جامعة سطيف 1 - فرحات عباس

Setif 1 University - Ferhat ABBAS

SETIF 1

UNIVERSITY

FERHAT ABBAS

IMMUNOLOGY

Dr. Hanane KHITHER

L2 Biology

2024-2025

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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Chapter 07 : Cellular and humoral cooperation

Chapter 08 : Immune dysfunction

Chapter 09 : Main tests in immunology

Background

This course is designed for second-year students in biology and biotechnology. It serves as a pedagogical resource to help students understand the fundamental mechanisms of the immune system and thereby acquire a solid foundation in immunological principles. To this end, both a general objective and specific learning objectives have been defined.

General Objective

The learner will acquire a foundational body of knowledge in immunology, with a focus on the functioning of the immune system as well as the properties of its effectors and their targets.

Specific Objectives

1. The learner will build a knowledge base regarding:

- The histology of primary and secondary lymphoid organs and their functions.
- The different types of immune cells, along with their properties and functions.

2. The learner will be able to:

- Define the concept of an antigen, identify the criteria of immunogenicity, and distinguish between different types of antigens.
- Recognize the structure and function of Major Histocompatibility Complex (MHC) molecules.
- Understand the mechanisms of action of the effectors of innate, cellular, and humoral immunity.
- Comprehend the principles of cellular and humoral cooperation, immune dysfunctions, and the main immunological tests.

Chapter 01

Introduction on immunology

I. Basic Concepts in Immunology

I.1. Immunology

Immunology is the branch of biology that studies the functioning of the immune system in both **physiological** and **pathological contexts**. It enables the investigation of the properties of immune effectors and their targets both ***in vivo*** and ***in vitro***.

I.2. The Immune System

The immune system comprises organs, tissues, cells (such as T and B lymphocytes, macrophages, and leukocytes), and molecules (including antibodies and interleukins) that participate in immunity.

Lymphoid organs and tissues are distributed throughout the body. Immune cells circulate within and between these organs via blood and lymph. They communicate either through direct contact (receptor–ligand interactions) or at a distance via secreted molecules (mediator–receptor interactions). These soluble, secreted molecules are called cytokines substances produced by certain immune cells that act on other immune cells to regulate their activity. This general term encompasses lymphokines, monokines, chemokines, and interleukins.

I.3. Immunity

Immunity refers to the set of biological mechanisms that allow an organism to:

- Recognize and tolerate what belongs to itself ("self"), and
- Identify and eliminate what is foreign ("non-self").

Two major types of immunity are distinguished:

- **Innate (non-specific) immunity**: physiological barriers and inflammation.
- **Adaptive (specific) immunity**: cellular or humoral responses.

During an infection, the immune response unfolds in three phases:

1. **Early response (0–4 hours)** mediated by innate immunity (physiological barriers).
2. **Intermediate response (4–96 hours)** also involving innate immunity (inflammation).
3. **Late response (after 96 hours)** involving adaptive immunity, characterized by the clonal expansion of B and T lymphocytes specific to the pathogen's antigens. This pro-

cess generally eliminates the infectious agent and "educates" the immune system by generating memory lymphocytes. After antigen clearance, the immune response gradually declines.

I.4. Self

"Self" refers to all molecules derived from the normal genetic expression of the organism's genome molecules that define the biological identity of the immunized organism.

I.5. Non-Self

"Non-self" encompasses all molecules that do not belong to the organism's biological identity, including:

- Altered self-components (e.g., tumor cells not derived from normal genomic expression), and
- Foreign substances or infectious agents (bacteria, viruses, fungi).

Non-self molecules can trigger an immune response and are therefore considered **antigens**.

I.6. Antigen (Antibody Generator)

An antigen is a substance foreign to the organism, typically a natural or synthetic macromolecule, capable of inducing a specific immune response (immunogenicity), either humoral or cellular. In addition, it can react with recognition molecules (TCRs/BCRs) and the products of the immune response (antibodies), thereby exhibiting reactivity.

II. Immunity in Daily Life and Major Discoveries

Immunity plays a crucial role in controlling many diseases. **Immunotherapy** is a therapeutic approach that modulates a patient's immune system to combat disease. It is already applied in several medical contexts:

- **Infectious diseases**: Preventive vaccines train the immune system to recognize and eliminate an infectious agent before an actual infection occurs. Vaccination relies on memory lymphocytes, which provide long-term protection. Furthermore, early trials with immunomodulators (drugs that either stimulate or suppress the immune system) have shown promising results against HIV. Inhibitory receptors such as **PD-1 (Programmed cell Death-1)** have been identified on T lymphocytes in AIDS patients. Blocking this inhibition with anti-PD-1 antibodies improves antiviral responses.
- **Allergic and autoimmune inflammatory diseases**: These result from dysregulated immune responses, either to harmless allergens or to self-components. Allergies can be treated through immunotherapy (desensitization), which progressively induces

tolerance to the allergen. Autoimmune diseases are managed with immune modulation, including immunosuppressants and monoclonal antibodies (e.g., anti–TNF-α, anti–IL-1, anti–IL-6, anti–IL-12/23).

- **Neurodegenerative diseases**: In Alzheimer's disease, therapeutic trials with monoclonal antibodies or vaccines aimed at clearing β-amyloid peptides have so far been unsuccessful. However, these studies suggest an important role for immunity and inflammation in disease onset, making the immune system a potential therapeutic target.
- **Cancer immunotherapy**: This strategy seeks to "awaken" and retrain the immune system to eliminate cancer cells. It employs vaccines, monoclonal antibodies, and immunomodulators.

II.1. Different Approaches to Immunotherapy

II.1.1. Stimulating the Global Immune Response

One strategy is to enhance the overall immune response by using cytokines proteins synthesized by certain cells in response to stimuli, which act at a distance to regulate the activity and function of other cells. Increasing their number or activity strengthens immune responses.

Two cytokines already used clinically include:

- **Interleukin-2 (IL-2)**: used in advanced renal cancer; modified (pegylated) forms reduce adverse effects without diminishing efficacy.
- **Interferon-α2b**: applied in certain leukemias, myelomas, and melanomas.

Another example is the **BCG vaccine (against tuberculosis)**, which also serves as a therapeutic agent in bladder cancer, inducing prolonged antitumor responses despite an incompletely understood mechanism.

II.1.2. Blocking Specific Tumor Signals

Monoclonal antibodies can target specific proteins on cancer cells or in their microenvironment, disrupting interactions that promote tumor growth.

Table I. Selected Monoclonal Antibodies Used in Cancer Immunotherapy

Monoclonal Antibody	Target Protein	Mechanism / Indication
Rituximab	CD20 (on B lymphocytes); certain lymphomas	Exerts toxic effects on B cells (lymphatic cancers).
Trastuzumab	HER2 (on ~15% of breast cancers)	Blocks receptor signaling and inhibits tumor growth.
Bevacizumab	VEGF (vascular endothelial growth factor)	Inhibits angiogenesis in lung, breast, and colon cancers.

Recently, **bispecific antibodies** have been developed. These recognize two distinct molecules simultaneously, allowing them to link cancer cells with cytotoxic T lymphocytes. For example, blinatumomab is used in acute lymphoblastic leukemia.

II.1.3. Arming the Immune System Against Tumors

- **Cell Therapy with CAR-T Cells**

  A breakthrough approach introduced in 2017 combines gene therapy and cell therapy. Patient-derived T lymphocytes are genetically engineered in vitro to express **Chimeric Antigen Receptors (CARs)** that recognize tumor antigens. Expanded in the laboratory, these **CAR-T cells** are reinfused into the patient, where they specifically attack and destroy cancer cells.

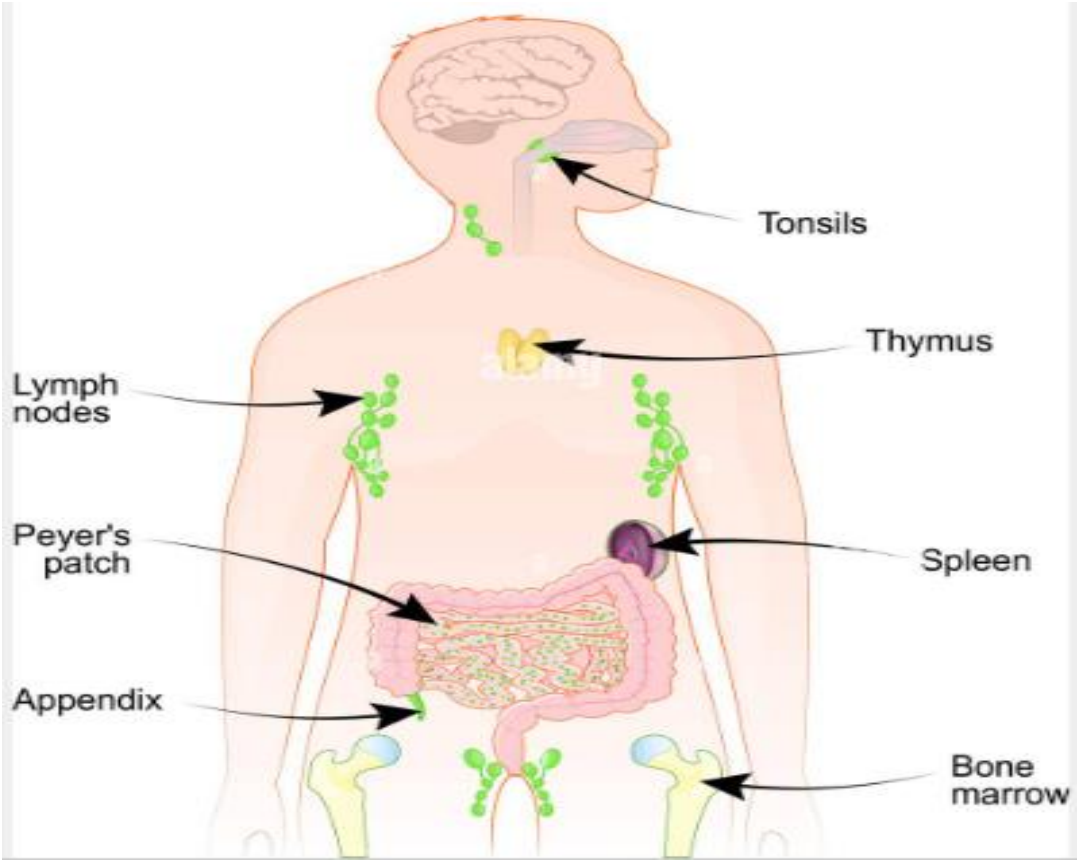
- **Therapeutic Cancer Vaccines**

  These vaccines aim to stimulate a targeted immune response against cancer cells by presenting tumor antigens. They are personalized to the patient's tumor profile. The only currently marketed vaccine is **Sipuleucel-T**, indicated for prostate cancer. It involves isolating dendritic cells from the patient, exposing them in vitro to a tumor-associated antigen (prostatic acid phosphatase, expressed in ~95% of prostate cancers), and reinfusing them. This triggers T-cell cytotoxicity against the tumor. Treatment requires three administrations at two-week intervals.

Therapeutic vaccination has a major advantage: it induces a **memory immune response**, which may protect the patient against relapse.

Chapter 02

Immune system



I. Lymphoid organs and tissues

Lymphoid organs and tissues correspond to the place of production and residence of lymphocytes and other cells of the immune system. There are two groups (**Fig. 01**):

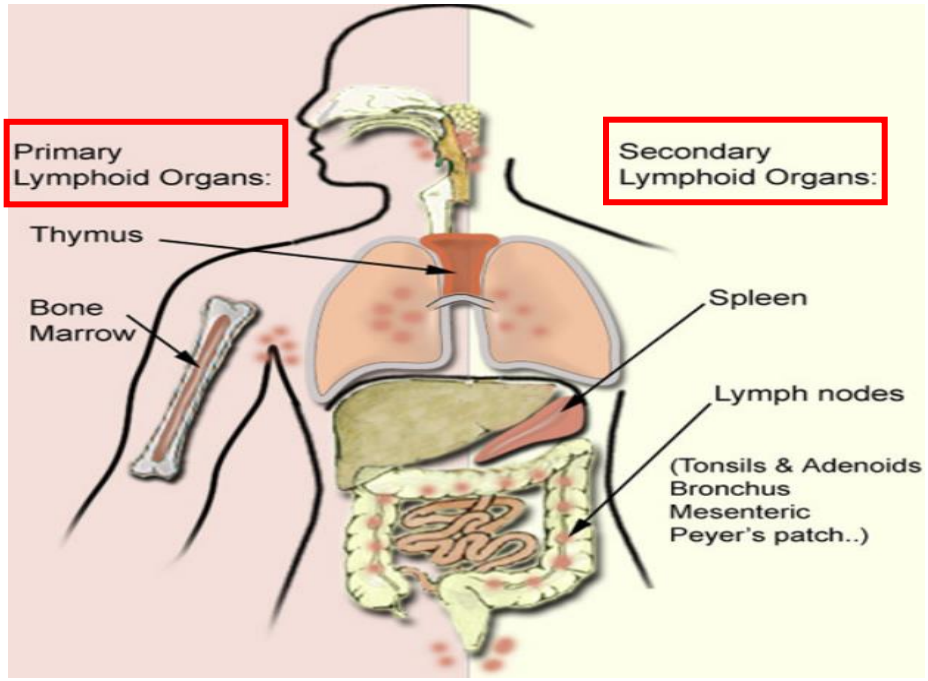


Fig. 01: Lymphoid organs and tissues

I.1. Primary or central lymphoid organs

Primary lymphoid organs are immune organs responsible for the production **and/ or** maturation of immune cells. They correspond to the **red bone marrow** and **thymus**.

I.1.1. Bone marrow

The bone marrow corresponds to the tissue present in the central part of both long and short bones; but essentially the bone marrow present in the **short and flat bones** (sternum, ribs, vertebrae, skull, etc.), in fact only these bones still have **red bone marrow (Fig.02).** Schematically, there are **several functional compartments:**

- **Multipotent hematopoietic stem cells** (**HSC**) that will multiply and differentiate into all immune cells, These are the mother cells of all bone marrow cells and ultimately blood. They have three main functions:

✓ They are capable of **self-renewal** to maintain the blood cell stock for life;
✓ They are said to be **pluripotent**, that is to say that they are capable of giving rise to the precursors of **all hematopoietic lines**;

- ✓ They are endowed with a capacity for engagement in **differentiation** to lead in several stages to immune cells.

- **Progenitors**

  These are cells engaged in a lineage but not recognizable under a microscope.

- **Precursors**

  Recognisable under a microscope, they will give rise to mature differentiated blood cells.

- **Stromal cells**

  Responsible for the maturation of BL (BCR acquisition) and other blood cells.

As a result, the bone marrow is the **birthplace** of the **progenitor** cells of the different populations of lymphocytes and phagocytic cells. It is the site of **hematopoiesis and B-cell maturation**.

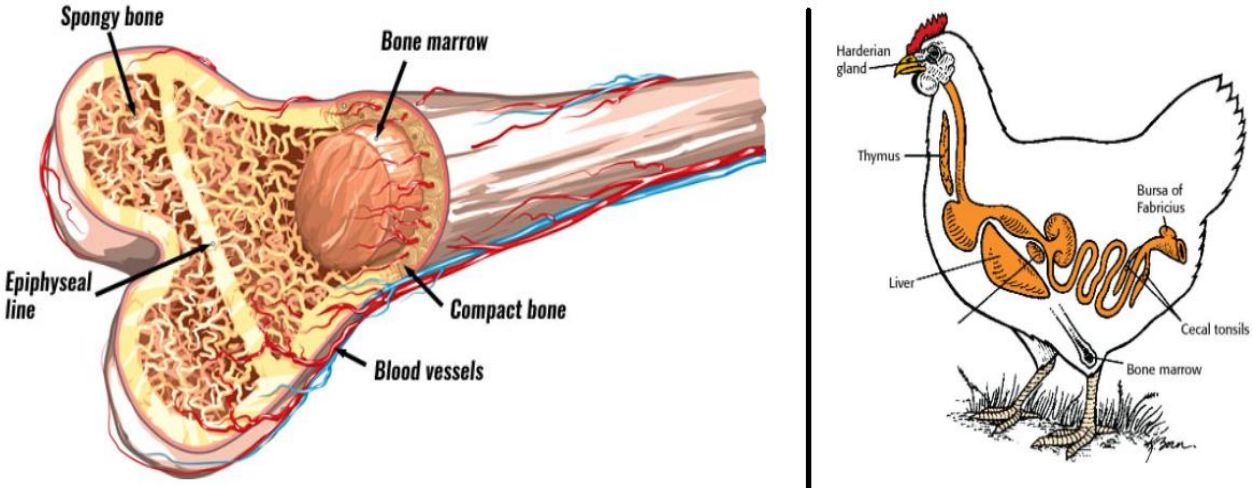


Fig. 02: Primary lymphoid organs (Bone marrow and Fabricius bursa)

Note: Note that the **Bursa of Fabricius** is the equivalent of the bone marrow in birds, serving as the site of **B lymphocyte maturation**.

I.1.2. Thymus

The thymus is a lympho-epithelial **organ** located above the heart (the anterior-upper part of the thoracic cavity). The thymus is very active in the perinatal period (15 g at birth), its maximum development at puberty (40 g), then regresses in adulthood but does not disappear entirely (10 g in the elderly). It consists of **two lobes**. Each lobe is subdivided by connective septa into **lobules (Fig. 03)**.

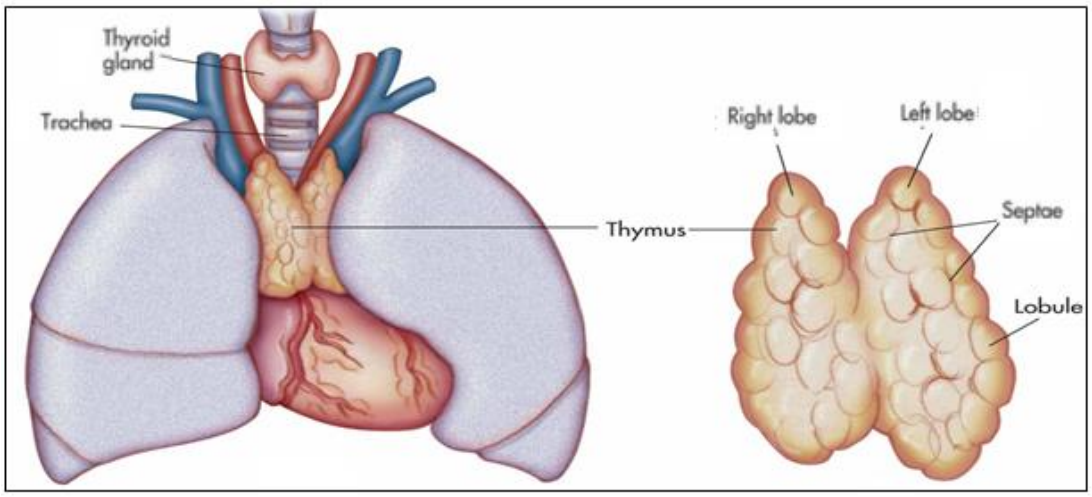


Fig. 03: Structure of the thymus

Each **lobule** contains **(Fig. 04)**:

- **Peripheral area**, the **cortex** is the **outermost area (below the capsule)**. There are mostly **cortical epithelial cells**, **immature thymocytes** and some **macrophages**. At the level of which the **positive selection** of T progenitors by **cortical** epithelial cells occurs: this is the survival of all pro T cells (from the bone marrow via the bloodstream) that can recognize one of the **MHC molecules (I or II)** presented on the surface of **cortical epithelial cells**, therefore the death by **apoptosis** of thymocytes that do not recognize the **MHC** molecules.
- The **cortico-medullary junction** is the **entry** point for progenitors coming from the bone marrow and the **exit** point for mature cells passing through the upper endothelium of the post capillary venules (**HEV**).
- The **medulla** is the innermost area where **mature cell accumulation** and **negative selection** by **medullary epithelial cells** and **dendritic cells** occur: this is the death by **apoptosis** of all pro T (**self-reactive**) that recognize **the self-peptide** presented in association with MHC molecules. In the medullary area, there are **mature thymocytes** , **macrophages** and **dendritic cells**.

The medulla gives the impression of being lobulated, and each of these lobules is centered by a **Hassall corpuscle** (thymic corpuscle); a keratinizing differentiation of epithelial cells.

Pro T at the end differentiate into **LT_4 CD4 carriers and TCR** or **LT_8 CD8** carriers with a **TCR**. As a result, the thymus is **the site of T-cell maturation**.

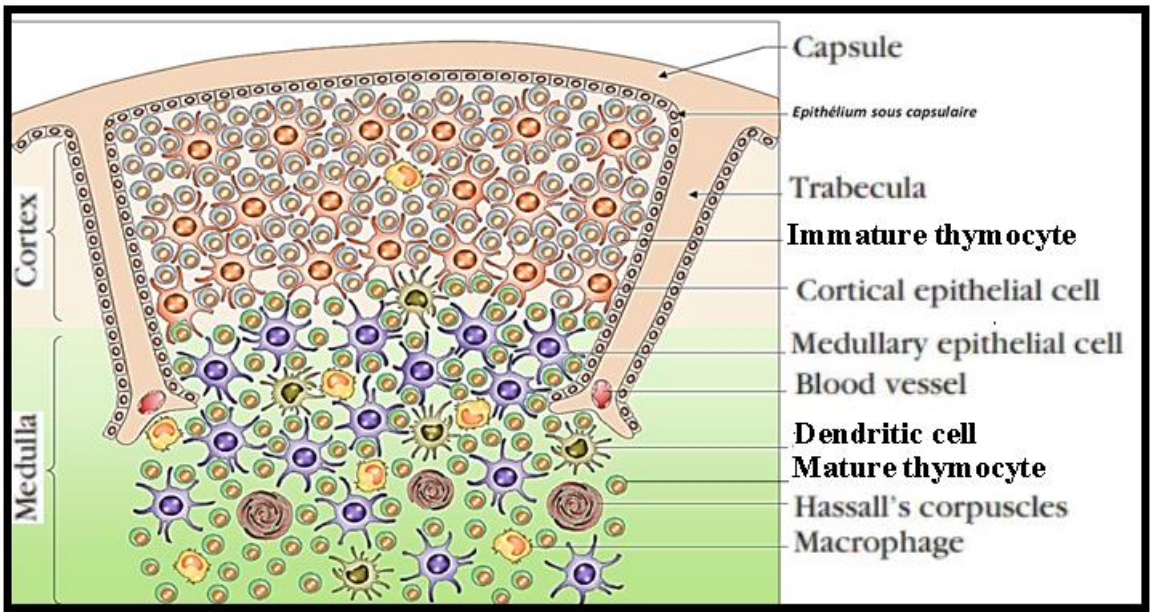


Fig. 04: Structure of a thymic lobule

I.2. Secondary or peripheral lymphoid organs

The **secondary lymphoid organs** are places of concentration (storage) of lymphocytes, at which the **triggering of the specific immune response** takes place, in other words the activation of lymphocytes which will differentiate into effector cells and memory cells (**seat of the specific immune response**). Among them are **lymph nodes**, **spleen** and **MALT** (for *"**M**ucosa **A**ssociated **L**ymphoid **T**issue"* including **tonsils** and **Peyer's patches** and others).

I.2.1. Lymph nodes

Lymph nodes or **lymphoid nodules** are small rounded or reniform organs with a diameter of between 5 and 20 mm, numbering between 500 and 1000 in adults. They are arranged at the **openings** of the **lymphatic pathways**, usually grouped in chains forming a complex network that drains the skin as well as the internal organs. They are surrounded by a **connective fibrous capsule**, pierced by an **efferent lymphatic channel** responsible for the **exit** of the **lymph**, **afferent lymphatic channels** that allow the lymph to **enter** the lymph node **(Fig. 05)**.

The nodes play a main role in the immune response because they are the place of proliferation and differentiation of activated immune cells (**seat of the specific immune response**), and also because they play the role of an antigen **filter** of the **lymphatic circulation (interstitial fluid and lymph)**.

They are formed of 3 regions **(Fig.05)**:

- **Cortical area:** is a **dependent B zone,** rich in **B lymphocytes,** follicular dendritic cells and macrophage. BLs form oval clusters called **primary follicles** rich in **naïve B lymphocytes** (in the absence of antigenic stimulation), and **secondary follicles** with a **germinal center**: **seat of B lymphocyte proliferation** (after antigenic stimulation).
- **Paracortical area** (deep cortex): is a **T-dependent** zone, rich in **T** lymphocytes, **antigen-presenting cells**; **dendritic cells** (interdigitated cells) and **macrophages**. This is the area of the **postcapillary venules** also called **high endothelial** venules (**HEV**), which express **specific** surface **receptors** for **lymphocytes**, allowing blood B and T lymphocytes to enter the lymph node (**homing**).
- **Medullary area** : **mixed zone** comprising **B** lymphocytes, **T** lymphocytes, **plasma** cells and **macrophages.**

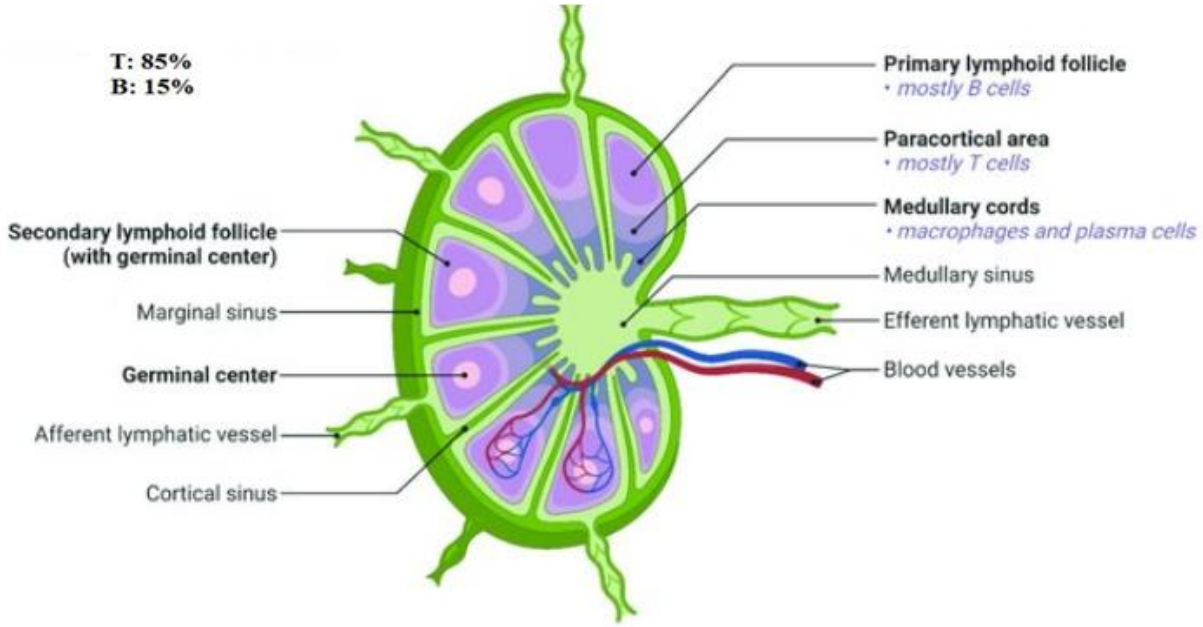


Fig. 05: Structure of a lymph node

I.2.2. Spleen

The **spleen** is the largest oval-shaped **intraperitoneal abdominal** organ (12 cm long). It is not connected to the lymphatic circulation, but placed on the **blood circulation**. It is both:

- A **haemolytic organ**: where the destruction of aged red blood cells is ensured.
- A **lymphoid organ**: ensuring the recognition and capture of circulating antigens in the blood and triggering the differentiation of immunocompetent cells.

The spleen is surrounded by a capsule from which start connective partitions delimiting **lobules** at which the **splenic pulp** is organized. This includes **red pulp** and **white pulp (Fig. 06)**.

a. Red pulp: occupies the largest space, it is a blood antigen **filter** .

- Area of macrophages, T and B lymphocytes, plasma cells, erythrocytes, and granulocytes.

- Place of destruction of senescent (old) red blood cells.

b. White pulp: locus of the specific immune response, including:

- **An area** of **T lymphocytes** and dendritic cells around the central arteriole, called periarterial lymphoid sheath; **PALS**: periarterial lymphoid sheath (sleeve) (**P**eri**a**rterial **L**ymphatic **S**heaths).
- **A follicular area** of **B lymphocytes** organized into **primary follicles** and **secondary follicles**.
- **A less dense marginal area** surrounding the pulp: rich in T lymphocytes, B lymphocytes and macrophages.

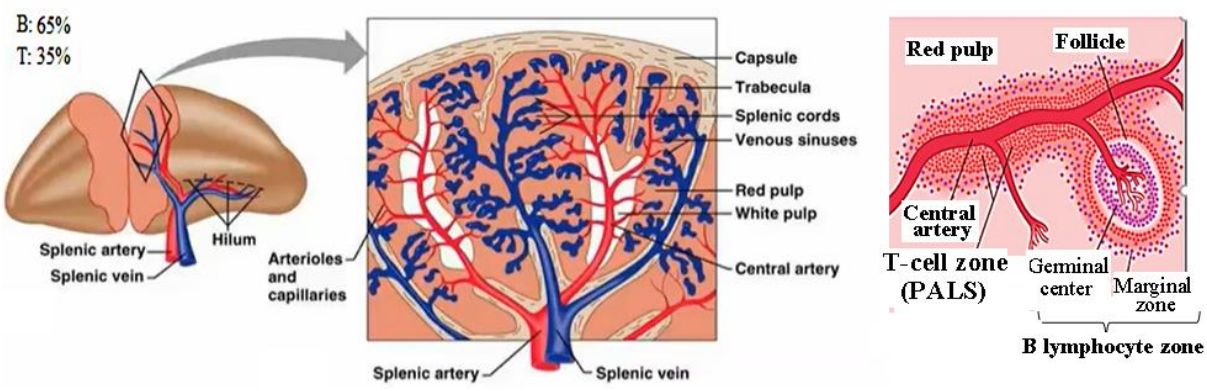


Fig. 06: Structure of the spleen.

I.2.3. Mucosal Associated Lymphoid Tissues

All mucous membranes contain **diffuse lymphoid** tissue or well individualized lymphoid formations, bearing the name **MALT** (mucosal associated lymphoid tissue) closely associated with the coating epithelia, and which provides immunological protection. It protects more than 400 ^{m2} of mucous membranes exposed to environmental risks. It contains small clusters of **B and T lymphocytes and plasma cells** (mainly secreting **IgA**). This system includes:

- **Bronchus** associated lymphoid tissue or **BALT** (bronchus associated lymphoid tissue).
- **Gut** associated lymphoid tissue (**GALT**).
  - Pay Plate
  - The Appendix

- The Tonsils

- Lymphoid tissue of the **nasal** mucosa or **NALT** (nasopharynx associated lymphoid tissue).

- Lymphoid tissue associated with the **eye**: **EALT (Eye Associated Lymphoid Tissue)** comprising:

  - Tear drainage: **LDALT (Lacrymal Drainage Associated Lymphoid Tissue)**
  - Conjunctiva: **CALT (Conjunctiva Associated Lymphoid Tissue).**

1. Gut Associated Lymphoid Tissue (GALT)

Gastrointestinal Tract Associated Lymphoid Tissue located in the mucosa and sub-mucosa of the intestinal wall of the ileum which loses its villi at this level. **GALT lymphoid tissue (Fig. 07)** includes:

- The villous epithelium: contains **intra-epithelial lymphocytes** (IELs)
- **Peyer's plaques**: described for the first time by Peyer (1677): from 40 to 100 in the fetus, up to 250 at puberty, dependent on antigenic stimulation. Located in the mucosa and sub-mucosa of the intestinal wall of the ileum which loses its villi at this level. It constitutes the **inducing** site, in which the immune response is triggered. It is organized into highly variant tissue structures including:

- **Non villous epithelium**: contains the **M cells (M = Microfolds)** responsible for the **endocytosis** of antigens present in the intestinal lumen.

- **Dome**: very rich in LB, LT, lots of macrophages and dendritic cells

. - L**ymphoid follicles** rich in B lymphocytes

- I**nter-follicular area;** between lymphoid follicles: rich in LT and dendritic cells.

- **Lamina propria**: this is the digestive mucosa: contains a lot of **activated LT**, **activated LB**, **plasma cells** producing **IgA** class antibodies. It constitutes an **effector site.**

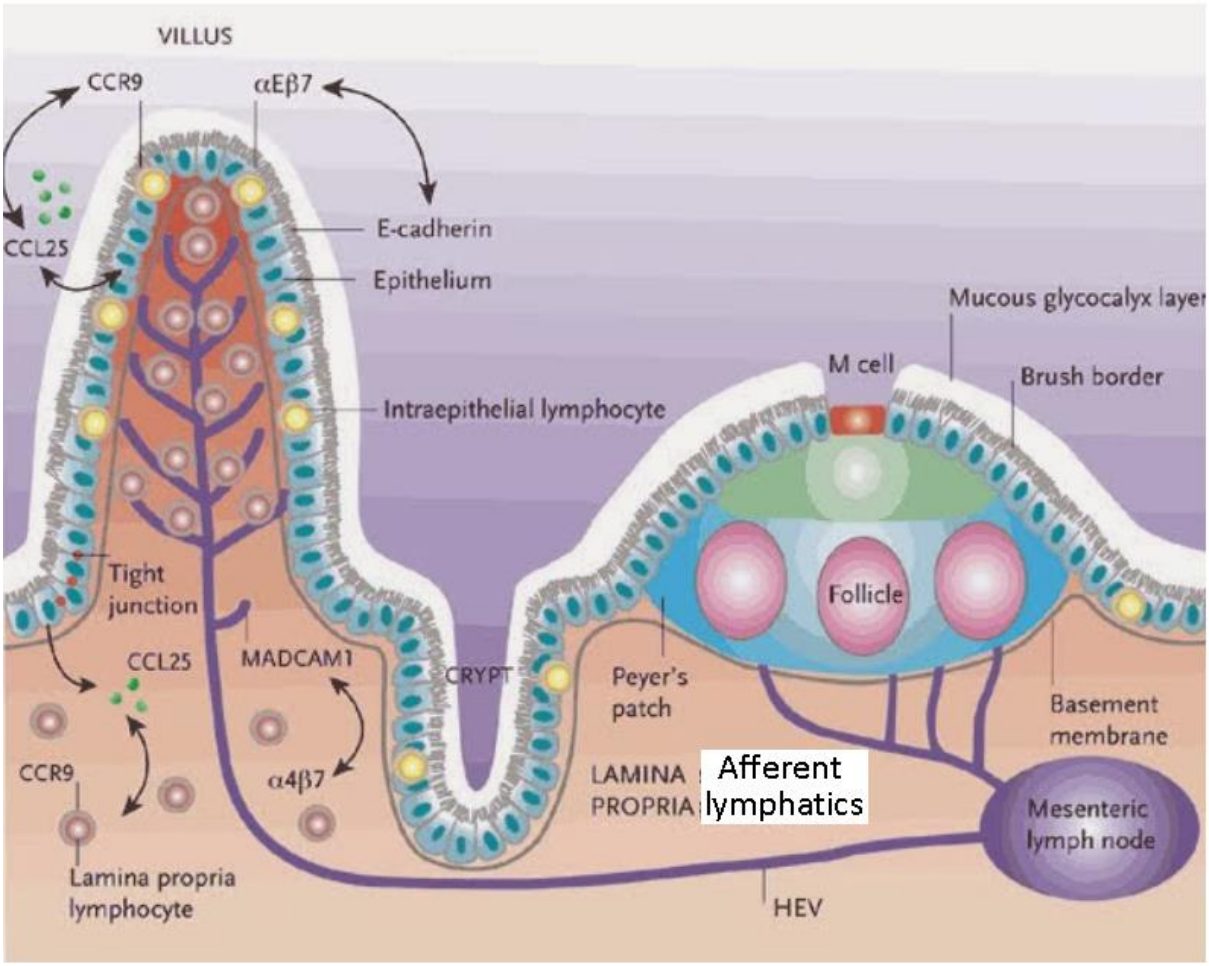


Fig. 07: Tissue organization of the GALT.

2. Tonsil

Are masses of lymphoid tissue, embedded in the chorion of the mucosa of the organ where they are located. The whole of which constitutes **Waldeyer's amygdala ring or circle**. Divided into four groups **(Fig. 08)**:

- **Palatine tonsils** located between the pillars of the soft palate (the largest).
- T**ubal tonsils** (in the pharynx).
- **Pharyngeal tonsil** (on the posterior side of the pharynx).
- **Lingual amygdala** (at the dorsal side of the tongue).

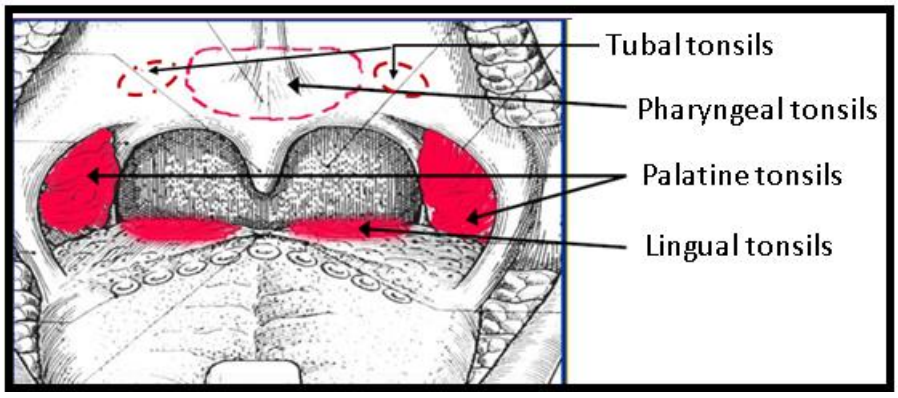


Fig. 08: Waldeyer's ring.

They are surrounded by a **stratified, non-keratinized buccal-type squamous epithelium** that forms **crypts** (deep and narrow invaginations). The underlying **chorion** is rich in **secondary lymphoid follicles**, surrounded by a sheet of lymphoid tissue. The lymphoid tissue of the tonsils is rich: in **B lymphocytes** and other immune cells: **T lymphocytes**, **macrophages** and **dendritic cells**.

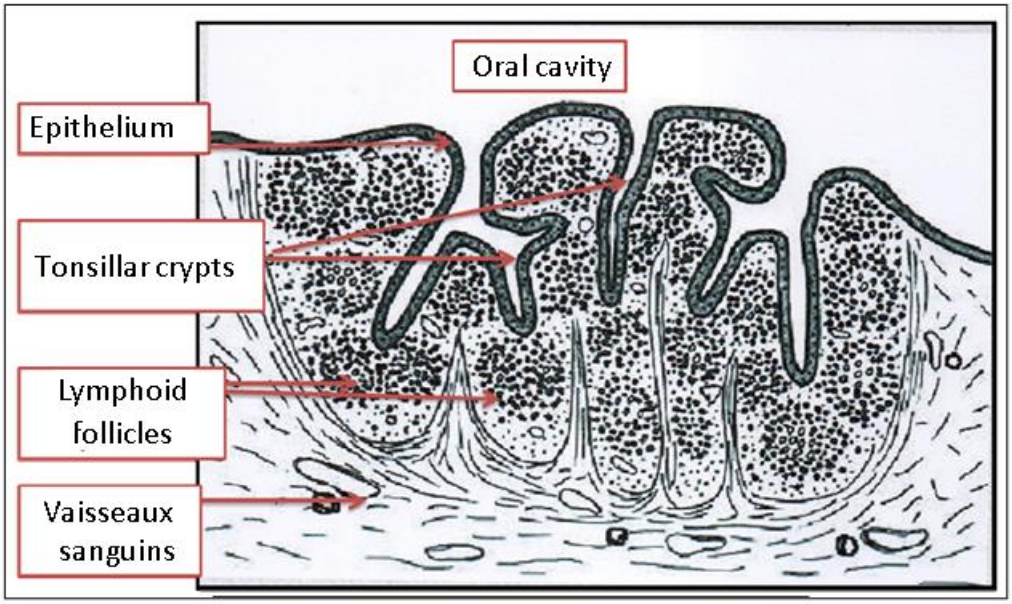


Fig. 09: Palatine tonsils.

II. Immune cells

Immune cells are derived from the proliferation and differentiation of hematopoietic stem cells, they have both **lymphoid** and **myeloid** origins **(Fig. 10)**.

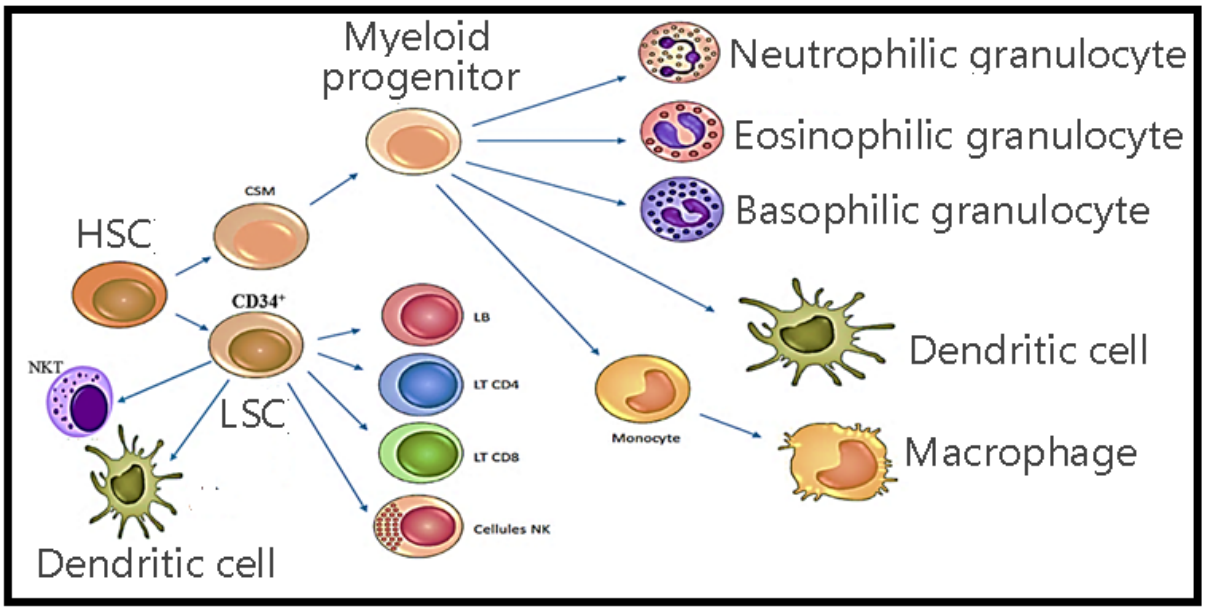


Fig. 10: Immune cells according to their origin.

From a multipotent hematopoietic stem cell (HSC) (which can also give rise to red blood cells or platelets) are generated **Lymphoid Stem Cells** (LSC) (CD_{34} carriers) and **Myeloid Stem Cells** (MSC). The first give rise mainly to **B lymphocytes**, **CD_4 or CD_8 T lymphocytes** and **NK cells**. The latter are the origin in principle of the three types of **granulocytes**: neutrophils, eosinophils and basophils, as well as **dendritic cells** and **monocytes** which subsequently differentiate into **macrophages** and **dendritic cells**.

II.1. Innate immunity cells and their receptors

II.1.1. Innate immunity cells

II.1.1.1. Polynuclear cells or granulocytes

This group of cells has common characteristics. They contain a multilobed core. The lobes are connected to each other by thin chromatin bridges. In the cytoplasm, there are **two types of granulations**: **primary non-specific granulations**, rich in **hydrolases** and **peroxidases**, common to all polynuclear cells and **secondary granulations specific** to each group. In the mature cell, non-specific granulations decrease.

