جامعة سطيف 1 - فرحات عباس

PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA
MINISTRY OF HIGHER EDUCATION
AND SCIENTIFIC RESEARCH

Faculty of Natural and Life Sciences
Department of Biotechnology
Specialty: Biotechnology and Health

COURSE HANDBOOK

CELLULAR & MOLECULAR IMMUNOLOGY

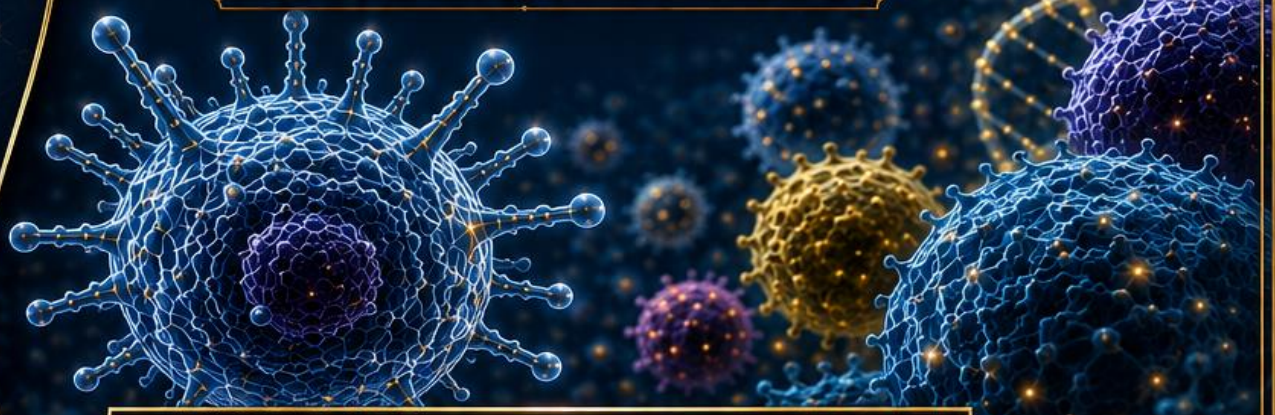
ADVANCED ACADEMIC EDITION

DISCOVER
THE IMMUNE
SYSTEM

UNDERSTAND
THE MECHANISMS
OF DEFENSE

EXPLORE
CELLULAR
INTERACTIONS

ADVANCE
IMMUNOLOGY
FOR BETTER
HEALTH



DESIGNED FOR
MASTER'S DEGREE
STUDENTS

SECOND YEAR
MASTER PROGRAM

BIOTECHNOLOGY AND HEALTH

FOR THE ADVANCEMENT OF IMMUNOLOGY

PREPARED BY

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2025 – 2026

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I. Course handout

Faculty: Faculty of Natural and Life Sciences

Department: Biotechnology

Master Program: Biotechnology and Health

Target Audience: Second-Year Master's Students (M2)

Teaching Unit (UE): Fundamental Unit

Course Title: Cellular and Molecular Immunology

Credits: 6 ECTS

Coefficient: 3

Total Semester Workload: 67.5 Hours

Schedule:

- Monday: 11:00 AM – 12:30 PM
- Wednesday: 08:00 AM – 09:30 AM

Classroom: SAM 2

Instructor: Dr. Selma HOUCHI

Teaching Activities: Lectures

Email: houchi.selma@univ-setif.dz

Office Hours: Monday, Tuesday, and Wednesday 08:00 AM – 12:30 PM; Office A13

Communication Policy

Discussion Forum

All course-related questions should be posted on the dedicated discussion forum to allow all students to benefit from the answers provided. Responses will normally be given within 48 hours.

Email Communication

Emails are reserved for urgent matters, particularly technical issues such as access problems to the online learning platform. Responses are generally provided within 48 hours, except in unforeseen circumstances. Students are encouraged to use the discussion forum as the primary communication channel.

II. Course description

Immunity encompasses the biological mechanisms that enable living organisms to recognize, resist, and eliminate foreign substances, particularly infectious agents, while maintaining tissue integrity and physiological homeostasis. The coordinated network of organs, tissues, cells, and soluble mediators involved in these protective processes constitutes the immune system. Together, these components generate highly regulated responses that protect the host against pathogens and other potentially harmful challenges.

Immunology is the branch of biomedical science devoted to the study of the immune system, its components, and its functions. The origins of modern immunology are commonly traced to Edward Jenner, whose pioneering work on vaccination against smallpox in 1796 laid the foundation for one of the most significant advances in medical history. Since then, immunology has evolved into a multidisciplinary field that integrates molecular biology, genetics, microbiology, biotechnology, and clinical medicine.

Although initially focused on host defense against infectious diseases, immunology now plays a central role in understanding a wide range of physiological and pathological processes. Advances in immunological research have revealed the involvement of the immune system in allergy, autoimmunity, chronic inflammation, transplantation, cancer, metabolic disorders, and tissue repair. Consequently, immunology has become an essential discipline for both fundamental biological research and modern healthcare.

The immune system represents a remarkably sophisticated and dynamic network that has evolved to provide efficient protection while maintaining self-tolerance. Understanding its cellular and molecular mechanisms is essential for deciphering the pathogenesis of numerous human diseases and for developing innovative diagnostic, preventive, and therapeutic strategies. This course provides an in-depth study of the cellular and molecular mechanisms governing immune responses. Particular emphasis is placed on the development, activation, regulation, and functional specialization of immune cells, as well as on their applications in biotechnology and health sciences.

By the end of this course, students will possess a comprehensive understanding of immune system organization and function, enabling them to apply immunological concepts to research, biotechnology, and biomedical innovation.

III. Course content

According to the Master's curriculum in Biotechnology and Health, the course *Cellular and Molecular Immunology* provides a comprehensive overview of the architecture, mechanisms, and functions of the immune system.

The course is organized into the following major topics:

1. Organization and Compartmentalization of the Immune System: Hematopoiesis, Immune Cells, and Lymphoid Organs
2. Fundamentals of Innate and Adaptive Immunity
3. Inflammatory Responses
4. Structure, Functions, and Signaling of Toll-Like Receptors (TLRs)
5. Mucosal Immune System
6. Structure of Major Histocompatibility Complex (MHC) Molecules and Antigen Presentation
7. Structure of Antigen Receptors (TCR and BCR)
8. Antibody Synthesis and Diversity
9. Immune Activation: Cellular Cooperation During Immune Responses
    - Antigen-Presenting Cells and T Cells
    - T Cells and B Cells
10. Regulation of the Immune System
11. Immunological Memory and Vaccination
12. Immunology of Pregnancy
13. Aging and the Immune System
14. Role of Apoptosis in Immune Homeostasis
15. Feto-Maternal Tolerance
16. Immuno-Endocrine Interactions

Note: In accordance with the official course template, all required topics are listed separately in the course content. However, some topics have been integrated into broader chapters for pedagogical coherence. Specifically, the topic *"Mucosal Immune System"* (Item 5) is covered within Chapter 1: Organization and Compartmentalization of the Immune System, under the section devoted to Mucosa-Associated Lymphoid Tissue (MALT). Likewise, the topics "Immunology of Pregnancy" (Item 12) and "Feto-Maternal Tolerance" (Item 15) are presented

together in Chapter 11: Immunology of Pregnancy and Feto-Maternal Tolerance, as these subjects are closely interconnected and are more effectively addressed within a single integrated chapter.

Prerequisites

Students are expected to possess basic knowledge of:

- Human anatomy and physiology;
- Cell biology;
- Molecular biology;
- General microbiology.

A self-assessment test is available on the university e-learning platform:

https://snv-courses.univ-setif.dz/course/view.php?id=591

Students should log in using the credentials provided by the instructor and access the course entitled **Cellular and Molecular Immunology**.

The test remains available throughout the semester and may be repeated as often as necessary. Students with insufficient scores will be directed toward supplementary self-learning resources available on the same platform.

IV. Learning outcomes

The main objective of this course is to provide students with an advanced understanding of modern immunology and its applications in biotechnology and health sciences.

Upon successful completion of the course, students will be able to:

Knowledge

- Describe the organization and function of the immune system.
- Explain the cellular and molecular mechanisms involved in innate and adaptive immunity.
- Understand immune regulation, tolerance, inflammation, and immunological memory.
- Analyze immune responses in physiological and pathological conditions.

Practical and Analytical Skills

- Interpret immunological phenomena using contemporary molecular and cellular concepts.
- Apply immunological principles to biotechnology and health-related applications.
- Understand the biological and medical engineering techniques commonly used in immunological research and diagnostics.
- Develop scientific reasoning for solving immunology-related problems.

This course provides a strong foundation for advanced studies and professional careers in biotechnology, biomedical research, clinical laboratories, immunodiagnostics, vaccine development, and health-related industries.

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Introduction

Immunity refers to the set of defense mechanisms that enable living organisms to recognize, resist, and eliminate foreign agents, particularly infectious microorganisms, as well as other potentially harmful factors that may threaten normal physiological functions or survival. The organs, tissues, cells, and molecules involved in these protective processes collectively constitute the immune system, while the coordinated actions of these components against pathogens are known as the immune response.

Immunology is the scientific discipline dedicated to the study of the immune system and its functions. The foundations of modern immunology are generally attributed to Edward Jenner, who demonstrated in 1796 that vaccination could provide protection against smallpox. Since then, immunology has evolved into a major branch of life sciences and biomedical research.

For many years, immunological research focused primarily on the mechanisms of host defense against infectious diseases. However, advances in cellular and molecular biology have revealed that the immune system plays a central role in a wide range of physiological and pathological processes. Today, immunology is essential for understanding allergies, autoimmune diseases, chronic inflammatory disorders, cancer, transplantation, immunodeficiencies, and many other human diseases.

The immune system can be viewed as a highly sophisticated and dynamic network composed of numerous interacting cells, tissues, and molecular mediators. Its remarkable organization is the result of millions of years of evolution, leading to a defense system that is both highly efficient and tightly regulated. The ability of the immune system to distinguish self from non-self, eliminate pathogens, maintain tissue homeostasis, and generate immunological memory is fundamental for the survival of all multicellular organisms.

Understanding how this complex system functions is one of the major challenges of modern biomedical sciences. A thorough knowledge of immunological mechanisms is essential not only for understanding disease pathogenesis but also for developing innovative diagnostic tools, vaccines, immunotherapies, and biotechnological applications.

The aim of this handbook is to provide a comprehensive overview of the cellular and molecular mechanisms that govern immune responses. It presents the fundamental concepts of modern immunology, including the organization of the immune system, innate and adaptive immunity, antigen recognition and presentation, lymphocyte development and activation, immune

regulation, immunological memory, tolerance mechanisms, and their applications in health and biotechnology.

The following chapters are designed to progressively guide students through the fundamental and advanced concepts of immunology, providing the theoretical foundation necessary for further studies and research in biotechnology and health sciences.

Chapter 1. Organization and Compartmentalization of the Immune System

The immune system is composed of numerous organs and tissues distributed throughout the body. Based on their functions, these structures can be classified into two major groups. Primary lymphoid organs provide the specialized microenvironments required for the development, differentiation, and maturation of lymphocytes. In contrast, secondary lymphoid organs are responsible for capturing antigens originating from tissues and vascular compartments and serve as the sites where mature lymphocytes encounter and respond to these antigens.

The blood vascular and lymphatic systems connect these organs, integrating them into a coordinated functional network. Circulating through the blood and lymph and residing within lymphoid organs, various types of white blood cells, or leukocytes, participate in immune responses. Among these cells, only lymphocytes possess the fundamental characteristics of adaptive immunity, including diversity, specificity, immunological memory, and the ability to distinguish self from non-self.

All other immune cells play supportive roles in adaptive immunity by activating lymphocytes, enhancing antigen elimination through phagocytosis, or secreting a variety of effector molecules that contribute to immune defense. Together, these cellular and molecular components ensure effective protection against pathogens while maintaining immune homeostasis.

This chapter describes the process of blood cell formation (hematopoiesis) and presents the structural and functional compartmentalization of the immune system.

1. Hematopoiesis

All blood cells originate from a specialized type of cell known as the hematopoietic stem cell (HSC). Stem cells are characterized by their ability to differentiate into multiple cell types and by their capacity for self-renewal, allowing them to maintain their population through continuous cell division.

In humans, hematopoiesis, the process by which red blood cells and white blood cells are formed and developed, begins in the yolk sac during the early weeks of embryonic development. At this stage, yolk sac stem cells differentiate into primitive erythroid cells that contain embryonic hemoglobin. During the third month of gestation, hematopoietic stem cells

migrate from the yolk sac to the fetal liver and subsequently to the spleen. These organs serve as the principal sites of hematopoiesis from the third to the seventh month of fetal development.

Later, hematopoietic stem cell differentiation takes place in the bone marrow, which becomes the primary site of hematopoiesis. Following birth, hematopoietic activity in the liver and spleen is greatly reduced or completely absent under normal physiological conditions.

A pluripotent hematopoietic stem cell differentiates into either a common lymphoid progenitor (CLP) or a common myeloid progenitor (CMP). Progenitor cells have lost their capacity for self-renewal and are committed to specific cellular lineages.

Common lymphoid progenitors give rise to T lymphocytes, B lymphocytes, and a specialized population of immune cells known as natural killer (NK) cells. Common myeloid progenitors generate precursors of red blood cells (erythrocytes), most white blood cells (including neutrophils, eosinophils, basophils, monocytes, and mast cells), as well as megakaryocytes, the large bone marrow cells responsible for platelet production **(Figure 1)**.

Within the bone marrow, hematopoietic stem cells proliferate and mature in close association with a network of stromal cells, including adipocytes, endothelial cells, fibroblasts, and macrophages. These stromal cells provide a specialized hematopoietic microenvironment composed of extracellular matrix components, growth factors, and differentiation signals that regulate the survival, proliferation, and maturation of hematopoietic stem cells.

Hematopoietic stem cells (HSCs), characterized by the expression of the CD34 surface marker, possess the ability to self-renew and differentiate into all blood cell lineages. Under the influence of the bone marrow stromal microenvironment and various growth factors and cytokines, HSCs give rise to two major progenitor populations: the common lymphoid progenitor (CLP) and the common myeloid progenitor (CMP). The CLP generates cells involved primarily in adaptive and innate immunity, including T lymphocytes, B lymphocytes, plasma cells, and natural killer (NK) cells. In contrast, the CMP gives rise to erythrocytes, platelets, granulocytes (neutrophils, eosinophils, and basophils), monocytes/macrophages, dendritic cells, and mast cells. The figure also highlights the essential role of cytokines such as IL-7, IL-15, EPO, TPO, G-CSF, GM-CSF, and M-CSF in regulating lineage commitment, proliferation, maturation, and differentiation of hematopoietic cells. Together, these processes ensure the continuous production and renewal of blood and immune cells throughout life.

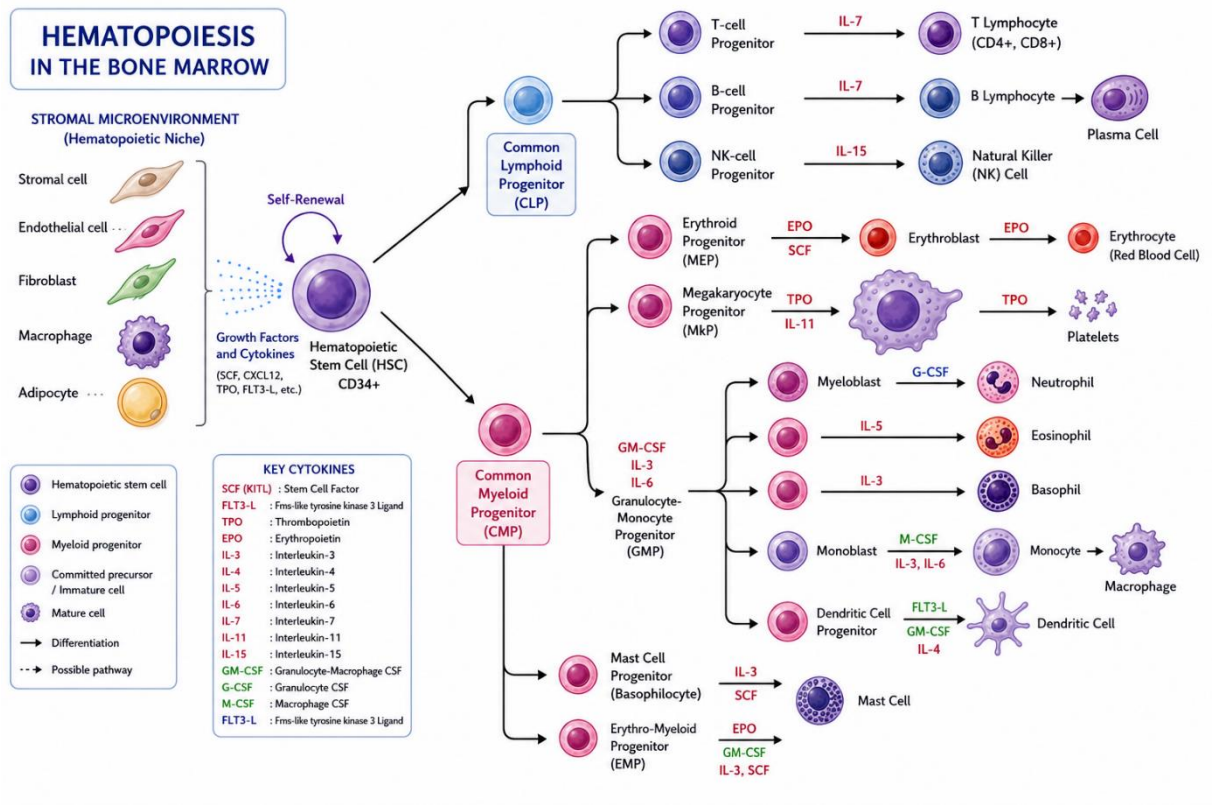


Figure 1. Hematopoiesis and differentiation of Immune cells in the Bone Marrow

2. Cells and Organs of the Immune System

Lymphoid organs and tissues are distributed throughout the body, and immune cells continuously circulate within and between these organs through the blood and lymphatic systems.

Immune cells communicate either through direct cell-to-cell contact, involving specific receptor–ligand interactions, or through soluble molecules secreted into the extracellular environment. These soluble signaling molecules are collectively known as cytokines. The term cytokine encompasses several groups of mediators, including lymphokines, monokines, and chemokines. Some cytokines are also classified as interleukins (ILs) and are designated according to an internationally recognized nomenclature.

Organs of the Immune System

The immune system is composed of two major categories of lymphoid organs: primary lymphoid organs (PLOs) and secondary lymphoid organs (SLOs).

Primary lymphoid organs are the sites where immunocompetent cells are generated and mature. These organs include the bone marrow, where B lymphocytes develop and mature in mammals,

and the thymus, where T lymphocytes undergo maturation and selection. In birds, B-cell development occurs in a specialized organ known as the bursa of Fabricius.

Secondary lymphoid organs are the sites where naïve lymphocytes encounter antigens and become activated, thereby initiating adaptive immune responses. These organs include highly organized lymphoid structures such as the spleen and lymph nodes, which are distributed throughout the lymphatic circulation. In addition, secondary lymphoid tissues include non-encapsulated accumulations of lymphoid cells associated with mucosal surfaces, collectively referred to as mucosa-associated lymphoid tissues (MALT) **(Figure 2).**

Together, primary and secondary lymphoid organs provide the structural framework necessary for the development, activation, and coordination of immune responses.

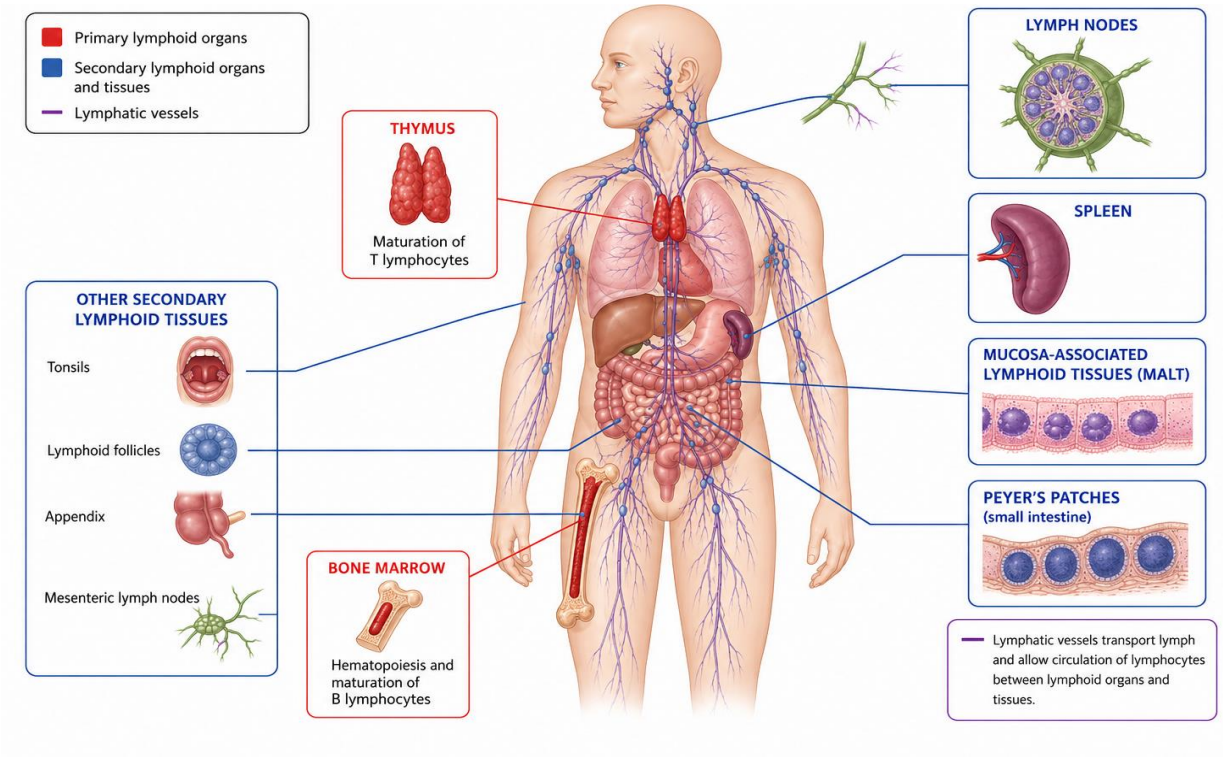


Figure 2. Organization of the Human Lymphoid System

Primary lymphoid organs (bone marrow and thymus) are shown in red, whereas secondary lymphoid organs and tissues are shown in blue. The lymphatic vessels (purple) ensure the circulation of lymphocytes between the different compartments of the immune system.

2.1.1. Primary Lymphoid Organs

2.1.1.1. Bone Marrow

The bone marrow, also known as red marrow or hematopoietic marrow, is the primary site of hematopoiesis. It should not be confused with the spinal cord, which is part of the central nervous system.

Bone marrow is the soft tissue located within the cavities of bones throughout the body. Two types of bone marrow can be distinguished: red bone marrow and yellow bone marrow **(Figure 3).** Red bone marrow is a highly active tissue that plays a crucial role in the production of precursor cells for the various populations of red blood cells, platelets, and immune cells involved in host defense. Through the process of hematopoiesis, these precursor cells undergo proliferation, differentiation, and maturation to generate the circulating blood cells required for oxygen transport, hemostasis, and immune protection.

In adults, active red bone marrow is primarily found in the vertebrae, ribs, sternum, pelvis, skull, and the proximal ends of long bones, whereas yellow bone marrow consists mainly of adipose tissue and serves as an energy reserve. Under certain pathological conditions, yellow marrow may revert to active hematopoietic tissue to increase blood cell production.

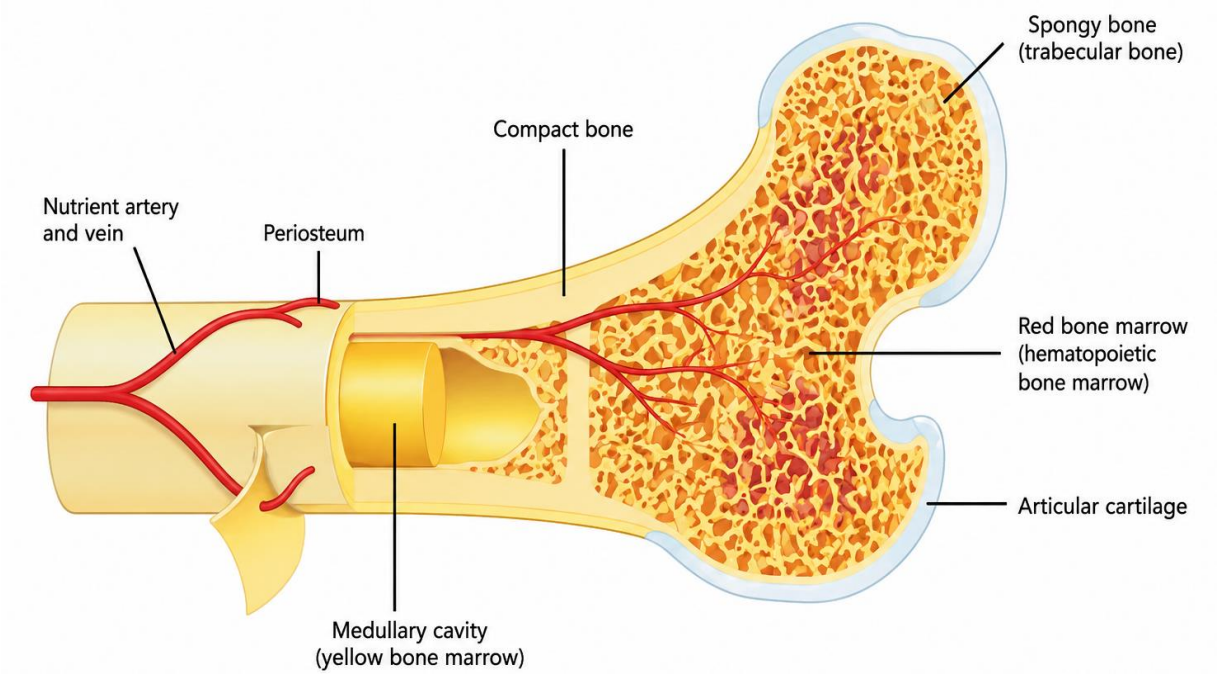


Figure 3. Anatomical organization of red and yellow Bone Marrow in a long bone

2.1.1.2. Thymus

The thymus is the primary site for the maturation and education of T lymphocytes through a series of selection processes that ensure the development of a functional and self-tolerant T-cell repertoire. It is a bilobed organ located in the anterior mediastinum, behind the sternum and above the heart.

Histologically, each thymic lobe is subdivided into functional units known as lobules, which are separated by connective tissue extensions of the capsule called trabeculae. Within each lobule, two distinct regions can be identified: an outer cortex and an inner medulla. These regions differ in their cellular composition and provide specialized microenvironments that support the successive stages of T-cell development.

Lymphoid progenitor cells originating from the bone marrow enter the thymus through postcapillary venules located at the corticomedullary junction. They subsequently migrate through the cortex toward the medulla while undergoing a series of differentiation and selection events. The primary objective of these processes is to ensure the survival of thymocytes expressing a functional T-cell receptor (TCR) while eliminating cells that are unable to recognize self-major histocompatibility complex (MHC) molecules appropriately or that exhibit excessive reactivity toward self-antigens.

In addition to thymocytes at various stages of development, the thymus contains several stromal cell populations that contribute to T-cell maturation. These include thymic epithelial cells and fibroblasts in both the cortex and medulla, as well as macrophages and dendritic cells, which are particularly abundant in the medullary region. Together, these cells create a specialized microenvironment that regulates thymocyte differentiation, positive and negative selection, and the establishment of central immune tolerance **(Figure 4)**.

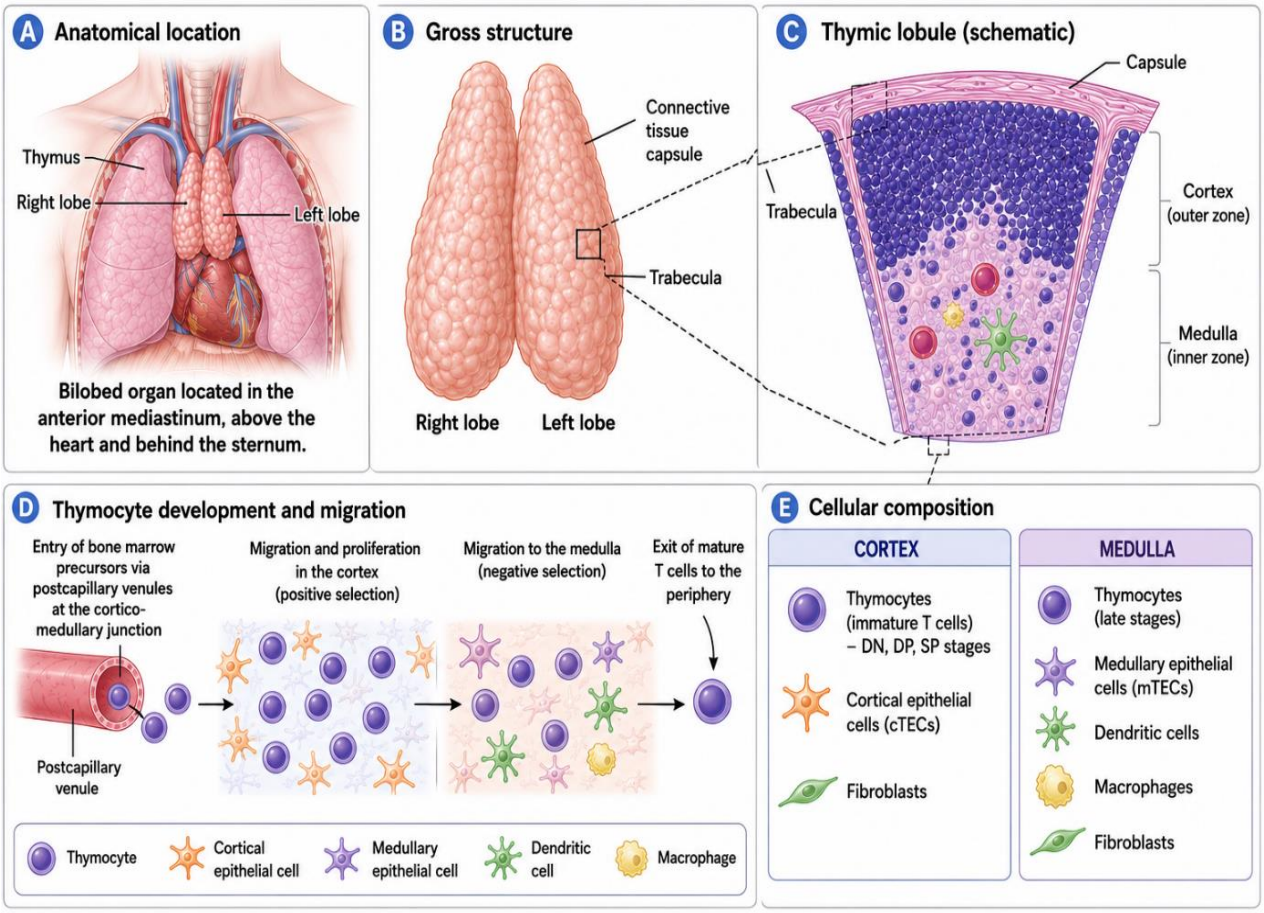


Figure 4. Structure and functional organization of the Human Thymus

2.1.1.3. Bursa of Fabricius

The Bursa of Fabricius is a primary lymphoid organ located at the terminal portion of the cloaca in birds **(Figure 5)**. It is a specialized lymphoepithelial organ that plays a crucial role in the differentiation and maturation of precursor B lymphocytes into mature B lymphocytes.

In birds, the Bursa of Fabricius serves as the primary site of B-cell development and the acquisition of immunocompetence. In mammals, including humans, this function is performed by the bone marrow, which is considered the functional equivalent of the Bursa of Fabricius. Consequently, B-cell development, maturation, and selection occur within the bone marrow before mature B lymphocytes migrate to secondary lymphoid organs.

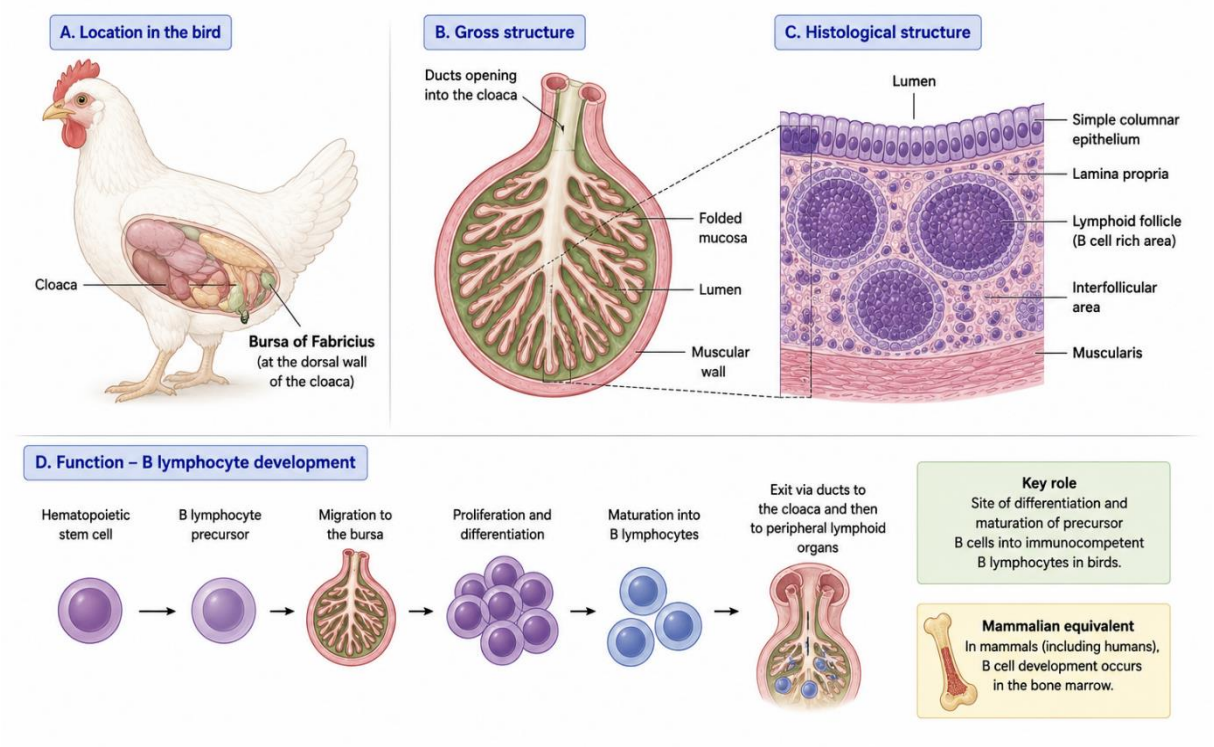


Figure 5. Bursa of Fabricius: Primary Lymphoid Organ for B-cell development in birds

2.1.2. Secondary Lymphoid Organs

The lymph nodes and the spleen are the most highly organized secondary lymphoid organs (SLOs). They contain not only lymphoid follicles but also distinct regions dedicated to T-cell and B-cell activities and are surrounded by a fibrous capsule. Less organized lymphoid tissues, collectively known as mucosa-associated lymphoid tissue (MALT), are found in various regions of the body. These tissues include Peyer's patches in the small intestine, the tonsils, the appendix, and numerous lymphoid follicles located within the lamina propria of the intestinal mucosa and the mucosal surfaces lining the upper respiratory tract, bronchi, and genital tract.

2.1.2.1. Lymph Nodes

Lymph nodes are the sites where immune responses are initiated against antigens carried in the lymph. They are encapsulated, bean-shaped structures containing a reticular network populated by lymphocytes, macrophages, and dendritic cells. Located at strategic points along the lymphatic vessels, lymph nodes are the first organized lymphoid structures to encounter antigens entering tissue spaces. As lymph flows through a lymph node, particulate antigens are trapped and processed by a network of phagocytic cells and dendritic cells, including follicular and interdigitating dendritic cells.

Morphologically, a lymph node can be divided into three roughly concentric regions: the cortex, the paracortex, and the medulla, each providing a specialized microenvironment.

The outermost region, the cortex, contains numerous lymphocytes, predominantly B cells, together with macrophages and follicular dendritic cells organized into primary follicles. Following antigenic stimulation, primary follicles enlarge and develop into secondary follicles, each containing a germinal center where B-cell proliferation, somatic hypermutation, and affinity maturation occur.

Beneath the cortex lies the paracortex, a region rich in T lymphocytes. It also contains interdigitating dendritic cells that have migrated from peripheral tissues into the lymph node. These dendritic cells express high levels of major histocompatibility complex class II (MHC II) molecules, enabling efficient antigen presentation to helper T cells and initiation of adaptive immune responses.

The innermost region, the medulla, contains fewer lymphoid cells than the cortex and paracortex. Many of the cells present are plasma cells, which actively synthesize and secrete antibodies into the lymphatic circulation. The increase in lymphocyte numbers within the lymph leaving a lymph node is partly due to lymphocyte proliferation in response to antigenic stimulation. However, most of this increase results from lymphocytes entering the lymph node directly from the bloodstream. This migration occurs through specialized postcapillary venules known as high endothelial venules (HEVs). Unlike ordinary venules, HEVs are lined by plump endothelial cells that facilitate the extravasation of circulating lymphocytes into the lymph node. HEVs play a critical role in lymphocyte recirculation, as most lymphocytes enter lymph nodes through these specialized vessels. During an immune response, antigenic stimulation can increase lymphocyte migration into the lymph node by as much as tenfold. Consequently, the number of lymphocytes within an activated lymph node rises dramatically, causing visible enlargement of the node. This enhanced migration is thought to be mediated by factors released within the lymph node during antigenic stimulation **(Figure 6)**.

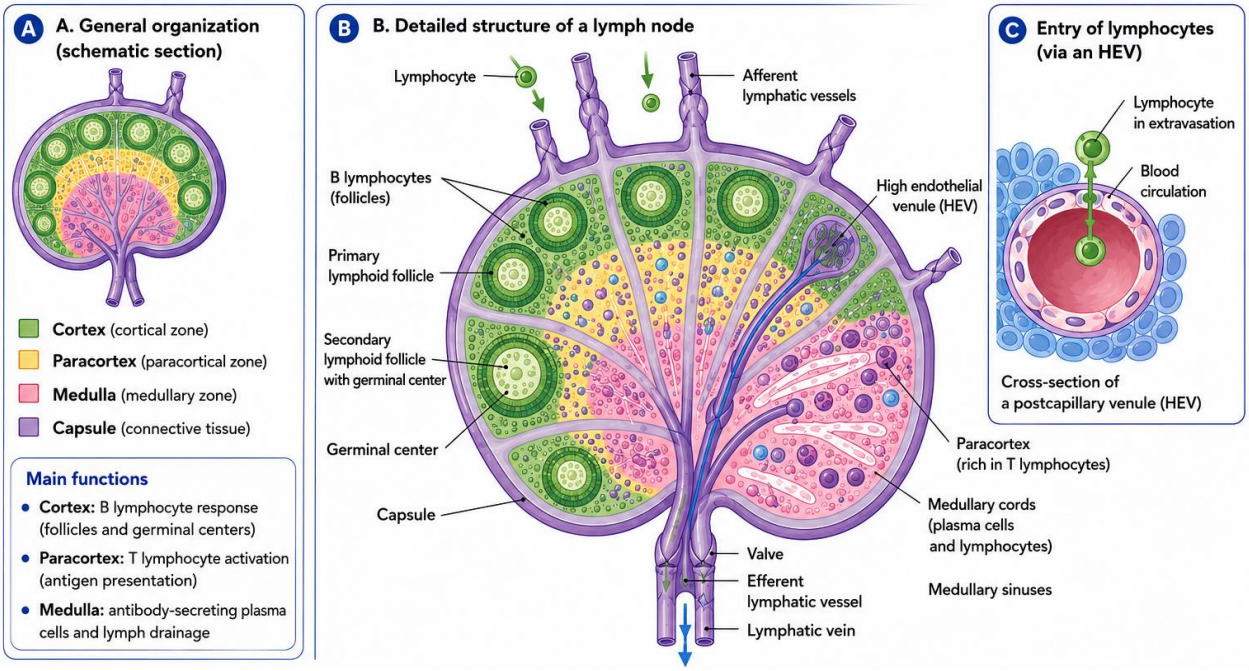


Figure 6. Structure of a lymph node

2.1.2.2. Spleen

The spleen is a large, oval-shaped secondary lymphoid organ located in the upper left region of the abdominal cavity. It is specialized in the filtration of blood and the capture of blood-borne antigens, enabling it to mount immune responses against systemic infections. Unlike lymph nodes, the spleen is not supplied by lymphatic vessels. Instead, antigens and lymphocytes are delivered to the spleen through the splenic artery.

The spleen is surrounded by a fibrous capsule that extends inward through numerous projections known as trabeculae, creating a compartmentalized structure. Two major compartments can be distinguished: the red pulp and the white pulp, separated by a diffuse marginal zone **(Figure 7)**.

The red pulp consists of a network of vascular sinuses populated by macrophages and large numbers of erythrocytes. It is the principal site for the removal and destruction of aged, damaged, or defective red blood cells. Many macrophages in the red pulp contain phagocytosed erythrocytes or iron-containing pigments derived from degraded hemoglobin.

The white pulp surrounds branches of the splenic artery, forming structures known as periarteriolar lymphoid sheaths (PALS), which are composed predominantly of T lymphocytes. Associated with the PALS are primary lymphoid follicles, which are rich in B lymphocytes, and some of these follicles develop germinal centers following antigenic stimulation.

The marginal zone, located at the periphery of the PALS, contains abundant B lymphocytes and macrophages. It serves as an important interface between the circulation and the immune compartments of the spleen.

Blood-borne antigens and lymphocytes enter the spleen through the splenic artery and are released into the marginal zone. In this region, antigens are captured by interdigitating dendritic cells, which transport and present them within the PALS. Circulating lymphocytes also enter the marginal sinus system and subsequently migrate into the white pulp, where they participate in adaptive immune responses.

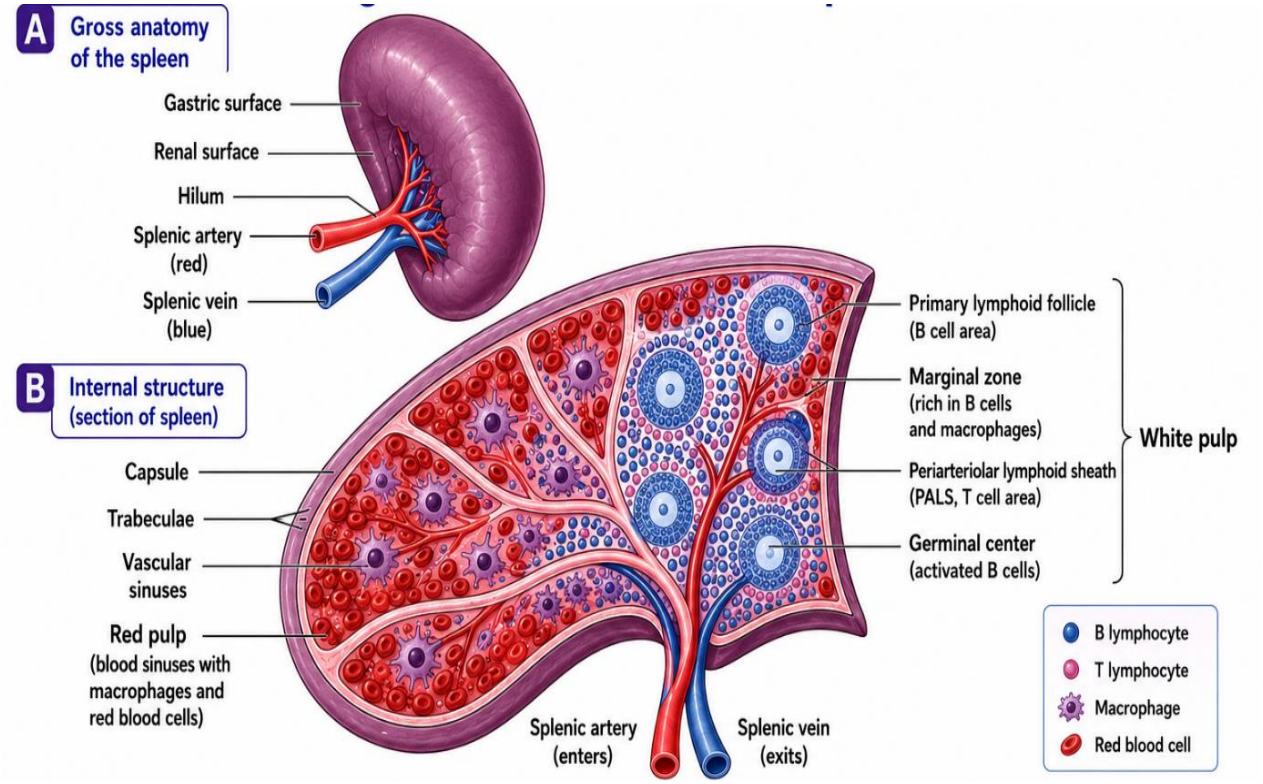


Figure 7. Gross anatomy and histological organization of the human spleen

2.1.2.3. Mucosa-Associated Immune System

The mucosal surfaces lining the gastrointestinal, respiratory, and urogenital tracts cover a total area of approximately 400 m² and represent the major entry sites for most pathogens. These vulnerable surfaces are protected by a specialized network of organized lymphoid tissues collectively known as Mucosa-Associated Lymphoid Tissue (MALT).

