ALAGAPPA UNIVERSITY

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(A State University Established by the Government of Tamilnadu)
KARAIKUDI-630 003

DIRECTORATE OF DISTANCE EDUCATION

M.Sc. (MICROBIOLOGY)

36431–IMMUNOLOGY

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SYLLABI – BOOK MAPPING TABLE
IMMUNOLOGY

Syllabi	Mapping in Book
BLOCK-1: IMMUNE SYSTEM AND IMMUNE CELLS	
UNIT I Basic concepts and terminologies in immunology. Haematopoiesis.	**Pages 1 - 9**
UNIT II Immune system- primary and secondary lymphoid organs: structure and functions and cellsof immune system.	**Pages 10 - 46**
UNIT III Innate and acquired immune system- cells and molecules involves. Cell mediated andhumoral mediated response. Role of Toll like receptors in innate immunity.	**Pages 47 – 64**
UNIT IV Maturation and Differentiation of T-cell and B-cell.T-cell and B-cell receptors.	**Pages 65 – 76**
BLOCK-2: CYTOKINES, ANTIGEN ANTIBODY INTERACTIONS	
UNIT V Characteristics and functions of cytokines, chemokines.	**Pages 77 – 83**
UNIT VI Immunoglobulins- class, subclass, structure and function, Immunoglobulins genes-Organization and expression.Generation of antibody diversity.	**Pages 84 - 96**
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BLOCK-1: IMMUNE SYSTEM AND IMMUNE CELLS

BLOCK - 1 IMMUNE SYSTEM AND IMMUNE CELLS

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UNIT- I

1.1: INTRODUCTION

The term ***immunity*** is derived from the Latin word "*immunis*" (exempt), which was originally referred to the protection from legal prosecution offered to the Roman senators during theirtenures in office. This term was adopted subsequently to designate the naturally acquired protection against diseases, suchas measles or smallpox. It indicated that an individual candevelop lifelong resistance to a certain disease after having contractedit only once. The cells and molecules responsible for immunity constitute the ***immune system***, and their collective and coordinated response to foreign substances is called the ***immune response***.

1.2: OBJECTIVES

After going through this unit, you will be able:

- To know the Cells of the Immune System,
- To study the Basic Concepts in Immunology System, its components and the functions involved in the immune response.
- To know the Hematopoiesis process

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1.3: BASIC CONCEPTS IN IMMUNOLOGY

The concept of immunity has existed since the ancient times. An example is the Chinese custom of making children resistant to smallpox by making them inhale powders made from the skin lesions of patients recovering from the disease. The first European mention of immunity is recorded by Thucydides in Athens during the fifth century BC. In describing plague in Athens, he wrote in 430 BC that only those who had recovered from plague could nurse the sick, because they would not contract the disease a second time.

Once the concept of existence of immunity was established, it was not long before manipulation of immunity under controlled conditions followed. First, it was Edward Jenner who in a successful experiment injected the material from a cowpox pustule into the arm of an 8-year-old boy and demonstrated the lack of development of disease after subsequent exposure to smallpox. This was based on his observations that milkmaids who had suffered from cowpox never contracted the more serious smallpox. Jenner's technique of inoculating with cowpox to protect against smallpox spread quickly throughout Europe. However, for many reasons, including lack of knowledge of obvious disease targets and their causes, it was after nearly hundred years that this technique was applied to prevent smallpox.

The experimental work of Emil von Behring and Shibasaburo Kitasato in 1890 gave the first insights into the mechanism of immunity, earning von Behring the Nobel Prize in Medicine in 1901. Von Behring and Kitasato demonstrated that serum (the liquid, noncellular component of coagulated blood) from animals previously immunized to diphtheria could transfer the immune state to unimmunized animals. Since then, immunology as a field of study has come a long way. It has been and remains one of the hottest fields of research as shown by the statistic that about 17 Nobel Prizes have been awarded to scientists involved in immunological research.

1.3.1: Physical and Chemical Barriers

Before any immune factors are triggered, the skin functions as a continuous, impassable barrier to potentially infectious pathogens. Pathogens are killed or inactivated on the skin by desiccation (drying out) and by the skin's acidity. In addition, beneficial microorganisms that coexist on the skin compete with invading pathogens, preventing infection. Regions of the body that are not protected by skin (such as the eyes and mucus membranes) have alternative methods of defense, such as tears and mucus secretions that trap and rinse away pathogens, and cilia in the nasal passages and respiratory tract that push the mucus with the pathogens out of the body. Throughout the body are other defenses, such as the low pH of the stomach (which inhibits the growth of pathogens), blood proteins that bind and disrupt bacterial cell membranes, and the process of urination (which flushes pathogens from the urinary tract).

Despite these barriers, pathogens may enter the body through skin abrasions or punctures, or by collecting on mucosal surfaces in large numbers that overcome the mucus or cilia. Some pathogens have evolved specific mechanisms that allow them to overcome physical and chemical barriers. When pathogens do enter the body, the innate immune system responds with inflammation, pathogen engulfment, and secretion of immune factors and proteins.

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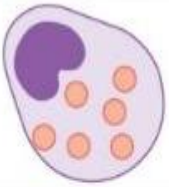
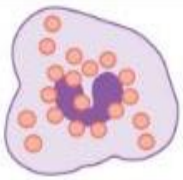
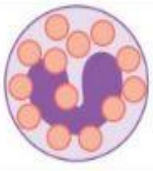
Table 1.1: The characteristics and location of cells involved in the innate immune system are described. (Credit: modification of work by NIH)

Cell type	Characteristics	Location	Image
Mast cell	Dilates blood vessels and induces inflammation through release of histamines and heparin. Recruits macrophages and neutrophils. Involved in wound healing and defense against pathogens but can also be responsible for allergic reactions.	Connective tissues, mucous membranes	
Macrophage	Phagocytic cell that consumes foreign pathogens and cancer cells. Stimulates response of other immune cells.	Migrates from blood vessels into tissues.	
Natural killer cell	Kills tumor cells and virus-infected cells.	Circulates in blood and migrates into tissues.	
Dendritic cell	Presents antigens on its surface, thereby triggering adaptive immunity.	Present in epithelial tissue, including skin, lung and tissues of the digestive tract. Migrates to lymph nodes upon activation.	
Monocyte	Differentiates into macrophages and dendritic cells in response to inflammation.	Stored in spleen, moves through blood vessels to infected tissues.	
Neutrophil	First responders at the site of infection or trauma, this abundant phagocytic cell represents 50-60 percent of all leukocytes. Releases toxins that kill or inhibit bacteria and fungi and recruits other immune cells to the site of infection.	Migrates from blood vessels into tissues.	
Basophil	Responsible for defense against parasites. Releases histamines that cause inflammation and may be responsible for allergic reactions.	Circulates in blood and migrates to tissues.	
Eosinophil	Releases toxins that kill bacteria and parasites but also causes tissue damage.	Circulates in blood and migrates to tissues.	

1.4: TERMINOLOGIES

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Antigen A toxin or other foreign substance which induces an immune response in the body, especially the production of antibodies

Antibody A protective protein produced by the immune system in response to the presence of antigen.

Bone marrow Unlike most other tissues or organs, the haemopoetic system is constantly renewing itself. In the adult, the development of haemopoetic cells occurs predominantly in the bone marrow. In the fetus, before bones develop, haemopoeisis occurs first in the yolk sac and then in the liver.

Haematopoiesis The generation of all cellular elements of blood and in humans occur in bone lymphocytes.

Memory cells, also generated from the progeny of antigen-stimulated lymphocytes, do survive for long periods in the absence of antigen.

Stroma Epithelial and endothelial cells that provide support and secrete growth factors for haemopoiesis.

***S* Stem cell**, the totipotent and self-renewing marrow cell. Stem cells are found in low numbers in blood as well as bone marrow and the numbers can be boosted by treatment with appropriate growth factors (e.g. G-CSF), which greatly facilitates the process of bone marrow transplantation (see Fig. 39).

***LS* Lymphoid stem cell**, presumed to be capable of differentiating into T or B lymphocytes. Very recent data suggest that the distinction between lymphoid and myeloid stem cells may in fact be more complex.

***HS* Haemopoietic stem cell**: the precursor of spleen nodules and probably able to differentiate into all but the lymphoid pathways, i.e. granulocyte, erythroid, monocyte, megakaryocyte; often referred to as CFU-GEMM.

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***ES* Erythroid stem cell**, giving rise to erythrocytes. Erythropoietin, a glycoprotein hormone formed in the kidney in response to hypoxia, accelerates the differentiation of red cell precursors and thus adjusts the production of red cells to the demand for their oxygen-carrying capacity, a typical example of 'negative feedback'.

***GM* Granulocyte–monocyte** common precursor; the relative proportion of these two cell types is regulated by 'growth-' or 'colonystimulating' factors.

Binding: If an antigen is bound to a solid matrix (latex particles or a plastic dish, for example), and if the antibody is labeled in some way (with a visible, radioactive or enzyme molecule), binding of the antibody to its antigen can be easily and sensitively measured. If a radioactive label is used, the assay is called a solid-state.

Radio Immuno Assay (RIA): With an enzyme-based label, on the other hand, it becomes an **E**nzyme-**L**inked **I**mmuno **S**orbent **A**ssay (**ELISA**). These solid state assays (particularly ELISA's) have largely replaced precipitation and agglutination assays in a wide variety of clinical and research applications.

Immobilization: An antibody directed against components of the flagellae of motile bacteria or protozoa can cause these flagellae to stop moving. These results in the loss of the organisms' ability to move around, and this loss of motility can be detected by microscopic examination.

Cytolysis: If the target antigen is an integral component of the membrane of certain sensitive cells, antibodies may cause disruption of the membrane and death of the cell. This requires the participation of a collection of other serum components collectively known as COMPLEMENT, and binding of these components to antibodies is referred to as **"Complement Fixation".** If the antigen target is a red blood cell, this effect is known as **hemolysis**, which can be readily detected visually. In the case of a bacterial cell target, the effect is referred to as **bacteriolysis**. If the target is a nucleated cell the effect is referred to

as **cytotoxicity**, and may be measured by release of a radioactive label incorporated into the cell (such as 51Cr), exclusion of "vital" dyes such as Trypan Blue, or any of several other measures of cell viability.

Opsonization: If the target antigen is particulate (e.g. a bacterium, or an antigen- coated latex particle), bound antibodies may greatly increase the efficiency with which the particles are phagocytosed by macrophages and other "scavenger" cells. This improvement of phagocytosis is known as opsonization, and may be facilitated even further by the presence of complement. As will be discussed later, opsonization is the result of antibodies' increasing the degree to which antigenic particles will "stick" to phagocytic cells. This phenomenon has therefore been referred to as immune adherence, and depends on the presence in the membranes of white blood cells of specific receptors either for antibody (**FcR**, or "Fc-receptors") or for complement (**CR**, or "complement receptors").

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Cloning The potential of individual stem cells to give rise to one or more types of haemopoetic cells has been explored by isolating single cells and allowing them to divide many times, and then observing what cell types can be found among the progeny. This process is known as cloning (a clone being a set of daughter cells all arising from a single parent cell). Evidence suggests that in certain conditions a single stem cell can give rise to all the fully differentiated cells of an adult haemopoetic system.

1.5: Hematopoiesis

Hematopoiesis is the process by which mature blood and immune cells are produced from hematopoietic stem and progenitor cells (HSCs and HSPCs). The last several decades of research have shed light on the origin of HSCs, as well as the heterogeneous pools of fetal progenitors that contribute to lifelong hematopoiesis. The overarching concept that hematopoiesis occurs in dynamic, overlapping waves throughout development, with each wave contributing to both continuous and developmentally limited cell types, has been solidified over the years. However, recent advances in our ability to track the production of

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hematopoietic cells *in vivo* have challenged several long-held dogmas on the origin and persistence of distinct hematopoietic cell types.

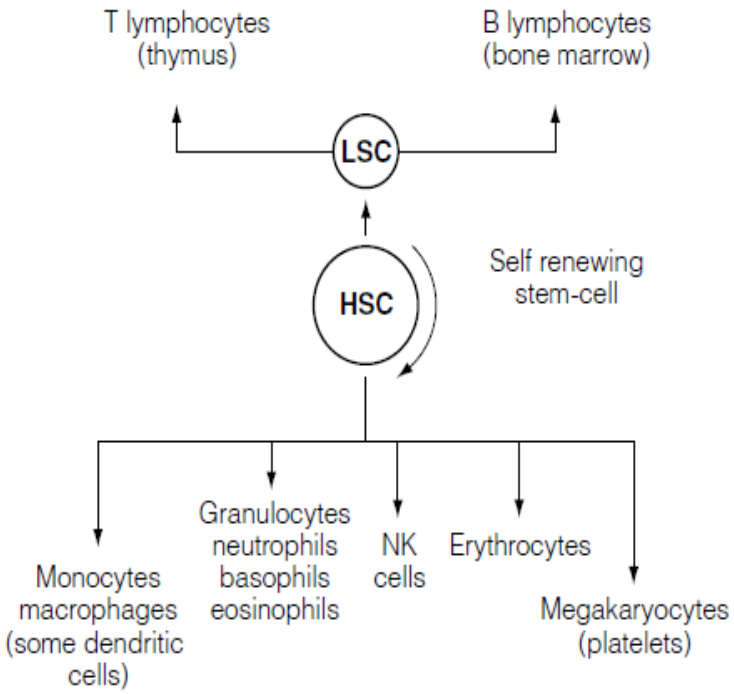

Fig.1.1. Origin of blood cells (hemopoiesis); LSC, lymphoid stem cell; HSC, hemopoietic stem cell.

1.6: CHECK YOUR PROGRESS

1. The cells and molecules responsible for immunity constitute……..
2. …… a protective protein produced by the immune system in response to the presence of antigen
3. A clone being a set of daughter cells all arising from a single parent cell is called ……
4. …….. is the process by which mature blood and immune cells are produced from hematopoietic stem and progenitor cells.

1.7: LET US SUM UP

In this unit, you have learnt about the meaning, definition, need, objectives, importance and basic concept of Immunology. This Knowledge would make you understand what is Immunology and terminologies of Immunology. Hematopoietic stem cells (HSCs) source of

all blood cells. Blood-forming cells first found in the yolk sac (primarily primitive RBC production). HSCs arise in distal aorta ~3-4 weeks. HSCs migrate to the liver (primary site of hematopoiesis after 6 wks gestation). Bone marrow hematopoiesis starts ~5 months of gestation.

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1.8: UNIT - END EXERCISES

1. Write short notes on Granulocytic Cells.
2. Write in detail about the Immune System.
3. With a neat sketch write about Hematopoiesis.
4. Describe the role of thymus in immune response
5. What are physical and chemical barriers?

1.9: ANSWERS TO CHECK YOUR PROGRESS

1. Immune system
2. Antibody
3. Cloning
4. Hematopoiesis

1.10: SUGGESTED READINGS

1. Peter J. Delves, Seamus J. Martin, Dennis R. Burton, Ivan M. Roitt. (2016). **Roitt's Essential Immunology** (13th Edition), Wiley-Blackwell Publications.

2. .Kenneth Murphy, Casey Weaver. (2016). **Janeway's Immunobiology** (9^{th} Edition), Garland Sciences.

3. Abul K. Abbas & Andrew H. H. Lichtman & Shiv Pillai. (2015). **Basic Immunology, Functions and Disorders of the Immune System** (5^{th} Edition), Elsevier.

4. Abul K. Abbas & Andrew H. Lichtman & Shiv Pillai. (2014). **Cellular and Molecular Immunology** (8th Edition), Elsevier.

5. Jeffrey K. Actor. (2011). **Review Immunology and Microbiology** (2nd Edition), Elsevier

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UNIT - II

2.1: INTRODUCTION

The immune system is the body's defense against infectious organisms and other invaders. Through a series of steps called the immune response, the immune system attacks organisms and substances that invade body systems and cause disease. The immune system is made up of a network of cells, tissues, and organs that work together to protect the body. The cells involved are white blood cells, or leukocytes, which come in two basic types that combine to seek out and destroy disease-causing organisms or substances. Leukocytes are produced or stored in many locations in the body, including the thymus, spleen, and bone marrow. For this reason, they're called the lymphoid organs. There are also clumps of lymphoid tissue throughout the body, primarily as lymph nodes, that house the leukocytes. The leukocytes circulate through the body between the organs and nodes via lymphatic vessels and blood vessels. In this way, the immune system works in a coordinated manner to monitor the body for germs or substances that might cause problems.

2.2: OBJECTIVES

After going through this unit, you will be able:

- To know the Cells of the Immune System

- To study the basic Structure of organs of the Immune System and the functions involved in the immune response.

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2.3: PRIMARY LYMPHOID ORGANS

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. The thymus and bone marrow are the primary (or central) lymphoid organs, where maturation of lymphocytes takes place. The lymph nodes, spleen, and various mucosal associated lymphoid tissues (MALT) such as gut-associated lymphoid tissue (GALT) are the secondary (or peripheral) lymphoid organs, which trap antigen and provide sites for mature lymphocytes to interact with that antigen. In addition, tertiary lymphoid tissues, which normally contain fewer lymphoid cells than secondary lymphoid organs, can import lymphoid cells during an inflammatory response. Most prominent of these are cutaneous-associated lymphoid tissues. Once mature lymphocytes have been generated in the primary lymphoid organs, they circulate in the blood and lymphatic system, a network of vessels that collect fluid that has escaped into the tissues from capillaries of the circulatory system and ultimately return it to the blood.

Immature lymphocytes generated in hematopoiesis mature and become committed to a particular antigenic specificity within the primary lymphoid organs. Only after a lympho- cyte has matured within a primary lymphoid organ is the cell **immuno competent** (capable of mounting an immune response). T cells arise in the **thymus** and in many mammals humans and mice for example, B cells originate in **bone marrow.**

2.3.1: 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 separated from each other by strands of connective tissue called trabeculae. Each lobule is organized into two compartments: the outer compartment, or *cortex,* is densely

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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

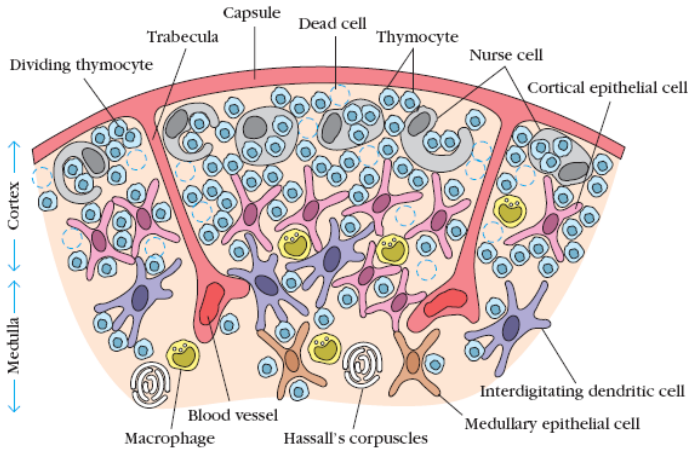

Figure 2.1: Diagrammatic cross section of a portion of the thymus, showing several lobules separated by connective tissue strands (trabeculae). The densely populated outer cortex is thought to contain many immature thymocytes (blue), which undergo rapid proliferation coupled with an enormous rate of cell death. Also present in the outer cortex are thymic nurse cells (gray), which are specialized epithelial cells with long membrane extensions that surround as many as 50 thymocytes. The medulla is sparsely populated and is thought to contain thymocytes that are more mature. During their stay within the thymus, thymocytes interact with various stromal cells, including cortical epithelial cells (light red), medullary epithelial cells (tan), interdigitating dendritic cells (purple), and macrophages (yellow). These cells produce thymic hormones and express high levels of class I and class II MHC molecules. Hassalls corpuscles, found in the medulla, contain concentric layers of degenerating epithelial cells. (Adapted from Immunology Janis Kubay)

Some thymic epithelial cells in the outer cortex, called **nurse cells,** have long membrane extensions that surround as many as 50 thymocytes, forming large multicellular complexes. Other cortical epithelial cells have long interconnecting cytoplasmic extensions that form a network and have been shown to interact with numerous thymocytes as they traverse the cortex. The function of the thymus is to generate and select a repertoire of T cells that will protect the body from infection. As thymocytes develop, an enormous diversity of T-cell receptors is generated by a random process that produces some T cells with receptors capable of recognizing antigen-MHC complexes. However, most of the T-cell receptors produced by this random process are incapable of recognizing antigen-MHC complexes and a small portion react with combinations of self antigen-MHC complexes. The thymus induces the death of those T cells that cannot recognize antigen- MHC complexes and those that react with self-antigen– MHC and pose a danger of causing autoimmune disease. More than 95% of all thymocytes die by apoptosis in the thymus without ever reaching maturity.

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2.3.1.1: THE THYMUS AND IMMUNE FUNCTION

The role of the thymus in immune function can be studied in mice by examining the effects of neonatal thymectomy, a procedure in which the thymus is surgically removed from newborn mice. These thymectomized mice show a dramatic decrease in circulating lymphocytes of the T-cell lineage and an absence of cell-mediated immunity. Other evidence of the importance of the thymus comes from studies of a congenital birth defect in humans **(DiGeorge's syndrome)** and in certain mice **(nude mice)** in which the thymus fails to develop. In both cases, there is an absence of circulating T cells and of cell-mediated immunity and an increase in infectious disease. Aging is accompanied by a decline in thymic function. This decline may play some role in the decline in immune function during aging in humans and mice. The thymus reaches its maximal size at puberty and then atrophies, with a significant decrease in both cortical and medullary cells and an increase in the total fat content of the organ. Whereas the average weight of the thymus is 70 g in infants,

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its age-dependent involution leaves an organ with an average weight of only 3 g in the elderly.

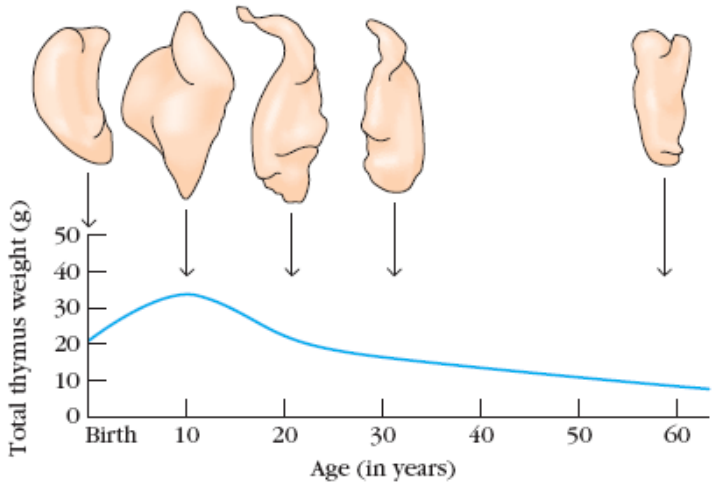

Figure 2.2: Changes in the thymus with age. The thymus decreases in size and cellularity after puberty.(Adapted from Immunology Janis Kubay). A number of experiments have been designed to look at the effect of age on the immune function of the thymus. In one experiment, the thymus from a 1-day-old or 33-monthold mouse was grafted into thymectomized adults. (For most laboratory mice, 33 months is very old.) Mice receiving the newborn thymus graft showed a significantly larger improvement in immune function than mice receiving the 33- month-old thymus.

2.3.2: 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. Like thymic selection during T-cell maturation, a selection process within the bone marrow eliminates B cells with self-reactive antibody receptors. 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 tissue hosting the maturation, proliferation, and diversification of B cells early in gestation is the fetal spleen. Later in gestation, this function is assumed by a patch of tissue embedded in the wall of the intestine called the ileal Peyer's patch, which contains a large number (>10^{10}) B cells. The rabbit, too, uses gut-associated tissues such as the appendix as primary lymphoid tissue for important steps in the proliferation and diversification of B cells.

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2.3.3: LYMPHATIC SYSTEM

As blood circulates under pressure, its fluid component **(plasma)** seeps through the thin wall of the capillaries into the surrounding tissue. Much of this fluid, called **interstitial fluid,** returns to the blood through the capillary membranes.

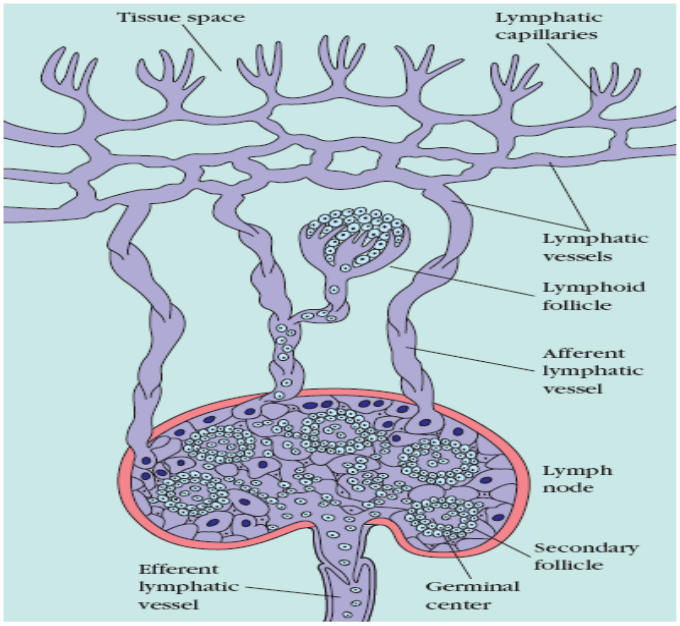

Figure 2.3: Lymphatic vessels. Small lymphatic capillaries opening into the tissue spaces pick up interstitial tissue fluid and carry it into progressively larger lymphatic vessels, which carry the fluid, now called lymph, into regional lymph nodes. As lymph leaves the nodes, it is carried

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through larger efferent lymphatic vessels, which eventually drain into the circulatory system at the thoracic duct or right lymph duct. (Adapted from Immunology Janis Kubay).

The remainder of the interstitial fluid now called **lymph,** flows from the spaces in connective tissue into a network of tiny open lymphatic capillaries and then into a series of progressively larger collecting vessels called **lymphatic vessels.**

The largest lymphatic vessel, the **thoracic duct,** empties into the left subclavian vein near the heart. In this way, the lymphatic system captures fluid lost from the blood and returns it to the blood, thus ensuring steady-state levels of fluid within the circulatory system. The heart does not pump the lymph through the lymphatic system; instead the flow of lymph is achieved as the lymph vessels are squeezed by movements of the body's muscles. A series of one-way valves along the lymphatic vessels ensures that lymph flows only in one direction. When a foreign antigen gains entrance to the tissues, it is picked up by the lymphatic system (which drains all the tissues of the body) and is carried to various organized lymphoid tissues such as lymph nodes, which trap the foreign antigen. As lymph passes from the tissues to lymphatic vessels, it becomes progressively enriched in lymphocytes. Thus, the lymphatic system also serves as a means of transporting lymphocytes and antigen from the connective tissues to organized lymphoid tissues where the lymphocytes may interact with the trapped antigen and undergo activation.

2.4: 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 non-lymphoid cells surrounded by a network of draining lymphatic capillaries. Until it is activated by antigen, a lymphoid follicle called a **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 non dividing B cells, and some helper T cells interspersed with macrophages and follicular dendritic cells. Most antigen-activated B cells divide and differentiate into antibody-producing plasma cells in lymphoid follicles, but only a few B cells in the antigen-activated population find their way into germinal centers. Those that do undergo one or more rounds of cell division, during which the genes that encode their antibodies mutate at an unusually high rate. Following the period of division and mutation, there is a rigorous selection process in which more than 90% of these B cells die by apoptosis. In general, those B cells producing antibodies that bind antigen more strongly have a much better chance of surviving than do their weaker companions. The small numbers of B cells that survive the germinal center's rigorous selection differentiate into plasma cells or memory cells and emerge.

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Lymph nodes and the **spleen** are the most highly organized of the secondary lymphoid organs; they comprise not only lymphoid follicles, but additional distinct regions of T cell and B-cell activity, and they are surrounded by a fibrous capsule. Less-organized lymphoid tissue, collectively called mucosal-associated lymphoid tissue (MALT), is found in various body sites. MALT includes Peyer's patches (in the small intestine), the tonsils, and the appendix, as well as numerous lymphoid follicles within the lamina propria of the intestines and in the mucous membranes lining the upper airways, bronchi, and genital tract.

2.4.1: LYMPH NODES

Lymph nodes are the sites where immune responses are mounted to antigens in lymph. They are encapsulated bean shaped structures containing a reticular network packed with lymphocytes, macrophages, and dendritic cells. Clustered at junctions of the lymphatic vessels, lymph nodes are the first organized lymphoid structure to encounter antigens that enter the tissue spaces. As lymph percolates through a node, any

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particulate antigen that is brought in with the lymph will be trapped by the cellular network of phagocytic cells and dendritic cells (follicular and interdigitating). The overall architecture of a lymph node supports an ideal microenvironment for lymphocytes to effectively encounter and respond to trapped antigens. Morphologically, a lymph node can be divided into three roughly concentric regions: the cortex, the paracortex, and the medulla, each of which supports a distinct microenvironment

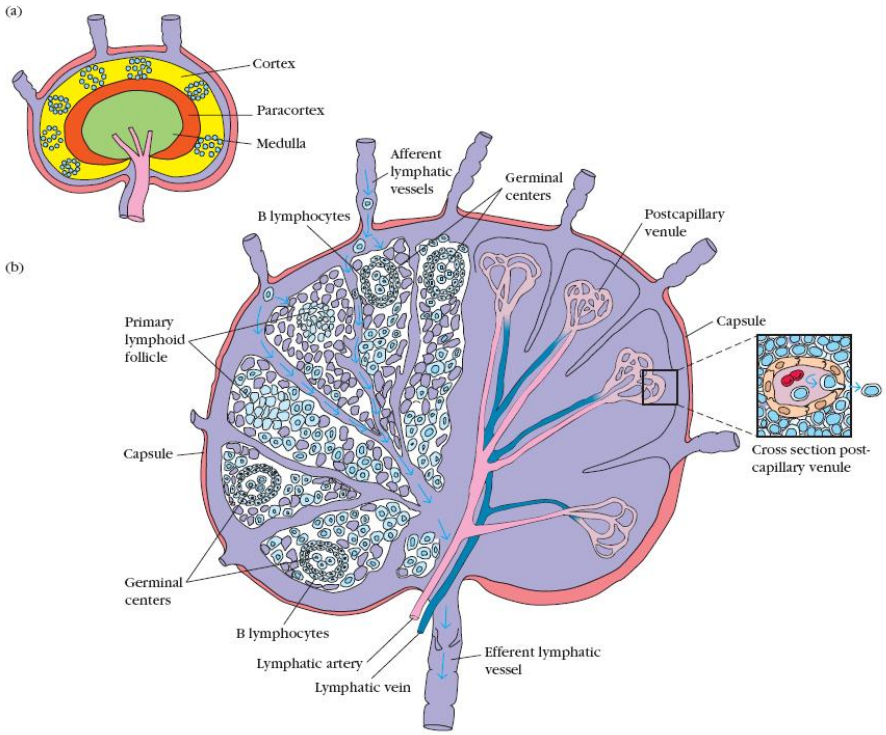

Figure 2.4: Structure of a lymph node. (a) The three layers of a lymph node support distinct microenvironments. (b) The left side depicts the arrangement of reticulum and lymphocytes within the various regions of a lymph node. Macrophages and dendritic cells, which trap antigen, are present in the cortex and paracortex. TH cells are concentrated in the paracortex; B cells are located primarily in the cortex, within follicles and germinal centers. The medulla is populated largely by antibody-producing plasma cells. Lymphocytes circulating in the lymph are carried into the node by afferent lymphatic vessels; they either enter the reticular matrix of the node or pass through it and leave by the efferent lymphatic vessel. The right side of (b) depicts the lymphatic artery and vein and the

postcapillary venules. Lymphocytes in the circulation can pass into the node from the post-capillary venules by a process called extravasation (*inset*). (Adapted from Immunology Janis Kubay).

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The outermost layer, the **cortex,** contains lymphocytes (mostly B cells), macro-phages, and follicular dendritic cells arranged in primary follicles. After antigenic challenge, the primary follicles enlarge into secondary follicles, each containing a germinal center. In children with B-ell deficiencies, the cortex lacks primary follicles and germinal centers. Beneath the cortex is the **paracortex,** which is populated largely by T lymphocytes and also contains inter-digitating dendritic cells thought to have migrated from tissues to the node. These interdigitating dendritic cells express high levels of class II MHC molecules, which are necessary for presenting antigen to TH cells. Lymph nodes taken from neonatally thymectomized mice have unusually few cells in the paracortical region; the paracortex is therefore sometimes referred to as a **thymus-dependent area** in contrast to the cortex, which is a **thymus-independent area.** The innermost layer of a lymph node, the **medulla,** is more sparsely populated with lymphoid-lineage cells; of those present, many are plasma cells actively secreting antibody molecules. As antigen is carried into a regional node by the lymph, it is trapped, processed, and presented together with class II MHC molecules by inter digitating dendritic cells in the paracortex, resulting in the activation of TH cells.

The initial activation of B cells is also thought to take place within the T-cell-rich paracortex. Once activated, TH and B cells form small foci consisting largely of proliferating B cells at the edges of the paracortex. Some B cells within the foci differentiate into plasma cells secreting IgM and IgG. These foci reach maximum size within 4–6 days of antigen challenge. Within 4–7 days of antigen challenge, a few B cells and TH cells migrate to the primary follicles of the cortex. It is not known what causes this migration. Within a primary follicle, cellular interactions between follicular dendritic cells, B cells, and TH cells take place, leading to development of a secondary follicle with a central germinal center.

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Some of the plasma cells generated in the germinal center move to the medullary areas of the lymph node, and many migrate to bone marrow. Afferent lymphatic vessels pierce the capsule of a lymph node at numerous sites and empty lymph into the sub-capsular sinus.

Lymph coming from the tissues percolates slowly inward through the cortex, paracortex, and medulla, allowing phagocytic cells and dendritic cells to trap any bacteria or particulate material (e.g., antigen-antibody complexes) carried by the lymph. After infection or the introduction of other antigens into the body, the lymph leaving a node through its single efferent lymphatic vessel is enriched with antibodies newly secreted by medullary plasma cells and also has a fifty fold higher concentration of lymphocytes than the afferent lymph. The increase in lymphocytes in lymph leaving a node is due in part to lymphocyte proliferation within the node in response to antigen. Most of the increase, however, represents blood-borne lymphocytes that migrate into the node by passing between specialized endothelial cells that line the **postcapillary venules** of the node. Estimates are that 25% of the lymphocytes leaving a lymph node have migrated across this endothelial layer and entered the node from the blood. Because antigenic stimulation within a node can increase this migration tenfold, the concentration of lymphocytes in a node that is actively responding can increase greatly, and the node swells visibly. Factors released in lymph nodes during antigen stimulation are thought to facilitate this increased migration.

2.4.2: 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, blood-borne antigens and lymphocytes are carried into the spleen through the splenic artery. Experiments with radioactively labeled lymphocytes show that more recirculating

lymphocytes pass daily through the spleen than through all the lymph nodes combined. 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.

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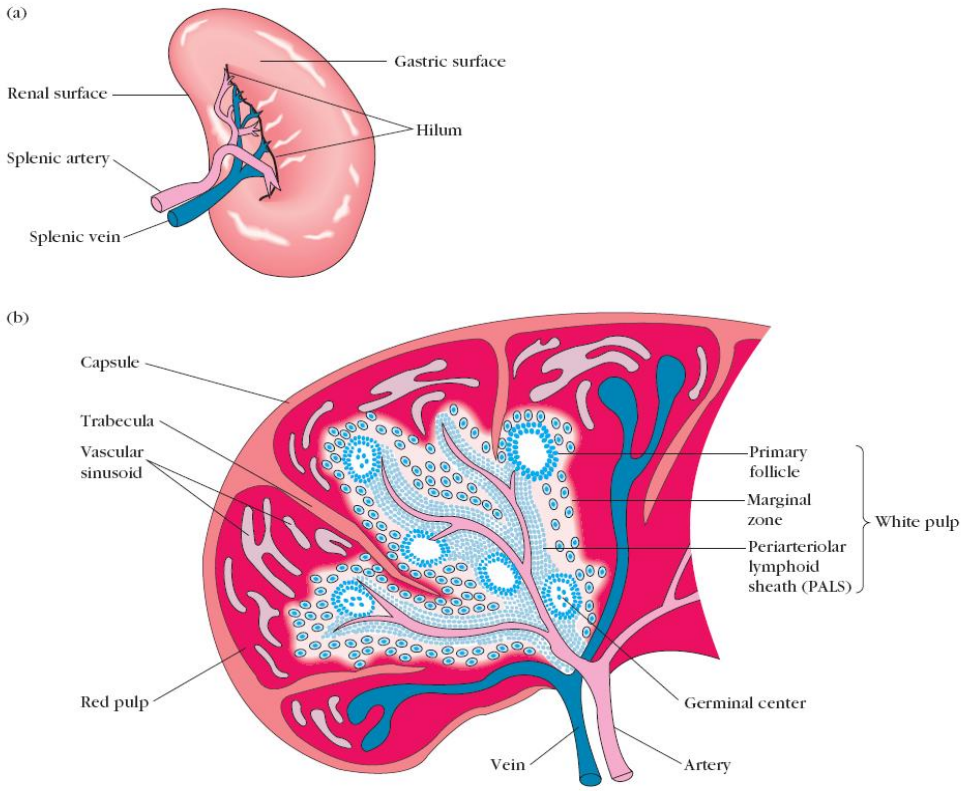

Figure 2.5: Structure of the spleen. (a) The spleen, which is about 5 inches long in adults, is the largest secondary lymphoid organ. It is specialized for trapping blood-borne antigens. (b) Diagrammatic cross section of the spleen. The splenic artery pierces the capsule and divides into progressively smaller arterioles, ending in vascular sinusoids that drain back into the splenic vein. The erythrocyte-filled red pulp surrounds the sinusoids. The white pulp forms a sleeve, the periarteriolar lymphoid sheath (PALS), around the arterioles; this sheath contains numerous T cells. Closely associated with the PALS is the marginal zone, an area rich in B cells that contains lymphoid follicles that can develop into secondary follicles containing germinal centers. (Adapted from Immunology Janis Kubay)

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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. Many of the macrophages within the red pulp contain engulfed red blood cells or iron pigments from degraded hemoglobin. The splenic **white pulp** surrounds the branches of the splenic artery, forming a **periarteriolar lymphoid sheath (PALS)** populated mainly by T lymphocytes. Primary lymphoid follicles are attached to the PALS. These follicles are rich in B cells and some of them contain germinal centers. The **marginal zone,** located peripheral to the PALS, is populated by lymphocytes and macrophages. Blood-borne antigens and lymphocytes enter the spleen through the splenic artery, which empties into the marginal zone. In the marginal zone, antigen is trapped by inter-digitating dendritic cells, which carry it to the PALS. Lymphocytes in the blood also enter sinuses in the marginal zone and migrate to the PALS.

The initial activation of B and T cells takes place in the T cell-rich PALS. Here interdigitating dendritic cells capture antigen and present it combined with class II MHC molecules to TH cells. Once activated, these TH cells can then activate B cells. The activated B cells, together with some TH cells, then migrate to primary follicles in the marginal zone. Upon antigenic challenge, these primary follicles develop into characteristic secondary follicles containing germinal centers (like those in the lymph nodes), where rapidly dividing B cells (centroblasts) and plasma cells are surrounded by dense clusters of concentrically arranged lymphocytes. The effects of splenectomy on the immune response depend on the age at which the spleen is removed. In children, splenectomy often leads to an increased incidence of bacterial sepsis caused primarily by *Streptococcus pneumoniae, Neisseria meningitidis,* and *Haemophilus influenzae.* Splenectomy in adults has less adverse effects, although it leads to some increase in blood-borne bacterial infections **(bacteremia).**

2.4.3: MUCOSAL-ASSOCIATED LYMPHOID TISSUE

The mucous membranes lining the digestive, respiratory, and urogenital systems have a combined surface area of about 400 m2 (nearly

the size of a basketball court) 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).** Structurally, these tissues range from loose, barely organized clusters of lymphoid cells in the lamina propria of intestinal villi to well-organized structures such as the familiar tonsils and appendix, as well as Peyer's patches, which are found within the sub-mucosal layer of the intestinal lining. The functional importance of MALT in the body's defense is attested to by its large population of antibody-producing plasma cells, whose number far exceeds that of plasma cells in the spleen, lymph nodes, and bone marrow combined. The **tonsils** are found in three locations: lingual at the base of the tongue; palatine at the sides of the back of the mouth; and pharyngeal (adenoids) in the roof of the nasopharynx.

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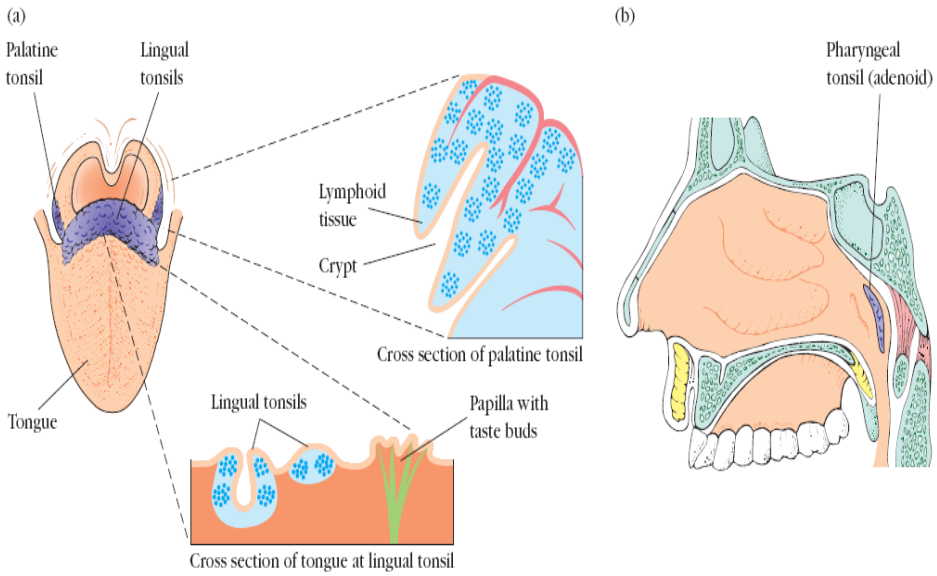

Figure 2.6: Three types of tonsils. (a) The position and internal features of the palatine and lingual tonsils; (b) a view of the position of the nasopharyngeal tonsils (adenoids). (Adapted from Immunology Janis Kubay)

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All three tonsil groups are nodular structures consisting of a meshwork of reticular cells and fibers interspersed with lymphocytes, macrophages, granulocytes, and mast cells. The B cells are organized into follicles and germinal centers; the latter are surrounded by regions showing T-cell activity. The tonsils defend against antigens entering through the nasal and oral epithelial routes. The best studied of the mucous membranes is the one that lines the gastrointestinal tract. This tissue, like that of the respiratory and urogenital tracts, has the capacity to endocytose antigen from the lumen. Immune reactions are initiated against pathogens and antibody can be generated and exported to the lumen to combat the invading organisms.

