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University of Mustapha Stambouli – Mascara

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UNIVERSITY OF MASCARA

كلية علوم الطبيعة والحياة

Faculty of Science of Nature and Life

قسم البيولوجيا

Department of Biology

Tutorials (travaux dirigés)

Immunology

Tutorial for License students

Stream: Biological Sciences

Level: License 2 (all the groups)

Presented by: Dr. Walid Hassene HAMRI

Tutorial information

Module: Immunology

Fundamental learning unit

Level: Second year License

Specialty: Common core

Coefficient: 2

Credits: 4

Semester: 4

Hours per week: 1.5 hours lecture / 1.5 hours tutorials

Assessment methods: A written test + assessment of work

The immunology tutorial combines the study of the body's defense reactions to a pathogen, regardless of its nature—virus, bacteria, fungi, or protozoa. Autoimmune diseases, allergies, and transplant rejection constitute the medical aspect of this science. The mechanisms of antibody synthesis and maturation, complement system activation, and the mobilization and coordination of defense cells constitute its fundamental and mechanistic aspects.

Tutorial presentation

To familiarize students with the role of immunity, immune defense systems, types of immune response, and immune system dysfunctions. Presentations on this topic could include information on the structure of antibodies, HLA, immune cells, cytokines, and the molecular mechanisms of immune deficiency.

Recommended prerequisites

Students should have a general knowledge of basic cell biology.

Assessment of learning

Assess the skills acquired throughout the tutorial through written exams and practical work.

Teaching and learning activities

Tutorials are a form of teaching that allows students to apply the knowledge learned in lectures or to introduce new concepts. They serve to assess their level of understanding by placing them in research-oriented situations and encouraging them to strive for excellence. Tutorial sessions involve a small number of students to ensure better monitoring and facilitate concentration.

The tutorial worksheet is presented in the form of exercises. The instructor carefully prepares each worksheet and must give it to the student in advance or at the beginning of the session to allow them time to prepare. Furthermore, the instructor may divide the tutorial session into two parts: the first for a brief review of the relevant chapter and the second for solving the exercises, while providing clarifications or guidance on the approach to follow to encourage independent student work.

The tutorial mark is submitted at the end of the semester and is calculated by adding together the following four (4) marks:

- Two marks for continuous assessment (each out of five (5) points): Two tests are given during the semester to evaluate each student's knowledge.
- A mark for participation (out of five (5) points).
- A mark out of five (5) points for attendance.

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LIST OF ABBREVIATIONS

APC	Antigen-presenting cell
BCR	B cell receptor
CD	Cluster of differentiation
CLPs	Common lymphoid progenitors
CSF	Colony-stimulating factors
DC	Dendritic cells
Fc	crystallizable fraction
HLA	Human leukocyte antigen
HSCs	Haematopoietic stem cells
ICAM-1	Intracellular Adhesion Molecule 1
Ig	Immunoglobulin
IL	Interleukin
IFN-γ	Interferon gamma
ITAM	Immunoreceptor tyrosine-based activation motif
LB	Lymphocytes B
LFA-1	Leukocyte Function Associated Antigen 1
LT	Lymphocytes T
LTh	Lymphocytes T helper
MALT	Mucosa-Associated Lymphoid Tissue
MBL	Mannose-binding lectin
MHC	Major Histocompatibility Complex
NK	Natural killer
TCR	T cell receptor
TLR	Toll-Like Receptors
TNF	Tumor necrosis factor

1- Introduction

The immune system is a complex biological system composed of a coordinated set of recognition and defense elements that distinguish between self and non-self. It is inherited at birth, but autonomous, adaptive, and highly plastic; it subsequently evolves in response to its interactions with microbes or foreign environmental substances **(Marshall *et al*., 2024)**.

From Edward Jenner's pioneering work in the 18th Century that would ultimately lead to vaccination in its modern form (an innovation that has likely saved more lives than any other medical advance), to the many scientific breakthroughs in the 19th and 20th centuries that would lead to, amongst other things, safe organ transplantation, the identification of blood groups, and the now ubiquitous use of monoclonal antibodies 1hroughout science and healthcare, immunology has changed the face of modern medicine. Immunological research continues to extend horizons in our understanding of how to treat significant health issues, with ongoing research efforts in immunotherapy, autoimmune diseases, and vaccines for emerging pathogens, such as Ebola **(Marshall *et al*., 2024)**.

Advancing our understanding of basic immunology is crucial for both clinical and commercial applications, as it has facilitated the discovery of new diagnostics and treatments to manage a wide range of diseases. In addition to the above, coupled with advancing technology, immunological research has provided critically important research techniques and tools, such as flow cytometry and antibody technology **(Marshall *et al*., 2024)**.

2- Ontogenesis of the immune system and lymphoid organs

The multipotent stem cell produces the lymphoid progenitor in the bone marrow (a central lymphoid organ) to give rise to various cell types **(García-Gil *et al.*, 2019)**.

2.1 Primary lymphoid organs

Primary lymphoid organs are the bone marrow and the thymus, crucial sites where immune cells (such as lymphocytes) are generated, proliferate, and mature (T cells in the thymus, B cells in bone marrow) to form the body's immune defenses **(Guillaume *et al.*, 2025)**.

These organs provide the essential environment for developing the specific receptor-bearing lymphocytes (T and B cells) that form the adaptive immune system **(Guillaume *et al.*, 2025)**.

2.1.1 Bone marrow

The bone is made up of compact bone, spongy bone, and bone marrow. Compact bone makes up the outer layer of the bone. Spongy bone is found mostly at the ends of bones and contains red marrow. Bone marrow is found in the center of most bones and has many blood vessels. There are two types of bone marrow: red and yellow. Red marrow contains blood stem cells that can become red blood cells, white blood cells, or platelets. Yellow marrow is made mostly of fat **(Del Fattore *et al.*, 2010)**.

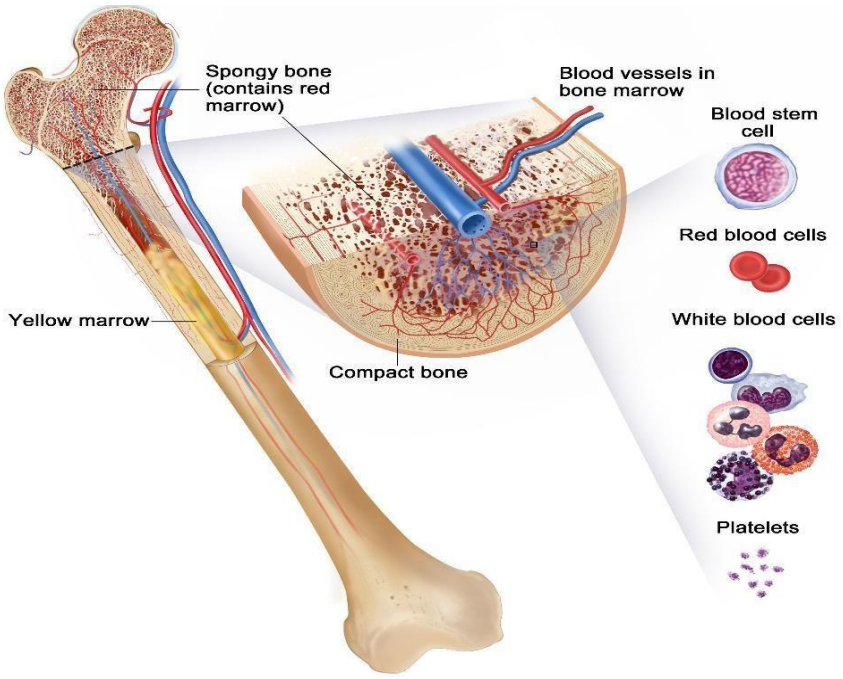


Figure 1: Anatomy of the bone **(Del Fattore *et al.*, 2010)**.

2.1.2 Thymus

The thymus gland is a vital, butterfly-shaped organ in the upper chest, part of the immune and endocrine systems, responsible for producing and maturing T-cells (lymphocytes) that fight infections; it's largest in childhood, shrinking after puberty as fatty tissue replaces it, though it remains crucial for immune system development and function **(Nowell *et al.*, 2007)**.

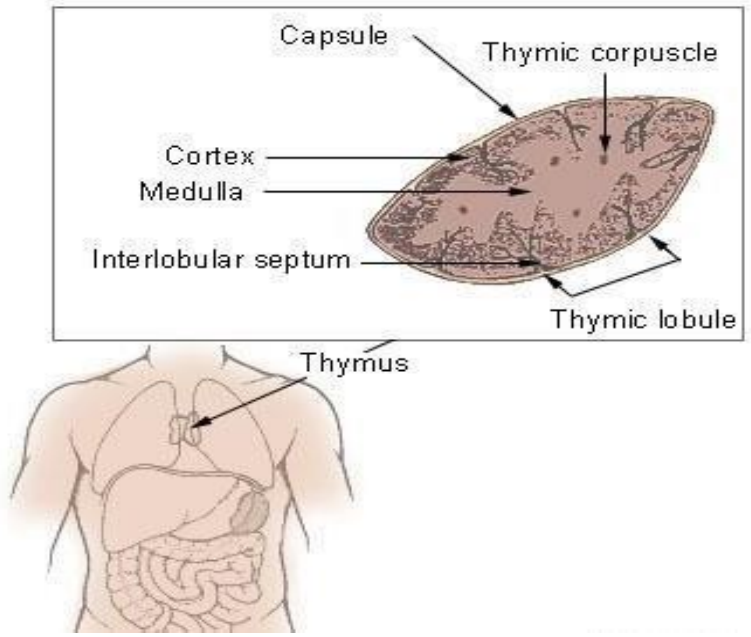


Figure 2: Anatomy of the thymus **(Nowell *et al.*, 2007)**.

2.2 Secondary lymphoid organs

Secondary lymphoid organs are immune system sites like lymph nodes, spleen, tonsils, and Peyer's patches (MALT) where mature lymphocytes (B and T cells) encounter antigens from lymph, blood, or tissues, triggering adaptive immune responses (activation, proliferation) to fight infections and maintain tolerance. They act as monitoring stations, filtering fluids, and activating immune cells in organized structures **(Bar-Ephraïm *et al.*, 2016)**.

2.2.1 Lymph nodes

Lymph nodes are small, bean-shaped glands in the lymphatic system that filter lymph fluid, trapping germs, damaged cells, and cancer cells using infection-fighting white blood cells (lymphocytes) to help the body fight illness **(Woods *et al.*, 2019)**.

They screen lymph fluid for harmful substances, waste, viruses, bacteria, and abnormal cells. They are major sites for lymphocytes (B & T cells) to multiply and launch attacks against invaders, destroying them before the fluid returns to the bloodstream **(Woods *et al*., 2019)**.

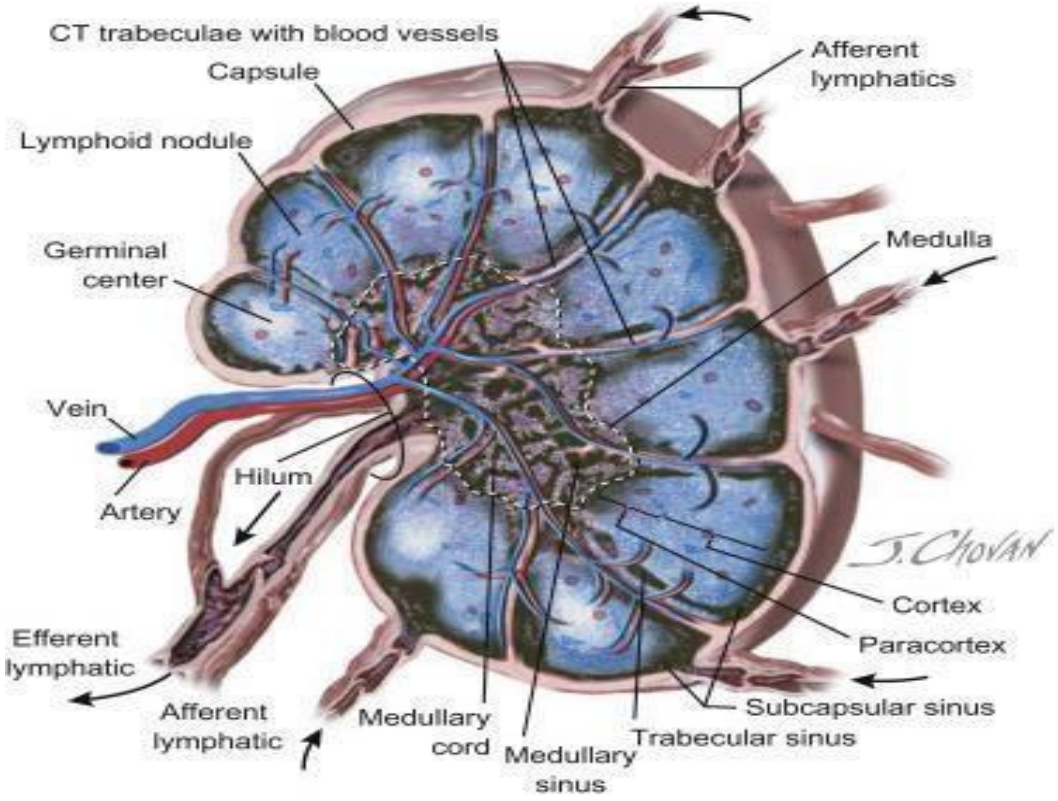


Figure 3: Anatomy of a lymph node **(Woods *et al*., 2019)**.

2.2.2 Spleen

The spleen is a small organ inside your left rib cage, just above the stomach. It's part of the lymphatic system (which is part of the immune system). The spleen stores and filters blood and makes white blood cells that protect from infection **(He *et al*., 2023)**.

There are two parts of the spleen. They each do different jobs. The types of tissue in the spleen are:

- **White pulp:** As part of the immune system, the white pulp produces white blood cells. These blood cells make antibodies. Antibodies fight infection.
- **Red pulp:** The red pulp acts like a filter. It removes waste from the blood and gets rid of old or damaged blood cells. Red pulp also destroys bacteria and viruses **(He *et al*., 2023)**.

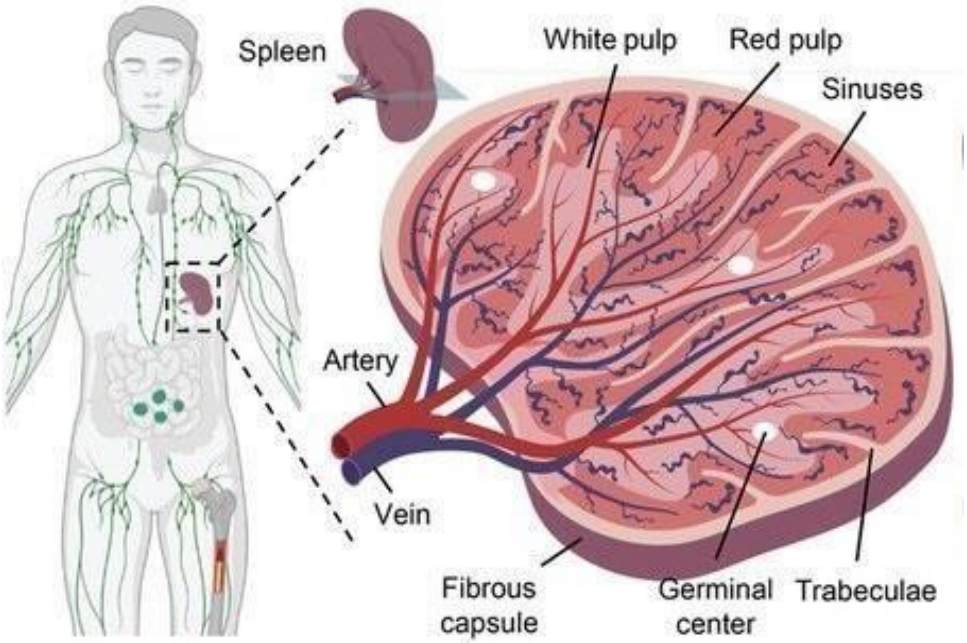


Figure 4: Anatomy of a spleen **(He *et al*., 2023)**.

2.2.3 Mucosa-Associated Lymphoid Tissue

Mucosa-Associated Lymphoid Tissue (MALT) is a crucial, widespread immune system component lining body surfaces like the gut, lungs, and eyes, acting as a first defense by sampling antigens and triggering immune responses where pathogens enter, consisting of organized lymphoid tissues like tonsils, Peyer's patches, and the appendix, and serving as the origin for MALT lymphomas when abnormal **(Kogame *et al*., 2021)**.

The components of MALT are sometimes subdivided into the following: GALT (gut-associated lymphoid tissue). Peyer's patches are a component of GALT found in the lining of the small intestines; BALT (bronchus-associated lymphoid tissue); NALT (nasal-associated lymphoid tissue); CALT (conjunctival-associated lymphoid tissue); LALT (larynx-associated lymphoid tissue); SALT (skin-associated lymphoid tissue); VALT (vulvo-vaginal-associated lymphoid tissue); TALT (testis-associated lymphoid tissue) **(Kogame *et al*., 2021)**.

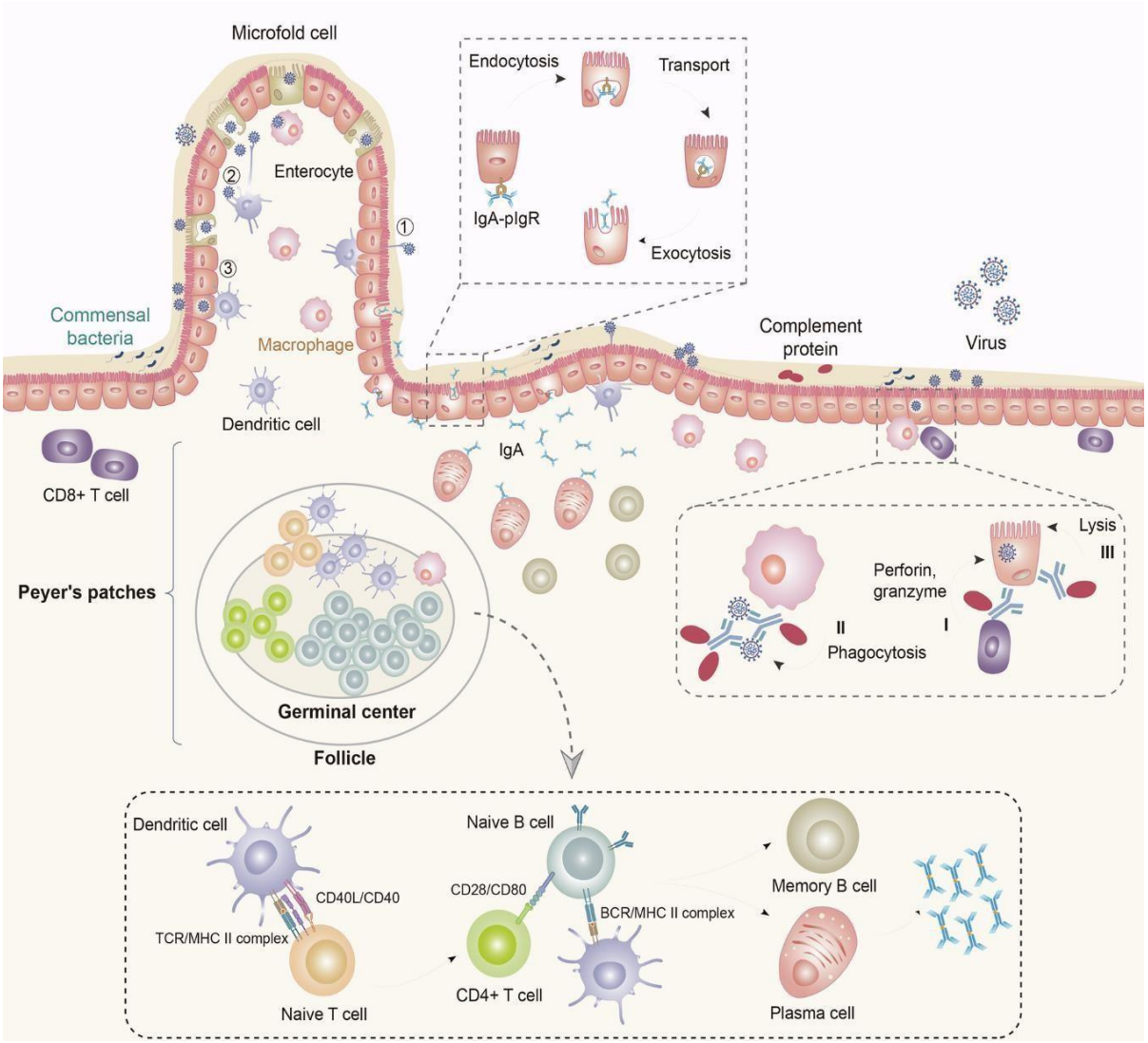


Figure 5: Mucosal immune system in the gastrointestinal tract **(Kogame *et al.*, 2021)**.

2.3 Immune cells

Immune cells, also called white blood cells (leukocytes), are the body's defenders, originating from bone marrow stem cells to fight infections, diseases, and abnormal cells like cancer, categorized into innate (quick, general response like neutrophils, macrophages, natural killer "NK" cells) and adaptive (specific, memory-based response like T cells, B cells) systems, working together in blood, lymph, and tissues. Key types include neutrophils (first responders), macrophages (engulf pathogens), lymphocytes (B & T cells for targeted immunity), and dendritic cells (present antigens) **(Gray *et al.*, 2022)**.

2.3.1 Innate immune cells

2.3.1.1 Granulocytes

Granulocytes include basophils, eosinophils, and neutrophils. Basophils and eosinophils are important for host defense against parasites. They are also involved in allergic reactions. Neutrophils, the most numerous innate immune cells, patrol for problems by circulating in the bloodstream. They can phagocytose, or ingest, bacteria, degrading them inside special compartments called vesicles **(Medina, 2016)**.

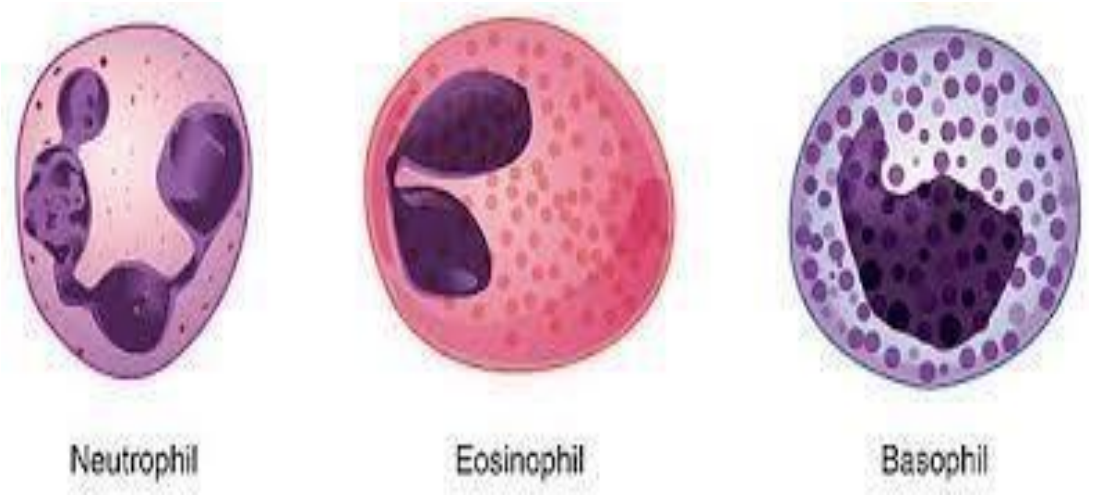


Figure 6: Microscopic view of granulocytes **(Medina, 2016)**.

2.3.1.2 Mast cells

Mast cells are also important for defense against parasites. Mast cells are found in tissues and can mediate allergic reactions by releasing inflammatory chemicals like histamine **(Medina, 2016)**.

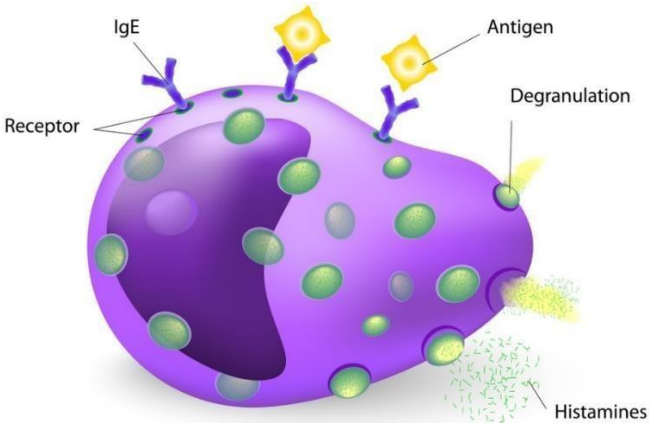


Figure 7: Microscopic view of a mast cell **(Medina, 2016)**.

2.3.1.3 Monocytes

Monocytes, which develop into macrophages, also patrol and respond to problems. They are found in the bloodstream and in tissues. Macrophages, “big eater” in Greek, are

named for their ability to ingest and degrade bacteria. Upon activation, monocytes and macrophages coordinate an immune response by notifying other immune cells of the problem. Macrophages also have important non-immune functions, such as recycling dead cells, like red blood cells, and clearing away cellular debris. These "housekeeping" functions occur without activation of an immune response **(Medina, 2016)**.

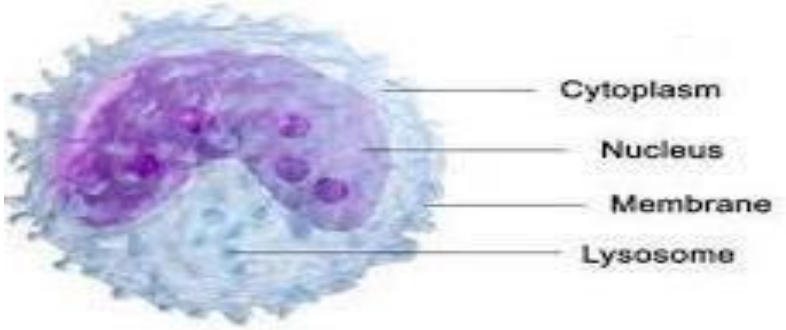


Figure 8: Microscopic view of a monocyte **(Medina, 2016)**.

2.3.1.4 Dendritic cells

Dendritic cells (DC) are an important antigen-presenting cell (APC), and they can also develop from monocytes. Antigens are molecules from pathogens, host cells, and allergens that may be recognized by adaptive immune cells. APCs like DCs are responsible for processing large molecules into "readable" fragments (antigens) recognized by adaptive B or T cells. However, antigens alone cannot activate T cells. They must be presented with the appropriate major histocompatibility complex (MHC) expressed on the APC. MHC provides a checkpoint and helps immune cells distinguish between host and foreign cells **(Medina, 2016)**.