A- Neutrophils: 10 to 12 µm in diameter, **multilobed nucleus**, 60-70% of leukocytes. The cytoplasm contains two types of granulations: **non-specific or primary, azurophilic granulations** that contain a **myeloperoxidase**, **acid hydrolases** and **lysozyme** and **specific secondary, neutrophilic, small granulations** (0.3 to 0.8 µm) scattered throughout the cytoplasm. These granules contain **lysozyme** and **collagenase**.

The **function** of these neutrophils is the **nonspecific defense of the body.** They are mainly involved in **antibacterial and antifungal** control. They **phagocytose small** elements.

B- Eosinophils: 10 to 12 µm in diameter, **bilobed** nucleus, 1-2% of the total leukocyte population. The **specific, eosinophilic granulations** are large, 0.5 to 1.5 µm in diameter and contain **peroxidase** (different from neutrophil myeloperoxidase) and **acid hydrolases**.

These cells participate in synergy with other cells in immediate and delayed **hypersensitivity reactions**. They have to a lesser degree than neutrophils **bactericidal and phagocytic** properties. They are mainly involved in **the destruction of parasites** via **high molecular weight proteins** (Eosinophil Cationic Protein - **ECP** and Major Basic Protein - **MBP**). The plasma membrane has **receptors** for IgE-type **immunoglobulins** and **histamine**.

C- Basophils: 9 to 10 µm in diameter, horseshoe core, less than 1% of leukocytes. They are not phagocytic. They have large **granulations** containing vasoactive mediators (histamine, heparin). They intervene in **allergic reactions** of the immediate type. Because the plasma membrane of basophils has **receptors** for the **Fc** fragment of **IgE-type** immunoglobulins.

II.1.1.2. Mast cells

Connective tissue cells, often grouped around capillaries, of 20-30 µm, possessing granulations containing **vasoactive** amines (Histamine, serotonin, kinins, etc.). Mast cells **initiate the immune response** (**inflammation**) and intervene **in allergic reactions.** The mast cell expresses membrane **receptors** at the constant fragments **(Fc) of immunoglobulin E(IgE)**

II.1.1.3. Monocytes/ Macrophages

Cells with the ability to **phagocyte** large elements.

Monocytes: **14-17 µm** in diameter, 6-8% of circulating leukocytes, very mobile. Their nucleus is roughly kidney-shaped. Their cytoplasm is rich **in lysosomes** with varied enzymatic activities. They leave the blood circulation compartment, to reach the different tissues. They are then called **macrophages (17 to 40 µm** in diameter), characterized by **cytoplasmic expansions** that

form true **pseudopods**. They are located in almost all tissues, including connective tissue (**histiocytes**), liver (**Kupffer cells**), nervous system (**microglia**), kidneys (**mesangial cells**), bones (**osteoclasts**), lung (**alveolar macrophages**). The main function of macrophages is **phagocytosis**. They play an accessory role in specific immunity by presenting antigenic immunogenic peptides to previously activated T lymphocytes.

II.1.1.4. Dendritic cells

They are of two different origins, myeloid and lymphoid.

* **Conventional myeloid dendritic cells** are found in the **blood** and in **non-lymphoid** tissues. They are in an **immature state**. There are three populations: **Langerhans dendritic** cells in the **epidermis**, **interstitial dendritic cells** in **the dermis** of most organs (heart, kidneys, lungs, liver, gastrointestinal tract) and **dendritic cells derived from monocytes** (circulating) in the blood and lymph.

* **Plasmacytoid dendritic** cells of lymphoid origin migrate directly from the blood into the lymphoid organs (thymus, T-zone of secondary lymphoid organs). They are mature, they then have a function of induction of the adaptive response and typically have long cytoplasmic extensions. They have the unique ability to produce large amounts **of interferons (IFN *α*)** in response to viral infection.

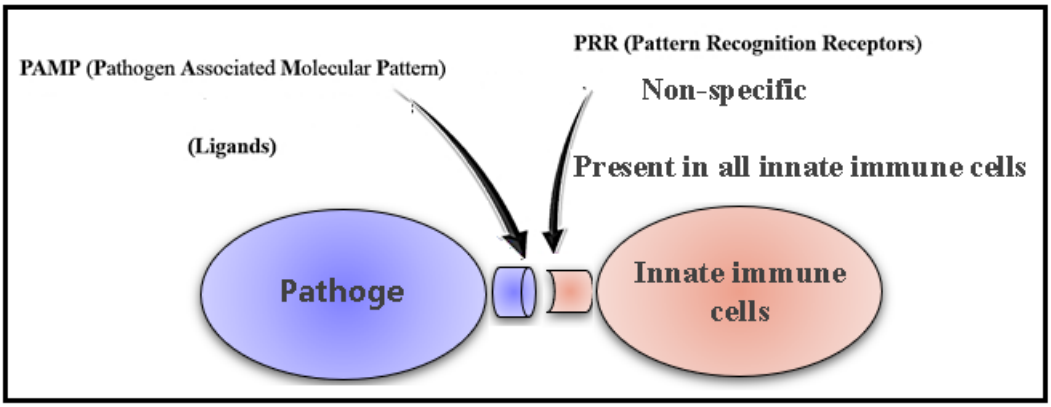
* **Macrophages** and **dendritic cells** with **BL** are **antigen presenting cells**.

II.1.1.5. Natural Killer cells (NK).

They are large granular lymphocytes that belong to the innate immune system, neither **T nor B.** They are capable of **killing tumor** cells, cells **infected with** viruses or **foreign** cells, characterized by **CD_{16} and CD_{56}** markers. They express inhibitory receptors (**KIR**) and activator receptors (**KAR**). These cells are very rich in granules that contain **perforin** and **granzyme**. NK cells also secrete cytokines (interferon gamma "IFN-γ") that participate in the orientation of the adaptive immune response.

II.1.2. Receptors of innate immunity cells for pathogens

The microorganisms are recognized by receptors of the resident cells, mast cells, macrophages and dendritic cells. These receptors are called **PRRs (Pattern Recognition Receptors**) of pathogens. These receptors recognize conserved patterns or structures, shared by many pathogens, called PAMPs (**P athogen A**ssociated **M**olecular **P**attern). These receptors are of two types.



II.1.2.1. Endocytosis receptors (phagocytosis)

They activate phagocytosis by stimulating the ingestion and destruction of the pathogens they recognize (Fig. 11).

A- Lectin receptors: CLR (C-type-Lectin Receptor), membrane receptors.

- **Receptors for mannose** surface constituent of bacteria and yeast.
- **-Receptors for glucan** component of mushroom wall glucose polymers

B- LPS receptor (CD_{14}): lipo-polysaccharide receptor constituting the walls of Gram-negative bacteria.

C- Scavenger receptors or garbage collectors: membrane receptors that bind to various pol-yanions of certain bacterial walls.

D- N-Formyl-Methionine receptors: membrane receptors that bind to proteins starting with an *N -formyl-methionine* residue, the latter cannot be synthesized by the nuclear genome of eukaryotes. These receptors are used to trigger the phagocytosis process.

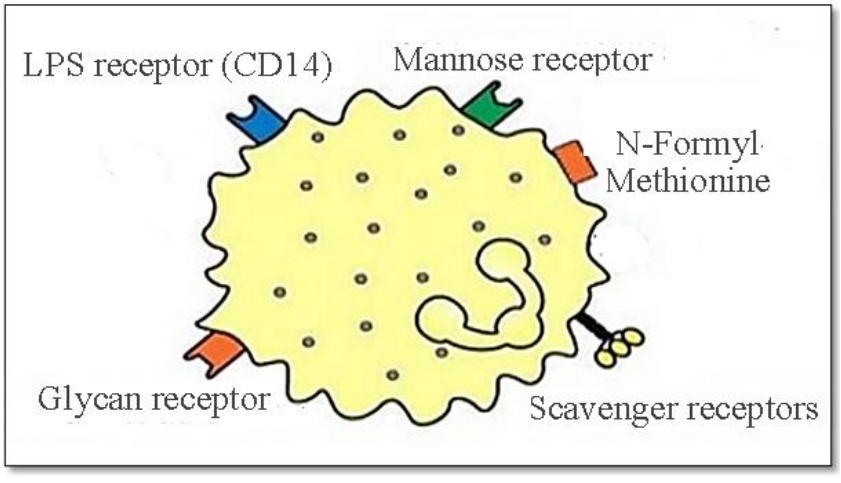


Fig. 11: Endocytosis receptors.

II.1.2.2. Signalling receptors

The binding of these receptors to microbial ligands activates the cells that carry them and triggers the secretion by these cells of pro-inflammatory factors and cytokines and chemokines. These receptors are of several types (**Fig. 12**):

A- Toll-like receptors (TLR)

These are proteins homologous to **Toll** that protect Drosophila from infection. LRTs recognize repeated structures (patterns) present on the surface of microorganisms. There are **12** of them, from TLR1 to TLR12 in humans. Some are **membrane** (1,2, 4, 5, 6, 10, 11 and 12) others are **endosomal** (TLR 3, 7,8 and 9).

- **TLR membranes : TLRs 1**, **2**, **4**, **5**, **6** are located at the plasma membrane and are involved in the recognition of wall components of infectious agents. Specifically, the following forms can be mentioned:
    - **TLR-4** which recognizes **LPS** (*Lipo Polysaccharides*), endotoxins present in Gram-negative bacteria.
    - **TLR-5** which recognizes flagellin, structural proteins of bacterial flagella.
    - **TLR-1**, **TLR-2** and **TLR-6** which recognize peptidoglycan, lipoproteins and glycophospholipids; they form **heterodimers** TLR-1/TLR-2 and TLR-2/TLR-6.
- **TLR endosomal :** TLRs-3**, 7**, **8**, **9** are located at the endosomes and recognize viral and bacterial components, especially nucleic acids. Specifically, the following forms can be mentioned:
    - **TLR-3** mainly recognizes viral double-stranded RNA.
    - **TLR-7** mainly recognizes viral single-stranded RNA.
    - **TLR-9** which recognizes bacterial DNA.

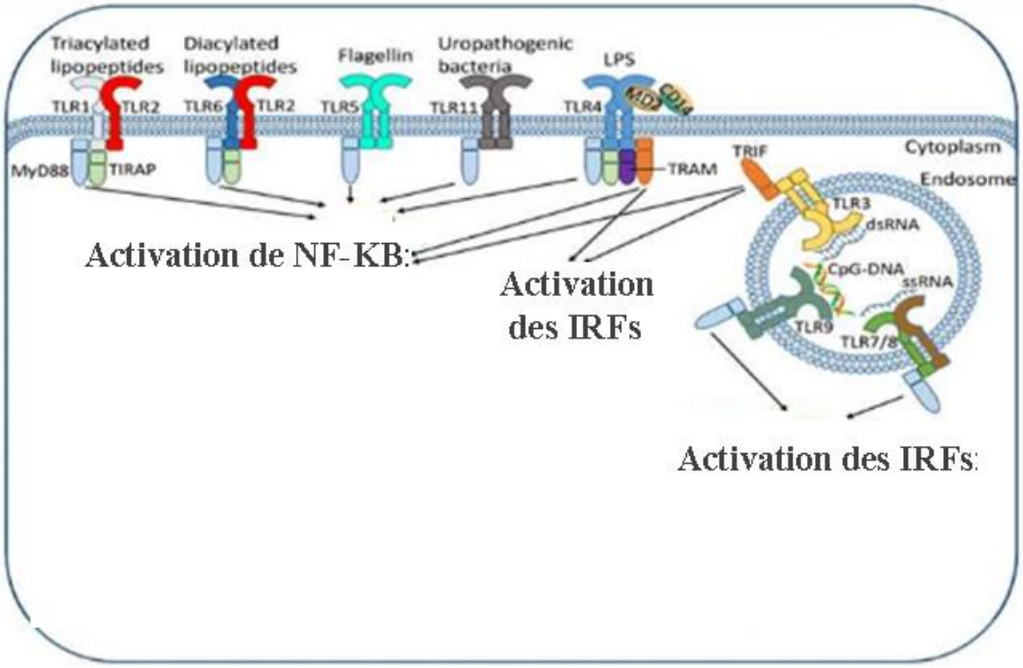


Fig. 12: TLRs and their ligands. **Sb** : simple brin. **Db**: double strand.

B- NLR receptors

These **NOD Like Receptors or NLRs** were characterized in 2002, they are a family of **about twenty cytoplasmic** proteins, they detect bacterial components, such as bacterial walls (**peptidoglycan**) or bacterial motifs (**flagellin, toxin**) and endogenous danger signals (damage, stress, necrosis). They are subdivided into **3 subfamilies**: **NOD (Nucleotide-binding Oligomerization Domains**), **NALP** (or NLRP) and **NAID**.

C- RLR-like receptors (RIG-Like Receptor)

They are **cytoplasmic,** they recognize **viral components** (**double-stranded RNA**).

II.2. Acquired immunity cells and their receptors

B and T lymphocytes make up 25-30% of circulating leukocytes. They are at the centre of immune function. By optical microscope they are identical. The lymphocytes are rounded cells of variable size, small lymphocytes (5 to 8 µm in diameter), medium lymphocytes (8 to 12 µm) and large lymphocytes (12 to 16 µm). This is related to their activation state.

II.2.1. T-cells

Are characterized by surface differentiation molecules **CD_3** with **CD_4** for **LT4** or **CD8** for **LT8**, receptors specific for **TCR antigens (T cell receptor)** which is a **glycoprotein** formed of 2 chains (**heterodimer**) **α and β** or **γ and δ** linked together by a disulfide bridge. They comprise a **variable region** on the terminal NH_2 side corresponding to recognition and **specific binding**

to the antigen. More than **95%** of mature T cells express **α/β TCR (TCR-2)** on their surface and functional properties: antibacterial and antiviral immunity, transplant rejection, anti-tumor and anti-parasitic immunity, regulation of antibody synthesis. The differentiation surface molecules of the T line are **CD2, CD5 and CD7**. T cells only recognize immunogenic peptides presented by antigen-presenting cells.

II-2.2. B Cell Lymphocyte

B lymphocytes represent approximately 5 to 15% of circulating lymphocytes and are defined by the presence on their surface of **glycoproteins** of the surface immunoglobulin type whose role is the specific recognition of the antigen (B cell receptor, BCR). These immunoglobulins, produced by the cell itself. The naive B lymphocyte carries **monomeric IgM**, **and IgD**. There are 10^5 molecules of surface immunoglobulins on a lymphocyte, which recognize the same antigenic determinant. Immunoglobulins are protein heterodimers composed of two identical heavy 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**. BLs have the property of transforming under the action of an antigenic stimulus into **plasma** cells; antibody-producing cells, carrying the same specificity (the same paratope: same variable part) with respect to the antigen as surface immunoglobulin. B lymphocytes are characterized by surface differentiation molecules of the B lineage (**CD19, CD20 and CD21**) and two heterodimers CD79a (Igα) and CD79b (Igβ) responsible for signal transduction after contact with the antigen.

LBs have the property of recognizing antigens in their native form (without any prior modification).

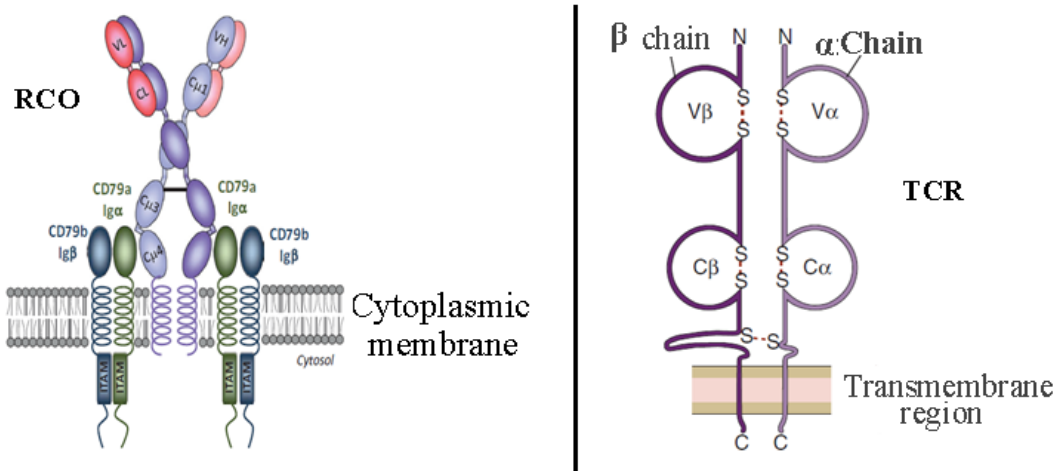


Fig. 13: Structure of a BCR and a TCR

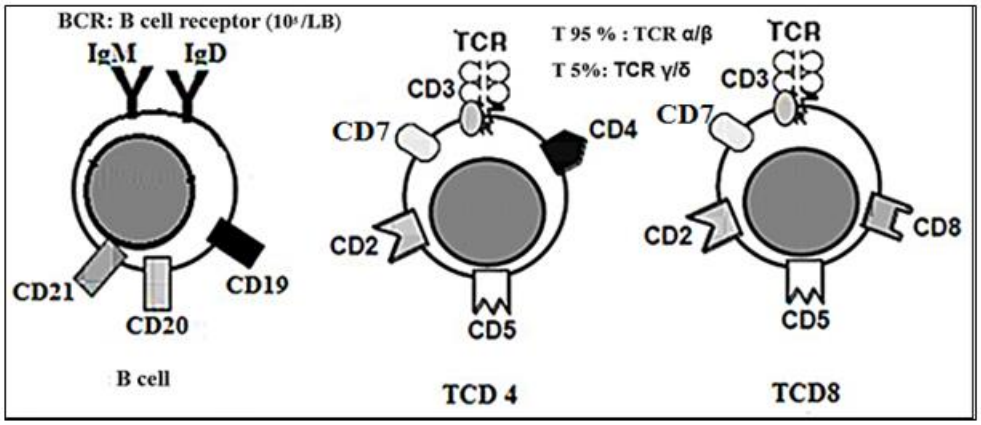


Fig. 14: Acquired immunity cell receptors.

II.3. Cells at the interface between innate and acquired immunity

II.3.1. NKT Lymphocytes (Natural Killer T)

It is an intermediate cell between the NK lymphocyte and the T lymphocyte. NKTs make up about 0.2% of peripheral blood T lymphocytes. It has an α/β TCR **(**TCR-2), as well as T cell CD3 and NK cell markers (expression of CD56 and CD16 molecules). By their TCR, although it is **almost invariant**, in other words it is the same on all NKT cells. NKTs recognize **lipids and glycolipids** presented by molecules, called **CD1d**, which are also **invariant,** structurally close to **MHC-1 molecules.**

II.3.2. γ/δ T-cells

LT-γ/**δ** are particular T cells characterized by the expression of a **TCR-1** (presents only a weak polymorphism) associated with a CD3 but having neither CD4 nor CD8. It is much rarer (05% of T cells) than LTs have an α/β TCR. They recognize the antigen in their original form, i.e. without presentation by the MHC. They are found mainly in the skin and mucous membranes.

II.3.3. Mait Lymphocytes

Mucosal-Associated *Invariant T cells (*MAIT) are a subpopulation of **CD3**$^{+}$ cells with semi-invariant **TCR** and preferentially found in mucous membranes, with antimicrobial properties. These cells are activated by **cells infected with** various strains of **bacteria and yeast**, but **not** by cells infected with the virus. Mucosal-associated invariant T cells are known as **unconventional T cells** in part because they recognize the **non-peptide** antigens presented by the MHC **MR1** molecule.

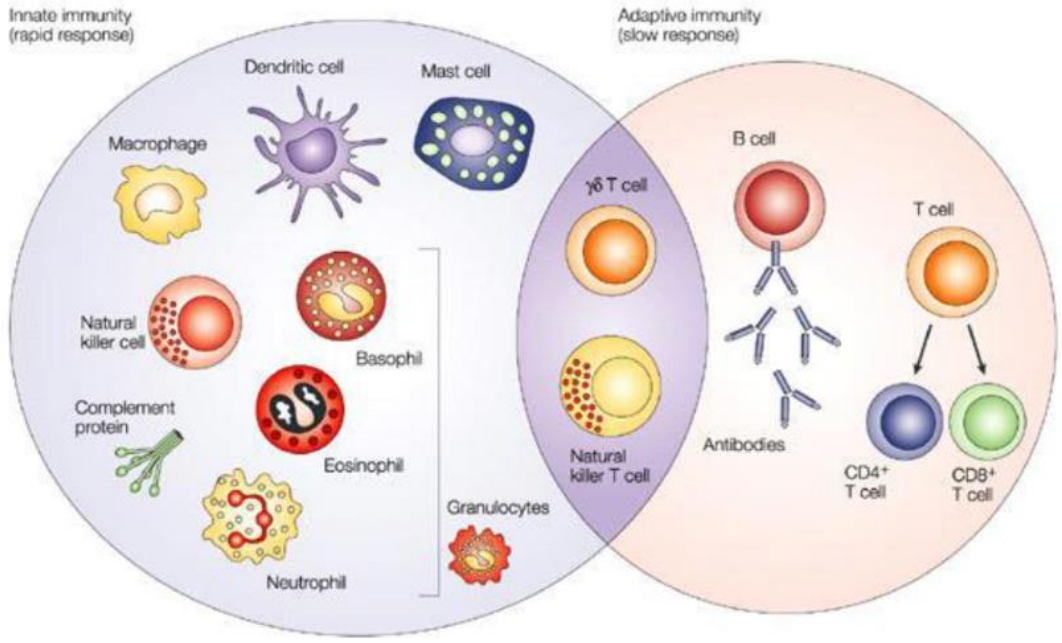


Fig. 15: Immunes cell according to type of immunity.

III. Lymphocyte Circulation

Mature lymphocytes migrate via the bloodstream from the **primary lymphoid organs** (bone marrow for B cells and thymus for T cells) to the **secondary lymphoid organs** (spleen and lymph nodes) in search of potential antigens. In the lymph nodes, they reside for a few hours. If they do not encounter their specific antigens, they exit within **24 hours** through the **efferent lymphatic vessel**. From there, they enter the **thoracic duct**, which empties them into the bloodstream at the **left subclavian vein**.

They spend no more than **one hour** in circulation before returning via the bloodstream to the lymph nodes by crossing the **high endothelial venules (HEVs)** at the junction between the **cortex** and **paracortex**. If they encounter their specific antigen, they remain in the lymph nodes for several days to develop an **adaptive immune response** (**Fig. 16**). In the **spleen**, there is no lymphatic vasculature. **B lymphocytes** enter (via the **artery**) and exit (via the **vein**) through the bloodstream. In the spleen, if they encounter their specific antigen, the corresponding lymphocytes respond and develop an **immune response** that lasts for several days. If no antigen is encountered, the lymphocytes, after a few hours of residence, exit the spleen and re-enter the circulation, repeating the cycle (**Fig. 16**).

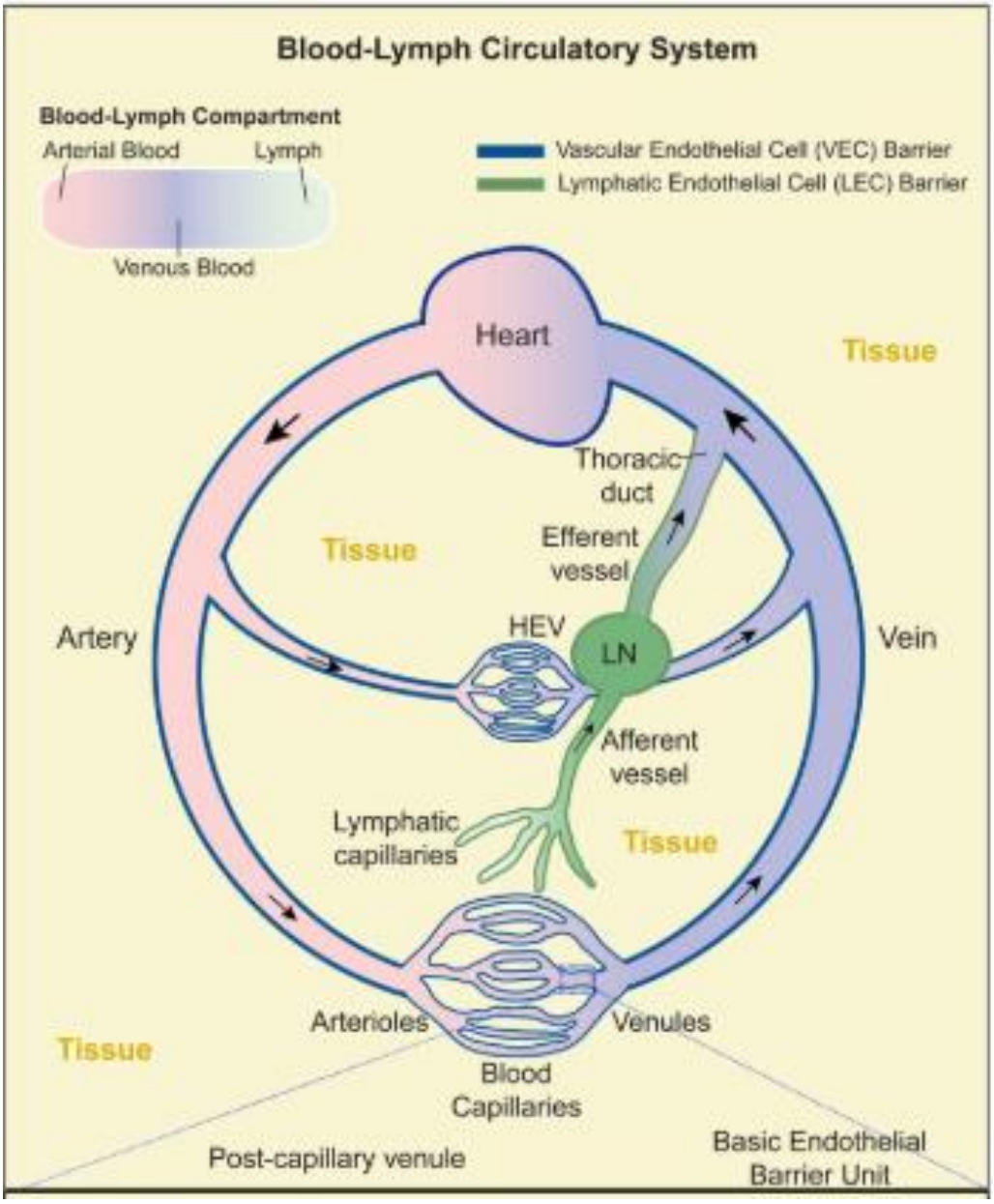


Fig. 16: Lymphocyte Circulation (follow the arrows).

Chapter 03

Lymphocytes Ontogeny

I. Generalities

Hematopoietic stem cells (HSCs) are the orgone of all immune cells. They differentiate in part into **lymphoid stem cells** or common lymphoid progenitors (CLPs) in the bone marrow of children and adults and in the liver of the fetus. These progenitors are of the CD34+ phenotype. They subsequently differentiate into different populations of **lymphocytes; T, B and NK** via a phenomenon called **lymphopoiesis** or lymphocyte **ontogenesis.**

Lymphopoiesis has two phases:

- **Primary or basal lymphopoiesis** leads to the production of mature lymphocytes (immunocompetent).
- **Secondary lymphopoiesis** corresponds to the **multiplication** of mature lymphocytes subjected to the activation of antigenic contact. It allows the adaptation of the immune response.

We are interested here in **primary lymphopoiesis** or **primary ontogenesis** of lymphocytes; corresponds on the one hand to the **development** of lymphocytes, to their **maturation** and finally to the **acquisition of self-tolerance**.

II. Primary B lymphopoiesis or B ontogenesis

B lymphocytes differentiate from $\mathbf{CD_{34}^{+}}$ stem cells in the **liver** microenvironment in the **fetus**, and then in **children and adults** in the **hematopoietic marrow**. In **birds**, B lymphocyte differentiation takes place in the **Bursa of Fabricius**, an individualized organ at the caudal end of the digestive tract.

Common lymphoid progenitors or **CLPs** develop into B lymphoid progenitors (**pro-B**) under the action of cytokines **IL3 and SCF** (stem cell factor) secreted by **stromal** cells. Pro-B lymphocytes proliferate and differentiate, in the presence of **IL7** and **SCF,** into immature B lymphocytes. It takes place in the bone marrow and progresses from the periphery to the center of the bone marrow. Differentiation occurs in several stages:

II.1. Progenitors - B

In this stage, we distinguish two stages:

- **An early step**, is characterized by the **proliferation** and expression of genes coding for **RAG** (Recombination Activating Genes) enzymes, responsible for the genetic rearrangement of the immunoglobulin **heavy chain** (H) locus on **chromosome 14**, first a **recombination** between a **D segment** and a **J segment**, then a **2nd rearrangement** between a **V segment** and the **DJ complex**. If this rearrangement is functional, a heavy chain of **isotype**

μ (mu) is synthesized and any other rearrangement of the **H chain** locus is **stopped (Fig. 01).** A low synthesis of **signal transduction** proteins CD_{79b} (Ig**β)** is thus observed.

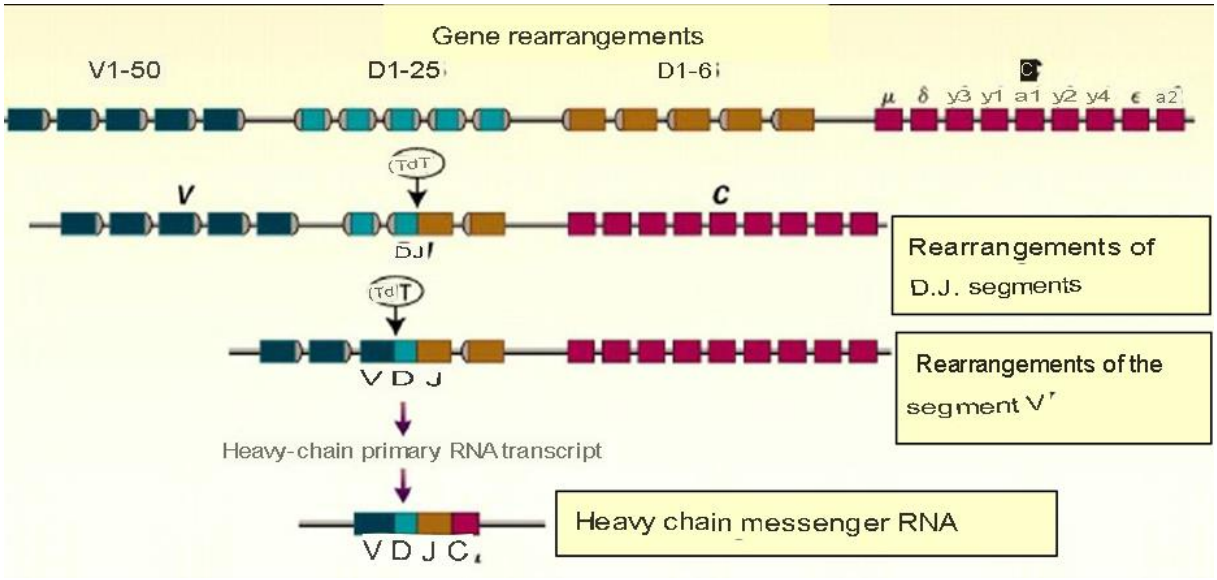


Fig. 01: Genes rearrangement of the heavy chain μ.

- **A late step**, is characterized by the membrane expression of differentiation molecules; **CD_{19}** and **signaling** molecules CD79a, b (Igα, Ig**β).** It is completed by the presence of the **intra-cytoplasmic μ heavy chain**.

II.2. Precursors - B.

In this stage the intracellular μ heavy chain associates with a **pseudo-light** chain, composed of the 2 proteins **λ5** and V preB, and the **Igα and Igβ** chains to form **the pre-B receptor**. This is made up of **2 rearranged μ heavy** chains, associated with 2 pseudo light chains. The expression of **the pre BCR** allows the cell to enter a phase of clonal expansion (proliferation). This inhibits the expression of the heavy chain of the 2nd **allele** (**allelic exclusion).**

A second expression of **RAG** will take place, which initiates another rearrangement, this concerns the genes of the **κ (kappa) light chain** carriedon **chromosome 2**. This rearrangement is carried out on a single allele of the κ genes (allelic exclusion), if it is not productive, it is carried out on the 2nd κ allele. If the latter is not productive, the rearrangement will continue on the **λ locus (lambda)**, genes located on **chromosome 22**.

Once the pre-BCR is expressed, the cell will be subjected to a first selection, called "**positive selection**". This will allow the pre-B cell, in the case where the expression is productive, to

receive a **survival signal** in order to continue its maturation, otherwise the cell will die by **apoptosis**.

This stage is also characterized by the expression **of B lineage** markers such as; **CD_{19}, CD_{20} and CD_{21}**.

II.3. Immature B

It is characterized by the membrane expression of **complete IgM** molecules forming the BCR (two heavy chains **μ** and two light chains **κ or λ**), giving the cell **specificity** in antigen recognition. The appearance on the cell surface of the markers: **CD_{22}, CD_{23}, and CD_{40}** is thus observed.

Immature B lymphocytes will undergo asecond selection, called "**negative selection**", which will allow the acquisition of **self-tolerance**. Immature B lymphocytes which **recognize** self-peptides (called autoreactive) presented on the surface of stromal cells; Some die by apoptosis, others **rearrange** the genes of **the variable domains** of the **light chain** to modify the **specificity** of the BCR with respect to the antigen (receptor editing). Lymphocytes whose BCR persists in being reactive to the self are eliminated by apoptosis, or inactivated (anergic), the other lymphocytes continue their differentiation.

II.4. Naive mature B lymphocytes

Immature B lymphocytes become **mature (immunocompetent)** and **naïve**, represent only 10% of the initial lymphocytes. They then express **IgM and IgD** on their surface. These mature cells will then migrate to the **secondary lymphoid organs**, and **circulate between them** (spleen, lymphoid nodes) in search of the specific antigen.

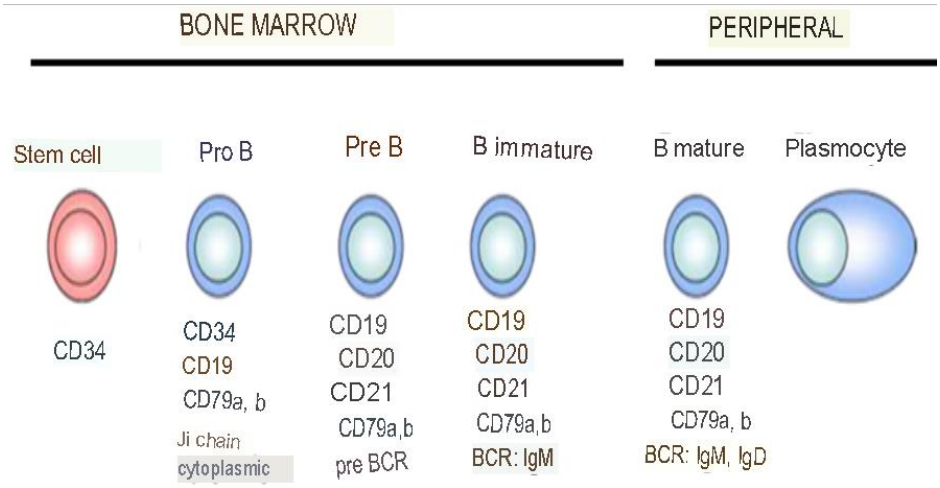


Fig. 02: Ontogenesis of B lymphocytes. **Pro B**: progenitor of B. **Pre B**: precursor of B.

III. Primary T lymphopoiesis (LT ontogenesis)

Common lymphoid progenitors (**lymphoid stem cells**) develop into T lymphoid progenitors (pro-T) under the action of cytokines **IL3 and SCF** (Stem Cell Factor) secreted by **stromal cells**. Pro-T proliferate and differentiate, in the presence of **IL7, SCF, IL2, IL3**, etc., into naive T lymphocytes in the **thymus.** T cell development goes through a series of distinct phenotypic stages characterized by the expression of several membrane molecules. T progenitors (pro-T) from the **bone marrow** access the thymus from blood vessels located near the thymic corti-medullary junction and from there migrate to the subcapsular region of the thymic cortex. At this point, they are **called thymocytes**. Their differentiation proceeds from the **cortex** to the **medulla** in **4 stages**, distinguishable on the basis of the expression of CD_4 and CD_8 coreceptors.

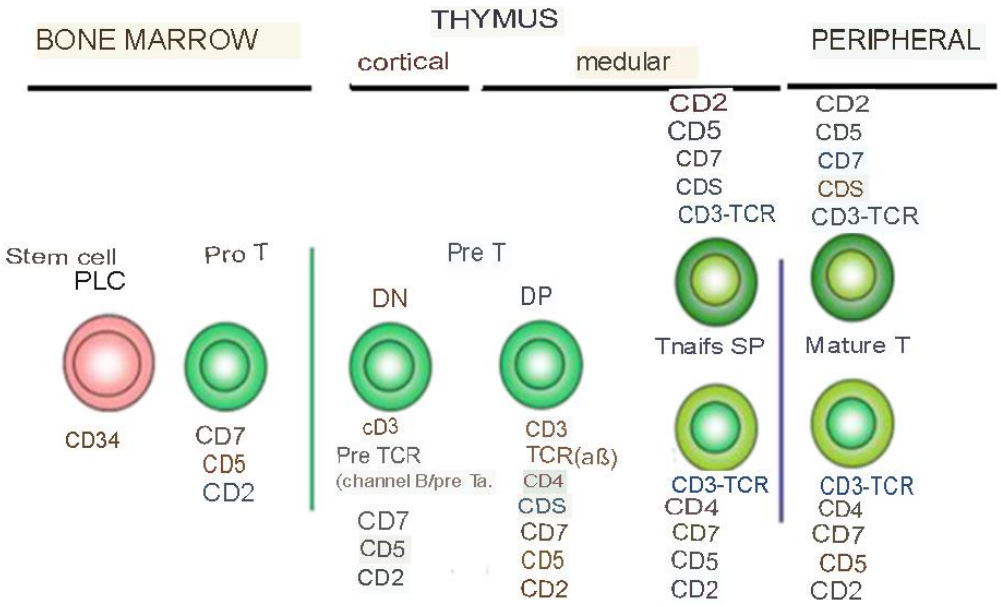


Fig. 03: T lymphocyte ontogenesis. **Pro T:** T progenitor. **Pre T:** T precursor. **cCD3:** cytoplasmic CD3. **DN:** double negative. **DP**: double positive CD_4^+ CD_8^+. **SP:** single positive CD_4^+ or CD_8^+.

III.1. T progenitors (pro-T) or double-negative T lymphocytes

In this stage, **pro T** cells interact with the **thymic stroma** (epithelial cells) and under the action of cytokines **IL3** and **SCF** (Stem Cell Factor). This leads to their rapid **proliferation**. Pro T cells are characterized by the expression of T **lineage markers** (CD_2, CD_5 and CD_7), then they will **express cytoplasmic** CD_3 (cCD_3). They are characterized by the **absence of the TCR**, **CD_4 and CD_8**. This leads to the appearance of so-called **double negative** thymocytes (CD_4^-, CD_8^-).

III.2. T precursors (pre-T)

Double negative (DN) lymphocytes undergo a VDJ-type **rearrangement** of the genes encoding the **90% β chain** of the **αβ TCR** and the **5% γ chain of the γδ TCR** (**Chromosome 07**). This allows the membrane expression of a **pre-TCR**, consisting of a rearranged β chain associated with an **invariant pre-T α** chain, and **membrane CD_3**.

The pre-TCR transmits signals for thymocyte survival and proliferation. More than 90% of DN cells that reach this stage die due to the absence of pre-TCR expression on their surface. This selection is **called β selection**.

Subsequently, **CD_4 and CD_8** will express on the surface of thymocytes, they are then **double positive** (CD_4+, CD_8+). Then the VJ type rearrangement of the **α locus (Chromosome 14),** which allows the membrane expression of the **TCR (αβ)**.

At this stage, **positive selection** occurs (restriction to self-MHC molecules). It takes place in **the deep cortex**. The TCRs of **double-positive** thymocytes are capable of interacting with MHC-I and II molecules. Thymocytes that have a TCR capable of interacting with MHC I and II molecules expressed by **cortical epithelial cells** survive and are therefore **selected**. They represent only 5% of the initial thymocytes. Double-positive thymocytes (CD_4+ and CD_8+) that have interacted with an **MHC-I** molecule become **CD_8+** T lymphocytes, while those that have interacted with an **MHC-II** molecule become **CD_4+** T lymphocytes. Consequently, they transform into single-positive thymocytes.

III.3. Immature T lymphocytes: single positives

In the medulla, selected **single-positive** thymocytes undergo **negative selection** (self-tolerance) to eliminate **self-reactive** thymocytes through the intervention of dendritic cells and macrophages. These cells capture antigens expressed by **medullary epithelial cells** and present them via their **MHC** to double-positive thymocytes. The crucial protein in this step is the **AIRE** (AutoImmune REgulator) protein present in medullary thymic epithelial cells and essential for the expression of tissue self-antigens.

Thymocytes whose TCRs recognize with **high affinity** self-peptides (**autoantigens**) associated with an MHC molecule on the surface of **dendritic cells** or macrophages are eliminated by apoptosis. Whereas, thymocytes whose TCRs **do not recognize self-peptides survive**. The surviving cells represent **only 1 to 2%** of the initial thymocytes.

III.4. Mature T lymphocytes

CD_4 and CD_8 T lymphocytes become mature (**immunocompetent**). These are **naive** T lymphocytes because they have not yet encountered the antigen; they are resting. They leave the thymus and enter the circulation via the blood, reaching secondary lymphoid organs.

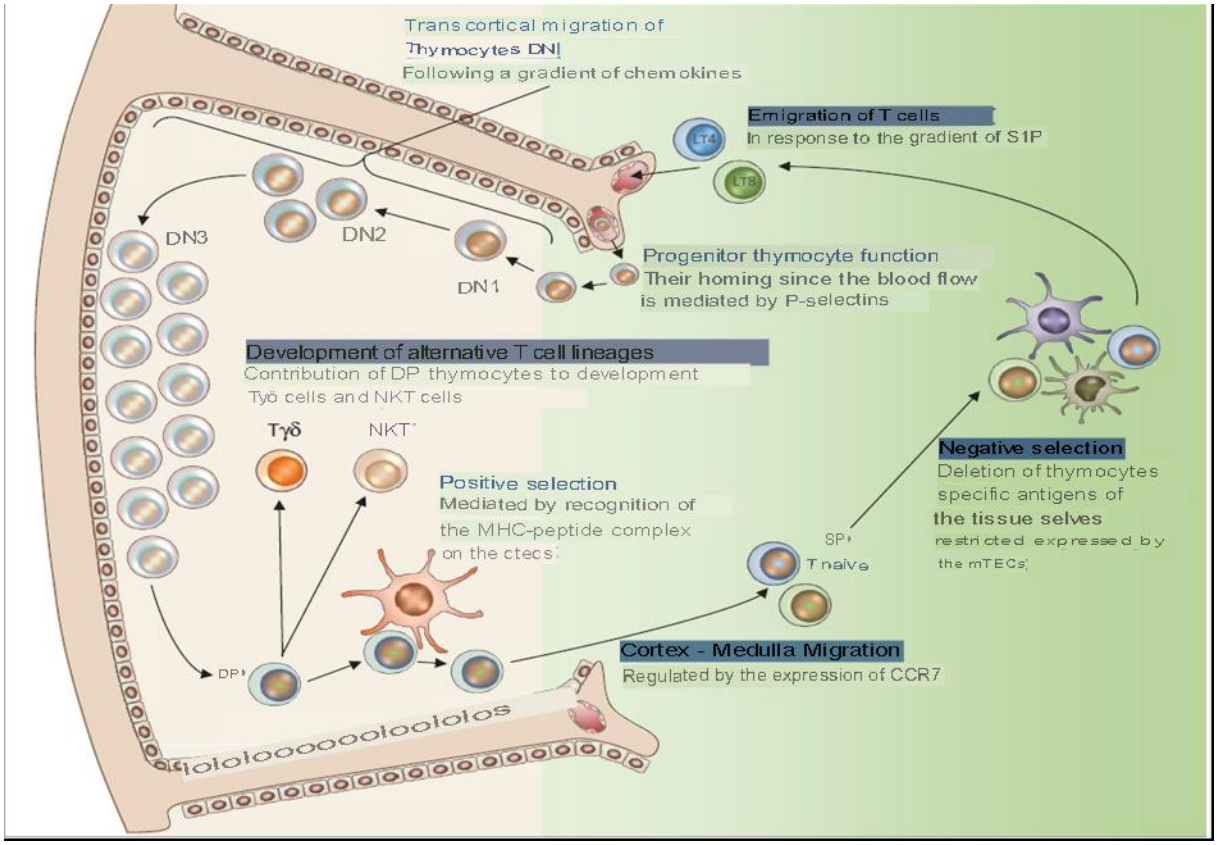


Fig. 04: Ontogenesis of T lymphocytes and negative selection.

