzymes.
- Increased surface expression of MHC class II molecules, co-stimulatory molecules, and Fc receptors for IgG antibodies, thereby enhancing the phagocytosis of antigen–IgG immune complexes.

V.2.3. Recognition and Activation of $CD8^+$ T Lymphocytes (T8 Cells)

$CD8^+$ T lymphocytes specifically recognize self-cells infected by viruses, tumor cells, as well as allogeneic or xenogeneic cells. They express the surface molecule CD8 in humans and Ly2 in mice. These cells recognize antigen-derived peptides presented in association with MHC class I molecules. They can also recognize foreign MHC class I molecules expressed on allogeneic or xenogeneic cells.

V.2.3.1 Direct Activation of $CD8^+$ T Lymphocytes

A dendritic cell presents an antigenic peptide (of viral or tumor origin) bound to MHC class I molecules, providing the first activation signal (Signal 1) to the naïve $CD8^+$ T lymphocyte. At the same time, the dendritic cell delivers a strong co-stimulatory signal (Signal 2) through the interaction of B7 molecules (CD80/CD86) with CD28 on the T cell (Fig. 31).

This strong co-stimulatory signal fully activates the naïve $CD8^+$ T lymphocyte. Once activated, the $CD8^+$ T cell produces interleukin-2 (IL-2) and expresses high-affinity IL-2 receptors. IL-2 stimulates the proliferation and clonal expansion of the activated $CD8^+$ T cell.

The resulting daughter cells differentiate into:

- Effector $CD8^+$ T lymphocytes (cytotoxic T lymphocytes, CTLs or Tc cells), which are responsible for killing infected or abnormal cells.
- Memory $CD8^+$ T lymphocytes, which provide long-term immunological memory and enable a faster response upon subsequent exposure to the same antigen.

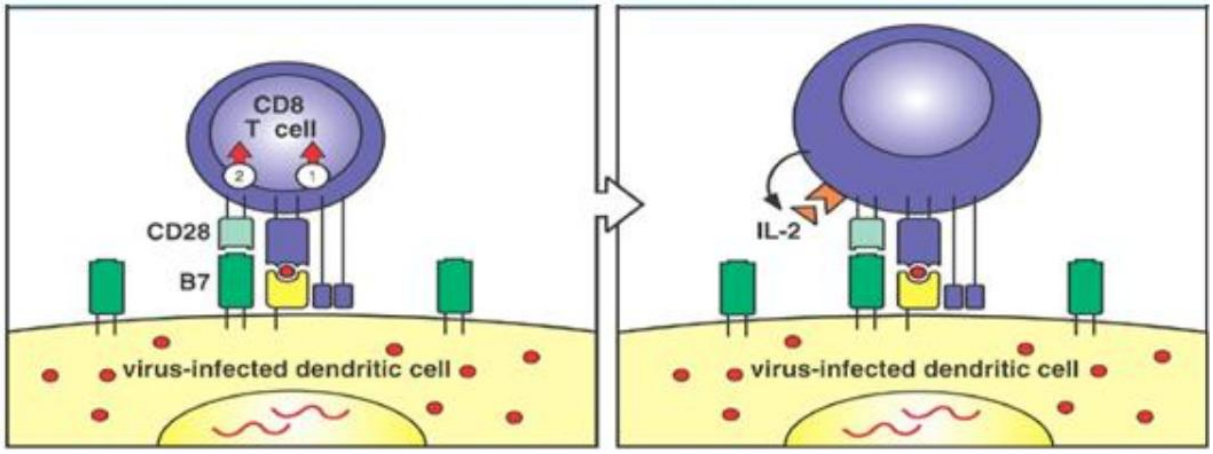


Figure 31 : Direct Activation of $CD8^+$ T Lymphocytes

V.2.3.2. Indirect Activation of $CD8^+$ T Lymphocytes

The activation of $CD8^+$ T lymphocytes in response to certain viral infections or during graft rejection often requires the assistance of **$CD4^+$ T lymphocytes**.

When a **naïve $CD4^+$ T lymphocyte** is activated by an antigen-presenting cell (APC), it produces **interleukin-2 (IL-2)**, which promotes the activation, proliferation, and differentiation of $CD8^+$ T lymphocytes.

Alternatively, when the $CD4^+$ T lymphocyte has already differentiated into an **effector Th1 cell**, its activation leads to the stimulation of the APC through interactions such as **CD40–CD40L.** As a result, the APC increases its expression of **co-stimulatory molecules (CD80/CD86)** and enhances its ability to activate naïve $CD8^+$ T lymphocytes.

In both cases, following clonal expansion and differentiation, the resulting **effector $CD8^+$ T lymphocytes (cytotoxic T cells, CTLs)** leave the lymph node and migrate through the bloodstream to the infected or tumor-bearing tissue, where they exert their cytotoxic functions. A small proportion of these activated cells differentiate into **memory $CD8^+$ T lymphocytes**, ensuring a more rapid and effective response upon subsequent exposure to the same antigen.

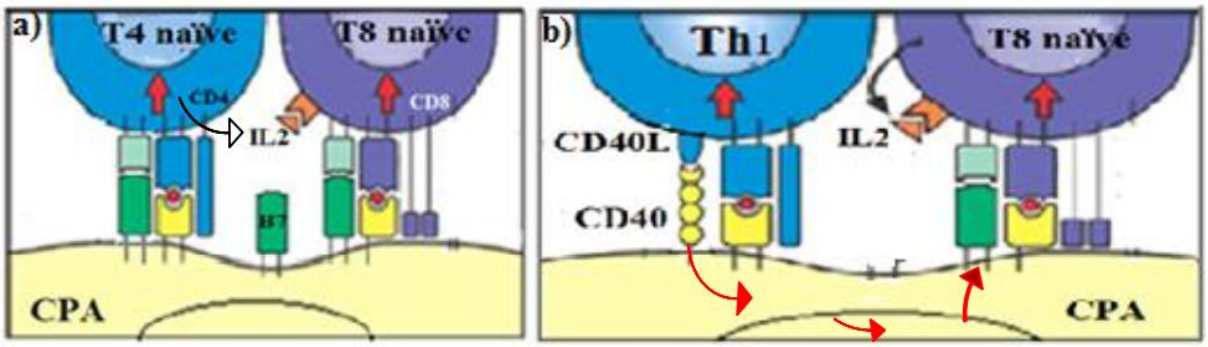


Figure 32 : Indirect Activation of $CD8^+$ T Cells.
(a) $CD4^+$ T cells produce **IL-2**, which activates $CD8^+$ T cells.
(b) Th1 cells activate the APC, which subsequently activates $CD8^+$ T cells.

V.2.4. Effector Mechanisms of $CD8^+$ T Lymphocytes

Within infected or tumor tissues, **effector $CD8^+$ T lymphocytes (cytotoxic T cells, CTLs or Tc cells)** specifically recognize viral peptides presented by **MHC class I molecules** on the surface of infected cells, or tumor-derived peptides associated with **MHC class I molecules** on tumor cells. This recognition activates the cytotoxic T lymphocytes and triggers the destruction of the target cells.

The cytotoxic mechanisms used by activated Tc cells are essentially the same as those employed by **natural killer (NK) cells**. These mechanisms include:

- **The perforin–granzyme pathway**, in which perforin forms pores in the target-cell membrane, allowing granzymes to enter the cell and initiate apoptosis.
- **The Fas–Fas ligand (FasL) pathway**, in which Fas ligand expressed on the activated Tc cell binds to the Fas receptor (CD95) on the target cell, triggering a cascade of intracellular signals that culminate in apoptosis.

Through these mechanisms, cytotoxic T lymphocytes induce **programmed cell death (apoptosis)** of infected or malignant cells while minimizing damage to surrounding healthy tissues.

- During a second or subsequent encounter with the same specific antigen, **memory T lymphocytes** recognize antigenic peptides presented by **MHC molecules** on antigen-presenting cells, particularly macrophages. Unlike naïve T cells, memory T cells respond much more rapidly. They quickly become activated, undergo proliferation, and differentiate into highly effective **effector T lymphocytes** as well as new **memory T**

lymphocytes. As a result, the **secondary immune response** is characterized by a shorter lag phase, greater intensity, and higher efficiency than the primary response. This enhanced response provides effective protection against reinfection by the same pathogen and constitutes the basis of long-term immunological memory.

V. 3. Humoral Adaptive Immune Response

V.3. 1. T-Independent Activation of B Lymphocytes

Most protein antigens require the assistance of helper T cells to induce an effective antibody response. However, certain non-protein antigens, particularly bacterial polysaccharides and lipopolysaccharides, can activate B lymphocytes without T-cell help. These antigens are called **T-independent (TI) antigens**. Two major categories of T-independent antigens are recognized. Type 1 T-Independent Antigens and Type 2 T-Independent Antigens.

- T-independent type 1 antigens are mainly components of bacterial cell walls. A typical example is **lipopolysaccharide (LPS)**, a major constituent of the cell wall of **Gram-negative bacteria**. In this response, the **first signal** is provided by the interaction between the **antigen and the B-cell receptor (Ag–BCR)**, while the **second signal** is delivered through the interaction between the **antigen and a mitogen receptor (Ag–mitogen receptor)**.
- **Type 2 T-Independent Antigens (TI-2) Type 2 T-independent antigens are characterized by highly repetitive epitopes, typically found in bacterial capsular polysaccharides. Their repetitive structure allows extensive cross-linking of B-cell receptors (BCRs), generating a strong activation signal.**

Unlike TI-1 antigens, TI-2 antigens activate B cells primarily through intense BCR cross-linking and require signaling molecules such as **Bruton's tyrosine kinase (Btk)**.

These antigens are particularly important for immunity against encapsulated extracellular bacteria whose polysaccharide capsules resist phagocytosis.

T-independent antigens induce little or no immunological memory and predominantly elicit IgM antibody responses.

V.3. 2. Response to T-Dependent Antigens

V.3. 2.1. Antigen Recognition by B and T Lymphocytes

T-dependent antigens are primarily protein antigens, such as diphtheria toxin and viral hemagglutinin. Their recognition by specific B lymphocytes, followed by activation, proliferation, and differentiation into antibody-secreting plasma cells, requires the cooperation of $CD4^+$ helper T lymphocytes.

B and T lymphocytes generally recognize different epitopes of the same antigen:

- B lymphocytes recognize the native (unprocessed) antigen directly through their B-cell receptor (BCR).
- $CD4^+$ T lymphocytes recognize only antigenic peptides generated by antigen processing and presented by antigen-presenting cells (APCs) in association with Major Histocompatibility Complex (MHC) class II molecules.

This cooperation between B and T lymphocytes is known as **linked recognition**.

V.3. 2.2. Activation of T and B Lymphocytes

The response begins when an antigen-presenting cell, primarily a dendritic cell, processes the antigen and presents antigenic peptides to a naïve $CD4^+$ T lymphocyte in association with MHC class II molecules. In the presence of appropriate co-stimulatory signals, the T cell becomes activated, proliferates, and differentiates into effector helper T cells and memory T cells.

At the same time, a specific B lymphocyte binds the native antigen through its BCR. This interaction provides the **first activation signal**. The B cell then internalizes the antigen, processes it into peptides, and presents these peptides on MHC class II molecules at its surface.

An activated helper T cell recognizes the peptide–MHC II complex displayed by the B cell through its T-cell receptor (TCR). This interaction induces the expression of CD40 ligand (CD40L) on the T-cell surface.

Binding of CD40L on the T cell to CD40 on the B cell delivers the **second activation signal**, which is essential for a full B-cell response. The helper T cell also secretes cytokines,

including IL-4, IL-5, IL-6, IL-10, IL-13, IL-21, and TGF-β, which promote B-cell proliferation and differentiation.

Following the reception of these two signals, the activated B cell enlarges and becomes a lymphoblast (Fig. 33).

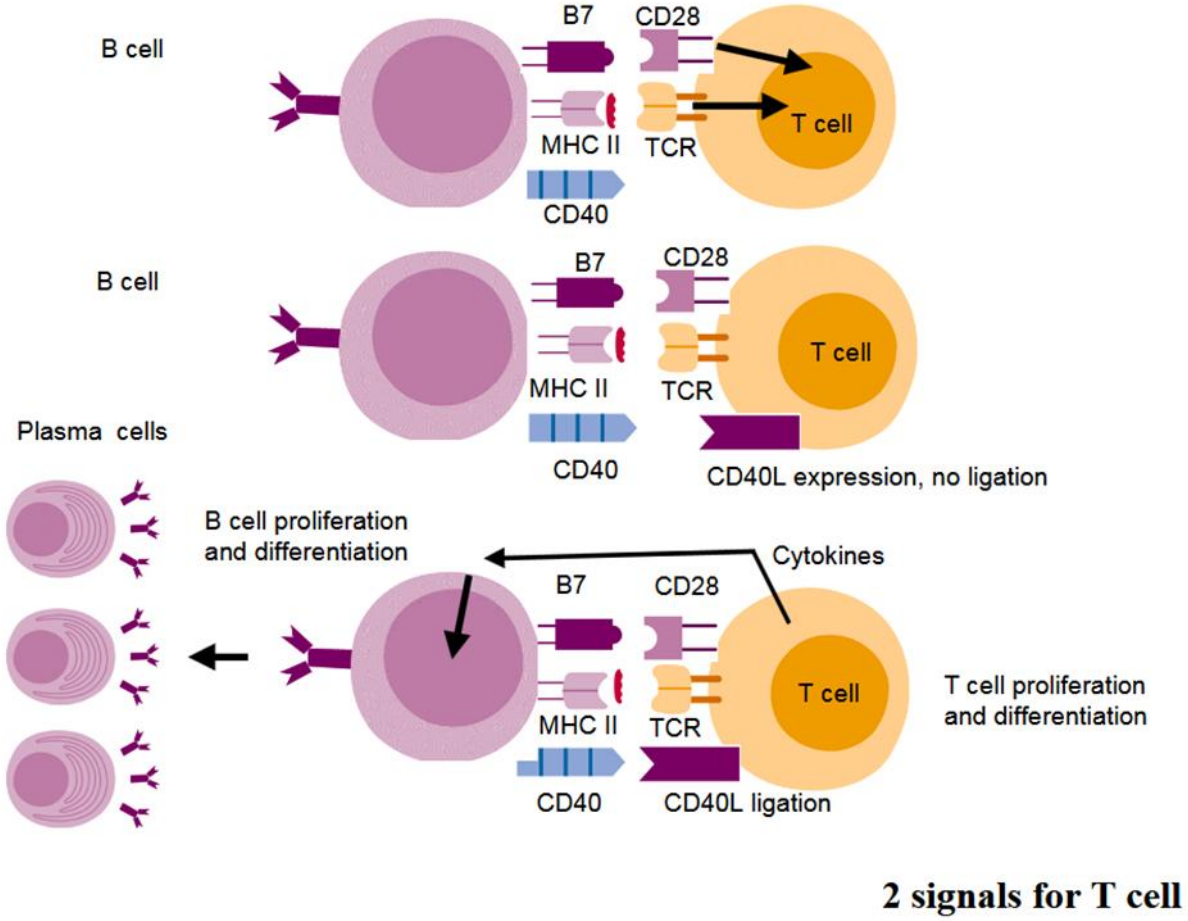


Figure 33 : Costimulatory Signals Required for T Cell Activation and B Cell Differentiation

V.3. 2.3. Proliferation and Differentiation

Under the influence of helper T-cell cytokines, the B lymphoblast undergoes multiple rounds of cell division. The resulting cells differentiate into:

- **Plasma cells**, which produce and secrete antigen-specific antibodies.
- **Memory B cells**, which provide a faster and stronger response upon subsequent exposure to the same antigen.

The primary antibody response is initially characterized by the production of IgM antibodies. Under the influence of CD40–CD40L interactions and cytokines produced by helper T cells, activated B lymphocytes undergo class-switch recombination and

Affinity maturation of antibodies subsequently produce other antibody isotypes, such as IgG, IgA, or IgE, depending on the cytokine environment.

A. The First Phase of the Humoral Response

1. The Spatiotemporal Challenge of Lymphocyte Encounter

One of the most puzzling features of the adaptive antibody response is how an antigen-specific B cell manages to successfully encounter a helper T cell with an appropriate corresponding antigen specificity. This biological dilemma arises primarily due to the extreme scarcity of naive lymphocytes specific for any single given antigen. It is estimated that the baseline frequency of naive lymphocytes responsive to a particular antigen lies between 1 × 10-4 and 1 × 10-6 (1 in 10,000 to 1 in 1,000,000).

Furthermore, compartmentalization worsens this challenge: under steady-state conditions, T cells and B cells occupy distinctly separate spatial zones within peripheral lymphoid organs. The resolution to this spatial paradox lies in antigen-specific trapping of migrating lymphocytes.

2. Mechanisms of Antigen-Specific Lymphocyte Trapping

When a foreign antigen enters an animal, it is captured, internalized, and processed by professional antigen-presenting cells (APCs). Crucially, tissue-resident dendritic cells migrate from the site of infection directly into the T-cell zones of local draining lymph nodes. Recirculating naive T cells continuously pass through these zones. Those rare, specific T cells whose T-cell receptors (TCR) bind the peptide-MHC complexes are efficiently trapped. This arrest is stabilized by the rapid activation of local adhesion molecules and chemokines.

An identical logic governs antigen-specific B-cell trapping within the T-cell zone:

Entry: Re-circulating B cells enter the lymphoid tissue from the bloodstream via high endothelial venules (HEVs), passing directly into the T-cell zone.

Pathways: While non-activated B cells rapidly move through the T-cell zone toward the primary follicles (B-cell zones), B cells that have bound their specific antigen are instantly arrested.

Molecular Mediators: This arrest and physical localization are driven by the engagement and upregulation of chemokine receptors, most notably CCR7, which senses the chemokines MIP-3β and SLC.

By selectively trapping antigen-binding B cells precisely within the T-cell zone, the lymphoid microenvironment maximizes the encounter probability between antigen-activated B cells and armed helper T cells. Once this specific interaction occurs, helper T cells deliver activation

signals, triggering the formation of a primary focus of clonal expansion located directly at the border between the T-cell and B-cell zones. Here, both populations undergo intensive proliferation for several days to drive the first phase of the primary humoral response (Fig.34).

