BHARATH COLLEGE OF SCIENCE AND MANAGEMENT

THANJAVUR -5

PG& RESEARCH DEPARTMENT OF BIOTECHNOLOGY

TITTLE OF THE PAPER : IMMUNOLOGY

SUBJECT CODE: 16SCCBT4

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SYLLABUS REVIEW

Unit I

- Antigen
- Immunoglobulin,
- Innate & acquired immunity
- Haematopoesis
- Primary lymphoid organs,
- Secondary lymphoid tissues

Unit II

- Natural built in barriers
- Cytokines
- Interferon
- Macrophages
- Phagocytosis
- MALT
- GALT

Unit III

- T cell development, maturation, activation and differentiation
- B cell development, maturation, activation and differentiation.

Unit IV

- Active, passive immunization

Unit V

- Hypersensitivity – Type I, II, III and IV.
- Immunodeficiency

PART -B (5 Mark questions with answers)

Antigens

The term antigen is derived from antibody generation, referring to any substance that is capable of eliciting an immune response (e.g., the production of specific antibody molecules). By definition, an antigen (Ag) is capable of combining with the specific antibodies formed by its presence.

Antigens are generally of high molecular weight, and commonly are proteins or polysaccharides. Polypeptides, lipids, nucleic acids, and many other materials can also function as antigens. Immune responses may also be generated against smaller substances, called haptens,

On basis of Source/Origin

1) **Exogenous antigens**: These are antigens which are foreign to host body hence are also called **foreign antigens**. These are antigens that enters the body of the organism from the outside, e.g. through inhalation, ingestion, or injection. These antigens enters the body or system and start circulating in the body fluids and trapped by the APCs (Antigen processing cells such as macrophages, dendritic cells etc.). The uptakes of these exogenous antigens by APCs are mainly mediated by the phagocytosis. E.g. Bacteria, Fungi, Viruses etc.
2) **Endogenous antigens:** These are antigens which originate from own body of host organisms. These are body's own cells or sub fragments or compounds or the antigenic products that are produced. The endogenous antigens are processed by the macrophages which are later accepted by the cytotoxic T – cells. E.g. Blood group antigens, HLA (Histocompatibility Leukocyte antigens) etc. **Endogenous antigens**are generated within normal cells as a result of normal cell metabolism, or because of viral or intracellular bacterial infection.
3) **Auto antigens:** These are usually a normal protein or protein complex (and sometimes DNA or RNA) that is recognized by the immune system of patients suffering from a specific autoimmune disease. These are not immunogenic under normal condition however due to genetic and environmental changes or factors immunological tolerance is lost and immune response is generated. E.g. Nucleoproteins, Nucleic acids, etc.

On the basis of immune response

Immunogens/ Complete antigens: A substance that induces specific immune response can be called as immunogen. Antigens which are able to generate immune response by themselves are known as complete antigens. These are generally molecules with high molecular weight (more than 10,000 Daltons). They possess antigenic properties denovo and are usually proteinaceous in nature. Some of them can be polysaccharide in chemical nature.

Haptens/ Incomplete antigens: Antigens which are unable to generate the immune response themselves are termed as incomplete antigens however on coupling with carrier proteins they can be immunogenic. They are also called haptens. When a molecule of haptens are coupled to carrier proteins they become accessible to immune system and function as an immunogen. They generally have low molecular weight (Less than 10,000 Daltons) and are usually non-protein substances. E.g. Capsular polysaccharide of pneumococcus, polysaccharide "C" of β-haemolytic streptococci, Cardiolipin antigens, etc.

Superantigens

- **Superantigens** (SAgs) are a class of antigens that cause non-specific activation of T-cells resulting in polyclonal T cell activation and massive cytokine release. SAgs are produced by some pathogenic viruses and bacteria most likely as a defense mechanism against the immune system.

- **Autoantigens**: These are antigens despite being a normal tissue constituent, is the target of a humoral or cell-mediated immune response, such as in autoimmune disease. E.g. Thyroglobulin, DNA, Corneal tissue, etc.
- **Alloantigens:** These are antigens found in different members of the same species. E.g. Red blood cell antigens A and B.
- **Heterophile antigens:** These are same or closely related antigens, sometimes present in tissues of different biological species, classes, or kingdoms. identical antigens found in the cells of different species. Examples: Forrssman antigen, Cross-reacting microbial antigens, etc.

IMMUNOGLOBULINS - STRUCTURE AND FUNCTION

Immunoglobulin (Ig)

Immunoglobulins are glycoprotein molecules that are produced by plasma cells in response to an immunogen and which function as antibodies. The immunoglobulins derive their name from the finding that they migrate with globular proteins when antibody-containing serum is placed in an electrical field.

GENERAL FUNCTIONS OF IMMUNOGLOBULINS

A.Antigenbinding

Immunoglobulins bind specifically to one or a few closely related antigens. Each immunoglobulin actually binds to a specific antigenic determinant. Antigen binding by antibodies is the primary function of antibodies and can result in protection of the host. The valency of antibody refers to the number of antigenic determinants that an individual antibody molecule can bind. The valency of all antibodies is at least two and in some instances more.

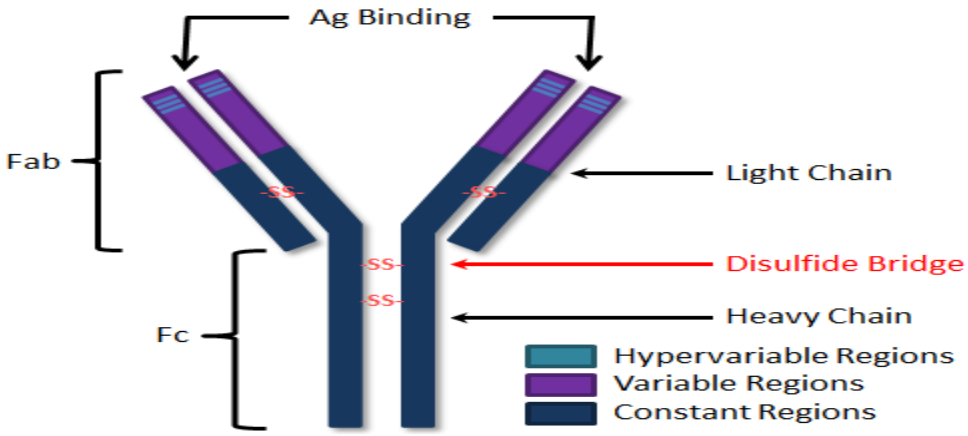
B.EffectorFunctions

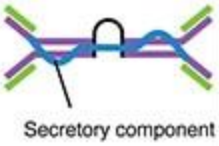
Frequently the binding of an antibody to an antigen has no direct biological effect. Rather, the significant biological effects are a consequence of secondary "effector functions" of antibodies. The immunoglobulins mediate a variety of these effector functions. Usually the ability to carry out a particular effector function requires that the antibody bind to its antigen. Not every immunoglobulin will mediate all effector functions. Such effector functions include:

1. Fixation of complement - This results in lysis of cells and release of biologically active molecules (see chapter two)

2. Binding to various cell types - Phagocytic cells, lymphocytes, platelets, mast cells, and basophils have receptors that bind immunoglobulins. This binding can activate the cells to perform some function. Some immunoglobulins also bind to receptors on placental trophoblasts, which results in transfer of the immunoglobulin across the placenta. As a result, the transferred maternal antibodies provide immunity to the fetus and newborn.

Immunoglobulin structure



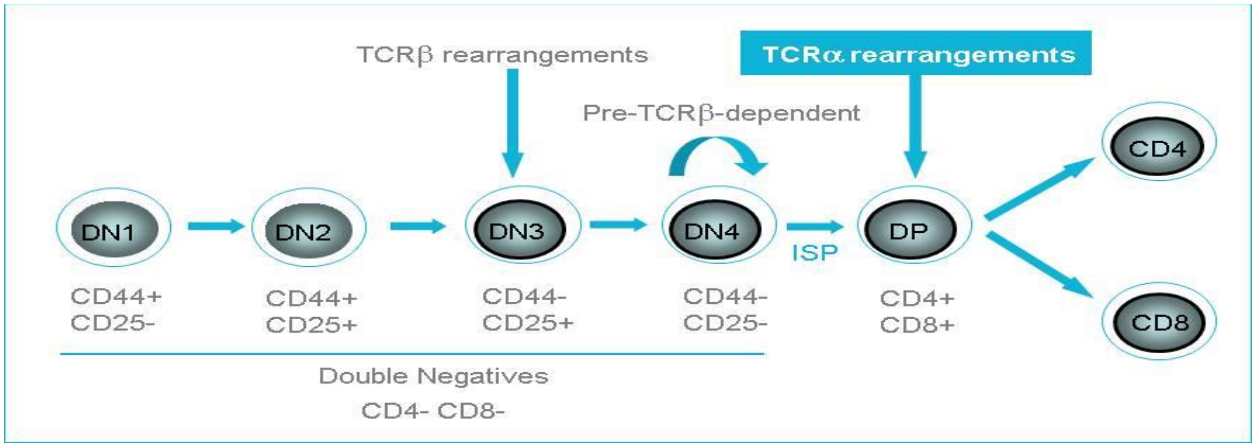
The Five Immunoglobulin (Ig) Classes					
	IgM pentamer	IgG monomer	Secretory IgA dimer	IgE monomer	IgD monomer
					
Heavy chains	μ	γ	α	ε	δ
Number of antigen binding sites	10	2	4	2	2
Molecular weight (Daltons)	900,000	150,000	385,000	200,000	180,000
Percentage of total antibody in serum	6%	80%	13%	0.002%	1%
Crosses placenta	no	yes	no	no	no
Fixes complement	yes	yes	no	no	no
Fc binds to		phagocytes		mast cells and basophils	
Function	Main antibody of primary responses, best at fixing complement; the monomer form of IgM serves as the B cell receptor	Main blood antibody of secondary responses, neutralizes toxins, opsonization	Secreted into mucus, tears, saliva, colostrum	Antibody of allergy and antiparasitic activity	B cell receptor

T-cell development in thymus

cells are derived from haematopoietic stem cells that are found in the bone marrow. The progenitors of these cells migrate to and colonise the thymus. The developing progenitors within the thymus, also known as thymocytes, undergo a series of maturation steps that can be identified based on the expression of different cell surface markers. The majority of cells in the thymus give rise to αβ T cells, however approximately 5% bear the γδ T cell receptor (TCR). Developing thymocytes interact with the thymus stromal (non-haematopoietic) cells, and undergo the process described below in distinct regions of the thymus. The thymus is made up of an outer **cortex** and an inner **medulla** region.

The earliest developing thymocytes lack the expression of the co-receptors CD4 and CD8 and are termed **double negative** (DN) cells. The DN population can be further sub-divided by the expression of CD44 (an adhesion molecule) and CD25 (Interleukin-2 receptor α chain),

Cells that lack expression of CD44, but express CD25 (DN3) undergo a process termed **beta-selection**. This process selects for cells that have successfully rearranged their TCR-β chain locus. The β chain then pairs with the surrogate chain, pre-Tα, and produces a pre-TCR, which forms a complex with CD3 molecules. This complex leads to the survival, proliferation, arrest in further β chain loci rearrangement, and further differentiation by up-regulation and expression of CD4 and CD8, these cells are termed **double positive** (DP) cells. Cells that do not undergo beta-selection die by apoptosis.



The above diagram shows αβ T cell development, showing the different cell surface markers expressed at the different stages of T cell development in the mouse.

Hematopoiesis

Hematopoiesis is the production of all of the cellular components of blood and blood plasma. It occurs within the hematopoietic system, which includes organs and tissues such as the bone marrow, liver, and spleen.

Hematopoiesis is the process through which the body manufactures blood cells. It begins early in the development of an embryo, well before birth, and continues for the life of an individual.

The blood is made up of more than 10 different cell types. Each of these cell types falls into one of three broad categories:

1. Red blood cells (erythrocytes): These transport oxygen and hemoglobin throughout the body.

2. White blood cells (leukocytes): These support the immune system. There are several different types of white blood cells:

- **Lymphocytes**: Including T cells and B cells, which help fight some viruses and tumors.
- **Neutrophils**: These help fight bacterial and fungal infections.
- **Eosinophils**: These play a role in the inflammatory response, and help fight some parasites.
- **Basophils**: These release the histamines necessary for the inflammatory response.
- **Macrophages**: These engulf and digest debris, including bacteria.

3. Platelets (thrombocytes): These help the blood to clot.

Current research endorses a theory of hematopoiesis called the monophyletic theory. This theory says that one type of stem cell produces all types of blood cells.