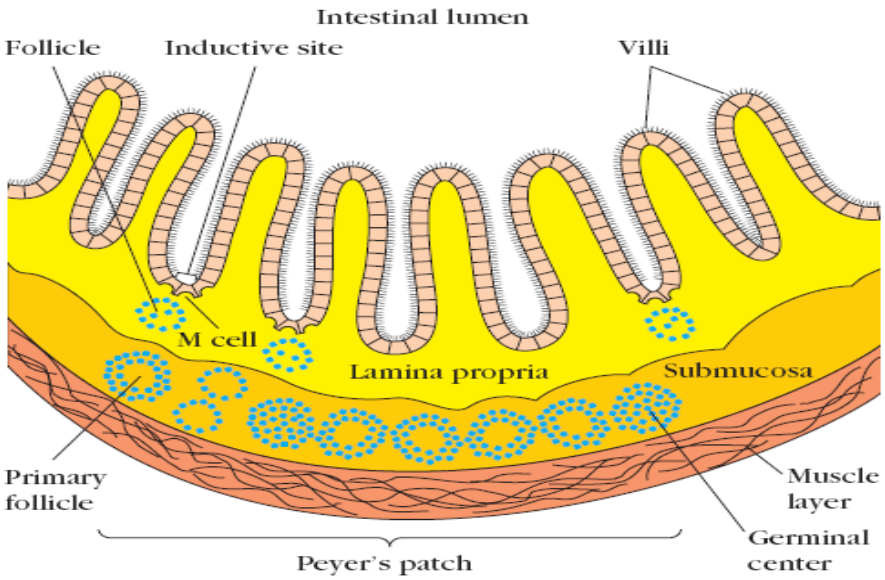

Figure 2.7: Cross-sectional diagram of the mucous membrane lining the intestine showing a nodule of lymphoid follicles that constitutes a Peyer's patch in the sub-mucosa. The intestinal lamina propria contains loose clusters of lymphoid cells and diffuse follicles. (Adapted from Immunology Janis Kubay).

Lymphoid cells are found in various regions within this tissue. The outer mucosal epithelial layer contains so-called **intraepithelial lymphocytes (IELs).** Many of these lymphocytes are T cells that express

unusual receptors (T-cell receptors, or TCRs), which exhibit limited diversity for antigen. Although this population of T cells is well situated to encounter antigens that enter through the intestinal mucous epithelium, their actual function remains largely unknown the lamina propria, which lies under the epithelial layer, contains large numbers of B cells, plasma cells, activated TH cells, and macrophages in loose clusters. Histologic sections have revealed more than 15,000 lymphoid follicles within the intestinal lamina propria of a healthy child.

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The sub-mucosal layer beneath the lamina propria contains Peyer's patches, nodules of 30–40 lymphoid follicles. Like lymphoid follicles in other sites, those that compose Peyer's patches can develop into secondary follicles with germinal centers. The epithelial cells of mucous membranes play an important role in promoting the immune response by delivering small samples of foreign antigen from the lumina of the respiratory, digestive, and urogenital tracts to the underlying mucosal-associated lymphoid tissue. This antigen transport is carried out by specialized **M cells.** The structure of the M cell is striking: these are flattened epithelial cells lacking the microvilli that characterize the rest of the mucous epithelium. In addition, M cells have a deep invagination, or pocket, in the basolateral plasma membrane; this pocket is filled with a cluster of B cells, T cells, and macrophages.

Luminal antigens are endocytosed into vesicles that are transported from the luminal membrane to the underlying pocket membrane. The vesicles then fuse with the pocket membrane, delivering the potentially response-activating antigens to the clusters of lymphocytes contained within the pocket. M cells are located in so-called **inductive sites**—small regions of a mucous membrane that lie over organized lymphoid follicles. Antigens transported across the mucous membrane by M cells can activate B cells within these lymphoid follicles. The activated B cells differentiate into plasma cells, which leave the follicles and secrete the IgA class of antibodies. These antibodies then are transported across the epithelial cells and released as **secretory IgA** into the lumen, where they can interact with antigens.

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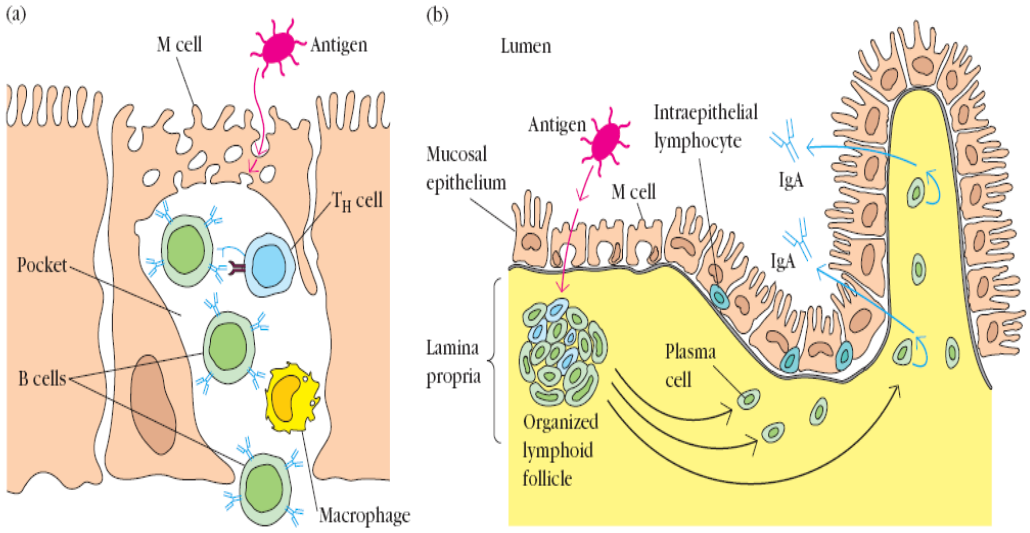

Figure 2.8: Structure of M cells and production of IgA at inductive sites. (a) M cells, located in mucous membranes, endocytose antigen from the lumen of the digestive, respiratory, and urogenital tracts. The antigen is transported across the cell and released into the large basolateral pocket. (b) Antigen transported across the epithelial layer by M cells at an inductive site activates B cells in the underlying lymphoid follicles. The activated B cells differentiate into IgA-producing plasma cells, which migrate along the submucosa. The outer mucosal epithelial layer contains intraepithelial lymphocytes, of which many are CD8_ T cells that express _ TCRs with limited receptor diversity for antigen. (Adapted from Immunology Janis Kubay).

Mucous membranes are an effective barrier to the entrance of most pathogens, which thereby contributes to nonspecific immunity. One reason for this is that the mucosal epithelial cells are cemented to one another by tight junctions that make it difficult for pathogens to penetrate. Interestingly, some enteric pathogens, including both bacteria and viruses, have exploited the M cell as an entry route through the mucous-membrane barrier. In some cases, the pathogen is internalized by the M cell and transported into the pocket. In other cases, the pathogen binds to the M cell and disrupts the cell, thus allowing entry of the pathogen. Among the

pathogens that use M cells in these ways are several invasive *Salmonella* species, *Vibrio cholerae*, and the polio virus.

2.4.4: CUTANEOUS-ASSOCIATED LYMPHOID TISSUE

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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. These cells express high levels of class II MHC molecules and function as potent activators of naive TH cells. The epidermis also contains so-called *intraepidermal lymphocytes.* These are similar to the intraepithelial lymphocytes of MALT in that most of them are CD8 T cells, many of which express _ T-cell receptors, which have limited diversity for antigen. These intraepidermal T cells are well situated to encounter antigens that enter through the skin and some immunologists believe that they may play a role in combating antigens that enter through the skin. The underlying dermal layer of the skin contains scattered CD4 and CD8 T cells and macrophages. Most of these dermal T cells were either previously activated cells or are memory cells.

2.5: CELLS OF THE IMMUNE SYSTEM

Lymphocytes are the central cells of the immune system, responsible for adaptive immunity and the immunologic attributes of diversity, specificity, memory, and self/non-self recognition. The other types of white blood cells play important roles, engulfing and destroying microorganisms, presenting antigens, and secreting cytokines. Lymphoid

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Cells Lymphocytes constitute 20%–40% of the body's white blood cells and 99% of the cells in the lymph. There are approximately 1011 (range depending on body size and age: ~1010–1012) lymphocytes in the human body. These lymphocytes continually circulate in the blood and lymph and are capable of migrating into the tissue spaces and lymphoid organs, thereby integrating the immune system to a high degree. The lymphocytes can be broadly subdivided into three populations: B cells, T cells, and natural killer cells on the basis of function and cell-membrane components.

2.5.1: NATURAL KILLER CELLS (NK CELLS)

The NK cells are large, granular lymphocytes that do not express the set of surface markers typical of B or T cells. Resting B and T lymphocytes are small, motile, nonphagocytic cells, which cannot be distinguished morphologically. B and T lymphocytes that have not interacted with antigen referred to as **naive,** or unprimed are resting cells in the G0 phase of the cell cycle. Known as small lymphocytes, these cells are only about 6 μm in diameter; their cytoplasm forms a barely discernible rim around the nucleus. Small lymphocytes have densely packed chromatin, few mitochondria, and a poorly developed endoplasmic reticulum and Golgi apparatus. The naive lymphocyte is generally thought to have a short life span.

These cells to enter the cell cycle by progressing from G0 into G1 and subsequently into S, G2, and M. As they progress through the cell cycle, lymphocytes enlarge into 15 _m-diameter blast cells, called **lymphoblasts;** these cells have a higher cytoplasm: nucleus ratio and more organellar complexity than small lymphocytes. Lymphoblasts proliferate and eventually differentiate into **effector cells** or into **memory cells.** Effector cells function in various ways to eliminate antigen. These cells have short life spans, generally ranging from a few days to a few weeks.

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Table 2.1: Types of Cells

Cell type	Cells/mm^3	%
Red blood cells	5.0×10^6	
Platelets	2.5×10^5	
Leukocytes	7.3×10^3	
Neutrophil		50–70
Lymphocyte		20–40
Monocyte		1–6
Eosinophil		1–3
Basophil		<1

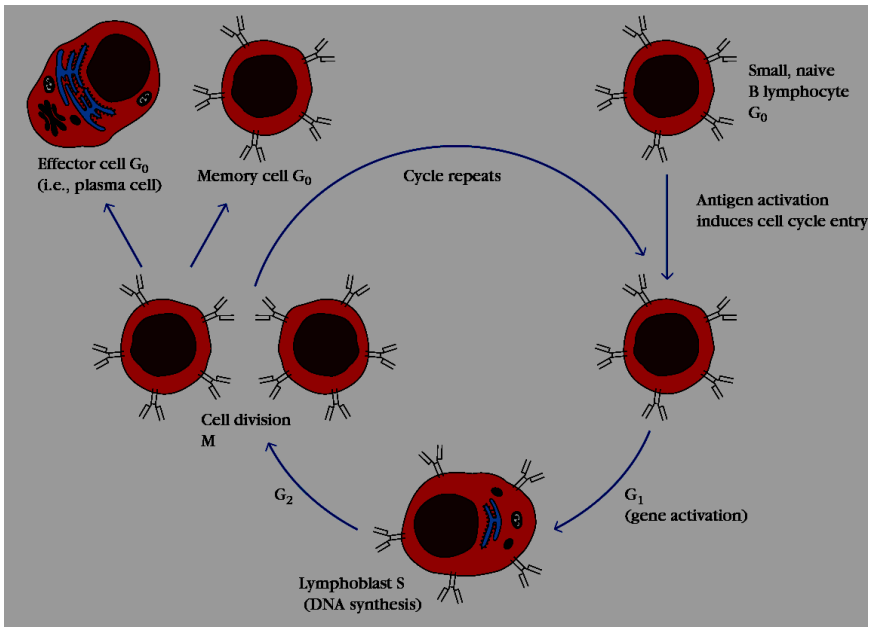

Figure 2.10: Fate of antigen-activated small lymphocytes. (a) A small resting (naive or unprimed) lymphocyte resides in the G0 phase of the cell cycle. At this stage, B and T lymphocytes cannot be distinguished morphologically. After antigen activation, a B or T cell enters the cell cycle and enlarges into a lymphoblast, which undergoes several rounds of cell division and, eventually, generates effector cells and memory cells.

Shown here are cells of the B-cell lineage. (Adapted from Immunology Janis Kubay).

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2.5.2: PLASMA CELLS

The antibody-secreting effector cells of the B cell lineage—have a characteristic cytoplasm that contains abundant endoplasmic reticulum (to support their high rate of protein synthesis) arranged in concentric layers and also many Golgi vesicles. The effector cells of the T-cell lineage include the cytokine-secreting T helper cell (TH cell) and the T cytotoxic lymphocyte (TC cell). Some of the progeny of B and T lymphoblasts differentiate into memory cells. The persistence of this population of cells is responsible for life-long immunity to many pathogens. Memory cells look like small lymphocytes but can be distinguished from naive cells by the presence or absence of certain cell membrane molecules. Different lineages or maturational stages of lymphocytes can be distinguished by their expression of membrane molecules recognized by particular monoclonal antibodies (antibodies that are specific for a single epitope of an antigen; see Chapter 4 for a description of monoclonal antibodies).All of the monoclonal antibodies that react with a particular membrane molecule are grouped together as a **cluster of differentiation (CD).**

Each new monoclonal antibody that recognizes a leukocyte membrane molecule is analyzed for whether it falls within a recognized CD designation; if it does not, it is given a new CD designation reflecting a new membrane molecule. Although the CD nomenclature was originally developed for the membrane molecules of human leukocytes, the homologous membrane molecules of other species, such as mice, are commonly referred to by the same CD designations. However, this is only a partial listing of the more than 200 CD markers that have been described.

2.5.3: B LYMPHOCYTES

The B lymphocyte derived its letter designation from its site of maturation, in the *b*ursa of Fabricius in birds; the name turned out to be

apt, for *b*one marrow is its major site of maturation in a number of mammalian species, including humans and mice. Mature B cells are definitively distinguished from other lymphocytes by their synthesis and display of membrane-bound immunoglobulin (antibody) molecules, which serve as receptors for antigen. Each of the approximately 1.5 X 10^5 molecules of antibody on the membrane of a single B cell has an identical binding site for antigen. Among the other molecules expressed on the membrane of mature B cells are the following: **B220** (a form of CD45) is frequently used as a marker for B cells and their precursors. However, unlike antibody, it is not expressed uniquely by B-lineage cells. **Class II MHC molecules** permit the B cell to function as an antigen-presenting cell (APC). **CR1** (CD35) and **CR2** (CD21) are receptors for certain complement products. **FcRII** (CD32) is a receptor for IgG, a type of antibody.**B7-1** (CD80) and **B7-2** (CD86) are molecules that interact with CD28 and CTLA-4, important regulatory molecules on the surface of different types of T cells, including TH cells.

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CD40 is a molecule that interacts with CD40 ligand on the surface of helper T cells. In most cases this interaction is critical for the survival of antigen stimulated B cells and for their development into antibody-secreting plasma cells or memory B cells. Interaction between antigen and the membrane-bound antibody on a mature naive B cell, as well as interactions with T cells and macrophages, selectively induces the activation and differentiation of B-cell clones of corresponding specificity. In this process, the B cell divides repeatedly and differentiates over a 4- to 5-day period, generating a population of plasma cells and memory cells. Plasma cells, which have lower levels of membrane-bound antibody than B cells, synthesize and secrete antibody. All clonal progeny from a given B cell secrete antibody molecules with the same antigen-binding specificity. Plasma cells are terminally differentiated cells, and many die in 1 or 2 weeks.

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Table: 2.2

Common CD markers used to distinguish functional lymphocyte subpopulations

CD designation*	Function	B cell	T cell		NK cell
			T_H	T_C	
CD2	Adhesion molecule; signal transduction	–	+	+	+
CD3	Signal-transduction element of T-cell receptor	–	+	+	–
CD4	Adhesion molecule that binds to class II MHC molecules; signal transduction	–	+ (usually)	– (usually)	–
CD5	Unknown	+	+ (subset)	+	–
CD8	Adhesion molecule that binds to class I MHC molecules; signal transduction	–	– (usually)	+ (usually)	+ (variable)
CD16 (FcγRIII)	Low-affinity receptor for Fc region of IgG	–	–	–	+
CD21 (CR2)	Receptor for complement (C3d) and Epstein-Barr virus	+	–	–	–
CD28	Receptor for co-stimulatory B7 molecule on antigen-presenting cells	–	+	+	–
CD32 (FcγRII)	Receptor for Fc region of IgG	+	–	–	–
CD35 (CR1)	Receptor for complement (C3b)	+	–	–	–
CD40	Signal transduction	+	–	–	–
CD45	Signal transduction	+	+	+	+
CD56	Adhesion molecule	–	–	–	+

*Synonyms are shown in parentheses.

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2.5.4: T LYMPHOCYTES

T lymphocytes derive their name from their site of maturation in the thymus. Like B lymphocytes, these cells have membrane receptors for antigen. Although the antigen binding T-cell receptor is structurally distinct from immunoglobulin, it does share some common structural features with the immunoglobulin molecule, most notably in the structure of its antigen-binding site. Unlike the membrane-bound antibody on B cells, though, the T-cell receptor (TCR) does not recognize free antigen. Instead the TCR recognizes only antigen that is bound to particular classes of self-molecules. Most T cells recognize antigen only when it is bound to a self-molecule encoded by genes within the major histocompatibility complex (MHC). Thus, a fundamental difference between the humoral and cell-mediated branches of the immune system is that the B cell is capable of binding soluble antigen, whereas the T cell is restricted to binding antigen displayed on self-cells. To be recognized by most T cells, this antigen must be displayed together with MHC molecules on the surface of antigen-presenting cells or on virus-infected cells, cancer cells, and grafts. The T-cell system has developed to eliminate these altered self-cells, which pose a threat to the normal functioning of the body. Like B cells, T cells express distinctive membrane molecules. All T-cell subpopulations express the T-cell receptor, a complex of polypeptides that includes CD3; and most can be distinguished by the presence of one or the other of two membrane molecules, CD4 and CD8. In addition, most mature T cells express the following membrane molecules:

CD28, a receptor for the co-stimulatory B7 family of molecules present on B cells and other antigen presenting cells. **CD45,** a signal-transduction molecule T cells that express the membrane glycoprotein molecule CD4 are restricted to recognizing antigen bound to class II MHC molecules, whereas T cells expressing CD8, a dimeric membrane glycoprotein, are restricted to recognition of antigen bound to class I MHC molecules. Thus the expression of CD4 versus CD8 corresponds to

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the MHC restriction of the T cell. In general, expression of CD4 and of CD8 also defines two major functional subpopulations of T lymphocytes.CD4, T cells generally function as T helper (TH) cells and are class-II restricted; CD8_ T cells generally function as T cytotoxic (TC) cells and are class-I restricted. Thus assaying the number of CD4_ and CD8_ T cells can approximate the ratio of TH to TC cells in a sample. This ratio is approximately 2:1 in normal human peripheral blood, but it may be significantly altered by immunodeficiency diseases, autoimmune diseases, and other disorders.

The classification of CD4_ class II–restricted cells as TH cells and CD8 class I–restricted cells as TC cells is not absolute. Some CD4_ cells can act as killer cells. Also, some TC cells have been shown to secrete a variety of cytokines and exert an effect on other cells comparable to that exerted by TH cells. The distinction between TH and TC cells, then, is not always clear; there can be ambiguous functional activities. However, because these ambiguities are the exception and not the rule, the generalization of T helper (TH) cells as being CD4_ and class-II restricted and of T cytotoxic cells (TC) as being CD8_ and class-I restricted is assumed throughout this text, unless otherwise specified. TH cells are activated by recognition of an antigen–class II MHC complex on an antigen-presenting cell. After activation, the TH cell begins to divide and gives rise to a clone of effector cells, each specific for the same antigen–class II MHC complex. These TH cells secrete various cytokines, which play a central role in the activation of B cells, T cells, and other cells that participate in the immune response. Changes in the pattern of cytokines produced by TH cells can change the type of immune response that develops among other leukocytes. The **TH1 response** produces a cytokine profile that supports inflammation and activates mainly certain T cells and macrophages, whereas the **TH2 response** activates mainly B cells and immune responses that depend upon antibodies. TC cells are activated when they interact with an antigen–class I MHC complex on the surface of an altered self-cell (e.g., a virus-infected cell or a tumor cell) in the presence of appropriate cytokines. This activation, which results in

proliferation, causes the TC cell to differentiate into an effector cell called a **cytotoxic T lymphocyte (CTL).**

In contrast to TH cells, most CTLs secrete few cytokines. Instead, CTLs acquire the ability to recognize and eliminate altered self-cells. Another subpopulation of T lymphocytes called **T suppressor (TS) cells** has been postulated. It is clear that some T cells help to suppress the humoral and the cell-mediated branches of the immune system, but the actual isolation and cloning of normal TS cells is a matter of controversy and dispute among immunologists. For this reason, it is uncertain whether TS cells do indeed constitute a separate functional subpopulation of T cells. Some immunologists believe that the suppression mediated by T cells observed in some systems is simply the consequence of activities of TH or TC subpopulations whose end results are suppressive.

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2.5.5: NATURAL KILLER CELLS

The natural killer cell was first described in 1976, when it was shown that the body contains a small population of large, granular lymphocytes that display cytotoxic activity against a wide range of tumor cells in the absence of any previous immunization with the tumor. NK cells were subsequently shown to play an important role in host defense both against tumor cells and against cells infected with some, though not all, viruses. These cells, which constitute 5%–10% of lymphocytes in human peripheral blood, do not express the membrane molecules and receptors that distinguish T- and B-cell lineages. Although NK cells do not have T-cell receptors or immunoglobulin incorporated in their plasma membranes, they can recognize potential target cells in two different ways. In some cases, an NK cell employs NK cell receptors to distinguish abnormalities, notably a reduction in the display of class I MHC molecules and the unusual profile of surface antigens displayed by some tumor cells and cells infected by some viruses. Another way in which NK cells recognize potential target cells depends upon the fact that some tumor cells and cells infected by certain viruses display antigens against which the immune system has made an antibody response, so that antitumor or antiviral antibodies are bound to their surfaces. Because NK

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cells express CD16, a membrane receptor for the carboxyl-terminal end of the IgG molecule, called the Fc region, they can attach to these antibodies and subsequently destroy the targeted cells. This is an example of a process known as antibody-dependent cell mediated cytotoxicity (ADCC).

Several observations suggest that NK cells play an important role in host defense against tumors. For example, in humans the Chediak-Higashi syndrome—an Autosomal recessive disorder—is associated with impairment in neutrophils, macrophages, and NK cells and an increased incidence of lymphomas. Likewise, mice with an Autosomal mutation called *beige* lack NK cells; these mutants are more susceptible than normal mice to tumor growth following injection with live tumor cells. There has been growing recognition of a cell type, the NK1-T cell that has some of the characteristics of both T cells and NK cells. Like T cells, NK1-T cells have T cell receptors (TCRs). Unlike most T cells, the TCRs of NK1-T cells interact with MHC-like molecules called CD1 rather than with class I or class II MHC molecules. Like NK cells, they have variable levels of CD16 and other receptors typical of NK cells, and they can kill cells. A population of triggered NK1-T cells can rapidly secrete large amounts of the cytokines needed to support antibody production by B cells as well as inflammation and the development and expansion of cytotoxic T cells. Some immunologists view this cell type as a kind of rapid response system that has evolved to provide early help while conventional TH responses are still developing.

2.5.6: MONONUCLEAR PHAGOCYTES

The mononuclear phagocytic system consists of **monocytes** circulating in the blood and **macrophages** in the tissues **(Figure 2.2).** During hematopoiesis in the bone marrow, granulocyte-monocyte progenitor cells differentiate into promonocytes, which leave the bone marrow and enter the blood, where they further differentiate into mature monocytes.

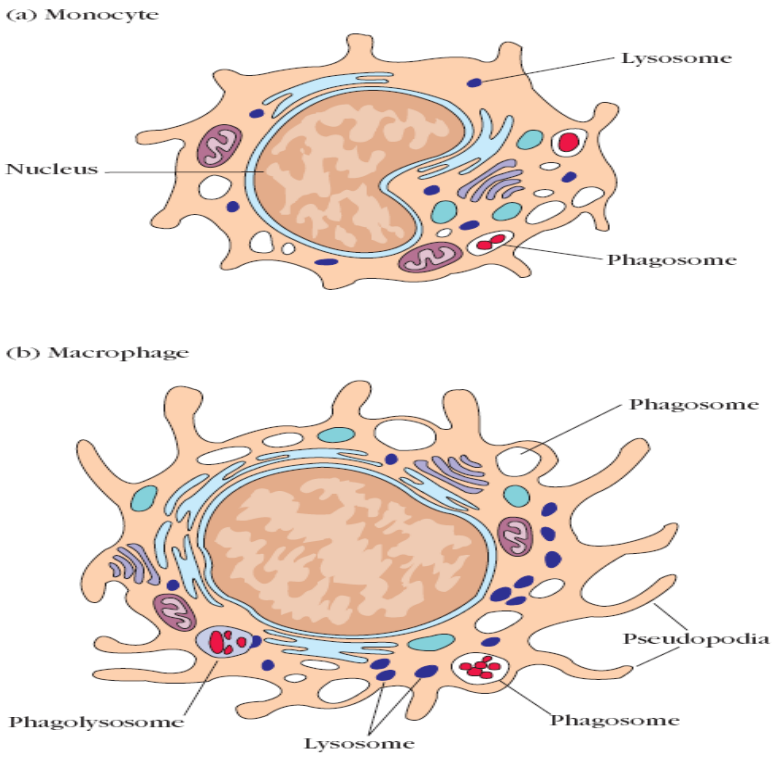

Figure 2.11: Typical morphology of a monocyte and a macrophage. Macrophages are five- to tenfold larger than monocytes and contain more organelles, especially lysosomes. (Adapted from Immunology Janis Kubay).

Monocytes circulate in the bloodstream for about 8 h, during which they enlarge; they then migrate into the tissues and differentiate into specific tissue macrophages or, as discussed later, into dendritic cells. Differentiation of a monocyte into a tissue macrophage involves a number of changes: The cell enlarges five- to tenfold; its intracellular organelles increase in both number and complexity; and it acquires increased phagocytic ability, produces higher levels of hydrolytic enzymes, and begins to secrete a variety of soluble factors. Macrophages are dispersed throughout the body. Some take up residence in particular tissues, becoming fixed macrophages, whereas others remain motile and are called free, or wandering, macrophages. Free macrophages travel by amoeboid movement throughout the tissues. Macrophage-like cells serve different functions in different tissues and are named according to their tissue location:

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- **Alveolar macrophages** in the lung
- **Histiocytes** in connective tissues
- **Kupffer cells** in the liver
- **Mesangial cells** in the kidney
- **Microglial cells** in the brain
- **Osteoclasts** in bone

Although normally in a resting state, macrophages are activated by a variety of stimuli in the course of an immune response. Phagocytosis of particulate antigens serves as an initial activating stimulus. However, macrophage activity can be further enhanced by cytokines secreted by activated TH cells, by mediators of the inflammatory response, and by components of bacterial cell walls. One of the most potent activators of macrophages is interferon gamma secreted by activated TH cells. Activated macrophages are more effective than resting ones in eliminating potential pathogens, because they exhibit greater phagocytic activity, an increased ability to kill ingested microbes, increased secretion of inflammatory mediators, and an increased ability to activate T cells. In addition, activated macrophages, but not resting ones, secrete various cytotoxic proteins that help them eliminate a broad range of pathogens, including virus-infected cells, tumor cells, and intracellular bacteria. Activated macrophages also express higher levels of class II MHC molecules, allowing them to function more effectively as antigen-presenting cells. Thus, macrophages and TH cells facilitate each other's activation during the immune response.

2.5.7: PHAGOCYTOSIS

Macrophages are capable of ingesting and digesting exogenous antigens, such as whole microorganisms and insoluble particles, and endogenous matter, such as injured or dead host cells, cellular debris, and activated clotting factors. In the first step in phagocytosis, macrophages are attracted by and move toward a variety of substances generated in an immune response; this process is called **chemotaxis.** The next step in

phagocytosis is adherence of the antigen to the macrophage cell membrane. Complex antigens, such as whole bacterial cells or viral particles, tend to adhere well and are readily phagocytosed; isolated proteins and encapsulated bacteria tend to adhere poorly and are less readily phagocytosed. Adherence induces membrane protrusions, called **pseudopodia,** to extend around the attached material. Fusion of the pseudopodia encloses the material within a membrane-bounded structure called a **phagosome,** which then enters the endocytic-processing pathway. In this pathway, a phagosome moves toward the cell interior, where it fuses with a **lysosome** to form a **phagolysosome.**

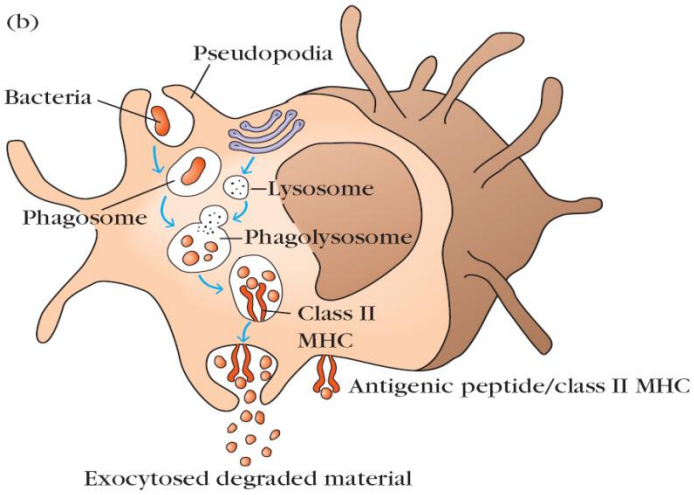

Figure 2.12: Macrophages can ingest and degrade particulate antigens, including bacteria. (b) Phagocytosis and processing of exogenous antigen by macrophages. Most of the products resulting from digestion of ingested material are exocytosed, but some peptide products may interact with class II MHC molecules, forming complexes that move to the cell surface, where they are presented to TH cells. [*Photograph by L. Nilsson, © Boehringer Ingelheim International GmbH.*]

Lysosomes contain lysozyme and a variety of other hydrolytic enzymes that digest the ingested material. The digested contents of the phagolysosome are then eliminated in a process called **Exocytosis.** The macrophage membrane has receptors for certain classes of antibody. If an

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antigen (e.g., a bacterium) is coated with the appropriate antibody, the complex of antigen and antibody binds to antibody receptors on the macrophage membrane more readily than antigen alone and phagocytosis is enhanced. In one study, for example, the rate of phagocytosis of an antigen was 4000-fold higher in the presence of specific antibody to the antigen than in its absence. Thus, antibody functions as an **opsonin,** a molecule that binds to both antigen and macrophage and enhances phagocytosis. The process by which particulate antigens are rendered more susceptible to phagocytosis is called **opsonization.**

2.5.8: GRANULOCYTIC CELLS

The **granulocytes** are classified as neutrophils, eosinophils, or basophils on the basis of cellular morphology and cytoplasmic staining characteristics. The **neutrophil** has a multilobed nucleus and a granulated cytoplasm that stains with both acid and basic dyes; it is often called a polymorphonuclear leukocyte (PMN) for its multilobed nucleus. The **eosinophil** has a bilobed nucleus and a granulated cytoplasm that stains with the acid dye eosin red (hence its name). The **basophil** has a lobed nucleus and heavily granulated cytoplasm that stains with the basic dye methylene blue. Both neutrophils and eosinophils are phagocytic, whereas basophils are not. Neutrophils, which constitute 50%–70% of the circulating white blood cells, are much more numerous than eosinophils (1%–3%) or basophils (1%).

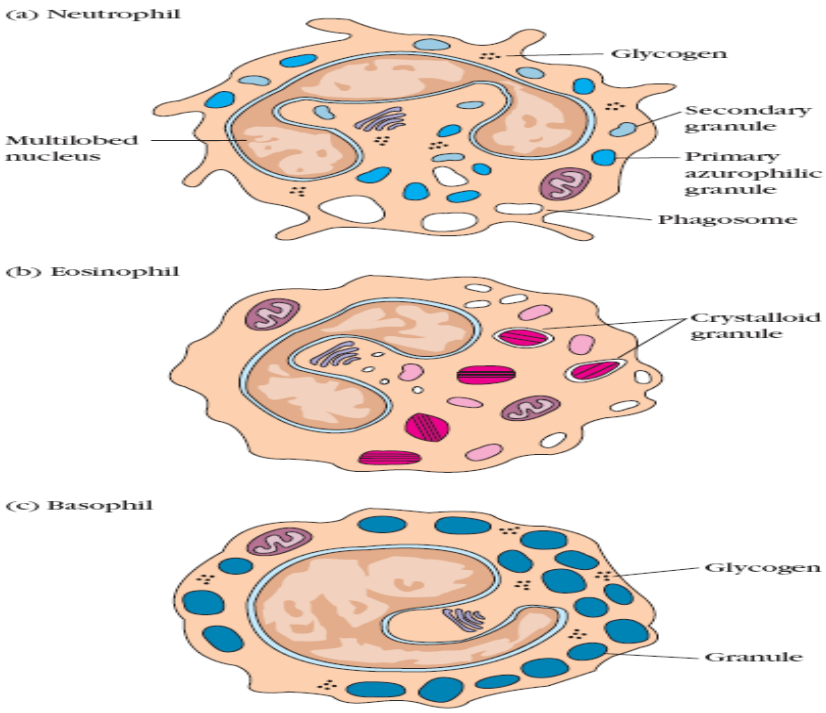

Figure 2.13: Drawings showing typical morphology of granulocytes. Note differences in the shape of the nucleus and in the number and shape of cytoplasmic granules. (Adapted from Immunology Janis Kubay).

2.5.8.1: NEUTROPHILS

Neutrophils are produced by hematopoiesis in the bone marrow. They are released into the peripheral blood and circulate for 7–10 h before migrating into the tissues, where they have a life span of only a few days. In response to many types of infections, the bone marrow releases more than the usual number of neutrophils and these cells generally are the first to arrive at a site of inflammation. The resulting transient increase in the number of circulating neutrophils, called **leukocytosis,** is used medically as an indication of infection. Movement of circulating neutrophils into tissues, called **extravasation,** takes several steps: the cell first adheres to the vascular endothelium, then penetrates the gap between adjacent endothelial cells lining the vessel wall, and finally penetrates the vascular basement membrane, moving out into the tissue spaces. A number of substances generated in an inflammatory reaction serve as **chemotactic factors** that promote accumulation of neutrophils at an inflammatory site.

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Among these chemotactic factors are some of the complement components, components of the blood-clotting system, and several cytokines secreted by activated TH cells and macrophages. Like macrophages, neutrophils are active phagocytic cells. Phagocytosis by neutrophils is similar to that described for macrophages, except that the lytic enzymes and bactericidal substances in neutrophils are contained within primary and secondary granules. The larger, denser primary granules are a type of lysosome containing peroxidase, lysozyme, and various hydrolytic enzymes. The smaller secondary granules contain collagenase, lactoferrin, and lysozyme. Both primary and secondary granules fuse with phagosomes, whose contents are then digested and eliminated much as they are in macrophages. Neutrophils also employ both oxygen-dependent and oxygen-independent pathways to generate antimicrobial substances. Neutrophils are in fact much more likely than macrophages to kill ingested microorganisms. Neutrophils exhibit a larger respiratory burst than macrophages and consequently are able to generate more reactive oxygen intermediates and reactive nitrogen intermediates. In addition, neutrophils express higher levels of defence than macrophages do.

2.5.8.2: EOSINOPHILS

Eosinophils, like neutrophils, are motile phagocytic cells that can migrate from the blood into the tissue spaces. Their phagocytic role is significantly less important than that of neutrophils, and it is thought that they play a role in the defense against parasitic organisms. The secreted contents of eosinophilic granules may damage the parasite membrane.

2.5.8.3: BASOPHILS

Basophils are nonphagocytic granulocytes that function by releasing pharmacologically active substances from their cytoplasmic granules. These substances play a major role in certain allergic responses.

2.5.8.4: MAST CELLS

Mast-cell precursors, which are formed in the bone marrow by hematopoiesis, are released into the blood as undifferentiated cells; they do not differentiate until they leave the blood and enter the tissues. Mast

cells can be found in a wide variety of tissues, including the skin, connective tissues of various organs, and mucosal epithelial tissue of the respiratory, genitourinary, and digestive tracts. Like circulating basophils, these cells have large numbers of cytoplasmic granules that contain histamine and other pharmacologically active substances. Mast cells, together with blood basophils, play an important role in the development of allergies.

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2.5.8.5: DENDRITIC CELLS

The **dendritic cell (DC)** acquired its name because it is covered with long membrane extensions that resemble the dendrites of nerve cells. Dendritic cells can be difficult to isolate because the conventional procedures for cell isolation tend to damage their long extensions. The development of isolation techniques that employ enzymes and gentler dispersion has facilitated isolation of these cells for study in vitro. There are many types of dendritic cells, although most mature dendritic cells have the same major function, the presentation of antigen to TH cells. Four types of dendritic cells are known: Langerhans cells, interstitial dendritic cells, myeloid cells, and lymphoid dendritic cells. Each arises from hematopoietic stem cells via different pathways and in different locations. Figure 2.14 shows that they descend through both the myeloid and lymphoid lineages. Despite their differences, they all constitutively express high levels of both class II MHC molecules and members of the co-stimulatory B7 family. For this reason, they are more potent antigen-presenting cells than macrophages and B cells, both of which need to be activated before they can function as antigen-presenting cells (APCs).

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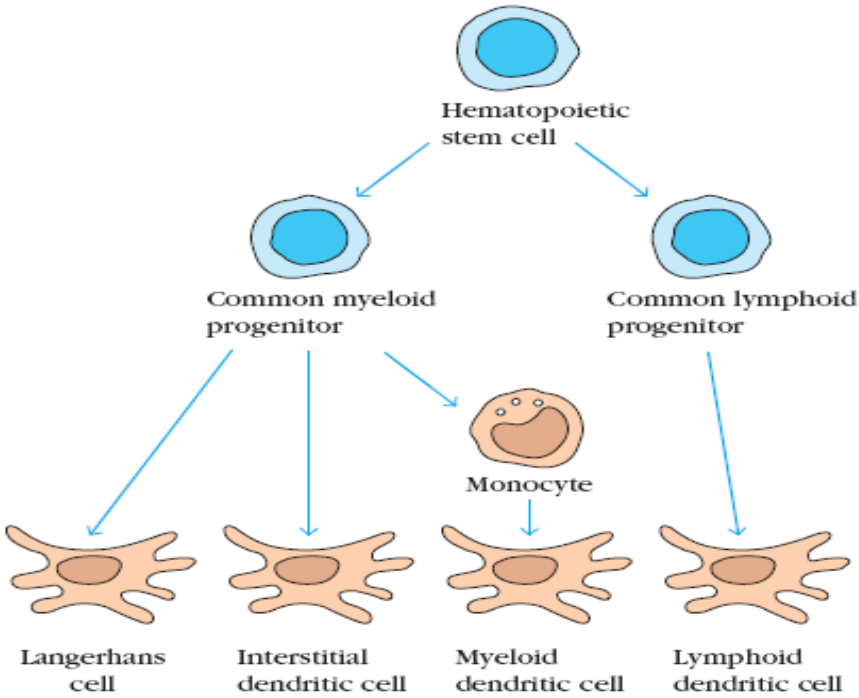

Figure 2.14: Dendritic cells arise from both the myeloid and lymphoid lineages. The myeloid pathway that gives rise to the monocyte/macrophage cell type also gives rise to dendritic cells. Some dendritic cells also arise from the lymphoid lineage. These considerations do not apply to follicular dendritic cells, which are not derived from bone marrow. (Adapted from Immunology Janis Kubay).

Immature or precursor forms of each of these types of dendritic cells acquire antigen by phagocytosis or endocytosis; the antigen is processed, and mature dendritic cells present it to TH cells. Following microbial invasion or during inflammation, mature and immature forms of Langerhans cells and interstitial dendritic cells migrate into draining lymph nodes, where they make the critical presentation of antigen to TH cells that is required for the initiation of responses by those key cells. Another type of dendritic cell, the **follicular dendritic cell**, does not arise in bone marrow and has a different function from the antigen-presenting dendritic cells described above. Follicular dendritic cells do not express class II MHC molecules and therefore do not function as antigen presenting cells for TH-cell activation. These dendritic cells were named for their exclusive location in organized structures of the lymph node

called lymph follicles, which are rich in B cells. Although they do not express class II molecules, follicular dendritic cells express high levels of membrane receptors for antibody, which allows the binding of antigen-antibody complexes. The interaction of B cells with this bound antigen can have important effects on B cell responses.

