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Intended for: Veterinary Medicine Students – 2nd Year Level

Essential Foundations of Immunology: A Comprehensive Overview to the Body's Defense System

Presented by: Dr. AICHE Souad

Grade MCB, Laghouat University

Email : so.aiche@lagh-univ.dz

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Preface

This academic handbook has been meticulously prepared for second-year students of the Doctor of Veterinary Medicine program, functioning as a foundational resource for the Immuno-Vaccinology module. This course aims to provide future veterinarians with the fundamental theoretical knowledge and practical skills in immunology, a fundamental aspect of veterinary medicine.

Comprehending the complex mechanisms of the immune system is more than a purely intellectual pursuit; it is a crucial skill for identifying diseases, halting the spread of outbreaks, and safeguarding the health of animals being treated by veterinarians. This module offers a detailed understanding of vaccine principles and mechanisms, which are one of the most effective means of protecting animal health at both the individual and population levels.

This guide will enable students to examine the relationship between host immunity and pathogens, the mechanisms involved in vaccine creation, and the essential function of immunological resources in contemporary veterinary medicine. This module aims to equip students with a thorough understanding of these subjects, enabling them to tackle the complexities of veterinary medicine with assurance and a robust scientific approach.

It is our expectation that this handbook will function not only as an academic resource but also as a lasting reference point throughout your career as a veterinary professional. As you begin this course, it is our hope that it will motivate your dedication to improving animal health and supporting the broader objectives of public and animal welfare.

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List of Abbreviations

ADCP: antibody-dependent cellular phagocytosis

APC: antigen-presenting cell

BCG: Bacillus Calmette–Guérin

BCR: B Cell Receptor

CD4+: helper T cells

CD8+: cytotoxic T

CRP: C-Reactive Protein

DTP: diphtheria-tetanus-pertussis

EPO: Erythropoietin

Fab: Fragment Antigen Binding

Fc: Fragment Crystallizable

GALT: like gut-associated lymphoid tissue

GM-CSF: Granulocyte-Macrophage Colony-Stimulating Factor

HLA: Human Leukocyte Antigen

HSC: Hematopoietic Stem Cells

IFN: Interferons

Ig: Immunoglobulin

IL: Interleukins

LPS: Lipopolysaccharide

MALT: mucosal-associated lymphoid tissues

MASP: Mannose-Associated Serine Proteases

MBL: mannose-binding lectin

M-CSF: Macrophage Colony-Stimulating Factor

MHC: Major Histocompatibility Complexes

NK: Natural Killer

PRR: pattern recognition receptors

TCC: terminal complement complex

TCR: T Cell Receptor

TCR: T cell receptors

TGF-β: Transforming Growth Factor-Beta

TNF: Tumor Necrosis Factors

TNF: Tumor Necrosis Factors

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INTRODUCTION

Introduction

The animal body is a dynamic and intricate organism, comprising all components essential for sustaining life. The warm, moist, and nutrient-rich surroundings make it an ideal target for microorganisms trying to utilise these resources. The vulnerability of animal tissues to microbial invasion becomes especially apparent when an animal dies, as the lack of defense mechanisms enables swift decomposition by bacteria. In living and healthy animals, strong systems of resistance effectively prevent microbial invasions, which are essential for survival **(Tizard, 2013).**

The study of these intricate defense systems falls under the discipline of immunology, a field that seeks to unravel the mechanisms through which the body protects itself from microbial invaders. Immunology is not merely an academic pursuit; it is essential for understanding how the immune system functions as an interactive network, initiating diverse responses to eliminate pathogens and bolster resistance to future infections **(Tizard, 2013; Murphy *et al.*, 2016).**

The discipline of immunology encompasses the study of these complex defense systems, with the goal of understanding the bodily mechanisms that prevent infection by microbial invaders. Immunology is not merely an academic discipline; it is crucial for grasping how the immune system operates as a dynamic network, triggering varied responses to eradicate pathogens and enhance resistance to subsequent infections **(Abbas *et al.*, 2021).**

This academic handbook on Immuno-Vaccinology for **second-year veterinary students** delves into these fundamental concepts, focusing on the immune system's structure, functions, and its role in vaccine-mediated protection. It emphasizes the interconnected nature of immune pathways and highlights the importance of immunology in diagnosing, preventing, and managing diseases. By understanding these mechanisms, future veterinarians will be better equipped to safeguard animal health, addressing both individual cases and broader challenges in animal populations.

Module objectives

The objectives of immune-vaccinology module are as follows:

1. To provide the student with basic knowledge of immunology.
2. To enable the student to distinguish between the various factors involved in an immune response.
3. To help the students understand the immuno-infectious status of a host and the mechanisms employed to combat infection.

Targeted competencies

1. **Understanding antigens and antibodies:** Students will develop a comprehensive understanding of antigens and antibodies, including their structure, functions, and interactions within the immune system. This foundational knowledge is crucial for understanding how the body recognizes and responds to pathogens, forming the basis for immunological processes.
2. **Mechanisms of immune response:** Students will explore the various types of immune reactions involving antigens and antibodies. This includes an in-depth study of immune response pathways and their roles in the body's defense mechanisms against infections. Emphasis will be placed on how these pathways contribute to overall immune system efficacy.
3. **Analyzing infection outcomes:** Through guided tutorials, students will enhance their ability to analyze how immune responses shape the outcomes of infections caused by microorganisms. This involves critical evaluation of immune system dynamics in the context of host-pathogen interactions.
4. **Application to vaccinology:** Students will acquire the ability to apply core immunological concepts to the field of vaccinology. This skill will enable them to understand and contribute to the design, development, and evaluation of vaccines as tools for disease prevention and control.

Evaluation methods

1. **Continuous assessment:** The continuous assessment (CA) grade will be based on the scores obtained in tutorials, quizzes, attendance, participation, and any other criteria deemed appropriate by the instructor, in agreement with the administration.
2. **Midterm exam (EMD):** A midterm exam of moderate duration will be scheduled at the end of the course.
3. **Make-up Exam:** A make-up exam will be provided for students who require it.

The final grade for the course will be weighted as follows:

- **Midterm Exam (EMD):** 70%
- **Continuous Assessment (CA):** 30%

History of Veterinary Immunology

History of veterinary immunology

The history of veterinary immunology traces back to early observations of immunity during infectious disease outbreaks. In the 12th century, the Chinese practiced variolation, deliberately infecting individuals with smallpox material to confer immunity. This technique, which involved inoculating infants with scabs from mild cases, reduced mortality and laid the groundwork for later vaccination practices. By the 18th century, this practice had spread to Europe, where it was used for rinderpest in cattle, demonstrating the potential for immunization in animal diseases **(Tizard, 2013).**

In 1798, Edward Jenner revolutionized immunology with his discovery of vaccination. He found that material from cowpox lesions could safely protect against smallpox, marking a significant advance over variolation. This discovery not only reduced the risks of inoculation but eventually led to the global eradication of smallpox. Jenner's work sparked further exploration into vaccination, which led to new methods for preventing other animal diseases, such as sheep pox and anthrax **(Tizard, 2013).**

Pasteur's research in the late 19th century was based on these principles. Pasteur showed that immunity could be created by exposing animals to virulent bacterial strains without making them sick. The development of vaccines for anthrax and rabies by him, coupled with contributions from individuals like Daniel Salmon and Theobald Smith, who used heat-killed bacteria, established the foundation for contemporary immunology. Advances in understanding microbial immunity have significantly impacted veterinary medicine, with immune mechanisms being pivotal in curbing and controlling infectious diseases in animals **(Rosset, 1985).**

CHAPTER I:

IMMUNE SYSTEM

I.1 Self and non-self: The fundamentals of immunity

The body's immune system functions as an internal defense system that safeguards against invaders including bacteria, viruses, and cancer cells. To accomplish this task, the system needs to distinguish between elements that are part of the body (referred to as one's own "self") and those that are external (the "non-self") **(Abbas *et al.*, 2021).**

I.1.1 The self

The "self" comprises all the molecules, cells, and tissues that constitute the body. The immune system recognises these components as being intrinsic to the organism, which helps to form its biological identity **(Murphy *et al.*, 2016).** For easier recognition, cells in the body show distinct molecules on their exterior called major histocompatibility complexes (MHCs). These MHC molecules act as identity markers, enabling immune cells to distinguish friendly cells from potential threats **(Alberts *et al.*, 2015).**

I.1.2 The non-self

The "non-self" encompasses all entities external to the body, including bacteria, viruses, parasites, tumour cells, and transplanted tissues. Foreign substances contain molecules that are chemically distinct from the body's own and are consequently recognised as invaders by the immune system **(Abbas *et al.*, 2021).** Pathogens have foreign molecules on their surfaces, which immune cells can detect using specialized receptors. Upon discovering a "non-self" entity, the immune system initiates a sequence of reactions designed to neutralise the danger **(Murphy *et al.*, 2016).**

I.2 Major Histocompatibility Complex

The major histocompatibility complex (MHC) was initially identified as the genetic region that encodes glycoproteins involved in the rejection of tissue grafts between individuals with different genetic makeups. In 1930, Gorer identified the antigens associated with allograft rejection in mice, specifically two blood group antigens—antigen 1 and antigen 2. Antigen 1 was present in all mouse strains, whereas antigen 2, only found in specific strains, caused allograft rejection and was subsequently referred to as the H2 antigen. This antigen was associated with a cluster of genes, now termed the MHC, which plays a crucial role in the immune response. The equivalent of the MHC in humans is the human leukocyte

antigen (HLA) complex, located on the surfaces of leukocytes. These antigens, along with blood group carbohydrates and membrane glycoproteins, are vital for transplant compatibility. The discovery of MHC and its part in immune responses led to Snell, Dausset, and Benacerraf being awarded the Nobel Prize in 1980 for their pioneering work on genetic control of immune functions **(Parija, 2012).**

I.2.1 MHC class I molecules

Class I proteins are surface glycoproteins present on virtually all nucleated cells. Approximately 20 proteins are found at the A locus, 40 at the B locus, and eight at the C locus. Each Class I protein is made up of a 45,000 Da transmembrane glycoprotein heavy chain, which is linked to a 12,000 Da beta-2 microglobulin without a covalent bond. The heavy chain comprises both variable and constant regions, with the variable region showing considerable diversity. This diversity is crucial in distinguishing self from nonself. The constant region binds to CD8 proteins on cytotoxic T cells, and Class I proteins play a significant role in graft rejection and cell-mediated cytotoxicity **(Parija, 2012).**

I.2.2 MHC class II molecules

Class II proteins primarily consist of glycoproteins and are typically located on macrophages, B cells, and other antigen-presenting cells, including dendritic cells in the spleen and Langerhans cells in the skin. Their expression on other cells, such as endothelial cells, can be triggered by gamma interferon. The proteins in question are comprised of two transmembrane glycoproteins, measuring approximately 33,000 and 29,000 Da, and are noncovalently associated. Class II proteins are crucial in graft-versus-host reactions and mixed leukocyte responses. Specifically, CD4 proteins on helper T cells interact with Class II proteins. When a Class II protein presents an antigen, a CD4+ T cell identifies it and initiates an immune response, which is crucial for combating infections and modulating the immune system **(Ishina, et al., 2023; Parija, 2012).**

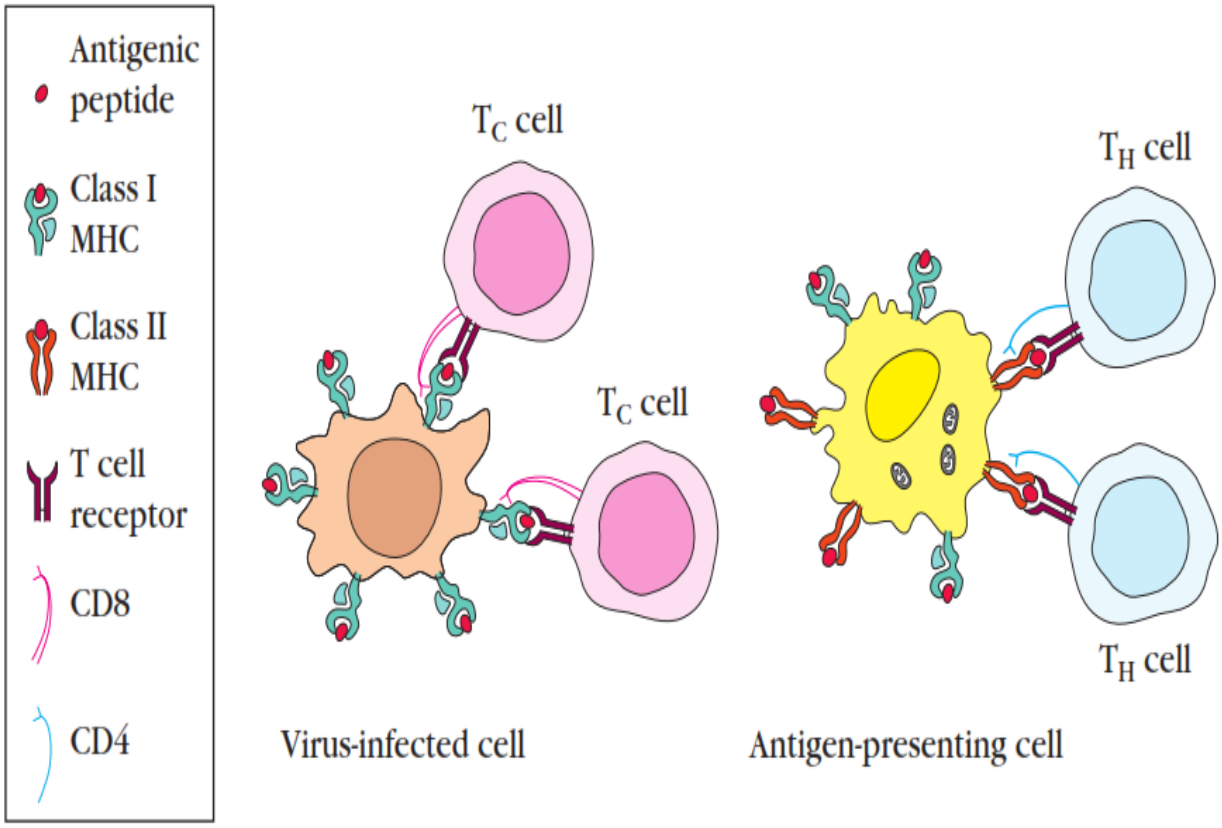

Figure 1.The role of MHC molecules in antigen recognition by T cells (Kindt, 2004)

I.3 Organs of the immune system

The immune system relies on a number of organs and tissues, with each one having a distinct role in generating immune responses. These organs are classified into primary, secondary, and tertiary lymphoid organs based on their roles. The primary lymphoid organs are the thymus and bone marrow, in which lymphocytes undergo maturation to prepare for their immune roles **(Sautès-Fridman *et al.*, 2019).** Secondary lymphoid organs like lymph nodes, the spleen, and mucosal-associated lymphoid tissues, including gut-associated lymphoid tissue, act as key centres. Within this region, antigens are captured, and mature lymphocytes interact with them, triggering an immune response **(Sautès-Fridman *et al.*, 2019).**

I.3.1 Primary lymphoid organs

Lymphocytes which are immature, develop during the process of hematopoiesis, mature, and acquire specific antigen recognition capabilities in the primary lymphoid organs. Once fully developed, these cells gain the ability to launch a complete immune response. In many mammals, including humans and mice, B cells originate in the bone marrow, whereas T cells develop in the thymus **(Kindt, 2004).**

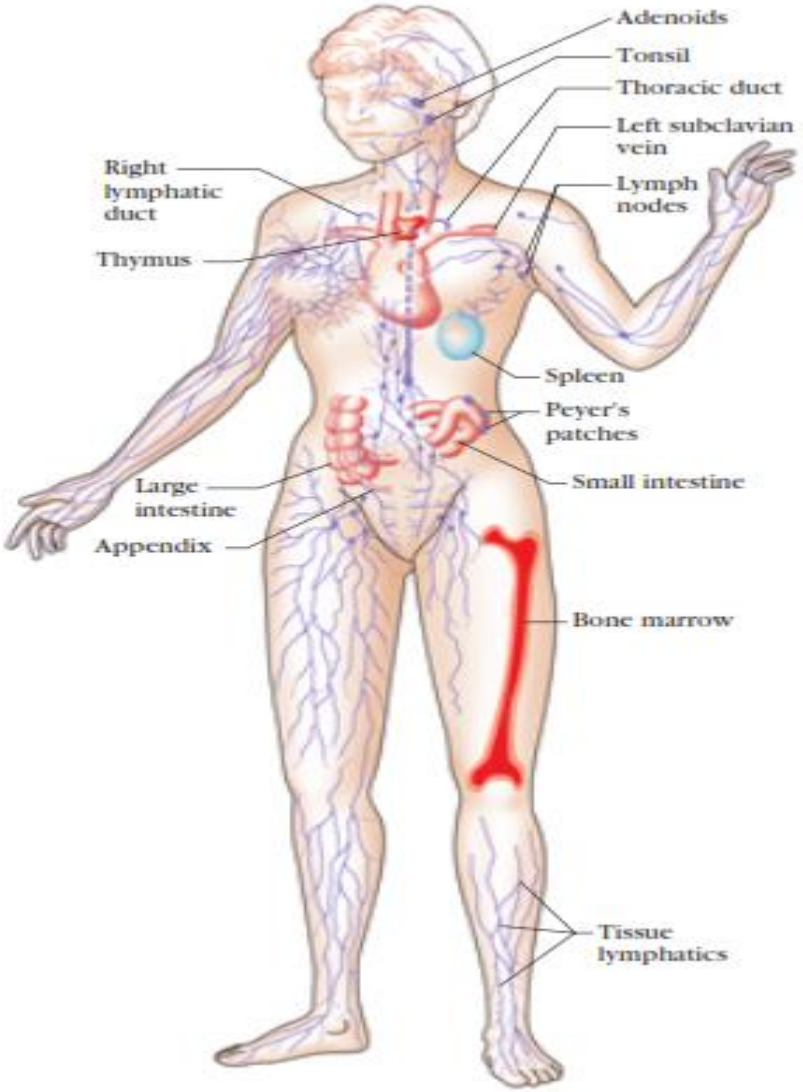

Figure 2. The human lymphoid system, the primary organs (bone marrow and thymus) are shown in red; secondary organs andtissues, in blue (Kindt, 2004).

I.3.1.1 Thymus

Above the heart, the thymus, a bilobed organ, serves as the location for T-cell development and maturation. The thymus is divided into lobules, with an outer cortex that is densely packed with immature thymocytes, and an inner medulla that contains fewer thymocytes. A network of epithelial cells, dendritic cells, and macrophages promotes thymocyte growth and maturation, interacting closely with specialized cells such as nurse cells. More than 95% of thymocytes undergo apoptosis, with the remaining thymic cells producing a diverse array of T cells that can identify infections via T-cell receptors. This rigorous selection process eliminates non-functional cells and those reacting with self-antigens, thereby preventing autoimmune diseases and ensuring that only effective, safe T cells are released to defend the body **(Kindt, 2004).**

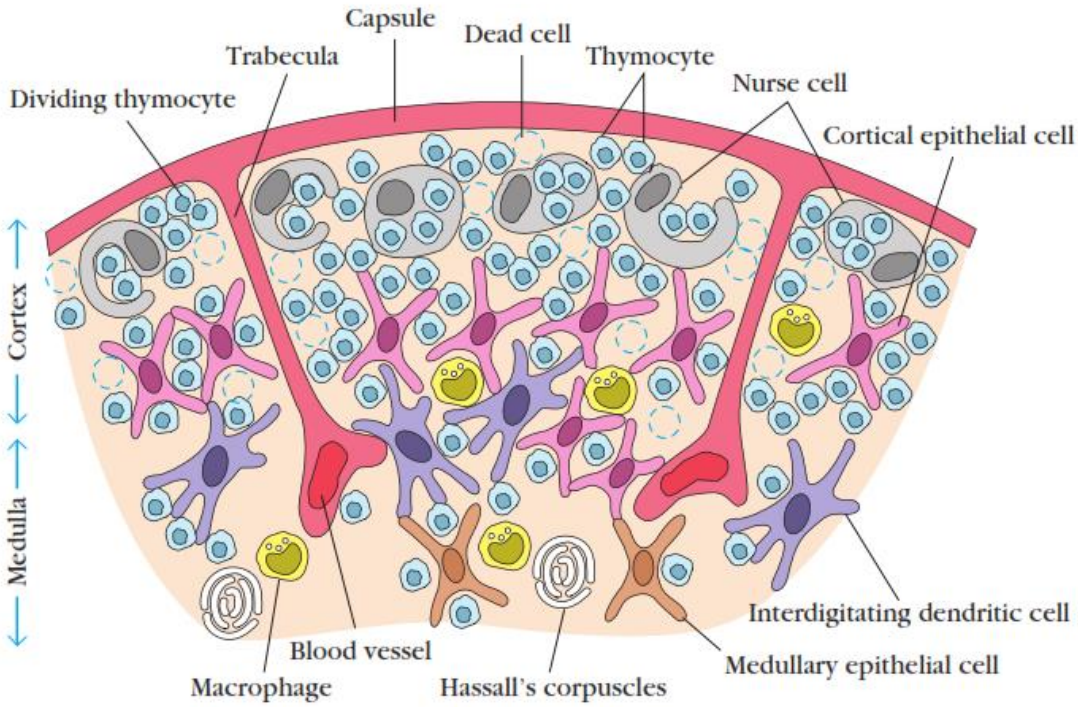

Figure 3. Diagrammatic cross-section of a portion of the thymus (Kindt, 2004)

I.3.1.2 Bone marrow

In humans and mice, the bone marrow serves as the primary site for B-cell development, where immature B cells, originating from lymphoid progenitors, proliferate and undergo differentiation. Bone marrow stromal cells offer vital support by interacting with B cells and secreting cytokines crucial for B cell development. The bone marrow employs a selection process to remove self-reactive B cells and thereby prevent autoimmune responses. The site of B-cell maturation differs among species. It occurs in the bursa of Fabricius, a gut-associated lymphoid organ in birds. Early B-cell development in cattle and sheep starts in the fetal spleen and then moves to the ileal Peyer's patch in the intestine. Rabbits also rely on gut-associated tissues, such as the appendix, for B-cell maturation and diversification **(Carcelain *et al.*, 2023).**

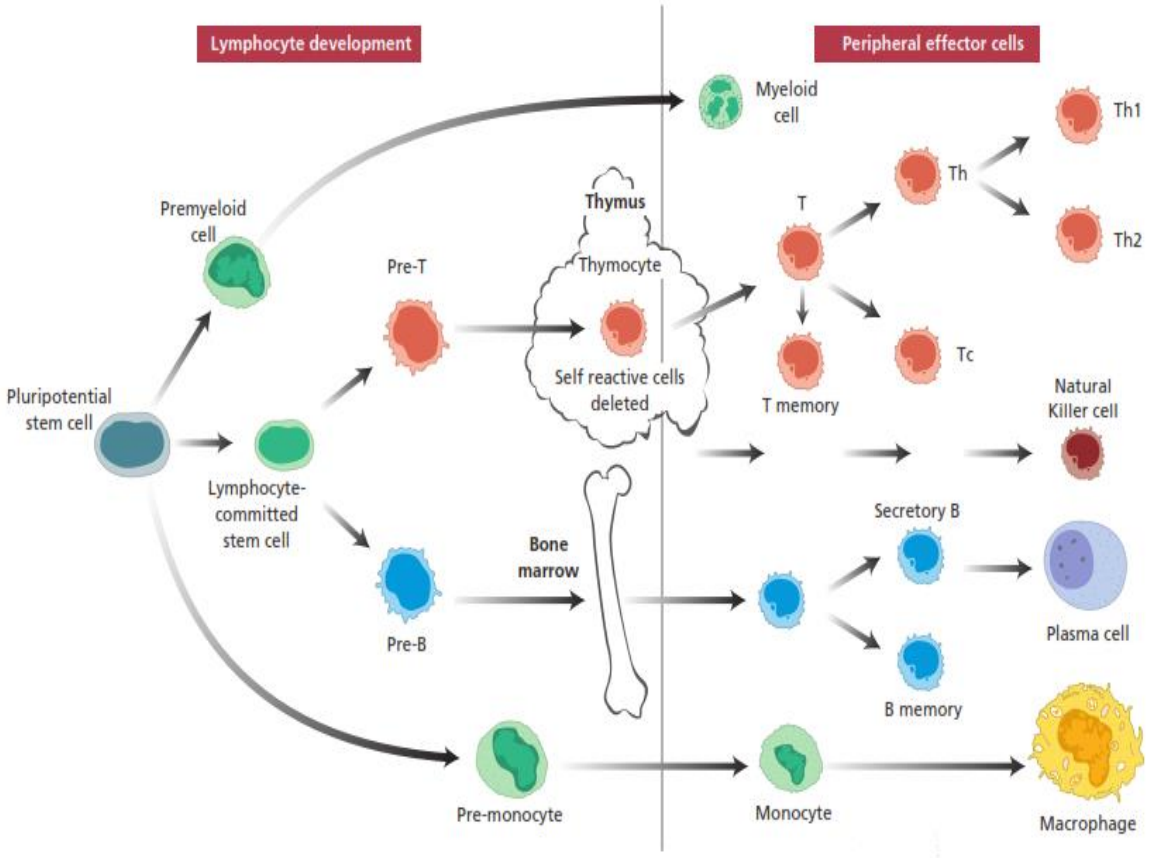

Figure 4. Development of different types of blood from a pluripotential stem cell in the bone marrow (Chapel *et al.*, 2013)

The developmental pathway for natural killer (NK) cells is shown separately because it is thought NK cells may develop in both the thymus and the bone marrow.