Secondary lymphoid tissues associated with the respiratory epithelium are referred to as Bronchus-Associated Lymphoid Tissue (BALT), whereas those associated with the digestive tract are known as Gut-Associated Lymphoid Tissue (GALT). Structurally, these tissues range

from loosely organized clusters of lymphoid cells within the intestinal lamina propria to highly organized structures such as the tonsils, appendix, and Peyer's patches, which are located within the submucosal layer of the intestinal wall. The functional importance of MALT is reflected by its exceptionally large population of antibody-producing plasma cells, whose total number exceeds that of the plasma cells found in the spleen, lymph nodes, and bone marrow combined.

As illustrated in **Figures 8** and **9**, lymphoid cells are distributed throughout these tissues. The outer mucosal epithelial layer contains intraepithelial lymphocytes (IELs), most of which are T lymphocytes. Beneath the epithelium lies the lamina propria, which contains numerous B cells, plasma cells, activated helper T cells, and macrophages organized in loose aggregates. The submucosa, located below the lamina propria, contains Peyer's patches, which are lymphoid nodules composed of 30 to 40 lymphoid follicles. Like lymphoid follicles found elsewhere in the body, these follicles may develop into secondary follicles containing germinal centers following antigenic stimulation.

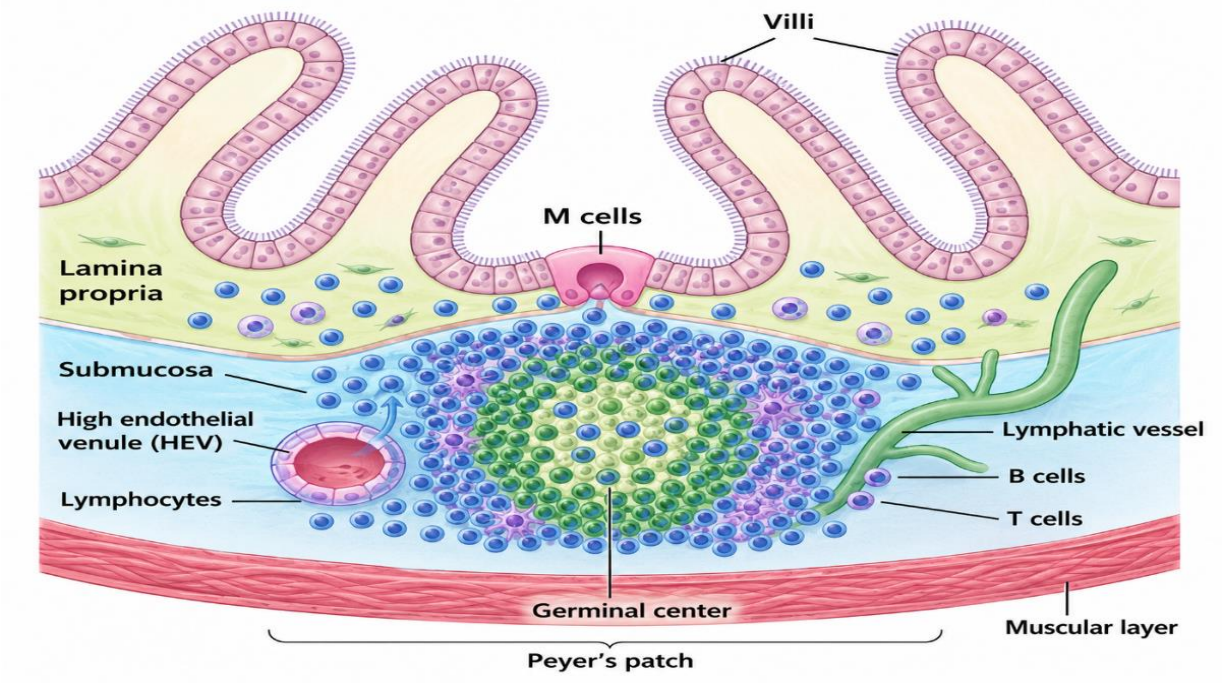


Figure 8. Schematic section of the intestinal mucosa showing a cluster of lymphoid follicles forming a Peyer's patch within the submucosa.

Mucosal epithelial cells play a crucial role in promoting immune responses by delivering small samples of foreign antigens from the lumen of the respiratory, gastrointestinal, and genitourinary tracts to the underlying mucosa-associated lymphoid tissue. This transport is carried out by specialized epithelial cells known as M cells (microfold cells).

M cells possess distinctive structural features. They are flattened epithelial cells lacking the microvilli that characterize most mucosal epithelial cells. Furthermore, M cells contain a deep invagination, or pocket, within their basolateral plasma membrane. This pocket houses clusters of B lymphocytes, T lymphocytes, macrophages, and dendritic cells **(Figure 9a)**. Luminal antigens are internalized through endocytosis into vesicles that are transported from the apical membrane to the basolateral pocket. These vesicles subsequently fuse with the pocket membrane, releasing antigens directly to immune cells capable of initiating an immune response.

Antigens transported across the mucosal epithelium by M cells can activate B lymphocytes, which differentiate into plasma cells and secrete Immunoglobulin A (IgA) antibodies. IgA is the principal antibody class involved in mucosal immunity and represents a critical defense mechanism against a wide variety of infections occurring at mucosal surfaces **(Figure 9b)**.

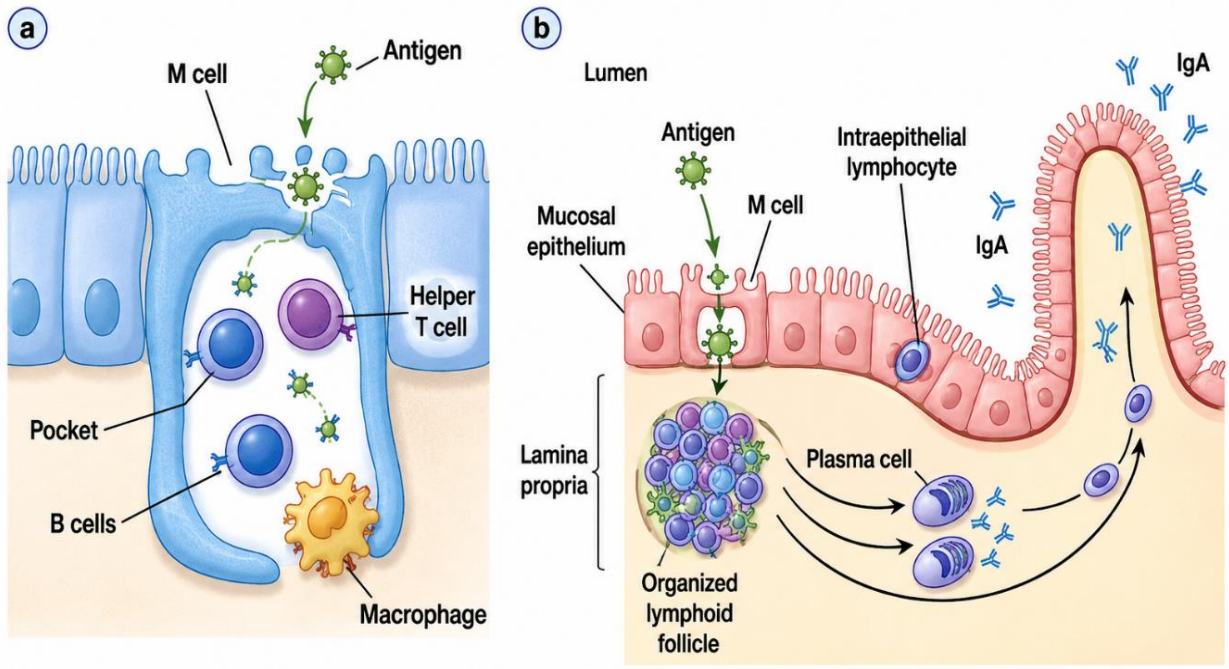


Figure 9. Structure of M Cells and IgA production at inductive sites. **(a)** M cells located within mucosal epithelia endocytose antigens originating from the gastrointestinal, respiratory, and genitourinary tracts. The antigens are transported across the M cell and released into a large basolateral pocket containing immune cells.

(b) Antigens transported across the epithelial layer by M cells at inductive sites activate B lymphocytes within the underlying lymphoid follicles. Activated B cells differentiate into IgA-secreting plasma cells, which migrate throughout the submucosal tissue. The outer epithelial layer of the mucosa contains intraepithelial lymphocytes (IELs), many of which are $CD8^+$ T cells expressing γδ T-cell receptors (γδ TCRs) characterized by limited antigen receptor diversity.

2.1.2.4. Skin-Associated Lymphoid Tissue (SALT)

The skin constitutes a fundamental anatomical barrier against the external environment, and its extensive surface area makes it a major component of the body's innate (natural) defense mechanisms. The outermost layer of the skin, the epidermis, is composed primarily of specialized epithelial cells known as keratinocytes. These cells secrete numerous cytokines that can initiate and regulate local inflammatory responses.

Scattered throughout the epidermal epithelial matrix are Langerhans cells, a specialized type of dendritic cell capable of capturing antigens through phagocytosis and endocytosis. Following antigen uptake, Langerhans cells undergo maturation and migrate from the epidermis to regional lymph nodes, where they serve as potent antigen-presenting cells capable of activating naïve helper T (Th) cells.

The underlying dermis contains dispersed populations of $CD4^+$ and $CD8^+$ T lymphocytes, as well as macrophages. Most dermal T cells are either previously activated effector cells or memory T cells, enabling the skin to mount rapid and efficient immune responses upon re-exposure to pathogens or other foreign antigens.

Chapter 2. Fundamentals of Innate and Adaptive Immunity

Vertebrate organisms are protected by two major immune systems: the innate immune system and the adaptive immune system **(Table 1)**. Innate immunity consists of a set of defense mechanisms against infection that are activated immediately following invasion by a pathogenic organism. The innate immune system includes physical, chemical, and cellular barriers. When the protective barriers of the skin and mucosal membranes are breached, the response to invading pathogens is rapid and begins within minutes after infection.

Despite the multiple levels of protection provided by innate immunity, some pathogens are able to evade these defenses. In such cases, a second system, known as adaptive immunity (or acquired immunity), is activated. Adaptive immunity develops following exposure to microorganisms and combats infection through highly specific responses tailored to each pathogen. This system involves populations of B and T lymphocytes, which recognize pathogens with remarkable specificity.

Unlike innate immunity, the adaptive immune response requires time to develop and may take one week or longer before becoming fully effective. A defining characteristic of adaptive immunity is its capacity for immunological memory. Following an initial infection caused by a particular pathogen, subsequent exposures to the same pathogen trigger faster, stronger, and more efficient immune responses, thereby providing enhanced protection against reinfection.

Table 1. Innate and adaptive immunity

ATTRIBUTE	INNATE IMMUNITY	ADAPTIVE IMMUNITY
Response time	Minutes to hours	Days
Specificity	Specific for molecules and molecular patterns associated with pathogens	High specificity: discriminates even minor differences; details of microbial or non-microbial structures recognized with high specificity
Diversity	Limited number of receptors encoded by non-rearranged genes	High diversity: a large number of receptors generated by genetic recombination of the genes encoding these receptors
Memory responses	None	Persistent memory, with a faster and more robust response to subsequent infections
Self/non-self discrimination	Perfect, absence of specific microbial patterns in the host	Very good; occasional failures in discrimination between self and non-self, leading to autoimmune diseases
Soluble components in blood or tissue fluids	Most antimicrobial peptides and proteins	Antibodies
Main types of cells	Phagocytes (monocytes, macrophages, neutrophils), natural killer (NK) cells, dendritic cells	T cells, B cells, antigen-presenting cells (APCs)

1. Innate Immunity (Non-Specific Immunity)

Innate immunity relies on both humoral and cellular mechanisms. The humoral component of innate immunity is mediated by the complement system, cytokines, and acute-phase proteins involved in inflammation. The cellular component is carried out by cells with phagocytic or cytolytic functions, including neutrophils, macrophages, and natural killer (NK) cells. Activation of these cells constitutes a critical step in the inflammatory response, which represents one of the two major forms of innate immune defense against microbial infections.

- Inflammation is the process by which leukocytes and circulating plasma proteins are recruited and activated at sites of infection to destroy and eliminate invading pathogens. Inflammation also serves as the primary response to damaged or dead cells and to the accumulation of abnormal substances within tissues.
- Antiviral defense consists of inducing cellular changes that prevent viral replication. In addition, antiviral mechanisms increase the susceptibility of infected cells to destruction by cytotoxic lymphocytes, thereby eliminating viral reservoirs.

1.1. Physical, Chemical, and Biological Barriers

The most evident components of innate immunity that protect against microbial invasion are the physical, chemical, and biological barriers that constitute the first line of non-specific defense **(Table 2)**. Physical barriers include the impermeability of the skin and epithelial surfaces, as well as mechanical clearance mechanisms such as the action of cilia, tears, saliva, nasal and bronchial mucus, gastric secretions, bile, and the continuous flow of fluids across mucosal surfaces. Chemical barriers further reinforce host protection through factors such as body temperature, acidic pH, the antifungal activity of certain fatty acids, the antibacterial properties of lactic acid present in sebum and sweat, and antimicrobial molecules including lysozyme, interferons, defensins, digestive enzymes, and components of the complement system. Biological barriers are primarily represented by the host's normal microbiota, which colonizes the skin, respiratory tract, gastrointestinal tract, and genitourinary mucosa. This commensal flora contributes significantly to innate defense by producing antimicrobial substances, inhibiting pathogen colonization, competing with invading microorganisms for nutrients and ecological niches, and helping maintain immune homeostasis. Together, these physical, chemical, and biological barriers provide continuous protection against infection and

greatly reduce the likelihood of pathogen invasion before the activation of cellular and adaptive immune responses.

Table 2. Physical, chemical, and biological barriers of innate immunity.

BARRIER TYPE	MAIN COMPONENTS	PROTECTIVE FUNCTIONS
PHYSICAL BARRIERS	• Skin • Epithelial surfaces • Mucus • Cilia • Tears • Saliva • Gastric secretions • Bile	Prevent pathogen entry and promote mechanical removal of microorganisms
CHEMICAL BARRIERS	• Gastric acid • Digestive enzymes • Lysozyme • Defensins • Interferons • Complement proteins • Fatty acids • Lactic acid	Inhibit microbial growth, neutralize pathogens, and destroy invading microorganisms
BIOLOGICAL BARRIERS	• Commensal microbiota of the skin, respiratory tract, gastrointestinal tract, and genitourinary tract	Compete with pathogens for nutrients and ecological niches, produce antimicrobial substances, and maintain immune homeostasis

These physical, chemical, and biological barriers constitute the first line of innate immune defense. Together, they prevent microbial colonization, limit pathogen invasion, and maintain tissue homeostasis before the activation of cellular and adaptive immune responses.

1.2. Cellular Components

The cellular arm of innate immunity consists of a diverse group of immune cells that provide rapid, non-specific protection against invading pathogens. These cells recognize conserved microbial structures through pattern-recognition receptors (PRRs) and initiate immediate defense mechanisms, including phagocytosis, cytotoxicity, cytokine production, and antigen presentation. The principal cellular components of innate immunity include neutrophils, which are the first leukocytes recruited to sites of infection and are highly efficient phagocytes; monocytes and macrophages, which engulf and destroy microorganisms while producing inflammatory mediators; dendritic cells, which act as sentinel cells and bridge innate and adaptive immunity through antigen presentation; and natural killer (NK) cells, which eliminate virus-infected and transformed cells through cytotoxic mechanisms. Other important innate immune cells include mast cells, eosinophils, and basophils, which contribute to inflammatory responses, allergic reactions, and defense against parasites. Together, these cellular components coordinate the early immune response and play a crucial role in controlling infections before the activation of adaptive immunity **(Figure 10)**.

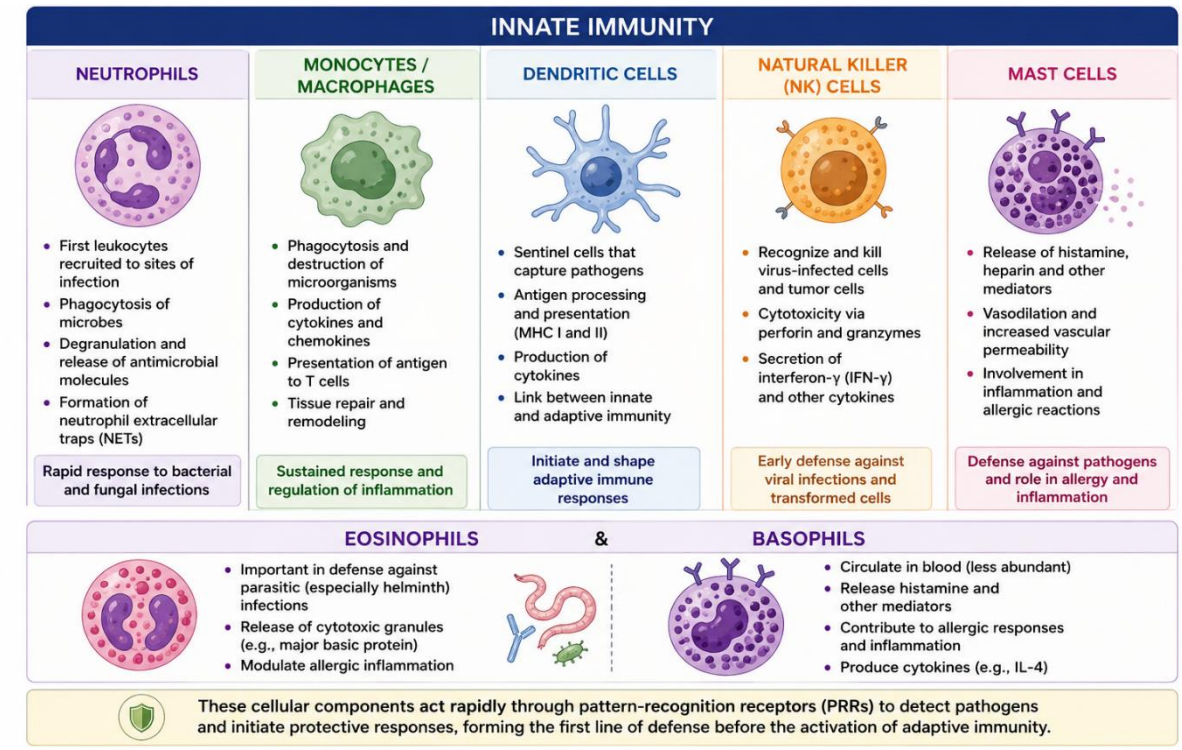


Figure 10. Major Cellular Components and Functions of the Innate Immune System.

1.3. Soluble mediators

Soluble mediators are essential components of innate immunity that coordinate the recognition, elimination, and containment of invading pathogens. These molecules are produced by immune cells, epithelial cells, endothelial cells, and other tissue-resident cells in response to infection or tissue damage. They contribute to the initiation and amplification of inflammation, recruitment of immune cells, pathogen destruction, and regulation of immune responses. The principal soluble mediators of innate immunity include the complement system, cytokines and chemokines, antimicrobial peptides and enzymes, and various inflammatory mediators such as histamine, prostaglandins, leukotrienes, and platelet-activating factor (PAF). Together, these molecules provide rapid protection against microbial invasion and help establish the conditions necessary for the development of adaptive immune responses.

1.3.1. Complement System

The complement system is a major component of innate immunity and consists of more than 30 plasma and membrane-associated proteins that work together to protect the host against infections. These proteins circulate in the blood in an inactive form and become activated through a cascade of enzymatic reactions when microorganisms invade the body. Complement

proteins are primarily synthesized by the liver, although several immune cells can also produce them locally at sites of infection.

Once activated, the complement system contributes to host defense through three principal mechanisms. First, it promotes opsonization, whereby complement fragments such as C3b coat the surface of pathogens, facilitating their recognition and phagocytosis by macrophages and neutrophils. Second, complement activation generates inflammatory mediators (anaphylatoxins), including C3a and C5a, which recruit leukocytes to sites of infection, increase vascular permeability, and amplify the inflammatory response. Third, the terminal components of the complement cascade assemble into the Membrane Attack Complex (MAC), which forms pores in the membranes of target cells, leading to their lysis and destruction. The complement system can be activated through three distinct pathways: the classical pathway, triggered mainly by antigen-antibody complexes; the lectin pathway, initiated by the recognition of microbial carbohydrates; and the alternative pathway, which is activated directly on microbial surfaces. Although these pathways differ in their initiation mechanisms, they converge at the activation of C3 and ultimately generate the same effector functions. Thus, the complement system serves as a critical link between innate and adaptive immunity, enhancing pathogen elimination and contributing to immune surveillance **(Figure 11)**.

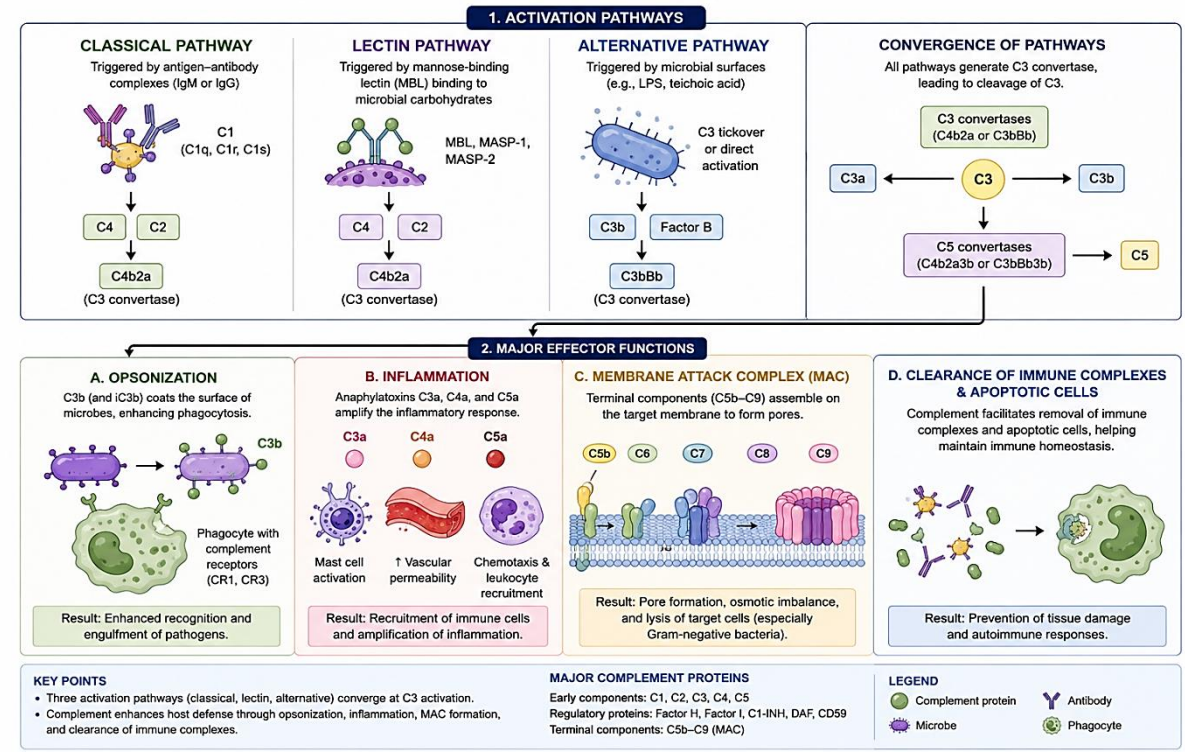


Figure 11. Complement system: activation pathways and effector functions.

1.3.2. Cytokines and Chemokines

Cytokines and chemokines are small soluble proteins that play essential roles in cell-to-cell communication during immune responses. They are produced by a wide variety of cells, including macrophages, dendritic cells, lymphocytes, endothelial cells, epithelial cells, and fibroblasts, in response to infection, inflammation, or tissue injury. These mediators regulate the activation, proliferation, differentiation, migration, and effector functions of immune cells, thereby coordinating both innate and adaptive immune responses. Cytokines can be broadly classified according to their biological functions. Pro-inflammatory cytokines, such as tumor necrosis factor (TNF), interleukin-1 (IL-1), and interleukin-6 (IL-6), promote inflammation and enhance host defense mechanisms. Antiviral cytokines, including interferon-α (IFN-α) and interferon-β (IFN-β), induce an antiviral state in infected and neighboring cells. Regulatory cytokines, such as interleukin-10 (IL-10) and transforming growth factor-β (TGF-β), limit excessive inflammation and contribute to immune homeostasis. Chemokines are a specialized family of cytokines that primarily regulate leukocyte trafficking. They create concentration gradients that direct immune cells toward sites of infection, inflammation, or tissue damage. A prominent example is CXCL8 (IL-8), which recruits neutrophils to inflammatory sites. Through their coordinated actions, cytokines and chemokines orchestrate the recruitment and activation of immune cells, ensuring an effective and controlled immune response.

1.3.3. Antimicrobial Peptides and Enzymes

Antimicrobial peptides and enzymes are important soluble components of innate immunity that provide rapid protection against invading microorganisms. They are produced by epithelial cells, neutrophils, macrophages, mast cells, and other immune cells, and are found in body secretions such as saliva, tears, mucus, sweat, and gastrointestinal fluids. These molecules act as a first line of defense by directly inhibiting microbial growth, disrupting pathogen membranes, or enhancing the activity of other immune mechanisms.

Among the most important antimicrobial peptides are defensins and cathelicidins, which possess broad-spectrum activity against bacteria, fungi, viruses, and certain parasites. These peptides disrupt microbial membranes, leading to pathogen death. Innate immunity also relies on several antimicrobial enzymes, including lysozyme, which degrades the peptidoglycan layer of bacterial cell walls, and myeloperoxidase (MPO), produced by neutrophils to generate reactive antimicrobial compounds. Other molecules, such as lactoferrin, contribute indirectly to antimicrobial defense by sequestering iron, an essential nutrient required for microbial

growth. Together, antimicrobial peptides and enzymes play a crucial role in limiting pathogen colonization and supporting the early phases of immune defense **(Figure 12)**.

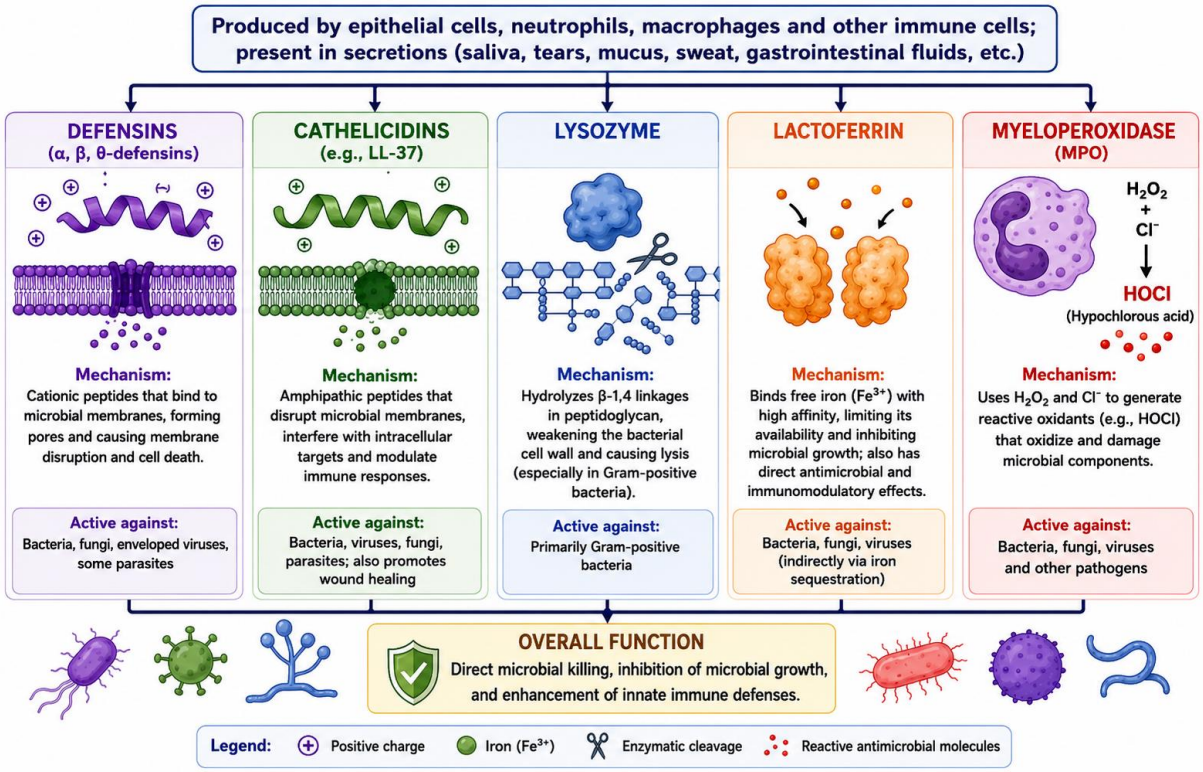


Figure 12: Major Antimicrobial Peptides and Enzymes of Innate Immunity and Their Mechanisms of Action.

1.3.4. Inflammatory Mediators

Inflammatory mediators are soluble molecules released by immune cells, endothelial cells, platelets, and damaged tissues in response to infection or injury. They play a central role in initiating, amplifying, and regulating the inflammatory response by promoting vasodilation, increasing vascular permeability, recruiting leukocytes, and facilitating pathogen elimination. These mediators ensure the rapid delivery of immune cells and plasma proteins to sites of infection or tissue damage.

Among the most important inflammatory mediators are histamine, prostaglandins, leukotrienes, and platelet-activating factor (PAF). Histamine, released primarily by mast cells and basophils, induces vasodilation and increases capillary permeability. Prostaglandins contribute to inflammation, fever, and pain, whereas leukotrienes promote leukocyte recruitment and bronchoconstriction. PAF is involved in platelet activation, leukocyte adhesion, and vascular responses. In addition, neuropeptides such as substance P contribute to pain perception and neurogenic inflammation. Together, these mediators coordinate the vascular and cellular events

required for an effective inflammatory response while also participating in tissue repair and immune regulation **(Figure 13)**.

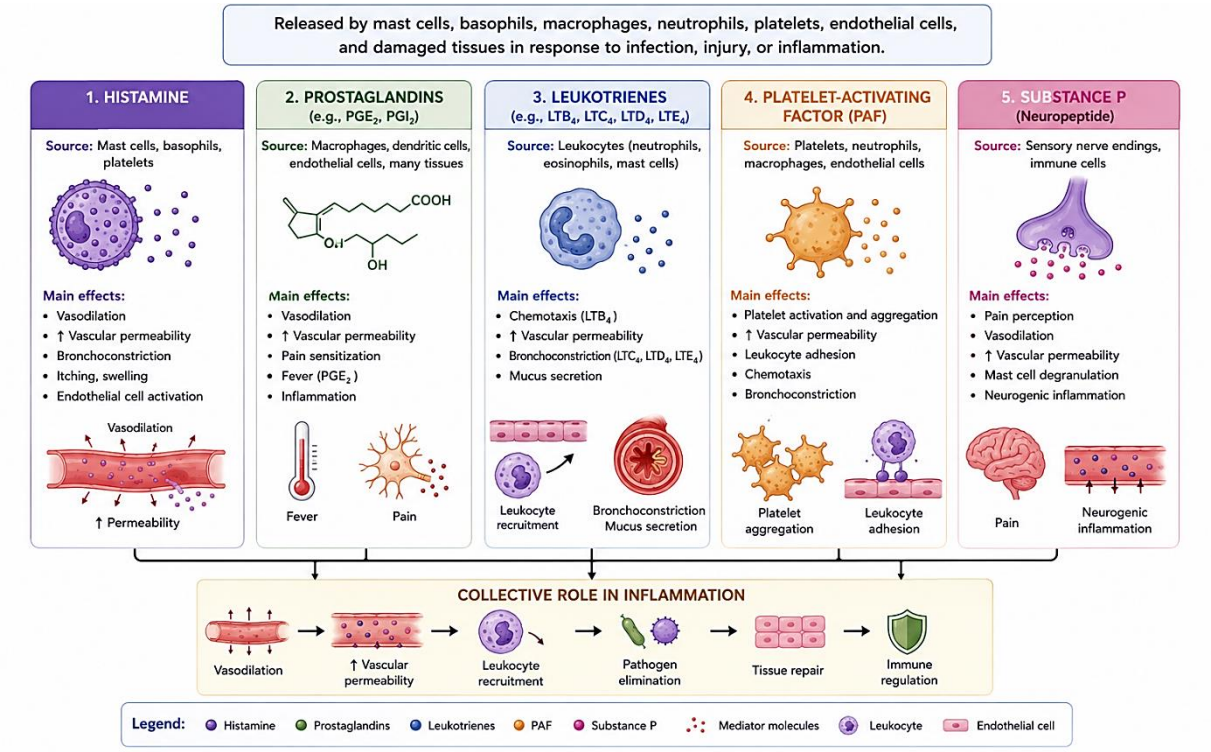


Figure 13. Major inflammatory mediators and their biological effects

1.4. Effector Mechanisms of Innate Immunity

The innate immune system employs several effector mechanisms that provide rapid protection against invading pathogens and tissue damage. These mechanisms are activated immediately after infection and act to eliminate microorganisms before the development of adaptive immune responses. The major effector mechanisms include inflammation, phagocytosis, natural killer (NK) cell-mediated cytotoxicity, complement activation, and antiviral responses induced by interferons. Inflammation promotes the recruitment of leukocytes and plasma proteins to sites of infection, facilitating pathogen destruction and tissue repair. Phagocytic cells, such as neutrophils and macrophages, engulf and eliminate microorganisms through intracellular killing mechanisms. NK cells recognize and destroy virus-infected and transformed cells by releasing cytotoxic granules containing perforin and granzymes. The complement system contributes through opsonization, inflammation, and direct lysis of target cells via the membrane attack complex (MAC). In addition, type I interferons (IFN-α and IFN-β) induce an antiviral state in infected and neighboring cells, limiting viral replication and spread. Together, these mechanisms provide an effective first line of defense against infection**.**

1.4. Link Between Innate and Adaptive Immunity

Although innate and adaptive immunity are often described as distinct systems, they function in close coordination. The innate immune response provides the first defense against pathogens and generates signals that initiate and shape adaptive immune responses. This connection is mediated primarily by dendritic cells, which serve as a bridge between the two arms of immunity.

Upon recognizing pathogens through pattern-recognition receptors (PRRs), dendritic cells undergo activation and migrate to secondary lymphoid organs. There, they process microbial antigens and present peptide fragments to naïve T lymphocytes through major histocompatibility complex (MHC) molecules. In addition to antigen presentation, dendritic cells express co-stimulatory molecules and secrete cytokines that influence T-cell activation and differentiation. Macrophages and B lymphocytes can also function as antigen-presenting cells under certain conditions. Through these interactions, innate immunity not only provides immediate protection but also directs the specificity, magnitude, and quality of adaptive immune responses, ensuring efficient long-term immunity and immunological memory (**Figure 14)**.

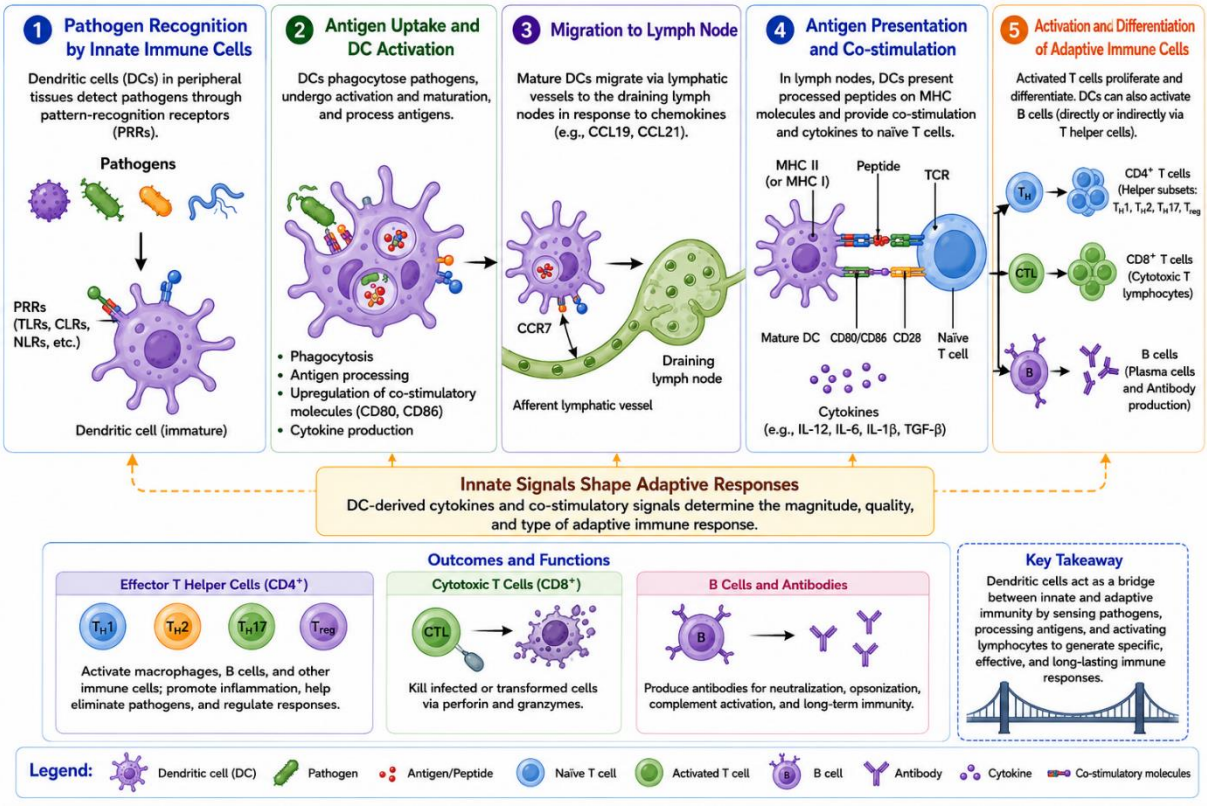


Figure 14. Dendritic Cells as the link between innate and adaptive immunity.

2. Adaptive Immunity

2.1. Specificity and Diversity

Adaptive immunity is characterized by its ability to recognize and respond specifically to an almost unlimited variety of antigens. This remarkable specificity is mediated by antigen receptors expressed on B and T lymphocytes, namely the B-cell receptor (BCR) and the T-cell receptor (TCR). Unlike innate immune receptors, which recognize conserved molecular patterns, BCRs and TCRs are highly variable and can distinguish subtle differences between antigens.

The extraordinary diversity of adaptive immunity results from the random rearrangement of gene segments encoding antigen receptors during lymphocyte development, a process known as V(D)J recombination. Additional diversity is generated through junctional modifications and, in B lymphocytes, through somatic hypermutation. Together, these mechanisms enable the immune system to generate millions of distinct antigen receptors, ensuring effective recognition of a vast array of pathogens and foreign substances.

2.2. Humoral and Cell-Mediated Responses

Adaptive immunity operates through two complementary mechanisms: humoral immunity and cell-mediated immunity. Humoral immunity is mediated by B lymphocytes, which differentiate into plasma cells that produce antibodies. These antibodies circulate in blood and body fluids, where they neutralize pathogens and toxins, promote phagocytosis, and activate the complement system. Humoral immunity is particularly effective against extracellular microorganisms and their products.

Cell-mediated immunity is mediated primarily by T lymphocytes. $CD4^+$ helper T cells coordinate immune responses by secreting cytokines that activate and regulate other immune cells, whereas $CD8^+$ cytotoxic T cells directly eliminate virus-infected and transformed cells. Cell-mediated immunity is especially important for the control of intracellular pathogens, including viruses and certain bacteria and parasites. Together, humoral and cell-mediated responses provide comprehensive protection against a wide range of infectious agents **(Figure 15)**.

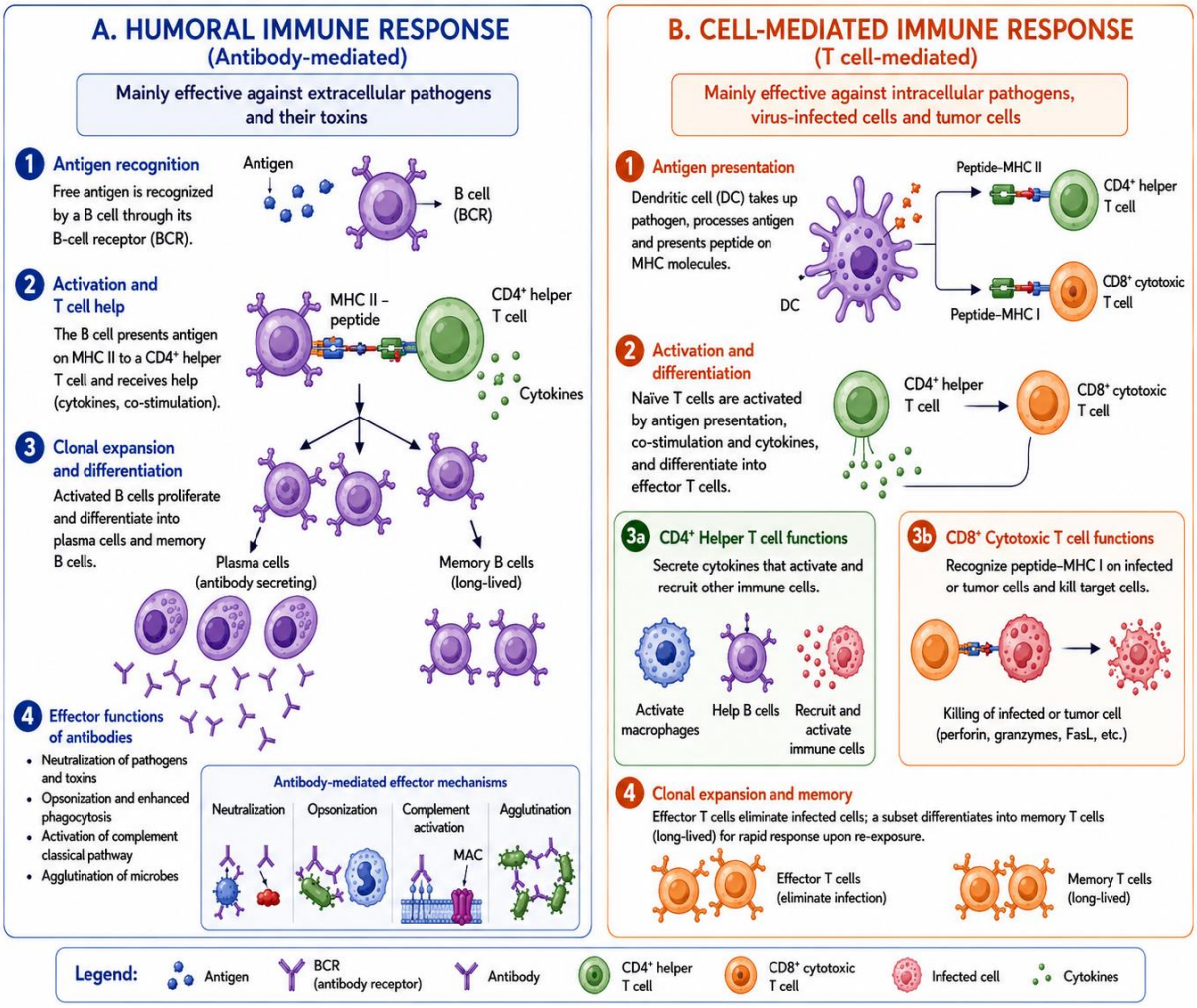


Figure 15. Humoral and cell-mediated adaptive immune responses.

2.3. Immunological Memory

One of the defining characteristics of adaptive immunity is its ability to generate immunological memory. Following primary exposure to an antigen, a fraction of activated B and T lymphocytes differentiates into long-lived memory cells. These cells persist for years or even decades after the initial infection has been eliminated.

Upon subsequent exposure to the same antigen, memory lymphocytes respond more rapidly and more effectively than naïve lymphocytes. Memory B cells rapidly differentiate into antibody-secreting plasma cells, while memory T cells quickly acquire effector functions. As a result, secondary immune responses are faster, stronger, and often more protective than primary responses. Immunological memory forms the biological basis of vaccination and provides long-term protection against previously encountered pathogens **(Figure 16)**.