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2.6: CHECK YOUR PROGRESS

1. ……. is the body's defense against infectious organisms
2. Lymphoblast proliferate and eventually differentiate into……
3. The process by which particulate antigens are rendered more susceptible to phagocytosis is called ……
4. The ……. plays a major role in mounting immune responses to antigens in the blood stream
5. Neutrophils are produced by hematopoiesis in……

2.7: LET US SUM UP

In this unit, you have learnt about the meaning, definition, need, objectives, importance and functions of primary and secondary lymphoid organs Immunology. This Knowledge would make you understand structure and functions of Immune system. Humoral immunity involves combating pathogens via antibodies, which are produced by B cells and can be found in bodily fluids. Antibodies can be transferred between individuals to provide passive immune protection. Cell-mediated immunity involves primarily antigen specific T lymphocytes, which act to eradicate pathogens or otherwise aid other cells in inducing immunity. Clonal selection is the process by which individual T and B lymphocytes are engaged by antigen and cloned to create a population of antigen-reactive cells. Memory cells are residual B

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and T cells that remain after antigen exposure and that pick up where they left off during a subsequent or secondary response.

2.8: UNIT – END EXERCISES

1. Write short notes on the organs of the Immune System.
2. Write in detail about the Granulocytic Cells.
3. With a neat sketch write about the lymph nodes and its function.
4. Describe the role of thymus in immune response.
5. Write short notes on Phagocytosis?
6. Write in detail about the NK cells.

2.9: ANSWERS TO CHECK YOUR PROGRESS

1. The immune system
2. Effector cells or into memory cells.
3. Opsonization
4. Spleen
5. Bone marrow

2.10: SUGGESTED READINGS

1. Peter J. Delves, Seamus J. Martin, Dennis R. Burton, Ivan M. Roitt. (2016). **Roitt's Essential Immunology** (13th Edition), Wiley-Blackwell Publications.

2. Kenneth Murphy, Casey Weaver. (2016). **Janeway's Immunobiology** (9th Edition), Garland Sciences.

3. Abul K. Abbas & Andrew H. H. Lichtman & Shiv Pillai. (2015). **Basic Immunology, Functions and Disorders of the Immune System** (5th Edition), Elsevier.

4. Abul K. Abbas & Andrew H. Lichtman& Shiv Pillai. (2014). **Cellular and Molecular Immunology** (8th Edition), Elsevier.

5. Jeffrey K. Actor. (2011). **Review Immunology and Microbiology** (2nd Edition), Elsevier.

UNIT - III

NOTES

3.1: INTRODUCTION

The adoptive immune system is developed in a host primarily to protect the host from harmful effects of pathogens and other foreign substances. The adoptive response can be antibody- mediated (humoral), cell-mediated (cellular), or both. An encounter with a microbial or viral agent usually elicits a complex variety of responses. There are two main sites where pathogens may reside in an infected host—extracellularly in tissue spaces or intracellularly within a host cell; the immune system has different ways of dealing with pathogens at these sites. Humoral immunity acts mainly against extracellular pathogens, while cell-mediated immunity (CMI) acts against intracellular pathogens.

3.2: OBJECTIVES

After going through this unit, you will be able:

- To know the Cells of the Immune System
- To study the basic Structure of types of the Immunity
- System and the functions involved in the immune response.

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3.3: INNATE IMMUNITY AND ACQUIRED IMMUNITY

Host defenses are grouped under innate immunity, which provides immediate protection against microbial invasion, and adaptive immunity, which develops more slowly and provides more specialized defense against infections. Innate immunity, also called natural immunity or native immunity, is always present in healthy individuals (hence the term *innate*), prepared to block the entry of microbes and to rapidly eliminate microbes that do succeed in entering host tissues. Adaptive immunity, also called specific immunity or acquired immunity, requires expansion and differentiation of lymphocytes in response to microbes before it can provide effective defense; that is, it adapts to the presence of microbial invaders.

In innate immunity, the first line of defense is provided by epithelial barriers of the skin and mucosal tissues and by cells and natural antibiotics present in epithelia, all of which function to block the entry of microbes. If microbes do breach epithelia and enter the tissues or circulation, they are attacked by phagocytes, specialized lymphocytes called innate lymphoid cells, which include natural killer cells, and several plasma proteins, including the proteins of the complement system. All these mechanisms of innate immunity specifically recognize and react against microbes. In addition to providing early defense against infections, innate immune responses enhance adaptive immune responses against the infectious agents.

The adaptive immune system consists of lymphocytes and their products, such as antibodies**.** Adaptive immune responses are especially important for defense against infectious microbes that are pathogenic for humans (i.e., capable of causing disease) and may have evolved to resist innate immunity. Whereas the mechanisms of innate immunity recognize structures shared by classes of microbes, the cells of adaptive immunity (lymphocytes) express receptors that specifically recognize a much wider variety of molecules produced by microbes as well as noninfectious

substances. Any substance that is specifically recognized by lymphocytes or antibodies is called an **antigen.** Adaptive immune responses often use the cells and molecules of the innate immune system to eliminate microbes, and adaptive immunity functions to greatly enhance these antimicrobial mechanisms of innate immunity. For example, antibodies (a component of adaptive immunity) bind to microbes, and these coated microbes avidly bind to and activate phagocytes (a component of innate immunity), which ingest and destroy the microbes. Differences between innate and adaptive immunity are listed in the Table 3.1. By convention, the terms *immune response* and *immune system* generally refer to adaptive immunity, and that is the focus of most of this chapter.

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3.3.1: TYPES OF ADAPTIVE IMMUNITY

The two types of adaptive immunity, called humoral immunity and cell-mediated immunity, are mediated by different cells and molecules and provide defense against extracellular microbes and intracellular microbes, respectively (Table 3.2).

• **Humoral immunity** is mediated by proteins called **antibodies,** which are produced by cells called **B lymphocytes**. Secreted antibodies enter the circulation and mucosal fluids, and they neutralize and eliminate microbes and microbial toxins that are present outside host cells, in the blood, extracellular fluid derived from plasma, and in the lumens of mucosal organs such as the gastrointestinal and respiratory tracts. One of the most important functions of antibodies is to stop microbes that are present at mucosal surfaces and in the blood from gaining access to and colonizing host cells and connective tissues. In this way, antibodies prevent infections from ever being established. Antibodies cannot gain access to microbes that live and divide inside infected cells.

• Defense against such intracellular microbes is called **cell-mediated immunity** because it is mediated by cells, which are called **T lymphocytes**. Some T lymphocytes activate phagocytes to destroy microbes that have been ingested by the phagocytes into intracellular vesicles. Other T lymphocytes kill any type of host cells that are harboring infectious microbes in the cytoplasm. In both cases, the T cells recognize microbial

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antigens that are displayed on host a cell surface, which indicates there is a microbe inside the cell.

The specificities of B and T lymphocytes differ in important respects. Most T cells recognize only protein antigens, whereas B cells and antibodies are able to recognize many different types of molecules, including proteins, carbohydrates, nucleic acids, and lipids.

Immunity may be induced in an individual by infection or vaccination (active immunity) or conferred on an individual by transfer of antibodies or lymphocytes from an actively immunized individual (passive immunity).

• In **active immunity,** an individual exposed to the antigens of a microbe mounts an active response to eradicate the infection and develops resistance to later infection by that microbe. Such an individual is said to be immune to that microbe, in contrast with a naive individual, not previously exposed to that microbe's antigens.

• In **passive immunity,** a naive individual receives antibodies or cells (e.g., lymphocytes, feasible only in animal experiments) from another individual already immune to an infection. The recipient acquires the ability to combat the infection for as long as the transferred antibodies or cells last. Passive immunity is therefore useful for rapidly conferring immunity even before the individual is able to mount an active response, but it does not induce long-lived resistance to the infection. The only physiologic example of passive immunity is seen in newborns, whose immune systems are not mature enough to respond to many pathogens but who are protected against infections by acquiring antibodies from their mothers through the placenta and breast milk. Clinically, passive immunity is limited to treatment of some immunodeficiency diseases with antibodies pooled from multiple donors, and for emergency treatment of some viral infections and snakebites using serum from immunized donors.

3.3.2 PROPERTIES OF ADAPTIVE IMMUNE RESPONSES

Several properties of adaptive immune responses are crucial for the effectiveness of these responses in combating infections (Fig. 3.1).

3.3.2.1: Specificity and Diversity

The adaptive immune system is capable of distinguishing among millions of different antigens or portions of antigens. **Specificity** is the ability to distinguish between many different antigens. It implies that the total collection of lymphocyte specificities, sometimes called the **lymphocyte repertoire,** is extremely diverse**.** The basis for this remarkable specificity and diversity is that lymphocytes express clonally distributed receptors for antigens, meaning that the total population of lymphocytes consists of many different clones (each made up of one cell and its progeny), and each clone expresses an antigen receptor that is different from the receptors of all other clones. The **clonal selection hypothesis,** formulated in the 1950s, correctly predicted that clones of lymphocytes specific for different antigens develop before an encounter with these antigens, and each antigen elicits an immune response by selecting and activating the lymphocytes of a specific clone.

3.3.2.2: Memory

The adaptive immune system mounts larger and more effective responses to repeated exposures to the same antigen. This feature of adaptive immune responses implies that the immune system remembers exposure to antigen, and this property of adaptive immunity is therefore called **immunologic memory**. The response to the first exposure to antigen, called the **primary immune response,** is initiated by lymphocytes called naive lymphocytes that are seeing antigen for the first time. The term *naive* refers to these cells being immunologically inexperienced, not having previously responded to antigens. Subsequent encounters with the same antigen lead to responses called **secondary immune responses** that usually are more rapid, larger, and better able to eliminate the antigen than primary responses.

Secondary responses are the result of the activation of memory lymphocytes, which are long-lived cells that were induced during the primary immune response. The term *memory* arose because of the realization that these cells must remember previous encounter with antigen since they respond better upon subsequent encounters. Immunologic

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memory optimizes the ability of the immune system to combat persistent and recurrent infections, because each exposure to a microbe generates more memory cells and activates previously generated memory cells. Memory also is one of the reasons why vaccines confer long-lasting protection against infections.

3.3.3 Other Features of Adaptive Immunity

Adaptive immune responses have other characteristics that are important for their functions.

• When lymphocytes are activated by antigens, they undergo proliferation, generating many thousands of clonal progeny cells, all with the same antigen specificity. This process, called **clonal expansion,** rapidly increases the number of cells specific for the antigen encountered and ensures that adaptive immunity keeps pace with rapidly proliferating microbes.

• Immune responses are specialized, and different responses are designed to defend best against different classes of microbes.

• All immune responses are self-limited and decline as the infection is eliminated, allowing the system to return to a resting state, prepared to respond to another infection.

• The immune system is able to react against an enormous number and variety of microbes and other foreign antigens, but it normally does not react against the host's own potentially antigenic substances—so-called self antigens. This unresponsiveness to self is called **immunological tolerance**, referring to the ability of the immune system to coexist with (tolerate) potentially antigenic self molecules, cells, and tissues.

Table 3.1 - Difference between Innate and Acquired immunity

Feature	Innate immunity	Acquired immunity
Definition	The resistance to infection that an individual possesses by virtue of genetic and constitutional makeup	The resistance that an individual acquires during life
Types	Nonspecific and specific	Active and passive
Time taken to develop	Hours	Days
Specificity	For structures shared by groups of related microbes	For antigens of microbes and for nonmicrobial antigens
Memory	None; repeated exposure brings response like primary response	Yes; secondary response much faster than primary response
Components		
Physical and chemical barriers	Skin, mucosal epithelia, and antimicrobial chemicals	Lymphocytes in epithelia and antibodies secreted at epithelial surfaces
Blood and tissue antimicrobial substances	Complement; leukins from leukocytes, plakins from platelets, lactic acid found in muscle tissue, lactoperoxidase in milk, and interferons (antiviral)	Antibodies
Cells	Phagocytes (macrophages and neutrophils) and natural killer cells	Lymphocytes

3.4: HUMORAL IMMUNITY

Humoral immunity is based on the action of antibodies and complement. It is directed primarily against:

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■ Extracellular bacteria, in particular exotoxin-producing bacteria, such as *Corynebacterium diphtheriae*, *Clostridium tetani*, etc.

■ Bacteria whose virulence is due to polysaccharide capsules (e.g., *Haemophilus influenzae*, *Neisseria meningitidis*, *Streptococcus pneumoniae*, etc.), and

■ Certain viruses that cause infection through respiratory or intestinal tract. The humoral immunity also participates in the pathogenesis of hypersensitivity reactions and certain autoimmune diseases.

Production of antibodies is the main feature of humoral immune responses. The production of antibodies follows a characteristic pattern as follows:

1. Lag phase: This is the immediate phase following exposure to antigen. During this phase, no antibodies are detected in circulation.

2. Log phase: This is the next phase characterized by a steady rise in antibody titers in the circulation.

3. Stationary phase: This is a phase of equilibrium between antibody synthesis and catabolism.

4. Phase of decline: This phase is characterized by an increase in the catabolism of antibodies compared to the production of antibodies, leading to a fall in antibody titer in the circulation. Humoral immune response is of two types: primary and secondary.

3.4.1: Primary Response

During the primary response, when an individual encounters an antigen for the first time, antibody response to that antigen is detectable in the serum after a longer lag period than occurs in the secondary response. The serum antibody concentration continues to rise for several weeks and then declines; it may drop to very low levels. During this primary response, a small clone of B cells and plasma cells specific for the antigen are formed. The lag period is typically of 7–10 days duration but can be longer, even for weeks, depending on the nature of the antigen. For example, the lag phase may be as long as 2–3 weeks with some antigens, such as diphtheria toxoid, while it may be a short as a few hours with

pneumococcal polysaccharide. The lag period also depends on dose of the antigen and the route of administration whether oral or parenteral. IgM is the first antibody to be formed, followed by IgG, IgA, or both. IgM levels tend to decline sooner as compared to IgG levels (Fig. 3.1).

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3.4.2: Secondary Response

The antibody response is typically more rapid in the secondary response, due to second encounter with the same antigen, or a closely related "cross-reacting" antigen, months or years after the primary response. The lag period is typically very short (only 3–5 days). The level of antibody is also much higher than that during the primary response. These changes in secondary response are attributed to the persistence of antigen-specific "memory cells" following the first contact with the antigen. These memory cells proliferate in large numbers to produce large clones of specific B cells and plasma cells that mediate the secondary response. In the secondary response:

■ The amount of IgM produced is qualitatively similar to that produced after the first contact with the antigen; however, much more IgG is produced and the level of IgG tends to persist much longer than in the primary response.

■ Furthermore, such antibody tends to bind antigen more firmly (i.e., to have higher affinity) and thus to dissociate less easily. Improved antibody binding is due to mutations that occur in the DNA that encodes the antigen-binding site. This process is called ***somatic hypermutation***.

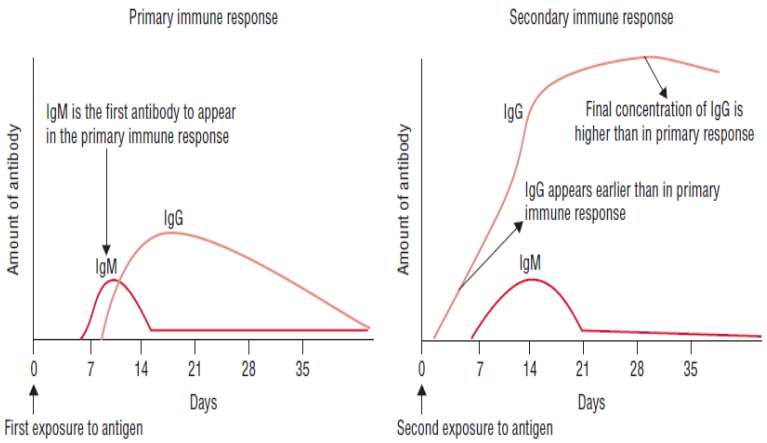

Fig 3.1: Primary and Secondary Immune Response produced by the antibody

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3.4.3: CELL-MEDIATED IMMUNITY (CMI)

Cell-mediated immunity (CMI) is a specific type of acquired immune response not mediated by antibodies but by sensitized T cells. This form of immunity is transferred from donor to recipient, not with antisera but with intact lymphocytes; hence it is called cell-mediated immune reaction. CMI performs the following immunological functions:

1. It confers immunity in diseases caused by obligate intracellular bacteria (*Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Brucella*, etc.), viruses (small pox, measles, mumps, etc.), fungi (*Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Coccidioides immitis*, etc.), and parasites (*Toxoplasma gondii*, *Leishmania donovani*, etc.).

2. It participates in immunological surveillance and immunity against cancer.

3. It plays an important role in pathogenesis of delayed hypersensitivity reactions and in pathogenesis of certain autoimmune diseases, such as autoimmune thyroiditis, encephalitis, etc.

3.4.3.1: Induction of CMI

Antigen processing and presentation are the means by which antigens become associated with self-MHC molecules for presentation to T cells with appropriate receptors. Proteins from exogenous antigens, such as bacteria, are internalized via endocytic vesicles into APCs, such as macrophages. Then, they are exposed to cellular proteases in intracellular vesicles. Peptides, approximately 10–30 amino acid residues in length, are generated in endosomal vesicles. The endosomal vesicles can then fuse with exocytic vesicles containing class II MHC molecules. Induction of CMI involves sequence of events, which is explained below.

A. **Presentation of foreign antigen by APCs to T lymphocytes**

Induction of CMI begins with presentation of foreign antigen by APCs to T lymphocytes. T-cell receptors (TCRs), which are antigen

recognition receptors, are present on T lymphocytes, and recognize foreign antigen and a self-MHC molecule on the surface of APCs. Subsequently, the sensitized T lymphocytes undergo blast transformation, clonal proliferation, and differentiation into memory cells and effector cells, such as Th, Tc, Td, and Ts. Finally, the lymphokines, which are biologically active products responsible for various manifestations of CMI, are released by the activated lymphocytes.

B. **Recognition of antigen by T cells**

T cells recognize antigens only when presented with MHC molecules. The combination of foreign antigen and class I MHC molecule is recognized by CD8_ cells. These CD8_ cells after recognition differentiate into Tc and Ts lymphocytes. On the other hand, CD4_ cells recognize the combination of antigen and class II MHC antigen, after which they are differentiated into Th and Td cells. The class II MHC molecules are synthesized, as for other membrane glycoprotein, in the rough endoplasmic reticulum and then proceed out through the Golgi apparatus. A third polypeptide, the invariant chain (Ii), protects the binding site of the class II dimer until the lowered pH of the compartment created after fusion with an endosomal vesicle causes a dissociation of the Ii chain. The MHC class II peptide antigen complex is then transported to the cell surface for display and recognition by a TCR of a CD4 T cell.

The lymphocyte recognizes antigen and class I MHC molecule and gets attached to the target cells. Endogenous antigens such as cytosolic viral proteins synthesized in an infected cell are processed for presentation by class I MHC molecule. In brief, cytosolic proteins are broken down by a peptidase complex known as the proteasome. The cytosolic peptides gain access to nascent MHC class I molecules in the rough endoplasmic reticulum via peptide transporter systems (transporters associated with antigen processing; TAPs). The TAP genes are also encoded in the MHC. The binding groove of the class I molecule is more constrained than that of the class II molecule; for that reason, shorter peptides are found in class I than in class II MHC molecules.

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Table 3.2: Difference between Humoral and cell mediated immunity

Cell-mediated immunity	Humoral immunity
Immune response mediated by cells	Immune response mediated by antibodies
Protects against fungi, viruses, and facultative intracellular bacterial pathogens	Protects against extracellular bacterial pathogens and viruses infecting respiratory or intestinal tract; and prevents recurrence of viral infections
Mediates delayed (type IV) hypersensitivity	Mediates immediate (types I, II, and III) hypersensitivity
Only T-cell-dependent antigens lead to cell-mediated immunity	B cells directly bind soluble antigens resulting in production of antibodies
Both CD4+ and CD8+ T cells are involved	Only T_H cells are involved
Provides immunological surveillance and immunity against cancer	No major role in immunological surveillance
Participates in rejection of homografts and graft-versus-host reaction	May be involved in early graft rejection due to preformed antibodies

C. **Release of cytokines by Tc lymphocytes**

This stimulates Tc lymphocytes to release cytokines, resulting in the lysis of the target cells. The T cells then detach from the target cells and attach with other target cells, and the same process is repeated. Interferon-gamma synthesized and secreted by Tc lymphocytes possibly also contributes for macrophage activation in some way.

3.5: ROLE OF TOLL-LIKE RECEPTORS (TLR) IN INNATE IMMUNITY

A major subset of the PRRs belong to the **Toll like receptor (TLR) family**, named on the basis of their similarity to the Toll receptor

in the fruit fly, Drosophila. The history of the discovery of the TLR family is interesting, as it perfectly illustrates the serendipitous nature of scientific discovery and illustrates how very important findings can originate in the most unlikely places. Lipopolysaccharide (LPS, also called endotoxin), a major component of the cell walls of Gram negative bacteria, was long known to provoke strong immune responses in animals and is a good example of a classical PAMP.

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Indeed, LPS is one of the major contributors to septic shock, the severe immune reaction that results when a bacterial infection reaches the bloodstream, and which is often fatal. For these reasons, immunologists tried to identify the LPS receptor in human and mouse for many years, largely without success. However, a major breakthrough came when the Toll receptor was found to be involved in sensing microbial infection in adult fruit flies. This in itself was quite a surprise because the Toll receptor had already been identified, many years before, as a major regulator of dorsal– ventral patterning (i.e., specifying which surface of the fly is the back and which the underside is) during early embryonic development of Drosophila.

A curious fact that emerged was that the intracellular domain of Drosophila Toll contained a motif, now known as the Toll/IL-1 receptor (TIR) signaling motif that was very similar to the cytoplasmic signaling domain identified in the IL-1 receptor, a molecule that was already well known to be involved in immune signaling in mammals. Putting two and two together, this led to the identification of the whole TLR family in mammals, as these receptors all possess a TIR domain within their cytoplasmic regions.

A series of TLRs have now been identified (there are 10 distinct TLRs in humans), all of which act as sensors for PAMPs (Figure 3.2). TLR ligands include peptidoglycan, lipoproteins, mycobacterial lipoarabinomannan, yeast zymosan, flagellin, microbial DNA, microbial RNAs, as well as other pathogen-derived ligands (Table 3.3). Although many TLRs are displayed on the cell surface, some, such as TLR3 and TLR7/8/9 that are responsive to intracellular viral RNA and unmethylated

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bacterial DNA, are located in endosomes and become engaged upon encounter with phagocytosed material (Figure 3.2). Engagement of TLRs with their respective ligands drives activation of nuclear factor kB (NFkB) and several members of the interferon-regulated factor (IRF) family of transcription factors, depending on the specific TLR. Combinatorial activation of TLRs is also possible, for example TLR2 is capable of responding to a wide diversity of PAMPs and typically functions within heterodimeric TLR2/TLR1 or TLR2/TLR6 complexes (Table 3.3).

Table 3.3: Ligands for Toll-like receptors (TLRs).

TLR	Ligand	Location
TLR1/ TLR2 heterodimer	Bacterial lipopeptides	Plasma membrane
TLR2/TLR6 heterodimer	Lipoteichoic acid (Gram-positive bacteria), zymosan (fungi)	Plasma membrane
TLR3	dsRNA	Endosomal
TLR4	LPS	Plasma membrane
TLR5	Flagellin (motile bacteria)	Plasma membrane
TLR7	Viral ssRNA	Endosomal
TLR8	Viral ssRNA	Endosomal
TLR9	Unmethylated CpG DNA (bacterial)	Endosomal
TLR10	Unknown	Plasma membrane
TLR11 (mouse only)	Profilin and profilin-like proteins	Plasma membrane

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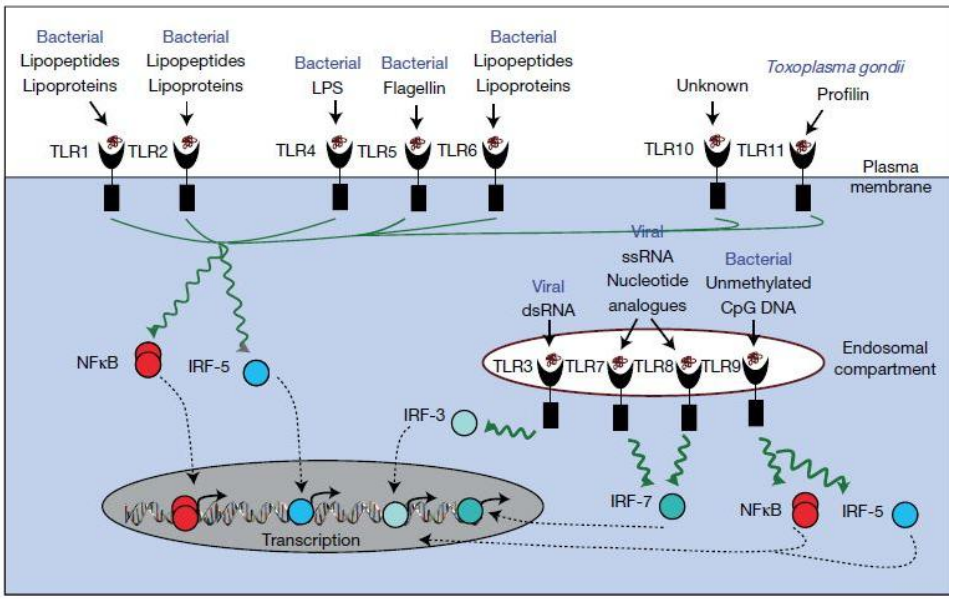

Fig 3.2: A family of Toll-like receptors (TLRs) act as sensors for pathogen associated molecular patterns (PAMPs). TLRs reside within plasma membrane or endosomal membrane compartments, as shown. Upon engagement of the TLR ectodomain with an appropriate PAMP (some examples are shown), signals are propagated into the cell that activate the nuclear factor κB (NFκB) and/or interferon-regulated factor (IRF) transcription factors, as shown. NFκB and IRF transcription factors then direct the expression of numerous antimicrobial gene products, such as cytokines and chemokines, as well as proteins that are involved in altering the activation state of the cell.

All TLRs have the same basic structural features, with multiple N-terminal leucine rich repeats (LRRs) arranged in a horseshoe- or crescent-shaped solenoid structure that acts as the PAMP-binding domain (Figure 3.2). Upon binding of a PAMP, TLRs transduce signals into the cell via their TIR domains, which recruit adaptor proteins within the cytoplasm (such as MyD88) that possess similar TIR motifs. These adaptors propagate the signal downstream, culminating in activation of NFκB and interferon regulatory family (IRF) transcription factors, which regulate the transcription of a whole battery of inflammatory cytokines and chemokines (Figure 3.2). As we will discuss later in this chapter, the IRF transcription factors control the expression of, among other things,

type I interferons. The latter cytokines are especially important in defense against viral infections as they can induce the expression of a series of proteins that can interfere with viral mRNA translation and viral replication, as well as induce the degradation of viral RNA genomes.

NOTES

3.6: CHECK YOUR PROGRESS

1. Any substance that is specifically recognized by lymphocytes or antibodies is called an ……
2. ……is based on the action of antibodies and complement
3. ……..is a specific type of acquired immune response not mediated by antibodies

3.7: LET US SUM UP

In this unit, you have learnt about the meaning, definition, need, objectives and importance of maturation and differentiation of T- cell and B- cell and its receptors or T-cell and B-cell. Pathogens fall into four major categories and come in many forms. The immune response quickly becomes tailored to the type of organism involved. The immune response relies on recognition molecules that can be germline encoded or randomly generated. The process of self-tolerance ensures that the immune system avoids destroying host tissue. The vertebrate immune response can be divided into two interconnected arms of immunity: innate and adaptive. Innate responses are the first line of defense, utilizing germline-encoded recognition molecules and phagocytic cells. Innate immunity is faster but less specific than adaptive responses, which take several days but are highly antigen specific. Innate and adaptive immunity operate cooperatively; activation of the innate immune response produces signals that stimulate and direct subsequent adaptive immune pathways. Adaptive immunity relies upon surface receptors, called B- and T-cell receptors, that are randomly generated by DNA rearrangements in developing B and T cells.

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3.8: UNIT - END EXERCISES

1. Write short notes on Immunity.
2. Write in detail about the Humoral response.
3. Explain about CMI.
4. Describe the types of adaptive immunity.
5. Write in detail about the TLR.

3.9: ANSWERS TO CHECK YOUR PROGRESS

1. Antigen
2. Humoral immunity
3. Cell-mediated immunity

3.10: SUGGESTED READINGS

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UNIT – IV

NOTES

4.1: INTRODUCTION

B cells are produced in the bone marrow, where the initial stages of maturation occur, and travel to the spleen for final steps of maturation into naïve mature B cells. B-cell receptors (BCRs) are membrane-bound monomeric forms of IgD and IgM that bind specific antigen epitopes with their Fab antigen-binding regions. Lymphoid progenitors which have developed from hematopoietic stem cells in the bone marrow migrate to the thymus to complete their antigen-independent maturation into functional T cells. In the thymus, T cells develop their specific T cell markers, including TCR, CD3, CD4 or CD8, and CD2.

4.2: OBJECTIVES

After going through this unit, you will be able:

- To know about the maturation and Differentiation of T-cell
- To know about the maturation and Differentiation of B-cell
- To study about T- cell and B – cell Receptor

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4.3: MATURATION AND DIFFERENTIATION OF T- CELL AND B – CELL

B cells, also known as B lymphocytes, develop from hematopoietic precursor cells which play a central role in the immune-pathogenesis of glomerulonephritis and transplant rejection. B cells can secrete antibodies through multiple mechanisms and their antibodies are the central elements of humoral immunity which against an almost unlimited variety of pathogens. In addition to that, B cells contribute to disease pathogenesis by providing costimulation and cytokines to T cells. B cells also play an immunomodulatory role in regulating the immune response by secreting cytokines that inhibit disease onset and progression. B cell–targeted approaches for treating immune diseases of the kidney and other organs have gained significant momentum. However, much remains to be understood about B cell biology in order to determine the timing, duration, and context of optimal therapeutic response to B cell–targeted approaches.

It takes about 1 or 2 weeks for original B cells to become mature B cells, during which they randomly rearrange their Ig genes to generate Ag-specific B-cell receptors (BCRs) capable of recognizing a wide variety of Ags. Each B cell produces a single species of antibody, each with a unique antigen-binding site. The sequence of expression of cell surface receptor and adhesion molecules which allows for differentiation of B cells, proliferation at various stages, and movement within the bone marrow microenvironment. Immature B cell leaves the bone marrow and undergoes further differentiation.

When a naive or memory B cell encounters antigen in an environment and the aid of a T cell, BCRs stimulation induces B cell differentiation into an antibody-secreting effector cell. Such cells make and secrete large amounts of soluble (rather than membrane-bound) antibody, which has the same unique antigen-binding site as the cell-surface antibody that served earlier as the antigen receptor. Effector B cells can begin secreting antibody while they are still small lymphocytes, but the end stage of their maturation pathway is a large plasma cell, which

continuously secretes antibodies at the astonishing rate of about 2000 molecules per second.

Plasma cells seem to have committed so much of their protein-synthesizing machinery to make an antibody that they are incapable of further growth and division. Although many die after several days, some survive in the bone marrow for months or years and continue to secrete antibodies into the blood. It is a general rule in adaptive immunity that naive antigen-specific lymphocytes are difficult to activate by antigen alone.

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Naive T cells require a co-stimulatory signal from professional antigen-presenting cells; naive B cells require accessory signals that can come either from an armed helper T cell or, in some cases, directly from microbial constituents. Antibody responses to protein antigens require antigen-specific T cell help. B cells can receive help from armed helper T cells when antigen bound by surface immunoglobulin is internalized and returned to the cell surface as peptides bound to MHC class II molecules. Armed helper T cells that recognize MHC complex then deliver activating signals to the B cell. Thus, protein antigens binding to B cells both provide a specific signal to the B cell by cross-linking its antigen receptors and allow the B cell to attract antigen-specific T cell help. These antigens are unable to induce antibody responses in animals or humans who lack T cells.

After several days, the primary focus of proliferation begins to involute. Many of the lymphocytes comprising the focus undergo apoptosis. However, some of the proliferating B cells differentiate into antibody-synthesizing plasma cells and migrate to the red pulp of the spleen or the medullary cords of the lymph node. The differentiation of a B cell into a plasma cell is accompanied by many morphological changes that reflect its commitment to the production of large amounts of secreted antibody. Plasma cells have abundant cytoplasm dominated by multiple layers of rough endoplasmic reticulum.

The purpose of the germinal center reaction is to enhance the later part of the primary immune response. Some germinal center cells

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differentiate first into plasmablasts and then into plasma cells. Plasmablasts continue to divide rapidly but have begun to specialize to secrete antibody at a high rate; they are destined to become nondividing, terminally differentiated plasma cells and thus represent an intermediate stage of differentiation. These plasma cells will migrate to the bone marrow, where a subset of them will live for a long period of time. Plasma cells obtain signals from bone marrow stromal cells that are essential for their survival. These plasma cells provide a source of long-lasting high-affinity antibody.

Other germinal center cells differentiate into memory B cells. Memory B cells are long-lived descendants of cells that were once stimulated by antigen and had proliferated in the germinal center. These cells divide very slowly if at all; they express surface immunoglobulin, but do not secrete antibody at a high rate. Since the precursors of memory B cells once participated in a germinal center reaction, memory B cells inherit the genetic changes that occurred in germinal center cells, including somatic mutations and the gene rearrangements that result in isotype switch. The signals that control which differentiation path a B cell takes, and even whether at any given point the B cell continues to divide instead of differentiating are unclear.

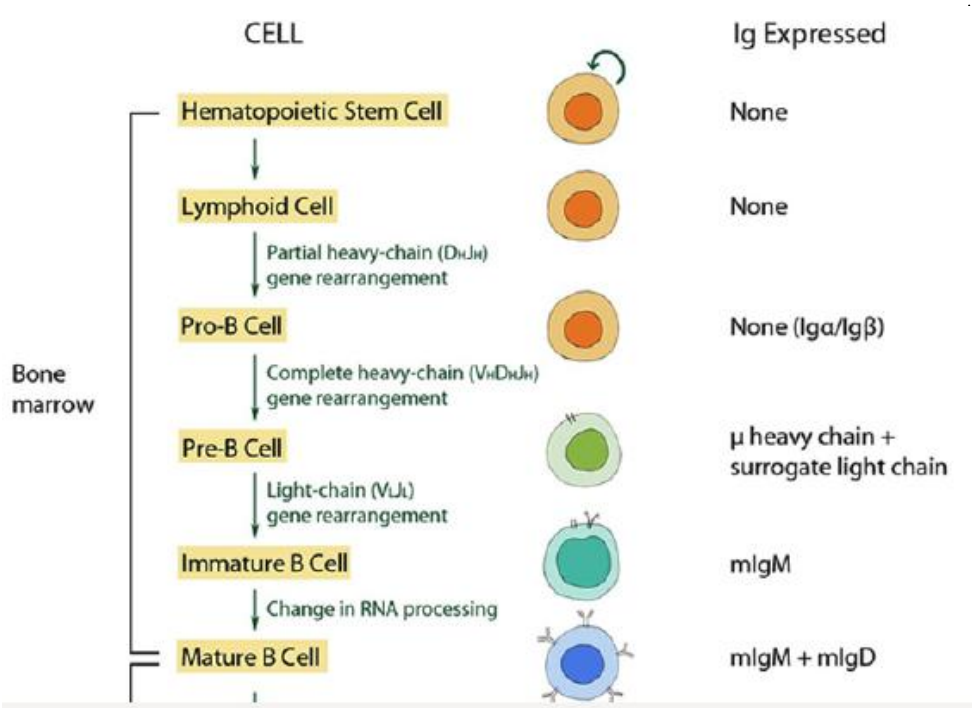

Fig.4.1: B cell Differentiation and Maturation

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4.3.1: T CELLS DIFFERENTIATION AND MATURATION

T cells are derived from hematopoietic stem cells that are found in the bone marrow. The progenitors of these cells migrate to and colonize 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-hematopoietic) 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), Figure 1 shows the ordered expression of these markers. Cells that lack expression of CD44, but express CD25 (DN3) undergo a process termed **beta-**

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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.

DP cells rearrange their TCR-α chain loci, to produce an αβ-TCR. These cells then undergo **positive selection**, in the cortex. DP cells interact with self-antigens in the context of major histocompatabilty complex (MHC) class I or class II molecules. Those cells that engage antigen/MHC with an appropriate affinity survive, whereas those cells that interact with a weaker affinity die by apoptosis. Thymocytes then migrate into the medulla to undergo **negative selection**. They are presented self-antigens on **antigen presenting cells** (**APCs**), such as dendritic cells and macrophages. Thymocytes that interact too strongly with antigen undergo apoptosis. The majority of developing thymocytes die during this process. Following selection, down-regulation of either co-receptor produces either naïve CD4 or CD8 **single positive** cells that exit the thymus and circulate the periphery

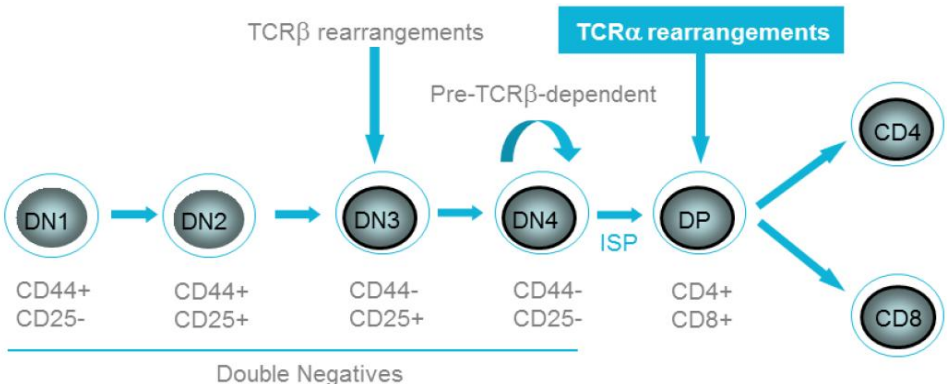

Fig. 4.2: αβ T cell development, showing the different cell surface markers expressed at the different stages of T cell development in the mouse.

4.4: T-CELL RECEPTOR

T- cell receptor (TCR) for antigen consists of two polypeptides: alpha and beta. These two peptides are associated with CD3 proteins. Each T cell has a unique TCR on its surface, thereby implying that hundreds of millions of different T cells occur in each person. Activated T cells as well as activated B cells produce large number of cells specific for those antigens. T-cell alpha and beta polypeptides show many similarities to immunoglobulin heavy chain in the following ways:

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■ The genes coding for T-cell polypeptides are formed by rearrangement of multiple regions of DNA.

■ There are V (variable), D (diversity), J (joining), and C (constant) segments that rearrange to provide diversity, thereby resulting in more than 107 different receptor proteins.

■ RAG-1 and RAG-2 are the two genes that encode the recombinase enzymes that catalyze these gene rearrangements and are similar in T cells and B cells. T cells, however, differ from immunoglobulins in the following

ways:

■ T cells have two chains rather than four chains in immunoglobulins.

■ T cells recognize antigen only in conjunction with MHC proteins, whereas immunoglobulins recognize free antigens.

4.4.1: ANTIGEN RECEPTORS OF LYMPHOCYTES

The antigen receptors of B and T lymphocytes have several features that are important for their functions in adaptive immunity (Fig. 4.3). Although these receptors have many similarities in terms of structure and mechanisms of signaling, there are fundamental differences related to the types of antigenic structures that B cells and T cells recognize.

• **Membrane-bound antibodies, which serve as the antigen receptors of B lymphocytes, can recognize many types of chemical structures, while most T cell antigen receptors recognize only peptides bound to major histocompatibility complex (MHC) molecules.** B lymphocyte antigen receptors and the antibodies that B cells secrete are able to recognize the shapes, or conformations, of

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macromolecules, including proteins, lipids, carbohydrates, and nucleic acids, as well as simpler, smaller chemical moieties. This broad specificity of B cells for structurally different types of molecules enables antibodies to recognize diverse microbes and toxins in their native form. In striking contrast, most T cells see only peptides displayed on antigen-presenting cells (APCs) bound to MHC molecules. This specificity of T cells restricts their recognition to only cell-associated microbes.

• **Antigen receptor molecules consist of regions (domains) involved in antigen recognition—therefore varying between clones of lymphocytes—and other regions required for structural integrity and effector functions—thus relatively conserved among all clones.** The antigen-recognizing domains of the receptors are called **variable (V) regions,** and the conserved portions are the **constant (C) regions.** Even within each V region, most of the sequence variability is concentrated within short stretches, which are called hypervariable regions, or complementarity-determining regions (CDRs), because they form the parts of the receptor that bind antigens (i.e., they are complementary to the shapes of antigens). By concentrating sequence variation in small regions of the receptor, it is possible to maximize the variability of the antigen-binding part, while retaining the basic structure of the receptors. As discussed later, special mechanisms exist in developing lymphocytes to create genes that encode different variable regions of antigen receptor proteins in individual clones.

• **Antigen receptor chains are associated with invariant membrane proteins whose function is to deliver intracellular signals following antigen recognition** (Fig. 4.3).

These signals, which are transmitted to the cytosol and the nucleus, may cause a lymphocyte to divide, to differentiate, or in certain circumstances to die. Thus, the two functions of lymphocyte receptors for antigen—specific antigen recognition and signal transduction—are mediated by different polypeptides. This again allows variability to be segregated in one set of molecules—the receptors themselves—while leaving the conserved function of signal transduction in other, invariant

proteins. The set of associated plasma membrane antigen receptor and signaling molecules in B lymphocytes is called the B cell receptor (BCR) complex, and in T lymphocytes it is called the T cell receptor (TCR) complex. When antigen molecules bind to antigen receptors of lymphocytes, the associated signaling proteins of the receptor complexes are brought into proximity. As a result, enzymes attached to the cytoplasmic portions of the signaling proteins catalyze the phosphorylation of other proteins. Phosphorylation triggers complex signaling cascades that culminate in the transcriptional activation of many genes and the production of numerous proteins that mediate the responses of the lymphocytes.

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• Antibodies exist in two forms—as membrane-bound antigen receptors on B cells or as secreted proteins—but TCRs exist only as membrane receptors on T cells. Secreted antibodies are present in the blood and mucosal secretions, where they function to defend against microbes (i.e., they are the effector molecules of humoral immunity). Antibodies are also called immunoglobulins (Igs), referring to immunity-conferring proteins with the characteristic slow electrophoretic mobility of globulins.