I.3.2 Secondary lymphoid organs

They are schematically classified into systemic organs and mucosal organs, sharing common characteristics:

- Their development depends on cells originating from the primary lymphoid organs.
- Their maturation occurs primarily after birth, through contact with environmental antigens.
- They contain distinct zones for T lymphocytes (e.g., the paracortical zone of lymph nodes) and B lymphocytes (e.g., germinal centers, also called lymphoid follicles).
- In these structures, high endothelial venules allow the controlled entry of lymphocytes.

These organs act as sites for draining and concentrating antigens from tissues, lymph (lymph nodes), blood (spleen), or mucosae (mucosa-associated lymphoid tissue, MALT). Additionally, the vascularization of secondary lymphoid organs ensures the continuous circulation of naïve lymphocytes **(Arroz-Madeira *et al.*, 2023).** Thus, they serve as a critical meeting point between antigens and the various cells involved in the adaptive immune response. Finally, once activated, the effectors of adaptive immunity are distributed from secondary lymphoid organs to peripheral tissues through the efferent lymphatic channel, thoracic duct, and then into the blood **(Carcelain *et al.*, 2023).**

I.3.2.1 Spleen

The spleen is the largest secondary lymphoid organ, weighing approximately 150 to 200 grams, with an oval shape situated in the left hypochondrium. Unlike other lymphoid organs, it is exclusively linked to the blood circulation, acting as a filter and performing immune surveillance by detecting antigens in the bloodstream **(Cesta, 2006).** During embryonic development, the spleen functions as a hematopoietic organ, similar to the fetal liver. After birth, it consists of two main components:

1. Red pulp (99% of its volume), which is rich in macrophages and specializes in breaking down old red blood cells.
2. White pulp (1% of its mass), located around arterioles, where immune responses are initiated.

The white pulp contains periarteriolar lymphoid sheaths, made up of lymphocytes:

1. The central zone (T zone) is rich in T lymphocytes.
2. The peripheral zone (B zone) contains B lymphocytes, organized into primary or secondary lymphoid follicles and a surrounding marginal zone.

This specialized structure enables the spleen to efficiently combine its roles in filtering blood and generating immune responses **(Carcelain et al., 2023).**

I.3.2.2 Lymph nodes

Lymph nodes are encapsulated structures with a round or kidney-shaped appearance, measuring between 1 to 15 mm in diameter, with approximately 500 to 1,000 nodes in humans. They are interconnected by lymphatic vessels, forming chains of lymph nodes.Each lymph node has a well-developed afferent lymphatic system (bringing lymph in) and a single efferent lymphatic vessel (carrying lymph out). Lymph nodes, distributed throughout the body, serve as monitors for different regions, draining lymph from the interstitial fluid surrounding tissues via their afferent vessels. They act as filters, concentrating soluble antigens or those captured by antigen-presenting cells (APCs) **(Carcelain *et al.*, 2023).**

Their strategic position at the crossroads of lymphatic and blood circulation enhances antigen detection by immune cells passing through them, thereby triggering adaptive immune responses.

Lymph nodes are divided into three main regions:

1. **Cortical zone (B zone):** Contains lymphoid follicles rich in B lymphocytes.
2. **Paracortical zone (T zone):** Primarily composed of T lymphocytes, which interact with dendritic cells presenting antigens.

3. **Medullary cords and sinuses:** Located in the center, rich in macrophages, and serve as sites for capturing particulate antigens brought in by lymph.

The lymph and the immune cells it contains leave the lymph nodes via the efferent vessel. The entire lymphatic network is ultimately drained by the thoracic duct, which discharges into the subclavian vein. This distinctive arrangement, combined with the hemolymphatic circulation, enables efficient communication and interaction among all the cellular components participating in immune responses **(Nicolas, 2007; Carcelain, et al., 2023).**

I.3.2.3 Mucosal lymphoid organs

Mucosal lymphoid organs, collectively referred to as Mucosa-Associated Lymphoid Tissue (MALT), encompass a diverse group of structures that represent 80% of the lymphoid tissue mass in the body **(Kuper *et al.*, 2017).** This makes them extremely important for protecting against antigens that penetrate through mucosal epithelium, which covers a surface area of over 400 square meters (including the respiratory, digestive, and urogenital mucosa). MALT consists of diffuse lymphoid tissue as well as specialized structures, such as Peyer's patches, the appendix, and the tonsils within the digestive tract. These tissues play a crucial role in the immune system's defense at mucosal barriers, where the body frequently encounters environmental antigens **(Nicolas 2007; Carcelain *et al.*, 2023).**

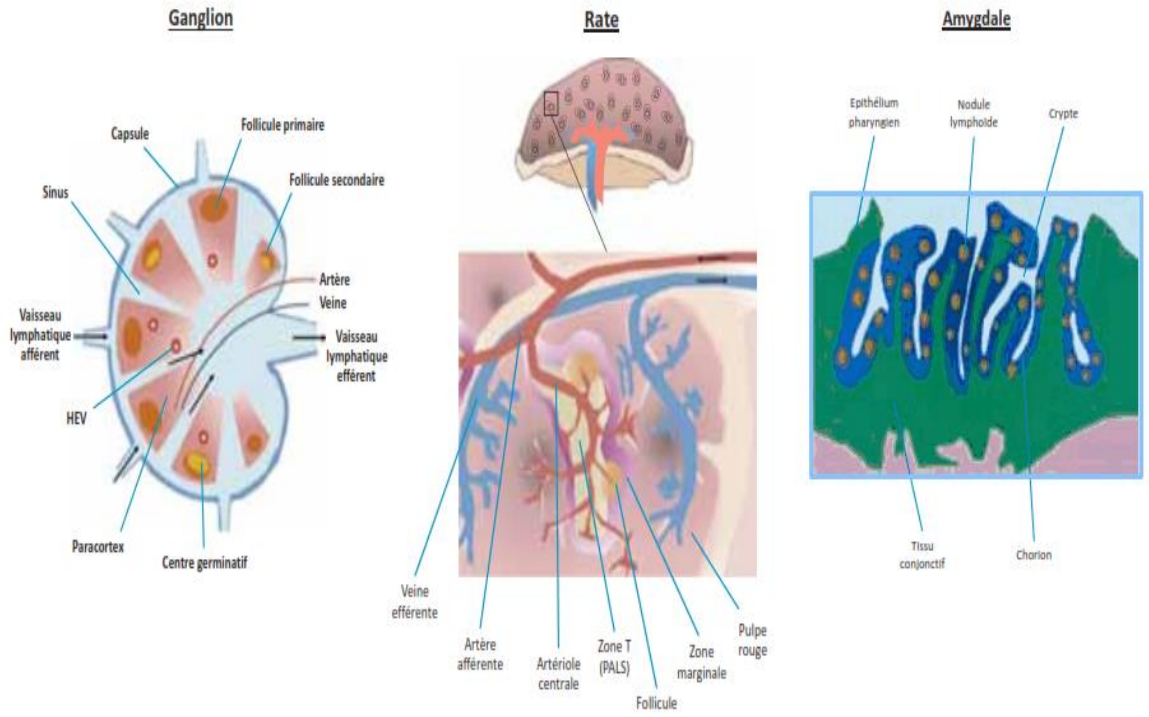

Figure 5. Examples of secondary lymphoid organ structures (Carcelain *et al.*, 2023)

I.4 Cells of the Immune System

I.4.1 Hematopoiesis

Hematopoiesis is the physiological process by which all blood cells, including red blood cells, white blood cells, and platelets, are produced. The process starts with hematopoietic stem cells (HSCs), which are specialized cells that can both multiply and transform into numerous different blood cell types. These stem cells form the basis for the entire blood cell lineage. Hematopoiesis commences early in embryonic development within the yolk sac. In the initial weeks, yolk-sac stem cells generate primitive red blood cells that contain embryonic hemoglobin **(Kindt, 2004).** Around the third month of gestation, hematopoietic stem cells migrate to the fetal liver and spleen, which become the primary sites of blood cell production until the seventh month. After this stage, the bone marrow takes over as the central site for hematopoiesis, and by birth, liver and spleen involvement in blood production ceases (**Klaus et al., 2017).**

HSCs differentiate into two main progenitor pathways: lymphoid and myeloid. Lymphoid progenitor cells give rise to B cells, T cells, natural killer (NK) cells, and some dendritic cells. On the other hand, myeloid progenitor cells form red blood cells, platelets, granulocytes (neutrophils, eosinophils, basophils), monocytes, mast cells, and other dendritic cells **(Purton, 2022).** This differentiation is influenced by the surrounding microenvironment and specific growth factors.The bone marrow serves as a nurturing environment for hematopoiesis. It contains stromal cells, such as fat cells, fibroblasts, endothelial cells, and macrophages, which create a cellular matrix and secrete growth factors. These growth factors, essential for regulating the differentiation and proliferation of hematopoietic cells, may function as soluble molecules or as membrane-bound signals that require direct cell-to-cell interaction **(Dzierzak *et al.*, 2018).**

During infections or stress, hematopoiesis is accelerated. Activated macrophages and T cells release additional hematopoietic growth factors to meet the increased demand for immune cells. This adaptive mechanism ensures the body can respond effectively to challenges **(Kindt, 2004).**

Once hematopoietic cells have matured, they leave the bone marrow and enter the bloodstream. Red blood cells, white blood cells, and platelets then perform their specialized functions, such as oxygen transport, immune defense, and blood clotting, ensuring the body’s systems work efficiently. This tightly regulated process sustains blood cell production throughout life, responding dynamically to the body's physiological needs **(Kindt, 2004; Dzierzak *et al.*, 2018).**

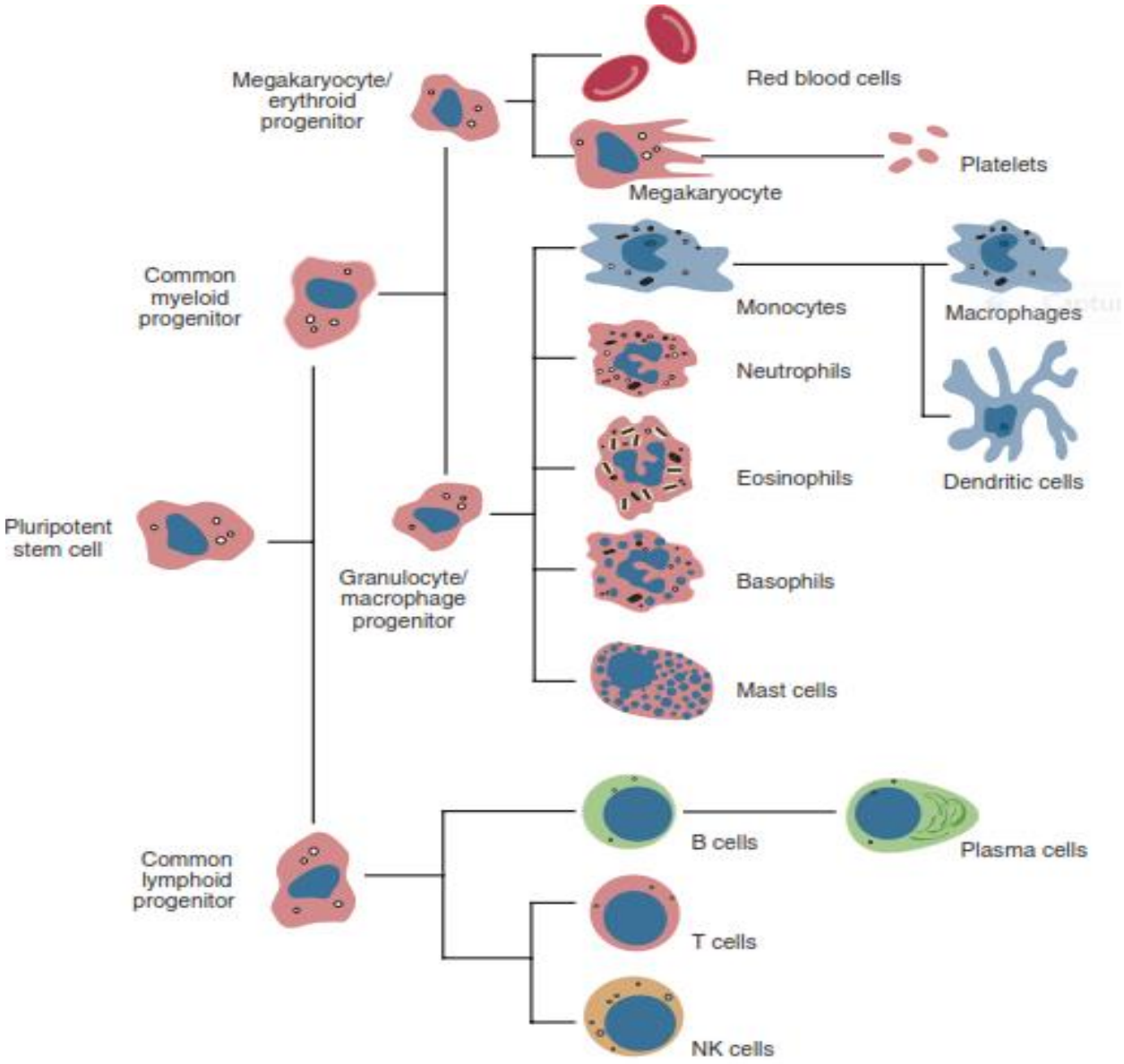

Figure 6. Hematopoiesis (Kindt, 2004)

I.4.2 Innate immunity cell

Innate immune cells, such as neutrophils, monocytes/macrophages, and dendritic cells, play a crucial role in phagocytosing and destroying foreign elements. Natural Killer (NK) cells, another component of innate immunity, target and eliminate virus-infected and tumor cells **(Carcelain *et al.*, 2023).**

I.4.2.1 Neutrophils

Neutrophils are the most abundant granulocytes in the bloodstream, distinguished by their multilobed nuclei. As the first responders to bacterial infections, they are quickly recruited to sites of infection by chemotactic signals. Neutrophils engage in phagocytosis and release more than 100 enzymes from their cytoplasm to fight microbes, although they have a relatively short lifespan once in tissues **(Carcelain et al., 2023; Visnyaiová et al., 2024).**

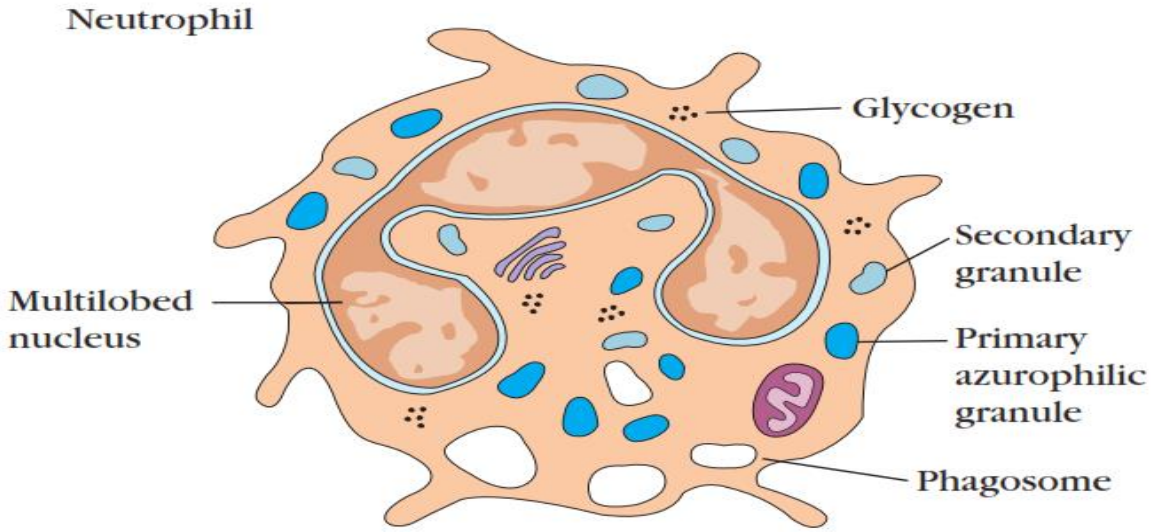

Figure 7.Neutrophil (Kindt, 2004)

I.4.2.2 Eosinophils

Eosinophils are characterized by their bilobed nuclei and orange-red granules. Primarily located in tissues, these cells play a crucial role in defending against parasites and in hypersensitivity reactions, thanks to the cytotoxic and pro-inflammatory substances contained in their granules **(Carcelain et al., 2023; Visnyaiová et al., 2024).**

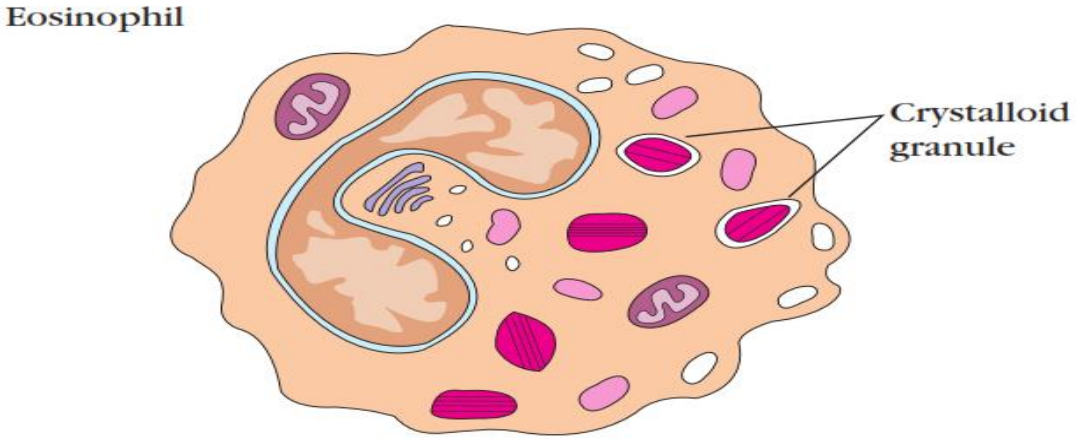

Figure 8. Eosinophil (Kindt, 2004)

I.4.2.3 Basophils

Basophils have bilobed nuclei that are often concealed by numerous granules filled with histamine and acidic, pro-inflammatory substances. They play a key role in immediate hypersensitivity reactions and anti-infective responses. Mast cells, their tissue counterparts, are found in abundance in mucosal areas **(Carcelain et al., 2023; Visnyaiová et al., 2024).**

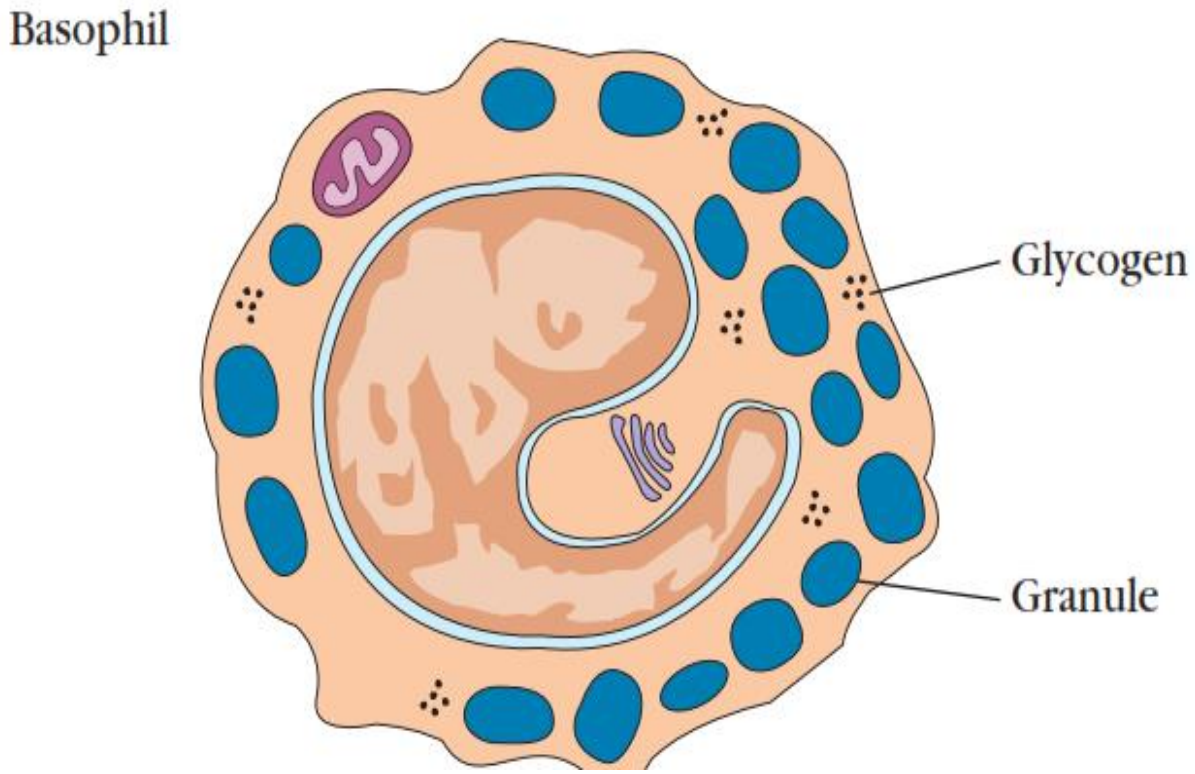

Figure 9. Basophil (Kindt, 2004)