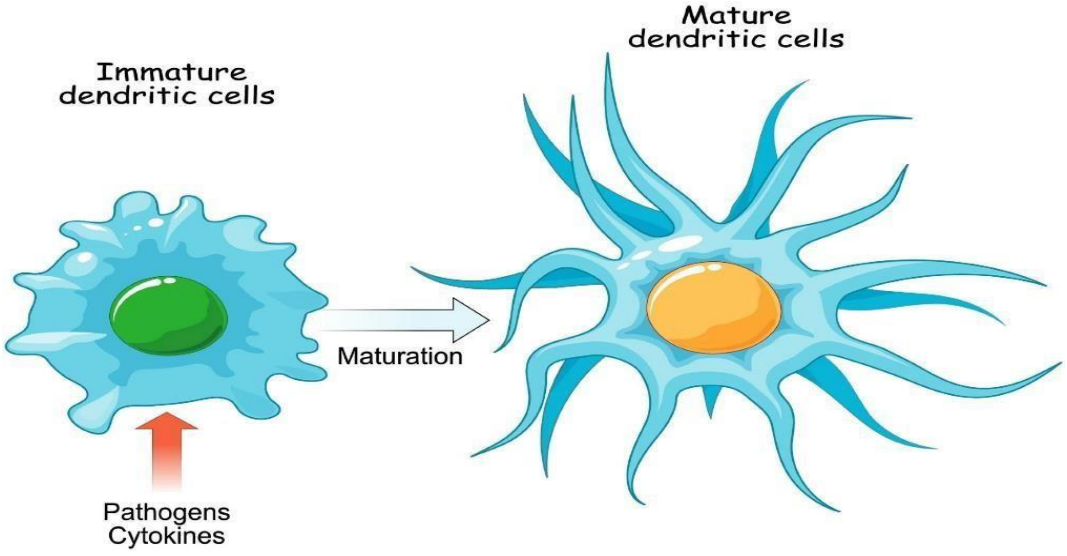


Figure 9: Microscopic view of a dendritic cell **(Medina, 2016)**.

2.3.1.5 Natural killer

Natural killer (NK) cells have features of both innate and adaptive immunity. They are important for recognizing and killing virus-infected cells or tumor cells. They contain intracellular compartments called granules, which are filled with proteins that can form holes in the target cell and also cause apoptosis, the process of programmed cell death. It is important to distinguish between apoptosis and other forms of cell death, like necrosis. Apoptosis, unlike necrosis, does not release danger signals that can lead to greater immune activation and inflammation. Through apoptosis, immune cells can discreetly remove infected cells and limit bystander damage. Recently, researchers have shown in mouse models that NK cells, like adaptive cells, can be retained as memory cells and respond to subsequent infections by the same pathogen **(Medina, 2016)**.

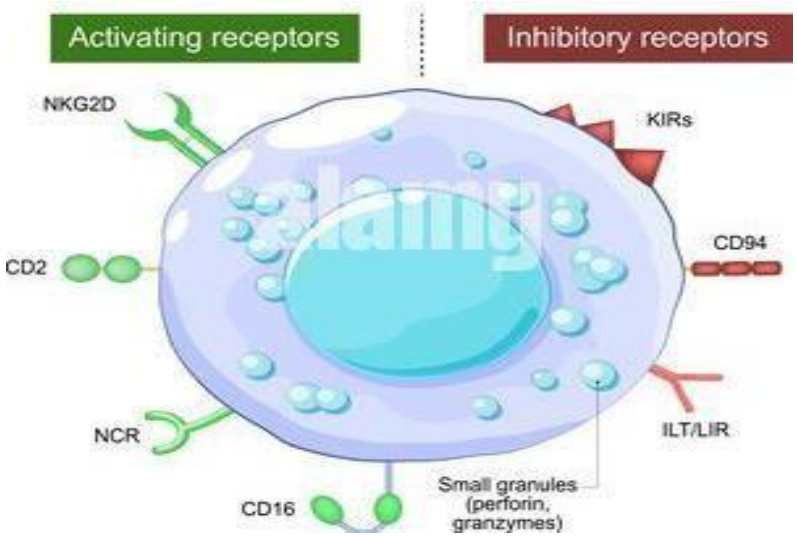


Figure 10: Microscopic view of a natural killer **(Medina, 2016)**.

2.3.2 Adaptive immune cells

Adaptive immune cells, primarily T cells and B cells, are lymphocytes that provide specific, long-lasting immunity by recognizing, targeting, and remembering pathogens, unlike the general innate system. B cells produce antibodies for humoral immunity, while T cells manage cell-mediated immunity, directly killing infected cells **(García-Gil *et al*., 2019)**.

2.3.2.1 B lymphocytes

These cells mature in the bone marrow and then differentiate into memory B cells and plasma cells, which secrete antibodies. These cells are a major component of the adaptive immune system **(García-Gil *et al*., 2019)**.

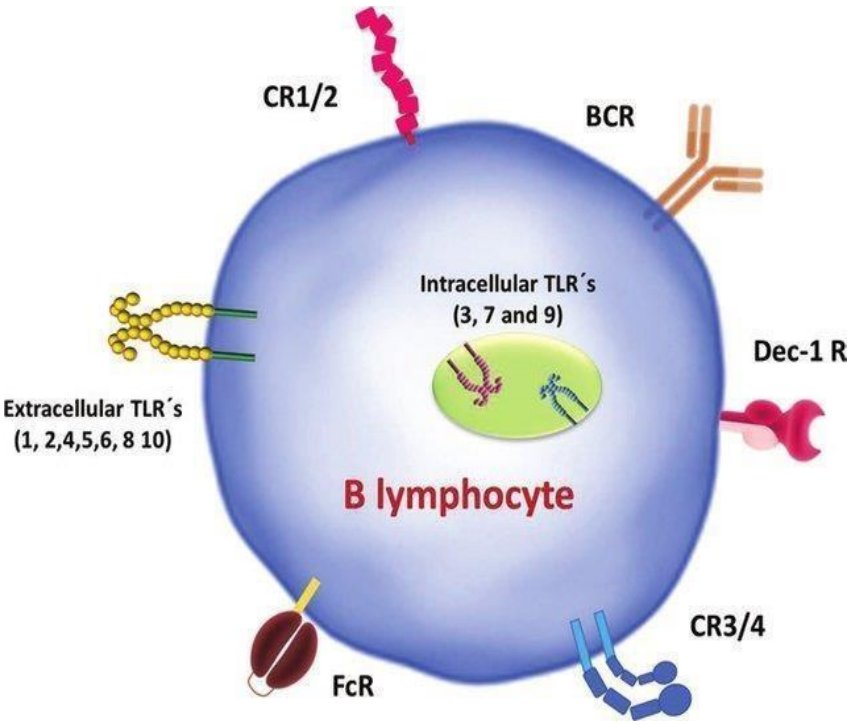


Figure 11: B lymphocytes display a wide number of receptors capable of recognizing and engulfing pathogens **(García-Gil *et al*., 2019)**

❖ **B lymphocytes development and maturation in the bone marrow**

B lymphocytes develop in the bone marrow through a process called hematopoiesis. It is also the site where B lymphocytes acquire immunocompetence **(Patel *et al*., 2021)**.

B lymphocytes develop in the bone marrow from haematopoietic stem cells (HSCs), progressing from pro-B cell stages to pre-B cells before migrating into the circulation as transitional B cells. Upon antigen recognition, activated B cells migrate to secondary lymphoid organs and enter germinal centres where they undergo clonal expansion and somatic hypermutation within the dark zones. B cells with disadvantageous mutations die by apoptosis, whereas those B cells with improved receptor affinity interact with follicular dendritic cells and T follicular helper cells, in the light zone. B cells undergo class switch recombination and receive survival signals to differentiate into memory B cells and long-lived plasma cells. Naïve B cells can differentiate into short-lived plasma cells through extrafollicular responses. Naïve B cells can also differentiate into age-associated B cells upon stimulation **(Patel *et al*., 2021)**.

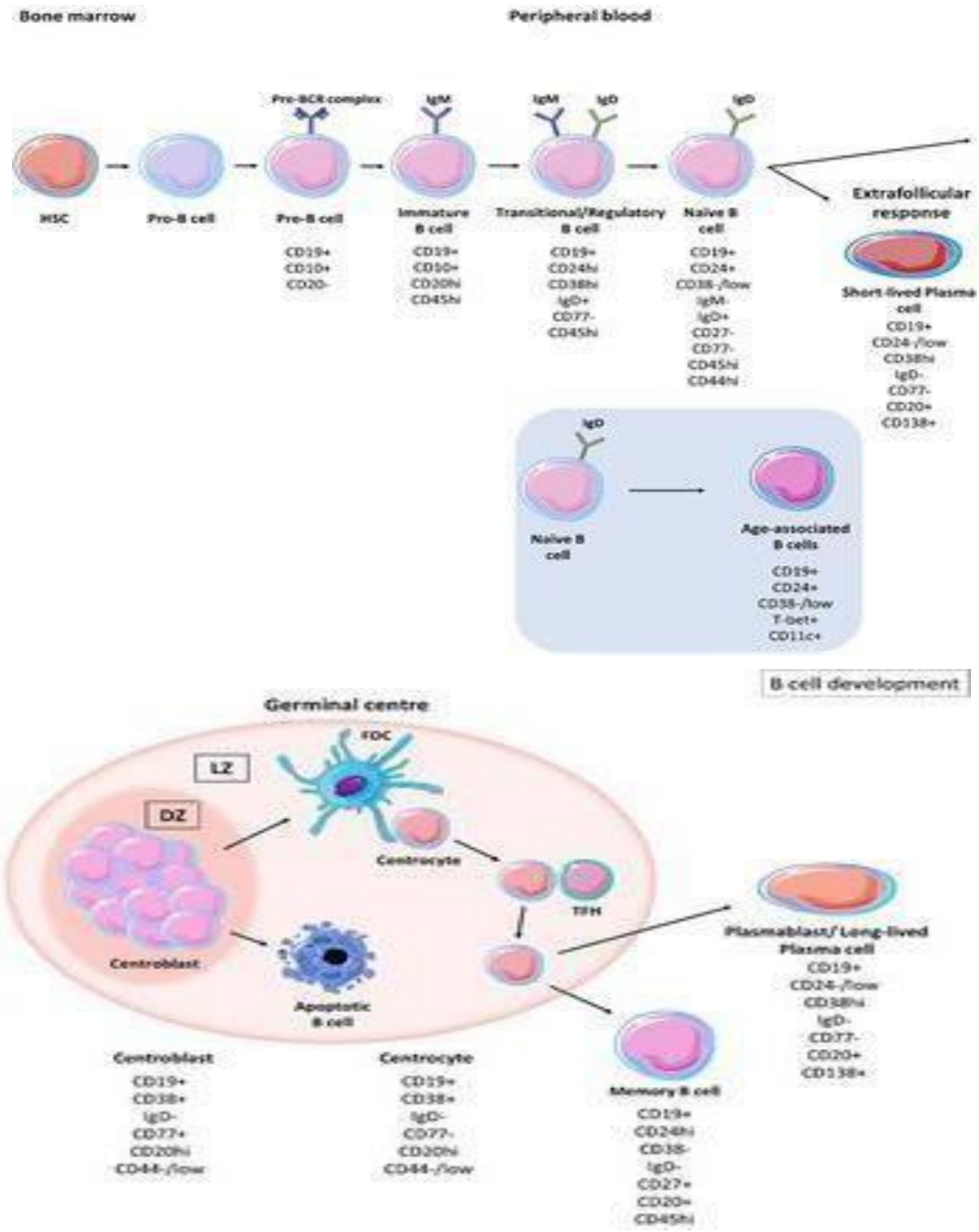


Figure 12: B lymphocyte development stages **(Patel *et al*., 2021)**

2.3.2.2 T lymphocytes

T lymphocytes are crucial for immune functions to maintain health and prevent disease. T lymphocytes develop in a stepwise process in the thymus and mainly generate $CD4^+$ and $CD8^+$ T cell subsets **(Sun et al., 2023)**.

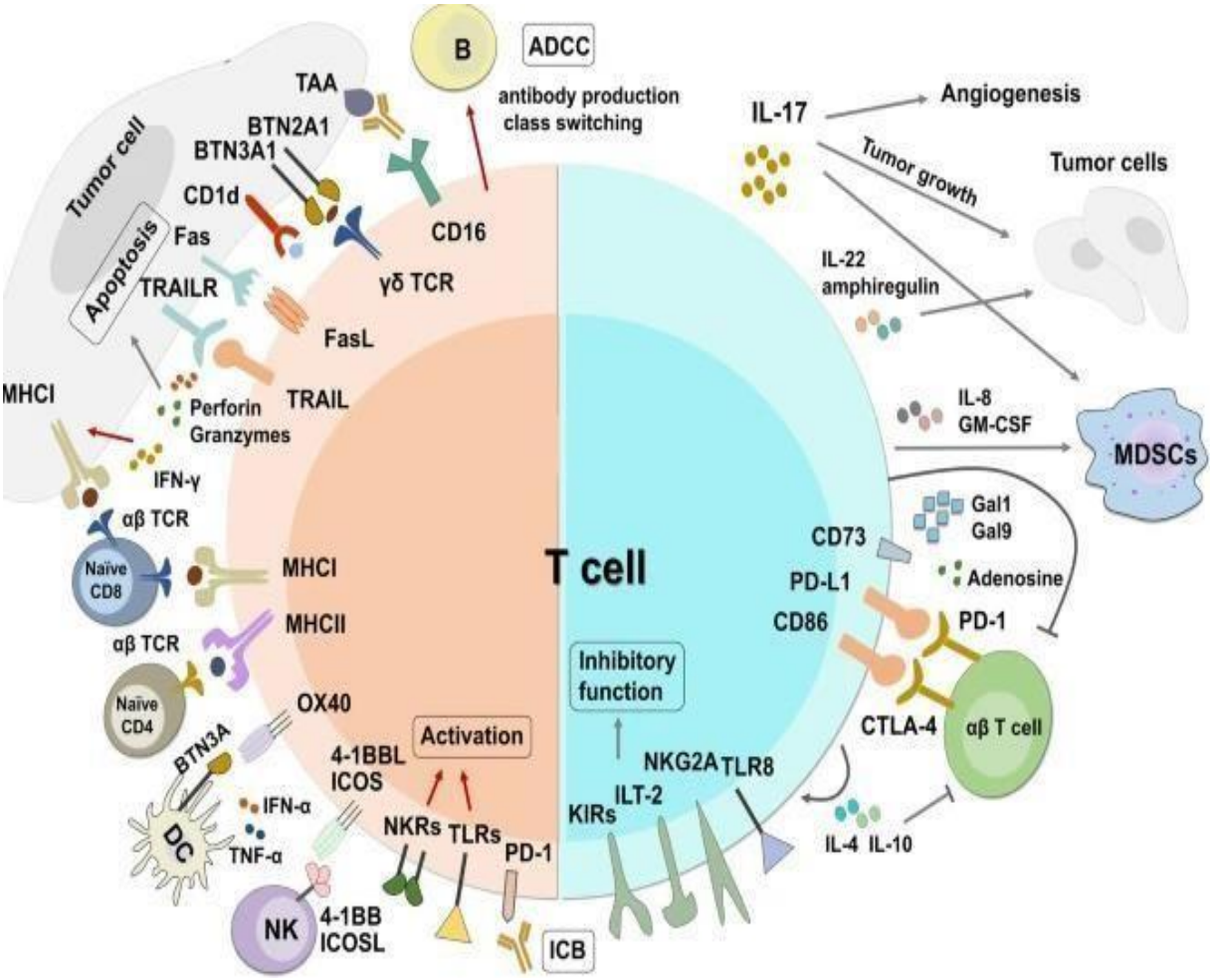


Figure 13: The immunity role of T lymphocytes **(Sun *et al*., 2023)**

❖ **T lymphocytes development in the bone marrow**

T-cell education starts in the bone marrow, where HSCs create common lymphoid progenitors (CLPs), which then migrate to the thymus for their real "education" (thymopoiesis) **(Sapkota, 2022)**.

❖ **T lymphocytes maturation in the thymus**

- Double Negative Stage: In the thymus cortex, cells start producing T-cell receptors (TCRs) and become double positive ($CD4^+$ $CD8^+$).
- Positive Selection: Cells that can weakly bind to self-MHC molecules (Major Histocompatibility Complex) survive, developing either CD4 or CD8.
- Negative Selection: Cells that strongly bind to self-antigens presented on MHC molecules are eliminated (apoptosis) to prevent autoimmunity.
- Final Selection: Cells that pass selection become either $CD4^+$ helper T-cells or $CD8^+$ cytotoxic T-cells and mature **(Sapkota, 2022)**.

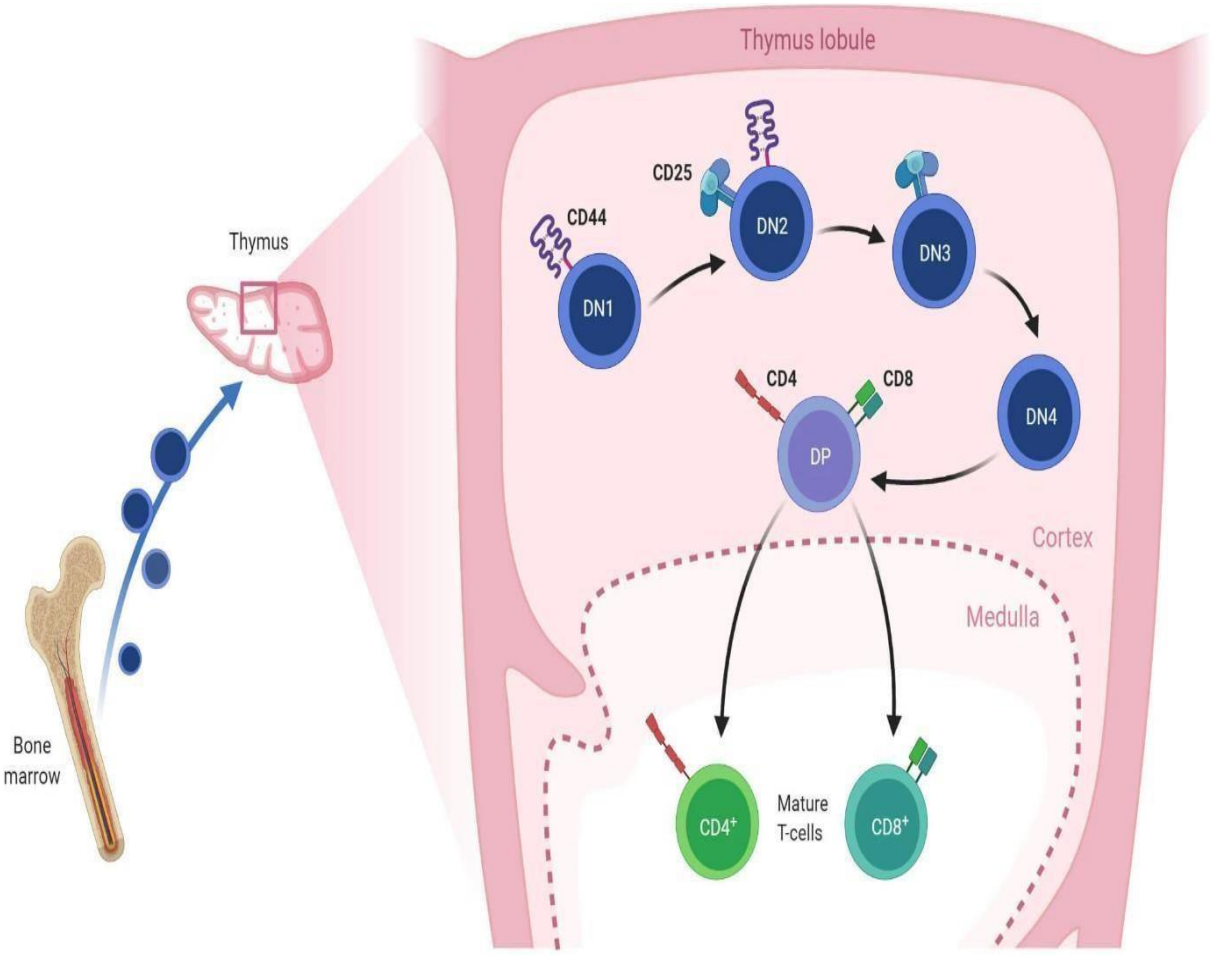


Figure 14: T lymphocytes maturation in the thymus **(Sapkota, 2022)**

❖ **Role of the bone marrow in mature T lymphocytes**

After maturation, the bone marrow serves as a major site for long-lived memory T lymphocytes, providing a continuous supply and long-term immunological memory. Mature T-lymphocytes in the bone marrow interact with other cells (like B-lymphocytes, osteoclasts) and receive signals that influence their function and maintenance **(Sapkota, 2022)**.

Tutorial objective: to assimilate the fundamental concepts of the immune system. It allows the identification of the key players in the body's defense (antigens, antibodies, lymphoid organs).

Immunology tutorials series N1

1. What is the body's defense system called? Can you list some of its components?

.....

2. What are the components of the lymphatic system? What are its two functions?

.....

.....

3. We call…… any foreign substance capable of being recognized by a subject's immune system as… ..... **and capable of triggering in that subject a …… which results in the** ..... **of that substance.**

- Multiple choice questions:

1. Give the name of the place where immune cells are formed.

a. tonsils, spleen
b. bone marrow
c. thymus
d. lymph nodes

2. Give the name of the storage site for immune cells

a. lymph nodes, tonsils, spleen
b. thymus
c. red bone marrow
d. yellow bone marrow

3. Which blood cells differentiate and multiply to a mature stage in the bone marrow?

a. Neutrophils
b. B lymphocytes
c. T lymphocytes
d. Red blood cells
e. Platelets

<u>Primary lymphoid organs:</u>

4. Which of these organs are considered primary lymphoid organs?

a. spleen b. thymus c. lymph nodes d. bone marrow
e. Mucosa-associated lymphoid tissue

5. Regarding intrathymic differentiation

a. The thymus is the central organ for lymphocyte differentiation.
b. Intrathymic differentiation occurs only in the cortex.

c. It includes the acquisition and genetic rearrangements of the TCR (thyroxine receptor).
d. The thymus is an organ where the rate of lymphocyte proliferation is low.

6. Regarding the role of bone marrow

a. It is hematopoietic tissue, the site of differentiation, multiplication, and maturation of all blood cells.
b. It is the site of formation of macrophages and leukocytes, except T lymphocytes.
c. It is the site of differentiation of B lymphocytes.
d. This differentiation includes the acquisition of receptors for the antigen.

7. Regarding the thymus

a. The thymus is a central lymphoid organ.
b. The prothymocytes that populate the thymus originate from the bone marrow.
c. It increases in size until puberty and then involutes without disappearing completely in the elderly.
d. Thymocytes multiply in the medullary zone.

8. What is the effect of thymectomy?

a. in a newborn mouse? b. in an adult mouse? Justify your answer

.....

.....

<u>Secondary lymphoid organs:</u>

1. In this list, which are the secondary lymphoid organs, formations, or tissues?

a. spleen.
b. lymph nodes.
c. bone marrow.
d. Peyer's patches.
e. lamina propria.

2. Regarding the functions of the lymph nodes

a. They filter antigens from interstitial fluids and lymph.
b. They are not capable of excluding pathogens.
c. Plasma cells complete their differentiation in the lymph nodes.
d. These are sites of development of specific immune responses.
e. They are the site of antibody production.

3. About the spleen

a. It is a lymphoid organ located in the right part of the abdomen, near the diaphragm.
b. It mainly contains macrophages, B cells, and cells specialized in antigen presentation.
c. It is composed of two main tissues, the red pulp and the white pulp.
d. It contains macrophages.

4. Mucosal-associated lymphoid tissue

a. comprises isolated lymphoid cells in the subepithelial connective tissue.
b. is organized into Peyer's patches in the small intestine.
c. includes the tonsils.
d. enables the immune response to inhaled antigens.

5. Which of the following is/are the correct answer(s)? Regarding Peyer's patches:

a. They are located in the small intestine.
b. At their level, the epithelium contains M cells that transport undegraded mucosal antigens to dendritic cells.
c. B lymphocytes activated at their level migrate to the mesenteric lymph nodes.
d. They are the site of induction of the immune response to ingested antigens.

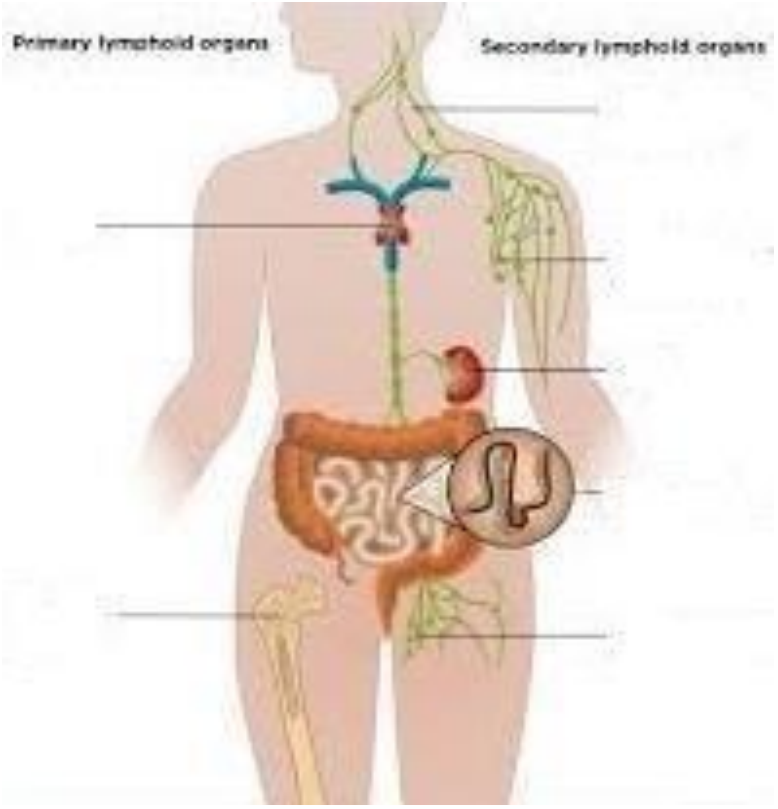
6. Which of the following properties are attributed to the lymph node?

a- It is one of the sites of development of specific immune responses.
b- It is the site of antibody production.
c- It is populated exclusively by B lymphocytes.
d- It ensures the filtration of antigens from interstitial fluids and lymph.

7. Regarding the role of the spleen

a. It is a blood-purifying organ.
b. Antigens are retained in the white pulp.
c. It is the primary site of the immune response to circulating antigens in the blood.
d. The antibody response develops particularly strongly there against polysaccharide antigens.