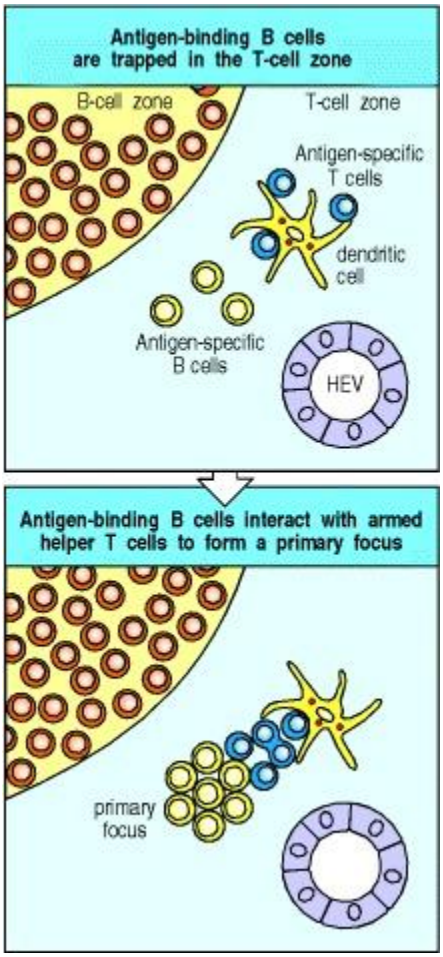


Figure 34 : Recruitment of Antigen-Specific B Cells to the T-Cell Zone and Formation of the Primary Focus

3. Terminal B-Cell Differentiation: Plasma Cells

After several days of rapid division, the primary focus begins to involute, and many of the constituent lymphocytes undergo programmed cell death (apoptosis). However, a portion of the proliferating B cells escape death by differentiating into antibody-synthesizing plasma cells. These cells leave the border zone and migrate to effector sites—specifically the red pulp of the spleen or the medullary cords of lymph nodes.

The differentiation into a plasma cell represents a profound morphological and functional commitment to high-rate protein synthesis. The table below show a detailed comparison of their cellular profiles (Table 8).

Table 8 : Phenotypic and Functional Changes During B-Cell Differentiation into Plasma Cells

	Property					
	Intrinsic			Inducible		
B-lineage cell	Surface Ig	Surface MHC class II	High-rate Ig secretion	Growth	Somatic hyper-mutation	Isotype switch
 Resting B cell	High	Yes	No	Yes	Yes	Yes
 Plasma cell	Low	No	Yes	No	No	No

While plasma cells no longer present antigen or interact dynamically with T cells, helper T cells continue to provide vital survival and differentiation factors, such as IL-6 and CD40L. Plasma cells exhibit variable lifespans: some survive for days to weeks, while a long-lived subset populates niches in the bone marrow to provide persistent, long-term humoral immunity.

4. The Second Phase of the Humoral Response: Germinal Centers

An alternative fate awaits a subset of the activated B cells and T cells proliferating within the primar yfocus: they migrate directly into primary lymphoid follicles to initiate the second phase of the primary response. Here, they proliferate exponentially to form a germinal center, transforming the primary follicle into a secondary follicle. A germinal center is structurally organized into distinct zones dictated by cell packing, division rates, and specialized cellular interactions:

Follicular Dendritic Cells (FDCs): FDCs form a dense network of cellular processes throughout the follicle. They secrete the chemokine BLC, which powerfully attracts both naive and activated B cells into the follicular microenvironment.

The Dark Zone (Centroblasts): This zone is characterized by densely packed, rapidly dividing B cells known as centroblasts. Centroblasts divide every 6 to 8 hours and dramatically downregulate their surface immunoglobulin expression (especially IgD).

The Light Zone (Centrocytes): As centroblasts reduce their division rate, they upregulate surface immunoglobulins and transition into centrocytes, migrating into the light zone. This

zone is rich in FDCs and antigen-specific helper T cells (which comprise ~10% of germinal center lymphocytes).

4. Somatic Hypermutation and Affinity Maturation

The core function of the germinal center reaction is to drastically optimize the quality of the ongoing and future antibody response. This is achieved via two tightly coupled processes: Somatic Hypermutation (SHM) and Affinity Maturation.

The Mechanics of Somatic Hypermutation

Unlike baseline DNA replication, somatic hypermutation introduces random single point mutations selectively into the rearranged immunoglobulin V-region genes.

Hypermutation Rate Kinetics:

The mutation rate in the V-region is approximately 1 × 10-3 base pair changes per cell division. In stark contrast, standard somatic cell DNA mutation rates are around 1 × 10-10. Because heavy- and light-chain V-region genes are each roughly 360 base pairs long, and ~75% of base changes alter an amino acid, virtually every second B cell acquires a new structural mutation during each cell division.

Selection and Macrophage Clearance

Because these mutations are random, their functional consequences vary widely:

Deleterious Mutations (Common): Many mutations create premature stop codons or disrupt critical structural framework folds, abolishing B-cell receptor (BCR) expression. These defective cells are rapidly driven into apoptosis. To handle this immense cellular waste, specialized tingible body macrophages engulf the dark-staining apoptotic debris, serving as a classic histological hallmark of active germinal centers.

Beneficial Mutations (Rare): A small minority of mutations alter the complementarity-determining regions (CDRs) in a manner that increases the binding affinity for the target antigen. Selection is strictly incremental. Centrocytes displaying higher-affinity receptors successfully compete to bind antigen presented on FDCs and receive survival signals from follicular helper T cells.

Surviving cells undergo subsequent rounds of division and mutation, continuously refining antibody affinity.

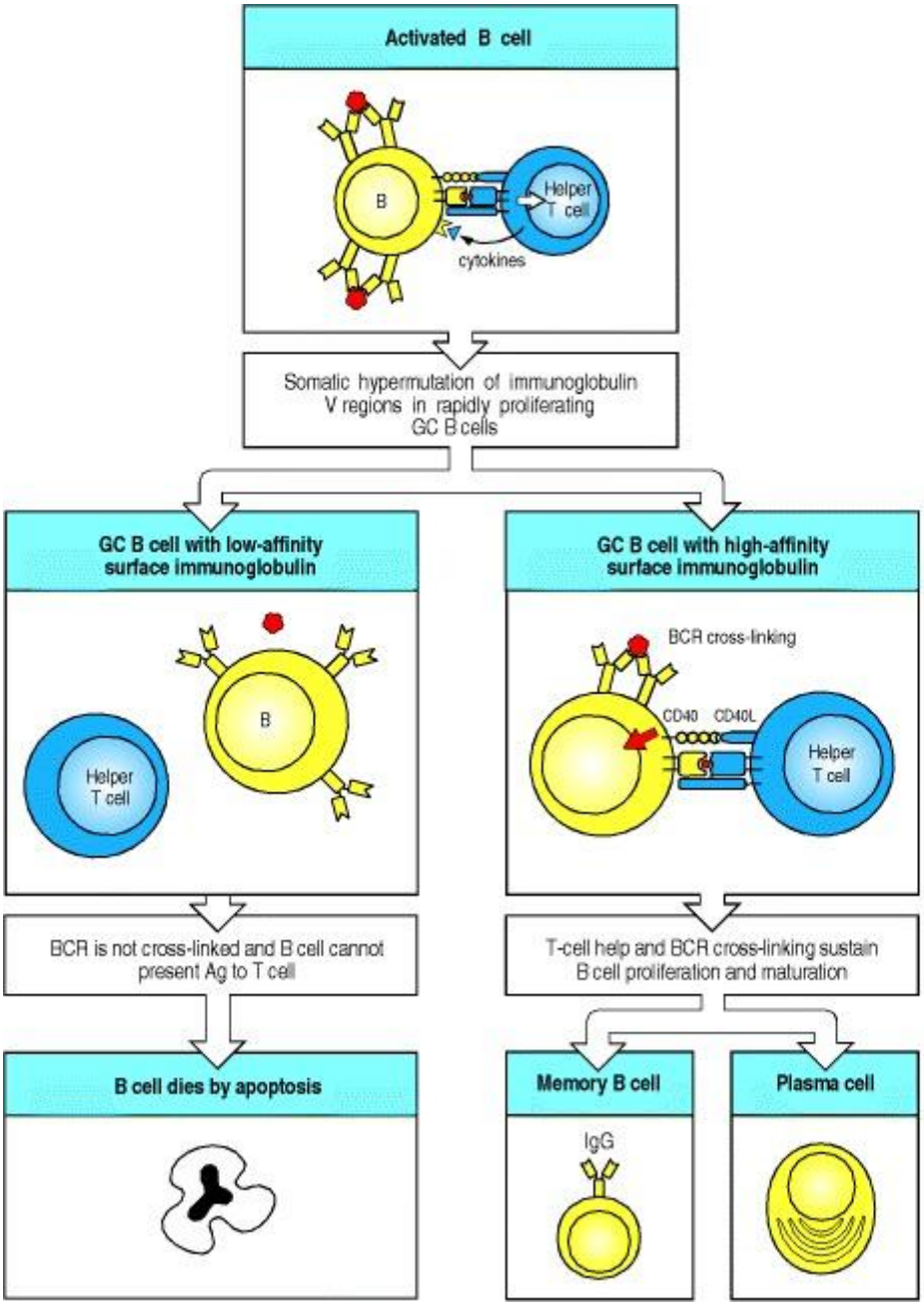


Figure 35 : Affinity Maturation and Selection of High-Affinity B Cells in the Germinal Center

After activation by antigen and helper T cells, B cells enter the germinal center (GC), where they proliferate rapidly and undergo somatic hypermutation in the variable (V) regions of their immunoglobulin genes. This process generates B cells with receptors of different affinities for the antigen

6. Regulation of Isotype Switching by T-Cell Cytokines

While naive B cells co-express surface IgM and IgD, a protective immune response requires diverse antibody effector functions. Isotype switching alters the heavy-chain constant (C) region while preserving antigen specificity. This process is strictly dependent on the physical expression of CD40L by helper T cells interacting with CD40 on B cells; individuals lacking functional CD40L suffer from severe hyper-IgM immunodeficiency.

The specific immunoglobulin class produced is directed by cytokines secreted by distinct helper T cell subsets, which activate transcription from specific switch recombination sites located 5′ to each heavy-chain C gene (Table 9).

Table 9 : Different cytokines induce switching to different isotypes

The individual cytokines induce (violet) or inhibit (red) production of certain isotypes. Much of the inhibitory effect is probably the result of directed switching to a different isotype. These data are drawn from experiments with mouse Cells.

Role of cytokines in regulating Ig isotype expression							
Cytokines	IgM	IgG3	IgG1	IgG2b	IgG2a	IgE	IgA
IL-4	Inhibits	Inhibits	Induces		Inhibits	Induces	
IL-5							Augments production
IFN-γ	Inhibits	Induces	Inhibits		Induces	Inhibits	
TGF-β	Inhibits	Inhibits		Induces			Induces

V.4. Antibody-mediated Immunity

V.4.1. Definition of Immunoglobulins (Igs)

Immunoglobulins (Igs) are antigen-binding glycoproteins produced by **plasma cells**, the differentiated form of **B lymphocytes**, in response to antigenic stimulation. They specifically recognize and bind foreign substances known as **antigens**, playing a central role in the **humoral immune response.** Immunoglobulins exist in two forms:

- As **membrane-bound molecules** on the surface of B lymphocytes, where they function as **B-cell receptors (BCRs).**

- As **soluble proteins** secreted by plasma cells into the blood and body fluids.

When immunoglobulins are secreted and participate in antigen recognition and immune effector mechanisms, they are referred to as **antibodies.**

V.4.2. General Organization of Immunoglobulins

Immunoglobulins (Igs) are Y-shaped glycoproteins composed of **four polypeptide chains** linked by disulfide bonds: **two identical heavy (H) chains** and **two identical light (L) chains**. The molecular structure of immunoglobulins is highly conserved and is organized into **variable (V) regions** and **constant (C) regions**.

The **amino-terminal portions** of both heavy and light chains form the **variable regions,** which are responsible for antigen recognition and binding. These regions exhibit considerable sequence diversity among different antibodies, allowing the immune system to recognize a vast array of antigens. The remainder of each chain constitutes the **constant regions**, which are relatively conserved and determine the biological properties and effector functions of the antibody.

Each heavy and light chain contains one variable domain composed of approximately **100–110 amino acids.** The variable regions of one heavy chain and one light chain combine to form an **antigen-binding site (paratope).** Sequence variations within these regions provide antibody specificity.

The constant regions exhibit limited variation and are responsible for: Determining the antibody isotype, Activating the complement system, Binding to Fc receptors on immune cells, Mediating biological effector functions.

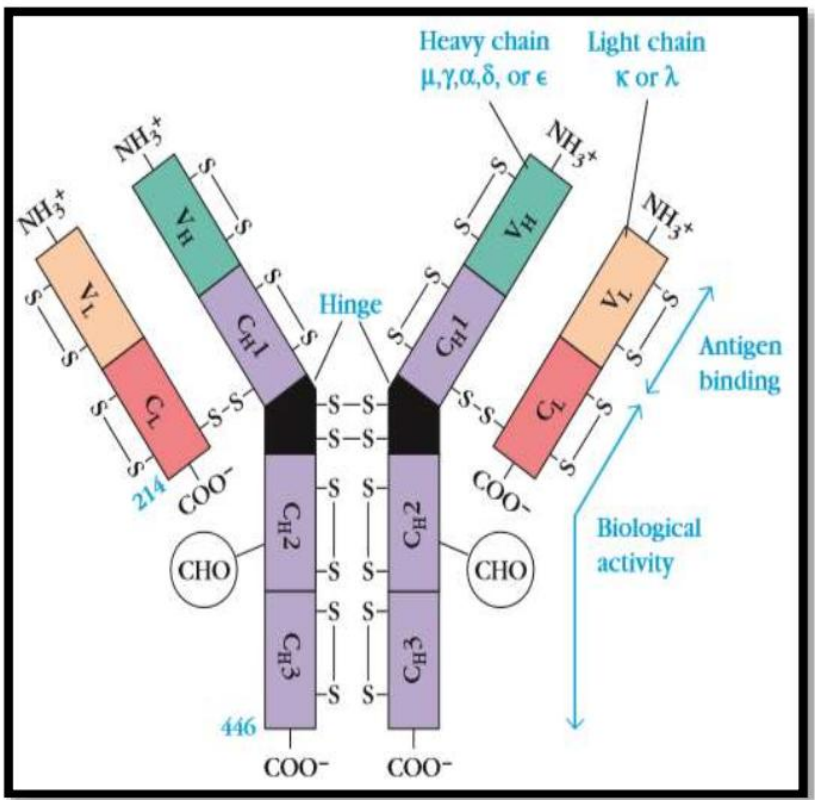


Figure 36 : shematic diagram of immunoglobilins

V.4.2.1. Types of light chain

All light chains have protein with molecular weight of about 23,000 and can be classified in to two distinct types, kappa (k) and lambda (λ), on the basis of their constant light region sequence. There is no known functional difference between these two types. A given immunoglobulin molecule always contains exclusively either k or λ chains never a mixture.

Similarly, any given B-lineage cell produces only one type of light chain.

The proportion of K to λ chain in the entire population of immunoglobulin in an individual is about 2:1 but it may vary from species to species.

V.4.2.2. Types of heavy chains

Human express five different classes of immunoglobulin heavy chains, which differ considerably in their constant heavy region, which in turn result difference in their physical and biologic properties. All of the heavy chains in a given immunoglobulin molecule are identical. The heavy chain polypeptides range in molecular weight from about 50,000- 70,000.

The classes of heavy chains are designated as μ, δ, α, γ and Σ. The immunoglobulins that contain these heavy chains are designated as IgM, IgD, IgG, IgA & IgE, classes, respectively.

V.4.3. Classes of immunoglobulins

Five different classes of immunoglobulins have been identified (IgG, IgM, IgA, IgD, and IgE), based on structural differences in the composition of their heavy chains. Some of the immunoglobulin classes contain subclasses.

V.4.3.1. IgG (Immunoglobulin G)

IgG is the most abundant class of immunoglobulin found in human serum, representing approximately 80% of the total serum immunoglobulins. It has a molecular weight of about 150,000 Da and contains gamma (γ) heavy chains, each weighing approximately 53,000 Da. Structurally, an IgG molecule is composed of two gamma heavy chains and either two kappa (κ) light chains or two lambda (λ) light chains. IgG exists in four subclasses: IgG1, IgG2, IgG3, and IgG4, which are classified according to their decreasing serum concentrations.

Among all antibodies, IgG has the longest half-life, which is about 23 days. It is also the only antibody capable of crossing the placenta, thereby providing passive natural immunity to the fetus and protecting the newborn for up to six months after birth. In addition, IgG can activate the complement system and is able to cross blood vessels, allowing it to play an important role in immune defense throughout body tissues.

IgG is the major antibody produced during the secondary immune response. Among its subclasses, IgG1, IgG3, and IgG4 can readily cross the placenta and therefore play an important role in protecting the fetus. IgG3 is the most effective activator of the complement system, followed by IgG1 and IgG2, whereas IgG4 is unable to activate complement. In addition, IgG1 and IgG3 bind with high affinity to Fc receptors present on phagocytic cells, thereby promoting opsonization and enhancing phagocytosis. IgG also contributes to bacterial immobilization and plays a crucial role in neutralizing toxins and viruses.

V.4.3.2. IgM (Immunoglobulin M)

IgM is a large immunoglobulin with a molecular weight of approximately 900,000 Da. Its heavy chain type is mu (μ), with a molecular weight of about 65,000 Da. IgM represents about 5–10% of the total serum immunoglobulins and has an average serum concentration of approximately

1.5 mg/dl. It is secreted by plasma cells and mainly exists in a pentameric form, in which five IgM monomers are linked together by disulfide bonds through a joining (J) chain. Due to its large size, IgM is often referred to as the “millionaire molecule.”