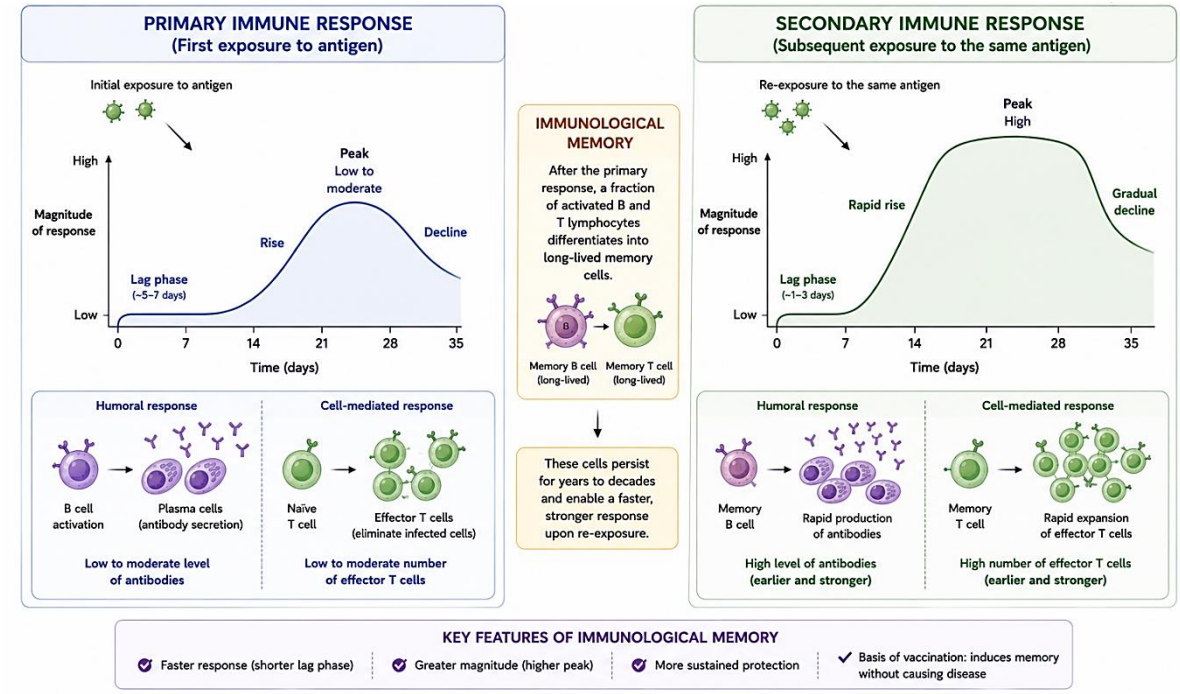


Figure 16. Primary and secondary immune responses and the generation of immunological memory.

Chapter 3. Inflammatory Reactions

Inflammation is the protective response of living, vascularized tissues to injury, infection, or other harmful stimuli. It represents one of the major mechanisms of innate immunity and constitutes an essential defense process aimed at eliminating the cause of tissue damage, removing injured cells, and initiating tissue repair. Tissues lacking blood vessels, such as cartilage and the cornea, are unable to develop a complete inflammatory response because inflammation depends on the vascular system.

Connective tissues play a central role in inflammation because they contain the microcirculation, resident macrophages, extracellular matrix components, and fibroblasts necessary for tissue repair and healing. Epithelial tissues, on the other hand, primarily function as protective barriers against the external environment, although they are often the first structures affected during inflammatory processes. Following injury, restoration of tissue integrity depends on regenerative mechanisms, which vary considerably among organs and may be limited in highly specialized tissues such as the nervous system.

Inflammation may be triggered by a wide variety of stimuli, including physical agents such as heat, cold, trauma, and ionizing radiation; chemical agents such as acids, alkalis, and bacterial toxins; infectious microorganisms including bacteria, viruses, fungi, and parasites; immune reactions such as allergies and hypersensitivity responses; and tissue necrosis resulting from ischemia or other pathological conditions.

One of the earliest events during inflammation is the recruitment of leukocytes from the bloodstream to the site of injury. Among these cells, neutrophils are the first to arrive and constitute the predominant cellular population during acute inflammation. Their migration from blood vessels into tissues, known as extravasation, occurs through a series of coordinated steps involving adhesion to vascular endothelial cells, passage through the endothelial junctions, and migration across the basement membrane into the affected tissue.

The accumulation of neutrophils at inflammatory sites is directed by chemotactic factors released during the inflammatory response. These include complement fragments, coagulation products, cytokines, and chemokines produced by activated macrophages and lymphocytes. Once they reach the site of infection or injury, neutrophils eliminate microorganisms through phagocytosis and the release of antimicrobial substances contained within their cytoplasmic granules.

Neutrophils possess two major types of granules. Primary granules, also known as azurophilic granules, contain lysosomal enzymes, myeloperoxidase, lysozyme, and various hydrolytic enzymes. Secondary granules contain collagenase, lactoferrin, lysozyme, and additional antimicrobial proteins. Following phagocytosis, these granules fuse with phagosomes, leading to the destruction and digestion of engulfed microorganisms. Through these mechanisms, neutrophils play a fundamental role in the early control of infection and the initiation of tissue repair.

1. Stages of Inflammation

The inflammatory response is a dynamic and coordinated process that develops through several successive stages. These stages include the vascular and exudative reaction, the cellular reaction, the removal of damaged tissue and debris, and finally tissue repair and healing.

1.1. Vascular and Exudative Reaction

The vascular and exudative phase is responsible for the four classical cardinal signs of acute inflammation: redness (*rubor*), heat (*calor*), swelling (*tumor*), and pain (*dolor*). These manifestations result from vascular changes occurring shortly after tissue injury.

1.1.1. Active Congestion

The earliest vascular event is arteriolar and capillary vasodilation within the affected area. This vasodilation increases local blood flow and slows the rate of blood circulation. Active congestion is rapidly induced through neural mechanisms involving vasomotor nerves and by chemical mediators released at the site of injury. The increased blood supply accounts for the redness and warmth characteristic of inflamed tissues.

1.1.2. Inflammatory Edema

Inflammatory edema results from the leakage of fluid rich in plasma proteins, known as an exudate, from the circulation into the interstitial connective tissue or serous cavities. Clinically, edema appears as tissue swelling and contributes significantly to pain by compressing local nerve endings.

The formation of inflammatory edema is caused by both increased hydrostatic pressure resulting from vasodilation and increased vascular permeability induced by inflammatory mediators such as histamine, bradykinin, and cytokines. This exudate carries antibodies,

complement proteins, and coagulation factors to the site of injury. In addition, it dilutes toxins, helps localize infectious agents through fibrin deposition, and promotes leukocyte migration by slowing blood flow within the microcirculation.

1.1.3. Leukocyte Diapedesis

Leukocyte diapedesis initially involves neutrophils, which predominate during the first 6 to 24 hours of acute inflammation, followed by monocytes and lymphocytes, which become more abundant between 24 and 48 hours. Diapedesis is an active process by which leukocytes leave the bloodstream and migrate into injured tissues. This process occurs in several sequential steps. First, leukocytes move toward the endothelial surface, a phenomenon known as margination, which is facilitated by the slowing of blood flow within inflamed vessels. Second, leukocytes adhere firmly to endothelial cells through interactions between adhesion molecules expressed on both leukocytes and the vascular endothelium. Finally, leukocytes cross the endothelial barrier by extending pseudopodia between adjacent endothelial cells. They subsequently penetrate the basement membrane through localized enzymatic degradation, allowing their migration into the surrounding tissues **(Figure 17).**

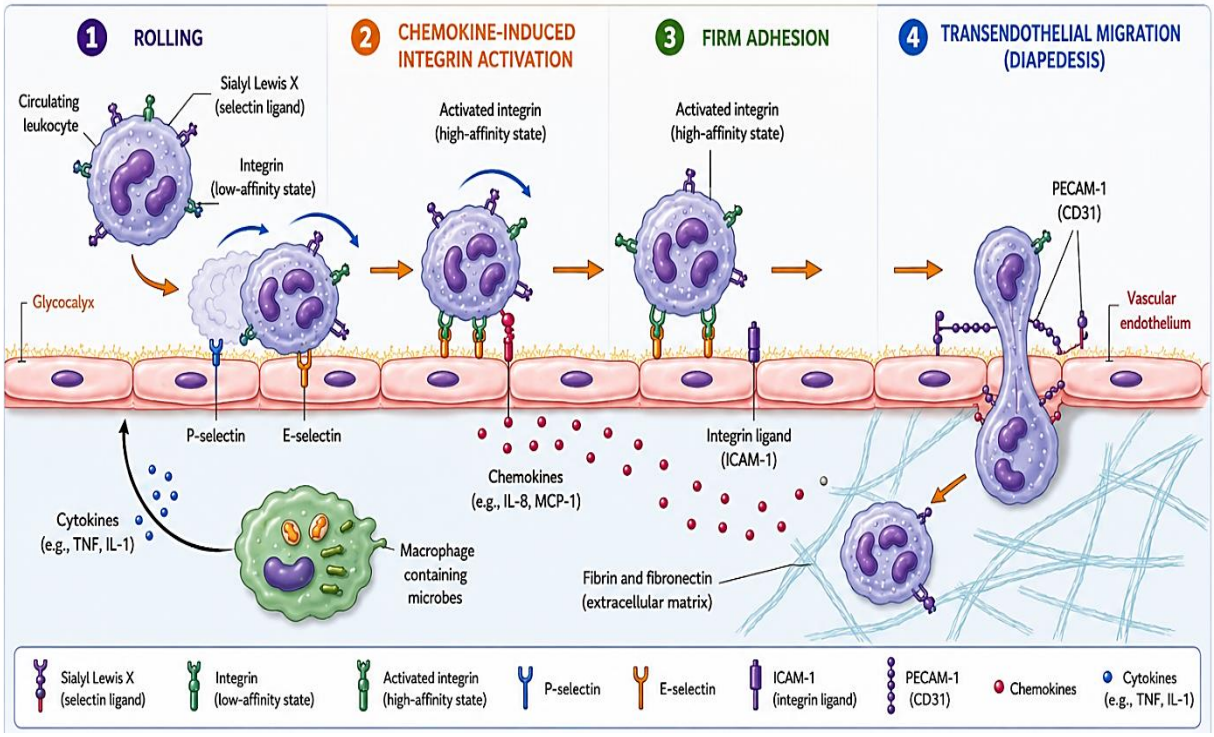


Figure 17. Molecular mechanisms of leukocyte recruitment and transendothelial migration during inflammation

1.2. Cellular Reactions

The cellular phase of inflammation is characterized by the accumulation and activation of cells originating either from the bloodstream or from resident tissue populations **(Figure 18)**.

1.2.1. Blood-Derived Cells

Neutrophils are the first inflammatory cells to arrive at the site of injury and are particularly abundant during the early hours of acute inflammation. Their numbers typically decline after approximately two days. Monocytes subsequently migrate into the affected tissue, where they differentiate into macrophages and become the predominant inflammatory cells. Lymphocytic infiltrates are generally associated with subacute and chronic inflammatory responses.

Following diapedesis, leukocytes leave the perivascular region and migrate toward the site of tissue damage through a process known as chemotaxis. Chemotactic factors released by injured tissues, invading microorganisms, and previously recruited leukocytes bind to specific receptors on leukocyte membranes. This interaction activates the cytoskeleton and promotes directed cell migration toward the inflammatory focus.

1.2.2. Tissue-Resident Cells

Several resident cells also participate actively in inflammatory reactions, including fibroblasts, endothelial cells, mast cells, and resident macrophages. Certain chronic inflammatory conditions, known as granulomatous inflammations, are characterized by the presence of epithelioid cells and multinucleated giant cells. Epithelioid cells are activated macrophages with reduced phagocytic activity and abundant endoplasmic reticulum. Giant cells arise either through the fusion of epithelioid cells or, less frequently, through repeated nuclear division without cytoplasmic division. The composition of granulation tissue changes over time. Neutrophils represent the hallmark of acute inflammation; however, after several days or weeks, the inflammatory infiltrate becomes dominated by mononuclear cells, particularly macrophages, lymphocytes, and plasma cells. As healing progresses, growth factors stimulate the proliferation of fibroblasts and endothelial cells, leading to the formation of new blood vessels and connective tissue. At this stage, the granulation tissue is often referred to as vascular granulation tissue. The cellular composition of the inflammatory infiltrate may also vary according to the underlying cause of inflammation, with certain cell populations predominating depending on the nature of the stimulus.

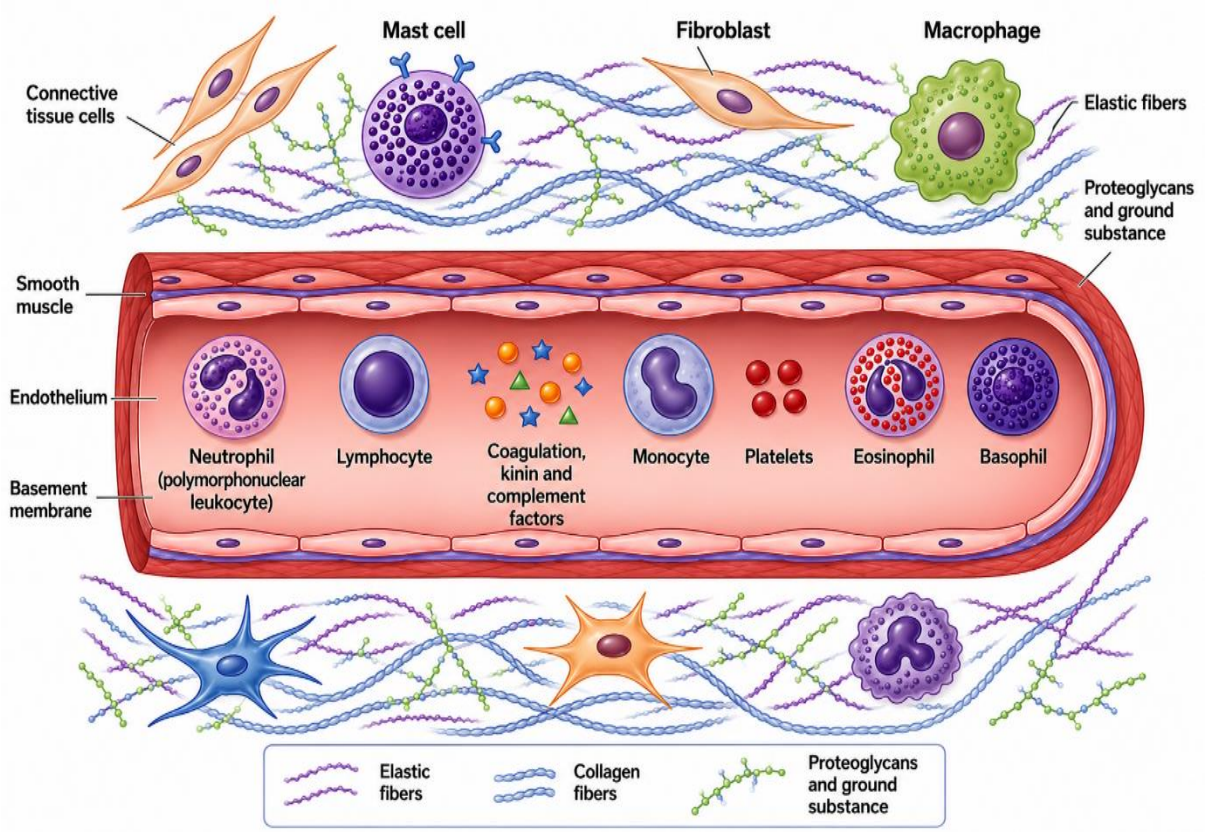


Figure 18. Cellular components involved in the inflammatory response

1.3. Debridement

Debridement is an essential step in tissue repair and healing. It consists of the removal of foreign materials, microorganisms, and necrotic tissue present within the inflammatory focus. Debridement is considered internal when it is entirely carried out by macrophages through phagocytosis and intracellular degradation. It is considered external when the inflammatory products are discharged through the skin or a natural body passage. For example, a subcutaneous abscess may drain through the skin, whereas a pulmonary abscess may empty into a bronchus.

In some situations, debridement occurs indirectly when the inflammatory focus is located far from the skin surface or a natural cavity. In such cases, a newly formed channel known as a fistula develops, connecting the inflammatory site to the external environment. Surgical debridement is a therapeutic procedure aimed at removing necrotic tissue and inflammatory debris in order to accelerate or facilitate healing.

1.4. Tissue Repair

Tissue repair occurs through two principal mechanisms: scar formation and regeneration. Scar formation results in the replacement of damaged tissue by newly formed connective tissue. Although this process restores tissue integrity, it may impair organ function because fibrous tissue replaces specialized functional parenchyma. The early phase of scar formation is characterized by intense angiogenesis, leading to the development of highly vascularized granulation tissue.

The second mechanism of repair is regeneration. When tissue destruction is limited and surviving cells retain proliferative capacity, damaged tissues may regenerate and recover their original structure and function. The regenerative potential varies among tissues. Historically, post-mitotic tissues such as nervous tissue were considered incapable of regeneration. However, the discovery of pluripotent stem cells within the central nervous system suggests that a limited degree of neural regeneration may be possible under certain conditions.

1.5. Molecules Involved in Inflammation

Inflammatory cells accumulate precisely at sites of injury through complex interactions involving adhesion molecules, chemotactic factors, and soluble mediators. Endothelial cells at the inflammatory focus express adhesion molecules that facilitate leukocyte recruitment and retention. The initiation, amplification, and propagation of inflammation depend on mediators that are either synthesized locally or circulate as inactive precursors and become activated during the inflammatory response.

1.5.1. Locally Produced Mediators

Among the earliest inflammatory mediators are vasoactive amines, particularly histamine and serotonin. These preformed mediators are stored within mast cells, basophils, and platelets and are rapidly released following tissue injury. Their principal effects include vasodilation and increased vascular permeability, which contribute to active congestion and inflammatory edema. Other important mediators are the eicosanoids, including prostaglandins and leukotrienes, which are synthesized from arachidonic acid released from cellular membranes. These newly formed mediators exert both local and systemic effects. Locally, they promote vasodilation, pain, vascular permeability, and leukocyte recruitment. Systemically, they contribute to fever and other manifestations of inflammation. Cytokines constitute another major group of inflammatory mediators. These small proteins are produced by numerous cell

types, particularly T lymphocytes, monocytes, and macrophages. Cytokines act through specific membrane receptors and may exert autocrine, paracrine, or endocrine effects. Some cytokines, such as IL-1, IL-6, and TNF-α, are predominantly pro-inflammatory, whereas others, including IL-4, IL-10, and IL-13, exert anti-inflammatory functions **(Figure 19)**.

a. Mediation of Innate Immunity

Certain cytokines contribute primarily to innate immune defense. Among these, interferons induce antiviral states in infected and neighboring cells, thereby limiting viral replication and spread. Their action constitutes a key component of the early, non-specific immune response against viral infections.

b. Regulation of Lymphocyte Activation, Growth, and Differentiation

Cytokines play a central role in determining the nature of adaptive immune responses. Infections caused by intracellular pathogens, such as viruses, mycobacteria, and certain parasites, predominantly stimulate cell-mediated immunity. In contrast, extracellular bacteria and parasites generally induce strong antibody-mediated responses. The polarization of immune responses depends largely on cytokines produced by helper T lymphocytes. Cytokines associated with Th1 responses promote cellular immunity, whereas Th2 cytokines favor B-cell activation and antibody production.

c. Stimulation of Hematopoiesis

Several cytokines, collectively known as colony-stimulating factors (CSFs), regulate hematopoiesis by stimulating the proliferation and differentiation of hematopoietic progenitor cells. These factors promote the production of specific blood cell lineages, including granulocytes, macrophages, and megakaryocytes, thereby ensuring an adequate supply of immune and inflammatory cells during host defense responses.

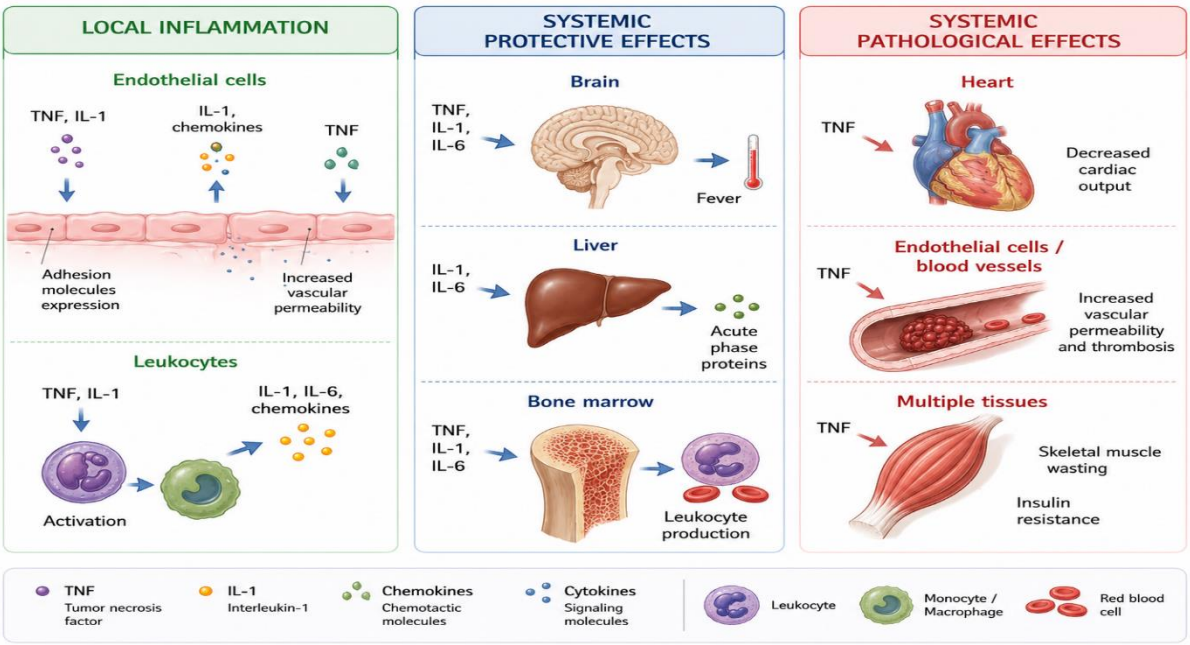


Figure 19. Local and systemic actions of pro-inflammatory cytokines during inflammation.

The major pro-inflammatory cytokines TNF-α, IL-1, and IL-6 exert both local and systemic effects. Locally, they activate endothelial cells, increase vascular permeability, induce the expression of adhesion molecules, and promote leukocyte recruitment. Systemically, they stimulate fever through actions on the hypothalamus, induce acute-phase protein synthesis by the liver, and enhance leukocyte production in the bone marrow. Excessive cytokine production may also lead to pathological effects, including vascular leakage, thrombosis, cardiac dysfunction, insulin resistance, and septic shock.

1.5.2. Circulating Plasma-Derived) Mediators

Circulating inflammatory mediators are present in the bloodstream as inactive precursors and become activated through enzymatic cascade reactions that tightly regulate their production and activity. These mediators play essential roles in amplifying and coordinating inflammatory responses.

The kinin system originates from kininogen, which is cleaved by kallikrein. Kallikrein itself is generated from circulating prekallikrein through proteolytic activation. One of the molecules responsible for this activation is activated Factor XII (Hageman factor). Kinins are potent vasoactive mediators that induce vasodilation and increase vascular permeability. Among them, bradykinin is particularly important because it is a major mediator of inflammatory pain.

Another major plasma-derived inflammatory system is the complement system, which consists of a group of serum proteins activated through sequential proteolytic reactions. Activation of

complement generates several biologically active fragments that contribute to host defense and inflammation.

a. C3a: Anaphylatoxin

C3a is an anaphylatoxin that stimulates the degranulation of mast cells and basophils. The release of histamine and other vasoactive mediators increases vascular permeability and promotes inflammation. Excessive activation may contribute to severe hypersensitivity reactions, including anaphylactic shock.

b. C3b: Opsonin

C3b functions as a powerful opsonin. It binds to the surface of microorganisms, particularly bacteria, facilitating their recognition and engulfment by phagocytic cells such as neutrophils and macrophages. This process, known as opsonization, significantly enhances phagocytosis and microbial clearance.

c. C5b–C9: Membrane Attack Complex (MAC)

The terminal complement components C5b, C6, C7, C8, and C9 assemble to form the Membrane Attack Complex (MAC). This complex inserts into the membrane of susceptible microorganisms, creating transmembrane pores that disrupt cellular integrity and ultimately lead to bacterial lysis.

Together, the kinin and complement systems represent major plasma-derived effector mechanisms that contribute to vasodilation, vascular permeability, leukocyte recruitment, pathogen elimination, and amplification of the inflammatory response.

Chapter 4. Structure, Functions, and Signaling of Toll-Like Receptors (TLRs)

1. Molecular Structures Recognized by the Innate Immune System

1.1. The Emergence of the Concept of Molecular Patterns Recognized by Innate Immunity

The receptors of the innate immune system and the molecular structures recognized by these receptors were identified much later than the receptors of adaptive immunity. Antibodies, for example, were recognized at the beginning of the twentieth century and are capable of recognizing an almost unlimited variety of molecular structures. Owing to their remarkable specificity, antibodies have become indispensable experimental and therapeutic tools. However, under certain circumstances, antibodies may also recognize self-components, raising important questions regarding the regulation and tolerance of adaptive immunity.

In 1949, Frank Burnet introduced the concept of **"self versus non-self"**, proposing that foreign elements constituting the "non-self" could trigger adaptive immune responses, whereas endogenous components of the "self" would be tolerated and would not induce immune activation. Several mechanisms of immune tolerance have since been identified, some of which involve interactions between the innate and adaptive immune systems.

In 1989, Charles Janeway proposed that the discrimination between self and non-self is largely mediated by the innate immune system. He suggested the existence of specialized receptors, termed Pattern Recognition Receptors (PRRs), capable of recognizing conserved molecular structures expressed by microorganisms. These microbial structures were initially designated Pathogen-Associated Molecular Patterns (PAMPs). Subsequent studies demonstrated that these molecular motifs are not restricted to pathogenic microorganisms but are present in a broad range of microbes, including non-pathogenic species. Consequently, the term Microbe-Associated Molecular Patterns (MAMPs) is now considered more appropriate than PAMPs. Examples of MAMPs include bacterial lipopolysaccharides (LPS), peptidoglycans, flagellin, unmethylated CpG DNA, and viral double-stranded RNA.

In 1994, Polly Matzinger introduced the "danger model", proposing that the immune system responds not only to microbial products but also to endogenous molecules released by stressed, injured, or dying cells. These host-derived molecules were termed Damage-Associated Molecular Patterns (DAMPs), also known as alarmins. Examples include HMGB1, ATP, uric acid crystals, heat-shock proteins, and DNA released from damaged cells.

The danger model provided an explanation for why immune responses induced by sterile tissue injury often resemble those triggered by infection. Indeed, both MAMPs and DAMPs can be recognized by the same pattern-recognition receptors and activate similar intracellular signaling pathways, leading to inflammation and immune activation. Importantly, the detection of MAMPs and DAMPs by PRRs represents the first step in the initiation of virtually all immune responses. Through this recognition process, innate immunity serves as the primary sensor of danger and infection, while simultaneously orchestrating the activation of adaptive immune responses **(Figure 20)**.

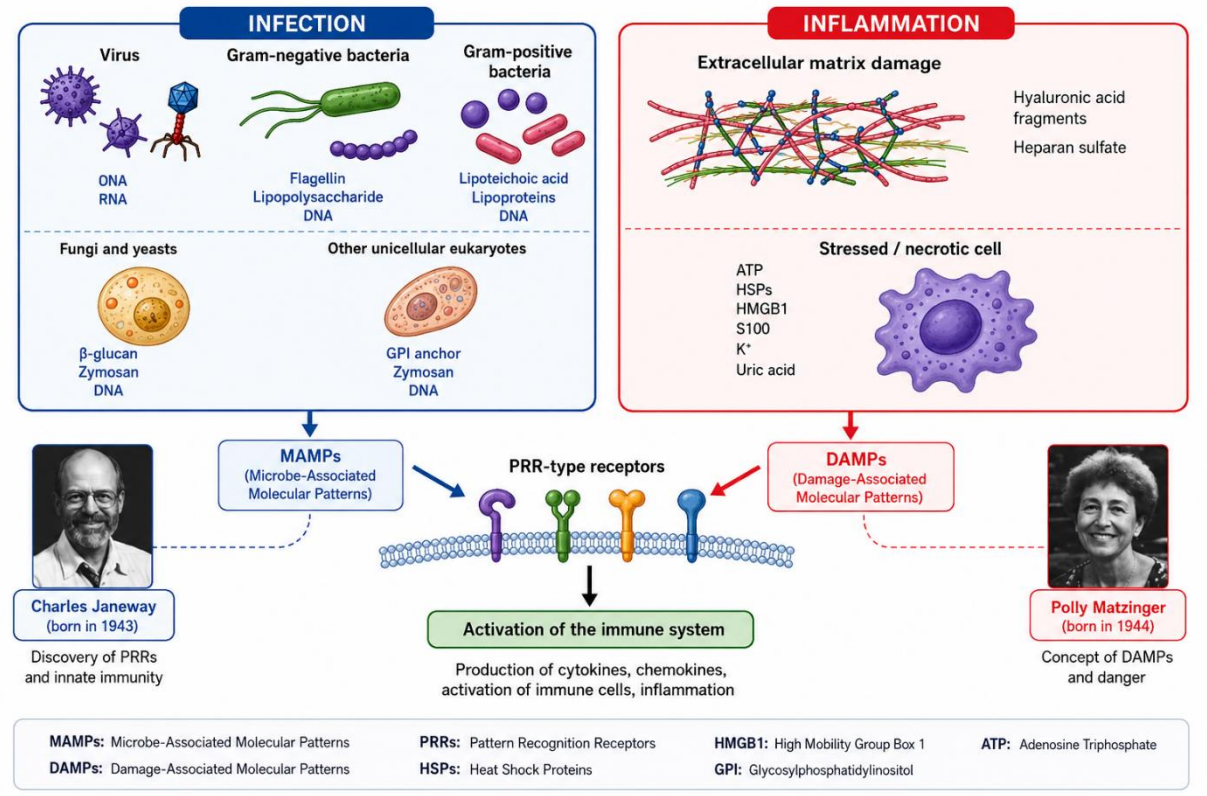


Figure 20. Differences between MAMPs and DAMPs

1.2. Microbe-Associated Molecular Patterns (MAMPs)

Many MAMPs correspond to structures located on the surface of microorganisms. Bacterial lipopolysaccharides (LPS), formerly known as endotoxins, are found on the outer membrane of Gram-negative bacteria. Other MAMPs include bacterial flagellin, lipoteichoic acid, and peptidoglycans. In unicellular fungi such as yeasts and molds, important MAMPs include β-glucans and α-mannans. Other unicellular eukaryotes express glycosylphosphatidylinositol (GPI)-anchored proteins that can also be recognized by the innate immune system. When microorganisms are degraded, for example during phagocytosis, intracellular components

become exposed and can also function as MAMPs. Microbial nucleic acids are particularly important targets. The DNA of bacteria, unicellular eukaryotes, and some viruses contains unmethylated CpG motifs that are much less methylated than those found in mammalian DNA. These unmethylated CpG sequences constitute important MAMPs that enable the immune system to detect foreign DNA. Recognition of unmethylated CpG DNA and endosomal single-stranded RNA is crucial for antiviral immunity. In addition, viruses can be detected through the presence of double-stranded RNA or cytosolic DNA generated during their replication cycle.

1.3. Damage-Associated Molecular Patterns (DAMPs or Alarmins)

Certain molecules normally confined within cells may be released into the extracellular environment either passively from dying cells or actively in response to cellular stress. Once outside the cell, molecules such as heat-shock proteins (HSPs), HMGB1 (High Mobility Group Box 1), S100 proteins, nucleic acids, ATP, and uric acid become DAMPs that can be recognized by pattern-recognition receptors. Tissue injury may also generate extracellular matrix-derived DAMPs, including hyaluronic acid fragments and heparan sulfate. Furthermore, disruption of plasma membrane integrity, such as that induced by bacterial toxins, can lead to the release of intracellular ions. Potassium efflux is therefore considered another form of DAMP and can be detected by intracellular PRRs.

2. Pattern Recognition Receptors (PRRs): The Immunoreceptors of Innate Immunity

In his 1989 theory, Charles Janeway proposed that microorganisms are detected by specialized receptors known as Pattern Recognition Receptors (PRRs), even before the first PRRs were experimentally identified. In 1996, the research group led by Jules Hoffmann demonstrated that the Toll signaling pathway, originally described in Drosophila embryonic development, also played a role in antifungal immunity. Subsequently, Ruslan Medzhitov and Charles Janeway showed that a human homolog of Toll was involved in initiating adaptive immune responses. Further studies by Paul Godowski and Bruce Beutler established that human Toll homologs recognize microbial products. These discoveries led to the identification of the Toll-Like Receptor (TLR) family, one of the most important classes of PRRs in mammals. Since then, additional PRR families have been identified. PRRs are highly conserved throughout evolution and are found in nearly all eukaryotic organisms.

In vertebrates, PRRs play essential roles in inflammation and in the initiation of adaptive immunity, particularly through the activation and maturation of dendritic cells.

PRRs can be divided into three major categories: soluble PRRs (opsonins), endocytic receptors, and signaling receptors. They may be membrane-bound, cytosolic, or extracellular.

Scavenger Receptors (SRs) are PRRs that promote the phagocytosis of apoptotic cells and microorganisms. Their signaling primarily regulates actin cytoskeleton remodeling and target internalization. Most other PRRs function as signaling receptors, transmitting information to neighboring or distant cells. Some TLRs are expressed on the plasma membrane, where they recognize structures present on intact microorganisms, whereas others are located within endosomal membranes and recognize degradation products such as microbial nucleic acids. C-type Lectin Receptors (CLRs) recognize carbohydrate structures predominantly expressed on fungi. Intracellular bacteria and viruses are detected by cytosolic PRRs. Nod-Like Receptors (NLRs) recognize both bacterial MAMPs and endogenous DAMPs. Viral nucleic acids are detected by several classes of cytosolic receptors, including Protein Kinase R (PKR), Oligoadenylate Synthetase (OAS), RIG-I-Like Receptors (RLRs), and cytosolic DNA sensors such as cGAS, IFI16, and AIM2. In the extracellular environment, soluble PRRs such as C-reactive protein (CRP) and Mannose-Binding Protein (MBP) contribute to pathogen recognition. MBP is notably involved in activating the complement system through the lectin pathway. Recognition of a PAMP by a PRR may trigger phagocytosis and activate intracellular signaling pathways that initiate antimicrobial and inflammatory responses.

2.1. Toll-Like Receptors (TLRs)

Toll-Like Receptors (TLRs) are proteins homologous to the Drosophila Toll receptor, which protects fruit flies against infection. TLRs recognize conserved molecular structures repeatedly expressed on microorganisms. In humans, ten functional Toll-like receptors (TLR1–TLR10) have been identified. TLR11 is functional in several animal species, particularly mice, but is not functional in humans. Some are located on the cell membrane (TLR1, TLR2, TLR4, TLR5, TLR6, TLR10, and TLR11), whereas others are found within intracellular endosomal compartments (TLR3, TLR7, TLR8, and TLR9).

2.1.1. Localization

TLRs are expressed by numerous immune cells, including macrophages, dendritic cells, B and T lymphocytes, neutrophils, natural killer (NK) cells, monocytes, and eosinophils. They are also found on non-immune cells such as fibroblasts, synoviocytes, keratinocytes, and epithelial cells lining the intestinal, respiratory, and urogenital tracts. These receptors recognize conserved microbial structures known as Pathogen-Associated Molecular Patterns (PAMPs). PAMPs possess three defining characteristics: they are absent from host cells, shared by many microbial species, and essential for microbial survival, limiting the emergence of escape mutants. TLR1, TLR2, TLR4, TLR5, TLR6, TLR10, and TLR11 are located on the plasma membrane, whereas TLR3, TLR7, TLR8, and TLR9 are localized within endosomes.

2.1.2. Structure

TLRs are type I transmembrane glycoproteins characterized by an extracellular domain containing multiple Leucine-Rich Repeats (LRRs) flanked by cysteine-rich motifs. This N-terminal region contains between 16 and 28 LRRs responsible for ligand recognition and binding. Each LRR consists of approximately 20–30 amino acids and includes the conserved sequence LxxLxLxxN, where L represents leucine, x any amino acid, and N asparagine. The intracellular C-terminal region is approximately 150 amino acids long and shares strong homology with members of the Interleukin-1 Receptor (IL-1R) family. This conserved intracellular region is known as the TIR domain (Toll/IL-1 Receptor/Resistance domain). TIR domains are also found in several plant resistance proteins and in the cytoplasmic tails of the IL-18 receptor, highlighting their evolutionary conservation and central role in innate immune signalling **(Figure 21)**.

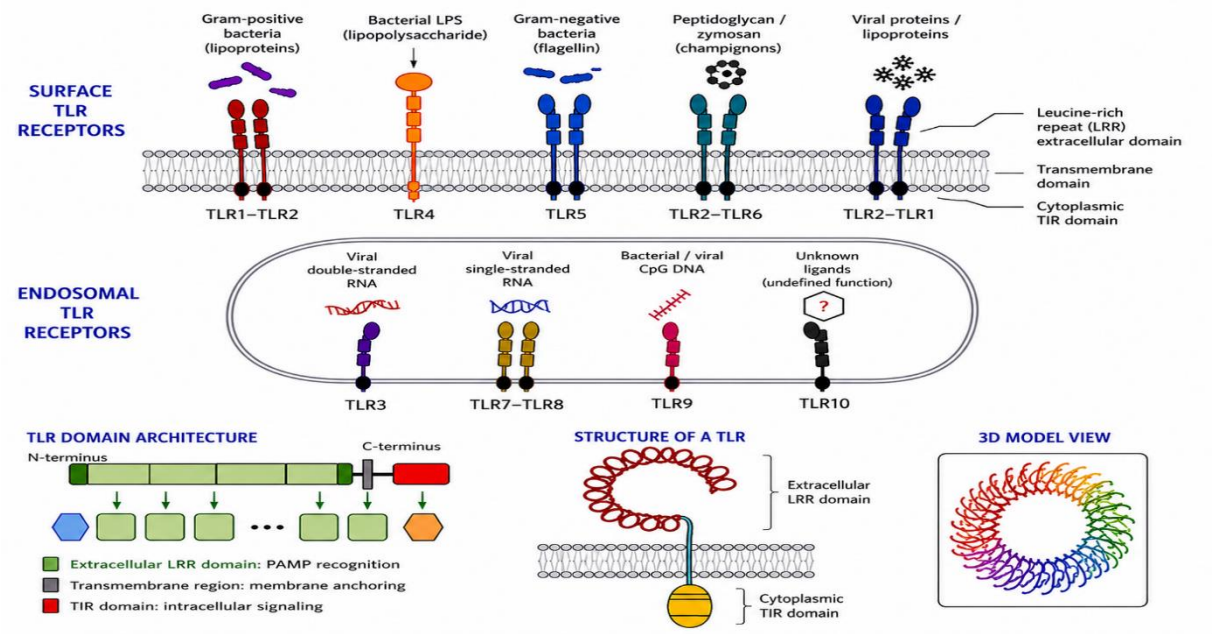


Figure 21. Localization, ligand recognition, and structural organization of human toll-like receptors (TLRs).

2.1.3. Ligands

TLR activation is initiated through the binding of microbial molecules known as Pathogen-Associated Molecular Patterns (PAMPs). Typical examples of PAMPs include lipopolysaccharides (LPS) from Gram-negative bacteria, peptidoglycans from Gram-positive bacteria, viral double-stranded RNA (dsRNA), and unmethylated CpG DNA motifs that are characteristic of bacterial genomes and certain DNA viruses. The discovery of endogenous molecules that resemble PAMPs, known as Damage-Associated Molecular Patterns (DAMPs), has provided important insights into the involvement of TLRs in the pathophysiology of various inflammatory diseases. DAMPs are endogenous molecules released during tissue injury, cellular stress, hemorrhagic shock, ischemia, or cell death. Examples include High-Mobility Group Box 1 (HMGB1), Heat Shock Proteins (HSPs), extracellular DNA and RNA, hyaluronan fragments, heparan sulfate, fibronectin, and fibrinogen.

The various endogenous and exogenous ligands recognized by TLRs are summarized in **Table 3.** Engagement of TLRs by their ligands initiates a cascade of molecular events that ultimately results in the production of cytokines and other mediators capable of stimulating both innate and adaptive immune responses, including interferons, TNF-α, and several interleukins such as IL-1, IL-6, and IL-12.

Table 3. Distribution, localization, and ligand specificity of human toll-like receptors (TLRs)

TLR	Localisation	PAMP (Pathogen-Associated Molecular Patterns)	DAMPs (Damage-Associated Molecular Patterns)
TLR1	Membrane cellulaire	• Lipopeptides des mycobactéries	• β-défensines humaines
TLR2	Membrane cellulaire	• Lipoprotéines des bactéries • Peptidoglycanes de bactéries Gram+ • Acide lipotéichoïque des streptocoques B • Porines de *Neisseria* • Lipoarabinomannane des mycobactéries • Zymozan des *Saccharomyces cerevisiae* • Phospholipomannane de *Candida albicans* • Glucuronoxylomannane de *Cryptococcus neoformans* • Mutin-type transmembrane glycoprotein des trypanosomes • Hémagglutinines du virus de la rougeole	• Protéine du choc thermique (HSP) • HMGB1
TLR3	Endosome	• Polyinosinic-polycytidylic acid (poly IC)	• ARNm • HSP • HMGB1 • Fragments de hyaluronane
TLR4	Membrane cellulaire	• Lipopolysaccharides (LPS) bactériens • Mannanes des champignons • Glycoinositolphospholipides des parasites • Protéines d'enveloppes virales	• HMGB1 • Fibronectine • Surfactant A • Lipoprotéines
TLR5	Membrane cellulaire	• Flagelline des bactéries flagellées	
TLR6	Membrane cellulaire	• Lipoprotéines des mycoplasmes • LTA des streptocoques B • Zymozan des *Saccharomyces cerevisiae*	• HSP • HMGB1
TLR7 et 8	Endosome	• ARN simple brin des virus	• ARN simple brin
TLR9	Endosome	• Cytosine-phosphate-guanine (CpG) des bactéries • Hémozoïne du plasmodium falciparum	• ADN
TLR10	Membrane cellulaire	• Inconnu	• Inconnu
TLR11	Membrane cellulaire	• Bactéries à tropisme urologique • Profilin-like de Toxoplasma gondii	• Inconnu

2.1.4. TLR Signaling Pathways

The interaction between TLRs and their ligands triggers intracellular signaling pathways responsible for cellular activation. Some TLRs function as heterodimers, thereby expanding the spectrum of ligands they can recognize. For example, the recognition of triacylated lipopeptides requires the formation of a TLR1/TLR2 heterodimer, whereas the detection of diacylated lipopeptides depends on the TLR2/TLR6 heterodimer.

Other TLRs, including TLR3, TLR4, TLR5, and TLR9, generally function as homodimers, although optimal ligand recognition and signaling often require accessory molecules such as CD14 and MD-2. Activation of TLRs initiates multiple signaling cascades that ultimately activate transcription factors responsible for regulating the expression of inflammatory genes. These pathways induce the production of pro-inflammatory cytokines such as TNF-α, IL-1, IL-6, and IL-12, as well as the co-stimulatory molecules CD80 and CD86, which are essential for adaptive immune responses.

Signal transduction is mediated by the intracellular TIR (Toll/IL-1 Receptor) domain. TLR signaling involves a complex network of cytoplasmic proteins that also contain TIR domains. The adaptor protein MyD88 (Myeloid Differentiation Primary Response 88) is the central

mediator of most TLR signaling pathways. Through TIR-TIR interactions, MyD88 is recruited to activated TLRs and subsequently engages members of the IRAK (Interleukin-1 Receptor-Associated Kinase) family via its N-terminal death domain (DD).

Studies using MyD88-deficient mice demonstrated that this adaptor protein is essential for NF-κB-dependent transcription of pro-inflammatory cytokines such as TNF-α and IL-6. However, these studies also revealed the existence of MyD88-independent signaling pathways. In MyD88-deficient animals, signaling downstream of the TLR4-LPS and TLR3-dsRNA complexes is not completely abolished but only delayed. Furthermore, induction of Interferon-β (IFN-β) by TLR3 and TLR4 occurs largely through a MyD88-independent mechanism.

These observations led to the identification of a second major adaptor molecule, TRIF (TIR-domain-containing adaptor-inducing interferon-β), also known as TICAM-1. TRIF is essential for the activation of NF-κB, AP-1, and antiviral signaling pathways. In addition, activation of the transcription factor IRF3 (Interferon Regulatory Factor 3) is required for IFN-β gene expression.

Two additional TIR-domain-containing adaptor proteins contribute to TLR signaling. The first, TIRAP (TIR-domain-containing adaptor protein), also known as MAL, functions as a cofactor for MyD88 and is specifically involved in signaling through TLR2 and TLR4. The second, TRAM (TRIF-related adaptor molecule), acts as a cofactor for TRIF and is required specifically for TLR4 signaling. Once adaptor proteins are recruited to the TIR domain of activated TLRs, they undergo conformational changes and activate downstream signaling molecules, including members of the IRAK family. These kinases subsequently trigger signaling cascades leading to the activation of transcription factors such as NF-κB, AP-1, and IRF3, resulting in the expression of inflammatory cytokines, chemokines, co-stimulatory molecules, and antiviral mediators that coordinate innate and adaptive immune responses **(Figure 22)**.