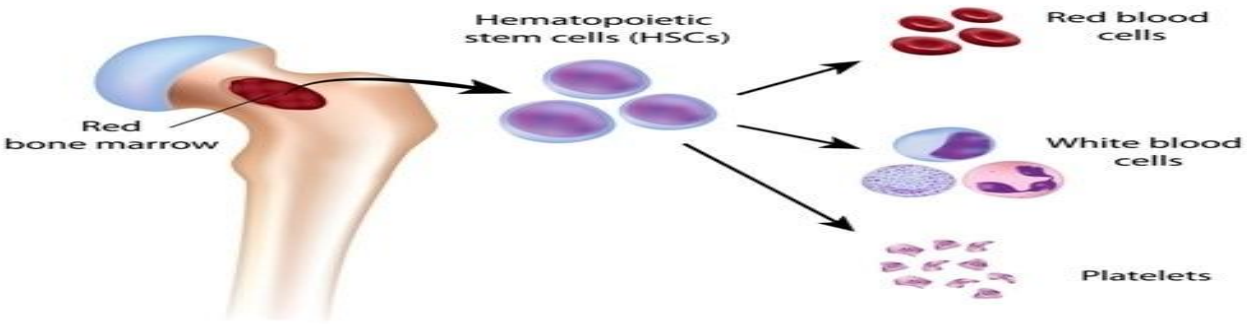
Hematopoiesis occurs in many places:

- **Hematopoiesis in the embryo** Sometimes called primitive hematopoiesis, hematopoiesis in the embryo produces only red blood cells that can provide developing organs with

oxygen. At this stage in development, the yolk sac, which nourishes the embryo until the placenta is fully developed, controls hematopoiesis.

As the embryo continues to develop, the hematopoiesis process moves to the liver, the spleen, and bone marrow, and begins producing other types of blood cells.

The process of hematopoiesis



The rate of hematopoiesis depends on the body's needs. The body continually manufactures new blood cells to replace old ones. About 1 percent of the body's blood cells must be replaced every day.

White blood cells have the shortest life span, sometimes surviving just a few hours to a few days, while red blood cells can last up to 120 days or so.

The process of hematopoiesis begins with an unspecialized stem cell. This stem cell multiplies, and some of these new cells transform into precursor cells. These are cells that are destined to become a particular type of blood cell but are not yet fully developed. However, these immature cells soon divide and mature into blood components, such as red and white blood cells, or platelets.

Phagocytosis role in immune system

Phagocytosis is a process wherein a cell binds to the item it wants to engulf on the cell surface and draws the item inward while engulfing around it. The process of phagocytosis often happens when the cell is trying to destroy something, like a virus or an infected cell, and is often used by immune system cells.

Phagocytosis differs from other methods of endocytosis because it is very specific and depends on the cell being able to bind to the item it wants to engulf by way of cell surface receptors. Phagocytosis won't happen unless the cell is in physical contact with the particle it wants to engulf.

Phagocytosis and the immune system

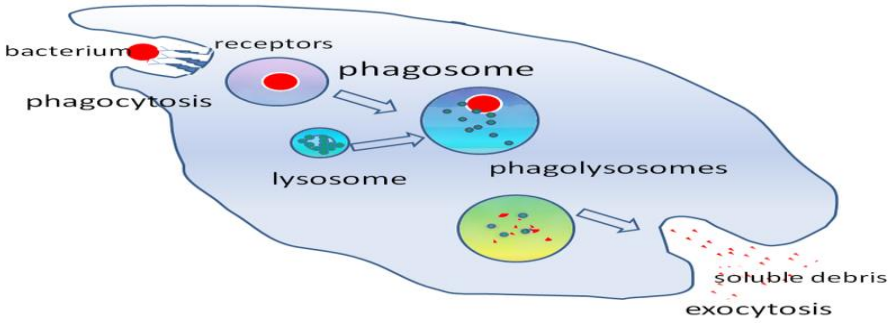
Another function of phagocytosis in the immune system is to ingest and destroy pathogens (like viruses and bacteria) and infected cells. By destroying the infected cells, the immune system limits how quickly the infection can spread and multiply. We mentioned before that the phagolysosome creates an acidic environment to destroy or neutralize its contents. The immune system cells that perform phagocytosis can also use other mechanisms to destroy pathogens inside the phagolysosome, such as:

1) **Oxygen Radicals**: Oxygen radicals are highly reactive molecules that react with proteins, lipids and other biological molecules. During physiological stress, the amount of oxygen radicals in a cell can increase dramatically, causing oxidative stress, which can destroy cell structures.

2) **Nitric Oxide**: Nitric oxide is a reactive substance, similar to oxygen radicals, that reacts with superoxide to create further molecules that damage various biological molecules.

3) **Antimicrobial Proteins**: Antimicrobial proteins are proteins that specifically damage or kill bacteria. Examples of antimicrobial proteins include proteases, which kill various bacteria by destroying essential proteins, and lysozyme, which attacks the cell walls of gram positive bacteria.

4) **Antimicrobial Peptides**: Antimicrobial peptides are similar to antimicrobial proteins in that they attack and kill bacteria. Some antimicrobial peptides, like defensins, attack bacterial cell membranes.



This diagram represents the phacytic process against microbial invasion

Activated factors in Macrophages

S.NO	FACTORS	FUNCTIONS
1	Interleukin 1 (IL-1)	Promotes inflammatory responses and fever
2	Interleukin 6 (IL-6)	Promote innate immunity and TNF-_ elimination of pathogens
3	Complement proteins	Promote inflammatory response and elimination of pathogens
4	Hydrolytic enzymes	Promote inflammatory response
5	Interferon alpha	Activates cellular genes, resulting in the production of proteins that confer an antiviral state on the cell
6	Tumor necrosis factor	Kills tumor cells
7	GM-CSF G-CSF M-CSF	Promote inducible hematopoiesis

Influenza viral Infections

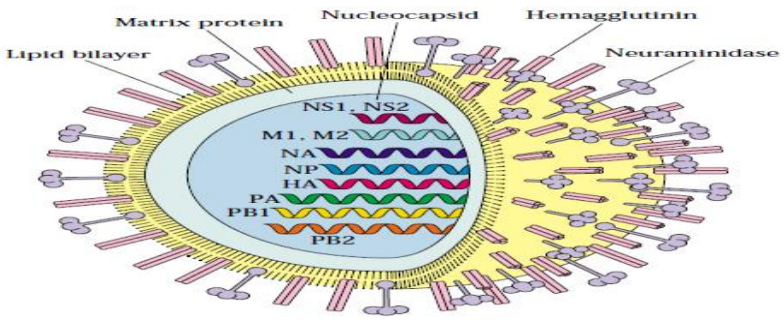
The viral restricted genome size, a number of viruses have been found to encode proteins that interfere at various levels with specific or nonspecific host defenses. Presumably, the advantage of such proteins is that they enable viruses to replicate more effectively amidst host antiviral defenses.

Influenza Has Been Responsible for Some of the Worst Pandemics in History

The influenza virus infects the upper respiratory tract and major central airways in humans, horses, birds, pigs, and even seals. In 1918–19, an influenza pandemic (worldwideepidemic) killed more than 20 million people, a toll surpassing the number of casualties in World War I.

PROPERTIES OF THE INFLUENZA VIRUS

Influenza viral particles, or virions, are roughly spherical or ovoid in shape, with an average diameter of 90–100 nm. The virions are surrounded by an outer envelope—a lipid bilayer acquired from the plasma membrane of the infected host cell during the process of budding. Inserted into the envelope are two glycoproteins, **hemagglutinin (HA)** and **neuraminidase (NA),** which form radiating projections that are visible in electron micrographs. Three basic types of influenza (A, B, and C), can be distinguished by differences in their nucleoprotein and matrix proteins. Type A, which is the most common, is responsible for the major human pandemics.



Structure of Influenza virus

Active immunization

Immunity to infectious microorganisms can be achieved by active or passive **immunization**. In each case, immunity can be acquired either by natural processes(usually by transfer from mother to fetus or by previous infect ion by the organism) or by artificial means such as injection of antibodies or vaccines The agents used for inducing passive immunity include antibodies from humans or animals, whereas active immunization is achieved by inoculation with microbial pathogens that induce immunity butdo not cause disease or with antigenic components from the pathogens. This section describes current usage of passive and active immunization techniques

Passive immunization Passive Immunization Involves Transfer of Preformed Antibodies

Jenner and Pasteur are recognized as the pioneers of vaccination, or induction of active immunity, but similar recognition is due to Emil von Behring and Hidesaburo Kitasato for their contributions to passive immunity. These investigators were the first to show that immunity elicited in one animal can be transferred to another by injecting it with serum from the first.

Passive immunization, in which preformed antibodies aretransferred to a recipient, occurs naturally by transfer of maternal antibodies across the placenta to the developing fetus. Maternal antibodies to diphtheria, tetanus, streptococci, rubeola, rubella, mumps, and poliovirus all afford passively acquired protection to the developing fetus.

Passive immunization can also be achieved by injecting a recipient with preformed antibodies. In the past, before vaccines and antibiotics became available, passive immunization provided a major defense against various infectious diseases. Despite the risks.

S.NO	TYPE OF IMMUNIZATION	ACQUIRED THROUGH
1	**Passive immunity**	Natural maternal antibody Immune globulin
		Humanized monoclonal antibody Antitoxin†
2	**Active immunity**	Natural infection Vaccines‡
		Attenuated organisms
		Inactivated organisms
		Purified microbial macromolecules

PART-C 10 Mark questions with Answers

Innate and Acquired immune system

Over view

Innate Immunity

Adaptive immunity

Components of Innate immune system

- **Anatomic barrier**
- **Physiologic barrier**
- **Phagocytic barrier**
- **Inflammatory barrier**

Anatomic barrier Skin

Anatomic barrier Mucous membrane

Physiologic barrier PH,Temperature

Phagocytic barrier Phagosomes

Inflammatory responses

INNATE AND ADAPTIVE IMMUNE RESPONSES

Innate Immunity

Immunity—the state of protection from infectious disease has both a less specific and more specific component. The less specific component, **innate immunity,** provides the first line of defense against infection

Adaptive immunity

Adaptive immunity responds to the challenge with a high degree of specificity as well as the remarkable property of "memory." Typically, there is an adaptive immune response against an antigen within five or six days after the initial exposure to that antigen.

Adaptive immune responses require some time to marshal, innate immunity provides the first line of defense during the critical period just after the host's exposure to a pathogen.

Components of Innate immune system

Innate immunity can be seen to comprise four types of defensive barriers:

1. Anatomic barrier.
2. Physiologic barrier.
3. Phagocytic barrier.
4. Inflammatory barrier.

Anatomic and Physiologic barriers

Anatomic barrier(Table:1 shows anatomic barrier and mechanisms)

S.NO	Anatomic barriers	Mechanism
1	Skin	Mechanical barrier retards entry of microbes. Acidic environment (pH 3–5) retards growth of microbes.
		Normal flora compete with microbes for attachment

2	Mucous membranes	sites and nutrients.

S.NO	Physiologic barriers	Mechanisms
		Normal body temperature inhibits growth of some pathogens. Fever

Physiologic barriers(Table:2 shows the Physiologic barriers and mechanisms)

1	Temperature	response inhibits growth of some pathogens.
2	Low pH	Acidity of stomach contents kills most ingested microorganisms.
3	Chemical mediators	Lysozyme cleaves bacterial cell wall. Interferon induces antiviral state in uninfected cells.
4	Phagocytic/endocytic barriers	Various cells internalize (endocytose) and break down foreign macromolecules. Specialized cells (blood monocytes, neutrophils, tissue macrophages) internalize (phagocytose),kill,and digest whole microorganisms.
5	Inflammatory barriers	Tissue damage and infection induce leakage of vascular fluid, containing serum proteins with antibacterial activity, and influx of phagocytic cells into the affected area.

Anatomic barrier

Skin

Physical and anatomic barriers that tend to prevent the entry of pathogens are an organism's first line of defense against infection.

The skin consists of two distinct layers

- **Epidermis layer**
- **Dermis layer**

The epidermis contains several layers of tightly packed epithelial cells. The outer epidermal layer consists of dead cells and is filled with a waterproofing protein called keratin.

The dermis, which is composed of connective tissue, contains blood vessels, hair follicles, sebaceous glands, and sweat glands.

The skin may also be penetrated by biting insects (e.g., mosquitoes, mites, ticks, fleas, and sandflies); if these harbor pathogenic organisms, they can introduce the pathogen into the body as they feed.

Mucous membrane

Although many pathogens enter the body by binding to and penetrating mucous membranes, a number of nonspecific defense mechanisms tend to prevent this entry. For example, saliva, tears, and mucous secretions act to wash away potential invaders and also contain antibacterial or antiviral substances. The viscous fluid called mucus, which is secreted by epithelial cells of mucous membranes, entraps foreign microorganisms. In the lower respiratory tract, the mucous membrane is covered by **cilia,** hairlike protrusions of the epithelial-cell membranes.

Physiologic barriers

The physiologic barriers that contribute to innate immunity include temperature, pH, and various soluble and cellassociated molecules. Many species are not susceptible to certain diseases simply because their normal body temperature inhibits growth of the pathogens. Chickens, for example, have innate immunity to anthrax because their high body temperature inhibits the growth of the bacteria. Gastric acidity is an innate physiologic barrier to infection because very few ingested microorganisms can survive the low pH of the stomach contents. One reason newborns are susceptible to some diseases that do not afflict adults is that their stomach contents are less acid than those of adults.