Pentameric IgM possesses ten antigen-binding sites (Fab regions); however, because of steric hindrance, it cannot bind ten complete antigens simultaneously. IgM is the major antibody produced during the primary immune response, making it the first immunoglobulin to appear after initial exposure to an antigen. In addition, the monomeric form of IgM, with a molecular weight of approximately 180,000 Da, is expressed as a membrane-bound receptor on B lymphocytes.

IgM) is the very first antibody produced during a primary immune response, and it also holds the distinction of being the first immunoglobulin synthesized by a neonate. Because it naturally forms a pentamer, IgM possesses a higher valency, meaning it features a greater number of antigen-binding sites. While your notes contain a small typo attributing this next feature to IgG, it is actually this unique pentameric structure that makes **IgM** highly effective in agglutination reactions. Furthermore, IgM is significantly more efficient than IgG when it comes to complement activation. Thanks to its J-chain, IgM also plays an essential accessory role as a secretory immunoglobulin.

V.4.3.3. Immunoglobulin A (IgA)

Immunoglobulin A (IgA) has a molecular weight of approximately 320,000 Da and features an Alpha-type heavy chain (H-chain) weighing 55,000 Da. Making up about 10% to 15% of the total serum immunoglobulins, IgA serves as the predominant antibody found in external bodily secretions. It can be found protecting the body within breast milk, saliva, tears, and the mucus linings of the bronchial, genitourinary, and digestive tracts. Structurally, IgA primarily exists in a monomeric form, though dimeric, trimeric, and occasional tetrameric variations can also be found.

Immunoglobulin A (IgA) exhibits different structural forms depending on its location in the body; it occurs as a monomer within the bloodstream, whereas it takes on dimeric or multimeric forms when found in bodily secretions. The dimeric form of IgA is characterized by the presence of a J-chain and a secretory chain, the latter of which plays a crucial role in facilitating **transcytosis**. Transcytosis is the specific biological process that allows IgA to cross the

epithelial cell layer so it can successfully enter body secretions and provide localized mucosal immunity. Additionally, IgA is classified into two distinct subclasses, namely **IgA1** and **IgA2**.

By successfully crossing the epithelial cell layer, Immunoglobulin A (IgA) enters into body secretions where it provides vital localized immunity within the gastrointestinal (GI), respiratory, and genital tracts, among others. Once present in these external secretions, IgA functions as a primary line of defense by neutralizing viruses and preventing pathogens from attaching to host cell surfaces, effectively blocking the initial steps of infection.

V.4.3.4. Immunoglobulin D (IgD)

Immunoglobulin D (IgD) has a molecular weight of 180,000 Da and features a Delta-type heavy chain (H-chain) weighing 70,000 Da. It is found in extremely low concentrations in the body, accounting for only about 0.2% of the total serum immunoglobulins. Alongside IgM, IgD serves as a major membrane-bound immunoglobulin expressed on the surface of mature B-cells. Structurally, it is divided into two subclasses—**IgD1** and **IgD2**—and it plays a critical role in the overall maturation and proliferation of B-cells.

While the exact biological function of IgD is not fully established, it is thought to be involved in lymphocyte activation and suppression. Unlike other immunoglobulins, IgD is incapable of activating the complement system, crossing the placenta, or causing mast cell degranulation.

V.4.3.5. Immunoglobulin E (IgE)

Immunoglobulin E (IgE) has a molecular weight of 200,000 Da and features an epsilon-type heavy chain (H-chain) weighing 73,000 Da. It is present in very small quantities, accounting for just 0.3% of the total serum immunoglobulins in a normal individual. IgE is famously known as the reagenic antibody due to its direct involvement in allergic reactions. It mediates immediate hypersensitivity reactions and is responsible for typical clinical symptoms such as hay fever, asthma, and severe anaphylactic shocks.

Mechanistically, the Fc region of IgE binds specifically to blood basophils and tissue mast cells. When an antigen subsequently cross-reacts with this bound IgE, it triggers the degranulation of these mast cells and basophils. This process releases histamine into the body, which is the primary chemical mediator responsible for producing allergy symptoms.

In terms of defense, IgE provides essential immunity against parasitic infections by driving Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC). While the baseline level of IgE antibody in the blood of a healthy individual is remarkably low, these levels noticeably spike during active parasitic infections and allergic reactions.

Table 10 : Properties of immunoglobulins.

Property	IgA	IgD	IgE	IgG	IgM
Molecular weight	160,000 or 385,000 (dimer)	184,000	188,000	146,000	970,000(pentamer)
Heavy chain	α	δ	ε	γ	μ
Light chain	λ, κ	λ, κ	λ, κ	λ, κ	λ, κ
Serum conc. (mg/ml)	4	0.03	0.005	12	1.2
Hlaf-life (days)	6	3	2	23	5
Subclass isotypes	2	1	1	4	1
Carbohydrate (%)	10	13	10	3	10
Complement fixation	No	No	No	Yes	Yes

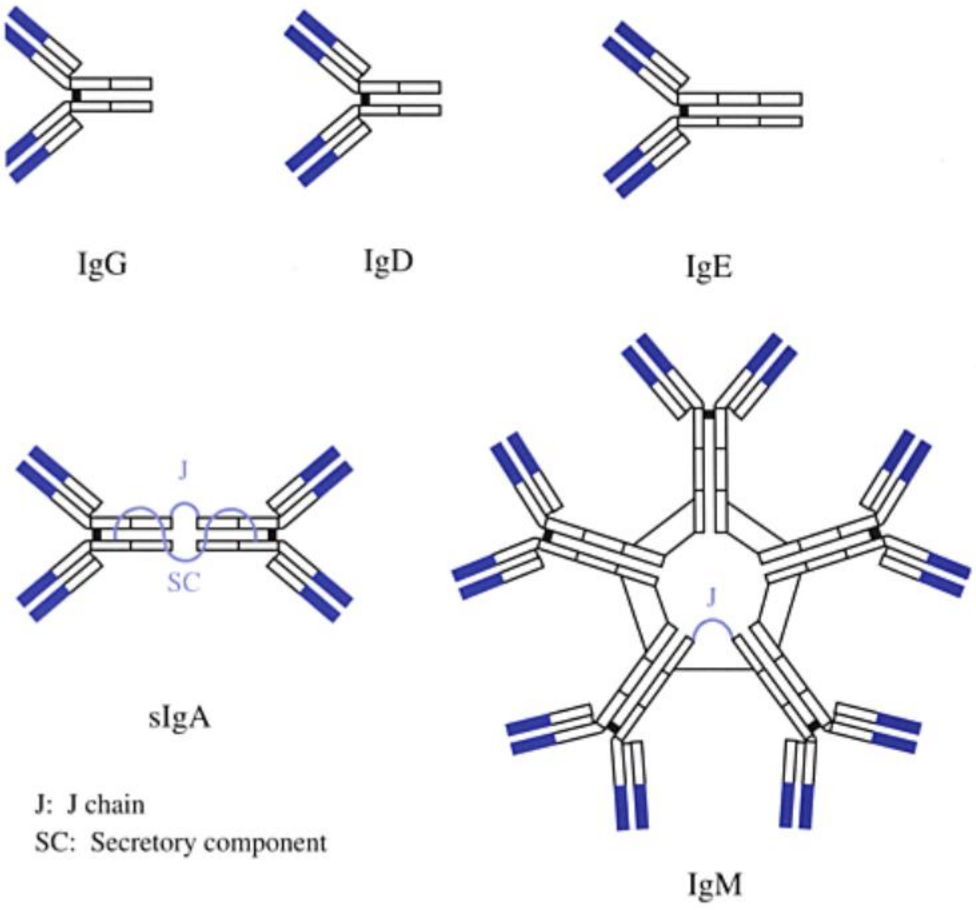


Figure 37 : Structural models of IgG, sIgA, IgD and IgM.

V.4.4. Functions of immunoglobulins

The primary function of immunoglobulins is to serve as antibodies. This is accomplished through the molecule's antigen binding (Fab) portion.

The size of the immunoglobulin molecule is one of the factors determining its tissue distribution. IgG is the major immunoglobulin in the bloodstream and accounts for about 80% of the total immunoglobulin pool. IgG is also present in the tissue spaces. It easily passes through the placenta. IgG is responsible for neutralization of viruses and bacterial toxins, facilitating phagocytosis, and lysing (destroying) bacteria.

IgM, the largest immunoglobulin, is confined mainly to the bloodstream and is less able to pass through capillary walls. IgM does not pass through the placenta. With its 10-valent antigen-combining site, IgM has a high affinity, i.e. a strong ability to bind firmly with antigen. IgM is particularly effective in the complement-mediated lysis of microorganisms.

IgA is the second most abundant immunoglobulin in serum. IgA is the predominant immunoglobulin in secretions of the gastrointestinal and respiratory tracts as well as in human colostrum and milk. IgA provides local mucosal immunity against viruses and limits bacteria overgrowth on mucosal surfaces. IgA also functions in the gastrointestinal tract and shows a greater resistance to proteolytic enzymes that other classes of antibodies.

V.4.5. Placental transfer of immunoglobulins

Maternal IgG (but not IgM or IgA) is transported across the placenta from about 16 weeks onwards. This reflects passive transport, which increases progressively with gestation and is proportional to the maternal IgG concentration. It also reflects active transport, which tends to normalize neonatal IgG concentration, suggesting that low maternal values stimulate and high maternal values inhibit transport. At full term, umbilical cord levels of IgG can be equal to or even higher than maternal levels. Premature infants have lower IgG levels than those delivered at term. Passively-acquired IgG antibodies are responsible for the protection of newborns and young infants against viral and bacterial diseases. The transfer of IgG antibodies from mother to fetus across the placenta provides the newborn with a portion of the mother's immunologic experience.

This experience is different in areas where infectious agents circulate at high levels in the population and adults are naturally immune, compared to areas where the circulation of infectious agents is limited and adults have low levels of immunity. In developing countries,

passive transfer occurs for diphtheria, measles, polio, and rubella antibodies. Also, tetanus antibodies induced in the mother following immunization

with tetanus toxoid easily pass across the placenta providing protection against tetanus in the newborn. In developed countries, where women of child- bearing age may have low levels of polio and diphtheria antibodies, transfer of these antibodies is more limited. If protective maternal antibodies are not of the IgG type, as is usually the case for the gramnegative pathogens such as ***Escherichia coli*** and ***Salmonella,*** the fetus does not receive antibodies from the mother and the neonate is not passively protected against these infections.

V.4.6. Normal development of serum immunoglobulins

Immunoglobulin synthesis starts before birth. IgM has been shown to be present at 10 weeks, IgG at 12 weeks, and IgA at 30 weeks of gestation. Most of the antibody synthesized by the fetus is IgM. Nevertheless, the fetus grows in a sterile environment and production of immunoglobulins by the healthy fetus is extremely limited until birth. In some fetuses immunoglobulin synthesis may be delayed or may not occur at all.

In the first year of life immunoglobulin levels increase quickly under the influence of antigenic challenge from the environment (infections) and through the contact with vaccine antigens. By one year of age, values for the IgG, IgM, and IgA concentrations are approximately 60%, 100%, and 30%, respectively, of those in adults.

The newborn infant is capable of responding to a number of diverse antigens, but to fewer and at a much reduced level compared with an adult. There is little or no response to polysaccharide antigens. The relative inefficiency of the fetal and newborn humoral immune response reflects both immaturity in the antibody-producing B cells and plasma cells and poor T cell — B cell cooperation.

Passively transferred antibody, especially at high levels, may transiently suppress the response of the infant to specific antigens. This phenomenon has influenced the schedule for some immunizations. Measles immunization, for example, is postponed until 9 months of age when placentally transferred antibodies have fallen to low concentrations . A high level of passively acquired diphtheria, tetanus, and pertussis antibodies may inhibit the response to all components of DPT vaccine during the first weeks of life. This is the reason for delaying the administration of the first dose of DPT vaccine until 6 weeks of age. This inhibitory effect is transient and it diminishes following subsequent doses of DPT vaccine.

Infants born prematurely and infants small for gestational age respond to immunization as well as term infants of a similar postnatal age.

V.5. Primary and Secondary Adaptive Immune Responses

When the immune system encounters an antigen, it can generate either a **primary immune response** or a **secondary immune response**, depending on whether the antigen has been encountered previously.

V.5.1. Primary Immune Response

The primary immune response occurs during the **first exposure** to a pathogen or antigen. Naïve B and T lymphocytes must first recognize the antigen, become activated, proliferate, and differentiate into effector and memory cells.

This response is characterized by:

- **Slow onset:** several days are required before detectable immune activity appears.
- **Low intensity:** relatively few effector cells and antibodies are produced.
- **Limited effectiveness:** pathogen elimination is less efficient than in subsequent exposures.
- **Short duration:** the response declines after antigen clearance.

Despite its relatively modest effectiveness, the primary response leads to the generation of **memory lymphocytes**, which are essential for long-term protection.

V.5.2. Secondary Immune Response

The secondary immune response occurs during **subsequent exposures** to the same antigen. Because memory B and T lymphocytes have already been generated during the primary response, immune activation is more rapid and effective.

This response is characterized by:

a. Faster Response

Memory lymphocytes are activated rapidly, and clonal expansion begins within approximately 24 hours, compared with several days during the primary response.

b. Greater Intensity

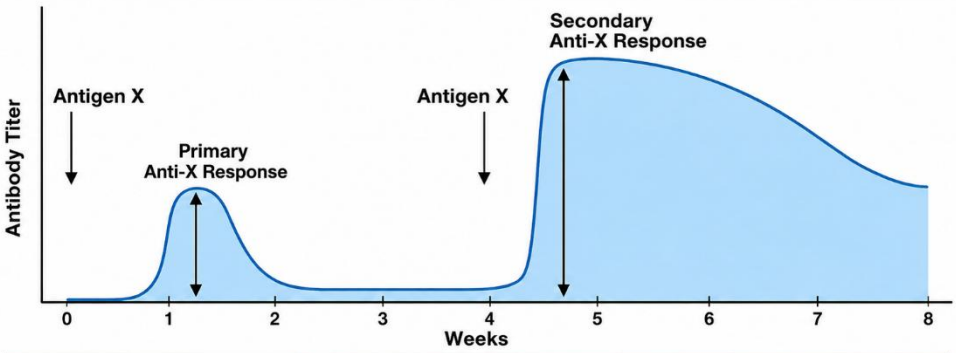
A much larger number of effector lymphocytes and antibodies are produced, resulting in more efficient pathogen elimination.

c. Longer Duration

The immune response persists for a longer period and may provide protection for years or even decades, forming the basis of vaccine-induced immunity.

d. Higher Specificity and Affinity

Memory lymphocytes possess receptors with increased affinity for the antigen due to affinity maturation, allowing more effective recognition and elimination of the pathogen.



Properties	Primary Response	Secondary Response
Types of B cells involved	Naïve B cells	Memory B cells
Latency period after antigen recognition by B cells	4–7 days	1–3 days
Time to reach maximum response	7–10 days	3–5 days
Magnitude of peak antibody response	Depends on the antigen	100–1000 times higher than the primary response
Predominant antibody isotypes produced	IgM predominant	IgG predominant
Antibody affinity	Lower	Higher

Figure 38 : Characteristics of the Primary and Secondary Immune Responses.

V.6. Classification of Adaptive Immunity

Adaptive immunity can be classified into **active immunity** and **passive immunity**, depending on how protection is acquired.

V.6.1. Active Immunity

Active immunity results from the activation of an individual's own immune system following exposure to an antigen. It is characterized by the production of antigen-specific lymphocytes and antibodies, as well as the development of immunological memory. Consequently, active immunity is generally long-lasting and becomes stronger with repeated antigen exposure.

a. Naturally Acquired Active Immunity

Naturally acquired active immunity develops following natural infection by a pathogen. Recovery from the infection leads to the formation of memory cells that provide protection against future encounters with the same pathogen. However, the duration and degree of protection vary among diseases.

b. Artificially Acquired Active Immunity

Artificially acquired active immunity is induced through **vaccination**. Vaccines expose the immune system to harmless forms or components of pathogens, stimulating the development of protective immunity without causing the disease itself. This approach exploits the immune system's capacity to generate memory cells for preventive purposes.

V.6.2. Passive Immunity

Passive immunity results from the transfer of preformed antibodies from one individual to another. Unlike active immunity, passive immunity provides **immediate protection** but does not induce immunological memory and is therefore temporary.

a. Naturally Acquired Passive Immunity

Naturally acquired passive immunity occurs when maternal antibodies are transferred to the child:

- Through the **placenta** during fetal development (mainly IgG).
- Through **colostrum and breast milk** after birth (mainly IgA).

These antibodies provide protection during the first months of life while the infant's immune system matures.

b. Artificially Acquired Passive Immunity

Artificially acquired passive immunity involves the administration of antibodies produced by another human or animal. It is used for the prevention or treatment of certain infections, toxin exposures, and other medical emergencies requiring immediate protection.

Chapter VI : Immune System Dysfunction

VI.1. Autoimmunity and Autoimmune Diseases

VI.1.1. Definition

Autoimmunity results from defects in the establishment or maintenance of **self-tolerance** by the immune system. In both animals and humans, the breakdown of self-tolerance leads to the activation of **autoreactive T and B lymphocytes**, resulting in the production of effector cells and **autoantibodies** that recognize the body's own constituents. These immune responses cause cellular and tissue damage and, in some cases, lead to clinical manifestations responsible for **autoimmune diseases**.

Autoimmune diseases are common, with an overall prevalence of approximately **5%**, and represent an important cause of morbidity and mortality in developed countries. They are highly heterogeneous and are generally classified into two major categories:

- Organ-Specific Autoimmune Diseases

In these diseases, autoantibodies or autoreactive T lymphocytes are directed against antigens restricted to a particular tissue or organ. Consequently, the damage is mainly confined to that specific organ. **Examples:** Type 1 diabetes mellitus, Hashimoto's thyroiditis

- Systemic Autoimmune Diseases

Also known as **non-organ-specific autoimmune diseases**, these disorders involve autoantigens that are widely distributed throughout the body. The formation of circulating immune complexes often contributes to the development of systemic disease, resulting in diffuse and diverse manifestations affecting multiple organs over time. **Example:** Systemic lupus erythematosus (SLE)

Autoimmune diseases are considered **multifactorial disorders**, resulting from the interaction of: Genetic predisposition, Environmental factors, Hormonal influences.