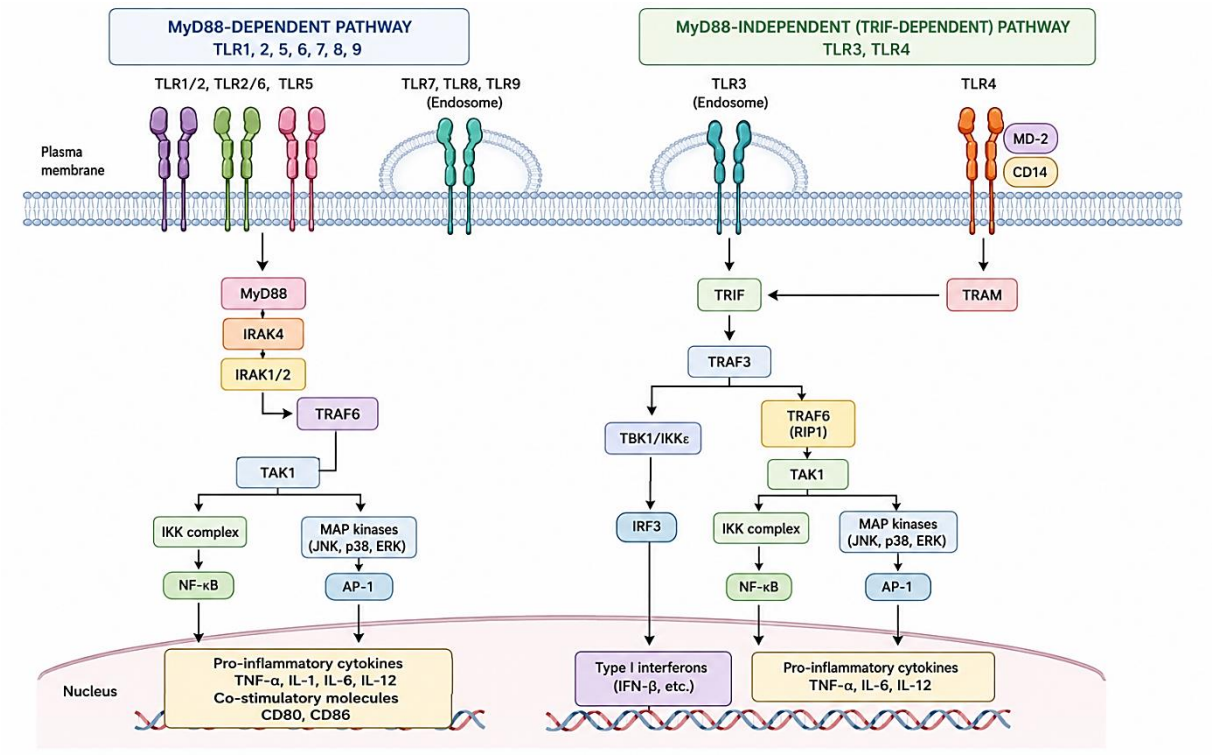


Figure 22. Major signaling pathways activated by toll-like receptors (TLRs): Myd88-dependent and trif-dependent pathways.

Chapter 5. Structure of Major Histocompatibility Complex (MHC) Molecules and Mechanisms of Antigen Presentation

Major Histocompatibility Complex (MHC) molecules were first described by Jean Dausset in 1952 and were named the Major Histocompatibility Complex because of their critical role in tissue compatibility and organ transplantation. Historically, the discovery of this system provided a molecular explanation for the almost inevitable rejection of transplanted organs between genetically unrelated individuals.

In humans, MHC molecules are known as Human Leukocyte Antigens (HLA). The HLA system consists of a cluster of closely linked genes encoding membrane glycoproteins that bind and present antigenic peptides to T-cell receptors (TCRs). Consequently, the HLA system plays a fundamental role in the initiation and regulation of adaptive immune responses. The HLA gene complex is located on the short arm of chromosome 6 (6p21.3). This genomic region contains numerous genes organized into three major classes. Class I and Class II HLA genes are directly involved in antigen presentation and immune responses, whereas Class III genes encode several complement proteins and inflammatory cytokines, including Tumor Necrosis Factor-alpha (TNF-α) and Tumor Necrosis Factor-beta (TNF-β).

The Class I HLA system primarily includes the genes HLA-A, HLA-B, and HLA-C. The Class II HLA system comprises several gene families, the most important of which are HLA-DR, HLA-DQ, and HLA-DP. Within each family, both functional genes and non-functional genes (pseudogenes) are present.

1. Structure of MHC Class I Molecules

MHC Class I molecules consist of two non-covalently associated polypeptide chains: a transmembrane heavy α-chain with a molecular weight of approximately 45 kDa and a light chain called β2-microglobulin with a molecular weight of approximately 12 kDa. Unlike the α-chain, β2-microglobulin is not anchored in the plasma membrane. The extracellular portion of the heavy α-chain contains three domains designated α1, α2, and α3. The α1 and α2 domains fold together to form a peptide-binding groove composed of a β-pleated sheet platform topped by two α-helices. This groove serves as the antigen-binding site and accommodates peptides that are presented to $CD8^+$ T lymphocytes. The α3 domain is structurally associated with β2-microglobulin and contributes to the overall stability of the molecule. The three-dimensional

structure of the MHC Class I molecule fits approximately within a cylinder measuring 7 nm in length and 4–5 nm in diameter .

The heavy α-chain is a glycoprotein composed of 348 amino acids, distributed as follows:

- 274 amino acids form the extracellular region, including: 90 amino acids in the α1 domain, 92 amino acids in the α2 domain,92 amino acids in the α3 domain.
- 27 amino acids constitute the transmembrane region, which is rich in hydrophobic amino acids and ensures stable anchoring of the molecule within the plasma membrane.
- The remaining amino acids form the intracellular (cytoplasmic) tail, which contains phosphorylation sites and represents the carboxy-terminal end of the molecule.

The β2-microglobulin chain is a polypeptide composed of 99 amino acids. It contains a single immunoglobulin-like domain and associates non-covalently with the α3 domain of the heavy chain. β2-microglobulin is essential for maintaining the proper conformation and stability of the MHC Class I molecule. In its absence, the α-chain cannot fold correctly and therefore cannot be expressed on the cell surface. Thus, the association between the heavy α-chain and β2-microglobulin is indispensable for the assembly, stability, and antigen-presenting function of MHC Class I molecules **(Figure 23)**. Crystallographic studies have elucidated the three-dimensional structure of MHC Class I molecules and their antigen-binding site. The peptide-binding groove is formed at the junction of the α1 and α2 domains of the heavy chain. The floor of the groove consists of an eight-stranded β-sheet, with four β-strands contributed by each domain, while the sides are formed by two α-helices. This groove accommodates antigenic peptides approximately 8–10 amino acids in length, allowing their presentation to $CD8^+$ T lymphocytes.

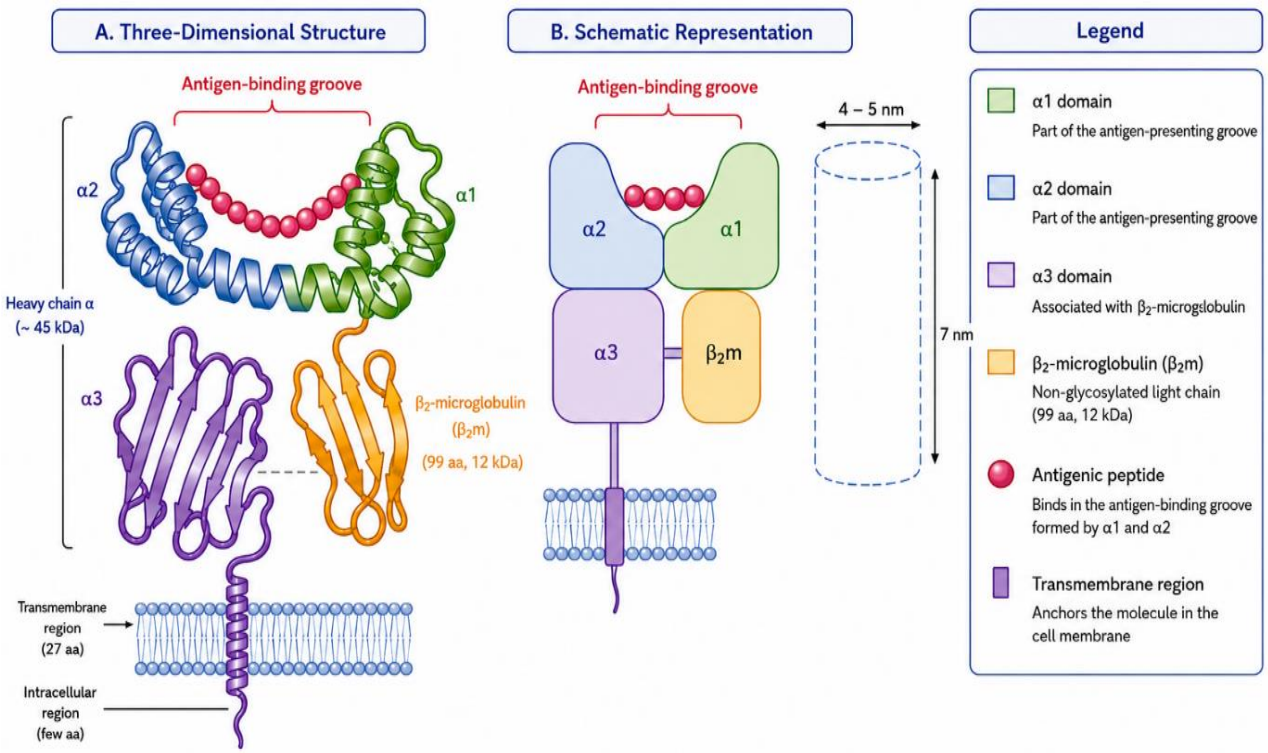


Figure 23. Structure of MHC Class I molecules

2. Structure of MHC Class II Molecules

MHC Class II molecules are transmembrane glycoproteins structurally related to MHC Class I molecules. They are composed of two non-covalently associated polypeptide chains: an α-chain with a molecular weight of approximately 34 kDa and a β-chain with a molecular weight of approximately 29 kDa. Unlike MHC Class I molecules, the α and β chains of MHC Class II molecules are structurally homologous. Each chain consists of an extracellular N-terminal region of approximately 190 amino acids, a transmembrane segment of about 20 amino acids, and a short cytoplasmic C-terminal tail of 4–5 amino acids.

The extracellular portions of both chains contain two domains of approximately 90 amino acids each. Sequence polymorphisms are primarily concentrated within the α1 and β1 domains, which contain hypervariable regions responsible for peptide recognition. In contrast, the α2 and β2 domains are relatively conserved **(Figure 24)**. The peptide-binding groove is formed through the interaction of the α1 and β1 domains and serves as the site where antigenic peptides are presented to $CD4^+$ T lymphocytes. Structural studies have shown that these two domains are highly symmetrical. Each domain folds into a four-stranded antiparallel β-sheet topped by a long α-helix of approximately 30 amino acid residues. Hydrogen bonds between the β-sheets of the α1 and β1 domains generate a single eight-stranded β-sheet platform, while the two α-

helices form the walls of a deep groove. This groove constitutes the functional peptide-binding site of MHC Class II molecules.

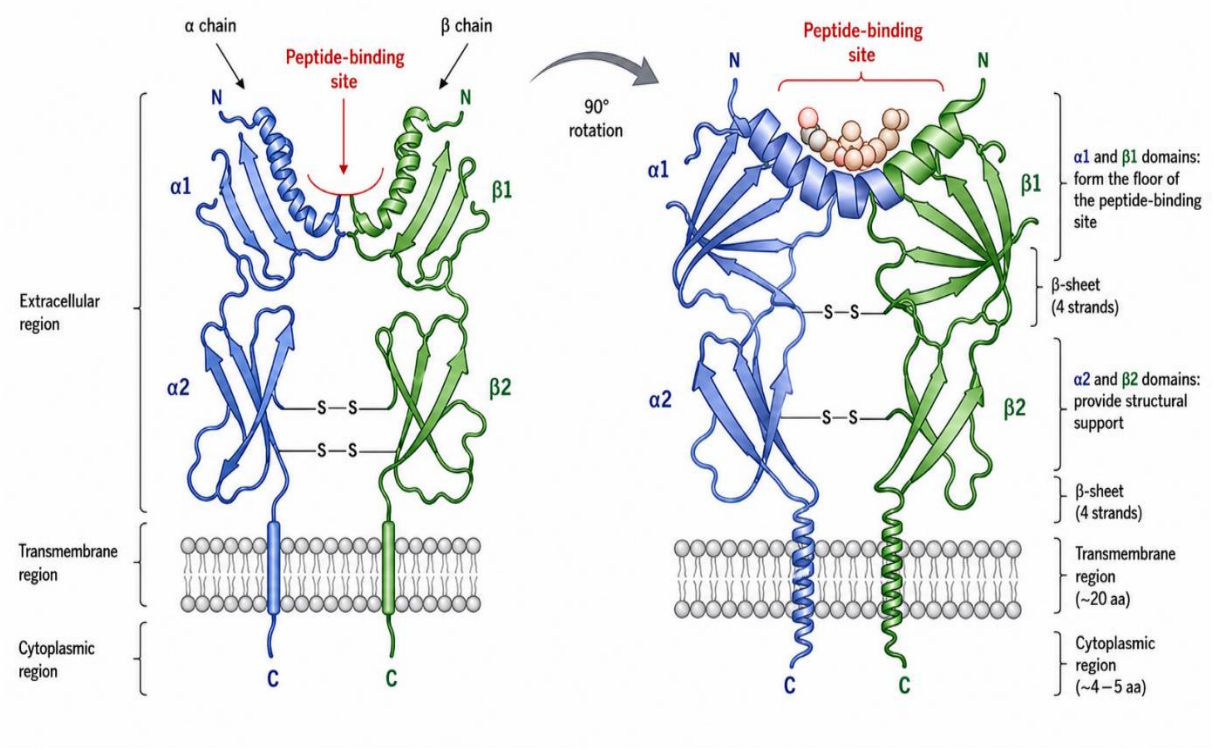


Figure 24. Three-dimensional structure of the antigen-binding site of MHC class II molecules

3. Peptide Loading onto MHC Molecules

3.1. MHC Class I Antigen Presentation Pathway (Endogenous Pathway)

MHC Class I molecules present peptides generated within the cell. These peptides may originate from normal cellular proteins, tumor-associated proteins, or viral proteins synthesized by infected cells. Therefore, MHC Class I molecules primarily present endogenous antigens derived from the cytoplasm. Intracellular proteins are first degraded into peptide fragments by the proteasome. Additional trimming may occur within the endoplasmic reticulum (ER) through the action of Endoplasmic Reticulum Aminopeptidases (ERAPs). The heavy α-chain and β2-microglobulin are synthesized independently in the rough endoplasmic reticulum of nucleated cells. Their proper assembly requires molecular chaperones, including calnexin and calreticulin, which maintain correct protein folding. The resulting complex then associates with the peptide-loading machinery, including the TAP1 and TAP2 transporters (Transporters Associated with Antigen Processing), which form a channel within the ER membrane. The

adaptor protein tapasin facilitates the interaction between MHC Class I molecules and TAP. Peptides generated in the cytoplasm are transported into the ER lumen by TAP, where they may undergo further processing by ERAP before binding to the peptide-binding groove of MHC Class I molecules. Once a suitable peptide is loaded, the chaperone proteins dissociate, and the stable peptide-MHC Class I complex is transported through the Golgi apparatus to the plasma membrane for presentation to $CD8^+$ T cells **(Figure 25)**.

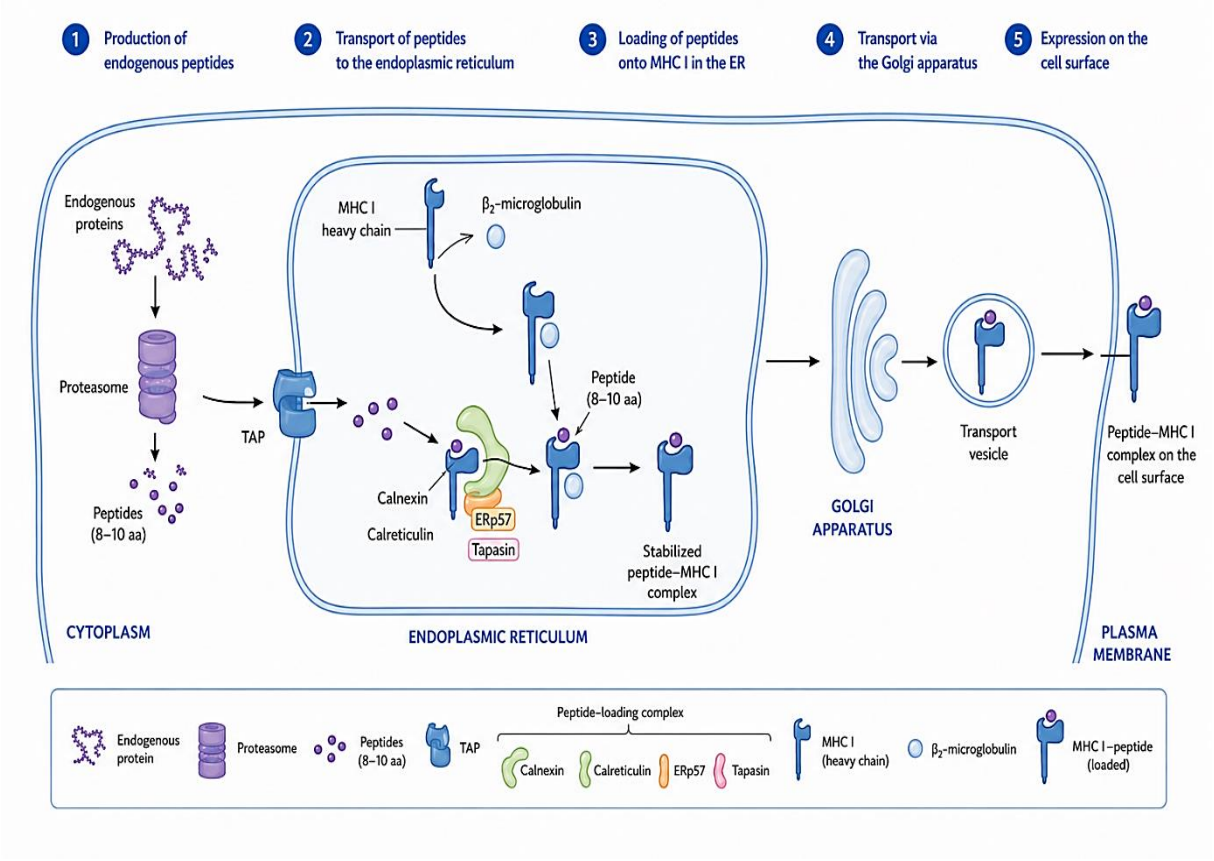


Figure 25. Endogenous antigen presentation pathway via MHC class I molecules

3.2. MHC Class II Antigen Presentation Pathway (Exogenous Pathway)

MHC Class II molecules present peptides derived from extracellular antigens. These antigens are internalized by antigen-presenting cells through endocytosis or phagocytosis and are subsequently degraded within endosomal and lysosomal compartments into peptides generally ranging from 12 to 25 amino acids in length. The α and β chains of MHC Class II molecules are synthesized independently in the endoplasmic reticulum. During assembly, they associate with a third molecule known as the invariant chain (Ii). The invariant chain contains a transmembrane region and a fragment called CLIP (Class II-Associated Invariant Chain Peptide), which occupies the peptide-binding groove and prevents premature peptide loading.

The MHC Class II–invariant chain complex is transported from the ER to the Golgi apparatus and subsequently packaged into vesicles. Exit from the ER requires the assembly of three invariant chains with three MHC Class II heterodimers, forming a nonameric complex composed of nine polypeptide chains. Within endosomal compartments, proteolytic enzymes known as cathepsins degrade the invariant chain while leaving CLIP temporarily bound to the peptide-binding groove. Specialized molecules called HLA-DM then facilitate the removal of CLIP and its replacement with antigenic peptides generated within the endosomal pathway. Because the peptide-binding groove of MHC Class II molecules is open at both ends, peptides of variable lengths can be accommodated. Once peptide loading is complete, the peptide-MHC Class II complex is transported to the plasma membrane, where it presents antigenic peptides to $CD4^+$ T lymphocytes **(Figure 26)**.

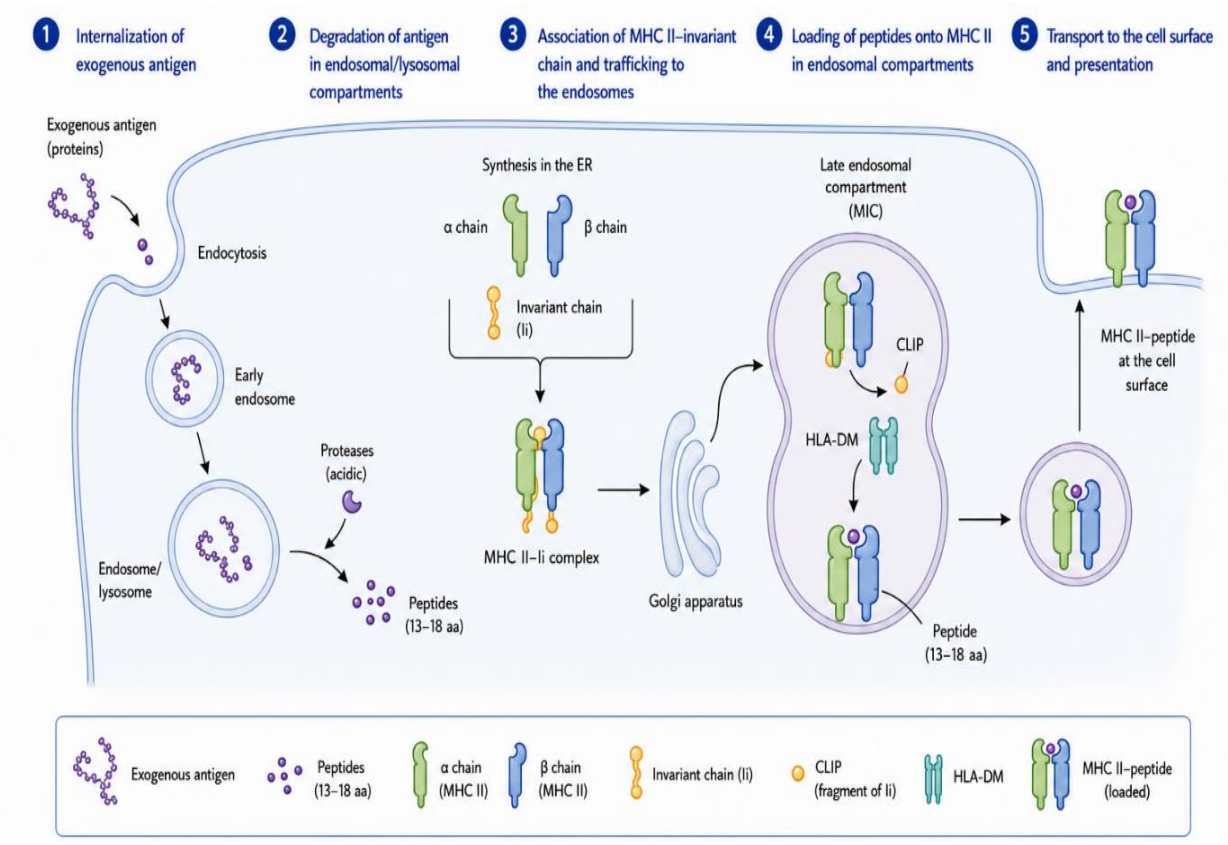


Figure 26. Exogenous antigen presentation pathway via MHC class II molecules.

Chapter 6. Structure of Lymphocyte Receptors (TCR and BCR)

The B-cell receptor (BCR) and the T-cell receptor (TCR) are characterized by their remarkable diversity, which results from the recombination of gene segments encoding the heavy and light chains that constitute these receptors. The large number of antigens that may be encountered by the immune system requires the genome to be capable of synthesizing several million different receptor molecules.

However, the constant regions of the various heavy and light chains remain invariant, whereas the variable regions differ from one receptor to another and are each specific for a particular antigenic epitope. Several gene segments contribute to the formation of these variable regions.

1. T-Cell Antigen Receptor (TCR)

The T-cell antigen receptor (TCR) consists of two polypeptide chains, either α and β or γ and δ, associated with the CD3 complex. The αβ and γδ heterodimers are responsible for the specific antigen-recognition function of T lymphocytes. These heterodimers do not possess a sufficiently long cytoplasmic domain to contain the signaling motifs required for intracellular signal transduction. Consequently, they are always associated with the CD3 complex, whose function is to transmit the signal generated by the interaction between the TCR and the peptide–MHC complex. The TCR is expressed clonally on the surface of T lymphocytes. In approximately 95% of cases, T lymphocytes express an αβ TCR.

1.1. Structure of the TCR

Whether αβ or γδ, the TCR heterodimer consists of two polypeptide chains linked together by a disulfide bond. Each chain contains two domains, one variable and one constant, arranged in a loop structure stabilized by an intrachain disulfide bond, thereby forming an immunoglobulin-like domain. Approximately twenty amino acids organized into an α-helix ensure anchoring of each protein chain within the plasma membrane. Most of these amino acids are hydrophobic, whereas certain charged residues probably interact with oppositely charged residues present in the CD3 chains. The transmembrane region is extended by a very short intracellular segment. The cytoplasmic domains of the CD3 chains contain a total of ten Immunoreceptor Tyrosine-based Activation Motifs (ITAMs). Each ITAM consists of two conserved amino acid sequences

(Tyr–X–X–Leu/Ile) separated by six to seven amino acids. These motifs play a crucial role in initiating intracellular signaling cascades following TCR engagement (**Figure 27)**.

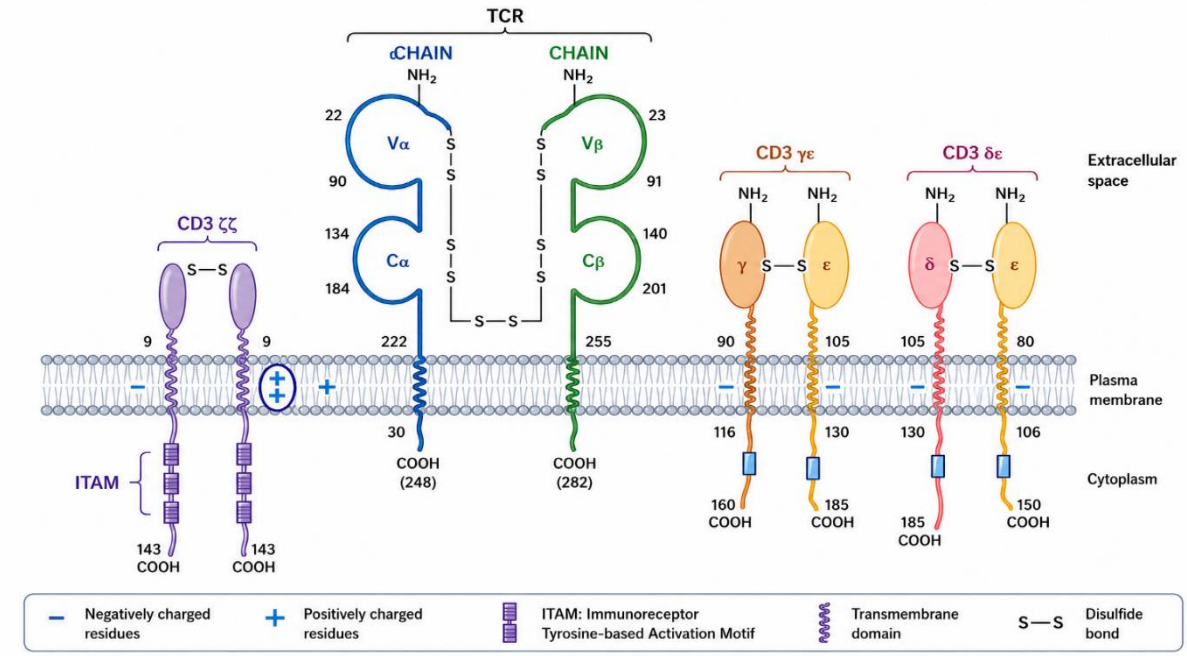


Figure 27. Structure of the T-cell receptor (TCR).

The TCR is permanently associated with the CD3 complex, which is composed of the CD3γε, CD3δε, and CD3ζζ dimers. While the TCR α and β chains provide antigen specificity, the CD3 molecules are responsible for signal transduction. The ITAM motifs present within the cytoplasmic domains of the CD3 chains become phosphorylated following antigen recognition, thereby initiating the intracellular signaling pathways required for T-cell activation. The variable domain is responsible for specific antigen recognition. The variable region of the TCR contains three hypervariable regions known as complementarity-determining regions (CDRs). While CDR1 and CDR2 are germline encoded by V genes, CDR3 is generated through the rearrangement and juxtaposition of V, D, and J gene segments to form the corresponding TCR chain gene. The crystallographic resolution of TCR–peptide–MHC complexes has highlighted the crucial role of these regions in TCR function. CDR1 interacts directly with both the antigenic peptide and the MHC molecule. CDR2 primarily interacts with the MHC molecule, whereas CDR3, the most hypervariable region, directly recognizes the antigenic peptide. This structural organization enables the TCR to recognize a vast diversity of antigenic peptides while maintaining specificity for self-MHC molecules (**Figure 28)**.

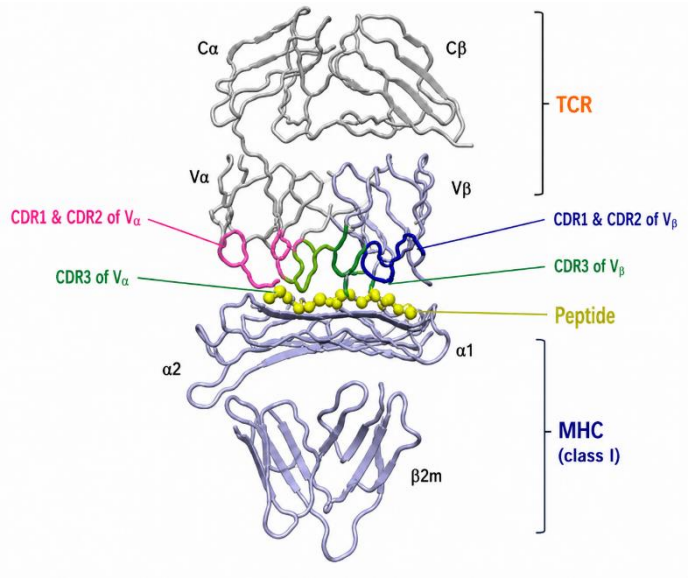


Figure 28. Three-dimensional representation of the interaction between the hypervariable regions of the TCR and an antigenic peptide associated with an MHC class I molecule.

The antigen-binding site of the TCR is formed by six CDR loops, three contributed by the α chain and three by the β chain. The CDR1 and CDR2 loops mainly contact the α-helices of the MHC molecule, whereas the highly diverse CDR3 loops establish direct interactions with the antigenic peptide bound within the peptide-binding groove. This dual recognition of both the peptide and the MHC molecule constitutes the molecular basis of MHC restriction, a fundamental feature of adaptive immunity. Upon formation of a stable TCR–peptide–MHC complex, activation signals are transmitted through the associated CD3 complex, leading to T-cell activation, proliferation, and differentiation.

1.2. Organization of TCR Genes

In their germline configuration, the genes encoding T-cell receptor (TCR) chains are fragmented and must undergo rearrangement through a process of directed somatic recombination before they can be expressed. This process, known as V(D)J recombination, occurs in the thymus during the maturation of lymphoid precursors. The genes encoding the TCRα, TCRβ, TCRδ, and TCRγ chains are designated TCRA, TCRB, TCRD, and TCRG, respectively. The loci containing the TCRB and TCRG genes are referred to by the same names, whereas the locus containing both TCRA and TCRD genes is designated TCRAD **(Figure 29)**.

The TCRB locus is located on chromosome 7 in humans and spans approximately 600 kb. It contains a large number of BV gene segments, approximately sixty, grouped into about thirty

families. Most BV gene segments are located upstream of the locus, with a few exceptions. The diversity (BD) and joining (BJ) gene segments are organized into two duplicated clusters, each containing one BD gene, six to eight BJ genes, and one constant region gene (BC). In humans, four BJ segments and twelve BV segments are pseudogenes.

The TCRG locus, also located on chromosome 7, has an organization similar to that of the TCRB locus. It contains a region composed of V gene segments and two duplicated regions, each comprising several J genes and a single constant region gene (C).

The TCRAD locus exhibits a similar organization in humans and mice. It is located on chromosome 14 in both species and extends over approximately 1 to 1.5 Mb. Its unique organization places the genes encoding the TCRδ chain within the TCRα locus, between the TCRAV and TCRAJ gene segments. Consequently, rearrangement of the TCRα genes results in the deletion of the TCRδ locus, thereby preventing the expression of a TCRδ chain. Most V gene segments are located upstream of the locus and can be used interchangeably in the formation of either TCRα or TCRδ chains. However, some V genes (DV101 to DV105) are preferentially utilized in the generation of the δ chain. Depending on the relative orientation and location of the gene segments involved, rearrangement may occur either through deletion (excision) or inversion. Some AJ gene segments lack recombination signal sequences (RSS), functional splice sites, or a correct open reading frame and are therefore nonfunctional. A number of these pseudogenes have been identified in both humans and mice, although their exact number has not yet been precisely determined.

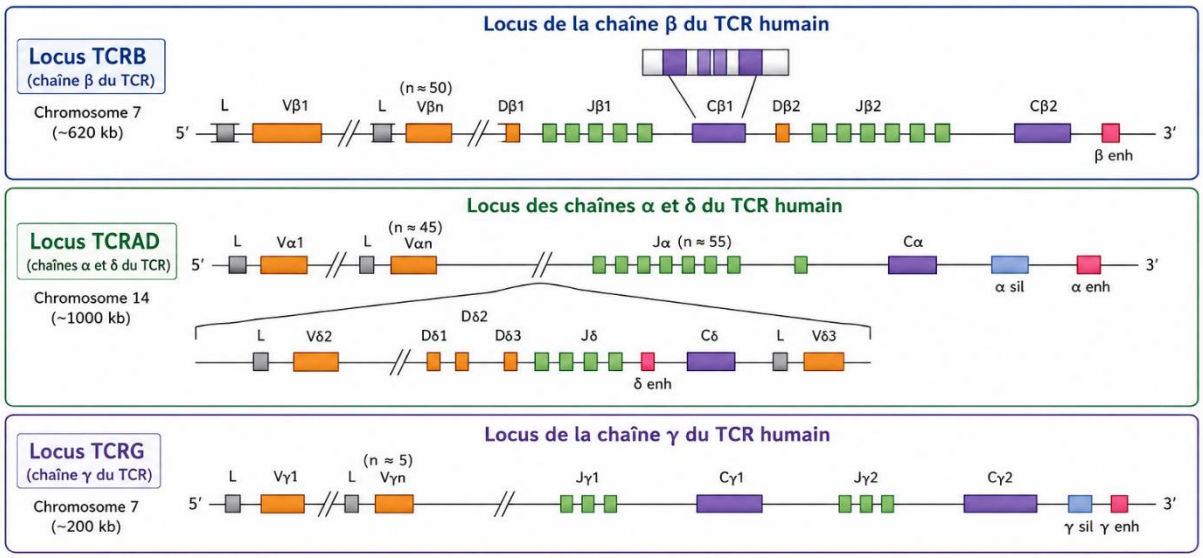


Figure 29. Genetic organization of the human TCR loci (TCRB, TCRG, and TCRAD).

2. B-Cell Antigen Receptor (BCR)

Specific antigen recognition is the hallmark of the adaptive immune response. In B lymphocytes, the molecule responsible for this function is a membrane-bound immunoglobulin known as the **B-cell receptor (BCR)**.

2.1. Structure of the BCR

The B-cell receptor (BCR) is a hetero-oligomeric structure **(Figure 30)**. It is a tetrameric complex composed of two membrane-anchored heavy chains and two light chains. Structurally, the BCR corresponds to a membrane-bound immunoglobulin capable of specifically recognizing antigens. The cytoplasmic tail of membrane immunoglobulins is relatively short, consisting of approximately three amino acid residues in IgM and IgD and twenty-eight residues in IgG. Consequently, this intracellular region does not play a direct role in signal transduction following antigen binding to the membrane immunoglobulin. Signal transduction is mediated by two proteins closely associated with the BCR, namely Igα (CD79a) and Igβ (CD79b). These molecules form a heterodimer that is non-covalently associated with the membrane immunoglobulin and is essential for the expression and function of the BCR at the cell surface. Like the CD3 complex associated with the T-cell receptor, Igα and Igβ contain Immunoreceptor Tyrosine-based Activation Motifs (ITAMs) within their cytoplasmic domains. Following antigen binding and receptor cross-linking, these ITAM motifs become phosphorylated by Src-family kinases, initiating a cascade of intracellular phosphorylation events. This signaling pathway ultimately leads to B-cell activation, proliferation, differentiation, and antibody production.

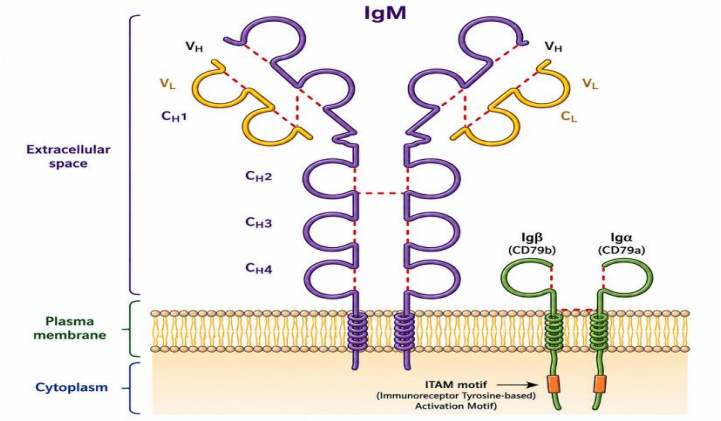


Figure 30. Molecular structure of the B-cell receptor (BCR) and its associated signaling molecules Igα (CD79a) and Igβ (CD79b)

2.2. Organization of Immunoglobulin Genes

Three distinct genetic complexes are involved in immunoglobulin synthesis: the Igκ and Igλ loci, which encode the light chains, and the IgH locus, which encodes the heavy chains. Each locus is composed of variable (V) gene segments, joining (J) gene segments, and genes encoding the constant regions (C). Diversity (D) segments are present only in the IgH locus. The immunoglobulin (Ig) repertoire is encoded by multiple germline gene segments that undergo somatic diversification during B-cell development. In humans, the λ light-chain locus is located on chromosome 22q11 and contains approximately 30 Vλ genes and 5 Jλ segments. The κ locus, located on chromosome 2p12, contains 34–40 Vκ genes and 5 Jκ genes. The heavy-chain (IgH) locus is located on chromosome 14q32 and contains approximately 300 VH genes, 30 DH genes, and 6 JH genes. Nine genes encode the constant regions corresponding to the different immunoglobulin classes and subclasses. In their chromosomal order, these constant-region genes are: Cμ, Cδ, Cγ3, Cγ1, Cε2, Cα1, Cγ2, Cγ4, Cε1, and Cα2. The Cε2 gene is considered a pseudogene **(Figure 31)**.

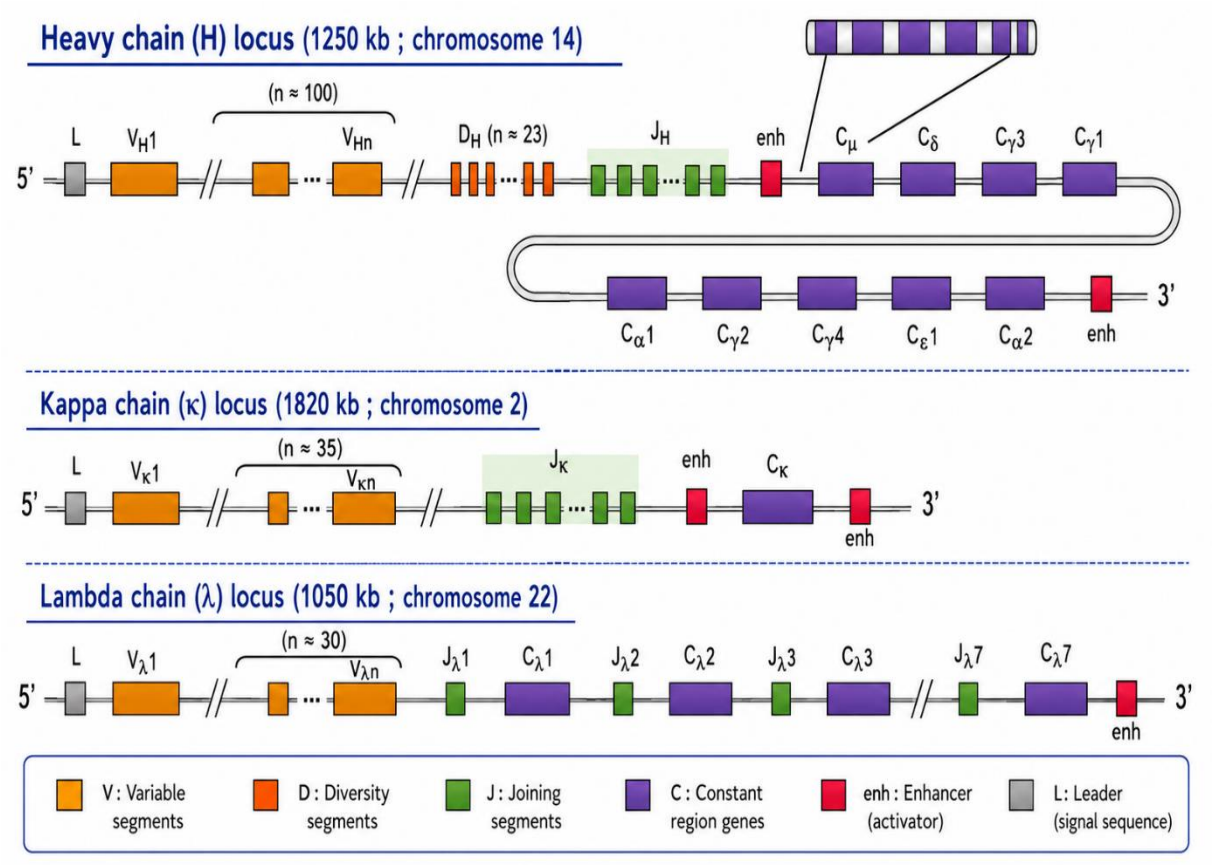


Figure 31. Genetic organization of immunoglobulin loci encoding heavy and light chains.

The light chains are encoded by the Igκ and Igλ loci. The κ light-chain genes are located on chromosome 2. In its germline configuration, the human Igκ locus contains 31 to 35 functional Vκ segments and 5 Jκ segments. The Vκ and Jκ segments encode the variable region of the light chain, whereas a single Cκ segment encodes the constant region.

The λ light-chain genes are located on chromosome 22. In its germline configuration, the human Igλ locus contains approximately 30 Vλ segments and 4 Jλ segments. At least six different Cλ genes have been identified, each preceded by its own Jλ segment. Recombination occurs randomly between one Vλ gene segment and one Jλ segment to generate the variable region of the λ light chain.

3. Comparison Between TCR and BCR

Although T-cell receptors (TCRs) and B-cell receptors (BCRs) are responsible for antigen recognition and play central roles in adaptive immunity, they differ in their structure, mode of antigen recognition, and biological functions. Despite these differences, both receptors share several structural and genetic characteristics that reflect their common evolutionary origin.

3.1. Structural Similarities

TCRs and BCRs belong to the immunoglobulin superfamily and are composed of polypeptide chains containing variable (V) and constant (C) domains with an immunoglobulin-like structure. The variable domains of both receptors are generated through V(D)J recombination, a process that creates a highly diverse repertoire of antigen-recognition molecules.

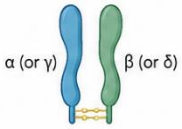
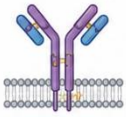
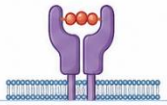
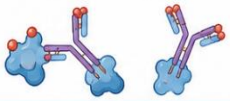
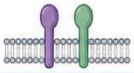
Both receptors contain complementarity-determining regions (CDRs) within their variable domains. These hypervariable regions form the antigen-binding site and determine antigen specificity. In addition, neither receptor can efficiently transmit activation signals on its own. Signal transduction requires association with accessory molecules containing ITAM motifs: the CD3 complex in T lymphocytes and the Igα/Igβ (CD79a/CD79b) complex in B lymphocytes.

3.2. Functional Differences

Despite their structural similarities, TCRs and BCRs differ considerably in the way they recognize antigens. The TCR recognizes only processed antigenic peptides presented by major histocompatibility complex (MHC) molecules on the surface of antigen-presenting cells. This phenomenon is known as MHC restriction. In contrast, the BCR can directly recognize native

antigens in their soluble or membrane-bound forms without the need for antigen processing or MHC presentation. Another major difference concerns their fate after activation. The TCR is expressed exclusively as a membrane-bound receptor and is never secreted. Conversely, the BCR is a membrane-bound immunoglobulin that can be converted into a secreted antibody following B-cell differentiation into plasma cells. Furthermore, BCR genes undergo additional diversification mechanisms after antigen stimulation, including somatic hypermutation and class-switch recombination, which increase antibody affinity and modify effector functions. These processes do not occur in TCR genes **(Table 4)**.