8. In the following image, identify the main components of the defense system.



Immunology tutorials series N1 (Solutions)

1. What is the body's defense system called? Can you list some of its components?

Immune system - white blood cells, macrophages, skin, mucous membranes, lymphatic system, etc.

2. What are the components of the lymphatic system? What are its two functions?

Organs, vessels, lymph nodes, lymph – 1) defense of the body against infections 2) circulation of fluids and their regulation in the body

3. We call an antigen **any foreign substance capable of being recognized by a subject's immune system as** non-self **and capable of triggering in that subject an** immune response **which results in the** neutralization **of that substance.**

- Multiple choice questions:

1. Give the name of the place where immune cells are formed and mature.

a. tonsils, spleen
b. bone marrow
c. thymus
d. lymph nodes

2. Give the name of the storage site for immune cells

a. lymph nodes, tonsils, spleen
b. thymus
c. red bone marrow
d. yellow bone marrow

3. Which blood cells differentiate and multiply to a mature stage in the bone marrow?

a. Neutrophils
b. B lymphocytes
c. T lymphocytes
d. Red blood cells
e. Platelets

Primary lymphoid organs:

4. Which of these organs are considered primary lymphoid organs?

a. spleen b. thymus c. lymph nodes d. bone marrow
e. Mucosa-associated lymphoid tissue

5. Regarding intrathymic differentiation

a. The thymus is the central organ for T lymphocyte differentiation.
b. Intrathymic differentiation occurs only in the cortex.

c. It includes the acquisition and genetic rearrangements of the TCR (thyroxine receptor).
d. The thymus is an organ where the rate of lymphocyte proliferation is low.

6. Regarding the role of bone marrow

a. It is hematopoietic tissue, the site of differentiation, multiplication, and maturation of all blood cells.
b. It is the site of formation of macrophages and leukocytes, except T lymphocytes.
c. It is the site of differentiation of B lymphocytes.
d. This differentiation includes the acquisition of receptors for the antigen.

7. Regarding the thymus

a. The thymus is a central lymphoid organ.
b. The prothymocytes that populate the thymus originate from the bone marrow.
c. It increases in size until puberty and then involutes without disappearing completely in the elderly.
d. Thymocytes multiply in the medullary zone.

8. What is the effect of thymectomy?

a. in a newborn mouse? b. in an adult mouse? Justify your answer

No LT production (animal death) - no effect because LTs are present.

Secondary lymphoid organs:

1. In this list, which are the secondary lymphoid organs, formations, or tissues?

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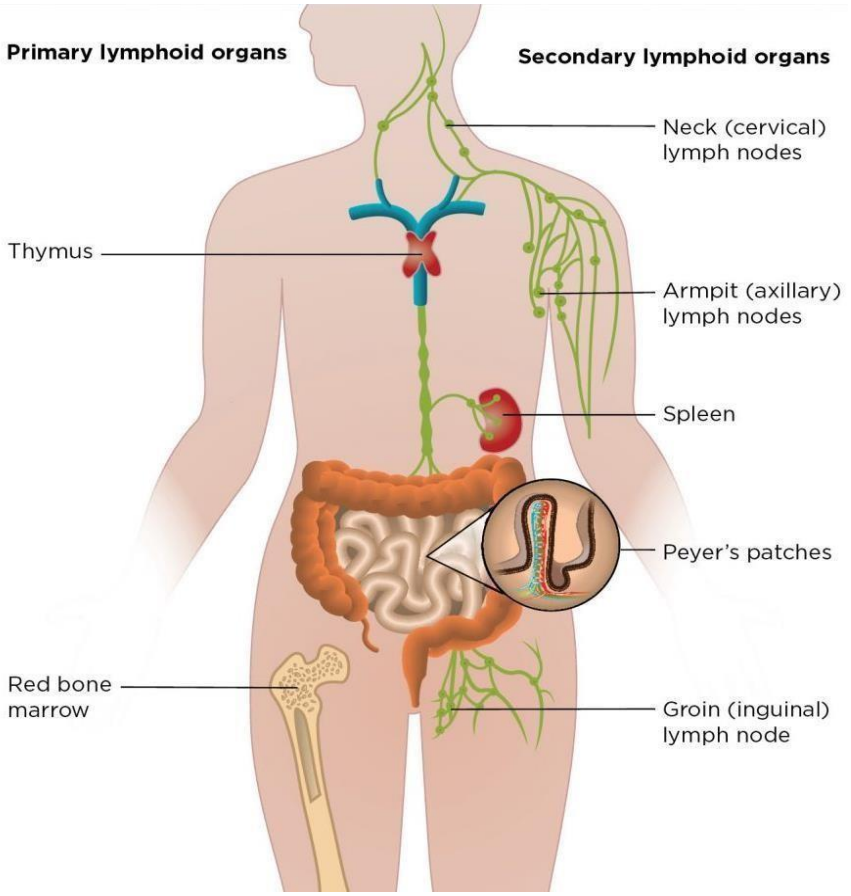
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8. In the following image, identify the main components of the defense system.



3- Immune responses

3.1 Nonspecific immune response

The non-specific immune response functions as the first line of defense against infection or injury. The innate immune system is always present at the site of infection and ready to fight the bacteria; it can also be referred to as the "natural" immune system. The cells of the innate immune system lack specificity and respond to each foreign invader using the same mechanism **(Marshall *et al*., 2018)**.

3.1.1 Anatomic barriers

Anatomic barriers to viruses exist at the body surfaces and within the body. At the body surfaces, the dead cells of the epidermis and any live cells that may lack viral receptors resist virus penetration and do not permit virus replication. However, this barrier is easily breached, for example, by animal bites (rabies virus), insect bites (togaviruses), and minor traumas (wart virus) **(Marshall *et al*., 2018)**.

At mucosal surfaces, only the mucus layer stands between the invading virus and live cells. The mucus layer forms a physical barrier that entraps foreign particles and carries them out of the body; it also contains nonspecific inhibitors. The mucus barrier is not absolute, however, since sufficient quantities of many viruses can overwhelm it and infect by this route. In fact, most viruses use mucous surfaces as the portal of entry and initial replication site **(Marshall *et al*., 2018)**.

3.1.2 Nonspecific inhibitors

Several viral inhibitors occur naturally in most body fluids and tissues. They vary chemically (lipids, polysaccharides, proteins, lipoproteins, and glycoproteins) and in the degree of viral inhibition and types of viruses affected. Some inhibitors are related to the viral receptors of the cell surface, but most are of unknown origin. Many inhibitors act by preventing the virus from attaching to cells, others by directly inactivating the virus, and a few by inhibiting virus replication **(Frischmeyer-Guerrerio & Schroeder, 2012)**.

In the gastrointestinal tract, some susceptible viruses are inactivated by acid, bile salts, and enzymes. Whereas most inhibitors block only one or a few viruses, some have a broad antiviral spectrum. Although the effectiveness of the inhibitors has not been fully established in vivo, their importance as host defenses is suggested by their antiviral activity in tissue culture and in vivo, and by the direct correlation between the virulence of some viruses and their resistance to certain inhibitors. Examples are the serum and mucus inhibitors of

influenza viruses during experimental infection **(Frischmeyer-Guerrerio & Schroeder, 2012)**.

However, even sensitive viruses may overwhelm these inhibitors when the infecting dose of virus is sufficiently high. Therefore, the presence of these inhibitors may explain the relatively high dose of virus required to initiate infection in vivo, compared with the dose needed in cell cultures **(Frischmeyer-Guerrerio & Schroeder, 2012)**.

3.1.3 Phagocytosis

Phagocytosis is a cellular process where a cell engulfs large particles (like bacteria, debris, or dead cells) by extending its membrane to form arm-like extensions (pseudopodia) that wrap around the particle, bringing it inside the cell within a vesicle called a phagosome, which then fuses with lysosomes for degradation, acting as a key defense mechanism for immune cells like macrophages and neutrophils. It's a vital form of endocytosis, crucial for immunity, tissue cleanup, and maintaining homeostasis **(Deshpande & Wadhwa, 2023)**.

3.1.3.1 Phagocytosis stages

- **Detection & binding:** The phagocyte's surface receptors recognize and bind to the target particle (bacteria, dead cells).
- **Engulfment:** The cell membrane extends, forming pseudopodia that surround the particle, a process involving actin cytoskeleton remodeling.
- **Phagosome Formation:** The membrane pinches off, internalizing the particle into a membrane-bound vesicle (phagosome).
- **Maturation & degradation:** The phagosome fuses with lysosomes, becoming a phagolysosome, which contains enzymes and acidic conditions to break down the engulfed material **(Deshpande & Wadhwa, 2023)**.

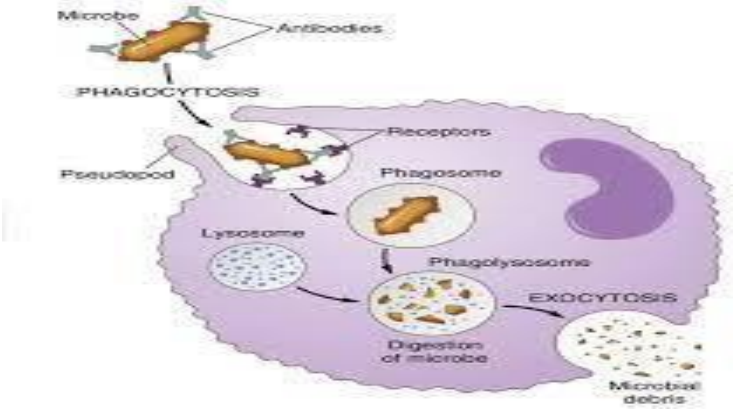


Figure 15: Different phagocytosis stages **(Deshpande & Wadhwa, 2023)**.

Tutorial objective: to understand the fundamental mechanisms of non-specific innate immunity.

This involves analyzing how the body detects an attack, deploys its effectors, and triggers the immune response while preparing tissue repair mechanisms.

Immunology tutorials series N2

EXERCICE 1:

A - A foreign microorganism that has just triggered an immune response is called an agent ……. Give some examples:……..……..……..

B - Recall the entry routes of microbes into the body:

……...……

……...…….

C – Before the activation of the immune system, are there other means that oppose the penetration of foreign substances?

……...……

D – How, for example, do mucous membranes prevent the penetration of an antigen?

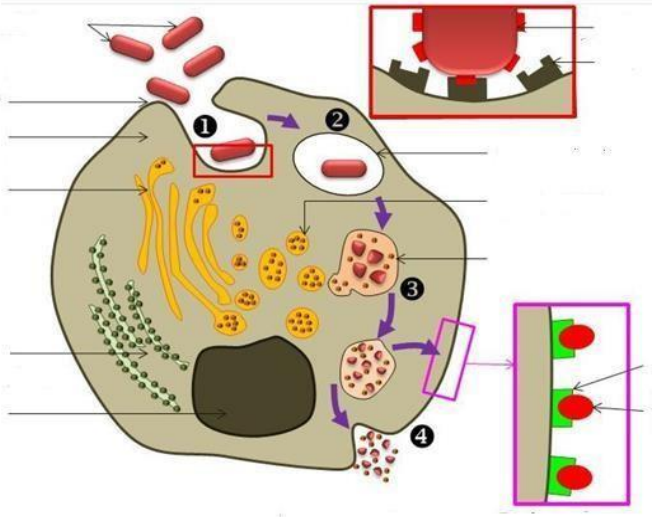
……...……

E – What are the four lines of defense of the body against pathogens?

……...……

EXERCICE 2:

Label the illustration below, indicating the different steps and giving it a title.



1- ……. 2 - ……. 3 - ……. 4- …….

Title: ……...……

EXERCICE 3: **- Multiple choice questions :**

1 – Innate immunity

a) – is characterized by an absence of immunological memory.
b) – develops within a few days.
c) – can be directed to the adaptive immune response.
d) – innate immunity against an infectious agent involves NK cells.

2 - Which component is part of the chemical barriers of innate immunity

a) – The movement of cilia in the respiratory epithelium.
b) – The stratum corneum of intact skin.
c) – Antibodies circulating in blood plasma.
d) – Lysozyme is present in tears and saliva.

3 – Dendritic cells

a) – are on the front line for capturing antigens.
b) – possess molecules of the major histocompatibility complex (HLA).
c) – largely differentiate between "self" and "non-self" through recognition structures such as TLRs (Toll-Like Receptors).
d) – are elements of the interface between innate and adaptive immunity.

4 – NK cells

a) – are part of the innate immune system.
b) – are produced in the bone marrow.
c) – the stimulation of KAR receptors induces NK cell cytotoxicity.
d) – have cytotoxicity restricted by the HLA complex
e) – their activity is increased by interferon gamma (IFN).

5 – Neutrophils

a) – are part of the adaptive immune system.
b) – are capable of phagocytosis.
c) – are capable of secreting pro- and anti-inflammatory cytokines.
d) – express Toll-like receptors (TLRs) on their surface.

6 - Anti-inflammatory drugs:

a) - prevent the migration of innate immune sentinel cells to an injury.
b) - completely stop the inflammatory response.
c) - prevent the release of certain chemical mediators of inflammation.
d) - prevent the multiplication of innate immune cells.

7 – Monocytes

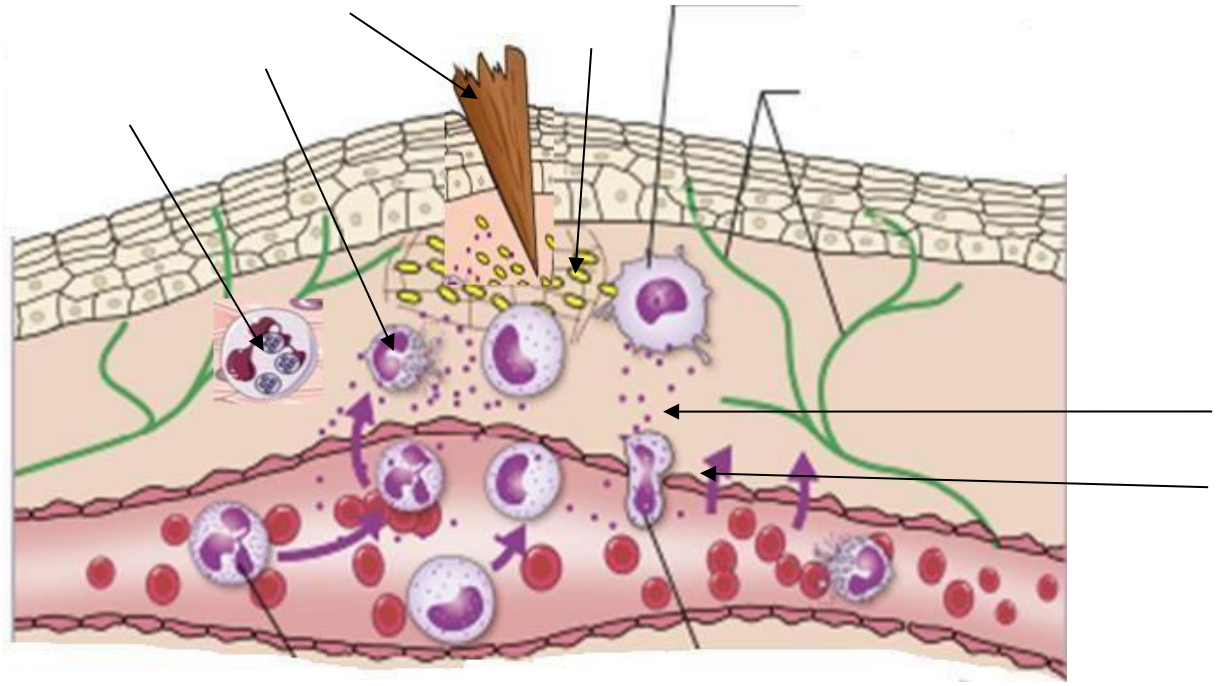
a) – are produced in the thymus.
b) – are capable of migrating to an inflammatory site.
c) – do not express PRRs (Pathogen Recognition Receptors) on their surface.
d) – can secrete significant amounts of cytokines.

8 – When antibody-producing cells are taken from an actively immunized individual and transferred to a new individual, is the immunization:

a) active.
b) passive.
c) adoptive.

EXERCICE 4:

The inflammatory response is a type of innate immune response. Using your knowledge, label the following diagram and list the four symptoms of this response.



Immunology tutorials series N2 (Solutions)

A - A foreign microorganism that has just triggered an immune response is called a pathogenic **agent**. Give **some examples:** viruses, bacteria, fungi.

B - Recall the entry routes of microbes into the body:

respiratory, digestive, cutaneous, and genital tracts.

C – Before the activation of the immune system, are there other means that oppose the penetration of foreign substances?

Skin and mucous membranes

D – How, for example, do mucous membranes prevent the penetration of an antigen?

Explanation of an example of a mucous membrane.

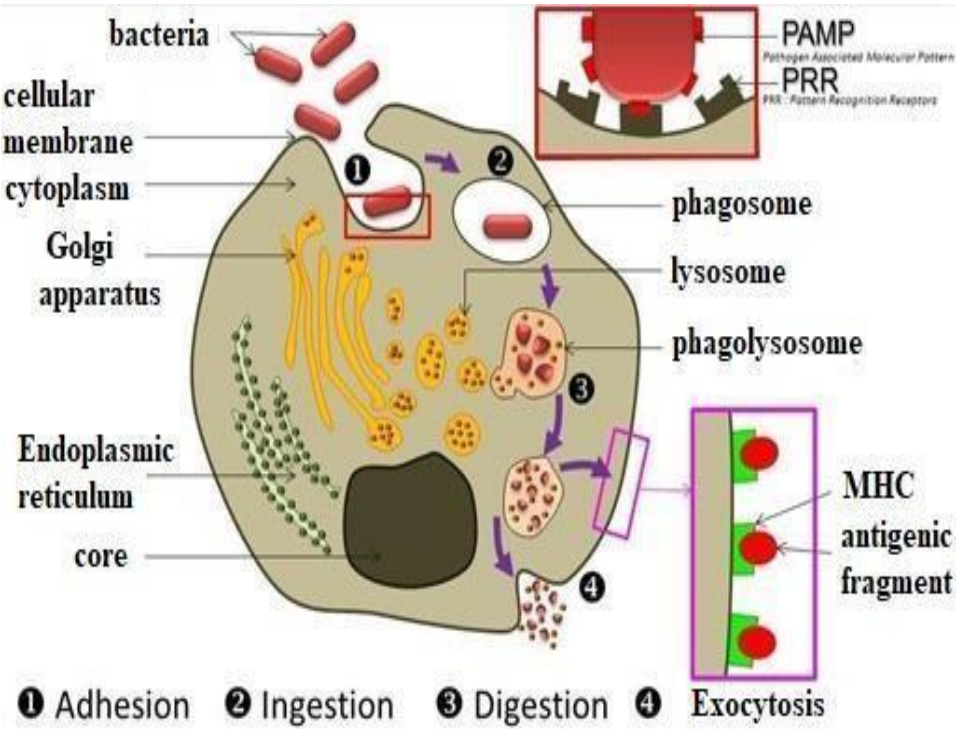
E – What are the four lines of defense of the body against pathogens?

Skin and mucous membranes, and inflammatory reaction ⟶ non-specific immunity

Humoral and cellular immune responses ⟶ specific immunity

EXERCICE 2:

Label the illustration below, indicating the different steps and giving it a title.



Title: The stages of phagocytosis and antigen presentation.

EXERCICE 3: - Multiple choice questions:

1 – Innate immunity

a) – is characterized by an absence of immunological memory.
b) – develops within a few days.
c) – can be directed to the adaptive immune response.
d) – innate immunity against an infectious agent involves NK cells.

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d) – The movement of cilia in the respiratory epithelium.
e) – The stratum corneum of intact skin.
f) – Antibodies circulating in blood plasma.
g) – Lysozyme is present in tears and saliva.

a) – Dendritic cells

b) – are on the front line for capturing antigens.
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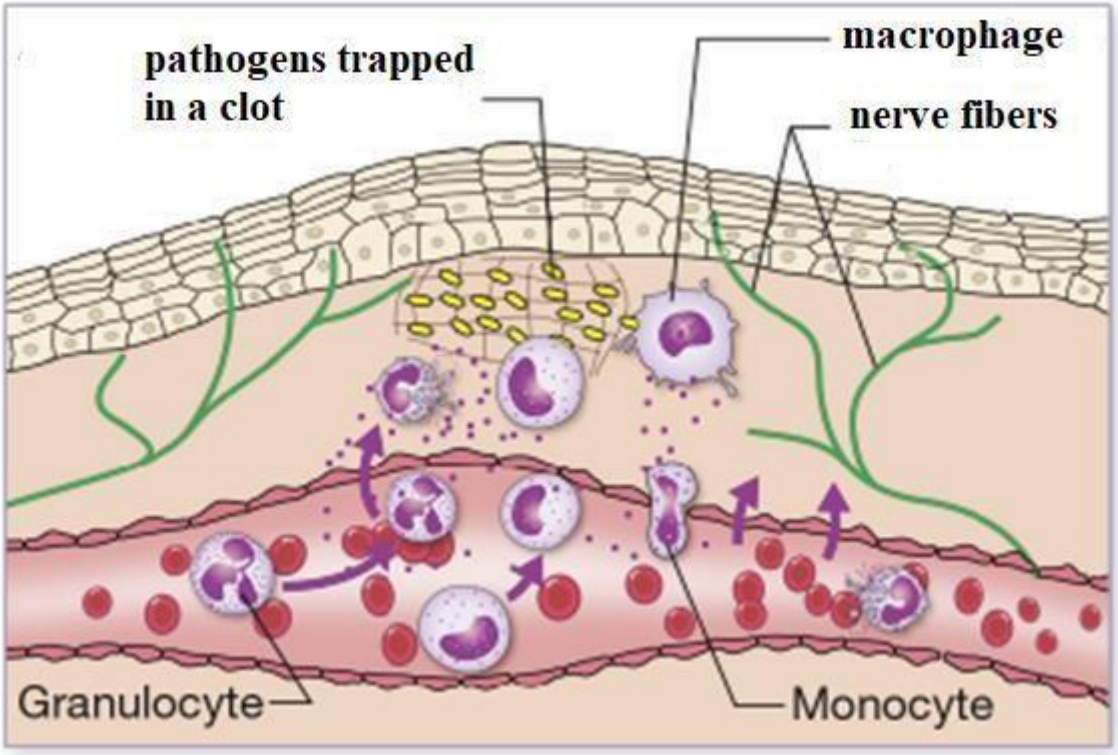
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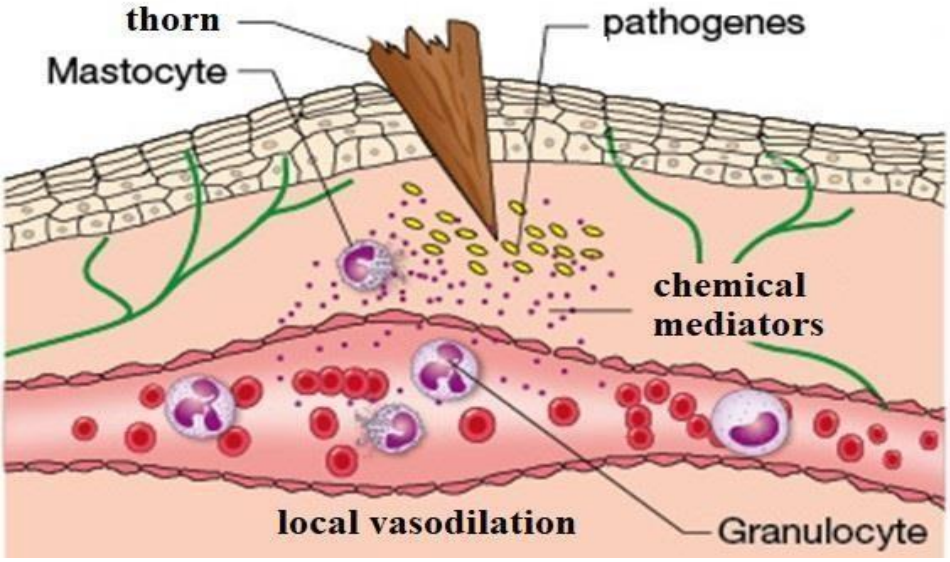
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c) adoptive.

EXERCICE 4:

The inflammatory response is a type of innate immune response. Using your knowledge, label the following diagram and list the four symptoms of this response.





3.2 Specific immune response

The specific immune response, or adaptive immunity, is the body's tailored defense against particular pathogens, involving lymphocytes (T and B cells) that recognize specific antigens, create memory for future encounters, and mount targeted attacks through cell-mediated (T-cells killing infected cells) and humoral immunity (B-cells producing antibodies) for faster, more effective defense over time **(Weisberg *et al.*, 2021)**.

3.2.1 Humoral immunity

Humoral immunity refers to antibody production and the coinciding processes that accompany it, including: Th2 activation and cytokine production, germinal center formation and isotype switching, and affinity maturation and memory cell generation. It also refers to the effector functions of antibodies, which include pathogen and toxin neutralization, classical complement activation, and opsonin promotion of phagocytosis and pathogen elimination **(Weisberg *et al.*, 2021)**.

3.2.1.1 B lymphocyte activation

When a B cell encounters an antigen, a signal is activated, the antigen binds to the receptor, and is taken inside the B cell by endocytosis. The antigen is processed and presented on the B cell's surface again by MHC-II proteins. The MHC-II proteins are recognized by helper T cells, stimulating the production of proteins, allowing for B cells to multiply and the descendants to differentiate into antibody-secreting cells circulating in the blood. B cells can be activated through certain microbial agents without the help of T-cells and can work directly with antigens to respond to pathogens present **(Sebina & Pepper, 2018)**.

3.2.1.2 B lymphocyte proliferation

The B cell waits for a lymphocyte T helper cell (LTh) to bind to the complex. This binding will activate the LTh cell, which then releases cytokines that induce B cells to divide rapidly, making thousands of identical clones of the B cell. These daughter cells either become plasma cells or memory cells. The memory B cells remain inactive here; later, when these memory B cells encounter the same antigen due to reinfection, they divide and form plasma cells. On the other hand, the plasma cells produce a large number of antibodies, which are released freely into the circulatory system **(Sebina & Pepper, 2018)**.

3.2.1.3 Antibody-antigen reaction

These antibodies will encounter antigens and bind with them. This will either interfere

with the chemical interaction between host and foreign cells or may form bridges between their antigenic sites, hindering their proper functioning. Their presence might also attract macrophages or killer cells to attack and phagocytose them **(Sebina & Pepper, 2018)**.