However, despite extensive research, the exact cause remains unknown in the majority of cases.

VI.1.2. Components of the Autoimmune Response

Autoimmune diseases occur when the breakdown of **self-tolerance** leads to cellular or tissue damage mediated by **autoreactive T lymphocytes** and/or **B lymphocytes** that produce **autoantibodies** directed against self-antigens (**autoantigens**). These immune responses mistakenly target the body's own tissues, resulting in chronic inflammation and progressive tissue injury.

1. Autoantigens

Autoantigens are self-components recognized by the immune system during autoimmune diseases. They may be **organ-specific**, being restricted to a particular organ or cell type, such as thyroperoxidase in thyroid cells in Hashimoto's thyroiditis, insulin-producing β-cells in type 1 diabetes mellitus, or red blood cell surface antigens in autoimmune hemolytic anemia. Alternatively, they may be **ubiquitous**, being present in most or all cells of the body, such as DNA, nuclear proteins, and mitochondrial proteins, which are common targets in systemic autoimmune diseases.

2. Autoreactive T and B Lymphocytes

A small population of autoreactive T and B lymphocytes is normally present even in healthy individuals because some cells escape the mechanisms of central tolerance in the thymus and bone marrow. However, their activation is tightly controlled by peripheral tolerance mechanisms, including suppression by regulatory T cells (Tregs), induction of anergy, and deletion of activated autoreactive cells. In healthy individuals, these lymphocytes usually possess insufficient affinity for self-antigens to become fully activated. Autoimmune diseases develop when these tolerance mechanisms fail, allowing autoreactive lymphocytes to proliferate and attack self-tissues. In the case of sequestered antigens, which are normally hidden from the immune system, tissue injury or inflammation may suddenly expose these antigens and trigger an autoimmune response.

3. Physiological Autoimmunity and Natural Autoantibodies

A certain degree of autoimmunity is physiological and contributes to immune surveillance. Natural autoantibodies are generally non-pathogenic, of low affinity, and often polyreactive, recognizing not only self-antigens but also foreign antigens and conserved structures shared

among different species. These antibodies commonly react with polysaccharides and glycolipids present on the membranes of both eukaryotic and prokaryotic cells.

4. Pathogenic Autoantibodies

In autoimmune diseases, autoantibodies are usually produced by activated autoreactive B2 lymphocytes with the assistance of follicular helper $CD4^+$ T cells (Tfh cells). These high-affinity autoantibodies contribute directly to tissue damage by activating the complement system, recruiting inflammatory cells such as macrophages, neutrophils, and NK cells, or by blocking or altering the normal function of cellular receptors.

5. Role of Dendritic Cells in Autoimmunity

Dendritic cells play a crucial role in both maintaining immune tolerance and promoting autoimmune responses. In their immature state, they are considered tolerogenic because they can induce the deletion of autoreactive T lymphocytes and promote the expansion of regulatory T cells. However, under inflammatory conditions, dendritic cells undergo maturation and acquire an immunogenic phenotype capable of activating autoreactive T and B lymphocytes, thereby contributing to the initiation and progression of autoimmune diseases.

VI.1.3. Genetic Factors and Genetic Predisposition

The development of autoimmunity depends on susceptibility genes, as suggested by the occurrence of autoimmune diseases within families. Most autoimmune diseases are **polygenic**, meaning they are associated with multiple susceptibility loci, each of which individually confers only a small increase in the risk of developing autoimmunity. However, the combination of several susceptibility factors, together with specific environmental conditions, can lead to the development of clinically evident autoimmune diseases.

In most cases, the identification of these genetic factors is not routinely used in the diagnostic strategy of autoimmune diseases in clinical practice. Their main interest lies in improving our understanding of the pathophysiology of autoimmune disorders.

A. Polygenic Autoimmune Diseases

The association between the **Human Leukocyte Antigen (HLA) system** and autoimmune diseases is well established. HLA molecules may influence **central tolerance** by affecting

positive and negative selection of T lymphocytes in the thymus. They may also act at the **peripheral level** through the ability of certain HLA alleles to present specific self-antigens to autoreactive T cells.

Examples of HLA associations include:

- **Ankylosing spondylitis** → HLA-B*27
- **Type 1 diabetes mellitus** → HLA-DR3 (HLA-DRB1*03) and HLA-DR4 (HLA-DRB1*04)
- **Rheumatoid arthritis (RA)** → HLA-DR4 (HLA-DRB1*04) and HLA-DR1 (HLA-DRB1*01)
- **Celiac disease** → HLA-DQ2 (HLA-DQB1*02) and HLA-DQ8 (HLA-DQB1*03:02)

In rheumatoid arthritis and celiac disease, high-risk HLA alleles have been shown to present modified self-antigens for prolonged periods to autoreactive T lymphocytes. These modified antigens include **citrullinated epitopes** in rheumatoid arthritis and **deamidated epitopes** in celiac disease.

In addition to HLA genes, several **non-HLA genes** are involved in autoimmune susceptibility. For example, homozygous deficiencies of certain complement proteins, such as **C1q, C4, and C2**, are strongly associated with an increased risk of developing **systemic lupus erythematosus (SLE)**. This association may result from the role of the complement system in the clearance of apoptotic bodies; impaired clearance can promote the breakdown of immune tolerance.

Polymorphisms in genes involved in the regulation of immune responses are also linked to various autoimmune diseases. These include genes encoding:

- Cytokines and their receptors
- Receptors for the Fc portion of immunoglobulin G (IgG), particularly **FcγRIIb**

Some of these receptors possess **Immunoreceptor Tyrosine-based Inhibitory Motifs (ITIMs)** in their intracellular domains, which provide important regulatory signals. Genetic polymorphisms may alter these regulatory pathways, leading to defective immune control and increased susceptibility to autoimmunity.

B. Monogenic Autoimmune Diseases

As previously discussed, the most common autoimmune diseases are **polygenic**. However, rare **monogenic mutations** can also cause autoimmune disorders, often affecting multiple organs and systems. Mutations in genes encoding key transcription factors may result in severe autoimmune syndromes in humans.

One example is mutations in the **AIRE (Autoimmune Regulator)** gene. AIRE is essential for the ectopic expression of tissue-specific antigens in the thymus and therefore plays a crucial role in **negative thymic selection**. Defects in this gene lead to **Autoimmune Polyendocrine Syndrome Type 1 (APS-1/APECED)**, characterized by multiple autoimmune endocrine disorders.

Mutations in the **FOXP3** gene cause a deficiency of **regulatory T cells (Tregs)**, resulting in **IPEX syndrome (Immune Dysregulation, Polyendocrinopathy, Enteropathy, X-linked syndrome)**. This severe disorder is characterized by widespread autoimmunity due to the inability of the immune system to maintain self-tolerance.

Similarly, mutations affecting the **Fas** or **Fas Ligand (FasL)** genes disrupt the normal process of apoptosis, leading to the accumulation of autoreactive lymphocytes. These defects are responsible for **Autoimmune Lymphoproliferative Syndrome (ALPS)**, a disorder characterized by lymphocyte proliferation and autoimmune manifestations

VI.1.4. Environmental Triggering Factors

Genetic factors are estimated to account for approximately **30% of the overall risk** of developing an autoimmune disease. In genetically predisposed individuals, various environmental factors can trigger the onset of pathological autoimmunity.

1. Infectious Agents

Infectious agents have long been suspected of initiating autoimmune diseases. Experimental studies have shown that infections can activate autoreactive lymphocytes through several mechanisms. Both acute infections, such as *Mycoplasma pneumoniae*–associated cold agglutinin hemolytic anemia, and chronic infections may trigger autoimmune responses.

Several pathogens have been implicated in specific autoimmune diseases:

- **Enteroviruses (Coxsackievirus B4)** in type 1 diabetes through molecular mimicry with **Glutamic Acid Decarboxylase (GAD)**.
- **Reoviruses** in Hashimoto's thyroiditis.
- **Epstein–Barr virus (EBV)** in systemic lupus erythematosus.

Although these associations are supported by experimental and epidemiological data, definitive proof of causation remains lacking.

2. Intestinal Microbiota

The intestinal microbiota plays a major role in the development and regulation of the immune system. Recent studies have demonstrated that a healthy microbial community is essential for proper immune maturation from early life.

Certain microbial species promote the generation of **regulatory immune cells**, whereas others stimulate highly inflammatory responses, particularly **IL-17-producing cells (Th17 cells)**. Experimental models of **multiple sclerosis (MS)** have shown that gut microbiota composition influences both the initiation and maintenance of demyelinating processes, highlighting its importance in autoimmune pathogenesis.

VI.1.5. Physical and Chemical Factors

Various physical and chemical agents can trigger or aggravate autoimmune diseases:

- **Ultraviolet radiation (UV)** → systemic lupus erythematosus.
- **Drugs** → drug-induced lupus (β-blockers, tetracyclines), autoimmune hemolytic anemia, and thrombocytopenia (α-methyldopa).
- **Smoking** → rheumatoid arthritis, thyroiditis, multiple sclerosis, and inflammatory bowel diseases.
- **Silica exposure** → systemic sclerosis.
- **Dietary proteins** such as **gliadin** → celiac disease.

VI.1.6. Trauma and Cancer

Some autoimmune diseases may be initiated by physical trauma. For example, **post-traumatic uveitis** can occur following exposure of previously sequestered self-antigens to the immune system.

Autoimmunity may also develop in association with cancer. Oncogenic processes can generate **neo-autoantigens**, contributing to autoimmune encephalitis, autoimmune myopathies, and systemic sclerosis.

VI.1.7. Hormonal Factors and Sex Differences

Autoimmune diseases are generally more common in women. Experimental studies have demonstrated an important role for **estrogens** in regulating immune responses. Estrogens influence the expression of numerous genes involved in dendritic cell function, activation, and **type I interferon production**.

Female susceptibility may also be related to genes located on the **X chromosome**, many of which participate in immune activation. Incomplete X-chromosome inactivation (lyonization) may lead to overexpression of these genes. For example, increased expression of **TLR7**, encoded on the X chromosome, may enhance **IFN-α production** and increase the risk of systemic lupus erythematosus in women.

Pregnancy can also influence autoimmune diseases:

- **Multiple sclerosis** often improves during pregnancy, with fewer relapses.
- **Systemic lupus erythematosus** may worsen during pregnancy.

VI.1.8. Vitamin D

Recent studies have highlighted the relationship between **vitamin D** and immune regulation. Vitamin D plays an important role in establishing and maintaining a balanced immune response. Insufficient vitamin D levels may impair immune regulation and increase susceptibility to autoimmune diseases. Consequently, vitamin D deficiency is considered a potential risk factor for the development of autoimmune disorders.

VI.1.9. Hypothetical Mechanisms Triggering Autoimmunity

As early as 1897, Paul Ehrlich proposed that any defect in immune tolerance mechanisms could impair self-recognition and trigger an inappropriate immune response against one or more components of the body, leading to tissue destruction. This concept, known as **horror autotoxicus**, laid the foundation for our understanding of autoimmune diseases. Although animal models have greatly improved our knowledge of autoimmune mechanisms, the precise causes responsible for initiating autoimmune reactions remain largely unknown.

A. Activation of Ignorant Autoreactive Cells

1. Sequestered Self-Antigens

Certain self-antigens remain hidden from the immune system because their anatomical location prevents contact with immunocompetent cells. This phenomenon is known as **immune ignorance**. Examples include antigens found in the lens of the eye and in sperm cells.

Following trauma, these normally hidden antigens may enter the bloodstream or lymphatic circulation and become accessible to antigen-presenting cells (APCs). APCs process and present these antigens to T lymphocytes, activating autoreactive clones that had escaped central tolerance because these antigens are not normally expressed in the thymus.

For example, injury to one eye can release intraocular antigens that are transported to regional lymph nodes, where they activate autoreactive T cells. These activated T cells subsequently migrate to both the injured and the healthy eye, producing a destructive inflammatory process known as **sympathetic ophthalmia (sympathetic uveitis)**.

2. Cryptic Antigens

Normally, only a limited number of peptide fragments generated during antigen processing are presented by **Major Histocompatibility Complex (MHC)** molecules. Consequently, T cells become tolerant only to these presented epitopes.

Other epitopes, known as **cryptic epitopes**, remain hidden from immune recognition because they are not efficiently processed or presented. During inflammation, however, these cryptic epitopes may become exposed and presented by MHC molecules, allowing activation of autoreactive T cells that have never acquired tolerance to them.

A classical example involves cryptic peptides derived from **myelin basic protein (MBP)** in **multiple sclerosis (MS)**. Once an autoimmune response begins, a phenomenon called **epitope spreading** may occur, whereby immune responses expand to recognize additional self-antigens, thereby perpetuating tissue damage and chronic inflammation.

B. Activation of Anergic Autoreactive Cells

1. Molecular Mimicry

The theory of **molecular mimicry** proposes that certain microbial antigens share structural similarities with self-antigens. As a result, an immune response directed against a pathogen may inadvertently target host tissues.

During infection, vigorous immune activation occurs because lymphocytes are not tolerant to microbial antigens. The inflammatory environment promotes the presentation of self-antigens together with costimulatory signals and cytokines, thereby overcoming the anergic state of autoreactive lymphocytes.

Examples include:

- Similarities between **Glutamic Acid Decarboxylase (GAD)** and antigens of **Coxsackievirus B4**, potentially contributing to autoimmune type 1 diabetes.
- Cross-reactivity between **Streptococcus pyogenes** antigens and cardiac tissues, leading to rheumatic heart disease and valvular damage following streptococcal infections.

2. Activation of Antigen-Presenting Cells (APCs)

Experimental autoimmune diseases can be induced by immunizing animals with self-antigens mixed with **Complete Freund's Adjuvant**. This adjuvant enhances antigen uptake by APCs and contains killed mycobacteria that stimulate innate immune receptors, particularly **Toll-like receptors (TLRs)**.

TLR activation promotes APC maturation, resulting in:

- Increased production of pro-inflammatory cytokines.
- Enhanced expression of costimulatory molecules.
- More efficient presentation of self-antigens.

These changes can break T-cell anergy and trigger autoimmune disease.

In humans, **interferon-alpha (IFN-α)** can exert similar effects by activating dendritic cells and increasing MHC class I antigen presentation. This may favor the emergence of autoreactive T-cell clones and induce autoimmune diseases, particularly autoimmune thyroiditis in some patients receiving IFN-α therapy.

3. Non-Specific Polyclonal Stimulation

Autoimmunity may also arise through **non-specific polyclonal activation of B lymphocytes**. Unlike molecular mimicry or enhanced antigen presentation, this mechanism does not depend on a particular self-antigen.

A sufficiently strong polyclonal stimulus can activate numerous B-cell clones simultaneously, including potentially autoreactive cells. This may lead to the production of autoantibodies and, in some cases, clinical manifestations of autoimmune disease.

4. Failure of Deletion of Autoreactive Cells

The **Fas (CD95)–Fas Ligand (FasL)** pathway plays a critical role in eliminating autoreactive lymphocytes and in terminating immune responses after antigen stimulation through apoptosis.

Mutations affecting **CD95 (Fas)**, **FasL**, or components of their signaling pathways impair apoptosis and allow autoreactive lymphocytes to survive and accumulate.

Experimental evidence comes from **MRL-lpr** and **gld** mice, which carry mutations in Fas-related genes and develop lupus-like autoimmune disease characterized by activation of autoreactive B cells and production of autoantibodies.

In humans, defects in the Fas/FasL pathway cause **Autoimmune Lymphoproliferative Syndrome (ALPS)**, characterized by:

- Chronic lymphoproliferation
- Autoimmune hemolytic anemia
- Autoimmune thrombocytopenia
- Persistence of autoreactive lymphocytes due to defective apoptosis

Thus, impaired deletion of autoreactive cells represents an important mechanism contributing to the development and maintenance of autoimmune diseases.

5. Defects in Regulatory Cells

Deficiencies in the number or function of **regulatory lymphocytes** have been reported in several autoimmune diseases. Experimental studies have demonstrated that depletion of **$CD4^+CD25^+FoxP3^+$ regulatory T cells (Tregs)**, or neonatal thymectomy performed three days

after birth (thereby preventing their thymic development), leads to the appearance of multiple autoimmune disorders, including **type 1 diabetes mellitus, thyroiditis, and gastritis**.

In these animal models, the transfer of **$CD4^+CD25^+FoxP3^+$ regulatory T cells** suppresses the autoimmune response and prevents disease development, highlighting their essential role in maintaining self-tolerance. Deficiencies in **regulatory B cells (Bregs)** have also been described and may contribute to the loss of immune regulation observed in autoimmune diseases.

VI.1.10. Tissue Damage Mechanisms Mediated by Autoimmune Effectors

A. Autoantibodies: Major Mediators of Tissue Injury

In many autoimmune diseases, although not all autoantibodies are pathogenic, autoantibodies represent the principal mediators of tissue damage. Their pathogenic role has been demonstrated by their ability to transfer disease experimentally through serum transfer in animals or through transplacental transfer of maternal IgG autoantibodies to the fetus.

Examples include:

- **Myasthenia gravis**
- **Graves' disease**
- **Pemphigus**

These diseases can be reproduced experimentally by transferring purified IgG autoantibodies from affected individuals.

Autoantibodies induce tissue damage through four major mechanisms.

1. Induction of Target Cell Cytolysis

Autoantibodies may induce destruction of target cells through activation of the **complement system**. In autoimmune hemolytic anemia, antibodies bound to erythrocyte surface antigens activate the classical complement pathway, resulting in formation of the **Membrane Attack Complex (MAC)** and lysis of red blood cells.

Cell destruction may also occur through **phagocytosis by monocytes and macrophages**. Fcγ receptors on macrophages recognize IgG-coated target cells, leading to their engulfment and

destruction. This mechanism is responsible for platelet destruction in **autoimmune thrombocytopenia**.

Another mechanism is **Antibody-Dependent Cellular Cytotoxicity (ADCC)**. In this process, **Natural Killer (NK) cells** recognize antibody-coated target cells through Fcγ receptors and release perforin and granzymes, inducing apoptosis. ADCC is thought to contribute to cardiomyocyte destruction during autoimmune myocarditis.