Table 4. Comparison between TCR and BCR

Characteristic	TCR	BCR
Main cell type	T lymphocyte	B lymphocyte
Structure	αβ or γδ heterodimer 	Membrane immunoglobulin (2 heavy chains + 2 light chains) 
Antigen recognition	Peptide presented by MHC 	Native antigen 
MHC restriction	Yes	No
Signaling molecules	CD3 complex 	Igα/Igβ (CD79a/CD79b) 
Secreted form	No	Yes (antibodies) 
Somatic hypermutation	No	Yes
Class-switch recombination	No	Yes
Main function	Cellular immunity	Humoral immunity

Chapter 7. Antibody Synthesis and Generation of Immune Receptor Diversity

One of the most remarkable features of the adaptive immune system is its ability to recognize and respond to an almost unlimited variety of antigens. This extraordinary capacity relies on the generation of a highly diverse repertoire of antigen receptors expressed by B and T lymphocytes. Although the human genome contains a limited number of genes, specialized genetic mechanisms allow the production of millions of distinct antigen receptors and antibodies capable of recognizing a vast array of pathogens and foreign molecules. The diversity of antigen receptors is primarily generated during lymphocyte development through V(D)J recombination, a process that randomly assembles variable (V), diversity (D), and joining (J) gene segments. Additional diversity is introduced through junctional modifications, including nucleotide insertions and deletions at gene segment junctions. Together, these mechanisms generate a unique antigen receptor repertoire for each individual. Following antigen stimulation, B lymphocytes undergo further diversification processes within germinal centers. These include somatic hypermutation, which increases antibody affinity for the antigen, and class-switch recombination, which modifies the effector functions of antibodies while preserving their antigen specificity. As a result, activated B cells can differentiate into plasma cells that secrete large quantities of antibodies or into memory B cells that provide long-term immune protection. This chapter describes the molecular mechanisms responsible for antibody synthesis and the generation of immune receptor diversity, highlighting their essential role in the effectiveness and specificity of adaptive immune responses.

1. T- and B-Cell Repertoire

The repertoire refers to the complete set of T-cell receptors (TCRs) and B-cell receptors (BCRs) with different antigen specificities expressed by peripheral T and B lymphocytes. The diversity of TCR and BCR repertoires results from two major mechanisms: combinatorial diversity and junctional diversity. These mechanisms are essential for generating a repertoire broad enough to recognize a virtually unlimited number of antigens. Without junctional diversity, the number of gene segments required to generate an equivalent repertoire would likely be too large to be accommodated within the genome.

1.1. Combinatorial Diversity

The diversity of antigen receptors is largely generated through the random recombination of variable (V), diversity (D), and joining (J) gene segments during lymphocyte development. In immunoglobulin heavy chains, the variable region is formed through the sequential rearrangement of a DH segment with a JH segment, followed by the joining of a VH segment, resulting in the formation of a complete VDJ exon. In contrast, the variable regions of light chains are generated by the direct association of VL and JL segments to produce a VJ exon. During these rearrangements, the intervening DNA sequences are excised and removed as circular DNA molecules known as episomes. The random selection of V, D, and J segments from a large pool of available gene segments generates an enormous number of possible combinations, thereby contributing significantly to the diversity of both T-cell receptors (TCRs) and B-cell receptors (BCRs). Consequently, the greater the number of available gene segments within a locus, the higher the level of combinatorial diversity that can be achieved.

1.2. Junctional Diversity

Junctional diversity represents one of the most important mechanisms contributing to the vast diversity of antigen receptors. While combinatorial diversity results from the random selection and rearrangement of V, D, and J gene segments, junctional diversity arises from modifications introduced at the junctions of these gene segments during V(D)J recombination. DNA cleavage and repair mechanisms can lead to the deletion of nucleotides and the addition of new nucleotides at recombination sites, generating unique coding sequences. These modifications primarily affect the complementarity-determining region 3 (CDR3), the most variable region of T-cell receptors (TCRs) and immunoglobulins. Consequently, junctional diversity greatly expands the antigen-recognition repertoire beyond that generated by combinatorial diversity alone.

1.2.1. P Nucleotides

Palindromic (P) nucleotides are generated during the early stages of V(D)J recombination. Following cleavage of DNA by the recombination machinery, the coding ends form hairpin structures. When these hairpins are opened asymmetrically, short single-stranded overhangs are produced. DNA repair enzymes then fill in the complementary bases, generating short palindromic nucleotide sequences known as P nucleotides. Because these nucleotides are derived from the original coding sequence, they are referred to as templated nucleotides. The

addition of P nucleotides contributes to variability at the coding junctions and increases receptor diversity.

1.2.2. N Nucleotides

Non-templated (N) nucleotides are added randomly to coding junctions by the enzyme terminal deoxynucleotidyl transferase (TdT). Unlike P nucleotides, N nucleotides are not encoded by the germline DNA sequence and are inserted independently of any template. TdT is expressed during the early stages of T- and B-cell development, when V(D)J recombination occurs. On average, approximately three nucleotides are added per junction in TCRβ chains, whereas up to fifteen nucleotides may be added in immunoglobulin heavy and light chains. These random insertions significantly increase receptor diversity and are a major source of variability within the CDR3 region **(Figure 32)**.

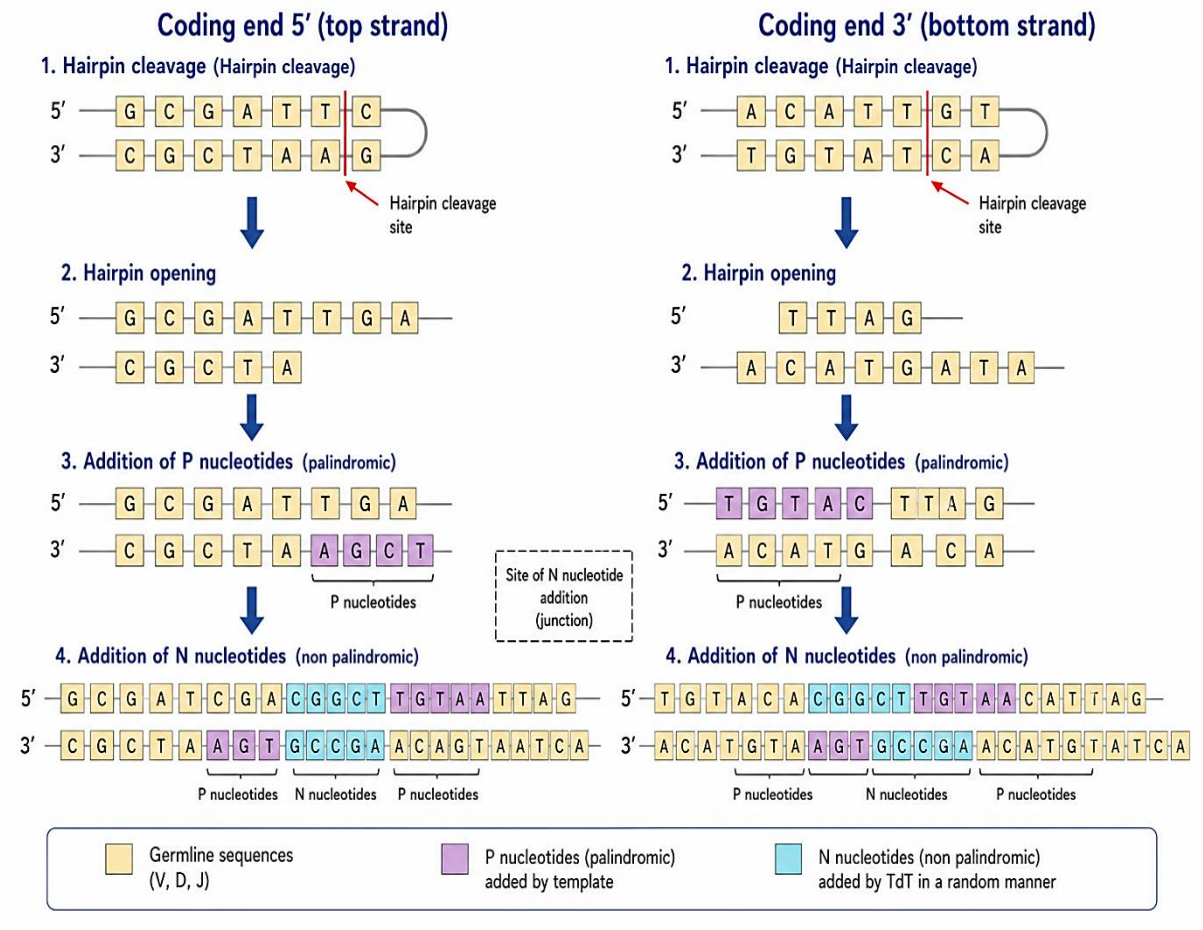


Figure 32. Generation of junctional diversity during V(D)J recombination through the addition of P and N nucleotides

1.2.3. Deletions

In addition to nucleotide insertions, deletions frequently occur at the coding ends during V(D)J recombination. After the hairpin structures have been opened, exonucleases may remove nucleotides from the coding segments before ligation takes place. These deletions alter the nucleotide sequence at V–D, D–J, or V–J junctions and further increase receptor diversity. The combined effects of nucleotide deletions, imprecise DNA cleavage, and the addition of P and N nucleotides allow the generation of an enormous repertoire of antigen receptors. It is estimated that these mechanisms enable an individual to produce theoretically up to 10^9 different immunoglobulins. However, because nucleotide additions and deletions occur randomly, they often disrupt the reading frame of the rearranged gene. As a result, only approximately one-third of recombination events generate a productive rearrangement capable of encoding a functional antigen receptor.

2. Germinal Center

2.1. Structure of the Germinal Center

The lymph node cortex is generally divided into a superficial region and a deeper region. The superficial region contains lymphoid follicles, which may develop into germinal centers following antigenic stimulation. A germinal center is a specialized microenvironment formed within secondary lymphoid organs, including lymph nodes, the spleen, and mucosa-associated lymphoid tissues. It serves as the principal site for B-cell proliferation, differentiation, and affinity maturation during T-dependent immune responses. A mature germinal center is organized into two anatomically and functionally distinct compartments: the dark zone and the light zone. The dark zone contains rapidly proliferating B cells known as centroblasts, which undergo intense clonal expansion and somatic hypermutation of their immunoglobulin genes. The light zone contains centrocytes, follicular dendritic cells (FDCs), and T follicular helper (Tfh) cells. This compartment is responsible for the selection of B cells expressing high-affinity antigen receptors **(Figure 33)**.

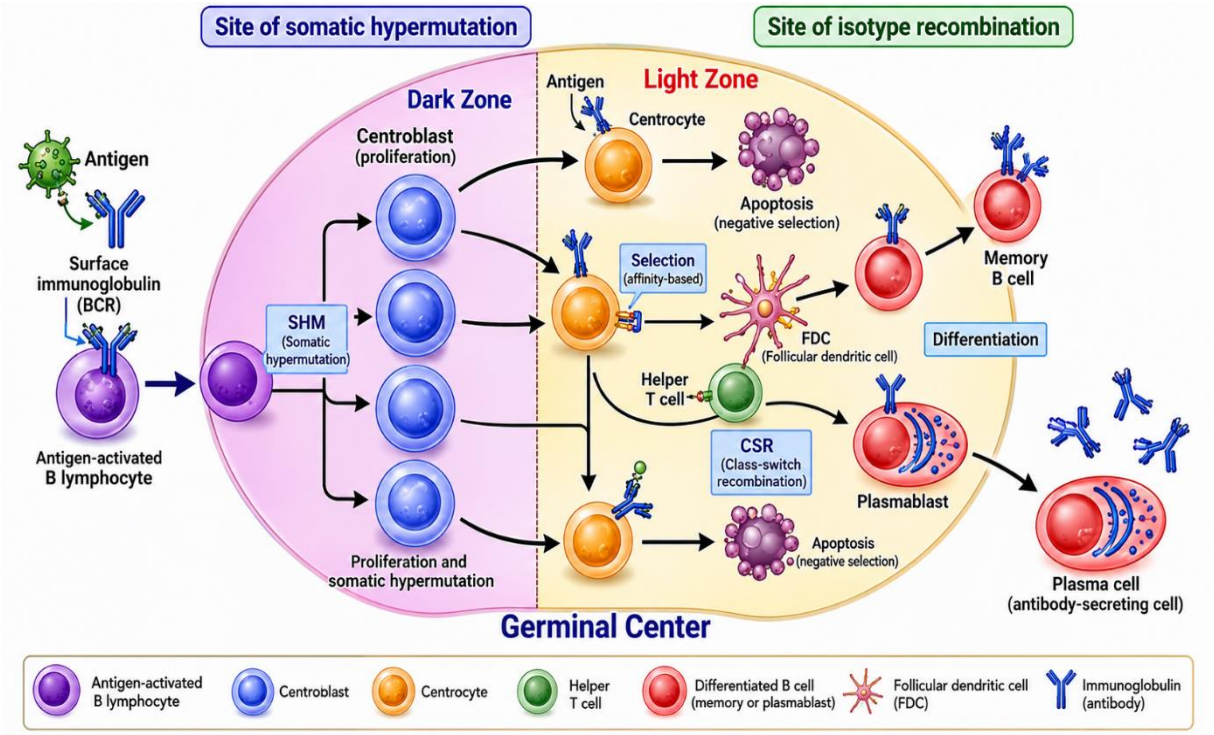


Figure 33. Schematic structure of an active germinal center.

2.2. B-Cell Selection

Following somatic hypermutation in the dark zone, centroblasts differentiate into centrocytes and migrate to the light zone. There, they compete for binding to antigens displayed on the surface of follicular dendritic cells. B cells expressing high-affinity B-cell receptors capture and internalize greater amounts of antigen and subsequently present antigen-derived peptides to T follicular helper cells. Interactions with T follicular helper cells provide essential survival and proliferation signals through molecules such as CD40L and cytokines including IL-4 and IL-21. B cells that successfully receive these signals are positively selected and survive. In contrast, B cells expressing low-affinity or nonfunctional receptors fail to obtain sufficient stimulation and undergo apoptosis. This process, known as affinity maturation, progressively enriches the immune response with B cells producing antibodies of increasingly higher affinity **(Figure 33)**.

2.3. Plasma Cells and Memory B Cells

Selected B cells can follow two major differentiation pathways. Some cells differentiate into plasma cells, specialized effector cells responsible for the synthesis and secretion of large quantities of antibodies. Plasma cells may remain within secondary lymphoid organs or migrate

to the bone marrow, where long-lived plasma cells can continue antibody production for extended periods.

Other selected B cells differentiate into memory B cells. These cells do not actively secrete antibodies but persist in the organism for many years, providing rapid and enhanced responses upon subsequent exposure to the same antigen. Memory B cells constitute the cellular basis of long-term humoral immunity and are essential for the effectiveness of vaccination. Within the germinal center, B cells may also undergo class-switch recombination (CSR), allowing the replacement of the IgM constant region with other immunoglobulin isotypes such as IgG, IgA, or IgE while preserving antigen specificity. Together, affinity maturation, plasma-cell differentiation, memory-cell generation, and class-switch recombination ensure the production of highly effective and long-lasting antibody-mediated immune responses. Within the germinal center, the first plasma cells generated during the primary immune response secrete soluble pentameric IgM antibodies rather than membrane-bound IgM directed against the encountered antigen. This difference is controlled at the level of pre-mRNA processing. Within the Cμ gene segment, two polyadenylation sites are present: one located at the end of the constant region and another situated upstream, immediately before the TM and CY exons that encode the transmembrane and cytoplasmic portions of the molecule. Naïve B lymphocytes use the distal polyadenylation site, allowing the TM and CY exons to be retained in the mature mRNA, resulting in the synthesis of membrane-bound IgM (BCR). In contrast, plasma cells utilize the proximal polyadenylation site, leading to the removal of the TM and CY exons during mRNA maturation and consequently producing secreted IgM antibodies **(Figure 34)**.

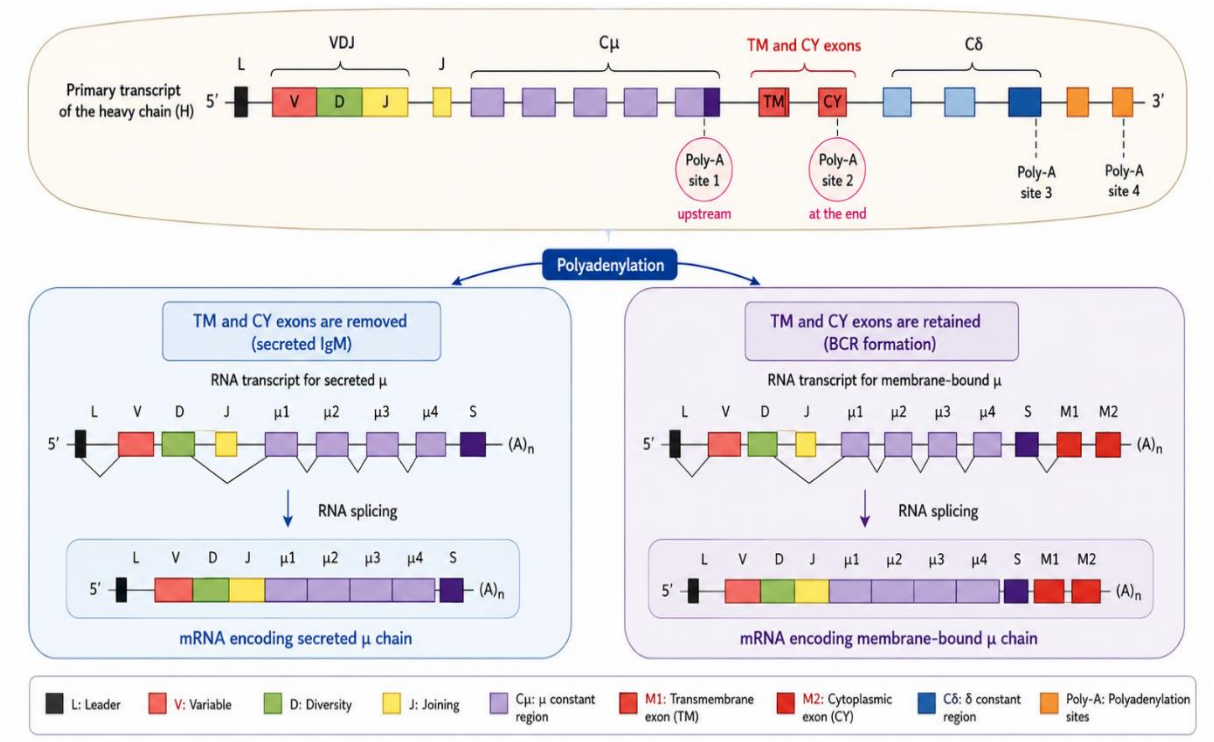


Figure 34. Immunoglobulin expression: membrane-bound and secreted forms.

3. Somatic Hypermutation (SHM)

Somatic hypermutation (SHM) is a specialized mechanism that introduces point mutations into the variable regions of immunoglobulin genes after B-cell activation. This process occurs primarily within the dark zone of germinal centers and plays a crucial role in the generation of high-affinity antibodies **(Figure 33)**. SHM is initiated by the enzyme activation-induced cytidine deaminase (AID), which converts cytosine residues into uracil within immunoglobulin variable-region genes. Subsequent DNA repair mechanisms introduce mutations at a very high frequency, approximately one mutation per 10^3 base pairs per cell division, a rate that is several orders of magnitude higher than the normal mutation rate of cellular genes. As a result, a population of B cells expressing slightly different antigen receptors is generated. Following migration to the light zone of the germinal center, these mutated B cells undergo affinity-based selection. Cells expressing receptors with increased affinity for the antigen receive survival signals from follicular dendritic cells and T follicular helper (Tfh) cells, whereas cells expressing low-affinity or nonfunctional receptors are eliminated by apoptosis. This process, known as affinity maturation, progressively enriches the immune response with B cells capable of producing antibodies of increasingly higher affinity, thereby improving the effectiveness of humoral immunity.

4. Isotype Switching (Class-Switch Recombination)

Class-switch recombination (CSR), also known as isotype switching, is a mechanism that enables activated B cells to change the constant region of the immunoglobulin heavy chain without altering antigen specificity. Consequently, antibodies maintain the same variable region and antigen-binding capacity while acquiring different biological functions. Initially, naïve B lymphocytes express IgM and IgD. Following activation, B cells may switch to the production of IgG, IgA, or IgE antibodies through recombination between specific switch (S) regions located upstream of heavy-chain constant-region genes. This process is also initiated by activation-induced cytidine deaminase (AID). During CSR, the DNA segment located between two selected switch regions is excised, bringing a new constant-region gene adjacent to the rearranged VDJ exon. Because the antigen-binding region remains unchanged, the resulting antibody retains the same specificity while acquiring distinct effector functions such as complement activation, placental transfer, mucosal protection, or allergic responses.

Class-switch recombination therefore allows the immune system to adapt antibody functions to different types of pathogens and immunological environments **(Figure 35)**.

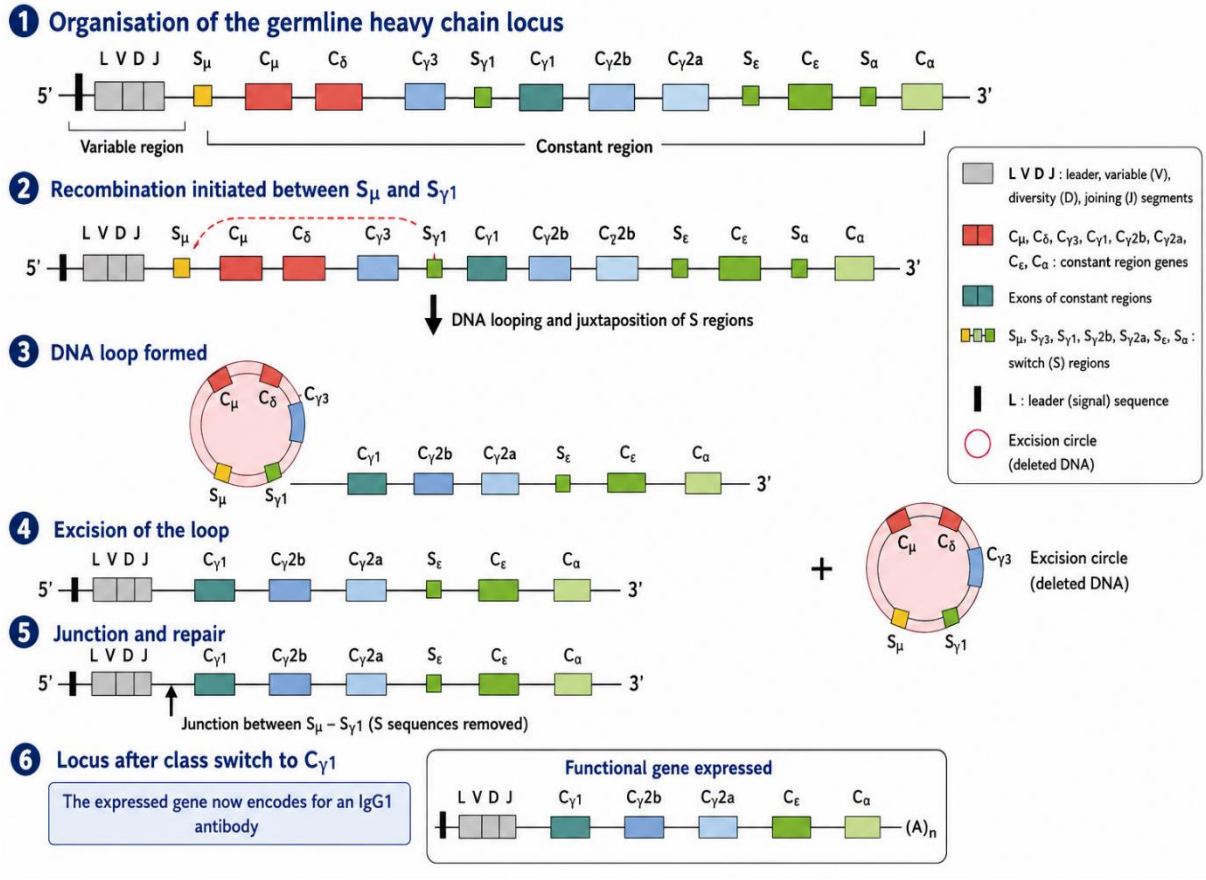


Figure 35. Mechanism of class-switch recombination in activated B lymphocytes.

5. Cytokine Regulation of Antibody Class Switching

The choice of immunoglobulin isotype produced during class-switch recombination is largely determined by cytokines secreted by T follicular helper cells and other immune cells. These cytokines regulate the transcription of specific heavy-chain constant-region genes and direct switching toward particular antibody classes. Different cytokines promote the production of distinct immunoglobulin isotypes:

- **IL-4** stimulates switching to **IgE** and certain subclasses of **IgG**.
- **IFN-γ** promotes switching to opsonizing and complement-fixing **IgG subclasses**.
- **TGF-β** is a major inducer of **IgA** production, particularly at mucosal surfaces.
- **IL-5** and **IL-6** support IgA production and plasma-cell differentiation.
- **IL-21**, produced by T follicular helper cells, enhances germinal-center responses, plasma-cell formation, and antibody production.

The cytokine environment therefore plays a fundamental role in shaping the quality and function of the humoral immune response. Through the coordinated action of cytokines, activated B cells generate antibody classes best suited to eliminate specific pathogens while minimizing tissue damage **(Figure 36)**.

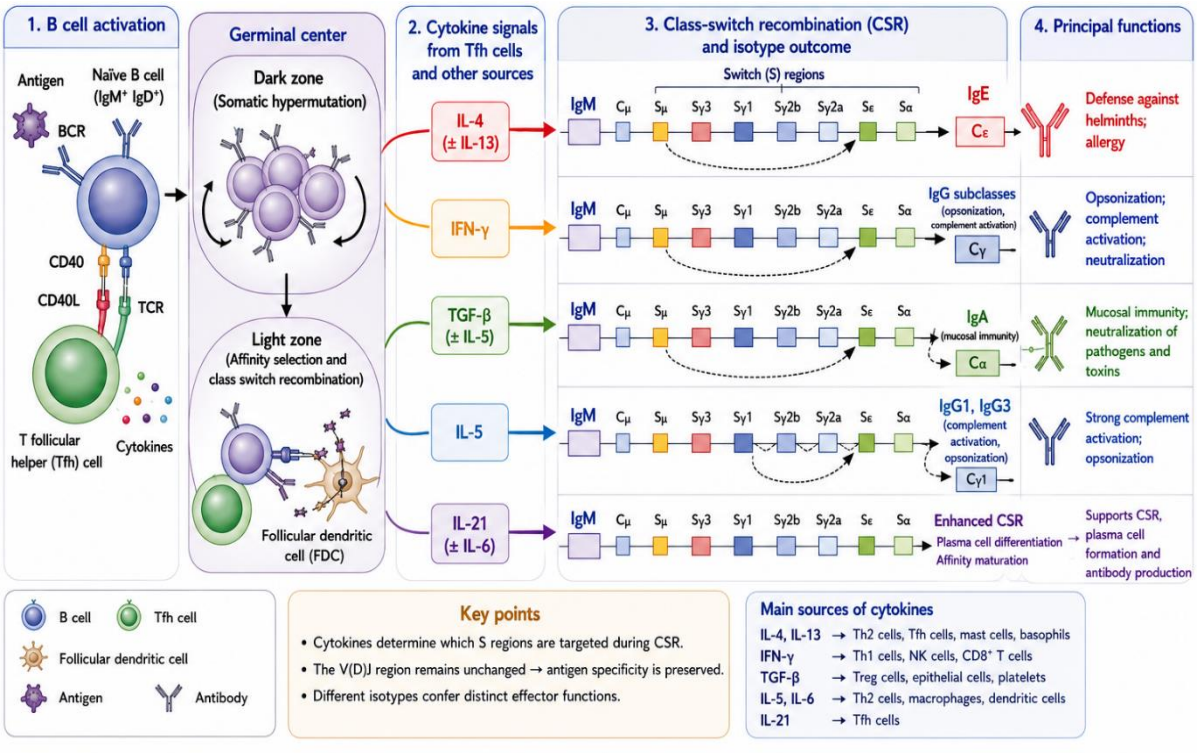


Figure 36. Cytokine-mediated regulation of immunoglobulin class switching.

Chapter 8. Immune Activation: Cellular Cooperation During Immune Responses

The initiation and development of adaptive immune responses require close cooperation between different immune cell populations. Effective immune activation depends on a series of coordinated cellular interactions involving antigen-presenting cells (APCs), T lymphocytes, and B lymphocytes. These interactions ensure antigen recognition, lymphocyte activation, clonal expansion, differentiation into effector cells, and the establishment of immunological memory.

1. Activation of T Lymphocytes

The molecular interaction between the T-cell receptor (TCR) and the major histocompatibility complex (MHC) expressed on antigen-presenting cells (APCs) initiates a cascade of intracellular events known as cellular signaling. These signaling pathways ultimately activate several transcription factors that regulate the expression of genes involved in T-cell activation and effector functions, including proliferation, cytokine production, and cellular differentiation.

1.1. Contact Between Antigen-Presenting Cells and $CD4^+$ T Lymphocytes

Dendritic cells (DCs) capture antigens in peripheral tissues and subsequently migrate to secondary lymphoid organs, where they become competent antigen-presenting cells capable of initiating an antigen-specific immune response. Naïve $CD4^+$ T lymphocytes are activated by antigenic peptide fragments associated with MHC class II molecules presented on the surface of dendritic cells. Among all antigen-presenting cells, dendritic cells possess the greatest capacity to activate naïve T cells and induce their differentiation into effector cells. For example, peptide–MHC complexes are present at levels approximately 10- to 100-fold higher on dendritic cells than on other APCs such as B lymphocytes and macrophages. When the TCR specifically recognizes its cognate antigen, a specialized contact zone known as the immunological synapse is formed **(Figure 37)**. This structure results from the dynamic reorganization of proteins on both the dendritic-cell membrane and the T-cell membrane. The immunological synapse is stabilized by numerous adhesion molecules, including CD2/LFA-3 (Lymphocyte Function-Associated Antigen-3), ICAM-1 (Intercellular Adhesion Molecule-1, CD54) and ICAM-2 (CD50)/LFA-1, as well as ICAM-3/DC-SIGN interactions. Together, these adhesion molecules strengthen the contact between the dendritic cell and the T lymphocyte, thereby facilitating the interaction between the TCR and the peptide–MHC complex.

Simultaneously, engagement of the co-receptors CD4 or CD8, which bind respectively to the β2 domain of MHC class II molecules or the α3 domain of MHC class I molecules, increases the affinity of the TCR for the peptide–MHC complex. This co-receptor engagement significantly enhances T-cell activation, increasing its efficiency by approximately one hundred-fold.

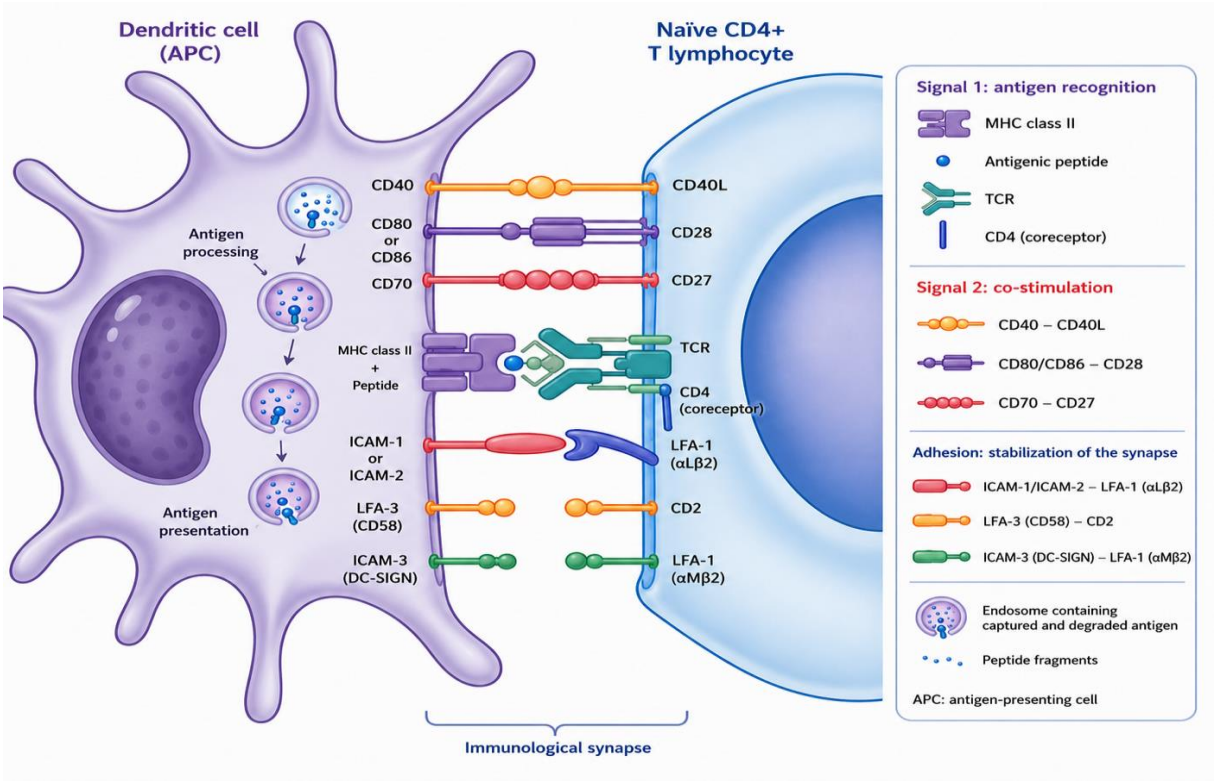


Figure 37. Formation of the immunological synapse between a dendritic cell and a naïve CD4⁺ T lymphocyte.

1.2. Signal Transduction and Signaling Pathways

Once the immunological synapse has been established between the dendritic cell and the T lymphocyte, T-cell activation requires different types of signals: an activation signal (Signal 1) and a co-stimulatory signal (Signal 2). Recognition of the peptide–MHC complex displayed by the dendritic cell constitutes Signal 1 of T-cell activation. The second signal is provided by co-stimulatory molecular interactions, primarily CD28/B7 (CD80/CD86) and CD40L/CD40. Together, these signals ensure complete activation of T lymphocytes, leading to proliferation, cytokine production, and differentiation into effector and memory cells **(Figure 29)**.

1.2.1. Activation Signal (Signal 1)

Binding of the T-cell receptor (TCR) to the peptide–MHC complex induces the formation of a lipid raft, a specialized membrane microdomain that concentrates signaling molecules. This event promotes the activation of the phosphatase CD45, a transmembrane protein that activates members of the Src-family tyrosine kinases, particularly Lck and Fyn, through dephosphorylation. In resting T cells, Lck is maintained in an inactive conformation through phosphorylation of its regulatory tyrosine residue. Following TCR engagement, CD45 dephosphorylates Lck associated with the CD4 co-receptor, resulting in a conformational change that activates the kinase. The phosphorylation state of Lck is regulated by the opposing activities of CD45, which activates Lck, and C-terminal Src kinase (Csk), which inhibits it. Similarly, Fyn, which is associated with the CD3 ζ chains, is activated through a CD45-dependent mechanism. Activated Lck and Fyn phosphorylate tyrosine residues within the Immunoreceptor Tyrosine-based Activation Motifs (ITAMs) located in the cytoplasmic domains of the CD3 complex. These phosphorylated ITAMs serve as docking sites for the tyrosine kinase ZAP-70 (Zeta-chain Associated Protein of 70 kDa). Upon binding, ZAP-70 becomes activated and subsequently phosphorylates the transmembrane adaptor protein LAT (Linker for Activation of T Cells) and the cytosolic adaptor protein SLP-76 (SH2-domain-containing Leukocyte Protein of 76 kDa).

LAT and SLP-76 play central roles in assembling signaling complexes and coordinating multiple downstream signaling pathways. Their phosphorylation on numerous tyrosine residues creates binding sites for various signaling proteins, leading to activation of pathways dependent on phospholipase C-γ1 (PLC-γ1) and phosphatidylinositol 3-kinase (PI3K). Following TCR activation, PLC-γ1 is recruited to a signaling complex containing LAT, SLP-76, and Vav1, where it is phosphorylated and activated by Itk (IL-2-inducible T-cell kinase). Activated PLC-γ1 hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) into two important second messengers: inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 diffuses into the cytoplasm and triggers the release of calcium ions (Ca^{2+}) from intracellular stores, leading to activation of the phosphatase calcineurin and the transcription factor NFAT (Nuclear Factor of Activated T Cells). In parallel, DAG remains associated with the plasma membrane and activates protein kinase Cθ (PKCθ) and the Ras/MAPK pathway, resulting in the activation of additional transcription factors such as NF-κB and AP-1. The coordinated activation of NFAT, NF-κB, and AP-1 induces the transcription of numerous genes involved in T-cell activation,

including those encoding interleukin-2 (IL-2), cytokine receptors, and proteins required for proliferation, survival, and differentiation **(Figure 38)**.

a) Calcium Signaling Pathway

IP_3 binds to receptors located on the membrane of the endoplasmic reticulum, triggering the opening of calcium channels and the release of calcium ions into the cytoplasm. This rapid but transient increase in intracellular calcium concentration induces the opening of plasma membrane calcium channels known as CRAC (Calcium Release-Activated Calcium) channels. The opening of CRAC channels allows sustained calcium influx from the extracellular environment, thereby maintaining elevated intracellular calcium levels. Calcium ions subsequently bind to calmodulin, forming a calcium–calmodulin complex. This complex activates calcineurin, a serine/threonine phosphatase that dephosphorylates the transcription factor NFAT (Nuclear Factor of Activated T Cells), enabling its translocation into the nucleus **(Figure 38)**. Within the nucleus, NFAT cooperates with other transcription factors to regulate the expression of numerous genes, including the IL-2 gene. In addition, calcium signaling contributes to cytoskeletal reorganization, promoting T-cell immobilization during contact with an antigen-presenting cell and facilitating the formation of a mature immunological synapse.

b) PKCθ Signaling Pathway

Protein kinase C theta (PKCθ) is recruited to the plasma membrane of the T lymphocyte at the site of the immunological synapse. Its activation is promoted by both diacylglycerol (DAG) and Lck. Once activated, PKCθ stimulates another kinase known as IKK (IκB kinase). IKK phosphorylates IκB, the inhibitory protein associated with the transcription factor NF-κB (Nuclear Factor-kappa B).

Phosphorylation of IκB results in its degradation, releasing NF-κB and allowing its translocation into the nucleus. Activation of NF-κB, together with the transcription factor complex AP-1, induces the expression of genes involved in T-cell survival, differentiation, homeostasis, and effector functions **(Figure 38)**.

c) MAP Kinase Signaling Pathway

Following TCR engagement, the adaptor protein LAT recruits and binds the adaptor molecule GRB2 (Growth Factor Receptor-Bound Protein 2). GRB2 is constitutively associated with SOS

(Son of Sevenless), a guanine nucleotide exchange factor (GEF) that activates the small GTPase Ras.

Activated Ras initiates the MAP kinase cascade by stimulating MAP kinase kinase kinases (MAPKKKs), ultimately leading to the activation and nuclear translocation of ERK1 and ERK2 (Extracellular Signal-Regulated Kinases). ERK kinases activate the transcription factor Elk-1, which contributes to the formation of the AP-1 transcription complex, composed primarily of c-Jun and c-Fos. Co-stimulation through CD28 further enhances this pathway by activating Vav-1, which promotes phosphorylation of c-Jun by JNK (c-Jun N-terminal kinase). Phosphorylated c-Jun subsequently associates with c-Fos to form the functional AP-1 complex **(Figure 38)**.

d) PI3K Signaling Pathway

A fourth signaling pathway involves the lipid kinase phosphatidylinositol 3-kinase (PI3K). This enzyme phosphorylates membrane-bound PIP_2 (phosphatidylinositol 4,5-bisphosphate) to generate PIP_3 (phosphatidylinositol 3,4,5-trisphosphate). Following TCR activation, PI3K is recruited to the plasma membrane, where it binds to phosphorylated tyrosine residues within the cytoplasmic tails of co-stimulatory molecules such as CD28 and ICOS (Inducible Co-Stimulator). PIP_3 serves as a docking platform for proteins containing Pleckstrin Homology (PH) domains, including the serine/threonine kinase AKT (Protein Kinase B). AKT plays a central role in regulating cell growth, proliferation, survival, metabolism, and cytokine production. Members of the Tec kinase family, including Itk, also contain PH domains and can be recruited to the plasma membrane through PIP_3. These kinases activate PLC-γ1, thereby linking the PI3K pathway to calcium signaling. The coordinated action of the transcription factors NFAT, AP-1, and NF-κB ultimately induces the transcription of numerous genes, including those encoding IL-2, the α-chain of the IL-2 receptor (CD25), and other molecules involved in T-cell activation and proliferation. TCR signaling also induces the expression of CD40L (CD154) on the surface of activated T lymphocytes. Binding of CD40L to CD40 expressed on antigen-presenting cells enhances the expression of the co-stimulatory molecules CD80 and CD86, thereby amplifying the co-stimulatory signal.

1.2.2. Co-stimulatory Signal (Signal 2)

The signal delivered through the TCR alone is insufficient for complete T-cell activation. Indeed, TCR engagement in the absence of co-stimulation generally results in apoptosis or a state of anergy, in which T lymphocytes become unable to produce IL-2 or proliferate.

Optimal activation of T lymphocytes therefore requires a second, antigen-independent signal known as the co-stimulatory signal. The interaction between CD28, constitutively expressed on T cells, and its ligands B7-1 (CD80) and B7-2 (CD86) expressed on antigen-presenting cells strongly promotes cell survival, clonal expansion, cytokine production, and differentiation. CD28 signaling significantly lowers the threshold required for T-cell activation.

Co-stimulatory molecules can be classified according to their effects on lymphocyte activation. Molecules such as CD28 and LFA-1 deliver positive signals that enhance activation following ligand binding. In contrast, CTLA-4 (Cytotoxic T-Lymphocyte Antigen-4) delivers inhibitory signals that negatively regulate T-cell responses. Expression of CTLA-4 is primarily restricted to activated and memory T cells. Although CD28 plays a major role in co-stimulation, some immune responses remain relatively unaffected in its absence, indicating that additional co-stimulatory pathways exist. One important alternative pathway involves the interaction between CD40L (CD154) expressed on activated T cells and CD40 expressed on antigen-presenting cells. This interaction contributes significantly to co-stimulation and enhances T-cell activation and immune responses.

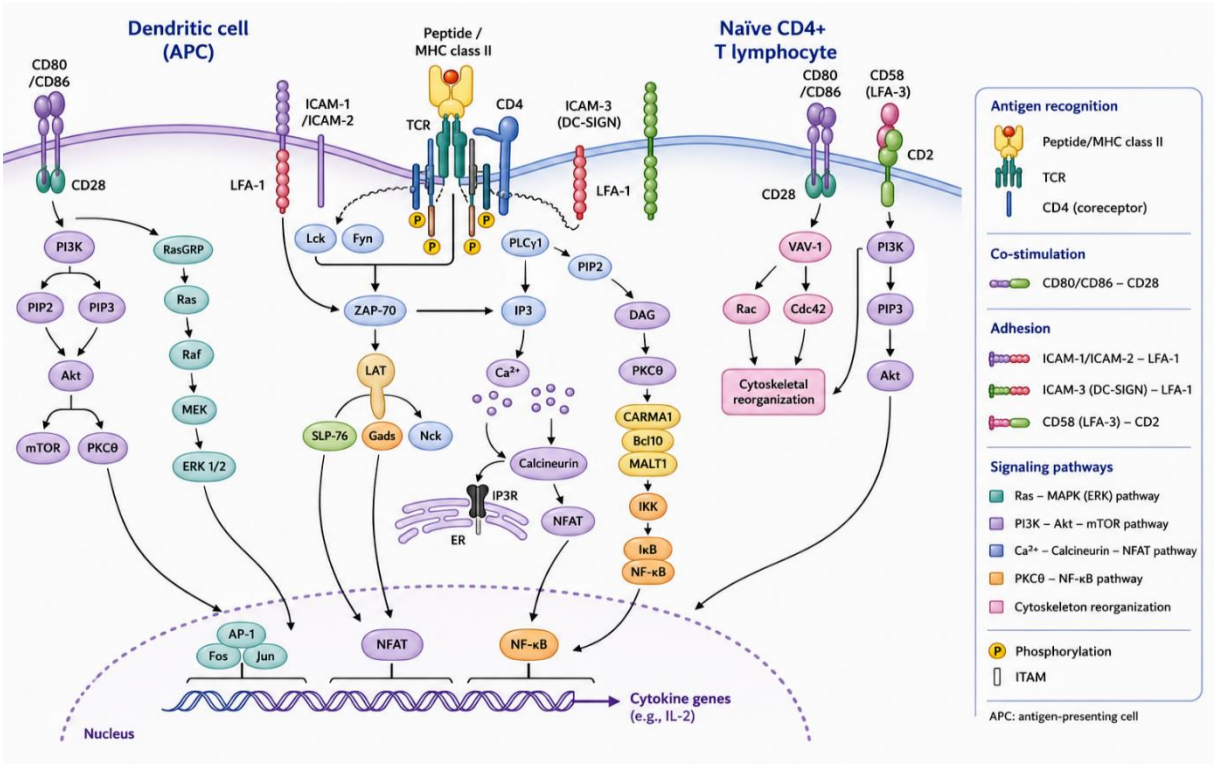


Figure 38. TCR signaling pathways initiated following antigen recognition.