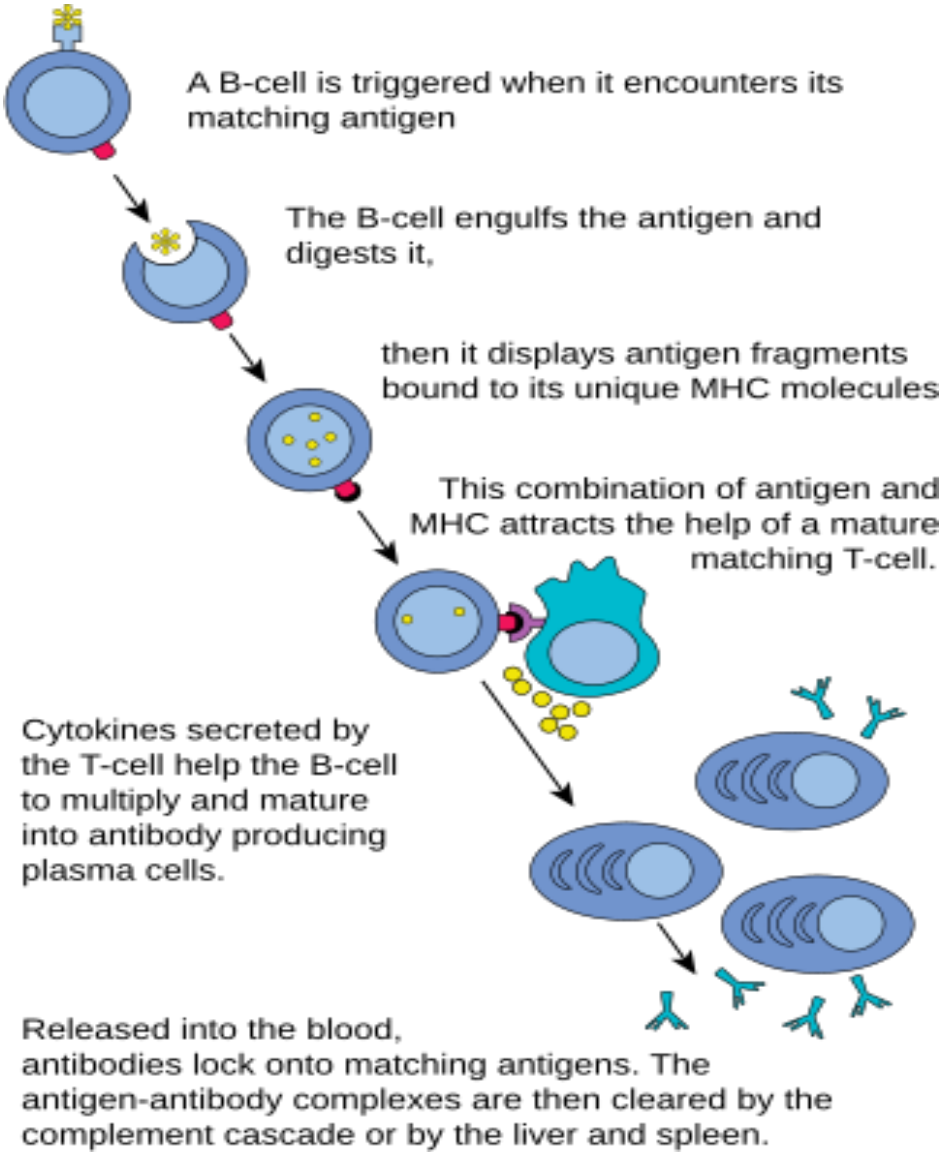


Figure 16: B lymphocyte activation is a large part of the humoral immune response **(Sebina & Pepper, 2018)**.

3.2.2 Cellular immunity

In cell-mediated immune responses, the second class of adaptive immune responses, activated T cells react directly against a foreign antigen presented to them on the surface of a host cell. The T cell, for example, might kill a virus-infected host cell that has viral antigens on its surface, thereby eliminating the infected cell before the virus has had a chance to replicate. In other cases, the T cell produces signal molecules that activate macrophages to destroy the invading microbes that they have phagocytosed **(Schmidt *et al.*, 2021)**.

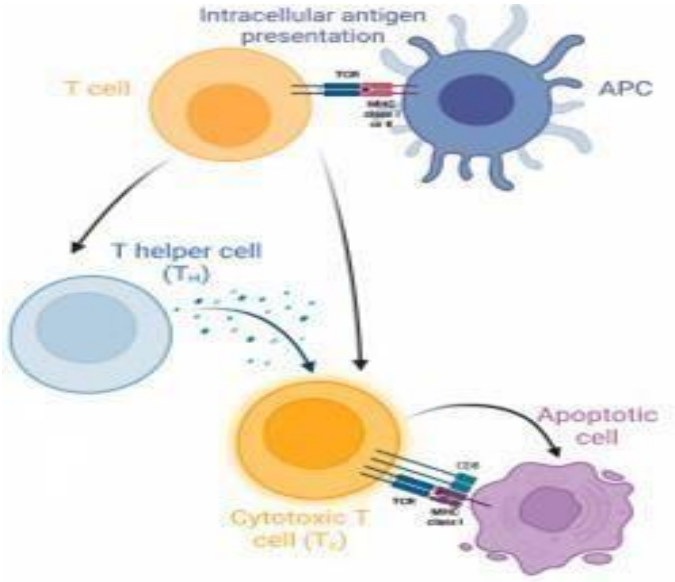


Figure 17: Interactions between the antigen-presenting cell (APC) and the T cell **(Schmidt *et al.*, 2021)**.

3.2.2.1 Cytotoxic $CD8^+$ T cells

These cells migrate throughout the body and are critical for eliminating viruses and bacteria that may reside in the cellular cytoplasm. They react to cells that display MHC I complexes. They will release molecules that kill target cells directly **(Schmidt *et al.*, 2021)**.

3.2.2.2 Differentiation of LTh

During an immune response, APCs endocytose antigens (typically bacteria or viruses), which undergo processing, then travel from the infection site to the lymph nodes. Typically, the APC responsible is a dendritic cell. If the antigen expresses appropriate molecular patterns, it can induce maturation of the dendritic cell, which results in enhanced expression of costimulatory molecules needed to activate T cells and MHC Class II.[12] Once at the lymph nodes, the APCs begin to present antigen peptides that are bound to Class II MHC, allowing $CD4^+$ T cells that express the specific TCRs against the peptide/MHC complex to activate **(Basu *et al.*, 2021)**.

Having received the first TCR/CD3 signal, the naïve T cell must activate a second independent biochemical pathway, known as Signal 2. This verification step is a protective measure to ensure that a T cell is responding to a foreign antigen. If this second signal is not present during initial antigen exposure, the T cell presumes that it is auto-reactive. This results in the cell becoming anergic (anergy is generated from the unprotected biochemical changes of Signal 1). Anergic cells will not respond to any antigen in the future, even if both signals

are present later on. These cells are generally believed to circulate throughout the body with no value until they undergo apoptosis **(Basu *et al*., 2021)**.

Once the two-signal activation is complete, the Th then allows itself to proliferate. It achieves this by releasing a potent T cell growth factor called interleukin 2 (IL-2), which acts upon itself in an autocrine fashion. Activated T cells also produce the alpha subunit of the IL-2 receptor (CD25 or IL-2R), enabling a fully functional receptor that can bind with IL-2, which in turn activates the T cell's proliferation pathways **(Basu *et al*., 2021)**.

The autocrine or paracrine secretion of IL-2 can bind to that same Th cell or neighboring Th's via the IL-2R, thus driving proliferation and clonal expansion. The Th cells receiving both signals of activation and proliferation will then become Th0 (T helper 0) cells that secrete IL-2, IL-4, and interferon gamma (IFN-γ). The Th0 cells will then differentiate into Th1 or Th2 cells depending on the cytokine environment. IFN-γ drives Th1 cell production while IL-10 and IL-4 inhibit Th1 cell production. Conversely, IL-4 drives Th2 cell production, and IFN-γ inhibits Th2 cells. These cytokines are pleiotropic and carry out many other functions of the immune response **(Basu *et al*., 2021)**.

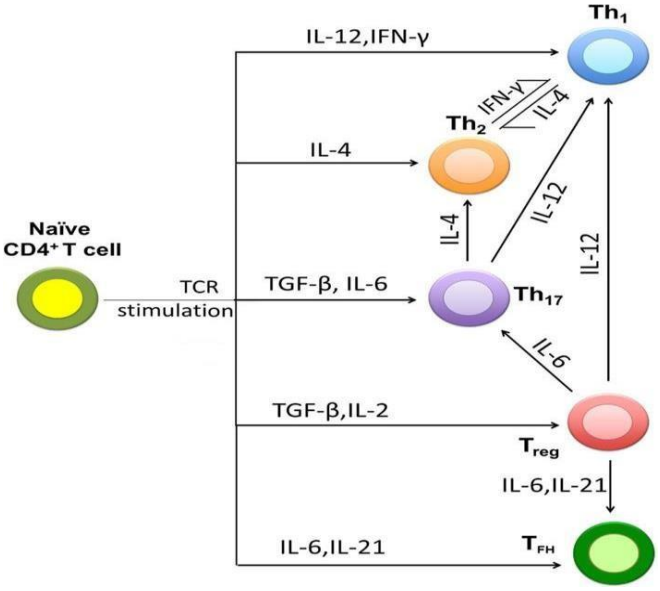


Figure 18: Major pathways of naïve $CD4^+$ T cell differentiation into effectors **(Basu *et al*., 2021)**.

3.2.2.3 LTh1 cells

These cells express CD4+. These cells will migrate through tissues and will recognize MHC II/Peptide complexes as recognized and displayed on macrophages. Gamma interferon is produced and is encountered by the macrophages. This tends to enhance the killing ability of the macrophages. This cell also has chemokine receptors (CXCR3A, CCR5), which allow for enhanced mobility to inflammation sites or areas **(Harrison, 2016)**.

3.2.2.4 LTh2 cells

These cells also express $CD4^+$. However, in this case, they migrate to the lymph nodes, where they encounter activated B cells that are expressing microbial peptide MHC on their surface. This is a result of the B cell essentially phagocytizing at least some protein fragment from the microbe, if not the whole organism, and is now expressing and displaying the processed microbial peptides by MHC II. Th2 cells, upon encounter with B cells (with the peptide-MHC II on their surface) will produce IL-4 and IL-5. These cytokines induce target B-cells to differentiate into plasma cells with the result of antibody production. This is the best example in immunology of "collaboration" between humoral and cell-mediated immunity **(Harrison, 2016)**.

Immune Memory. Immune memory, or as it is referred to in some of the older literature, as the anamnestic response, T memory cells are produced. They circulate in the blood (effector T memory) or are found in tissues (resident T memory). They may be either T8 or T4 cells. T memory cells (CD8) may be activated by a virus and engage an inflammatory cytokine response. Circulating T memory cells are found associated with macrophages in the mucosa. The most important response that is elicited is the humoral immune response. This response is activated to a much faster extent and to a greater degree than the primary response to an antigen. This is due to the programming of these memory T cells. The anamnestic response also applies to non-specific immunity, where an antigen is responded to more rapidly **(Harrison, 2016)**.

3.2.3 Adhesion and costimulatory molecules

3.2.3.1 Adhesion molecules

Adhesion molecules allow the dendritic cell to remain attached long enough to transmit peptide recognition and danger signals to the T lymphocyte. Adhesion molecules are found on T cells alongside ligands on the dendritic cell **(Verma & Kelleher, 2017)**.

Adhesion molecules belong to the family of heterodimeric proteins called integrins; for example, the protein LFA-1 (Leukocyte Function Associated Antigen 1) binds to the protein ICAM-1 (Intracellular Adhesion Molecule 1) on the dendritic cell **(Verma & Kelleher, 2017)**.

3.2.3.2 Costimulatory molecules

Research has identified a host of co-signaling molecules that modulate the immune responses by T and B lymphocytes. Co-signaling molecules have been shown to have both

positive and negative modulatory effects on T and B cell activation. R&D Systems offers products for both co-stimulatory and co-inhibitory molecules in both the Immunoglobulin superfamily (IgSF) and the Tumor Necrosis Factor (TNF) receptor superfamily. Molecules in the IgSF are further classified as members of the B7/CD28 family, T cell immunoglobulin and mucin (TIM) family, signaling lymphocyte activation molecule (SLAM) family, butyrophilin family, and nectin and nectin-like binding receptor family **(Chen & Flies, 2013)**.

There are two classes of co-stimulatory molecules:

- The CD80/CD86 class, sometimes called B7-1/B7-2, interacts with CD28 or CTLA4 present on T cells, reducing the signal strength needed to activate the T cell.
- The CD40/CD40 ligand class, belonging to the tumor necrosis factor receptor family, ensures a regulatory mechanism on CD80/CD86 and is involved in the release of interleukin-12.

In the absence of co-stimulation, the T cell does not undergo clonal expansion. The T cell is therefore unable to inhibit infection. Co-stimulation is a mechanism that ensures the T cell is only activated by pathogens **(Chen & Flies, 2013)**.

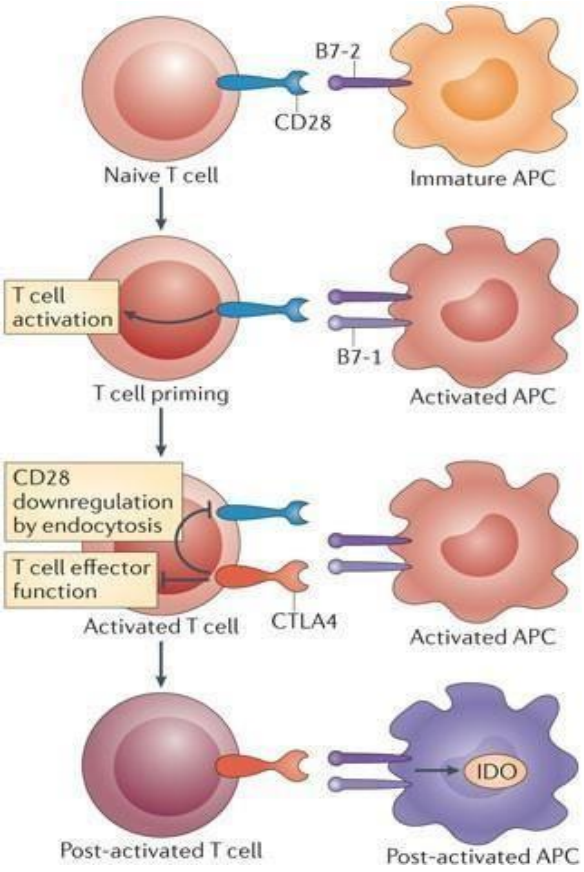
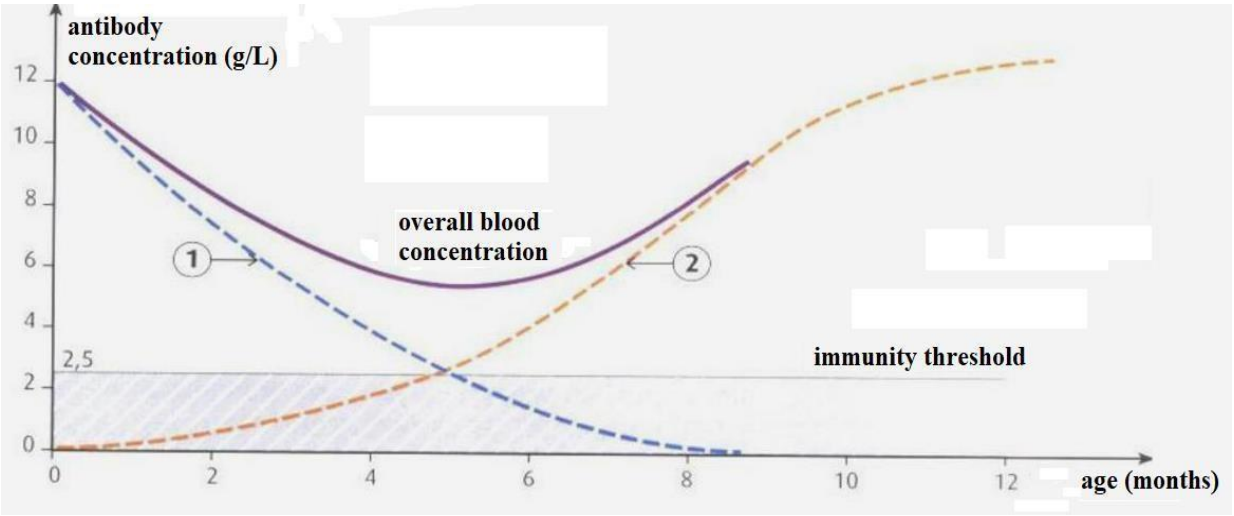


Figure 19: Molecular mechanisms of T cell co-stimulation and co-inhibition **(Chen & Flies, 2013)**.

Tutorial objective: to comprehend how the body recognizes and eliminates specific pathogens in a targeted manner. It aims to master the cellular and molecular mechanisms that give the immune system its learning, specificity, and memory properties.

Immunology tutorials series N3

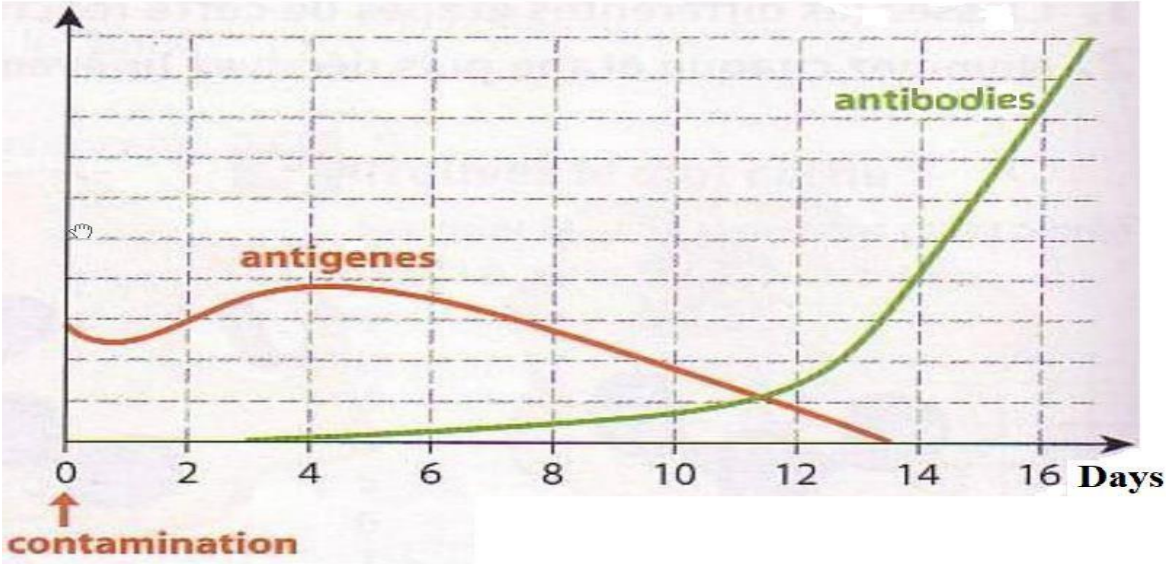
EXERCICE 1: - Consider the following graph: **curve 1: mother** and **curve 2: infant**



1. **Indicate what represents the ordinate axis, as well as the unit used**
2. **Indicate what represents the ordinate axis, as well as the unit used**
3. **Give a title to this graph.**
4. **Show how the number of antibodies transmitted by the mother evolves.**
5. **Show how the number of antibodies produced by the infant evolves.**
6. **Indicate the number of antibodies with which the infant is immunized.**

EXERCICE 2:

"Following contamination by pathogenic bacteria (which cause disease), the infected person's reaction is studied by taking regular blood samples. The quantity of antigens and antibodies present in these blood samples is measured. The results are shown in the two graphs below."



1. **Label the two variables on the y-axis (vertical axis).**
2. **Tag these two graphs.**
3. **Determine when the infection is at its peak. Justify your answer.**
4. **Describe the change in the antibody levels in the blood.**
5. **Determine when antibody production is sufficient for this person to recover. Justify your answer.**
6. **Determine when this person is completely cured. Justify your answer.**
7. **Determine at what point this person can be considered seropositive for this pathogenic bacterium?**

EXERCICE 3:

An allergic reaction is an abnormal response of the body to the introduction of a toxic substance. For the following four statements, fill in the missing words:

a) In type I hypersensitivity, the immune response is mediated by ……..and involves type ..... antibodies.

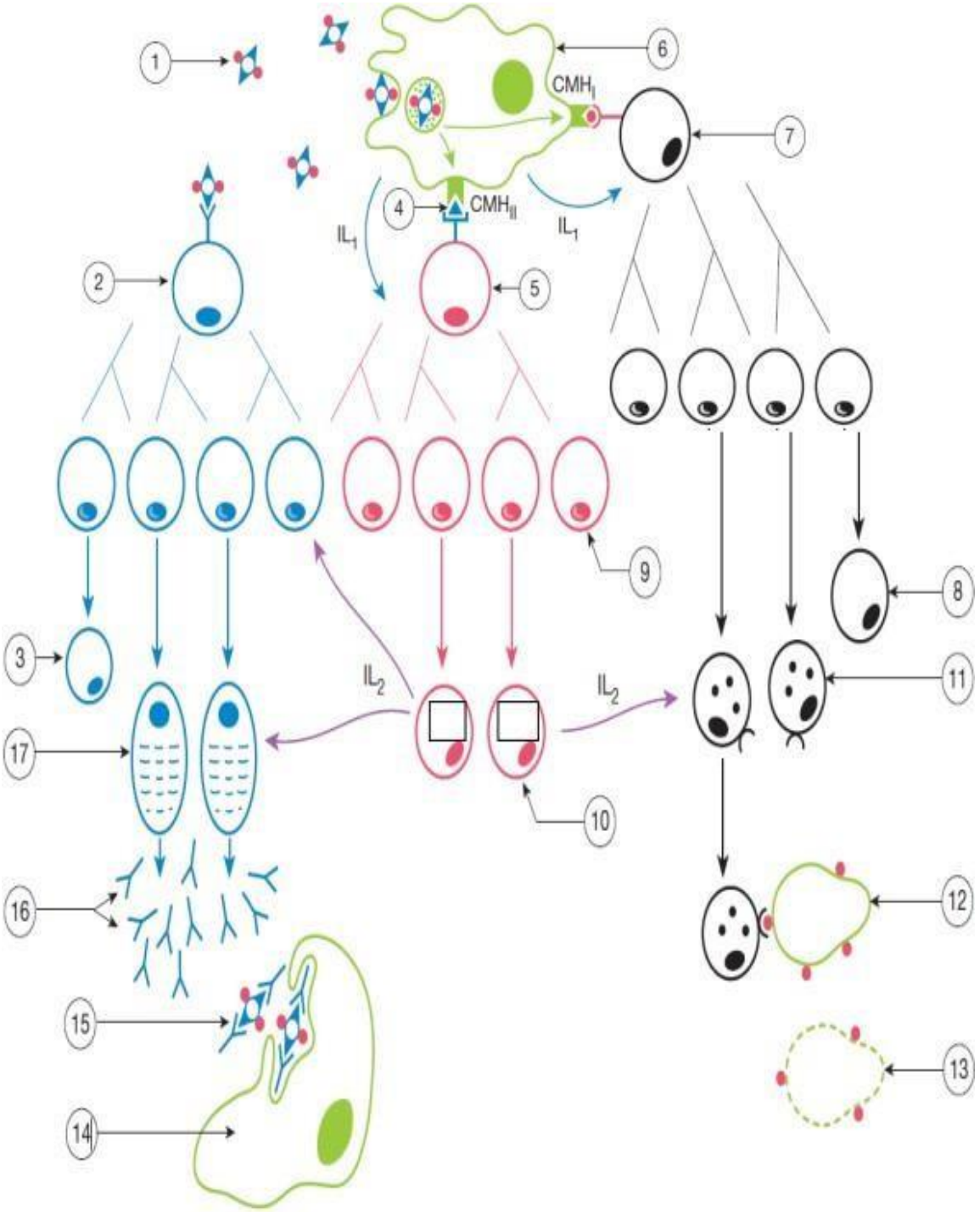
b) The antigen that triggers the reaction is then called ……

c) The manifestations of this type of allergy are due to the release of certain mediators, the main one being……

d) The cells that release this mediator are essentially the …….

EXERCICE 4:

The document below summarizes the main events of the specific immune response. Complete the document using your knowledge.


1
2
3
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11
12
13
14
15
16
17
CMH I
CMH II
IL1
IL1
IL2
IL2
19
18

Immunology tutorials series N3 (Solutions)

EXERCICE 1:

1. The x-axis represents the infant's age in months.
2. The y-axis represents the infant's antibody concentration in g/L.
3. Title: Graph of the evolution of the infant's antibody concentration.
4. The concentration of maternally derived antibodies decreases from birth, dropping from 12 g/L at birth to 0 g/L at 9 months.
5. The concentration of antibodies produced by the infant increases from birth, rising from 0 g/L at birth to 12 g/L at 12 months.
6. The antibodies produced by the infant allow them to maintain their own immunity from 5 months of age.

EXERCICE 2:

1.

- For the red curve, the vertical axis represents the quantity of antigens present in this person's blood.
- For the green curve, the vertical axis represents the quantity of antibodies present in this person's blood. (No unit specified; arbitrary unit).

2.

- The red curve shows the change in the quantity of antigens present in the blood of a person infected with pathogenic bacteria over the days following this infection.
- The green curve shows the change in the quantity of antibodies present in the blood of the same infected person over the days following this infection.

3. Infection is the multiplication of bacteria within the body. The graph shows that the number of bacteria reaches its maximum 4 days after infection.

4. Antibody production by B lymphocytes begins around the 6th day after infection and increases very gradually until the 11th day. From the 12th day onward, antibody production accelerates: a very large quantity of antibodies is produced in just a few days (4 days).

5. From 8 to 10 days after infection, the quantity of antigens decreases, which proves that the antibodies are beginning to be sufficient to eliminate them.

6. This person is cured from the 13th day onward because the red curve shows that their antigen level has become zero. This person is no longer carrying the pathogenic bacteria.

7. A person is said to be seropositive for an antigen when they have specific antibodies to that antigen in their blood. Since antibodies begin to appear in the blood around the 4th day after infection, it can be said that this is when they become seropositive.

EXERCICE 3:

An allergic reaction is an abnormal response of the body to the introduction of a toxic substance. For the following four statements, fill in the missing words:

a) In type I hypersensitivity, the immune response is humoral-mediated and involves type IgE antibodies.

b) The antigen that triggers the reaction is then called an allergen.

c) The manifestations of this type of allergy are due to the release of certain mediators, the main one being histamine.

d) The cells that release this mediator are essentially the mastocytes.

Definition of type 1 hypersensitivity: (or immediate), caused by mast cell degranulation induced by specific IgE antibodies, leading to the release of vasoactive mediators.