I.4.2.4 Monocytes/ Macrophages

Monocytes circulate in the blood and migrate to tissues, where they differentiate into macrophages. These phagocytic cells capture and destroy diverse elements, including microbes, antigens, and cellular debris. They also present antigens to adaptive immune cells and produce cytokines critical for immune responses and tissue repair. Tissue-specific macrophages include Kupffer cells (liver), microglia (brain), and osteoclasts (bone) **(Kindt, 2004).**

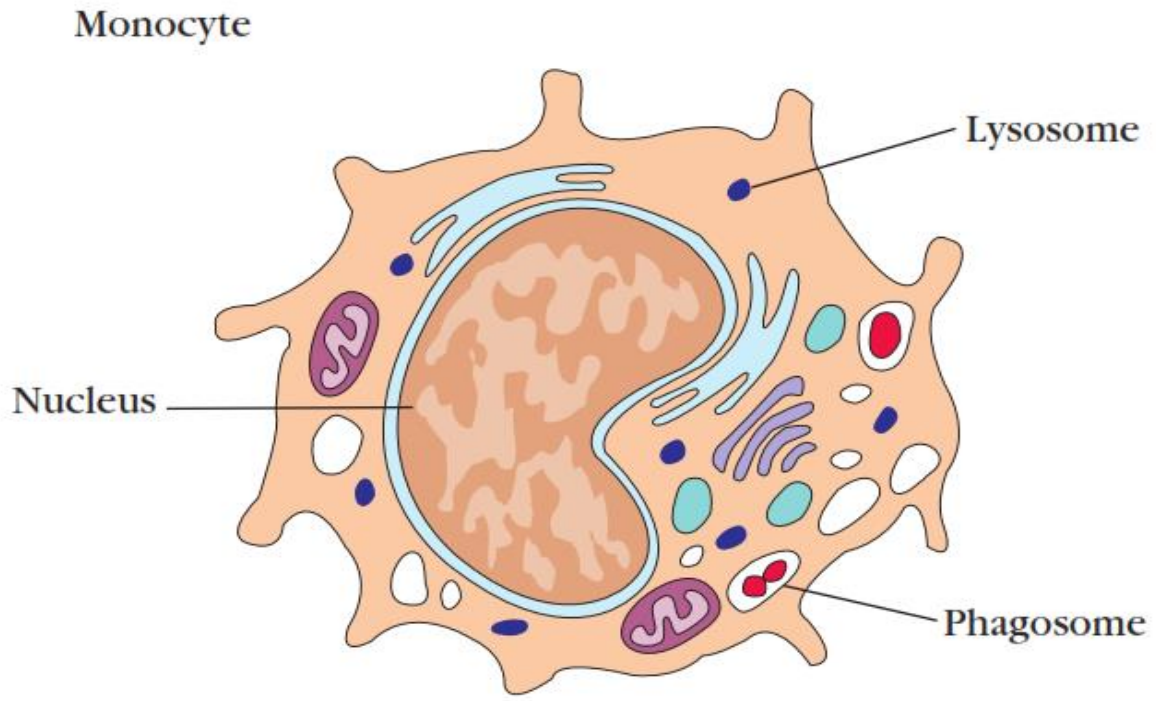

Figure 10. Monocytes (Kindt, 2004)

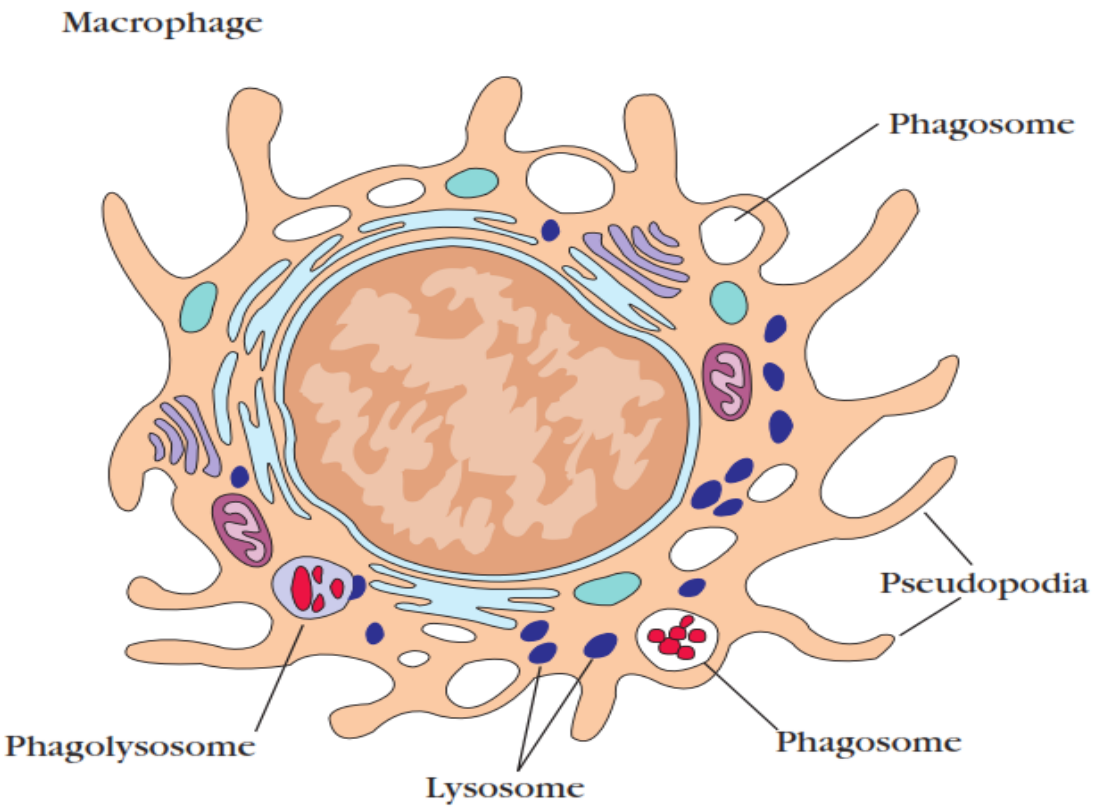

Figure 11. Macrophage (Kindt, 2004)

I.4.2.5 Dendritic Cells

These cells are present in various tissues and are highly efficient at capturing antigens while in their immature form. Upon migrating to lymphoid tissues, they undergo maturation and specialize in presenting antigens to naive T cells, thereby initiating adaptive immune responses. As the most powerful antigen-presenting cells (APCs), they exist in different subtypes, each with unique functions **(Carcelain et al., 2023).**

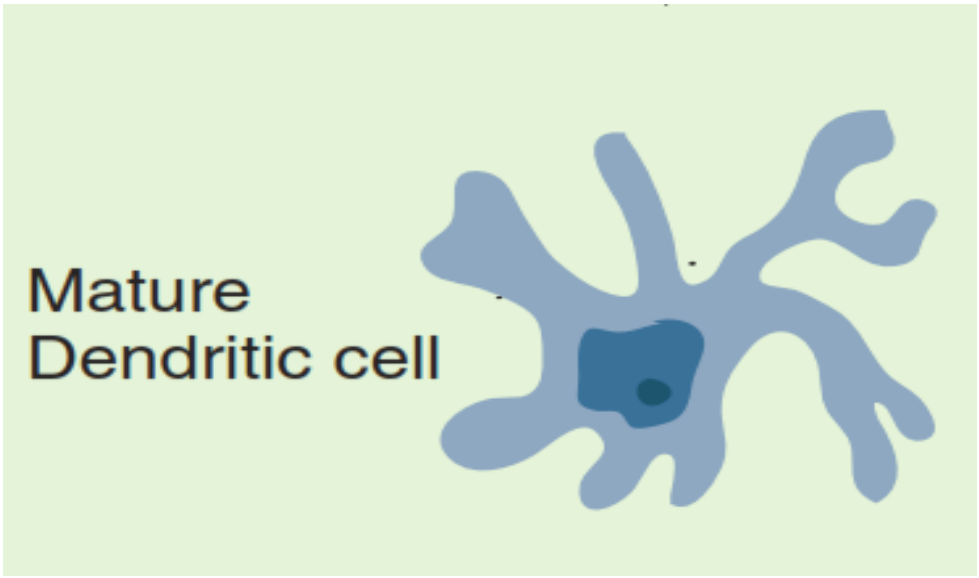

Figure 12.Dendritic cells (Tizard, 2013)

I.4.2.6 Natural Killer (NK) Cells

These cytotoxic cells, found in blood and peripheral lymphoid organs, destroy infected or damaged cells and those marked by IgG antibodies. They also secrete cytokines, such as IFN-γ, to enhance immune responses **(Carcelain et al., 2023; Visnyaiová et al., 2024).**

I.4.2.7 Mast cells

Mast cells are tissue-resident immune cells found primarily in mucosal tissues, such as the skin, lungs, and gastrointestinal tract. They contain granules rich in histamine, proteases,

and other pro-inflammatory mediators, which are released during immune responses. Mast cells play a crucial role in allergic reactions, wound healing, and defending against pathogens. They can be activated by various stimuli, including allergens and pathogens, leading to the release of their granules, which contribute to inflammation and tissue protection **(Visnyaiová *et al.*, 2024).**

I.4.3 Adaptive immunity cells

Lymphocytes B and T are the key players in adaptive immunity, with distinct roles in humoral and cellular immune responses.

I.4.3.1 B Lymphocytes

B lymphocytes are responsible for the humoral immune response, which involves the production of antibodies that target and neutralize foreign pathogens. These cells are initially naïve, circulating in the blood and lymphatic systems, and they become activated when they encounter their specific antigen in secondary lymphoid organs. Upon activation, B lymphocytes differentiate into plasma cells, which secrete antibodies, and memory B cells, which provide long-term immunity. B cells recognize antigens through their unique B cell receptors (BCR), and their activation plays a pivotal role in defending against extracellular pathogens, including bacteria and viruses **(Carcelain *et al.*, 2023).**

I.4.3.2 T Lymphocytes

T lymphocytes are central to cellular immunity and are responsible for recognizing and responding to infected or abnormal cells. These cells are divided into subtypes, including helper T cells (which assist other immune cells), cytotoxic T cells (which kill infected or tumor cells), and regulatory T cells (which modulate the immune response to prevent autoimmunity). T lymphocytes mature in the thymus, where they undergo a selection process to ensure they can recognize specific antigens through their T cell receptors (TCR) while maintaining tolerance to self-antigens. Once matured, T cells circulate and migrate to secondary lymphoid organs, where they become activated by their specific antigen, initiating a targeted immune response **(Carcelain *et al.*, 2023).**

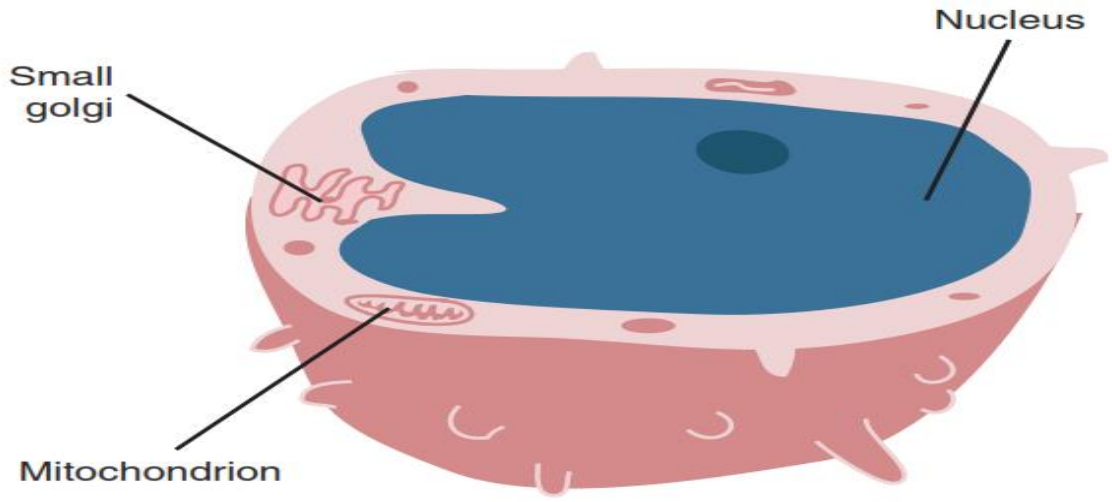

Figure 13. Essential structural features of a lymphocyte (Tizard, 2013)

Table 1. Identifying Features of T and B Cells (Kindt, 2004)

Property	B Cells	T Cells
Development Site	Bone marrow, bursa, Peyer's patches	Thymus
Distribution	Lymph node cortex, splenic follicles	Lymph node paracortex, spleen periarteriolar sheath
Circulation	No	Yes
AntigenReceptors	BCR (B-cell receptor) – immunoglobulin	TCR (T-cell receptor) – protein heterodimer, associated with CD3, CD4, or CD8
Important Surface Antigens	Immunoglobulins	CD2, CD3, CD4, CD8
Mitogens	Pokeweed, lipopolysaccharide	Phytohemagglutinin, concanavalin A, BCG vaccine, pokeweed
RecognizedAntigens	Free foreignproteins	Processed foreign proteins presented in MHC antigens
Tolerance Induction	Difficult	Easy
ProgenyCells	Plasma cells, memory B cells	Effector T cells, memory T cells
SecretedProducts	Immunoglobulins	Cytokines

I.5 Molecules of the Immune System

The immune system relies on a wide range of molecules that play essential roles in defending the body against pathogens (bacteria, viruses, fungi, parasites) as well as regulating the immune response. These molecules include proteins, cytokines, receptors, and other soluble factors **(Nicolas, 2007).**

I.5.1 Antibodies (Immunoglobulins)

B lymphocytes produce antibodies that specifically target and attach to antigens on pathogens, flagging them for removal by other immune system components. There are multiple classes of antibodies, each with specific functions **(Goldberg *et al.*, 2020):**

1. **IgG:** The most abundant class in blood and body fluids, responsible for long-term immunity.
2. **IgA**: Found in mucous membranes and secretions like saliva and breast milk, playing a key role in mucosal immunity.
3. **IgM:** The first antibody produced in response to an infection.
4. **IgE:** Involved in allergic reactions and defense against parasites.
5. **IgD**: Functions primarily in the activation of B lymphocytes.

I.5.2 Cytokines

These messenger proteins facilitate communication between immune cells, regulating their growth, differentiation, and activation. Key cytokines include **(Torrado et al., 2013):**

1. **Interleukins (IL):** Each interleukin has specific functions, such as activating lymphocytes (e.g., IL-2) or promoting inflammation (e.g., IL-1, IL-6).
2. **Interferons (IFN):** Play a pivotal role in antiviral responses, notably IFN-α and IFN-β.
3. **Tumor Necrosis Factors (TNF):** Involved in inflammation regulation, with TNF-α being a major pro-inflammatory cytokine.

4. **Chemokines:** Attract immune cells to sites of infection or tissue damage.

I.5.3 Complement System

According to **Ostrycharzet al., (2022),** this group of circulating proteins, when activated, leads to pathogen destruction through:

1. **Opsonization:** Marking pathogens to enhance their uptake by phagocytic cells.
2. **Cell Lysis:** Forming membrane attack complexes that create pores in pathogen membranes.
3. **Inflammation:** Releasing pro-inflammatory molecules that recruit and activate other immune cells.

Table 2. Effector molecules in immunity (Kindt, 2004)

<table>
<tr><th colspan="2">InnateImmunity</th><th colspan="2">Adaptive Immunity</th></tr>
<tr><td colspan="2">Complement components for opsonization or lysis</td><td>Humoral</td><td>Specific antibodies for opsonization and phagocytosis or lysis with complement</td></tr>
<tr><td colspan="2">Perforin in NK cells creates pores in target cell membranes</td><td rowspan="2">Cellular</td><td>Perforin in cytolytic (CD8) T cells creates pores in
specific target cell membranes, allowing entry of
granzymes to cause apoptosis</td></tr>
<tr><td colspan="2">Granzymes in NK cells induce apoptosis in target cells</td><td>NKT cells induce apoptosis by perforin production</td></tr>
<tr><td colspan="2">Lysosomes in phagocytic vacuoles result in death of
ingested microbes</td><td colspan="2" rowspan="2">/</td></tr>
<tr><td colspan="2">Preformed histamine and related vasoactive substances as well as leukotrienes in mast cells</td></tr>
</table>

CHAPTER II:

THE IMMUNE RESPONSE

Immunity is the state of protection against infectious diseases and includes both a non-specific component and a more specific component **(Nicolas, 2007).**

The non-specific component, known as innate immunity, serves as the first line of defense against infections. Most elements of innate immunity are present before the onset of infection and represent a set of disease resistance mechanisms that are not specific to a particular pathogen. These include cellular and molecular components that recognize molecular patterns commonly found on pathogens. Key players in innate immunity include phagocytic cells such as macrophages and neutrophils, physical barriers like the skin, and a variety of antimicrobial compounds synthesized by the host **(Nicolas, 2007).**

Unlike the broad reactivity of the innate immune system, which is uniform across all members of a species, the specific component, known as adaptive immunity, is activated only in response to an antigenic challenge. Adaptive immunity responds to such challenges with a high degree of specificity and an extraordinary property called "memory" **(Nicolas, 2007; Murphy et al., 2016).**

An adaptive immune response usually begins within five to six days following the first exposure to an antigen. When the immune system encounters the same antigen again, it mounts a swift, forceful, and typically more successful memory response to rapidly neutralize and eliminate the pathogen. The primary mediators of adaptive immunity are lymphocytes, which also produce antibodies and other molecules **(Keselowsky et al., 2020).**

Since adaptive immune responses take time to develop, innate immunity provides the first line of defense during the critical period immediately following pathogen exposure. In most cases, the innate immune system eliminates the majority of microorganisms encountered by a healthy individual within a few days, often before the activation of the adaptive immune system **(Nicolas, 2007).**

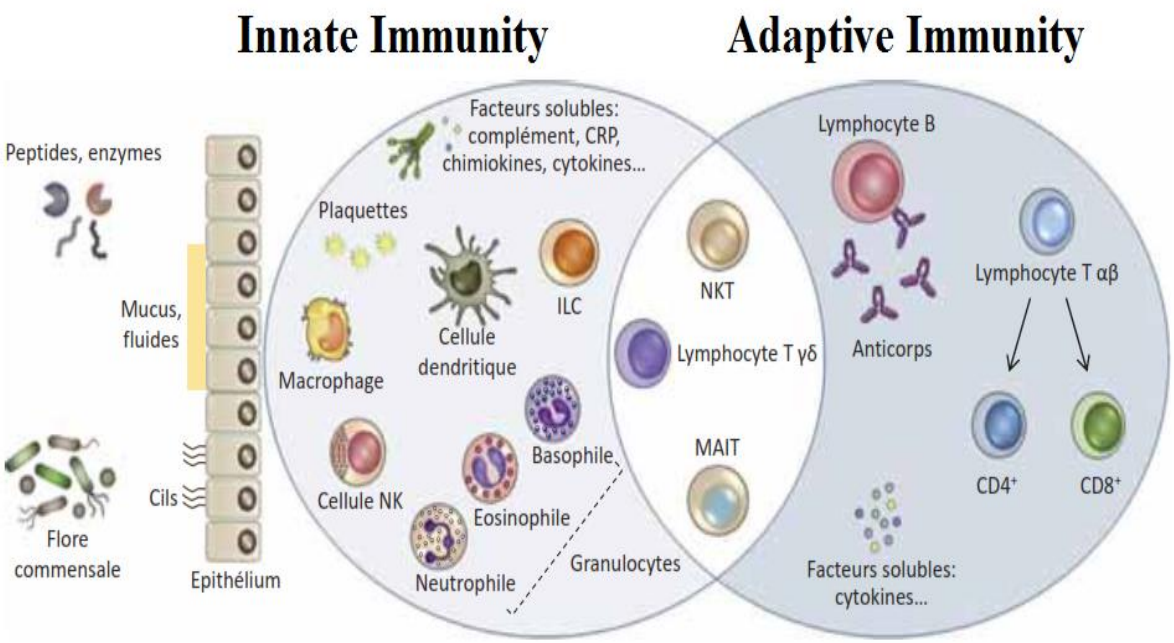

Figure 14. General organization of the immune system (Carcelainet al., 2023)

II.1 Innate Immunity (Non-Specific)

The body's innate immunity acts as the first line of defence against infections. It is viewed as non-specific because it reacts to pathogens in a broad way, rather than focusing on particular invaders. This type of immunity relies on physical barriers (for example, the skin and mucous membranes), cellular components (such as macrophages, neutrophils, and natural killer cells), and chemical mediators (including cytokines and antimicrobial peptides). Innate immunity offers immediate defence and triggers the adaptive immune system if necessary, despite lacking the precision and recall found in adaptive immunity **(Kindt, 2004; Carcelainet al., 2023).**

II.1.1 Surface Barriers

The physical and anatomical barriers that work to prevent the entry of pathogens constitute the first line of defense of an organism against infections. The skin and the surface of mucous membranes are included in this category as they serve as effective barriers against the entry of most microorganisms **(Kindt, 2004).**

II.1.1.1 Skin and mucous surfaces

The skin plays a crucial role in innate immunity by acting as both a physical and chemical barrier against infections. The epidermis, with its layers of dead cells filled with keratin, prevents microorganisms from entering the body. Sebum produced by sebaceous glands contains fatty acids and lactic acid, which maintain an acidic pH, inhibiting the growth of many pathogens **(Turvey et al., 2010).**

On the other hand, mucous membranes also play a vital role in innate immunity by protecting the internal surfaces of the body, such as the respiratory, digestive, and urogenital tracts. These membranes are lined with epithelial cells that secrete mucus, a viscous substance that traps microorganisms and foreign particles. This mucus, combined with the cilia in the respiratory tract, helps eliminate pathogens by expelling them through sweeping motions. Additionally, mucous membranes contain immune cells that respond quickly to infections, further preventing their spread **(Turvey et al., 2010).**

II.1.2 Internal defense: cells and molecules

The body uses several non-specific defence mechanisms, such as phagocytes, natural killer cells, antimicrobial proteins, and fever. The inflammatory response involves cells like macrophages, mast cells, and different types of leukocytes, as well as numerous chemical substances that eliminate pathogens and aid in tissue repair **(Nicolas, 2007).**

II.1.2.1 Phagocytes

Pathogens that penetrate tissues beneath the skin and mucous membranes encounter phagocytes, primarily macrophages, which are white blood cells derived from monocytes. Macrophages circulate freely within tissues, scavenging for debris or intruders **(Patel *et al.*, 2021).** The primary immune cells responsible for phagocytosis include:

1. **Monocytes:** Found in the blood, monocytes differentiate into macrophages or dendritic cells when they migrate into tissues.
2. **Macrophages:** Present in tissues, macrophages play a critical role in eliminating pathogens and dead cells.