Chapter 04

Antigens & MHC

I. Generalities

Antigens and the Major Histocompatibility Complex (MHC) constitute fundamental elements in the mechanisms of immune recognition. Antigens represent molecular structures capable of eliciting specific immune responses, while the MHC ensures the processing and presentation of these antigens to lymphocytes. This interaction forms the basis of immune specificity and self–non-self discrimination. Through their coordinated action, antigens and MHC molecules maintain immunological surveillance and contribute to the regulation of adaptive immune responses essential for the preservation of organismal integrity.

II Antigens

II.1. Definition

The term Antigen is an acronym that means Generating Antibody. An antigen is any natural or synthetic molecular species capable of inducing an immune response in a living organism (immunogenicity property) and of recognizing and reacting specifically with the products of this response such as antibodies, etc. (antigenicity or reactogenicity property).

II.2. Antigenic determinant or Epitope

An epitope corresponds to an area of 1 to 3 nm in diameter, immunologically active, or 15 to 18 amino acids for a protein, or 5 to 6 oses for a polysaccharide. It is the part complementary to the Ag binding site in the antibody (paratope).

Antigens usually have on their surface a large number of determinants, which can be different from each other, each being capable of inducing the production of a specific antibody, or on the contrary be identical repetitive structures.

Some epitopes can be exposed (accessible), others are hidden, they are released enzymatically. Depending on their structurewe distinguish (Fig. 01):

- Linear epitopes corresponding to a sequence of consecutive amino acids on the protein.
- Conformational epitopes corresponding to the spatial proximity of amino acids located at different places in the protein but dependent on its folding to be accessible.

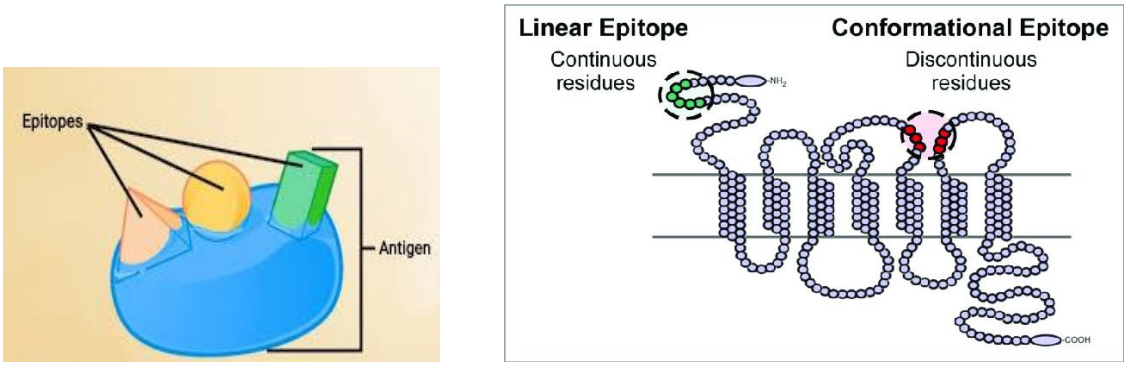


Fig. 01: Type of epitopes according to structure.

II.3. Types of antigens

Ag can be classified into several types according to the following criteria:

1)-Depending on the structure of the Ag, two types of antigens can be distinguished:

- **Soluble antigens:** include soluble macromolecules such as; toxins, proteins, glyco-proteins, polysaccharides, lipopolysaccharides (LPS), glycolipids and nucleic acids.
- **Particulate antigens (solid or cellular):** These are cells such as bacteria, viruses, parasites, fungi and foreign cells (sheep red blood cells (RBCs)). They are divided into infectious (pathogenic) antigens and non-infectious (non-pathogenic) antigens.

2)-Depending on the origin of the Ag, several types of antigens can be distinguished:

- **Natural:** Produced by the genetic expression of genes, such as viruses, bacteria, fungi, parasites, ABO and MHC system. Among the natural antigens, we distinguish:
  - **Xenoantigens**: These are exogenous antigens originating from a species different from the immunized species; present in all individuals of one or more species distinct from that of the immunized subject.
    Example :Bacteria, virus, fungus or parasite in relation to humans.
  - **Alloantigens**: These are exogenous antigens; antigens that characterize groups of genetically different individuals within the same species, leading to the formation of antibodies in an individual who does not possess the alloantigen in question.
    Example : **MHC, ABO and Rhesus system**in humans.
  - **Autoantigens**: These are endogenous antigens originating from the same immunized individual; present in the cells or tissues of the immunized subject themselves. Inducing an abnormal immune response such as autoimmune diseases.
- **Synthetics:** Produced by purely chemical means, widely used in vaccines.
- **Artificial:** (chemically modified natural) such as anatoxins.

3)-Depending on the reactivity with the immune system, we distinguish:

- **Immunogens**: Macromolecules capable of inducing a specific immune response in vivo and of reacting specifically, in vivo and in vitro, with the products of the response thus induced.
- **Haptens**: Low molecular weight (<10kD), univalent (containing a single epitope) substances that possess antigenic reactivity are not immunogenic by themselves, but can become so when coupled to carrier macromolecules, generally proteins. They are considered reactogens.

NB: All immunogens are reactogens, but not all reactogens are necessarily immunogens.

4)-Depending on whether or not the help of T lymphocytes is needed for the activation of B lymphocytes, and therefore for the production of antibodies. A distinction is made between:

- **Thymus-dependent immunogens:** Most natural antigens are thymus-dependent. The immune response against this type of Ag requires the intervention of LT4 for the activation and differentiation of LBs. These are proteins.
- Thymus-independent immunogens: However, a minority of so-called thymus-independent antigens are known, capable of directly activating B lymphocytes after attachment to their surface receptor, without the intervention of LT4. There are two types:
  - **Thymus-independent antigens type 1:** have physicochemical characteristics that make them stimulators of mature and immature B lymphocytes at high doses. We then speak of mitogens among the thymus-independent antigens of type 1, we cite lipopolysaccharides.
  - **Thymus-independent antigens type 2:** are polysaccharides with a repetitive structure, found in bacterial walls. They are devoid of mitogenic activity and can only stimulate mature B lymphocytes.

II.4. Conditions of immunogenicity

Immunogenicity is determined partly by intrinsic properties of the antigen and others are extrinsic.

II.4.1. Intrinsic conditions

a)- Foreign character

Also called phylogenetic distance, it corresponds to the degree of strangeness of the antigen. When an Ag is introduced into an organism, the degree of its immunogenicity depends on the degree of its foreign character (generally the greater the phylogenetic "taxonomic" distance

between 2 species, the greater the structural disparity between them and the strangeness is high, therefore the more the immunogenicity is increased).

Example: Bovine Serum Albumin (BSA); a common experimental antigen is not immunogenic in a cow, but is highly immunogenic in rats.

b)- Molecular size

The larger the size of a molecule, the more powerful its immunogenicity, because there will be more epitopes so it is easy to recognize. The best immunogens tend to have a molecular mass approaching 100 kD. Generally, substances with a molecular mass of 5 to 10 kD are poor immunogens.

Example :- Ovalbumin: molecular weight (MW) = 44000: 5 antigenic determinants

- Tetanus toxin: PM = 69000: 8 antigenic determinants

c)- Heterogeneity, chemical nature and complexity of the structure

Some diversity in Ag structure is required to achieve immunogenicity. The more heterogeneous and complex the structure, the more immunogenic the antigen.

- Proteins are the most powerful immunogens; Proteins are highly antigenic molecules due to the polymorphism of their structure and the differences between species and between individuals within the same species.
- Polysaccharides and polysaccharides are weakly immunogenic compared to proteins.
- Lipids by themselves are not immunogenic, because their structure is substantially identical in many animal species: they are haptens.
- Pure isolated DNA is not immunogenic.

Example :

- Homopolymers (only 1 type of amino acid): not very immunogenic.
- Copolymers (2 or more types of amino acids): more immunogenic.
- Copolymer + aromatic amino acids: highly immunogenic.
- Copolymer + glutamic acid + lysine: is immunogenic at a molecular weight of 30-40 Kd.
- Previous copolymer + tyrosine: immunogen at a low molecular weight (10-20 Kd).

d)- Sensitivity to the preparation and presentation of the Ag

Macromolecules that cannot be degraded and presented in association with MHC molecules are poor immunogens. This can be explained by D-amino acid polymers which are stereoisomers of L-amino acids.

The degrading enzymes present inside Antigen Presenting Cells can only degrade proteins containing L-AAs. D-AA polymers cannot be processed and are therefore poor immunogens.

II.4.2 Extrinsic conditions

Even if a molecule possesses the characteristics that contribute to immunogenicity, its ability to induce an immune response will depend on certain extrinsic properties of the biological system that the antigen encounters.

a)- Dose of immunogen

To induce a specific immune response an **optimal dose** is required, which varies from one antigen to another. An insufficient dose will not stimulate an immune response, either because it cannot activate a sufficient number of lymphocytes or because it induces a state of tolerance.

Conversely, an excessively high dose may also fail to induce a response because it forces lymphocytes into a state of anergy. A possible explanation is death by apoptosis of macrophages (indigestion) due to excessively high macrophagic activity.

Generally, a single dose of most Ags will not induce an effective response, in fact, repeated administration over a period of several weeks is necessary to induce an effective immune response (principle of vaccine boosters).

b)- Route of administration

The route of administration of the Ag can modify the immunogenic character of a substance, parenteral routes (by injection) are the most immunogenic, an Ag administered subcutaneously or intradermally or intramuscularly is the most immunogenic. It reaches the lymph nodes first, while an Ag administered intravenously is delivered first to the spleen. While the oral route (Per OS) and the cutaneous route (topical) are poorly immunogenic.

c)- Adjuvant

These are inert, non-immunogenic substances that amplify the immune response (humoral and cellular) of the individual, when administered simultaneously with the Ag, used in vaccines. They act essentially by transforming soluble antigens into particulate material, reducing their diffusion, which promotes their capture by the presenting cells and their slower release (release) by the latter: all this results in increasing the contact time between the Ag and the immunocompetent cells.

Examples:

- Aluminum hydroxide ($Al(OH)_3$).

- Calcium hydroxide ($Ca(OH)_2$).
- Muramyl-dipeptide: a constituent of the peptidoglycan of the bacterial wall.
- Freund's complete adjuvant and Freund's incomplete adjuvant (for animals).

d)- Genotype of the immunized subject

Within the same species, certain lineages are hereditarily capable of immunizing themselves with certain Ags while others respond very poorly to these same antigens.

III. Major Histocompatibility Complex

The **Major Histocompatibility Complex** (MHC) is a set of genes that encode **histocompatibility molecules** expressed on the surface of **nucleated cells and antigen-presenting cells**. These molecules perform two main functions:

- **Determine biological identity**cells: The MHC has been discovered to be the primary gene locus determining the **uptake** or **rejection** of **tissue grafts** between individuals. In other words, individuals whose MHC locus is **idental** will **accept grafts**, while individuals with **different MHC** loci will **reject** these grafts.
- **Ensure the presentation** peptides derived from protein antigens to specific **T lymphocytes** in order to activate them.

The major human histocompatibility complex is called the **HLA (Human Leukocyte Antigen)** system, the molecules resulting from its genetic expression are called **human leukocyte antigens**.

The HLA systemis located on the short arm of the pair of **chromosome 6**. These genes are extremely **polymorphic** (several alleles) (Fig. 01) and **co-dominant** within the human species and this explains why each individual has their **own MHC**. These genes are **closely linked**, they are passed on bloc from parents to children.

A given individual has **two different HLA haplotypes** (a haplotype is a set of genes carried by one chromosome of the pair), one parental and one maternal. **Recombination** events (crossing over) between these two haplotypes are **rare**.

Noticed : In mice, the MHC is called the **H–2 system** carried by chromosome 17 pair.

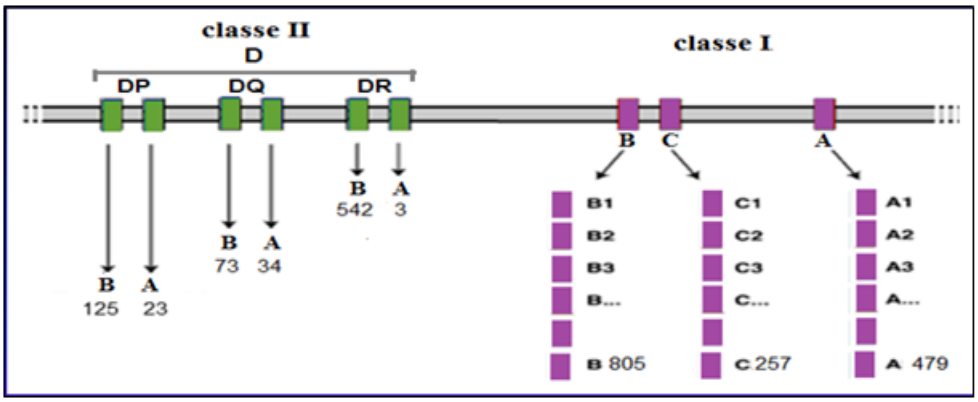


Fig. 01: Polymorphism of major histocompatibility complex (MHC) genes.

III.1. HLA genes

Human MHC genes are divided into **two** classes (Fig. 02):

- **Class I genes** encode **MHC-I** molecules. The most important are **HLA-A**, **HLA-B**, and **HLA-C**, each of which encodes a **polypeptide** chain **alpha** molecule of the same name. **MHC-I** molecules are expressed on the surface of **all nucleated cells** (except red blood cells).
- **Class II** genes encode **MHC-II** molecules. The most important are **HLA-DP**, **HLA-DQ**, **and HLA-DR**, Each of the **3 class II loci** carries **2 genes**; **A and B** coding respectively for an α heavy chain and a β light chain which associate and form class II **heterodimers**.

MHC class II molecules are expressed on the surface of antigen-presenting cells (APCs) (macrophages, dendritic cells, and LB).

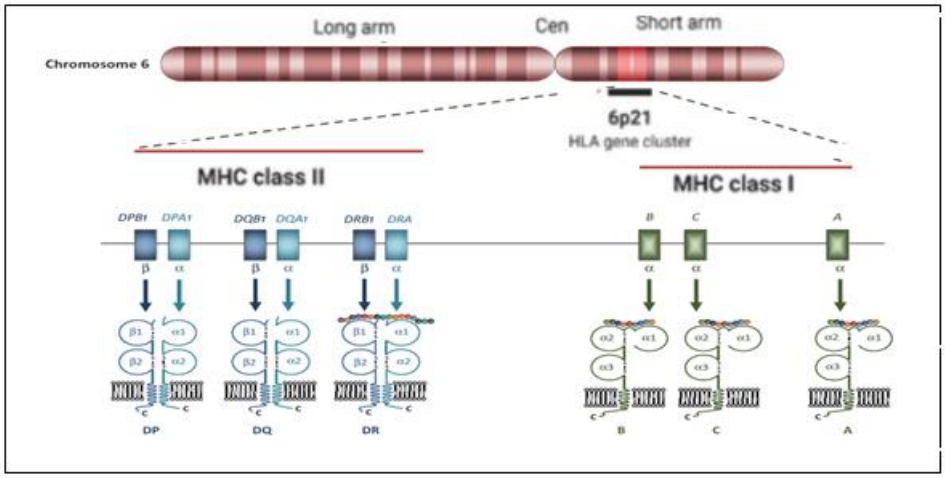


Fig. 02: Major histocompatibility complex (MHC) genes.

Remarks :

Additional genes have been identified, in the **HLA-II** region;

- Genes encode products involved in **exogenous** antigen presentation pathways (**DM genes and CD74 gene**); **DM genes** encode **HLA-DM** molecules responsible for **protecting HLA-II** molecules against **hydrolysis** by **phagolysosomal** enzymes. The **CD74** gene encodes the **invariant chain li(γ)**, a polypeptide enabling the formation, maturation and transport of MHC-II during its formation in APCs.
- Others are involved in the **endogenous** antigen presentation pathway (**TAP and LMP** genes).
  - **TAP**: Transporter Associated with Antigen Processing: transporter associated with the processing of antigens, allowing endogenous peptides (viral or tumor) to be transported from the cytoplasm to the rough endoplasmic reticulum. A distinction is made between **TAP 1** and **TAP 2**.
  - **LMP**: Large Multifunctional Proteases (proteasomes), responsible for the degradation of endogenous antigenic proteins into peptides in the cytoplasm of antigen-presenting cells. The most important of these are **LMP 2** and **LMP 7.**

There are MHC-III genes, which are located at loci between those of class I and class II genes. These genes mainly code for complement components C2, factor B, C4 (C4A and C4B), for tumor necrosis factors: TNF α and TNF β.

III.2. Structure and properties of MHC molecules

III.2.1. Class I molecules (HLA-I)

HLA-1 are composed of a **heavy polypeptide chain α** (45KD), it carries 3 domains. The outermost **amino-terminal domains α1 and α2** form a groove-shaped peptide binding site (cavity, niche), which is large enough to accommodate peptides of **8 to 10** amino acids. The **α3 domain** is conserved, it carries an interaction site with the **CD8** molecule expressed on the surface of CD8 T lymphocytes. The chain **alpha** is non-covalently associated with another polypeptide chain called **β2-microglobulin (β2m)** (12KD) encoded by **chromosome 15**, associated with the **α3 domain** of the heavy chain **alpha** (Fig. 03 and Fig. 05).

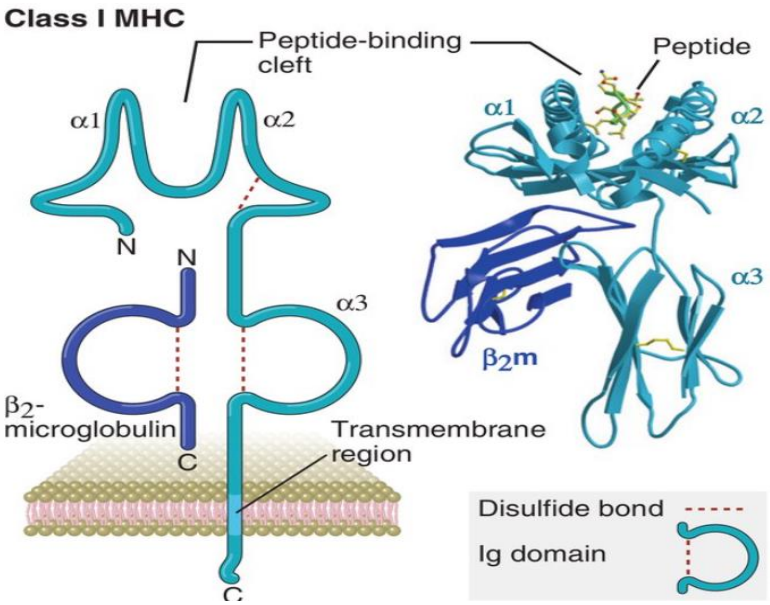


Fig. 03: Structure of MHC-I molecule.

III.2.1. Class II molecules (HLA-II)

HLA-II are formed from 2 different polypeptide chains, a **heavy α chain** (32 kDa) and a **light β chain** (28 kDa) non-covalently associated. The amino-terminal regions of both chains, called **α1 and β1 domains**, contain **polymorphic** residues that form a groove (cavity open at both ends) in which an immunogenic peptide of **12 to 18 amino** acids can be accommodated. The external part of the non-polymorphic β2 domain contains the **binding site** for the CD4 coreceptor of **TCD4 lymphocytes** (Fig. 04 and Fig.05).

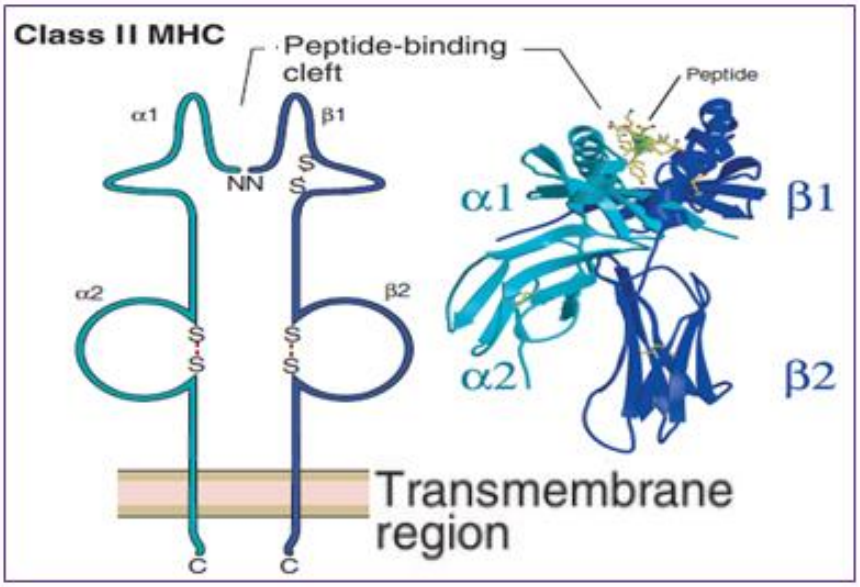


Fig. 04: Structure of an HLA-II molecule.

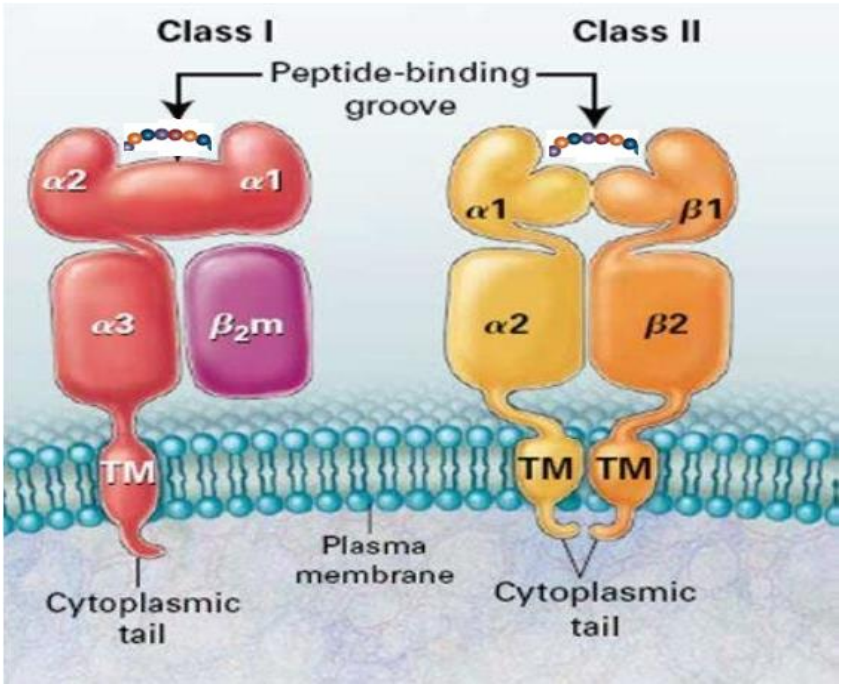


Fig. 05: Structure of HLA-I and HLA-II molecules in association with immunogenic peptides.

III.3. Presentations of immunogenic peptides by MHC molecules

III.3.1. Presentation of exogenous peptides for MHC-II molecules

Exogenous antigens (bacteria, proteins, etc.) phagocytosed by **macrophages** or captured by **dendritic cells** or internalized by **LBs** are degraded in phagolysosomes or endosomes (intracellular vesicles in which lysosomes have been degranulated) by proteases such as **cathepsin B and D** into peptides of **12 to 18** amino acids.

In parallel, α and β chains of class II molecules are synthesized and then assembled in the endoplasmic reticulum into heterodimers to which an **invariant Li chain (CD 74)** is then associated. **The Li** chain protects the groove of the α, β dimers by preventing the attachment to the grooves of peptides present in the cytosol and in the endoplasmic reticulum.

The complexes, class II-invariant **chain Li** molecules, migrate out of the endoplasmic reticulum to the **Golgi apparatus** and end up in Golgi **vesicles** (transport vesicles).
These vesicles fuse with endosomes. In these fusion vesicles obtained, the cleavage and dissociation of the invariant chain Li takes place by proteases (**the DM protein**), thus allowing the interaction of immunogenic peptides with the groove of MHC molecules (Fig. 06). The immunogenic **peptide-MHC class II** complexes are transported to the cell surface where they will be recognized by specific **TCD4 lymphocytes** (Fig. 07).

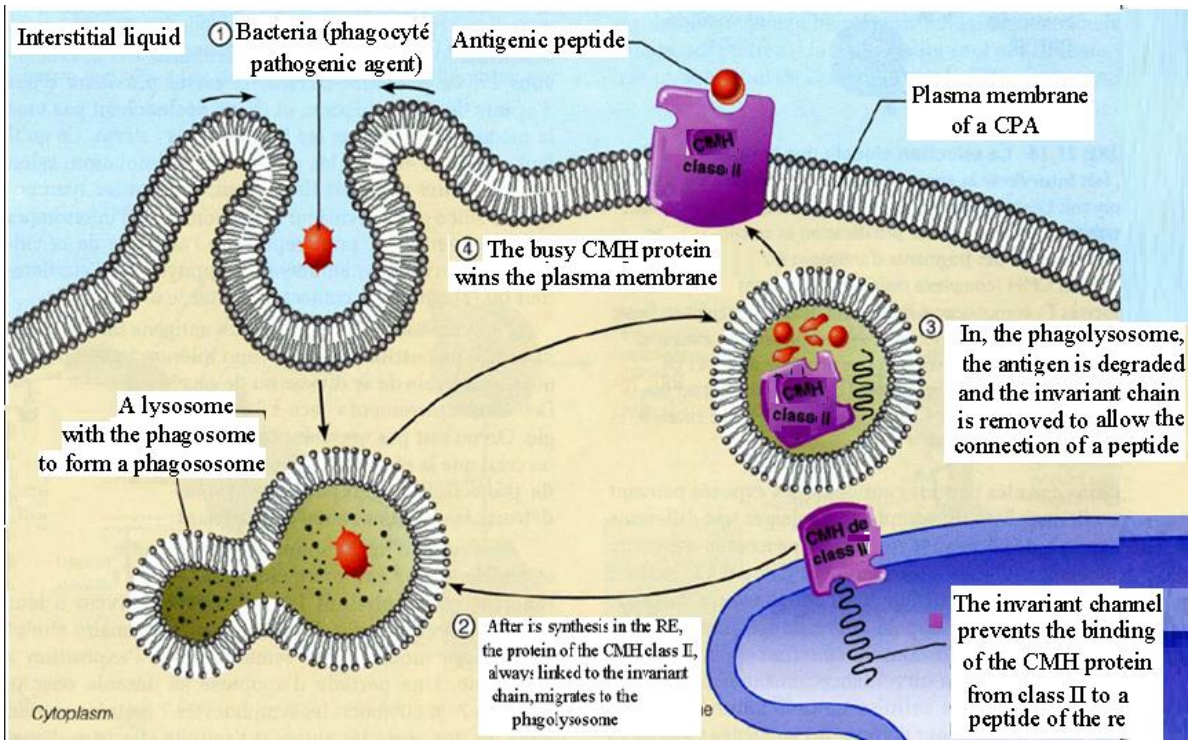


Fig. 06: Processing and transport of exogenous antigens. APC: antigen-presenting cell. P: peptide. Ag: antigen. RER: endoplasmic reticulum.

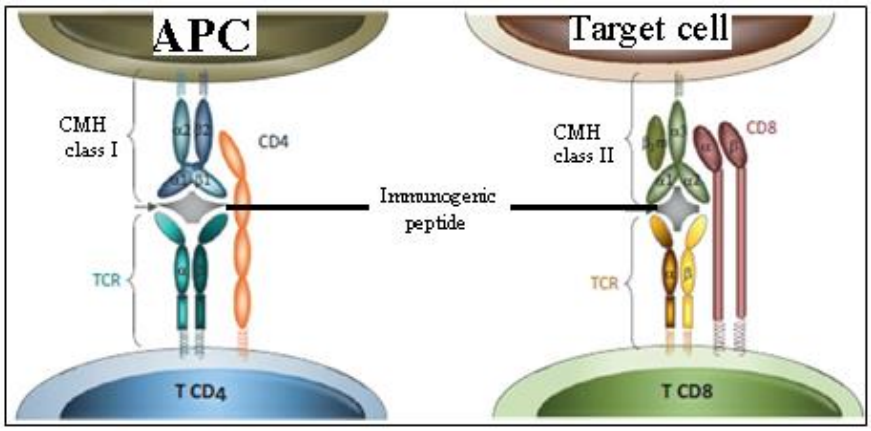


Fig. 07: Structures of HLA molecules interacting with the appropriate lymphocyte.

III.3.2. Presentation of endogenous peptides for MHC-I molecules

Endogenous antigens, proteins whose production is intracellular, encoded by genes specific to normally repressed **tumors** (**oncogenes)** or **viral** (encoded by viral genes integrated into the genome of the infected cell). These proteins synthesized in the cytoplasm are degraded into peptides of **8 to 10** amino acids by enzymatic proteins (**proteosomes or LMP**: Large Multi-functional Proteases) within the cytoplasm.

The **endogenous peptides** thus produced are transported by TAP (Transporter Associated with Antigen Processing) transporters to the **endoplasmic reticulum**. In this intracellular compartment, immunogenic peptides bind to newly synthesized MHC class I molecules (Fig. 08). The **peptide–MHC class I** complexes are transported through the Golgi apparatus to the cell surface for presentation to **specific CD8 T lymphocytes** (Fig. 07).

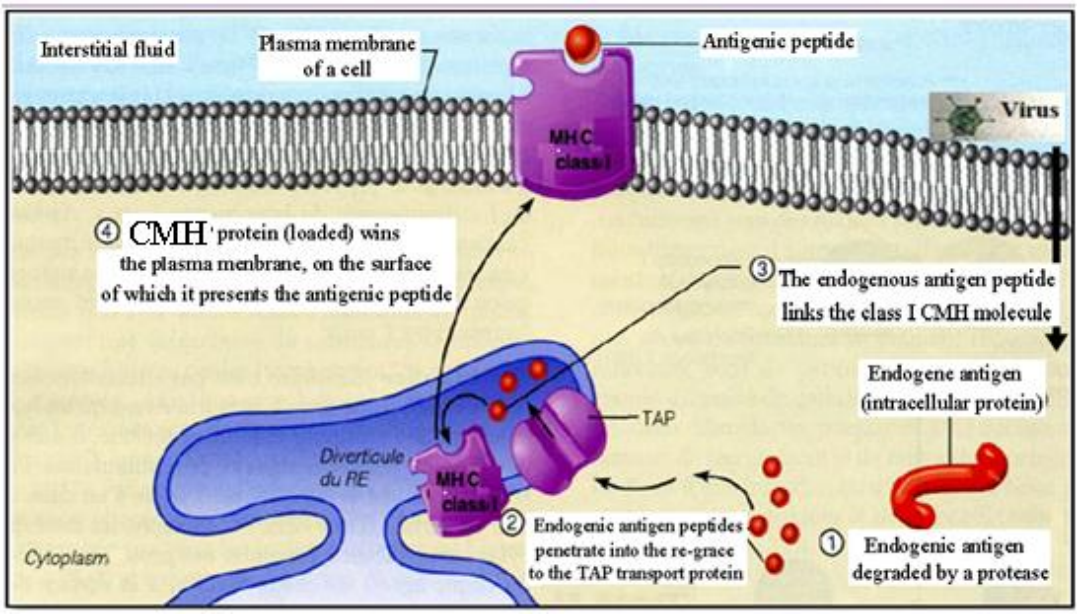
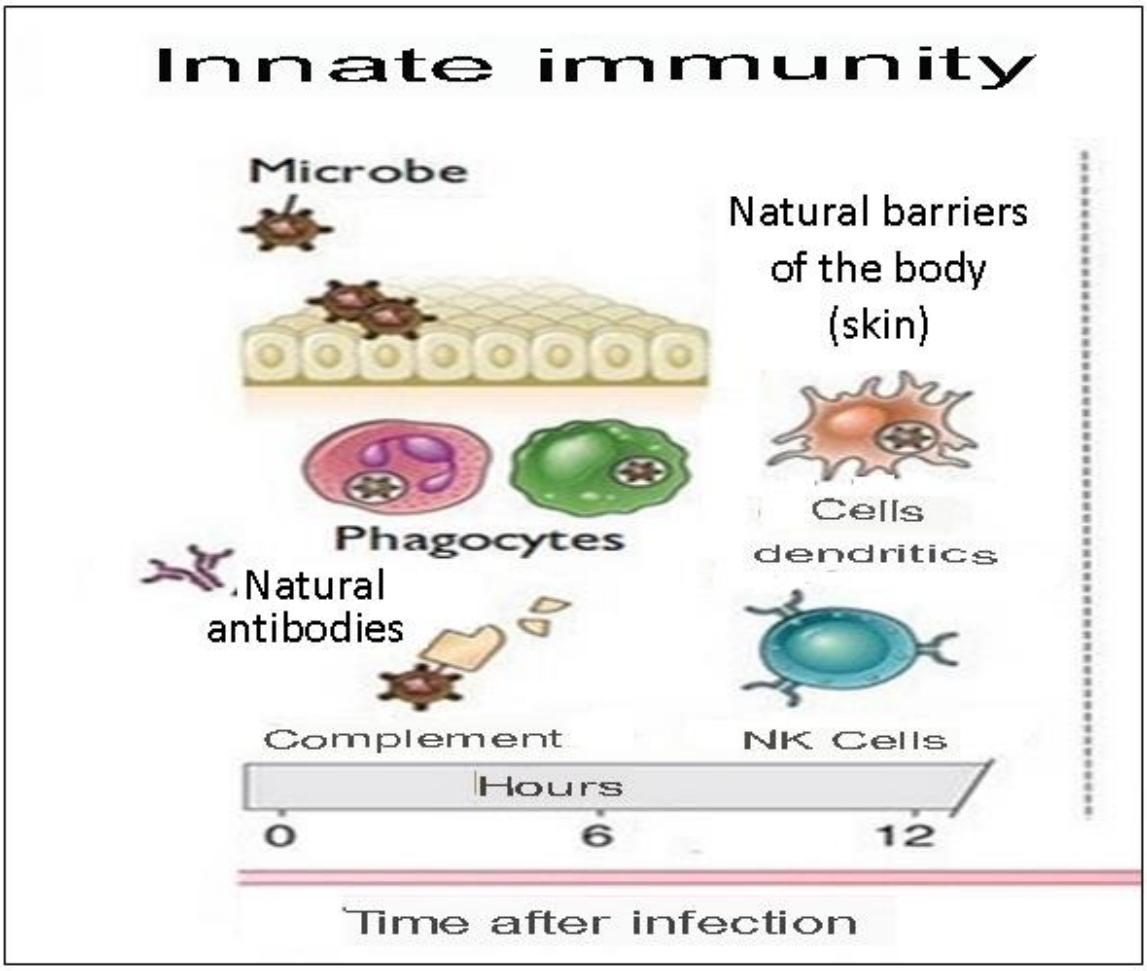


Fig. 08: Priming and transport of endogenous antigens.

Chapter 05

Innate Immunity



I. Generalities

Innate immunity (also called **natural**, **native** or **non-specific immunity**) is the first response put in place by the body following an attack (microbial invasion, tissue damage, physical or chemical burn, etc.), and this in all **multicellular organisms**. It is activated **immediately** and is functional for **4 days** (96 hours). It is **non-pathogen-specific**, non-adaptive, and based on a global distinction between self and non-self. The innate immune response can be **non-induced** (physiological barriers) or **induced** (inflammatory response) by a **danger signal** emitted following the **interaction** between self receptors called **PRR** (for "Pathern Recognition Receptors") and non-self molecules called **PAMP** (for "Pathogen Associated Molecular Patherns") present in microorganisms whether they are pathogenic or not.

PRRs are groups of receptors, whose genes are not polymorphic; they are all the same within a species. These receptors are expressed in different cells: macrophages, dendritic cells (DCs), NK ("natural killer") cells, polymorphonuclear leukocytes, mast cells and resident cells (fibroblasts, muscle cells, epithelial cells). They are of two types: **endocytosis** PRRs and **signalling** PRRs.

The function of innate immunity is to block the **penetration** of microbes. If microbes manage to pass through epithelia and enter tissues or the circulation, they are attacked by **phagocytes**, specialized lymphocytes called killer cells or **NK cells**, and several plasma proteins, including proteins of the **complement** system.

Innate immunity involves two lines of defense:

- Physiological (natural) barriers
- The inflammatory response (cell barrier)

II. Natural barriers

Natural barriers constitute the **first line** of defense of **natural immunity**. They are subdivided into three types.

II.1. Physical barriers

These barriers are essentially epithelial barriers. They include the skin and mucous membranes.

- **Skin**: very resistant barrier because of the **keratinized multi-stratified epithelium**, surrounding the entire external surface of the organism. Most microbial agents cannot pass through it in the normal state.

❖ **Mucous membranes:** have a **non-keratinized uni- or multi-stratified epithelium.** The epithelial cells of the mucous membranes of the respiratory, gastrointestinal, and genitourinary tracts are more susceptible to various infectious attacks.

These cells, at the level of the respiratory system, retain foreign elements (micro-organisms) by the **mucus** that they secrete, it forms a **viscous** substance that traps foreign elements and which will then be eliminated by expectoration. Then, by **beating of the eyelashes**, they make upward movements, moving the microorganisms that enter during breathing.

I.2. Chemical barriers

Chemical barriers encompass various **secretions**. These originate from the epithelial surfaces of mucous membranes and include **tears**, **nasal** and **bronchial mucus**, **saliva**, **earwax**, and **gastric juices** (acidic pH). They contain substances toxic to microorganisms and lytic enzymes such as **lysozyme** and **lactoferrin**.

Table I: The different chemical barriers.

Seat	Source	Secreted chemicals
Eye	Lacrimal glands (tears)	Lysozymes
Ear	Sebaceous glands	Wax-cerumen
Mouth	Salivary glands (Saliva)	Digestive enzymes; Lysozyme, lactoferrin
Skin	Sweat glands (Sweat)	Lysozyme, NaCl, medium chain fatty acid.
Stomach	Gastric juice	Acidic digestive enzymes (pepsin, renin, etc.), acidic pH (1-2).

I.3. Microbiological barriers

Microbiological barriers are represented by the **commensal bacterial flora**, which is a group of saprophytic bacteria hosted by the body, located on the skin and mucous membranes (nasal cavities, mouth, throat and in the gastrointestinal and genitourinary tracts) and playing an important role as a barrier.

The gastrointestinal flora prevents the colonization of the site by pathogenic germs by preventing their attachment to the mucosa, competing for essential nutrients, and releasing substances against these germs. Bacteria such as **lactobacilli** that colonize the vagina make its environment acidic (**pH 4.0-4.5**). This would prevent the growth of many pathogenic microorganisms.

NB: The role of physiological barriers is to prevent the entry of foreign bodies into the internal environment of the organism.

III. Cellular Barriers or Inflammation

Inflammation or inflammatory reaction is the response of **living tissues**, **vascularized**, to an attack (physical, chemical, microbiological), is a component of the immune response. It is involved in **innate immunity** in response to a **danger signal**. It constitutes **the second** line of natural defense. It is a usually beneficial process: its goal is to eliminate the pathogen and repair tissue damage.

In the event of loss of integrity of the **epithelial barriers**, micro-organisms (bacteria, viruses, parasites) manage to cross the natural barriers and find themselves in a tissue (notion of **contamination**), they will take advantage of the favorable environment (nutrients, optimal pH, etc.) to multiply, they have released from **toxic substances**, by inducing **lesions** at the local cell level **(**notion **of infection**), therefore an **inflammatory response is triggered**. This consists of:

III.1. Activation of innate immune cells

In the attacked tissue (inflammatory focus), microorganisms are recognized by innate immune cells, through **signaling PRRs** expressed on the surface of so-called **sentinel** immune cells (dendritic cells, macrophages and mast cells in addition to resident cells), which recognize the **PAMPs/MAMPs** of microorganisms. This recognition leads to the activation of these cells, which results in the **production** and then **release of soluble chemical mediators**; are the **cytokines** (soluble or membrane mediators produced by immune cells ensuring communication between cells):

- The release of **vasoactive** amines including **histamine** by **mast cells**.
- The production and secretion by **mast cells** and phagocytic cells of **lipid mediators** such as **prostaglandins** and **leukotrienes**, and platelet-activating factor (PAF) (lipid mediators are derivatives of arachidomic acid).

This induces **dilation of the capillaries**: **vasodilation**, responsible for **erythema** (redness) and **heat** (fever). This vasodilation is at the origin of the increase in **capillary permeability**, thus allowing:

- **Plasma exudation**: Corresponds to the passage of **fluid and molecules** such as coagulation factors, complement components, and acute phase proteins into the inflammatory focus. This explains the **swelling** or **edema** (Fig. 01 and 02).

- **Diapedesis:** Corresponds to the passage of **blood leukocytes** (neutrophils, eosinophils, monocytes, dendritic cells and/or NK) towards the inflammatory focus **(**Fig. 01 and 02), under the influence of **cytokines** called **chemokines** produced by **monocytes, macrophages, endothelium, fibroblasts and keratinocytes**:
  - **IL8** (interleukin 8) **and TNF α** (Tumor Necrosis Factor α) (TNF is thus called tumor necrosis factor because it can damage cancer cells by simple contact) to attract **neutrophils**;
  - **CCL2 or MCP-1**(monocyte chemotactic protein 1); to attract **monocytes** and later **immature dendritic cells**;
  - **Eotaxin 1 (CCL11)** and IL5; for **eosinophils**.

❖ The synthesis and secretion by **mast cells**, **macrophages** and **dendritic cells** of so-called **pro-inflammatory** cytokines**; IL1** (Interleukin 1), **IL6**, **TNFα**. These are the amplifiers of the inflammatory response.

In fact, these local circulatory modifications (**vasodilation** and **increase in vascular permeability**) at the site of the attack are caused by substances of **plasma** origin (products of the **coagulation**, **kinin** and **complement systems**) and others of **cellular origin** (histamine, serotonin, prostaglandins, leukotrienes and interleukins).