EXERCICE 4:

Key:
1- Antigen.
2- B lymphocyte: B cell.
3- Memory B cell.
4- Epitope.
5- T4 lymphocyte: CD4 cell.
6- APC: macrophage.
7- CD8+ T cell.
8- Memory CD8+ T cell: CD8 cell.
9- Memory CD4+ T cell.
10- Helper T cells (Th): there are two types of cells, left: Th2 and right: Th1.
11- Cytotoxic T cell.
12- Cell infected by a virus, bacterium, etc.
13- Cell lysis: destruction via CD8+ T cells.
14- Macrophage: phagocytosis and destruction of the immune complex.
15- Immune complex.
16- Antibody.
17- Plasma cell.
18- Cell-mediated immune response.
19- Humoral-mediated immune response.

4- Lymphocytes' receptors

Lymphocyte receptors are specialized proteins on T and B cells that recognize specific antigens to trigger adaptive immunity, primarily the T-Cell Receptor (TCR) for processed antigens via MHC molecules, and the B-Cell Receptor (BCR, essentially membrane-bound antibodies) for intact antigens, both generated through diverse V(D)J gene recombination for vast specificity. Other receptors, such as Toll-like receptors (TLRs) and Fc receptors, help modulate these responses by detecting pathogen patterns or antibody fragments, thereby linking innate and adaptive immunity **(Alcover *et al*., 2018)**.

4.1 B-cell receptor

The B-cell receptor (BCR) is a transmembrane protein on the surface of a B cell. A BCR is composed of a membrane-bound immunoglobulin molecule and a signal transduction moiety. The former forms a type 1 transmembrane receptor protein and is typically located on the outer surface of these lymphocyte cells. Through biochemical signaling and by physically acquiring antigens from the immune synapses, the BCR controls the activation of the B cell **(Yam-Puc *et al*., 2018)**.

The receptor's binding moiety is composed of a membrane-bound antibody that, like all antibodies, has two identical paratopes that are unique and randomly determined. A B cell is activated by its first encounter with an antigen (its "cognate antigen") that binds to its receptor, resulting in cell proliferation and differentiation to generate a population of antibody-secreting plasma B cells and memory B cells. The BCR has two crucial functions upon interaction with the antigen:

- One function is signal transduction, involving changes in receptor oligomerization.
- The second function is to mediate internalization for subsequent processing of the antigen and presentation of peptides to helper T cells **(Yam-Puc *et al*., 2018)**.

The BCR is composed of two parts:

- A membrane-bound immunoglobulin molecule of one isotype (IgD, IgM, IgA, IgG, or IgE). Except for the presence of a transmembrane alpha-helix, these are identical to their secreted forms.
- Signal transduction moiety: a heterodimer called Ig-α/Ig-β (CD79), bound together by disulfide bridges. Each member of the dimer spans the plasma membrane and has a cytoplasmic tail bearing an immunoreceptor tyrosine-based activation motif (ITAM) **(Yam-Puc *et al*., 2018)**.

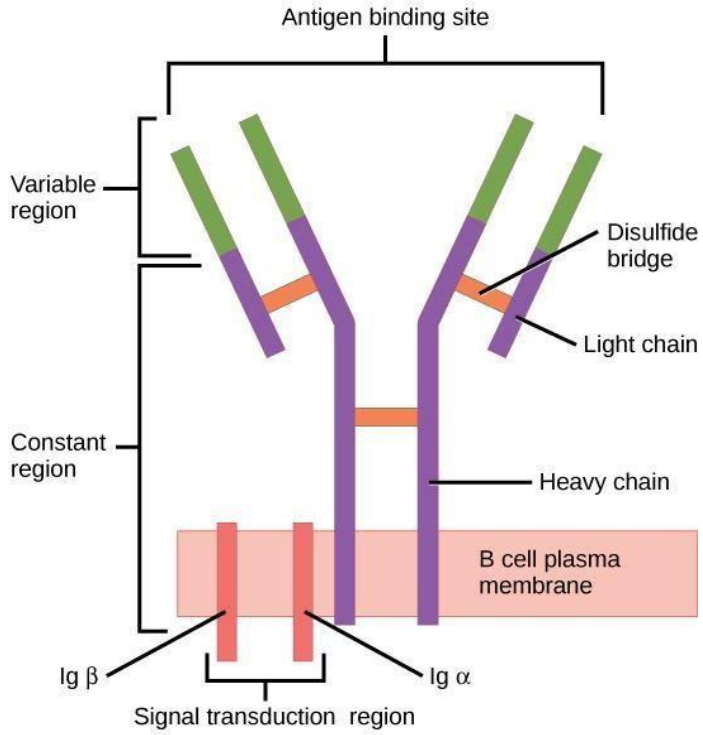


Figure 20: The general structure of the BCR **(Yam-Puc *et al*., 2018)**.

4.2 T-cell receptor

The T-cell receptor (TCR) is a protein complex, located on the surface of T cells (also called T lymphocytes). They are responsible for recognizing fragments of antigen as peptides bound to major histocompatibility complex (MHC) molecules. The binding between TCR and antigen peptides is of relatively low affinity and is biologically degenerate (that is, many TCRs recognize the same antigen peptide, and the same TCR recognizes many antigen peptides) **(Shah *et al*., 2021)**.

The TCR is composed of two different protein chains (that is, it is a heterodimer). In humans, in 95% of T cells, the TCR consists of an alpha (α) chain and a beta (β) chain, whereas in 5% of T cells, the TCR consists of gamma and delta (γ/δ) chains **(Shah *et al*., 2021)**.

When the TCR engages with antigenic peptide and MHC (peptide/MHC), the T lymphocyte is activated through signal transduction (that is, a series of biochemical events mediated by associated enzymes, co-receptors, specialized adaptor molecules, and activated or released transcription factors). Based on the initial receptor-triggering mechanism, the TCR is classified as belonging to the family of non-catalytic tyrosine-phosphorylated receptors (NTRs) **(Shah *et al*., 2021)**.

The TCR is a disulfide-linked membrane-anchored heterodimeric protein normally consisting of the highly variable alpha (α) and beta (β) chains expressed as part of a complex with the invariant CD3 chain molecules. T cells expressing this receptor are referred to as α:β (or αβ) T cells, though a minority of T cells express an alternate receptor, formed by variable gamma (γ) and delta (δ) chains, referred as γδ T cells **(Shah *et al.*, 2021)**.

The TCR is a member of the immunoglobulin superfamily, a large group of proteins involved in binding, recognition, and adhesion; the family is named after antibodies (also called immunoglobulins). The TCR is similar to a half-antibody consisting of a single heavy and single light chain, except the heavy chain is without its crystallizable fraction (Fc). The two main subunits of TCR (α- and β-chains) are twisted together. CD3 and zeta subunits are required to carry out the signal transduction. The MHC-TCR-CD3 interaction for T cells is functionally similar to the antigen(Ag)-immunoglobulin(Ig)-FcR interaction for myeloid leukocytes, and Ag-Ig-CD79 interaction for B cells **(Shah *et al.*, 2021)**.

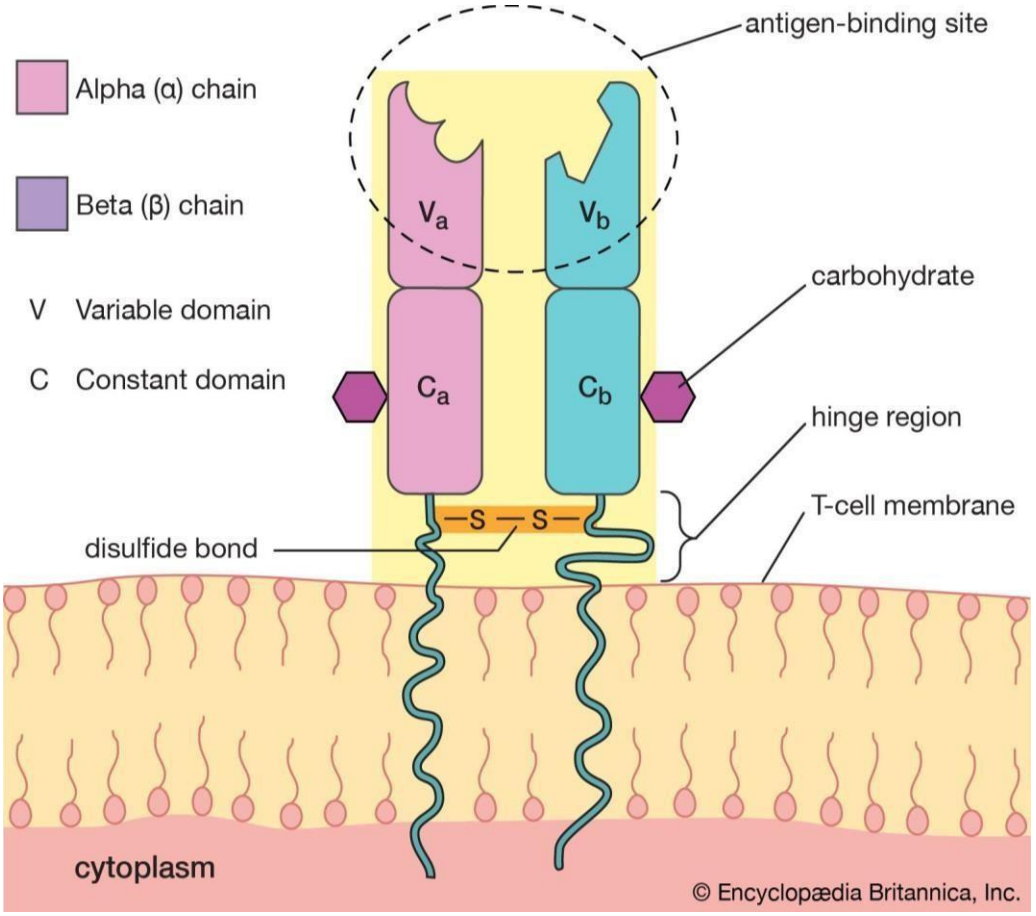


Figure 21: The general structure of the TCR **(Shah *et al.*, 2021)**.

5- Immunoglobulin

An antibody (Ab), or immunoglobulin (Ig), is a large protein belonging to the

immunoglobulin superfamily that is used by the immune system to identify and neutralize antigens such as bacteria and viruses, including those that cause disease. Each antibody recognizes one or more specific antigens **(Schroeder & Cavacini, 2010)**.

Each of the branching chains comprising the "Y" of an antibody contains a paratope (the antigen-binding site) that specifically binds to one particular epitope (a specific part of an antigen bound by the paratope) on an antigen, allowing the two molecules to bind together with precision. Using this mechanism, antibodies can effectively "tag" the antigen (or a microbe or an infected cell bearing such an antigen) for attack by cells of the immune system, or can neutralize it directly (for example, by blocking a part of a virus that is essential for its ability to invade a host cell) **(Schroeder & Cavacini, 2010)**.

Antibodies may be borne on the surface of an immune cell, as in a B cell receptor, or they may exist freely by being secreted into the extracellular space. The term antibody generally refers to the free (secreted) form, while the term immunoglobulin can refer to either form. Since they are, broadly speaking, the same protein, the terms are often treated as synonymous **(Schroeder & Cavacini, 2010)**.

To allow the immune system to recognize millions of different antigens, the antigen-binding paratopes at each tip of the antibody come in an equally wide variety. The rest of an antibody's structure is much less variable; in humans, antibodies occur in five classes or isotypes: IgA, IgD, IgE, IgG, and IgM. Human IgG and IgA antibodies are also divided into discrete subclasses (IgG1, IgG2, IgG3, and IgG4; IgA1 and IgA2) **(Schroeder & Cavacini, 2010)**.

The class refers to the functions triggered by the antibody (also known as effector functions), in addition to some other structural features. Antibodies from different classes also differ in where they are released in the body and at what stage of an immune response. Between species, while classes and subclasses of antibodies may be shared (at least in name), their function and distribution throughout the body may be different, which complicates the use of animal models in studying antibodies. For example, mouse IgG1 is closer to human IgG2 than to human IgG1 in terms of its function **(Schroeder & Cavacini, 2010)**.

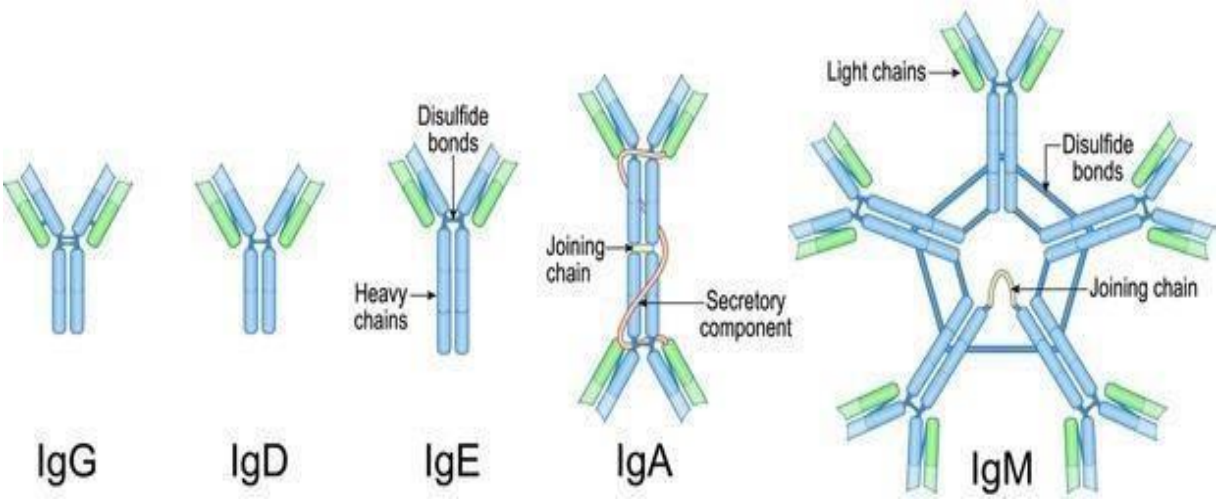


Figure 22: Structure of immunoglobulin **(Schroeder & Cavacini, 2010)**.

Antibodies arise from antibody-secreting cells, a term that encompasses plasmablasts and plasma cells. B cells themselves are not able to secrete antibodies. B cells can also differentiate into memory B cells, which can persist for decades, similarly to long-lived

plasma cells. These cells can be rapidly recalled in a secondary immune response, undergoing class switching, affinity maturation, and differentiating into antibody-secreting cells **(Schroeder & Cavacini, 2010)**.

Table 1: Five major classes of immunoglobulins with distinct roles in the immune response **(Schroeder & Cavacini, 2010)**.

Class	Primary Location	Functions
IgG	Blood, extracellular fluid	Long-term immunity, opsonization, neutralization of toxins, and complement activation.
IgA	Mucosal surfaces, secretions	Mucosal immunity protects the respiratory, gastrointestinal, and urogenital tracts.
IgM	Blood	First antibody produced during infection; efficient in agglutination and complement activation.
IgE	Blood, tissues	Mediates allergic responses and defense against parasites by activating mast cells and eosinophils.
IgD	B cell surface	Plays a role in B-cell activation; its function is not fully understood.

Tutorial objective: to assimilate the structure, function, and central role of immunoglobulins (Ig) in the body's defense. It allows for mastery of the mechanisms of humoral immunity.

Immunology tutorials series N4

1 - Restore the order in which the body's various defense mechanisms are activated during a serious infection: a) specific cellular/humoral immunity – b) mechanical defense – c) biochemical defense – d) cellular defense.

A - b, d, a, c. B - c, d, a, b. C - b, a, c, d. D - d, c, a, b. E - none of the above.

2 – What is the difference between an antigen and an immunogen?

……...……

……...……

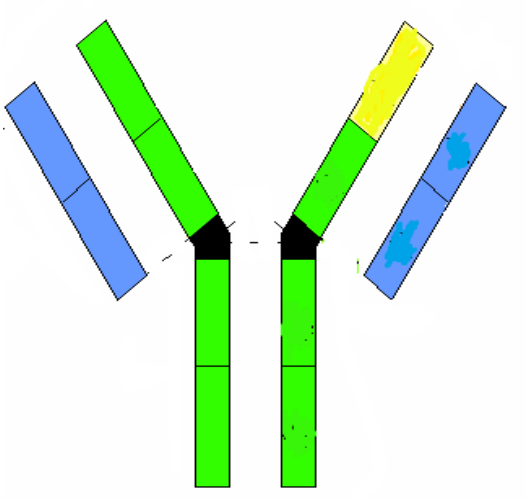
3 – What is the name given to the fraction of antigen that has the capacity to bind with specific antibodies? The fraction of the antibody binds to the antigen?

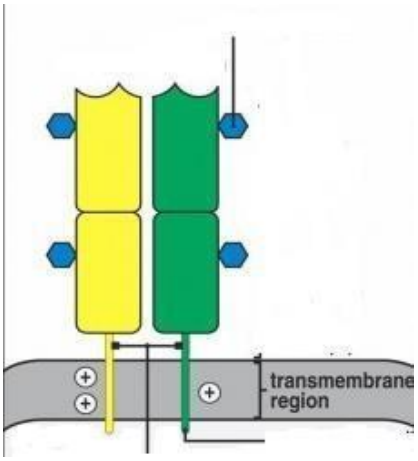
…… ……...……

4 – List the different classes and subclasses of immunoglobulins

……...……

5 – Annotate the following illustrations representing an immunoglobulin and a TCR





1)- What are the differences in the structure of a membrane antibody?

2)- What is the main difference between the types of antigens that bind to receptors on a B lymphocyte and those that bind to receptors on a T lymphocyte?

6 - What fragments can be obtained by the enzymatic degradation of an IgG molecule by papain?

a) – Two fragments lacking antibody activity.
b) – Fragments containing only the entire variable region of the heavy chains.
c) – Fragments containing only the entire variable region of the light chains.
d) – Two monovalent fragments possessing antibody activity.

7 - An antibody molecule (or molecules)

a) – can bind to two antigens of different types.
b) – form immune complexes.
c) – directly destroy bacteria.
d) – are polysaccharides belonging to the gammaglobulin family.

8 - To which part of an antibody does an epitope bind?

a) – To the effector domain of the antibody.
b) – To the constant regions of the heavy chain only.
c) – To the variable regions of both the heavy and light chains.
d) – To the constant regions of the light chain.

9 – Choose the correct statement(s) for each of the following statements:

- IgM

a) – are dimeric structures.
b) – are produced from the primary infection.
c) – cross the placental barrier.
d) – can be membrane-bound.
e) – correspond to natural antibodies.

- IgG

a) – cross the placental barrier.
b) – have two paratopes.
c) – are the most abundant in serum after a secondary immune response.
d) – have a γ-type heavy chain.

- IgA

a) – exist in two forms: serum and secretory.
b) – are present in serum at a higher concentration than IgG.
c) – are the main immunoglobulins in mucous secretions.
d) – are synthesized by the normal fetus.

6- B cells differ from T cells by the presence of:

a – CD3. b – CD4. c – CD8. d – Surface Ig. e – MHC class I antigen.

11 - Lymphocytes expressing CD3 and CD4 molecules on their surface:

a) – are T cells. b) – are always cytotoxic. c) – recognize MHC II. d) – CD3 is associated with the antigen receptor.

12 – T cell education: complete the following text

A - Immature T lymphocytes (pre-T lymphocytes), generated in..... travel towards the ……In the cortex of the latter, the pre-T cells acquire……, as well as two other receptors: ……. And ……. Then they come into contact with cortical epithelial cells which present them with class I and class II .....molecules.

B - T cells that do not recognize either of the two molecules..... die by apoptosis. A large proportion of T cells die in this way (positive selection).

C - The surviving T cells move to……, where cells present them with …… antigen. T cells that recognize these Ag with too much affinity are ….....(negative selection).

D - The surviving T cells (less than 1%!) lose their receptor …….. or .....The cells that react with the …….keep the.....molecule and lose the …….

Conversely, cells that bind with the ……. lose their ……. Mature T cells pass into the bloodstream and head towards the ……..where they may encounter potential ……...

13 – -Using a diagram, represent the immune response with Th1/Th2.

Immunology tutorials series N4 (Solutions)

1 - Restore the order in which the body's various defense mechanisms are activated during a serious infection: a) specific cellular/humoral immunity – b) mechanical defense – c) biochemical defense – d) cellular defense.

A - b, d, a, c. B - c, d, a, b. C - b, a, c, d. D - d, c, a, b. E - none of the above**.**

2 – What is the difference between an antigen and an immunogen?

An antigen is any molecule that can be recognized and bound by the immune system (like antibodies or T-cell receptors). At the same time, an immunogen is a specific type of antigen that can also trigger a full immune response (producing antibodies or T-cells). The key difference is that all immunogens are antigens. Still, not all antigens are immunogens, as some small molecules (haptens) are antigenic (bind) but need a carrier protein to become immunogenic (induce a response).

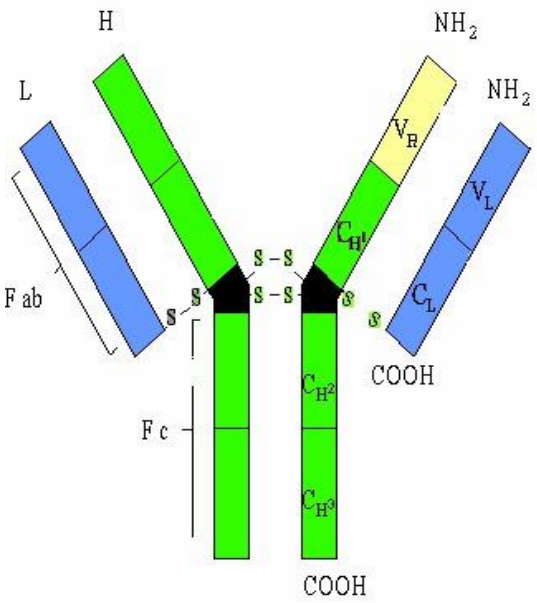
3 – What is the name given to the fraction of antigen that has the capacity to bind with specific antibodies? The fraction of the antibody binds to the antigen?

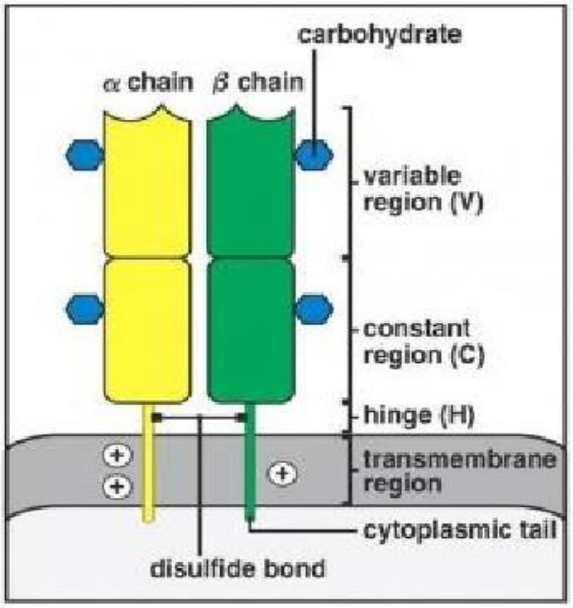
-EPITOPE -PARATOPE

4 – List the different classes and subclasses of immunoglobulins

There are five main classes of immunoglobulins (antibodies): IgG, IgM, IgA, IgD, and IgE, distinguished by their heavy chains, with IgG and IgA further divided into subclasses (IgG1-4, IgA1-2) due to variations in their heavy chains, while IgM, IgD, and IgE generally lack subclasses in humans.

5 – Annotate the following illustrations representing an immunoglobulin and a TCR





1) – A secreted antibody has neither a transmembrane region nor a cytoplasmic domain.
2) - B lymphocyte receptors bind intact extracellular antigens present on the surface of microorganisms or in a free state in bodily fluids.
- T lymphocyte receptors bind small fragments of intracellular antigens that form complexes with MHC class I or class II molecules.

6 - What fragments can be obtained by the enzymatic degradation of an IgG molecule by papain?

a) – Two fragments lacking antibody activity.
b) – Fragments containing only the entire variable region of the heavy chains.
c) – Fragments containing only the entire variable region of the light chains.
d) – Two monovalent fragments possessing antibody activity.

7 - An antibody molecule (or molecules)

a) – can bind to two antigens of different types.
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d) – have a γ-type heavy chain.

- IgA

a) – exist in two forms: serum and secretory.
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10- B cells differ from T cells by the presence of:

a – CD3. b – CD4. c – CD8. d – Surface Ig. e – MHC class I antigen.

11 - Lymphocytes expressing CD3 and CD4 molecules on their surface:

a) – are T cells. b) – are always cytotoxic. c) – recognize MHC II. d) – CD3 is associated with the antigen receptor.

12 – T cell education: complete the following text

A - Immature T lymphocytes (pre-T lymphocytes), generated in bone marrow, travel towards the thymus. In the cortex of the latter, the pre-T cells acquire TCR, as well as two other receptors: CD4 and CD8. Then they come into contact with cortical epithelial cells, which present them with class I and class II MHC molecules.

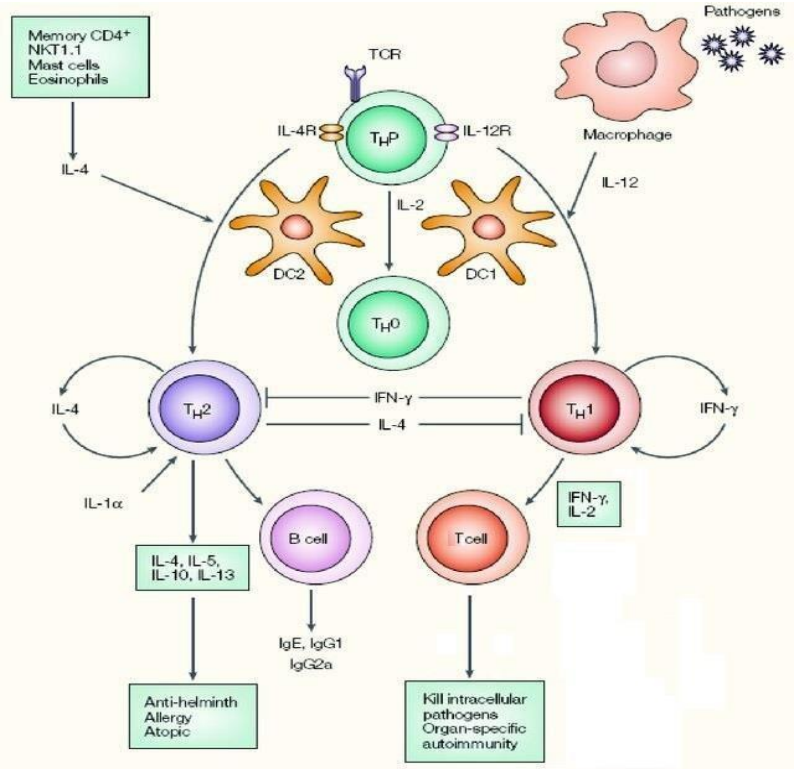
B - T cells that do not recognize either of the two molecules MHC die by apoptosis. A large proportion of T cells die in this way (positive selection).