Secreted antibodies recognize microbial antigens and toxins by their variable domains, the same as the membrane-bound antigen receptors of B lymphocytes. The constant regions of some secreted antibodies have the ability to bind to other molecules that participate in the elimination of antigens: these molecules include receptors on phagocytes and proteins of the complement system.

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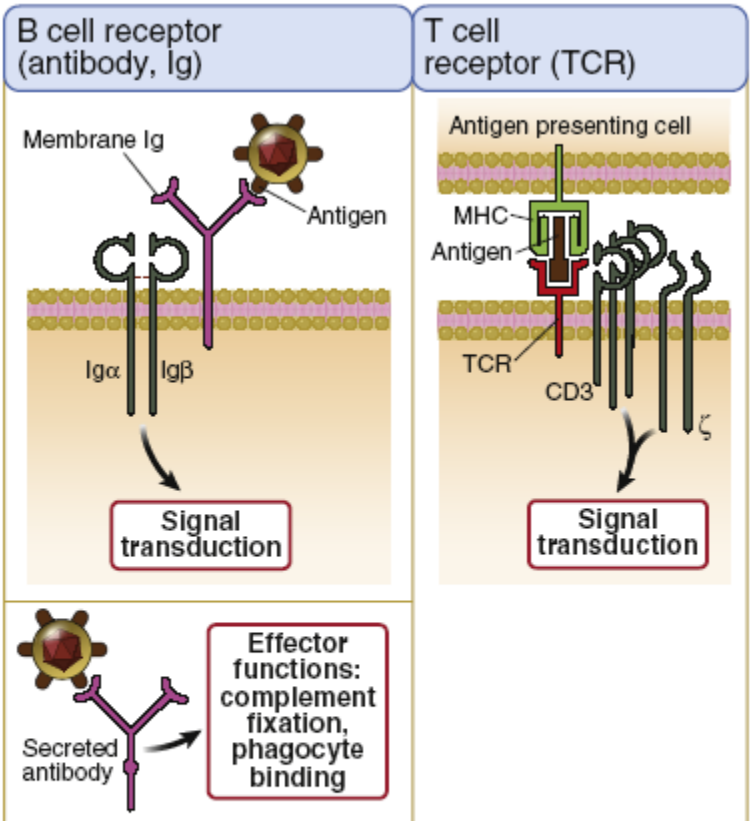

	B cell receptor (antibody, Ig)	T cell receptor (TCR)
Forms of antigens recognized	Macromolecules (proteins, polysaccharides, lipids, nucleic acids), small chemicals Conformational and linear epitopes	Mainly peptides displayed by MHC molecules on APCs Linear epitopes
Diversity	Each clone has a unique specificity; potential for $>10^9$ distinct specificities	Each clone has a unique specificity; potential for $>10^{11}$ distinct specificities
Antigen recognition is mediated by:	Variable (V) regions of heavy and light chains of membrane Ig	Variable (V) regions of α and β chains of the TCR
Signaling functions are mediated by:	Proteins (Igα and Igβ) associated with membrane Ig	Proteins (CD3 and ζ) associated with the TCR
Effector functions are mediated by:	Constant (C) regions of secreted Ig	TCR does not perform effector functions

Fig 4.3: Properties of antibodies and T cell antigen receptors (TCRs). Antibodies (also called immunoglobulins) may be expressed as membrane receptors or secreted proteins; TCRs only function as membrane receptors. When immunoglobulin (Ig) or TCR molecules recognize antigens, signals are delivered to the lymphocytes by proteins associated

with the antigen receptors. The antigen receptors and attached signaling proteins form the B cell receptor (BCR) and TCR complexes. Note that single antigen receptors are shown recognizing antigens, but signaling typically requires the binding of two or more receptors to adjacent antigen molecules. The important characteristics of these antigen-recognizing molecules are summarized. *APCs*, Antigen-presenting cells; *Ig*, immunoglobulin; *MHC*, major histocompatibility complex.

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Thus, antibodies serve different functions at different stages of humoral immune responses: membrane-bound antibodies on B cells recognize antigens to initiate the responses, and secreted antibodies neutralize and eliminate microbes and their toxins in the effector phase of humoral immunity. In cell-mediated immunity, the effector function of microbe elimination is performed by T lymphocytes themselves and by other leukocytes responding to the T cells. The antigen receptors of T cells are involved only in antigen recognition and T cell activation, and these proteins are not secreted and do not mediate effector functions.

4.5: CHECK YOUR PROGRESS

1. B cells also play an…… role in regulating the immune response by secreting cytokines
2. T cells are derived from haematopoietic stem cells that are found in…….
3. T- cell receptor (TCR) for antigen consists of …… polypeptides

4.6: LET US SUM UP

In this unit, you have learnt about the meaning, definition, need, objectives and importance of Maturation and Differentiation of T-cell and B-cell. T cell differentiation in the thymus has been subdivided into discrete stages of development based on thymocyte expression of the coreceptor molecules CD4 and CD8. B cell development and differentiation occurs in multiple phases. The initial, antigen-independent phase generates mature, immunocompetent B cells that can bind to a unique antigen. This stage of development occurs in the bone marrow and involves

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progenitor B cell proliferation and V-(D)-J gene rearrangement, which produces clonally-unique, immunoglobulin variable regions that specifically bind antigen. B cells complete further, antigen-independent maturation into immunocompetent naïve mature follicular B cells in the bone marrow and spleen.

4.7: UNIT - END EXERCISES

1. Write short notes on B cell.
2. Write in detail about the B cell differentiation and Maturation.
3. Explain about T cells Differentiation and maturation.

4.8: ANSWERS TO CHECK YOUR PROGRESS

1. Immunomodulatory
2. Bone marrow
3. Two

4.9: SUGGESTED READINGS

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BLOCK – 2 CYTOKINES, ANTIGEN ANTIBODY INTERACTIONS

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UNIT - V

5.1: INTRODUCTION

Molecules that communicate among cells of the immune system are referred to as cytokines. In general, cytokines are soluble molecules, although some also exist in membrane-bound forms. The interaction of a cytokine with its receptor on a target cell can cause changes in the expression of adhesion molecules and chemokine receptors on the target membrane, thus allowing it to move from one location to another. Cytokines can also signal an immune cell to increase or decrease the activity of particular enzymes or to change its transcriptional program, thereby altering and enhancing its effector functions. Finally, they can instruct a cell when to survive and when to die.

5.2: OBJECTIVES

After going through this unit, you will be able:

- To know the Cells of the cytokines
- To know the Cells of the chemokines

- To study the basic Structure and functions of the cytokines and chemokines

5.3: CYTOKINES

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The development of an effective response involves lymphoid cells, inflammatory cells, and hematopoietic cells. The complex interactions among these cells are mediated by a group of proteins collectively designated **cytokines** to denote their role in cell-to-cell communication. Cytokines are low-molecular weight regulatory proteins or glycoproteins secreted by white blood cells and various other cells in the body in response to a number of stimuli. These proteins assist in regulating the development of immune effector cells, and some cytokines possess direct effector functions of their own. This chapter focuses on the biological activity of cytokines, the structure of cytokines and their receptors, signal transduction by cytokine receptors, the role of cytokine abnormalities in the pathogenesis of certain diseases, and therapeutic uses of cytokines or their receptors.

5.3.1: PROPERTIES OF CYTOKINES

Cytokines bind to specific receptors on the membrane of target cells, triggering signal-transduction pathways that ultimately alter gene expression in the target cells. The susceptibility of the target cell to a particular cytokine is determined by the presence of specific membrane receptors. In general, the cytokines and their receptors exhibit very high affinity for each other, with dissociation constants ranging from 10–10 to 10–12 M. Because their affinities are so high, cytokines can mediate biological effects at picomolar concentrations. A particular cytokine may bind to receptors on the membrane of the same cell that secreted it, exerting **autocrine** action; it may bind to receptors on a target cell in close proximity to the producer cell, exerting **paracrine** action; in a few cases, it may bind to target cells in distant parts of the body, exerting **endocrine** action. Cytokines regulate the intensity and duration of the immune response by stimulating or inhibiting the activation, proliferation, and/ or differentiation of various cells and by regulating the secretion of antibodies or other cytokines. As described later, binding of a given cytokine to responsive target cells generally stimulates increased expression of cytokine receptors and secretion of other cytokines, which

affect other target cells in turn. Thus, the cytokines secreted by even a small number of lymphocytes activated by antigen can influence the activity of numerous cells involved in the immune response. For example, cytokines produced by activated TH cells can influence the activity of B cells, TC cells, natural killer cells, macrophages, granulocytes, and hematopoietic stem cells, thereby activating an entire network of interacting cells.

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Cytokines exhibit the attributes of pleiotropy, redundancy, synergy, antagonism, and cascade induction, which permit them to regulate cellular activity in a coordinated, interactive way. A given cytokine that has different biological effects on different target cells has a pleiotropic action. Two or more cytokines that mediate similar functions are said to be redundant; redundancy makes it difficult to ascribe a particular activity to a single cytokine. Cytokine synergism occurs when the combined effect of two cytokines on cellular activity is greater than the additive effects of the individual cytokines. In some cases, cytokines exhibit antagonism; that is, the effects of one cytokine inhibit or offset the effects of another cytokine. Cascade induction occurs when the action of one cytokine on a target cell induces that cell to produce one or more other cytokines, which in turn may induce other target cells to produce other cytokines.

The term *cytokine* encompasses those cytokines secreted by lymphocytes, substances formerly known as **lymphokines,** and those secreted by monocytes and macrophages, substances formerly known as **monokines.** Although these other two terms continue to be used, they are misleading because secretion of many lymphokines and monokines is not limited to lymphocytes and monocytes as these terms imply, but extends to a broad spectrum of cells and types. For this reason, the more inclusive term *cytokine* is preferred. Many cytokines are referred to as **interleukins,** a name indicating that they are secreted by some leukocytes and act upon other leukocytes. Interleukins 1–25 have been identified. There is reason to suppose that still other cytokines will be discovered and that the interleukin group will expand further. Some cytokines are known by common names, including the interferons and tumor necrosis factors. Recently gaining

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prominence is yet another subgroup of cytokines, the **chemokines,** a group of low-molecular weight cytokines that affect chemotaxis and other aspects of leukocyte behavior. Because cytokines share many properties with hormones and growth factors, the distinction between these three classes of mediators is often blurred. All three are secreted soluble factors that elicit their biological effects at picomolar concentrations by binding to receptors on target cells. Growth factors tend to be produced constitutively, whereas cytokines and hormones are secreted in response to discrete stimuli, and secretion is short-lived, generally ranging from a few hours to a few days. Unlike hormones, which generally act long range in an endocrine fashion, most cytokines act over a short distance in an autocrine or paracrine fashion. In addition, most hormones are produced by specialized glands and tend to have a unique action on one or a few types of target cell. In contrast, cytokines are often produced by, and bind to, a variety of cells.

The activity of cytokines was first recognized in the mid- 1960s, when supernatants derived from in vitro cultures of lymphocytes were found to contain factors that could regulate proliferation, differentiation, and maturation of allogeneic immune-system cells. Soon after, it was discovered that production of these factors by cultured lymphocytes as induced by activation with antigen or with nonspecific mitogens. Biochemical isolation and purification of cytokines was hampered because of their low concentration in culture supernatants immune-system cells. Soon after, it was discovered that production of these factors by cultured lymphocytes was induced by activation with antigen or with nonspecific mitogens. Biochemical isolation and purification of cytokines was hampered because of their low concentration in culture supernatants and the absence of well-defined assay systems for individual cytokines. A great advance was made with the development of gene-cloning techniques during the 1970s and 1980s, which made it possible to produce pure cytokines by expressing the protein from cloned genes. The discovery of cell lines whose growth depended on the presence of a particular cytokine provided researchers with the first simple assay systems. The derivation of monoclonal antibodies

specific for each of the more important cytokines has made it possible to develop rapid quantitative immunoassays for each of them.

5.4: CHEMOKINES

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A large family of structurally homologous low- molecular-weight cytokines that stimulate leukocyte chemotaxis, regulate the migration of leukocytes from the blood to tissues by activating leukocyte integrins, and maintain the spatial organization of different subsets of lymphocytes and antigen-presenting cells within lymphoid organs.

This designation is given to a group of cytokines with chemotactic properties. They are divided into two major groups, α and β, depending on their tertiary structure. In the α chemokines one amino acid separates the first two cysteine residues (Cys-X amino acidCys) and for that reason are also known as CXC chemokines. In β chemokines the first two cysteine residues are adjacent to each other and for that reason are also known as CC chemokines. Interleukin-8 (Neutrophil-Activating Factor) is the most important of the α chemokines. It is released by T lymphocytes and monocytes stimulated with TNF or IL-1. It functions as a chemotactic and activating factor for granulocytes, the cell population with the highest level of IL-8 receptor expression. IL-8 recruits granulocytes to areas of inflammation and increasing their phagocytic and pro-inflammatory abilities. It has also been demonstrated to be chemotactic for T lymphocytes. β Chemokines include four major cytokines, which act predominantly on mononuclear cells, the cells that predominantly express the receptors for this group of cytokines:

1. RANTES (regulated on activation, normal T-cell expressed and secreted), released by T cells, attracts T cells with memory phenotype, NK cells, eosinophils, and mast cells.

2. Macrophage inflammatory proteins (MIP), released by monocytes and macrophages, attract eosinophils, lymphocytes, and NK and LAK cells.

3. Macrophage chemotactic proteins (MCP), produced by monocytes, macrophages, and related cells, attract monocytes, eosinophils, and NK and LAK cells.

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4. Eotaxin, a chemokine induced by IL-4 that recruits eosinophils and TH2 CD41 T cells to the sites of allergic inflammation. Other cytokines and peptides with chemotactic activity, but structurally different from classical chemokines, have also been characterized.

Migration-inhibition factor (MIF), the structure of which is not fully known, is released by T cells, monocytes, and macrophages and keeps macrophages in the area where the reaction is taking place and contributes to their activation, promoting the release of TNF. Recently it has been found that endotoxin stimulates the release of MIF by the pituitary gland, and its release seems associated with increased mortality in the post-acute phase of septic shock. β -Defensins, released primarily by granulocytes, have also been characterized as chemotactic for T lymphocytes.

5.5: CHECK YOUR PROGRESS

1. Cytokines are low-molecular weight regulatory proteins or glycoproteins secreted by ……
2. ---------- is a group of low-molecular weight cytokines that affect chemotaxis
3. The activity of cytokines was first recognized in the mid……

5.6: LET US SUM UP

In this unit, you have learnt about the meaning, definition, need, objectives, importance and functions of cytokines and chemokines. Cytokines are proteins that mediate the effector functions of the immune system. Most cytokines are soluble proteins, but some for eg, members of the TNF family, may be expressed in a membrane-bound form. Chemokines act on GPCR-coupled receptors to promote chemoattraction, the movement of immune system cells into, within, and out of lymphoid organs.

5.7: UNIT - END EXERCISES

1. Write short notes on Cytokines.
2. Describe about the functions of Cytokines.
3. Explain about Chemokines

5.8: ANSWERS TO CHECK YOUR PROGRESS

1. White blood cells
2. Chemokines
3. 1960

NOTES

5.9: SUGGESTED READINGS

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NOTES

UNIT - VI

6.1: INTRODUCTION

Immunoglobulins, also known as antibodies, are glycoprotein molecules produced by plasma cells (white blood cells). They act as a critical part of the immune response by specifically recognizing and binding to particular antigens, such as bacteria or viruses, and aiding in their destruction. The antibody immune response is highly complex and exceedingly specific. The various immunoglobulin classes and subclasses (isotypes) differ in their biological features, structure, target specificity and distribution. Hence, the assessment of the immunoglobulin isotype can provide useful insight into complex humoral immune response. Assessment and knowledge of immunoglobulin structure and classes is also important for selection and preparation of antibodies as tools for immunoassays and other detection applications.

6.2: OBJECTIVES

After going through this unit, you will be able:

- To know the Cells of the Immunoglobulins
- To study the basic Structure and functions of the Immunoglobulins
- To know the generation of antibody diversity

6.3: IMMUNOGLOBULINS

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Immunoglobulins are proteins of animal origin, endowed with known antibody activity and for certain other proteins related to them by chemical structure. That means the Ig include, besides antibody globulin, the abnormal proteins found in myeloma, macroglobulinemia, cryoglobulinemia, etc. While Ig satisfies the structural and chemical concept, the antibody provides biological and functional concept. All antibodies are Ig, but all Ig are not antibodies. Immunoglobulins constitute 20% to 25% of the serum protein. Based on the physicochemical, antigenic differences and the types of heavy chain Igs are classified into five types. All Igs are made up of light (molecular weight 25,000) and heavy polypeptide chains (molecular weight 50,000). Light (L) chains are of one of the two, kappa (K) or lambda (l). Both types can occur in all classes of Ig (IgG, IgM, IgA, IgE and IgD), but any one Ig contains only one type of L chain. Both the L chain of one Ig molecule cannot have both kappa and lambda chain.

The amino-terminal portion of each L chain contains a part of antigen-binding site. Heavy (H) chains are distinct for each of the five Ig classes and are designated γ (gamma), μ (mu), α (alpha), δ (delta) and e (epsilon). The amino-terminal portion of each H chain participates in the antigen-binding site. The carboxy-terminal portion forms the fraction crystallizable (Fc) fragment, which has various biologic activities (complement activation, macrophage fixation, reactivity with rheumatoid factor and binding to cell-surface receptors). An individual antibody molecule consists of two H chains and L chains, covalently linked by disulfide bonds. Both the H chains and L chains are identical.

Proteolytic cleavage of IgG by Porter, Edelman and their colleagues led to a better understanding of the detailed structure of the Ig molecule. Pepsin treatment produces a dimeric F(ab)2 fragment. Papain treatment produces monovalent antigen-binding fragment (Fab) and Fc fragments. The F(ab)2 and Fab fragments bind antigen, but lack a functional Fc region (Fig. 6.1). Light and heavy chains are subdivided into variable regions and constant regions. A L chain consists of one variable domain (VL) and one

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constant domain (CL). Most H chains consist of one variable domain (VH) and three or more constant domains (CH). Each domain is approximately 110 amino acids long. Variable regions are for antigen-binding and the constant regions are responsible for other biologic functions. In the variable regions of both L and H chains, there are three extremely variable (hypervariable) amino acid sequences that form the antigen-binding site. The hypervariable region (HVR) form the complementary region of the antigenic determinant and therefore, known as complementary determining regions (CDRs) (Fig. 6.1).

6.3.1: CLASSES OF IMMUNOGLOBULIN

There are five classes of immunoglobulins, according to their properties (Table 6.1). They are Immunoglobulin G (IgG), Immunoglobulin A (IgA), Immunoglobulin M (IgM), Immunoglobulin D (IgD) and Immunoglobulin E (IgE).

6.3.1.1: Immunoglobulin *G*

Immunoglobulin G is the main class of immunoglobulin in serum. It exists as a molecule of molecular weight 146 to 160 kDa (7S) in serum and is abundant component of the secondary humoral response.

This class of Ig is not only found in the bloodstream, but also in extravascular spaces. It contains less carbohydrate than other Igs. It has a half-life of approximately 23 days. It is also transported across the placenta and is therefore, responsible for passive immunity in the fetus and neonate. Passively administered IgG, suppresses the homologous antibody synthesis by a feed back mechanism. This process is utilized in the immunization of women by the administration of anti-RhD IgG during delivery.

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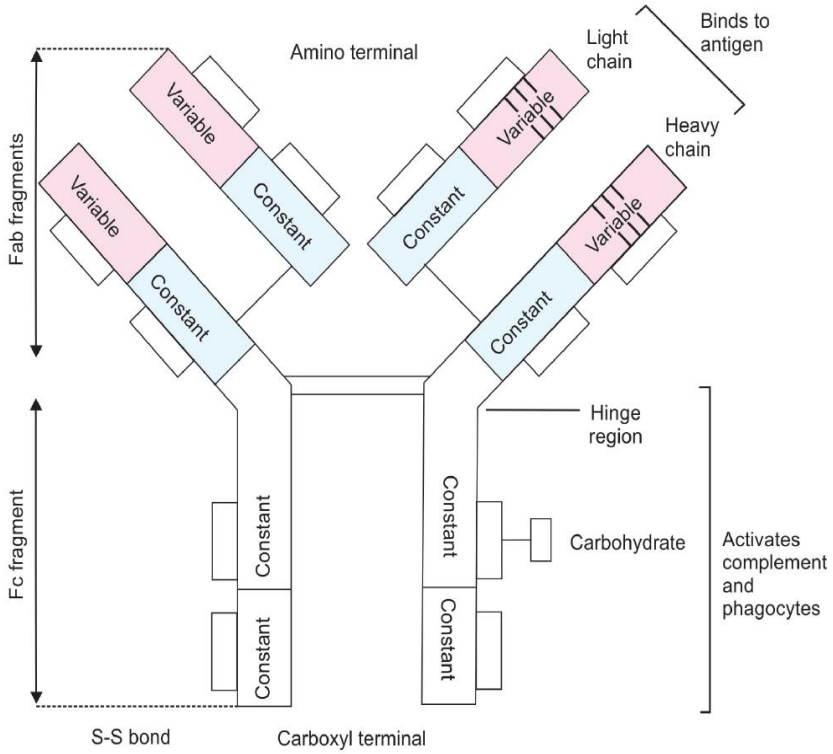

Fig. 6.1: Schematic representation of an IgG molecule, indicating the location of the constant and the variable regions on the light and heavy chains

There are four subclasses of IgG isotypes in man (IgG1, IgG2, IgG3 and IgG4), each one is distinguished by a minor variation in the amino acid sequences in the C-region and by the numbers and location of disulfide bridges. The four subclasses are distributed in human serum, IgG1 (65%), IgG2 (23%), IgG3 (8%) and IgG4 (4%). Immunoglobulin G participates in most immunological reactions such as complement fixation (IgG1 and IgG3), precipitation, neutralization of toxins and viruses. IgG1and IgG3 are capable of interacting with the Fc receptors on macrophages and therefore, acting as efficient opsonins.

6.3.1.2: Immunoglobulin A

Immunoglobulin A is the second most abundant class of immunoglobulin constitute about 10% to 13% of all serum immunoglobulins. The normal serum level is 0.6 to 4.2 mg per mL. It has a half-life of 6 to 8 days. IgA is found in two forms in the body—in serum, where it occurs principally as monomer (160 kDa, 7S) and on secretory

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surfaces, where it exists as a dimeric molecule (385 kDa, 11S). The dimeric form is known as secretory IgA (sIgA) and is found in association of J chain and with secretory component; the latter is involved in the transport of IgA to the secretory surfaces. Secretory component is non-covalently associated with the IgA molecules in the sIgA complex. sIgA is the main Ig in the secretions such as milk, saliva and tears and in the secretions of respiratory, intestinal and genital tracts. It protects mucous membranes from attack by bacteria and viruses.

There are two subclasses of IgA; IgA1 and IgA2 distinguished by their distribution and arrangement of disulfide bonds. IgA is the predominant form found in serum, where as IgA1 and IgA2 isotypes are present in roughly equal amounts in IgA. Immunoglobulin A is the component of the secondary humoral response. The principal antigens that elicit an IgA response are microorganisms in the gut or on the airways. IgA cannot cross the placental barrier, but however, sIgA can be passed to the neonate through milk. IgA does not fix complement, but can activate the alternative complement pathway. It promotes phagocytosis and intracellular killing of microorganisms.

6.3.1.3: Immunoglobulin M

Immunoglobulin M constitutes 5% to 8% of serum Ig with a normal level of 0.5 to 2 mg per mL. It has a half-life of about 5 days. It is a heavy molecule (19S; molecular weight 900,000 to 1,000,000, hence called the millionaire molecule). It has a pentameric structure comprising five identical four chain units (Fig. 5.6), i.e. it has 10 identical binding sites.The ‘mu’ heavy chains has five domains, VH plus 4 C regions (Cμ1, Cμ2, Cμ3, Cμ4) and lacks a hinge region. The pentameric structure is stabilized by disulfide bonding between adjacent Cμ3 domains and by the presence of Joinez (J) chain. Though theoretically 10 antigen-binding sites are there, only five antigen-binding sites react with antigen probably due to steric hindrance.

Immunoglobulin M is the principal component of primary immune response. Because of its large size (970 kDa, 19S), it is located mainly in the bloodstream. As it is not transported across the placenta, the presence of IgM in the fetus indicates intrauterine infection and its detection is useful to the

diagnosis of congenital infections such as syphilis, rubella, human immunodeficiency virus (HIV) infection and toxoplasmosis. IgM antibodies are relatively short lived, disappears earlier than IgG. Hence, their demonstration in serum indicates recent infection. Treatment of serum with 0.12 M 2-mercaptoethanol selectively destroys IgM without affecting IgG antibodies.

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The isohemagglutinins (anti-A, anti-B) and many other natural antibodies to microorganisms are IgM. Antibodies to typhoid O antigen (endotoxin) and Wassermann reaction (WR) antibodies in syphilis are also of this class. It is efficient in both opsonization and complement fixation.

6.3.1.4: Immunoglobulin D

Immunoglobulin D structurally resembles IgG. The concentration is about 0.03 mg per mL of serum. It has a half-life of about 3 days. IgD acts as an antigen receptor, when present on the surface of certain B lymphocyte. Two subclasses of IgD are IgD1 and IgD2.

6.3.1.5: Immunoglobulin E

Immunoglobulin E is 8S molecule (molecular weight is about 190,000) with a half-life of 2 days. Normal serum contains only traces. It exhibits unique properties such as heat lability and affinity towards surface of mast cells. The Fc region of IgE binds to the receptor for the antigen on the surface of mast cell and basophil. The resulting antigen-antibody complex triggers immediate (type 1) hypersensitivity reaction by releasing the mediators. Serum IgE increased in anaphylactic reaction and helminthic infection. IgE is also known as reagin.

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Table 6.1 Comparative features of immunoglobulin isotypes

Property	IgG	IgA	IgM	IgD	IgE
Light chain	Kappa or lambda	Kappa or lambda	Kappa or lambda	Kappa or lambda	Kappa or lambda
Heavy chain	Gamma (γ)	Alpha (α)	Mu (μ)	Delta (δ)	Epsilon (ε)
Serum concentration (mg/mL)	12	2	1.2	0.03	0.0003
Percentage	75%	10% –15%	5% –10%	–	–
Sedimentation coefficient	7S	7S, 11S	19S	7S	8S
Molecular weight	150,000	160,000	900,000–1,000,000	180,000	190,000
Half-life (day)	23	6	5	3	2
Placental transfer	+ (IgG1, IgG3, IgG4)	–	–	–	–
Presence in milk	+	+	–	–	–
Carbohydrate percentage (%)	3	8	12	13	12
Heat stability (56°C)	+	–	+	+	–
Location (mostly)	Serum, extravascular	Transport across epithelium	Serum (intravascular)	B cell membrane	Serum, extravascular
Principal biological activities					
Complement fixation	+	+ (alternate pathway)	+++	–	–
Neutralization	+	–	–	–	–
Opsonization	+	–	+++	–	–
Phagocytosis	+	+	–/+	–	–
Binds to mast cells and basophils	–	–	–	–	+
Local immunity	–	+ (secretory IgA)	–	–	–
Examples	Antiviral, antitoxic, opsonin, protects fetus and newborn	Immunity against enteric, polio and influenza virus	ABO red cell antibodies, rheumatoid factor, antibodies against OAg (Enterobacteriaceae)	–	Mediator of immediate-hypersensitivity reaction

6.4: GENERATION OF ANTIBODY DIVERSITY

Antibodies are the antigen binding proteins present on the B-cell membrane and secreted by plasma cells. Membrane-bound antibody confers antigenic specificity on B cells; antigen-specific proliferation of B-cell clones is elicted by the interaction of membrane antibody with antigen. Secreted antibodies circulate in the blood, where they serve as the effectors of humoral immunity by searching out and neutralizing antigens or marking them for elimination. All antibodies share structural features, bind to antigen, and participate in a limited number of effector functions. The antibodies

produced in response to a particular antigen are heterogeneous. Most antigens are complex and contain many different antigenic determinants, and the immune system usually responds by producing antibodies to several epitopes on the antigen. This response requires the recruitment of several clones of B cells. Their outputs are monoclonal antibodies, each of which specifically binds a single antigenic determinant. Together, these monoclonal antibodies make up the polyclonal and heterogeneous serum antibody response to an immunizing antigen.

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6.4.1: BASIC STRUCTURE OF ANTIBODIES

Blood can be separated in a centrifuge into a fluid and a cellular fraction. The fluid fraction is the plasma and the cellular fraction contains red blood cells, leukocytes, and platelets. Plasma contains all of the soluble small molecules and macromolecules of blood, including fibrin and other proteins required for the formation of blood clots. If the blood or plasma is allowed to clot, the fluid phase that remains is called serum. It has been known since the turn of the century that antibodies reside in the serum. The first evidence that antibodies were contained in particular serum protein fractions came from a classic experiment by A. Tiselius and E. A. Kabat, in 1939. They immunized rabbits with the protein ovalbumin (the albumin of egg whites) and then divided the immunized rabbits' serum into two aliquots. Electrophoresis of one serum aliquot revealed four peaks corresponding to albumin and the alpha, beta, and gamma globulins. The other serum aliquot was reacted with ovalbumin, and the precipitate that formed was removed; the remaining serum proteins, which did not react with the antigen, were then electrophoresed.

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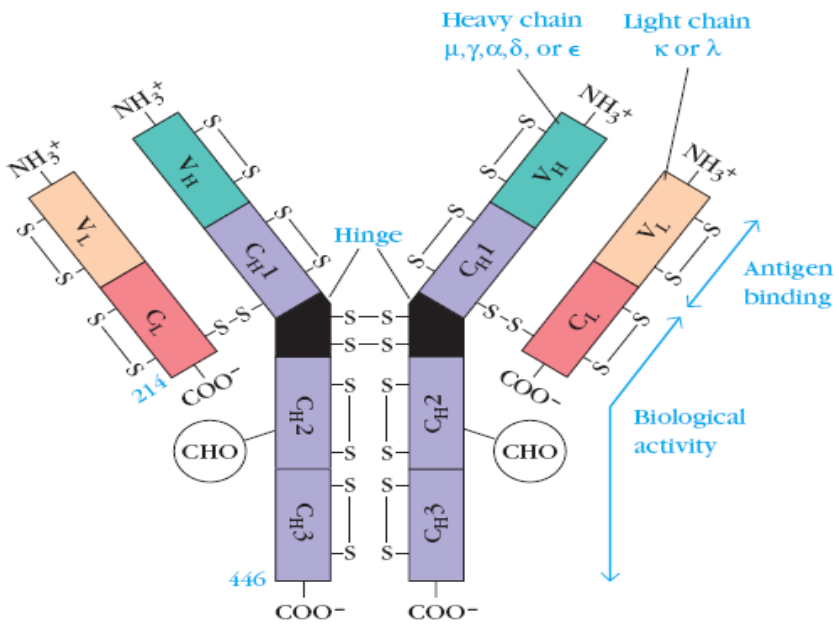

Fig 6.2: Schematic diagram of structure of immunoglobulins derived from amino acid sequencing studies. Each heavy and light chain in an immunoglobulin molecule contains an amino-terminal variable (V) region (aqua and tan, respectively) that consists of 100–110 amino acids and differs from one antibody to the next. The remainder of each chains in the molecule the constant (C) regions (purple and red)—exhibits limited variation that defines the two light-chain subtypes and the five heavy-chain subclasses. Some heavy chains also contain a proline-rich hinge region (black). The amino-terminal portions, corresponding to the V regions, bind to antigen; effector functions are mediated by the other domains. The light and heavy chains, which lack a hinge region, contain an additional domain in the middle of the molecule. (Adapted from Immunology Janis Kubay)

A comparison of the electrophoretic profiles of these two serum aliquots revealed that there was a significant drop in the globulin peak in the aliquot that had been reacted with antigen. Thus, the globulin fraction was identified as containing serum antibodies, which were called immunoglobulins, to distinguish them from any other proteins that might be contained in the globulin fraction.

6.4.3: ANTIBODIES ARE HETERODIMERS

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Antibody molecules have a common structure of four peptide chains. This structure consists of two identical light (L) chains, polypeptides of about 25,000 molecular weight, and two identical heavy (H) chains, larger polypeptides of molecular weight 50,000 or more. Like the antibody molecules they constitute, H and L chains are also called immunoglobulins. Each light chain is bound to a heavy chain by a disulfide bond, and by such non-covalent interactions as salt linkages, hydrogen bonds, and hydrophobic bonds, to form a heterodimer (H-L). Similar non covalent interactions and disulfide bridges link the two identical heavy and light (H-L) chain combinations to each other to form the basic four-chain (H-L)2 antibody structure, a dimer of dimers. As we shall see, the exact number and precise positions of these interchain disulfide bonds differs among antibody classes and subclasses.

The first 110 or so amino acids of the amino-terminal region of a light or heavy chain varies greatly among antibodies of different specificity. These segments of highly variable sequence are called *V regions:* VL in light chains and VH in heavy. All of the differences in specificity displayed by different antibodies can be traced to differences in the amino acid sequences of V regions. In fact, most of the differences among antibodies fall within areas of the V regions called *complementarity- determining regions (CDRs),* and it is these CDRs, on both light and heavy chains, that constitute the antigen binding site of the antibody molecule. By contrast, within the same antibody class, far fewer differences are seen when one compares sequences throughout the rest of the molecule. The regions of relatively constant sequence beyond the variable regions have been dubbed C regions, CL on the light chain and CH on the heavy chain. Antibodies are glycoproteins; with few exceptions, the sites of attachment for carbohydrates are restricted to the constant region. We do not completely understand the role played by glycosylation of antibodies, but it probably increases the solubility of the molecules. Inappropriate glycosylation, or its absence, affects the rate at which antibodies are cleared from the serum, and decreases the efficiency of

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interaction between antibody and the complement system and between antibodies and Fc receptors.

6.4.4: CHEMICAL AND ENZYMATIC METHODS REVEALED BASIC ANTIBODY STRUCTURE

Our knowledge of basic antibody structure was derived from a variety of experimental observations. When the _-globulin fraction of serum is separated into high- and low-molecular weight fractions, antibodies of around 150,000-MW, designated as immunoglobulin G (IgG) are found in the low molecular-weight fraction. In a key experiment, brief digestion of IgG with the enzyme papain produced three fragments, two of which were identical fragments and a third that was quite different.

The two identical fragments (each with a MW of 45,000), had antigen-binding activity and were called Fab fragments ("fragment, antigen binding"). The other fragment (MW of 50,000) had no antigen binding activity at all. Because it was found to crystallize during cold storage, it was called the Fc fragment ("fragment, crystallizable").Digestion with pepsin, a different proteolytic enzyme, also demonstrated that the antigen-binding properties of an antibody can be separated from the rest of the molecule. Pepsin digestion generated a single 100,000- MW fragment composed of two Fab-like fragments designated the F(ab)2 fragment, which binds antigen.

The Fc fragment was not recovered from pepsin digestion because it had been digested into multiple fragments. A key observation in deducing the multichain structure of IgG was made when the molecule was subjected to mercaptoethanol reduction and alkylation, a chemical treatment that irreversibly cleaves disulfide bonds. If the sample is chromatographed on a column that separates molecules by size following cleavage of disulfide bonds, it is clear that the intact 150,000-MW IgG molecule is, in fact, composed of subunits. Each IgG molecule contains two 50,000-MW polypeptide chains, designated as heavy (H) chains, and two 25,000-MW chains, designated as light (L) chains. Antibodies themselves were used to determine how the enzyme digestion products—Fab, F (ab_) 2, and Fc were related to the heavy-chain and light-chain reduction products. This question was answered by using antisera from goats that had been immunized with

either the Fab fragments or the Fc fragments of rabbit IgG. The antibody to the Fab fragment could react with both the H and the L chains, whereas antibody to the Fc fragment reacted only with the H chain. These observations led to the conclusion that the Fab fragment consists of portions of a heavy and a light chain and that Fc contains only heavy-chain components. According to this model, the IgG molecule consists of two identical H chains and two identical L chains, which are linked by disulfide bridges. The enzyme papain cleaves just above the interchain disulfide bonds linking the heavy chains, whereas the enzyme pepsin cleaves just below these bonds, so that the two proteolytic enzymes generate different digestion products. Mercaptoethanol reduction and alkylation allow separation of the individual heavy and light chains.

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6.5: CHECK YOUR PROGRESS

1. The hypervariable region (HVR) form the complementary region of the antigenic determinant and therefore, known as ……
2. IgE is also known as ……
3. Mercaptoethanol reduction and alkylation allow separation of the individual ……

6.6: LET US SUM UP

In this unit, you have learnt about the meaning, definition, need, objectives, importance and functions of Immunoglobulins and Generation of antibody diversity. Immunoglobulins are heterodimeric proteins composed of two heavy (H) and two light (L) chains. They can be separated functionally into variable (V) domains that binds antigens and constant (C) domains that specify effector functions such as activation of complement or binding to Fc receptors. The variable domains are created by means of a complex series of gene rearrangement events, and can then be subjected to somatic hypermutation after exposure to antigen to allow affinity maturation. Each V domain can be split into three regions of sequence variability,

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termed the complementarity determining regions, or CDRs, and four regions of relatively constant sequence termed the framework regions, or FRs. The three CDRs of the H chain are paired with the three CDRs of the L chain to form the antigen binding site, as classically defined. There are five main classes of heavy chain C domains. Each class defines the IgM, IgG, IgA, IgD, and IgE isotypes. IgG can be split into four subclasses, IgG1, IgG2, IgG3, and IgG4, each with its own biologic properties; and IgA can similarly be split into IgA1 and IgA2. The constant domains of the H chain can be switched to allow altered effector function while maintaining antigen specificity.

6.7: UNIT - END EXERCISES

1. Write short notes on Immunoglobulins.
2. Describe about the structure and function of Immunoglobulins.
3. Explain about antibody diversity

6.8: ANSWERS TO CHECK YOUR PROGRESS

1. Complementary determining regions (CDRs)
2. Reagin
3. Heavy and light chains.

6.9: SUGGESTED READINGS

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UNIT - VII

NOTES

7.1: INTRODUCTION

Immunogenicity is the ability of a particular substance, such as an antigen or epitope, to provoke an immune response in the body of a human and other animal. In other words, immunogenicity is the ability to induce a humoral and/or cell-mediated immune responses. An immunogen is any antigen that is capable of inducing humoral and/or cell-mediated immune response rather than immunological tolerance. This ability is called immunogenicity. Sometimes the term immunogen is used interchangeably with the term antigen. But only an immunogen can evoke an immune response. Adjuvants, a substance which enhances the body's immune response to an antigen. An epitope, also known as antigenic determinant, is the part of an antigen that is recognized by the immune system, specifically by antibodies, B cells, or T cells.

7.2: OBJECTIVES

After going through this unit, you will be able:

- To know about the Immunogencity and Immunogens

- To know about the Adjuvants and Epitope
- To know about the Haptens and Carrier

7.3 IMMUNOGENCITY

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Immunogenicity or immunoreactivity results from the biomaterial being detected by the body's immune system as a foreign object. Immunoreactive biomaterials, especially wear particles, are detected by antigenic reactions on cells. A biochemical cascade then occurs, whereby T-helper cells migrate towards the biomaterial. This immune response can result in rejection of the biomaterial, and non-union between the biomaterial and the wound site. For bio-integrative products, this would be a significant hindrance to successful functioning. Immunogenicity may also be brought about by biomaterials that have become damaged or oxidised and therefore no longer non-immunogenic.

Immunogenicity assays provide a way to measure the potential immune responses of biologics and biosimilars. Often a single biologic will require a panel of assays to produce a thorough picture of potential immunogenicity. The FDA stipulates that assays should be designed in such a way that they provide sufficient sensitivity, are free from confounding interference, can detect physiological consequences, and account for potential risks based on the profile of the therapeutic and the target patient population. By designing assays with these factors in mind, it is possible to gather predictive data about the strength and type of immune response that a drug may produce in humans.

7.4: IMMUNOGENS

Immunogen is a stimulus that produces a humoral or cell-mediated immune response, whereas antigens are any substance that binds specifically to an antibody or a T-cell receptor. All immunogens are antigens, but all antigens may not be immunogens, some very small molecules called haptens can bind to antibodies or B-cell receptor but they cannot initiate an immune response.

7.5 ADJUVANTS

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An adjuvant is a pharmacological or immunological agent that modifies the effect of other agents. Directly immunizing most antigens will lead to a poor immune response and rapid removal of the antigen from the body. To prevent this, the antigen is first combined with an adjuvant, which is a material that helps stimulate and enhance the immune response against the antigen through the creation of a depot effect. Adjuvants can act in various ways in presenting an antigen to the immune system. They can act as a depot for the antigen, presenting the antigen over a longer period of time, thus maximizing the immune response before the body clears the antigen.

Adjuvant immunobiological functions

- Improve the immunogenicity of highly purified or recombinant antigens (protein or peptide).
- Increase the innate immune response to antigen by interacting with pattern recognition receptors (PRRs) on or within accessory cells.
- Provide physical protection to antigens which grants the antigen a prolonged delivery.
- Increase the capacity to cause local reactions at the injection site (during vaccination), inducing greater release of danger signals by chemokine releasing cells such as helper T cells and mast cells.
- Help in the translocation of antigens to the lymph nodes where they can be recognized by T cells

7.6: EPITOPE

An **epitope**, also known as antigenic determinant, That part of an antigenic molecule to which the T-cell receptor responds, a site on a large molecule against which an antibody will be produced and to which it will bind. The part of an antibody that recognizes the epitope is called a paratope. Although epitopes are usually thought to be derived from nonself proteins, sequences derived from the host that can be recognized are also classified as epitopes. Most epitopes recognized by antibodies or B cells can be thought of as three-dimensional surface features of an antigen molecule; these features fit precisely and thus bind to antibodies. Exceptions are linear epitopes,

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which are determined by the amino acid sequence (the primary structure) rather than by the 3D shape (tertiary structure) of a protein. T cell epitopes are presented on the surface of an antigen-presenting cell, where they are bound to MHC molecules. T cell epitopes presented by MHC class I molecules are typically peptides between 8 and 11 amino acids in length, whereas MHC class II molecules present longer peptides, and non-classical MHC molecules also present non-peptidic epitopes such as glycolipids. Epitopes can be mapped using protein microarrays, and with the ELISPOT or ELISA techniques. Genetic sequences coding for epitopes that are recognized by common antibodies can be fused to genes, thus aiding further molecular characterization of the gene product. Common epitopes used for this purpose are c-myc, HA, FLAG, V5.