2. Modification of Target Antigen Function

Some autoantibodies alter the function of membrane receptors rather than destroying cells.

- *Inhibitory Effects*

In **myasthenia gravis**, antibodies against the acetylcholine receptor:

- Promote receptor internalization and degradation.
- Block acetylcholine binding through steric hindrance.

These effects impair neuromuscular transmission and cause muscle weakness.

- *Stimulatory Effects*

In **Graves' disease**, autoantibodies directed against the **TSH receptor** act as agonists, stimulating thyroid hormone production and causing hyperthyroidism.

Different antibodies targeting other receptor epitopes may instead inhibit receptor function.

- ***Neutralizing Effects***

Some autoantibodies neutralize soluble molecules. For example, antibodies against **intrinsic factor** in **pernicious anemia (Biermer disease)** impair vitamin B12 absorption.

3. Immune Complex Formation

Autoantibodies may combine with antigens to form **immune complexes** that circulate in the bloodstream and deposit in tissues. Alternatively, antigens may first become deposited within

tissues and subsequently bind circulating autoantibodies, resulting in **in situ immune complex formation**.

Immune complexes activate complement and generate the anaphylatoxins **C3a and C5a**, which recruit and activate neutrophils. These inflammatory cells release enzymes and reactive molecules that damage surrounding tissues.

A classic example is **lupus nephritis** in systemic lupus erythematosus (SLE). Autoantibodies against double-stranded DNA and nucleosomal components bind antigens deposited within the glomerular basement membrane ("planted antigens"), leading to glomerular injury.

Glomerular damage results from:

- Direct accumulation of immune complexes that alter basement membrane permeability.
- Complement activation and inflammation.
- Recruitment of neutrophils that release proteolytic enzymes, degrading the basement membrane.

These processes cause proteinuria and progressive loss of renal filtration function.

- **T-Lymphocyte-Mediated Tissue Damage**

Some autoimmune diseases are primarily mediated by autoreactive T lymphocytes rather than autoantibodies. Two classical examples are: **Type 1 diabetes mellitus and Multiple sclerosis (MS)**

- ***The Inflammatory Component of Autoimmune Diseases***

Most autoimmune diseases involve chronic inflammation characterized by infiltration of lymphocytes, plasma cells, macrophages, and other immune cells. These cells often organize into inflammatory lesions resembling granulomatous structures.

Such infiltrates are commonly observed in: Autoimmune thyroiditis, Autoimmune hepatitis, Rheumatoid arthritis, Multiple sclerosis

Attempts at tissue repair frequently occur but are often incomplete and accompanied by **fibrosis**, resulting in permanent organ damage.

Numerous inflammatory mediators contribute to tissue injury, including: Vasoactive amines, Nitric oxide (NO), Lipid mediators, Plasma proteases, Complement proteins (particularly C3a and C5a), Growth factors, Cytokines

Among these mediators, **cytokines** play a particularly important role because they represent major therapeutic targets. The treatment of **rheumatoid arthritis**, for example, was transformed by the development of biological agents such as monoclonal antibodies and soluble receptors that block pro-inflammatory cytokines, particularly **Tumor Necrosis Factor-alpha (TNF-α)**. Similar cytokine-targeted therapies are now widely used in many autoimmune and inflammatory diseases.

Table 11 : Polygenic Autoimmune Diseases and Their Main Pathophysiological Mechanisms

Disease	Predisposition / Triggers	Target Tissue(s)	Primary Mechanism	Secondary Mechanism	Major Autoantigens / Autoantibodies
Systemic Lupus Erythematosus (SLE)	Multifactorial genetic predisposition	Systemic	Autoantibody production; increased Type I interferons	Immune complex formation and complement activation	Nuclear antigens, especially double-stranded DNA (dsDNA)
Rheumatoid Arthritis (RA)	Multifactorial genetic disease; HLA-DR1/DR4	Mainly joints; may become systemic	T-cell-mediated immunity; granuloma formation	Systemic inflammation	Rheumatoid factor (RF); anti-citrullinated protein antibodies (ACPA)
Hashimoto's Thyroiditis	Multifactorial genetic disease; HLA-DR3	Thyroid gland (hypothyroidism)	Autoantibody production	T-cell infiltration	Thyroid peroxidase (TPO); thyroglobulin
Graves' Disease	Multifactorial genetic disease	TSH receptor (hyperthyroidism)	Autoantibody production	Receptor stimulation	TSH receptor-stimulating antibodies; anti-TPO; anti-thyroglobulin
Type 1 Diabetes Mellitus	HLA-DR3/DR4; viral infections (enteroviruses, Coxsackievirus)	Pancreatic β-cells	T-cell-mediated destruction	Autoantibody production	Insulin; proinsulin; GAD65; IA-2; ZnT8
Celiac Disease	HLA-DQ2/DQ8; gluten ingestion	Intestinal villi	Intraepithelial T-cell proliferation	Autoantibody production	Deamidated gliadin peptides; anti-tissue transglutaminase; anti-endomysial antibodies

Disease	Predisposition / Triggers	Target Tissue(s)	Primary Mechanism	Secondary Mechanism	Major Autoantigens / Autoantibodies
Myasthenia Gravis	Multifactorial genetic disease; thymoma association	Neuromuscular junction	Autoantibody production	Complement activation	Anti-acetylcholine receptor (AChR); anti-MuSK; anti-titin
Bullous Pemphigoid	Unknown	Hemidesmosomes (dermo-epidermal junction)	Autoantibody production	Complement activation	BP180 (BPAG2); BP230 (BPAG1)
Multiple Sclerosis (MS)	HLA-DR2; possible role of EBV	Oligodendrocytes / myelin	$CD4^+$ and $CD8^+$ T-cell-mediated immunity	Autoantibody production	MBP, MOG, MAG
Pernicious Anemia (Autoimmune Gastritis)	Unknown	Gastric parietal cells	$CD4^+$ and $CD8^+$ T-cell-mediated immunity	Autoantibody production	Anti-H^+/K^+ ATPase; anti-intrinsic factor antibodies
Autoimmune Hemolytic Anemia	Drugs; infections (*Mycoplasma pneumoniae*); Fas/FasL mutations	Red blood cells	Autoantibody production	Complement activation; phagocytosis	Rh, P, or I blood group antigens
Immune Thrombocytopenic Purpura (ITP)	Viral infections; medications; Fas/FasL mutations	Platelets	Autoantibody production	Complement activation; phagocytosis	Platelet glycoproteins GPIIb/IIIa

Table 12 : Monogenic autoimmune diseases and the associated pathophysiological mechanisms.

Name	Genetic abnormality	Pathophysiological mechanisms	Phenotype
IPEX (Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked)	FOXP3 gene mutation	Defect in regulatory T cells (Treg) function	Severe enteropathy, autoimmune diabetes, thyroiditis
APECED (Autoimmune Polyendocrinopathy Candidiasis Ectodermal Dysplasia)	AIRE gene mutation	Defective thymic expression of self-antigens → failure of central tolerance	Type 1 diabetes, thyroiditis, parathyroid autoimmunity, candidiasis
ALPS (Autoimmune Lymphoproliferative Syndrome)	Fas / Fas ligand mutation	Defective apoptosis (failure to eliminate autoreactive lymphocytes)	Autoimmune hemolytic anemia, thrombocytopenia, lymphoproliferation ($CD4^-$ $CD8^-$ T cells)

VI.2. HYPERSENSITIVITY REACTIONS (Immunopathology)

VI.2.1. Definition of Hypersensitivity

Hypersensitivity is defined as an immune response that is exaggerated, inappropriate, or misdirected, resulting in tissue damage instead of protection. These reactions represent

pathological immune responses and are classically divided into four types. Types I, II, and III are mediated mainly by antibodies, whereas Type IV is primarily mediated by T lymphocytes and macrophages.

A. Type I Hypersensitivity (Immediate, IgE-mediated)

Type I hypersensitivity is an allergic reaction that occurs rapidly after exposure to an antigen known as an allergen. It depends on the production of IgE antibodies, which bind to mast cells and basophils. Upon re-exposure to the same allergen, cross-linking of IgE triggers the release of inflammatory mediators such as histamine, leading to acute inflammation. This reaction develops within seconds to minutes and is responsible for allergic conditions such as asthma, hay fever, eczema, urticaria, and systemic reactions to bee venom.

B. Type II Hypersensitivity (Antibody-dependent cytotoxic)

Type II hypersensitivity occurs when IgG or IgM antibodies bind to antigens present on the surface of cells or tissues. This interaction leads to cell destruction through complement activation, phagocytosis, or antibody-dependent cellular cytotoxicity. A classical example is the hemolytic reaction observed after incompatible blood transfusion, where antibodies target and destroy red blood cells.

C. Type III Hypersensitivity (Immune complex–mediated)

Type III hypersensitivity results from the formation of antigen–antibody complexes in excessive amounts or their inadequate clearance. These immune complexes deposit in tissues, where they activate complement and induce inflammation, leading to tissue injury. This mechanism is involved in serum sickness, chronic infections such as streptococcal or staphylococcal endocarditis, malaria, hepatitis B infection, and reactions following hyperimmunization with tetanus toxoid.

D. Type IV Hypersensitivity (Delayed-type, T cell–mediated)

Type IV hypersensitivity is mediated by T lymphocytes and macrophages rather than antibodies. It occurs when persistent antigen is processed by macrophages and presented to T cells, leading to cytokine release and activation of macrophages that mediate inflammation. This reaction develops over 24 to 48 hours and is typically seen in conditions such as the

tuberculin skin test, tuberculosis, and various infections including viral, bacterial, fungal, protozoal, and helminth infections, as well as contact dermatitis.

Table 13 : Classification of hypersensibility Diseases

Type of Hypersensitivity	Pathologic Immune Mechanisms	Mechanisms of Tissue Injury and Disease
Immediate hypersensitivity: type I	IgE antibody	Mast cells and their mediators (vasoactive amines, lipid mediators, cytokines)
Antibody mediated: type II	IgM, IgG antibodies against cell surface or extracellular matrix antigens	Opsonization and phagocytosis of cells Complement- and Fc receptor–mediated recruitment and activation of leukocytes (neutrophils, macrophages) Abnormalities in cellular functions, e.g., hormone receptor signaling
Immune complex mediated: type III	Immune complexes of circulating antigens and IgM or IgG antibodies	Complement- and Fc receptor–mediated recruitment and activation of leukocytes
T cell mediated: type IV	$CD4^+$ T cells (cytokine-mediated inflammation) $CD8^+$ CTLs (T cell–mediated cytolysis)	Recruitment and activation of leukocytes Direct target cell killing, cytokine-mediated inflammation

This classification is useful because distinct types of pathologic immune responses show different patterns of tissue injury and may vary in their tissue specificity. As a result, the different immunologic mechanisms cause disorders with distinct clinical and pathologic features. However, immunologic diseases in the clinical situation are often complex and caused by combinations of humoral and cell-mediated immune responses and multiple effector mechanisms. This complexity is not surprising given that a single antigen may normally stimulate both humoral and cell-mediated immune responses, in which several types of antibodies and effector T cells are produced. Because multiple mechanisms may be involved and inflammation, typically chronic inflammation, is a major component of the pathology and clinical manifestations of these disorders, they are sometimes grouped under the rubric immune-mediated inflammatory diseases.

VI.2.2. Causes of Hypersensitivity Diseases

Hypersensitivity diseases arise from different sources including autoimmunity, immune responses against microbes, and reactions against environmental antigens. These diseases are chronic, often affect individuals between 20 and 40 years of age, and are more frequent in women. Immune responses against microbes may also cause tissue damage when infections are persistent or when the immune response is excessive; in such cases, T cell–mediated inflammation may lead to granuloma formation as seen in tuberculosis, immune complexes may deposit in tissues during chronic infections, or cytotoxic T lymphocytes may damage infected cells as in viral hepatitis. Environmental antigens can also trigger hypersensitivity in susceptible individuals; while most people tolerate harmless substances, about 20% develop

IgE-mediated allergic diseases or T cell–mediated contact hypersensitivity. In all these conditions, the same immune mechanisms that normally protect the body against pathogens become harmful when excessively activated or misdirected. Because the triggering antigens are often persistent or unavoidable, hypersensitivity diseases tend to be chronic, progressive, and difficult to control.

augmentation, sur une période aussi courte, ne peut être expliquée par une modification du génome. Par contre, une modification de la transcription du génome pourrait l'expliquer. Ce mécanisme, appelé épigénétique, est dépendant de facteurs environnementaux qui peuvent moduler la traduction de notre génome. L'augmentation des allergies est donc davantage en lien avec les nombreuses modifications qui sont intervenues au niveau de notre environnement depuis 50 ans. L'ensemble des facteurs environnementaux internant dans ces mécanismes épigénétiques est maintenant regroupé sous le terme d'exposome. Ce dernier est décomposé en 3 sous-unités. Le premier concerne les facteurs environnementaux externes généraux : mode de vie (campagne/ville), le climat, la pollution (particules diésel, industrie pétrolière…). Le deuxième concerne les facteurs environnementaux externes spécifiques comme le régime alimentaire, l'activité physique, l'exposition au tabac, les infections, l'hygiène ou l'exposition aux antibiotiques. Le troisième concerne l'exposition environnementale intérieure comme les polluants des meubles, les parfums d'intérieur, etc. Dans le cadre des maladies allergiques, il a été identifié des facteurs prépondérants comme les allergènes et les polluants. Il est probable que les modifications des habitudes alimentaires, associées à d'autres facteurs comme l'immaturité de l'immunité du système immunitaire des nouveaunés, puissent avoir facilité le développement des allergies. La pollution industrielle et domestique, qui s'est développée au cours des dernières années, aurait également une responsabilité importante sur l'augmentation des allergies. La pollution atmosphérique peut augmenter la production ou l'allergénicité des allergènes. C'est le cas des pollens qui voit leur production augmenter dans des zones de forte pollution (milieu citadin) ou leur allergénicité s'accroître après transformation au contact des particules diésel. Le facteur prépondérant pourrait cependant être le microbiote. Ce dernier se définit comme l'ensemble des organismes vivants, bactéries, champignons et virus, présent au niveau de nos muqueuses et de notre peau. Il existe ainsi un microbiote spécifique intestinal, cutané, urinaire, génital, ORL et même pulmonaire. Certains le considèrent comme faisant partie intégrale de notre organisme, et d'autres l'intègrent à l'exposome. Quel qu'il en soit, le microbiote est à l'interface entre l'exposome et notre organisme. Ce microbiote a diffé rentes fonctions dont une majeure qui est de permettre de stimuler, développer et orienter les réponses

immunes. Sans microbiote (souris axénique), le système immunitaire ne se développe pas normalement. Un microbiote anormal conduit à des anomalies de développement et de régulation de l'immunité qui pourraient conduire à des maladies chez l'Homme. Dans le cadre des maladies allergiques, des anomalies dans la composition du microbiote ont été décrites. Le microbiote des allergiques serait moins riche et moins complexe que ceux des sujets sains. Le microbiote humain est directement dépendant de notre environnement. Les facteurs environnementaux externes généraux, notamment le mode vie, un pays du sud/nord, développé ou moins développé, urbain ou rural, le climat, mais aussi les facteurs environnementaux externes spécifiques comme le régime alimentaire, l'exposition aux antibiotiques, aux polluants, interviennent directement sur l'abondance et la diversité du microbiote. De par sa capacité à modifier le microbiote, qui lui-même régule l'immunité, les facteurs environnementaux pourraient ainsi moduler les réponses immunes et de ce fait favoriser (ou protéger) le développement de maladies allergiques. La théorie hygiéniste qui avait été développée dans les années 1990–2000 s'intéressait au rôle des antigènes microbiens de l'environnement dans le développement des maladies allergiques. Il avait été ainsi rapporté que vivre à la campagne, où l'exposition antigénique bactérienne est plus forte (moins d'hygiène, plus d'animaux d'élevages…), protégeait contre le développement des maladies allergiques. La présence en grande quantité d'endotoxines de type LPS des bactéries Gram négatif a, via une réponse immune Th1 forte, la capacité de bloquer la réponse Th2 et d'empêcher le développement de l'allergie. Cette théorie est englobée maintenant dans le concept plus large de l'exposome et du microbiote. Aujourd'hui, le microbiote et certains facteurs environnementaux comme les régimes alimentaires, l'exposition au tabac ou la nature des allergènes sont identifiés comme des axes de recherche pour le développement de nouvelles stratégies de prévention ou de traitements de l'allergie.

VI.2.3. Factors involved in the pathophysiology of allergy

Genetic predisposition plays a role in the development of allergic diseases, which are more frequent in individuals with a family history of allergy. Although several susceptibility genes have been identified, no genetic testing is currently used for diagnosis or clinical management. The sharp increase in allergic diseases over the past 50 years cannot be explained by genetic changes alone, but rather by epigenetic mechanisms, where environmental factors influence gene expression without altering DNA sequences.

These environmental influences are grouped under the concept of the **exposome**, which includes: general external factors such as lifestyle (urban or rural living), climate, and pollution (diesel particles, industrial emissions); specific external factors such as diet, physical activity, smoking exposure, infections, hygiene practices, and antibiotic use; and internal environmental factors such as indoor pollutants and fragrances. Among these, allergens and pollutants are major contributors to allergic diseases. Changes in dietary habits and the immaturity of the neonatal immune system may also promote allergy development. In addition, industrial and domestic pollution can increase both the production and allergenicity of allergens, for example by increasing pollen levels in polluted urban areas or by enhancing pollen allergenicity after interaction with diesel particles.

A key factor is the **microbiota**, composed of bacteria, fungi, and viruses living on mucosal surfaces and skin (intestinal, cutaneous, respiratory, urogenital, etc.). The microbiota plays a crucial role in the development and regulation of the immune system; in its absence, immune development is impaired. Alterations in microbiota composition (dysbiosis), characterized by reduced diversity, have been observed in allergic patients and may contribute to immune dysfunction.

Environmental factors strongly influence microbiota composition, and through it, immune responses. This supports the idea that environment–microbiota interactions can either promote or protect against allergic diseases. The “hygiene hypothesis” proposed that reduced microbial exposure in modern urban lifestyles favors allergy development by limiting immune stimulation. Microbial components such as LPS from Gram-negative bacteria can promote Th1 responses, which suppress Th2-mediated allergic responses.