C - The surviving T cells move to the medulla, where cells present them with self-antigens. T cells that recognize these Ag with too much affinity are destroyed (negative selection).

D - The surviving T cells (less than 1%!) lose their receptor CD4 or CD8. The cells that react with the MHC class I keep the CD8 molecule and lose the CD4.

Conversely, cells that bind with the MHC class II lose their CD8. Mature T cells pass into the bloodstream and head towards the secondary lymphoid organs, where they may encounter potential antigen.

13 – -Using a diagram, represent the immune response with Th1/Th2.



6- Major histocompatibility complex

Major histocompatibility complex (MHC), or human leukocyte antigen (HLA), proteins serve two general roles **(Karpiński *et al.*, 2020)**.

MHC proteins function as carriers to present antigens on cell surfaces. MHC class I proteins are essential for presenting viral antigens and are expressed by nearly all cell types, except red blood cells. Any cell infected by a virus can signal the problem through MHC class I proteins. In response, $CD8^+$ T cells (also called CTLs) will recognize and kill infected cells. MHC class II proteins are generally only expressed by antigen-presenting cells like dendritic cells and macrophages. MHC class II proteins are important for presenting antigens to $CD4^+$ T cells. MHC class II antigens are varied and include both pathogen- and host-derived molecules **(Karpiński *et al.*, 2020)**.

MHC proteins also signal whether a cell is a host cell or a foreign cell. They are very diverse, and every person has a unique set of MHC proteins inherited from his or her parents. As such, there are similarities in MHC proteins between family members. Immune cells use MHC to determine whether or not a cell is friendly. In organ transplantation, the MHC or HLA proteins of donors and recipients are matched to lower the risk of transplant rejection, which occurs when the recipient's immune system attacks the donor tissue or organ. In stem cell or bone marrow transplantation, improper MHC or HLA matching can result in graft-versus-host disease, which occurs when the donor cells attack the recipient's body **(Karpiński *et al.*, 2020)**.

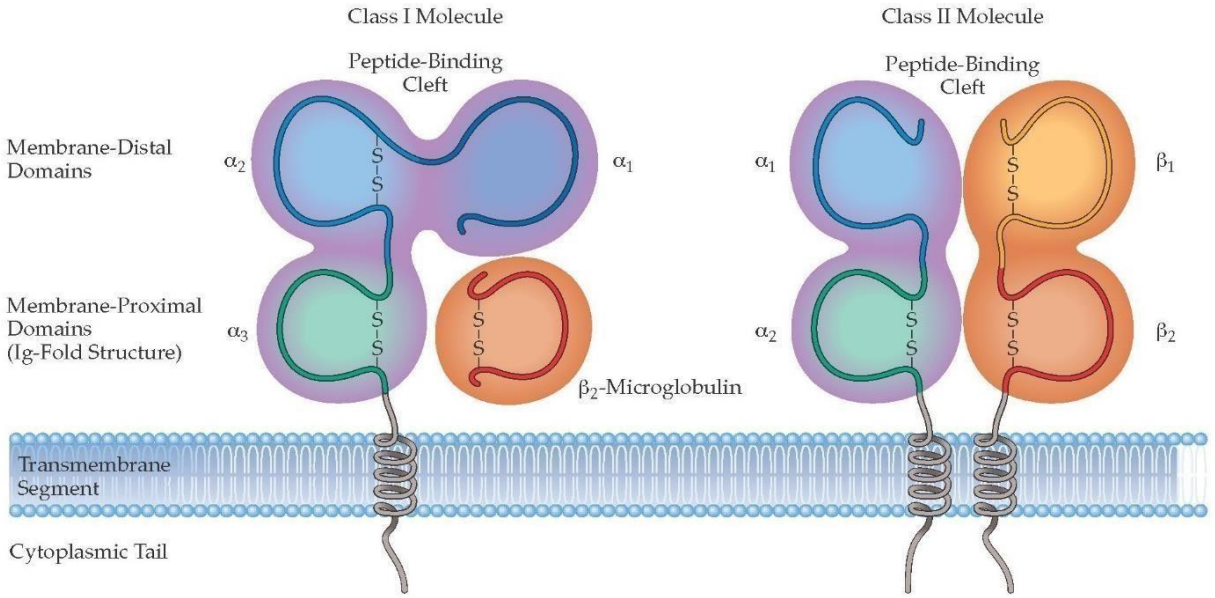


Figure 23: Major histocompatibility complex structure **(Karpiński *et al.*, 2020)**.

Tutorial objective: to assimilate the molecular and genetic mechanisms of antigen presentation, enabling the immune system to distinguish self from non-self and to coordinate the adaptive immune response.

Immunology tutorials series N5

1 - What do MHC class I and II proteins mean? Where are they found? What is their main role?

2 - MHC:

a. constitutes a set of cell markers specific to erythrocytes.
b. is not expressed on trophoblast cells.
c. is expressed abundantly on neurons, which explains the difficulties of nerve tissue transplantation.
d. is altered in tumor cells.
r. characterizes each individual.
f. is identical for individuals of the same family.

3 - MHC molecules are:

a- glycoproteins.
b- of the immunoglobulin family.
c- present only on immune cells.
d- present on all cells of the body.
e- responsible for blood groups.
f- encoded by highly polymorphic genes.

4 - Which molecule is not encoded by genes of the major histocompatibility complex (MHC)?

a – Beta-2 microglobulin.
b – HLA class I antigen.
c – HLA class I antigen.
d – C4.
e – C2.

5 – Human MHC molecules are glycoproteins encoded by genes:

a- Located on chromosomes 7 and 14.
b- Of which there are a large number of allelic variants.
c- Subject to regulation by interferons.
d- Undergoing gene rearrangements during fetal development.
e- Expressed only during the immune response.

6 – The following molecules are part of MHC class I:

a - HLA-A. b- HLA-F. c- HLA-DP. d- HLA-G. e- HLA-DM. f- HLA-E.

7 – An HLA haplotype is:

a – the entire group of MHC alleles present on each chromosome.
b – a specific segment of the MHC locus.
c – genes outside the MHC focus that contribute to rejection.
d – an HLA-A allele.

8 - The role of MHC (Major Histocompatibility Complex) class I molecules is to:

a - Present antigens to CD8 T lymphocytes.
b - Regulate the cytotoxic activity of NK cells.
c - Degrade antigens.
d - Stimulate the innate immune response.
e - Bind peptides of 8 to 10 amino acids.

9 - Class 1 molecules of the HLA system:

a- Are encoded by genes in the A, B, and C loci in humans.
b- Are involved in the recognition of target cells by cytotoxic T lymphocytes.
c- Do not contain beta2-microglobulin.
d- Only appears on T lymphocytes after activation.
e- A decrease in its expression on the cell surface triggers the activity of NK lymphocytes, which destroy the cell.

10 - a) What do the genes listed above represent?

A3C4B35DR5DQ1DP2 **A28C3B50DR14DQ9DP5**
A19C1B5DR4DQ1DP6 **A19C2B20DR7DQ10DP6**

b)- Indicate the possible genotype of a child of this couple in the most frequent case where there is no recombination between homologous chromosomes.
c)- How many different genotypes are possible for the children of this couple?
d)- What is the purpose of HLA typing?
e)- For these six genes, is there dominance/recessiveness or codominance? What is the consequence at the phenotype level?

11 - Class II HLA molecules:

a- are dimers of polypeptide chains.
b- are formed by a heavy chain associated with β2 microglobulin.
c- are the products of genes on chromosome 6.
d- are involved in antigen presentation mechanisms.
e- are present on the majority of cells in the body.

12 – The Nazim family consists of 5 children, including two twins. One of the twins is awaiting a kidney transplant. The mother would like to understand why the other twin is the best candidate for this organ donation.

13 - MHC class III genes encode for:

a. A component of the alternative complement pathway.
b. HLA-C.
c. TNF.

Immunology tutorials series N5 (Solutions)

1 - MHC proteins are divided into two categories: class I and class II.

- Class I proteins are encoded by the MHC genes A, B, and C. These proteins are found on the surface of all cells in the body.
- Class II proteins are encoded by the other MHC genes: DR, DQ, and DP. These proteins are found only on the surface of lymphocytes and macrophages (a type of leukocyte).
- Thanks to the wide diversity of alleles, MHC proteins are true self-markers, antigen displays, and play a role in thymic selection. They are primarily responsible for many organ transplant rejections and provide protection against autoimmune diseases (multiple sclerosis, type 1 diabetes, lupus, etc.).

2 - MHC:

a. constitutes a set of cell markers specific to erythrocytes.
b. is not expressed on trophoblast cells.
c. is expressed abundantly on neurons, which explains the difficulties of nerve tissue transplantation.
d. is altered in tumor cells.
r. characterizes each individual.
f. is identical for individuals of the same family.

3 - MHC molecules are:

a- glycoproteins.
b- of the immunoglobulin family.
c- present only on immune cells.
d- present on all cells of the body.
e- responsible for blood groups.
f- encoded by highly polymorphic genes.

4 - Which molecule is not encoded by genes of the major histocompatibility complex (MHC)?

a – Beta-2 microglobulin.
b – HLA class I antigen.
c – HLA class I antigen.
d – C4.
e – C2.

5 – Human MHC molecules are glycoproteins encoded by genes:

a- Located on chromosomes 7 and 14.
b- Of which there are a large number of allelic variants.
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c- Do not contain beta2-microglobulin.
d- Only appears on T lymphocytes after activation.
e- A decrease in its expression on the cell surface triggers the activity of NK lymphocytes, which destroy the cell.

10 - a)- the genes listed above represent:

Father's HLA $\frac{\text{A3C4B35DR5DQ1DP2}}{\text{A19C1B5DR4DQ1DP6}}$ x Mother's HLA $\frac{\text{A28C3B50DR14DQ9DP5}}{\text{A19C2B20DR7DQ10DP6}}$

It represents the genotypes of two people for their six HLA genes carried by chromosome 6.

b)- A possible genotype of a child of this couple could be:

$\frac{\text{A3C4B35DR5DQ1DP2}}{\text{A19C2B20DR7DQ10DP6}}$

c)- **Four different genotypes** are possible for the children of this couple since each of the two parents randomly provides one of their two chromosomes 6.

d)- HLA typing is absolutely crucial before any organ or tissue transplant. The molecules synthesized by these genes constitute, on the surface of nucleated cells, the MHC or major histocompatibility complex.

e) For these six genes, there is **codominance** of the different alleles. Consequently, at the phenotype level, all nucleated cells of a heterozygous organism will carry **six different proteins** (HLA A, B, C genes expressed), and the cells of the immune system will carry **twelve different proteins** (HLA A, B, C, DP, DQ, DR genes expressed).

11 - Class II HLA molecules:

a- are dimers of polypeptide chains.
b- are formed by a heavy chain associated with β2 microglobulin.
c- are the products of genes on chromosome 6.
d- are involved in antigen presentation mechanisms.
e- are present on the majority of cells in the body.

12 – Martin family: to avoid rejection, the tissue sought for the graft is one whose MHC system is as close as possible to that of the patient. That is to say, tissue that carries the same MHC markers as the cells of the transplant recipient. In this case, the twins have the same genetic material, their MHC systems are identical, and the graft will be accepted by the recipient's body (see: "Conditions for a successful transplant").

13 - MHC class III genes encode for:

a. A component of the alternative complement pathway.
b. HLA-C.
TNF.

7- Cytokines

Cytokines are signaling proteins that act as messengers for the immune system, coordinating responses to infection, inflammation, and injury by telling immune cells when to activate, proliferate, and differentiate. They regulate the intensity and duration of immune responses, promoting healing and fighting pathogens, but also have roles in fever, blood cell development (hematopoiesis), and even cancer, functioning through autocrine (self-signaling), paracrine (nearby cells), and endocrine (distant cells) pathways **(Kupsa *et al.*, 2012)**.

7.1 Basic classification of cytokines

Cytokines include different types of proteins that tell immune cells where to go and what to do to keep your immune system functioning correctly **(Kupsa *et al.*, 2012)**.

- **Chemokines:** Chemokines direct immune cells toward places in your body where they can fight infection.
- **Interferons:** Interferons signal cells to put up their defenses against viruses invading your body. In this way, interferons "interfere" in the process that allows viruses to replicate, or make more viruses once they've invaded a healthy cell.
- **Interleukins:** Interleukins get their name from "inter" which means between and "leukocyte," which is another name for a white blood cell. Originally, scientists thought that leukocytes alone released interleukins and only relayed messages to other leukocytes. But now we know that cells other than leukocytes release these proteins. Also, interleukins can relay messages between cells that aren't leukocytes.
- **Tumor necrosis factor (TNF):** TNF helps regulate inflammation in your body. TNF also signals to immune cells that kill tumor cells.
- **Colony-stimulating factors (CSF):** CSF signals hematopoietic stem cells to develop into specific cell types. Hematopoietic stem cells (HSC) are precursor cells that give rise to all blood cell types: white blood cells, red blood cells, and platelets. These changes take place during a process called hematopoiesis. For example, granulocyte-colony stimulating factor (G-CSF) signals an HSC to become a white blood cell called a neutrophil. Neutrophils help fight infection **(Kupsa *et al.*, 2012)**.

Table 2: Basic classification of cytokines **(Kupsa *et al.*, 2012)**.

Cytokine family	Functional subgroups	Physiologic effect
Interleukins	IL-3, IL-7, Flt3-ligand	- stimulate hematopoiesis
	IL-1, IL-6	- pluripotent, inflammatory
	IL-2, IL-4, IL-5, IL-12, IL-13	- regulate T and B cell cooperation
Chemokines	α: CXC	- regulate migration of granulocytes and lymphocytes - promote angiogenesis and inflammation
	β: CC	- regulate migration of monocytes - enable medullar homing
	γ: C, δ: CX_3C	- regulate migration of lymphocytes
Interferons	Type I: INF α, β, ω	- antiviral immunity - anti-proliferative effect
	Type II: INF γ	- antitumorous activity - response to intracellular pathogens
Tumor necrosis factors	TNFα:	- pro-inflammatory pyrogenic - activates non-specific immunity - adhesive molecule expression on endothelial surface - may cause apoptosis - - overproduction causes SIRS
	TNFβ:	- similar in effect, but produced by T- and B lymphocytes
Colony Stimulating Factors	G-CSF, GM-CSF, M-CSF Erythropoietin Thrombopoietin	- stimulate proliferation and maturation of myeloid precursors
TGF-β		- stimulate growth of fibroblasts and extracellular matrix production - lead to MMPs inhibition

7.2 Basic classification of cytokine receptors

Cytokine receptors are cell surface proteins that bind to cytokines, initiating intracellular signals that regulate immune responses, cell growth, and differentiation. They don't have intrinsic kinase activity but activate associated cytoplasmic kinases (like JAKs), triggering pathways (e.g., JAK-STAT) that change gene expression and cell behavior. Key types include Class I (e.g., EPO, IL-6), Class II (e.g., interferons, IL-10), Chemokine receptors (cell migration), and TNF receptors (cell death, inflammation) **(Cameron & Kelvin, 2013)**.

- **Class I Cytokine Receptors (Hematopoietin Family):** Single polypeptide chain receptors or complexes that share common subunits, like the common beta chain (βc) or gp130. Examples: Receptors for Erythropoietin (EPO), Growth Hormone (GH), IL-3, IL-5, GM-CSF, IL-6.
- **Class II Cytokine Receptors:** Includes interferon receptors and receptors for IL-10 family members.
- **Chemokine Receptors:** Crucial for guiding immune cell movement (chemotaxis).
- **TNF Receptor Family:** Involved in inflammation, cell death (apoptosis), and immune regulation **(Cameron & Kelvin, 2013)**.

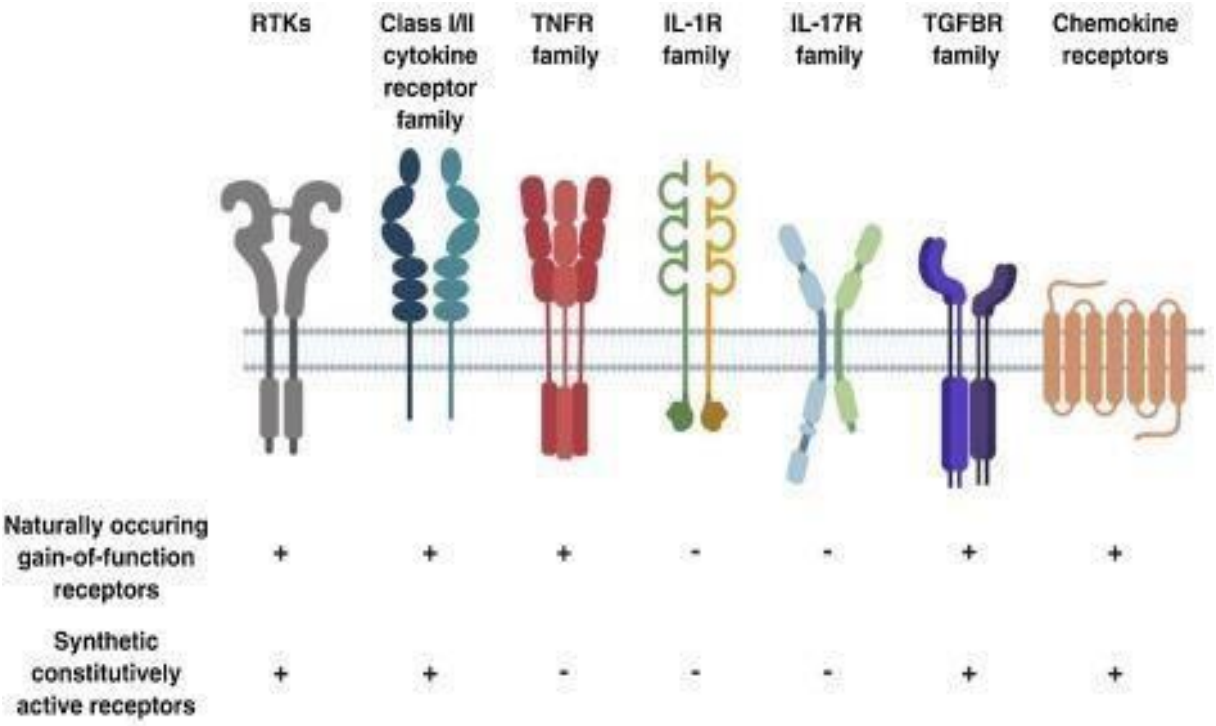


Figure 24: Cytokine receptors **(Cameron & Kelvin, 2013)**.

Tutorial objective: to understand the role of these proteins as chemical messengers ensuring communication between immune cells and regulating the intensity and duration of the immune response.

Immunology tutorials series N6

EXERCICE 1: - Among the cytokines, we find:

a. Interleukins (IL).
b. Lymphokines.
c. Interferons (IFN).
d. Growth factors.
e. Steroid hormones.

EXERCICE 2: **- What are the three main modes of action of cytokines?**

EXERCICE 3: **- Characteristics of cytokines include:**

a. Single cellular origin.
b. Pleiotropy.
c. Redundancy.
d. Synergy.
e. Antagonism.

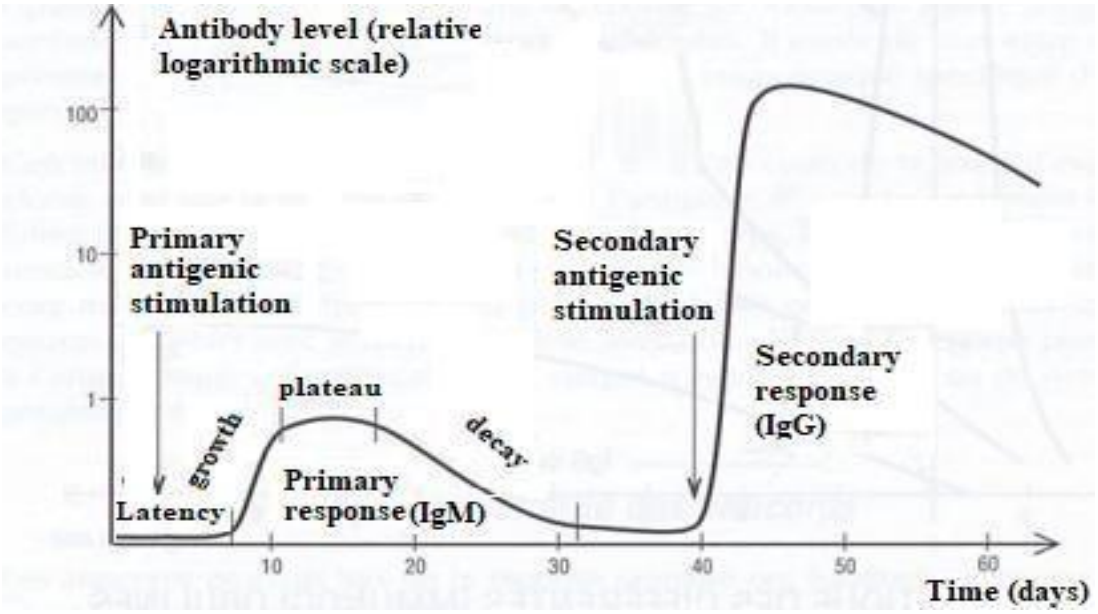
EXERCICE 4: **- What are the main cellular sources and effector properties of IFN?**

EXERCICE 5: **- Schematically represent the mechanisms of induction of Th, Tc, and B immune responses.**

EXERCICE 6: **- What are the consequences of these interactions?**

a. B7-CD28 of Th responses.
b. CPA-Th-Tc of Tc responses.
c. CD40-CD40L of B thymus-dependent responses.

EXERCICE 7: **- Explain the following graph:**



Immunology tutorials series N6 (Solutions)

EXERCICE 1: - Among the cytokines, we find:

a. Interleukins (IL).
b. Lymphokines.
c. Interferons (IFN).
d. Growth factors.
e. Steroid hormones.

EXERCICE 2: - the three main modes of action of cytokines:

Autocrine: exp: synthesis of IL2 by Th1 and autoregulation.
Paracrine: exp: release of IL1 by macrophage to stimulate the proliferation of LT4.
Endocrine: exp: release of INF y˝by Th1 to activate the macrophage.

EXERCICE 3: - Characteristics of cytokines include:

a. Single cellular origin.
b. Pleiotropy.
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d. Synergy.
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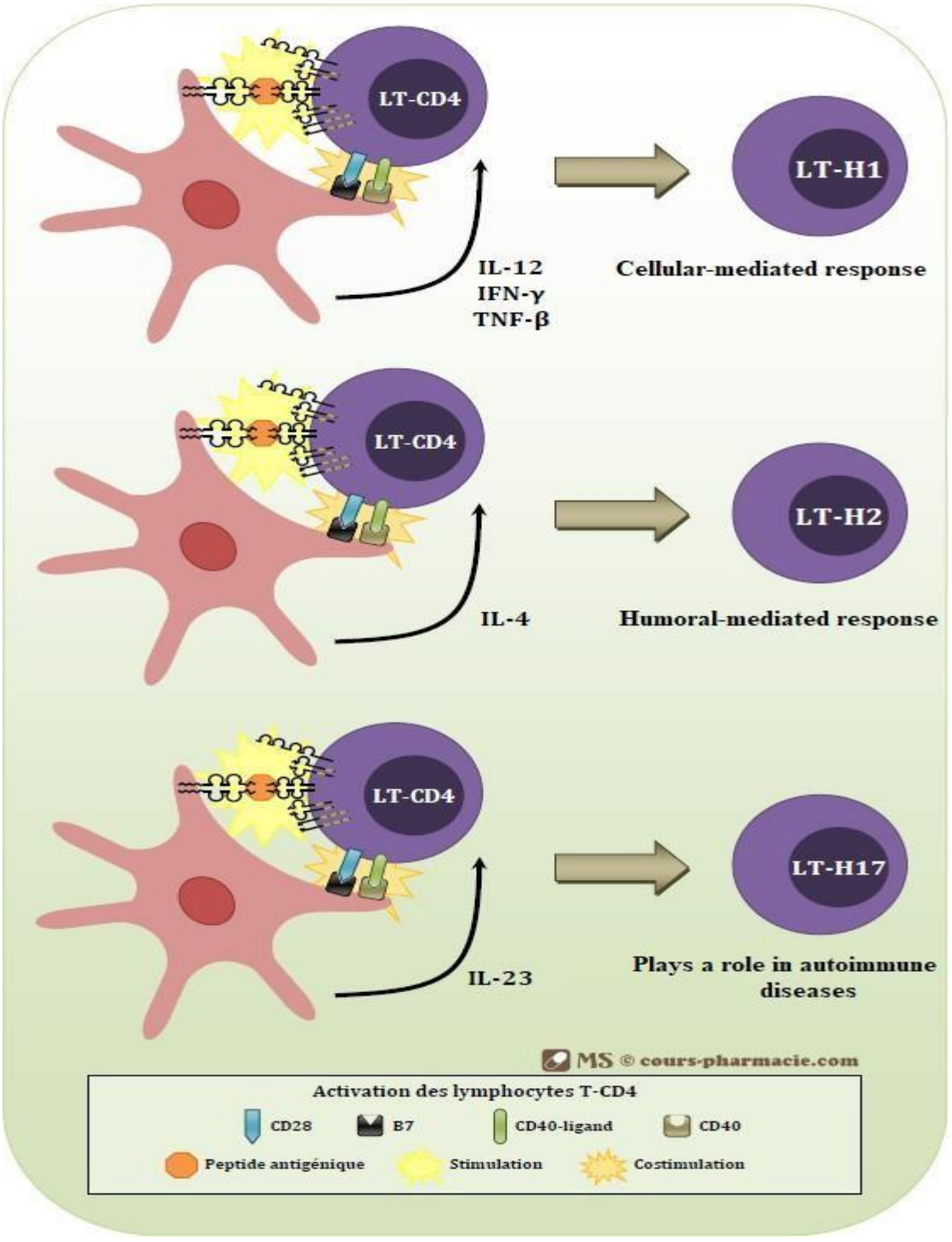
EXERCICE 4: - the main cellular sources and effector properties of IFN:

Cellular sources: Th1, LT8, NK. Effector properties: (+) CPA and (-) Th2.

EXERCICE 5: - Schematically represent the mechanisms of induction of Th, Tc, and B immune responses:

Once these cells are activated, a phase of proliferation and differentiation will be observed. Indeed, the fate of the CD4 cell will be different depending on the cytokines produced by the antigen-presenting cell that activates it. We thus distinguish:

- **T helper 1 lymphocytes** (or **LT-h1**) are obtained via the interleukins IL-12, IFN-γ, and TNF-β. LT-H1 is also responsible for increasing the expression of the B7 receptor, which is essential for the formation of cytotoxic T lymphocytes (see the rest of the course).
- **T helper 2 lymphocytes** (or **LT-h2**) are obtained using interleukin IL-4. They play a role in the activation of B lymphocytes.
- **T helper 17 lymphocytes** (or **LT-h17**) are obtained using interleukin IL-23.