Phagocytic barrier.

Another important innate defense mechanism is the ingestion of extracellular particulate material by **phagocytosis.** Phagocytosis is one type of **endocytosis,** the general term for the uptake by a cell of material from its environment. In phagocytosis, a cell's plasma membrane expands

around the particulate material, which may include whole pathogenic microorganisms, to form large vesicles called **phagosomes**

Inflammatory barrier

Tissue damage caused by a wound or by an invading pathogenic microorganism induces a complex sequence of events collectively known as the **inflammatory response.**, a molecular component of a microbe, such as LPS, may trigger an inflammatory response via interaction with cell surface receptors. The end result of inflammation may be the marshalling of a specific immune response to the invasion or clearance of the invader by components of the innate immune system.

Phagosome fuses with lysosome Lysosomal enzymes digest captured material Digestion products are released from cell of inflammation".

Adaptive Immunity

Adaptive immunity is capable of recognizing and selectively eliminating specific foreign microorganisms and molecules (i.e., foreign antigens). Unlike innate immune responses, adaptive immune responses are not the same in all members of a species but are reactions to specific antigenic challenges.

Adaptive immunity displays four characteristic attributes:

- Antigenic specificity
- Diversity
- Immunologic memory
- Self/nonself recognition

- The **antigenic specificity** of the immune system permits it to distinguish subtle differences among antigens. Antibodies can distinguish between two protein molecules that differ in only a single amino acid.
- Diversity The immune system is capable of generating tremendous *diversity* in its recognition molecules, allowing it to recognize billions of unique structures on foreign antigens. Once the immune system has recognized and responded to an antigen, it exhibits

- Immunologic memory; that is, a second encounter with the same antigen induces a heightened state of immune reactivity. Because of this attribute, the immune system can confer life-long immunity to many infectious agents after an initial encounter.
- Finally, theimmune system normally responds only to foreign antigens, indicating that it is capable of *self/nonself recognition*. The ability of the immune system to distinguish self from nonself and respond only to nonself molecules is essential, for, as described below, the outcome of an inappropriate response to self molecules can be fatal. Adaptive immunity is not

LYMPHOID ORGANS

OVER VIEW

ORGANA S OF THE IMMUNE SYSTEM

LYMPHOID ORGANS

PRIMARY YMPHOID ORGANS

BONE MARROW

THYMUS

SECONDARYLYMPHOID ORGANS

LYMPH NODE

SPLEEN

MALT

GALT

Organs of the Immune System

A number of morphologically and functionally diverse organs and tissues have various functions in the development of immune responses. These can be distinguished by function as the **primary** and **secondary lymphoid organs**

Primary lymphoid organs

1) Thymus

2) bone marrow

Secondary Lymphoid organs

1)lymph nodes

2)spleen

3)MALT

4)GALT

Primary Lymphoid Organs

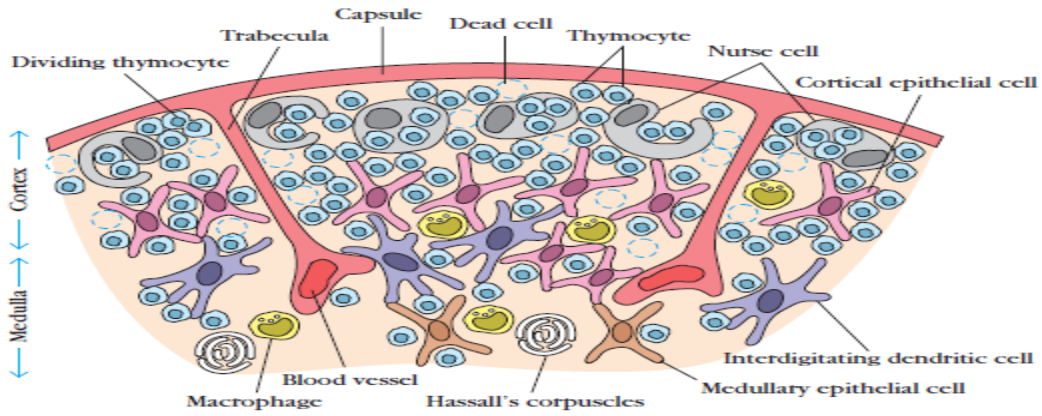
Immature lymphocytes generated in hematopoiesis mature and become committed to a particular antigenic specificity within the primary lymphoid organs.

Thymus

The thymus is the site of T-cell development and maturation. It is a flat, bilobed organ situated above the heart. Each lobe is surrounded by a capsule and is divided into lobules, which are from each other by strands of connective tissue called trabeculae. Each lobule is organized into two compartments: the outer compartment, or *cortex*, is densely packed with immature T cells, called thymocytes, whereas the inner compartment, or *medulla,* is sparsely populated with thymocytes.

Both the cortex and medulla of the thymus are crisscrossed by a three-dimensional stromal-cell network composed of epithelial cells, dendritic cells, and macrophages, which make up the framework of the organ and contribute to the growth and maturation of thymocytes. Many of these stromal cells interact physically with the developing thymocytes

The diagram represents the cross sectioned portion of Thymus



Bone marrow

In humans and mice, bone marrow is the site of B-cell origin and development. Arising from lymphoid progenitors, immature B cells proliferate and differentiate within the bone marrow, and stromal cells within the bone marrow interact directly with the B cells and secrete various cytokines that are required for development.

Bone marrow is not the site of B-cell development in all species. In birds, a lymphoid organ called the bursa of Fabricius, a lymphoid tissue associated with the gut, is the primary site of B-cell maturation. In mammals such as primates and rodents, there is no bursa and no single counterpart to it as a primary lymphoid organ. In cattle and sheep, the primary lymphoid tissuehosting the maturation, proliferation, and diversification of B cells early in gestation is the fetal spleen.

Secondary Lymphoid Organs

Various types of organized lymphoid tissues are located along the vessels of the lymphatic system. Some lymphoid tissue in the lung and lamina propria of the intestinal wall

consists of diffuse collections of lymphocytes and macrophages. Other lymphoid tissue is organized into structures called lymphoid follicles, which consist of aggregates of lymphoid and nonlymphoid cells surrounded by a network of draining lymphatic capillaries. Until it is activated by antigen

primary follicle comprises a network of follicular dendritic cells and small resting B cells. After an antigenic challenge, a primary follicle becomes a larger **secondary follicle** a ring of concentrically packed B lymphocytes surrounding a center (the **germinal center**) in which one finds a focus of proliferating B lymphocytes and an area that contains nondividing B cells, and some helper T cells interspersed with macrophages and follicular dendritic cells

LYMPH NODES

Lymph nodes are the sites where immune responses are mounted to antigens in lymph. They are encapsulated beanshaped structures containing a reticular network packed with lymphocytes, macrophages, and dendritic cells. Morphologically, a lymph node can be divided into three

roughly concentric regions

1 cortex

2 paracortex

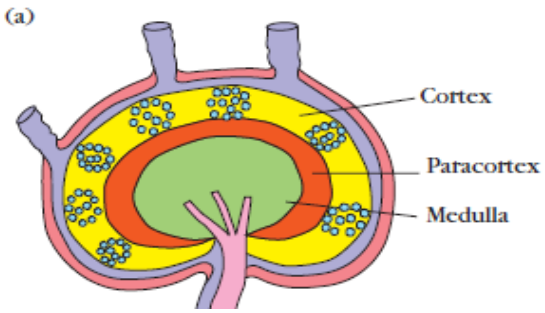
3 medulla

The outermost layer, the **cortex,** contains lymphocytes (mostly B cells), macro-phages, and follicular dendritic cells arranged in primary follicles.

Beneath the cortex is the **paracortex,** which is populated largely by T lymphocytes and also contains interdigitating dendritic cells thought to have migrated from tissues to the node,.the paracortex is therefore sometimes referred to as a **thymus-dependent area**

The **medulla,** is more sparsely populated with lymphoid-lineage cells; of those present, many are plasma cells actively secreting antibody molecules.

The diagram represents the structure of Lymph node

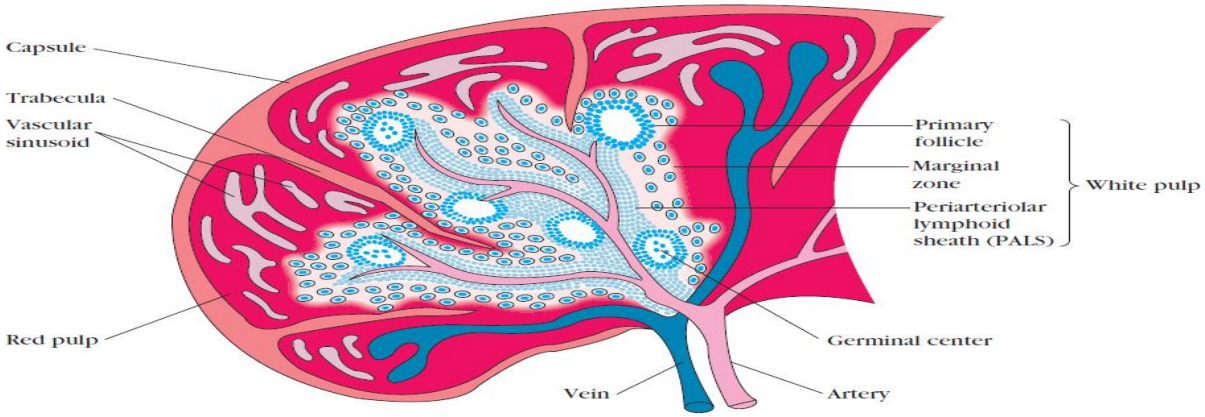


Spleen

The spleen plays a major role in mounting immune responses to antigens in the blood stream. It is a large, ovoid secondary lymphoid organ situated high in the left abdominal cavity. While lymph nodes are specialized for trapping antigen from local tissues, the spleen specializes in filtering blood and trapping blood-borne antigens; thus, it can respond to systemic infections.

Unlike the lymph nodes, the spleen is not supplied by lymphatic vessels. Instead, bloodborne antigens and lymphocytes are carried into the spleen through the splenic artery. The spleen is surrounded by a capsule that extends a number of projections (trabeculae) into the interior to form a compartmentalized structure. The compartments are of two types, the red pulp and white pulp, which are separated by a diffuse marginal zone The splenic **red pulp** consists of a network of sinusoids populated by macrophages and numerous red blood cells (erythrocytes) and few lymphocytes; it is the site where old and defective red blood cells are destroyed and removed The splenic **white pulp** surrounds the branches of the splenic artery, forming a **periarteriolar lymphoid sheath (PALS)** populated mainly by T cells

The diagram shows the structure of Spleen



MUCOSAL-ASSOCIATED LYMPHOID TISSUE

The mucous membranes lining the digestive, respiratory, and urogenital systems have a combined surface area of about 400 m2 and are the major sites of entry for most pathogens. These vulnerable membrane surfaces are defended by a group of organized lymphoid tissues mentioned earlier and known collectively as **mucosal-associated lymphoid tissue (MALT).**

Cutaneous-Associated Lymphoid Tissue

The skin is an important anatomic barrier to the external environment, and its large surface area makes this tissue important in nonspecific (innate) defenses. The epidermal (outer) layer of the skin is composed largely of specialized epithelial cells called keratinocytes. These cells secrete a number of cytokines that may function to induce a local inflammatory reaction. In addition, keratinocytes can be induced to express class II MHC molecules and may function as antigen-presenting cells. Scattered among the epithelial-cell matrix of the epidermis are

Langerhans cells, a type of dendritic cell, which internalize antigen by phagocytosis or endocytosis. The Langerhans cells then migrate from the epidermis to regional lymph nodes, where they differentiate into interdigitating dendritic cells.

T-Cell Maturation, Activation, and Differentiation

OVER VIEW

- **INTRODUCTION**
- **T-Cell Maturation and the Thymus**
- **Ontogeny**
- **T cell receptor (TCR) complex**
- **Activation of the naïve T cells**
    - **Ag recognition and signal transduction pathways in T cells**

CD4 T Cell subsets.

$CD8^+$ T cells

T-Cell Maturation, Activation, and Differentiation

INTRODUCTION

In most cases, both the maturation of progenitor T cells in the thymus and the activation of mature T cells in the periphery are influenced by the involvement of MHC molecules. The potential antigenic diversity of the T-cell population is reduced during maturation by a selection process that allows only MHC-restricted and nonself-reactive T cells to mature. The final stages in the maturation of most T cells proceed along two different developmental pathways, which generate functionally distinct CD4_ and CD8_ subpopulations that exhibit class II and class I MHC restriction, respectively.

Activation of mature peripheral T cells begins with the interaction of the T-cell receptor (TCR) with an antigenic peptide displayed in the groove of an MHC molecule. Although the specificity of this interaction is governed by the TCR, its low avidity necessitates the involvement of coreceptors and other accessory membrane molecules that strengthen the TCR-antigen-MHC interaction and transducer the activating signal. Activation leads to the proliferation and differentiation of T cells into various types of effector cells and memory T cells.