Today, this concept is integrated into the broader framework of the exposome and microbiota research. The microbiota, together with environmental factors such as diet, tobacco exposure, and allergen nature, represents a major target for future strategies in allergy prevention and treatment.

VI.3. Hereditary immunodeficiencies (HIDs)

VI.3.1. Introduction

Hereditary immunodeficiencies (HIDs) are rare genetic disorders with an estimated frequency of about 1 in 4,000 births in the general population. More than 340 HIDs have been described,

most of which have a known genetic cause. They are usually classified into six groups according to the underlying immunological defect:

- Combined immunodeficiencies (CIDs), affecting both cellular (T lymphocytes) and humoral (B lymphocytes) adaptive immunity
- Isolated humoral immunodeficiencies (B-cell defects), also affecting adaptive immunity
- Phagocytic cell defects (granulocytes, dendritic cells, monocytes, macrophages)
- Complement deficiencies (affecting opsonization)
- Innate immunity defects
- Defects of immune system homeostasis

HIDs are typically associated with increased susceptibility to infections caused by a wide range of pathogens (bacteria, viruses, fungi). However, some genetic defects lead to susceptibility to a restricted range of pathogens. In addition, certain HIDs are associated with non-infectious manifestations such as immune dysregulation, including macrophage activation syndrome, autoimmunity, autoinflammatory diseases, and cancer. From a scientific perspective, studying these genetic disorders helps to better understand the role of immune components in infection control, anti-tumor immunity, and immune homeostasis.

VI.3.2. Cellular and Combined Immunodeficiencies

Combined immunodeficiencies (CIDs) affect both cellular adaptive immunity mediated by T lymphocytes and humoral adaptive immunity mediated by B-cell production of immunoglobulins and specific antibodies. They are rare but among the most severe forms of immunodeficiency, usually presenting very early in life (within the first months).

A. Severe Combined Immunodeficiency (SCID)

Severe combined immunodeficiencies (SCIDs) are rare, with an estimated incidence of 1 in 50,000. Clinically, affected infants present within the first weeks of life with severe, recurrent infections involving bacteria, viruses (e.g., parainfluenza virus, RSV, CMV, adenovirus), fungi, and opportunistic pathogens such as *Pneumocystis*. These infections mainly affect the respiratory tract, gastrointestinal tract, and skin. In some cases, children develop disseminated BCG infection following BCG vaccination with the live attenuated vaccine. Lymphoid tissues

(thymus, spleen, lymph nodes) are typically hypoplastic. Early diagnosis is critical, as each infection can be life-threatening.

Immunologically, SCID patients present with lymphopenia on blood count. Immunophenotyping shows profound T-cell lymphopenia, often associated with B-cell and/or NK-cell deficiencies depending on the underlying genetic defect. Serum immunoglobulin levels (IgG, IgA, IgM) are low, and infectious serologies are negative.

About fifteen genetic causes are known, most with autosomal recessive inheritance (e.g., adenosine deaminase deficiency, ADA, or V(D)J recombination defects due to RAG1 mutations). The most common form is X-linked recessive SCID caused by mutations in the IL2RG gene encoding the common γ chain of cytokine receptors (IL-2, IL-4, IL-7, IL-9, IL-15, IL-21). This form affects only males, while females are asymptomatic carriers.

Five main mechanisms can explain the absence of T lymphocytes in SCID, depending on the genetic defect:

- Excess apoptosis of lymphocytes and thymocytes (e.g., complete ADA deficiency), leading to T−B−NK− SCID
- Defective T-cell development due to impaired cytokine signaling essential for lymphocyte ontogeny (e.g., IL2RG γ-chain defect), leading to T−B+NK− SCID
- A defect in V(D)J recombination, leading to impaired T- and B-cell development and resulting in the absence of both T and B lymphocytes (e.g., RAG1 deficiency), producing a T−B−NK+ SCID phenotype.
- A defect in T-cell ontogeny due to a genetic abnormality affecting one of the TCR co-receptor chains (e.g., CD3ε deficiency), resulting in a T−B+NK+ SCID phenotype.
- A defect in T-cell maturation secondary to complete thymic aplasia, as seen in some patients with DiGeorge syndrome (22q11 microdeletion).

The absence of adaptive immunity leads to primary immunodeficiency with very early clinical presentation, characterized by a broad spectrum of severe infections that can rapidly become life-threatening if the child is not protected in a sterile environment (“bubble” isolation) while awaiting immune reconstitution, most often achieved through hematopoietic stem cell transplantation.

B. Combined Immunodeficiencies (CIDs)

Combined immunodeficiencies are defined by a less profound T-cell lymphopenia than in SCID, but with impaired T-cell proliferative function. More than 100 different genetic defects have been identified, and diagnosis is guided by the clinical presentation, inheritance pattern, and immunological investigations.

CIDs typically present later in life than SCID, ranging from several months to several years of age. Clinically, they are characterized by recurrent infections involving bacteria, viruses, fungi, and opportunistic pathogens, mainly affecting the respiratory and gastrointestinal tracts, and may also be associated with autoimmune manifestations. In some cases, CIDs may occur as part of syndromic diseases.

C. Wiskott–Aldrich Syndrome

Wiskott–Aldrich syndrome is an X-linked recessive disorder (frequency ~1/250,000) characterized in boys by a triad of severe eczema, thrombocytopenia with small-sized platelets (microthrombocytopenia), and recurrent bacterial and/or viral infections. The thrombocytopenia is associated with a bleeding tendency, and patients are at high risk of hemorrhages, including cerebral bleeding. The disease may also be associated with inflammatory complications such as vasculitis, nephropathies, and sometimes lymphoproliferative disorders.

The immunodeficiency is characterized by CD8+ T-cell lymphopenia and variable hypogammaglobulinemia. The responsible gene is located on the X chromosome (WAS gene, Wiskott–Aldrich syndrome protein). This protein is involved in actin cytoskeleton polymerization, playing a key role in cell signaling and migration.

D. Hyper-IgM Syndromes due to CD40L and/or CD40 Deficiency

Hyper-IgM syndromes are genetically heterogeneous disorders characterized by hypogammaglobulinemia (low IgG and IgA) with elevated IgM levels and an absence of specific antibody production (negative serologies). They result from defects in immunoglobulin class-switch recombination.

Several genetic forms exist (X-linked, autosomal recessive, and autosomal dominant). Among them, CD40 ligand (CD40L) deficiency (X-linked) and CD40 deficiency (autosomal recessive)

are classified as combined immunodeficiencies because they disrupt T–B cell cooperation. This defect affects the CD40–CD40L signaling pathway between activated T cells and B cells.

Clinically, in addition to recurrent bacterial infections due to impaired IgG production (humoral defect), patients also develop opportunistic infections such as toxoplasmosis, pneumocystosis, and cryptosporidiosis, reflecting an associated cellular (T-cell) immune defect. In contrast, other genetic forms of hyper-IgM syndromes affecting only B cells are not associated with opportunistic infections, although autoimmune manifestations may occur.

VI.3.3. Humoral Immunodeficiencies

Humoral immunodeficiencies are the most frequent primary immunodeficiencies. They are typically revealed by recurrent bacterial infections of the upper respiratory tract and lungs. A hereditary humoral immunodeficiency is a genetic disorder characterized by an inability to produce normal immunoglobulin responses during infection. These patients show impaired antibody production ranging from complete agammaglobulinemia (profound B-cell deficiency with absence of immunoglobulins) to selective defects such as impaired anti-polysaccharide antibody responses. Some humoral immunodeficiencies may be secondary to T-cell defects, in which case they are classified as combined immunodeficiencies.

A. Agammaglobulinemia (Absence of B Lymphocytes)

Agammaglobulinemia typically becomes clinically apparent after maternal antibodies disappear, during the first year of life, with recurrent bacterial infections (sepsis, meningitis, arthritis, pneumonia, otitis media). Later in childhood, patients may develop gastrointestinal parasitic infections such as giardiasis. In rare cases, chronic viral meningoencephalitis caused by enteroviruses may occur.

Immunologically, agammaglobulinemia is characterized by the absence of peripheral B lymphocytes on immunophenotyping, very low or absent serum immunoglobulins (IgG, IgA, IgM), and absent specific antibody responses (negative serologies). Several genetic forms exist, all involving defects in early B-cell differentiation in the bone marrow.

The most common form (about 85% of cases) is X-linked Bruton agammaglobulinemia (frequency ~1/250,000), caused by mutations in the BTK (Bruton tyrosine kinase) gene, which

is essential for B-cell maturation. Other genetic forms may be autosomal recessive or autosomal dominant and affect different stages of B-cell development.

B. Common Variable Immunodeficiency (CVID)

Common variable immunodeficiency (CVID) is the most frequent primary immunodeficiency, with an estimated prevalence of about 1 in 30,000. It usually has a later onset, typically after adolescence. CVID is a heterogeneous group of disorders defined by hypogammaglobulinemia and impaired production of specific antibodies.

Patients usually have a normal number of B lymphocytes, but a reduced population of class-switched memory B cells. T-cell dysfunction is variable and often mild.

Clinically, CVID is mainly characterized by recurrent bacterial infections affecting the upper and lower respiratory tracts (sinusitis, otitis, bronchitis, pneumonia) and the gastrointestinal tract (e.g., *Giardia, Campylobacter, Salmonella*). These infections may lead to chronic sinusitis and bronchiectasis, reflecting the underlying defect in humoral immunity.

CVID is also associated in about 40% of cases with non-infectious complications such as lymphadenopathy, splenomegaly, autoimmune disease, tissue granulomas, and an increased risk of lymphoma.

The genetic cause is identified in fewer than 10% of patients. CVID is genetically very heterogeneous, with more than 20 genes described. For example, autosomal recessive ICOS deficiency (Inducible T-cell COStimulator) impairs follicular helper T-cell function. Similarly, defects in molecules such as CD19, CD20, CD21, or CD81 affect B-cell activation, leading to impaired antibody secretion in response to antigen stimulation.

C. Other Humoral Immunodeficiencies

Selective IgA deficiency is the most common immunodeficiency in the general population, affecting approximately 1 in 600 individuals. Most are asymptomatic, but some may present with recurrent respiratory infections.

IgG subclass deficiencies, particularly IgG2 deficiency, are associated with impaired specific antibody responses. Clinical presentation ranges from asymptomatic cases to recurrent viral or bacterial respiratory and ENT infections. These deficiencies may be transient, stable, or may progress over time. For example, selective IgA deficiency may evolve into IgG subclass deficiency and eventually into CVID.

Selective antibody deficiency to polysaccharide antigens is a rare immunodeficiency characterized by a reduced antibody response to encapsulated bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, and *Neisseria meningitidis*, while responses to protein antigens remain normal. Total immunoglobulin levels and subclasses are normal. Diagnosis can only be made after the age of 2 years, as younger children naturally have poor responses to polysaccharide antigens.

VI.3.4. Phagocyte Deficiencies

Phagocytosis is a key component of innate immunity involved in defense against microorganisms. It is performed by neutrophils, blood monocytes, tissue macrophages, and dendritic cells. Quantitative and/or functional defects in phagocytosis lead to primary immunodeficiencies characterized by recurrent tissue infections caused by pyogenic bacteria (abscesses) and fungi (notably *Aspergillus*).

A. Quantitative Phagocyte Defects (Neutropenias)

Congenital chronic neutropenias result from bone marrow failure (defective production or differentiation of cells). They may be isolated, such as Kostmann agranulocytosis (autosomal recessive mutation in the HAX1 gene), or syndromic, such as Shwachman–Diamond syndrome (autosomal recessive mutation in the SBDS gene), which is associated with pancreatic insufficiency and skeletal abnormalities. Other congenital neutropenias are linked to metabolic diseases such as glycogen storage disease type 1b.

Cyclic neutropenia is characterized by recurrent episodes of severe neutropenia lasting 3–6 days, recurring approximately every 3 weeks. Autosomal dominant mutations in the ELA2 gene (encoding neutrophil elastase) are implicated in most chronic and cyclic forms.

Clinically, chronic neutropenias present with fever and recurrent infections of the skin, oral mucosa, and periorificial regions (e.g., perianal abscesses), often caused by Gram-negative bacteria and sometimes fungi.

B. Qualitative Phagocyte Defects

Chronic granulomatous disease (CGD) is a functional defect of phagocytes that are unable to generate reactive oxygen species required to kill ingested pathogens. Neutrophils, macrophages, and dendritic cells normally eliminate microbes through the production of reactive oxygen species generated by NADPH oxidase during the oxidative burst.

In CGD, mutations in components of the NADPH oxidase complex impair this respiratory burst, leading to defective microbial killing. The disease has an estimated frequency of 1 in 200,000 and may be X-linked (CYBB gene) or autosomal recessive (CYBA, NCF1, NCF2, NCF3).

Clinically, CGD is characterized by recurrent deep tissue infections caused by *Staphylococcus aureus*, Enterobacteriaceae, mycobacteria, and fungi, particularly *Aspergillus* species. Patients may also develop granuloma formation in various organs due to persistence of ingested pathogens, sometimes leading to complications such as colitis.

The diagnostic reference test is the dihydrorhodamine (DHR) flow cytometry assay, which evaluates hydrogen peroxide production after phagocyte activation; this is abnormal in affected patients.

Chapter VII: Main Immunological Tests and Their Applications

VII.1. Immunological tests application

Immunological tests are based on the specific interaction between antibodies (Ab) and antigens (Ag), which enables the detection, identification, and quantification of a wide variety of biological molecules. These techniques have numerous applications in clinical diagnosis and laboratory medicine.

VII.1.1. Diagnosis of Infectious Diseases

Antigen–antibody interactions are widely used in the diagnosis of infectious diseases caused by bacteria, viruses, parasites, and fungi. **Indirect diagnosis** relies on the detection of pathogen-specific antibodies in a patient's serum, indicating current or past exposure to the infectious agent. In contrast, **direct diagnosis** involves the detection of microbial antigens in biological fluids, tissues, or biopsy specimens, providing evidence of the presence of the pathogen itself.

VII.1.2. Diagnosis of Immune System Disorders

Immunological assays are essential for the investigation of disorders affecting the immune system. They help identify **immunodeficiencies** through the evaluation of immune components, detect **autoimmune diseases** by measuring autoantibodies directed against self-antigens (e.g., rheumatoid arthritis), assess **hypersensitivity reactions** by identifying allergen-specific antibodies, and contribute to the diagnosis and monitoring of **lymphoproliferative disorders** such as lymphomas.

VII.1.3. Quantitative Measurement of Biological Molecules

Antibody-based techniques are also employed for the quantitative determination of numerous substances in biological samples. These include **hormones**, **vitamins**, **inflammatory proteins**, **therapeutic drugs**, and many other clinically important molecules. Their high specificity and sensitivity make immunological assays valuable tools for diagnosis, disease monitoring, and therapeutic follow-up.

VII.2. Molecular Basis of the Antigen–Antibody Interaction

The interaction between an antigen (Ag) and an antibody (Ab) is the fundamental principle underlying immunological techniques used for the detection and quantification of antigens and

antibodies. Understanding the molecular characteristics of this interaction is essential because they determine the sensitivity, specificity, and performance of immunological assays.

VII.2.1. Antigen–Antibody Binding

The interaction occurs between the **epitope** (antigenic determinant) on the antigen and the **paratope** (antigen-binding site) on the antibody. Effective binding requires a high degree of steric and chemical complementarity between these two structures.

The antigen–antibody complex is stabilized by multiple **non-covalent and reversible interactions**, including: Van der Waals forces, Hydrophobic and hydrophilic interactions , Electrostatic interactions, Hydrogen bonds

Because these interactions are non-covalent, antigen–antibody binding remains reversible and can be influenced by environmental conditions.

- ***Concept of Affinity***

Affinity refers to the strength of the interaction between a single epitope and a single paratope. It represents the sum of all attractive and repulsive forces acting at the binding site.

Affinity is measured by the **affinity constant (Ka)**, expressed in L/mol:

- **Low-affinity antibodies:** Ka ≈ 10^4 L/mol
- **High-affinity antibodies:** Ka ≈ 10^{12} L/mol

The higher the affinity constant, the stronger and more stable the antigen–antibody interaction.

- ***Concept of Avidity***

Avidity is the overall binding strength between a complete antibody, which possesses at least two antigen-binding sites, and a multivalent antigen containing multiple epitopes.

Unlike affinity, which concerns a single binding site, avidity reflects the combined strength of all antigen–antibody interactions. It determines the stability and rate of formation of immune complexes and depends on:

- The intrinsic affinity of individual binding sites

- The valency of the antibody and antigen
- Physicochemical conditions such as temperature, pH, and ionic strength

Therefore, an antibody with moderate affinity may exhibit high avidity if multiple binding sites interact simultaneously with the antigen.

➢ ***Specificity and Cross-Reactivity***

Specificity is the ability of an antibody to recognize and bind a particular antigenic determinant. It reflects the capacity of an antibody, or a population of polyclonal antibodies, to distinguish one antigen from another based on structural differences.

However, antigen–antibody specificity is not absolute. **Cross-reactivity** may occur when an antibody generated against one antigen (Ag1) also recognizes related antigens that possess identical or structurally similar epitopes (Ag2 or Ag3). In such cases, the antibody binds these related antigens with lower or comparable affinity, producing cross-reactions.

Cross-reactivity is an important consideration in immunological testing because it can influence the accuracy and interpretation of diagnostic assays.