Epitopes are sometimes cross-reactive. This property is exploited by the immune system in regulation by anti-idiotypic antibodies (originally proposed by Nobel laureate Niels Kaj Jerne). If an antibody binds to an antigen's epitope, the paratope could become the epitope for another antibody that will then bind to it. If this second antibody is of IgM class, its binding can upregulate the immune response; if the second antibody is of IgG class, its binding can down regulate the immune response.

7.7: HAPTENS AND CARRIERS

A Hapten is a substance that is non-immunogenic but which can react with the products of a specific immune response. It is a small molecule which can elicit an immune response only when attached to a large carrier such as a protein, the carrier may be one which also does not elicit an immune response by itself (Generally, only large molecules, infectious agents, or insoluble foreign matter can elicit an immune response in the body.). On their own they can never induce an immune response. Free haptens, however, can react with products of the immune response after such products have been elicited. Haptens have the property of antigenicity but not immunogenicity. Once the body has generated antibodies to a hapten-carrier adduct, the small-molecule hapten may also be able to bind to the antibody, but it will usually not initiate an immune response; usually only the

hapten-carrier adduct can do this. Sometimes the small-molecule hapten can even block immune response to the hapten-carrier adduct by preventing the adduct from binding to the antibody. A well known example of a hapten is urushiol, which is the toxin found in poison ivy. When absorbed through the skin from a poison ivy plant, urushiol undergoes oxidation in the skin cells to generate the actual hapten, a reactive molecule called a quinone which then reacts with skin proteins to form hapten adducts.

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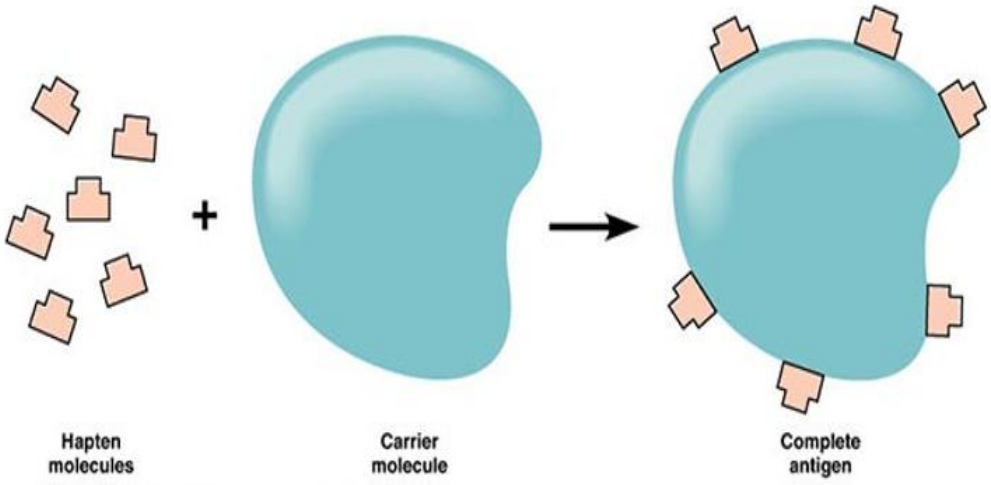

Fig 7.1: Complete Antigen

Typically the first exposure only causes sensitization, in which there is a proliferation of B cells able to make antibody to the hapten adducts. After a second exposure later, the proliferated B cells can become activated, generating an immune reaction producing the typical blisters of poison ivy exposure. Some haptens can induce autoimmune disease. An example is hydralazine, a blood pressure lowering drug which occasionally can produce drug-induced lupus erythematosus in certain individuals. This also appears to be the mechanism by which the anaesthetic gas halothane can cause a life-threatening hepatitis, as well as the mechanism by which penicillin-class drugs causes' autoimmune hemolytic anemia.

7.8: CHECK YOUR PROGRESS:

1. ……is a pharmacological or immunological agent

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2. ……..is a substance that is non-immunogenic but which can react with the products of a specific immune response
3. A well known example of a hapten is …….

7.9: LET US SUM UP

In this unit, you have learnt about the meaning, definition, need, objectives, importance and functions of Immunogencity, Immunogens, Adjuvants, Epitope, Haptens and Carriers. Immunogenicity or immunoreactivity results from the biomaterial being detected by the body's immune system as a foreign object. Immunoreactive biomaterials. Immunogen is a stimulus that produces a humoral or cell-mediated immune response, whereas antigens are any substance that binds specifically to an antibody or a T-cell receptor. An adjuvant is a pharmacological or immunological agent that modifies the effect of other agents. An epitope, also known as antigenic determinant. A Hapten is a substance that is non-immunogenic but which can react with the products of a specific immune response. The small-molecule hapten may also be able to bind to the antibody i.e Carrier.

7.10: UNIT - END EXERCISES

1. Write short notes on Immunogencity.
2. Describe about an adjuvants.
3. Explain about Haptens and carriers

7.11: ANSWERS TO CHECK YOUR PROGRESS:

1. An adjuvant
2. Hapten
3. Urushiol

7.12: SUGGESTED READINGS

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UNIT - VIII

8.1: INTRODUCTION

Antigen, substance that is capable of stimulating an immune response, specifically activating lymphocytes, which are the body's infection-fighting white blood cells. In general, two main divisions of antigens are recognized: foreign antigens (or heteroantigens) and autoantigens (or self-antigens). Foreign antigens originate from outside the body. Examples include parts of or substances produced by viruses or microorganisms (such as bacteria and protozoa), as well as substances in snake venom, certain proteins in foods, and components of serum and red blood cells from other individuals. Autoantigens, on the other hand, originate within the body. Normally, the body is able to distinguish self from nonself, but in persons with autoimmune disorders, normal bodily substances provoke an immune response, leading to the generation of autoantibodies. An antigen that induces an immune response—i.e., stimulates the lymphocytes to produce antibody or to attack the antigen directly—is called an immunogen.

8.2: OBJECTIVES

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After going through this unit, you will be able:

- To know about the T dependent and independent Antigens
- To study the Strengths of Antibody-antigen interaction
- To know about Affinity, Precipitation, Agglutination Avidity and Valency

8.3: ANTIGEN CLASSIFICATION

Antigen (Ag) can be described as a substance that reacts with the products of a specific immune response. The word originated from the notion that they can stimulate antibody generation. This phenomenon is known as antigenicity of a molecule.

8.3.1: Classification by origin -Exogenous antigens

Exogenous antigens are antigens that have entered the body from the outside, for example by inhalation, ingestion, or injection. By endocytosis or phagocytosis, these antigens are taken into the antigen-presenting cells (APCs) and processed into fragments. APCs then present the fragments to T helper cells ($CD4^+$) by the use of class II histocompatibility molecules on their surface. Some T cells are specific for the peptide: MHC complex. They become activated and start to secrete cytokines. Cytokines are substances that can activate cytotoxic T lymphocytes (CTL), antibody-secreting B cells, macrophages and other particles.

8.3.2: Endogenous antigens

Endogenous antigens are antigens that have been generated within the cell, as a result of normal cell metabolism, or because of viral or intracellular bacterial infection. The fragments are then presented on the cell surface in the complex with MHC class I molecules. If activated cytotoxic $CD8^+$ T cells recognize them, the T cells begin to secrete different toxins that cause the lysis or apoptosis of the infected cell. In order to keep the cytotoxic cells from killing cells just for presenting self-proteins, self-reactive T cells are

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deleted from the repertoire as a result of tolerance (also known as negative selection which occurs in the thymus).

8.3.3: Autoantigens

An autoantigen is usually a normal protein or complex of proteins (and sometimes DNA or RNA) that is recognized by the immune system of patients suffering from a specific autoimmune disease. These antigens should under normal conditions not be the target of the immune system, but due to mainly genetic and environmental factors the normal immunological tolerance for such an antigen has been lost in these patients.

8.4: CLASSIFICATION ACCORDING TO THE CELLULAR RESPONSE GENERATED T-INDEPENDENT ANTIGENS

T-independent antigens are antigens which can directly stimulate the B cells to produce antibody without the requirement for T cell help In general, polysaccharides are T-independent antigens. The responses to these antigens differ from the responses to other antigens.

8.4.1: PROPERTIES OF T-INDEPENDENT ANTIGENS

1. Polymeric structure

These antigens are characterized by the same antigenic determinant repeated many times.

2. Polyclonal activation of B cells

Many of these antigens can activate B cell clones specific for other antigens (polyclonal activation). T-independent antigens can be subdivided into Type 1 and Type 2 based on their ability to polyclonally activate B cells. Type 1 T-independent antigens are polyclonal activators while Type 2 is not.

3. Resistance to degradation

T-independent antigens are generally more resistant to degradation and thus they persist for longer periods of time and continue to stimulate the immune system.

8.5: T-DEPENDENT ANTIGENS

T-dependent antigens are those that do not directly stimulate the production of antibody without the help of T cells. Proteins are T-dependent antigens. Structurally these antigens are characterized by a few copies of many different antigenic determinants.

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8.5.1: FACTORS INFLUENCING IMMUNOGENICITY OF AN ANTIGEN

1. Foreignness

The immune system normally discriminates between self and non-self such that only foreign molecules are immunogenic.

2. Size

There is not absolute size above which a substance will be immunogenic. However, in general, the larger the molecule the more immunogenic it is likely to be.

3. Chemical Composition

In general, the more complex the substance is chemically the more immunogenic it will be. The antigenic determinants are created by the primary sequence of residues in the polymer and/or by the secondary, tertiary or quaternary structure of the molecule.

4. Physical form

In general particulate antigens are more immunogenic than soluble ones and denatured antigens more immunogenic than the native form.

5. Degradability

Antigens that are easily phagocytosed are generally more immunogenic. This is because for most antigens (T-dependant antigens, see below) the development of an immune response requires that the antigen be phagocytosed, processed and presented to helper T cells by an antigen presenting cell (APC).

8.5.2: ANTIGENIC DETERMINANTS -DETERMINANTS RECOGNIZED BY B CELLS

1. Composition

Antigenic determinants recognized by B cells and the antibodies secreted by B cells are created by the primary sequence of residues in the

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polymer (linear or sequence determinants) and/or by the secondary, tertiary or quaternary structure of the molecule (conformational determinants).

2. Size

In general antigenic determinants are small and are limited to approximately 4-8 residues. (Amino acids and or sugars). The combining site of an antibody will accommodate an antigenic determinant of approximately 4-8 residues.

3. Number

Although, in theory, each 4-8 residues can constitute a separate antigenic determinant, in practice, the number of antigenic determinants per antigen is much lower than what would theoretically be possible. Usually the antigenic determinants are limited to those portions of the antigen that are accessible to antibodies.

8.5.3: DETERMINANTS RECOGNIZED BY T CELLS

1. Composition

Antigenic determinants recognized by T cells are created by the primary sequence of amino acids in proteins. T cells do not recognize polysaccharide or nucleic acid antigens. This is why polysaccharides are generally T-independent antigens and proteins are generally T-dependent antigens. The determinants need not be located on the exposed surface of the antigen since recognition of the determinant by T cells requires that the antigen be proteolytically degraded into smaller peptides. Free peptides are not recognized by T cells, rather the peptides associate with molecules coded for by the major histocompatibility complex (MHC) and it is the complex of MHC molecules + peptide that is recognized by T cells.

2. Size

In general antigenic determinants are small and are limited to approximately 8-15 amino acids.

3. Number

Although, in theory, each 8-15 residues can constitute a separate antigenic determinant, in practice, the number of antigenic determinants per antigen is much less than what would theoretically be possible. The antigenic

determinants are limited to those portions of the antigen that can bind to MHC molecules. This is why there can by differences in the responses of different individuals.

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8.6: STRENGTH OF ANTIBODY-ANTIGEN INTERACTION

An antibody is an immunoglobulin protein, secreted by B lymphocytes, that is present in serum or body fluid and combines specifically with an antigen. Antigens are classically defined as any foreign substance that elicits an immune response. An antigen that produces an adaptive immune response after injection into an animal is an immunogen. Immunogens can be designed so that antibodies are generated against specific proteins. Because antibodies can be made against specific proteins, they are very useful tools in science and can be used to investigate specific protein function and location in a dynamic biological system.

The specific region on an antigen that an antibody recognizes and binds to is the epitope, or antigenic determinant. An epitope is usually 5-8 amino acids long on the surface of the protein. Proteins are three dimensional folded structures and an epitope may only be recognized in its form as it exists in solution, or its native form. When an epitope is made up of two or more regions of the protein that are brought together by the three-dimensional structure, the epitope is conformational, or discontinuous. If the epitope exists on a single polypeptide chain, it is a continuous or linear epitope. Depending on the epitope an antibody recognizes, it may bind only fragments or denatured segments of a protein or it may also be able to bind the native protein. Antibodies can be generated against specific peptide sequences or proteins by being conjugated to a carrier protein that is known to be strongly immunogenic. Common carrier proteins are keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA). When the immunizing peptide sequence or protein is conjugated to the carrier protein, the antibodies generated by the immunized animal will be specific to epitopes across the surface of the whole carrier complex.

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8.7: AFFINITY

Interactions between antigen and antibody involve non-covalent binding of an antigenic determinant (epitope) to the variable region (complementarity determining region, CDR) of both the heavy and light immunoglobulin chains. These interactions are analogous to those observed in enzyme-substrate interactions and they can be defined similarly. To describe the strength of the antigen-antibody interaction, one can define the affinity constant (K) as shown:

$$\text{Affinity } K = \frac{[Ab - Ag]}{[Ab] \times [Ag]} = 10^4 \text{ to } 10^{12} \text{ L/mol}$$

If the interaction between antigen and antibody were totally random, one would expect the concentrations of free antigen, free antibody and bound Ag-Ab complex to all be equivalent. In other words,

Therefore, the greater the K, the stronger the affinity between antigen and antibody. These interactions are the result of complementarity in shapes, hydrophobic interactions, hydrogen bonds and Van der Waals forces.

8.8: PRECIPITATION

Interaction of antibody with a soluble antigen to form an insoluble complex, e.g., with BSA (bovine serum albumin).In liquid - the precipitate can be recovered by centrifugation and analyzed. If either the antigen or antibody is radioactively labeled, it can be used in a **R**adio **I**mmuno **P**recipitation (RIP) assay, first developed in the 1950s. In agarose - if the antigen-antibody interaction takes place in a semi-solid medium such as agarose, the resulting precipitate can be easily visualized. This is of special significance in a configuration known as Ouchterlony Analysis. Precipitation and agglutination are both consequence of cross-linking of antigens by antibody into large complexes. The ability of antibodies to carry out this process implies that each antibody can bind at least two antigen molecules,

and that it can only occur if the antigen molecule has two or more determinants ("epitopes"), which can be recognized by that antibody.

When antigen and antibody are mixed in a test tube in their soluble forms, one of two things may happen: both components will remain soluble or variable amounts of Ag-Ab precipitate will be formed. If progressively increasing amounts of antigen are mixed with a fixed amount of antibody, a precipitin curve can be constructed (Fig. 8.5). There are three areas to consider in a precipitin curve:

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• Antibody excess—free antibody remains in solution after centrifugation of Ag-Ab complexes.

• Equivalence—no free antigen or antibody remains in solution. The amount of precipitated Ag-Ab complexes reaches its peak at this point.

• Antigen excess—free antigen is detected in the supernatant after centrifugation of Ag-Ab complexes.

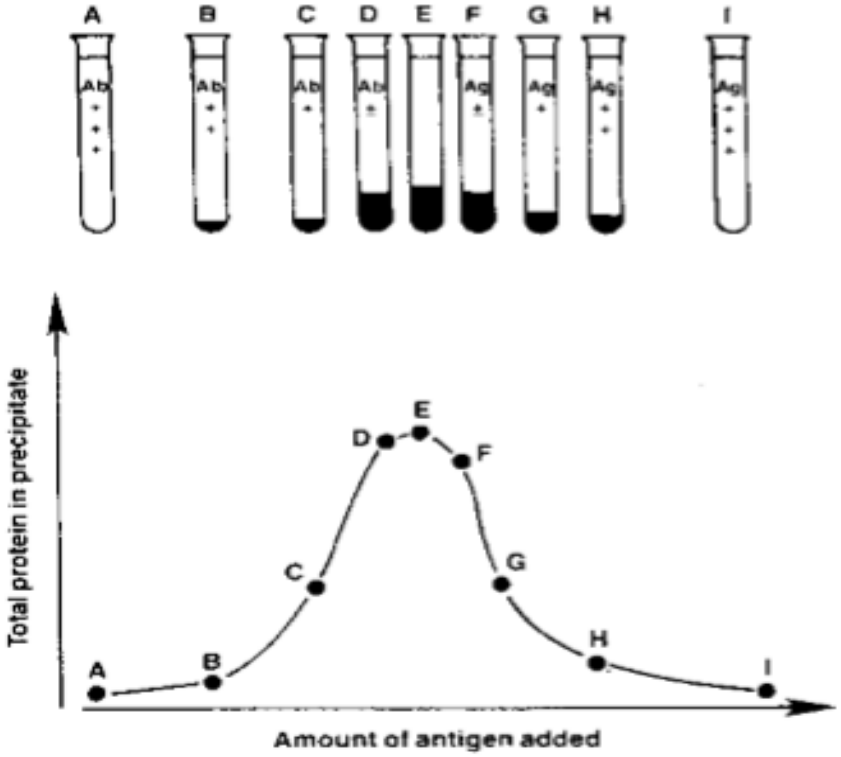

Fig. 8.1: The precipitation curve. When increasing amounts of antigen are added to a fixed concentration of antibody, increasing amounts of precipitate

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appear as a consequence of the antigen-antibody interaction. After a maximum precipitation is reached, the amounts of precipitate begin to decrease. Analysis of the supernatants reveals that at low antigen concentrations there is free antibody left in solution (antibody excess); at the point of maximal precipitation, neither antigen nor antibody are detected in the supernatant (equivalence zone); with greater antigen concentrations, antigen becomes detectable in the supernatant (antigen excess).

8.9: AGGLUTINATION

"Clumping" of a particulate antigen, e.g. bacteria or SRBC (sheep red blood cells). Agglutination of red blood cells is a technique, which has been widely used in clinical and basic research as well as in the clinical laboratory, and is called **hemagglutination**. Many soluble antigens can be made effectively particulate by coating them onto SRBC or latex or other particles; the resulting clumping by antibody is known as passive agglutination.

Agglutination reactions result from the cross-linking of cells and insoluble particles by specific antibodies. Due to the relatively short distance between the two Fab fragments, 7S antibodies (such as IgG) are usually unable to bridge the gap between two cells, each of them surrounded by an electronic “cloud” of identical charge that will tend to keep them apart. IgM antibodies, on the other hand, are considerably more efficient in inducing cellular agglutination (Fig. 8.1). The visualization of agglutination reactions differs according to the technique used for their study. In slide tests, the nonagglutinated cell or particulate antigen appears as a homogeneous suspension, while the agglutinated antigen will appear irregularly clumped. If antibodies and cells are mixed in a test tube, the cross-linking of cells and antibodies will result in the diffuse deposition of cell clumps in the bottom and walls of the test tube, while the nonagglutinated red cells will precipitate in a very regular fashion, forming a compact red button on the bottom of the tube.

Agglutination reactions follow the same basic rules of the precipitation reaction. When cells and antibody are mixed at very high antibody concentrations (low dilutions of antisera), antibody excess may

result, no significant cross-linking of the cells is seen, and, therefore, the agglutination reaction may appear to be negative. Dilutions in which antibody excess prevents agglutination constitute the prozone. With increasing antibody dilutions, more favorable ratios for cross-linking are reached, and very fine clumps cover the walls of the test tube or microtitration wells. When equivalence is approached, larger clumps of cells can be distinguished. At still higher dilutions, when the concentration of antibody is very low, the zone of antigen excess is reached and agglutination is no longer seen.

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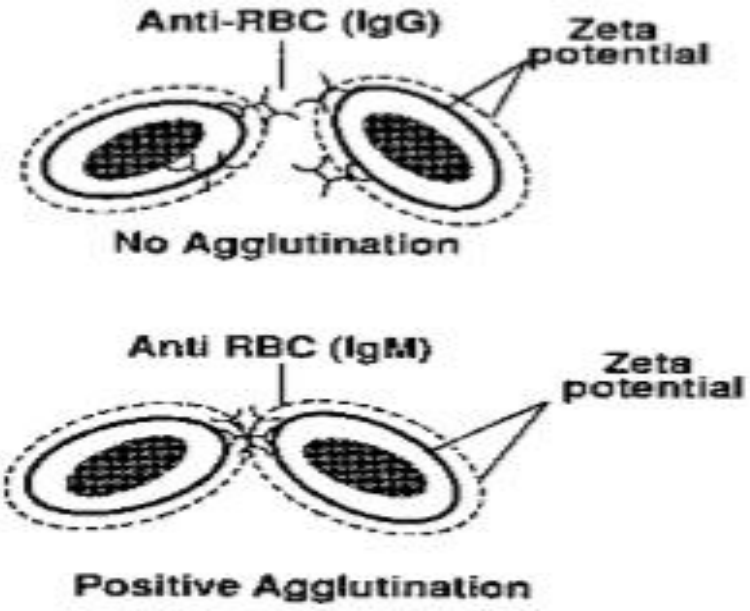

Fig 8.2: IgM antibodies are more efficient in inducing red cell agglutination.

Red cells remain at the same distance from each other due to their identical electrical charge (zeta potential). IgG antibodies are not large enough to bridge the space between two red cells, but IgM antibodies, due to their polymeric nature and size, can induce red blood cell agglutination with considerable ease.

8.10: AVIDITY

Antibody avidity can be defined as the strength of the binding of the several different antibodies produced in response to an immunogen, which

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presents several different epitopes to the immune system. The strength of the Ag-Ab reaction is enhanced when several different antibodies bind simultaneously to different epitopes on the antigen molecule, crosslinking antigen molecules very tightly. Thus, a more stable bonding between antigen and antibody will be established due to the "bonus effect" of multiple antigen-antibody bonds (Fig. 8.3); the increased stability of the overall antigen-antibody reaction corresponds to an increased avidity.

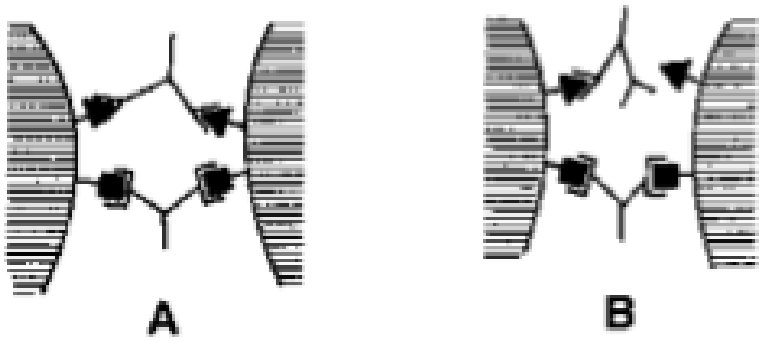

Fig 8.3: Diagrammatic representation of the avidity concept. The binding of antigen molecules by several antibodies of different specificities (A) stabilizes the immune complex, since it is highly unlikely that all Ag-Ab reactions dissociate simultaneously at any given point of time (B). (Redrawn from Roitt, I. Essential Immunology, 4th ed. Blackwell, Oxford, 1980.)

8.11: VALENCY

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.

8.12: CHECK YOUR PROGRESS

1. ……..which induces an immune response in the body
2. ……. is the clumping of particles
3. ……. is replaced precipitation and agglutination assays in a wide variety of clinical and research applications.

8.13: LET US SUM UP

NOTES

In this unit, you have learnt about the meaning, definition, need, objectives, importance and functions of T dependent and T independent antigens, Immunogens, Strength of antigen –antibody interactions, affinity, avidity, valency, agglutination and Precipitation. T-dependent antigens are those that do not directly stimulate the production of antibody without the help of T cells. Proteins are T-dependent antigens. Structurally these antigens are characterized by a few copies of many different antigenic determinants. Agglutination of red blood cells is a technique, which has been widely used in clinical and basic research as well as in the clinical laboratory, and is called hemagglutination. Antibody avidity can be defined as the strength of the binding of the several different antibodies produced in response to an immunogen, which presents several different epitopes to the immune system.

8.14: UNIT - END EXERCISES

1. Write short notes on the Affinity.
2. Write in detail about the Precipitation.
3. Explain about cytolysis
4. Describe about T dependent and T independent Antigens
5. Write in detail about the antigen-antibody interactions.

8.15: ANSWERS TO CHECK YOUR PROGRESS

1. Toxin or foreign substance
2. Agglutination
3. ELISA

NOTES

8.16: SUGGESTED READINGS

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BLOCK – 3COMPLEMENT SYSTEM AND MAJOR HISTOCOMPATIBILITY COMPLEX

NOTES

UNIT - IX

9.1: INTRODUCTION

Complement was discovered by Jules Bordet as a heat-labile component of normal plasma that causes the opsonisation and killing of bacteria. The complement system refers to a series of >20 proteins, circulating in the blood and tissue fluids. Most of the proteins are normally inactive, but in response to the recognition of molecular components of microorganisms they become sequentially activated in an enzyme cascade – the activation of one protein enzymatically cleaves and activates the next protein in the cascade. Complement can be activated via three different pathways, which can each cause the activation of C3, cleaving it into a large fragment, C3b, that acts as an opsonin, and a small fragment C3a (anaphylatoxin) that promotes inflammation. Activated C3 can trigger the lytic pathway, which can damage the plasma membranes of cells and some bacteria. C5a, produced by this process, attracts macrophages and neutrophils and also activates mast cells.

NOTES

9.2: OBJECTIVES

After going through this unit, you will be able:

- To know about the Complement System
- To study the structure and functions of Complement Activation and pathways

9.3: COMPLEMENT SYSTEM

The complement system is a collection of circulating and membrane-associated proteins that are important in defense against microbes. Many complement proteins are proteolytic enzymes, and complement activation involves the sequential activation of these enzymes. The complement system includes serum and membrane-bound proteins that functions in both natural and acquired defense system. These proteins are highly regulated and interact via a series of proteolytic cascades.

The term 'complement' refers to the ability of these proteins, to complement (augment) the effects of other components of the immune system (e.g. antibody). Complement has several main effects:

1. Lysis of cells (e.g. bacteria, tumor cells and enveloped virus directly).

2. Production of peptide fragments that participate in inflammation and attract phagocytes.

3. Opsonization of organisms and immune complexes for clearance by phagocytosis and enhancement of antibody-mediated immune responses.

Further more the complement goes to work, as soon as an invading microbe is detected; the system makes up an effective host immune defense long before specific host defenses are mobilized.

9.4: COMPLEMENT ACTIVATION

The complement system works as a cascade. A cascade is a set of reactions that amplify some effects, i.e. more products are formed in the second reaction than the first, still more in the third and so on. Of the proteins, so far identified in the complement system, 13 participate in the cascade itself, seven activate or inhibit reactions in the cascade. The components of the classical pathway are numbered from C1 to C9 and the

reaction sequence is C1-C4-C2-C3-C5-C6-C7- C8-C9. Up to C5, activation involves proteolytic cleavage, liberating smaller fragments from C2 through C5 except for C2, where for historical reasons the larger fragment that remains bound to the complex is termed C2a, the smaller fragments are by the letter 'a' (e.g. C4a) and the larger fragments by letter 'b' (e.g. C5b). Activation of the complement system can be initiated, either by antigen antibody complexes or by a variety of nonimmunological molecules.

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9.5: PATHWAYS IN COMPLEMENT SYSTEM

The complement cascade may be activated by any of three pathways (Fig. 9.1):

• The **alternative pathway** is triggered when some complement proteins are activated on microbial surfaces and cannot be controlled, because complement regulatory proteins are not present on microbes (but are present on host cells). The alternative pathway is a component of innate immunity.

• The **classical pathway** is most often triggered by antibodies that bind to microbes or other antigens and is thus a component of the humoral arm of adaptive immunity.

• The **lectin pathway** is activated when a carbohydrate-binding plasma protein, mannose-binding lectin (MBL), binds to terminal mannose residues on the surface glycoproteins

9.5.1: CLASSICAL PATHWAY

Only, immunoglobulin M (IgM) and immunoglobulin G (IgG) (IgG1, IgG2, IgG3 not IgG4) activate or fix complement via the classical pathway. The formation of an antigen antibody complex induces conformational changes in the Fc portion of the IgM molecule that expose a binding site for the C1 component of the complement system. C1 in serum is a macromolecular complex consisting of C1q and two molecules of each of C1r and C1s, held together in a complex (C1qr2, s2) stabilized by Ca2+ (Fig. 9.1). One molecule of IgM or two molecules of IgG can initiate the process.

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C1q binding in the presence of calcium ions, leads to sequential activation of C1r and C1s. C1s cleaves C4 and C2 to form C4bC2a, the latter is an active C3 convertase, which cleaves C3 molecule into two fragments as C3a and C3b. C3a is an anaphylatoxin. C3b forms complex with C4bC2a producing a new enzyme called C5 convertase (C4bC2aC3b). C5 convertase cleaves C5 to form C5a and C5b. C5a is an anaphylatoxin and chemotactic factor. The C5b component is extremely labile and becomes inactive within 2 minutes, unless C6 binds to it and stabilizes its activity. Attachment of C5b to the bacterial membrane initiates formation of the membrane attack complex (MAC) and lysis of the cell. The attachment of C5b leads to the addition of components C6, C7 and C8. C8 provides a strong anchor into the membrane and facilitates the subsequent addition of multiple C9 molecules to form a pore in the membrane. Loss of membrane integrity results in the unregulated flow of electrolytes and causes lysis and death of cell.

9.5.2: ALTERNATIVE PATHWAY

The alternative pathway is otherwise called as Properdin pathway which does not involve in immune complex. Many unrelated substances such as bacterial endotoxin, IgA and IgD antibodies, cobra venom factor, nephritic factor (protein present in the serum of glomerulonephritis) initiate alternative pathway. This pathway does not involve C1, C2 or C4. C3 is cleaved so that C3 convertase is generated via the action of factors B, D and properdin. The alternative C3 convertase (C3bBb) generates more C3b. The additional C3b binds to the C3 convertase to form C3bBbC3b, which is the C5 convertase of the alternative pathway that generates C5b, leading to production of MAC described earlier (Fig. 9.1).

9.5.3: MANNAN-BINDING LECTIN PATHWAY

Lectins are proteins that bind to specific carbohydrate. Mannose-binding lectin (MBL) or mannan-binding lectin is an acute phase protein, which binds sugar residues like mannose, found on microbial surface *(e.g. Listeria* species, *Salmonella* species, *Candida albicans).* MBL level can rise rapidly in response to infection, inflammation and other forms of stress. MBL once bound to appropriate mannose containing residues, can interact

with MBL-activated serum protease (MASP). Activation of MASP leads to subsequent activation of components C2, C4 and C3 (Fig. 9.1).

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Table 9.1: Comparison of Classical, Alternative and Lectin Pathway

Feature	C activation pathway		
	Classical	Alternative	Lectin
Main activators	Ag complexed IgM & IgG molecules	Bacterial & viral structures, polyanions	Mannose- & N-acetyl glucosamine containing bacterial polysaccharides
Ag-dependence	Yes	No	No
Pathway initiation	C1q binding to IgM/ IgG complexed to Ag	Deposition of C3b deposition onto foreign (unprotected) surface	MBL binding to mannans on bacterial surface
C4 & C2 activation	C1s	Not involved	MASP-2
C3 convertase	C4b2a	C3bBbP	C4bC2a
Stabilization of C3 convertase	-	By properdin	-
C5 convertase	C4b2a3b	C3bBbP3b	C4b2a3b
Inhibitors of early stage	C1 INH, C4-bp	FI, FH, CR1, DAF, MCP	C1 INH, C4-bp
Role	Effector arm of the adaptive immunity	Innate immunity	Innate immunity
Effect of Ig deficiency	Activation prevented	Nil	Nil
Involvement in tissue injury in autoimmune disease & organ transplant	Yes, Ab-mediated	-	-
Evolution	About 400 millions years old, parallel to emergence of Igs	About 700 million years old	About ≥700 million years old
Present in	Only vertebrates, starting from cartilaginous fishes	Echinoderms	Echinoderms, sponges (?)

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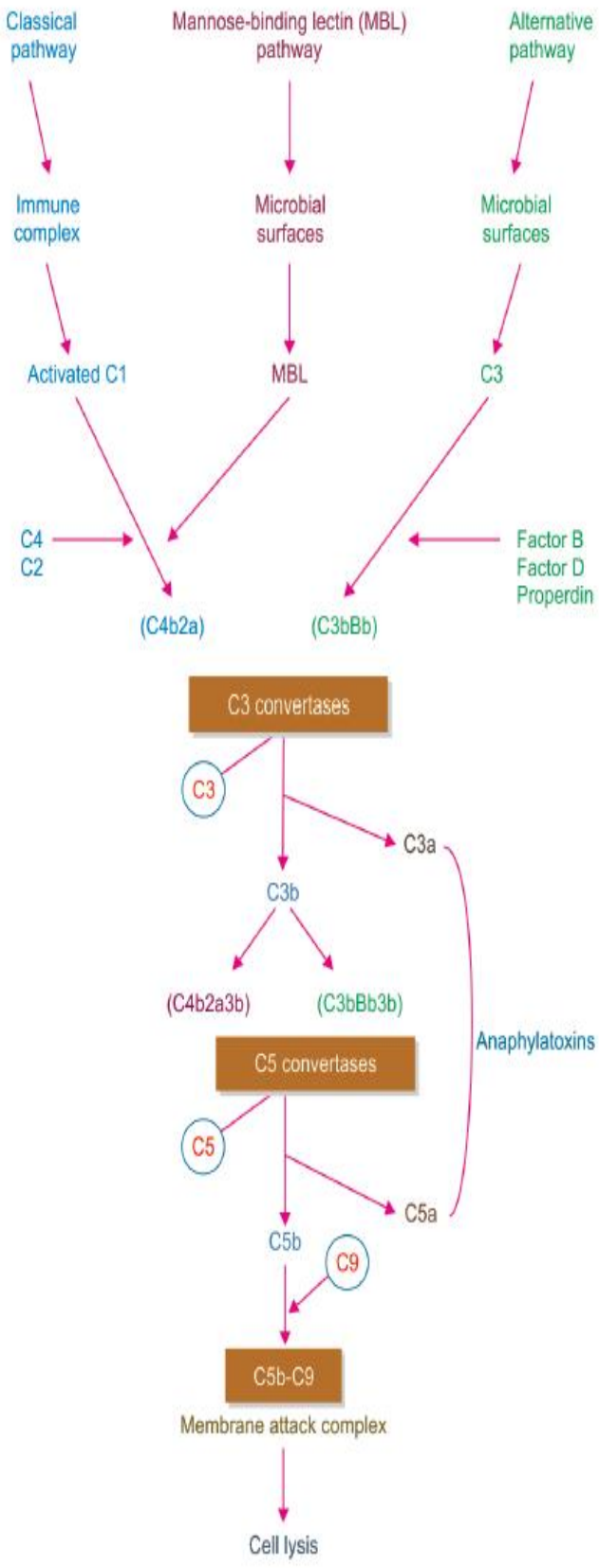

Fig 9.1: Classical, Alternate and Lectin Pathways.

9.6: CHECK YOUR PROGRESS

1. …….. is triggered when some complement proteins are activated on microbial surfaces

2. …….. is most often triggered by antibodies

3. ……. is activated when a carbohydrate-binding plasma protein, mannose-binding lectin (MBL)

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9.7: LET US SUM UP

In this unit, you have learnt about the meaning, definition, need, objectives, importance and functions of Complement. Classical Pathway involves complement components C1, C2 and C4. The pathway is triggered by antibody-antigen complexes binding to C1, which itself has three subcomponents C1q, C1r and C1s. The pathway forms a C3 convertase, C4b2a, which splits C3 into two fragments; the large fragment, C3b, can covalently attach to the surface of microbial pathogens and opsonise them; the small fragment, C3a, activates mast cells, causing the release of vasoactive mediators such as histamine. Alternative Pathway involves various factors, B, D, H & I, which interact with each other, and with C3b, to form a C3 convertase, C3bBb, that can activate more C3, hence the pathway is sometimes called 'the amplification loop'. Activation of the loop is promoted in the presence of bacterial and fungal cell walls, but is inhibited by molecules on the surface of normal mammalian cells. Mannose-binding Lectin Pathway is activated by the binding of mannose-binding lectin (MBL) to mannose residues on the pathogen surface. This in turn activates the MBL-associated serine proteases, MASP-1 and MASP-2, which activate C4 and C2, to form the C3 convertase, C4b2a.

9.8: UNIT - END EXERCISES

4. Write short notes on Complement.
5. Describe about Classical pathway.
6. Explain about Alternative pathway.

9.9: ANSWERS TO CHECK YOUR PROGRESS

1. Alternative pathway

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2. Classical pathway
3. Lectin pathway

9.10: SUGGESTED READINGS

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UNIT - X

NOTES

10.1: INTRODUCTION

Antigen processing and presentation is the process by which protein antigen is ingested by an antigen-presenting cell (APC), partially digested into peptide fragments and then displayed on the surface of the APC associated with an antigen-presenting molecule such as MHC class I or MHC class II, for recognition by certain lymphocytes such as T cells.

10.2: OBJECTIVES

After going through this unit, you will be able:

- To know about the mechanisms of antigen processing and presentation
- To study the Cytosolic and Endocytic pathways.
- To know about the Antibody engineering

10.3: MECHANISMS OF ANTIGEN PROCESSING AND PRESENTATION

Antigen processing or cytosolic' pathway is an immunological process that prepares antigens for presentation to special cells of the immune system called T lymphocytes. It is considered to be a stage of antigen presentation pathways. This process involves two distinct pathways for

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processing of antigens from an organism's own (self) proteins or intracellular pathogens (e.g. viruses), or from phagocytosed pathogens (e.g. bacteria); subsequent presentation of these antigens on class I or class II major histocompatibility complex (MHC) molecules is dependent on which pathway is used. Both MHC class I and II are required to bind antigen before they are stably expressed on a cell surface. MHC I antigen presentation typically (considering cross-presentation) involves the endogenous pathway of antigen processing, and MHC II antigen presentation involves the exogenous pathway of antigen processing. Cross-presentation involves parts of the exogenous and the endogenous pathways but ultimately involves the latter portion of the endogenous pathway (e.g. proteolysis of antigens for binding to MHC I molecules). While the joint distinction between the two pathways is useful, there are instances where extracellular-derived peptides are presented in the context of MHC class I and cytosolic peptides are presented in the context of MHC class II (this often happens in dendritic cells).

10.3.1: THE ENDOGENOUS PATHWAY

The endogenous pathway is used to present cellular peptide fragments on the cell surface on MHC class I molecules. If a virus had infected the cell, viral peptides would also be presented, allowing the immune system to recognize and kill the infected cell. Worn out proteins within the cell become ubiquitinated, marking them for proteasome degradation. Proteasomes break the protein up into peptides that include some around nine amino acids long (suitable for fitting within the peptide binding cleft of MHC class I molecules). Transporter associated with antigen processing (TAP), a protein that spans the membrane of the rough endoplasmic reticulum, transports the peptides into the lumen of the rough endoplasmic reticulum (ER). Also within the rough ER, a series of chaperone proteins, including calnexin, calreticulin, ERp57, and Binding immunoglobulin protein (BiP) facilitates the proper folding of class I MHC and its association with β2 microglobulin. The partially folded MHC class I molecule then interacts with TAP via tapasin (the complete complex also contains calreticulin and Erp57 and, in mice, calnexin). Once the peptide is

transported into the ER lumen it binds to the cleft of the awaiting MHC class I molecule, stabilizing the MHC and allowing it to be transported to the cell surface by the golgi apparatus.

10.3.2: THE EXOGENOUS PATHWAY

The exogenous pathway is utilized by specialized antigen presenting cells to present peptides derived from proteins that the cell has endocytosed. The peptides are presented on MHC class II molecules. Proteins are endocytosed and degraded by acid-dependent proteases in endosomes; this process takes about an hour.

The nascent MHC class II protein in the rough ER has its peptide-binding cleft blocked by Ii (the invariant chain; a trimer) to prevent it from binding cellular peptides or peptides from the endogenous pathway. The invariant chain also facilitates MHC class II's export from the ER in a vesicle. This fuses with a late endosome containing the endocytosed, degraded proteins. The invariant chain is then broken down in stages, leaving only a small fragment called "Class II-associated invariant chain peptide" (CLIP) which still blocks the peptide binding cleft. An MHC class II-like structure, HLA-DM, removes CLIP and replaces it with a peptide from the endosome. The stable MHC class-II is then presented on the cell surface.

10.3.3: CROSS-PRESENTATION PROCESSING

In Cross-presentation, peptides derived from extracellular proteins are presented in the context of MHC class I. The cell starts off with the exogenous pathways but diverts the antigens (cytosolic diversion) to the endogenous pathway. This can allow the cell to skip the parts of the endogenous pathway that involve synthesis of antigens from the antigenic genes with cellular machinery upon infection, because the endogenous pathway can involve infection before being able to present antigens with MHC I, and cross-presentation saves them the effort needed for that and allows the professional antigen-presenting cells (dendritic cells) to process and present antigens without getting infected, which does not tend to happen to dendritic cells and is quite common scenario of antigen-processing using the endogenous pathway. Not all antigen-presenting cells utilize cross-presentation.