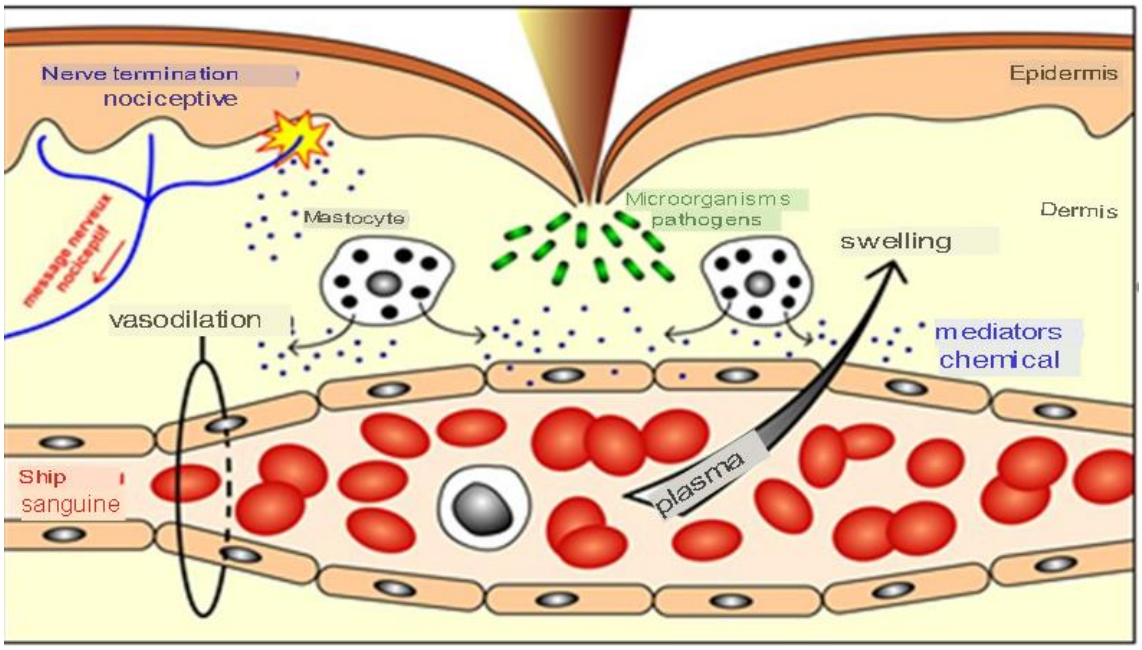


Fig. 01: Vascular activity of the inflammatory reaction.

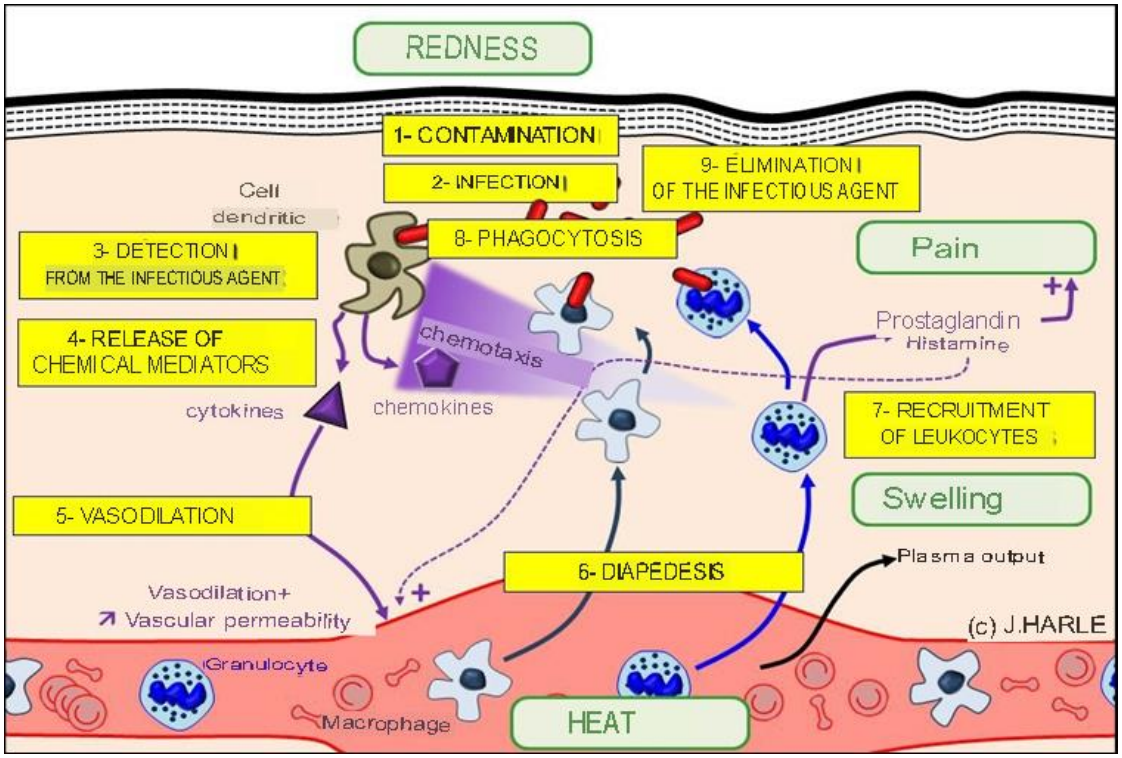


Fig. 02: Mechanisms and cardinal signs of inflammation.

III.2. Mechanism of diapedesis

The phenomenon of diapedesis begins with the induction of adhesion molecules on the endothelium. To do this, **TNFα** and **IL1** activate the vascular **endothelium** of the injured tissue to express adhesion molecules (**receptors**), called **P** and **E selectins,** allowing **leukocytes** to interact via **ligands** and **roll over the inflamed endothelium** (Fig. 03).

The chemokines IL8 and MCP-1 transform this **rolling** into **a firm and stable bond** by causing a conformational change of **integrins** (**LFA1 receptors**: lymphocyte function associated antigen 1) of **leukocytes** with induced **ICAM** (**I**nter **C**ellular **A**dhesion Molecules) cell adhesion molecules on the **activated endothelium** (Fig. 3).

The **leukocytes** involved in this phenomenon are first **neutrophils**, later **monocytes**, and then immature **dendritic cells** are recruited last. They **stop rolling** and **cross** the vascular endothelium (**diapedesis**) by slipping between the **endothelial cells**. The neutrophils that arrive at the site are then activated by IL8 and/or TNFα. This gives them a high capacity for phagocytosis (Fig. 03).

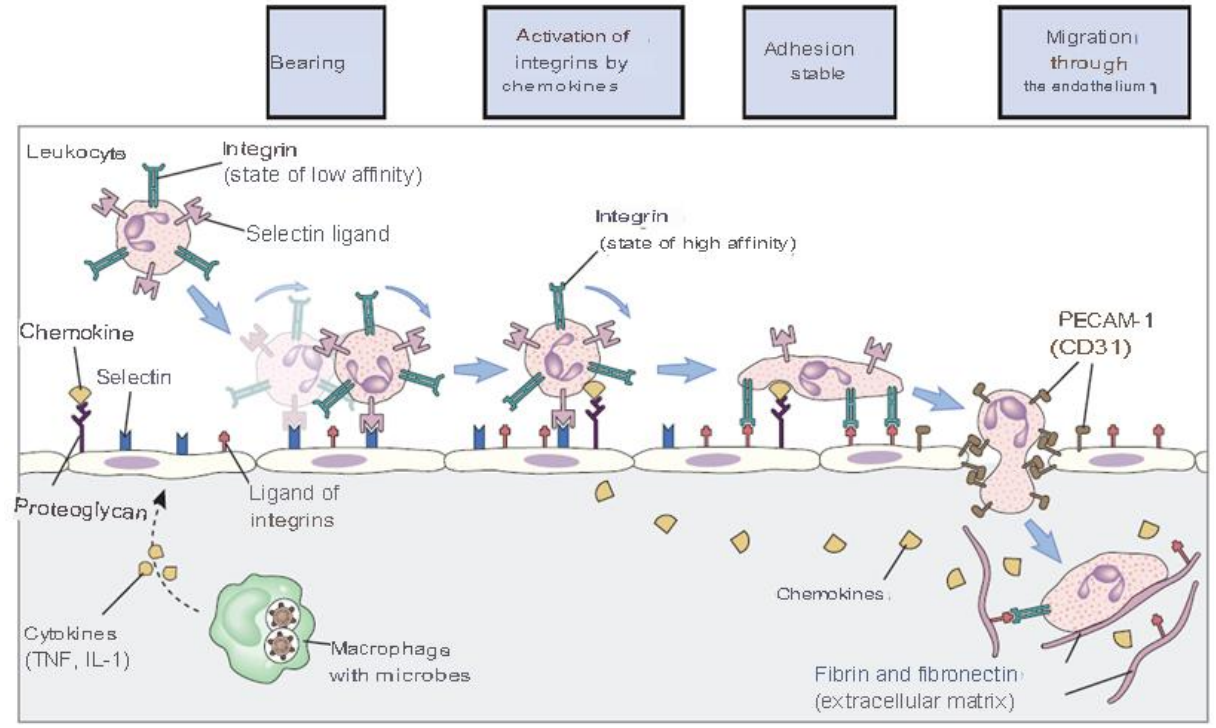


Fig. 03: Mechanism of neutrophil diapedesis.

Noticed: N-formyl peptides (with an N-formyl methionine at the amino terminal) produced by bacteria guide neutrophils to the site of infection.

III.3. Remote effects of cytokines

In addition to the local effects of cytokines, they exert other remote effects, such as:

- **Increased blood leukocytes** (leukocytosis): **IL-6** and **TNFα** induce the production of growth factors, **CSF** (colony stimulating factors), in the bone marrow by **stromal cells** and **macrophages**. CSF stimulates the production by hematopoietic stem cells of large numbers of **leukocytes** (leukocytosis) which will be recruited to the inflammatory site.
- **Fever:** It is caused by **endogenous pyrogens** (**TNFα, IL1 and IL6**) that stimulate the nervous center responsible for regulating body temperature (at the hypothalamus), and under the influence of **prostaglandins**. Fever is beneficial for the host, most pathogens are sensitive to high temperatures (Fig. 04).
- **Production of acute phase proteins: Pro-inflammatory** cytokines (**IL1, IL6 and TNFα)** act on **hepatocytes**, significantly stimulating the production of complement **system components** and so-called **acute phase proteins** including **CRP** (C reactive protein), **MBL** (mannose-binding lectin) and **pulmonary surfactants A and D**.

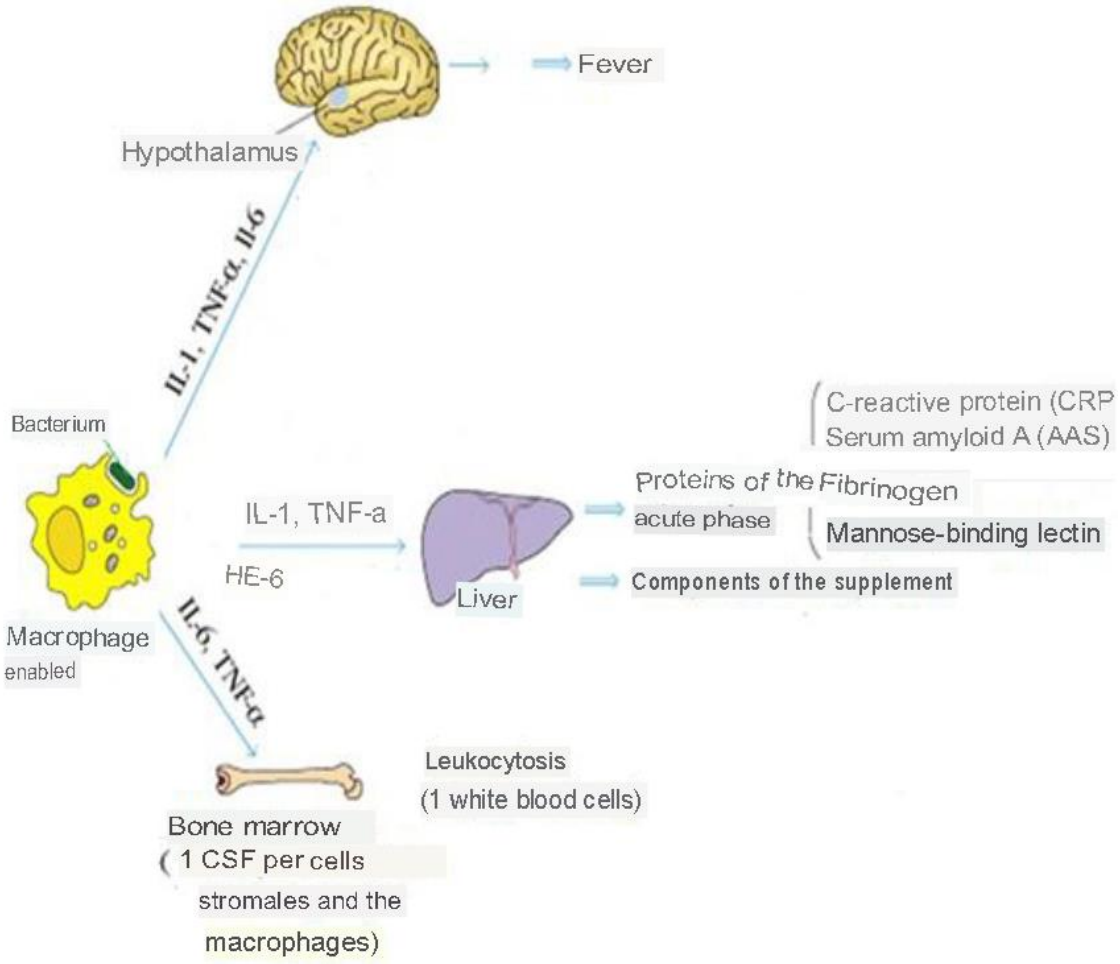


Fig. 04: Distant effect of cytokines.

II.4. Cardinal signs of inflammation

The following table summarizes the four cardinal signs of inflammation and their causes.

Table II: Manifestations of inflammation

Demonstrations	Causes
Redness (Erythema)	Dilation of the capillaries (**vasodilation:** dilation of the blood vessel) (the vessel is so dilated that it becomes transparent) and influx of blood.
Heat (Fever)	-Slowing of blood flow and activity or movement of the different cell types involved (phagocytic cells that try to pass from the blood into the tissue) - Effect of **endogenous pyrogens** (IL1, IL6 and TNF alpha) on the hypothalamus and prostaglandins
Edema (swelling)	**Increased vascular permeability**, resulting in **plasma exudation** (passage of plasma fluid) and **diapedesis** (passage of leukocytes) into the attacked tissue (inflammatory focus).

Pain	Excitement of nerve endings due to: *mechanical pressure due to edema. *Bacterial toxins *Chemical mediators: prostaglandins, leukotrienes, IL-1, TNFα, etc.

II.5. Phagocytosis

A cellular defense process by which **phagocytes** can **ingest** and **destroy** solid foreign particles (microorganisms, etc.). Phagocytosis is usually considered a specific form of endocytosis. It is generally triggered by an interaction between **endocytic PRRs** expressed on the surface of phagocytes and **PAMPs** of microorganisms. It is mainly carried out by **neutrophils** and **macrophages**. It occurs in 4 phases (Fig. 5).

II.5.1. Oriented movement or chemotaxis

Phagocytes (monocytes/macrophages, neutrophils and dendritic cells) are attracted to the **inflammatory focus** by substances produced by bacteria, degradation products of damaged tissues, **chemokines** and **other substances** such as **complement** activation products; **C5a** and **C3a**.

II.5.2. Adhesion of the particle to the membrane of the phagocytic cell

Phagocytic cells move by emitting **pseudopodia** and come into contact with the material to be phagocytosed (microorganisms, etc.). The adhesion of the phagocyte to the microorganism involves membrane receptors such as **endocytosis PRRs**.

II.5.3. Ingestion of the phagocytosed particle

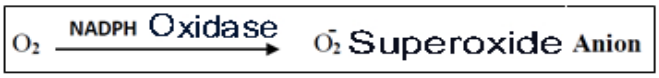
Phagocytes enclose the particle to be ingested in a phagocytic pocket called a **phagosome**. This detaches from the cell periphery and then ends up in the center of the phagocytic cell.

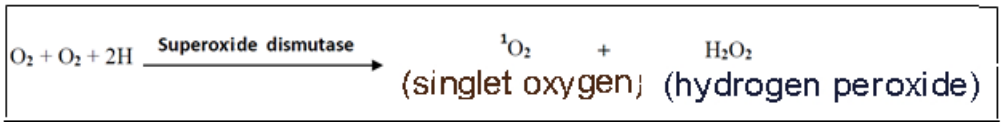
II.5.4. Effector step

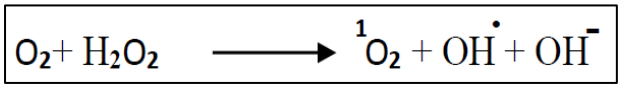
a-1)- Production of reactive oxygen species

Once the microorganism is encompassed, it ends up in the phagosome. It stimulates the phagocyte to increase its oxygen consumption, consequently the generation of toxic molecules derived from oxygen called "**reactive oxygen species**", or **ROS** (reactive oxygen species) occurred, by enzymes called**: oxidase** (NADPH oxidase, Superoxide dismutase, ...). Of which the most toxic ROS; the **superoxide anion ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2)** and the **hydroxyl radical ($OH^{\cdot}$).**

The production of these **ROS** is a common function of most phagocytic cells. These **ROS** are **hyperactive bactericidal** agents. They are produced through the following reactions:

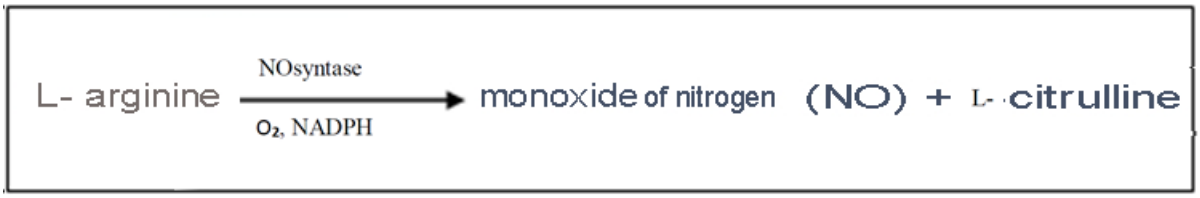






a-2)- Production of nitrogen monoxide

Nitric oxide ($NO^{\bullet}$) is generated from the conversion of **L-arginine** to **L-citrulline**, catalyzed by **NO synthase** (reaction below), using NADPH and O_2 as co-substrates. It is highly toxic. It is the source of the generation of other derivatives nitrogen dioxide **(NO_2)** and peroxynitrite (**$OONO^-$**); an even more toxic oxidant.



b)- Formation of the phagosome and degranulation

The **phagosomes** thus formed will fuse with the **lysosomes** to form **phagolysosomes**. **Degranulation** corresponds to the release of granules from the **lysosomes** inside the **phagolysosomes**. The pH of the phagosome, which was initially neutral, becomes acidic (pH = 4) in the phagolysosome; this pH is optimal for the activity of **lysosomal enzymes**.

c)- Destruction

Phagocytes destroy microorganisms inside phagolysosomes (Fig. 05) via:

- ✓ **Reactive oxygen species (ROS).**
- ✓ **Nitric oxide**.
- ✓ **Lytic proteins and enzymes** such as:
  - **Cationic proteins**: They attach to the bacteria inside the phagolysosome and damage the wall and membrane of the bacteria.
  - **Lactoferrins:** Proteins perform an antimicrobial function, binding and retaining **iron** needed by ingested bacteria.
  - **Lysozyme:** An enzyme that hydrolyzes **mucopeptides (peptidoglycans**) from the cell walls of various bacteria. It has a **digestive** rather than a bactericidal action.

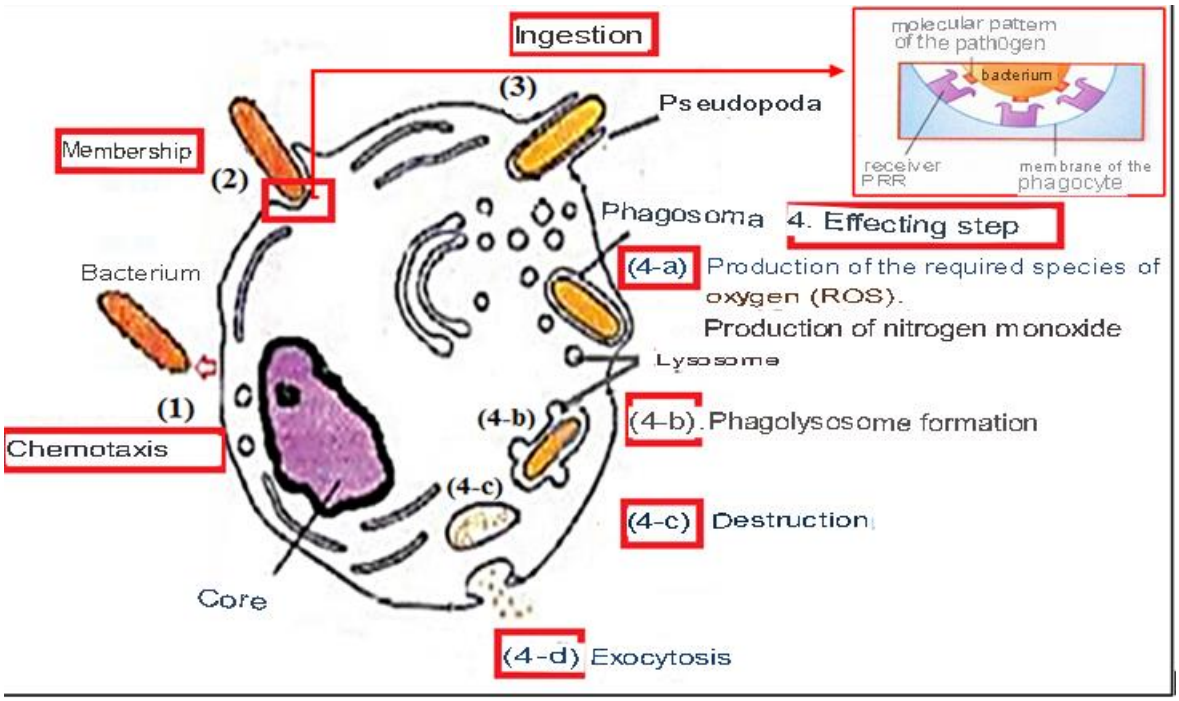


Fig. 05: Stages of phagocytosis.

d)- Exocytosis

This involves the release of the products of the **destruction** of microorganisms into the external environment of the **phagocyte**.

Noticed

Neutrophils and **macrophages** can **directly** phagocytose pathogens that they recognize through their surface receptors (PRR) and **indirectly** through their **receptors** for certain complement fragments and for other opsonization proteins such as **CRP** and **MBL**.

These innate phagocyte responses, induced by pathogens, succeed either in **completely eliminating** the infection or in **limiting** it while the adaptive response takes place.

III.6. Type of inflammation

Depending on the evolution of the inflammatory reaction in time and space, two types of inflammation can be distinguished.

III.6.1. Acute inflammation

Acute inflammation represents the immediate response to an aggressive agent, of **short duration** (a few days or weeks), often of sudden onset and characterized by intense **vasculo-exudative** phenomena. Acute inflammations **heal spontaneously** or with treatment, but can leave after-effects if tissue destruction is significant.

III.6.2. Chronic inflammation

Chronic inflammation is inflammation that has no tendency to heal **spontaneously** and that progresses by persisting or worsening over several months or years.

IV. Natural Killer Cells

NK cells (*Natural Killer*) are lymphocytes historically called "natural killer cells" because of their apparently spontaneous ability to cause death by apoptosis (programmed death) cells tumorous, infected by viruses or cells foreigners in the absence of prior specific immunization. NK cells are one of the components of innate immunity.

NK cells intervene in inflammation by producing IFN γ (interferon) a cytokine that activates phagocytes (for the amplification of the inflammatory reaction), following a activation by the IL12(produced by the dendritic cells) And IFN α and βproduced by the infected cells.

VI.1. Mechanism of action of NK cells

The NK cell is characterized by the expression of **inhibitory receptors (KIR)** recognize **molecules of MHC I** of self, and **activating receptors (KAR)** recognize surface **ligands** that are distress molecules overexpressed by infected, foreign, or tumor-prone cells. It also expresses **receptors for the Fc fragment of IgG (CD16)** and ligands for **Fas death receptors (CD95)**. It induces the death of target cells through two pathways; one way involves the perforins and the granzymes and another involves the death receiver Fas (Fas: apoptotic stimulation fragment).

VI.1.1. Induction of programmed death by perforins/granzymes

This apoptosis induction pathway is the result of two activation pathways:

a)- Activation by inhibitory receptors and activating receptors

In this activation pathway, the activity of NK lymphocytes is determined by the resultant (sum) of inhibitory and activating signals. The inhibitory signals come from the interaction between receptors inhibitors (KIR) and the molecules of CMH-I of oneself, and the activating signals come from recognition between receptors activators (KAR)and the molecules of stress expressed by the target cells (**Fig. 06**). Several cases are distinguished depending on the type of target cell (Table III).

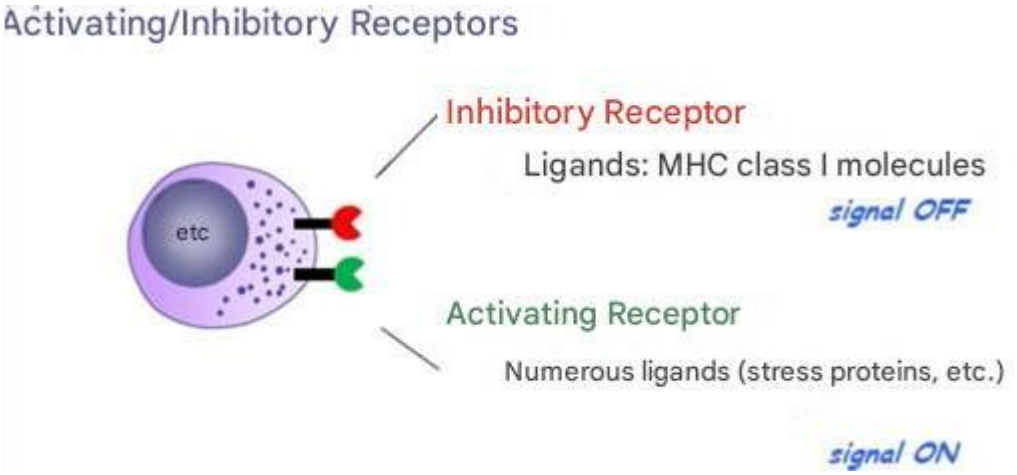
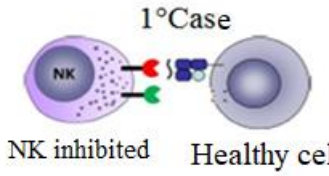
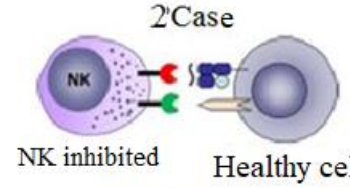
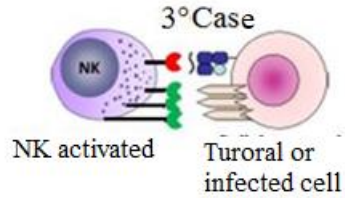
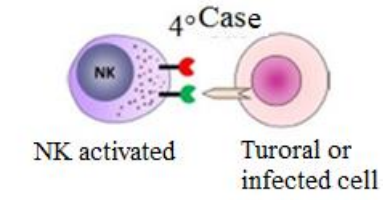
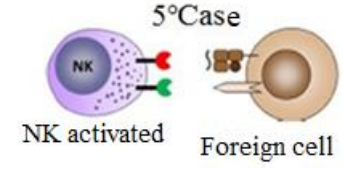


Fig. 06: Inhibitory receptors and activating receptors of NK.

Table III: Activation of NK lymphocytes determined by the sum of signals received.

Cells targets	**Receptors and ligands Intervene**	**Types of signals**	**Results**
Healthy cell		* Receives inhibitory signals * No activating signals	There are only inhibitory signals, NK inhibits itself
Healthy cell little stressed		* Receives inhibitory signals and activating signals	Sum of signals is zero, NK inhibits itself
Cell Tumor or infected expresses little of CMH-I		* Receives little inhibitory signals * Receives several activating signals	More signals activators, NK is active
Cell Tumor or infected loses his CMH-I		* No inhibitory signals * Receives activating signals	There are only activating signals, NK is active
Cell foreign CMH-I of the no self		* No inhibitory signals * Receives only activating signals	only activating- signals NK is active

b)- **Activation by receptors of the Fc fragment of IgG**

This activation is the result of the recognition between theCD16; a receptor for the Fc fragment of IgG previously bound to their specific antigen (target cell) (**Fig. 07**).

This activation leads to an Antibody-Dependent Cellular Cytotoxicity or ADCC.

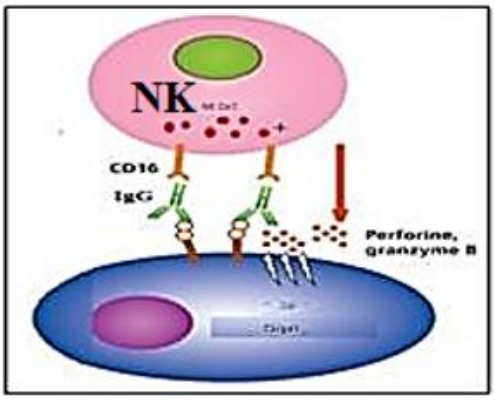


Fig. 07: Antibody-dependent activation of NK lymphocytes.

Induction of apoptosis via perforins and granzymes is the most dominant mechanism in the cytotoxic activity of NK. Once the NK cell is activated by the resultant of inhibitory signals and activating signals in favor of the latter and/or by antibody-dependent activation (IgG) via the receptorCD16, THE perforins and the granzymes (serine esterases stored in lytic granules) are released in the contact zone between the NK and the target cell.

In the presence of the calcium, the perforins polymerize on the cytoplasmic membrane of the target cell, forming channels (pores), These latter facilitate the penetration of granzymes in the target cell, inactivating subsequently proteases called caspases, thus promoting the programmed death (apoptosis) of the target cell (**Fig. 08**).

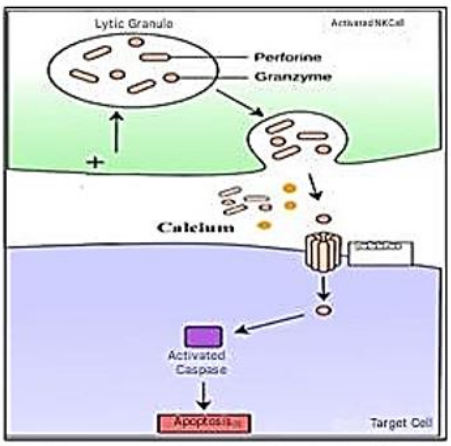


Fig. 08: Induction of programmed cell death by the perforin/granzyme pathway.

VI.1.2. Induction of programed death by Fas Receptor

The NK cell activated expresses on its surface Fas ligand (Fas L or CD178). The interaction of this ligand with the Fas receptor (CD95) (Fas: apoptotic stimulation fragment) expressed on the surface of certain infected or tumor target cells transmits the apoptotic death signal has the target cell by also activating caspases (Fig. 09).

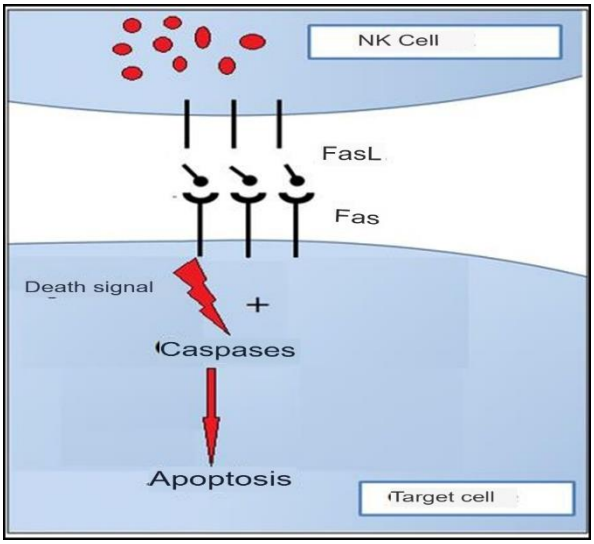


Fig. 09: Induction of programmed death by the Fas death receptor.

VI.2. Mechanism of apoptosis

Apoptosis is a process of programmed cell death. It begins with the activation of caspases; proteases which cleave enzymes that are important in the maintaining integrity of the nucleus, cell structure (cytoskeletal proteins) and DNA fragmentation. These cleavages have the consequence to inactivate proteins that promote the survival of target cells. Apoptosis of a cell is manifested by a decrease in cytoplasmic volume which is accompanied by the appearance of membrane budding cytoplasmic and condensation of the nucleus. Therefore, DNA is fragmented. It results in vesicles each containing a fragment of DNA surrounded by a cytoplasmic membrane. These vesicles are called apoptotic bodies, which will be eliminated by the macrophages (Fig.10).

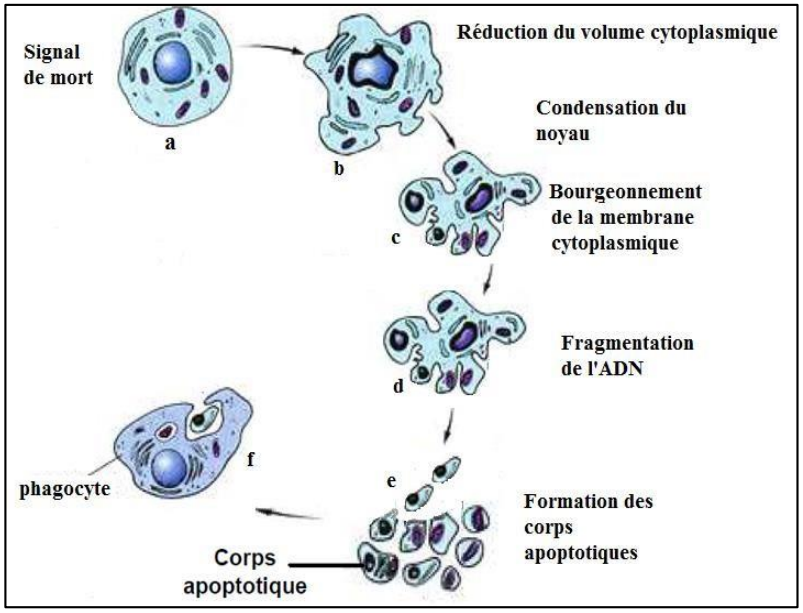


Fig. 10: Apoptotic events.

V. Complement System

The complement system is a component of the innate immune system that plays a key role in eliminating pathogens. It consists of thirties serum proteins. They are naturally present in serum, in the form inactive (zymogens) in the basic state (C1, C2,…, C9, Factor B,…) with the exception of Factor D. They are non-specific. The name complement comes from the fact that it complete in a way the action of the antibody. In fact, it can act alone and in many cases, without the intervention of antibodies.

V.1. Tissue origin of complement proteins

Most of the complement components are synthesized by the monocytes – macrophages and by the hepatocytes. Some are produced by the epithelial cells of the thymus and small intestine.

V.2. Activation of the complement

The activation of the complement is carried out in cascade, it is that the activation by cleavage of a first compound leads to the activation of a second compound which in turn activates another compound. For example, cleavage of the component C3 allows the formation of two fragments; a large fragmentC3b (endowed with enzymatic activity) and one small fragment C3a.Activating a compound gives it a enzymatic activity (Serine protease). It can be done via three-way:

- The Classic pathway: the recognition protein (the componentC1 of the complement) most often detects the antibodies (2 IgG or IgM)in complex with the cellular antigen;
- The alternates pathway: complement recognition proteins (fragmentC3b) directly recognize the pathogen via its PAMPs;
- The pathway of the MBL (Mannose-binding lectin). The recognition protein MBL recognizes the mannose of the walls of microorganisms.

V.2.1. Classical pathway

It is a way of antibody dependent. It involves the following components of the complement (C1, C4, C2, C3, C5, C6, C7, C8, C9). The activation of these components and consequently the cytolysis of antigen passes by several steps:

a)- Recognition and Initiation of activation

The triggering of this pathway begins with the formation of a immune complex

(Antigen-Antibody).

The component C1complement is a macromolecular complex with six globular heads consisting of a molecule of recognition; The C1q, And two serine protease dimers (2 C1rand 2C1s). The C1 binds to the Ag-Ac complex at the fragment Fc of 2 neighboring molecules IgG (IgG1, IgG2, IgG3) or a single molecule of IgM. This fixation causes a conformational change in C1, which activates theC1q, this one active the C1r, which in turn active The C1s. These reactions require the presence of Calcium (**Fig.11**).

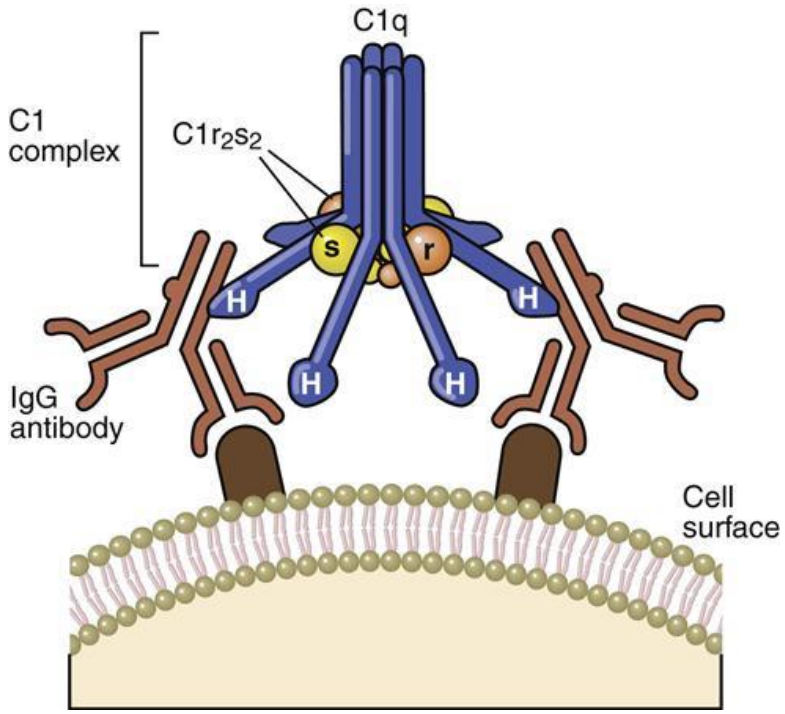


Fig. 11: Structure of the 1st complement component C1 and triggering of the classical pathway

The C1 (1st enzyme) Thus activated expresses enzymatic activity via the C1s. The latter cleaves the C4 in C4a free and C4b. The C4b binds covalently to the surface of the pathogen. It can then bind to a molecule ofC2making its cleavage possible by C1s in C2b free and in C2a which is it self a serine protease.

b. Formation of C3 and C5 convertases

The complex, formed byC4bwith theserine protease C2aactive, remains attached to the surface of the pathogen and forms theClassic C3 convertase(C4b2a)(2nd enzyme). This cleaves a large number of molecules ofC3inC3a and C3b.

The fragmentsC3b, bind to the antigen and allow phagocytosis of the Ag-Ac complex. C3b can also associate with the enzymeC3 convertase(C4b2a) to form a new enzyme, there C4b2a3b active, called theC5 convertase (3rd enzyme). The fragments C3aremain free. The C5 convertase(C4b2a3b) (attached to the Ag of the Ag-Ac complex) cleaves the C5in 2 fragments C5a and C5b (Fig. 12).

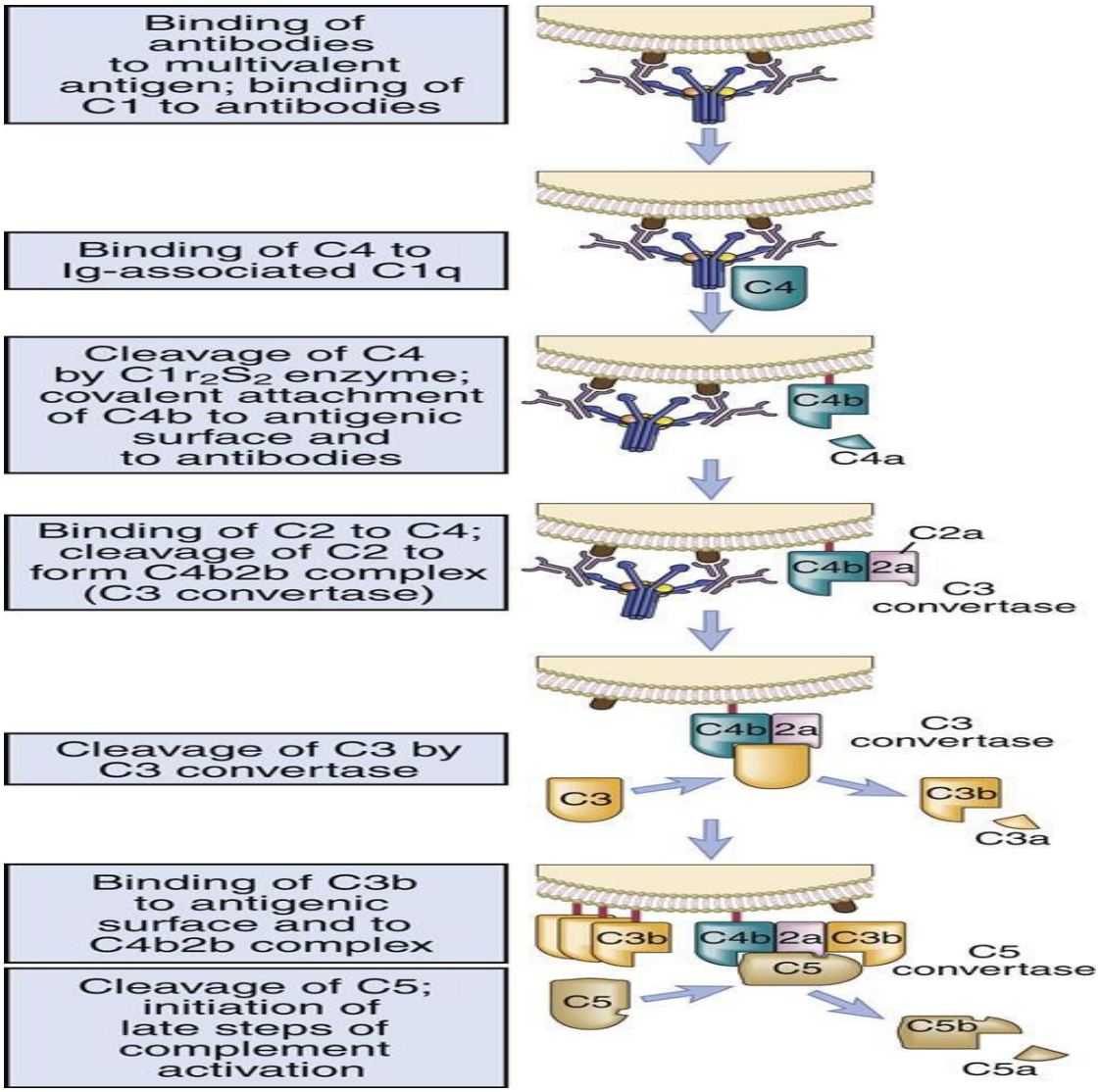


Fig. 12: Training of C3 And C5 convertase.

c)- Formation of the membrane attack complex (MAC)

The C5b associates with the antigen of the complexAg-Ac- C14b2a3band associates with proteinsC6AndC7to form a complex that integrates into the cytoplasmic membrane. The proteinC8binds to this complex, allowing several molecules of C9 to form a multienzyme complex C5bC6C7C8PolyC9 (4th enzyme). This is the lytic complex, calledmembrane attack complex (MAC) fixed on the cell wall of the antigen which perforatedby training ofchannels (pores). These will allow the entry of theThat2+and electrolyteswith H2O, which induces an osmotic shock, resulting in the lysisof the cellular Ag of the immune complex (Fig. 13).