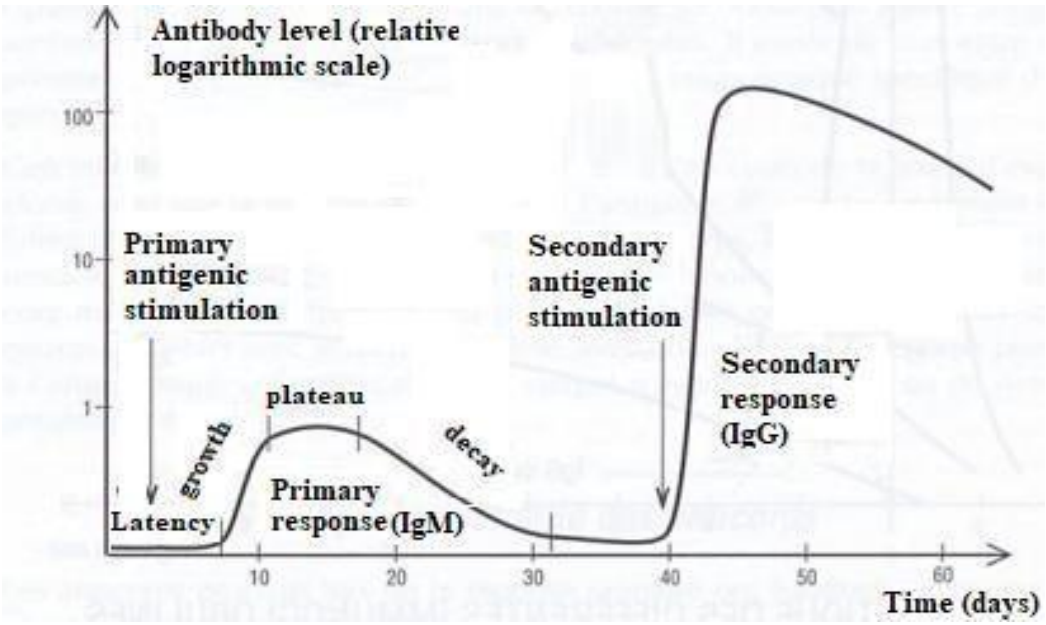
EXERCICE 6: - the consequences of the following interactions:

a. B7-CD28 of Th responses: activation of lymphocytes.

b. CPA-Th-Tc of Tc responses: Cell-mediated immune response leads to destruction of infected cells.

c. CD40-CD40L of B thymus-dependent responses: proliferation signals: the interaction between the CD40 receptor ligand (CD40-ligand) present on the surface of LT-h2 and the CD40 cluster of differentiation present on the surface of B cells, the IL-4 interleukins produced by LT-h2.

EXERCICE 7: - Explain the following graph:



The graph above shows the evolution of the antibody level over time:

We see in this graph that during the first contact with the antigen (primary antigenic stimulation); there are almost no antibodies in the blood for 8 days this is the **latency phase**.

The latency phase: this is the time from exposure to the initial antigen when antibodies are detected in the blood, and takes about a week. During this time, specialized B and T cells are activated by contact with the antigen. Two weeks after this contact there is a significant number of additional antibodies in the body. This is the **primary response**.

The Steady State Phase (Plateau): Antibody levels remain relatively constant due to the continued secretion of antibodies to replenish those that have been degraded.

We also notice that during the second contact with the antigen, the antibodies are approximately twice as numerous as during the first contact and approximately a few days later the level of antibodies has become very high, almost three times more than 10 days after the first contact. This is the **secondary response**.

During the secondary response, the production of antibodies is greater but also faster than during the primary response. However, we know that during a first contact with an antigen; we have ***memory* B lymphocytes**.

We therefore deduce that it is they who are capable, during the second contact with the same antigen, of recognizing it much more quickly and therefore of allowing a much more effective response.

8- Complement system

The complement system, a crucial part of innate immune defense, involves ~60 plasma proteins that work in a sequential cascade to "complement" antibody action, tagging pathogens for destruction, triggering inflammation, and clearing cellular debris, effectively linking innate and adaptive immunity. This cascade activates zymogens (inactive precursors) like C1-C9 into enzymes, leading to pathogen opsonization (marking for phagocytosis), direct pathogen lysis, and inflammation via anaphylatoxins (C3a, C5a) **(Mastellos *et al.*, 2024)**.

Three different pathways trigger the complement system:

- **Classical pathway:** The classical pathway is triggered by activation of the C1-complex. The C1-complex is composed of 1 molecule of C1q, 2 molecules of C1r and 2 molecules of C1s, or $C1qr_2s_2$. This occurs when C1q binds to IgM or IgG complexed with antigens. A single pentameric IgM can initiate the pathway, while several, ideally six, IgGs are needed. This also occurs when C1q binds directly to the surface of the pathogen. Such binding leads to conformational changes in the C1q molecule, which leads to the activation of two C1r molecules. C1r is a serine protease. They then cleave C1s (another serine protease). The $C1r_2s_2$ component now splits C4 and then C2, producing C4a, C4b, C2a, and C2b (historically, the larger fragment of C2 was called C2a but is now referred to as C2b). C4b and C2b bind to form the classical pathway C3-convertase (C4b2b complex), which promotes cleavage of C3 into C3a and C3b. C3b later joins with C4b2b to make C5 convertase (C4b2b3b complex) **(Mastellos *et al.*, 2024)**.
- **Alternative pathway:** The alternative pathway is continuously activated at a low level, analogous to a car engine at idle, as a result of spontaneous C3 hydrolysis due to the breakdown of the internal thioester bond (C3 is mildly unstable in an aqueous environment). The alternative pathway does not rely on pathogen-binding antibodies like the other pathways. C3b, which is generated from C3 by a C3 convertase enzyme complex in the fluid phase, is rapidly inactivated by factor H and factor I, as is the C3b-like C3 that is the product of spontaneous cleavage of the internal thioester. In contrast, when the internal thioester of C3 reacts with a hydroxyl or amino group of a molecule on the surface of a cell or pathogen, the C3b that is now covalently bound to the surface is protected from factor H-mediated inactivation. The surface-bound C3b may now bind factor B to form C3bB. This complex, in the presence of factor D will

be cleaved into Ba and Bb. Bb will remain associated with C3b to form C3bBb, which is the alternative pathway C3 convertase. The C3bBb complex is stabilized by binding oligomers of factor P (properdin). The stabilized C3 convertase, C3bBbP, then acts enzymatically to cleave much more C3, some of which becomes covalently attached to the same surface as C3b. This newly bound C3b recruits more B, D, and P activity and greatly amplifies the complement activation. When a complement is activated on a cell surface, the activation is limited by endogenous complement regulatory proteins, which include CD35, CD46, CD55, and CD59, depending on the cell. Pathogens, in general, don't have complement regulatory proteins (there are many exceptions, which reflect adaptation of microbial pathogens to vertebrate immune defenses). Thus, the alternative complement pathway can distinguish self from non-self on the basis of the surface expression of complement regulatory proteins. Host cells don't accumulate cell surface C3b (and the proteolytic fragment of C3b called iC3b) because this is prevented by the complement regulatory proteins, while foreign cells, pathogens, and abnormal surfaces may be heavily decorated with C3b and iC3b. Accordingly, the alternative complement pathway is one element of innate immunity. Once the alternative C3 convertase enzyme is formed on a pathogen or cell surface, it may bind covalently another C3b, to form C3bBbC3bP, the C5 convertase. This enzyme then cleaves C5 to C5a, a potent anaphylatoxin, and C5b. The C5b then recruits and assembles C6, C7, C8 and multiple C9 molecules to assemble the membrane attack complex. This creates a hole or pore in the membrane that can kill or damage the pathogen or cell **(Mastellos *et al*., 2024)**.

- **Lectin pathway:** The lectin pathway is homologous to the classical pathway, but with the opsonin, mannose-binding lectin (MBL), and ficolins, instead of C1q. This pathway is activated by binding of MBL to mannose residues on the pathogen surface, which activates the MBL-associated serine proteases, MASP-1, and MASP-2 (very similar to C1r and C1s, respectively), which can then split C4 into C4a and C4b and C2 into C2a and C2b. C4b and C2b then bind together to form the classical C3-convertase, as in the classical pathway. Ficolins are homologous to MBL and function via MASP in a similar way. Several single-nucleotide polymorphisms have been described in M-ficolin in humans, with effect on ligand-binding ability and serum levels. Historically, the larger fragment of C2 was named C2a, but it is now referred to as C2b. In invertebrates without an adaptive immune system, ficolins are expanded

and their binding specificities diversified to compensate for the lack of pathogen-specific recognition molecules **(Mastellos *et al.*, 2024)**.

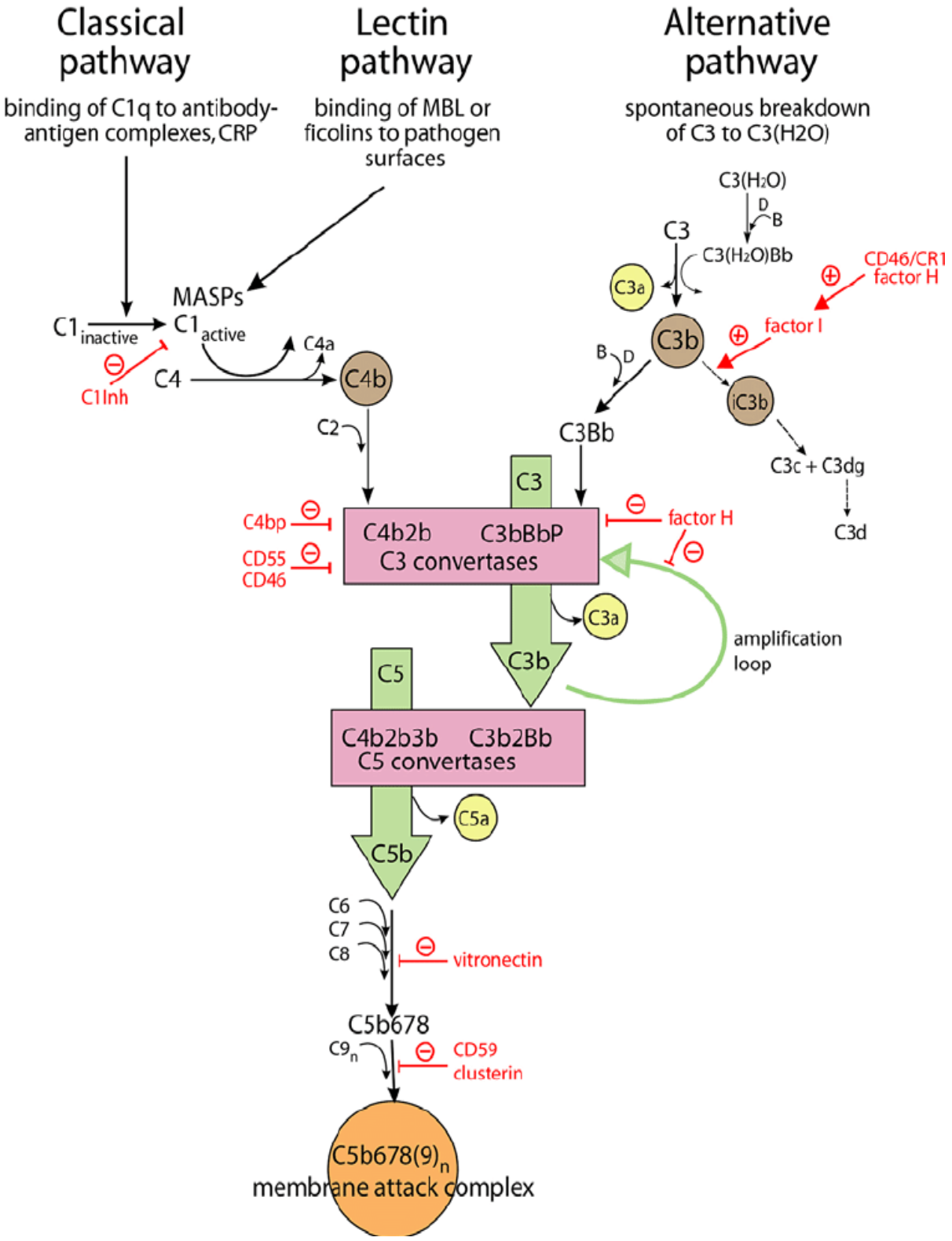


Figure 25: Schematic representation of the complement system and its regulators **(Mastellos *et al.*, 2024)**.

Tutorial objective: to study this major biochemical cascade of the immune system, which links innate and adaptive immunity. It aims to understand how these inactive blood proteins become activated to destroy pathogens, stimulate inflammation, and eliminate cellular waste.

Immunology tutorials series N7

1- The complement system:

a – Represents a complex system of at least 30 plasma proteins.
b – Represents a glycosylated compound insoluble in plasma.
c – Present in inactive form in the blood.
d – Involved in humoral and cellular immunity.
e - Involved in specific and non-specific immunity.

2- The complement intervenes by:

a – classic pathway and associated with specific immunity.
b- classic pathway and associated with non-specific immunity.
c – lectin pathway.
d - alternate pathway associated with non-specific immunity.

3- The complement:

a – is sensitive to heat.
b – participates in cell lysis reactions.
c – is present in normal human serum.
d – increases quantitatively in case of immunization.

4- The classical pathway of complement activation:

a – requires the presence of an antigen-antibody complex.
b – involves C1.
c – involves the C4.
d – can be activated by bacteria.
e – involves C2.

5- The C3b complement fragment can be fixed by:

a – Gram-negative bacteria
b – yeasts
c – Gram-positive bacteria
d – viral envelopes

6- What are the constituent elements of the common terminal complement activation pathway?

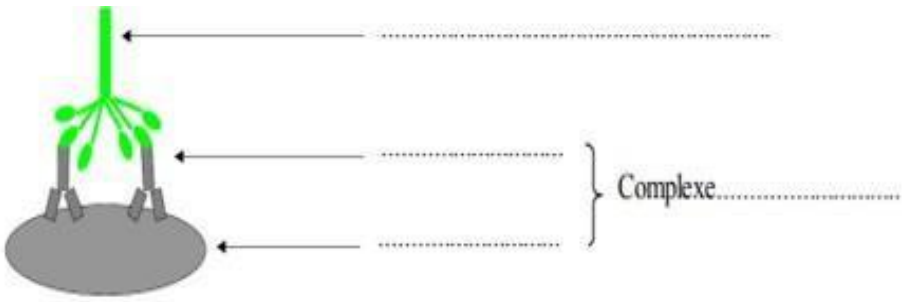
a – C1. b – C3b. c – C5b. d – C6. e – C9.

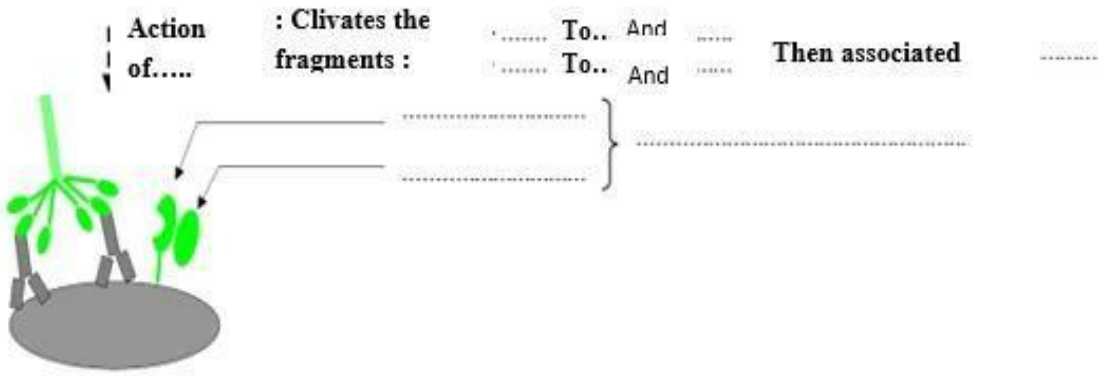
7- Which complement activation products are anaphylatoxins?

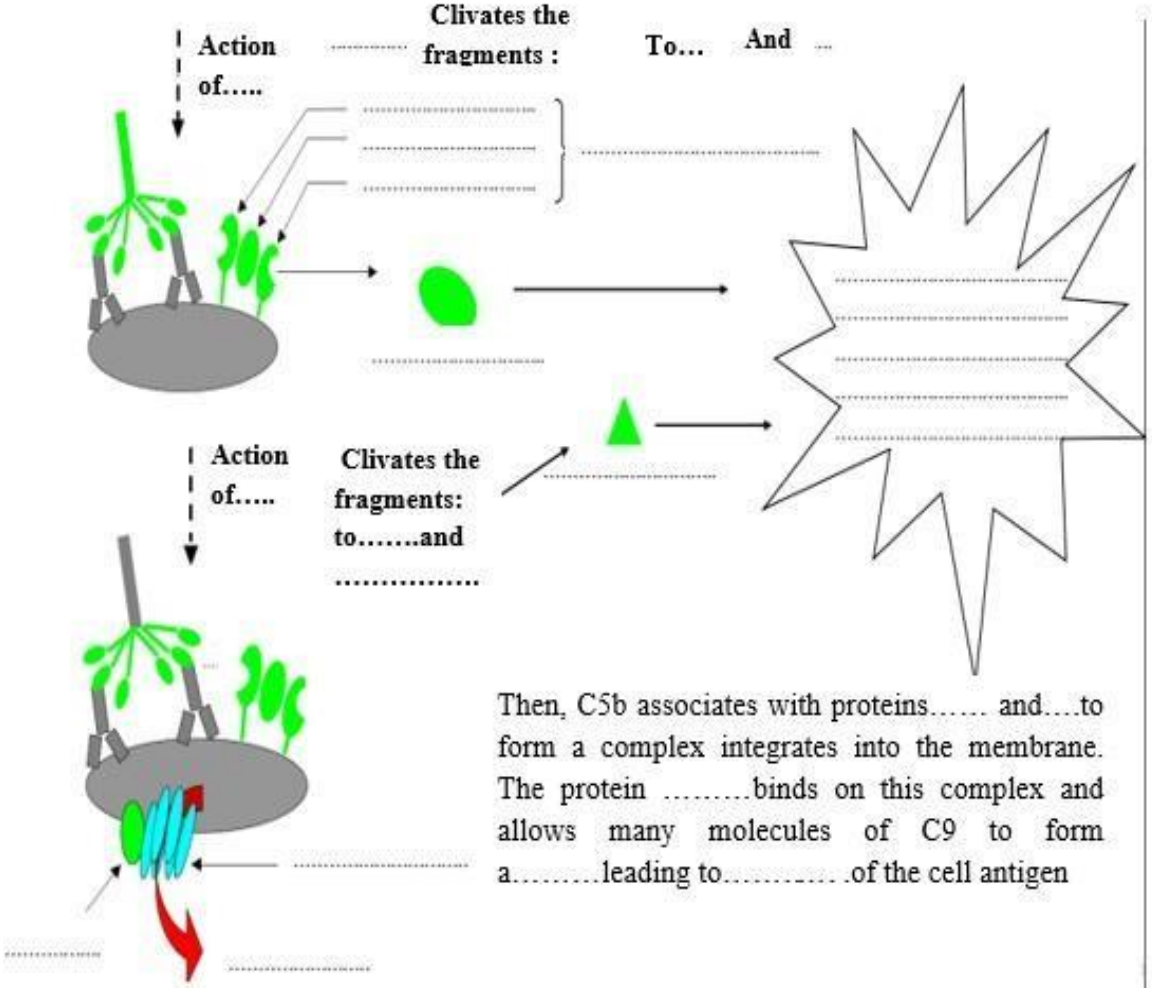
a – C2a. b – C3a. c – C4a. d – C5a. e – C5b.

8- Explain why serum IgM cannot activate complement by itself?

9- The document below summarizes the main events of the complement system activation. Complete the document using your knowledge.


Complexe.....


Action of.....
: Clivates the fragments :
..... To.. And .....
..... To.. And .....
Then associated .....


Action of.....
..... Clivates the fragments :
To... And ...
Action of.....
Clivates the fragments: to.....and .....
Then, C5b associates with proteins..... and....to form a complex integrates into the membrane. The protein .....binds on this complex and allows many molecules of C9 to form a.....leading to..... .of the cell antigen

Immunology tutorials series N7 (Solutions)

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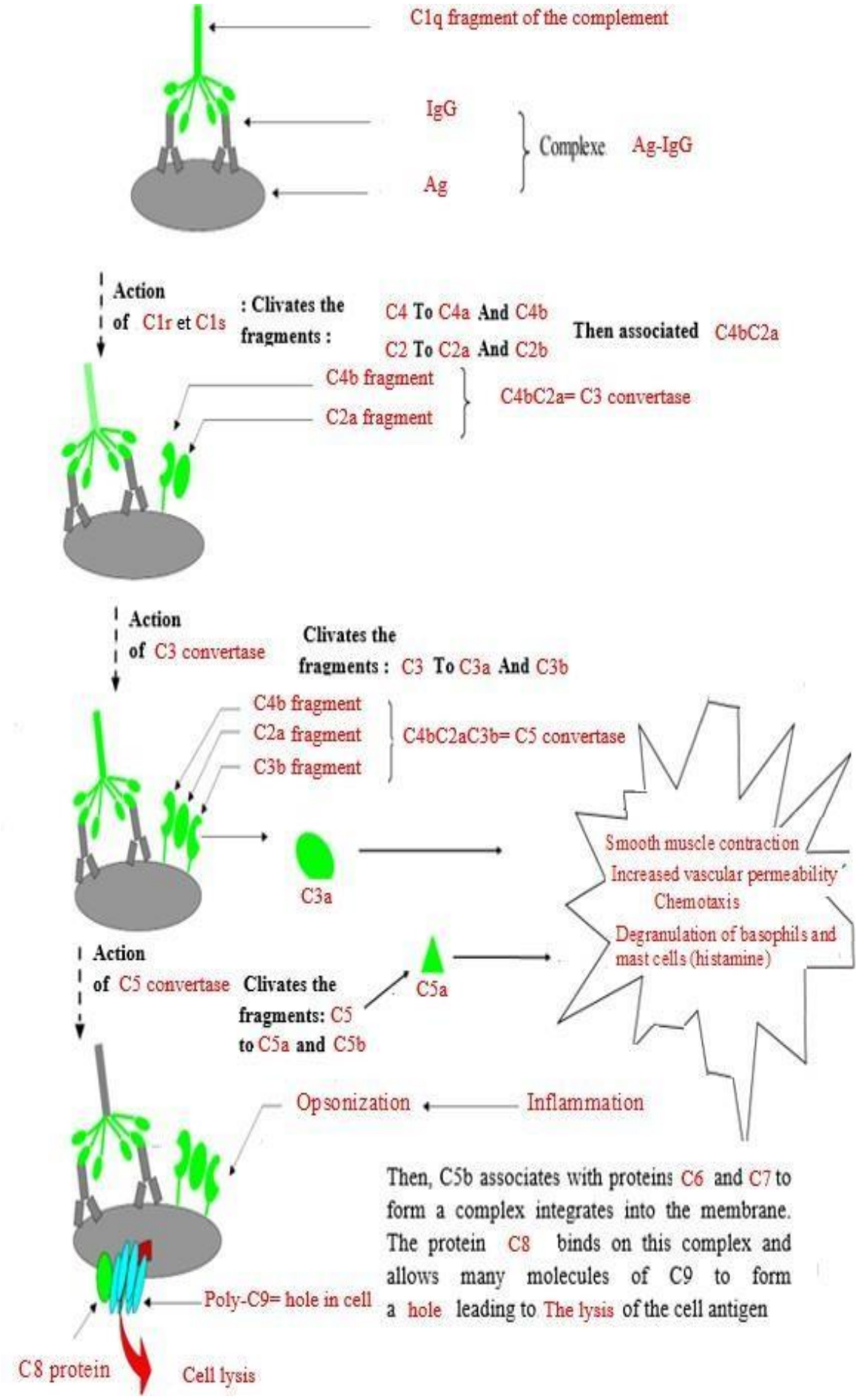
a – C1. b – C3b. c – C5b. d – C6. e – C9.

7- Which complement activation products are anaphylatoxins?

a – C2a. b – C3a. c – C4a. d – C5a. e – C5b.

8- Serum IgM to a form in which the complement binding sites of the Fc region are not accessible. Only after the addition of Ag does IgM assume a conformation in which its complement binding sites become accessible.

9- The document below summarizes the main events of the complement system activation. Complete the document using your knowledge.


C1q fragment of the complement
IgG
Ag
Complexe Ag-IgG
Action of C1r et C1s
: Clivates the fragments :
C4 To C4a And C4b
C2 To C2a And C2b
Then associated C4bC2a
C4b fragment
C2a fragment
C4bC2a= C3 convertase
Action of C3 convertase
Clivates the fragments : C3 To C3a And C3b
C4b fragment
C2a fragment
C3b fragment
C4bC2aC3b= C5 convertase
C3a
Smooth muscle contraction
Increased vascular permeability
Chemotaxis
Degranulation of basophils and mast cells (histamine)
Action of C5 convertase
Clivates the fragments: C5 to C5a and C5b
C5a
Opsonization
Inflammation
Poly-C9= hole in cell
C8 protein
Cell lysis
Then, C5b associates with proteins C6 and C7 to form a complex integrates into the membrane. The protein C8 binds on this complex and allows many molecules of C9 to form a hole leading to The lysis of the cell antigen

9. Introduction to immunologic techniques

Immunological techniques are based on an antigen-antibody reaction. These techniques are used to detect antigens or antibodies. When antigens are to be detected, specific antibodies corresponding to the desired antigenic specificity are required. These antibodies can be polyclonal sera prepared in animals, purified immunoglobulins, or, more frequently today, monoclonal antibodies **(Shitara, 2023)**.

The Ag-Ac reaction is exothermic, reversible, and specific.

- Exothermic: the reaction is characterized by the formation of a bond releasing energy, which results in temperature influencing the proper course of the reaction.
- Reversible: the bond that forms between Ac and Ag are weak bonds (electrostatic, hydrogen, hydrophobic...), so it can be broken quite easily by varying physico-chemical parameters (pH, temperature, ionic strength).
- Specific: the antibody site of an immunoglobulin (paratope) can combine with one and only one epitope; there is stereochemical specificity between the paratope and the epitope **(Shitara, 2023)**.

The Ag-Ab reaction results in the formation of an immunocomplex. The immunocomplex is the product that will be detected in all immunological techniques.