3. **Neutrophils:** As the first responders to infections, neutrophils rapidly engulf pathogens through phagocytosis.
4. **Dendritic Cells:** Specialized in antigen presentation, these cells can also phagocytose particles to trigger an adaptive immune response.

II.1.2.1.1 Mechanism of Phagocytosis

The innate immune response relies on phagocytosis as a vital process, enabling phagocytic cells to capture and eliminate pathogens. The process entails a sequence of synchronised actions aimed at assimilating, breaking down, and removing foreign substances **(Kindt, 2004):**

1. **Attachment:** The bacterium becomes attached to membrane extensions of the phagocytic cell, called pseudopodia. These extensions surround the bacterium, initiating the engulfment process.
2. **Ingestion:** The bacterium is engulfed by the phagocyte, forming an internal compartment known as the phagosome.
3. **Phagosome-Lysosome Fusion:** The phagosome fuses with a lysosome, an organelle rich in digestive enzymes. This fusion creates a structure called the phagolysosome.
4. **Digestion:**Lysosomal enzymes within the phagolysosome degrade the captured bacterium into small, digestible fragments.
5. **Exocytosis:** The digestion products, now waste material, are expelled from the phagocytic cell via exocytosis, completing the process.

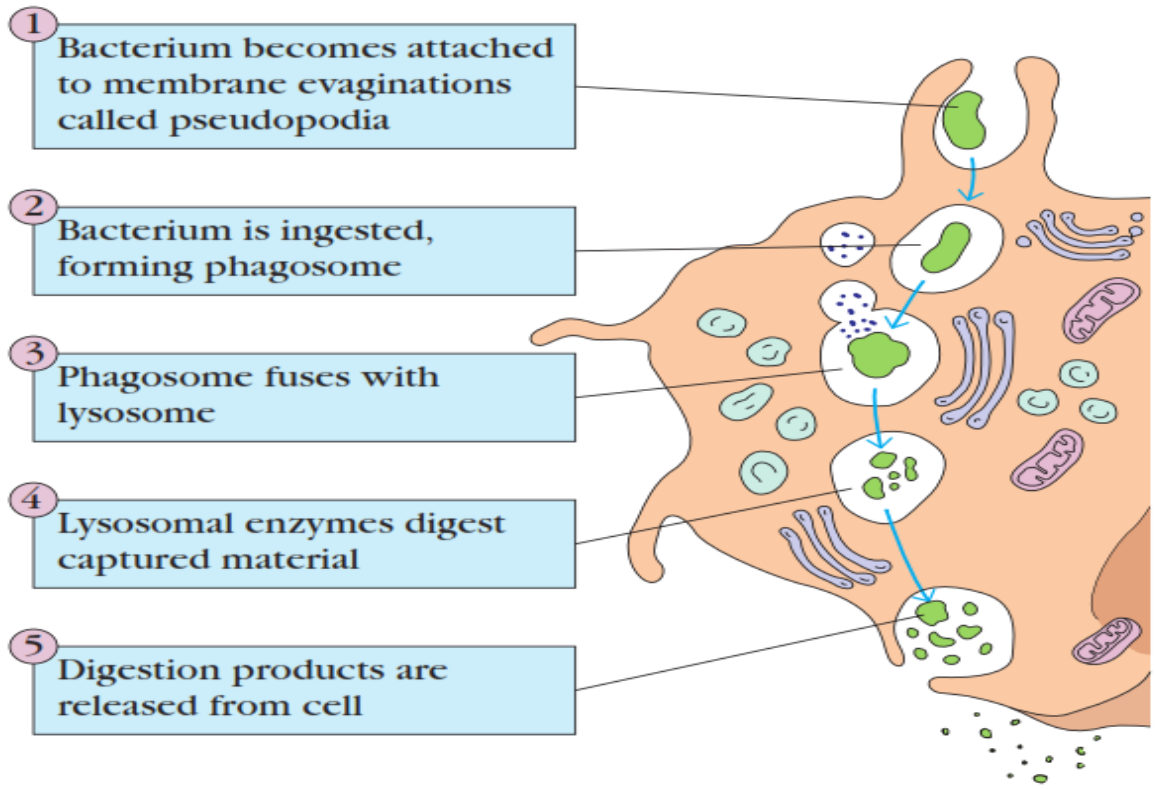

Figure 15. Mechanism of Phagocytosis (Kindt, 2004)

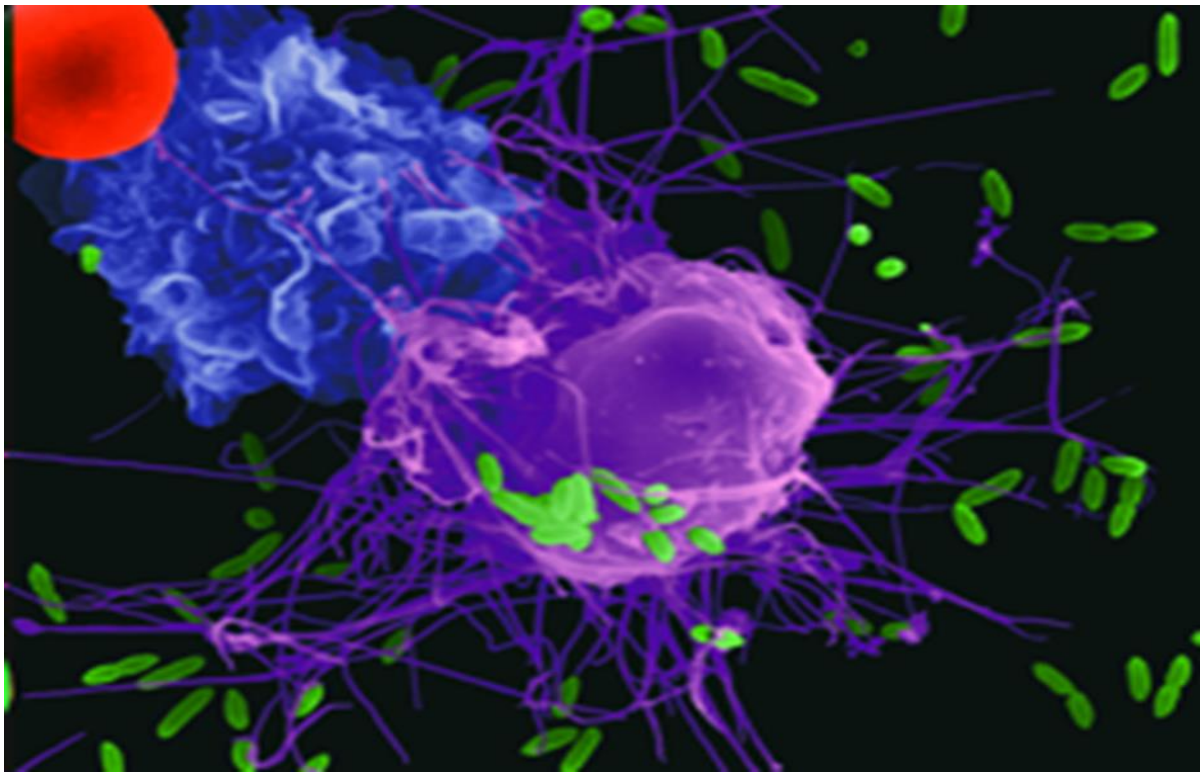

Figure 16.Electron micrograph of a macrophage (pink) attacking *Escherichia coli* (green). The bacteria are being phagocytosed (Kindt, 2004)

II.1.2.2 Natural Killer Cells

Natural Killer (NK) cells are essential components of the innate immune system, responsible for identifying and eliminating virus-infected and cancerous cells. They detect stress signals or abnormalities on the surface of cells, such as the absence of class I major histocompatibility complex (MHC) molecules, a common feature in infected or tumor cells. NK cells do not contribute to immune memory; once an infection or tumor is eliminated, they do not retain a "memory" of the event to respond more efficiently to future infections. They destroy infected cells through cytotoxicity, releasing perforins that create pores in the target cell's membrane, allowing granzymes to enter and induce apoptosis **(Hazeldine *et al.*, 2013).**

II.1.2.3 Inflammatory reaction

Tissue damage caused by a wound or an invading pathogenic microorganism triggers a complex sequence of events collectively known as the inflammatory response. As mentioned earlier, a molecular component of a microbe, such as lipopolysaccharides (LPS), can initiate the inflammatory response by interacting with cell surface receptors **(Page *et al.*, 2022).** The outcome of inflammation may involve the activation of a specific immune response to combat the invasion or the clearance of the invader by components of the innate immune system **(Carcelain *et al.*, 2023).**

The classic signs of inflammation rubor (redness), tumor (swelling), calor (heat), and dolor (pain) were first described by the Roman physician Celsus in the first century AD. Later, in the second century AD, Galen added a fifth sign: functiolaesa (loss of function). These signs correspond to the three major events of the inflammatory response (Figure 17) **(Carcelain *et al.*, 2023).**

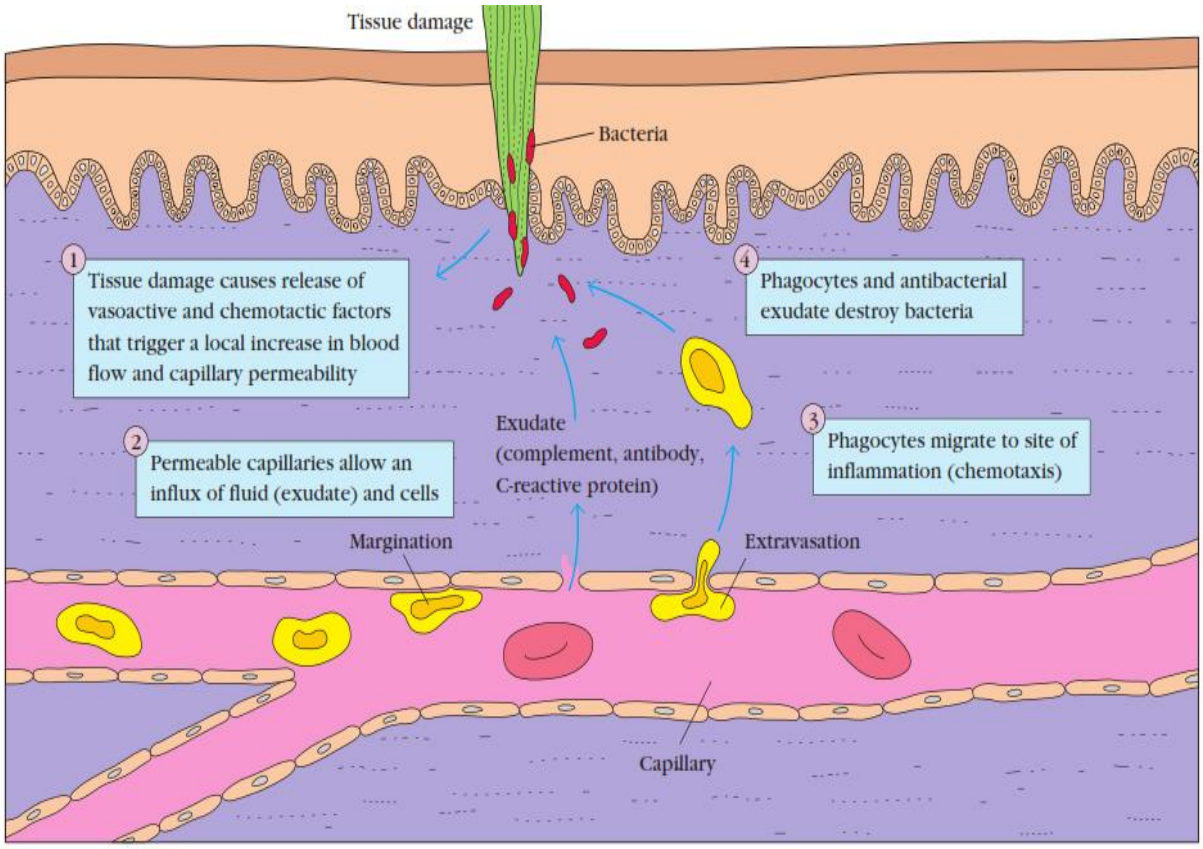

Figure 17. Major events in the inflammatory response (Kindt, 2004)

1. Vasodilation: An increase in the diameter of nearby blood vessels, particularly capillaries, occurs as vessels that carry blood away from the affected area constrict. This causes the capillary network to become engorged, which leads to tissue redness (erythema) and an increase in tissue temperature **(Nicolas, 2007).**

2. Increased capillary permeability: This allows fluid and cells to flow from the engorged capillaries into the surrounding tissue. The fluid, known as exudate, has a higher protein content than normal tissue fluid, contributing to tissue swelling (edema) **(Nicolas, 2007).**

3. Phagocyte influx: The increased permeability of the capillaries enables the movement of phagocytes from the bloodstream into the tissue. The emigration of these cells occurs in a series of steps: first, the cells adhere to the endothelial wall of the blood vessels (margination); next, they pass between the endothelial cells into the tissue (diapedesis or extravasation); finally, they migrate through the tissue to the site of infection (chemotaxis). As phagocytic cells accumulate and begin to engulf bacteria, they release lytic enzymes that can damage healthy cells nearby. The accumulation of dead cells, digested substances, and fluid

leads to the development of pus. The inflammatory response is triggered by a complex series of events involving various chemical mediators, whose interactions are only partially understood. Some of these mediators are derived from invading microorganisms, others are released from damaged cells in response to tissue injury, some are generated by plasma enzyme systems, and others come from white blood cells involved in the response.Among the chemical mediators released are acute-phase proteins, which increase significantly in response to tissue-damaging infections. C-reactive protein (CRP) is a major acute-phase protein produced by the liver in response to tissue damage. Its name reflects its ability to bind to C-polysaccharides found on the surface of bacteria and fungi, which activates the complement system and leads to enhanced pathogen clearance via complement-mediated lysis or increased phagocytosis **(Kindt, 2004; Ahmed, 2011).**

Histamine is another key mediator of the inflammatory response, released by various cells in response to tissue injury. Histamine binds to receptors on nearby capillaries and venules, causing vasodilation and increased permeability.Kinins, small peptides that are normally present in blood plasma in an inactive form, are another important group of inflammatory mediators **(Thangam *et al.*, 2018).** When activated by tissue injury, these peptides cause vasodilation and increased capillary permeability. A specific kinin, bradykinin, also stimulates pain receptors in the skin, which likely serves a protective function, as pain typically leads individuals to avoid using the injured area **(Nicolas, 2007).**

Enzymes from the blood-clotting system are also able to enter the tissue due to increased vasodilation and capillary permeability. The enzymes trigger a series of reactions that ultimately lead to the formation of fibrin strands, the main constituent of a blood clot. The injured area is effectively sealed off by the fibrin strands, thereby preventing the spread of infection. Once the inflammatory response has subsided and most of the debris has been cleared away by phagocytic cells, the process of tissue repair commences. As the fibrin clot forms, capillaries begin to infiltrate it, and fibroblasts, a type of connective tissue cell, subsequently replace the fibrin as the clot disintegrates (**Tizard, 2013; Agita *et al.*, 2017).**

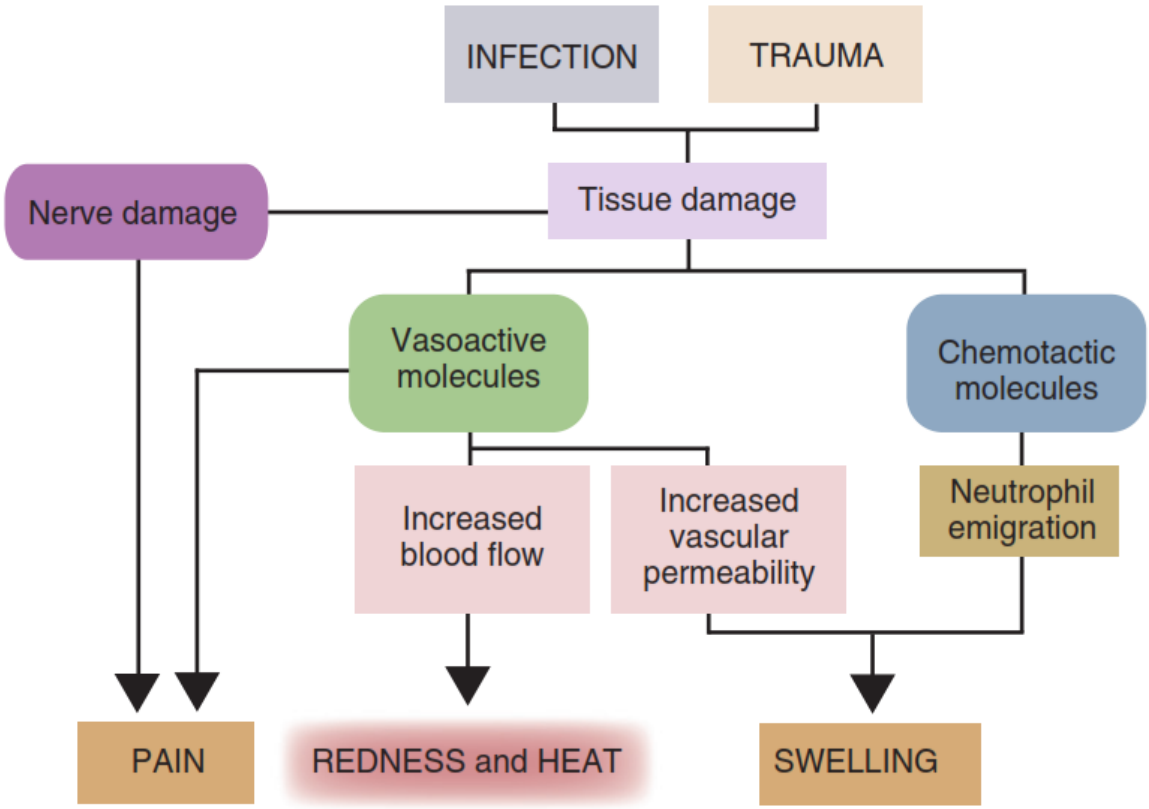

Figure 18. The major signs of acute inflammation and how they are generated (Tizard, 2013).

II.1.2.4 Antimicrobial proteins

Les protéines antimicrobiennes renforcent les défenses naturelles du corps en attaquant directement les microbes ou en empêchant leur multiplication. Les plus importantes sont l'interféron, les protéines du complément et la protéine C-réactive **(Nicolas, 2007).**

II.1.2.4.1 Interferon

Viruses are simply nucleic acids surrounded by proteins, unable to produce energy or proteins on their own. They cause damage by invading cells and using their machinery to reproduce, acting like parasites. While infected cells cannot defend themselves, some produce interferons, proteins that protect neighboring cells by preventing viral replication. This

interferon protection is not specific to a particular virus, as it works against several types of viruses **(De Andrea *et al.*, 2002).**

Interferons are a group of small proteins produced by different cells, with slightly different effects. T lymphocytes and NK cells produce interferon gamma (γ), while most white blood cells produce interferon alpha (α), which includes several substances. Fibroblasts produce interferon beta (β), which is particularly effective in reducing inflammation. In addition to their antiviral effects, interferons activate macrophages and stimulate NK cells, helping to fight cancer **(Yu *et al.*, 2022).**

II.1.2.4.2 Complement

More than twenty proteins, mainly produced by the liver, circulate in the blood in an inactive form. These proteins, which include C1 to C9 as well as factors B, D, and P, play a vital role in the body's immune response by identifying and neutralizing foreign invaders and enhancing the body's inflammatory reactions. Upon activation, the complement system facilitates the destruction of pathogens like bacteria via a process known as cytolysis. It plays a crucial role in boosting both innate and adaptive immune responses, enabling a swift and organised reaction to various threats **(Liszewski *et al.*, 2015).**

II.1.2.4.3 C-reactive protein

C-reactive protein (CRP) is produced by the liver in response to inflammation. CRP binds to various ligands, playing an important role in the immune system. It tags pathogens and damaged cells in the body, making it easier for phagocytes and the complement system to eliminate them. Clinically, CRP is used to diagnose acute infections or inflammatory diseases and to monitor the effectiveness of treatments **(Du Clos, 2000; Volanakis, 2001).**

Tableau 1. Summary of non-specific host defenses (Kindt, 2004)

Type	Mechanism
Anatomic barriers	- **Skin**: Mechanical barrier retards entry of microbes.
	- **Acidic environment (pH 3–5)**: Retards growth of microbes.
	- **Mucus**: Entraps foreign microorganisms.
	- **Cilia**: Propel microorganisms out of the body.
Physiologic barriers	- **Temperature**: Normal body temperature inhibits growth of some pathogens.
	- **Fever response**: Inhibits growth of some pathogens.
	- **Low pH**: Acidity of stomach contents kills most ingested microorganisms.
Chemical mediators	- **Lysozyme**: Cleaves bacterial cell wall.
	- **Interferon**: Induces antiviral state in uninfected cells.
	- **Complement**: Lyses microorganisms or facilitatesphagocytosis.
	- **C-reactive protein:** marking pathogens and damaged cells for elimination and aiding in the inflammatory response.
Phagocytic/endocytic barriers	- Various cells internalize (endocytose) and break down foreign macromolecules.
	- Specialized cells (e.g., blood monocytes, neutrophils, tissue macrophages) internalize (phagocytose), kill, and digest whole microorganisms.
Inflammatory barriers	- Tissue damage and infection induce leakage of vascular fluid containing serum proteins with antibacterial activity and influx of phagocytic cells.