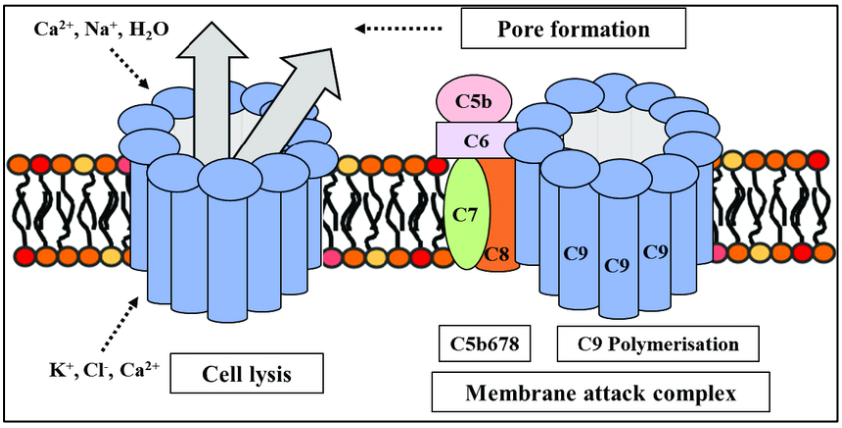


Fig. 13: Action of the membrane attack complex (MAC).

Noticed : the classic route can also be brought into play by the C-reactive protein (CRP). It attaches to the protein C of the pneumococci, this complex "CRP-protein C" is the equivalent of the immune complex; antigen-antibody in the classical route.

V.2.2. Lectins pathway

The way of the lectins also called the MBL way. Mannose-binding lectin (MBL), occurs at low concentration in normal plasma. Its production by the liver is increased during the acute phase of inflammation. The MBL recognizes certain constituents of bacterial walls (mannose s). This pathway involves the following components:C4, C2, C3, C5, C6, C7, C8 and C9.

This way is initiated by serum protein binding MBL to mannoses contained in the polysaccharides of the walls of certain bacteria. The structure of the MBL resembles that of the C1q, it is a molecule with6 globular heads which forms a complex with2 zymogenic proteases MASP1 and MASP2 (MBL Associated Serine Proteases). These2 serine proteases have practically analogy has C1r and C1s (Fig.14).

MBL is the recognition molecule of mannose of the wall of certain bacteria. This fixation active the 2 serine proteases MASP1 and MASP2 Who cleave the C4 then the C2. The next step of the activation is similar to that of the classic route (Fig 14).

Fig. 14: Structure of the mannose-binding lectin (MBL) complex.

V.2.3. Alternative pathway

The alternative pathway acts as a surveillance system by maintaining a low level of complement system activation. It involves the following components (C3, C5, C6, C7, C8, C9, factor B, factor D, and factor P). This pathway involves several steps:

a)- Preparatory stage of the alternative route

This step is initiated by the spontaneous hydrolysis of C3 in C3(H_2O). The latter in the fluid phase can attach to the factor B, which is then cleaved the factor D (this factor always circulates in active form) in two fragments; Bb and Ba, to form the initial C3 convertase ($C3H_2OBb$) in the fluid phase. This enzyme cleaves the C3 in 2 fragments; C3a and C3b (**Fig. 15**).

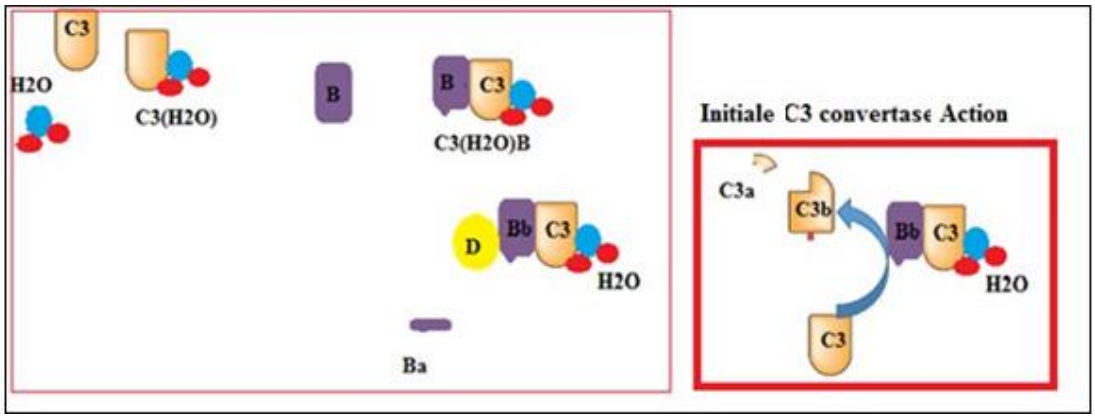


Fig. 15: Preparatory stage of the alternative route.

b)- Initial stage of activation of the complement of the alternative pathway

The initiation of this pathway is due to the fixation of the factor B to C3b, already associated with the pathogen membrane. Then factor B can be cleaved by the factor D, by detaching the fragment Ba and give the complex C3bB

C)- Formation of C3 and C5 convertase of the alternative pathway

The complex C3bBb is an enzyme, it's theC3 convertase alternates. This enzyme activates, is stabilized by theP factor, to cleave several C3 molecules. It cleaves C3 also into C3b and C3a, the C3b associates with the C3bBb complex to give the C5 convertase alternate active (C3bBb3b). This enzyme cleaves C5 into 2 fragments C5a and C5b. The continuation of this path is common with that of the classical route, it ends with the formation of the CAM and the lysis of the pathogen (Fig. 16).

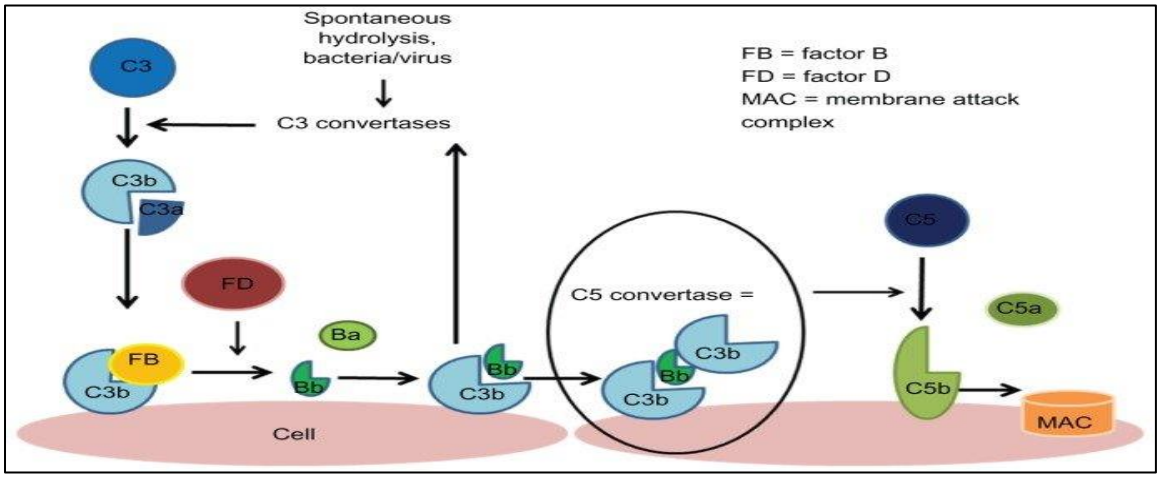


Fig. 16: The steps of complement activation by the alternative pathway.

A summary of the three complement activation pathways is shown in Figure 17.

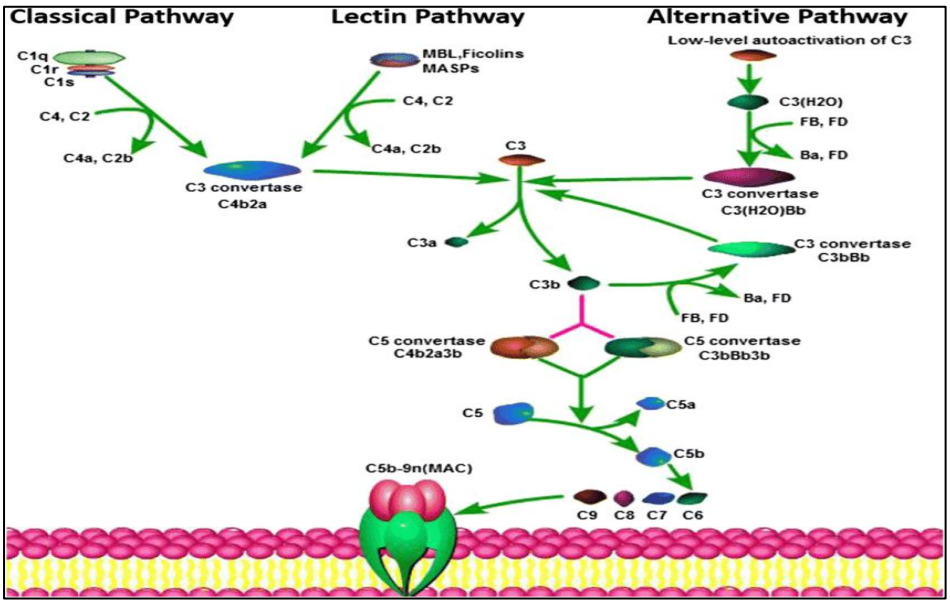


Fig. 17: The three pathways of complement activation.

V.3. Complement Activation Regulators

To prevent potential damage to healthy tissues, the complement system is tightly regulated. This regulation occurs at three main levels:

1. Inhibition of protease activity involved in the complement activation cascade;

2. Facilitation of protease degradation, thereby limiting their activity;

3. Control of membrane attack complex (MAC) formation, preventing lysis of host cells.

Complement regulators can be either soluble or membrane-bound. Soluble regulators include C1 inhibitor (C1-INH), I Factor, and H Factor. Membrane-bound regulators include CD35 (complement receptor 1, CR1), CD46 (membrane cofactor protein, MCP), CD55 (decay-accelerating factor, DAF), and CD59 (Fig. 18 & table IV).

Table IV: key complement regulatory proteins along with their respective mechanisms of action.

Localization	Regulator	Mechanism of Action
Plasma	**C1 Inhibitor (C1-INH)**	• Irreversibly inhibits the enzymatic activity of **C1r** and **C1s**.• Promotes dissociation of **C1q** from **C1** in immune complexes.
	C1q Inhibitor (C1q INH)	• Binds to **C1q**, preventing **C1r** and **C1s** from attaching to their binding sites.
	C4-Binding Protein (C4BP)	• Induces dissociation of **C4b2a** complexes (C3 convertase).• Prevents binding of **C2** to **C4b** fragments.• Acts as a cofactor for **Factor I**, facilitating the cleavage of **C4b** into **C4d** and **C4c**.
	Factor H	• Binds to **C3b**, making it susceptible to inactivation by **Factor I**, forming **C3bi** (inactive C3b). • Promotes dissociation of the **Bb** fragment from **C5 convertase** (**C3bBb3b**).
	Factor I	• Proteolytically cleaves **C3b** and **C4b**, leading to their inactivation.
	Protein S (Vitronectin) and **Clusterin**	• Bind to the **C5b67** complex, preventing its insertion into cell membranes.
Membrane-bound	**CR1 (CD35) – Complement Receptor 1**	• Binds **C3b** with a regulatory function similar to **Factor H**.
	MCP (CD46) – Membrane Cofactor Protein	• Binds to **C4b** and **C3b** within convertases, allowing their cleavage by **Factor I**.
	DAF (CD55) – Decay-Accelerating Factor	• Dissociates **C3** and **C5 convertases**, halting complement activation.
	CD59	• Blocks **C9** binding, thereby preventing **membrane attack complex (MAC)** formation.

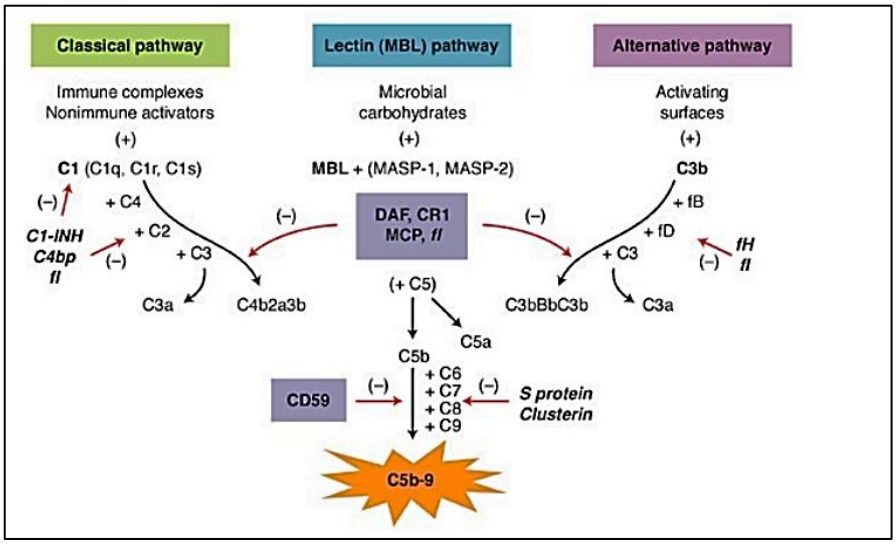


Fig.18: Sites of action of complement regulatory proteins.

V.4. Other Biological Functions of the Complement System

In addition to cytolysis mediated by the membrane attack complex (MAC), the complement system and its cleavage fragments perform a variety of other biological functions. These include opsonization of antigens (Fig. 19), clearance of immune complexes, as well as chemotactic and anaphylatoxic activities (Table V).

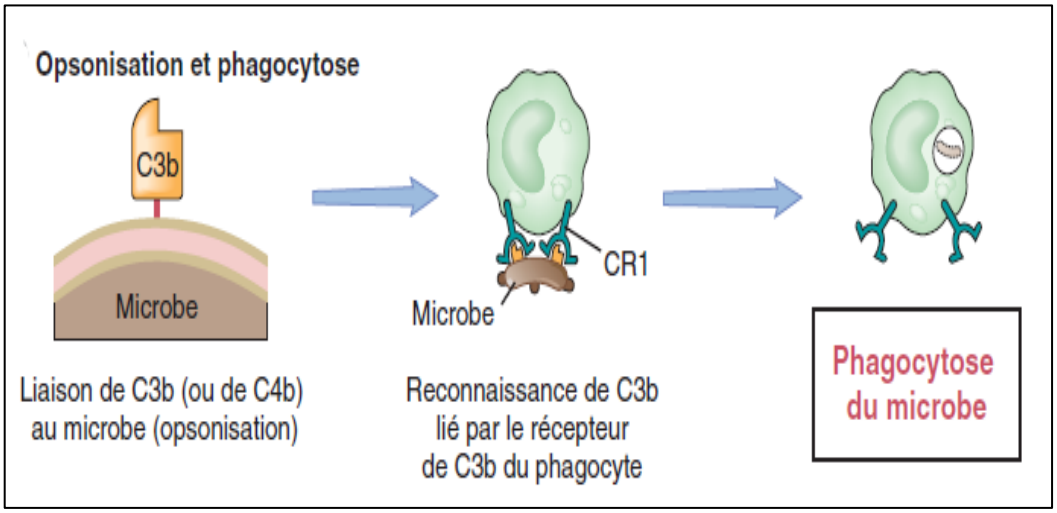


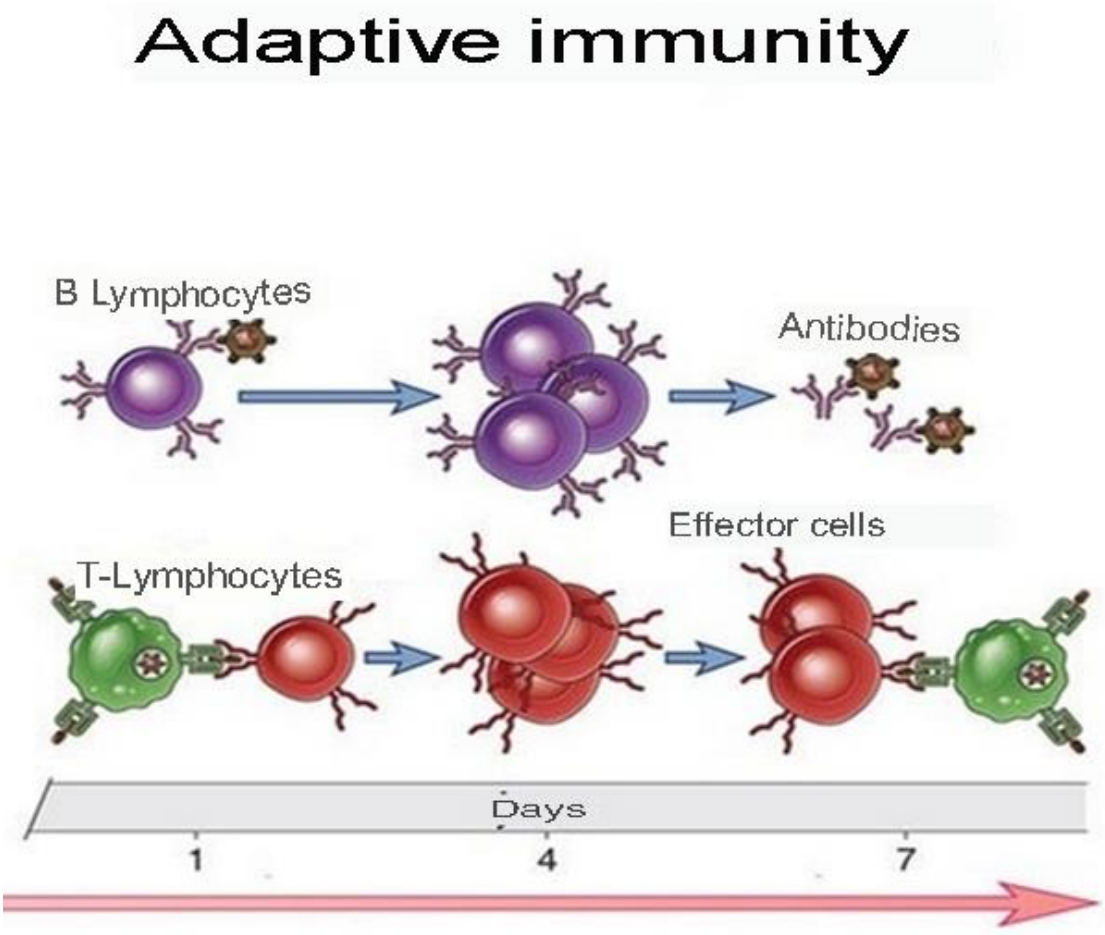
Fig. 19: Opsonization of antigens via C3b.

Table V : Biological Activities of the Complement System.

Biological Activity	**Complement Components**	**Mechanism of Action**
Opsonization and Clearance of Immune Complexes (Fig. 19)	**C3b**	Phagocytes (neutrophils and macrophages) express the **CR1 receptor** for C3b. The coating of free antigens or immune complexes with **C3b fragments** facilitates their adhesion to phagocytic cells, promoting ingestion and subsequent clearance.
Chemotactic Activity	**C5a, Ba, and free C5b67**	These components act as chemical attractants, guiding neutrophils to the site of inflammation.
Anaphylatoxin Activity (Inflammation)	**C5a, C3a, and C4a**	Induce the release of **histamine** from mast cells and basophils. Histamine triggers **smooth muscle contraction** (in the trachea, bronchi, and bronchioles), **capillary vasodilation**, and **increased vascular permeability**, amplifying the inflammatory response.

Chapiter 06

Specific Immunity



I. Generalities

Specific immunity also known as **acquired** immunity or **adaptive** immunity. It is the set of **humoral** and **cellular** biological mechanisms directed **specifically** against a given antigen, it is a **transmissible immunity** that **retains the memory** of the first contact with Ag, involving **T and B** lymphocytes **specific to the Ag**, possessing **TCRs** or **BCRs** that recognize a single type of **epitope**. There are two types of **response: a cell-mediated response** and a **humoral-mediated response**.

I. Specific cell-mediated response

The **TCD4** and **TCD8** lymphocytes are the actors of the **specific cellular** response. These lymphocytes are unable to recognize the antigens in their native form. They then recognize **immunogenic peptides** presented in association with **MHC** molecules.

I.1. Antigen capture and presentation

During the inflammatory reaction, **monocytes** and **immature dendritic cells** are recruited at the inflammatory site under the action of **chemokines** produced by local cells (epithelial, endothelial, or fibroblast cells) and macrophages under the effect of proinflammatory cytokines **IL-1 and TNFα**. In parallel, after microbial contact or after stimulation by **inflammatory cytokines**, **immature dendritic cells** are activated and become capable of **capturing** and **processing** a wide variety of molecules and microorganisms.

In these inflammatory circumstances, **dendritic cells** have become **antigen-presenting cells to naive T lymphocytes**, in case of a **primary response** of these lymphocytes to their specific antigens. They **present** these antigens captured after priming in association with **MHC-I** molecules for **endogenous immunogenic peptides <u>or</u> MHC-II** molecules for **exogenous immunogenic peptides** (**see MHC course**). This expresses the capacity of dendritic cells **to cross-present antigens**.

At the same time, once the antigen is captured, several events follow one another in the life of the dendritic cells to lead to their **maturation.** This maturation results in the following characteristics **(Fig. 01)**:

- Changes in cytoskeletal morphology and mobility, Volume increase with emission of many extensions or dendrites justifying their name.
- Loss of **phagocytosis/endocytosis** capacity and secretion of **chemokines**.

- Loss of **inflammatory chemokine** receptors that keep them in place in the inflammatory focus. On the other hand, they acquire other receptors such as **CCR7**.This allows them **to leave the inflammatory focus**, loaded with their antigens and to respond to the attraction by the **chemokine SLC** (secondary lymphoid-tissue chemokine) or **CCL21** emitted by the **endothelial cells** of the **afferent lymphatic channels** of the lymph nodes.
- Expression, increased **MHC-II** molecules especially but also **MHC-I**, necessary to ensure the **presentation of antigens**.
- Abundant appearance of the co-stimulatory molecules**, CD80 /CD86 and CD40**, essential for the complete activation of **naive** T lymphocytes.

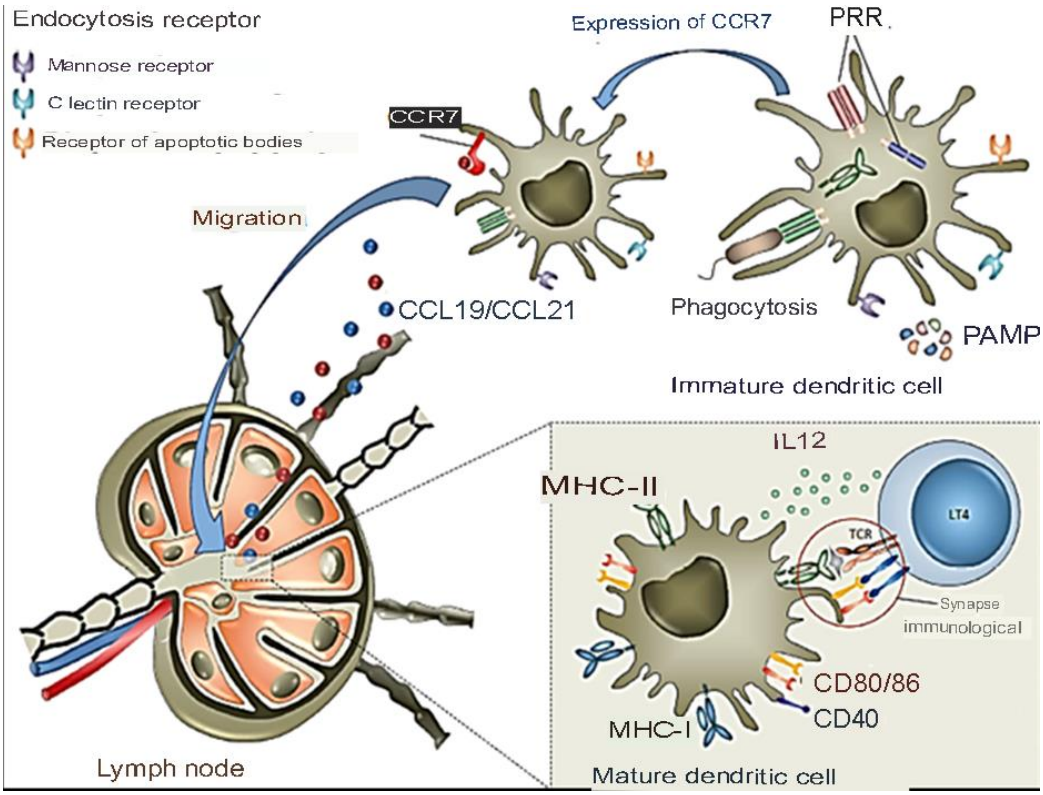


Fig. 01: Maturation and migration of dendritic cells.

Mature dendritic cells leave the inflammatory focus, to access the lymph node, through the **afferent duct**. They will settle around the high endothelial venules (**HEV**) of the **paracortex**. The latter is very rich in T lymphocytes, which considerably increases the chances of mature dendritic cells meeting naive T lymphocytes.

These mature dendritic **cells (antigen-presenting cells or APCs**) that express **exogenous immunogenic peptides** in association with **MHC-II** molecules will present them to naïve **CD4 T** cells**.** Whereas, those who express **endogenous immunogenic peptides** in association with

MHC-I molecules will present them to **naive CD8 T cells**. T4 and T8 lymphocytes will recognize specific immunogenic peptides via their **specific TCR** and via **CD4 and CD8** which will recognize the **corresponding MHC molecules.**

I.2. Recognition and activation of TCD4 lymphocytes

Activation of naïve LT4s in the lymph node requires three activation signals;

- **First signal:** Corresponds to recognition and binding by **TCR - CD4** (receptor for T4 lymphocyte antigen) and **exogenous immunogenic peptide** presented in association with **MHC-I** molecules on the surface of dendritic cells (**APC**). This, activates the **naïve TCD4 lymphocyte,** through the **CD3** molecule which transmits **an activator signal** (**1 st signal**) to the CD4 T lymphocyte (Th0 cell), **(Fig. 02)**.

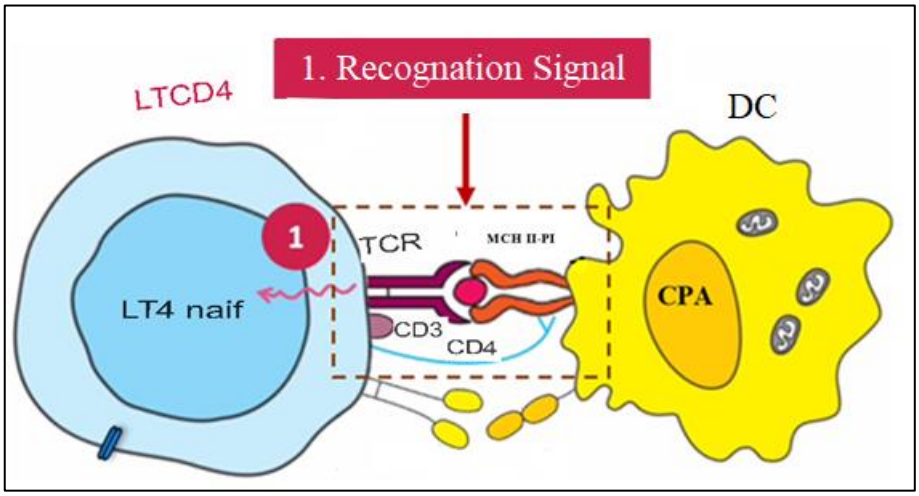


Fig. 02: First signal of activation of naïve LT4 by dendritic cells.

- **Second signal:** Via the interaction of the **co-stimulation** molecules CD80 or CD86 **(B7-1 or B7-2)** expressed on the **APC** cell, with the **CD28 (receptor)** molecule of the **T4** lymphocytes, provides the **2nd signal** (**co-stimulation signal**) necessary for the complete activation of **naïve LT4 (Fig. 03)**.

If the T cell receives the **1st signal** and does not receive the **2nd signal** (Co-stimulation), it becomes **anergic**. It then becomes unable to respond to any further stimulation by the same antigen.

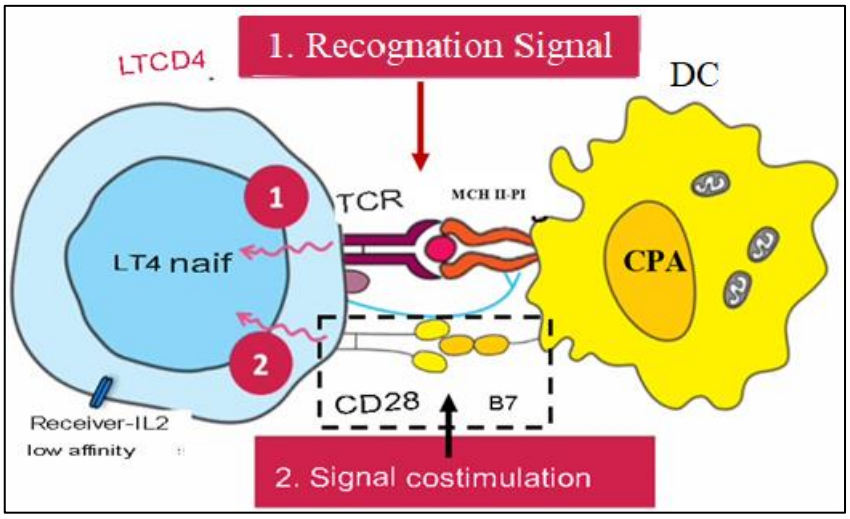


Fig. 03: Second activation signal of naïve LT4 by the APC cell.

Following these **2 signals**, the **activated** TCD4 lymphocyte produces **IL2** and then expresses **high affinity IL2 (IL2-R) receptors**. IL2 interacts with its specific receptor, thus transforming this same lymphocyte into a large **lymphoblast** (22µm). This **self-activation** of LT4 subsequently induces the **proliferation of T4 lymphocytes** which will divide a dozen times **(Fig. 04)**.

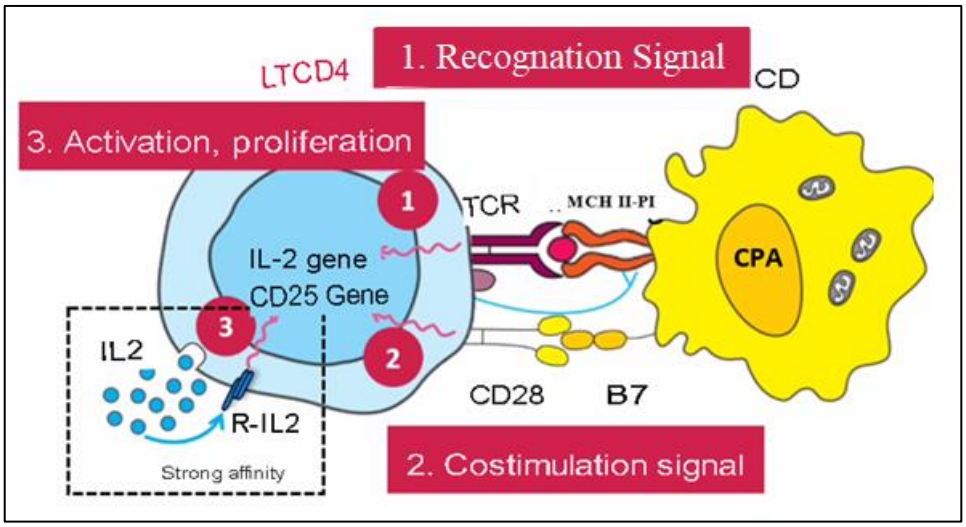


Fig. 04: Auto-activation of IL2-activated T4 lymphocytes.

- **Third signal:** In addition, the **APC** cell is stimulated, by interaction of its **CD40** surface **receptor** with the **ligand CD40-L** (**CD154**) expressed by **activated T4 lymphocyte**, to produce interleukin 12 (**IL-12**) and **IFNγ** (Interferon gamma) and others. These **polarizing cytokines** constitute the **3rd signal** for **activated LT4**, which promote the differentiation of activated TCD4 lymphocytes into **effector T and LT4m** (Fig. 05).

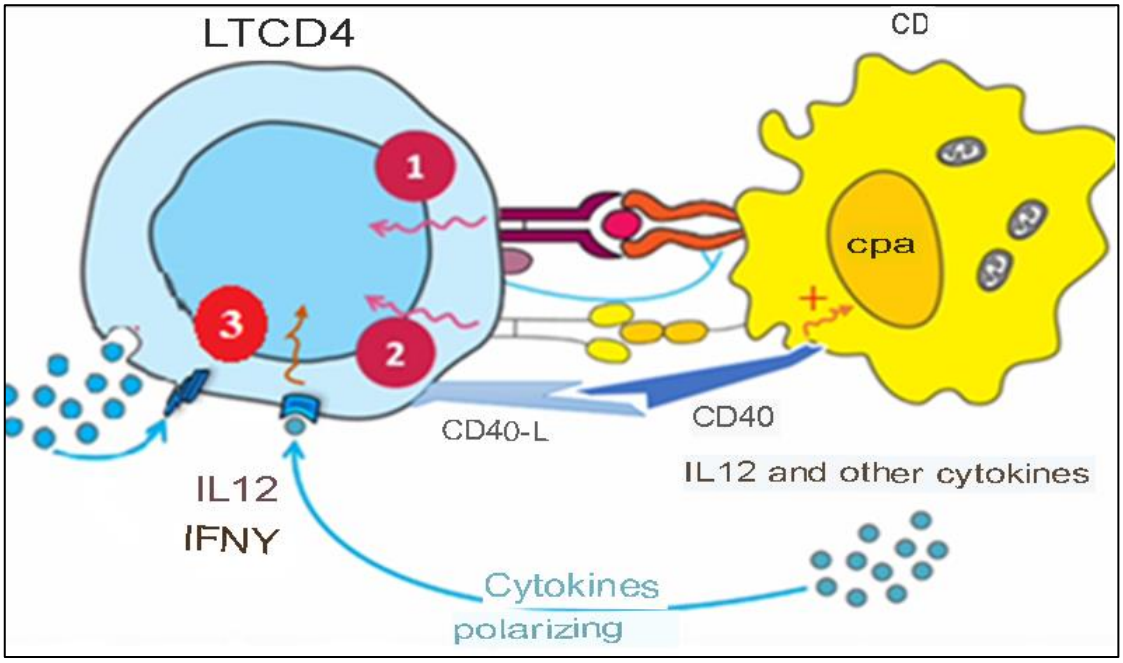


Fig. 05: Third activation signal of LT_4 by the APC cell.

The lymphocytes obtained, after mitotic division, **will differentiate into Th1 lymphocytes (helper 1 or helper 1)** or **LT4 effectors** of the **specific cellular** response). A small part of the **Th1** lymphocytes will keep the memory of this first contact with the antigen in question. So they are the **LT4m** (memories), (Fig. 06).

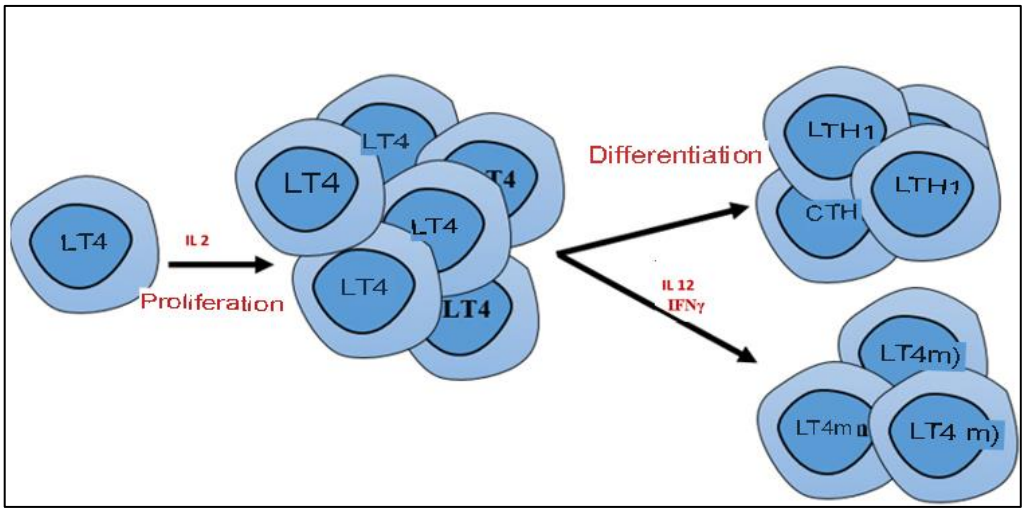


Fig. 06: Proliferation and differentiation of TCD4 lymphocytes.

Notes:

- After activation via the CD40 receptor, APCs produce other polarizing cytokines, such as **IL4**, in addition to **IL12**. Under the action of the latter, the **activated LT4** differentiate into **LTh-2** (**LT4 effectors** of the **humoral** specific response) and **LT4m**. Figure 07, summarizes the different **polarizing cytokines** and the corresponding **effector lymphocytes** with their **effector cytokines**.

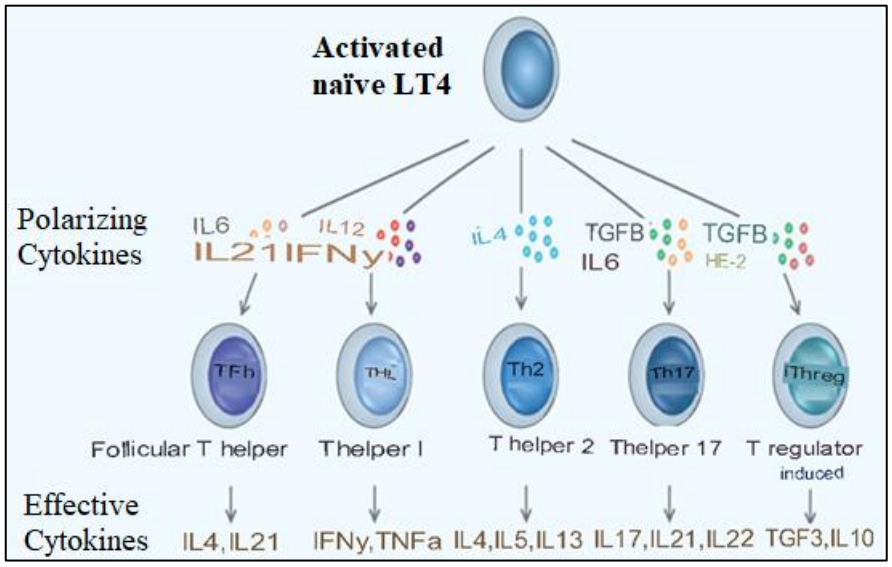


Fig. 07: Effector T lymphocytes come from the differentiation of activated LT4.

I.2.1. Th1 lymphocyte migration

Th1 lymphocytes will leave the **secondary lymphoid organs**; lymph node through the efferent duct and then through the thoracic lymphatic duct enter the bloodstream to access the **infected tissue**; in which the antigen was initially captured by the dendritic cells.

I.2.2. Regulation of activated T4 lymphocyte activity

After 48 hours of naïve LT4 activation, they will express **CTLA-4** (**C**ytotoxic T **L**ymphocyte **A**ntigen -4) or **CD152** molecules on their surface. Interaction of these **receptors** with **APC CD80 or CD86 ligands** provides an **inhibitory signal** that **inhibits** T4 cell activation and prevents **over-response** (Fig. 08).

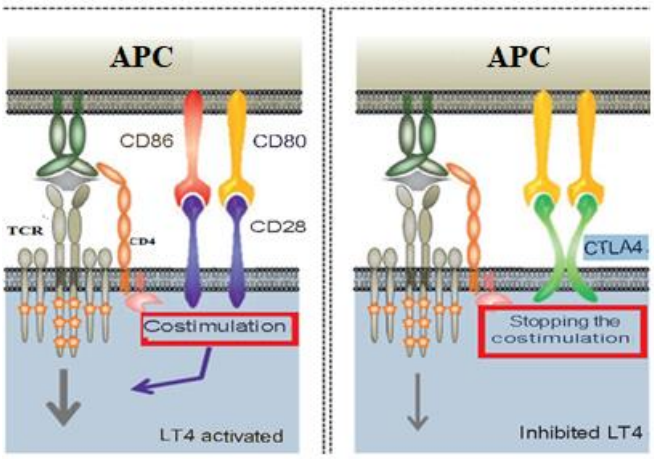


Fig.08: Inactivation of T4 lymphocyte activated by CTLA-4.

I.2.2. Effector functions of Th1 lymphocytes

At the level of the infected tissues, the **Th1** lymphocytes will recognize the immunogenic peptides of the antigen on the surface of the **macrophages** and activate again. They will then express **CD40-L** on their surface and produce **lymphokines**, the most important of which are **interferon γ** (**INF γ**) and **TNFα**, by which they **activate macrophages** in order to amplify their response by increasing:

- ✓ Their **bactericidal power** through an overproduction **of reactive oxygen** and **nitric oxide** species and increased synthesis of **enzymes**: hydrolases, proteases, etc.
- ✓ Their expression, on their surface, of **MHC II** molecules, **costimulation** molecules and **receptors for the IgG** Fc fragment and thereby increase the **phagocytosis** of the Ag – IgG complexes.

I.3. Recognition and activation of CD8 T cells

T8 lymphocytes have the ability **to specifically recognize virus-infected self** cells and **tumor** cells. They are characterized by the **antigen-specific receptor** (TCR), which recognizes the **endogenous immunogenic peptide** presented in association with the **MHC-I** molecules, the latter are recognized by **CD8.** Their activation can be carried out **directly** in the **absence** of **LT4 or LTh1**. As it can be done **indirectly** through them.

I.3.1. Direct activation of naive T8 lymphocytes

In the secondary lymphoid organs, the **dendritic cell** has the **exogenous immunogenic peptide (PI)** (tumor or viral) associated with **MHC-I** molecules, this complex (MHC-I-PI**)** is recognized by the complex **(TCR-CD8)** of **naive T8 lymphocytes**. This is the **first signal** to activate **naive T8 lymphocytes**.

A **second costimulation** signal is required for LT8 activation, via recognition between the **dendritic cell B7** (**CD80/CD86**) molecules and the **naive LT8 CD28** receptor. In the absence of this second signal, naïve LT8s who have engaged their TCR enter **anergy or apoptosis**. The **second signal** activates the **naive T8** lymphocyte, stimulating the production and secretion **of IL2** (Fig. 09).