1.3. Differentiation of T Helper (Th) Lymphocytes

The different subsets of T helper (Th) lymphocytes are characterized by their specific cytokine secretion profiles, which enable them to orchestrate immune responses against distinct classes of pathogens. Each subset is capable of promoting its own development while simultaneously inhibiting the differentiation of alternative Th lineages through the cytokines it produces.

Numerous factors influence differentiation toward a particular T-helper lineage, including the cytokine environment, the nature and dose of the antigen, the type of antigen-presenting cell (APC), and its degree of maturation or activation. Among these factors, cytokines are the best characterized and play a central role in directing lineage commitment. Following activation by dendritic cells, naïve $CD4^+$ T lymphocytes can differentiate into several effector subsets, including Th1, Th2, and Th17 cells.

1.3.1. Th1 Lymphocytes

Following activation by antigen-presenting cells through antigen recognition and co-stimulatory signals, activated $CD4^+$ T lymphocytes differentiate into Th1 cells under the influence of interleukin-12 (IL-12). This cytokine is secreted by activated APCs such as

macrophages, monocytes, and dendritic cells that have encountered intracellular pathogens. Binding of IL-12 to its receptor activates Janus kinase 2 (JAK2) and subsequently Signal Transducer and Activator of Transcription 4 (STAT4), both of which are required for optimal Th1 differentiation. In parallel, interferon-gamma (IFN-γ) binds to its receptor and activates JAK1 and JAK2, leading to activation of STAT1. Activated STAT1 induces expression of T-bet, the master transcription factor responsible for Th1 lineage commitment. T-bet promotes chromatin remodeling at the IFN-γ gene locus, facilitating its transcription. It also induces expression of the IL-12 receptor β2 chain (IL-12Rβ2), thereby increasing cellular responsiveness to IL-12 produced by APCs and enhancing STAT4 activation. Together, STAT4 and T-bet stimulate IFN-γ production, which further reinforces Th1 differentiation through a positive feedback loop involving STAT1 **(Figure 39)**. At the same time, T-bet suppresses expression of GATA-3, the master transcription factor of Th2 differentiation, as well as the IL-4 gene, thereby inhibiting development of the Th2 lineage.

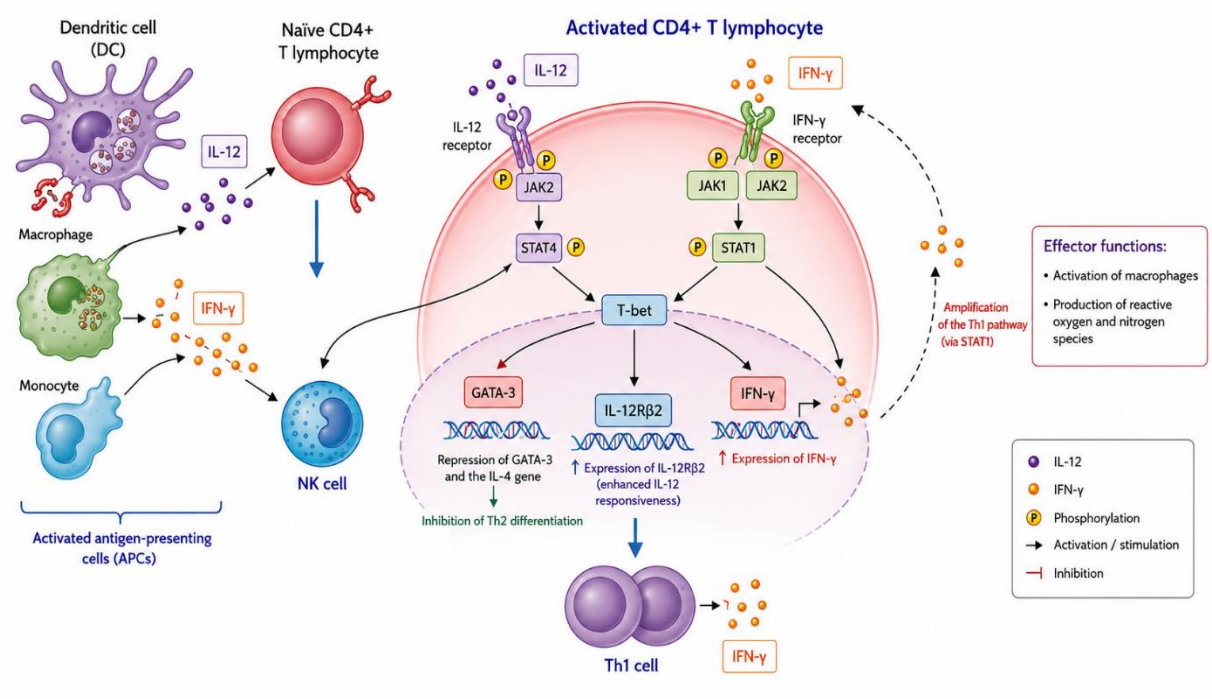


Figure 39. Development and differentiation of Th1 lymphocytes.

Functions of Th1 Cells

Th1 lymphocytes primarily secrete IFN-γ, IL-2, TNF-α, and TNF-β. These cytokines promote cell-mediated immunity, which is essential for protection against intracellular pathogens such as viruses, intracellular bacteria, and certain intracellular parasites. IFN-γ is the hallmark cytokine of Th1 cells and plays a central role in macrophage activation. It enhances macrophage

phagocytic activity, increases expression of MHC class I and class II molecules, stimulates IL-12 production, and promotes the generation of nitric oxide (NO) and superoxide anions, all of which contribute to the elimination of intracellular microorganisms. In addition, IFN-γ promotes the production of opsonizing IgG antibodies, facilitating phagocytosis by macrophages, and complement-fixing antibodies, enhancing pathogen destruction through complement activation. Th1-derived cytokines also support the differentiation and activation of $CD8^+$ cytotoxic T lymphocytes (CTLs), strengthening cellular immune responses. Despite their essential protective functions, Th1 cells are also involved in certain pathological immune reactions. In particular, they contribute to delayed-type hypersensitivity (DTH) responses, in which excessive production of IFN-γ and TNF-β can result in intense inflammation and tissue damage.

1.3.1. Th2 Lymphocytes

Differentiation of T helper cells into the Th2 lineage requires interleukin-4 (IL-4), which can be produced by Th2 cells themselves. Other cellular sources of IL-4 include mast cells, basophils, eosinophils, and natural killer T (NKT) cells. Unlike the IL-12 receptor, the IL-4 receptor is constitutively expressed on naïve T lymphocytes. IL-4 inhibits IL-12 production by dendritic cells and suppresses the expression of the IL-12 receptor β2 chain (IL-12Rβ2) on $CD4^+$ T cells, thereby preventing differentiation toward the Th1 lineage. Activation of naïve $CD4^+$ T lymphocytes induces the production of IL-4 and the transcription factor GATA-3 (GATA-binding protein 3). Binding of IL-4 to its receptor activates the transcription factor STAT6. In cooperation with NFAT, AP-1, and NF-κB, STAT6 promotes the expression of IL-4 and GATA-3. Furthermore, STAT6 modifies the chromatin structure of the IL-4 gene locus, facilitating access by transcription factors. GATA-3 reinforces Th2 differentiation by inducing the transcription of IL-5 and IL-13, while simultaneously inhibiting Th1 development through suppression of IL-12 receptor expression.

- **Functions of Th2 Cells**

Th2 lymphocytes are primarily involved in the elimination of extracellular pathogens, particularly helminths and certain protozoan parasites. They are characterized by the secretion of IL-4, IL-5, and IL-13. IL-4 stimulates the proliferation and differentiation of B lymphocytes into plasma cells producing IgE antibodies, which play a critical role in mast cell degranulation. IL-5 is the principal cytokine responsible for the differentiation, activation, and recruitment of

eosinophils, cells that are essential for antiparasitic immunity. IL-13 contributes to mucus production, epithelial barrier protection, and alternative macrophage activation. Although Th2 responses are protective against parasites, excessive activation of this pathway can lead to allergic diseases. IgE antibodies and mast cells generated following allergen-specific Th2 responses are major contributors to allergic reactions and asthma **(Figure 40)**.

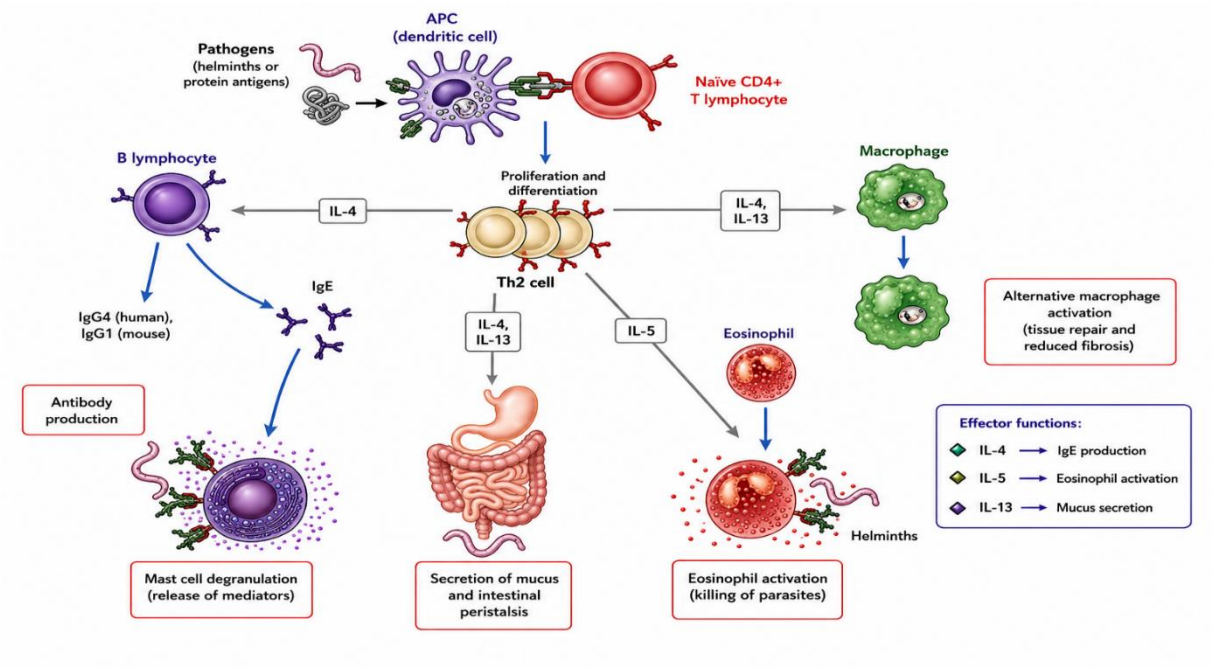


Figure 40. Effector functions of Th2 lymphocytes.

1.3.3. Th17 Lymphocytes

The combination of transforming growth factor-beta (TGF-β) and interleukin-6 (IL-6) is essential for the differentiation of naïve $CD4^+$ T cells into the Th17 lineage. IL-6 is produced by numerous cell types, including dendritic cells, monocytes, macrophages, mast cells, B lymphocytes, activated T cells, fibroblasts, endothelial cells, and keratinocytes. In contrast, the precise cellular source of TGF-β during Th17 differentiation remains less clearly defined. IL-6 activates STAT3, which cooperates with RORγt (Retinoic Acid Receptor-Related Orphan Receptor Gamma t), the master transcription factor of the Th17 lineage, to induce transcription of the IL-17 and IL-21 genes. Complete induction of RORγt requires the presence of TGF-β, which simultaneously suppresses differentiation toward both the Th1 and Th2 lineages. In addition, IL-21, together with TGF-β, can promote Th17 differentiation through RORγt activation. Experimental studies have also shown that IL-17 can directly inhibit Th1

differentiation by suppressing the expression of T-bet, the master regulator of Th1 development **(Figure 33)**.

Functions of Th17 Cells

Th17 cells play an important role in the elimination of pathogens that require a strong inflammatory response and are not effectively controlled by Th1 or Th2 mechanisms. Their characteristic cytokines are IL-17, IL-21, and IL-22. These cytokines stimulate fibroblasts, macrophages, endothelial cells, and epithelial cells to produce numerous pro-inflammatory mediators, including IL-1, IL-6, TNF-α, nitric oxide (NO), metalloproteinases, and chemokines. These mediators promote the recruitment and activation of neutrophils, which themselves can produce IL-17, thereby amplifying the inflammatory response. The appearance of Th17 cells at sites of inflammation occurs rapidly. Through the induction of chemokine production, Th17 cells establish an important link between innate and adaptive immunity and facilitate the recruitment of additional immune cell populations to inflamed tissues. While essential for host defense against extracellular bacteria and fungi, Th17 cells are also implicated in the pathogenesis of infectious diseases, autoimmune disorders, alloreactive responses, allergic diseases, and cancer **(Figure 41)**.

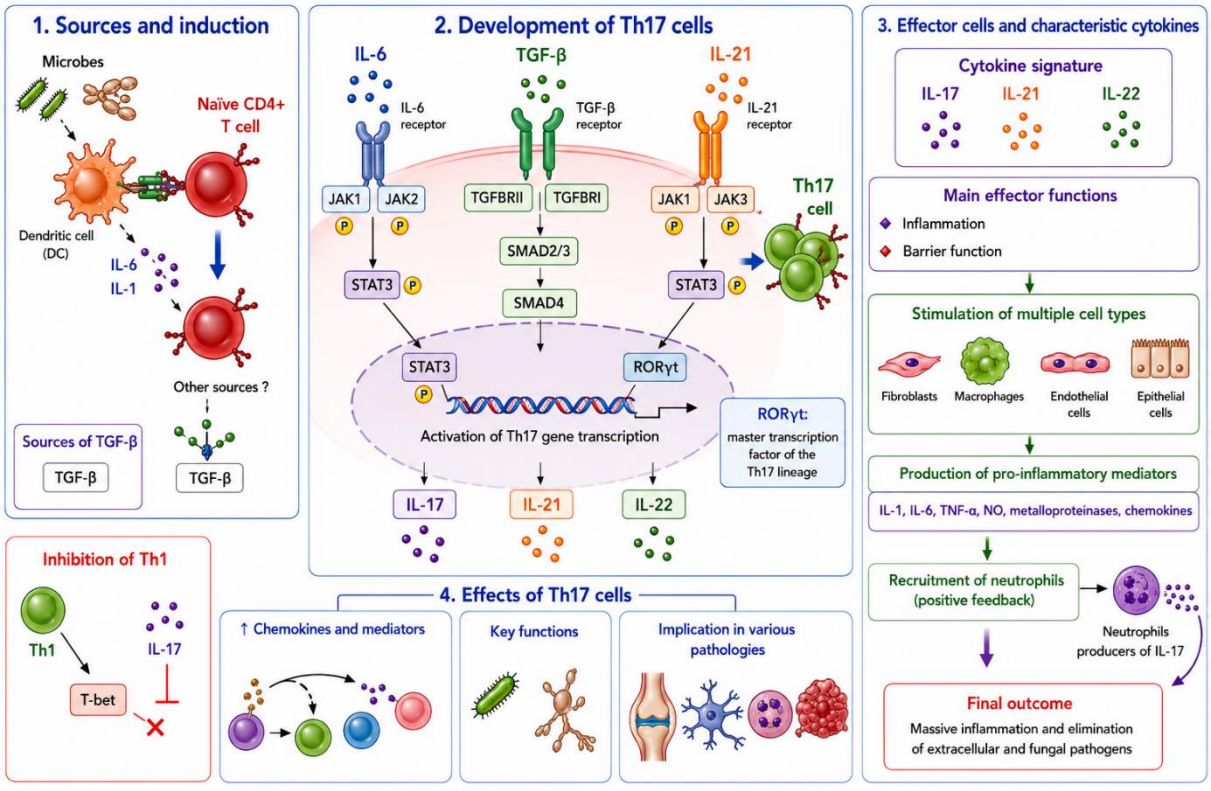


Figure 41. Development, functions and effector roles of Th17 lymphocytes.

1.3.4. Memory T Lymphocytes

Once an immune response has been successfully controlled, the majority of activated T lymphocytes undergo apoptosis. However, a small population persists and forms the memory T-cell compartment. Memory T cells provide long-term protection and are responsible for the rapid and robust secondary immune response observed upon re-exposure to previously encountered antigens. Two major memory T-cell subsets have been identified: effector memory T cells (TEM) and central memory T cells (TCM). Effector memory T cells migrate preferentially to inflamed peripheral tissues, where they exert immediate effector functions. In contrast, central memory T cells preferentially recirculate through secondary lymphoid organs and possess limited immediate effector activity. TCM cells constitutively express the homing receptors CCR7 and CD62L, which direct their migration to T-cell zones within secondary lymphoid organs. Compared with naïve T cells, TCM cells:

- Exhibit greater sensitivity to antigenic stimulation.
- Depend less on co-stimulatory signals.
- Express higher levels of CD40L.

Following TCR engagement, TCM cells primarily produce IL-2, undergo rapid proliferation, and differentiate into effector cells capable of producing large amounts of IFN-γ or IL-4. TEM cells express a broad repertoire of chemokine receptors and adhesion molecules that facilitate migration into inflamed tissues. They display rapid effector functions and can be activated by antigens presented by non-professional antigen-presenting cells. The relative proportions of TCM and TEM differ among T-cell subsets. Central memory cells predominate among helper T cells (Th cells), whereas effector memory cells are more abundant among cytotoxic T lymphocytes (CTLs).

2. Activation of B Lymphocytes and germinal center

2.1. Activation of B Lymphocytes

Antigen-dependent maturation of B lymphocytes differs from that of T lymphocytes for several reasons. First, apart from memory B cells, activated B cells have a single ultimate fate: differentiation into plasma cells. In addition, B-cell maturation involves rearrangement and selection among the different immunoglobulin (Ig) classes, whereas T lymphocytes express only a single class of T-cell receptor (TCR).

B lymphocytes are located within the primary follicles of secondary lymphoid organs. They do not have direct access to most antigens. Instead, antigens reach them either through lymphatic drainage from infected tissues or via dendritic cells. Indeed, dendritic cells migrating from sites of infection to present antigens to T lymphocytes often carry numerous native microbial antigens on their surface. B-cell activation can occur through different mechanisms depending on whether T-cell help is involved.

2.1.1. T-Independent Activation

Activation of B lymphocytes by certain pathogens occurs independently of T-cell assistance. These T-independent antigens primarily induce the production of low-affinity IgM antibodies. Two major mechanisms of T-independent activation have been described **(Figure 42)**:

2.1.1.1. Type 1 T-Independent Activation

This form of activation requires signaling through both the B-cell receptor (BCR) (Signal 1: antigen–BCR interaction) and mitogenic receptors shared by all B cells (Signal 2: antigen–mitogenic receptor interaction). This mechanism results in polyclonal B-cell activation. A classic example is bacterial lipopolysaccharide (LPS), which activates B cells through Toll-like receptors.

2.1.1.2. Type 2 T-Independent Activation

In this case, activation occurs exclusively through the BCR, which recognizes highly repetitive antigenic determinants. Extensive cross-linking of BCR molecules induces activation and leads to monoclonal B-cell expansion. Typical examples include pneumococcal polysaccharides.

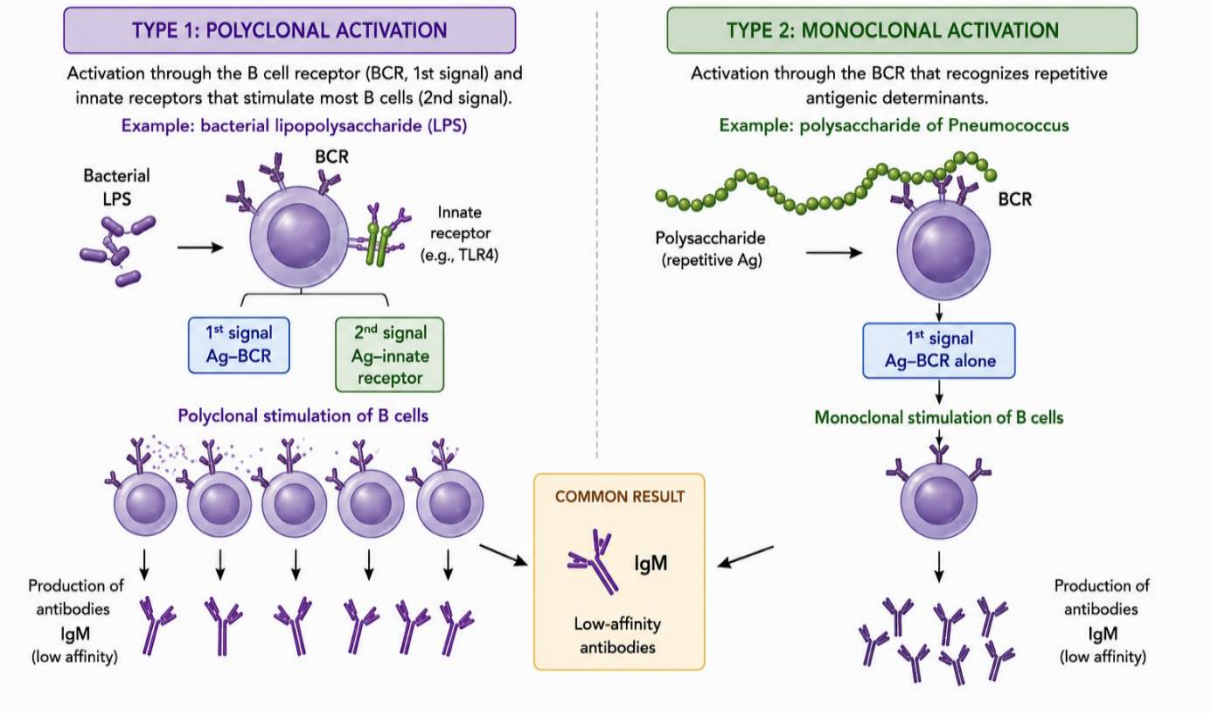


Figure 42. T-independent activation of B lymphocytes. Type 1 activation involves both BCR and mitogenic receptors, whereas Type 2 activation results from extensive BCR cross-linking by repetitive antigens.

2.1.2. T-Dependent Activation

As with T-cell activation, complete activation of B lymphocytes requires two signals. The first signal is delivered through antigen binding to the B-cell receptor (BCR). This interaction triggers internalization of the antigen–BCR complex through endocytosis, followed by antigen degradation within the endosomal compartment. The resulting peptide fragments are loaded onto MHC class II molecules, allowing the B lymphocyte to function as an antigen-presenting cell. Simultaneously, BCR engagement activates intracellular signaling pathways. Cross-linking of BCR molecules induces phosphorylation of the conserved tyrosine residues present within the ITAM motifs of the associated signaling proteins CD79a (Igα) and CD79b (Igβ). These phosphorylations are mediated by Src-family protein tyrosine kinases, including Fyn, Lyn, and Blk, whose activation depends on the phosphatase CD45. Activated Src-family kinases recruit the tyrosine kinase Syk, which translocates from the cytoplasm to the plasma membrane and binds phosphorylated ITAM motifs. Another kinase, Bruton's tyrosine kinase (Btk), is recruited simultaneously. Syk performs a function analogous to that of ZAP-70 in T lymphocytes. Activated Syk phosphorylates the adaptor protein BLNK (B-cell linker protein), which serves as a scaffold for the recruitment of signaling molecules including PLCγ2, Vav,

and Btk**.** BLNK activates the MAP kinase pathway through recruitment of the adaptor proteins Shc and Grb2, which activate SOS, a guanine nucleotide exchange factor for Ras. Activated Ras initiates the MAP kinase cascade, ultimately leading to activation of transcription factors involved in B-cell proliferation and differentiation. At the same time, BLNK links PLCγ2 to Btk. Activated PLCγ2 hydrolyzes PIP_2 (phosphatidylinositol 4,5-bisphosphate) into IP_3 (inositol 1,4,5-trisphosphate) and DAG (diacylglycerol). These second messengers trigger signaling pathways similar to those observed during T-cell activation, leading to calcium mobilization, PKC activation, and transcription factor activation **(Figure 43)**.

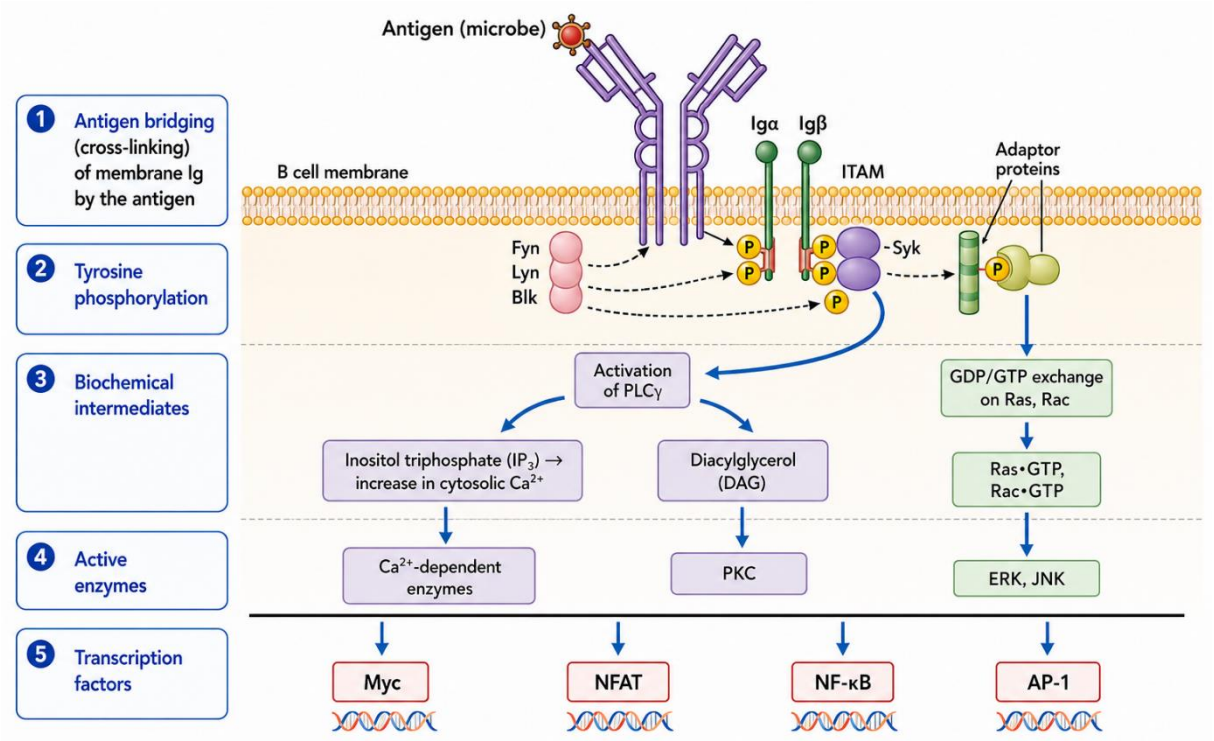


Figure 43. Intracellular signaling pathways activated following BCR engagement.

Co-stimulatory signals are also essential for complete B-cell activation. These signals are amplified by the B-cell co-receptor complex composed of CD19, CD21, and CD81. Activation induces expression of numerous molecules, including cytokine receptors **(**IL-4R, IL-6R, IL-10R, and IFN-γR**)**, co-stimulatory molecules **(**CD80 and CD86**),** MHC class II molecules, and adhesion molecules such as ICAM-1 and CD58. The activated B cell is thus prepared to interact with helper T cells as a professional antigen-presenting cell. Antigen internalization and processing enable presentation of peptide–MHC II complexes to antigen-specific helper T cells. IL-4 further enhances MHC II expression, increasing antigen presentation efficiency. Induction

of B7 (CD80/CD86) expression on activated B cells ultimately provides all of the properties required for professional antigen presentation.

2.2. Germinal Center Reaction

Among the B cells activated during an immune response, some differentiate locally into short-lived plasma cells that survive for approximately two weeks and secrete low-affinity IgM antibodies, characteristic of the primary antibody response.

Although these antibodies display relatively low affinity, they provide an important first line of defense against infection and generate circulating immune complexes that can be captured by follicular dendritic cells (FDCs) within secondary lymphoid organs. Other activated B cells migrate into B-cell zones of lymphoid tissues and initiate the formation of germinal centers, transforming primary follicles into secondary follicles. Germinal centers appear a few days after antigen exposure and persist for several weeks **(Figure 44)**.

Two major processes occur within germinal centers:

1. Affinity maturation through somatic hypermutation.
2. Class-switch recombination (isotype switching).

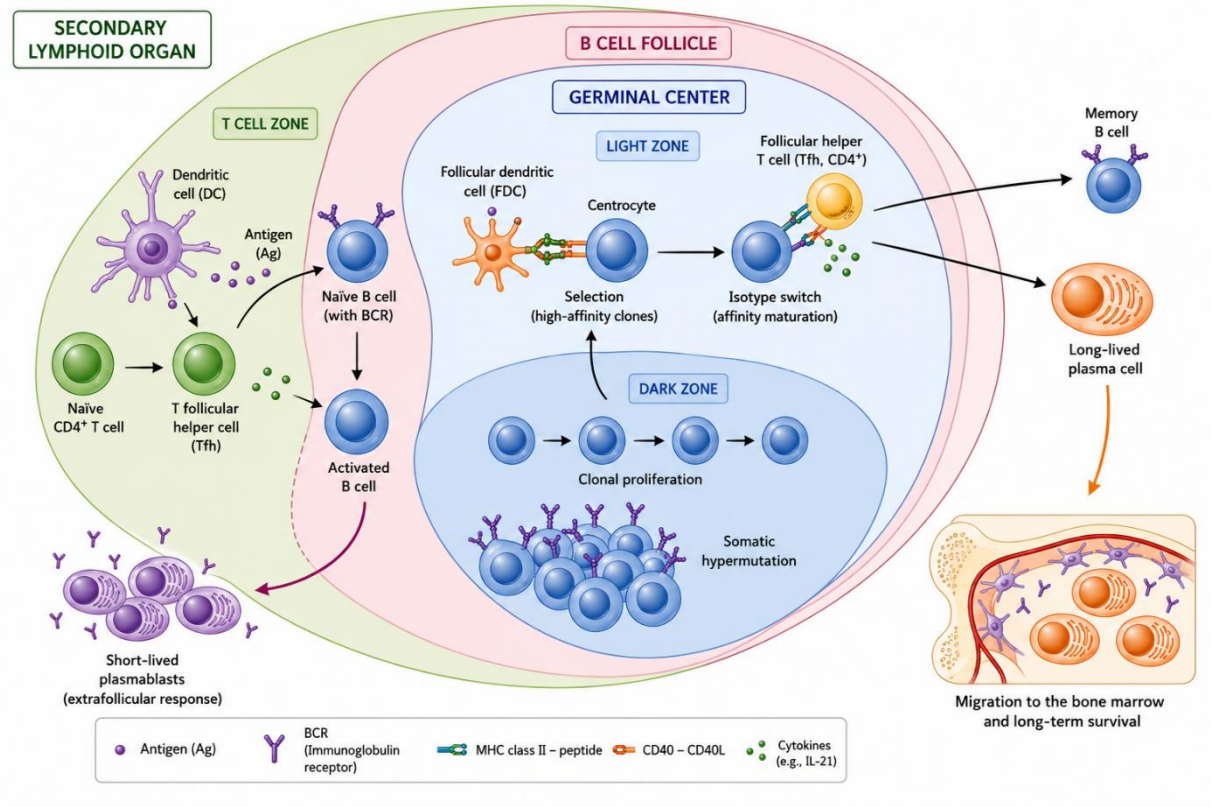


Figure 44. Germinal center reaction.

2.3. Affinity Maturation and Selection

The interaction between CD40 on B cells and CD154 (CD40L) on follicular helper T cells promotes intense proliferation of centroblasts and induces somatic hypermutation within the variable regions of immunoglobulin genes. These mutations occur in the V(D)J regions encoding the variable domains of heavy and light chains. Typically, 10–30 nucleotides may be altered relative to the original sequence of the naïve B cell, leading on average to changes in up to ten amino acids.

During this phase, transcription of immunoglobulin genes is transiently interrupted, and centroblasts temporarily lose surface immunoglobulin expression. Centroblasts subsequently migrate into the light zone of the germinal center and differentiate into centrocytes, which re-express membrane immunoglobulins. Antigens are presented to them as immune complexes displayed on follicular dendritic cells. The susceptibility of centrocytes to apoptosis plays a critical role in selecting B cells expressing high-affinity antibodies. Only centrocytes capable of binding antigen with high affinity receive survival signals. These signals are provided primarily through CD40–CD40L interactions with follicular helper T cells. In contrast, low-affinity centrocytes are eliminated through apoptosis. These cells express CD95 (Fas), and

engagement with Fas ligand (FasL) triggers programmed cell death. Follicular helper T cells secrete cytokines such as IL-2, IL-4, IL-10, and IL-13, which promote B-cell proliferation and survival while also determining the immunoglobulin isotype produced. Through class-switch recombination, activated B cells replace the constant region of the heavy chain while preserving antigen specificity. This process enables the production of IgG, IgA, or IgE antibodies instead of IgM. Different cytokines favor different isotypes **(Figure 45)**:

- IFN-γ promotes switching toward specific IgG subclasses.
- IL-4 favors switching to IgE and certain IgG subclasses.
- TGF-β promotes switching to IgA.

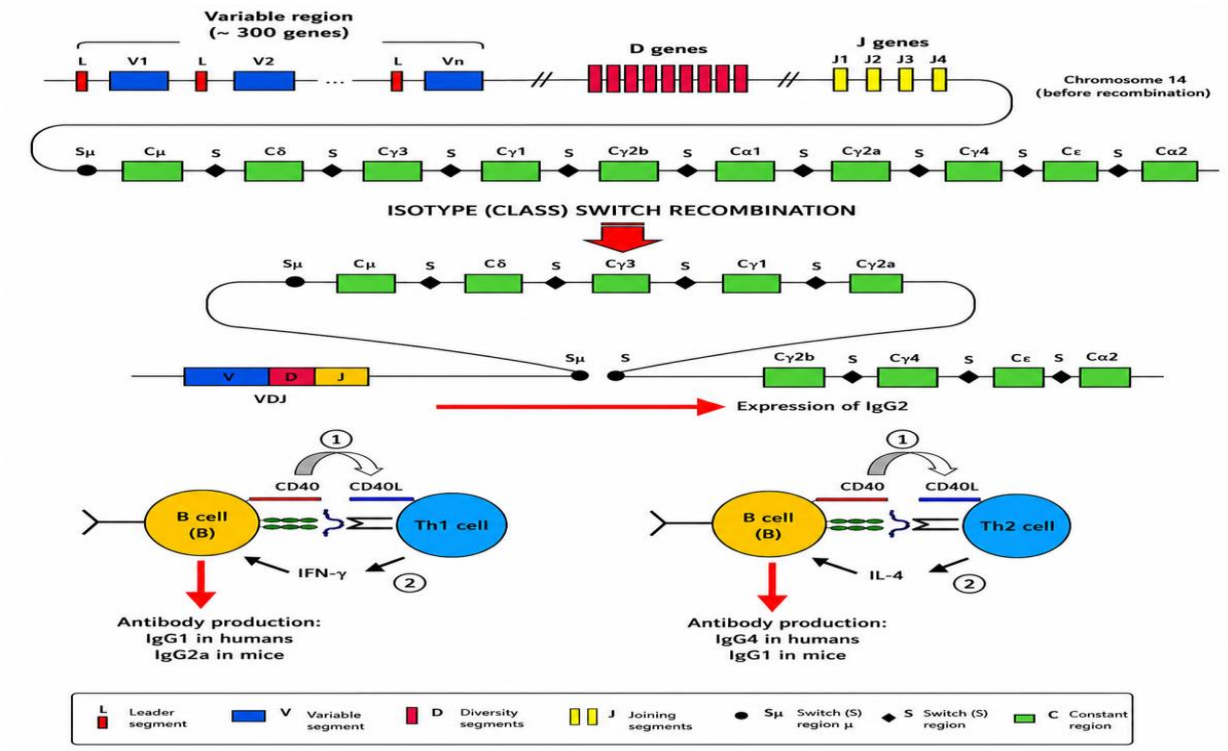


Figure 45. Immunoglobulin Class-Switch recombination and cytokine-mediated regulation of antibody isotype production.

2.4. Differentiation of B Lymphocytes

B-cell differentiation is regulated through the induction and activation of various transcription factors that control the development, survival, and specialized functions of mature B cells. Following affinity maturation and class switching, germinal-center B cells differentiate into either:

- Memory B cells, which provide long-term immunological memory.
- Long-lived plasma cells, which migrate primarily to the bone marrow and continuously secrete high-affinity antibodies for extended periods **(Figure 46)**.

2.4.1. Plasma Cells

Two types of plasma cells can be generated during an immune response: short-lived plasma cells and long-lived plasma cells.

Short-lived plasma cells are produced during T-independent immune responses and during the early phases of T-dependent responses within extrafollicular B-cell foci. These cells rapidly secrete antibodies, primarily IgM, providing immediate protection against invading pathogens. Long-lived plasma cells are generated during the germinal center reaction. Within germinal centers, signals delivered through CD40 and the IL-21 receptor induce the expression of the transcriptional repressor Bcl-6. Bcl-6 antagonizes another transcriptional regulator, Blimp-1 (B lymphocyte-induced maturation protein-1), which is essential for plasma-cell differentiation. Consequently, Bcl-6 prevents germinal center B cells from differentiating prematurely into plasma cells during the intense proliferative phase that characterizes the germinal center reaction. In contrast, B cells located in extrafollicular foci express low levels of Bcl-6 and therefore differentiate more readily into short-lived plasma cells. The precise mechanisms that determine whether a germinal center B cell becomes a memory B cell or a long-lived plasma cell are not yet fully understood. However, this decision is influenced by the strength of antigen stimulation, interactions with follicular helper T cells (Tfh cells), cytokine signals, and the balance between transcription factors such as Bcl-6 and Blimp-1. Long-lived plasma cells and their precursors, known as plasmablasts, migrate to the bone marrow, where they are maintained by survival-promoting cytokines and stromal-cell-derived signals. This specialized microenvironment allows plasma cells to survive for extended periods. Typically, two to three weeks after immunization with a T-dependent antigen, the bone marrow becomes a major site of antibody production. Bone marrow plasma cells can continue secreting antibodies for months or even years after the antigen has been eliminated. It is estimated that nearly half of the antibodies circulating in the blood of a healthy adult are produced by long-lived plasma cells and are directed against antigens encountered in the past. The antibodies secreted by plasma cells enter the bloodstream and mucosal secretions to provide long-term protection. In contrast, mature plasma cells themselves do not recirculate through the body.

2.4.2. Memory B Cells

Memory B cells are specialized lymphocytes capable of mounting rapid and efficient responses upon subsequent exposure to the same antigen. Because memory B cells are generated primarily within germinal centers, they are mainly associated with T-dependent immune responsesand generally arise in parallel with memory helper T cells. Memory B cells express high levels of the anti-apoptotic protein Bcl-2, which contributes significantly to their long lifespan and persistence in the immune system. These cells can survive for many years while retaining the ability to respond quickly upon antigen re-exposure. Some memory B cells remain within the lymphoid organ where they were generated, providing local immunological surveillance. Others exit germinal centers and continuously recirculate between the bloodstream and secondary lymphoid organs. Upon re-encounter with their specific antigen, memory B cells respond much more rapidly than naïve B cells. They proliferate extensively and differentiate into antibody-secreting plasma cells, producing large amounts of high-affinity antibodies. This accelerated response constitutes the basis of immunological memory and explains the enhanced effectiveness of secondary immune responses and vaccination.

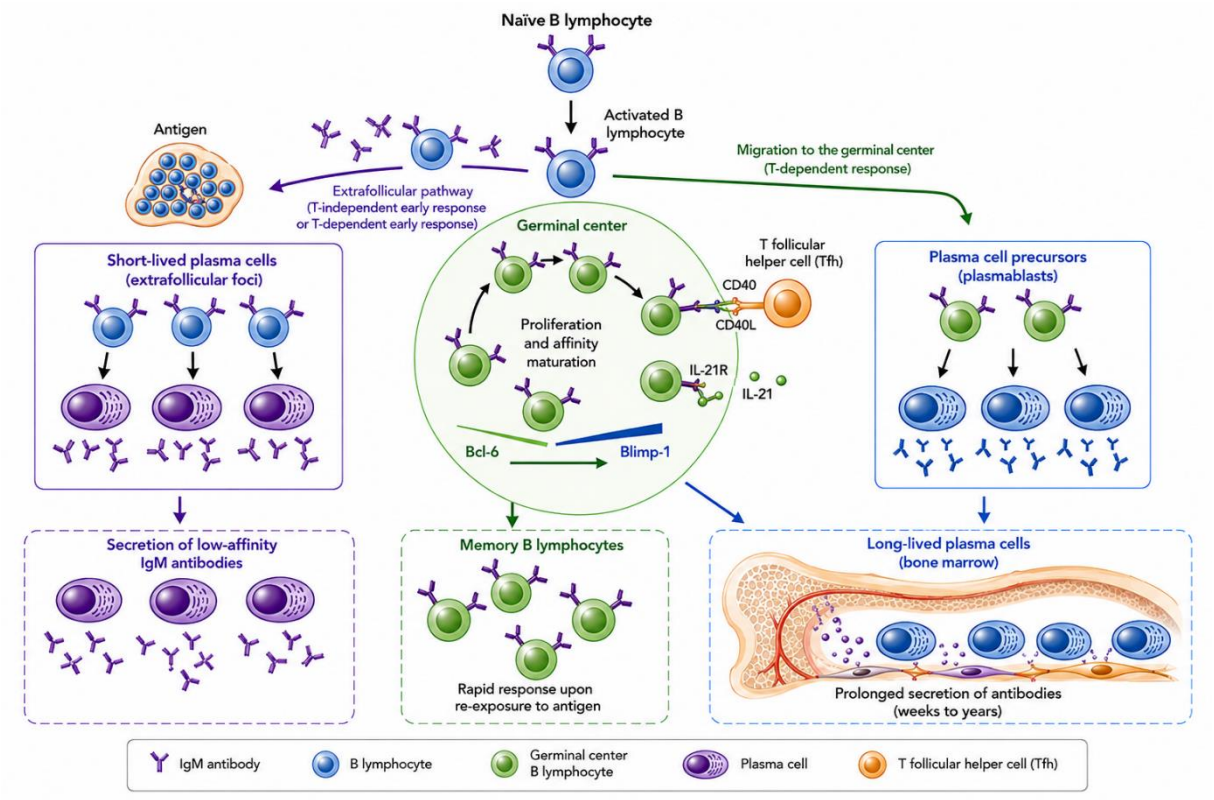


Figure 46. Differentiation pathways of activated B cells into plasma cells and memory B cells during the germinal center reaction.

Chapter 9. Regulation of the Immune System

Immune regulation refers to the collection of mechanisms that control the intensity, duration, and specificity of immune responses. These mechanisms not only ensure the efficient elimination of infectious agents but also prevent excessive immune reactions that may lead to chronic inflammation, allergies, or autoimmune diseases. Therefore, immune regulation is essential for maintaining self-tolerance while preserving effective host defense. This chapter aims to provide a comprehensive overview of the mechanisms involved in immune regulation from both cellular and molecular perspectives. It introduces the concepts of central and peripheral tolerance and describes the major regulatory cell populations, including regulatory T cells (Tregs), regulatory B cells (Bregs), myeloid-derived suppressor cells (MDSCs), and regulatory macrophages, highlighting their phenotypic markers, mechanisms of action, and physiological and pathological roles. The chapter also examines the principal inhibitory molecular pathways involved in immune regulation, including CTLA-4, PD-1/PD-L1, IL-10, TGF-β, FOXP3, AIRE, mTOR, and NF-κB, with emphasis on their signaling pathways and clinical relevance, such as their applications in cancer immunotherapy and immunosuppressive treatments. In addition, the roles of cellular metabolism and the microbiota in maintaining immune tolerance are discussed, together with the epigenetic regulation of key immune genes. Finally, the mechanisms responsible for the resolution of inflammation and their specialized mediators are presented, illustrating how immune homeostasis is restored following an immune response.

1. Central and peripheral tolerance

1.1. Central tolerance

Central tolerance refers to the mechanisms that eliminate or modify self-reactive lymphocytes during their development in the primary lymphoid organs. Its main objective is to prevent the emergence of immune cells capable of recognizing and attacking self-antigens **(Figure 47)**.

1.1.1. T-Cell Selection in the Thymus

T lymphocytes originate from hematopoietic stem cells in the bone marrow and migrate to the thymus, where they undergo a rigorous selection process. During positive selection, thymocytes capable of recognizing self-MHC molecules with low affinity receive survival signals, whereas those unable to recognize self-MHC undergo apoptosis. Subsequently, negative selection

eliminates thymocytes that bind strongly to self-peptide–MHC complexes. This process occurs primarily in the thymic medulla and is mediated by medullary thymic epithelial cells and dendritic cells. The transcription factor AIRE (Autoimmune Regulator) promotes the expression of tissue-specific self-antigens in the thymus, allowing the deletion of potentially autoreactive T cells before they enter the circulation.

1.1.2. B-Cell Selection in the Bone Marrow

B lymphocytes develop in the bone marrow, where they are also subjected to mechanisms of central tolerance. Immature B cells expressing B-cell receptors (BCRs) with high affinity for self-antigens may undergo clonal deletion through apoptosis. Alternatively, some self-reactive B cells can modify their antigen receptors through a process known as receptor editing, which involves additional rearrangements of immunoglobulin light-chain genes. Cells that remain weakly self-reactive may survive but become functionally unresponsive, contributing to the maintenance of self-tolerance.