II.2 Adaptive immunity (specific)

We all dream of a perfect defense system for our body, capable of identifying and eliminating any invader. This is the role of the adaptive immune system, which can specifically target different types of pathogens and eliminate them effectively. However, unlike the ever-ready innate defenses, the adaptive system needs to encounter a foreign agent (an antigen) for the first time to become fully operational, which takes some time **(Carcelain *et al.*, 2023).**

The adaptive immunity has three essential qualities **(Nicolas, 2007):**

1. **Specificity:** It identifies and targets specific invaders.
2. **Systemicity:** It acts throughout the body, not just at the site of infection.
3. **Memory:** After an initial encounter, it responds more quickly and more strongly to the same agents.

This immunity has two complementary branches **(Wang *et al.*, 2024):**

1. **Humoral immunity:** Antibodies circulate in the blood and lymph, temporarily neutralizing bacteria and viruses before they are destroyed by phagocytes.
2. **Cellular immunity:** T lymphocytes target infected, cancerous, or transplanted cells, either destroying them directly or activating other cells to strengthen the defense.

II.2.1 Humoral immune response

Antigenic stimulation, which refers to the activation of a naïve lymphocyte by a foreign antigen, typically occurs in the spleen or a lymph node but can also happen in other lymphatic tissue clusters. When a B lymphocyte (B cell) is stimulated by an antigen, it triggers the humoral immune response, where specific antibodies are produced and released to target that antigen **(Kindt, 2004; Carcelain *et al.*, 2023; Wang *et al.*, 2024).**

II.2.1.1 Clonal selection and differentiation of B lymphocytes

A B lymphocyte is activated when antigens bind to its receptors (called BCRs). This binding initiates a process where the B cell rapidly multiplies to produce a group of identical cells, all capable of recognizing the same antigen. Most of these cells become plasma cells, which produce and release large amounts of antibodies to bind and neutralize the antigens. A smaller number of these cells become memory cells, which remain ready to respond quickly if the same antigen is encountered again **(Nicolas, 2007; Syeda *et al.*, 2024).**

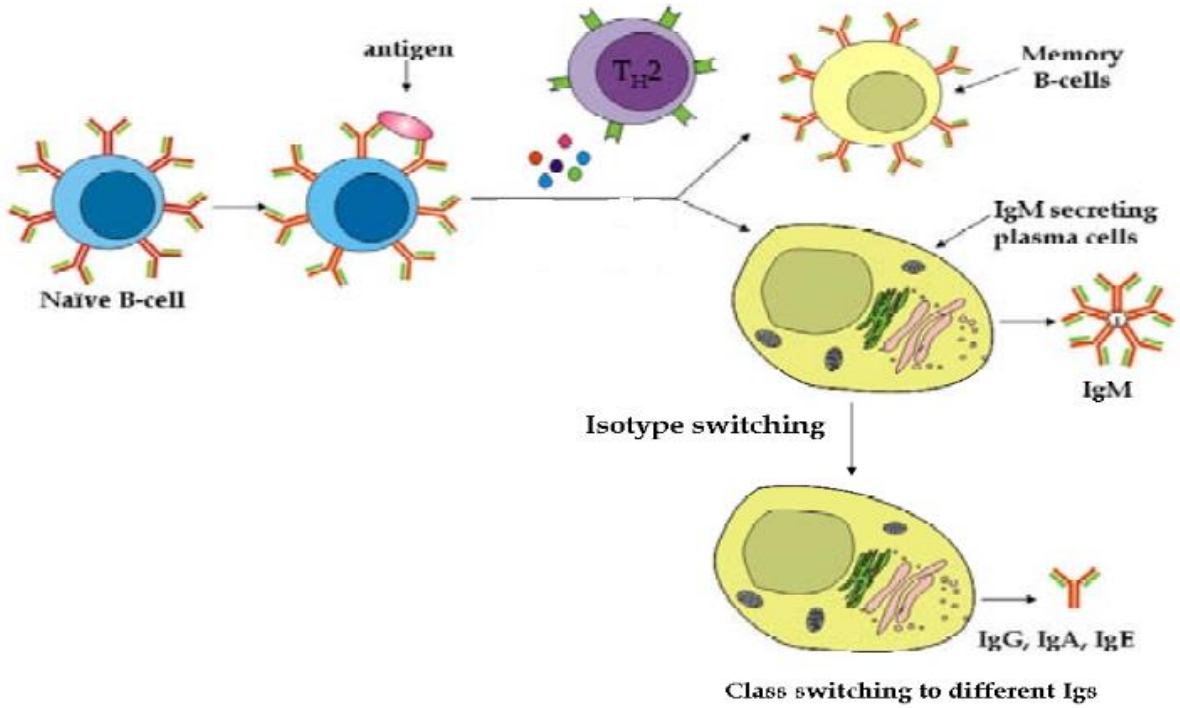

Figure 19. Summary of cellular processes involved in the development of the humoral response (Ahmed *et al.*, 2009)

II.2.1.2 Immune memory

During the first encounter with an antigen, the immune system takes a few days to respond. During this time, specific B lymphocytes multiply and transform into plasma cells, which produce antibodies. This response peaks around ten days before gradually declining. If the same antigen appears again, the response is much faster and stronger due to memory cells

formed during the first exposure. These cells provide a more effective and long-lasting defense, known as immune memory **(Lanzavecchia *et al.*, 2009).**

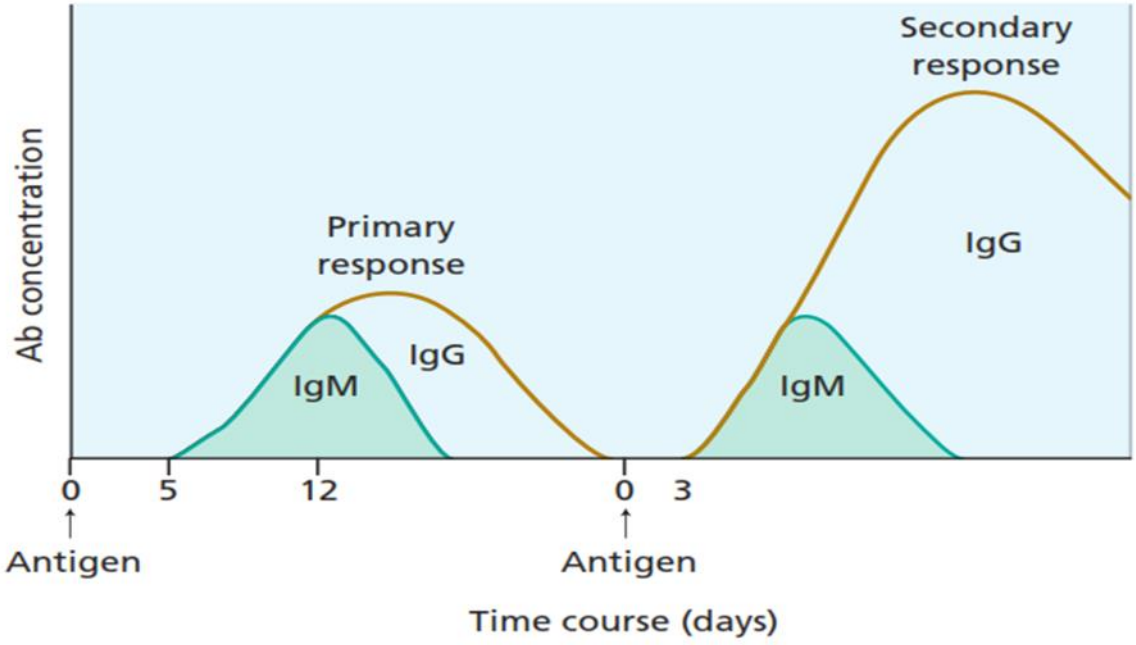

Figure 20. Primary and secondary antibody (Ab) responses following antigenic stimulation (Chapel *et al.*, 2013)

II.2.1.3 Active and passive humoral immunity

II.2.1.3.1 Active humoral immunity

Active humoral immunity develops when B lymphocytes encounter an antigen and produce antibodies. It can be acquired naturally through an infection or artificially through vaccination. Vaccines allow the body to recognize the antigen without causing the disease, exposing it to harmless or weakened pathogens. This prepares the immune system for a quick response during future encounters with the antigen. While highly beneficial, some vaccines may cause allergic reactions or fail to establish as strong a memory response as that acquired during a natural infection **(Pollard *et al.*, 2021).**

II.2.1.3.2 Passive humoral immunity

Immediate but temporary protection is achieved by receiving antibodies directly from another organism, as no antibodies are produced and no immune memory is formed. This can

happen naturally, for example with maternal antibodies being passed to the fetus via the placenta, or artificially via injections of immune serums, such as gamma globulins. It is often employed for serious infections, like rabies, or following venomous bites. This immunity only persists for a few weeks, since the introduced antibodies naturally break down over time **(Nicolas, 2007).**

II.2.2 Cell-mediated immune response

Antibodies are very useful for fighting certain infections, but their protection is limited, especially against agents like the tuberculosis bacillus, which quickly enter cells. In such cases, the cell-mediated immune response takes over. This type of immunity is driven by T lymphocytes, which are more diverse than B lymphocytes. There are two main types of T lymphocytes: T4 cells (CD4), which help activate other immune cells, and T8 cells (CD8), which destroy infected cells **(Kindt, 2004; Nicolas, 2007).**

Before diving into the details of cell-mediated immunity, it is important to note that antibodies, produced by plasma cells, primarily target bacteria and foreign molecules in body fluids. They do not directly attack solid tissues unless these are damaged. Essentially, antibodies and pathogens engage in a race where each tries to outcompete the other. The complexes formed between antibodies and antigens help flag antigens for destruction by other immune defenses **(Keselowsky *et al.*, 2020).**

Unlike B lymphocytes, T lymphocytes cannot detect free-floating or natural antigens. They only recognize antigen fragments (peptides) displayed on the surface of body cells by proteins called MHC (Major Histocompatibility Complex). For T lymphocytes to become activated, they must recognize both the foreign antigen and an MHC protein on the host cell.MHC class I proteins, found on almost all nucleated cells, are recognized by CD8 T cells, while MHC class II proteins, found mainly on certain immune cells, interact with CD4 T cells. These MHC proteins play a crucial role by signaling to T lymphocytes that microorganisms are hiding inside cells. Without this signaling, infections caused by viruses or bacteria could multiply undetected. When T lymphocytes encounter cells presenting antigens bound to MHC proteins, they trigger an immune response to attack infected, abnormal, or transplanted cells **(Chapel *et al.*, 2013).**

II.2.2.1 Activation of T lymphocytes

The activation of T lymphocytes occurs in two steps: antigen binding and costimulation.

Step 1: Antigen Binding

1. T cell receptors (TCRs) on T lymphocytes bind to a complex made up of an antigen and an MHC protein on the surface of a cell. These TCRs differ from B cell receptors in that they have two polypeptide chains and only one antigen-binding site.
2. Helper T cells (CD4) bind to antigens presented by MHC class II proteins, which are found mainly on antigen-presenting cells (APCs) such as dendritic cells. These APCs migrate to lymph nodes to alert the immune system quickly.
3. Cytotoxic T cells (CD8) are activated by antigens bound to MHC class I proteins, present on nearly all nucleated cells. While these cells do not need specialized antigen-presenting cells, they still require costimulation signals for full activation.

In summary, although both CD4 and CD8 T lymphocytes rely on antigen presentation, the type of presenting cells and the class of MHC proteins involved differ **(Fabbri *et al.*, 2003).**

Step 2: Costimulation

Costimulation is essential for a T lymphocyte to divide and form a clone. This often involves the T lymphocyte binding to additional receptors on the antigen-presenting cell (APC). For instance, macrophages present B7 proteins that bind to the CD28 receptor on the T lymphocyte, providing a critical costimulatory signal. Other signals, such as cytokines, further enhance this activation. If a T lymphocyte binds to an antigen without receiving a costimulatorysignal, it becomes tolerant to the antigen and cannot divide. This state, known as anergy, helps protect the body's healthy cells from unnecessary immune attacks **(Chapel *et al.*, 2013; Mayer *et al.*, 2019).**

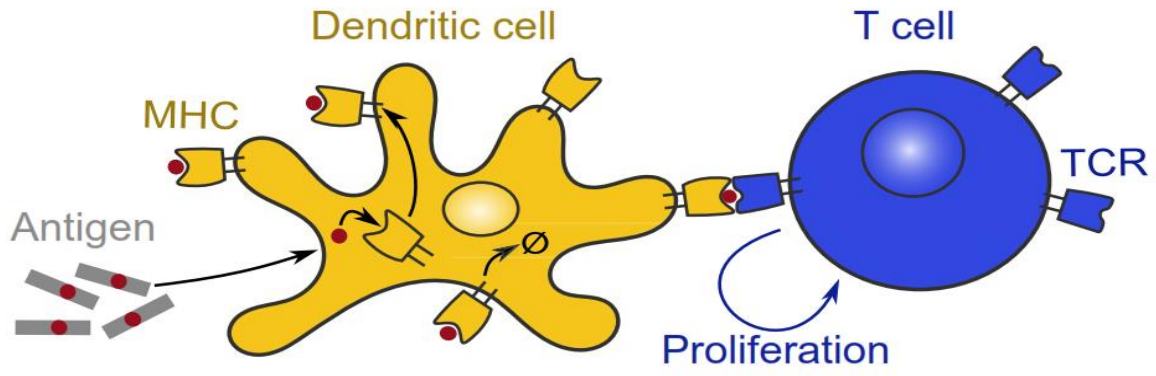

Figure 21. Activation and prolifération of T lymphocytes (Mayer *et al.*, 2019)

Table 3.Differences between Humoral Immunity and cell-mediated immunity (Tizard, 2013)

Characteristic	Humoral Immunity	Cell-Mediated Immunity
Main Mediators	Antibodies (produced by B lymphocytes)	T lymphocytes
Primary Targets	Soluble antigens (extracellular bacteria, toxins)	Virus-infected cells, cancer cells, grafts
Mechanism of Action	Antibodies specifically bind to antigens, neutralizing pathogens or marking them for destruction by other immune cells	T lymphocytes recognize infected cells and either kill them directly or activate other immune cells to destroy them
Response	Rapid response, with long-term memory	Slower to develop, but provides long-term memory
Protection	Protection against extracellular infections	Protection against intracellular infections, tumors, and grafts
Examples of Diseases	Bacterial infections, certain autoimmune diseases	Viral infections, certain autoimmune diseases, graft rejection

II.3 Immune tolerance

Immune tolerance refers to the immune system's ability to recognize and ignore the body's own cells and tissues, preventing attacks on them while still defending against harmful

invaders like pathogens. This process is essential for maintaining the balance between immunity and autoimmunity **(Romagnani, 2006).**

There are two main types of immune tolerance:

1. **Central Tolerance:** This occurs in the primary lymphoid organs (bone marrow and thymus), where immature immune cells that recognize and react strongly to the body's own antigens are either destroyed or modified to prevent self-reactivity **(Romagnani, 2006).**

2. **Peripheral Tolerance:** This takes place in the body's peripheral tissues. It involves mechanisms that stop mature immune cells from attacking the body's own tissues if they escape central tolerance (**Romagnani, 2006).**

CHAPTER III:

ANTIGENS

III.1 Definition

Antigens are substances, generally macromolecules such as proteins, polysaccharides, or lipids, capable of eliciting an immune response by being recognized as foreign by the immune system. They specifically bind to receptors on immune cells, such as lymphocytes, or to antibodies, thereby stimulating the production of antibodies. Antigens can originate externally (bacteria, viruses, toxins) or internally (modified proteins in certain pathologies), and their recognition is essential for the body's defense against pathogens **(Pandey *et al.*, 2022).**

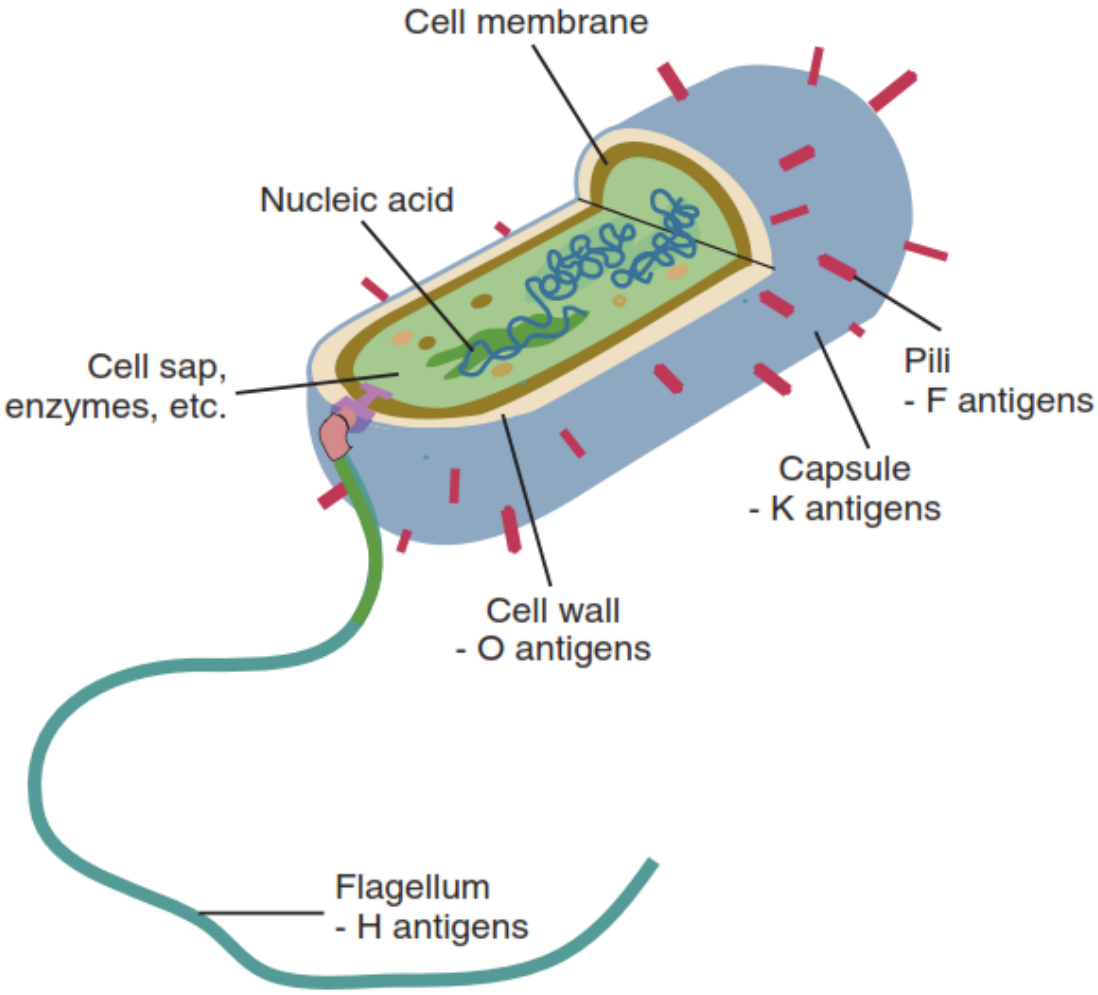

Figure 22.The structure of a typical bacterium and the location of its most important antigens (Tizard, 2013)

III.2 Classification of antigens

Antigens are substances that can activate the immune system and trigger an immune response because they are recognized as foreign **(Nicolas, 2007).**

Antigens can be classified into two main categories:

III.2.1 Complete antigens

These are complex molecules that possess both immunogenicity (the ability to provoke an immune response) and reactivity (the ability to interact with specific antibodies or immune cells). Examples of complete antigens include foreign proteins, nucleic acids, certain lipids, and polysaccharides. They can elicit a strong immune response by stimulating the proliferation of specific lymphocytes and the production of antibodies **(Parham, 2009).**

III.2.2 Haptens or incomplete antigens

Haptens are small molecules that, on their own, cannot trigger an immune response. However, when they bind to host proteins, they can be recognized as foreign and induce a response, often an allergic reaction. Examples of haptens include certain drugs, cosmetics, and chemicals **(Nicolas, 2007; Sakamoto *et al.*, 2023)**

III.3 Antigenicity and immunogenicity

III.3.1 Immunogenicity

Immunogenicity refers to the ability of an antigen to stimulate the proliferation of specific lymphocytes and the production of antibodies. This ability depends on the size and complexity of the molecule. Large molecules, especially proteins, have a strong immunogenic potential **(Nicolas, 2007).**

III.3.2 Reactivity

Reactivity is the ability of an antigen to bind to antibodies or immune cells (lymphocytes) that are already present in the body due to a previous immune response. However, this does not necessarily mean the antigen can trigger a new immune response on its own. For example, small molecules like haptens can react with antibodies but do not directly induce an immune response **(Nicolas, 2007; Gefen *et al.*, 2014).**

III.4 Antigen structure

The structure of an antigen plays a crucial role in its recognition by the immune system. Below are the key structural elements:

Antigenic determinants (Epitopes)

These are the specific sites on an antigen where antibodies or lymphocyte receptors bind. Most natural antigens have multiple epitopes, allowing the same antigen to activate different types of lymphocytes and generate a variety of antibodies **(Nicolas, 2007).**

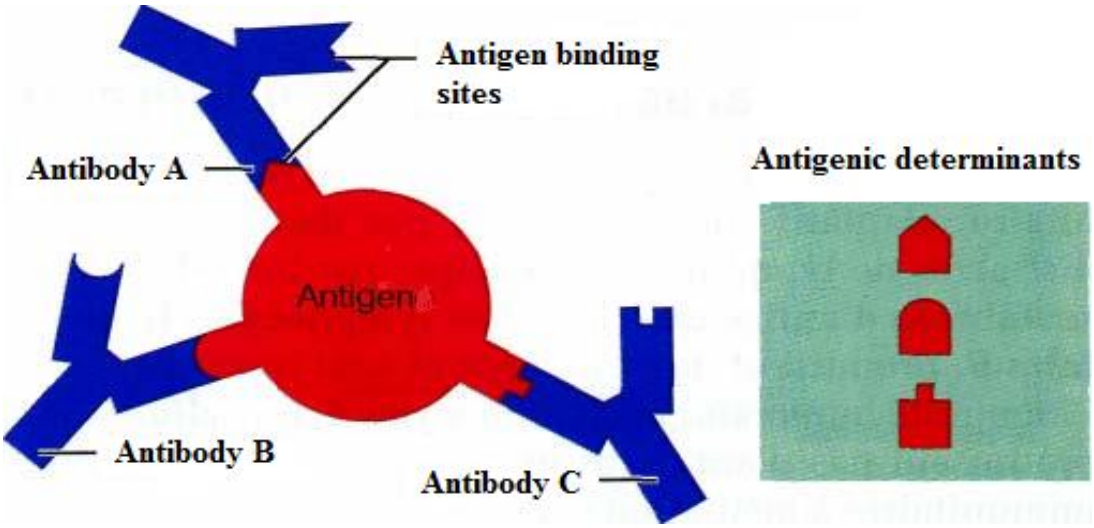

Figure 23. Antigenic determinants (Nicolas, 2007)

Antibodies bind to specific antigenic determinants (unique molecules) on the surface of antigens. Most antigens carry several different antigenic determinants, allowing multiple antibodies to bind to the same antigen. In the example illustrated, three types of antibodies interact with three different antigenic determinants on the same antigen molecule **(Nicolas, 2007).**

III.4.1 Major histocompatibility complex (MHC) antigens

MHC proteins, encoded by genes called HLA, act as cellular identity markers, enabling the immune system to distinguish between "self" and "non-self." They are divided into two classes:

1. **Class I MHC:** Found on most cells, these molecules signal infected cells.