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10.3.4: VIRAL EVASION OF ANTIGEN PROCESSING

Certain species in the Cytolomega virus family can cause the infected-cell to produce proteins like US2, 3, 6, and/or 11. US11 and US2 mislead MHC I to the cytoplasm; US3 inhibits the transportation of MHC I in the ER (a part of the endogenous pathway and cross-presentation); US6 blocks peptide transportation by TAP to MHC I.

Mycobacterium tuberculosis inhibits phagosome-endosome fusion, thus avoiding being destroyed by the harsh environment of the phagosome.

ICP47 from some herpesvirus block transport of the peptide by TAP. U21 from some human herpesvirus 7 binds and targets certain MHC I molecules for lysosomal degradation. E19 from some adenoviruses block the movement of MHC I to the proper locations for the endogenous pathway.

Nef from some HIV strains enhance the movement of MHC molecules back into the cytoplasm, preventing them from presentating antigens.

10.3.5: THE ROLE OF LANGERHANS' DENDRITIC CELLS IN ANTIGEN PROCESSING

Langerhans' cells are particular type of dendritic cells present in non lymphoid tissues together with interstitial cells. When these cells (in an immature state) come in contact with antigenic cells or disease causing viruses etc. these cells produce an inflammatory stimulus and start antigen processing and move toward lymph nodes where these APCs present antigen to mature T lymphocytes.

10.4: ANTIGEN PRESENTATION

Antigen presentation describes a vital immune process which is essential for T cell immune response triggering. Because T cells recognise only fragmented antigens displayed on cell surfaces, antigen processing must occur before the antigen fragment, now bound to the major histocompatibility complex (MHC), is transported to the surface of the cell, a process known as presentation, where it can be recognized by a T cell receptor. If there has been an infection with viruses or bacteria, the cell will present an endogenous or exogenous peptide fragment derived from the antigen bound to MHC molecules. There are two types of MHC molecules which differ in the of the antigens: MHC class I molecules (MHC-I) bind peptides from the cell cytosol,

while peptides generated in the endocytic vesicles after internalisation are bound to MHC class II (MHC-II). Cellular membranes separate these two cellular environments - intracellular and extracellular. Each T cell can finally recognise only ten to hundreds copies of a unique sequence of a single peptide among thousands of other peptides presented on the very same cell because MHC molecule in one cell can bind quite a large range of peptides.

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10.4.1: PRESENTATION OF INTRACELLULAR ANTIGENS: CLASS I

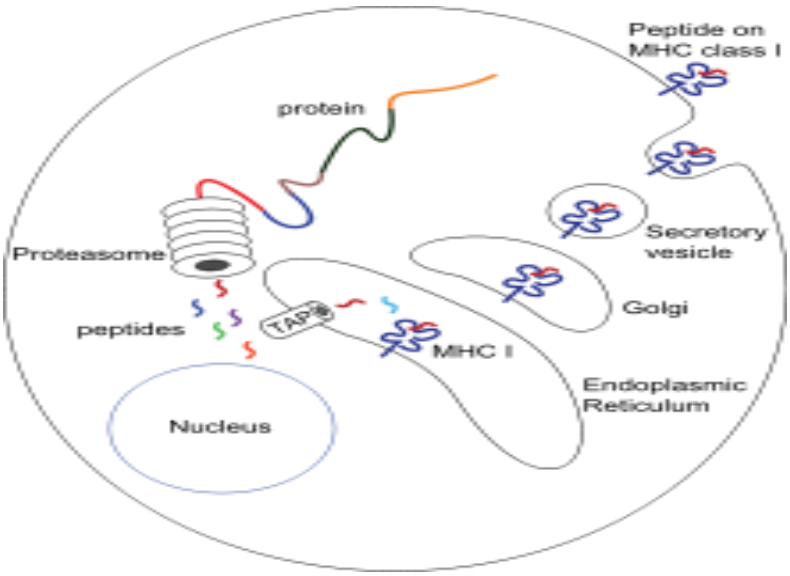

Fig 10.1: Antigen processing and presentation in MHC-I pathway

Cytotoxic T cells (also known as T_c, killer T cell, or cytotoxic T-lymphocyte (CTL)) express CD8 coreceptor are a population of T cells that are specialised for inducing programmed cell death of other cells. Cytotoxic T cells regularly patrol all body cells to maintain the organismal homeostasis. Whenever they encounter signs of disease, caused for example by the presence of viruses or intracellular bacteria or a transformed tumor cell, they initiate processes to destroy the potentially harmful cell. All nucleated cells in the body (along with platelets) display class I major histocompatibility complex (MHC-I molecules). Antigens generated endogenously within these cells are bound to MHC-I molecules and presented on the cell surface. This antigen presentation pathway enables the immune system to detect

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transformed or infected cells displaying peptides from modified-self (mutated) or foreign proteins.

In the presentation process, these proteins are mainly degraded into small peptides by cytosolic proteases in the proteasome, but there are also other cytoplasmic proteolytic pathways. Then, peptides are distributed to the endoplasmic reticulum (ER) via the action of heat shock proteins and the transporter associated with antigen processing (TAP) which translocates the cytosolic peptides into the ER lumen in an ATP-dependent transport mechanism. There are several ER chaperones involved in MHC-I assembly, such as calnexin, clareticulin and tapasin. Peptides are loaded to MHC-I peptide binding groove between two alpha helices at the bottom of the α1 and α2 domains of the MHC class I molecule. After releasing from tapasin, peptide-MHC-I complexes (pMHC-I) exit the ER and are transported to the cell surface by exocytic vesicles.

Naïve anti-viral T cells (CD8+) cannot directly eliminate transformed or infected cells. They have to be activated by the pMHC-I complexes of antigen-presenting cells (APCs). Here, antigen can be presented directly (as described above) or indirectly (cross-presentation) from virus-infected and non-infected cells. After the interaction between pMHC-I and TCR, in presence of costimulatory signals and/or cytokines, T cells are activated, migrate to the peripheral tissues and kill the target cells (infected or damaged cells) by inducing cytotoxicity.

Cross-presentation is a special case in which MHC-I molecules are able to present extracellular antigens, usually displayed only by MHC-II molecules. This ability appears in several APCs, mainly plasmacytoid dendritic cells in tissues that stimulate CD8+ T cells directly. This process is essential when APCs are not directly infected, triggering local antiviral and anti-tumor immune responses immediately without trafficking the APCs in the local lymph nodes.

10.4.2: PRESENTATION OF EXTRACELLULAR ANTIGENS: CLASS II

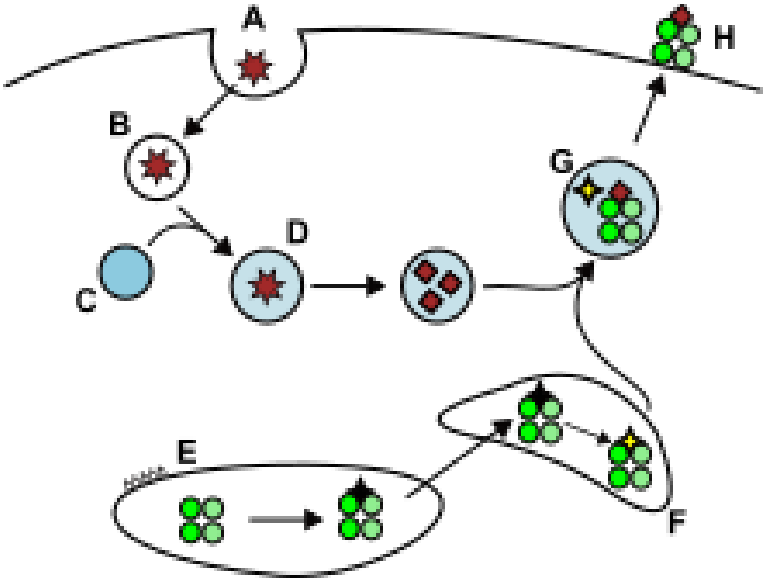

Fig 10.2: MHC II antigen processing pathway

A Foreign protein; B Endosome; C Lysosome; D Late endosome/Endolysosome; E ER; F Golgi apparatus; G CLIP for antigen exchange; H Antigen presentation at plasma membrane.

Antigens from the extracellular space and sometimes also endogenous ones, are enclosed into endocytic vesicles and presented on the cell surface by MHC-II molecules to the helper T cells expressing CD4 molecule. Only APCs do express the class II of MHC molecules on the surface in large quantity, such as dendritic cells, B cells or macrophages, so expression of MHC-II molecules is more cell-specific than MHC-I.

APCs usually internalise exogenous antigens by endocytosis, but also by pinocytosis, macroautophagy, endosomal microautophagy or chaperone-mediated autophagy. In the first case, after internalisation, the antigens are enclosed in vesicles called endosomes. There are three compartements involved in this antigen presentation pathway: early endosomes, late endosomes or endolysosomes and lysosomes, where antigens are hydrolized by lysosome-associated enzymes (acid-dependent hydrolases, glycosidases, proteases, lipases). This process is favored by gradual reduction of the pH. The main proteases in endosomes are cathepsins and the result is the degradation of the antigens into oligopeptides.

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MHC-II molecules are transported from the ER to the MHC class II loading compartment together with the protein Invariant chain (Ii, CD74). A non classical MHC-II molecule (HLA-DO and HLA-DM) catalyses the exchange of part of the CD74 (CLIP peptide) with the peptide antigen. Peptide-MHC-II complexes (pMHC-II) are transported to the plasma membrane and the processed antigen is presented to the helper T cells in the lymph nodes.

APCs undergo a process of maturation while migrating, via chemotactic signals, to lymphoid tissues, in which they lose the phagocytic capacity and develop an increased ability to communicate with T-cells by antigen-presentation. As well as in CD8+ cytotoxic T cells, APCs need pMHC-II and additional costimulatory signals to fully activate naive T helper cells.

Alternative pathway of endogenous antigen processing and presentation over MHC-II molecules exists in medullary thymic epithelial cells (mTEC) via the process of autophagy. It is important for the process of central tolerance of T cells in particular the negative selection of autoreactive clones. Random gene expression of the whole genome is achieved via the action of AIRE and a self-digestion of the expressed molecules presented on both MHC-I and MHC-II molecules.

10.4.3: PRESENTATION OF NATIVE INTACT ANTIGENS TO B CELLS

B-cell receptors on the surface of B cells bind to intact native and undigested antigens of structural nature, rather than to a linear sequence of a peptide which has been digested into small fragments and presented by MHC molecules. Large complexes of intact antigen are presented in lymph nodes to B cells by follicular dendritic cells in the form of immune complexes. Some APCs expressing comparatively lower levels of lysosomal enzymes are thus less likely to digest the antigen they have captured before presenting it to B cells.

10.5: ANTIBODY ENGINEERING

Today, therapeutic monoclonal antibodies (MAbs) represent one of the fastest growing area of the pharmaceutical industry, with exceptional

48.1% growth between 2003 and 2004. There are currently 19 therapeutic antibodies in clinical use that have been approved by the FDA and over 150 antibodies in clinical trials. The MAbs market is expected to almost triple in value over the next six years from $10.3 billion in 2004 to $30.3 billion in 2010, driven by technological evolution from chimeric and humanized to fully human antibodies. Oncology products will continue to dominate the market. However, sales of arthritis, immune and inflammatory disorders products are forecast to grow strongly and account. for 40.1% of the market by 2010. The development focus of the industry is moving away from murine and chimeric antibodies, to humanized and, in particular, fully human antibodies. A wave of fully human antibodies is expected to launch from 2007 onwards. The features that make antibodies attractive drug candidates are high target specificity and their organization into distinct structural and functional domains.

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The characteristic domain structure of antibody has facilitated protein engineering for the development of therapeutic antibodies. When an antibody is designed as a drug, all of its different features including immunogenicity, affinity, stability, effector functions, half-life, and tissue penetration and distribution should be taken into consideration and optimized accordingly. From a manufacturing standpoint, ease of production and stability must also be considered. Nowadays, natural antibodies are tailored by a variety of methods to suit a particular therapeutic use. This review describes the structure and function of antibody, and antibody engineering for the generation of humanized antibodies, antibody fragments, and fully human MAbs. In addition, the technologies for enhancing their biological activities such as affinity maturation, improvement of effector functions, and altering pharmacokinetics are reviewed.

10.5.1: HUMANIZATION OF MURINE ANTIBODIES

Since the hybridoma technology for the production of murine MAb was established in 1975, an anti-CD3 murine MAb (OKT3) was approved as the first therapeutic antibody in 1986. Despite the high expectations of MAb therapy, OKT3 failed as a good treatment for transplantation rejection primarily because OKT3 induced severe human anti-murine antibody

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(HAMA) response in patients. To reduce the immunogenicity of murine antibodies in humans, chimeric antibodies with mouse variable regions and human constant regions were constructed by genetic engineering. In 1994, ReoPro, a chimeric Fab, was introduced as the second therapeutic antibody. Since then, five more chimeric antibodies have been marketed and clinically used (Table 10.3). However, although chimeric antibodies were less immunogenic than murine MAbs, human anti-chimeric antibody (HACAs) responses have been observed.

Table 10.3: Approved therapeutic antibodies

Year (FDA)	Trade name (Generic)	Type of antibody (Target antigen)	Disease indication	Company
1986	OKT 3 (muromanab-CD3)	Murine (CD3)	Allograft rejection	Ortho Biotech
1994	ReoPro (abciximab)	Chimeric Fab (GPIIb/IIIa)	Adjunct to PTCA	Centocor
1995	Panorex (edrecolomab)	Chimeric (CA17-1A)	Colorectal cancer	GSK/Centocor
1997	Rituxan (rituximab)	Chimeric (CD20)	Non-Hodgkins lymphoma	IDEC
1997	Zenapax (daclizumab)	Humanized (IL2R)	Prevention of kidney transplant rejection	PDL
1998	Herceptin (trastuzumab)	Humanized (Her2/neu)	Metastatic breast cancer	Genentech
1998	Synagis (palivizumab)	Humanized (RSV F)	RSV propylaxis	MedImmune
1998	Simulect (basiliximab)	Chimeric (IL2R)	Prevention of kidney transplant rejection	Norvatis
1998	Remicade (infliximab)	Chimeric (TNF-α)	Rheumatoid arthritis, Crohn's disease	Centocor
2000	Mylotarg (gemtuzumab ozogamicin)	Humanized-calicheamicin(CD33)	CD33-acute myeloid leukemia	Celltech
2001	Campath (alemtuzumab)	Humanized (CD52)	B-cell Chronic Lymphocytic Leukemia	Millennium
2002	Zevalin (ibritumomab tiuxetan)	Murine-Y-90 (CD20)	Non-Hodgkins lymphoma	IDEC
2002	Humira (adalimumab)	Human (TNF-α)	Crohn's disease, RA	CAT/BASF
2003	Xolair(omalizumab)	Humanized (IgE)	Asthma	Tanox/Genentech/ Novartis
2003	Raptiva (efalizumab)	Humanized (CD11a)	Psorisis	Xoma/Genentech
2003	Bexxar (tositumomab)	Murine-I-131 (CD20)	Non-Hodgkins lymphoma	Corixa/GSK
2004	Erbitux (cetuximab)	Chimeric (EGFR)	Colorectal cancer	Imclone
2004	Avastin (bevacizumab)	Humanized (VEGF)	CRC, breast, renal, NSCL cancer	Genentech

10.5.2: GENERATION OF FULLY HUMAN MONOCLONAL ANTIBODIES

While the production of murine MAbs is routine, the production of human MAbs by conventional hybridoma technology has been difficult

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because human hybridomas and immortalized cell lines do not stably produce high levels of antibody and in vivo immunization of humans is 20 Antibody Engineering is not feasible for many antigens. However, the development of methods for the expression of antibody fragments such as Fab or ScFv in bacteria and the display of antibody fragments on filamentous bacteriophage as well as powerful techniques for the screening of antibody libraries made it possible to generate human MAbs. This phagedisplay technology is the most used and well-established technology for the development of new human antibodies. As an alternative strategy for producing human MAbs, transgenic mice containing human immunoglobulin germ line locus are used.

Immunization of such transgenic mice results in a human antibody response, from which hybridomas that produce human antibodies can be generated, as in traditional hybridoma technology. In 2002, the first human MAb, Humira, developed by phage display, was approved by the FDA and marketed (Table 10.3). The human MAbs derived from transgenic mice are in clinical trials. Recently, human MAbs are more developed than chimeric or humanized antibodies. Phage display technology In the mammalian immune system, antibodies with high affinities are created by combinatorial recombination of germline antibody gene segments and somatic hypermutation in antibody genes, preferentially within the CDRs. B-lymphocytes expressing affinity-matured antibodies are then selected and expanded. Phage antibody technology can successfully mimic the immune system by cloning large libraries of antibody genes and selecting for binding to a desired target. Immune library Humans exposed to a desired antigen. The display and screening system of antibody libraries through vaccination or disease have high levels of circulating antibodies to the antigen. Therefore, even small libraries (~10^5) from immunized donors give rise to specific antibodies. In addition, because the antibody genes have experienced affinity maturation in vivo, antibodies that do not require further affinity maturation can potentially be isolated. However, isolation of human MAbs from immune library has certain limitations because human antibodies in general cannot be generated by immunization for ethical reasons. Also,

immunological tolerance mechanisms make it very difficult and often impossible to isolate antibodies against self antigens, many of which are potentially important therapeutic targets, particularly in cancer.

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Naïve library Naïve libraries represent the V-gene repertoire created by cloning the antibody genes found in nonimmunized individuals. mRNA is isolated from peripheral lymphocytes, bone marrow, tonsils, and cadaver spleens. Subsequently, IgM and/or IgG variable regions are amplified from mRNA by PCR using degenerate oligonucleotide primer sets and cloned into vectors suitable for screening. IgM repertoires are preferred to IgG because they have not been subjected to tolerance or antigen selection and are thus more diverse. In contrast, IgG chains can be biased by host immune responses and will not react to self antigens. The affinity and specificity of antibodies that can be isolated from naïve libraries is intrinsically linked to library size.

For example, a library consisting of 1010 different clones yielded antibodies with affinities in the low nanomolar range whereas a similar library of 3×10^7 clones only resulted in antibodies with micromolar affinities. Once a library has been made, it can be propagated and used repeatedly to isolate antibodies against numerous antigens. However, poor expression and toxicity to the host bacteria are often issues with antibodies isolated from naïve libraries. These problems may be circumvented by using VH VL VL CL VH CH1. 21 synthetic antibody repertoire libraries. Synthetic library In principle, synthetic libraries have potentials of encoding antibodies to self-antigens. To create fully synthetic repertoires, germline antibody gene segments, VH, DH, and JH or Vκ/λ and Jκ/λ are cloned and arranged combinatorially in vitro so as to reconstitute genes encoding complete VH and VL chains. Such synthetic libraries of 107 −1010 clones gave rise to antibodies with specificity to self-antigens but their affinities are generally not high.

Semi-synthetic libraries have also been generated by selecting one or more antibody frameworks as a scaffold and randomizing sequences within the CDR loops. Because CDR3 contributes most of the antigen-binding contacts, it has been predominantly used as a target for randomization. Early

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semi-synthetic libraries gave rise to antibodies with rather low KD values for antigen binding, typically in the 100 nM range. Randomization of key residues in the light chain CDRs, in addition to the heavy chain CDR3, has been employed to improve diversity, resulting in the recovery of higher affinity antibodies. Recently, developed fully synthetic Human Combinatorial Antibody Library (HuCAL) and constructed 49 combinations of seven VH and seven VL consensus frameworks that represented each of the human V gene families and were optimized for expression in *E. coli*, then randomized the heavy and light chain CDR3 residues of the 49 master genes to build the synthetic library. This highly diverse library has been used to isolate numerous antibodies with high affinities and good expression characteristics in *E. coli***.**

10.5.3: ANTIBODY LIBRARY SCREENING

The screening of combinatorial antibody libraries is one of the most important tools in antibody engineering. Efficient high throughput screening of large libraries has enabled the isolation of specific antibody clones and engineering of antibodies with high affinity, increased stability and improved effector functions. Currently, the most widely used technique for library screening is based on the display of antibodies on the surface of filamentous bacteriophages.

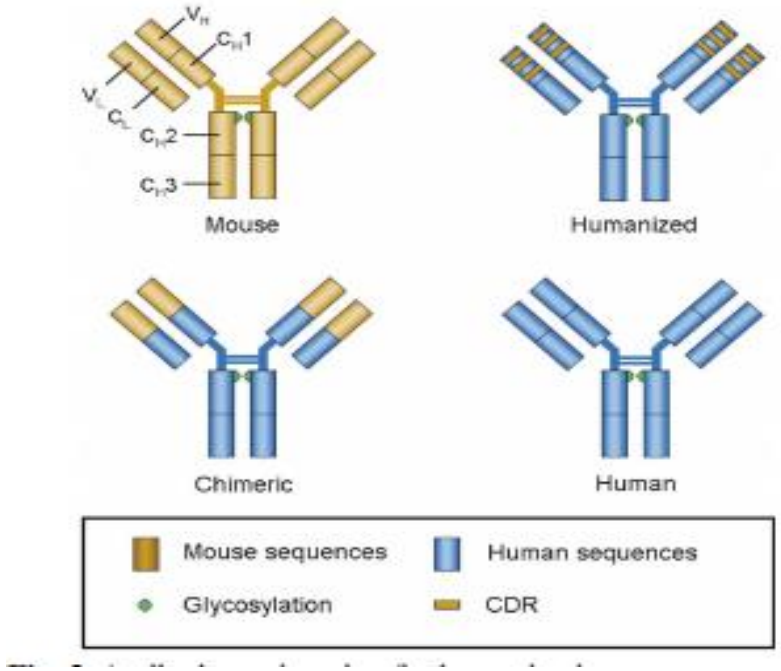

Fig 10.4: Antibody engineering for humanization.

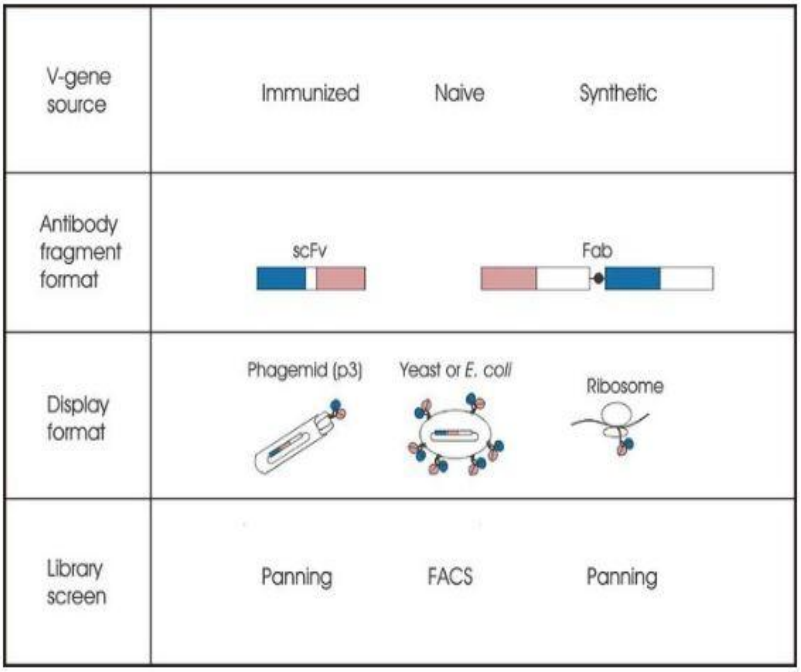

Fig. 10.5: The display and screening system of antibody libraries.

Antibody library in the Fab or ScFv format is fused to a surface protein of phages, most commonly pIII encoded by the gene III. Phages displaying an antibody specific for an antigen can be readily enriched by selective adsorption onto immobilized antigen, a process known as panning. Then the bound phage is eluted from the surface and amplified through infection of E. coli cells. Usually, 5−8 rounds of panning, elution, and amplification are sufficient to select for phages displaying specific antibodies, even from very large libraries (up to 1011 clones). A variety of techniques for the efficient enrichment of specific and high-affinity clones have been developed.

Cell display Antibodies can also be displayed on the surface of microbial cells such as *E. coli* and *Saccharomyces cerevisiae*. For screening purposes, a library of cells, each displaying multiple copies of a different antibody variant, is incubated with a fluorescently tagged ligand in buffer. Cells displaying antibodies that bind the ligand become fluorescently labeled and are isolated by fluorescence-activated cell sorting (FACS). With flow cytometry, the binding of each clone in the library to a particular ligand is determined quantitatively. Parameters such as ligand concentration or time for the dissociation of antibody: ligand complexes can be easily optimized to ensure the isolation of only the highest affinity antibodies.

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At present, cell surface display and FACS cannot be used for the de novo isolation of antibodies from natural or synthetic repertoire libraries because the maximum library diversity that can be realistically screened by FACS is about 5×10^8. These features are particularly significant for antibody affinity maturation. However, repertoire libraries can be screened by 1−2 rounds of panning to reduce the library diversity, followed by cell display technologies that guarantee the isolation of high-affinity, well expressed antibodies. Ribosome display Ribosome display relies on the formation of a ternary complex between ribosomes, mRNA, and the polypeptide. Complexes containing folded antibodies with a desired specificity are enriched by panning against immobilized ligand. The mRNA of the ribosome-mRNA-protein complexes is reverse transcribed to produce the DNA encoding the antibodies responsible for the binding of the complexes to the immobilized ligand. The DNA is then transcribed by RNA polymerase to begin another cycle of ternary complex formation and selection. Alternatively, a covalent link may be established directly between mRNA and the protein it encodes. Covalent mRNA-protein complexes are formed via the reactive amino acid analogue puromycin. The advantages of this technology are that covalent mRNA-protein is more stable and can be subjected to harsh screening conditions to enrich antibodies or other proteins with increased stability and the entire process is performed entirely in vitro without transformation. Using this technology, obtained picomolar affinity antibodies from the synthetic HuCAL library.

10.6: CHECK YOUR PROGRESS

1. …….. a vital immune process which is essential for T cell immune response triggering.
2. mTEC …….
3. FACS ……..

10.7: LET US SUM UP

In this unit, you have learnt about the meaning, definition, need, objectives, importance and functions of Antigen processing and presentation. *Antigen processing* or cytosolic' pathway is an immunological process that

prepares antigens for presentation to special cells of the immune system called T lymphocytes. It is considered to be a stage of antigen presentation pathways. Antigen presentation describes a vital immune process which is essential for T cell immune response triggering. Because T cellsrecognise only fragmented antigens displayed on cell surfaces, antigen processing must occur before the antigen fragment, now bound to the major histocompatibility complex (MHC), is transported to the surface of the cell, a process known as presentation. The screening of combinatorial antibody libraries is one of the most important tools in antibody engineering. Efficient high throughput screening of large libraries has enabled the isolation of specific antibody clones and engineering of antibodies with high affinity, increased stability and improved effector functions. Currently, the most widely used technique for library screening is based on the display of antibodies on the surface of filamentous bacteriophages.

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10.8: UNIT - END EXERCISES

1. Write short notes on antigen processing.
2. Describe about antigen presentation.
3. Explain in detail account on name of approved antibiotics
4. Ellaborate about antiboby engineering.

10.9: ANSWERS CHECK YOUR PROGRESS

1. Antigen presentation
2. Medullary thymic epithelial cells
3. Fluorescence-activated cell sorting

10.10: SUGGESTED READINGS

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UNIT - XI

11.1: INTRODUCTION

Major histocompatibility complex (MHC), group of genes that code for proteins found on the surfaces of cells that help the immune system recognize foreign substances. MHC proteins are found in all higher vertebrates. In human beings the complex is also called the human leukocyte antigen (HLA) system**.** MHC molecules are important components of the immune system because they allow T lymphocytes to detect cells, such as macrophages, that have ingested infectious microorganisms. When a macrophage engulfs a microorganism, it partially digests it and displays peptide fragments of the microbe on its surface, bound to MHC molecules. The T lymphocyte recognizes the foreign fragment attached to the MHC molecule and binds to it, stimulating an immune response. In uninfected healthy cells, the MHC molecule presents peptides from its own cell (self peptides), to which T cells do not normally react.

11.2: OBJECTIVES

After going through this unit, you will be able:

- To know about the mechanisms of Major histocompatibility complex
- To study the Structure and its interactions with Peptide

11.3: MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

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The major histocompatibility complex (MHC) is a large genetic complex with multiple loci. The MHC loci encode two major classes of membrane-bound glycoproteins: **class I** and **class II MHC molecules.** TH cells generally recognize antigen combined with class II molecules, whereas TC cells generally recognize antigen combined with class I molecules (Fig 11.1). MHC molecules function as antigen-recognition molecules, but they do not possess the fine specificity for antigen characteristic of antibodies and T-cell receptors. Rather, each MHC molecule can bind to a spectrum of **antigenic peptides** derived from the intracellular degradation of antigen molecules. In both class I and class II MHC molecules the distal regions (farthest from the membrane) of different alleles display wide variation in their amino acid sequences. These variable regions form a cleft within which the antigenic peptide sits and is presented to T lymphocytes (Fig 11.1). Different allelic forms of the genes encoding class I and class II molecules confer different structures on the antigen-binding cleft with different specificity. Thus the ability to present an antigen to T lymphocytes is influenced by the particular set of alleles that an individual inherits.

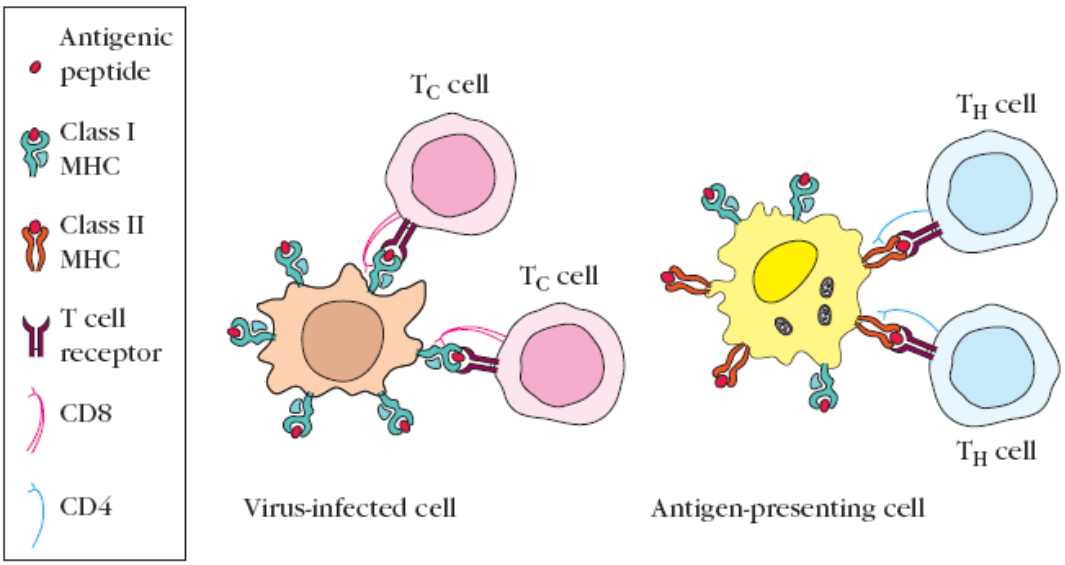

Figure 11.1: The role of MHC molecules in antigen recognition by T cells. (a) Class I MHC molecules are expressed on nearly all nucleated cells. Class II MHC molecules are expressed only on antigen presenting cells. T cells that recognize only antigenic peptides displayed with a class II MHC

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molecule generally function as T helper (TH) cells. T cells that recognize only antigenic peptides displayed with a class I MHC molecule generally function as T cytotoxic (TC) cells.

Complex Antigens Are Degraded (Processed) and Displayed (Presented) with MHC Molecules on the Cell Surface. In order for a foreign protein antigen to be recognized by a T cell, it must be degraded into small antigenic peptides that form complexes with class I or class II MHC molecules. This conversion of proteins into MHC-associated peptide fragments is called *antigen processing and presentation.* Whether a particular antigen will be processed and presented together with class I MHC or class II MHC molecules appears to be determined by the route that the antigen takes to enter a cell (Figure 11.2).

11.3.1: EXOGENOUS ANTIGEN

Exogenous antigen is produced outside of the host cell and enters the cell by endocytosis or phagcytosis. Antigen presenting cells (macrophages, dendritic cells, and B cells) degrade ingested exogenous antigen into peptide fragments within the endocytic processing pathway. Experiments suggest that class II MHC molecules are expressed within the endocytic processing pathway and that peptides produced by degradation of antigen in this pathway bind to the cleft within the class II MHC molecules. The MHC molecules bearing the peptide are then exported to the cell surface. Since expression of class II MHC molecules is limited to antigen- presenting cells, presentation of exogenous peptide– class II MHC complexes is limited to these cells. T cells displaying CD4 recognize antigen combined with class II MHC molecules and thus are said to be *class II MHC restricted.* These cells generally function as T helper cells.

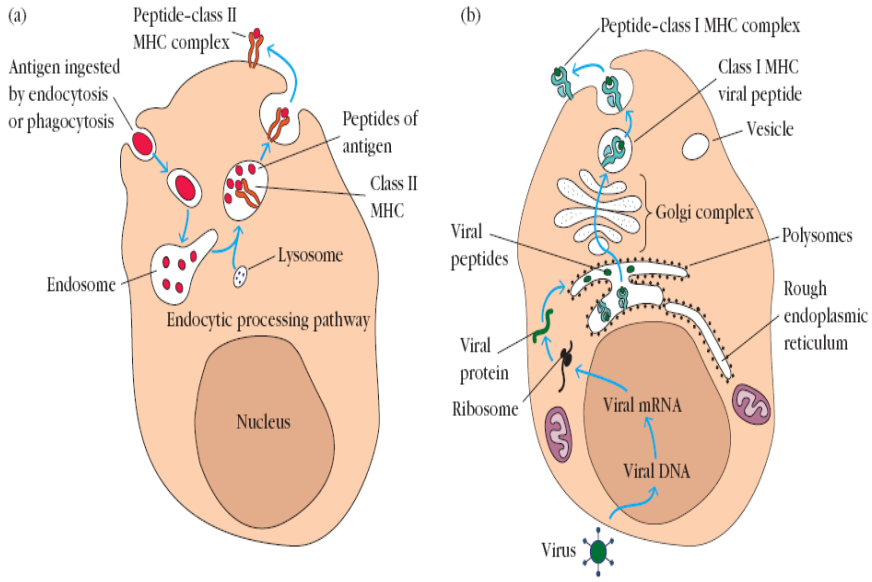

Figure 11.2: Processing and presentation of exogenous and endogenous antigens

11.3.2: ENDOGENOUS ANTIGEN

Endogenous antigen is produced within the host cell itself. Two common examples are viral proteins synthesized within virus-infected host cells and unique proteins synthesized by cancerous cells. Endogenous antigens are degraded into peptide fragments that bind to class I MHC molecules within the endoplasmic reticulum. The peptide–class I MHC complex is then transported to the cell membrane. Since all nucleated cells express class I MHC molecules, all cells producing endogenous antigen use this route to process the antigen. T cells displaying CD8 recognize antigen associated with class I MHC molecules and thus are said to be *class I MHC restricted.* These cytotoxic T cells attack and kill cells displaying the antigen–MHC class I complexes for which their receptors are specific.

11.4 : STRUCTURE AND ITS INTERACTIONS WITH PEPTIDE

Major histocompatibility complex class I (MHC I) deficiency is rare and has a variable clinical phenotype that ranges from totally asymptomatic to a condition similar to that resulting from severe combined immunodeficiency (SCID). Mutations in the genes coding for the transporter associated with antigen presentation (TAP) have been found in patients with this disorder. TAP mediates the translocation of foreign peptide from the

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proteasome into the endoplasmic reticulum so that it can combine with HLA-I molecules, an essential step in the presentation of MHC I–peptide complexes on the cell surface. Patients deficient in TAP have reduced cell-surface expression of MHC I. Immunity to viruses appears intact, with normal antibody titers, but chronic recurrent bacterial sinopulmonary infections are a major problem and patients have a course similar to those of patients with cystic fibrosis and ciliary dyskinesia. Chronic pulmonary infection may cause a reactive cutaneous leukocytoclastic vasculitis and a polyarthritis.

Necrotizing granulomatous inflammation of the nose and skin may resemble manifestations of Wegener granulomatosis or lethal midline granuloma. Histologic assessment of the granuloma shows large numbers of activated natural killer (NK) cells. Unlike patients with Wegener granulomatosis, patients with MHC I deficiency do not have glomerulonephritis or proteinase 3–antineutrophil cytoplasmic antibodies. Immunosuppressive therapy using steroids and cyclophosphamide worsens the clinical condition because it dampens the host immune response to the chronic infection. Care of these patients should include regular chest physiotherapy and antibiotic therapy.

Antibody production appears to be intact in MHC I deficiency, since patients have normal viral titers and a polyclonal hypergammaglobulinemia. Cellular immunity also appears to be unaffected: NK-cell–mediated and T-cell–mediated immunity to viruses is normal and skin testing using purified tuberculin may yield positive results. Thus, it is not known why patients with this disorder are susceptible to pyogenic bacterial infections.

11.5: CHECK YOUR PROGRESS

1. …….. is a large genetic complex with multiple loci
2. The conversion of proteins into MHC-associated peptide fragments is called……
3. MHC is a set of genes that code for cell surface proteins essential for……..

11.6: LET US SUM UP

In this unit, you have learnt about the meaning, definition, need, objectives, importance and functions of Major histocompatibility complex. The major histocompatibility complex (MHC) is a large genetic complex with multiple loci. The MHC loci encode two major classes of membrane-bound glycoproteins: class I and class II MHC molecules. Major histocompatibility complex class I (MHC I) deficiency is rare and has a variable clinical phenotype that ranges from totally asymptomatic to a condition similar to that resulting from severe combined immunodeficiency (SCID). Mutations in the genes coding for the transporter associated with antigen presentation (TAP) have been found in patients with this disorder.

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11.7: UNIT - END EXERCISES

1. Give brief note on MHC.
2. Describe about the types of MHC.
3. Explain about the functions of MHC

11.8: ANSWERS TO CHECK YOUR PROGRESS:

1. MHC
2. Antigen processing and presentation
3. The acquired immune system

11.9: SUGGESTED READINGS

1. Ajoy Paul, **Textbook of Immunology**. Arunabha Sen Publishing, 2016.
2. Ivan M. Roitt, J. Brostoff and D. K. Male, **Immunology**, Gower Medical Publishing, London.1993.
3. Clark WR, **The experimental foundations of modern immunology**. John Wiley and Sons Inc. New York. 1991.
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BLOCK – 4 HYPERSENSITIVITY, TRANSPLANTATION AND VACCINES

UNIT - XII

12.1: INTRODUCTION

Hypersensitivity reactions occur when the normally protective immune system responds abnormally, potentially harming the body. Various autoimmune disorders as well as allergies fall under the umbrella of hypersensitivity reactions, the difference being that allergies are immune reactions to exogenous substances (antigens or allergens), whereas autoimmune diseases arise from an abnormal immune response to endogenous substances (autoantigens). A symptomatic reaction only occurs in sensitized individuals, i.e., they must have had at least one prior asymptomatic contact with the offending antigen.

12.2: OBJECTIVES

After going through this unit, you will be able:

- To know about the Hypersensitivity reactions
- To study the Structure and types of Hypersensitivity reactions

12.3: HYPERSENSITIVITY REACTIONS

Hypersensitivity refers to undesirable (damaging, discomfort producing and sometimes fatal) reactions produced by the normal immune system. Hypersensitivity reactions require a pre-sensitized (immune) state of the host. Hypersensitivity reactions can be divided into four types: type I, type II, type III and type IV, based on the mechanisms involved and time taken for the reaction. Frequently, a particular clinical condition (disease) may involve more than one type of reaction.

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Table 12.1: IAP recommended vaccines

Age	Vaccines
Birth	BCG OPV0 HepB 1
6 weeks	DTwP1/DTaP1 OPV1*/OPV1+IPV1 Hib1 HepB2
10 weeks	DTwP2/DTaP2 OPV2/OPV2+IPV2 Hib 2
14 weeks	DTwP3/DTaP3 OPV3/OPV3+IPV3 Hib3 HepB3**
9 months	Measles
15-18 months	DTwP B1/DTaP B1 OPV4/OPV4+IPVB1 Hib B1 MMR1
2 years	Typhoid#
5 years	DTwP B2/DTaP B2 OPV5 MMR2$
10 years	Tdap/Td& HPV^

* OPV alone if IPV cannot be given

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**The third dose of Hepatitis B can be given at 6 months

Revaccination every 3 years

$The second dose of MMR vaccine can be given at any time 4-8 weeks after the first dose & Tdap preferred to Td, followed by repeat Td every 10 years

^ Only females, three doses at 0, 1-2 and 6 months.

Table 12.2: Vaccines to be given after one to one discussion with parents

Age	*Vaccine*
≥ 6 weeks	Rotavirus vaccine*
	PCV 7#
≥ 15 months	Varicella$
≥ 18 months	Hepatitis A^

*Rotavirus vaccine (2/3 doses (depending on brand) at 4-8 weeks interval)
#PCV 7 (three doses at 6, 10 and 14 weeks and 1 booster at 15-18 months)
$ Varicella (< 13 years single dose, ≥ 13 years two doses at 4-8 weeks interval)
^ Hepatitis A (2 doses at 6 months interval).

(Ref: http://www.iapcoi.com/chapter03immunizationschedule.pdf)

12.3.1: Type I Hypersensitivity

It is also known as immediate or anaphylactic hypersensitivity. The reaction may involve skin (urticaria and eczema), eyes (conjunctivitis), nasopharynx (rhinorrhea, rhinitis), bronchopulmonary tissues (asthma) and gastrointestinal tract (gastroenteritis). The reaction may cause from minor inconvenience to death. The reaction takes 15-30 minutes from the time of exposure to the antigen. Sometimes the reaction may have a delayed onset (10-12 hours). Immediate hypersensitivity is mediated by IgE. The primary cellular component in this hypersensitivity is mast cell or basophil. The reaction is amplified and/or modified by platelets, neutrophils and eosinophils. A biopsy of the reaction site demonstrates mainly mast cells and eosinophils. The mechanism of reaction involves preferential production of IgE, in response to certain antigens, allergens (Fig 12.1). IgE has very high affinity for its receptor on mast cells and basophils. A subsequent exposure

to the same allergen cross links the cell-bound IgE and triggers the release of various pharmacologically active substances (Figure 12.1). Cross-linking of IgE Fc-receptor is important in mast cell triggering. Mast cell degranulation is preceded by increased Ca++ influx, which is a crucial process; ionophores which increase cytoplasmic Ca++ also promote degranulation, whereas, agents which deplete cytoplasmic Ca++ suppress degranulation.