T-Cell Maturation and the Thymus

Progenitor T cells from the early sites of hematopoiesis begin to migrate to the thymus at about day 11 of gestation in mice and in the eighth or ninth week of gestation in humans. In a manner similar to B-cell maturation in the bone marrow, Tcell maturation involves rearrangements of the germ-line TCR genes and the expression of various membrane markers. In the thymus, developing T cells, known as **thymocytes,** proliferate and differentiate along developmental

pathways that generate functionally distinct subpopulations of mature T cells.. the thymus occupies a central role in T-cell biology.

Ontogeny

The process of development and maturation of the T Cells in mammals begins with the haematopoietic stem cells (HSC) in the fetal liver and later in the bone marrow where HSC differentiate into multipotent progenitors. A subset of multipotent progenitors initiates the transcription of recombination activating gene 1 and 2 (RAG 1 and RAG2) and become lymphoid-primed multipotent progenitors and then common lymphoid progenitors (CLP). Only a small subset of pluripotent cells migrates to the thymus and differentiates into early thymic progenitors (ETP). The thymus does not contain self-renewing progenitors; and therefore, long-term thymopoiesis depends on the recruitment of thymus-settling progenitors throughout the life of the individual. These progenitors must enter the thymus to become gradually reprogrammed into fully mature and functional T Cells. The T Cell's distinct developmental steps,

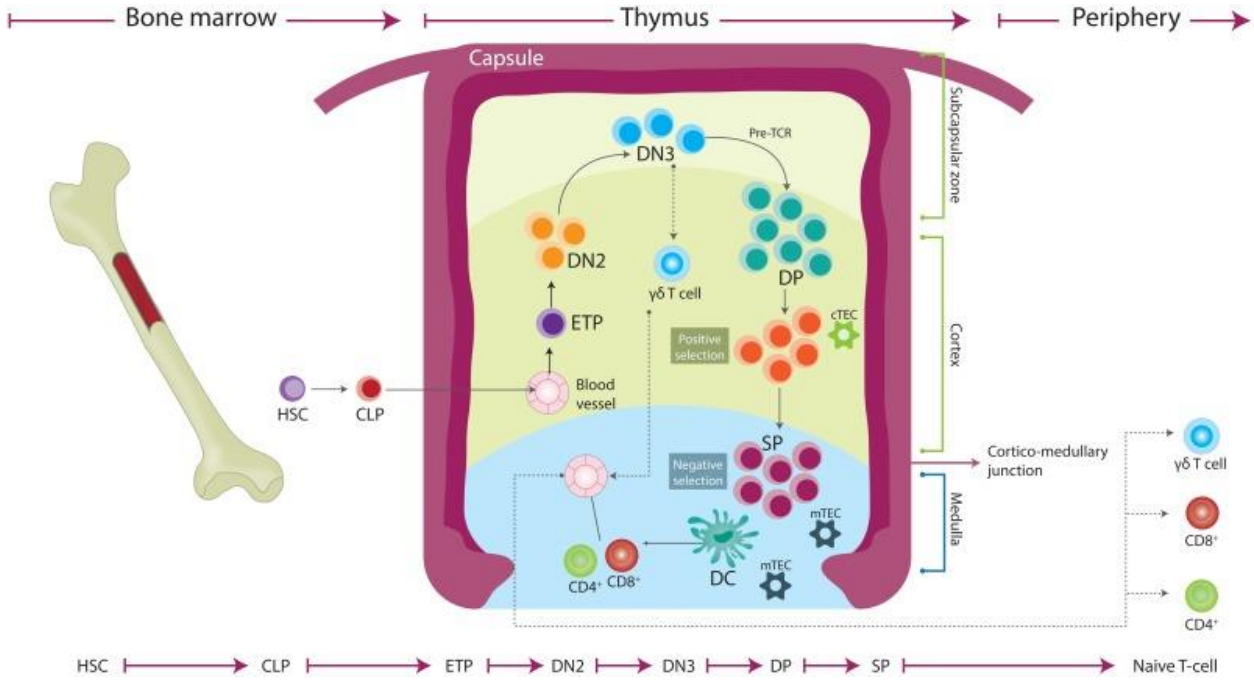


Figure T Cell development and maturation.

The ETP are multipotent and can generate T Cells, B Cells, Natural killer cells (NK), myeloid cells, and dendritic cells (DC). ETP represent a small and heterogenous subset, have the ability to proliferate massively, and can be identified by the phenotype, CD25 as well as by their expression of, CD24, and CCR9. These cells, which are attracted by the chemokines CCL19 and CCL21, enter the thymus via the corticomedullar junction. In the stroma of the thymus, the ETP encounter a large number of ligands for the Notch receptors as well as growth factors such as ligand and IL-7 which trigger and support the differentiation and proliferation of these cells in the initial stages of T Cell development.

Within the thymic cortex, ETP differentiate into double negative (DN) cells that do not express either CD4 or CD8 (i.e., $CD4^-$ and $CD8^-$). Some authors consider the ETP a DN1 cell that later differentiates into DN2 when it acquires the $CD25^+$ and $CD44^+$ receptors. At this stage of development, the cells lose the B potential and begin to express proteins that are critical for the subsequent T Cell receptor (TCR) gene rearrangement such as RAG1 and RAG2. They also begin to express proteins necessary for TCR assembly and signaling as CD3 chains, kinases, and phosphatases.

DN3 cells can take two divergent routes of differentiation. A cell can either express the αβ chains of the TCR and follow the process of selection to generate $CD4^+$ or $CD8^+$ T Cells or express the γ, δ chains to generate a subpopulation of γ δ lymphocytes with special functional characteristics.

T cell receptor (TCR) complex

During the maturation process, T Cells acquire a receptor called TCR that recognizes a specific Ag. TCR is a multiprotein complex composed of two variable antigen-binding chains, αβ or γδ, which are associated with invariant accessory proteins (CD3γε, CD3δε, and CD247 ζζ chains) that are required for initiating signaling when TCR binds to an Ag.

The αβ-TCR does not recognize Ag in its natural form but recognizes linear peptides which have been processed and presented in the HLA-I or HLA-II context. The peptides presented by HLA-I molecules are small (8–10 aminoacids) and have an intracellular origin while those presented by

HLA-II molecules are longer (13–25 aminoacids) and are generally of extracellular origin. Nevertheless, the αβ-TCR of NKT cells and the γδ-TCR can recognize glycolipids and phospholipids presented by CD1 molecules.

TCR gene rearrangement is essential during T Cell development. Multiple gene segments dispersed in the genomic DNA must bind and transcribe to produce a functional TCR. This process occurs independently for each chain beginning with the recombination of genes for the β chains.Genes that code for the TCR chains in humans map to four *loci*: TCRA and TCRD on

Activation of the naïve T cells

T Cell activation and differentiation will only be sucessfull if three signals are present: i) interaction of the TCR with the peptide presented by the HLA molecule, ii) signaling through co-stimulatory molecules, and iii) participation of cytokines that initiate clonal expansion.

Additionally, the cytokine microenvironment that accompanies the activation defines the type of response that will be generated later.

Ag recognition and signal transduction pathways in T cells

In the lymph nodes, T Cells establish temporary contact with a great number of dendritic cells (DC) but only halt and bind to those which present an Ag which is compatible and specific to their receptor,

T Cells within lymph nodes migrate at high speeds of about 11–14 μ per minute. This is in contrast to DCs which transit through lymph nodes at speeds of about 3–6 μ per minute and then stop. This allows DCs to constantly establish new contacts with T Cells. In the absence of Ag, T Cells do not stop, but in the presence of an Ag, the duration of the interaction with the DC may be transitory (3 - 11 min) or stable (several hours) depending on the affinity for the Ag Stable unions are favored by the high presence of peptides in the DC, highly antigenic ligands, mature DC, and expression of molecules such as ICAM-1,

Antigen recognition by TCR induces the formation of several "*TCR microclusters*" that accompany the reorganization and approach of other membrane molecules and signaling proteins towards the contact zone with the DC. This contact zone between the T Cell and DC membranes is known as an *immunological synapse* and consists of a highly organized and dynamic molecular complex divided into three concentric zones known as the central, peripheral, and distal supramolecular activation clusters. The central region is composed of the TCR complex, co-stimulatory and co-inhibitory molecules, and co-receptors. These co-receptors are known as primary and secondary activation signals. The peripheral zone is mainly made up of the adhesion molecules LFA-1-ICAM-1 and CD2-LFA-3 that, due to their affinity, maintain and stabilize binding between the cells. The distal zone consists of F-actin and phosphatase CD45.

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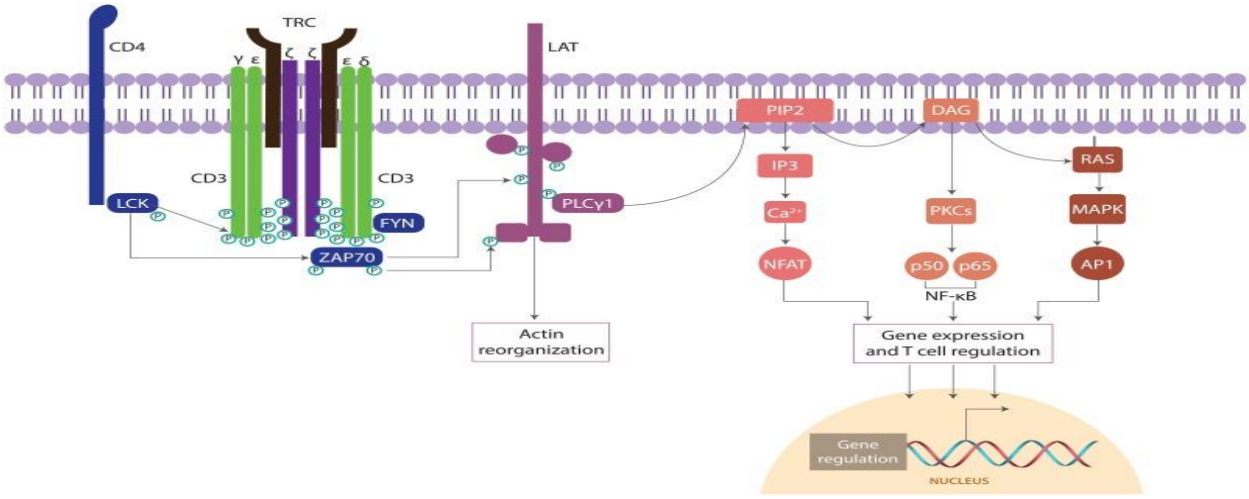
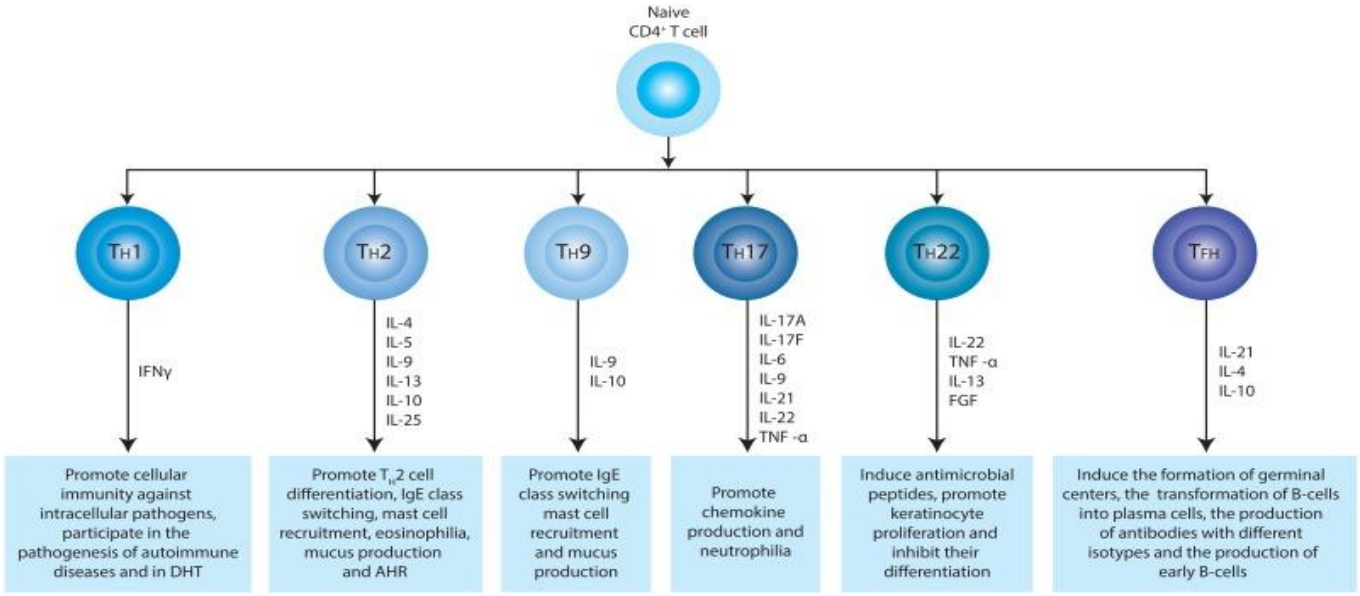


CD4+ T cell subsets

The differentiation of a $CD4^+$ T Cell into distinct subpopulations or cell phenotypes is determined by the nature and concentration of the Ag, the type of APC and its activation state, the cytokine microenvironment that accompanies the antigenic presentation, and the presence and quantity of co-stimulatory molecules, along with other variables.