2. **Class II MHC:** Specific to cells involved in the immune response, they play a key role in activating lymphocytes.

III.5 Criteria for immunogenicity

For a substance to be immunogenic, it must meet certain criteria:

III.5.1 Molecular size and complexity

The larger and more complex a molecule, the more immunogenic it is. Large proteins with multiple epitopes are highly immunogenic, unlike plastics, which, despite their size, have a simple repetitive structure **(Delves *et al.*, 2017).**

III.5.2 Degree of foreignness

The immune system recognizes molecules as foreign if they differ significantly from the body's own components. Natural antigens, such as microbial proteins, fulfill this criterion **(Delves *et al.*, 2017).**

III.5.3 Ability to be degraded

Antigenic molecules must be degraded by antigen-presenting cells to display fragments to lymphocytes, a process essential for their activation **(Delves *et al.*, 2017).**

III.5.4 Presentation on MHC molecules

The antigen must be able to bind to MHC molecules to be recognized by T lymphocytes. Antigen fragments fit into the peptide-binding grooves of MHC molecules, ensuring proper antigen presentation to the immune system **(Nicolas, 2007).**

CHAPTER IV:

ANTIBODIES

IV.1 Immunoglobulins

Antibodies, also known as immunoglobulins (Ig), are a class of serum proteins, specifically gamma globulins. They are produced by activated B lymphocytes or plasma cells in response to the presence of an antigen. Antibodies can specifically bind to these antigens and play a crucial role in immune defense. Their genetic variability and different classes allow them to recognize and target a wide range of antigens **(Tizard, 2013).**

IV.1.1 General Structure of Antibodies

Antibodies, also called immunoglobulins, have a symmetrical Y-shaped structure **(Tizard, 2013).**

They consist of:

1. **Two Fab regions (the arms of the Y):** which help recognize and bind to specific antigens.
2. **One Fc region (the stem of the Y):**This interacts with other parts of the immune system.

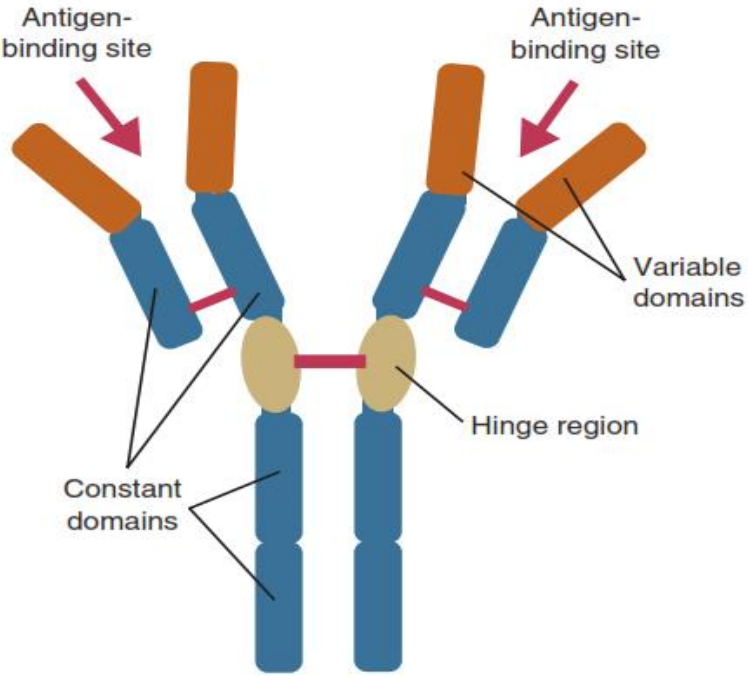

Figure 24.The structure of IgG, the prototypical immunoglobulin molecule (Tizard, 2013).

Each polypeptide chain in an antibody has two main regions:

1. **Variable Region (V):** Located at the end of the chains, this region is unique to each antibody. It allows the antibody to specifically recognize a particular antigen. The amino acid sequences in this region vary from one antibody to another **(Jaszczyszyn *et al.*, 2023).**
2. **Constant Region (C):** This region is the same for all antibodies of the same type. It does not participate in antigen recognition but plays a role in the antibody's function, such as activating the complement system or binding to cell receptors (**Jaszczyszyn *et al.*, 2023).**

The chains are connected by disulfide bonds (sulfur-sulfur links), forming a Y-shaped or T-shaped structure. Both heavy and light chains have a variable region (V) at one end and a constant region (C) at the other.

IV.1.1.1 Fab region and Fc region

The structure of antibodies is generally divided into two main functional parts:

1. **Fab Region (Fragment Antigen Binding):** Located at the two upper ends of the Y-shaped structure, the Fab region contains the variable region and is responsible for specifically binding to an antigen **(Vidarsson et al., 2014).**
2. **Fc Region (Fragment Crystallizable):** The stem of the Y is the Fc region, which consists of constant parts of the heavy chains. The Fc region allows the antibody to bind to various cell receptors and plays a role in antibody effector functions such as opsonization (marking pathogens for destruction) and complement activation (enhancing immune response) **(Vidarsson *et al.*, 2014).**

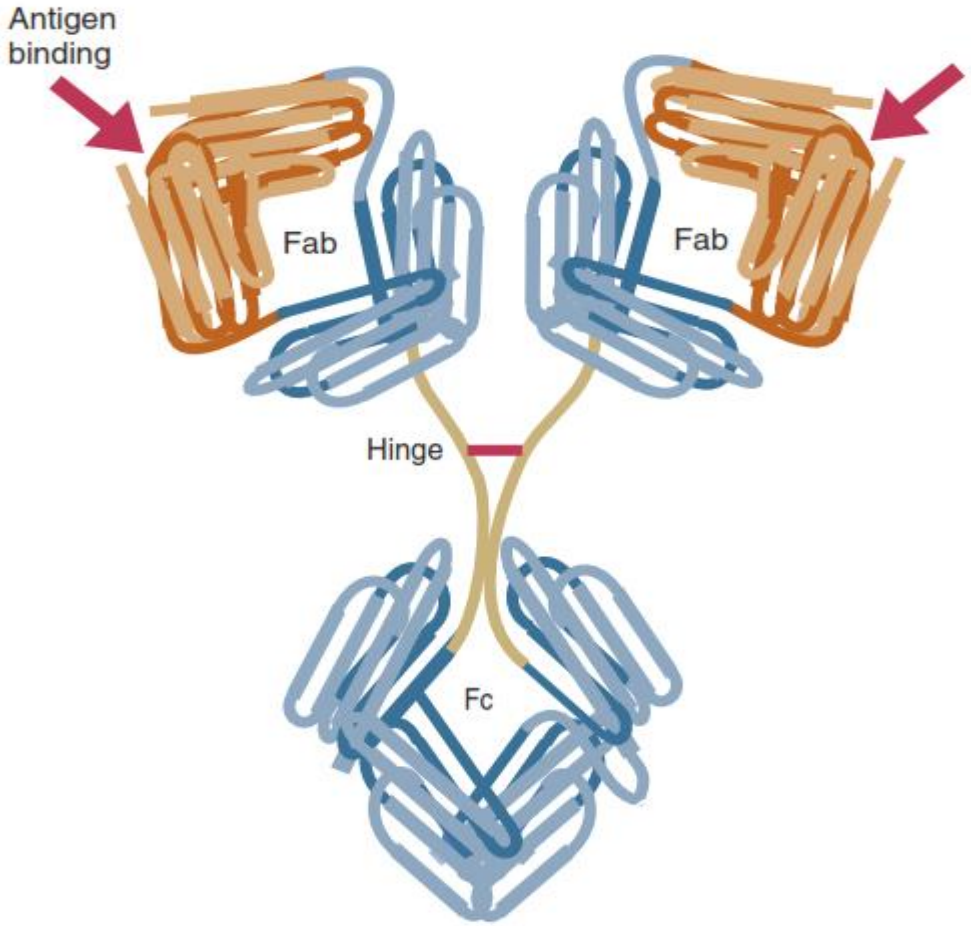

Figure 25. Fab and Fc Regions in an antibody (Tizard, 2013)

IV.1.2 Antibody variability

The diversity of antibodies is ensured by several genetic mechanisms.

IV.1.2.1 Isotypic variation

Isotypic variations refer to differences between antibody classes (IgG, IgA, IgM, etc.). Each class has a specific function and localization within the body **(Crowley et al., 2023).**

IV.1.2.2 Allotypic variation

Allotypic variations are subtle differences in the amino acid sequences within the constant region of the heavy chains, occurring between individuals of the same species **(Crowley *et al.*, 2023).**

IV.1.2.3 Idiotypic variation

Idiotypic variation refers to differences in the variable regions of antibodies, which are responsible for their ability to bind to specific antigens. These variations give each antibody a unique antigen specificity, allowing it to recognize and target a particular molecular structure. The idiotypic diversity of antibodies is essential for the immune system to effectively respond to a wide range of pathogens, as it contributes to the generation of a diverse repertoire of antibodies capable of binding to numerous antigens **(Crowley *et al.*, 2023).**

IV.1.3 Classes of immunoglobulins

There are five main classes of immunoglobulins **(Tizard, 2013):**

1. **IgM:** The first class produced during a primary immune response. It has a pentameric structure and is particularly effective at activating the complement system.
2. **IgG:** The most abundant antibody in serum, IgG is the only immunoglobulin that can cross the placenta, providing passive immunity to the fetus.
3. **IgA:** Found in mucosal secretions (such as saliva and mucus) in its dimeric form, IgA plays a protective role on body surfaces, especially in the respiratory and intestinal tracts.
4. **IgD:** Present on B lymphocytes, IgD functions as an antigen receptor and is crucial for initiating immune responses.
5. **IgE:** Involved in allergic reactions, IgE binds to mast cells and basophils, triggering the release of histamine and other chemicals that mediate allergic responses.

Each class is specialized for different functions, and some immunoglobulins have subclasses that further refine their roles in immune defense. Given your interest in the diversity of antibodies, you might find the mechanisms behind these classes particularly relevant in understanding how adaptive immunity operates—both in humans and animals **(Tizard, 2013).**

Table 4. Major Immunoglobulin Classes in the Domestic Mammals (Tizard, 2013)

Immunoglobulin Class	Molecularweight	Subunits	Heavy chain	Largelysynthesized in:
IgM	900	5	μ	Spleen and lymphnodes
IgG	180	1	γ	Spleen and lymphnodes
IgA	360	2	α	Intestinal and respiratory tracts
IgE	200	1	ε	Intestinal and respiratory tracts
IgD	180	1	δ	Spleen and lymphnodes

IV.1.4 Antigen-antibody interactions

Antibodies specifically bind to antigens to form immune complexes, a process governed by the compatibility between the antigenic site (epitope) and the antibody's binding site. This interaction is fundamental to the immune response, as it enables the immune system to recognize and target pathogens effectively.Once formed, these immune complexes do not directly eliminate antigens. Instead, they mark the antigens for destruction by immune cells such as macrophages **(Wen *et al.*, 2016).** This process, known as antibody-dependent cellular phagocytosis (ADCP), involves the recognition of the Fc region of antibodies by Fc receptors on phagocytic cells, leading to the ingestion and degradation of the antigen-antibody complex. The interaction between antibodies and antigens, leading to the formation of immune complexes, is a critical aspect of the adaptive immune response. These complexes facilitate the clearance of pathogens and play a role in activating other components of the immune system, such as the complement system, further enhancing the body's defense mechanisms **(Tay *et al.*, 2019).**

IV.1.5 Effector functions of antibodies

Antibodies play a crucial role in the immune system through several effector functions **(Carcelain *et al.*, 2023):**

1. **Neutralization**: Antibodies can block the attachment sites of pathogens, preventing them from entering host cells.
2. **Activation of the complement system**: By activating the complement system, antibodies lead to the lysis of target cells, enhancing the immune response.

3. **Agglutination**: Antibodies can bind multiple antigens together, forming clumps that facilitate their elimination by immune cells.
4. **Precipitation**: By forming soluble immune complexes, antibodies can facilitate the removal of antigens through phagocytosis.

IV.1.6 Polyclonal and monoclonal antibodies

Polyclonal and monoclonal antibodies are two distinct types of antibodies, each with unique characteristics and applications:

1. **Polyclonal Antibodies:** These antibodies are produced by multiple clones of B lymphocytes, allowing them to recognize and bind to various epitopes on the same antigen. This diversity enables a broader immune response **(Lipman *et al.*, 2005).**
2. **Monoclonal Antibodies**: Originating from a single clone of B lymphocytes, monoclonal antibodies are specific to a single epitope on an antigen. This specificity makes them highly valuable in diagnostic and therapeutic applications, as they can target specific molecules with precision **(Lipman *et al.*, 2005).**

IV.1.7 Nanobodies

A nanobody, also known as a single-domain antibody, is an extremely small antibody fragment composed of a single protein chain. Unlike conventional antibodies, which are much larger and more complex, nanobodies are derived from the variable region of antibodies found in certain animals, such as camels and llamas **(Vincke *et al.*, 2012).**

CHAPTER V:

THE COMPLEMENT SYSTEM

V.1 Definition

The complement system is a key part of the body's natural defense, primarily fighting infections. It also helps control inflammation, remove damaged cells, and support immune responses **(Azoulay *et al.*, 2024).** Furthermore, it plays a role in tissue repair and metabolism. However, if it is activated incorrectly, it can cause immune and inflammatory diseases. The system is triggered when it detects harmful invaders, like pathogens or antibodies, and binds to them to destroy them. It involves a range of proteins, enzymes, and regulators that work together in a complex way. Because it is so powerful, the complement system must be carefully controlled to avoid harming the body. The complement system can either disrupt microbial membranes or trigger inflammation. In both cases, it accelerates the removal of microbial invaders, making it a crucial element of the innate immune system. Additionally, the complement system has several other important functions (Figure below) **(Tizard, 2013).**

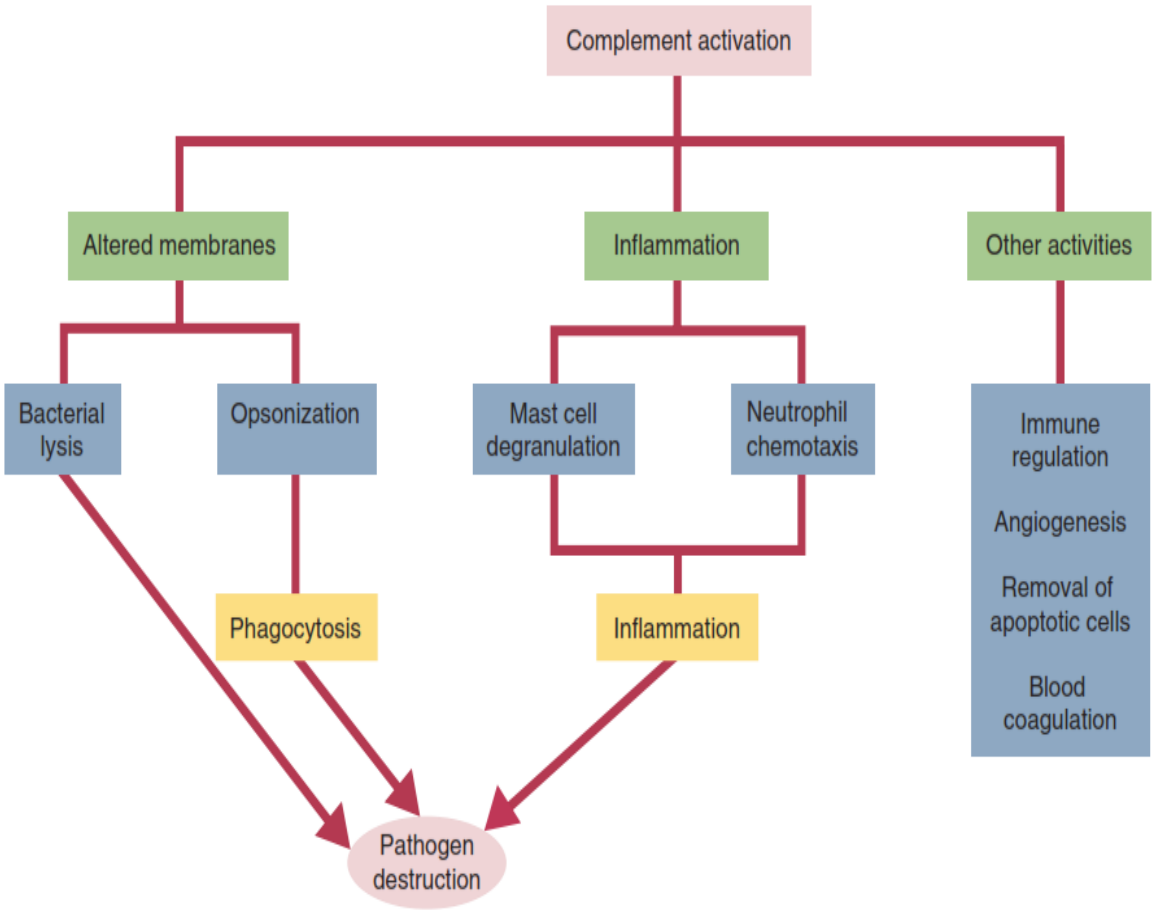

Figure 26. The functions of the complement system (Tizard, 2013)

V.2 Complement proteins

The complement system is comprised of over 30 proteins, some of which are identified with a 'C' prefix (e.g. C1, C2, C3) while others are designated with letters (e.g. B, D, P). Some proteins are freely present in the serum, whereas others are found on the surface of cells. Components that complement make up approximately 5% to 10% of the total proteins found in blood serum. The sizes of these components span from 24 kDa for factor D to 460 kDa for C1q, and their concentrations in human serum differ from 20 µg/mL for C2 to 1300 µg/mL for C3. These components are manufactured in various parts of the body. Majority of the C3, C6, C8, and B components are synthesised in the liver, whereas C2, C3, C4, C5, B, D, P, and I are produced by macrophages. Neutrophil granules can store substantial quantities of C6 and C7, enabling these components to be quickly available for immune defence at locations where macrophages and neutrophils congregate **(Tizard, 2013).**

V.3 Activation pathways of the complement system

The complement system is made up of inactive proteins that become active in a step-by-step process **(Merle *et al.*, 2015).**

There are three main steps:

1. Activation – The complement system must first be triggered.
2. C3b Generation – A key protein called C3b is produced.
3. Complex Formation – A final complement complex is assembled through an amplification process.

Once activated, the complement system produces several molecules that help fight infections. This process is tightly controlled.

The complement system can be activated through three pathways. The classical pathway is a more recent evolutionary mechanism that begins when antibodies bind to a pathogen, connecting it to the adaptive immune system. In contrast, the alternative and lectin pathways are triggered directly by microbial carbohydrates and function as part of the innate immune system. While the complement system was once considered a linear sequence of events, it is now recognized as a complex network with multiple points of interaction **(Tizard, 2013; Azoulay *et al.*, 2024).**

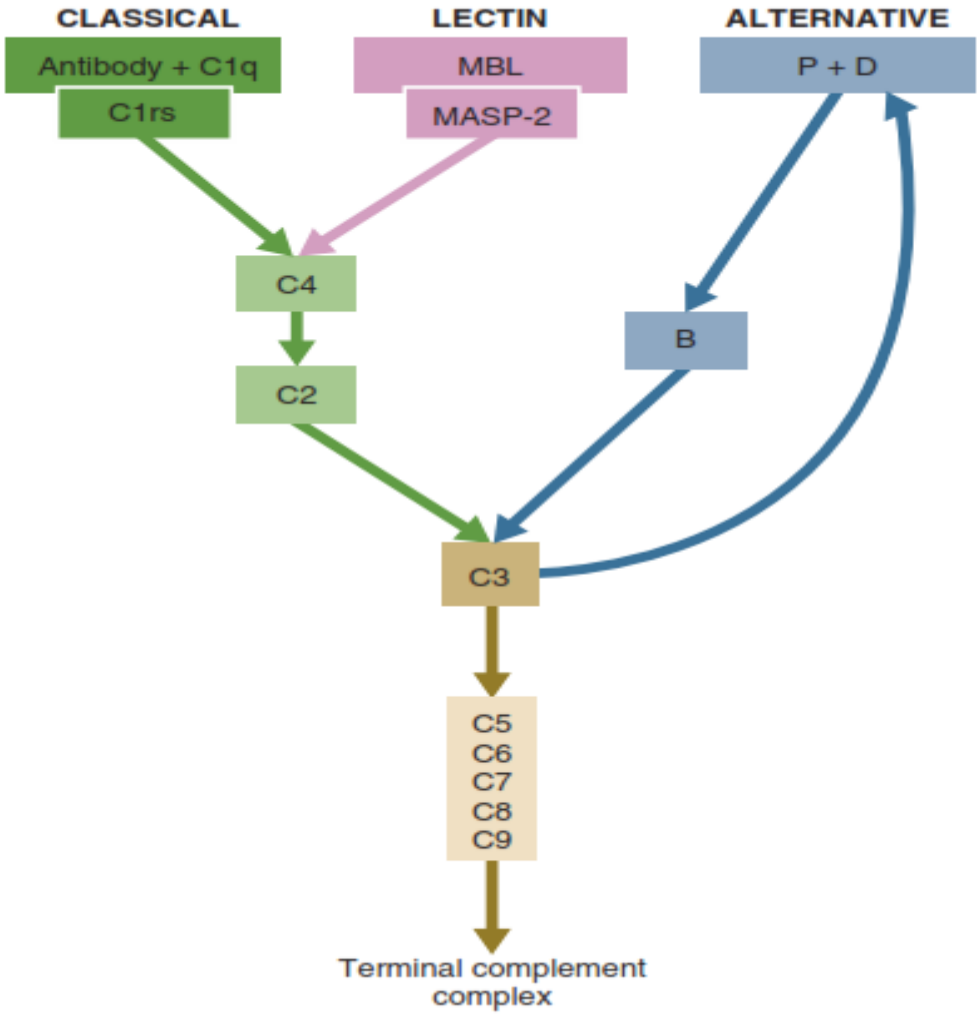

Figure 27. The three pathways by which the complement system (Tizard, 2013)

V.3.1 Classical Pathway

The classical complement pathway is triggered when antibodies bind to the surface of a pathogen, which is part of the adaptive immune response. Unlike the alternative and lectin pathways, the classical pathway only activates after antibodies are produced, typically 7 to 10 days after infection. Once antibodies bind to an antigen, they expose active sites that can trigger the complement system **(Lintner *et al.*, 2016).** The first component, C1, is a protein complex made up of C1q, C1r, and C1s. When C1q binds to antibody clusters, it activates C1r, which then activates C1s. This activation leads to the cleavage of C4 and C2, forming the complex C4b2b, which acts as a protease to cleave C3. The cleaved C3b binds to and activates C5, leading to the formation of the terminal complement complex and the destruction of the pathogen **(Hurler *et al.*, 2025).** In addition to immune complexes, C1q can

also bind to other molecules, such as apoptotic cells or CRP, but these bindings can be regulated by complement inhibitors to prevent excessive activation and inflammation.