The Ag-Ac reaction has two main types of applications:

- The detection and quantification of antigens (using methods whose specificity, reproducibility, and sensitivity must be verified).
- Antibody detection and titration (against an Ag or a mixture of Ag) **(Shitara, 2023)**.

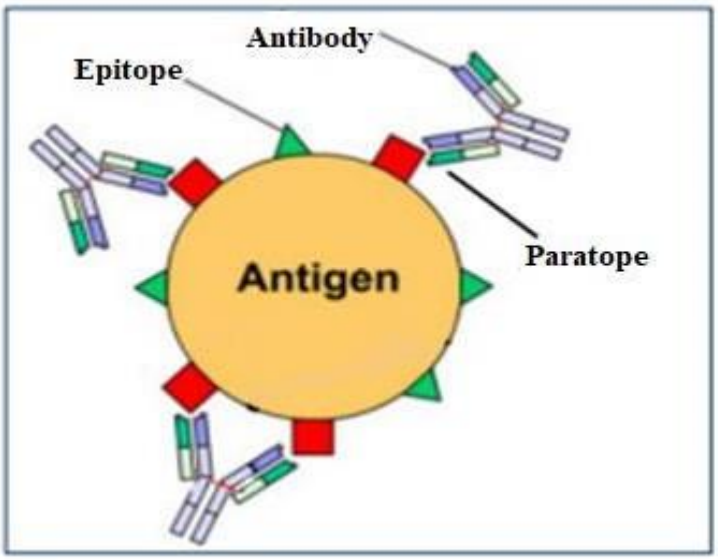


Figure 26: Detection of antigen by antibodies **(Shitara, 2023)**.

9.1 Immunoprecipitation techniques

9.1.1 Precipitation in liquid medium

The experiment involves dispensing equal amounts of a multivalent antigen solution with increasing dilutions of an immune serum **(Garashchenko *et al.*, 2012)**.

The equivalence point (the point where the curve reaches its maximum) corresponds to the formation of an antigen-antibody network **(Garashchenko *et al.*, 2012)**.

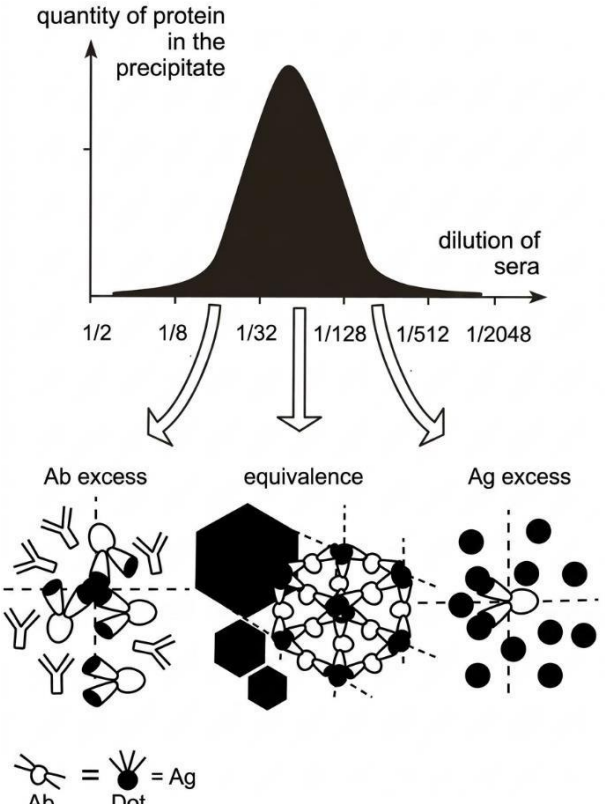


Figure 27: Antibody-Antigen precipitation curve **(Garashchenko *et al.*, 2012)**.

9.1.2 Precipitation in a gelled medium

Purpose: Precipitation methods in gel medium are applied to the qualitative analysis of an antigen (Ag) mixture in a solution or to the immunochemical assay of an antigen **(Ezzeddine & Sultan, 2022)**.

9.1.2.1 Double immunodiffusion (Ouchterlony reaction)

Solutions of antigen (Ag) and antibody (Ab) are placed in wells drilled at intervals in an agarose gel. The molecules diffuse through the gel according to their size and form precipitation lines for each Ag-Ab system **(Mujtaba *et al.*, 2021)**.

Each precipitation line corresponds to the respective equivalence zone, i.e., the formation of an Ag-Ab network **(Mujtaba *et al.*, 2021)**.

This method allows the analysis of an antigen mixture and the identification of its components. When two proteins diffuse through a gel and encounter antibodies, identity, non-identity, or partial identity reactions are observed **(Mujtaba *et al.*, 2021)**.

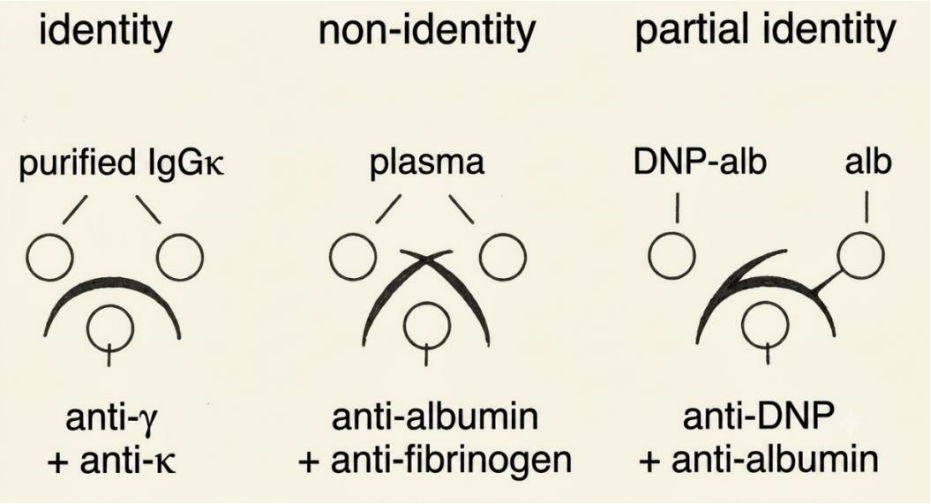


Figure 28: Ouchterlony double immunodiffusion patterns of immunological identity and cross-reactivity **(Mujtaba *et al.*, 2021)**.

9.1.2.2 Radial immunodiffusion (Mancini technique)

This method involves incorporating a specific antiserum into the agar and loading the antigen solution into wells. At equilibrium, a precipitation ring forms, the square of whose diameter is proportional to the antigen concentration. The concentration is expressed by reference to a standard curve with an antigen of known concentration.

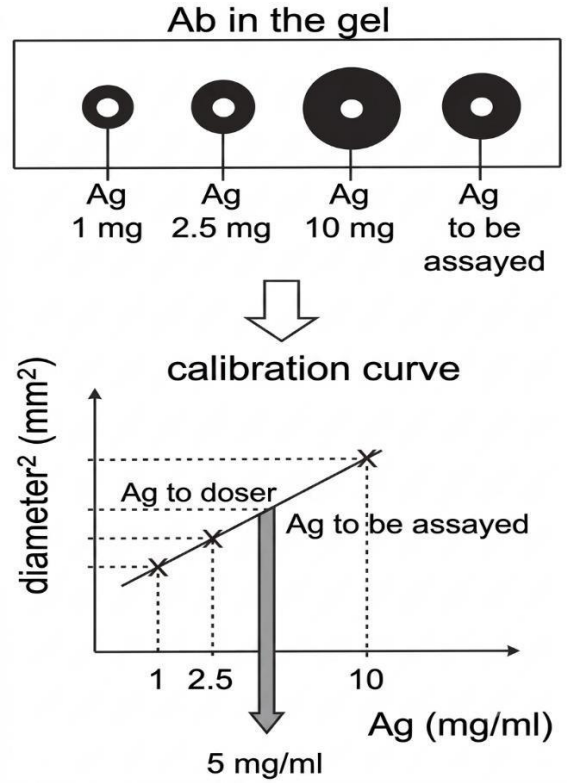


Figure 29: Radial immunodiffusion assay **(Thompson *et al.*, 2023)**.

9.3 Immunoelectrophoresis

This technique involves separating proteins by electrophoresis in an agarose gel, followed by double diffusion against specific antibodies in a direction perpendicular to the electrophoretic migration axis. Each equivalence zone corresponds to an antigen-antibody precipitate, which is represented by a precipitation arc. Immunoelectrophoresis allows for the characterization or identification of antigens (it is not a quantitative method) **(Csako, 2019)**.

9.4 Rocket electrophoresis

The Ac (anticoagulant) incorporated into the agarose gel is immobile (due to the gel's pH), while the negatively charged Ag (silver) migrates in an electric field **(Csako, 2019)**.

The resulting precipitation arc is rocket-shaped, with its height proportional to the Ag concentration **(Csako, 2019)**.

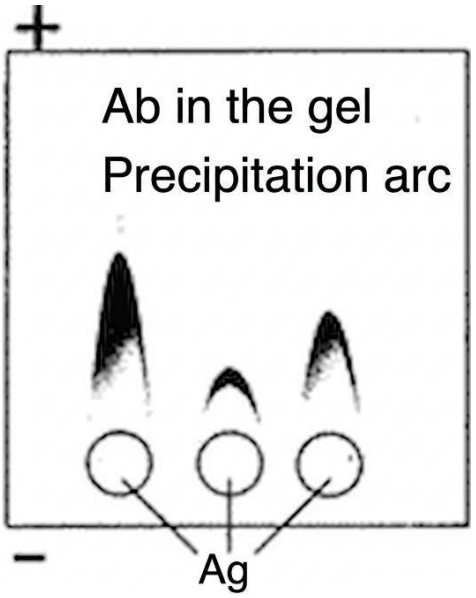


Figure 30: Rocket electrophoresis assay **(Csako, 2019)**.

9.5 Immunofixation

After separation of the antigenic solution by electrophoresis, a specific antibody is deposited on the gel surface, penetrates it, and precipitates in the presence of the corresponding antigen. This method is more sensitive and faster than immunoelectrophoresis; however, immunoblotting is preferred **(Karfo *et al*., 2018)**.

9.6 Immunoblotting or Western blot

Immunotransfer, or Western blot, is a technique for analyzing and identifying protein antigens. Proteins are separated by polyacrylamide gel electrophoresis and then electrophoretically transferred to a membrane (such as nitrocellulose). The bands of the proteins for which antigens are sought are detected by applying a specific antibody, followed by a second antibody labeled with an isotope, a fluorochrome, or an enzyme. The term 'Western blot' was coined by analogy to the Southern blot (used for DNA fragments) and the Northern blot (used for RNA) **(Sule *et al*., 2023)**.

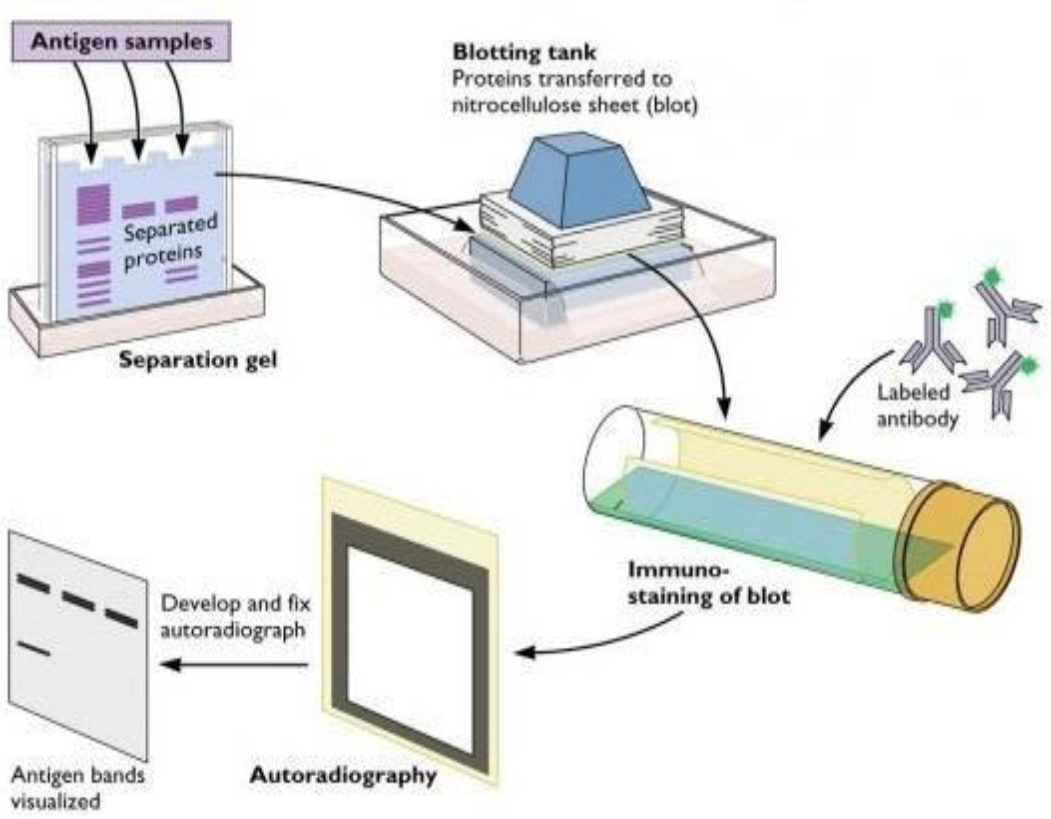


Figure 31: Western blotting technique **(Sule *et al*., 2023)**.

9.7 Agglutination reactions

The agglutination reaction brings together Ag on a formed element (cells, erythrocytes, particles, bacteria) and an antiserum containing specific agglutinating Antibodies **(Kretsch *et al*., 2023)**.

9.7.1 Direct agglutination reaction

This method is widely applied to blood group determination and antibody detection **(Kretsch *et al*., 2023)**.

9.7.2 Passive agglutination reaction

Passive agglutination involves fixing a soluble antibody to an inert, non-compounded support that does not participate in the antigen-antibody reaction. The presence of the antibody is detected by the agglutination of the particles to which the antigen is bound **(Kretsch *et al*., 2023)**.

Applications are numerous:

- The detection and titration of rheumatoid factors (autoantibodies directed against IgG) are often performed using the Waaler-Rose test or the latex agglutination test.
- The detection of antibodies present on the patient's red blood cells is performed using the direct Coombs test.

- The detection of anti-red blood cell antibodies in the patient's serum is performed using the indirect Coombs test **(Kretsch *et al*., 2023)**.

9.7.3 Passive hemagglutination inhibition reaction

This reaction allows the detection of an antigen in a biological fluid **(Kretsch *et al*., 2023)**.

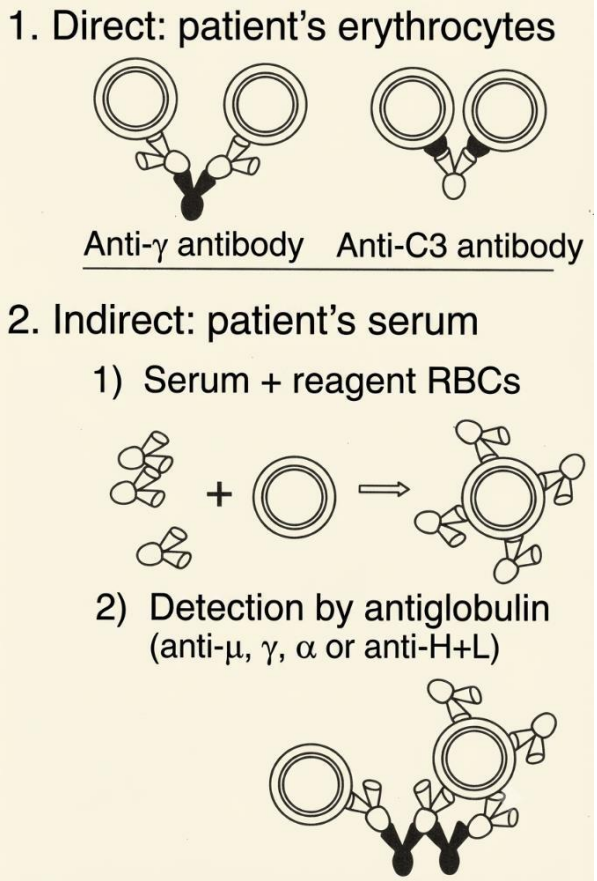


Figure 32: Agglutination reactions **(Kretsch *et al*., 2023)**.

9.8 Immunostaining techniques

In immunolabeling techniques, the antigen, or more often the antibody, is labeled, meaning it is coupled to an isotope (e.g., 125I), a fluorescent compound (fluorochrome), or an enzyme (e.g., horseradish peroxidase, alkaline phosphatase). Detection is performed using scintillation counters, UV microscopy (immunofluorescence), or optical microscopy (enzyme-linked immunosorbent assays) **(Piña *et al*., 2022)**.

9.8.1 Detection of an antigen or antibody in a heterogeneous suspension

Immunostaining techniques allow the identification of an Ag or an Ab within a heterogeneous population **(Piña *et al*., 2022)**.

9.8.2 Detection of an antigen on a tissue section

Immunostaining techniques allow the localization of an antigen (antigen) within a tissue on a tissue section or cell smear (after fixation and membrane permeabilization if necessary). If the

antigen-specific antibody is coupled to a fluorochrome or an enzyme, this is called direct labeling. Sensitivity can be increased by indirect labeling, in which the labeled antibody is not the antigen-specific antibody but a second anti-Fc antibody that recognizes the constant (Fc) region of the primary antigen-specific antibody. The second antibody is coupled to a fluorochrome (indirect immunofluorescence) or to an enzyme **(Piña *et al*., 2022)**.

9.9 Enzyme-linked immunosorbent assays (ELISA)

The enzyme-linked immunosorbent assay (ELISA) is an immunological assay commonly used to measure antibodies, antigens, proteins and glycoproteins in biological samples. Some examples include: diagnosis of HIV infection, pregnancy tests, and measurement of cytokines or soluble receptors in cell supernatant or serum. ELISA assays are generally carried out in 96 well plates, allowing multiple samples to be measured in a single experiment. These plates need to be special absorbant plates (e.g. NUNC Immuno plates) to ensure the antibody or antigen sticks to the surface. Each ELISA measures a specific antigen, and kits for a variety of antigens are widely available **(Hayrapetyan *et al*., 2023)**.

9.9.1 Direct ELISA

The antigen (Ag) is immobilized onto the solid phase (a plate or multi-well plate) and to this a complementary antibody, which is conjugated to a detection molecule or enzyme such as HRP, directly binds **(Hayrapetyan *et al*., 2023)**.

Direct ELISAs are particularly useful in a variety of applications. For example, they can be used to study how the immune system responds to an antigen, to detect specific biomolecules or pollutants, as a serological diagnostic method, in the immunohistochemical staining of cells, and for epitope mapping and antibody screening. Although direct ELISAs typically have lower sensitivity when compared to the other types of ELISA, they have the advantage that they do not use a secondary antibody. Consequently, the risk of secondary antibody cross-reactivity is reduced **(Hayrapetyan *et al*., 2023)**.

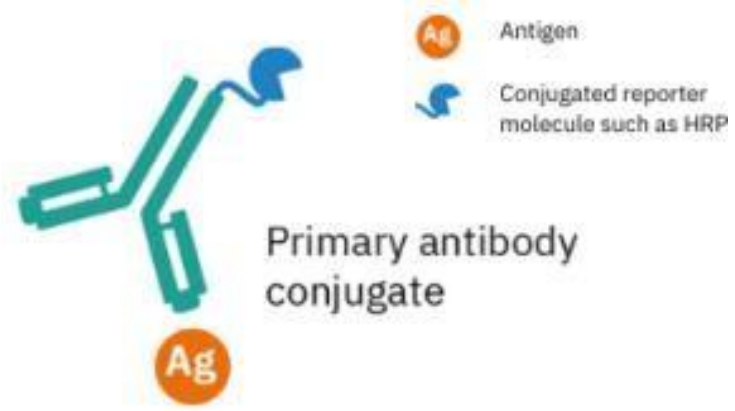


Figure 33: Direct ELISA reaction **(Hayrapetyan *et al*., 2023)**.

9.9.2 Indirect ELISA

Indirect ELISAs also have an antigen coated onto an ELISA plate, but this is recognised first by a primary antibody that is in turn recognized by a complementary secondary antibody which is labelled to provide a read-out signal **(Hayrapetyan *et al*., 2023)**.

Like the direct ELISA, the indirect ELISA can be used to investigate an immunological response; however, it can do so with higher sensitivity and with greater flexibility, as the secondary antibody can be used for a wider number of primary antibodies. Although it is important to note this method carries a risk of secondary antibody cross-reactivity that can be minimised by optimising the experimental conditions **(Hayrapetyan *et al*., 2023)**.

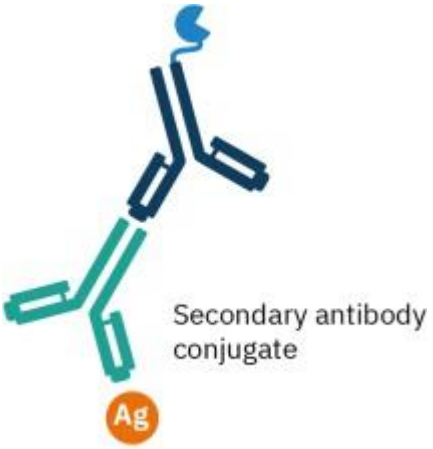


Figure 34: Indirect ELISA reaction **(Hayrapetyan *et al*., 2023)**.

9.9.3 Sandwich ELISA

It relies on having ‘matched antibody pairs’, which in essence are two antibodies that recognize and bind to two different epitopes of the same antigen. One of these antibodies is immobilized on the ELISA plate and captures the antigen, while the second antibody is added in a second step after antigen binding and carries a conjugated detection molecule. This results in the antigen being trapped in a ‘sandwich’ between the capture and detection antibodies **(Tabatabaei & Ahmed, 2022)**.

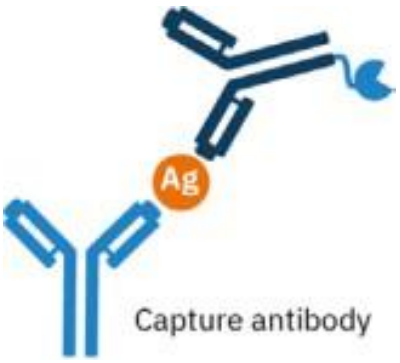


Figure 35: Sandwich ELISA reaction **(Hayrapetyan *et al*., 2023)**.

9.9.4 Competitive ELISA

The competitive ELISA form can have different variations. One of these variations is based on signal interference and uses a labelled purified antigen and an unlabelled form of the same antigen which is present in the sample. The unlabelled antigen in the test sample competes with the labelled antigen added in solution to bind to a capture antibody. If there is limited or no signal from the labelled antigen, it shows that the antigen of interest is present in the sample. Generally, this type of immunoassay is used to detect and quantify molecules that are smaller and when there is only one antibody available with specificity to the molecule of interest **(Tabatabaei & Ahmed, 2022)**.

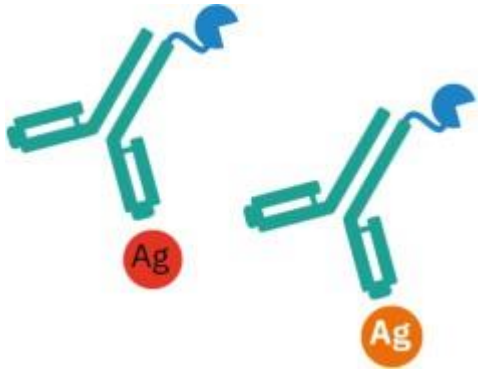


Figure 36: Competitive ELISA reaction **(Hayrapetyan *et al*., 2023)**.

9.10 Radioimmunoassay (RIA)

Radioimmunoassay (RIA) is an immunological analysis technique used to measure the concentrations of antigens (such as hormones, drugs, or proteins) in a sample by the use of antibodies. The RIA is based on the competition between an iodine radio-labelled antigen (emitting gamma radiation) and a non-labelled antigen present in a sample to bind to a specific antibody. This competition allows for the measurement of the concentration of the target analyte in a biological sample with high sensitivity and specificity. The bound antigens are then separated from the unbound ones, and the radioactivity of the bound antigen is measured using a gamma counter **(Roggenbuck *et al*., 2021)**.

Besides the RIA, the sandwich IRMA technique uses two antibodies, both specific for the same antigen, but binding to two different epitopes. One of the antibodies is coated onto the solid phase. A second antibody labelled with is added to the solid phase. This labelled antibody binds with the antigen, which is already bound to the first antibody. At the end of the reaction, unreacted labelled antibody is aspirated out and the solid phase washed with a wash solution. The radioactivity in the solid phase is directly proportional to the concentration of analyte present **(Roggenbuck *et al*., 2021)**.

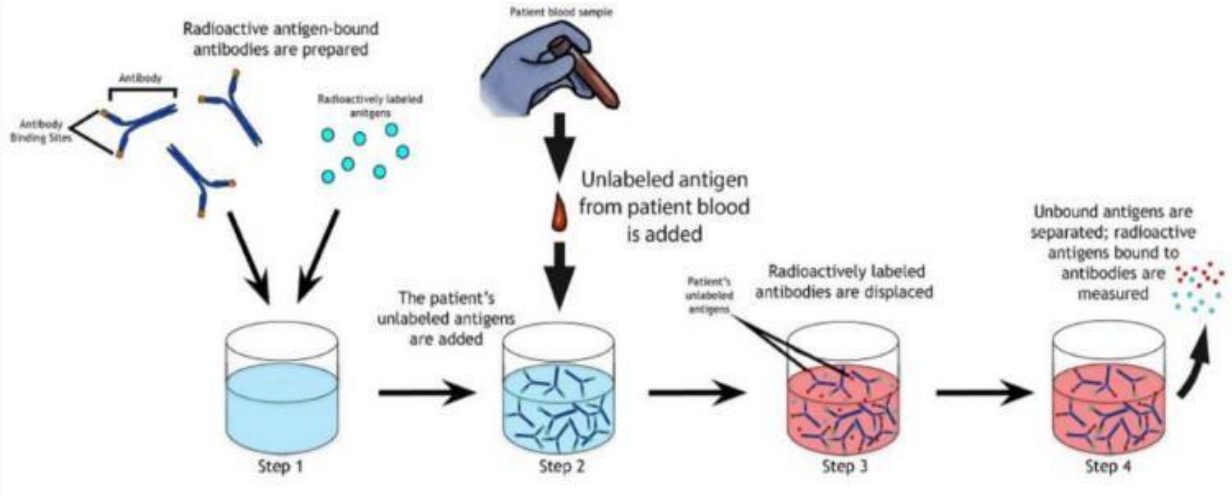


Figure 37: Radioimmunoassay reaction **(Roggenbuck *et al*., 2021)**.

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