IL2 induces the **proliferation** of this activated **naive T8** lymphocyte. The lymphocytes obtained will **differentiate:** a large part into **effector TCD8** lymphocytes (**Tc: cytotoxic T**) and a small part into **memory T8 lymphocytes (LT8m).**

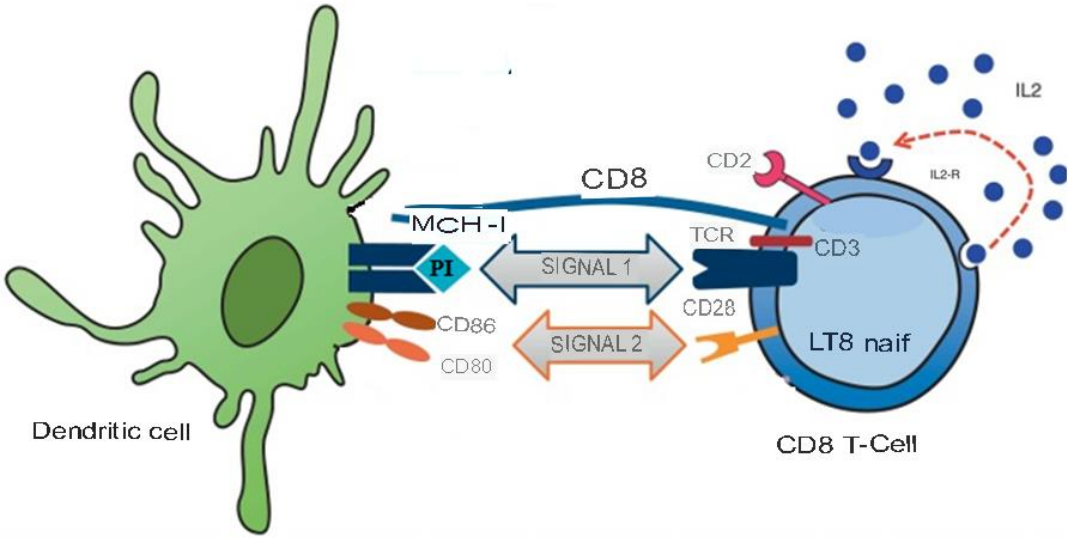


Fig. 09. Direct activation of TCD8 by an infected dendritic cell.

I.3.2. Indirect activation of naive T8 lymphocytes

The response of naive **T8 lymphocytes** to certain viruses or tumor cells, is in need of the **cooperation** of **activated TCD4 or LTh1 lymphocytes**. The naive TCD4 lymphocyte activated by the APC cell produces **IL2,** which stimulates the **activated T8** lymphocyte (received the **2 activation signals**) to proliferate and then differentiate.

Whereas, the **TCD4** effector or **Th1** lymphocyte, activated by the **infected APC** cell, can instead activate the APC cell to express **more costimulatory** molecules and thus amplify the activation of the naive T8 lymphocyte **(Fig. 10)**.

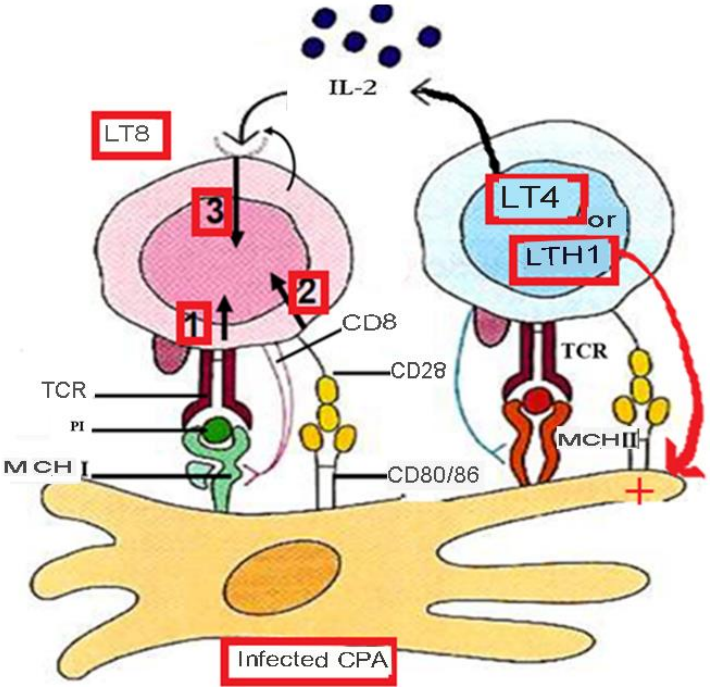


Fig. 10: Indirect activation of the TCD8 lymphocyte by LTCD4 or LTh1.

I.3.3. Migration of T8 effector lymphocytes

After the differentiation of the **TCD8** lymphocytes into **cytotoxic** T lymphocytes, the latter will leave the secondary lymphoid organs to reach the infected or tumor tissues via the bloodstream **(Fig. 11)**.

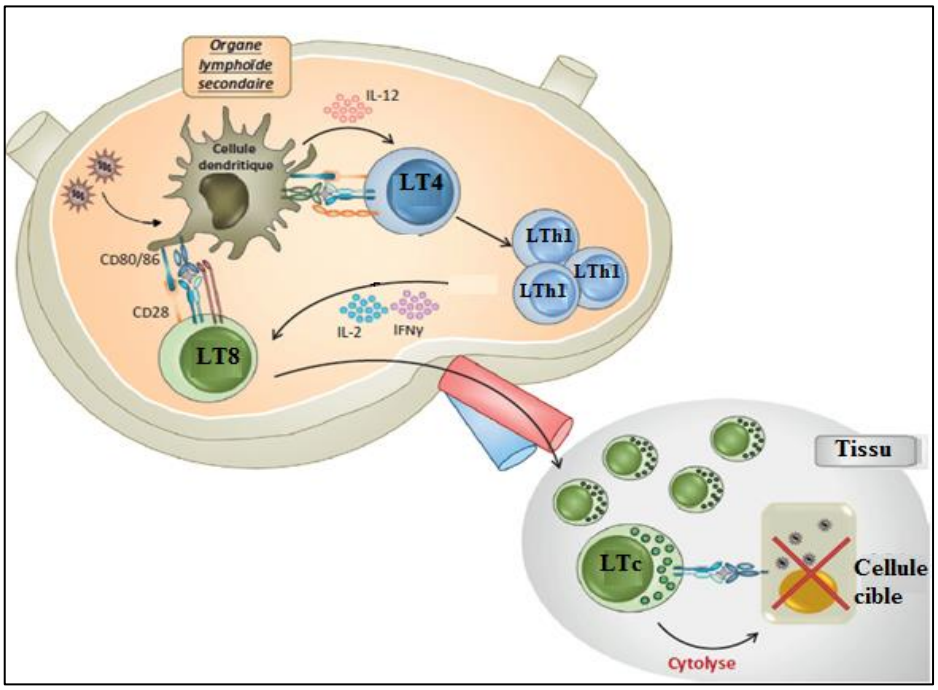


Fig. 11: Migration of cytotoxic T cells from the lymph node to infected tissue.

I.3.4. Mechanisms of action of cytotoxic lymphocytes

In the infected or tumor tissue, the **effector T8** lymphocytes (cytotoxic T cells) will specifically recognize the **immunogenic peptide**, associated with a **MHC-I** molecule, on the surface of the **target cells** (infected or tumor), which activate them to induce their **cytotoxicity** and cause their death by **apoptosis**. The mechanisms of cytotoxicity of activated lymphocytes (Tc) are the same as those of **NK lymphocytes**. They are done in two ways **(Fig. 12)**:

- The **perforin/granzyme** pathway;
- The **Fas death receptor pathway**.

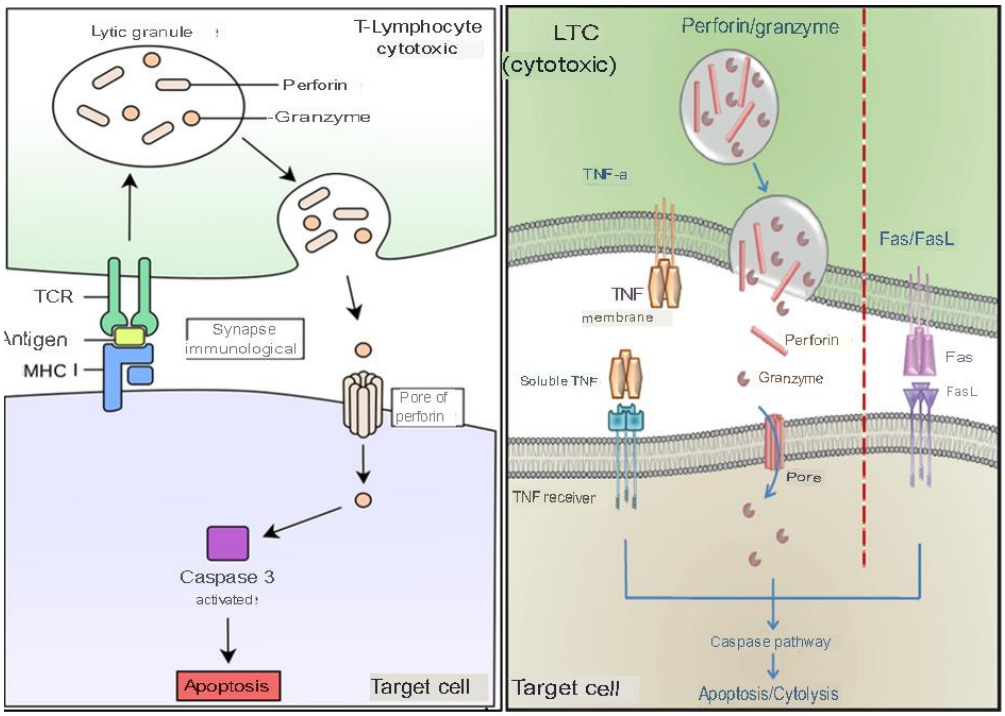


Fig. 12: Mechanisms of LTc cytotoxicity via the **perforin / granzyme** pathway and **Fas death** receptor.

I.4. Primary and secondary cellular response

The **primary** cell-mediated response is triggered in the event of **1st contact** with the antigen, carried out by the **naive** T4 and T8 **lymphocytes**, whose antigen-presenting cell (APC) is essentially the **dendritic cell**.

Whereas, the **secondary** cellular response is triggered upon **2nd or subsequent** contact with the same antigen. This response is orchestrated by **memory T cells, whose antigen-presenting cells** are **essentially macrophages.** Activation of memory T cells **requires less antigen** and fewer **B7** molecules.

In the secondary response, memory lymphocytes respond, dividing and differentiating into more efficient effector T cells and memory lymphocytes. This response is much **faster**, more **intense** and **more effective** than the primary response.

II. Specific humoral mediated response

The **B lymphocyte** is the lymphocyte responsible for humoral immunity. It specifically recognizes the antigen in its **native** form, by the **membrane immunoglobulins** it carries (**BCR**: B cell receptors). These BCRs are associated with heterodimers **Igα and Igβ**. These heterodimers are **signaling** molecules, they transmit the **activation signal** delivered by the binding of the **BCR** to the **antigen**. LB activation can be carried out in two ways depending on the type of antigen. Thyme-dependent **activation and thyme-independent activation are distinguished**.

II.1. Thymoindependent activation of B cells

Thymoindependent **antigens (TIDs)**, are antigens that do not require the cooperation of **T lymphocytes** to activate Bs lymphocytes to **proliferate** and **differentiate** into **plasma cells** producing **specific antibodies**. There are not many of them, they are of **2 types**:

1. Thymoindependent antigens 1: These are components of bacterial walls, they are **lipopolysaccharides (LPS)**, Gram-**negative** bacteria. Activation of **naïve B cells** requires **two activation signals**, the **first** of which is due to recognition between the **BCR and the epitope** of the specific antigen. Whereas the **second signal** corresponds to the recognition between a **mitogen** of the antigen and the **mitogen receptor** expressed by **LB** (Fig. 13A).

The B lymphocyte that has received these **two signals** transforms into a large **lymphoblast** and then activates to **proliferate** and **differentiate** into **plasma cells (LBp)**, producers and secretors of specific **IgM** type **antibodies** only. It is that there is no **immune memory** against this type of antigen.

2. Thymoindependent antigens 2: These are **repetitive antigenic determinants**, on the surface of the antigen, such as **polysaccharides** of the bacterial walls. This type of antigen activates the specific B-cell via a **single strong signal**, in which **several BCRs** recognize at the same time **several identical epitopes**. This **activates** the specific **naive B lymphocyte (Fig. 13B)**. The activated LB transforms into a large lymphoblast, then it will **proliferate** and **differentiate** into **specific** antibody-producing and secreting LBp, of the **IgM** type only. Although activation is independent of T cells, **cytokines** produced by T cells can amplify this response. In this case, there is little or no **immune memory** against this type of antigen.

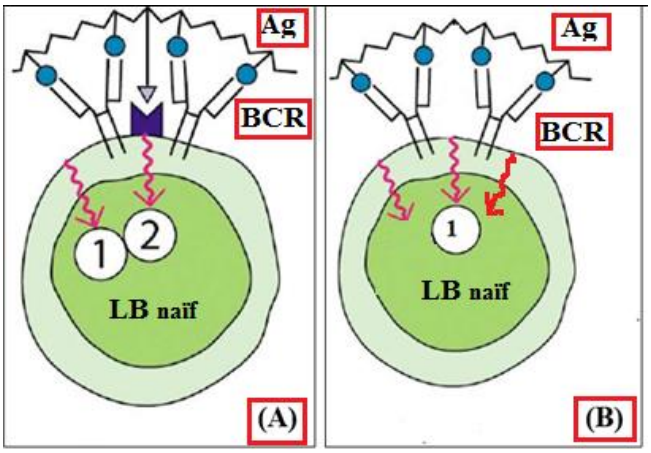


Fig. 13: Thymo-independent activation **1 (A)** and thymo-independent activation **2 (B)** of B lymphocytes.

II.2. Thyme-dependent activationof naif B cells

T-dependent antigens are antigens of a **protein** nature. Once recognized by specific naïve B lymphocytes, the activation, proliferation and differentiation of LB into plasma cells producing and secreting specific antibodies requires the **cooperation** of **CD4 T** lymphocytes. During this cooperation, the B lymphocyte and the T lymphocyte can recognize **different antigenic** determinants. Thymus-dependent activation is carried out in several stages.

II.2.1. Primary Follicular Phase

The naive B lymphocyte, at the level of the **primary follicle**, recognizes and fixes the native **antigenic determinant**, via its **BCR**. This link stimulates him and provides him with the **first activation signal**. It internalizes the antigen and then primes it. The immunogenic peptides obtained are associated with the MHC-II molecules and then, expressed on the surface of the **B lymphocyte** for those presented to the **Th2** lymphocyte (**TCD4 effector**) **(Fig.14)**.

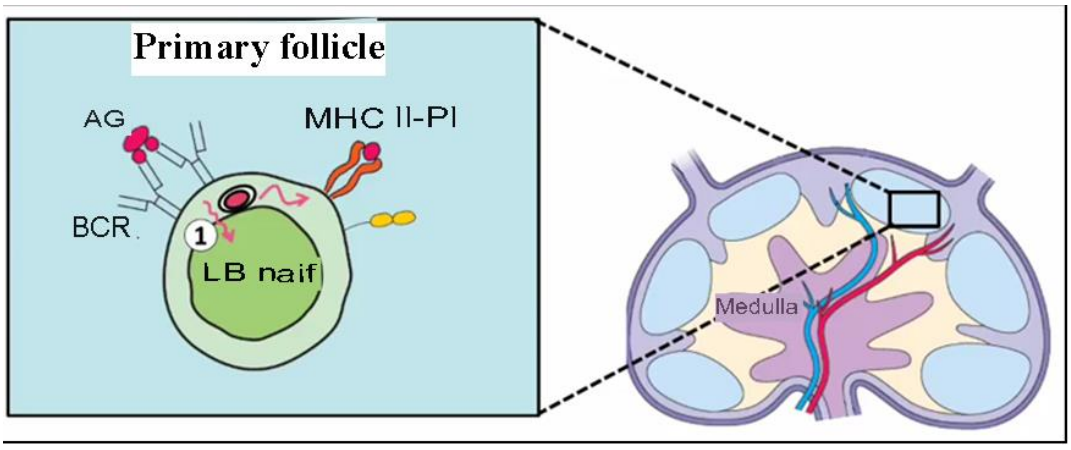


Fig. 14: First naive LB activation signal at the primary follicle.

II.2.2. Extra-follicular phase

The B lymphocytes having received the **first signal**, leave the primary follicle to reach the cortex-para-cortex junction of the lymph node (T Zone).

At the level of the T zone, the CPA (dendritic) cell presents the primed antigen to the TCD4 cell and activates it. The activated TCD4 lymphocyte divides and differentiates into effector or helper T4 cells (**Th2**) and memory T4 cells**,** under the action of **IL4**.

The **Th2** lymphocyte activates via its **TCR receptor, and CD4** which specifically recognizes the **immunogenic peptide** presented in association with the **MHC-II** molecules, on the surface of the **B lymphocytes** (having received the first signal), and come from the primary follicle (**1st signal of activation for LTh2**). Then, via the **CD28** receptor of **LTh2** which recognizes the **B7** molecules (CD80/86) of LB (**2nd signal for LTh2**) **(Fig.15)**.

Under the action of these two signals, the Th2 lymphocyte is activated. It then synthesizes and expresses on its surface the CD40 ligand (**CD40L**), and produces and secretes **lymphokines** (cytokines): **IL2, IL4, IL5, IL6, IL10, IL13 and TGF β**. It then triggers the activation of the **B lymphocyte** via the **CD40** receptor which will recognize the **CD40-L** ligand (**Second activation signal** for B). The LB thus activated expresses receptors for the lymphokines produced by the Th2 lymphocyte **(Fig.15)**. It transforms into a large **lymphoblast** (22µm in diameter).

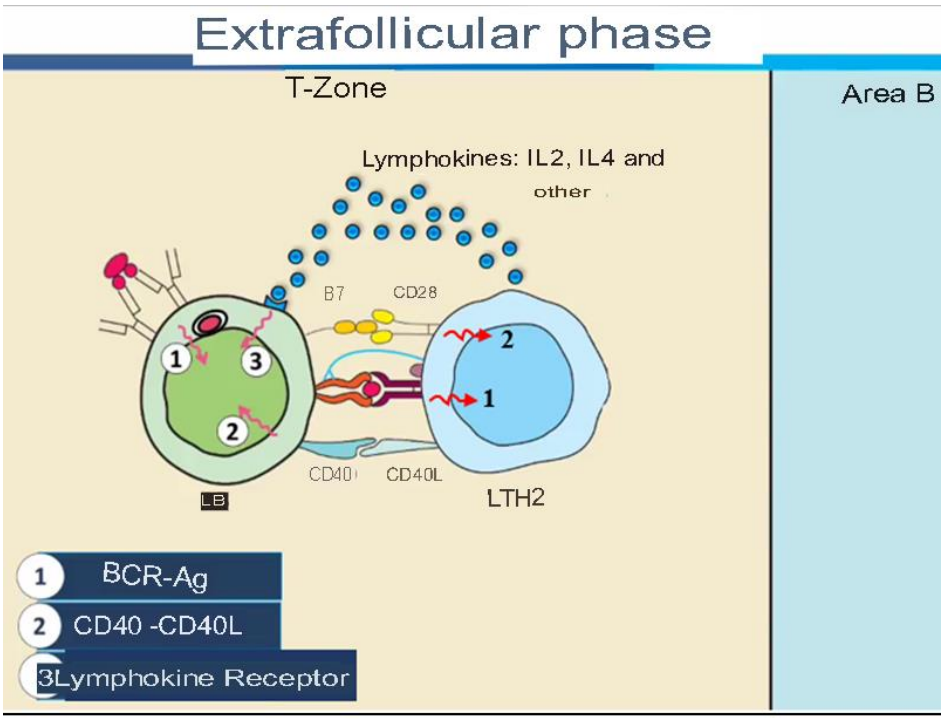


Fig. 15: B-cell activation signals.

Proliferation and differentiation of B lymphocytes in the extra follicular area

The B lymphoblast, and under the action of lymphokines produced by LTh2, divides a number of times to proliferate, the cells obtained **differentiate**: a **large part**, in short-lived plasma cells, producers and secretors of antibodies specific for the stimulating antigen. The first antibodies produced are **low affinity** IgMs and will be responsible for the **"primary"** antibody response. A **small part** of the activated B lymphocytes leaves the extrafollicular T zone to join the follicles again.

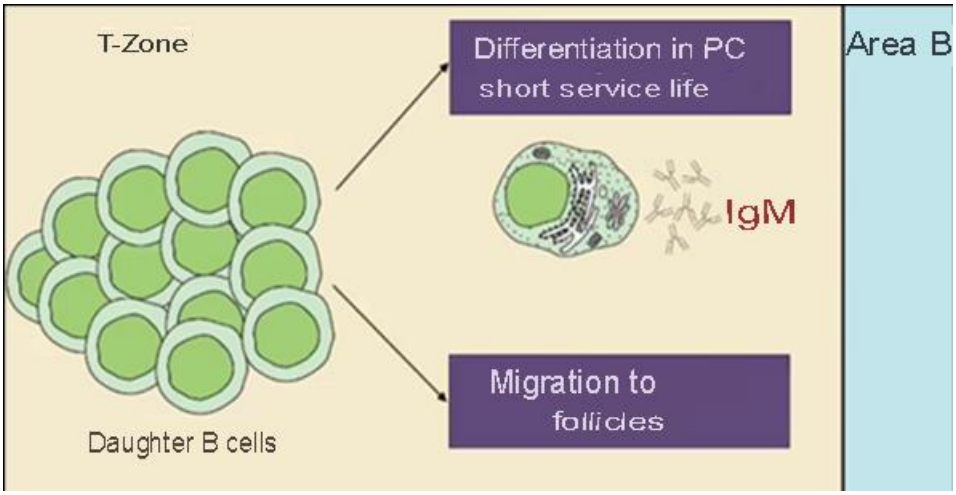


Fig.16: Differentiation of activated B cells in the extrafollicular area.

II.2.3. Secondary follicular phase

In the primary follicle, the daughter B cells from the extrafollicular T zone will proliferate at the marginal zone, forming a **germinal center**, this transforms the primary follicle into a **secondary follicle**. They are then called **centroblasts**.

In the **marginal (dark) area** of the germinal center, LBs (**centroblasts**) proliferate again. This intense proliferation is accompanied by **somatic hypermutation**, the purpose of which is to **have a maturation of affinity (increase the affinity of the BCR with respect to the specific Ag)**. Then they will migrate to the **central (clear) area** of the germinal center. At this level, these lymphocytes express their new surface immunoglobulins, which are called "**centrocytes**".

Centrocytes **(LB)** will undergo **affinity selection** by **follicular dendritic cells** that present them with native Ag (without having captured or primed it) attached to their membrane via complement receptors.

The B lymphocytes (centrocytes) which have received a **survival signal**, and which carry the same immunogenic peptide in association with the MHC-II molecules, on their surface, will present it to the LTFh **lymphocytes** (follicular helper T lymphocyte). They will activate again

via the **three activation signals,** in cooperation with **LTFh**, This will provide them with a survival **signal** and also a **switch** signal i.e. a **class switching (change of BCR isotype from IgM to IgG, IgA or IgE)** under the effect **of differentiation cytokines**.

Beyond that, the B lymphocytes will differentiate: a part differentiates into LBp producers of IgM with **high affinity** and **LBm with IgM**. And another part differs in LBp producing **another isotype** (IgG, IgA, IgE, ..) of antibodies according to the cytokines provided by the LTFh **and** in **LBm to IgG** or another class **(Fig. 17.18 and Table II).**

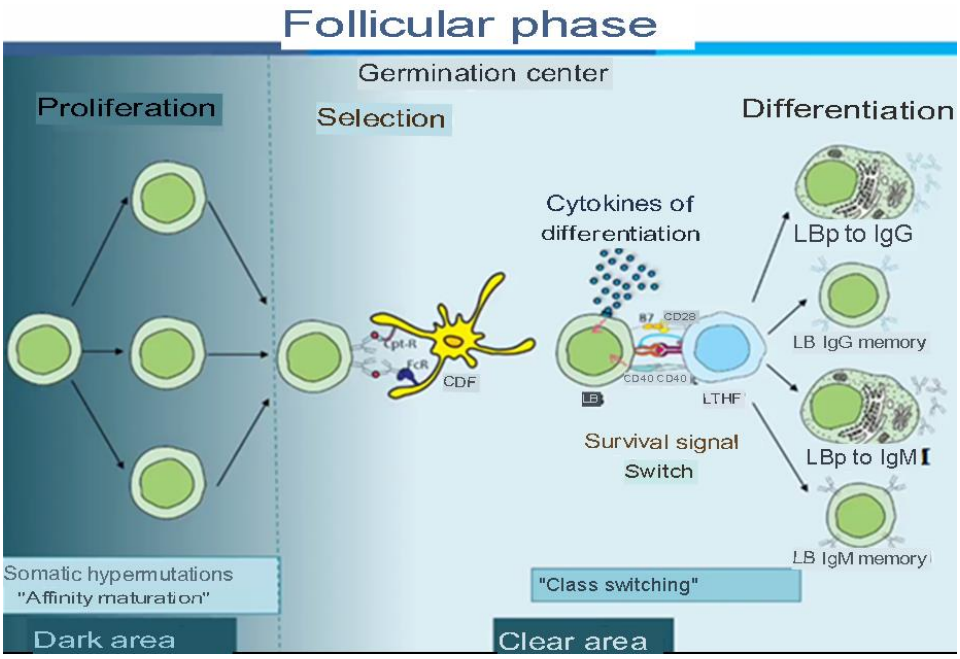


Fig. 17: proliferation and differentiation of B lymphocytes in the secondary follicle.

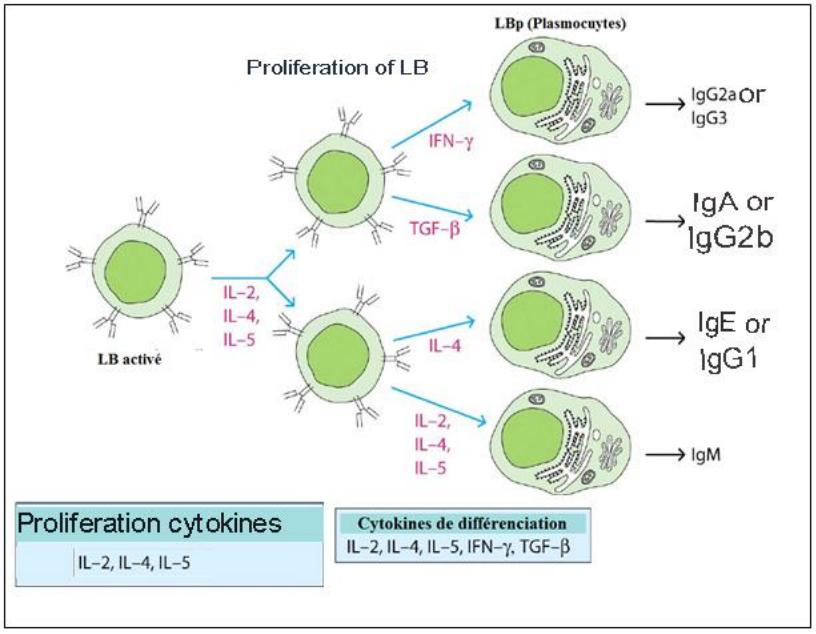


Fig. 18: Nature of cytokine-dependent secreted immunoglobulins present.

Table I: Nature of the immunoglobulins secreted according to the differentiation cytokines.

Cytokines	Source	Isotype
IL-4	LTh 2	IgE
IL-4, IL5 and IL-6	LTh 2	IgG1
IL-4 and IL-13	LTh 2	IgE and IgG4
IL-10	LTh 2	IgG1 and IgG3
IL-10 and TGF-β	LTh 2, LTh 3	IgA
IL-2 and IFN-γ	LTh 1	IgG2 and IgG3

II.3. Immunoglobulins

Immunoglobulins (Ig) are **membrane** or **soluble glycoproteins** in serum, extravascular fluids and secretions. They are able to recognize and react, via their **paratope**, specifically with a single antigenic determinant, or epitope. They are produced by B lymphocytes (**membrane immunoglobulins**) or by plasma cells (**soluble immunoglobulins**). The first are **BCRs**. While the latter are the soluble effectors of specific humoral immunity, endowed with **antibody** activity, they are of **five main classes**: **IgG**, **IgA**, **IgM**, **IgD** and **IgE**.

II.3.1. General structure of an immunoglobulin

An immunoglobulin consists of **four polypeptide chains**, two identical so-called **heavy chains (H: Heavy)** and two identical so-called **light chains (L: Light**). The two **heavy chains** are connected to each other by one or more **intercatenary disulfide bridges**. Each of the **light chains** is linked to a **heavy** chain by an intercatenary disulfide **bridge**. Each chain consists of a **constant part** on the **C-terminal** side and a **variable part** on the **N-terminal side.** Heavy chains have a short linear sequence, called a flexible **hinge region** that allows for the conformational changes necessary to perform effector functions. The heavy and light chains of the immunoglobulin molecule consist of **domains** of about **110 amino acids** stabilized by **intracatenary disulfide bridges** (Fig. 19).

a) Heavy chains

There are **five types** of heavy chains, designated by the Greek letters **γ (gamma)**, **α (alpha)**, **μ (mu)**, **δ (delta)**, and **ε (epsilon)** that define the **five classes** of immunoglobulins, **IgG**, **IgA**, **IgM**, **IgD**, and **IgE**, respectively. Some classes are divided into **subclasses** as for **IgG** (**IgG1 to IgG4**) and **IgA** (**IgA1** and **IgA2**). Heavy chains have **four domains** (CH1, CH2, CH3 and

VH) for IgD, IgG, IgA, or **five domains** for IgM and IgE, the additional domain is **CH4**. Knowing that, **CH** = **Constant heavy**, and **VH**= *Heavy variable* **(Fig. 19)**.

b) Light chains

There are **two types** of light chains, called **κ (kappa)** and **λ (lambda)** that can associate with any type of **heavy chain**. For a given immunoglobulin, the two light chains are always **identical**. Light chains have **two domains (CL=** *Constant light* **and VL=** *variable light***)**.

The VH-VL (*variable heavy-variable light*) combination constitutes the antibody binding site for the antigen or **paratope**. **Fab** is the association between the VH-VLCH1-CL domains. Each **immunoglobulin monomer** therefore has **two Fab fragments**. The constant part of the two associated heavy chains comprising the CH2-CH3 domains, or even CH4, constitutes the **Fc** fragment (Crystallizable Fragment), **(Fig. 19)**.

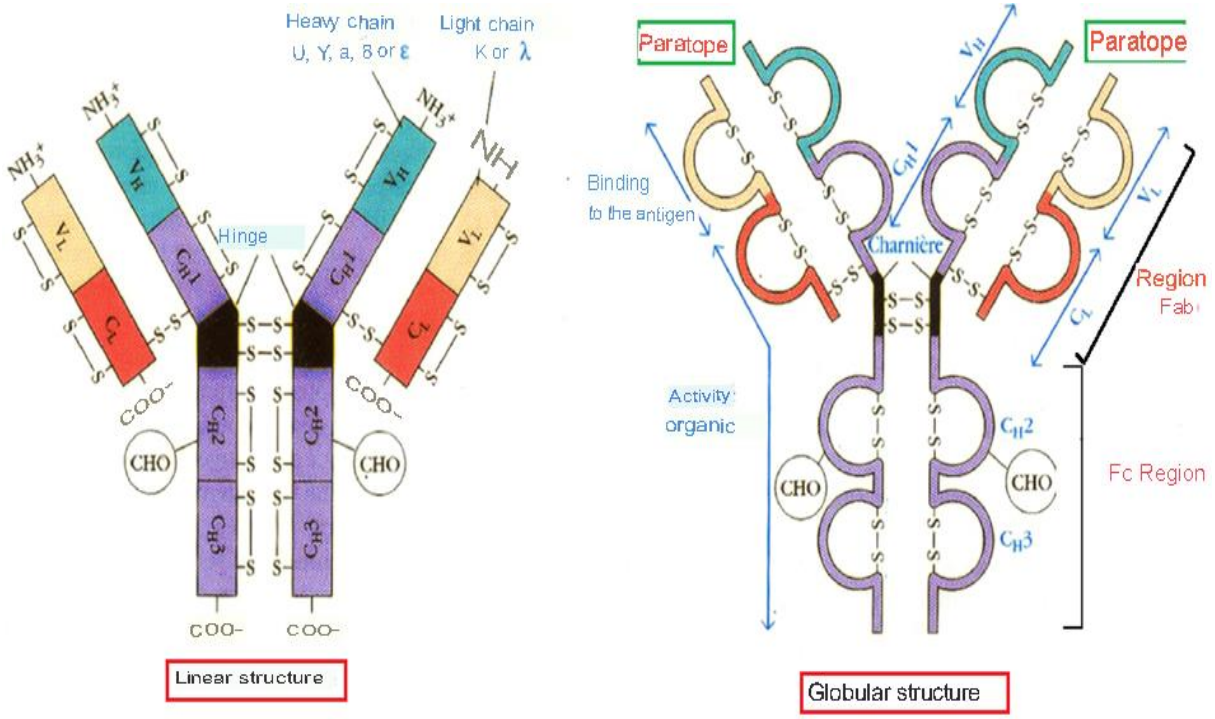


Fig 19: General structure of an immunoglobulin G: IgG1.

II.3.2. Enzymatic digestion of immunoglobulins

Enzymatic digestion of immunoglobulins by **papain** (in blue) cleaves the immunoglobulin into **3 fragments**: **2 Fab** fragments each fixing the antigen, formed of a light chain L, and half of a heavy chain H. And **1 Fc** fragment, not fixing the antigen, it comprises half of the two heavy chains. While digestion by **pepsin**, gives a fragment **F(ab')$_2$** (in brown), **(Fig. 20)**.

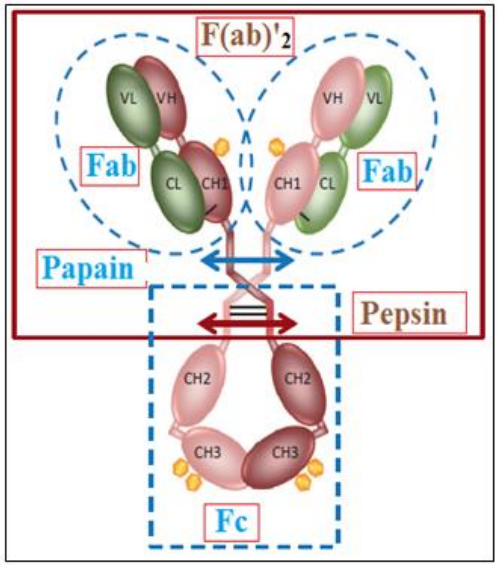


Fig.20: Enzymatic digestion of an immunoglobulin.

II.3.3. Classes and subclasses of immunoglobulins

1. Immunoglobulins G: IgG

IgGs are **monomeric** glycoproteins, consisting of two heavy chains (**γ: gamma**) (400-450 amino acids) and two light chains (**κ or λ**) (200-220 amino acids). They are predominantly present in serum, constituting **70 to 75%** of total circulating immunoglobulins, with a molecular weight (MW) of **150 KDa**. They are divided into **4 subclasses**; IgG 1, IgG 2, IgG 3 and IgG 4. They have a valence of **2 (2 paratopes**); the **valence** of the antibody refers to the number of antigenic determinants that each individual antibody molecule can bind.

IgG can cross the placenta (**IgG1, IgG4, IgG3 and weakly IgG2**), bind via the Fc fragment, complement **(IgG1, IgG2 and IgG3)** and phagocytic cells, neutralize viruses, toxins, and bacteria. Their shelf life is **21 days**, with the exception of IgG 3, which is 7 days (Fig. 21).

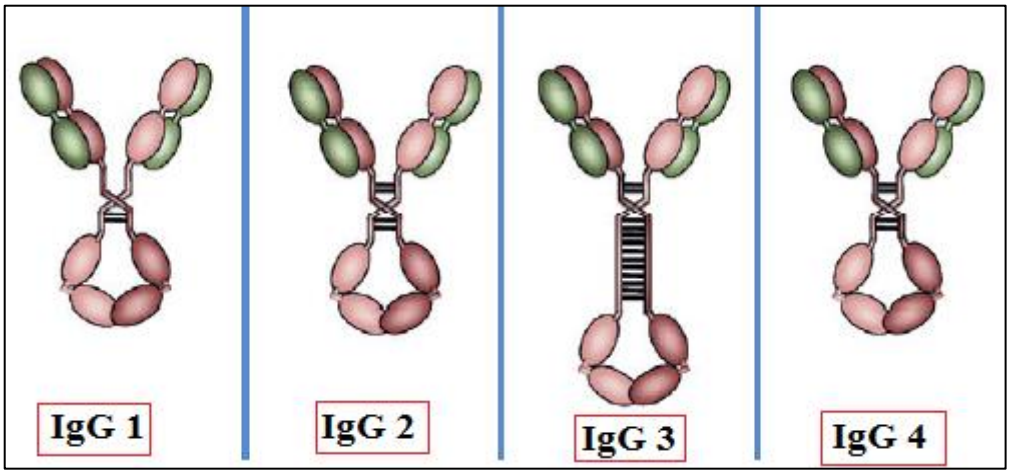


Fig. 21: IgG subclasses.

2. Immunoglobulin A: IgA

IgAs represent **15-20%** of total circulating immunoglobulins, bind via the Fc fragment to neutrophils. Their heavy chains **(α: alpha)** have 1 variable domain and 4 constant domains. They have a half-life of **6 days**. They have a role in agglutination, neutralization of bacteria, viruses. IgAs come in two forms, a **serum monomeric** form and a **secretory dimeric** form.

- **Serum IgA: IgA1** (93%) (α1), **IgA2** (7%) (α2), have a MW of **150 KDa to 160 KDa**. They have a **valence** of **2**.

- **Secretory IgA**: **IgA1 dimer** (40%) and **IgA2 dimer** (60%), have a MW of **380 KDa**. They are found in **secretions**; saliva, tears, breast milk, nasal, bronchial, genital and gastrointestinal secretions. The IgA1 or IgA2 dimers are joined together by a J-piece (MW of 15 KDa) and a glycoprotein **secretory component** (MW of 70 KDa). They have a **valence** of **4**.

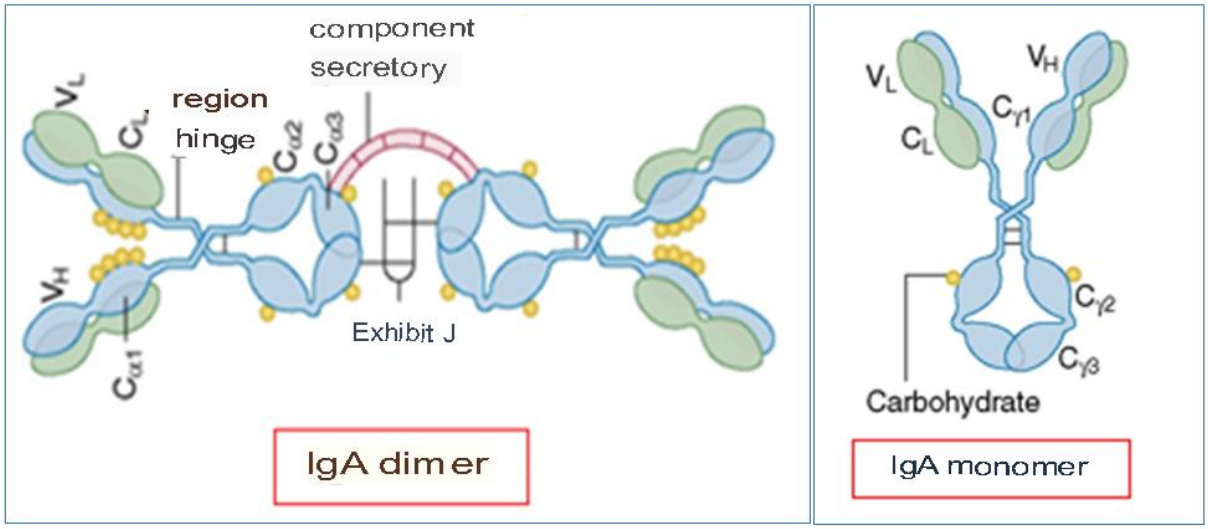


Fig. 22: Class of IgA. (a) Monomeric IgA. (b) Dimeric IgA.

3. Immunoglobulins M: IgM

They are star-shaped **pentamers**, with a MW of **900 KDa**. They have **10%** of the total circulating immunoglobulins, fix the complement via the Fc fragment, their heavy chains (**μ:mu)** comprise 5 domains: 1 variable and 4 constant (Fig. 23). They have a valence of **10**, have a half-life of **10 days**. IgMs are the first immunoglobulins to be produced by the fetus as well as the **first immunoglobulins** produced during a specific humoral response. These antibodies play a role in agglutination, and complement activation (classical pathway). Their **monomeric** form is present on the surface of naive B lymphocytes, acting as membrane receptors for the antigen (BCR). The BCR has a **valence** of **2**.

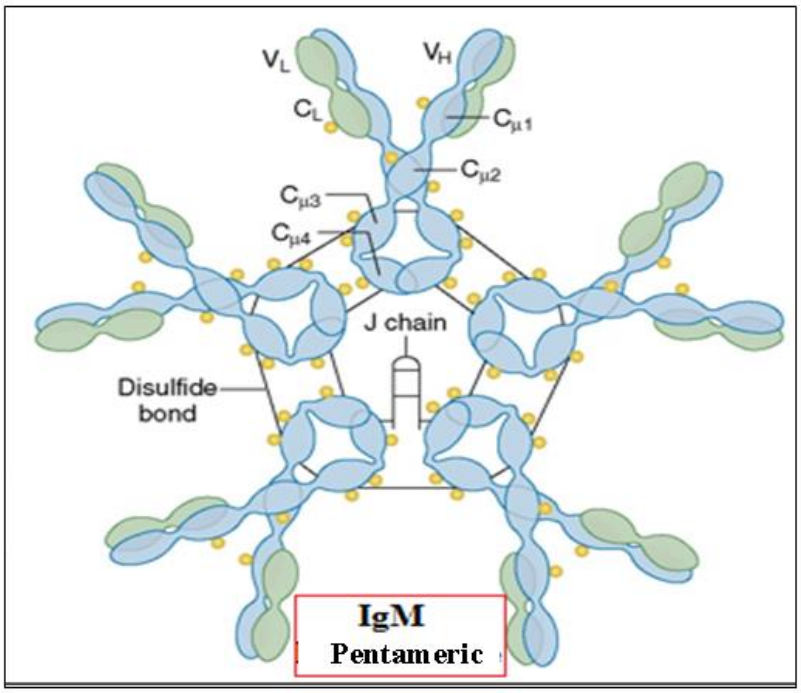


Fig. 23: Structure of a pentameric IgM.

4. Immunoglobulins E: IgE

IgE is **monomeric**, represents **0.1%** of total circulating immunoglobulins, has a MW of **190 KDa**. Their valence is **2**. Their heavy chains **(ε: epsilon)** have 1 variable domain and 4 constant domains. They bind, via the Fc fragment, to **mast cells** and **basophils** (allergies). Their half-life is **2 days**. They are involved in allergies and the neutralization of parasites **(Fig. 24)**.

5. Immunoglobulins D: IgD

IgD is **monomeric**, representing **0.5%** of total circulating immunoglobulins. Their MP is **170 KDa.** They are also present on the surface of naive B lymphocytes, associated with monomeric IgMs where they act as antigen receptors. Their half-life is **3 days**. They have a valence of **2**. Their heavy chains **(δ: delta)** have 1 variable domain and 3 constant domains **(Fig. 24)**.