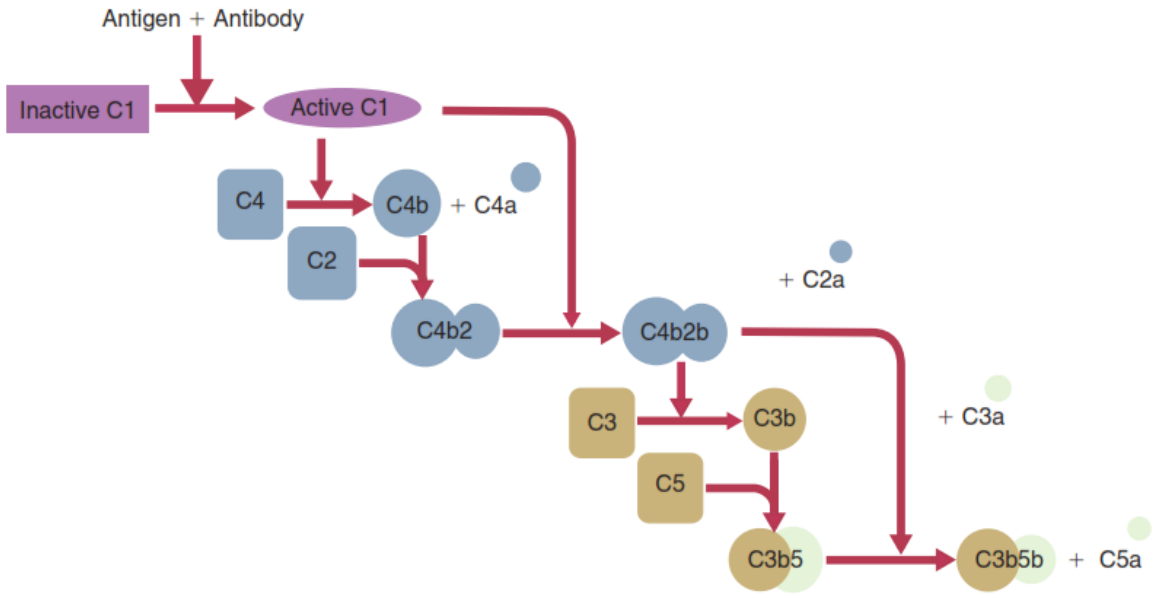

Figure 28. The basic features of the classical complement pathway (Tizard, 2013)

V.3.2 Alternative pathway

The alternative pathway is the oldest pathway in phylogeny. It is a surveillance system that operates continuously at low noise levels, with spontaneous hydrolysis of a small amount of C3 into hydrolyzed C3. This pathway can be activated by bacterial-derived activators, such as the Lipopolysaccharide (LPS) from Gram-negative bacteria, as well as by Gram-positive bacteria, viruses, or infected or transformed cells. The interactions of the proteins in the alternative pathway lead to the formation of the alternative C3 convertase, which enables the amplification of C3 cleavage **(Carcelain *et al.*, 2023).**

V.3.3 Lectin pathway

The lectin pathway is activated by specific carbohydrate or acetylated groups found on microorganisms or apoptotic cells. It shares similarities with the classical pathway. The recognition proteins, considered soluble pattern recognition receptors (PRRs), are primarily mannose-binding lectin (MBL) and ficolins. These are associated with serine esterases called MASP-1, MASP-2, and MASP-3 (Mannose-Associated Serine Proteases), which are highly homologous to C1s and C1r, reflecting the adaptation of the lectin pathway to the emergence

of immunoglobulins, leading to the creation of the classical pathway in phylogeny. Once activated, the MASPs gain the ability to cleave C4 and C2, contributing to the formation of a C3 convertase, C4b2a, which is identical to the one formed through activation by the classical pathway **(Matsushita *et al.*, 2013; Carcelain *et al.*, 2023).**

V.4 Regulation of complement activation

The consequences of complement activation are so critical and potentially harmful that each activation pathway is tightly regulated by soluble and cell-bound regulatory proteins (Figure blow) **(Tizard, 2013).** The key regulator of the classical pathway is C1-inhibitor, a glycoprotein that inhibits several proteases in both the classical and lectin pathways, including the active C1r and C1s components. Additional regulatory proteins control the activity of the C3 and C5 convertases. Some of these proteins compete with MASPs for binding sites on MBL and ficolins. CD55, or decay-accelerating factor, is a glycoprotein found on the surface of red blood cells, neutrophils, lymphocytes, monocytes, platelets, and endothelial cells. CD55 binds to convertases, accelerating their decay to protect normal cells from complement attack. Other proteins that promote the degradation of convertases include factor H and C4-binding protein (C4BP) in plasma, as well as CD35 (CR1) and CD46 on cell membranes. Regulation of the amplification pathway is managed by three glycoproteins: vitronectin, clusterin, and most importantly, CD59 (protectin), all of which inhibit the assembly of the terminal complement complex by blocking C5b678 insertion and C9 polymerization **(Ghosh *et al.*, 2015).**

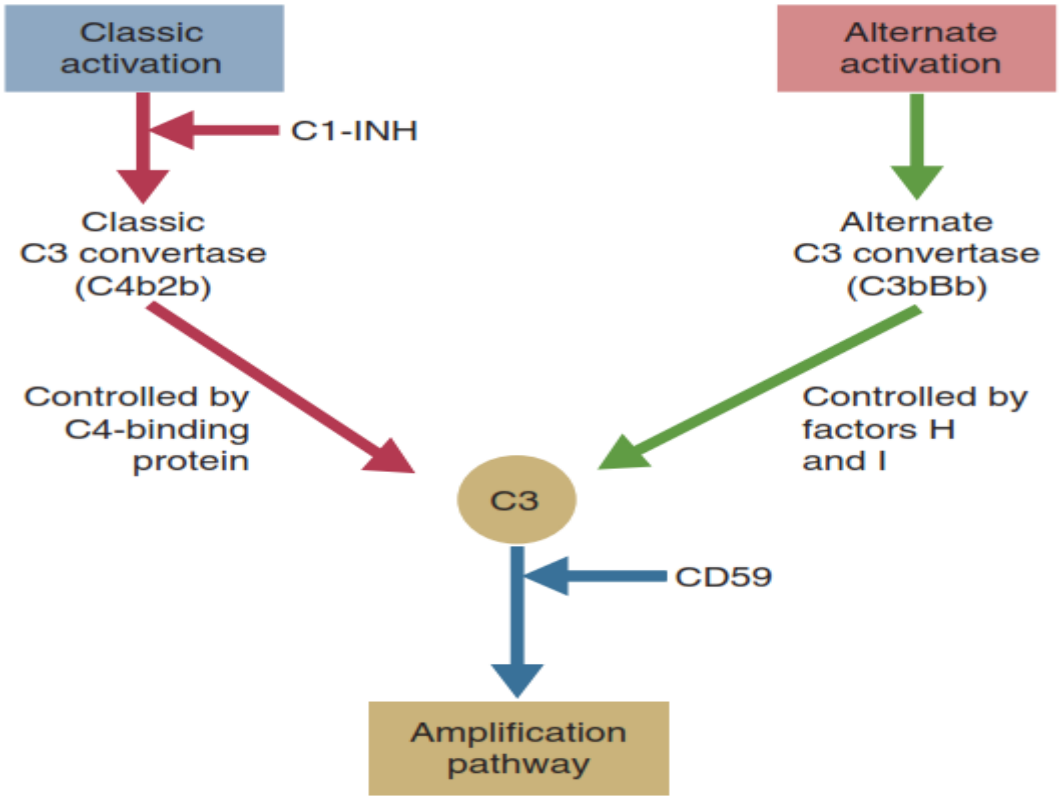

Figure 29. Basic control mechanisms of the complement system (Tizard, 2013)

V.5 Biological activities of the complement system

The complement system plays an essential role in the immune response, with several key biological activities, including:

Opsonization: Complement proteins, particularly C3b, bind to the surface of pathogens and mark them for recognition and destruction by phagocytes. This process enhances the efficiency of phagocytosis, a key part of innate immunity **(Nicolas, 2007).**

Inflammation: Activation of complement proteins generates small peptides such as C3a, C4a, and C5a, which act as anaphylatoxins. These molecules increase vascular permeability, promote the recruitment of immune cells to infection sites, and enhance the inflammatory response. C5a, in particular, is a potent chemotactic factor that attracts neutrophils, macrophages, and other immune cells to sites of infection or injury (**Kindt, 2004; Tizard, 2013).**

Cell lysis: The terminal complement complex (TCC), or membrane attack complex (MAC), is formed when complement components C5b, C6, C7, C8, and C9 assemble on the surface of pathogens, leading to the formation of pores in the target cell membrane. This pore formation results in osmotic imbalance and ultimately leads to the lysis (destruction) of the target cell. This is a crucial mechanism for the destruction of pathogens **(Nicolas, 2007; Tizard, 2013).**

Tissue repair: Complement activation also aids in tissue repair following infection or injury. C3a and C5a can promote the release of factors involved in tissue regeneration and healing (**Nielsen et al., 2000).**

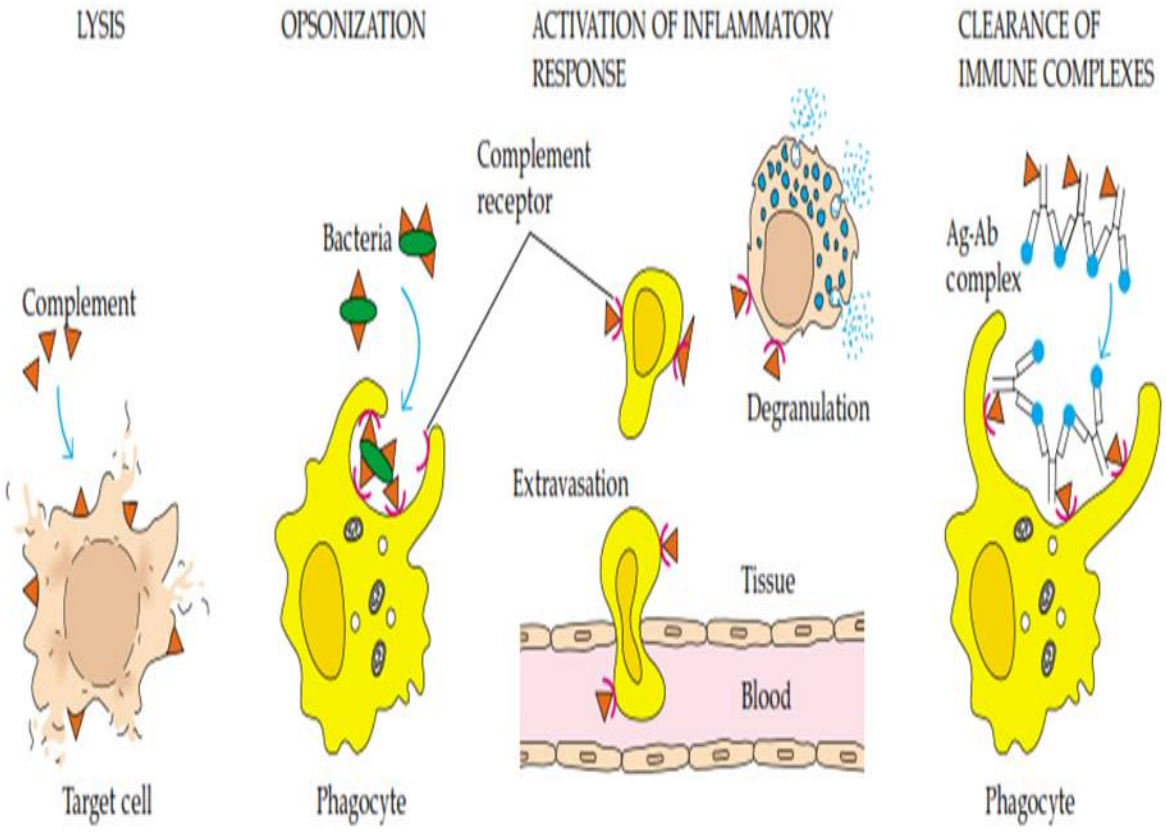

Figure 30. Biological activities of the complement system (Kindt, 2004)

CHAPTER VI:

CYTOKINES

VI.1 Definition

Cytokines are low-molecular-weight proteins or glycoproteins secreted primarily by white blood cells and various other cells in the body in response to different stimuli. They play a crucial role in cell-to-cell communication and regulate the development of immune effector cells. In addition to their regulatory function, some cytokines have direct effector activities **(O'Shea *et al.*, 2019).** They are involved in complex biological interactions among lymphoid, inflammatory, and hematopoietic cells. Dysregulation of cytokine production or function can contribute to the pathogenesis of certain diseases, and their modulation represents a promising therapeutic approach **(Fitzgerald *et al.*, 2001; Kindt, 2004).**

VI.2 Properties of cytokines

Cytokines bind to specific receptors on target cell membranes, activating signal transduction pathways that alter gene expression. The susceptibility of a target cell to a cytokine depends on the presence of specific receptors. Cytokines and their receptors exhibit high affinity, with dissociation constants ranging from 10^{-10} to 10^{-12} M, allowing them to exert biological effects at picomolar concentrations **(Kindt, 2004).**

Cytokines can act in different ways **(Kindt, 2004):**

1. **Autocrine action:** They bind to receptors on the same cell that secreted them.
2. **Paracrine action:** They affect nearby cells.
3. **Endocrine action:** In rare cases, they act on distant cells in the body.

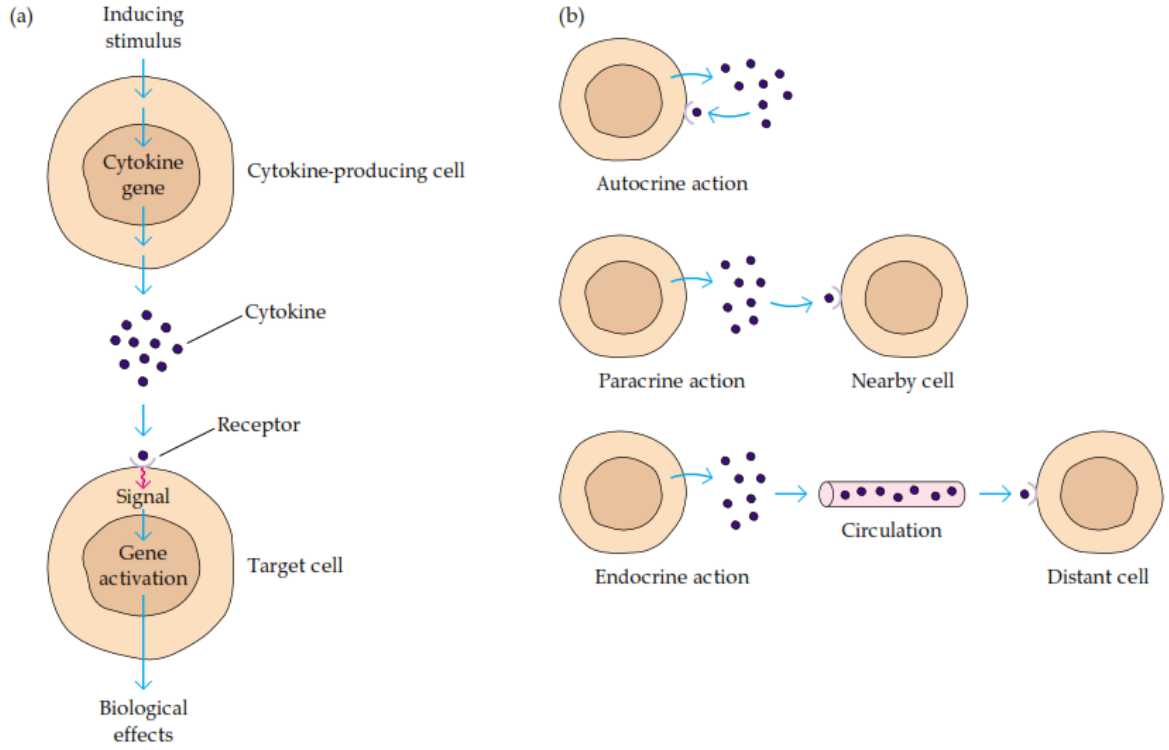

Figure 31. (a) Overview of the induction and function of cytokines. (b) Most cytokinesexhibitautocrine and/or paracrineaction; fewer exhibit endocrine actions (Kindt, 2004)

Cytokines regulate immune responses by influencing activation, proliferation, differentiation, and secretion of antibodies or other cytokines. Their effects can lead to the activation of numerous immune cells, such as B cells, T cells, natural killer cells, macrophages, and hematopoietic stem cells **(Kindt, 2004).**

Cytokines exhibit several key characteristics:

1. **Pleiotropy:** A single cytokine has different effects on various target cells.
2. **Redundancy:** Multiple cytokines perform similar functions.
3. **Synergy**: The combined effect of two cytokines is greater than their individual effects.
4. Antagonism: Some cytokines counteract the effects of others.
5. Cascade Induction: One cytokine induces the production of additional cytokines, amplifying immune responses.

Cytokines include interleukins, interferons, tumor necrosis factors, and chemokines. While cytokines share similarities with hormones and growth factors, they are usually

secreted in response to stimuli and act over short distances, whereas hormones generally function in an endocrine manner and originate from specialized glands **(Kindt, 2004).**

VI.3 Types of cytokines

Cytokines are a diverse group of small proteins crucial for cell signaling in the immune system. They can be classified based on their structure and function, with each type originating from specific cells and performing distinct roles **(You *et al.*, 2021):**

VI.3.1 Interleukins (IL)

Interleukins are a diverse group of cytokines primarily produced by leukocytes and other immune cells, playing a crucial role in regulating immune responses, inflammation, and hematopoiesis. They function as signaling molecules that mediate communication between cells, orchestrating various aspects of the immune system, including activation, proliferation, and differentiation of immune cells such as T lymphocytes, B lymphocytes, and macrophages. Interleukins are classified into different families based on their structure and function, each with distinct roles in immunity. Some interleukins promote inflammation (e.g., IL-1, IL-6, IL-17), while others have anti-inflammatory properties (e.g., IL-10, IL-4). They act by binding to specific receptors on target cells, triggering intracellular signaling pathways that modulate gene expression and immune activity. Dysregulation of interleukin signaling is associated with various diseases, including autoimmune disorders, chronic inflammation, and cancer **(Weber *et al.*, 2010).**

VI.3.2 Interferons (IFN)

Interferons (IFNs) are a family of cytokines with the unique ability to induce cell intrinsic programs that enhance resistance to viral infection. Induction of an antiviral state at the cell, tissue, organ, and organismal level is performed by three distinct IFN families, designated as Type-I, Type-II, and Type-III IFNs.Type I interferons are primarily involved in antiviral immunity, inducing the expression of genes that inhibit viral replication and enhancing the activity of natural killer (NK) cells and antigen-presenting cells. Type II interferon (IFN-γ) is mainly produced by T cells and NK cells, playing a key role in immune regulation, macrophage activation, and defense against intracellular pathogens. Type III interferons share antiviral properties with Type I but act on a more limited range of cells, mainly epithelial tissues.Interferons exert their effects by binding to specific receptors on cell

surfaces, activating intracellular signaling pathways, which leads to the expression of antiviral and immunomodulatorygenes. Due to their vital role in immunity, interferons are used therapeutically to treat viral infections (hepatitis B and C), autoimmune diseases, and certain cancers. However, excessive or dysregulated interferon responses can contribute to inflammatory and autoimmune disorders **(Walter, 2020).**

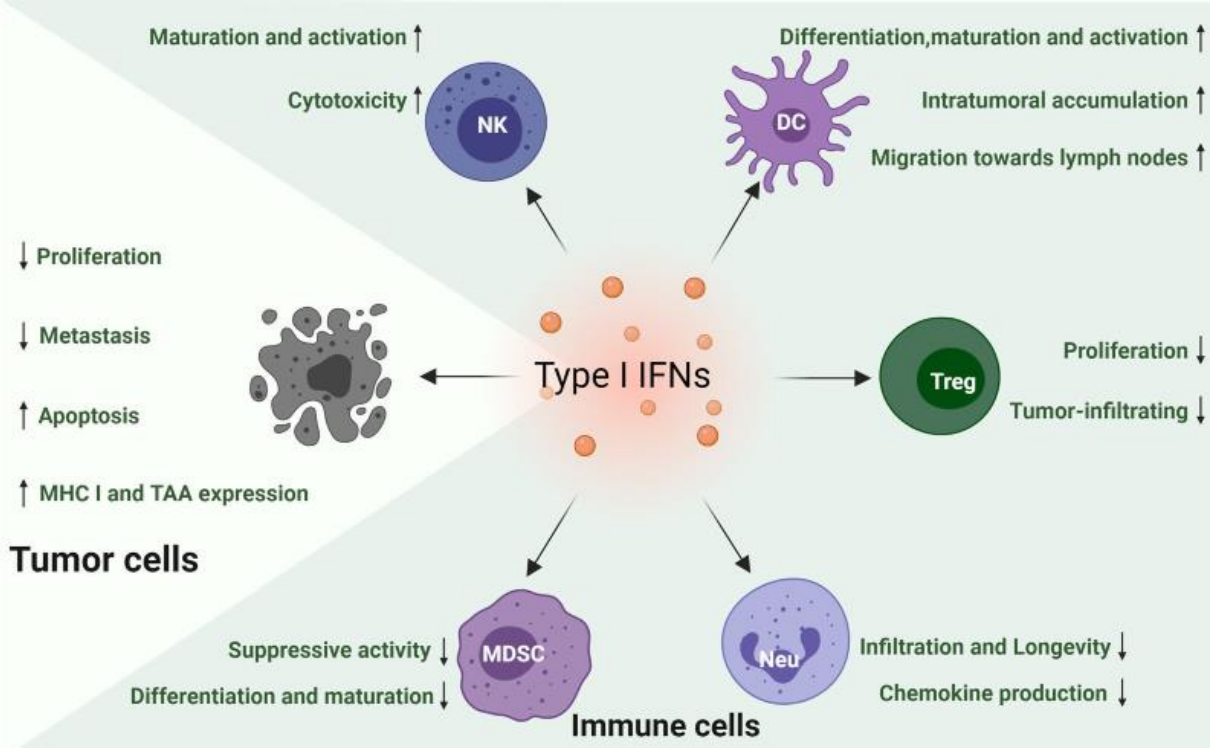

Figure 32. The efects of type I IFNs (Yu *et al.*, 2022)