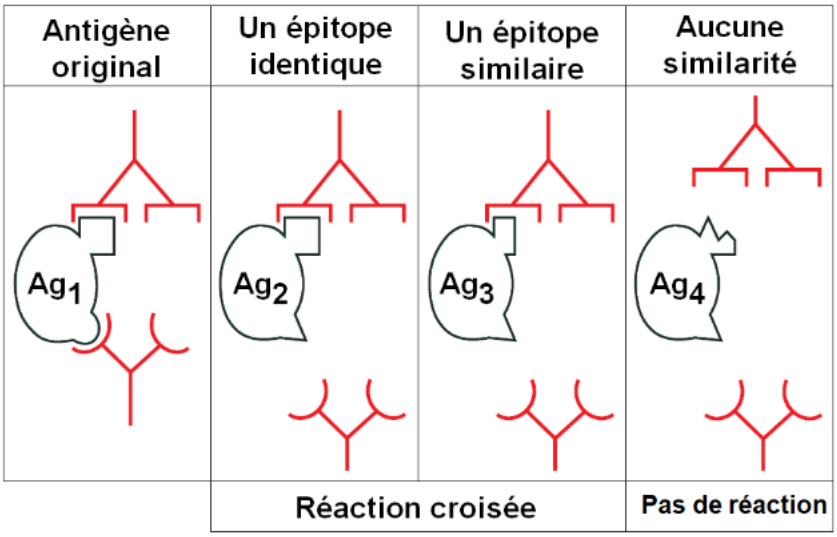


Figure 39 : Specificity and cross-reactions

During the Ab–Ag reaction, two situations may occur:

1. **Techniques without labeling**
2. **Techniques with labeling**

VII.3. Techniques without labeling

In these techniques, detection of the immune complex (Ab–Ag complex) does not require the use of a marker or label.

VII.3.1. Precipitation

Precipitation transforms a soluble antigen (Ag), when placed in the presence of its specific antibody (Ab), into an insoluble antigen–antibody (Ag–Ab) complex. This precipitation occurs only under specific antigen and antibody concentration conditions, known as the **optimal concentrations** or the **zone of equivalence**.

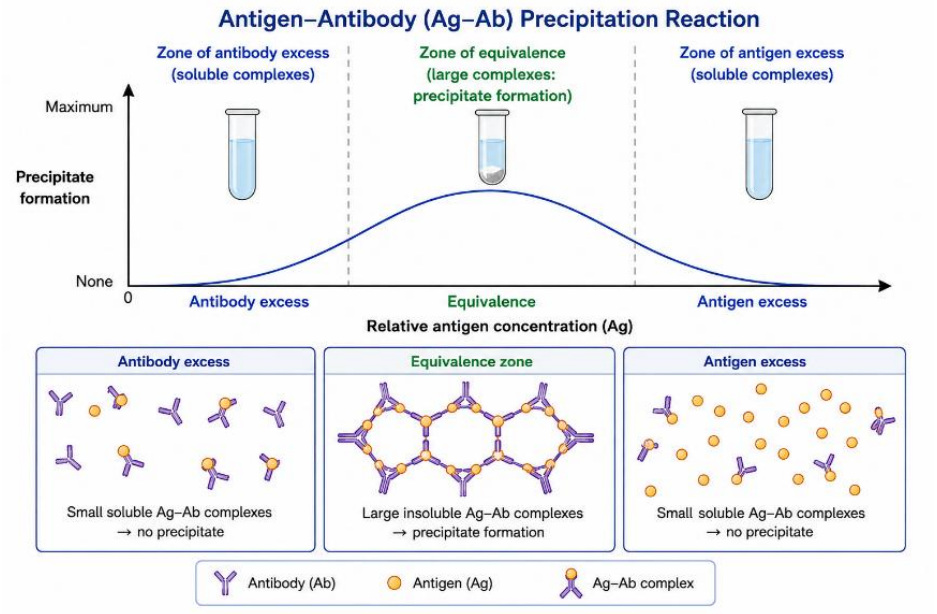


Figure 40 : Immunoprecipitation *(the amount of antibody used is fixed)*

VII.3.1.1. Precipitation in a liquid medium

A. The Ring Test

1. Qualitative Detection: The Ring Test is a rapid and simple technique used to determine whether a serum contains specific antibodies.

- The serum to be tested is introduced into a test tube.

The antigen (Ag) solution is carefully layered into the tube, avoiding mixing with the serum. If the serum contains antibodies (Ab) directed against the antigen present in the solution, a precipitate forms as an opaque ring at the interface between the two liquids within a few minutes. This is a qualitative method. The liquid precipitate can be used in test tubes by measuring the precipitates collected at the bottom of the tubes after centrifugation.

- **Quantitative assays:** Immunonephelometry:

This involves measuring light scattering. There is therefore a relationship between the intensity of the light measured by nephelometry and the amount of Ag-Ac precipitate .

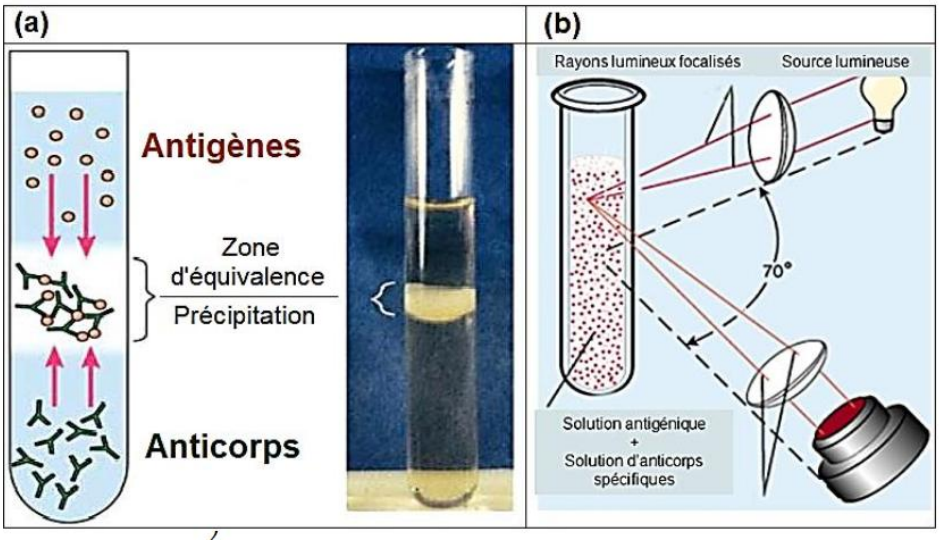


Figure 41 : Liquid-phase precipitation (a) Ring test; (b) Immunonephelometry

VII.3.1.2. Immunoprecipitation in Gel Media (Immunodiffusion)

In this diagnostic configuration, solutions of soluble antigens (Ag) and antibodies (Ac) are allowed to passively diffuse through a semisolid gel matrix. When the migrating fronts meet and achieve the **zone of equivalence** (optimal molecular proportions), the cross-linked antigen-antibody complexes form an insoluble lattice that drops out of solution, manifesting as a distinct visual band or ring of precipitation (*precipitin arc*). To enhance visibility and allow for precise measurement, the gel typically undergoes a post-diffusion chemical staining step.

A. Radial Immunodiffusion (Mancini Technique) This method consists of incorporating a specific antiserum into a gel (agarose) and placing the antigen (Ag) solution into wells. At equilibrium, a precipitation ring forms, the square of whose diameter is proportional to the

antigen concentration. The concentration is determined by reference to a standard curve using an antigen of known concentration.

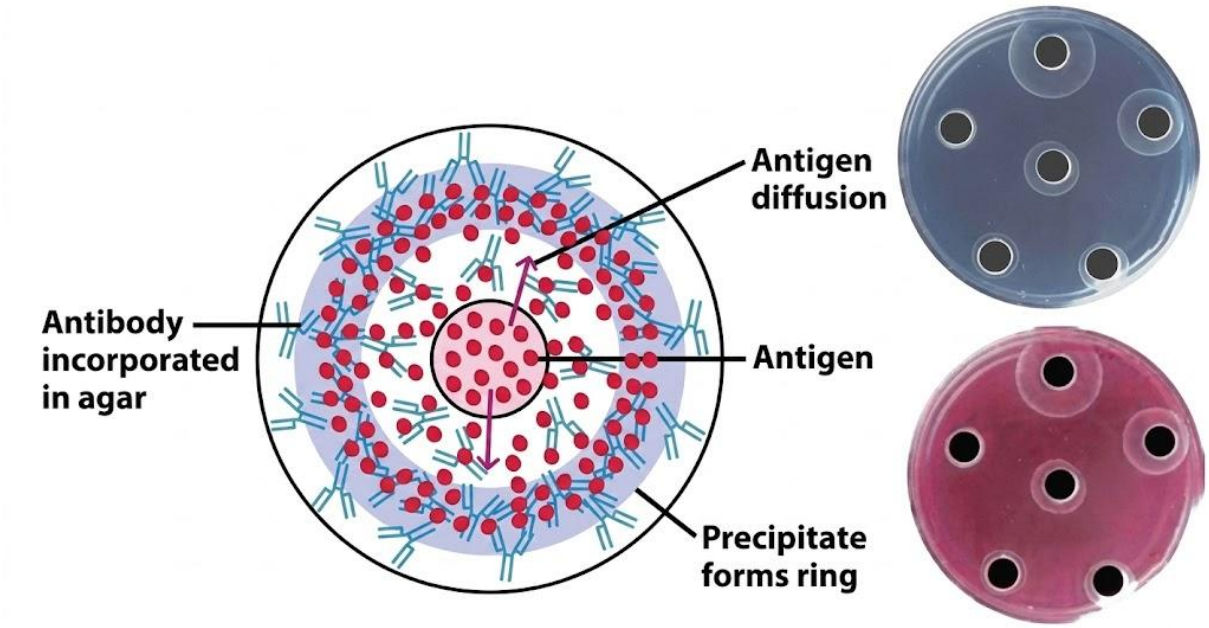


Figure 42 : Mechanism of Radial Immunodiffusion.

B. The Ouchterlony Technique (Double Immunodiffusion)

An agar gel is poured and cast into a Petri dish (or onto a glass microscope slide). Once solidified, a series of small cavities or wells are punched into the gel matrix. Typically, a central well is loaded with the serum sample containing the specific antibodies (Ab), while surrounding peripheral wells are filled with solutions of known target antigens (Ag).

The serum antibodies and the respective antigen solutions diffuse passively and radially outward through the porous agar network. When a diffusing antigen front meets its complementary migrating antibody front, they physically interact. Upon reaching the **zone of equivalence** (where the molecules exist in optimal homeostatic proportions), they cross-link into a stable, macromolecular lattice that precipitates out of the gel solution. This manifests macroscopically as a sharp, white **precipitin line**. To enhance contrast and preserve the assay for analytical profiling, this precipitation line can be stained a deep blue hue using **Coomassie Brilliant Blue**.

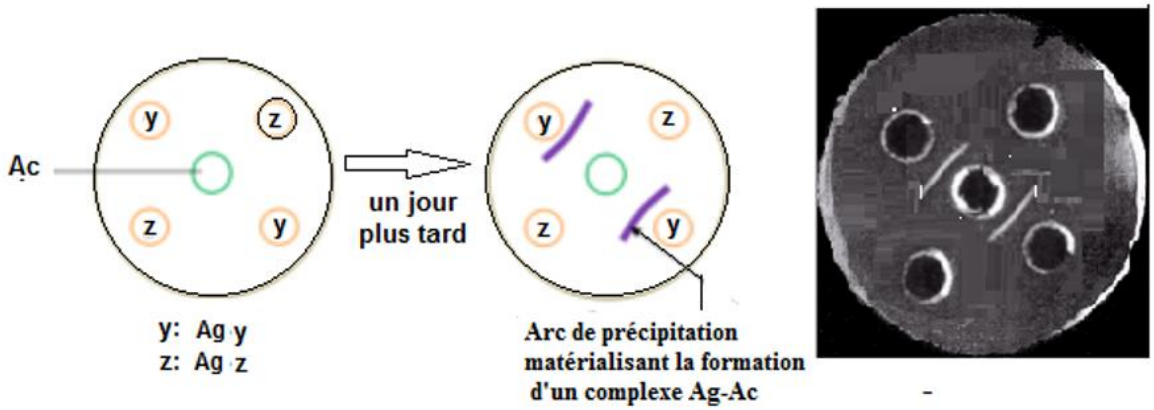


Figure 43 : Immunodiffusion: Ouchterlony Technique

VII.3.1.2. Agglutination Techniques

In agglutination reactions, the antigen is particulate (microorganisms, red blood cells, latex particles onto which the antigen is bound, etc.). When these cells or particles are mixed with the specific antiserum, they form clumps that aggregate to form large, visible structures.

The term **agglutinin** is used to describe antibodies that agglutinate particulate antigens. When the antigen is an erythrocyte (red blood cell), the term **hemagglutination** is used. In theory, all antibodies can agglutinate particulate antigens; however, **IgM** molecules, due to their high valency, are particularly effective agglutinins (10 to 100 times more effective than IgG). Consequently, it is often concluded that the antibody belongs to the IgM class when strong agglutination is detected.

Agglutination is used for blood typing, for the serodiagnosis of microbial diseases (for example, the Widal test for diagnosing typhoid fever), etc.

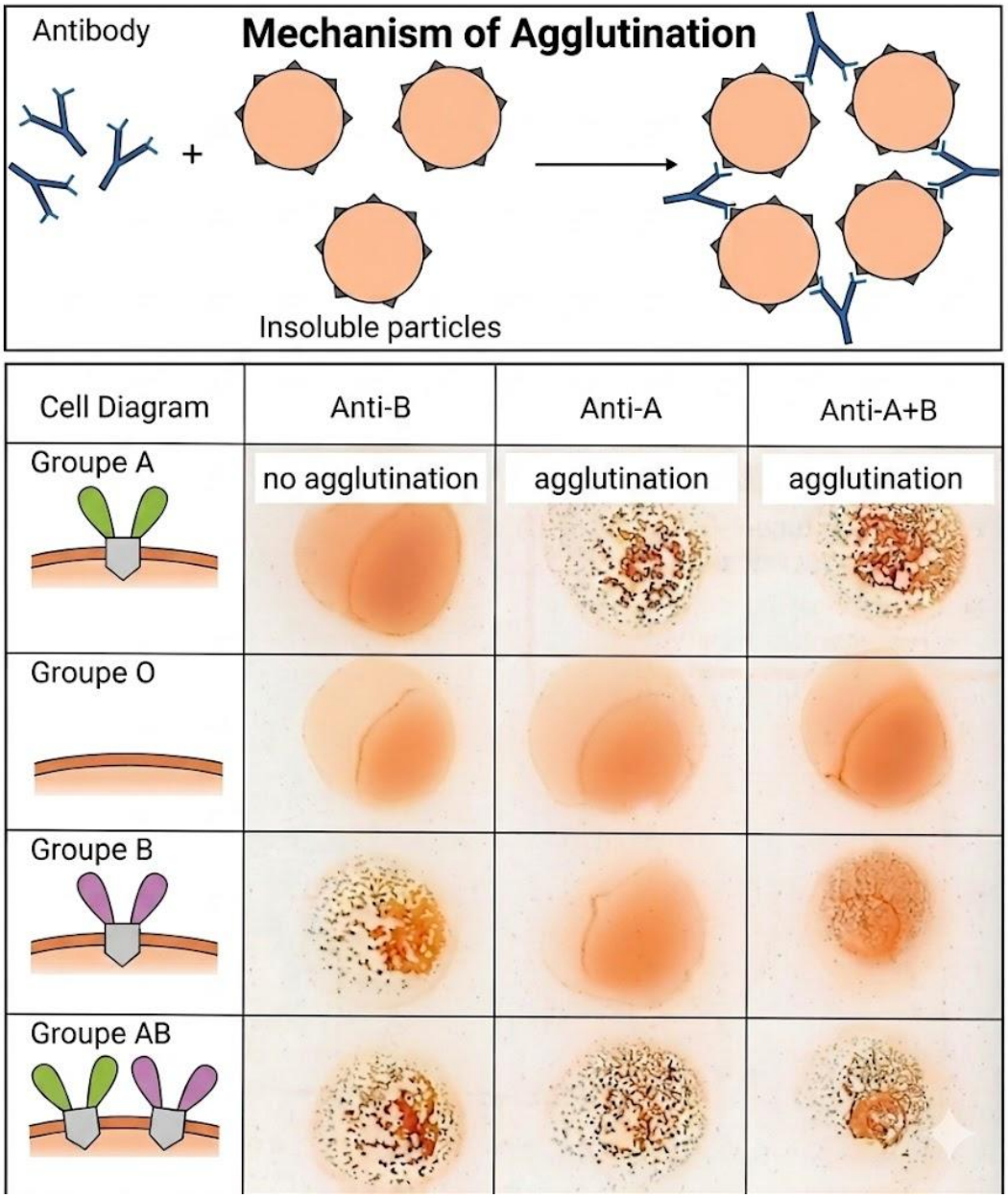


Figure 44 : Identification of Blood Groups by an Agglutination Test

VII.3.1.3. Techniques Using the Complement System

These techniques are based on the fact that antigen–antibody (Ag–Ab) complexes are capable of activating and therefore "consuming" complement proteins. They are used for the detection of antibodies in patients' sera.

After thermal inactivation of the complement present in the patient's sera, exogenous complement and the specific antigen corresponding to the antibody being investigated are added. If the antibody is present in the sera, the complement will be fixed and consumed.

Subsequently, antibody-coated erythrocytes (the indicator system) are added. The absence of hemolysis (which is complement-dependent) in the indicator system indicates complement consumption and therefore corresponds to a positive test.

In the case of a negative test, the added complement remains available to lyse the erythrocytes of the indicator system.