The agents released from mast cells and their effects are listed in Table 6. Mast cells may be triggered by other stimuli such as exercise, emotional stress, chemicals (*e.g.*, photographic developing medium, calcium ionophores, codeine, *etc.*), anaphylotoxins (*e.g.*, C4a, C3a, C5a, *etc.*). These reactions mediated by agents without IgE-allergen interaction are not hypersensitivity reactions, although they produce the same symptoms.

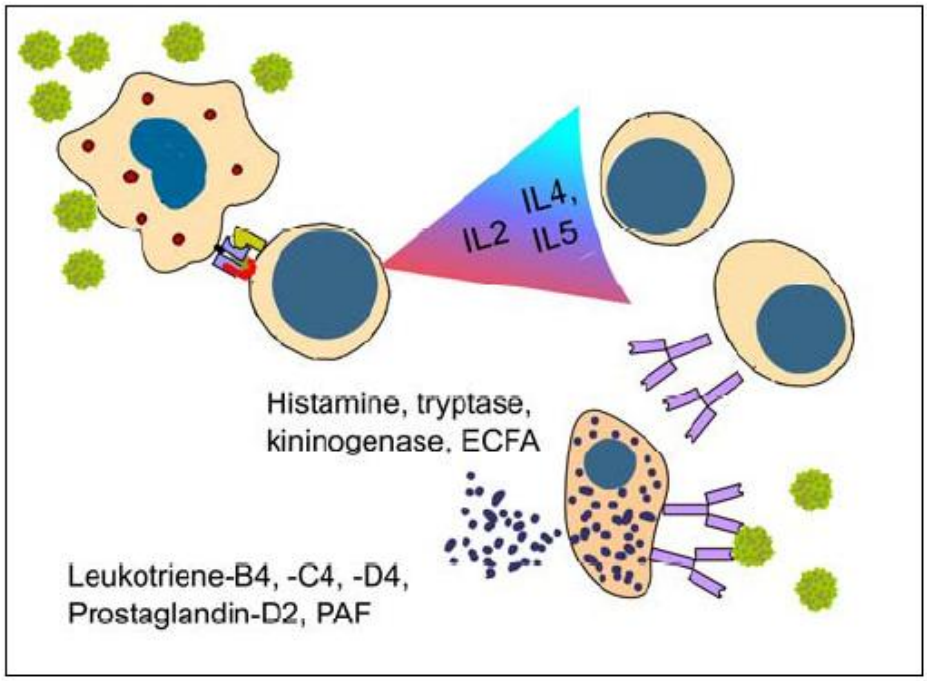

Fig 12.1: Induction and effector mechanisms in type I hypersensitivity

The reaction is amplified by PAF (platelet activation factor) which causes platelet aggregation and release of histamine, heparin and vasoactive amines. Eosinophil chemotactic factor of anaphylaxis (ECF-A) and neutrophils chemotactic factors attract eosinophils and neutrophils, respectively, which release various hydrolytic enzymes that cause necrosis. Eosinophil may also control the local reaction by releasing arylsulphatase,

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histaminase, phospholipase-D and prostaglandin-E, although this role of eosinophils is now in question. Cyclic nucleotides appear to play a significant role in the modulation of immediate hypersensitivity reaction, although their exact function is ill understood. Substances which alter cAMP and cGMP levels significantly alter the allergic symptoms. Thus, substances that increase intracellular cAMP seem to relieve allergic symptoms, particularly broncho-pulmonary ones, and are used therapeutically. Conversely, agents that decrease cAMP or stimulate cGMP aggravate these allergic conditions.

Table 12.3: Pharmacologic Mediators of Immediate Hypersensitivity

Mediator	
Preformed mediators in the granules	
Histamine Tryptase Kininogenase ECF-A (Tetrapeptidase)	Bronchoconstruction, mucus secretion, vasodialtion, vascular permeability , proteolysis, kinins and vasodialtion, vascular permeability, edema Attract esinophil and neutrophils
Newly formed Mediators	
Leukotrine B4 Leukotrine C4, D 4 Prostaglandins D2 PAF	Basophil attractant Same as histamine but 1000X more potent Edema and pain Platelet aggregation and heparin release; Microthrombi

Diagnostic tests for immediate hypersensitivity include skin (prick and intradermal) tests, measurement of total IgE and specific IgE antibodies against the suspected allergens. Total IgE and specific IgE antibodies are

measured by a modification of enzyme immunoassay (ELISA). Increased IgE levels are indicative of atopic condition, although IgE may be elevated in some non atopic diseases (*e.g.*, myelomas, helminthic infection, *etc.*). Symptomatic treatment is achieved with antihistamines which block histamine receptors. Chromolyn sodium inhibits mast cell degranulation, probably, by inhibiting Ca++ influx. Late onset allergic symptoms, particularly bronchoconstriction which is mediated by leukotrienes are treated with leukotriene receptor blockers (Singulair, Accolate) or inhibitors of cyclooxygenase pathway (Zileutoin). Symptomatic, although short term relief from broncho constriction is provided by bronchodilators (inhalants) such as isoproterenol derivatives (Terbutaline, Albuterol). Thophylline elevates cAMP by inhibiting cAMP-phosphodiesterase and inhibits intracellular Ca++ release is also used to relieve bronchopulmonary symptoms. There appears to be a genetic predisposition for atopic diseases and there is evidence for HLA (A2) association.

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Hyposensitization (immunotherapy or desensitization) is another treatment modality which is successful in a number of allergies, particularly to insect venoms and, to some extent, pollens. The mechanism is not clear, but there is a correlation between appearance of IgG (blocking) antibodies and relief from symptoms. Suppressor T cells that specifically inhibit IgE antibodies may play a role.

12.3.2: TYPE II HYPERSENSITIVITY

It is also known as cytotoxic hypersensitivity and may affect a variety of organs and tissues. The antigens are normally endogenous, although exogenous chemicals (haptens) which can attach to cell membranes can also lead to type II hypersensitivity. Drug-induced hemolytic anemia, granulocytopenia and thrombocytopenia are such examples.

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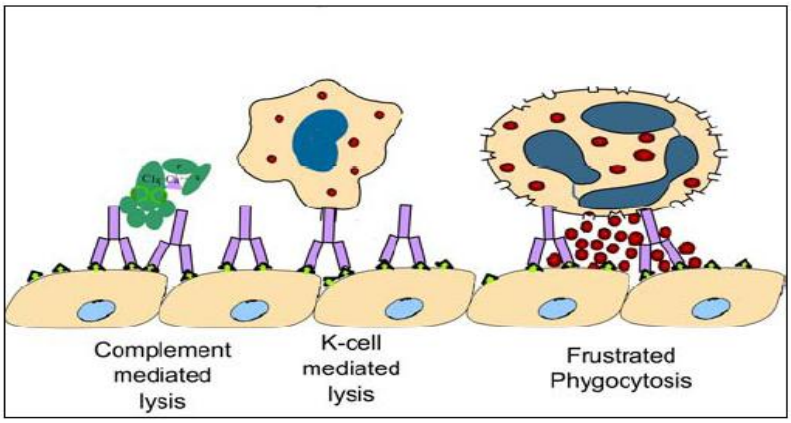

Figure 4.4: Type II hypersensitivity mechanisms

The reaction time is minutes to hours. It is primarily mediated by antibodies of IgM or IgG class and complement. Phagocytes and K cells may also play a role (ADCC). The lesion contains antibody, complement and neutrophils. Diagnostic tests include detection of circulating antibody against tissues involved and the presence of antibody and complement in the lesion (biopsy) by immunofluorescence. The staining pattern is normally smooth and linear, such as that seen in Good pasture's nephritis (renal and lung basement membrane) and pemphigus (skin intercellular protein, desmosome). Treatment involves anti-inflammatory and immuno-suppressive agents.

12.3.3: TYPE III HYPERSENSITIVITY

It is also known as **immune complex hypersensitivity**. The reaction may be general (*e.g.*, serum sickness) or may involve individual organs including skin (*e.g.*, systemic lupus erythematosus, Arthus reaction), kidneys (*e.g.*, lupus nephritis), lungs (*e.g.*, aspergillosis), blood vessels (*e.g.*, polyarteritis), joints (*e.g.*, rheumatoid arthritis) or other organs. This reaction may be the pathogenic mechanism of diseases caused by many microorganisms. The reaction may take 3-10 hours after exposure to the antigen (as in Arthus reaction). It is mediated by soluble immune complexes.

They are mostly of IgG class, although IgM may also be involved.

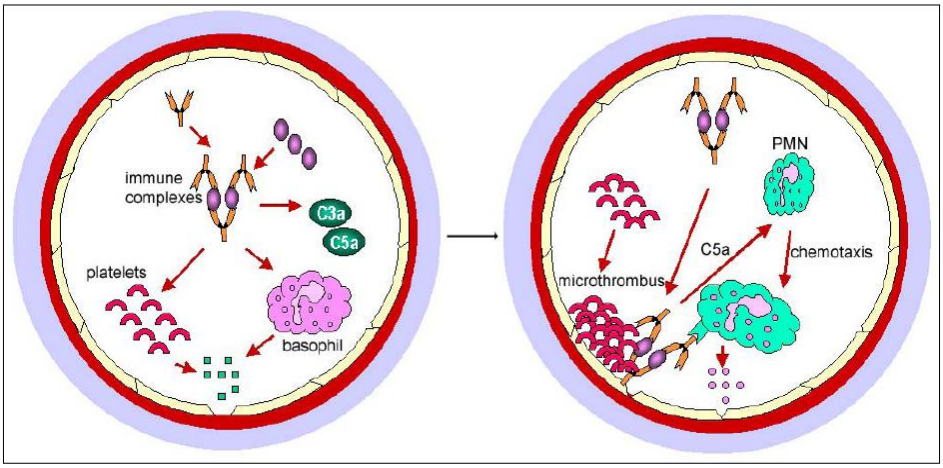

Fig 12.2: Mechanism of damage in type-III hypersensitivity

The antigen may be exogenous (chronic bacterial, viral or parasitic infections), or endogenous (non-organ specific autoimmunity: *e.g.*, systemic lupus eythematosus, SLE). The antigen is soluble and not attached to the organ involved. Primary components are soluble immune complexes and complement (C3a, 4a and 5a).

The damage is caused by platelets and neutrophils (Figure 12.2). The lesion contains primarily neutrophils and deposits of immune complexes and complement. Macrophages infiltrating in later stages may be involved in the healing process. The affinity of antibody and size of immune complexes are important in production of disease and determining the tissue involved. Diagnosis involves examination of tissue biopsies for deposits of Ig and complement by immunofluorescence. The immunofluorescent staining in type III hypersensitivity is granular (as opposed to linear in type II: Goodpasture). Presence of immune complexes in serum and depletion in complement level are also diagnostic. Polyethylene glycol mediated turbidity (nephelometry), binding of C1q and Raji cell test are utilized to detect immune complexes. Treatment includes anti-inflammatory agents.

12.3.4: TYPE IV HYPERSENSITIVITY

It is also known as **cell mediated** or **delayed type hypersensitivity**. The classical example of this hypersensitivity is **tuberculin** (**Montoux**)

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reaction which peaks 48 hours after the injection of antigen (PPD or old tuberculin). The lesion is characterized by induration and erythema. Type IV hypersensitivity is involved in the pathogenesis of many autoimmune and infectious diseases (tuberculosis, leprosy, blastomycosis, histoplasmosis, toxoplasmosis, leishmaniasis, *etc.*) and granulomas due to infections and foreign antigens. Another form of delayed hypersensitivity is **contact dermatitis** (poison ivy, chemicals, heavy metals, *etc.*) in which the lesions are more papular.Type IV hypersensitivity can be classified into three categories depending on the time of onset and clinical and histological presentation (Table 12.3). Mechanisms of damage in delayed hypersensitivity include T lymphocytes and monocytes and/or macrophages.

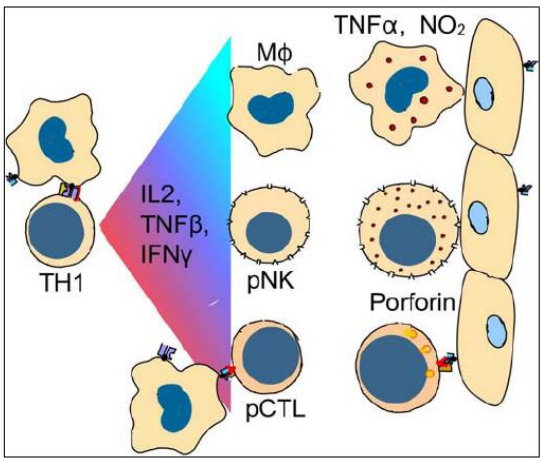

Fig 12.3: Mechanisms of damage in delayed hypersensitivity

Cytotoxic T cells (Tc) cause direct damage whereas helper T (TH1) cells secrete cytokines which activate cytotoxic T cells and recruit and activate monocytes and macrophages, which cause the bulk of the damage (Fig 12.3). The delayed hypersensitivity lesions mainly contain monocytes and a few T cells.

Table 12.3: Delayed hypersensitivity reactions

Type	Reaction time	Clinical appearance	Histology	Antigen site
Contact	48-72hr	Eczema	Lymphocytes, followed by macrophages; edema of epidermis	Epidermal (Organic chemicals, poison , heavy metals *etc*)
Tuberculin	48-72hr	Local induration	Lymphocytes, monocytes, macrophages	Intradermal (tuberculin, lepromin, *etc)*
Granuloma	21-28 days	Hardening	Macrophages, epitheloid and giant cells, fibrosis	Persistent antigen or foreign body presence (tuberculosis, leprosy, *etc)*

Major lymphokines involved in delayed hypersensitivity reaction include monocyte chemotactic factor, interleukin-2, interferon-(, TNF "/$, *etc.* Diagnostic tests *in vivo* include delayed cutaneous reaction (*e.g.* Montoux test) and patch test (for contact dermatitis).

In vitro tests for delayed hypersensitivity include mitogenic response, lympho-cytotoxicity and IL-2 production. Corticosteroids and other immunosuppressive agents are used in treatment.

12.4: AUTO IMMUNE DISORDERS

The purpose of the immune system is to keep infections, caused by certain bacteria and viruses, out of the body, and to destroy any infections that do invade the body. When the immune system does not function properly, a number of diseases can occur. Allergies and increased hypersensitivity to certain substances are considered immune system disorders. An autoimmune disorder occurs when the immune system attacks its own healthy cells. Autoimmune disorders are seen more often in women than men, and are also seen more frequently in certain populations. For example, lupus is more common in African-American and Hispanic women than in Caucasian women of European ancestry. Rheumatoid arthritis and

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scleroderma affect a higher percentage of Native Americans than the general U.S. population.

Table 12.4: Comparison of Different Types of hypersensitivity

Characteristics	Type-1 (Anaphylactic)	Type 2 (Cytotoxic)	Type 3 (Immune complex))	Type 4 (Delayed type)
Antibody	IgG	IgG, IgM	IgG, IgM	None
Antigen	Exogenous	Cell surface	Soluble	Tissue and Organs
Response time	15-30 mins	Minutes- hours	3-8hrs	48-72hrs
Appearance	Weal & flare	Lysis & necrosis	Erythema and edema, necrosis	Erythema and induration
Histology	Basophil and Esinophil	Antibody and Complement	Complement & neutrophils	Monocyte & lymphocyte
Transferred with	Antibody	Antibody	Antibody	T-cells
Examples	Allergic asthma, hay fever	Erythroblastosis fetalis, Goodpasture's nephritis	SLE farmerslung disease	Tuberculin test, Poison ivy, granuloma.

12.4.1: CAUSES OF AUTOIMMUNE DISORDERS

Most autoimmune disorders are thought to be multifactorial. Multifactorial inheritance means that "many factors" are involved in causing a health problem. The factors are usually both genetic and environmental. A combination of genes from both parents, in addition to unknown environmental factors, produce the trait or condition. Multifactorial traits do recur in families because they are partly caused by genes. The environmental factors are generally thought to trigger an immune response to certain environmental influences such as viral infections or sunlight. Once an autoimmune disease is present in a family, other relatives may be at risk to

develop the same autoimmune disease, or a different autoimmune disease. For example, a mother may have rheumatoid arthritis, and one of her siblings may develop lupus. Genes and family history are not the only factors involved in determining who will get an autoimmune disease. In other words, if autoimmune diseases are in your family, it does not automatically mean that all relatives will develop one of these conditions. A positive family history of autoimmune disorders means that there is a genetic predisposition that may increase your risk or your child's risk to develop an autoimmune disease.

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A group of genes on chromosome 6 codes for the HLA (human leukocyte antigens) which play a major role in predisposition and resistance to disease. Specific HLA influence the development of many common disorders, which may be autoimmune related. A person who has the specific HLA type associated with the disease may have a genetic predisposition to develop the condition. It is important to understand that a person without these antigens may also develop an autoimmune disease, so that HLA testing is not diagnostic or accurate for prediction of these conditions. Presymptomatic and prenatal testing is not available for autoimmune disorders.

12.4.2: RISKS FOR D ENVELOPING AUTOIMMUNE DISORDERS

The table below gives examples of autoimmune disorders and risks for developing these disorders depending on your family history (Table 12.5).

Table 12.5: Examples of Autoimmune Disorders

Autoimmune Disease	Risks
Behcet's Disease	Up to a 10% risk for first degree relatives
Crohn Disease*	10-20% of cases appear to run in families; several genes at different locations may contribute to this disease. Risk for first degree

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	relatives depends on the study (10-40 fold increase).
Dermatitis herpetiformis	Autosomal dominant inheritance (50% recurrence risk) has been reported, however, females are more often affected than males.
Grave's Disease	Clusters in families with other autoimmune disorders; recurrence risks not specified.
Hashimoto's thyroiditis .	Clusters in families with other autoimmune disorders; recurrence risks not specified
Multiple Sclerosis	3-5% for first degree relatives; 38% for monozygotic twins; 30% for offspring of 2 affected parents; 0.5-3% for brothers of an affected sibling; 13% for a brother to have MS if he has an affected sibling and one parent with MS (age of onset 21-30 yrs); 1.5-8% for sisters of an affected sibling; 7-50% for a sister to have MS if she has an affected sibling and one affected parent.
Myasthenia Gravis	Genetically heterogeneous. Usually occurs by chance; 1 to 4 % of cases cluster in a family; familial predisposition may be due to autoimmunity in general; there is also an autosomal recessive (congenital/infantile form; 25% RR) and autosomal dominant form (50% RR).

Pemphigus vulgaris	Some studies show autosomal dominant inheritance with up to 50% recurrence risk
Pernicious Anemia	20% of relatives with pernicious anemia, have pernicious anemia, especially first-degree female relatives; more common in Caucasians; recurrence risks not specified.
Polymyositis/Dermatomyositis .	Clusters in families with other autoimmune disorders; recurrence risks not specified
Psoriasis	One-third of cases appear to run in families. More commonly seen in Caucasians, and more common in women. Lifetime risk if one parent has psoriasis = 0.28; if both parents=0.65. If one parent and one affected child, RR=0.51; if both parents and one affected child, RR=0.83. If one affected child (parents unaffected), RR=0.24.
Rheumatoid Arthritis	Females are 2-3 times more likely to be affected than males; risk for parents and siblings of an affected individual is about 2-4.5%. For an affected individual to have an affected child, risk is 0.7%.
Scleroderma	1% RR for first degree relatives
Sjogren syndrome	9/10 pts. are women; 50% of cases occur alone while 50% of cases

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	occur in the presence of another autoimmune disease; recurrence risks not specified.
Spondyloarthropathies (such as ankylosing spondylitis)	For first degree relatives, general RR 4% for ankylosing spondylitis; w/HLAB27 antigen RR 9%; w/out HLA-B27 antigen, RR <1%
Systemic lupus erythematosus (SLE)	9/10 pts. are women; first degree relatives have a 8-9 fold increased risk over the general population.
Type I or Immune Mediated Diabetes Mellitus.	RR=4%; up to 50% if multiple affected Relatives
Ulcerative Colitis*	10-20% of cases appear to run in families; several genes at different locations may contribute to this disease. Recurrence risks range depending on the study (10-40 fold increase).
Vitiligo	20% of individuals with vitiligo have a positive family history of vitiligo; relative risk for vitiligo is 7 for parents, 12 for siblings and 36 for children. Relative risk for second degree relatives varies from 1-16.

*Inflammatory bowel disease (IBD) is a group of chronic disorders that causes inflammation or ulceration in the small and large intestines. Most often, IBD is classified either as ulcerative colitis or Crohn's disease. While ulcerative colitis affects the inner lining of the colon and rectum, Crohn's disease extends into the deeper layers of the intestinal wall. It is a chronic condition and may recur at various times over a lifetime.

RR=recurrence risks

Relative risk = a measure of comparative risk of developing a disease or condition; a relative risk of 2 means you are twice as likely to develop the condition.

First degree relatives = parents, siblings, children Second degree relatives = aunts/uncles, nieces/nephews, grandparents.

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12.4.3: AUTO IMMUNE DISORDERS AND IMMUNODEFICIENCY DISEASE

The basis of autoimmunity in primary immunodeficiency diseases and methods of classification

For many primary immunodeficiency diseases, the basis of the autoimmunity is the inability of the host to eradicate microbial pathogens and their antigens completely through the usual immune pathways. The result is a compensatory, often exaggerated and chronic inflammatory response by less effective alternative immune pathways, which damage not only infected cells but also surrounding tissue (Figure 12.4). Thus, in many affected patients, autoimmunity is not a breakdown of tolerance to self-antigens; rather, it is tissue damage incurred as the host attempts to rid itself of foreign immunogens.

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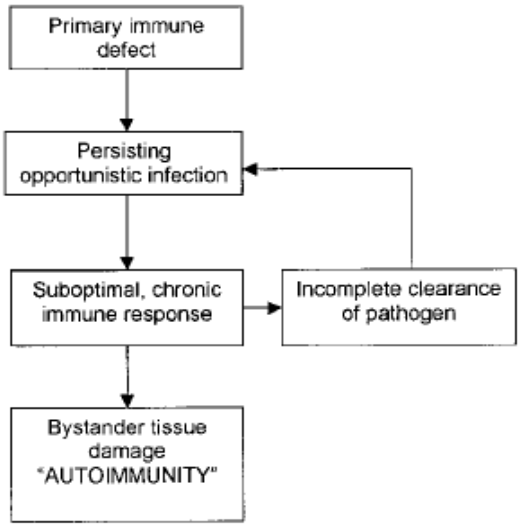

Figure 12.4: Common mechanism of autoimmunity in several primary immunodeficiency diseases.

Classification of autoimmune phenomena in primary immunodeficiency diseases is problematic. One method of classification is based on Classification of autoimmune phenomena in primary immunodeficiency diseases is problematic. One method of classification is based on the underlying genetic defect. This results in a long list that does little to help clinicians plan treatment strategy (Table 12.4). An alternative classification system is based on the proposed immune pathway causing the autoimmunity. This may seem more logical because it indicates the part of the immune system that must be suppressed to control the problem. However, suppression of compensatory immune pathways may not only decrease the inflammation causing the autoimmunity but may also leave the patient open to overwhelming sepsis and death. Furthermore, the problem is likely to recur after the immune-suppression has been reduced because the underlying primary immunodeficiency has not been corrected. A third method of classification—and that used here is to organize autoimmune phenomena according to which functional component of the immune system is defective (Table 12.4). This method is based on the idea that treatment should ideally focus on replacing the defective immune component. For many severe primary immunodeficiency diseases, BMT is currently the only effective treatment. Specific gene therapy is still in its infancy.

Table 12.6: Genetic defects underlying primary immune deficiencies associated with autoimmunity

Disease	Genes
Autoimmune lymphoproliferative syndrome (ALPS)	Fas, caspase 10
Autoimmune polyglandular syndrome I (APECED)	Autoimmune regulator
Hyper-IgM syndrome	CD40L, activation-induced cytidine deaminase
Chediak-Higashi syndrome (CHS)	Lysosomal trafficking regulator
Chronic granulomatous disease (CGD)	NADPH oxidase
Complement deficiencies	C1q, C1r, C1s, C2, C4, mannose-binding protein
Familial hemophagocytic lymphohistiocytosis (FHL)	Perforin
Familial Hibernian fever	Type I tumor necrosis factor (TNF) receptor
Familial Mediterranean fever	MEFV
Griscelli syndrome	RAB27A
Hyper-IgD and periodic fever syndrome	Mevalonate kinase
MHC I deficiency	Transporter associated with antigen presentation
MHC II deficiency	CIITA, RFXANF, RFX5, RFXAP
Leukocyte adhesion deficiency (LAD)	CD18
Omenn syndrome	Recombinase-activating genes 1 and 2
Wiskott-Aldrich syndrome (WAS)	Wiskott-Aldrich syndrome protein
X-linked lymphoproliferative disease (XLP)	SLAM-associated protein (SAP)

CD40L indicates CD40 ligand; NADPH, nicotinamide adenine dinucleotide phosphate; MHC, major histocompatibility complex; CIITA, class II transactivator protein; RFX, regulatory factor X; and SLAM, signaling lymphocyte activation molecule.

NOTES

12.4.4: SPECIFIC PRIMARY IMMUNODEFICIENCY DISEASES ASSOCIATED WITH AUTOIMMUNITY

Phagocyte disorders

Chronic granulomatous disease: Chronic granulomatous disease (CGD) consists of a group of X-linked and autosomal recessive disorders of neutrophil production of nicotinamide adenine dinucleotide phosphate oxidase that affect 1 in 200 000 children. In these disorders, neutrophils are

NOTES

incapable of completely eradicating phagocytosed catalase-positive bacteria and fungi. Patients have recurrent bacterial and fungal (especially *Aspergillus*) infections that may affect any part of the body, including the skin, lungs, liver, and bone. Death is usually caused by respiratory failure. The average patient survives to the age of 20 years. Along with infective complications, chronic inflammation of the gut (similar to Crohn disease) is a common (occurring in 50% of patients) although less well-recognized cause of morbidity in patients with CGD. Gastrointestinal symptoms of abdominal pain, diarrhea, and malabsorption respond variably to immunemodulatory agents (prednisolone, cyclosporin A, and interferon-g), and there is an increased risk of reactivating latent infection. Inflammation can occur anywhere from mouth to anus.

Widespread granuloma formation and fibrosis may result in stomatitis and oral ulcers; esophagitis associated with dysphagia, dysmotility, and obstruction; gastric outlet obstruction and eosinophilic gastritis; intestinal villous atrophy or granulomatous colitis; and liver fibrosis and cirrhosis. Chronic inflammation in other systems, such as the lungs, may produce fibrosis and corpulmonale. Specific pathogens are usually not isolated. The pathogenesis of this chronic inflammation is unknown, but one possibility is that the inability to eradicate bacterial and fungal immunogens completely promotes a chronic inflammatory reaction that destroys surrounding tissue. Carriers of the defective genes involved may not be entirely asymptomatic: 10% of X-linked recessive kindred and 3% of autosomal recessive kindred have family members with discoid lupus. Because of the poor long-term prognosis in CGD, early BMT is now being recommended by many specialist centers if a matched sibling donor is available. Although gene therapy for CGD has been attempted, it has not been successful.

12.4.5: LEUKOCYTE ADHESION MOLECULE DEFICIENCY

Leukocyte adhesion molecule deficiency (LAD) is due to defects in integrin family adhesion molecules (CD18) that are essential for binding of neutrophils to the endothelial surface as a prerequisite to infiltration into inflamed tissues. Consequently, patients with LAD have high circulating

neutrophil counts, resulting in severe, recurrent life-threatening infections associated with a lack of pus formation. Persistence of bacterial antigens and the inability of neutrophils to escape from the circulation may produce a persistent leukocytoclastic vasculitis. Early death can be averted only by BMT.

NOTES

12.5: CHECK YOUR PROGRESS

1. …….. is also known as cell mediated or delayed type hypersensitivity
2. SLE…….
3. CGD……
4. What is LAD…….

12.6: LET US SUM UP

In this unit, you have learnt about the meaning, definition, need, objectives and importance of Hypersenstivity reactions and Autoimmune disorders. Hypersensitivity reactions are commonly classified into four types. Type I hypersensitivity reactions are immediate allergic reactions (e.g., food and pollen allergies, asthma, anaphylaxis). Type II hypersensitivity reactions are referred to as cytotoxic, as they involve antibodies that are specific to particular tissues within the body and cause destruction of cells in these tissues (e.g., autoimmune hemolytic anemia, Good pasture syndrome). Type III hypersensitivity reactions are immune complex-mediated, with tissue damage caused by antigen-antibody complex deposition (e.g., many vasculitides and glomerulonephritides). Type IV hypersensitivity reactions (e.g., TB skin tests, contact dermatitis) are delayed and cell-mediated and are the only hypersensitivity reaction that involves sensitized T lymphocytes rather than antibodies. Unlike true hypersensitivity reactions, which occur after sensitization, nonallergic hypersensitivity reactions (e.g., pseudoallergies) cause mast cell activation and histamine release after initial exposure to a trigger substance (e.g., radiocontrast media).

12.7: UNIT - END EXERCISES

1. Write in Detail about the Hypersensitivity

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2. Define autoimmune disorder? Explain the causes for autoimmune disorder.
3. Write a brief note on types of Hypersensitivity

12.8: ANSWERS TO CHECK YOUR PROGRESS

1. Type IV Hypersensitivity.
2. Systemic lupus erythematosus
3. Chronic granulomatous disease.
4. Leukocyte adhesion molecule deficiency

12.9: SUGGESTED READINGS

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UNIT - XIII

NOTES

13.1: INTRODUCTION

Organ transplantation is a medical procedure in which an organ is removed from one body and placed in the body of a recipient, to replace a damaged or missing organ. The donor and recipient may be at the same location, or organs may be transported from a donor site to another location. Organs and/or tissues that are transplanted within the same person's body are called autografts. Transplants that are recently performed between two subjects of the same species are called allografts. Allografts can either be from a living or cadaveric source.

13.2: OBJECTIVES

After going through this unit, you will be able:

- To know about the Organ Transplantation
- To study the Structure of HLA Tissue Typing
- To know about the Oncogenes and Antioncogenes

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13.3: ORGAN TRANSPLANTATION

Transplantation can be defined as the transfer of cells, tissues, or organs from one site in an individual to another, or between two individuals. In the latter case, the individual who provides the transplant organ is termed a *donor* and the individual receiving the transplant is known as the *recipient*.

13.3.1: TYPES OF TRANSPLANTS

There are four different basic types of transplants. These reflect the genetic relationship of the donor to the recipient. The degree of immune response to a graft varies with the type of graft (Fig. 13.1).

1. **Autograft**: An autograft is the transfer of individual's own tissue or organ from one site to another site in the body. In other words, the recipient is also the donor. Common examples of autografts include skin transplants in burn patients and bypass surgery in patients suffering from coronary heart disease.
2. **Syngraft**: A syngraft is a transfer of tissue between two genetically identical individuals, i.e., identical twins. The first successful human kidney transplant was a syngraft, carried out in 1954 between identical twins.
3. **Allograft**: An allograft is the transfer of tissue or an organ between genetically different members of the same species, i.e., from one human to another. This is the predominant form of transplantation today, and allografts have dominated transplant research for many years.
4. **Xenograft**: A xenograft is the transfer of tissues or organs between members of different species. It represents the most disparate of genetic relationships and is always rejected by an immunocompetent recipient.

A major limitation in the success of transplantation is the immune response of the recipient to the donor tissue. Problem of rejection with autografts is usually minimal or absent. It is only when tissues from "others" are used, as in allografts and xenografts, the problem of rejection arises.

Transplantation immunology is the study of the events that occur after an allograft or a xenograft is removed from a donor and then transplanted into a recipient.

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Allografts are rejected by a process called allograft reaction. Graft rejection is the consequence of an immune response mounted by the recipient against the graft as a consequence of the incompatibility between tissue antigens of the donor and recipient. The problem of rejection was first recognized when attempts to replace damaged skin on burn patients with skin from unrelated donors were found to be relatively unsuccessful.

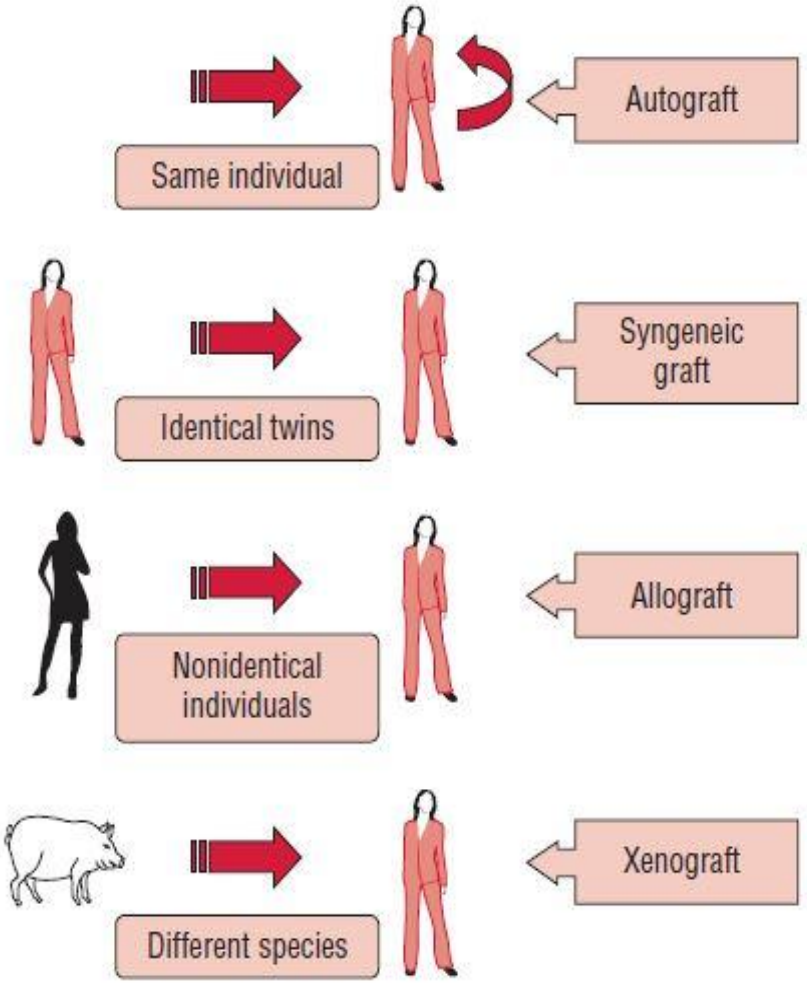

Fig 13.1: Grafts in transplantation

During a period of 1–2 weeks, the skin would undergo necrosis and peel off. The failure of such grafts led scientists like Peter Medawar and many others to study skin transplantation in animal models. These experiments established that the failure of skin grafting was caused by an

inflammatory reaction, now called as rejection. Results of several experimental studies imply that adaptive immune response is responsible for rejection.

13.4: HLA TISSUE TYPING

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The genetic loci involved in the rejection of foreign organs are known as the major histocompatibility complex (MHC), and highly polymorphic cell surface molecules are encoded by the MHC. The human MHC is called the HLA (Human Leukocyte Antigen) system because these antigens were first identified and characterized using alloantibodies against leukocytes. Leukocyte-agglutinating antibodies (leukoagglutinins) were observed in sera from multiparous women and previously transfused patients. Graft rejection was found to be associated with the development of antibodies against allogeneic leukocytes. The HLA system has been well known as transplantation antigens, but the primary biological role of HLA molecules is in the regulation of immune response

13.4.1: Histocompatibility antigens:

Cells expressing class II MHC (major histocompatibility complex) antigens play a major role in sensitizing the immune system of the recipient. The sensitization of alloreactive helper T lymphocytes from the recipient is followed by their clonal expansion. This in turn causes multiple immunological and inflammatory phenomena. Some of these phenomena are mediated by activated T lymphocytes and also by antibodies, which eventually result in graft rejection. Recognition of transplanted cells as self or foreign is determined by polymorphic genes that are inherited from both parents and are expressed codominantly. MHC molecules are responsible for almost all strong rejection reactions. The rejection reactions are mediated by T cells. Both CD4 and CD8 cells coordinate to bring about an effective and pronounced rejection reaction. Nude mice, which lack a thymus, are incapable of launching an allogeneic immune response.

Histocompatibility is tissue compatibility as demonstrated in the transplantation of tissues or organs from one member to another of the same species (an *allograft*), or from one species to another (a *xenograft*).

13.5: MECHANISMS OF GRAFT REJECTION

Allogeneic MHC molecules are presented for recognition by the T cells of a graft recipient in two distinctly different ways:

(*a*) Direct presentation and (*b*) Indirect presentation.

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13.5.1: Direct presentation:

Direct presentation involves recognition of an intact MHC molecule displayed by donor antigen-presenting cells (APCs) in the graft. It depends on the similarity in the structure of an intact foreign (allogeneic) molecule and self-MHC molecules. Direct recognition of foreign MHC molecules is a cross reaction of a normal T-cell receptor, which is selected to recognize a self-MHC molecule and foreign peptide, with an allogeneic MHC molecule and peptide. This is because an allogeneic MHC molecule with a bound peptide can mimic the determinant formed by a self-MHC molecule and a particular foreign peptide.

As many as 2% of an individual's T cells are capable of recognizing and responding to a single foreign MHC molecule, and this high frequency of T cells reactive with allogeneic MHC molecules is one reason that allografts elicit strong immune responses *in vivo.*

13.5.2: Indirect presentation:

The "indirect presentation" involves the recognition of processed allogeneic MHC molecules but not an intact MHC molecule. It involves processing of donor MHC molecules by recipient APCs and presentation of derived peptides from the allogeneic MHC molecules in association with self-MHC molecules. Here the processed MHC molecules are recognized by T cells like conventional foreign protein antigens. Indirect presentation may result in allorecognition by CD4_T cells. This is because alloantigen is acquired primarily through the endosomal vesicular pathway and is therefore presented by class II MHC molecules. Some antigens of phagocytosed graft cells appear to enter the class I MHC pathway of antigen presentation and are indirectly recognized by CD8_ T cells.

13.6: STAGES OF CELL-MEDIATED GRAFT REJECTION

Cell-mediated graft rejection could occur in two stages:

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(a) A sensitization phase, in which antigen-reactive lymphocytes of the recipient proliferate in response to alloantigens on the graft and

(b) An effector stage, in which immune destruction of the graft takes place.

During the sensitization phase, CD4_and CD8_ T cells recognize alloantigens expressed on the cells of foreign graft and proliferate in response. The response to major histocompatibility antigens involves recognition of both the donor MHC molecule and an associated peptide ligand in the cleft of the MHC molecule. The peptides present in the groove of allogeneic class I MHC molecules are derived from proteins synthesized within the allogeneic cell. The peptides present in the groove of allogeneic class II MHC molecules are generally proteins that are taken up and processed through the endocytic pathway of the allogeneic APC.

Recognition of the alloantigens expressed on the cells of a graft induces vigorous T-cell proliferation in the host. This proliferation can be demonstrated *in vitro* in a mixed lymphocyte reaction. Both dendritic cells and vascular endothelial cells from an allogeneic graft induce host T-cell proliferation. The CD4_ T cell is the major proliferating cell that recognizes class II alloantigens directly or alloantigen peptides presented by host APCs. This amplified population of activated TH cells is believed to play a key role in inducing the various effector mechanisms of allograft rejection.

13.7: CLINICAL FEATURES OF GRAFT REJECTION

Rejection episodes, based primarily on the time elapsed between transplantation and the rejection episode, are traditionally classified as (*a*) hyperacute, (*b*) acute, and (*c*) chronic rejections.

13.7.1: Hyperacute rejection

Hyperacute rejection occurs usually within the first few hours post-transplantation and is mediated by preformed antibodies against ABO or MHC antigens of the graft. Possibly, antibodies directed against other alloantigens, such as vascular endothelial antigens, also play a role in this type of rejection. Once the antibodies bind to the transplanted tissues, rejection can be caused either (*a*) by activation of the complement system,

which results in the chemotactic attraction of granulocytes and the triggering of inflammatory circuits, or (*b*) by ADCC.

Pathological features of hyperacute rejection are following:

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■ This is associated with the formation of massive intravascular platelet aggregates leading to thrombosis, ischemia, and necrosis.

■ The hyperacute rejection episodes are irreversible and invariably results in graft loss. With proper cross-matching techniques, this type of rejection is almost 100% avoidable.

■ The hyperacute rejection by antibodies to all human cellular antigens is the major limitation of xenogeneic transplantation (e.g., pig to human).

13.7.2: Acute rejection

Acute rejection occurs mostly in the first few days or weeks after transplantation:

■ When acute rejection takes place in the first few days after grafting, it may correspond to a secondary (second set) immune response. This indicates that the patient had been previously sensitized to the HLA antigens present in the organ donor (as a consequence of a previous transplant, pregnancy, or blood transfusions).

■ When graft rejection occurs first week after grafting, it usually corresponds to a first-set (primary) response. Up to 70% of graft recipients experience one or more acute rejection episodes.

Acute rejection is predominantly mediated by T lymphocytes. CD4_ helper T lymphocytes are believed to play the key role in acute rejection of the graft. This is because they release growth factors like IL-2 and IL-4 for the promotion of clonal expansion of CD8_ lymphocytes and B cells. In rejected organs, the cellular infiltrates contain mostly monocytes and T lymphocytes of both helper and cytotoxic phenotypes, and lesser numbers of B lymphocytes, NK (natural killer) cells, neutrophils, and eosinophils. All these cells have the potential to play significant roles in the rejection process.

The initial diagnosis of acute rejection is usually based on clinical suspicion:

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■ Functional deterioration of the grafted organ is the main basis for considering the diagnosis of acute rejection.

■ Confirmation usually requires a biopsy of the grafted organ.