VI.3.3 Tumor Necrosis Factors (TNF)

Tumor Necrosis Factors (TNFs) are a cytokine superfamily involved in inflammation, apoptosis, and immune responses. TNF-alpha (TNFα), also known as cachectin, is the most studied member. It is primarily produced by activated macrophages, and also secreted by T lymphocytes, natural killer (NK) cells, and mast cells. TNFα plays a vital role in inflammation by promoting adhesion molecule expression on endothelial cells, which helps recruit leukocytes to sites of infection or tissue damage. It also regulates cell proliferation and differentiation and supports immune defense against infections and tumor cells. Excessive or dysregulated TNFα production is linked to various inflammatory and autoimmune diseases, such as rheumatoid arthritis, Crohn's disease, and psoriasis. TNF inhibitors, targeting TNFα, are commonly used to treat these conditions **(Jang *et al.*, 2021).** Tumor Necrosis Factor-beta

(TNF-β), or lymphotoxin-alpha (LTα), is mainly produced by activated lymphocytes. Structurally similar to TNFα, TNF-β binds to TNFR1 and TNFR2 receptors to mediate its effects. TNF-β plays a crucial role in immune function, especially in tumor immunity, by inducing cytotoxic effects on tumor cells. Additionally, it helps regulate immune responses by activating neutrophils, endothelial cells, and osteoclasts, and by enhancing the expression of Major Histocompatibility Complex (MHC) and adhesion molecules, thus improving immune surveillance **(Jang *et al.*, 2021).**

VI.3.4 Chemokines

Chemokines play a vital role in the immune system by regulating the migration and positioning of immune cells. They guide leukocytes to sites of infection, inflammation, or injury, enhancing immune responses and promoting tissue repair **(Sokol *et al.*, 2015).** Additionally, chemokines contribute to the development and organization of lymphoid organs, ensuring efficient immune surveillance. Beyond their role in inflammation, they help maintain immune homeostasis by directing immune cells in normal physiological conditions. By binding to specific receptors, chemokines trigger signaling pathways that control cell movement, activation, and function, making them essential for both protective immunity and immune system balance **(Kindt, 2004).**

VI.3.5 Growth factors

Growth factors are a specialized group of cytokines that regulate cell proliferation, differentiation, survival, and tissue repair by binding to specific receptors and activating intracellular signaling pathways. They play crucial roles in hematopoiesis, immune responses, wound healing, and angiogenesis. For example, Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) promotes myeloid cell differentiation **(Hamilton, 2008),** while Erythropoietin (EPO) regulates red blood cell production **(Metcalf, 2008).** Transforming Growth Factor-beta (TGF-β) is essential for tissue regeneration and fibrosis (Blobe et al., 2000), and Vascular Endothelial Growth Factor (VEGF) stimulates blood vessel formation Additionally, Interleukin-7 (IL-7) supports lymphocyte survival, influencing adaptive immunity. These growth factors are fundamental in maintaining immune homeostasis and facilitating recovery from injury or disease **(Mazzucchelli *et al.*, 2007).**

VI.3.6 Transforming Growth Factor-Beta (TGF-β)

Transforming Growth Factor-Beta (TGF-β) is a multifunctional cytokine that regulates numerous cellular processes, including immune response modulation, cell proliferation, differentiation, apoptosis, and tissue homeostasis. It is a key mediator in maintaining immune tolerance and controlling inflammation by balancing pro-inflammatory and anti-inflammatory signals **(Sanjabi *et al.*, 2017).** In the immune system, TGF-β plays a dual role: it suppresses excessive immune activation to prevent autoimmune diseases while also promoting regulatory T cell differentiation and immune tolerance. Additionally, TGF-β is involved in tissue repair and fibrosis by stimulating extracellular matrix production and modulating fibroblast activity **(Biernacka *et al.*, 2011).** In cancer, it exhibits context-dependent effects, acting as a tumor suppressor in early stages but promoting tumor progression and metastasis in advanced stages. TGF-β also plays a role in angiogenesis by regulating endothelial cell proliferation and vascular remodeling **(Goumans *et al.*, 2009).**

Table 5.Key characteristics of major Cytokine families (Kindt, 2004)

Cytokine Type	Key Functions	OriginatingCells
Interleukins (IL)	Regulate immune responses, inflammation, hematopoiesis; Mediate communication between immune cells (T cells, B cells, macrophages); Somepromote inflammation (IL-1, IL-6, IL-17), others are anti-inflammatory (IL-10, IL-4)	Leukocytes and other immune cells
Interferons (IFN)	Induce antiviral state; Enhance NK cell and antigen-presenting cell activity; IFN-γ (Type II) activates macrophages and defends against intracellular pathogens	Various, depending on type (Type I: many cell types, Type II: T cells, NK cells, Type III: epithelial tissues)
TumorNecrosisFactors (TNF)	Involved in inflammation, apoptosis, immune responses; TNFα promotes adhesion molecule expression, regulates cell proliferation and differentiation; TNFβ (Lymphotoxin-alpha) induces cytotoxic effects on tumor cells	TNFα: Activated macrophages, T cells, NK cells, mast cells; TNFβ: Activated lymphocytes
Chemokines	Regulate immune cell migration and positioning; Guide leukocytes to sites of infection/inflammation/injury; Contribute to lymphoid organ development	Various immune cells
GrowthFactors	Regulate cell proliferation, differentiation, survival, tissue repair; Involved in hematopoiesis, immune responses, wound healing, angiogenesis (e.g., GM-CSF, EPO, TGF-β, VEGF, IL-7)	Variouscell types
Transforming Growth Factor-Beta (TGF-β)	Regulates immune responses, cell proliferation, differentiation, apoptosis, tissue homeostasis; Maintains immune tolerance, controls inflammation; Involved in tissue repair, fibrosis, angiogenesis; Dual role in cancer (tumor suppressor vs. promoter)	Manycell types

VI.4 Classification of cytokines

Cytokines are signaling molecules that play pivotal roles in the immune system, and they can be classified based on their biological functions and structural characteristics.

VI.4.1 Classification by function

VI.4.1.1 Pro-inflammatory cytokines

Molecules such as Tumor Necrosis Factor-alpha (TNF-α), Interleukin-1 (IL-1), and Interleukin-6 (IL-6) are crucial in initiating and propagating inflammatory responses. They activate immune cells, increase vascular permeability, and promote the production of other inflammatory mediators, thereby defending against infections. However, dysregulation can lead to chronic inflammatory diseases **(Cui *et al.*, 2021).**

VI.4.1.2 Anti-inflammatory cytokines

Cytokines like Interleukin-10 (IL-10) and Transforming Growth Factor-beta (TGF-β) modulate and suppress inflammatory responses. IL-10 inhibits the production of pro-inflammatory cytokines and the maturation of antigen-presenting cells, while TGF-β regulates cell growth and induces immune tolerance, aiding in the resolution of inflammation **(Esen, 2015).**

VI.4.1.3 Adaptive immune cytokines

Cytokines such as Interleukin-2 (IL-2), Interleukin-4 (IL-4), Interleukin-12 (IL-12), and Interferon-gamma (IFN-γ) are essential for regulating specific immune responses. For instance, IL-2 supports the proliferation of activated T cells, IL-4 is vital for the differentiation of T helper 2 cells, IL-12 promotes IFN-γ production, and IFN-γ activates macrophages to eliminate intracellular pathogens **(Esen, 2015).**

VI.4.1.4 Hematopoietic cytokines

Cytokines like Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), Macrophage Colony-Stimulating Factor (M-CSF), and Erythropoietin (EPO) regulate blood cell production. GM-CSF and M-CSF stimulate the differentiation and maturation of myeloid cells, including macrophages and granulocytes, while EPO is essential for red blood cell production in response to physiological signals **(Cui *et al.*, 2021).**

VI.4.1.5 Antiviral cytokines

Interferons alpha (IFN-α) and beta (IFN-β) are key in antiviral responses. They induce the production of antiviral proteins that inhibit viral replication, activate immune cells (notably Natural Killer cells), and enhance the presentation of viral antigens, contributing to rapid and effective defense against viral infections **(Lei *et al.*, 2024).**

VI.4.2 Classification by structural family

VI.4.2.1 Interferon family

This family includes type I interferons (IFN-α and IFN-β) and type II interferon (IFN-γ). Type I interferons are primarily produced by virus-infected cells and play a central role in antiviral defense by inducing an antiviral state in neighboring cells and activating innate immune cells. IFN-γ, produced mainly by T cells and NK cells, is crucial for macrophage activation and regulation of adaptive immune responses **(Lee *et al.*, 2018).**

VI.4.2.2 Tumor necrosis factor (TNF) family

Cytokines such as TNF-α and TNF-β are important mediators of inflammation and immune regulation. TNF-α, produced mainly by macrophages and dendritic cells in response to infection, initiates inflammatory responses, induces cell death, and regulates cell survival. TNF-β, also known as lymphotoxin, is produced primarily by T cells and has similar effects to TNF-α, with a more pronounced role in tissue destruction and long-term immune regulation **(McDevitt *et al.*, 2002).**

VI.4.2.3 Interleukin family

This group includes cytokines like IL-1, IL-2, and IL-6, which are central to communication between immune cells. IL-1 is a pro-inflammatory cytokine essential in innate immunity, produced mainly by macrophages and dendritic cells, activating other immune cells and inducing the production of inflammatory mediators. IL-2, produced by activated T cells, is crucial for T cell proliferation and adaptive immune activation. IL-6, both pro-inflammatory and immunoregulatory, plays a significant role in acute responses by activating hepatocytes to produce acute-phase proteins and promoting B cell differentiation into antibody-producing plasma cells **(Garlanda *et al.*, 2013).**

CHAPTER VII:

VACCINOLOGY

VII.1 History of vaccinology

Variolation, an empirical method, marks the beginning of vaccinology, which dates back to at least the 10th century in both China and India, where it was used to prevent smallpox. Edward Jenner introduced modern vaccination in 1796 by using the cowpox virus to guard against human smallpox. This breakthrough discovery triggered the start of scientific endeavors to avert infectious illnesses, motivating notable individuals like Louis Pasteur. In the 1880s, Pasteur created weakened vaccines against anthrax and rabies, thereby establishing the groundwork for scientific vaccinology **(Plotkin, 2014).**

The 20th century witnessed significant growth in vaccine development, encompassing breakthroughs such as the introduction of the Bacillus Calmette–Guérin (BCG) vaccine for tuberculosis in 1923, the polio vaccine in 1955, and the diphtheria-tetanus-pertussis (DTP) combination vaccine in 1940. During this time, advancements in biotechnology led to the creation of recombinant vaccines, such as the hepatitis B vaccine developed in 1986, and conjugate vaccines designed to shield children from severe infections. The ultimate achievement in vaccination was the complete elimination of smallpox by 1980 **(Greenwood, 2014).**

VII.2 Acquisition of immune memory

VII.2.1 Initial activation of the immune system

During the first exposure to an antigen, antigen-presenting cells (APCs), such as dendritic cells and macrophages, capture the antigen. These APCs digest the antigen into fragments and present them on their surface via major histocompatibility complex (MHC) molecules. Naïve T lymphocytes recognize these fragments through their specific receptors (TCR), while naïve B lymphocytes detect antigens in their native form via their receptors (BCR) **(Lee-Chang *et al.*, 2023).**

1. Helper T cells ($CD4^+$), activated by APCs, orchestrate the immune response by releasing cytokines to activate other cells.
2. Cytotoxic T cells ($CD8^+$) eliminate infected or cancerous cells.
3. B lymphocytes, activated by the antigen and helper T cell signals, differentiate into plasma cells that produce antigen-specific antibodies.

VII.2.2 Formation of memory cells

After antigen elimination, most activated lymphocytes die by apoptosis to prevent excessive immune reactions. However, a small population of lymphocytes survives and differentiates into memory cells. These cells persist in the body for years or even a lifetime **(Abbas *et al.*, 2021).**

VII.2.2.1 Memory T cells

They are divided into two main subpopulations:

1. **Central memory T cells:** Reside mainly in lymph nodes, where they can be rapidly reactivated upon re-exposure.
2. **Effector memory T cells:** Circulate in the blood and peripheral tissues, ready to act immediately.

VII.2.2.2 Memory B cells

They retain a highly specific antibody repertoire and enable an accelerated humoral response upon re-exposure **(Kaech *et al.*, 2012).**

VII.2.3 Secondary immune response

When the same antigen is encountered again, memory cells are activated quickly and efficiently, conferring enhanced adaptive immunity **(Pulendran *et al.*, 2011).** This secondary response is characterized by:

1. Immediate activation of memory T cells.
2. Rapid and abundant production of antibodies by memory B cells, often with higher affinity due to affinity maturation.
3. Faster antigen elimination, reducing disease severity and duration.

VII.3 Objectives of vaccinology

The primary goal of a vaccine is to stimulate the immune system to develop long-lasting protection against a specific disease without exposing the body to its severe forms. This

process relies on inducing immune memory, allowing a rapid and effective response upon future exposure to the pathogen **(Rappuoli *et al.*, 2011).**

VII.4 Vaccine composition

Vaccines consist of several components, each playing a specific role in inducing an effective and safe immune response **(Rappuoli *et al.*, 2011).**

VII.4.1 Antigen

The key component of the vaccine, responsible for stimulating the immune response, can be in various forms **(Rappuoli *et al.*, 2011):**

- Inactivated or attenuated viruses or bacteria.
- Specific proteins.
- Polysaccharides from bacterial capsules.

VII.5 Different types of vaccines

VII.5.1 Live attenuated vaccines

Contain live microorganisms that are weakened so they do not cause disease but still stimulate the immune system **(Pardi *et al.*, 2018).**

VII.5.2 Inactivated vaccines

Contain dead microorganisms incapable of causing disease, but their components can still trigger an immune response **(Pardi *et al.*, 2018).**

VII.5.3 Subunit vaccines

Contain only specific parts of the microorganism, such as proteins **(Pardi *et al.*, 2018).**

VII.5.4 DNA or RNA vaccines

These newer vaccines introduce genetic instructions into our cells to produce a portion of the virus, which triggers an immune response **(Pardi *et al.*, 2018).**

LEARNING ACTIVITIES

LEARNING ACTIVITIES

Activity one: Choose the correct answer(s)

1.All of the following are part of the innate (non-specific) defenses of the body except:

- **A.** The complement
- **B.** Phagocytosis
- **C.** Antibodies
- **D.** Lysozyme
- **E.** Inflammation

2. Which statements about the expression and functions of MHC molecules are correct?

- **A.** All nucleated cells express class I MHC molecules.
- **B.** Professional antigen-presenting cells (macrophages, dendritic cells, B lymphocytes) express both class I and class II MHC molecules.
- **C.** CD8+ cytotoxic T lymphocytes mainly express class II MHC molecules.
- **D.** CD4+ helper T lymphocytes recognize antigens presented by class I MHC molecules.
- **E.** Activated T lymphocytes express class I MHC molecules but not class II MHC molecules.

3. Bone marrow

- **A.** The bone marrow is a primary lymphoid organ located in the medullary cavity of bones and is the main site of maturation for T lymphocytes.
- **B.** It is responsible for hematopoiesis, producing all blood cells, including immune cells.
- **C.** It is the site of B lymphocyte maturation, where they acquire the ability to recognize specific antigens.
- **D.** The bone marrow consists only of mature immune cells ready to be released into the circulation.
- **E.** Stromal cells of the bone marrow play a key role in providing signals for the maturation of T lymphocytes.

4. What are the roles of the thymus in immunology?

- **A.** The thymus is the site of maturation for B lymphocytes.

B. The thymus plays a crucial role in the development and differentiation of T lymphocytes.

C. The thymus produces antibodies to fight infections.

D. The thymus contributes to the establishment of self-tolerance by eliminating autoreactive T cells.

E. The thymus is responsible for the production of red blood cells.

5. Which of the following statements about hematopoiesis are correct?

A. Hematopoiesis involves the differentiation of stem cells into various types of blood cells.

B. Hematopoiesis is a process that only occurs in the spleen in adults.

C. Hematopoiesis begins in the yolk sac during early embryonic development.

D. Hematopoiesis involves the production of only red blood cells.

E. Hematopoiesis occurs exclusively in the bone marrow after birth.

6. Which of the following statements about Natural Killer (NK) cells and perforin are correct?

A. Perforin is a protein produced by NK cells that creates pores in the membranes of target cells.

B. NK cells use perforin to activate the immune response against bacterial infections.

C. Perforin allows NK cells to induce apoptosis in infected or cancerous cells.

D. NK cells release perforin to directly neutralize pathogens in the bloodstream.

E. Perforin is released from NK cells to form pores in the target cell membrane, facilitating the entry of antibodies.

7. Which of the following are steps involved in phagocytosis?

A. Recognition and attachment of the pathogen to the phagocyte.

B. Engulfment of the pathogen into the phagosome.

C. Release of antibodies from the phagocyte to neutralize the pathogen.

D. Destruction of the pathogen within the phagosome by enzymes and reactive oxygen species.

E. Activation of helper T cells to increase phagocyte activity.

8. Which of the following statements about inflammation and its role in immunity are correct?

A. Inflammation helps to eliminate pathogens by recruiting immune cells to the site of infection.
B. Inflammation only occurs in response to bacterial infections.
C. Inflammation promotes the repair of damaged tissues after an injury or infection.
D. Inflammation suppresses the activity of immune cells to prevent excessive tissue damage.
E. Inflammation can enhance the effectiveness of adaptive immune responses by presenting antigens to lymphocytes.

9. Which of the following statements about Tumor Necrosis Factor (TNF) in immunity are correct?

A. TNF is primarily involved in the regulation of blood pressure during an immune response.
B. TNF promotes inflammation by activating immune cells and increasing vascular permeability.
C. TNF is produced exclusively by T lymphocytes during infection.
D. TNF plays a role in inducing apoptosis of infected or damaged cells.
E. TNF inhibits the activation of other cytokines in the immune response.

10. Which of the following statements about vaccines are correct?

A. Vaccines stimulate the immune system to develop memory cells for long-term protection.
B. All vaccines contain live pathogens to induce a stronger immune response.
C. Vaccines can be composed of inactivated pathogens, protein subunits, or mRNA.
D. Vaccines provide immediate immunity by directly neutralizing pathogens.
E. The primary goal of vaccination is to train the immune system to recognize and respond to specific antigens.

Activity two: Answer the following questions briefly.

1. List the essential functions of inflammation in tissue defense and repair.

2. Why do passive forms of immunity not establish immune memory?

3. What is the main role of CRP in the destruction of pathogens and damaged cells?

4. What is the difference between a complete antigen and a hapten?

5. What is the role of the variable and constant regions of an antibody?

6. Which antibodies are capable of fixing complement by activating the classical pathway via binding with C1?

7. What is humoral immunity and what role do antibodies play in it?

8. What is the role of interferons in the immune response?

9.What is the role of chemokines in the immune system?

10. What is cellular immunity and which cells are primarily involved in it?

Activity Three: Complete the following table.

Type of Blood Cell	Main Role
White Blood Cells (Leukocytes)	
*** Neutrophils**	
*** Lymphocytes**	
*** B Lymphocytes**	
*** T Lymphocytes**	
*** Monocytes**	
	Involved in allergic reactions and parasitic infections.
	Release histamine during allergic reactions.

Answers of activities

Activity one: The correct answers

Answer 1: C

Answer2: A and B

Answer3: B and C

Answer4: B and D

Answer5: A and C

Answer6: A and C

Answer7: A and B

Answer8: A and C

Answer9: B and D

Answer10: A and C

Activity two: Answer the following questions briefly.

1. List the essential functions of inflammation in tissue defense and repair.

It prevents the spread of toxins to neighboring tissues, removes cellular debris and pathogens, and initiates the repair process.

2.Why do passive forms of immunity not establish immune memory?

Passive forms of immunity do not establish immune memory because they involve the direct transfer of antibodies or immune cells, rather than the activation of the recipient's own immune system to produce long-lasting responses.

3.What is the main role of CRP in the destruction of pathogens and damaged cells?

It marks pathogens and damaged cells to facilitate their removal by phagocytes.

4.What is the difference between a complete antigen and a hapten?

A **complete antigen** can induce an immune response on its own, while a **hapten** requires a carrier to become immunogenic, and the **variable region** of an antibody binds to specific antigens, while the **constant region** determines its class and mediates immune system interactions.

5.What is the role of the variable and constant regions of an antibody?

The **variable region** of an antibody binds to specific antigens, while the **constant region** determines the antibody's class and facilitates interactions with other components of the immune system.

6. Which antibodies are capable of fixing complement by activating the classical pathway via binding with C1?

IgG and IgM class antibodies.

7. What is humoral immunity and what role do antibodies play in it?

Humoral immunity is a branch of the adaptive immune system where B cells produce antibodies that recognize and neutralize specific pathogens or toxins, facilitating their destruction or removal.

8. What is the role of interferons in the immune response?

Interferons are signaling proteins produced by infected cells that help protect neighboring cells from viral infection by enhancing their antiviral defenses and activating immune cells like macrophages and NK cells.

9.What is the role of chemokines in the immune system?

Chemokines are signaling molecules that guide the migration of immune cells to sites of infection or inflammation by binding to specific receptors on target cells, facilitating immune responses.

10. What is cellular immunity and which cells are primarily involved in it?

Cellular immunity is a type of adaptive immune response that involves T lymphocytes, particularly cytotoxic T cells, which recognize and destroy infected or abnormal cells, and helper T cells, which coordinate the immune response.

Answer three: Complete the following table.

Type of Blood Cell	Main Role
White Blood Cells (Leukocytes)	Defend the body against infections.
*** Neutrophils**	Engulf and destroy bacteria.
*** Lymphocytes**	Ensurespecific immune response.
*** B Lymphocytes**	Produceantibodies.
*** T Lymphocytes**	Destroy infected cells and coordinate the immune response.
*** Monocytes**	Transform into macrophages to engulf cellular debris and pathogens.
*** Eosinophils**	Involved in allergic reactions and parasitic infections.
*** Basophiles**	Release histamine during allergic reactions.

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