If the T Cell expresses CD4, it is converted into a T-helper cell (Th) which has a double function: to produce cytokines and to stimulate B Cells to generate Abs. Until recently, only four distinct phenotypes had been identified: Th1, Th2, Th17, and T-regulatory cells (Treg) each of

which secretes a different cytokine profile. However, in the last few years, new T-helper subsets such as Th9, Th22, and follicular helpers (Tfh) have been identified.



CD4 T Cell subsets.

CD8+ T cells

When a $CD8^+$ T Cell develops its effector functions, it is converted into a cytotoxic T Cell able to attack cells directly and destroy those that are malignant or infected with virus. In order to exert this function, a cytotoxic T Cell induces apoptosis in its target cells by the liberation of cytolytic granules or by the expression of ligands for death receptors such as FasL (CD95),

The cytolytic granules contain pore-forming proteins called perforins or cytolysins, proteases known as granzymes or fragmentins, granulolysins which participate in the degradation of membrane lipids, inhibitors of perforins that protect the cytotoxic T Cell from autolysis (calreticulin, catepsin G), and FasL.

Once the immunological synapse between the cytotoxic T Cell and the target cell has been established, the content of these granules is liberated. Perforins polymerize in the plasma membrane and produce pores, which act as channels that allow water entry and generate an

osmotic disequilibrium in the cell. Furthermore, they facilitate the passage of granzymes to the cytosol and to the nucleus of the target cell which favors their proteolytic action on the mitochondria and fragmentation of the DNA.

Cytotoxic T Cells also liberate IFN- γ and TNF-α, which are important in the defence against viral infections and in controlling the proliferation of tumoral cells

B-Cell Generation, Activation, and Differentiation

OVER VIEW

INTRODUCTION

B-Cell Maturation

Progenitor B Cells Proliferate in Bone Marrow

Ontogeny

B cell receptor (BCR)

Signaling mechanisms

Co-receptors of the BCR

Co-stimulatory molecules

B cell activating factor (BAFF)

APRIL (*A proliferation-inducing ligand*)

B cell subsets

B-Cell Generation, Activation, and Differentiation

INTRODUCTION

Many B cells are produced in the bone marrow throughout life, but very few of these cells mature. In mice, the size of the recirculating pool of B cells is about 2 _ 108 cells. Most of these cells circulate as naive B cells, which have short life spans (half lives of less than 3 days to about 8 weeks) if they fail to encounter antigen or lose in the competition with other B cells for residence in a supportive lymphoid environment. Given that the immune system is able to generate a total antibody diversity that exceeds 109, clearly only a small fraction of this potential repertoire is displayed at any time by membrane immunoglobulin on recirculating B cells. Indeed, throughout the life span of an animal, only a small fraction of the possible antibody diversity is ever generated.

B-Cell Maturation

The generation of mature B cells first occurs in the embryo and continues throughout life. Before birth, the yolk sac, fetal liver, and fetal bone marrow are the major sites of B-cell maturation; after birth, generation of mature B cells occurs in the bone marrow.

Progenitor B Cells Proliferate in Bone Marrow

B-cell development begins as lymphoid stem cells differentiate into the earliest distinctive B-lineage cell—the **progenitor B cell (pro-B cell)**—which expresses a transmembrane tyrosine phosphatase called CD45R (sometimes called B220 in mice). Pro-B cells proliferate within the bone marrow, filling the extravascular spaces between large sinusoids in the shaft of a bone. Proliferation and differentiation of pro-B cells into **precursor B cells (pre-B cells)** requires the microenvironment provided by the bone-marrow stromal cells. If pro-B cells are removed from the bone marrow and cultured in vitro, they will not progress to more mature B-cell stages unless stromal cells are present. The stromal cells play two important roles: they interact directly with pro-B and pre-B cells, and they secrete various cytokines, notably IL-7, that support the developmental process.

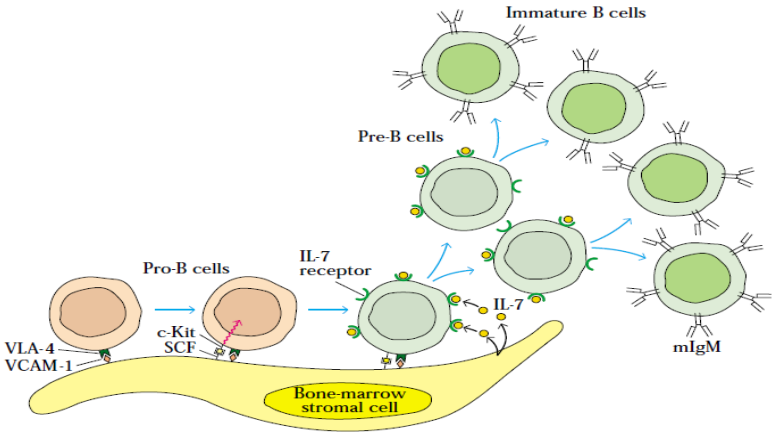


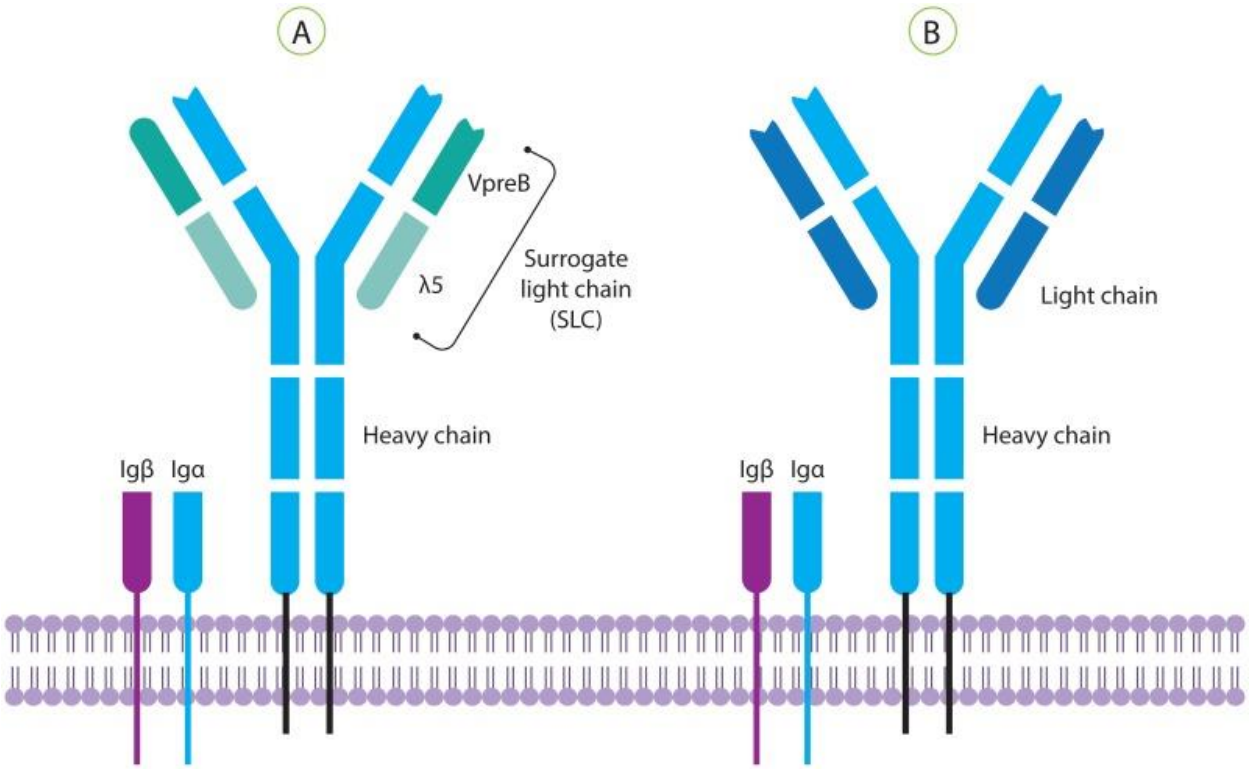
Figure maturation of Progenitor B cells

Ontogeny

The first stages of B Cell development take place in complex microenvironments created by the stromal cells of the bone marrow known as “niches” from which come the stimuli and factors required to initiate a series of cell signals. These, in turn, activate transcription factors that induce, or repress, the expression of different target genes that modulate cell survival, proliferation, and differentiation. IL-7 is critical to the development of the B Cells and is produced by the cells of the stroma

During the differentiation of the B Cells, a process of gene recombination is structured initially that codes for segments V (*Variable*), D (*Diversity*), and J (*Joining*) of the heavy chain (chain H) together with that of the genes for segments V and J of the light chain (chain L) of the membrane-bound immunoglobulin (mIg). This recombination process is initiated by the complex of proteins RAG1- RAG2 that generate the rupture of the double chain of DNA between segments of genes and specific recognition sites that are also known as “*recombination signal sequences*.” This process leads to the generation of B Cells that express a wide repertoire of mIg. Allelic Exclusion: During its development, the B Cell generates a wide diversity of BCR for the gene recombination process mentioned above. Although each cell has many allelic *loci* for the

different BCR chains (two *loci* for the heavy chain and multiple loci for the light chain), each mature cell eventually expresses only a single type of receptor. This is achieved by restricting the gene expression of the BCR of a single allele in a process known as allelic exclusion, which involves a monoallelic activation and feedback inhibition.



A) pre-B Cell receptor. B) B cell receptor.

B cell receptor (BCR)

The BCR is a macromolecular complex that is built in the membrane by *IgM/ IgD* with two additional *Ig* accessories denominated *Igα* (CD79a) and *Igβ* (CD79b) The membrane-bound immunoglobulins (mIgs) are glycoproteins with a basic monomer. Each of these is made up of four polypeptidic chains of which two are *heavy or H chains* with a molecular weight of approximately 65 kDa, and the others are *light or L chains* with a molecular weight of 25 kDa. Each L chain is linked to an H chain by a disulphur bridge. The H chains are linked to each other by at least one other disulphur bridge. These IgM or IgD monomers correspond to the extracellular segments of the BCR. However, the mIg also have another two segments, the transmembranal and the intracytoplasmic, which result from an extension of the carboxy-

terminal portion of the two H chains. The two V domains, which form the active sites allowing each BCR to bind specifically to an antigenic determinant, are found in the amino-terminal (H and L) portions of each peptide chain of the BCR. The antigen-BCR interaction is a non-covalent reaction.

The intracytoplasmic region of mIg, which presents only 3 aminoacids (lysine-valine-lysine), is very small and does not permit mlg to carry out the signaling process *per se*. The transmembranal segment of the C-terminal portion of the H chain of mIg consists of 25 aminoacids found close to the tyrosine kinase (PTK) enzymes which, in turn, are close to the heterodimers (Igα and Igβ). The latter are responsible for the signaling process since they can provide the substrate for the tyrosinases in their ITAM regions.