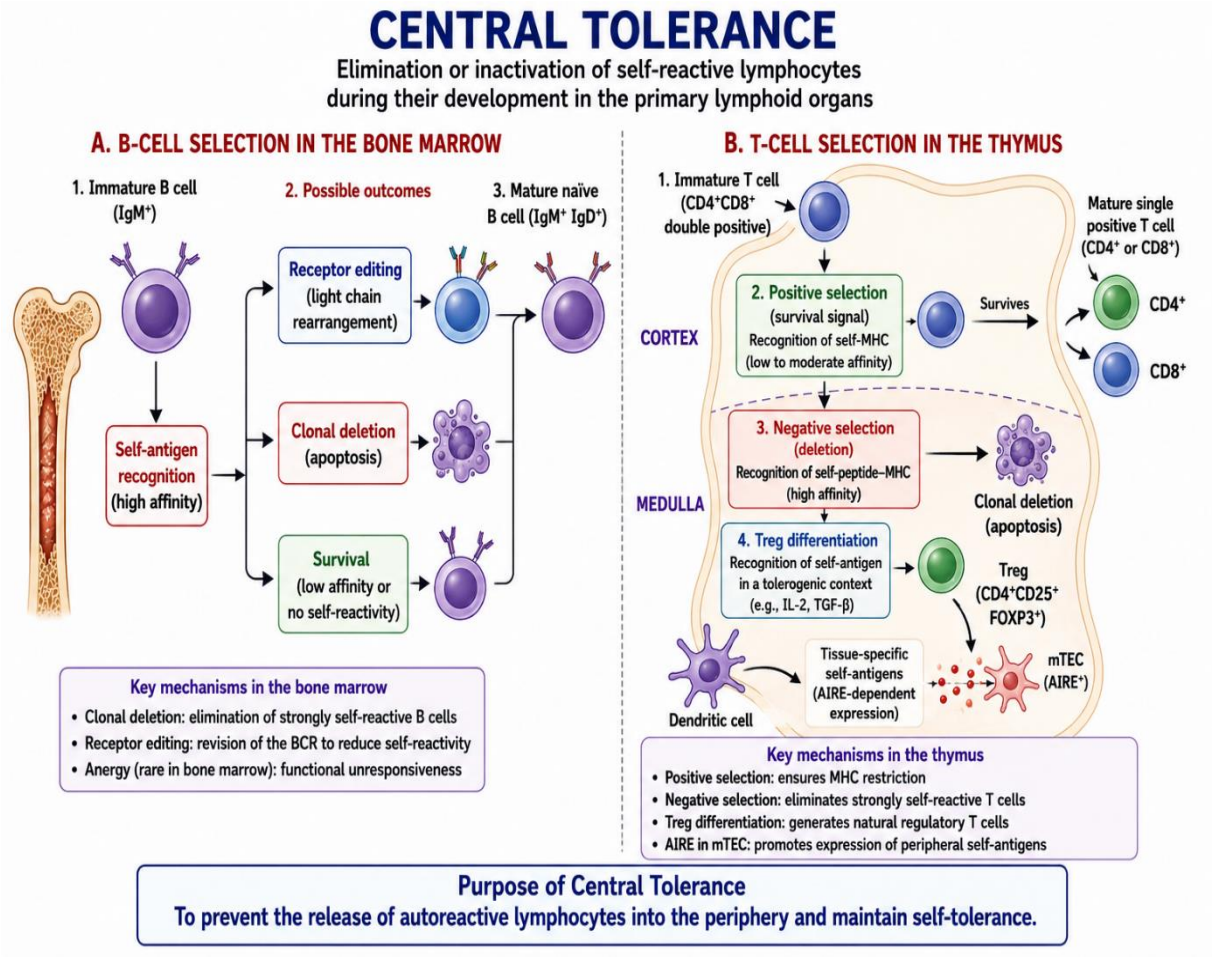


Figure 47. Mechanisms of central immune tolerance.

1.2. Peripheral Tolerance

Although central tolerance eliminates most autoreactive lymphocytes, some self-reactive cells escape into the peripheral tissues. Peripheral tolerance mechanisms are therefore essential to prevent inappropriate immune activation and maintain immune homeostasis **(Figure 48)**.

1.2.1. Anergy

Anergy is a state of long-lasting functional unresponsiveness in which lymphocytes remain alive but fail to respond to antigen stimulation. In T cells, anergy typically occurs when antigen recognition through the T-cell receptor takes place in the absence of adequate co-stimulatory signals, particularly those mediated by CD28. Similarly, B cells can become anergic when they encounter self-antigens without receiving appropriate activation signals from helper T cells.

1.2.2. Deletion (Apoptosis)

Autoreactive lymphocytes that become activated in peripheral tissues may be eliminated through programmed cell death, or apoptosis. This process is mediated by pro-apoptotic pathways involving molecules such as Fas (CD95) and Fas Ligand (FasL), as well as members of the Bcl-2 family. Peripheral deletion plays a critical role in terminating immune responses and preventing the accumulation of potentially harmful autoreactive lymphocytes.

1.2.3. Immune Ignorance

Immune ignorance occurs when self-reactive lymphocytes remain unaware of the presence of self-antigens. This may happen because certain self-antigens are present in very low concentrations, are anatomically sequestered in immune-privileged sites such as the brain, eye, or testes, or are inaccessible to circulating lymphocytes. Under normal conditions, these autoreactive cells remain harmless; however, tissue injury or inflammation may expose previously hidden antigens and trigger autoimmune responses.

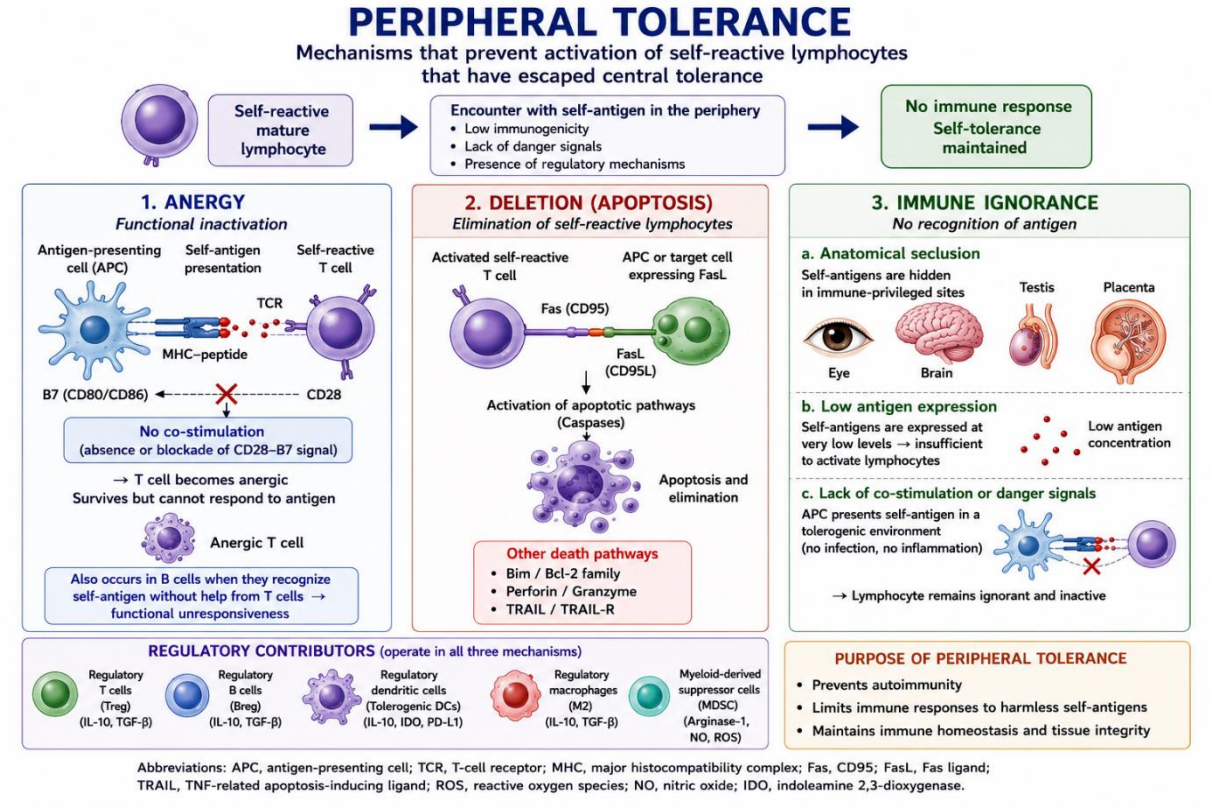


Figure 48. Peripheral tolerance and maintenance of immune homeostasis.

2. Regulatory T Cells (Treg)

Regulatory T cells (Tregs) constitute a specialized subset of T lymphocytes responsible for maintaining immune tolerance and preventing excessive immune responses. They play a central role in preserving self-tolerance by suppressing the activation and proliferation of autoreactive lymphocytes that escape central and peripheral tolerance mechanisms **(Figure 49)**.

2.1. Development and Markers ($CD4^+CD25^+FOXP3^+$)

Tregs originate through two main pathways. Natural Treg cells (nTregs) develop in the thymus during T-cell maturation following the recognition of self-antigens with intermediate affinity. Induced Treg cells (iTregs) are generated in peripheral tissues from conventional $CD4^+$ T cells under tolerogenic conditions, particularly in the presence of transforming growth factor-β (TGF-β). Regulatory T cells are characterized by the expression of several markers, including CD4, CD25 (the α-chain of the IL-2 receptor), and the transcription factor FOXP3, which is considered the master regulator of Treg development and function. Mutations in the FOXP3 gene result in severe autoimmune disorders, highlighting its critical role in immune regulation.

2.2. Mechanisms of Suppression

Tregs suppress immune responses through multiple complementary mechanisms. They secrete anti-inflammatory cytokines such as IL-10, TGF-β, and IL-35, which inhibit the activation and proliferation of effector T cells, B cells, macrophages, and dendritic cells. Tregs also express high levels of CD25, allowing them to consume available IL-2 and deprive effector T cells of this essential growth factor. Furthermore, Tregs inhibit antigen-presenting cells through the expression of CTLA-4, which reduces the expression of co-stimulatory molecules on dendritic cells. Through these mechanisms, Tregs contribute to immune homeostasis, prevent autoimmunity, and limit tissue damage during inflammatory responses.

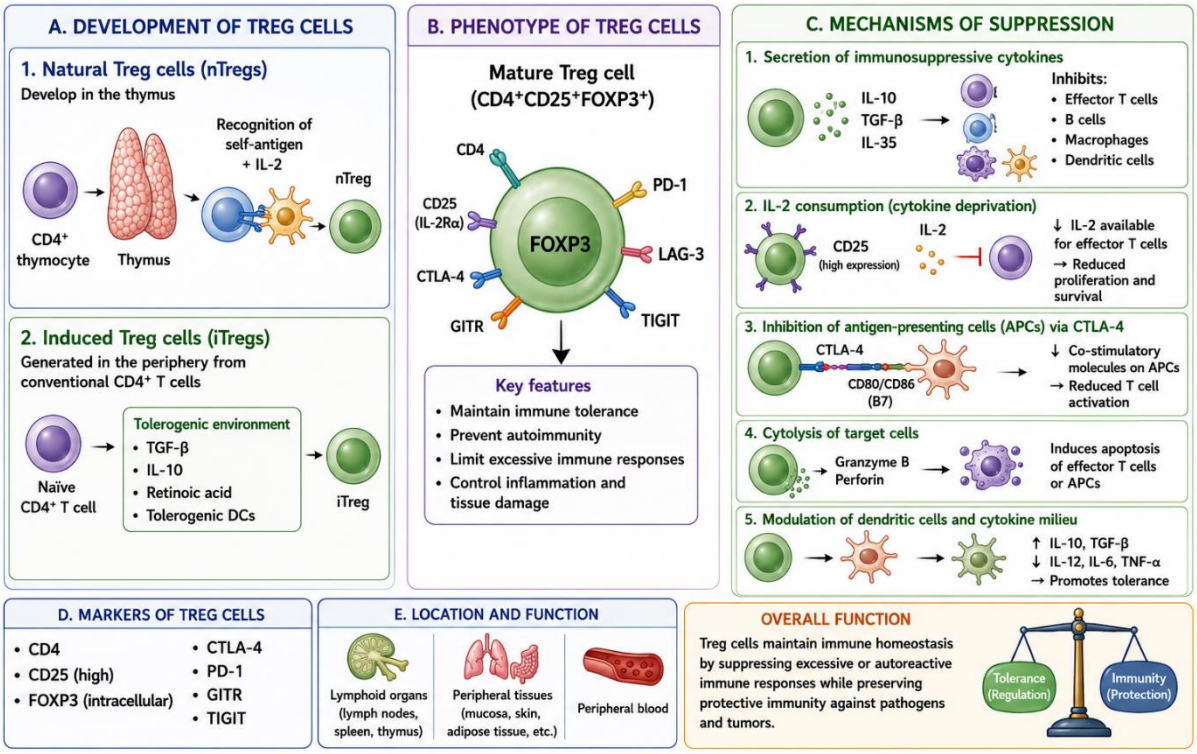


Figure 49. Development and suppressive mechanisms of regulatory T Cells (Tregs)

3. Immune Checkpoints

Immune checkpoints are inhibitory pathways that regulate immune responses and prevent excessive activation of lymphocytes. They are essential for maintaining self-tolerance and limiting tissue damage during inflammation. Among the most important immune checkpoint molecules are CTLA-4 and PD-1 **(Figure 50)**.

3.1. CTLA-4

Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) is expressed mainly on activated T cells and regulatory T cells. It competes with CD28 for binding to the co-stimulatory molecules CD80 (B7-1) and CD86 (B7-2) expressed on antigen-presenting cells. Because CTLA-4 has a higher affinity for these ligands than CD28, it inhibits T-cell activation and proliferation. CTLA-4 therefore acts during the early stages of immune responses, primarily within lymphoid organs.

3.2. PD-1 / PD-L1

Programmed Cell Death Protein 1 (PD-1) is expressed on activated T cells, B cells, and natural killer cells. Its ligands, PD-L1 and PD-L2, are expressed on antigen-presenting cells and many peripheral tissues. Engagement of PD-1 by its ligands transmits inhibitory signals that reduce T-cell proliferation, cytokine production, and cytotoxic activity. This pathway is particularly important in peripheral tissues, where it limits excessive immune responses and contributes to peripheral tolerance. Many tumors exploit the PD-1/PD-L1 pathway to evade immune surveillance.

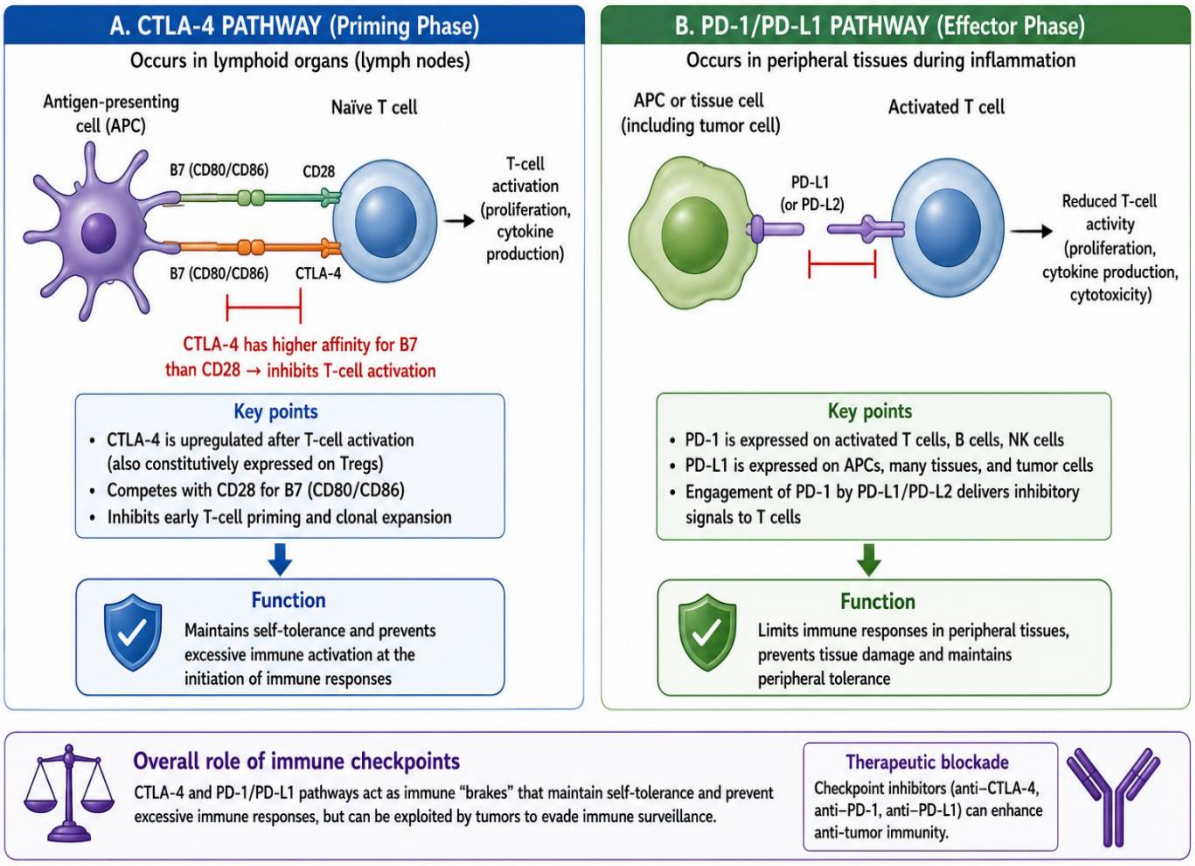


Figure 50. CTLA-4 and PD-1/PD-L1 immune checkpoint pathways

4. Cytokines in Immune Regulation

Cytokines are soluble proteins that coordinate communication between immune cells and regulate the balance between immune activation and suppression. Depending on their biological effects, cytokines can be broadly classified as pro-inflammatory or anti-inflammatory **(Figure 51)**.

4.1. Pro-Inflammatory Cytokines

Pro-inflammatory cytokines promote the initiation and amplification of immune responses. Major examples include tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), and interferon-gamma (IFN-γ). These cytokines stimulate leukocyte recruitment, increase vascular permeability, activate phagocytic cells, and contribute to systemic manifestations of inflammation such as fever and acute-phase protein production.

4.2. Anti-Inflammatory Cytokines

Anti-inflammatory cytokines limit immune responses and promote the restoration of tissue homeostasis. The most important are IL-10, TGF-β, and IL-35. These cytokines inhibit the production of inflammatory mediators, suppress antigen presentation, and promote the differentiation and function of regulatory immune cells. Their activity is essential for preventing chronic inflammation and autoimmune diseases.

5. Dysregulation of the Immune System

Defects in immune regulatory mechanisms may result in pathological conditions characterized by either excessive or insufficient immune responses.

5.1. Autoimmune Diseases

Autoimmune diseases occur when self-tolerance mechanisms fail and the immune system attacks the body's own tissues. Examples include Rheumatoid Arthritis, Systemic Lupus Erythematosus, and Type 1 Diabetes. These disorders involve autoreactive lymphocytes, autoantibody production, and chronic inflammation.

5.2. Hypersensitivity

Hypersensitivity reactions are exaggerated immune responses that cause tissue damage. They are traditionally classified into four types: immediate hypersensitivity (Type I), antibody-mediated cytotoxic reactions (Type II), immune complex-mediated reactions (Type III), and delayed-type hypersensitivity (Type IV). Allergic diseases such as asthma and allergic rhinitis are common examples of hypersensitivity disorders.

5.3. Immunodeficiency

Immunodeficiency results from defects in one or more components of the immune system, leading to increased susceptibility to infections and certain cancers. Immunodeficiencies may be primary (genetic) or secondary (acquired). A well-known example of acquired immunodeficiency is Acquired Immunodeficiency Syndrome, caused by infection with the Human Immunodeficiency Virus Infection. Impaired immune regulation may also contribute to chronic infections and reduced vaccine responses.

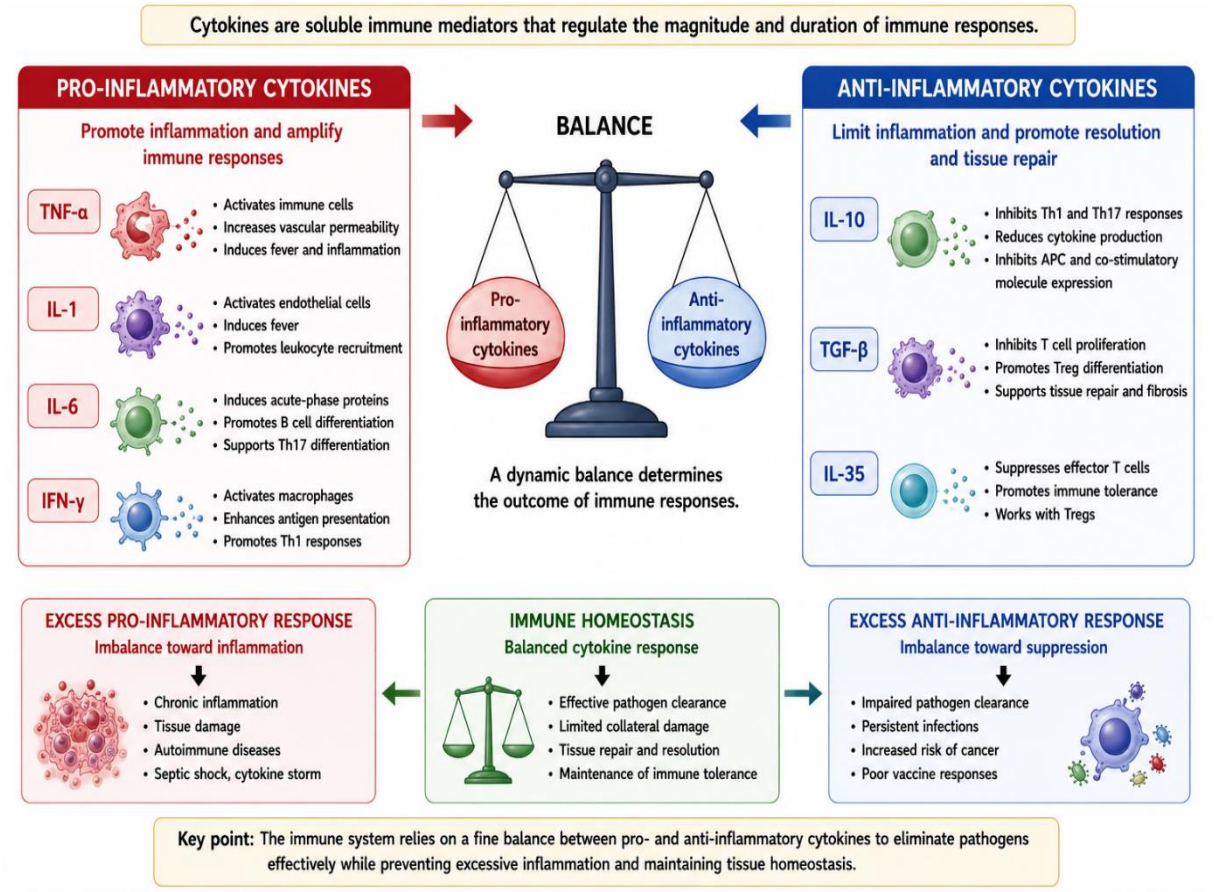


Figure 51. Balance between pro-inflammatory and anti-inflammatory cytokines

Chapter 10. Immunological Memory and Vaccination

Immunological memory is a hallmark of the adaptive immune system and refers to its ability to respond more rapidly and effectively to a previously encountered antigen. Following a primary immune response, a small population of antigen-specific lymphocytes survives and differentiates into long-lived memory cells. These cells persist in the body for years, or even decades, and can be rapidly reactivated upon subsequent exposure to the same pathogen.

The development of immunological memory provides enhanced protection against recurrent infections. During a secondary immune response, memory B and T lymphocytes expand more quickly and generate stronger effector functions than naïve lymphocytes. As a result, pathogens are eliminated more efficiently, often before clinical symptoms appear. This protective mechanism constitutes the biological basis of vaccination, whereby exposure to a harmless form of an antigen induces memory cell formation without causing disease.

Immunological memory is therefore essential for long-term immunity and contributes significantly to individual and population health. By maintaining a pool of antigen-specific memory lymphocytes and long-lived plasma cells, the immune system ensures rapid protection against previously encountered microorganisms and reduces the severity of future infections.

1. Immunological Memory

Immunological memory is established after the primary immune response and is maintained by long-lived populations of memory lymphocytes. These cells remain in the body after antigen clearance and can respond rapidly upon re-exposure to the same pathogen. Compared with naïve lymphocytes, memory cells are more numerous, have lower activation thresholds, and display enhanced effector functions. Consequently, secondary immune responses are faster, stronger, and more effective than primary responses.

1.1. Memory Cells

1.1.1. Memory T Cells

Memory T cells are generated following antigen stimulation and clonal expansion of naïve T lymphocytes. After pathogen elimination, most effector T cells undergo apoptosis, whereas a small fraction survives and differentiates into memory T cells. These cells provide long-term cellular immunity and can persist for many years. Memory T cells are heterogeneous and are

generally classified into two major subsets: central memory T cells (TCM) and effector memory T cells (TEM) **(Figure 52)**.

1.1.1.1. Central Memory T Cells (TCM)

Central memory T cells are characterized by the expression of lymphoid homing receptors such as CCR7 and CD62L, which allow them to circulate through secondary lymphoid organs, including lymph nodes and the spleen. These cells possess a high proliferative capacity and can rapidly expand following antigen re-exposure. Upon activation, they differentiate into effector T cells capable of producing large numbers of cytokines and mediating protective immune responses. Because of their longevity and proliferative potential, TCM cells are considered an important reservoir of long-term immunological memory.

1.1.1.2. Effector Memory T Cells (TEM)

Effector memory T cells lack the expression of CCR7 and CD62L and therefore preferentially migrate to peripheral tissues rather than lymphoid organs. They are strategically positioned at sites commonly exposed to pathogens, such as the skin and mucosal surfaces. TEM cells exhibit immediate effector functions, including rapid cytokine production and cytotoxic activity, allowing them to provide a first line of adaptive immune protection upon reinfection. Although they generally possess a lower proliferative capacity than TCM cells, their ability to respond rapidly makes them crucial for early pathogen control.

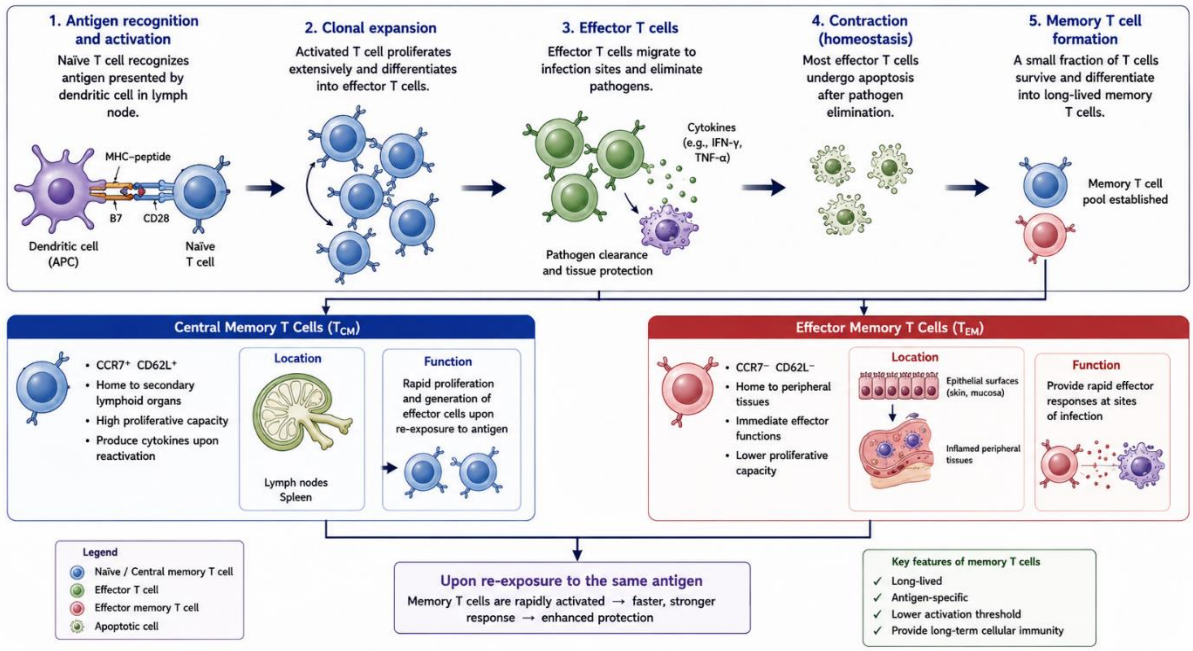


Figure 52. Generation and differentiation of memory t cells.

1.1.2. Memory B Cells

Memory B cells are long-lived lymphocytes generated during the primary immune response following antigen exposure. After activation by antigen and interaction with helper T cells in germinal centers of secondary lymphoid organs, activated B cells undergo clonal expansion, affinity maturation, and class-switch recombination. While some activated B cells differentiate into antibody-secreting plasma cells, others become memory B cells capable of persisting for many years **(Figure 53)**.

1.1.2.1. Generation and Functions

Memory B cells are produced primarily in germinal centers located within lymph nodes, the spleen, and mucosal lymphoid tissues. During their development, these cells undergo somatic hypermutation and selection processes that favor clones expressing high-affinity B-cell receptors (BCRs). As a result, memory B cells possess enhanced antigen recognition capabilities compared with naïve B cells.

Unlike plasma cells, memory B cells do not continuously secrete antibodies. Instead, they remain in a resting state and circulate through lymphoid tissues and the bloodstream. Upon re-exposure to the same antigen, they rapidly proliferate and differentiate into plasma cells, producing large amounts of high-affinity antibodies. This rapid response contributes significantly to long-term protective immunity.

1.1.2.2. Secondary Antibody Responses

The secondary immune response occurs when an individual encounters an antigen previously recognized by the immune system. Memory B cells respond much faster than naïve B cells because they have already undergone antigen selection and maturation. Consequently, the lag phase is shorter, antibody production is more rapid, and antibody concentrations are substantially higher than during the primary response.

In addition, antibodies produced during secondary responses generally exhibit higher affinity due to previous affinity maturation. Immunoglobulin G (IgG) is the predominant antibody class generated during secondary responses, although IgA and IgE may also be produced depending on the nature of the antigen and the site of infection. These characteristics allow the immune system to neutralize pathogens more efficiently and often prevent the development of clinical disease.

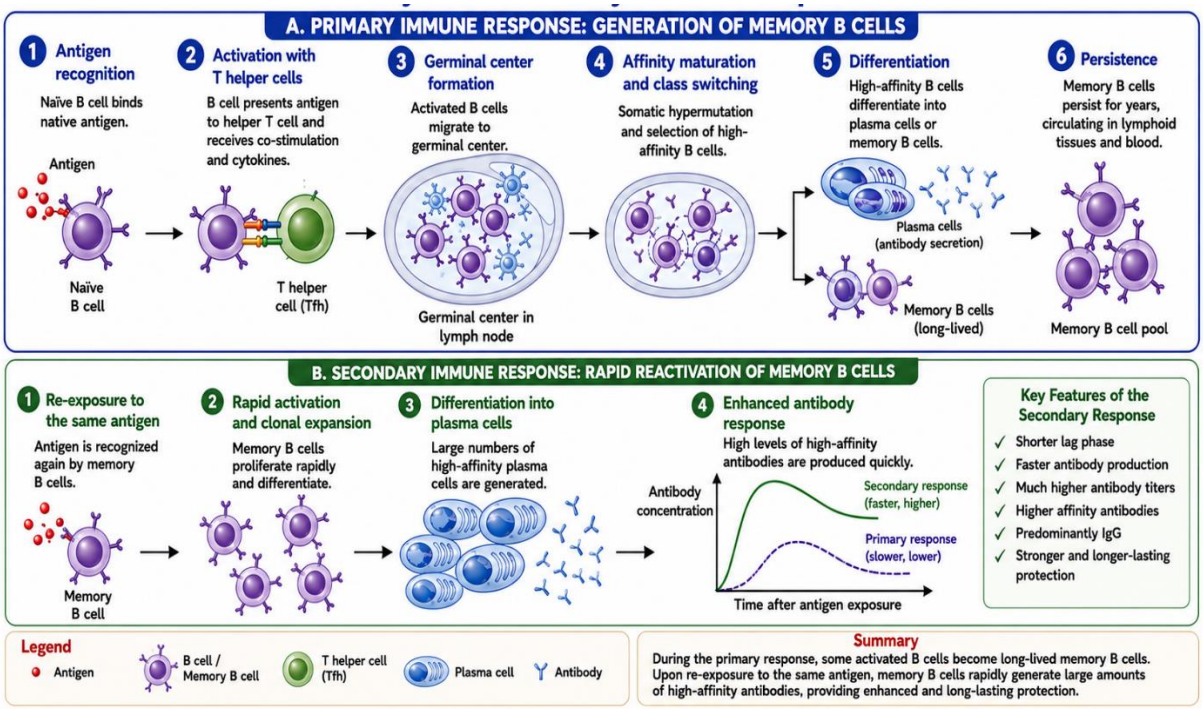


Figure 53. Generation and function of memory b cells during primary and secondary immune responses.

1.2. Primary and Secondary Immune Responses

The adaptive immune response can be divided into two phases: the primary immune response, which occurs upon first exposure to an antigen, and the secondary immune response, which develops following subsequent encounters with the same antigen. The secondary response is more rapid, more intense, and more effective because of the presence of immunological memory **(Figure 54)**.

1.2.1. Characteristics of the Primary Immune Response

The primary immune response is initiated when a pathogen or foreign antigen enters the body for the first time. Naïve B and T lymphocytes recognize the antigen and become activated after receiving appropriate co-stimulatory signals. This phase is characterized by a relatively long lag period, during which antigen processing, lymphocyte activation, clonal expansion, and differentiation take place. During the primary humoral response, IgM antibodies are produced first, followed by class switching to other immunoglobulin isotypes, particularly IgG. Antibody concentrations increase gradually, reach a peak, and then decline as the antigen is eliminated. At the end of the response, most effector cells undergo apoptosis, while a fraction differentiates into long-lived memory B and T cells.

1.2.2. Characteristics of the Secondary Immune Response

The secondary immune response occurs when the same antigen is encountered again. Memory lymphocytes generated during the primary response rapidly recognize the antigen and become activated. Consequently, the lag phase is significantly shorter, and the immune response develops more quickly.

The secondary humoral response is characterized by the rapid production of large amounts of high-affinity antibodies, predominantly IgG, although IgA or IgE may also be produced depending on the nature of the antigen. Antibody levels are substantially higher and persist longer than during the primary response. Similarly, memory T cells respond more rapidly and efficiently, providing enhanced cellular immunity.

1.2.3. Role of Memory Cells

Memory B and T cells constitute the cellular basis of long-term protective immunity. These cells survive long after the elimination of the antigen and remain capable of mounting a rapid and robust response upon re-exposure.

Memory B cells rapidly differentiate into antibody-secreting plasma cells, producing high-affinity antibodies that neutralize pathogens before disease develops. Memory T cells, including central memory (TCM) and effector memory (TEM) subsets, provide long-lasting cellular protection through rapid proliferation, cytokine production, and cytotoxic activity. Together, these memory populations ensure faster pathogen elimination and form the immunological foundation of vaccination.

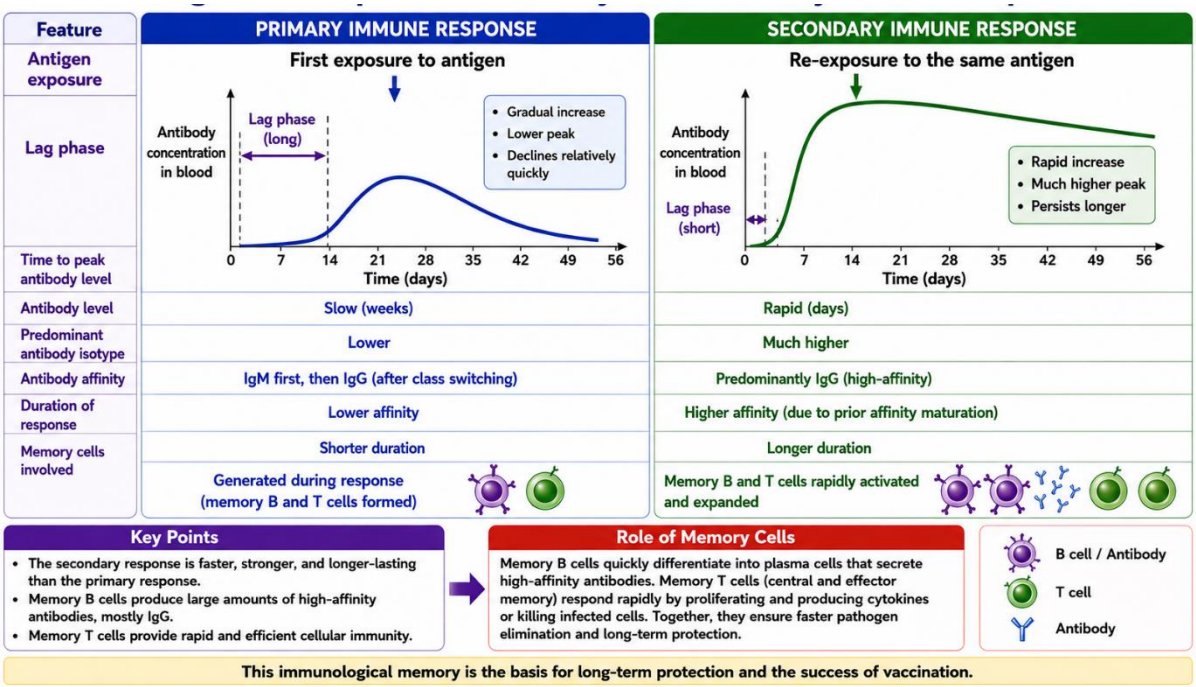


Figure 54. Comparison of primary and secondary immune responses.

2. Vaccination

Vaccination is a preventive medical strategy that aims to induce protective immunity against infectious diseases without causing the disease itself. It relies on the administration of antigens derived from pathogens or their components to stimulate the immune system and generate immunological memory. Following vaccination, memory B and T lymphocytes are produced, enabling a rapid and effective immune response upon future exposure to the pathogen.

2.1. Principles of Vaccination

The fundamental principle of vaccination is to expose the immune system to a harmless form of an antigen in order to stimulate adaptive immunity. This controlled exposure leads to the activation of antigen-specific lymphocytes, the production of antibodies, and the generation of memory cells. As a result, subsequent encounters with the pathogen trigger a faster and stronger immune response, preventing infection or reducing disease severity.

2.1.1. Active and Passive Immunization

Active immunization results from exposure to an antigen through natural infection or vaccination. It stimulates the host's immune system to produce antibodies and memory cells, providing long-lasting protection. In contrast, passive immunization involves the transfer of preformed antibodies from an immune individual to a non-immune recipient. Protection is

immediate but temporary because no immunological memory is generated. Examples include maternal antibody transfer through the placenta and the administration of immunoglobulins following exposure to specific pathogens.

2.1.2. Herd Immunity

Herd immunity occurs when a sufficiently large proportion of a population becomes immune to an infectious disease, thereby reducing pathogen transmission. This indirect protection benefits individuals who cannot be vaccinated, such as immunocompromised patients or newborn infants. The level of vaccination coverage required to achieve herd immunity depends on the transmissibility of the pathogen.

2.2. Types of Vaccines

Modern vaccines are produced using different technologies, each designed to stimulate protective immunity while maintaining an acceptable safety profile.

2.2.1. Live Attenuated Vaccines

Live attenuated vaccines contain living microorganisms that have been weakened so that they no longer cause disease in healthy individuals. Because they closely mimic natural infection, they induce strong and long-lasting humoral and cellular immune responses. However, they are generally contraindicated in immunocompromised individuals.

Examples: Measles, mumps, rubella (MMR), varicella, and yellow fever vaccines.

2.2.2. Inactivated Vaccines

Inactivated vaccines contain pathogens that have been killed by physical or chemical methods. Since they cannot replicate, they are safer than live attenuated vaccines but usually induce weaker immune responses and often require booster doses.

Examples: Inactivated polio vaccine, hepatitis A vaccine, and rabies vaccine.

2.2.3. Subunit and Conjugate Vaccines

Subunit vaccines contain only selected antigenic components of a pathogen, such as proteins or polysaccharides. Because they do not contain the entire microorganism, they are highly safe and well tolerated. Conjugate vaccines are a specialized form of subunit vaccine in which

polysaccharide antigens are linked to carrier proteins to enhance immunogenicity, particularly in young children.

Examples: Hepatitis B vaccine, human papillomavirus (HPV) vaccine, and pneumococcal conjugate vaccine.

2.2.4. Viral Vector Vaccines

Viral vector vaccines use a modified virus as a carrier to deliver genetic material encoding a pathogen antigen into host cells. The host cells then produce the antigen, stimulating both antibody-mediated and cellular immune responses.

Examples: Adenovirus-based vaccines developed against COVID-19 and Ebola virus disease.

2.2.5. mRNA Vaccines

mRNA vaccines contain messenger RNA encoding a specific pathogen antigen. After administration, host cells translate the mRNA into protein antigens that stimulate immune responses. These vaccines do not contain live pathogens and do not integrate into the host genome. mRNA technology offers rapid development, high efficacy, and flexible adaptation to emerging pathogens.

Examples: Several COVID-19 vaccines based on mRNA technology.

2.3. Vaccine Adjuvants and Boosters

Vaccines often contain additional components designed to enhance and prolong immune responses. Among these, adjuvants and booster doses play essential roles in maximizing vaccine efficacy and ensuring long-term protection.

2.3.1. Role of Adjuvants

Adjuvants are substances added to vaccines to enhance the immune response to an antigen. By stimulating innate immunity and promoting antigen presentation, adjuvants increase the magnitude and duration of adaptive immune responses. They improve antibody production, enhance T-cell activation, and may reduce the amount of antigen required in a vaccine formulation. Many adjuvants act by activating pattern-recognition receptors (PRRs) on antigen-presenting cells, leading to the production of cytokines and co-stimulatory molecules that

facilitate lymphocyte activation. Commonly used adjuvants include aluminum salts (alum), oil-in-water emulsions, and more recently developed adjuvants targeting Toll-like receptors (TLRs). The incorporation of adjuvants is particularly important in vaccines containing purified antigens, such as subunit vaccines, which are often less immunogenic than live attenuated vaccines.

2.3.2. Booster Immunization

A booster dose is an additional vaccine administration given after the primary vaccination series. Its purpose is to re-expose the immune system to the antigen and stimulate memory lymphocytes generated during the initial immunization. Booster immunization induces a strong secondary immune response characterized by rapid activation of memory B and T cells, increased production of high-affinity antibodies, and prolonged immune protection. Booster doses are particularly important when immunity declines over time or when pathogen variants emerge that may partially evade pre-existing immunity. The timing and number of booster doses depend on the vaccine type, the pathogen involved, and the duration of protective immunity generated by the primary vaccination schedule.

2.4. Applications and Challenges

Vaccination has transformed global public health by significantly reducing the incidence, severity, and mortality associated with many infectious diseases. Despite these successes, several scientific and logistical challenges remain.

2.4.1. Vaccine-Preventable Diseases

Vaccination programs have successfully controlled or eliminated numerous infectious diseases worldwide. Diseases such as measles, poliomyelitis, diphtheria, tetanus, pertussis, hepatitis B, and human papillomavirus-associated infections can be effectively prevented through vaccination. The widespread use of vaccines has resulted in substantial reductions in morbidity and mortality, particularly among children. In some cases, extensive vaccination campaigns have led to disease eradication, as demonstrated by smallpox, which remains the only human infectious disease completely eradicated through global immunization efforts.

2.4.2. Emerging Infectious Diseases

The emergence of new pathogens continues to represent a major challenge for global health. Recent outbreaks have highlighted the need for rapid vaccine development platforms capable of responding to newly emerging infectious agents. Advances in molecular biology, genomics, and biotechnology have facilitated the development of innovative vaccine technologies, allowing accelerated responses to epidemics and pandemics. Nevertheless, antigenic variation, pathogen evolution, and unequal global vaccine access remain important obstacles to disease control.

2.4.3. Future Vaccine Strategies

Future vaccine development aims to improve efficacy, safety, accessibility, and duration of protection. Novel approaches include mRNA vaccines, self-amplifying RNA vaccines, universal vaccines targeting conserved antigens, personalized vaccines, and next-generation adjuvant systems. Research is also focused on improving mucosal immunity, enhancing cellular immune responses, and developing vaccines against challenging pathogens such as HIV, malaria parasites, tuberculosis, and emerging zoonotic viruses. Artificial intelligence, systems biology, and genomic technologies are expected to accelerate vaccine discovery and optimization in the coming years.