■ Mononuclear cell infiltration in tissues of rejected graft tissue is characteristic finding.

■ The measurement of cytokines (such as IL-2) in serum and in urine (in the case of renal transplants) is another diagnostic approach. In most cases, acute rejection, if detected early, can be reversed by increasing the dose of immunosuppressive agents or by briefly administering additional immunosuppressants.

13.7.3: Delayed or chronic rejection

This is characterized by an insidiously progressive loss of function of the grafted organ. The functional deterioration associated with chronic rejection appears to be due to both immune and nonimmune processes. Vascular endothelial injury is the most common feature. Granulocytes, monocytes, and platelets are found to increasingly adhere to injured vascular endothelium. The damaged endothelium is covered by a layer of platelets and fibrin, and eventually by proliferating fibroblasts and smooth muscle cells. The end result is a proliferative lesion in the vessels, which progresses toward fibrosis and occlusion.

13.8: PREVENTION OF GRAFT REJECTION

Immunosuppression of the host prevents graft rejection (Tabl13.1). It is achieved by treatment with radiation, corticosteroids, and antilymphocyte serum. Cyclosporin A and rapamycin are also used, which cause immunosuppression by specific inhibition of T cells.

13.9: GRAFT-VERSUS-HOST REACTION

Whenever a patient with a profound immunodeficiency (primary, secondary, or iatrogenic) receives a graft of an organ rich in immunocompetent cells, there is a considerable risk that a graft-versus-host (GVH) reaction may develop. The probability of developing a GVH reaction is greatest in the 2-month period immediately following transplantation. GVH reactions require three important components, which are:

■ the donor graft must contain immunocompetent T cells,

■ the host must be immunocompromised, and

■ the recipient should express antigens, such as MHC proteins, which will be identified as foreign to the donor. For example, donor T cells recognize the recipient cells as foreign.

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Transplantation of organs, such as the heart and kidneys—poor in endogenous lymphoid tissue—very rarely results in a GVH reaction. GVH reactions occur because the donor T lymphocytes become activated, proliferate, and differentiate into helper and effector cells in the irradiated, immunocompromised host. These activated T cells attack the host cells and tissues, producing the signs and symptoms of GVH disease. The donor's cytotoxic T cells play a key role in destroying recipient's cells. The crucial role played by the donor T cells is demonstrated by the fact that removal of these T cells from a bone marrow graft prevents GVH reactions.

Table 13.1: Immunosuppressive Agents used in Transplantation

Immunosuppressive Agents	Mode of action
Azathioprine	Inhibition of nucleotide synthesis of multiple cells
Cyclophosphamide	Inhibition of nucleotide synthesis of multiple cells
Cyclosporine	Inhibition of transcription of cytokines in lymphocytes
Corticosteroids	Inhibition of transcription for cytokines and products involved in inflammation in multiple cells
Sirolimus	Inhibition of transduction induced by cytokines in T cells
Irradiation	DNA damage in all rapidly proliferating cells
Anti-CD4 and CD8 antibodies	Interference with T-cell receptor binding of CD4 and CD8 T cells

NOTES

The initial proliferation of donor T cells takes place in lymphoid tissues, particularly in the liver and spleen leading to hepatomegaly and splenomegaly. Later, at the peak of the proliferative reaction, the skin and intestinal walls are heavily infiltrated leading to severe skin rashes or exfoliative dermatitis and severe diarrhea. Finally, many GVH reactions end in overwhelming infections and death. All immunosuppressive drugs used in the prevention and treatment of rejection have been used for treatment of the GVH reaction. Thalidomide, the tranquilizer drug that achieved notoriety due to its teratogenic effects, has been used successfully for the control of chronic GVH unresponsive to traditional immunosuppressants.

13.10: ONCOGENES AND ANTIONCOGENES

An oncogene is defined as a gene that encodes a protein that is capable of transforming cells in culture or inducing cancer in animals leads to alterations in critical regulatory genes that control cell proliferation, differentiation, and survival. However, the majority (approximately 80%) of human cancers is not induced by viruses and apparently arises from other causes, such as radiation and chemical carcinogens. Therefore, in terms of our overall understanding of cancer, it has been critically important that studies of viral oncogenes also led to the identification of cellular oncogenes, which are involved in the development of non-virus-induced cancers. The key link between viral and cellular oncogenes was provided by studies of the highly oncogenic retroviruses.

The anti-oncogenes or tumor suppressor genes or recessive oncogenes are normally implicated in a negative regulation of cellular proliferation. The loss of their activity contributes to tumorigenesis in a recessive manner. Genetic events activating proto-oncogenes or inactivating anti-oncogenes accumulate in the same cell during tumor progression and co-operate to determine the malignant invasive phenotype of advanced tumors.

13.11: CHECK YOUR PROGRESS

1. ……is the transfer of individual's own tissue or organ from one site to another site in the body
2. GVH reaction…….
3. **……..**is defined as a gene that encodes a protein that is capable of transforming cells

13.12: LET US SUM UP

In this unit, you have learnt about the meaning, definition, need, objectives and importance of organ transplantation and Clinical features of graft rejection. Transplantation can be defined as the transfer of cells, tissues, or organs from one site in an individual to another, or between two individuals. In the latter case, the individual who provides the transplant organ is termed a donor and the individual receiving the transplant is known as the recipient. Histocompatibility is tissue compatibility as demonstrated in the transplantation of tissues or organs from one member to another of the same species (an allograft), or from one species to another (a xenograft).

NOTES

13.13: UNIT - END EXERCISES

1. Write in detail account on transplantation.
2. Explain the Clinical features of graft rejection
3. Give a brief note on Oncogenes and Antioncogenes.

13.14: ANSWERS TO CHECK YOUR PROGRESS

1. An autograft
2. Graft-Versus-Host Reaction
3. An oncogene

13.15: SUGGESTED READINGS

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NOTES

UNIT - XIV

14.1: INTRODUCTION

Hybridoma technology is a method for producing large numbers of identical antibodies (also called monoclonal antibodies). This process starts by injecting a mouse (or other mammal) with an antigen that provokes an immune response. A type of white blood cell, the B cell, produces antibodies that bind to the injected antigen. These antibody producing B-cells are then harvested from the mouse and, in turn, fused with immortal B cell cancer cells, a myeloma,[clarification needed] to produce a hybrid cell line called a hybridoma, which has both the antibody-producing ability of the B-cell and the exaggerated longevity and reproductivity of the myeloma. The hybridomas can be grown in culture, each culture starting with one viable hybridoma cell, producing cultures each of which consists of genetically identical hybridomas which produce one antibody per culture (monoclonal) rather than mixtures of different antibodies (polyclonal). The myeloma cell line that is used in this process is selected for its ability to grow in tissue culture and for an absence of antibody synthesis. In contrast to polyclonal antibodies, which are mixtures of many different antibody molecules, the monoclonal antibodies produced by each hybridoma line are all chemically identical.

14.2: OBJECTIVES

After going through this unit, you will be able:

- To know about the Hybridoma and Monoclonals
- To know the Vaccine and its types
- To know about the stem cells

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14.3: HYBRIDOMA AND MONOCLONAL ANTIBODIES

Most antigens offer multiple epitopes and therefore induce proliferation and differentiation of a variety of B-cell clones, each derived from a B cell that recognizes a particular epitope. The resulting serum antibodies are heterogeneous, comprising a mixture of antibodies, each specific for one epitope (Fig 14.1). Such a polyclonal antibody response facilitates the localization, phagocytosis, and complement-mediated lysis of antigen; it thus has clear advantages for the organism in vivo.

Unfortunately, the antibody heterogeneity that increases immune protection in vivo often reduces the efficacy of an antiserum for various in vitro uses. For most research, diagnostic, and therapeutic purposes, monoclonal antibodies, derived from a single clone and thus specific for a single epitope, are preferable. Direct biochemical purification of a monoclonal antibody from a polyclonal antibody preparation is not feasible. In 1975, Georges Kohler and Cesar Milstein devised a method for preparing monoclonal antibody, which quickly became one of immunology's key technologies. By fusing a normal activated, antibody-producing B cell with a myeloma cell (a cancerous plasma cell), they were able to generate a hybrid cell, called a hybridoma, that possessed the immortal growth properties of the myeloma cell and secreted the antibody produced by the B cell (Fig 14.2).

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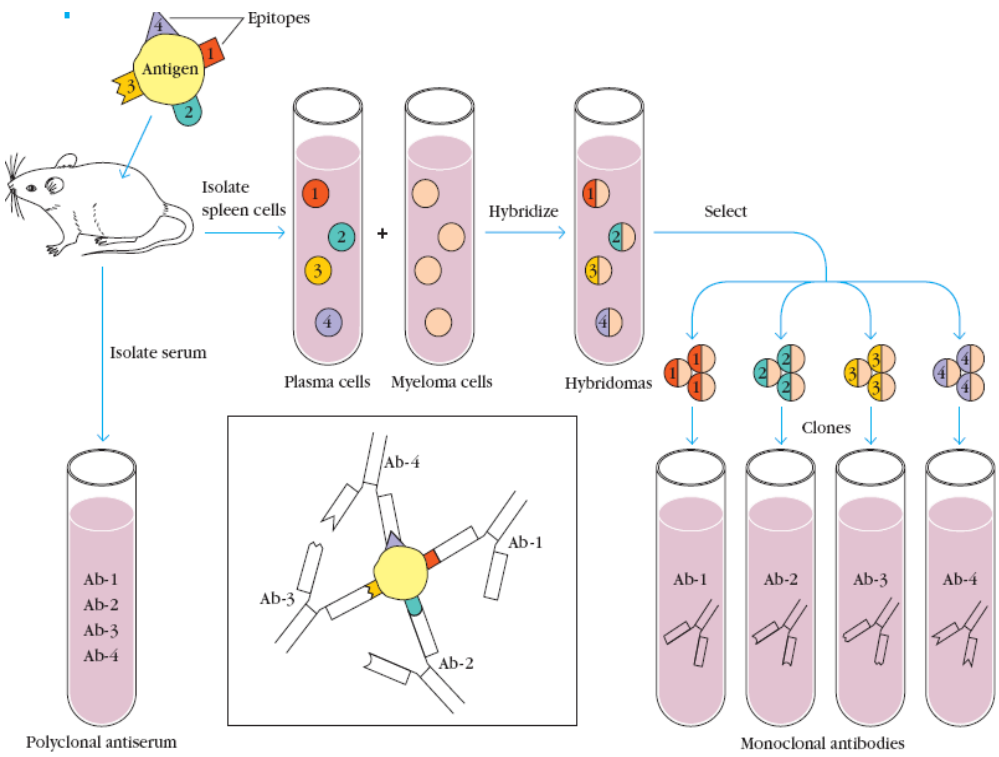


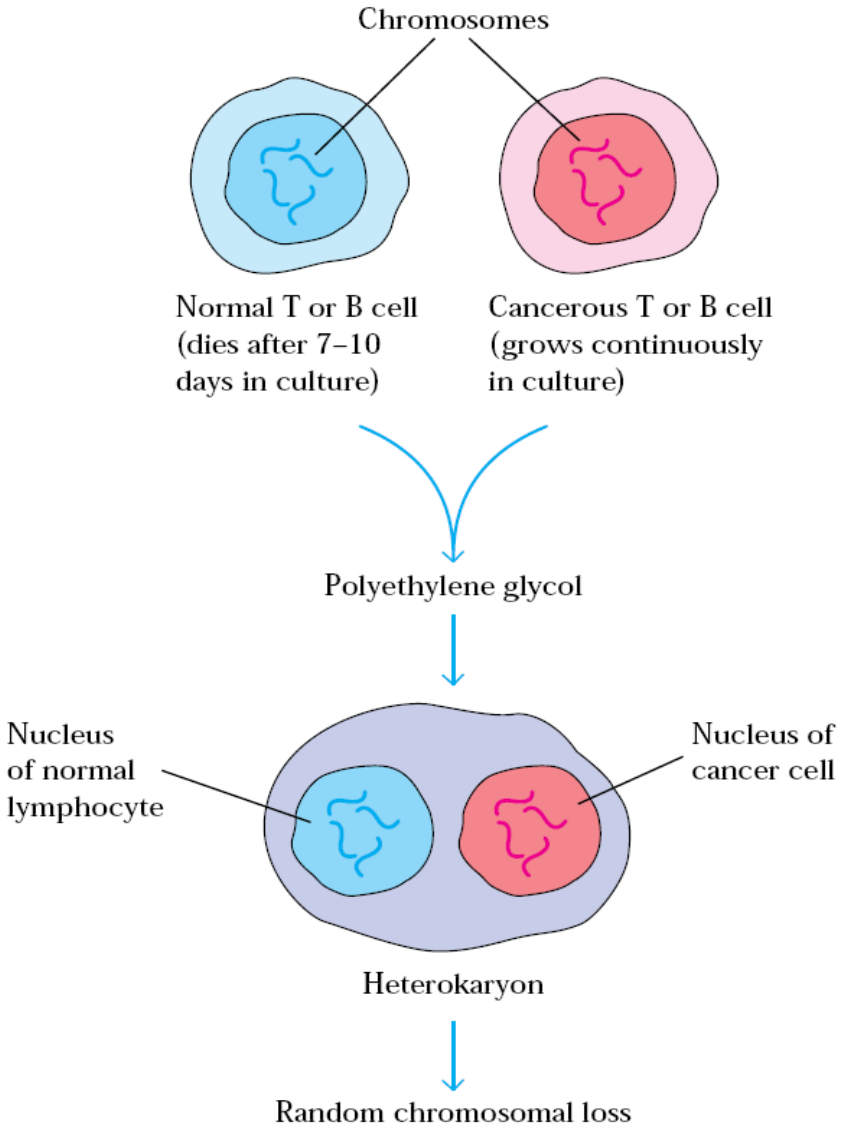
Figure 14.1: The conventional polyclonal antiserum produced in response to a complex antigen contains a mixture of monoclonal antibodies, each specific for one of the four epitopes shown on the antigen (inset). In contrast, a monoclonal antibody, which is derived from a single plasma cell, is specific for one epitope on a complex antigen. The outline of the basic method for obtaining a monoclonal antibody is illustrated here. [Thomas J. Kindt, Richard A. Goldsby, Barbara Anne Osborne, Janis Kuby. *Immunology, 2007, W.H. Freeman, ed.*,]

The resulting clones of hybridoma cells, which secrete large quantities of monoclonal antibody, can be cultured indefinitely. The development of techniques for producing monoclonal antibodies, gave immunologists a powerful and versatile research tool. The significance of the work by Kohler and Milstein was acknowledged when each was awarded a Nobel Prize.

14.3.1 Hybrid Lymphoid Cell Lines

In somatic-cell hybridization, immunologists fuse normal B or T lymphocytes with tumor cells, obtaining hybrid cells, orheterokaryons, containing nuclei from both parent cells. Random loss of some chromosomes and subsequent cell proliferation yield a clone of cells that contain a single

nucleus with chromosomes from each of the fused cells; such a clone is called a hybridoma. Historically, cell fusion was promoted with Sendai virus, but now it is generally done with polyethylene glycol. Normal antigen-primed B cells can be fused with cancerous plasma cells, called myeloma cells (Fig 14.2). The hybridoma thus formed continues to express the antibody genes of the normal B lymphocyte but is capable of unlimited growth, a characteristic of the myeloma cell. B-cell hybridomas that secrete antibody with a single antigenic specificity, called monoclonal antibody, in reference to its derivation from a single clone, have revolutionized not only immunology but biomedical research as well as the clinical laboratory. The production and uses of monoclonal antibodies in detail.



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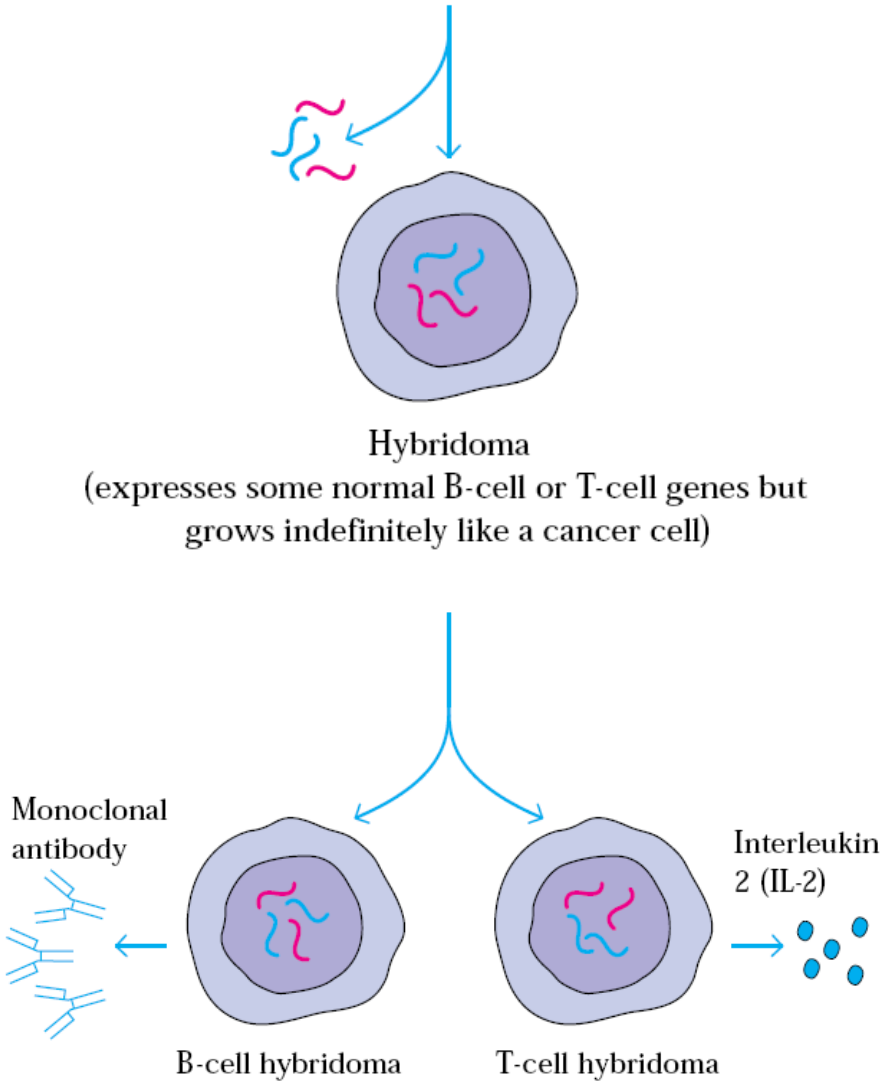

Figure 14.2: Production of B-cell and T-cell hybridomas by somatic-cell hybridization. The resulting hybridomas express some of the genes of the original normal B or T cell but also exhibit the immortal-growth properties of the tumor cell. This procedure is used to produce B-cell hybridomas that secrete monoclonal antibody and T-cell hybridomas that secrete various growth factors. [Thomas J. Kindt TJ, Richard A. Goldsby RA, Barbara Anne Osborne BA, Janis Kuby. *Immunology, 2007, W.H. Freeman, ed.*,]

T-cell hybridomas can also be obtained by fusing T lymphocytes with cancerous T-cell lymphomas. Again, the resulting hybridoma continues to express the genes of the normal T cell but acquires the immortal-growth properties of the cancerous T lymphoma cell. Immunologists have generated

a number of stable hybridoma cell lines representing T-helper and T-cytotoxic lineages.

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14.4: VACCINES

Vaccines may be live organisms, killed organisms, or modified toxins. Although no vaccine is ideal and each has its problems, the problems of live vaccines are generally related to their safety, while the problems of killed vaccines are related mainly to their effectiveness.

14.4.1 LIVE OR ATTENUATED VACCINES:

Live or attenuated vaccines are useful because they infect, replicate, and immunize in a manner similar to natural infection but with milder clinical symptoms. Examples include many of the childhood infections such as measles, mumps, and rubella (MMR vaccine), chicken pox (varicella) and Bacille Calmette-Guérin (BCG) for tuberculosis. Although millions of doses have been administered with no complications, if given to an immunocompromised host (such as primary immunodeficiency or secondary to HIV infection), these live vaccines may cause serious disease.

14.4.2 KILLED VACCINES

Killed vaccines consist of suspensions of killed organisms such as typhoid, cholera, and pertussis (although there is now an acellular vaccine) or one of the products or fractions of the organism. These include toxoids of diphtheria and tetanus and subunits of viruses such as surface hepatitis B antigen. Among the most successful of these types of vaccines has been the use of polysaccharides in the pneumococcal, meningococcal, and *Haemophilus influenza* vaccines. In general, the killed vaccines are not as effective as the live viruses because they do not give long-lasting immunity as a live infection does. For example, although the tetanus toxoid vaccine is effective, it requires a booster dose every ten years. The immunological response to the killed organism or product thereof has been enhanced by the use of adjuvants. Although the most common adjuvant for animal studies has been the complete Freund's adjuvant, it cannot be used in humans because it causes liver, skin, and spleen dysfunction. The most common adjuvant forhumans is aluminum compounds, which are generally safe for human use.

Others include muramyl dipeptide, biodegradable polymers, and a glycoside adjuvant called Quil A from the bark of an Amazon oak tree. However, many others are being developed or will probably be given U.S. Food and Drug Administration (FDA) acceptance in the future.

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The key feature will be their immunogenic enhancement and their strength of safety for use in humans. Although we have mainly been discussing various forms of vaccination to protect against the invading organism, one of the most interesting new vaccines has not been developed to eliminate the infectious agent but rather to prevent the development of another far more serious disease – a complication of the initial infection. This is the Gardisal vaccine manufactured by Merck to protect against human papilloma virus (HPV). HPV infection is usually sexually acquired, and it is estimated that currently 20 million people are infected in the United States.

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The infection has no real signs or symptoms, and HPV may lead to cervical cancer. It is estimated that ten of the thirty different serotypes of the virus can induce cervical cancer, so the vaccine has been directed at eliminating those serotypes. Thus, if 10,000 women are infected with one of the high-risk viral serotypes, approximately 3,900 of them will die of cervical cancer. The new vaccine, if given before active sexual activity in women, can prevent the viral infection and thereby markedly diminish the risk of cervical cancer. Because of the possible success of this vaccine, it may be worthwhile to look at how to prevent the Epstein-Barr virus in at-risk children to prevent or diminish the risk of Burkett's lymphoma in children infected with the virus.

14.4.3: DNA VACCINES

The first demonstration of the usefulness of the vaccine DNA was done by injection of a human growth hormone-encoding plasmid (HGH) to the mouse. In this first test, the HGH gene was injected into the skin of the ear of the mouse to produce its protein for treatment. Several immunised mice showed remarkable levels of antibodies. Similar to the immune response observed in the viral infection or the weakened virus vaccination, intracellular production of peritoneal or peptide antigen induces a high Th1 cellular response. However, due to low protein production (coded by a DNA

vaccine), the Th2 immune response is low. Considering the usefulness of DNA vaccines in small animal models, further clinical trial studies were carried out. One of several Phase I trials, which began about two decades ago, is about evaluating the DNA vaccine of the HIV-1 virus for the purpose of therapeutic and preventative use. Further studies have included DNA vaccines for other HIV-1 antigens, cancer antigens, influenza, HPV, hepatitis and malaria. However, the results of these early studies were not satisfactory. DNA vaccines are safe and well tolerated, but proved to be poorly immunised. The induced antibody titre is very low or absent, the CD + 8 T-cell response is rarely present, and the response of T-type CD + 4s is also low. However, these studies have shown that DNA vaccines can safely produce a human immune response.

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Table: 14.1: Types of Vaccines

Types of vaccine	Examples	Form of protection
Live attenuated, or killed, bacteria	BCG, cholera	Antibody response
Live attenuated viruses	Polio, rabies	Antibody response; cell-mediated immune response
Subunit (antigen) vaccines	Tetanus toxoid, diphtheria toxoid	Antibody response
Conjugate vaccines	*Haemophilus influenzae* infection	Helper T cell–dependent antibody response to polysaccharide antigens
Synthetic vaccines	Hepatitis virus (recombinant proteins)	Antibody response
Viral vectors	Clinical trials of HIV antigens in canary pox vector	Cell-mediated and humoral immune responses
DNA vaccines	Clinical trials ongoing for several infections	Cell-mediated and humoral immune responses

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14.5: STEM CELLS

All cells of the lymphoid and myeloid lineages are derived from a ***common hematopoietic stem cell progenitor*** in the bone marrow (Fig 14.1). These stem cells, which are self-renewing, give rise to a ***common lymphoid progenitor*** as well as a ***common myeloid progenitor***, from which the various types of lymphoid and myeloid cells differentiate (Fig 14.1). This process, called ***hematopoiesis***, is complex and takes place under the guidance of multiple factors within the bone marrow, including stromal cells, the factors they produce and the influence of the extracellular matrix. Indeed, the study of this process is a whole research discipline in itself (hematology) and it has taken many years to unravel the multiplicity of cues that dictate the production of the formed elements of the blood. However, the basic scheme is that the various soluble and membrane-bound hematopoietic factors influence the differentiation of the various myeloid and lymphoid cell types in a stepwise series of events that involve the switching on of different ***transcriptional programs*** at each stage of the hierarchy, such that immature precursor cells are guided towards a variety of specific terminally differentiated cellular phenotypes (monocytes, neutrophils, mast cells, etc.).

This process can also be influenced by factors external to the bone marrow (such as cytokines that are produced in the context of immune responses), to ramp up the production of specific cell types according to demand. Make no mistake, this is a large-scale operation with the average human requiring the production of almost 4×10^{11} leukocytes (400 billion) per day. One of the reasons for this prodigious rate of cell production is that many of the cells of the immune system, particularly the granulocytes (neutrophils, basophils, and eosinophils), have half-lives of only a day or so. Thus, these cells require practically continuous replacement. Upon differentiation to specific mature lymphoid and myeloid cell types, the various leukocytes exit the bone marrow and either circulate in the bloodstream until required or until they die (granulocytes), or migrate to the peripheral tissues where they differentiate further under the influence of

tissue specific factors (monocytes, mast cells, dendritic cells), or undergo further selection and differentiation in specialized compartments

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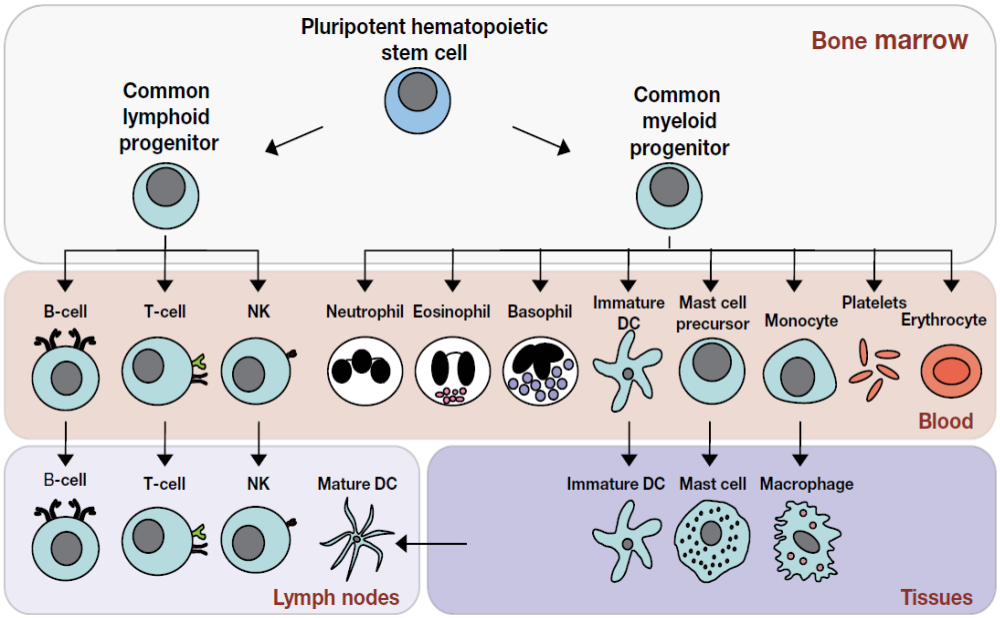

Fig 14.1: The cells of the immune system originate in the bone marrow from pluripotent hematopoietic stem cells. Pluripotent hematopoietic stem cells give rise to a common lymphoid progenitor, which gives rise to all of the major lymphoid cell types (T-cells, B-cells, and NK cells) or a common myeloid progenitor, which gives rise to all of the major myeloid cell types (neutrophils, eosinophils, basophils, dendritic cells [DCs], mast cells, and monocytes/macrophages) as well as the erythrocytes and megakaryocytes (which generate platelets).

14.5.1: STEM CELLS—CLINICAL USES AND POTENTIAL

Stem Cells transplantation holds great promise for the regeneration of diseased, damaged, or defective tissue. Hematopoietic stem cells are already used to restore hematopoietic cells, and their use is described in the clinic below. However, rapid advances in stem-cell research have raised the possibility that other stem-cell types, too, may soon be routinely employed for replacement of other cells and tissues. Two properties of stem cells underlie their utility and promise. They have the capacity to give rise to more differentiated cells, and they are self-renewing, because each division of a stem cell creates atleast one stem cell. If stem cells are classified according

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to their descent and developmental potential, four levels of stem cells can be recognized: totipotent, pluripotent, multipotent, and unipotent.

Totipotent cells can give rise to an entire organism. A fertilized egg, the zygote, is a totipotent cell. In humans the initial divisions of the zygote and its descendants produce cells that are also totipotent. In fact, identical twins, each with its own placenta, develop when totipotent cells separate and develop into genetically identical fetuses.

Pluripotent stem cells arise from totipotent cells and can give rise to most but not all of the cell types necessary for fetal development. For example, human pluripotent stem cells can give rise to all of the cells of the body but cannot generate a placenta. Further differentiation of pluripotent stem cells leads to the formation of multipotent and unipotent stem cells.

Multipotent stem cells can give rise to only a limited number of cell types, and **unipotent cells** to a single cell type. Pluripotent cells, called embryonic stem cells, or simply ES cells, can be isolated from early embryos, and for many years it has been possible to grow mouse ES cells as cell lines in the laboratory. Strikingly, these ES cells can be induced to generate many different types of cells. Mouse ES cells have been shown to give rise to muscle cells, nerve cells, liver cells, pancreatic cells, and, of course, hematopoietic cells.

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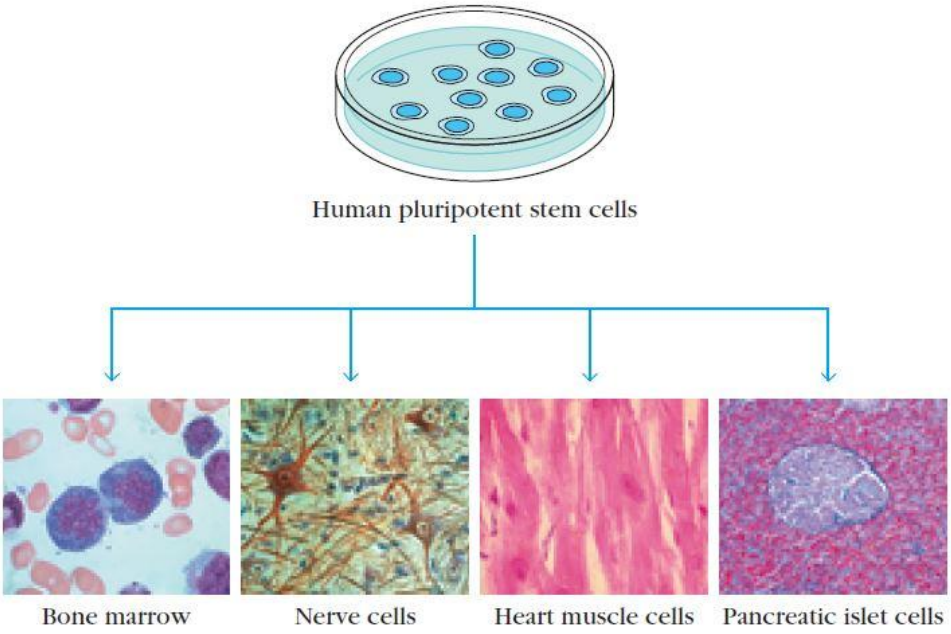

Human pluripotent stem cells can differentiate into a variety of different cell types, some of which are shown here. [*Adapted from* Stem Cells: A Primer, *NIH web site* http://www.nih.gov/news/stemcell/primer.htm. *Micrographs (left to right): Biophoto Associates/Science Source/Photo Researchers; Biophoto Associates/Photo Researchers; AFIP/Science Source/Photo Researchers; Astrid & Hanns-Frieder Michler/Science Photo Library/Photo Researchers.*]

Fig 14.2: Human Pluripotent Stem Cell

Recent advances have made it possible to grow lines of human pluripotent cells. This is a development of considerable importance to the understanding of human development, and it has great therapeutic potential. In vitro studies of the factors that determine or influence the development of human pluripotent stem cells along one developmental path as opposed to another will provide considerable insight into the factors that affect the differentiation of cells into specialized types. There is also great interest in exploring the use of pluripotent stem cells to generate cells and tissues that could be used to replace diseased or damaged ones. Success in this endeavor would be a major advance because transplantation medicine now depends totally upon donated organs and tissues. Unfortunately, the need far exceeds the number of donations and is increasing. Success in deriving practical quantities of cells, tissues, and organs from pluripotent stem cells would provide skin replacement for burn patients, heart muscle cells for those with

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chronic heart disease, pancreatic islet cells for patients with diabetes, and neurons for use in Parkinson's disease or Alzheimer's disease.

The transplantation of hematopoietic stem cells (HSCs) is an important therapy for patients whose hematopoietic systems must be replaced. It has three major applications:

1. Providing a functional immune system to individuals with a genetically determined immunodeficiency, such as severe combined immunodeficiency (SCID).

2. Replacing a defective hematopoietic system with a functional one to cure some patients who have a life threatening nonmalignant genetic disorder in hematopoiesis, such as sickle-cell anemia or thalassemia.

3. Restoring the hematopoietic system of cancer patients after treatment with doses of chemotherapeutic agents and radiation so high that they destroy the system. These high-dose regimens can be much more effective at killing tumor cells than are therapies that use more conventional doses of cytotoxic agents. Stem-cell transplantation makes it possible to recover from such drastic treatment. Also, certain cancers, such as some cases of acute myeloid leukemia, can be cured only by destroying the source of the leukemia cells, the patient's own hematopoietic system.

Restoration of the hematopoietic system by transplanting stem cells is facilitated by several important technical considerations. First, HSCs have extraordinary powers of regeneration. Experiments in mice indicate that only a few—perhaps, on occasion, a single HSC—can completely restore the erythroid population and the immune system. In humans it is necessary to administer as little as 10% of a donor's total volume of bone marrow to provide enough HSCs to completely restore the hematopoietic system.

Once injected into a vein, HSCs enter the circulation and find their own way to the bone marrow, where they begin the process of engraftment. There is no need for a surgeon to directly inject the cells into bones. In addition, HSCs can be preserved by freezing. This means that hematopoietic cells can be "banked." After collection, the cells are treated with a cryopreservative, frozen, and then stored for later use. When needed, the

frozen preparation is thawed and infused into the patient, where it reconstitutes the hematopoietic system. This cell-freezing technology even makes it possible for individuals to store their own hematopoietic cells for transplantation to themselves at a later time. Currently, this procedure is used to allow cancer patients to donate cells before undergoing chemotherapy and radiation treatments and then to reconstitute their hematopoietic system from their own stem cells. Hematopoietic stem cells are found in cell populations that display distinctive surface antigens. One of these antigens is CD34, which is present on only a small percentage (~1%) of the cells in adult bone marrow. An antibody specific for CD34 is used to select cells displaying this antigen, producing a population enriched in CD34_ stem cells. Various versions of this selection procedure have been used to enrich populations of stem cells from a variety of sources.

Transplantation of stem cell populations may be **autologous** (the recipient is also the donor), **syngeneic** (the donor is genetically identical, i.e., an identical twin of the recipient), or **allogeneic** (the donor and recipient are not genetically identical). In any transplantation procedure, genetic differences between donor and recipient can lead to immune-based rejection reactions. Aside from host rejection of transplanted tissue (host versus graft), lymphocytes in the graft can attack the recipient's issues, thereby causing **graft versus- host disease (GVHD),** a life threatening affliction.

In order to suppress rejection reactions, powerful immunosuppressive drugs must be used. Unfortunately, these drugs have serious side effects, and immunosuppression increases the patient's risk of infection and further growth of tumors. Consequently, HSC transplantation has fewest complications when there is genetic identity between donor and recipient. At one time, bone-marrow transplantation was the only way to restore the hematopoietic system. However, the essential element of bone-marrow transplantation is really stem-cell transplantation. Fortunately, significant numbers of stem cells can be obtained from other tissues, such as peripheral blood and umbilical-cord blood ("cord blood"). These alternative sources of

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HSCs are attractive because the donor does not have to undergo anesthesia and the subsequent highly invasive procedure that extracts bone marrow. Many in the transplantation community believe that peripheral blood will replace marrow as the major source of hematopoietic stem cells for many applications.

To obtain HSC-enriched preparations from peripheral blood, agents are used to induce increased numbers of circulating HSCs, and then the HSC containing fraction is separated from the plasma and red blood cells in a process called leukopheresis. If necessary, further purification can be done to remove T cells and to enrich the CD34_ population. Umbilical cord blood already contains a significant number of hematopoietic stem cells. Furthermore, it is obtained from placental tissue (the "afterbirth") which is normally discarded. Consequently, umbilical cord blood has become an attractive source of cells for HSC transplantation. Although HSCs from cord blood fail to engraft somewhat more often than do cells from peripheral blood, grafts of cord blood cells produce GVHD less frequently than do marrow grafts, probably because cord blood has fewer mature T cells.

Beyond its current applications in cancer treatment, many researchers feel that autologous stem-cell transplantation will be useful for gene therapy, the introduction of a normal gene to correct a disorder caused by a defective gene. Rapid advances in genetic engineering may soon make gene therapy a realistic treatment for genetic disorders of blood cells, and hematopoietic stem cells are attractive vehicles for such an approach. The therapy would entail removing a sample of hematopoietic stem cells from a patient, inserting a functional gene to compensate for the defective one, and then reinjecting the engineered stem cells into the donor. The advantage of using stem cells in gene therapy is that they are self-renewing. Consequently, at least in theory, patients would have to receive only a single injection of engineered stem cells. In contrast, gene therapy with engineered mature lymphocytes or other blood cells would require periodic injections because these cells are not capable of self renewal.

14.6: CHECK YOUR PROGRESS

1. SCID …….
2. …….. vaccines are provided at the time of birth
3. What is GVHD…….
4. ……which are self-renewing, give rise to a common lymphoid progenitor as well as a common myeloid progenitor

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14.7: LET US SUM UP

In this unit, you have learnt about the meaning, definition, need, objectives and importance of Hybridoma and monoclonals, Vaccine types and stem cells and its clinical application. Hybridoma technology is a method for producing large numbers of identical antibodies (also called monoclonal antibodies). Vaccines may be live organisms, killed organisms, or modified toxins. Live or attenuated vaccines are useful because they infect, replicate, and immunize in a manner similar to natural infection but with milder clinical symptoms. The immunological response to the killed organism or product thereof has been enhanced by the use of adjuvants. All cells of the lymphoid and myeloid lineages are derived from a common hematopoietic stem cell progenitor in the bone marrow.

14.8: UNIT - END EXERCISES

1. Write in Detail about the Hybridoma and monoclonal antibodies
2. Define vaccine. Give brief note on types of vaccines
3. Ellaborate stem cells and its clinical applications
4. Give short notes on bone marroe and nerve cells

14.9: ANSWERS TO CHECK YOUR PROGRESS

1. Severe combined immunodeficiency.
2. BCG.
3. Graft versus- host disease.
4. Stem cells

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14.10: SUGGESTED READINGS

1. Jenni Punt, Sharon, A., Stranford; Patricia P Jones; Janis Kuby. (2013). **Kuby Immunology,** New York, W.H. Freeman.
2. Joseph A. Bellanti. (2016). **Immunology IV: Clinical Applications in Health and Disease.** Georgetown University School of Medicine, Washington, DC.
3. Michael J. Day, Ronald D. Schultz. (2014). **Veterinary Immunology: Principles and Practice** (2nd Edition), CRC Press.
4. Raif Geha, Luigi Notarangelo. (2016). **Case Studies in Immunology. A Clinical Companion** (7th Edition), ASM Press.
5. Rao, C. V. (2013). **Immunology** (2nd Edition), Narosa Publishing House. New Delhi.
6. Richard Coico and Geoffrey Sunshine. (2015). **Immunology: A Short Course** (7th Edition). Wiley-Blackwell.
7. Singh R.P. (2015). **Immunology and Medical Microbiology** (2nd Edition), Kalyani Publishers, New Delhi.

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MODEL QUESTION PAPER
Paper – 36431: IMMUNOLOGY

Time: 3 hours **Max Marks: 75**

PART– A **(10x 2= 20 Marks)**

Answer all questions

1. Immunity
2. Hematopoiesis
3. T Cell
4. Cytokines
5. Immunoglobulins
6. Epitope
7. Avidity
8. Antibody
9. Antigen
10. Nerve Cell

PART– B **(5x5=25 Marks)**

Answer all questions choosing either (a) or (b)

11. a) Discuss the Basic Concepts in Immunology.

(or)

b) Write in detail about any one Primary Lymphoid Organs.

12. a) Difference between Innate and Acquired Immune system.

(or)

b) Write short note on B Cell Receptor.

13. a) What are the functions of cytokines?

(or)

b) Give an account of Immunoglobulin G.

14. a) Write short note on Precipitation.

(or)

b) Discuss about Complement Activation.

15. a) Write an account of Antigen Presentation?

(or)

b) Discuss about Hypersensitivity Reactions.

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PART – C **(3x10 = 30 Marks)**

Answer any 3 out of 5 questions.

16. Describe in detail about Cells of the Immune System.
17. Define autoimmune disorder? And the causes for it.
18. Elaborate about MHC.
19. Discuss in detail about Hybridoma Technology.
20. Detailed account of Hematopoietic Stem Cell.