Signaling mechanisms

Stimulation of the B Cells via antigenic BCR begins with the recognition and capture of the Ag through BCR molecules. This induces their aggregation and triggers the signaling process by activating the SRC family kinase (SFK) which then phosphorylates the ITAM moieties of the accessory chains Igα and Igβ. These carry out the same function as the δε chain (CD247) to activate the TCR and produce lipid-raft-associated calcium-signaling module forms. This complex contains 3 classes of activated protein tyrosine kinases (PTKs): i) Lyn, Fyn, and Blk of the Src family; ii) Syk/ZAP70; and iii) of Bruton (Btk) of the Tec family

Co-receptors of the BCR

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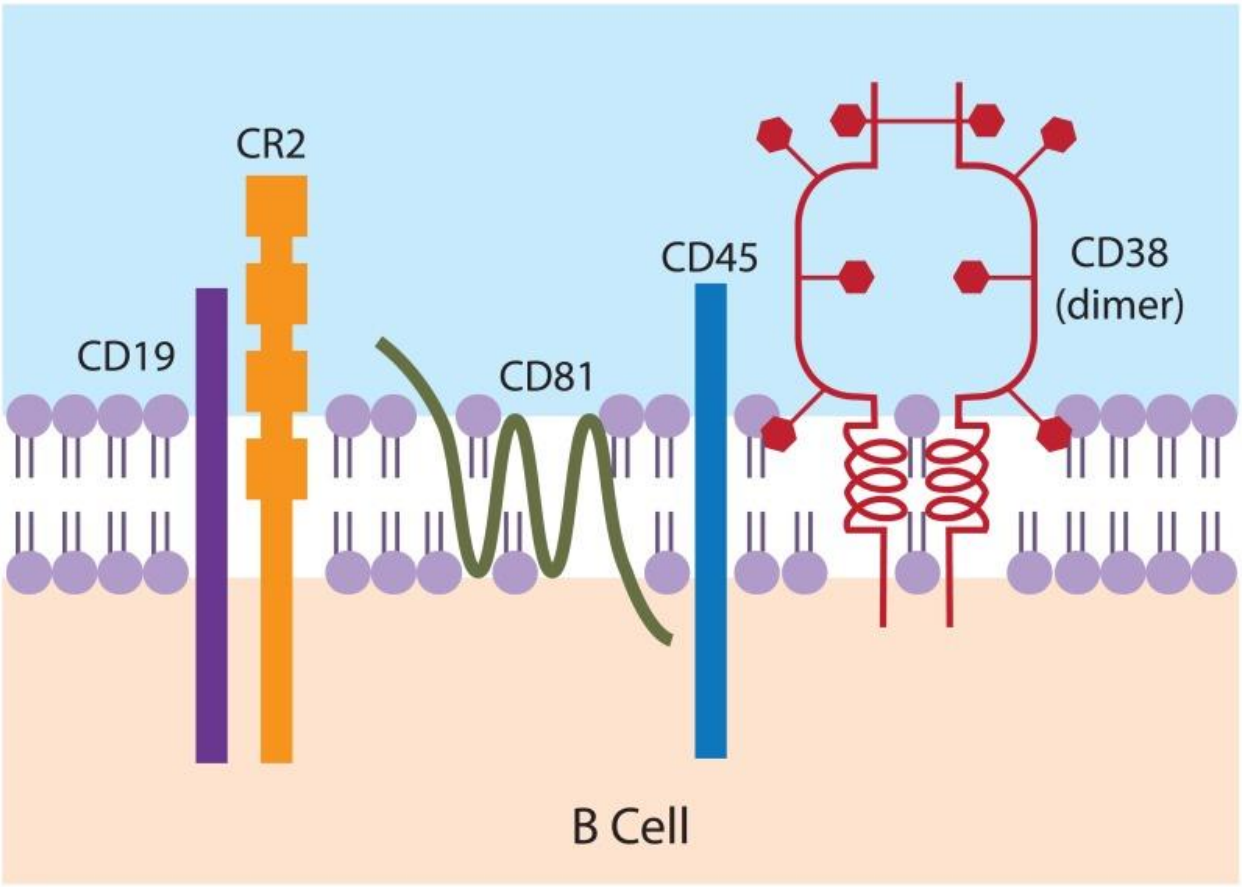


B Cell co-receptors.

Co-stimulatory molecules

Another important aspect in the activation of the B Cells is the presence of molecules which positively or negatively regulate the process. Together these are known as co-stimulatory molecules and some of them are described as follows.

B cell activating factor (BAFF) is a cytokine and member of the TNF family. It is produced by a wide variety of cells (neutrophils, monocytes, macrophages, DCs, and T Cells)t is essential for the maturation and survival of the B Cells since it participates in the processes of differentiation and proliferation. To date, three BAFF receptors have been identified: i) BAFF-R, ii) TACI (*transmembrane activator, calcium modulator, and cyclophilin ligand interactor*), and iii) BCMA (*B Cell maturation factor*).

APRIL (*A proliferation-inducing ligand*) is a BAFF homologue that binds to TACI and BCMA but does not interact with BAFF-R. In addition to its co-stimulatory function, APRIL improves the ability of B Cells to present Ag and to increase their survival time and also regulates tolerance. On the other hand, it promotes the proliferation and survival of malignant B Cells and other tumoral cells. As for BAFF, elevated levels of APRIL have been observed in the sera of patients with SLE.

B cell subsets

Mature B Cells can be divided into several subsets based on their location, cell surface phenotype, Ag specificity, and activation routes.

The transitional B Cells are considered to be the first stages of development of the B Cells once they leave the bone marrow to migrate to the secondary lymphoid organs. The lymphocytes $CD20^{+}$, $CD21^{\pm}$, $CD23^{\pm}$, IgM^{++}, and $IgD^{\pm}$ $CD38^{++}$ are designated B Cell transitional type-1 (T1) and differentiate from type 2 ($CD20^{+}$, $CD21^{++}$, $CD23^{\pm}$, IgM^{++}, and $IgD^{++}CD38^{\pm}$). The transitional B Cells T2 can evolve intomarginal zone B Cells or GC

The Follicular B Cells (FoB) or B-2. These are generated directly in the bone marrow and reach the follicules of secondary lymphoid organs and the circulation. They are considered to be resting (naïve) cells and constitute the largest subpopulation of B Cells. Their differentiation is influenced by a great variety of factors including chemokines, BCR signaling, and some Ags. They participate in T-dependent (TD) immune responses since they can use the BCR to engulf the Ag, process it, and present it to the Ag-specific T Cells.

Memory B Cells. There are several subsets of memory B Cells that are classified based on their origin, the differential expression of CD27, and the isotype of the mIg being expressed. Three different origins for the cells have been described: i) the spleen, ii) the germinal center, and iii) the intestine lamina propria outside the GC. In the spleen, they present $CD27^{-}IgG^{+}$ markers. At the GC, they are $CD27^{+}IgM^{+}IgD^{-}$and change from mIg to $CD27^{+}IgG/IgA^{+}$. Last of all, those generated in the intestine express $CD27^{-}IgA^{+}$.

HYPER SENSITIVITY REACTIONS

OVER VIEW

INTRODUCTION

- **IgE-mediated (type I),**
- **Antibody-mediated (type II),**
- **Immune complex–mediated (type III)**
- **Delayed-type hypersensitivity, or DTH (type IV)**

HYPER SENSITIVITY REACTIONS

INTRODUCTION

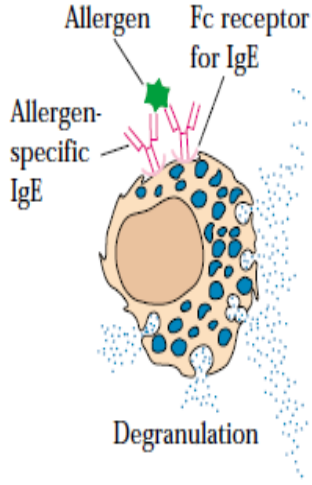
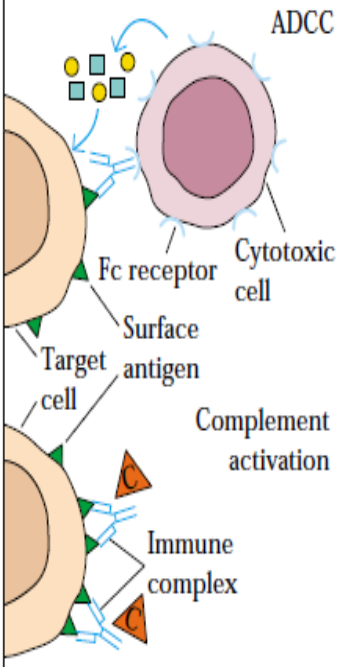
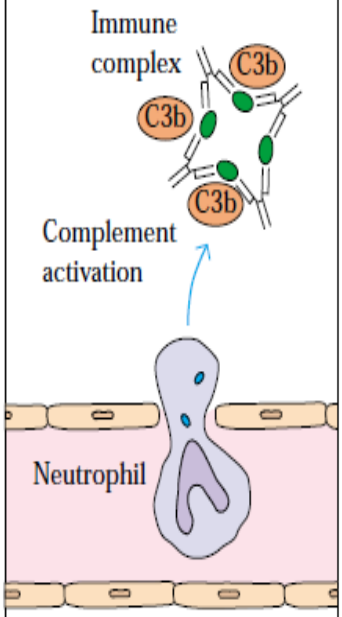
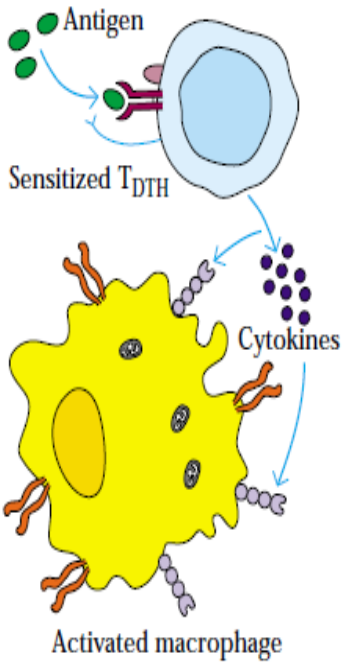
Under certain circumstances, however, this inflammatory response can have deleterious effects, resulting in significant tissue damage or even death. This inappropriate immune response is termed **hypersensitivity** or **allergy**.

Although the word *hypersensitivity* implies an increased response, the response is not always heightened but may, instead, be an inappropriate immune response to an antigen. Hypersensitive reactions may develop in the course of either humoral or cell-mediated responses.

The ability of the immune system to respond inappropriately to antigenic challenge was recognized early in this century. Two French scientists, Paul Portier and Charles Richet, investigated the problem of bathers in the Mediterranean reacting violently to the stings of Portuguese Man of War jellyfish.

We currently refer to anaphylactic reactions within the humoral branch initiated by antibody or antigen-antibody complexes as **immediate hypersensitivity,** because the symptoms are manifest within minutes or hours after a sensitized recipient encounters antigen. **Delayed-type hypersensitivity (DTH)** is so named in recognition of the delay of symptoms until days after exposure. As it became clear that several different immune mechanisms give rise to hypersensitive reactions, P. G. H. Gell and R. R. A. Coombs proposed a classification scheme in which hypersensitive reactions are divided into four types. Three types of hypersensitivity occur within the humoral branch and are mediated by antibody or antigen-antibody complexes:

- **IgE-mediated (type I),**
- **Antibody-mediated (type II),**
- **Immune complex–mediated (type III)**
- **Delayed-type hypersensitivity, or DTH (type IV)**

 Type I	 Type II	 Type III	 Type IV
IgE-Mediated Hypersensitivity	IgG-Mediated Cytotoxic Hypersensitivity	Immune Complex-Mediated Hypersensitivity	Cell-Mediated Hypersensitivity
Ag induces crosslinking of IgE bound to mast cells and basophils with release of vasoactive mediators	Ab directed against cell surface antigens meditates cell destruction via complement activation or ADCC	Ag-Ab complexes deposited in various tissues induce complement activation and an ensuing inflammatory response mediated by massive infiltration of neutrophils	Sensitized T_H1 cells release cytokines that activate macrophages or T_C cells which mediate direct cellular damage
Typical manifestations include systemic anaphylaxis and localized anaphylaxis such as hay fever, asthma, hives, food allergies, and eczema	Typical manifestations include blood transfusion reactions, erythroblastosis fetalis, and autoimmune hemolytic anemia	Typical manifestations include localized Arthus reaction and generalized reactions such as serum sickness, necrotizing vasculitis, glomerulnephritis, rheumatoid arthritis, and systemic lupus erythematosus	Typical manifestations include contact dermatitis, tubercular lesions and graft rejection

IgE-Mediated (Type I) Hypersensitivity

A type I hypersensitive reaction is induced by certain types of antigens referred to as **allergens,** and has all the hallmarks of a normal humoral response. That is, an allergen induces a humoral antibody response

ALLERGENS

The majority of humans mount significant IgE responses only as a defense against parasitic infections. After an individual has been exposed to a parasite, serum IgE levels increase and remain high until the parasite is successfully cleared from the body.

ALLERGIC RHINITIS

The most common atopic disorder, affecting 10% of the U.S. population, is allergic rhinitis, commonly known as hay fever. This results from the reaction of airborne allergens with sensitized mast cells in the conjunctivae and nasal mucosa to induce the release of pharmacologically active mediators from mast cells; these mediators then cause localized vasodilation and increased capillary permeability. The symptoms include watery exudation of the conjunctivae, nasal mucosa, and upper respiratory tract, as well as sneezing and coughing.

ASTHMA

Another common manifestation of localized anaphylaxis is asthma. In some cases, airborne or blood-borne allergens, such as pollens, dust, fumes, insect products, or viral antigens, trigger an asthmatic attack (allergic asthma); in other cases, an asthmatic attack can be induced by exercise or cold, apparently independently of allergen stimulation (intrinsic asthma). Like hay fever, asthma is triggered by degranulation of mast cells with release of mediators, butinstead of occurring in the nasal mucosa, the reaction develops in the lower respiratory tract. The resulting contraction of the bronchial smooth muscles leads to bronchoconstriction. Airway edema, mucus secretion, and inflammation contribute to the bronchial constriction and to airway obstruction. Asthmatic patients may have abnormal levels of receptors for neuropeptides. For example, asthmatic patients have been reported to have increased expression of receptors for substance P, a peptide that contracts smooth muscles, and decreased expression of receptors for vasoactive intestinal peptide, which relaxes smooth muscles.