Chapter 11. Immunology of Pregnancy and Feto-Maternal tolerance

Pregnancy represents a unique immunological challenge because the fetus carries genetic material inherited from both parents and therefore expresses paternal antigens that are foreign to the mother. From an immunological perspective, the fetus can be considered a semi-allogeneic graft. Under normal circumstances, the immune system is designed to recognize and eliminate foreign antigens; however, during pregnancy, a delicate balance must be achieved to protect the developing fetus while maintaining the mother's ability to defend against infections. This remarkable adaptation requires complex interactions between maternal immune cells, fetal tissues, and placental structures.

The concept of feto-maternal tolerance refers to the mechanisms that prevent maternal immune rejection of the fetus. Rather than a state of generalized immunosuppression, pregnancy is characterized by highly regulated immune modulation at the maternal-fetal interface. Specialized trophoblast cells, regulatory immune cells, and immunosuppressive cytokines cooperate to create a local environment that promotes fetal survival while preserving immune competence. These mechanisms include reduced expression of classical major histocompatibility complex (MHC) molecules, increased production of anti-inflammatory mediators, and the expansion of regulatory T cells. Feto-maternal tolerance is essential for successful implantation, placental development, fetal growth, and maintenance of pregnancy. Disruption of these regulatory pathways may contribute to pregnancy complications such as recurrent miscarriage, preeclampsia, preterm birth, and fetal growth restriction. Understanding the immunological basis of pregnancy has therefore become a major area of research, providing valuable insights into reproductive medicine, transplantation biology, and immune regulation.

1. Maternal-Fetal Interface

The maternal-fetal interface is the specialized region where maternal tissues come into direct contact with fetal-derived tissues. It is primarily composed of the placenta, trophoblast cells, and the maternal decidua. This interface serves not only as a site of nutrient and gas exchange but also as a highly regulated immunological environment that ensures fetal survival throughout pregnancy.

1.1. Placenta and Trophoblasts

The placenta is a transient organ that develops during pregnancy and functions as the primary interface between the mother and the fetus. It facilitates the transfer of oxygen, nutrients, hormones, and waste products while simultaneously acting as an immunological barrier.

Trophoblasts are specialized fetal cells that form the outer layer of the placenta and directly interact with maternal tissues. They are classified into different populations, including cytotrophoblasts, syncytiotrophoblasts, and extravillous trophoblasts. Unlike most nucleated cells, trophoblasts exhibit a unique pattern of major histocompatibility complex (MHC) expression. They do not express the highly polymorphic classical HLA-A and HLA-B molecules, thereby reducing recognition by maternal cytotoxic T lymphocytes. Instead, they express non-classical HLA molecules, particularly HLA-G, which contribute to immune tolerance and protect fetal tissues from maternal immune attack.

Beyond their structural role, trophoblasts actively participate in immune regulation by producing cytokines, chemokines, and growth factors that modulate maternal immune responses and promote placental development.

1.2. Decidual Immune Cells

The decidua, which corresponds to the modified endometrium during pregnancy, contains a diverse population of immune cells that account for a significant proportion of maternal cells at the implantation site. These immune cells play essential roles in tissue remodeling, placental development, and maintenance of fetal tolerance.

Decidual natural killer (dNK) cells represent the most abundant immune cell population during early pregnancy. Unlike peripheral NK cells, decidual NK cells display limited cytotoxic activity and primarily contribute to trophoblast invasion, vascular remodeling, and placental development through the secretion of cytokines and angiogenic factors.

Macrophages constitute the second largest immune cell population in the decidua. They participate in tissue repair, clearance of apoptotic cells, regulation of inflammation, and maintenance of immune tolerance. Most decidual macrophages exhibit an anti-inflammatory phenotype that supports pregnancy.

Regulatory T cells (Tregs), dendritic cells, and smaller populations of T and B lymphocytes are also present at the maternal-fetal interface. Together, these cells establish a tolerogenic environment that allows fetal development while preserving maternal immune competence against pathogens.

2. Mechanisms of Feto-Maternal Tolerance

Successful pregnancy depends on the establishment of multiple mechanisms that prevent maternal immune rejection of the semi-allogeneic fetus. These mechanisms involve specialized immune cells, non-classical HLA molecules, and immunoregulatory cytokines that collectively create a state of localized immune tolerance at the maternal-fetal interface **(Figure 55)**.

2.1. Role of Regulatory T Cells (Treg)

Regulatory T cells are among the most important mediators of feto-maternal tolerance. During pregnancy, the number of Treg cells increases both in peripheral blood and at the maternal-fetal interface. These cells suppress the activation and proliferation of potentially harmful maternal effector T lymphocytes that could recognize fetal antigens. Tregs exert their suppressive effects through direct cell-to-cell interactions and through the secretion of anti-inflammatory cytokines such as IL-10 and TGF-β. They also inhibit antigen-presenting cells and reduce the production of pro-inflammatory cytokines. Experimental and clinical studies have demonstrated that reduced Treg numbers or impaired Treg function are associated with recurrent pregnancy loss, preeclampsia, and other pregnancy complications.

2.2. HLA-G and Immune Evasion

HLA-G is a non-classical class I HLA molecule predominantly expressed by extravillous trophoblasts. It plays a central role in protecting fetal tissues from maternal immune attack. Unlike classical HLA molecules, HLA-G exhibits limited polymorphism and possesses strong immunosuppressive properties. HLA-G interacts with inhibitory receptors present on natural killer cells, T lymphocytes, dendritic cells, and macrophages. These interactions suppress cytotoxic responses, inhibit inflammatory cytokine production, and promote the development of tolerogenic immune cells. Through these mechanisms, HLA-G contributes significantly to fetal immune evasion and successful pregnancy maintenance.

2.3. Immunoregulatory Cytokines (IL-10 and TGF-β)

Anti-inflammatory cytokines are essential for maintaining immune balance during pregnancy. Among them, interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β) are particularly important. IL-10 inhibits the production of pro-inflammatory cytokines, reduces antigen presentation, and suppresses the activation of T helper 1 (Th1) responses. This cytokine contributes to the establishment of a tolerogenic microenvironment at the maternal-fetal interface. TGF-β promotes the differentiation and maintenance of regulatory T cells while inhibiting the activation and proliferation of effector lymphocytes. It also participates in tissue remodeling, placental development, and immune homeostasis throughout pregnancy.

Together, Treg cells, HLA-G expression, and immunoregulatory cytokines form an integrated network that protects the fetus from maternal immune rejection while ensuring normal fetal growth and development.

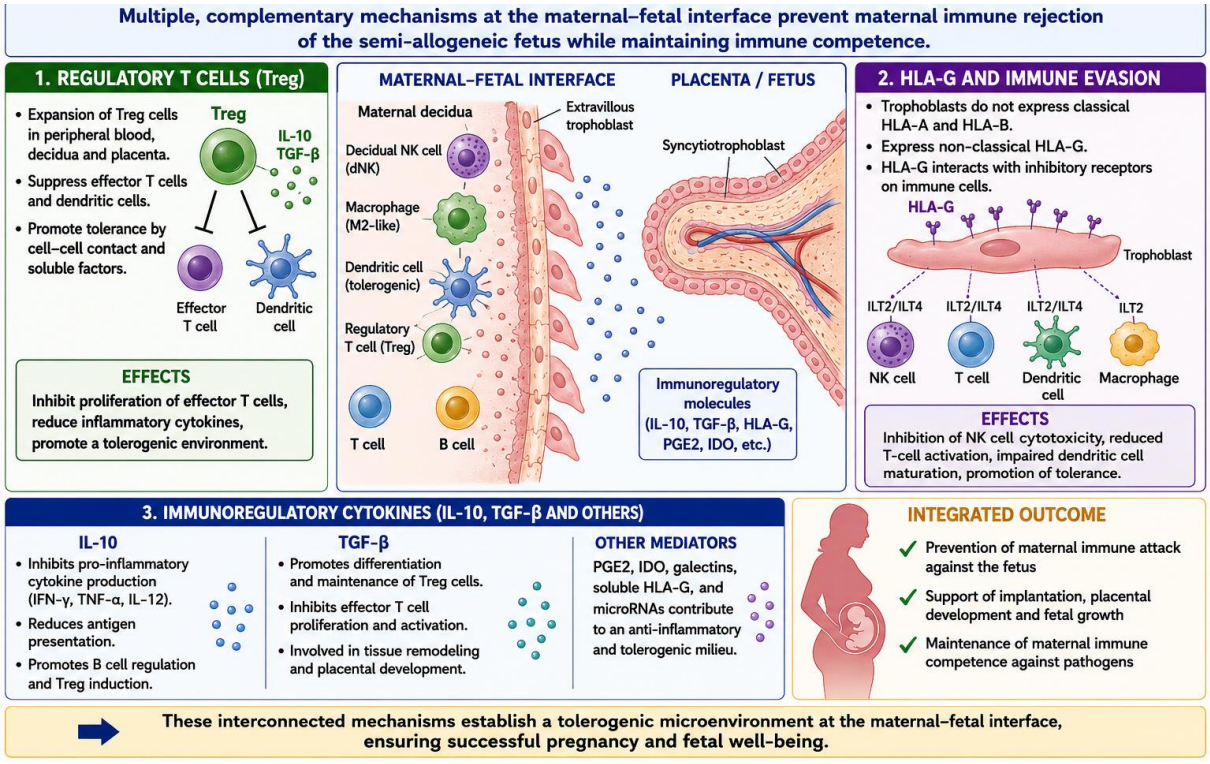


Figure 55. Mechanisms of Feto-Maternal immune tolerance

3. Immune Adaptations During Pregnancy

Pregnancy is associated with profound immunological adaptations that allow the maternal immune system to tolerate the semi-allogeneic fetus while maintaining protection against

infectious agents. These changes affect both innate and adaptive immunity and vary throughout the different stages of pregnancy. Rather than representing a state of generalized immunosuppression, pregnancy is characterized by a finely regulated modulation of immune responses that promotes fetal development and successful gestation.

3.1. Innate Immune Changes

The innate immune system undergoes significant modifications during pregnancy. Decidual natural killer (dNK) cells become the predominant immune cell population at the maternal-fetal interface, accounting for approximately 70% of decidual leukocytes during early pregnancy. Unlike peripheral NK cells, dNK cells exhibit reduced cytotoxic activity and instead secrete cytokines, chemokines, and angiogenic factors that support trophoblast invasion, placental development, and vascular remodeling.

Macrophages also play a crucial role in pregnancy. Most decidual macrophages display an anti-inflammatory (M2-like) phenotype, contributing to tissue remodeling, clearance of apoptotic cells, and maintenance of immune tolerance. Dendritic cells acquire tolerogenic properties that promote regulatory T-cell differentiation and limit excessive inflammatory responses.

In addition, innate immune cells produce growth factors and cytokines that facilitate implantation and placental development while preserving antimicrobial defense mechanisms.

3.2. Adaptive Immune Changes

The adaptive immune system is also modified during pregnancy. One of the most important changes is the expansion of regulatory T cells (Tregs), which suppress maternal immune responses directed against fetal antigens. Increased Treg activity contributes to the establishment and maintenance of feto-maternal tolerance. B-cell activity may also be altered during pregnancy, leading to changes in antibody production and immune regulation. Furthermore, maternal T lymphocytes become less responsive to fetal antigens, reducing the risk of immune-mediated fetal rejection. These adaptations ensure that maternal adaptive immunity remains capable of responding to pathogens while minimizing harmful responses against fetal tissues.

3.3. Th1/Th2 Balance

The balance between T helper 1 (Th1) and T helper 2 (Th2) immune responses is an important aspect of pregnancy immunology. Successful pregnancy is generally associated with a predominance of Th2-type responses, which favor antibody production and anti-inflammatory mechanisms. Th2 cytokines such as IL-4, IL-5, and IL-10 contribute to fetal tolerance and support pregnancy maintenance. In contrast, excessive Th1 responses characterized by the production of pro-inflammatory cytokines such as IFN-γ, TNF-α, and IL-2 may impair implantation and placental development and have been associated with pregnancy complications, including recurrent miscarriage and preeclampsia. Current evidence suggests that pregnancy is not simply a Th2-dominant condition but rather a dynamic immunological state involving a carefully regulated balance among Th1, Th2, Treg, and Th17 responses that changes throughout gestation **(Figure 56)**.

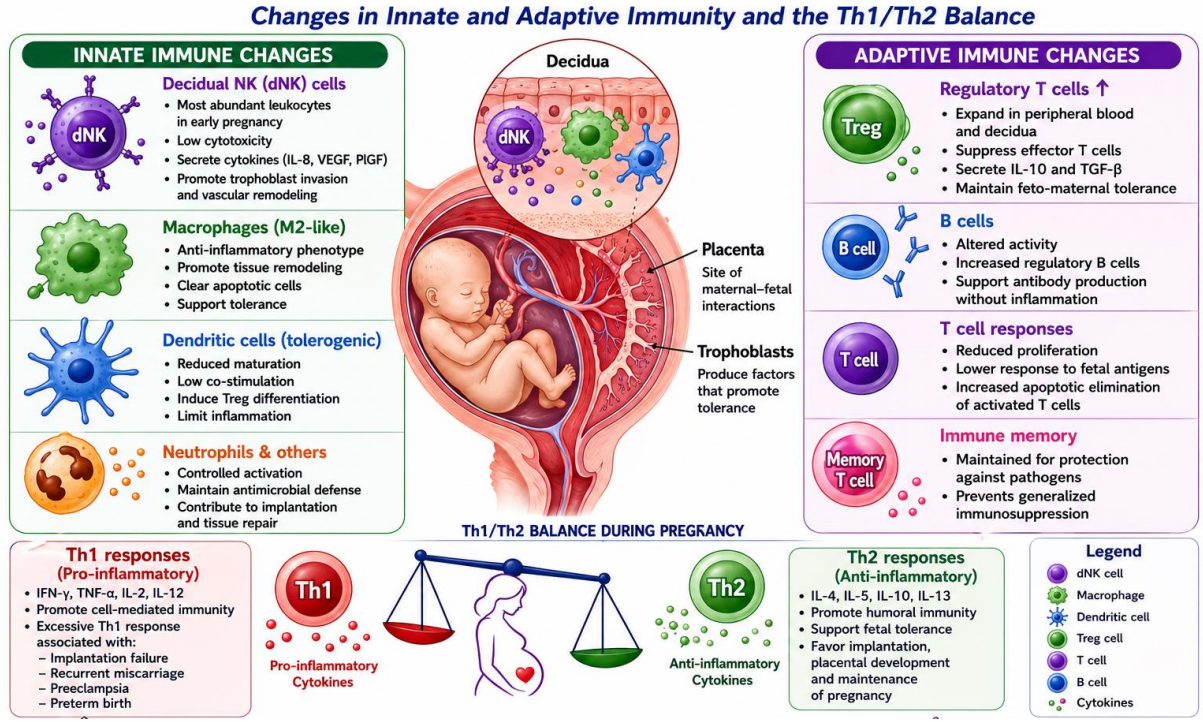


Figure 56. Immune adaptations during pregnancy: changes in innate and adaptive immunity and the Th1/Th2 balance.

4. Pregnancy-Related Immune Disorders

Successful pregnancy depends on the establishment and maintenance of a finely regulated immune balance at the maternal-fetal interface. Disruption of the mechanisms responsible for feto-maternal tolerance may result in pregnancy complications that threaten maternal health,

fetal development, or both. Among the most important immunologically mediated disorders are preeclampsia, recurrent pregnancy loss, and maternal-fetal infections.

4.1. Preeclampsia

Preeclampsia is a pregnancy-specific disorder characterized by hypertension and organ dysfunction, usually developing after the twentieth week of gestation. Although its exact cause remains incompletely understood, growing evidence suggests that abnormal immune responses play a central role in its pathogenesis.

Defective trophoblast invasion and inadequate remodeling of uterine spiral arteries result in placental hypoxia and the release of inflammatory mediators into the maternal circulation. Women with preeclampsia often exhibit reduced numbers or impaired function of regulatory T cells, increased activation of innate immune cells, and elevated levels of pro-inflammatory cytokines such as TNF-α, IL-6, and IFN-γ. These alterations contribute to endothelial dysfunction, systemic inflammation, and the clinical manifestations of the disease.

4.2. Recurrent Pregnancy Loss

Recurrent pregnancy loss is generally defined as the occurrence of two or more consecutive spontaneous miscarriages before fetal viability. Although genetic, anatomical, endocrine, and infectious factors may contribute to pregnancy loss, immunological abnormalities are recognized as important causes in many cases.

Defective feto-maternal tolerance has been associated with recurrent miscarriage. Reduced expansion of regulatory T cells, excessive natural killer cell activity, increased Th1-type immune responses, and insufficient production of anti-inflammatory cytokines may impair embryo implantation and placental development. Altered expression of HLA-G by trophoblast cells has also been linked to pregnancy failure. These findings emphasize the critical role of immune regulation during early pregnancy.

4.3. Maternal-Fetal Infections

Pregnancy requires a balance between immune tolerance toward the fetus and effective protection against infectious agents. Certain pathogens can cross the placental barrier and infect the fetus, resulting in congenital disease, developmental abnormalities, or fetal loss.

Viruses, bacteria, parasites, and other microorganisms may disrupt normal pregnancy by inducing inflammatory responses at the maternal-fetal interface. Excessive production of pro-inflammatory cytokines can impair placental function and contribute to adverse pregnancy outcomes, including preterm birth and fetal growth restriction. At the same time, some degree of immune modulation during pregnancy may increase maternal susceptibility to particular infections.

Understanding the interactions between pathogens, placental tissues, and the maternal immune system is essential for preventing and managing infectious complications during pregnancy.

Chapter 12. Aging and the Immune System

Aging is associated with progressive changes in the immune system that affect its ability to protect the body against infections, cancer, and other diseases. These age-related alterations, collectively known as **immunosenescence**, involve both innate and adaptive immunity and contribute to increased morbidity and mortality in older individuals. Immunosenescence is characterized by a decline in immune function, reduced responsiveness to new antigens, impaired vaccine efficacy, and the accumulation of chronic low-grade inflammation.

The aging immune system undergoes structural and functional modifications that influence immune cell production, activation, and communication. Although many immune responses become less effective with age, some inflammatory processes remain persistently activated, creating an imbalance that contributes to the development of chronic diseases. As a result, elderly individuals are more susceptible to infectious diseases, autoimmune disorders, cancer, and inflammatory conditions.

Understanding the mechanisms of immunosenescence is essential for developing strategies aimed at promoting healthy aging and improving immune protection in older populations.

1. Changes in Innate Immunity

The innate immune system represents the first line of defense against pathogens. Although many innate immune cells remain present throughout life, their functional capacity progressively declines with age. These alterations contribute to impaired pathogen recognition, reduced antimicrobial activity, and dysregulated inflammatory responses.

1.1. Neutrophils and Macrophages

Neutrophils from elderly individuals generally maintain normal cell numbers but exhibit reduced chemotaxis, phagocytosis, and microbial killing capacity. Their migration toward sites of infection becomes less efficient, resulting in delayed pathogen clearance.

Macrophages also undergo functional changes during aging. Their ability to recognize pathogens, phagocytose microorganisms, and present antigens to lymphocytes is diminished. In addition, aged macrophages often produce altered levels of inflammatory mediators, contributing to impaired immune regulation and tissue repair.

1.2. Dendritic Cells and Natural Killer Cells

Dendritic cells play a central role in linking innate and adaptive immunity through antigen presentation. Aging is associated with reduced dendritic cell function, impaired antigen uptake, and decreased activation of naïve T lymphocytes. These defects contribute to weaker adaptive immune responses.

Natural killer (NK) cells remain relatively abundant in older individuals; however, their cytotoxic activity and cytokine production may be altered. Although some NK cell subsets increase in number with age, their ability to eliminate virus-infected and tumor cells is often reduced.

1.3. Inflammaging

A characteristic feature of aging is the presence of chronic, low-grade systemic inflammation known as inflammaging. This condition is characterized by persistent elevation of inflammatory mediators, including IL-6, TNF-α, and C-reactive protein (CRP), even in the absence of infection.

Inflammaging results from multiple factors, including lifelong antigenic exposure, cellular senescence, accumulation of damaged molecules, and age-related alterations in immune regulation. Chronic inflammation contributes to the development of many age-associated diseases, including cardiovascular disease, type 2 diabetes, neurodegenerative disorders, osteoporosis, and cancer.

2. Changes in Adaptive Immunity

The adaptive immune system is particularly affected by aging. Progressive alterations in lymphocyte development, diversity, and function reduce the ability of older individuals to respond effectively to new pathogens and vaccines.

2.1. Thymic Involution

One of the earliest and most significant changes associated with aging is **thymic involution**. Beginning after puberty, the thymus gradually decreases in size and functional activity, with lymphoid tissue progressively replaced by adipose tissue. As thymic output declines, the

production of naïve T lymphocytes is reduced. Consequently, the repertoire of T-cell receptors becomes less diverse, limiting the capacity to recognize novel antigens and respond to emerging infections.

2.2. T-Cell Alterations

Aging is associated with a decrease in naïve T cells and an accumulation of memory and senescent T cells. These changes reduce immune flexibility and impair responses to previously unencountered pathogens. Older T cells exhibit decreased proliferative capacity, reduced cytokine production, and impaired cytotoxic activity. In addition, chronic antigenic stimulation throughout life may lead to the accumulation of dysfunctional T cells that contribute to persistent inflammation and reduced immune efficiency. These alterations result in weaker cellular immune responses, increased susceptibility to infections, and diminished vaccine-induced protection.

2.3. B-Cell Alterations and Antibody Responses

The aging process also affects B-cell development and function. Production of new naïve B cells declines, while the diversity of the B-cell receptor repertoire decreases. Consequently, older individuals generate fewer high-affinity antibodies in response to new antigens.

Age-related defects in germinal center formation, class-switch recombination, and affinity maturation further impair humoral immunity. As a result, antibody responses become weaker, less diverse, and shorter-lasting.

These changes contribute to reduced vaccine efficacy and increased vulnerability to bacterial and viral infections in the elderly population.

3. Clinical Consequences

The age-associated decline in immune function has important clinical implications. Immunosenescence affects the body's ability to prevent infections, respond effectively to vaccination, and maintain immune surveillance against malignant cells. At the same time, alterations in immune regulation may increase the risk of autoimmune disorders in older individuals.

3.1. Increased Susceptibility to Infections

Older adults are more vulnerable to bacterial, viral, and fungal infections due to impaired innate and adaptive immune responses. Reduced neutrophil and macrophage function, decreased T-cell diversity, and weaker antibody responses collectively compromise pathogen clearance. Respiratory infections such as influenza, pneumonia, and COVID-19 are particularly severe in elderly populations and are associated with higher hospitalization and mortality rates.

In addition, infections often present with atypical clinical manifestations in older individuals, which may delay diagnosis and treatment.

3.2. Reduced Vaccine Efficacy

Vaccination remains one of the most effective preventive strategies in older adults; however, immunosenescence significantly reduces vaccine responsiveness. Age-related defects in antigen presentation, T-cell activation, B-cell function, and antibody production lead to weaker and shorter-lasting protective immunity.

As a result, vaccines administered to elderly individuals may generate lower antibody titers and reduced cellular immune responses compared with younger adults. These observations have prompted the development of high-dose vaccines, adjuvanted formulations, and optimized vaccination schedules specifically designed for older populations.

3.3. Cancer and Autoimmunity in the Elderly

The immune system plays a critical role in detecting and eliminating transformed cells. Aging is associated with a decline in immune surveillance, allowing malignant cells to evade immune recognition and increasing the incidence of cancer. Many cancers, including those affecting the lung, colon, prostate, and hematopoietic system, become more common with advancing age.

Paradoxically, despite the overall decline in immune function, aging may also increase the risk of autoimmunity. Defects in immune regulation, altered tolerance mechanisms, and chronic inflammation can contribute to the production of autoreactive lymphocytes and autoantibodies. Consequently, several autoimmune disorders occur more frequently in elderly individuals.

4. Healthy Aging and Immune Interventions

Although aging cannot be prevented, several interventions can help preserve immune function and reduce the negative consequences of immunosenescence. Lifestyle modifications, preventive vaccination programs, and emerging therapeutic approaches have shown promise in promoting healthy immune aging.

4.1. Nutrition and Physical Activity

Adequate nutrition is essential for maintaining immune competence throughout life. Deficiencies in proteins, vitamins, and trace elements can impair immune cell development and function. Nutrients such as vitamins A, C, D, E, zinc, selenium, and omega-3 fatty acids play important roles in supporting immune responses and controlling inflammation. Regular physical activity has also been shown to improve immune function in older adults. Moderate exercise enhances immune surveillance, reduces chronic inflammation, improves metabolic health, and contributes to the maintenance of functional immune cell populations. Together, balanced nutrition and regular exercise represent fundamental strategies for healthy aging.

4.2. Vaccination in Older Adults

Vaccination remains a cornerstone of preventive healthcare in the elderly. Immunization against influenza, pneumococcal disease, COVID-19, herpes zoster, and other infectious diseases significantly reduces morbidity and mortality. To overcome reduced vaccine responsiveness associated with aging, newer vaccine formulations often include higher antigen doses or adjuvants designed to enhance immune activation. Booster vaccinations may also be necessary to maintain adequate protection over time.

4.3. Emerging Immunotherapies

Recent advances in immunology have led to the development of innovative approaches aimed at improving immune function in older individuals. These strategies include cytokine-based therapies, immune checkpoint modulation, enhancement of thymic function, cellular therapies, and interventions targeting chronic inflammation.

Research is also exploring the use of senolytic drugs, which selectively eliminate senescent cells, as well as therapies that modulate the gut microbiota and metabolic pathways involved in

immune regulation. Although many of these approaches remain experimental, they offer promising opportunities to delay immunosenescence and improve health outcomes in aging populations.

Chapter 13. Role of Apoptosis in Immune Homeostasis

Apoptosis is a highly regulated form of programmed cell death that plays a fundamental role in the development, maintenance, and regulation of multicellular organisms. Unlike necrosis, which results from acute cellular injury and is often associated with inflammation, apoptosis is an orderly process that eliminates unwanted, damaged, or potentially harmful cells without causing significant tissue damage or inflammatory reactions.

Within the immune system, apoptosis is essential for maintaining immune homeostasis, a state in which immune responses are effective against pathogens while remaining controlled to prevent excessive inflammation and autoimmunity. Throughout life, billions of cells undergo apoptosis every day, allowing tissues to renew themselves and preserve normal physiological function.

Apoptosis contributes to immune regulation at several levels. During lymphocyte development, it eliminates immature cells that recognize self-antigens too strongly, thereby promoting self-tolerance. Following an immune response, apoptosis removes excess activated lymphocytes, preventing prolonged inflammation and restoring immune equilibrium. It also participates in the elimination of infected, damaged, senescent, or malignant cells, protecting the organism from disease.

Defects in apoptotic pathways can have severe consequences. Insufficient apoptosis may allow the survival of autoreactive or transformed cells, contributing to autoimmune diseases and cancer. Conversely, excessive apoptosis may result in immunodeficiency and tissue damage. Therefore, the precise regulation of programmed cell death is critical for both immune function and overall health.

1. Molecular Mechanisms of Apoptosis

Apoptosis is mediated by a complex network of signaling pathways that ultimately lead to controlled cellular destruction. Two major pathways have been identified: the intrinsic (mitochondrial) pathway and the extrinsic (death receptor) pathway. Although initiated by different stimuli, both pathways converge on the activation of a family of proteolytic enzymes known as caspases, which execute the apoptotic program **(Figure 57)**.

1.1. Intrinsic (Mitochondrial) Pathway

The intrinsic pathway is activated by intracellular stress signals such as DNA damage, oxidative stress, growth factor deprivation, viral infection, or cellular aging. These stimuli disrupt mitochondrial integrity and trigger the release of pro-apoptotic molecules from the mitochondrial intermembrane space. A key event in this pathway is the release of cytochrome c, which associates with Apaf-1 (apoptotic protease-activating factor 1) and procaspase-9 to form a multiprotein complex called the apoptosome. This complex activates caspase-9, which subsequently activates downstream executioner caspases. The intrinsic pathway is tightly regulated by members of the Bcl-2 family. Anti-apoptotic proteins such as Bcl-2 and Bcl-xL preserve mitochondrial integrity, whereas pro-apoptotic proteins including Bax and Bak promote mitochondrial membrane permeabilization and apoptosis. The balance between these opposing proteins determines whether a cell survives or undergoes programmed death.

1.2. Extrinsic (Death Receptor) Pathway

The extrinsic pathway is initiated through the interaction of extracellular death ligands with specific cell-surface death receptors. Important receptors include Fas (CD95)**,** TNF receptor 1 (TNFR1)**,** and TRAIL receptors**.** Binding of Fas ligand (FasL) or other death ligands induces receptor trimerization and recruitment of adaptor proteins, leading to the formation of the death-inducing signaling complex (DISC). This complex activates initiator caspase-8, which then activates downstream executioner caspases. The extrinsic pathway plays a crucial role in immune regulation by eliminating activated lymphocytes after immune responses and by mediating cytotoxic T-cell and natural killer cell killing of infected or malignant cells.

1.3. Caspase Activation

Caspases are cysteine proteases that serve as the central effectors of apoptosis. They are synthesized as inactive precursors (procaspases) and become activated through proteolytic cleavage. Initiator caspases, including caspase-8 and caspase-9, are activated early in the apoptotic process and subsequently activate executioner caspases such as caspase-3, caspase-6, and caspase-7. These executioner caspases cleave numerous cellular proteins, leading to DNA fragmentation, chromatin condensation, cytoskeletal degradation, membrane blebbing, and formation of apoptotic bodies. The resulting apoptotic bodies are rapidly recognized and

engulfed by phagocytic cells, particularly macrophages, ensuring efficient cell removal without triggering inflammation.

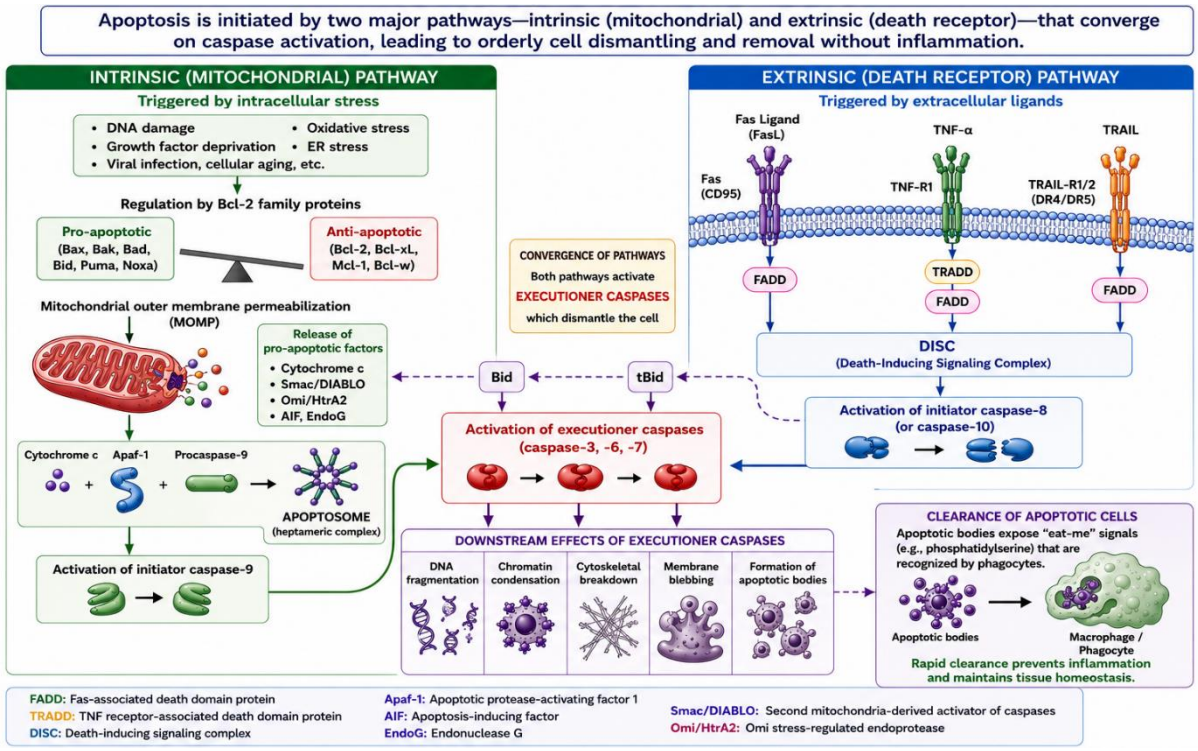


Figure 57. Molecular pathways of apoptosis: intrinsic and extrinsic signaling cascades converging on caspase activation.

2. Apoptosis in the Immune System

Apoptosis is essential for the development, regulation, and maintenance of the immune system. By eliminating unnecessary, damaged, infected, or potentially harmful cells, apoptosis ensures that immune responses remain effective while preventing excessive inflammation and autoimmunity. This process contributes to immune homeostasis throughout life and plays a central role in both central and peripheral tolerance mechanisms.

2.1. Elimination of Autoreactive Lymphocytes

During lymphocyte development, a large number of immature B and T cells are generated with randomly rearranged antigen receptors. Some of these receptors recognize self-antigens with high affinity and therefore pose a risk of autoimmunity. In the thymus, developing T lymphocytes undergo a process known as negative selection, during which autoreactive T cells are induced to undergo apoptosis. Similarly, immature B cells that strongly recognize self-

antigens in the bone marrow are eliminated through apoptotic mechanisms. This process, known as central tolerance, prevents the release of self-reactive lymphocytes into the peripheral circulation. Through the selective elimination of potentially dangerous lymphocytes, apoptosis contributes to the establishment of self-tolerance and protects the organism from autoimmune diseases.

2.2. Contraction of Immune Responses

Following pathogen elimination, most activated immune cells are no longer required. To restore homeostasis and prevent chronic inflammation, the immune system enters a contraction phase characterized by extensive apoptosis of effector lymphocytes. After an infection, the majority of activated T cells undergo activation-induced cell death (AICD), a process frequently mediated by Fas-FasL interactions and caspase activation. Similarly, many activated B cells and other immune effectors are eliminated once their function has been fulfilled. Only a small population of long-lived memory T and B cells survives, providing long-term protective immunity. Therefore, apoptosis is crucial for terminating immune responses and returning the immune system to a resting state.

2.3. Maintenance of Peripheral Tolerance

Despite central tolerance mechanisms, some autoreactive lymphocytes escape deletion and reach peripheral tissues. Peripheral tolerance mechanisms prevent these cells from causing tissue damage. Apoptosis contributes to peripheral tolerance by eliminating self-reactive lymphocytes that become activated outside primary lymphoid organs. Repeated recognition of self-antigens without adequate co-stimulatory signals can trigger apoptotic pathways and induce clonal deletion. In addition, regulatory T cells (Tregs) help maintain tolerance by suppressing autoreactive lymphocytes and promoting apoptotic elimination of potentially harmful immune cells. Together, these mechanisms prevent autoimmune reactions and maintain immune equilibrium.

3. Dysregulation of Apoptosis

The balance between cell survival and cell death is critical for normal immune function. Defects in apoptotic pathways can result in either excessive cell survival or excessive cell death, both

of which may contribute to disease. Dysregulation of apoptosis is implicated in autoimmune disorders, immunodeficiencies, and cancer development.

3.1. Autoimmune Diseases

Insufficient apoptosis can allow autoreactive lymphocytes to escape deletion and persist within the immune system. These cells may recognize self-antigens and initiate destructive immune responses against healthy tissues. Defects in Fas, Fas ligand, caspases, or other apoptotic regulators have been associated with autoimmune disorders such as systemic lupus erythematosus (SLE), autoimmune lymphoproliferative syndrome (ALPS), and rheumatoid arthritis. Failure to remove autoreactive cells results in chronic inflammation, autoantibody production, and progressive tissue damage. Thus, defective apoptotic elimination of self-reactive lymphocytes is a major mechanism contributing to autoimmunity.

3.2. Immunodeficiency

Excessive apoptosis can have the opposite effect by depleting essential immune cell populations and weakening host defenses. Increased lymphocyte apoptosis may impair immune responses against pathogens and increase susceptibility to infections. Several viral infections, including HIV infection, promote apoptosis of immune cells, particularly $CD4^+$ T lymphocytes, leading to profound immunodeficiency. Excessive apoptosis can also occur in severe systemic inflammatory conditions, contributing to immune exhaustion and impaired immune competence. Consequently, inappropriate activation of apoptotic pathways may result in reduced immune protection and increased vulnerability to opportunistic infections.

3.3. Cancer Development

Apoptosis serves as a critical defense mechanism against cancer by eliminating cells that have acquired potentially oncogenic mutations. When apoptotic pathways become defective, abnormal cells can survive, accumulate additional genetic alterations, and eventually develop into malignant tumors. Many cancers exhibit overexpression of anti-apoptotic proteins such as Bcl-2 or mutations in tumor suppressor genes such as p53, allowing transformed cells to evade programmed cell death. Tumor cells may also develop resistance to death receptor signaling and caspase activation. The ability to escape apoptosis is considered one of the fundamental hallmarks of cancer and contributes to tumor growth, progression, and resistance to therapy.

Chapter 14. Immuno-Endocrine Interactions

The immune and endocrine systems are two major regulatory networks that maintain homeostasis and coordinate the body's responses to internal and external challenges. Traditionally studied as separate systems, it is now well established that they communicate extensively through a complex network of hormones, cytokines, neurotransmitters, and cellular receptors. This bidirectional communication enables the organism to adapt efficiently to physiological changes, environmental stress, infection, inflammation, and tissue injury.

The immune system protects the body against pathogens and abnormal cells through innate and adaptive immune responses, while the endocrine system regulates growth, metabolism, reproduction, stress responses, and energy balance through the secretion of hormones. Increasing evidence indicates that hormones can profoundly influence immune cell development, differentiation, activation, and function. Conversely, immune mediators can affect endocrine gland activity and hormone production.

The concept of immuno-endocrine communication refers to this continuous exchange of signals between immune and endocrine organs. Immune cells express receptors for numerous hormones, including glucocorticoids, sex hormones, thyroid hormones, insulin, and growth hormone. Similarly, endocrine tissues respond to cytokines produced during immune responses. This reciprocal interaction plays a critical role in maintaining physiological balance and coordinating defense mechanisms during health and disease.

The relationship between these systems is particularly evident during stress, pregnancy, infection, inflammation, and autoimmune disorders. Dysregulation of immuno-endocrine interactions can contribute to the development of chronic inflammatory diseases, endocrine dysfunctions, metabolic disorders, and impaired immune responses.

1. Hormonal Regulation of Immunity

Hormones exert powerful regulatory effects on both innate and adaptive immune responses. Immune cells express specific hormone receptors that allow endocrine signals to modulate immune cell survival, proliferation, cytokine production, and effector functions. Among the hormones with the greatest immunological impact are glucocorticoids, sex hormones, and thyroid hormones.

1.1. Glucocorticoids

Glucocorticoids, primarily cortisol, are steroid hormones produced by the adrenal cortex under the control of the hypothalamic-pituitary-adrenal (HPA) axis. They are among the most potent endogenous regulators of immune function and inflammation.

Following stress or inflammation, glucocorticoid secretion increases and exerts predominantly anti-inflammatory and immunosuppressive effects. These hormones inhibit the production of pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6, reduce leukocyte migration to sites of inflammation, suppress antigen presentation, and limit lymphocyte proliferation. Glucocorticoids also promote apoptosis of certain immune cell populations and enhance the production of anti-inflammatory mediators.

Because of their powerful immunomodulatory properties, synthetic glucocorticoids are widely used in the treatment of autoimmune diseases, allergic disorders, transplantation, and chronic inflammatory conditions.

1.2. Sex Hormones

Sex hormones play a major role in shaping immune responses and contribute to many of the immunological differences observed between males and females.

1.2.1. Estrogens

Estrogens generally enhance immune responses, particularly at physiological concentrations. They stimulate antibody production, promote B-cell survival, and influence T-cell differentiation. Estrogens may increase the production of anti-inflammatory cytokines while supporting immune surveillance. These effects partly explain why females often develop stronger immune responses and exhibit higher vaccine responsiveness than males.

However, enhanced immune activity also contributes to the increased prevalence of autoimmune diseases among women.

1.2.2. Progesterone

Progesterone exerts predominantly immunosuppressive and anti-inflammatory effects. During pregnancy, elevated progesterone levels contribute to feto-maternal tolerance by inhibiting inflammatory responses and promoting regulatory T-cell development. Progesterone reduces Th1-mediated immunity and favors a more tolerogenic immune environment, thereby supporting fetal survival.

1.2.3. Androgens

Androgens, such as testosterone, generally suppress immune responses. They inhibit lymphocyte proliferation, decrease antibody production, and reduce the secretion of several pro-inflammatory cytokines. These effects contribute to the lower incidence of autoimmune diseases observed in males but may also be associated with increased susceptibility to certain infections.

1.3. Thyroid Hormones

Thyroid hormones, including triiodothyronine (T3) and thyroxine (T4), regulate cellular metabolism, growth, and development. They also influence immune system activity through direct effects on immune cells and indirect effects on metabolic pathways.

Thyroid hormones can modulate lymphocyte proliferation, macrophage activity, cytokine production, and antibody synthesis. Normal thyroid function is therefore important for maintaining effective immune responses. Both hypothyroidism and hyperthyroidism may alter immune regulation and are frequently associated with immune dysfunction.

The relationship between thyroid function and immunity is further illustrated by autoimmune thyroid diseases, such as Hashimoto's thyroiditis and Graves' disease, which result from dysregulated interactions between endocrine and immune mechanisms.

2. Cytokines and Endocrine Function

The immune and endocrine systems communicate continuously through a network of cytokines and hormones. Cytokines produced during immune responses not only regulate immune cell activity but also influence the function of endocrine glands. In turn, endocrine organs respond by adjusting hormone secretion, creating an integrated system that helps maintain homeostasis during health and disease.

2.1. Effects of Cytokines on Endocrine Glands

During infection and inflammation, immune cells release cytokines such as IL-1, IL-6, TNF-α, and interferons, which can directly affect endocrine tissues. These cytokines influence the hypothalamus, pituitary gland, adrenal glands, thyroid gland, pancreas, and gonads.

Pro-inflammatory cytokines stimulate the hypothalamic-pituitary-adrenal (HPA) axis, leading to increased secretion of cortisol. Cortisol then exerts anti-inflammatory effects that help limit excessive immune activation. Cytokines can also alter thyroid hormone production, insulin secretion, and reproductive hormone synthesis. During severe or chronic inflammation, these alterations may contribute to endocrine dysfunction and metabolic disturbances.

Thus, cytokines act as important messengers linking immune activation to hormonal adaptation.

2.2. Neuroendocrine-Immune Signaling

The nervous, endocrine, and immune systems form an interconnected regulatory network known as the neuroendocrine-immune axis. Communication occurs through neurotransmitters, hormones, cytokines, and their respective receptors expressed on immune and endocrine cells.

One of the best-characterized pathways is the hypothalamic-pituitary-adrenal axis. Stress, infection, or inflammation stimulates the hypothalamus to release corticotropin-releasing

hormone (CRH), which triggers adrenocorticotropic hormone (ACTH) secretion from the pituitary gland. ACTH subsequently stimulates cortisol production by the adrenal cortex. Cortisol then suppresses excessive inflammatory responses through negative feedback mechanisms.

This integrated signaling network allows the body to coordinate immune defense, stress adaptation, energy metabolism, and tissue repair.

3. Immuno-Endocrine Disorders

Disruption of normal communication between the immune and endocrine systems can contribute to the development of numerous diseases. Immune-mediated endocrine disorders, chronic stress-related immune dysfunction, and reproductive or metabolic abnormalities illustrate the importance of immuno-endocrine balance.

3.1. Autoimmune Endocrine Diseases

Autoimmune endocrine diseases occur when immune tolerance is lost and the immune system targets endocrine tissues. These disorders are among the most common examples of immuno-endocrine dysregulation.

In Hashimoto's thyroiditis, autoreactive lymphocytes and autoantibodies destroy thyroid tissue, resulting in hypothyroidism. In Graves' disease, stimulating autoantibodies target the thyroid-stimulating hormone receptor, causing hyperthyroidism. Other examples include type 1 diabetes mellitus, characterized by autoimmune destruction of pancreatic β-cells, and Addison's disease, resulting from immune-mediated adrenal gland damage. These conditions demonstrate how immune dysfunction can profoundly alter endocrine function.

3.2. Chronic Stress and Immune Dysfunction

Chronic psychological or physiological stress activates the hypothalamic-pituitary-adrenal axis and increases glucocorticoid production. While acute cortisol secretion is beneficial in controlling inflammation, prolonged exposure to elevated cortisol levels may impair immune function. Chronic stress is associated with reduced lymphocyte proliferation, decreased natural killer cell activity, impaired vaccine responses, and increased susceptibility to infections. Long-term stress may also contribute to chronic inflammatory diseases, metabolic disturbances, and impaired tissue repair. Therefore, sustained activation of stress pathways can significantly disrupt immune homeostasis.

3.3. Reproductive and Metabolic Disorders

Immuno-endocrine interactions play a crucial role in reproductive health and metabolic regulation. Abnormal immune responses may interfere with implantation, pregnancy

maintenance, and fetal development. Conditions such as recurrent pregnancy loss, preeclampsia, and infertility have been associated with altered immune regulation and hormonal imbalances. Similarly, chronic inflammation contributes to metabolic disorders including obesity, insulin resistance, metabolic syndrome, and type 2 diabetes. Pro-inflammatory cytokines such as TNF-α and IL-6 can impair insulin signaling and promote metabolic dysfunction. These examples highlight the close relationship between immune regulation, hormonal balance, reproduction, and metabolism.

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