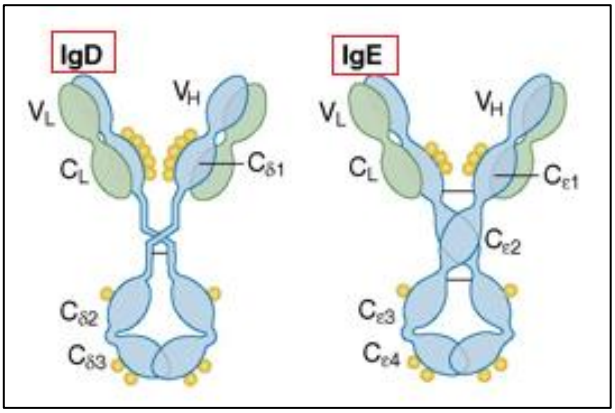


Fig. 24: Structure of IgD and IgE.

II.3.4. Variability of antibodies

The different forms of immunoglobulin variability are shown in Fig. 25. There are 3 types of goods:

a) Isotypic variation

The isotypic variation concerns the **five types of immunoglobulin heavy chains**, the structure of an **isotype** in amino acids is specific to each species.

b) Allotypic variation

Allotypic variation concerns **a few amino acids**, accounts for genetic variations (polymorphism) within the **same species** and most often involves domains of the **constant region** of **heavy chains**. A given **allotype** is therefore found for a subgroup of individuals in the **same species**.

c) Idiotypic variation

It concerns the modifications of the amino acid sequence of the **Fab** fragment at the **hypervariable** zones of the variable domains of the heavy and light chains. This region is directly responsible for the specificity of the **paratope**, determine the existence of idiotypes related to the VDJ and VJ rearrangements of the immunoglobulin genes occurring during the **maturation** of B lymphocytes in the bone marrow.

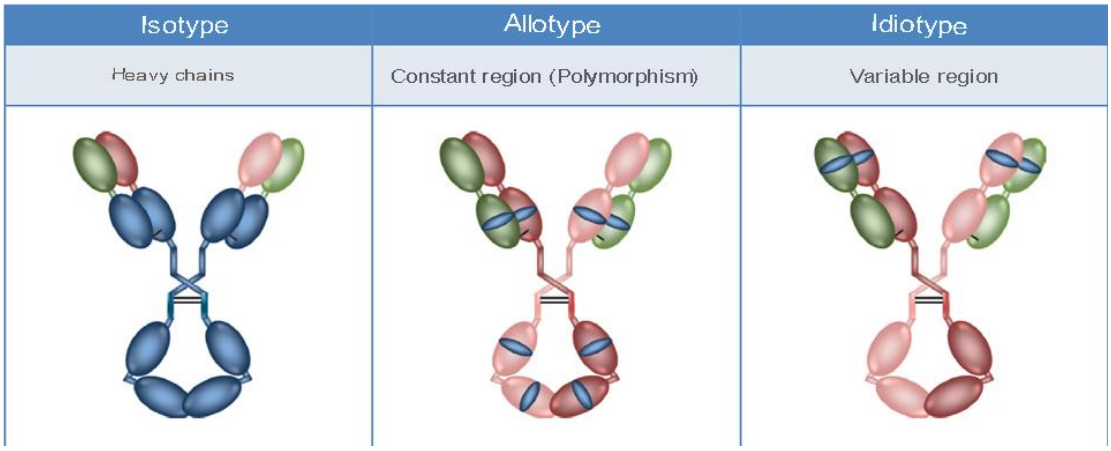


Fig. 25: Variability of antibodies, the sites of variability are shown in blue.

II.3.5. Reaction: antigen-antibody

Binding of the antibody to the specific antigen leads to the formation of an **immune complex**. This binding requires structural **complementarity** between the **paratope** of the antibody and the **epitope** of the antigen. This complementarity promotes the formation of attractive forces

between them. These forces are **non-covalent** forces of **4 types**: Hydrogen bonds, Hydrophobic forces, Electrostatic forces (Ionic forces) and VAN DER WAALS forces. The **immune complexes** thus formed are of two forms; **precipitation** in the case of **soluble Ag** and **agglutination** in the case of **cellular Ag.**

II.3.6. Effector functions of immunoglobulins

1. Function of membrane immunoglobulins

These are the monomeric IgMs and IgDs, on the surface of the B lymphocytes. These are the **BCRs** playing the role of **specific recognition of the antigen** and its **binding**, by facilitating their **internalization** inside the BLs to be primed into immunogenic peptides, which will present in association with the MHC-II molecules, in order to activate the Th2 lymphocytes.

2. *Function of circulating immunoglobulins*

Circulating immunoglobulins, or **antibodies**, provide various effector functions. In general, for an effector function to be implemented, the antibody must bind to the specific antigen, forming an **immune complex**. Not all immunoglobulins have all the effector functions (Fig.26).

a. Neutralization

Antibodies, by binding to microbes and bacterial toxins (toxins from diphtheria and tetanus bacilli) or viruses, **block** or **neutralize** the **infectivity** of microbes and the interactions of microbial toxins with host cells. The antibodies responsible are **IgG**, **IgM** and **IgA**.

b. Opsonization and phagocytosis

Antibodies **cover** soluble microorganisms or antigens, promoting their ingestion by **phagocytes** (the effect is multiplied by 1000). The process of coating particles to promote subsequent phagocytosis is called **opsonization**, and the molecules (here are the antibodies) that coat the antigens and promote their phagocytosis are called **opsonins**. The antibodies responsible are **IgG** and **IgA**. The Fc fragment binds to its high affinity **receptor** expressed on the surface of **neutrophils** and **macrophages**, thereby facilitating phagocytosis.

Opsonization can also be carried out by **C4b** and or **C3b** of the Ag – IgG complexes generated from complement activation and therefore increased phagocytosis.

c. Complement-dependent cytotoxicity (CDC)

Antibodies bound to their **cellular antigen** can activate the **classical complement pathway**. If the activation of this system is completed, the formation of the **membrane attack complex**

leads to the **lysis of the cell** to which the antibodies are initially fixed. The antibodies responsible are: **IgG1, 2, 3, and IgM**.

d. Antibody-dependent cytotoxicity (ADCC)

A cell **infected with a virus**, **tumor** or **foreign**, expresses antigenic proteins on its surface. The latter are recognized by **specific antibodies**. The Fc fragment of these antibodies can then interact with the IgG **Fc** fragment (**CD16**) **receptors** present on **NK** cells. NK lymphocytes then activate and induce apoptosis of the target cell via perforins / granzymes and the Fas death receptor. This process is called *Antibody Dependent Cell-mediated Cytotoxicity* (ADCC).

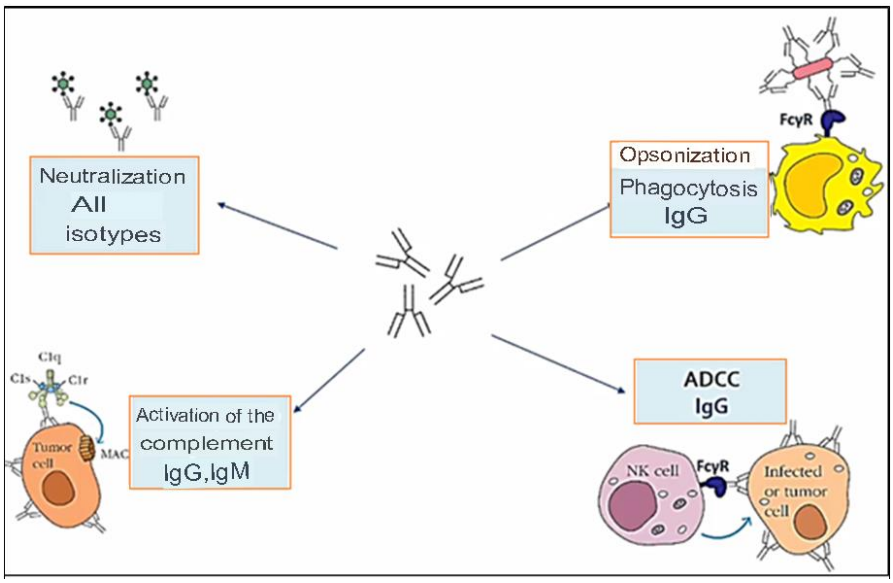


Fig. 26: Main functions of antibodies.

III. Primary and secondary humoral response

III.1. Primary humoral response

This response is triggered at the **first contact** between the antigen and the host. During the injection of an antigen, the evolution of the serum level of antibody produced against this antigen and the development of the **primary response** is done in 4 stages.

1. **latency phase**: corresponds to the time required for the recognition of the antigen by the specific lymphocytes, which will divide and differentiate into plasma cells. It is long. During this phase, antibodies are not detected in the serum.

2. **growth phase**: corresponds to the production and secretion of antibodies. This leads to a gradual (exponential) increase in serum antibody levels. The first antibodies produced are **IgM**.

3. **Platelet phase**: corresponds to the maximum production of serum antibodies.

4. Decay phase: corresponds to the binding of antibodies to antigens, therefore to the formation of immune complexes, which explains the decrease in serum antibody levels.

III.2. Secondary humoral response

The secondary response corresponds to the 2nd or nth administration of the same antigen, when the specific serum antibodies become zero. This response is characterized by; A much **shorter** latency period due to the presence of memory T and B lymphocytes, a faster increase in antibody levels, a maximum serum antibody level more than 10 times higher and persisting longer, and a much **slower** decrease in antibody levels allowing for residual antibody levels for long periods of time. The antibodies produced are of **better affinity** and almost entirely of the **IgG** class or another class (Fig. 27).

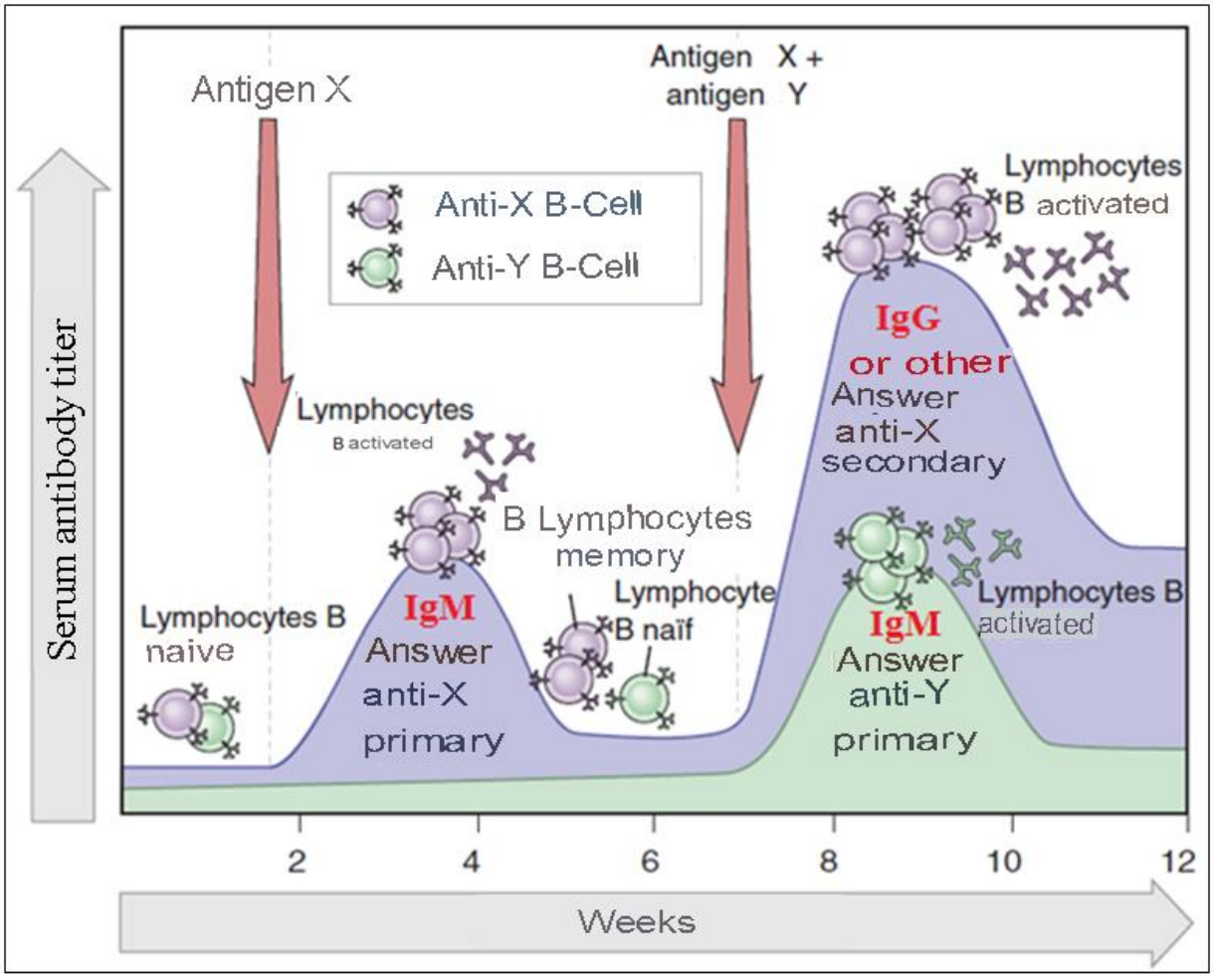


Fig. 27 : Primary and secondary immune responses. Antigens X and Y induce the production of different antibodies (specificity). The secondary response to antigen X is faster and more significant than the primary (memory) response, and it is different from the primary response to antigen Y (again reflecting specificity).

IV. Vaccination.

Vaccination is the process of **stimulating protective adaptive** immune responses against microorganisms by exposing the individual to non-pathogenic forms or components of the microorganisms. The active substance of a vaccine is an immunogen. Vaccination can be **prophylactic**, and therefore preventive of infection, or **therapeutic (curative)** for the treatment of chronically infected patients with cancers of autoimmune or infectious pathologies. Depending on the type of immune mechanisms it involves, vaccination can prevent infection by a pathogen or prevent the expression of clinical signs, and therefore of the disease.

IV.1. Prophylactic vaccines

There are currently three types of vaccines: live attenuated, inactivated, and purified vaccine antigens (infectious agent subunits and toxoids).

IV.1.1. Live attenuated vaccines

They are the best. They are generally obtained by successive passages of the infectious agent on cell cultures aimed at attenuating its virulence. These vaccines have the advantage of inducing **innate** immunity and an **adaptive** humoral and cellular response. The vaccine, being alive, is able to spread in the body, multiply and induce responses in different anatomical sites.

The major problems with these vaccines are the risk of **returning to** and **transmitting** from one individual to another when the recipient is immunocompromised.

IV.1.2. Inactivated vaccines

These are whole infectious agents **inactivated** by physical methods like heat. These vaccines are generally very well tolerated. However, the use of adjuvants to increase their effectiveness can pose tolerance problems. These inert agents do not diffuse. They induce an essentially **antibody-like** response, associated with a **CD4 T** response necessary for the B response to be optimal.

IV.1.3. Purified vaccine antigens

Vaccine antigens can be **proteins** responsible for pathogen activity (tetanus and diphtheria toxins), **inactivated** before administration (**toxoids**) but having the same **immunogenicity**. They may also be **proteins targeted** by protective antibodies (hepatitis B). The response to this type of vaccine is mainly of the **antibody** type. Some vaccine antigens require protein coupling to increase their immunogenicity. Thus, pneumococcal **polysaccharides** can directly stimulate B

lymphocytes in the spleen and induce the production of **IgM-type** antibodies. This type of vaccine does not induce a memory response (Pneumovax® vaccine). The coupling of polysaccharides to inactivated diphtheria toxoid makes it possible to obtain, on the other hand, both an **IgG-type** antibody response thanks to CD4 T lymphocytes stimulated by dendritic cells and a memory-type **B** response (Prevenar® 7 or 13 vaccine).

IV.2. Therapeutic vaccines or Serotherapy

Serotherapy is a technique for the **passive** transfer of **humoral** immunity, aimed at conferring **immediate curative**, but **transient** protection, pending the establishment of acquired immunity of vaccine origin. Preparations used for serotherapy are enriched with **antibodies** directed against a particular pathogen. They are obtained from animals or individuals hyper-immunized against the pathogen in question (inoculation of bacterial preparation or inactivated toxins).

Serotherapy with **animal-**derived antibodies can exceptionally cause **allergic** reactions. The use of purified antibodies (not serum) improved tolerance to these preparations. Specific immunoglobulins are usually administered **intramuscularly**.

The use of specific purified immunoglobulins of **human** origin has significantly reduced the frequency of allergic accidents caused by immunoglobulins of animal origin. Human-specific immunoglobulins are available for HBV (hepatitis B), rabies, and tetanus.

Note:

During the first months of life, the fetus as well as the newborn is protected by maternal antibodies, acquired by **placental transfer** (IgG), for the fetus and by intestinal absorption of **IgA** from milk, for the newborn. **These maternal antibodies** provide passive immunization to the fetus and newborn.

Chapter 07

Cellular-humoral Cooperation

I. Cooperation Between Different Immune Cells

The immune response, whether innate or adaptive, results from interactions and cooperation among various immune cells. These interactions occur through **immunological synapses** (receptor–ligand binding) and/or **cytokines** (mediator–receptor signaling) (Fig. 01).

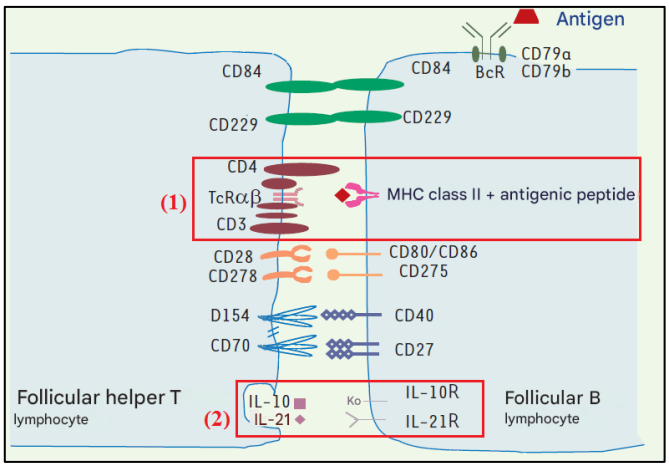


Fig. 1. Cooperation between a follicular helper T lymphocyte and a follicular B lymphocyte through the formation of (1) immunological synapses and (2) cytokine-mediated signaling.

I.1. Immunological Synapses

These are physical contacts based on receptor–ligand interactions, in which adhesion molecules (e.g., LFA1–ICAM1) play a crucial role. The formation of immunological synapses delivers either activating or inhibitory signals to the receptor-bearing cell, which subsequently adjusts its activity (Fig. 02).

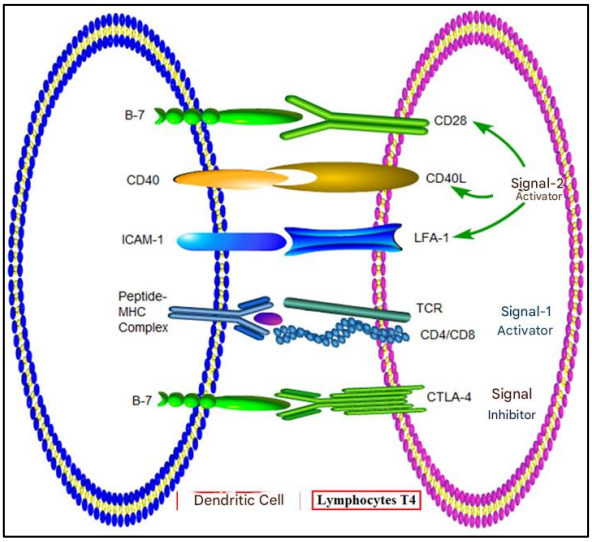


Fig. 02. Cellular cooperation between a $CD4^+$ T lymphocyte and a dendritic cell through immunological synapses.

I.2. Mediator–Receptor Interactions

These involve soluble factors, generally produced by immune cells, that act on the same cell (autocrine), a neighboring cell (paracrine), or distant cells via the bloodstream (endocrine). An example is **IL-2**, produced by $CD4^+$ T cells, which may act on the same T cell or on adjacent activated $CD8^+$ T cells. When IL-2 is secreted by Th1 lymphocytes, it may also act on activated B cells at a distance without direct contact. This type of cooperation is based on mediator–receptor interactions (Fig. 01), where the mediator is a cytokine (e.g., IL-2 binding to the IL-2 receptor).

Table I. Examples of immune cell cooperation mediated by cytokines in innate and adaptive immunity.

Source cells	Cytokines	Target cells	Functional outcome
Stromal cells of bone marrow	SCF, IL-3, IL-7	Common lymphoid progenitors	Ontogenesis of B and T lymphocytes
Macrophages	IL-8, TNF-α	Endothelial cells	Neutrophil diapedesis
Mast cells, macrophages, dendritic cells	IL-6, TNF-α	Bone marrow stromal cells, macrophages	CSF production (colony-stimulating factors)
Antigen-presenting cells (dendritic cells)	IL-12, IFN-γ	Activated $CD4^+$ T cells	Differentiation into Th1
Th1 cells	IFN-γ, TNF-α	Macrophages	Amplification of macrophage activity
Antigen-presenting cells (dendritic cells)	IL-4, TGF-β	Activated $CD4^+$ T cells	Differentiation into Th2
Endothelial cells of afferent lymphatic vessels	CCL21	Mature dendritic cells	Migration to lymph nodes (homing)
Activated $CD4^+$ T cells	IL-2	Activated $CD4^+$ T cells	Proliferation
Activated $CD8^+$ and $CD4^+$ T cells	IL-2	Activated $CD8^+$ T cells	Differentiation into cytotoxic T lymphocytes
Th2 / Tfh cells	IL-2, IL-4, IL-5	Activated B cells	Proliferation
Th2 / Tfh cells	IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, TGF-β	Activated B cells	Differentiation into plasma cells

II. Cytokines

The term *cytokine* derives from the Greek *cyto* (“cell”) and *kinos* (“movement”). Cytokines are **low-molecular-weight glycoproteins** (8–80 kDa), either soluble or membrane-bound, that me-

diate communication among cells involved in physiological and pathological immune processes. They belong to the extracellular signaling network that regulates innate and adaptive immune functions. Cytokines act in autocrine, paracrine, or endocrine fashions, display pleiotropic effects, and may induce diverse outcomes depending on the target cell. Overall, cytokines exert immunomodulatory functions.

Cytokines are classified into **seven categories** based on their functions or the types of cells capable of secreting them (Table II). The profile of cytokine secretion depends on the type of immune response initiated.

Table II. Cytokine classes.

Type	Abbreviation	Examples
Interleukins	IL	IL-1 to IL-38
Interferons	IFN	Type I IFNs (α, β, …), Type II IFN (γ), Type III IFNs (λ)
Chemokines	–	CCL1–28, CXCL1–17, XCL1–2, CX3CL1
Colony-stimulating factors	CSF	M-CSF, G-CSF
Tumor necrosis factors	TNF	TNF-α, TNF-β
Transforming growth factors	TGF	TGF-α, TGF-β
Growth factors	GF	NGF, EGF, VEGF

CCL = CC chemokine ligand; CXCL = CXC chemokine ligand; M-CSF = Macrophage colony-stimulating factor; G-CSF = Granulocyte colony-stimulating factor; NGF = Nerve growth factor; EGF = Epidermal growth factor; VEGF = Vascular endothelial growth factor; TNF = Tumor necrosis factor; TGF = Transforming growth factor.

- A cytokine may be produced by multiple cell types. For example, **IL-6** is secreted not only by hematopoietic cells (lymphocytes, monocytes/macrophages, dendritic cells) but also by fibroblasts, endothelial cells, and epithelial cells (Fig. 03).
- Conversely, a single immune cell can produce numerous cytokines. For example, Th1 lymphocytes secrete IFN-γ, IL-2, IL-3, IL-20, IL-21, TNF-α, FasL, CD40L, CXCL2, GM-CSF, among others (Fig. 04).

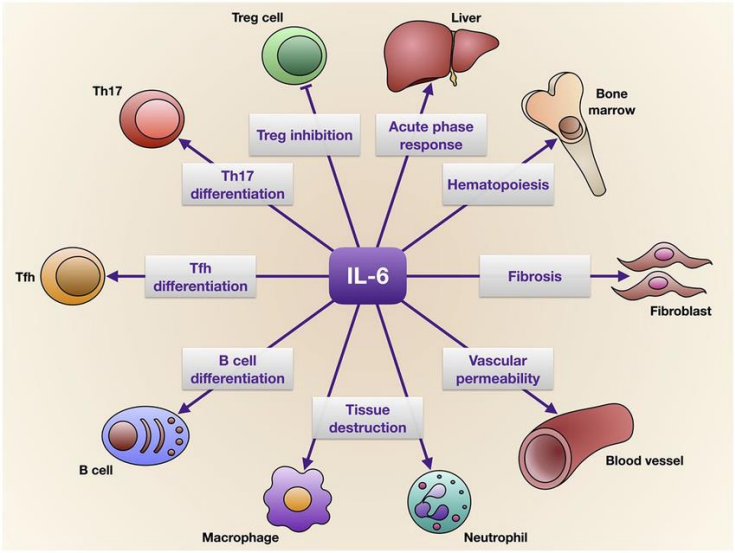


Fig. 03. Pleiotropic action of cytokines: example of IL-6.

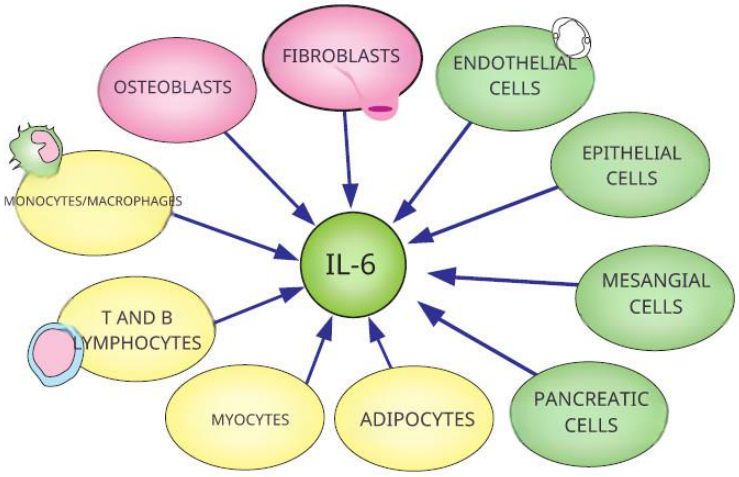


Fig. 04. A single cytokine may be secreted by multiple cell types: example of IL-6.

II.1. Interleukins

Interleukins are secreted by leukocytes. Those produced by lymphocytes are termed **lymphokines**, whereas those secreted by monocytes or macrophages are called **monokines**. To date, 40 interleukins have been identified (IL-1 to IL-38). They can be classified as **pro-inflammatory** (e.g., IL-1, IL-6, IL-17, IL-22) or **anti-inflammatory** (e.g., IL-1RA, IL-4, IL-10, IL-11, IL-13).

II.1.1. Interleukin-1 (IL-1)

IL-1 is a pro-inflammatory cytokine secreted by mast cells, macrophages, fibroblasts, and endothelial cells. It induces the expression of adhesion molecules on vascular endothelium, thereby promoting the migration of circulating leukocytes to the inflamed site, as well as lymphocyte activation.

II.1.2. Interleukin-2 (IL-2)

IL-2 is a pivotal cytokine in both cellular and humoral immunity. It stimulates the activation and proliferation of $CD4^+$ and $CD8^+$ T lymphocytes, enhances the cytotoxic activity of T cells, increases IFN-α production, induces natural killer (NK) cell activation, and promotes the differentiation of B lymphocytes into immunoglobulin-secreting plasma cells. Its receptor is composed of three subunits: **CD25, CD122, and CD132**.

II.1.3. Interleukin-4 (IL-4)

IL-4 has both pro- and anti-inflammatory properties. Produced by NKT cells, it drives the differentiation of $CD4^+$ T lymphocytes into **Th2 helper cells** and promotes B-cell differentiation into plasma cells. IL-4 is also secreted by Th2 lymphocytes.

II.1.4. Interleukin-6 (IL-6)

IL-6 is a pro-inflammatory cytokine secreted by phagocytes (macrophages and dendritic cells), mast cells, fibroblasts, and endothelial cells. It enhances phagocytosis, promotes monocyte recruitment to inflamed tissues, and induces the production of acute-phase proteins by hepatocytes.

II.1.5. Interleukin-8 (IL-8)

IL-8 is a pro-inflammatory chemotactic cytokine produced by monocytes, endothelial cells, fibroblasts, and T lymphocytes. It primarily mediates neutrophil attraction, adhesion, and activation, enhancing their enzymatic and phagocytic activities.

II.1.6. Interleukin-10 (IL-10)

IL-10 is an anti-inflammatory cytokine that regulates inflammatory responses. It is mainly produced by regulatory T cells (Treg, formerly iThreg).

II.2. Chemokines

Chemokines are cytokines with molecular weights ranging from 7 to 15 kDa, responsible for directing leukocyte chemotaxis. They are generally considered pro-inflammatory, and their release is often induced during immune responses. Chemokines are divided into **four main families** based on the spacing of cysteine residues at the N-terminal region:

- **C (γ family)**: contain a single cysteine, officially designated as **XCL** (ligand).
- **CC (β family)**: have two adjacent N-terminal cysteines, designated **CCL**.

- **CXC (α family)**: contain two cysteines separated by one amino acid, designated **CXCL**.
- **CX_3C (δ family)**: contain two cysteines separated by three amino acids, designated **CX_3CL**.

Table III. Some chemokines, their receptors, and their source and target cells.

Chemokine	Receptor	Producing Cells	Target/Responsive Cells
CCL2 (MCP-1)	CCR2	Monocytes, endothelial cells, fibroblasts	Monocytes, memory T cells
CCL3 (MIP-1α)	CCR1, CCR5	Macrophages, dendritic cells, T cells	Monocytes, T cells, NK cells
CCL5 (RANTES)	CCR1, CCR3, CCR5	T cells, platelets, endothelial cells	T cells, eosinophils, basophils
CXCL8 (IL-8)	CXCR1, CXCR2	Macrophages, epithelial cells, endothelial cells	Neutrophils
CXCL10 (IP-10)	CXCR3	Monocytes, endothelial cells, fibroblasts	T cells, NK cells
CXCL12 (SDF-1)	CXCR4	Stromal cells, endothelial cells	Hematopoietic stem cells, T cells, B cells
CX3CL1 (Fractalkine)	CX3CR1	Endothelial cells, neurons	Monocytes, NK cells, T cells

II.3. Interferons (IFNs)

Interferons are cytokines originally defined by their ability to interfere with viral replication. They are classified into three types:

- **Type I IFNs**: including IFN-α, IFN-β, IFN-ω, IFN-τ, IFN-δ, IFN-ε, IFN-κ, IFN-ζ. These play a critical role in innate immunity and are produced by both immune and non-immune cells (e.g., epithelial cells).
- **Type II IFN**: represented solely by IFN-γ, produced exclusively by immune cells (B cells, T cells, NKT cells) during adaptive immune responses.
- **Type III IFNs**: including IFN-λ1, IFN-λ2, and IFN-λ3.

II.4. Colony-Stimulating Factors (CSFs)

CSFs stimulate cell proliferation and play a central role in hematopoiesis. For example:

- **G-CSF (granulocyte-CSF)** specifically induces the proliferation and differentiation of stem cells into granulocytes.

- **M-CSF (macrophage-CSF)** drives the differentiation of progenitors into macrophages.

II.5. Tumor Necrosis Factors (TNFs)

TNFs are cytokines involved in systemic inflammation and acute-phase responses. Two main forms exist: **TNF-α** and **TNF-β**.

- **TNF-α** is secreted by macrophages, resident dendritic cells, and mast cells (the latter store pre-formed TNF-α in granules). Its roles include:

- Induction of adhesion molecule expression and chemokine production by endothelial cells, thereby recruiting leukocytes (neutrophils, eosinophils, monocytes, NK cells) to inflammatory sites.
- Activation of the microbicidal systems of phagocytes.
- Acting as a mitogen for T and B lymphocytes, supporting adaptive immune responses when innate immunity is insufficient.
- Stimulation of growth factor production, essential for tissue repair.

II.6. Transforming Growth Factors (TGFs)

TGFs are cytokines grouped into two categories:

- **TGF-α**, produced primarily by macrophages and keratinocytes, functions as a cell growth factor.
- **TGF-β** (isoforms TGF-β1, β2, β3) exerts anti-inflammatory effects and pleiotropic actions on adaptive immunity, notably regulating effector and regulatory $CD4^+$ T-cell responses. It also contributes to the differentiation of activated B cells.

II.7. Growth Factors

These cytokines regulate the formation and maintenance of connective tissues. Key examples include:

- **NGF** (nerve growth factor),
- **EGF** (epidermal growth factor), and
- **VEGF** (vascular endothelial growth factor), which promotes angiogenesis, i.e., the formation of new blood vessels.

Chapter 08

Immune Dysfunction

I. General Considerations

Under certain circumstances, the immune system may enter a state of immunosuppression or insufficiency, or act in such a way that it damages the host itself. Most of these problems arise from immunodeficiencies, autoimmune diseases, or hypersensitivities.

II. Autoimmunity

Autoimmunity refers to a breakdown (loss) of self-tolerance. As the term indicates, it is characterized by the production, by the host's adaptive immune system, of cellular effectors (T lymphocytes) and/or antibodies directed specifically against self-antigens. Two forms of tolerance exist: central and peripheral.

- **Central tolerance:** mediated by the negative selection of T and B lymphocytes with high affinity for self-peptides.
- **Peripheral tolerance:** maintained through several mechanisms:
  - **Indifference or ignorance:** not all self-cells act as professional antigen-presenting cells, which prevents activation of autoreactive T lymphocytes.
  - **Anergy:** functional inactivation of T cells that occurs when these cells recognize antigens in the absence of adequate costimulatory signals necessary for full activation.
  - **Deletion:** recognition of self-antigens may trigger signaling pathways leading to apoptosis, thereby eliminating autoreactive lymphocytes (activation-induced cell death).
  - **Immunoregulation:** mediated by regulatory T cells (Tregs), primarily through IL-10 and TGF-β.

II.1. Hypothetical Mechanisms of Autoimmunity Induction

The activation of autoreactive T lymphocytes requires the same steps as for T cells specific to foreign antigens:

- **Signal 1:** presentation of the antigenic peptide by an HLA molecule.
- **Signal 2:** expression of costimulatory molecules (CD80/86 and CD40) and the presence of soluble messengers such as cytokines.

In resting tissues, inflammation is absent; thus, costimulatory signals are lacking, and T lymphocytes recognizing HLA-peptide complexes are inactivated. However, inflammation can provide the second signal, leading to the activation of autoreactive T cells. Autoimmune diseases may result from several factors:

- Incomplete deletion of autoreactive "dangerous" lymphocytes in the thymus.
- Peripheral failure of Tregs.
- Mistaken recognition due to structural similarity between self and non-self (antigenic mimicry or cross-reactivity).
- Excessive or inappropriate presentation of self-antigens in an inflammatory context.
- Presentation of normally sequestered self-antigens.
- Modified self-antigens (e.g., induced by viruses or drugs).
- Other mechanisms, such as impaired clearance of immune complexes or apoptotic bodies.

II.1.1. Possible Causes of Inflammation

Target organ inflammation can originate from diverse causes. Microbial or viral infection may provide "danger signals" to innate immune cells, favoring the activation of autoreactive T lymphocytes alongside pathogen-specific T cells. Hence, the "immunological history" of patients (memory of past infections) is crucial in the onset of autoimmune disease.

For example, in rheumatoid arthritis, microbial fragments have been detected in the synovial fluid of inflamed joints. Similarly, pro-inflammatory or toxic products can trigger cytokine secretion and aberrant expression of costimulatory molecules by tissue antigen-presenting cells (APCs), enabling activation of autoreactive T cells. Poorly controlled inflammation therefore increases the likelihood of autoreactive T cell activation over time.

II.1.2. Microbial Antigen-Induced Autoimmunity

This refers to the concept of **molecular mimicry** or cross-reactivity. Some microbial antigens share structural motifs with self-antigens, thereby triggering activation of autoreactive cells. During infection by a bacterium, virus, or parasite expressing antigens similar to those of the host, the immune response may destroy both the pathogen and host cells.

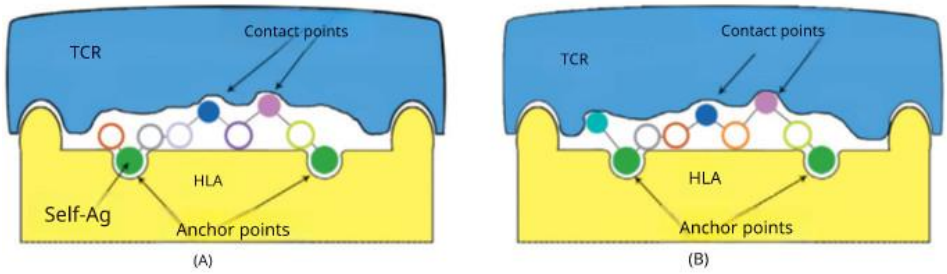


Fig. 01: Molecular mimicry between a self-peptide and a microbial peptide

The TCR may mistakenly recognize a self-peptide complexed with HLA that shares residues with a microbial peptide. Consequently, microbial antigens can activate autoreactive T cells.

This mechanism underlies several autoimmune diseases (see Table I). For instance, anti-DNA antibodies, anti-TSH receptor antibodies, and anti-citrullinated peptide antibodies (ACPA) are associated with systemic lupus erythematosus, Graves' disease, and rheumatoid arthritis, respectively.

Table I. Examples of Molecular Mimicry.

Disease	Infectious Antigen	Autoantigen
Acute rheumatic fever	M protein of β-hemolytic streptococcus	Myosin
Type 1 diabetes	Coxsackievirus B4	GAD (Glutamic Acid Decarboxylase)
Chagas disease	*Trypanosoma cruzi*	Cardiac tissue
Guillain–Barré syndrome	*Campylobacter jejuni*	Myelin gangliosides of peripheral nerves

II.1.3. Activation of Ignored Autoreactive Cells

Certain antigens are immunologically "ignored" due to their anatomical location (immune privilege), e.g., lens proteins or spermatozoa. Trauma or surgery may release these antigens into circulation, where they are processed by APCs, potentially activating autoreactive B and T cells.

For example, ocular trauma can release intraocular antigens, leading to activation of T cells that subsequently migrate to both the injured and healthy eye, causing autoimmune uveitis.

II.1.4. Breakdown of Tolerance Due to Regulatory Cell Deficiency

Animal studies show that thymectomy in neonatal mice (2–3 days after birth) or Treg depletion induces widespread autoimmune disease (affecting the stomach, prostate, thyroid, etc.). Thus, regulatory T cells play an essential role in controlling autoimmunity, and their dysfunction may result in tolerance breakdown.

II.1.5. Genetic Predisposition

Autoimmune susceptibility is influenced by specific HLA alleles.

- Presence of **HLA-DRB1** alleles increases the risk of rheumatoid arthritis.
- Type 1 diabetes risk is maximal in individuals carrying both **HLA-DR3** and **HLA-DR4** class II alleles.

In summary, autoimmune diseases are multifactorial, involving genetic factors (notably HLA) and environmental factors (e.g., smoking, infections, pollution, UV radiation), all within the context of immune dysfunction.

III. Hypersensitivities and Allergies

Hypersensitivity refers to an immune response that occurs after one or more exposures to an antigen and results in tissue damage. Hypersensitivity reactions are classified into two main categories:

1. Humoral-mediated hypersensitivities, including:

- **Type I hypersensitivity or anaphylaxis (IgE-mediated):** an immediate reaction occurring within minutes.
- **Type II hypersensitivity or cytotoxic reaction (IgG-mediated):** immediate onset.
- **Type III hypersensitivity or immune complex reaction (IgG-mediated):** delayed or semi-delayed response.

2. Cell-mediated hypersensitivity:

- **Type IV hypersensitivity or delayed-type reaction:** occurs within 48 to 72 hours.

III.1. Type I Hypersensitivity

This type of hypersensitivity is also known as **anaphylaxis** or **allergy**.

- **Anaphylaxis** literally means “opposite of protection” (*ana* = away from; *phylaxis* = protection).
- **Allergy** means “an altered reaction,” acquired by an organism following a first exposure to an allergen.

Type I hypersensitivity develops in **two phases**:

a) Sensitization Phase

This phase corresponds to the **first exposure** to the allergen, which induces the production of **allergen-specific IgE antibodies** in cooperation with **Th2 and T follicular helper (Tfh) cells**. These IgE antibodies bind via their Fc fragments to **Fc receptors** on circulating **basophils** and **tissue-resident mast cells**. This binding is referred to as **sensitization** of mast cells and basophils.

b) Effector Phase (Reaction Triggering)

Upon **subsequent exposure** to the same allergen, the allergen binds to the Fab fragments of cell-bound IgE antibodies, creating **cross-linking** between two IgE molecules. This **IgE-allergen bridging** triggers **mast cell and basophil activation**, resulting in the release of **preformed and newly synthesized mediators** from their granules:

- **Primary mediators:** histamine, serotonin, the slow-reacting substance of anaphylaxis (SRS-A), eosinophil chemotactic factor (ECF), neutrophil chemotactic factor (NCF), and proteases such as tryptase. These are responsible for the **early phase** of the reaction.
- **Secondary mediators:** newly synthesized from membrane phospholipids under the action of phospholipase A_2, including **arachidonic acid derivatives** (prostaglandins and leukotrienes) and the **platelet-activating factor (PAF)**. These mediators account for the **late phase**, characterized by cellular infiltration predominantly of **eosinophils and mononuclear cells** (monocytes and lymphocytes) (Fig. 02).

The biological effects of these mediators include smooth muscle contraction, vasodilation, and increased vascular permeability, leading to plasma leakage into tissues. These processes result in erythema, edema, and arterial hypotension.

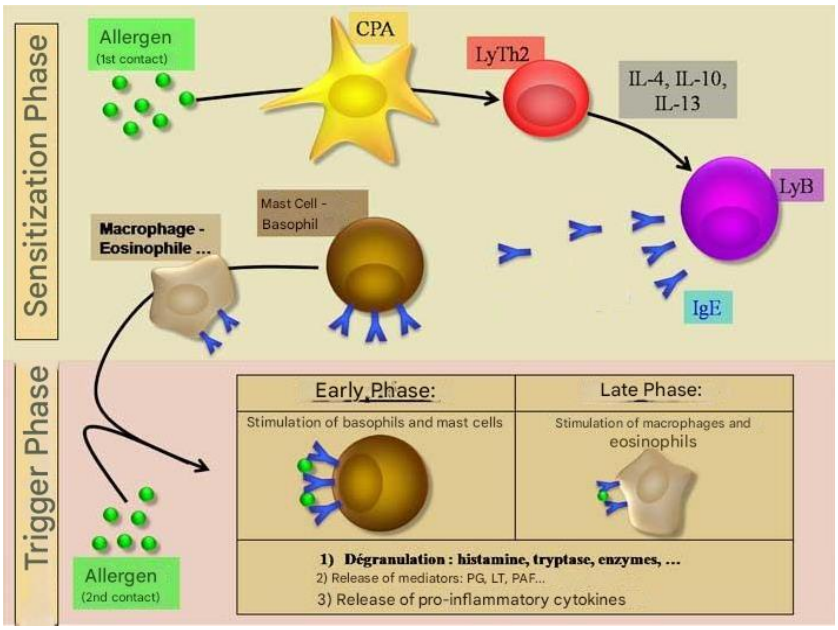


Fig. 02: Mechanism of Type I Hypersensitivity.