FOOD ALLERGIES

Various foods also can induce localized anaphylaxis in allergic individuals. Allergen crosslinking of IgE on mast cells along the upper or lower gastrointestinaltract can induce localized smooth-muscle contraction and vasodilation and thus such symptoms as vomiting or diarrhea. Mast-cell degranulation along the gut can increase the permeability of mucous membranes, so that the allergen enters the bloodstream. Various symptoms can ensue, depending on where the allergen is deposited. For example, some individuals develop asthmatic attacks after ingesting certain foods. Others develop atopic urticaria, commonly known as hives, when a food allergen is carried to sensitized mast cells in the skin, causing swollen (edematous) red (erythematous) eruptions; this is the wheal and flare response, or P-K reaction,mentioned earlier.

Immunotherapy

Immunotherapy with repeated injections of increasing doses of allergens (hyposensitization) has been known for some time to reduce the severity of type I reactions, or even eliminate them completely, in a significant number of individuals suffering from allergic rhinitis. Such repeated introduction of allergen by subcutaneous injections appears to cause a shift toward IgG production or to induce T-cell– mediated suppression (possibly by a shift to the TH1 subset and IFN-_ production) that turns off the IgE.

In this situation, the IgG antibody is referred to as *blocking antibody* because it competes for the allergen, binds to it, and forms a complex that can be removed by phagocytosis; as a result, the allergen is not available to crosslink the fixed IgE on the mast-cell membranes, and allergic symptoms decrease.

Antibody-Mediated Cytotoxic (Type II) Hypersensitivity

Type II hypersensitive reactions involve antibody-mediated destruction of cells. Antibody can activate the complement system, creating pores in the membrane of a foreign cell or it can mediate cell destruction by antibodydependent cell-mediated cytotoxicity (ADCC). In this process, cytotoxic cells with Fc receptors bind to the Fc region of antibodies on target cells and promote killing of the cells .Antibody bound to a foreign cell also can serve as an opsonin, enabling phagocytic cells with Fc or C3b receptors to bind and phagocytose the antibody-coated cell

Hemolytic Disease of the Newborn Is Caused by Type II Reactions

Hemolytic disease of the newborn develops when maternal IgG antibodies specific for fetal blood-group antigens cross the placenta and destroy fetal red blood cells. The consequences of such transfer can be minor, serious, or lethal. Severe hemolytic disease of the newborn, called **erythroblastosis fetalis,** most commonly develops when an Rh+ fetus expresses an **Rh antigen** on its blood cells that the Rh– mother does not express.

Drug-Induced Hemolytic Anemia Is a Type II Response

Certain antibiotics (e.g., penicillin, cephalosporin, and streptomycin) can adsorb nonspecifically to proteins on RBC membranes, forming a complex similar to a hapten-carrier complex. In some patients, such drug-protein complexes induce formation of antibodies, which then bind to the adsorbed drug on red blood cells, inducing complementmediated lysis and thus progressive anemia.When the drug is withdrawn, the hemolytic anemia disappears. Penicillin is notable in that it can induce all four types of hypersensitivity with various clinical manifestations

Immune Complex–Mediated (Type III) Hypersensitivity

The reaction of antibody with antigen generates immune complexes. Generally this complexing of antigen with antibody facilitates the clearance of antigen by phagocytic cells. In some cases, however, large amounts of immune complexes can lead to tissue-damaging type III hypersensitive reactions.

The magnitude of the reaction depends on the quantity of immune complexes as well as their distribution within the body.When the complexes are deposited in tissue very near the site of antigen entry, a localized reaction develops.When the complexes are formed in the blood, a reaction can develop wherever the complexes are deposited. In particular, complex deposition is frequently observed on blood-vessel walls, in the synovial membrane of joints, on the glomerular basement membrane of the kidney, and on the choroid plexus of the brain. The deposition of these complexes initiates a reaction that results in the recruitment of neutrophils to the site. The tissue there is injured as a consequence of granular release from the neutrophil. Formation of circulating immune complexes contributes to the pathogenesis of a number of conditions other than serum sickness. These include the following:

Autoimmune Diseases

Systemic lupus erythematosus

Rheumatoid arthritis

Goodpasture's syndrome

Drug Reactions

Allergies to penicillin and sulfonamides

Infectious Diseases

Poststreptococcal glomerulonephritis

Meningitis

Hepatitis

Mononucleosis

Malaria

Trypanosomiasis

Type IV or Delayed-Type Hypersensitivity (DTH)

When some subpopulations of activated TH cells encounter certain types of antigens, they secrete cytokines that induce a localized inflammatory reaction called delayed-type hypersensitivity (DTH).

The reaction is characterized by large influxes of nonspecific inflammatory cells, in particular,macrophages. This type of reaction was first described in 1890 by Robert Koch,

PRIMARY IMMUNODEFICIENCIES

OVER VIEW

INTRODUCTION

PRIMARY IMMUNO DEFICIENCIES

- **Lymphoid immune deficiencies**
- **SCID**
- **WAS**
- **Interferon gamma receptor defect**
- **X linked gamma globenia**
- **CVI**

SECONDARY IMMUNO DEFICIENCIES

- **Acquired Immuno Deficiency**
- **HIV Retro virus-I**

Immuno deficiencies

INTRODUCTION

A condition resulting from a genetic or developmental defect in the immune system is called a primary immunodeficiency. In such a condition, the defect is present at birth although it may not manifest itself until later in life. Secondary immunodeficiency, or acquired immunodeficiency, is the loss of immune function and results from exposure to various agents. By far the most common secondary immunodeficiency is **acquired immunodeficiency syndrome,** or **AIDS,** which results from infection with the human immunodeficiency virus 1 (HIV-1). In the year 2000, AIDS killed approximately 3 million persons, and HIV infection continues to spread to an estimated 15,000 persons per day.

Primary immunodeficiencies, examines progress in identifying the genetic defects that underlie these disorders, and considers approaches to their treatment, including innovative uses of gene therapy. Primary Immunodeficiencies

Primary Immuno deficiency

A primary immunodeficiency may affect either adaptive or innate immune functions. Deficiencies involving components of adaptive immunity, such as T or B cells, are thus differentiated from immunodeficiencies in which the nonspecific mediators of innate immunity, such as phagocytes or complement, are impaired. Immunodeficiencies are conveniently categorized by the type or the developmental stage of the cells involved.

The consequences of primary immunodeficiency depend on the number and type of immune system components involved. Defects in components early in the hematopoietic developmental scheme affect the entire immune system. In this category is reticular dysgenesis, a stem-cell defect that affects the maturation of all leukocytes; the resulting general failure of immunity leads to susceptibility to infection by a variety of microorganisms.

Lymphoid Immunodeficiencies

The combined forms of lymphoid immunodeficiency affect both lineages and are generally lethal within the first few years of life; these arise from defects early in developmental pathways.They are less common than conditions, usually less severe, that result from defects in more highly differentiated lymphoid cells.

B-cell immunodeficiency disorders make up a diverse spectrum of diseases ranging from the complete absence of mature recirculating B cells, plasma cells, and immunoglobulin to the selective absence of only certain classes of AIDS and Other Immunodeficiencies . Patients with these disorders usually are subject to recurrent bacterial infections but display normal immunity to most viral and fungal infections, because the Tcell branch of the immune system is largely unaffected.Most common in patients with humoral immunodeficiencies are infections by such encapsulated bacteria as staphylococci, streptococci, and pneumococci, because antibody is critical for the opsonization and clearance of these organisms. Immunoglobulin deficiencies are associated primarily with recurrent infections by extracellular bacteria, but those affected have normal responses to intracellular bacteria, as well s viral and fungal infections. By contrast, defects in the cellmediated are associated with increased susceptibility to viral, protozoan, and fungal infections. Intracellular

pathogens such as *Candida albicans, Pneumocystis carinii*, and *Mycobacteria* are often implicated, reflecting the importanceof T cells in eliminating intracellular pathogens. Infections with viruses that are rarely pathogenic for the normal individual (such as cytomegalovirus or even an attenuated measles vaccine) may be life threatening for those with impaired cell-mediated immunity.

SEVERE COMBINED IMMUNODEFICIENCY (SCID)

The family of disorders termed SCID stems from defects in lymphoid development that affect either T cells or both T and B cells. All forms of SCID have common features despite differences in the underlying genetic defects. Clinically, SCID is characterized by a very low number of circulating lymphocytes. There is a failure to mount immune responses mediated by T cells. The thymus does not develop, and the few circulating T cells in the SCID patient do not respond to

stimulation by mitogens, indicating that they cannot proliferate in response to antigens. Myeloid and erythroid (redblood- cell precursors) cells appear normal in number and function, indicating that only lymphoid cells are depleted in SCID.

WISKOTT-ALDRICH SYNDROME (WAS)

The severity of this X-linked disorder increases with age and usually results in fatal infection or lymphoid malignancy. Initially, T and B lymphocytes are present in normal numbers. WAS first manifests itself by defective responses to bacterial polysaccharides and by lower-than-average IgM levels. Other responses and effector mechanisms are normal in the early stages of the syndrome. As the WAS sufferer ages, there are recurrent bacterial infections and a gradual loss of humoral and cellular responses. The syndrome includes thrombocytopenia (lowered platelet count; the existing platelets are smaller than usual and have a short half-life), which may lead to fatal bleeding. Eczema (skin rashes) in varying degrees of severity may also occur, usually around one year of age. The defect in WAS has been mapped to the short arm of the X chromosome and involves a cytoskeletal glycoprotein present in lymphoid cells called sialophorin (CD43).The WAS protein is required for assembly of actin filaments required for the formation of microvesicles.

INTERFERON-GAMMA–RECEPTOR DEFECT

A recently described immunodeficiency that falls into the mixed-cell category involves a defect in the receptor for interferon gamma (IFN-_, see Chapter 12). This deficiency was found in patients suffering from infection with atypical mycobacteria (intracellular organisms related to the bacteria that cause tuberculosis and leprosy). Most of those carrying this autosomal recessive trait are from families with a history of inbreeding. The susceptibility to infection with mycobacteria is selective in that those who survive these infections are not unusually susceptible to other agents, including other intracellular bacteria. This immunodeficiency points to a specific role for IFN-_ and its receptor in protection from infection with mycobacteria.

X-LINKED GAMMAGLOBULINEMIA

A B-cell defect called X-linked agammaglobulinemia (XLA) or Bruton's hypogammaglobulinemia is characterized by extremely low IgG levels and by the absence of other immunoglobulin classes. Individuals with XLA have no peripheral B cells and suffer from recurrent bacterial infections, beginning at about nine months of age.

COMMON VARIABLE IMMUNODEFICIENCY (CVI)

CVI is characterized by a profound decrease in numbers of antibody-producing plasma cells, low levels of most immunoglobulin isotypes (hypogammaglobulinemia), and recurrentinfections. The condition is usually manifested later in life than other deficiencies and is sometimes called lateonset hypogammaglobulinemia or, incorrectly, acquired hypogammaglobulinemia. However,

CVI has a genetic component and is considered a primary immunodeficiency, although the exact pattern of inheritance is not known. Because the manifestations are very similar to those of acquired hypogammaglobulinemia, there is some confusion between the two forms (see below). Infections in CVI sufferers are most frequently bacterial and can be controlled by administration of immunoglobulin. In CVI patients, B cells fail to,mature into plasma cells; however in vitro studies show that CVI B cells are capable of maturing in response to appropriate differentiation signals. The underlying defect in CVI is not known, but must involve either an in vivo blockage of the maturation of B cells to the plasma-cell stage or their inability to produce the secreted form of immunoglobulins.

SECONDARY IMMUNO DEFICIENCIES

Acquired immuno deficiency

Avariety of defects in the immune system give rise to immunodeficiency. In addition to the primary immunodeficiencies, there are also acquired, or secondary, immunodeficiencies. One that has been known for some time is called acquired hypogammaglobulinemia.

Since its discovery in 1981, AIDS has increased to epidemic proportions throughout the world. As of December 2000, the cumulative total number of persons in the United States reported to have AIDS was 688,200, and of these approximately 420,000 have died. Although reporting of AIDS cases is mandatory, many states do not require reporting of cases of HIV infection that have not yet progressed to AIDS.

A Retrovirus, HIV-1, Is the Causative Agent of AIDS

Only one other human retrovirus, human T-cell lymphotropic virus I, or HTLV-I, This retrovirus is endemic in the southern part of Japan and in the Caribbean. Although most individuals infected with HTLV-I display no clinical signs of disease, a small percentage develop serious illness, either adult T-cell leukemia, which is aggressive and usually fatal, or a disabling progressive neurologic disorder called HTLV-I–associated myelopathy (called tropical spastic paraparesis in early reports). Although comparisons of their genomic sequences revealed that HIV-1 is not a close relative of HTLV-I, similarities in overall characteristics led to use of the name HTLV-III for the AIDS virus in early reports. There is also a related human virus called HIV-2, which is less pathogenic in humans than HIV-1. HIV-2 is similar to viruses isolated from monkeys; it infects certain nonhuman primates that are not infected by HIV-1.

Viruses related to HIV-1 have been found in nonhuman primates. These viruses, variants of simian immunodeficiency virus, or SIV, cause immunodeficiency disease in certain infected monkeys. Normally, SIV strains cause no disease in their normal host but produce immunodeficiency similar to AIDS when injected into another species. For example, the virus from African green monkeys (SIVagm) is present in a high percentage of normal healthy African green monkeys in the wild. However, when SIVagm is injected into macaques, it causes a severe, often lethal, immunodeficiency.