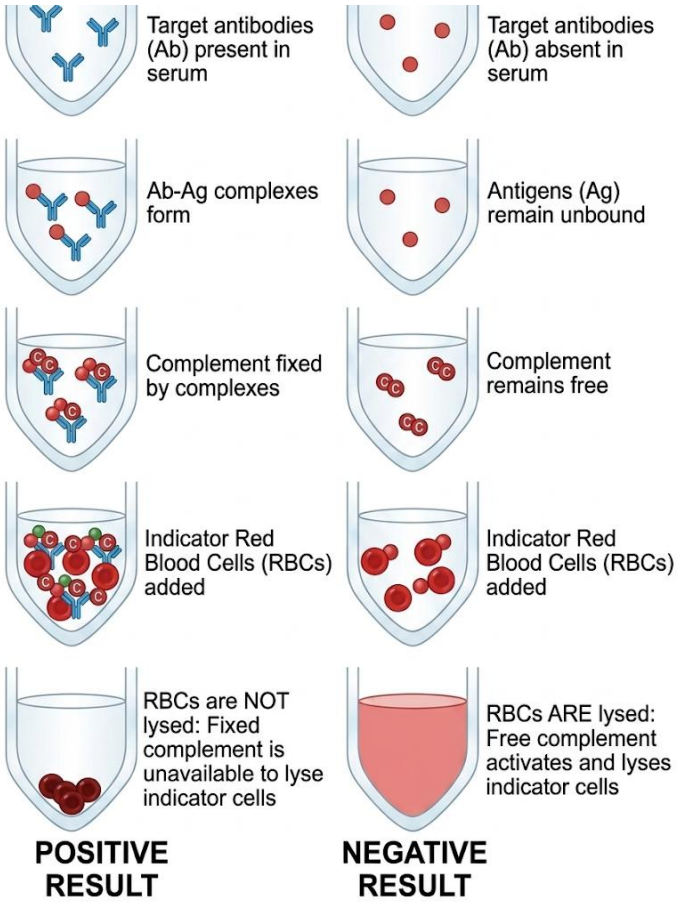


Figure 45 : Complement Fixation Technique.

VII.3.2. Labeled Techniques (Immunolabeling)

In these immunochemical techniques, detecting the immune complex (Ag-Ab) requires labeling with a tracer element. The antigen, or more commonly the antibody, is labeled—meaning it is conjugated to an isotope (e.g., ^{125}I, referred to as radioimmunoassays or RIA), a fluorescent compound (immunofluorescence techniques), or an enzyme (enzyme immunoassays). Detection is performed using a scintillation counter (RIA), a fluorescence microscope/spectrometer (immunofluorescence), or an optical microscope/spectrophotometer (enzyme immunoassays).

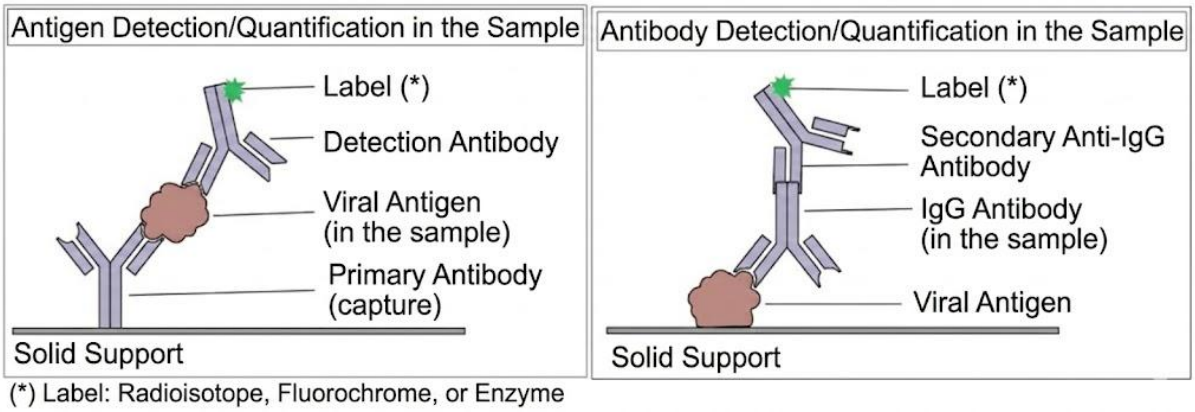


Figure 46 : Direct and Indirect Immunoassays for Antigen and Antibody Detection

VII.3.2.1. ELISA Test (Enzyme-Linked Immunosorbent Assay)

Enzyme immunoassays are the most frequently used methods, as they are simpler and less expensive than other immunochemical techniques. The most popular technique within this group is ELISA (Enzyme-Linked Immunosorbent Assay). The marker is an enzyme (such as horseradish peroxidase or alkaline phosphatase) that converts a colorless substrate (a chromogen) into a colored product detectable by visible light spectroscopy. The different variants of the ELISA technique are presented below.

A. *Indiret Method*

the enzyme used is either peroxidase or alkaline phosphatase. This is an indirect method used, for example, for the diagnosis of HIV infection in the body. HIV viral proteins are immobilized at the bottom of a microplate well. The patient’s serum is then added and incubated. The well is washed to remove unbound substances. Rabbit anti-human immunoglobulin antibodies conjugated to an enzyme (for example, alkaline phosphatase) are added, followed by incubation and another washing step. A colorless substrate (PNPP: p-nitrophenyl phosphate) is then added. In the presence of the enzyme, the substrate is converted into a colored product. The intensity of the coloration is proportional to the number of bound enzyme molecules and therefore to the antibody titer.

In the presence of the enzyme, this substrate is converted into a colored product. The intensity of the color reaction is directly proportional to the amount of bound enzyme complexes, and thus to the patient's antibody titer.

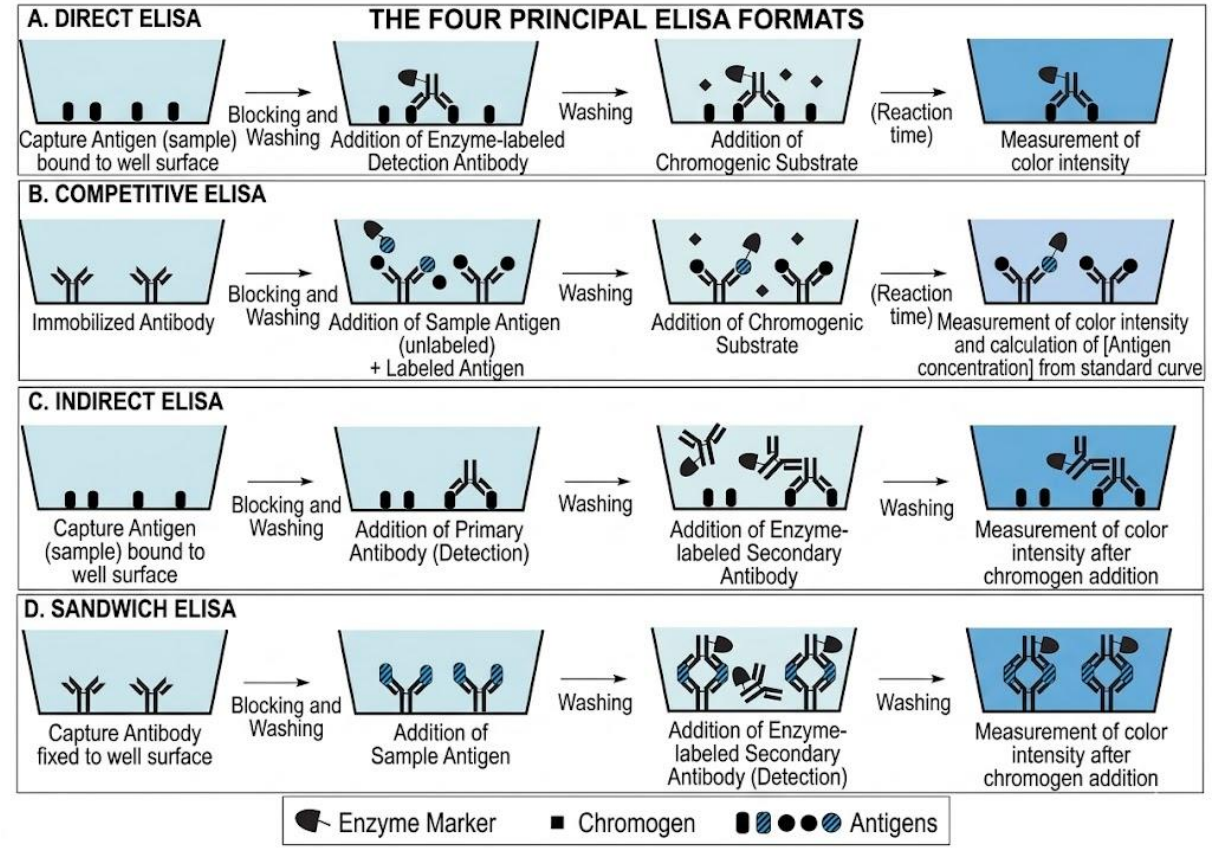


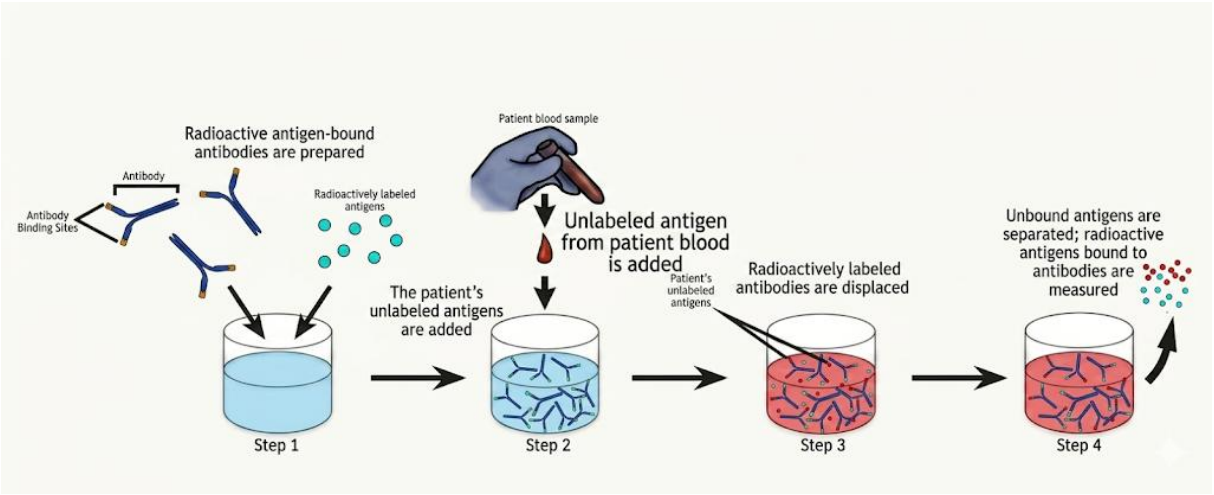
Figure 47 : the four principal ELISA format

VII.3.3. Sandwich" method

The "sandwich" method is applied to antigens possessing at least two epitopes, whether identical or different. It is commonly used for antigen quantification, in which both the antigen and the antibody are known. The result is determined by measuring the colored reaction using a spectrophotometer or an ELISA reader.

VII.3.4. Radioimmunoassay technique (Farr Test)

It uses double-stranded DNA labeled with a radioisotope (^125I). This method detects serum anti-DNA antibodies involved in autoimmunity. The patient's serum is incubated with the labeled double-stranded DNA. Immune complexes are then separated by precipitation and centrifugation. The supernatant containing labeled double-stranded DNA not bound to antibodies is removed. Finally, radioactivity is measured, and the measured radioactivity is proportional to the quantity of antibodies present in the serum.



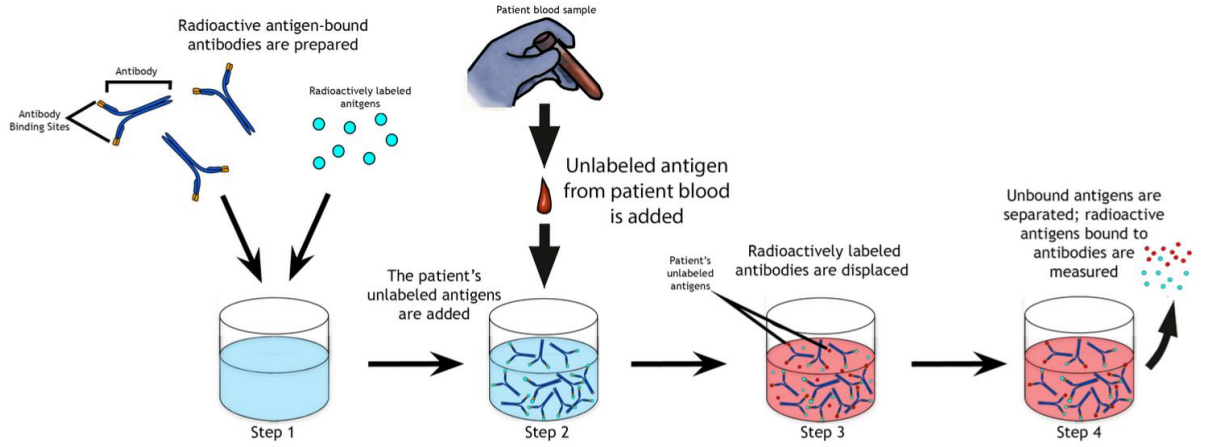


Figure 48 : Radioimmunoassay procedure

VII.3.5. Immunofluorescence

- **Direct Method:** This method enables the detection of a particulate antigen (such as bacteria or a tissue section) using a known, specific antibody labeled with fluorescein. When exposed to an ultraviolet light source (290 - 495nm), this substance emits green light at a longer wavelength (525nm). This method can be applied to the detection of hepatitis B virus & chlamydia. A fluorescent microscope is required to observe the production of color.

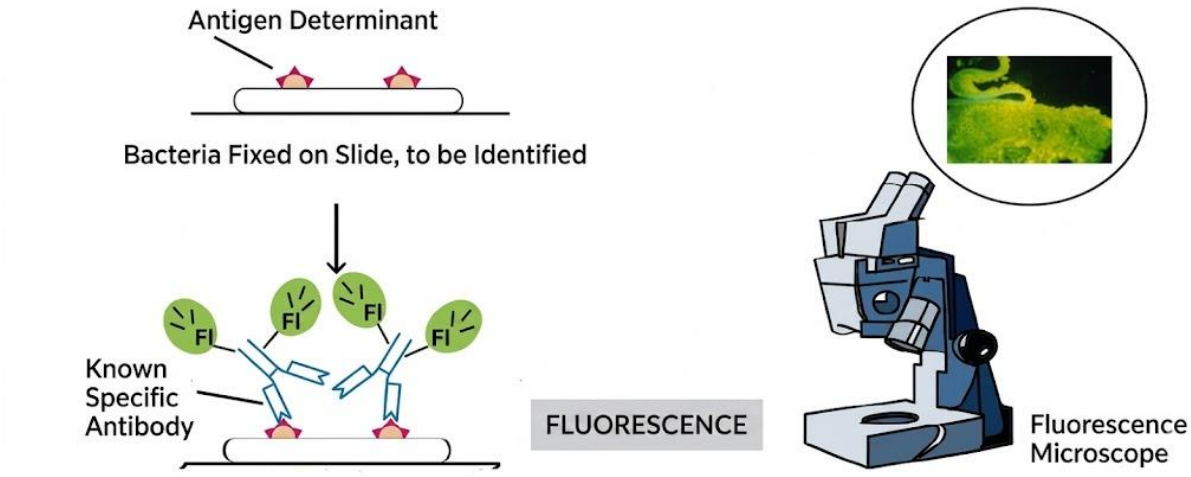


Figure 49 : Identification of an antigen using a fluorophore-labeled antibody

VII.3.6. Indirect Immunofluorescent Assay (IFA)

This method is based on the fact that antibodies not only react with homologous antigens but can act as antigens and react with antibody. In the indirect immunofluorescent assay, the antigen source to the specific antibody being tested is fixed to the surface of a microscopic slide. The patent's serum is diluted and placed on the slide to cover the antigen source. If antibody is present in the serum, it will bind to its specific antigen unbound antibody is then removed by washing the slide, finally antihuman globulin conjugated to a fluorescent substance that will fluoresce when exposed to a fluorescent substance that will fluoresce when exposed to ultraviolet light is placed on the slide. This conjugated marker of human antibody will bind to the antibody already bound to the antigen on the slide and will serve as a marker for the antibody when viewed under a fluorescent microscope.

VII.4. Factors Affecting Antigen Antibody Reactions

Many factors affect the interaction between antigen and antibody; these include specificity, cross reactivity, temperature PH, ionic strength, concentration, and intermolecular specificity.

Specificity: The ability of a particular antibody to combinewith one antigen instead of another is referred to as specificity. This property resides in the portion of the antigen- binding fragment of an immunoglobulin molecule. Antigen- antibody reactions can show a high level of specificity. Specificity exists when the binging sites of antibodies directed against determinants of one antigen.

Cross reactivity: When some of the determinants of an antigen are shared by similar antigenic determinants on the surface apparently unrelated molecules, a proportion of the antibodies directed against one kind of antigen will also react with the other kind of antigen. This is called cross reactivity. Antibodies directed against a protein in one species may also react in a detectable manner with the homologous protein in another species, which is another example of cross reactivity.

Temperature: The optimum temperature needed to reach equilibrium in an antibody-antigen reaction differs for different antibodies. Igm antibodies are cold reacting with thermal range 4-220C, and IgG antibodies are warm reacting, with an optimum temperature of reaction at 370C.

pH: Although the optimum pH for all reactions has not been determined, a pH of 7.0 is used for routine laboratory testing.

Ionic strength: The concentration of salt in the reaction medium has an effect on antibody uptake by the membrane bound erythrocyte antigens. Sodium and chloride ions in solution have inhibition effect. These ions cluster around and partially neutralize the opposite charges on antigen and antibody molecules, which hinders the association of antibody with antigen. Reducing or lowering the ionic strength of a reaction medium such as low-ionic strength salt can enhance antibody uptake.

Concentration: Under normal condition the concentration of antigen and antibody should be optimal but some time this thing fail to be happen in which excess antibody or antigen concentration will result in false reaction, some times knownas zonal reaction. When the concentration of antigen is excess it is known as post zone reaction; excess antibody is referred as prozone reaction. This phenomenon can by overcome by serial dilution until optimum amount of antigen and antibody will present.

Bond strength and inter molecular attractive force Bonding of an antigen to an antibody takes place because of the formation of multiple, reversible, intermolecular attraction between an antigen and amino acids of the binding site. The bonding of antigen to antibody is exclusively non covalent.

The attractive force of noncovalent bonds is weak when compared to covalent bonds, but the formation of multiple non covalent bonds produces considerable total- binding energy.

The strength of a single antigen- antibody bond is termed antibody affinity.

The strongest bonding develops when antigens and antibodies are close to each other and when the shapes of both the antigenic determinate and the antigen-binding site conform to each other. This complementary matching is referred to as goodness of fit.

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