III.1.1. Forms of Type I Hypersensitivity

Depending on the site of interaction between the allergen and its specific antibody, two forms of type I hypersensitivity can be distinguished:

- **Tissue form:** includes allergic conjunctivitis, allergic rhinitis, allergic bronchial asthma, food allergies, drug allergies, atopic dermatitis, urticaria, allergic gastroenteritis, and other related conditions.
- **Intravascular form:** includes **angioedema** (subcutaneous edema) and **anaphylactic shock**.

III.1.1.1. Seasonal and Perennial Allergic Conjunctivitis

Allergic conjunctivitis is a **type I hypersensitivity reaction** occurring at the level of the **ocular conjunctiva**. During this reaction, the activation of **mast cells** and **basophils** previously sensitized by specific IgE antibodies by the allergen triggers an **immediate conjunctival allergic response**.

This **early phase** is characterized by elevated levels of **histamine**, **tryptase**, **prostaglandins**, **leukotrienes**, and **platelet-activating factor (PAF)** in the tears. Clinically, this phase presents with **itching (pruritus)**, **tearing**, **edema**, and **redness** of the conjunctiva.

Following the release of **cytokines**, **chemokines**, **prostaglandins**, and **leukotrienes**, a **late-phase conjunctival allergic reaction** occurs, typically several hours after the initial mast cell

activation. This phase is characterized by **infiltration of inflammatory cells**, predominantly **eosinophils**.

Activated mast cells also release multiple **cytokines** that stimulate **epithelial cells** and **fibroblasts**, promoting the production of **pro-inflammatory cytokines** and **chemokines**, which sustain **chronic conjunctival inflammation**.

Activated **eosinophils** further release **cytotoxic proteins** such as **Major Basic Protein (MBP)** and **Eosinophil Cationic Protein (ECP)**, leading to **photophobia**, **ocular discharge**, **pain**, and **visual disturbances** (Fig. 03).

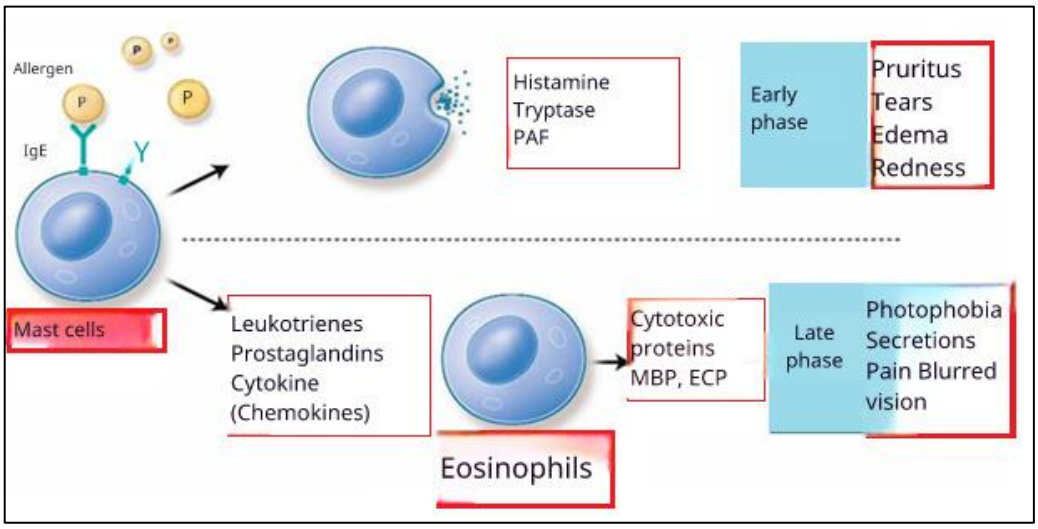


Fig. 03: Pathophysiological Effector Mechanisms of IgE-Mediated Hypersensitivity.

III.2. Type II Hypersensitivity: Cytotoxic Reaction

Type II hypersensitivity, also known as **cytotoxic hypersensitivity**, represents an exaggerated immune response directed against specific **endogenous or exogenous antigens** (for example, a drug bound to the surface of red blood cells and/or platelets). This reaction generally occurs **4 to 6 hours** after exposure to the antigen.

It develops when **circulating antibodies** (IgM or IgG) bind to **antigens present on the surface of target cells**, leading to **cell lysis** through one of the following **four mechanisms**:

- **Complement-mediated cytolysis** (Fig. 04-A);
- **Phagocytosis** of antibody-coated target cells (Fig. 04-B);
- **Antibody-dependent cellular cytotoxicity (ADCC)** (Fig. 04-C);
- **Binding of antibodies to cellular receptors** on target cells, thereby interfering with their normal function (Fig. 04-D).

The most well-known examples of Type II hypersensitivity include:

- **Hemolytic disease of the newborn**, which occurs in cases of **Rh incompatibility** (Rh-negative mother and Rh-positive fetus);
- **Drug-induced hemolytic anemia**;
- **Thrombocytopenia**;
- **Leukopenia**, among others.

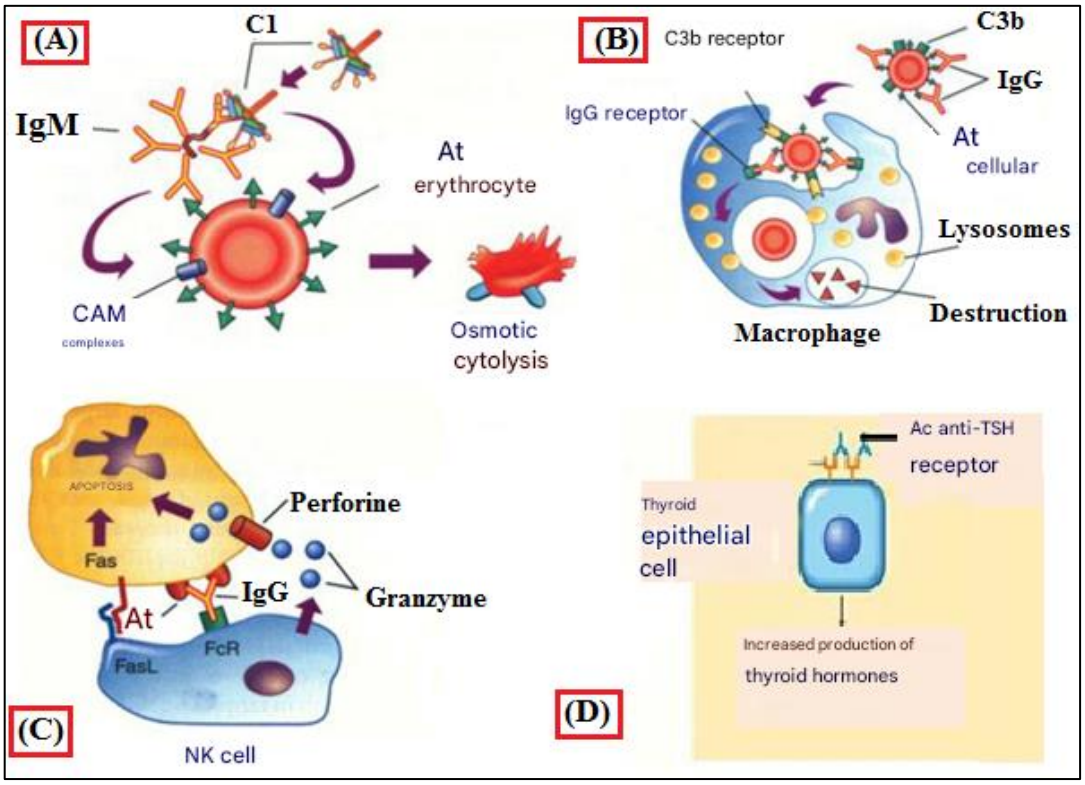


Fig. 04: Mechanisms of Type II Hypersensitivity.

III.3. Type III Hypersensitivity

Type III hypersensitivity involves the formation of **immune complexes** composed of **antibodies** (mainly IgG) bound to **soluble antigens**. These complexes become **deposited along basement membranes** and within tissues, where they **activate the complement system**, leading to the release of **anaphylatoxins** (C3a, C4a, and C5a) and **chemotactic factors** (C5a and C5b67).

This complement activation triggers an **inflammatory reaction**, characterized by **exudation** and infiltration of inflammatory cells rich in **phagocytes**. Activated phagocytes subsequently release **lysosomal proteases** and **reactive oxygen species (ROS)**, which cause **tissue damage**, resulting in various pathological conditions (Fig. 05). These reactions are of **semi-delayed onset**, typically appearing **4–6 hours** after exposure.

When immune complexes bind to Fc receptors on platelet membranes, they induce platelet adhesion and aggregation, leading to local microthrombus formation. These microthrombi worsen tissue injury by causing secondary ischemia due to vascular obstruction.

The most representative examples of Type III hypersensitivity include:

- **Extrinsic allergic alveolitis** (hypersensitivity pneumonitis);
- The **Arthus reaction**, as a localized manifestation;
- **Serum sickness**, and **drug-induced immune hemolytic anemia or thrombocytopenia**, as systemic manifestations.

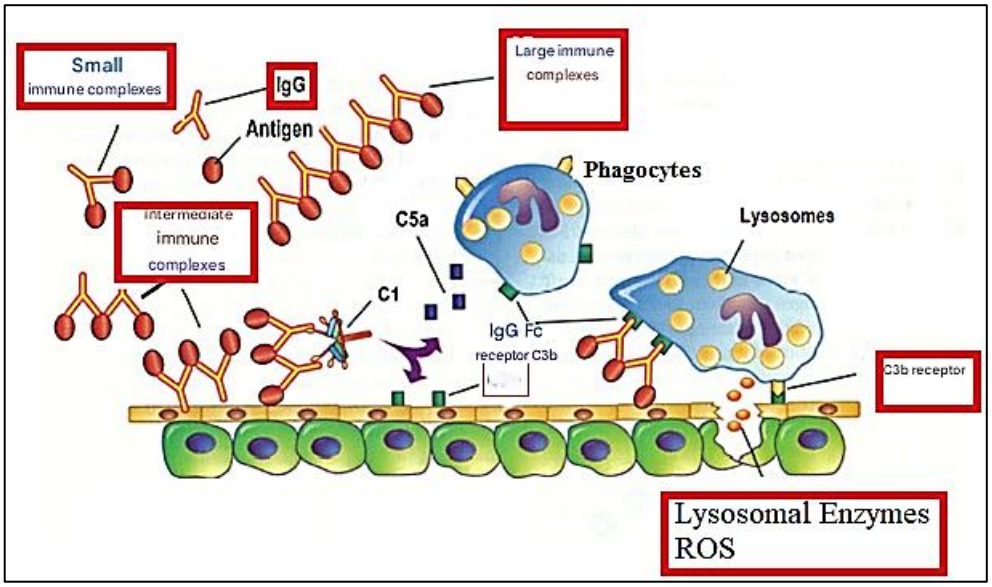


Fig. 05: Mechanisms of Type III Hypersensitivity.

III.4. Type IV or Delayed-Type Hypersensitivity

Type IV hypersensitivity is a **cell-mediated** immune response. It occurs after a **latent period of 48–72 hours** following exposure to the sensitizing antigen, which had previously induced the formation of **Th1 helper T cells** and **cytotoxic T lymphocytes (CTLs)** during the initial contact.

This reaction is **antigen-specific** and involves **T lymphocytes** in the **absence of antibodies**. It is characterized by the **accumulation of mononuclear cells** (Th1 lymphocytes, cytotoxic T cells, and macrophages) at the site of the reaction. Upon **re-exposure** to the antigen, **effector cytotoxic T cells** directly attack target cells by releasing **cytotoxic proteins** such as **perforin** and **granzyme**, leading to **target cell lysis** (Fig. 06).

Activated **Th1 cells** secrete **lymphokines** with chemotactic and activating functions, including **IL-8** (neutrophil chemotaxis), **IL-2**, **IFN-γ**, and **TNF-α**, which activate macrophages. Activated **macrophages** in turn release **lysosomal enzymes** and **reactive oxygen species (ROS)** that contribute to the destruction of target cells.

Because this reaction develops **after a second contact** and requires **more than 12 hours** to manifest, it is referred to as a **delayed-type hypersensitivity**, in contrast to the **immediate** type I response that occurs within minutes. The most typical example of this mechanism is **contact hypersensitivity**.

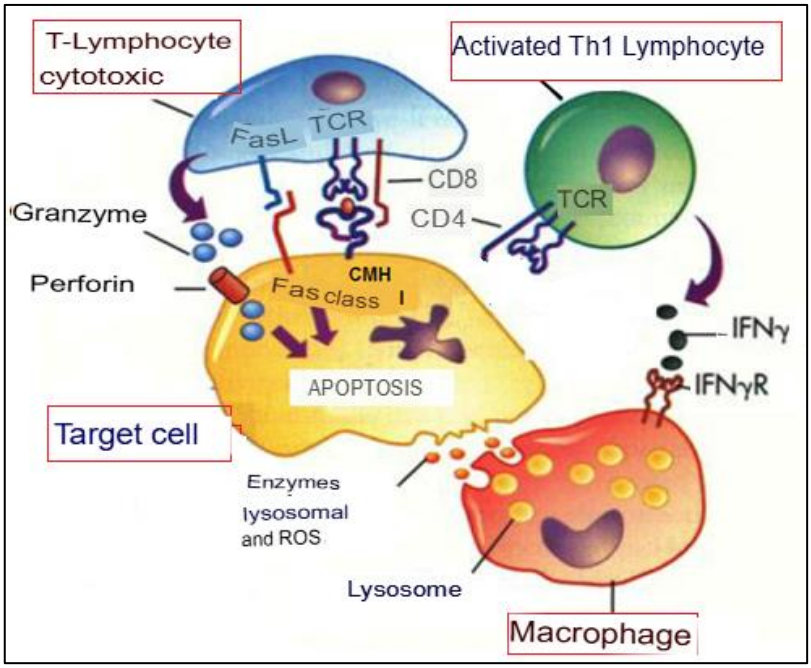


Fig. 06: Cell-Mediated Immune Response. Th1 = Effector $CD4^+$ T cell; Tc = Effector $CD8^+$ T cell.

IV. Immune Deficiencies

An **immune deficiency** is characterized by a weakening of the body's defenses against various infectious agents **bacteria, viruses, fungi,** or **cancerous cells**. Immune deficiencies may be **primary**, often of genetic origin, or **secondary**, resulting from various diseases or external factors.

IV.1. Primary Immune Deficiency

Primary immune deficiencies are **genetic disorders** characterized by defects in certain components of the immune system. They may result from **mutations** that interfere either with the **development** (absence or defective maturation of one or more immune cell types) or the **function** (defective activation or migration) of immune cells.

These deficiencies may involve either **innate immunity** such as **C2, C4, or C3 complement deficiencies**, **membrane attack complex (C5–C9) defects**, or **severe congenital neutropenia** or **adaptive immunity**, including **agammaglobulinemia** or **hypogammaglobulinemia** (humoral deficiencies).

Due to **quantitative or functional abnormalities** of specific immune cells, the body is unable to mount an adequate defense against **viral, fungal, parasitic,** or **bacterial infections**.

IV.2. Secondary Immune Deficiency

A **secondary immune deficiency** is **acquired**, resulting from an underlying condition that impairs the function of the immune system. Such deficiencies may occur as a consequence of:

- **Human Immunodeficiency Virus (HIV) infection**,
- **Type 2 diabetes mellitus**,
- **Corticosteroid therapy**,
- **Chemotherapy**, or
- The use of **immunosuppressive drugs**.

IV.3. Severe Combined Immunodeficiency (SCID)

Severe Combined Immunodeficiencies (SCID) are characterized by profound defects in the **function of immune cells**—including **T lymphocytes, B lymphocytes,** and **natural killer (NK) cells** which normally protect the body against microbial infections.

Immunologically, children with SCID typically present with **lymphopenia** (low T, B, and NK cell counts) on **hematological examination**, along with **low serum immunoglobulin levels** (IgG, IgA, and IgM). **Serologic tests for infectious agents** are usually **negative**, reflecting the absence of an effective immune response.

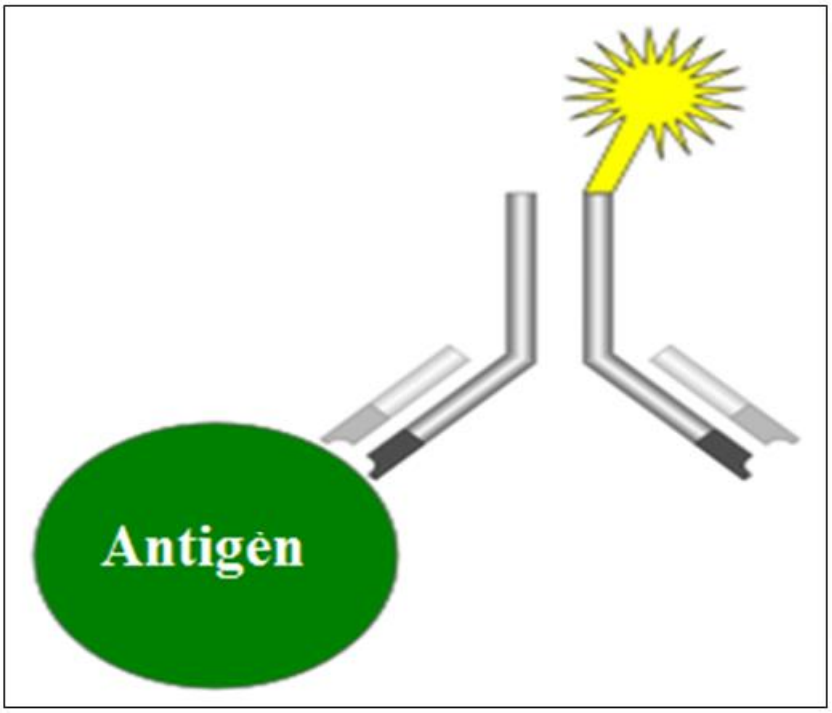
IV.4. Common Variable Immune Deficiency (CVID)

Common Variable Immune Deficiency (CVID) is a relatively frequent disorder characterized by low serum immunoglobulin (antibody) levels. Patients generally have a normal number of B lymphocytes, but exhibit a defect in class-switched memory B cells.

CVID is associated, in approximately 40% of cases, with additional complications such as lymphoid hyperplasia, splenomegaly, autoimmune diseases, tissue granulomas, and/or the development of lymphoma.

Chapter 09

Main immunological tests



I. General Concepts

An **immunological test** is a biochemical assay used to detect the presence or measure the concentration of a macromolecule or a small molecule in a solution through the use of an **antibody** or **immunoglobulin**. When the goal is to quantify the concentration, it is referred to as an **immunoassay**.

Two types of antibodies are commonly used in immunological tests:

- **Polyclonal antibodies**: These are mixtures of antibodies that recognize multiple epitopes of the same antigen (multivalent), produced by different clones of plasma cells.
- **Monoclonal antibodies**: These consist of a single type of antibody that binds to a single specific epitope on the antigen (univalent), produced by a single plasma cell clone.

Immunological tests are based on the **antigen–antibody (Ag–Ab) reaction**, which can be used to:

- Detect, identify, or quantify any molecule capable of inducing antibody production (**antigen detection**).
- Detect, identify, or quantify antibodies using their corresponding antigen (**antibody detection**).

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

1. **Visible but inconsistent phenomena (without labeling):**
   These reactions are directly observable, leading to techniques such as:
   - Immunoprecipitation
   - Immunoagglutination
   - Immunoelectrophoresis
2. **Constant but invisible phenomena (binding of antigen molecules to antibody Fab fragments):**
   These require visualization through labeling techniques, such as:
   - **Immunofluorescence** – using fluorescent dyes
   - **Radioimmunoassay (RIA)** – using radioactive isotopes
   - **Enzyme-linked immunoassays (ELISA)** – using enzyme markers

The visibility of the Ag–Ab reaction depends on the type of **label** used:

- **Fluorescent molecules** → Immunofluorescence
- **Enzymes** → Immunoenzymatic assays (ELISA)
- **Radioactive isotopes** → Radioimmunoassays

II. Immunoprecipitation

Immunoprecipitation and **immunodiffusion** exploit the specificity of the Ag–Ab reaction to analyze and/or quantify immune complexes.

When a solution of multivalent antigen is mixed with a polyclonal antiserum containing heterogeneous antibodies, a **three-dimensional lattice network** forms. This network becomes visible as a **precipitate** to the naked eye when the **zone of equivalence** between antigen and antibody concentrations is reached.

There are two main approaches:

II.1. Immunoprecipitation in Liquid Phase

II.1.1. Heidelberger and Kendall Technique

This is a qualitative method.

When increasing amounts of antigen are added to a fixed concentration of antibody in solution, a precipitate gradually forms.

The quantity of precipitate varies depending on the Ag–Ab ratio and reaches a maximum in the zone of **equivalence** (Fig. 01).

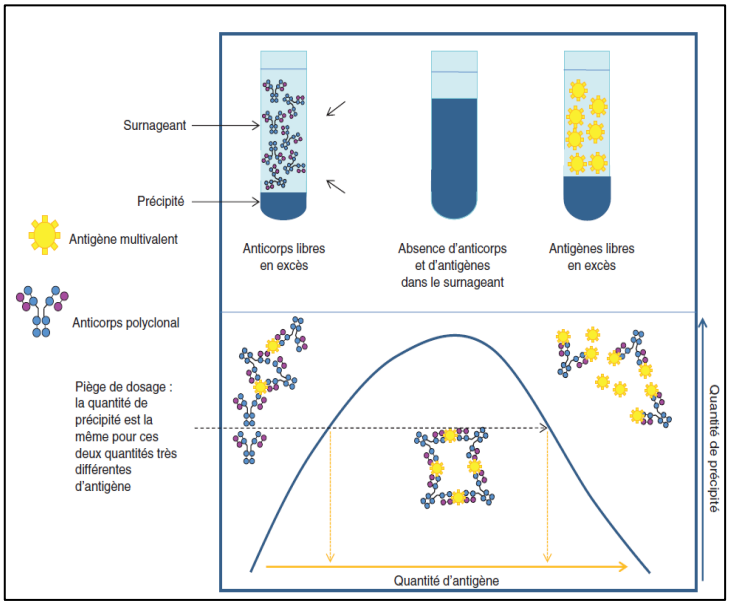


Fig. 01. Principle of liquid-phase immunoprecipitation showing the Heidelberger curve.

II.1.2. Ring Test (Precipitation in Ring Form)

This **qualitative technique** detects the presence of an antigen or antibody in solution.

- The **antiserum** (antibody) is placed at the bottom of a narrow tube (~1 cm).
- A solution containing the **antigen** (or antibody, in reverse testing) is gently layered above it without disturbing the interface.
- A **positive reaction** is indicated by the appearance of a **precipitation ring** at the interface within **5 minutes**.
- No precipitate should appear in **negative control tubes** (Fig. 02).

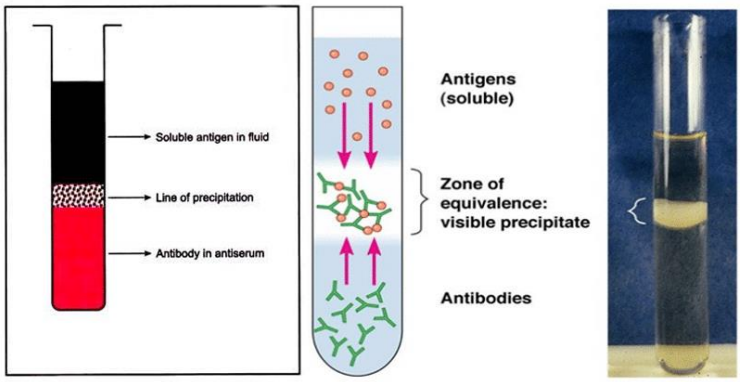


Fig. 02. Ring test for antigen–antibody precipitation.

II.2. Immunoprecipitation in Gel (Immunodiffusion)

II.2.1. Double Immunodiffusion (Ouchterlony Method)

This **qualitative technique** uses agar-filled Petri dishes.

- A **central well** is filled with serum containing antibodies.
- **Peripheral wells** are filled with known antigen solutions.
- Both antigen and antibody **diffuse through the gel**.
- When they meet in optimal proportions, they form **visible precipitation arcs**, which can be stained with **Coomassie blue** (Fig. 03).

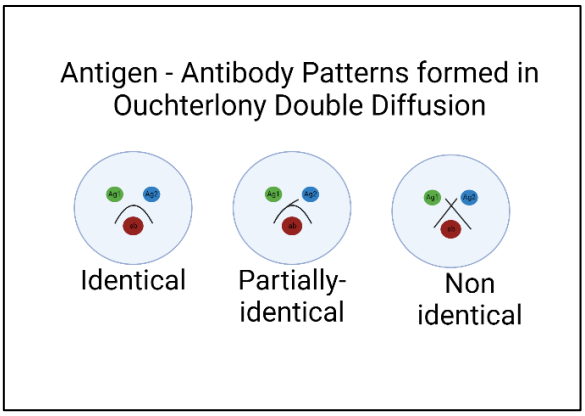


Fig. 03. Ouchterlony double immunodiffusion showing precipitation arcs formed by identical, partially identical, or different antigens.

II.2.2. Single Radial Immunodiffusion (Mancini Method)

This is a **quantitative technique**.

- A gel containing a **uniformly distributed antibody** is prepared.
- Antigen samples are placed into **small wells** cut into the gel.
- The antigen **diffuses radially** and is continuously diluted.
- When the **equivalence zone** is reached, **immune complexes precipitate**, forming visible rings (Fig. 04).

The **antigen concentration** is proportional to the **square of the precipitation ring diameter**, determined using a **calibration curve** prepared with standards.

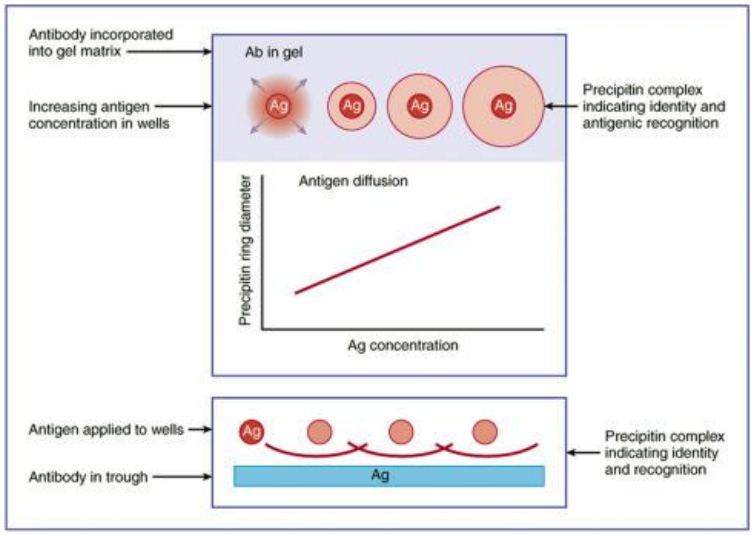


Fig. 04. Principle of radial immunodiffusion.

III. Immunoagglutination

This technique relies on the formation of **insoluble immune complexes** between antibodies and **particulate antigens**.
The reaction is **visible to the naked eye** and can be used to detect either **antibodies** or **antigens**.

There are two main types:

III.1. Active Immunoagglutination

- Occurs naturally when **particulate antigens** bind directly to their specific antibodies.
- Widely used for **blood group determination**.

Types of active agglutination:

- **Direct active agglutination:**
  - Occurs with **agglutinating antibodies** (usually IgM).

- **Indirect active agglutination:**
  - Involves **non-agglutinating antibodies** (usually IgG).
  - Requires macromolecules such as **SAB, dextran, or ficoll** to create **artificial bridges** between antibodies (Fig. 05).

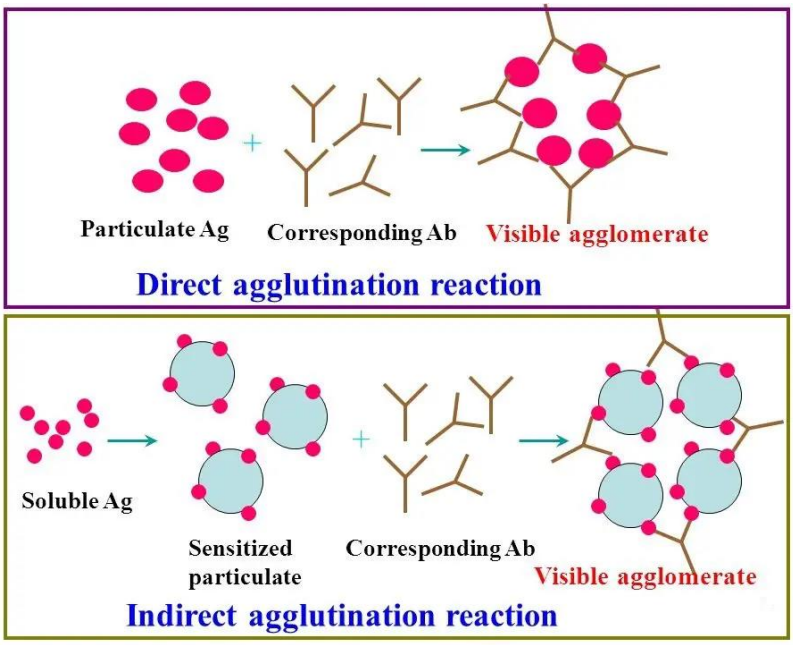


Fig. 05. Principle of direct and indirect active agglutination.

III.2. Passive Immunoagglutination

- A **soluble antigen** is converted into a **particulate antigen** by coupling it to an **inert carrier**, such as:
  - **Organic carriers:** e.g., human or animal red blood cells (*passive hemagglutination*).
  - **Synthetic carriers:** e.g., latex particles.
- Antibodies may also be bound to latex particles to detect specific antigens in biological samples (Fig. 06).

Example:
Detection of **rheumatoid factor** in rheumatoid arthritis (IgM anti-Fc of IgG).

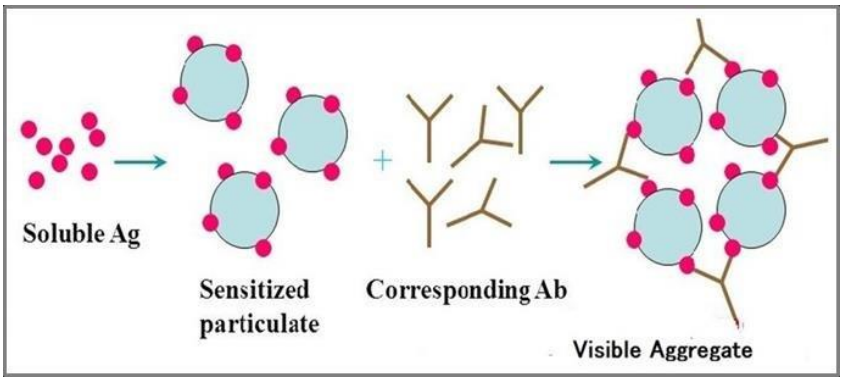


Fig. 06: Passive immunoagglutination principle.

IV. Immunoelectrophoresis

Also known as **immunoelectrodiffusion**, this method is derived from Ouchterlony's double immunodiffusion but uses an **electric field** to accelerate diffusion. It is a **qualitative immunoprecipitation technique** in gel, performed in two steps:

1. **Electrophoresis:**
    - A mixture of antigens is placed in a well and subjected to an electric field.
    - The antigens migrate based on their **charge and molecular weight** (Fig. 07).
2. **Antibody diffusion:**
    - A narrow **trench (~1 mm wide)** is cut parallel to the electrophoresis track and filled with **poly- or monospecific antiserum**.
    - The antibodies **diffuse perpendicularly** to the migration path.
3. **Incubation:**
    - After **48–72 hours**, **precipitation arcs** form at equivalence zones.
    - These arcs are interpreted similarly to those in the Ouchterlony technique.

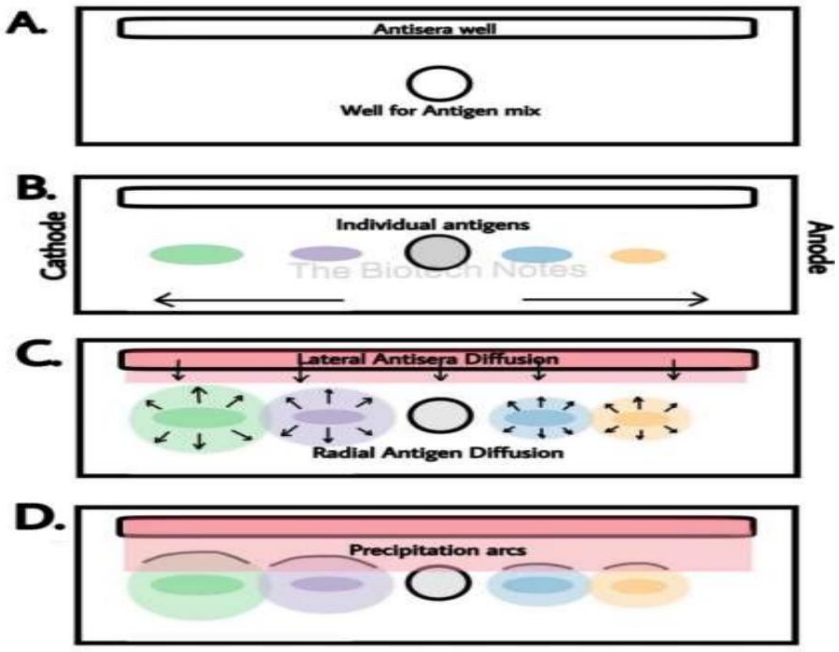


Fig. 07. Principle of immunoelectrophoresis.

V. Radioimmunoassay (RIA)

Radioimmunoassays use **radioisotopes** as highly sensitive tracers (e.g., **^{125}I** or **^{3}H**).

- **Sensitivity:** detection limits are in the **pmol/L** or **ng/mL** range.

- Applications: measurement of **steroid hormones**, **vitamins**, **drugs**, **tumor markers**, and **autoantibodies**.

Types of RIA methods:

- Immunoprecipitation in liquid phase (**Farr test**)
- Competitive RIA
- Sandwich RIA

Example: Farr Test

- Uses **radio-labeled double-stranded DNA (^{125}I)** to detect **serum anti-DNA antibodies**, particularly in autoimmune diseases (Fig. 08).

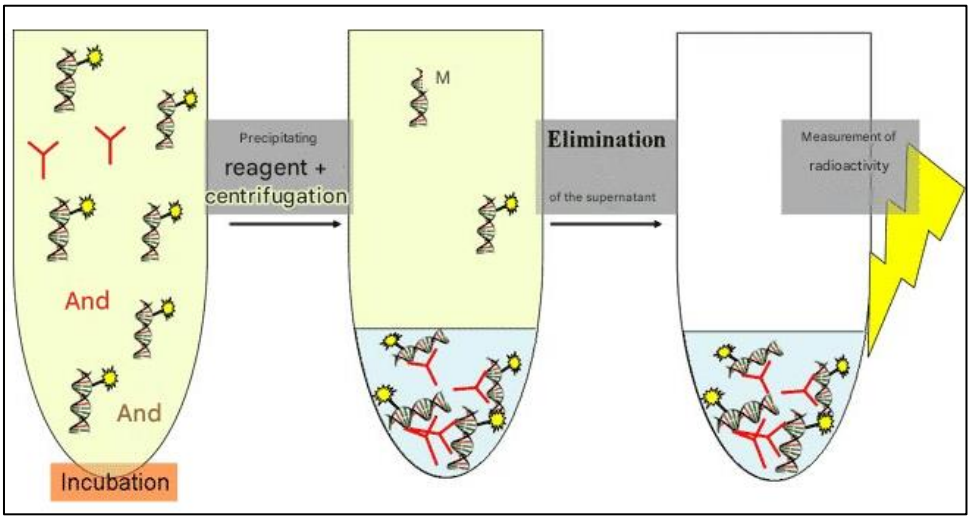


Fig. 08. Principle of the Farr radioimmunoassay.

VI. Immunofluorescence

Immunofluorescence detects the presence of a **specific antigen**, such as a bacterium or tissue section, using a **fluorescently labeled antibody**.

- **Excitation:** The fluorochrome is excited by **UV light (290–495 nm)**.
- **Emission:** It emits a **green light at 525 nm**.

Two main types (Fig. 09):

1. **Direct immunofluorescence:**
    - A fluorescent antibody directly binds to the target antigen.

2. **Indirect immunofluorescence:**
    - Used to detect antibodies.
    - Involves a known antigen and a **fluorescent anti-Fc antibody**.

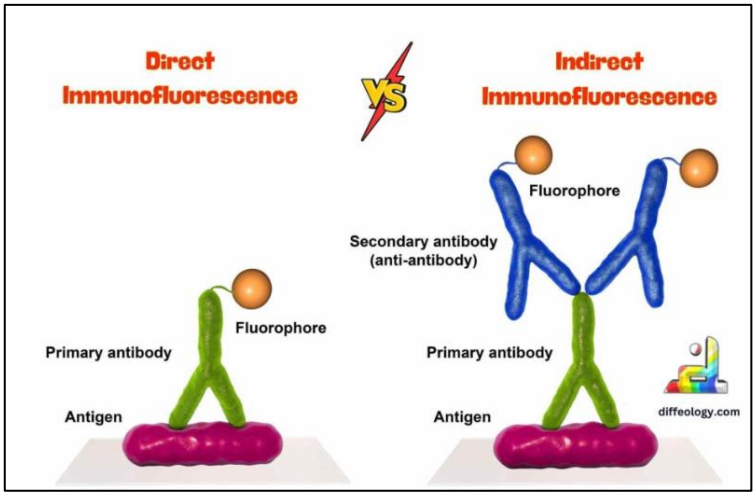


Fig. 09. Direct vs. indirect immunofluorescence.

VII. Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA detects **either antigens or antibodies** using an enzyme-labeled component such as peroxidase or alkaline phosphatase.

- The reaction is visualized by adding a **colorless substrate** (e.g., **p-nitrophenyl phosphate, PNPP**).
- The enzyme converts the substrate into a **colored product**, with color intensity proportional to the amount of bound enzyme and, therefore, the concentration of the analyte.

Detection limit:

- **pmol/L** or **ng/mL**, making it suitable for highly sensitive assays.

Types of ELISA:

- Direct ELISA
- Indirect ELISA
- Sandwich ELISA
- Competitive ELISA

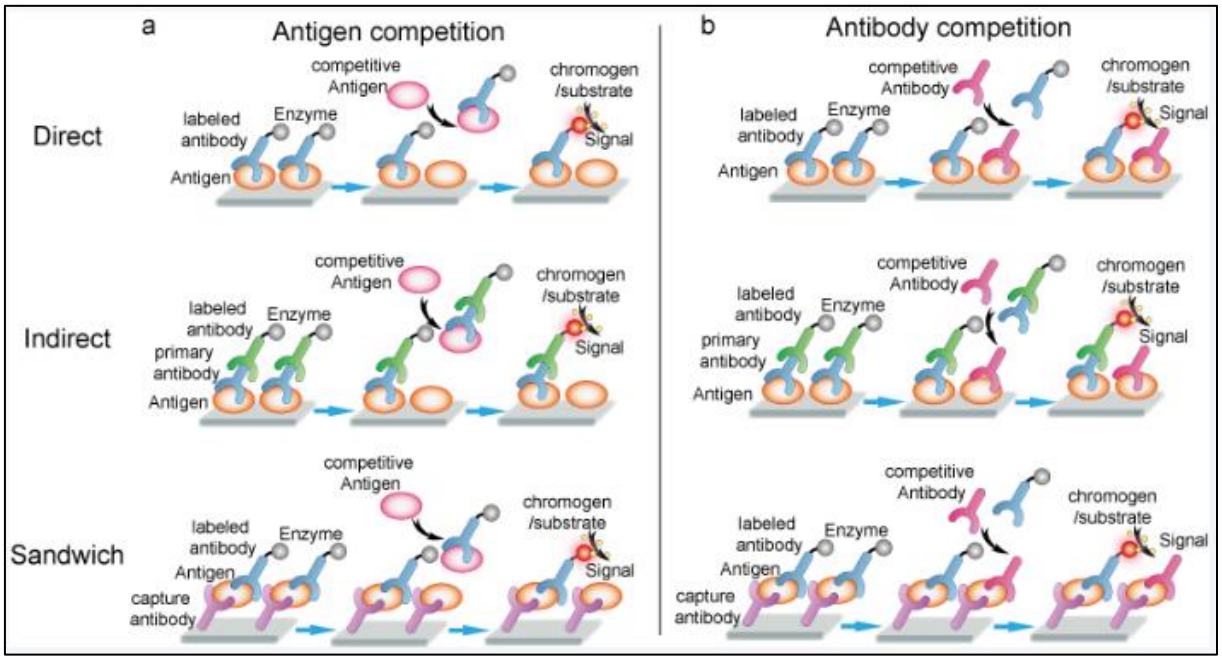


Fig. 10. Schematic representation of different ELISA types.

Table I. Summary of ELISA types according to target analyte (antigen or antibody).

Type of ELISA	Target Analyte	Principle	Typical Use
Direct ELISA	**Antigen**	Antigen is immobilized on plate; detected directly by enzyme-labeled antibody.	Detection and quantification of specific antigens.
Indirect ELISA	**Antibody**	Antigen is immobilized; primary antibody binds, then detected by labeled secondary antibody.	Measurement of specific antibodies in a sample (e.g., serological tests).
Sandwich ELISA	**Antigen (usually protein or cytokine)**	Capture antibody binds antigen; a second enzyme-linked antibody detects it.	Quantification of soluble antigens with high specificity.
Competitive ELISA	**Antigen or Antibody**	Sample antigen competes with labeled antigen for antibody binding sites.	Suitable for small molecules or when only one antibody is available.
Capture (Antibody) ELISA	**Antibody (e.g., IgE, IgM, autoantibody)**	Antibody is captured by anti-immunoglobulin antibody coated on plate, then detected by labeled antigen.	Measurement of specific antibody subclasses or isotypes.

Bibliographic References

- Abbas A.K., Lichtman A.H., & Pillai S. 2024. *Cellular and Molecular Immunology.* 11th ed., Elsevier, 576 p.
- Delves P.J., Martin S.J., Burton D.R., & Roitt I.M. 2021. *Essential Immunology.* 14th ed., Wiley-Blackwell, 560 p.
- Janeway C.A., Travers P., Walport M., & Shlomchik M.J. 2022. *Janeway's Immunobiology.* 10th ed., Garland Science, 912 p.
- Kindt T.J., Goldsby R.A., & Osborne B.A. 2022. *Kuby Immunology.* 9th ed., W.H. Freeman, 880 p.
- Male D.K. 2021. *Immunology: Illustrated Review.* 4th ed., Elsevier, 320 p.
- Murphy K., & Weaver C. 2023. *Case Studies in Immunobiology.* 7th ed., Garland Science, 384 p.
- Owen J.A., Punt J., & Stranford S.A. 2021. *Kuby Immunology Laboratory Manual.* 8th ed., W.H. Freeman, 432 p.
- Parham P. 2023. *The Immune System.* 5th ed., Garland Science, 512 p.
- Paul W.E. 2019. *Fundamental Immunology.* 8th ed., Wolters Kluwer, 1600 p.
- Rich R.R., Fleisher T.A., Shearer W.T., Schroeder H.W., Frew A.J., & Weyand C.M. 2023. *Clinical Immunology: Principles and Practice.* 6th ed., Elsevier, 1952 p.
- Roitt I.M., Brostoff J., & Male D.K. 2021. *Roitt's Essential Immunology.* 14th ed., Wiley-Blackwell, 560 p.
- Sompayrac L. 2022. *How the Immune System Works.* 7th ed., Wiley-Blackwell, 176 p.