ENZYME LINKED IMMUNOSORBENT ASSAY(ELISA)

OVERVIEW

INTRODUCTION

Numerous varients of ELISA

IndirectELISA

SandwichELISA

Competitive ELISA

Elispot Assay

Enzyme Linked Immunosorbent Assay(ELISA)

INTRODUCTION

Enzyme-linked immunosorbent assay, commonly known as **ELISA** (or EIA), is similar in principle to RIA but depends on an enzyme rather than a radioactive label. An enzyme conjugated with an antibody reacts with a colorless substrate to generate a colored reaction product. Such a substrate is called **a chromogenic substrate.** A number of enzymes have been employed for ELISA, including alkaline phosphatase, peroxidase, and _-galactosidase. These assays approach the sensitivity of RIAs and have the advantage of being safer and less costly.

Numerous Variants of ELISA

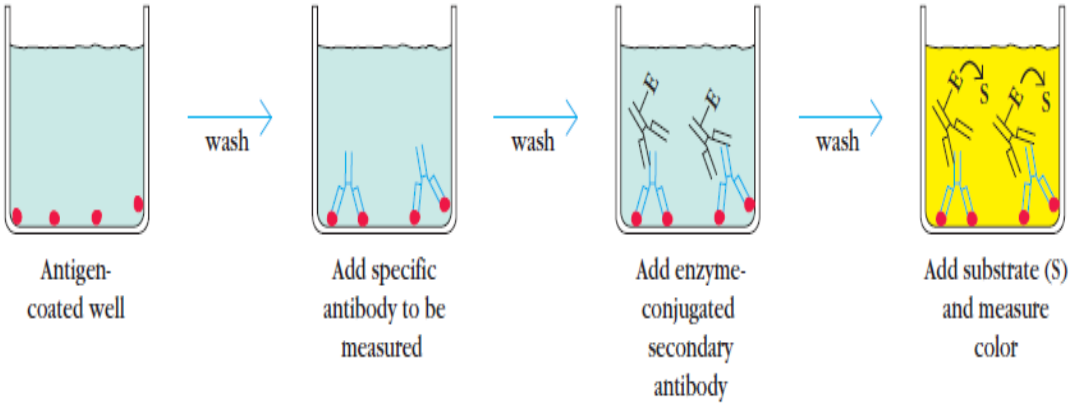
A number of variations of ELISA have been developed, allowing qualitative detection or quantitative measurement of either antigen or antibody. Each type of ELISA can be used qualitatively to detect the presence of antibody or antigen. Alternatively, a standard curve based on known concentrations of antibody or antigen is prepared, from which the unknown concentration of a sample can be determined.

INDIRECT ELISA

Antibody can be detected or quantitatively determined with an indirect ELISA (Figure 6-10a). Serum or some other sample containing primary antibody (Ab1) is added to an antigen-coated microtiter well and allowed to react with the antigen attached to the well. After any free Ab1 is washed away, the presence of antibody bound to the antigen is detected by adding an enzyme-conjugated secondary anti-isotype antibody (Ab2), which binds to the primary antibody. Any free Ab2 then is washed away, and a substrate for the enzyme is added. The amount of colored reaction product that forms is measured by specialized spectrophotometric plate readers, which can measure the absorbance of all of the wells of a 96-well plate in seconds.

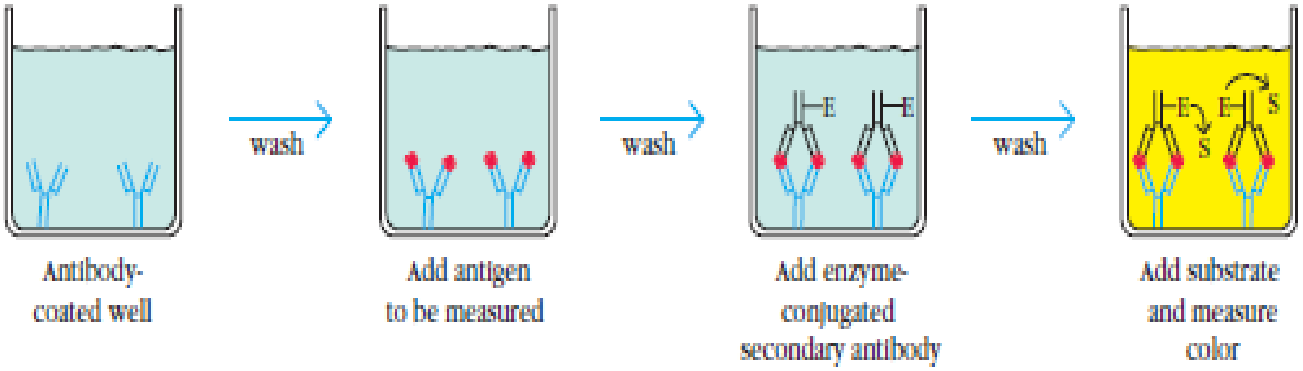
Indirect ELISA is the method of choice to detect the presence of serum antibodies against human immunodeficiency virus (HIV), the causative agent of AIDS. In this assay, recombinant envelope and core proteins of HIV are adsorbed

(a) Indirect ELISA



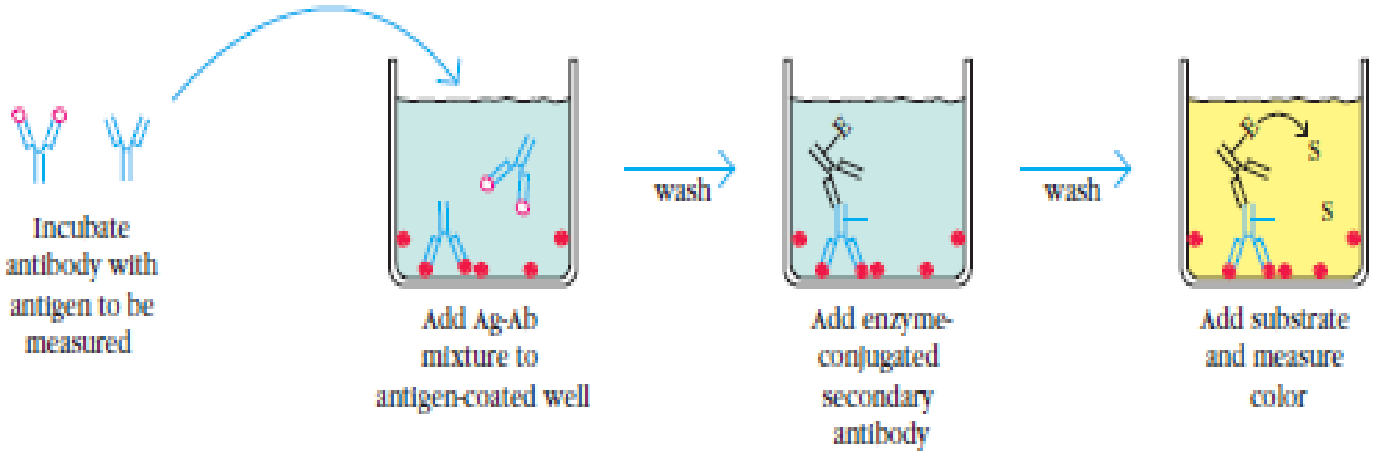
SANDWICH ELISA

Antigen can be detected or measured by a sandwich ELISA In this technique, the antibody (rather than the antigen) is immobilized on a microtiter well. A sample containing antigen is added and allowed to react with the immobilized antibody.After the well is washed, a second enzyme- linked antibody specific for a different epitope on the antigen is added and allowed to react with the bound antigen. After any free second antibody is removed by washing, substrate is added, and the colored reaction product is measured.



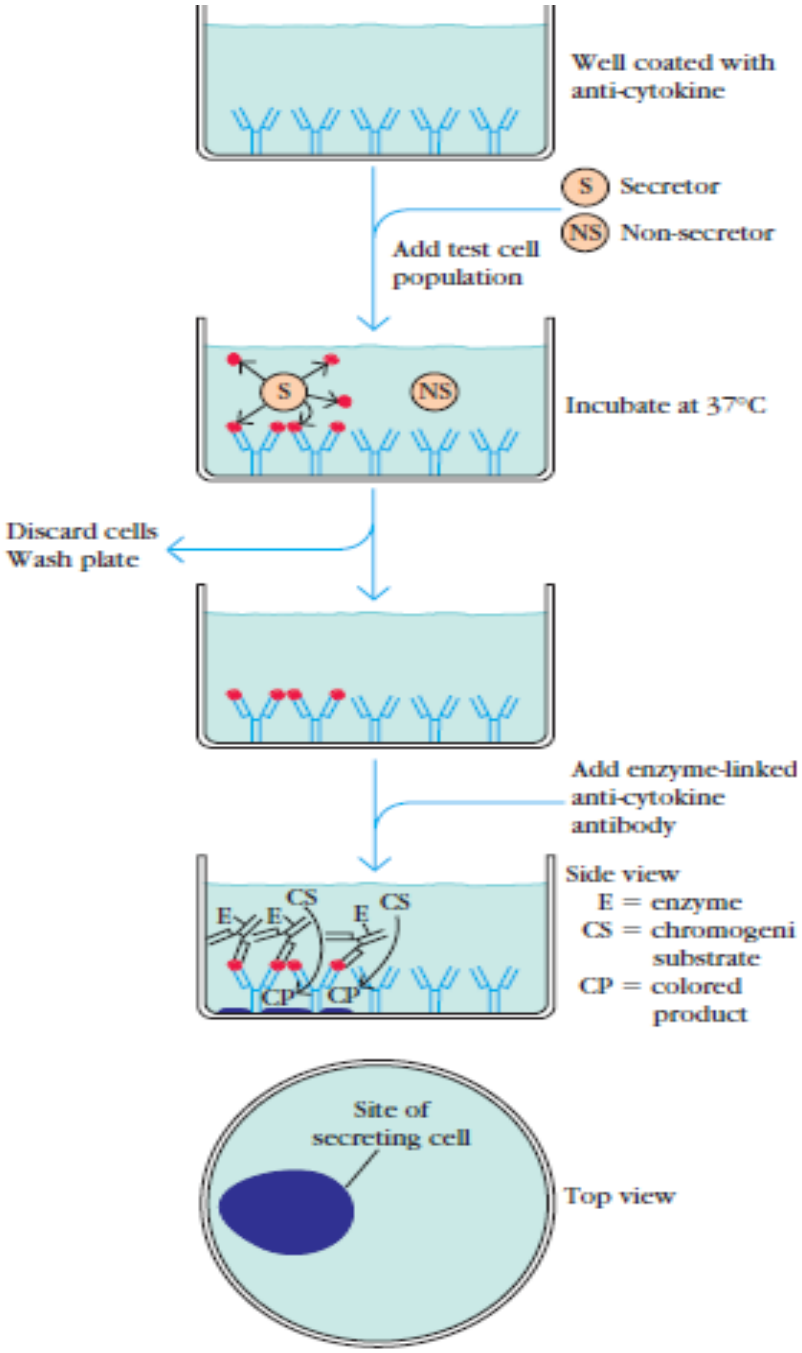
COMPETITIVE ELISA

Another variation for measuring amounts of antigen is competitive ELISA In this technique, antibody is first incubated in solution with a sample containing antigen The antigen-antibody mixture is then added to an antigencoated microtiter well. The more antigen present in the sample, the less free antibody will be available to bind to the antigen-coated well. Addition of an enzyme-conjugated secondary antibody (Ab2) specific for the isotype of the primary antibody can be used to determine the amount of primary antibody bound to the well as in an indirect ELISA. In the competitive assay, however, the higher the concentration of antigen in the original sample, the lower the absorbance.



ELISPOT ASSAY

A modification of the ELISA assay called the ELISPOT assay allows the quantitative of the number of cells in a population that are producing antibodies specific for a given antigen or an antigen for which one has a specific antibody. In this approach, the plates are coated with the antigen (capture antigen) recognized by the antibody of interest or with the antibody (capture antibody) specific for the antigen whose production is being assayed. A suspension of the cell population under investigation is then added to the coated plates and incubated. The cells settle onto the surface of the plate, and secreted molecules reactive with the capture molecules are bound by the capture molecules in the vicinity of the secreting cells, producing a ring of antigen-antibody complexes around each cell that is producing the molecule of interest. The plate is then washed and an enzyme-linked antibody specific for the secreted antigen or specific for the species (e.g., goat anti-rabbit) of the secreted antibody is added and allowed to bind. Subsequent development of the assay by addition of a suitable chromogenic or chemiluminescence-producing substrate reveals the position of each antibody- or antigen-producing cell as a point of color or light.


Well coated with anti-cytokine
S Secretor
NS Non-secretor
Add test cell population
S
NS
Incubate at 37°C
Discard cells
Wash plate
Add enzyme-linked anti-cytokine antibody
E
E
CS
E
CS
CP
CP
Side view
E = enzyme
CS = chromogeni substrate
CP = colored product
Site of secreting cell
Top view